Zachodniopomorski Uniwersytet Technologiczny
w Szczecinie

Wydział Kształtowania Środowiska i Rolnictwa

mgr inż. Arleta Kruczek

Rozprawa doktorska

# Wpływ zabiegów agrotechnicznych na plonowanie i jakość owoców kolcowoju chińskiego (*Lycium chinense* Mill.) oraz jego wzrost w kulturach *in vitro*

Influence of agrotechnical measures on yield, fruit quality and growth in *in vitro* cultures of *Lycium chinense* Mill.

Promotor
dr hab. Ireneusz Ochmian, prof. ZUT

Katedra Ogrodnictwa

Promotor pomocniczy
dr hab. Marcelina Krupa-Małkiewicz

Katedra Genetyki, Hodowli i Biotechnologii Roślin

SZCZECIN 2022

*Składam serdeczne podziękowania*

**Ireneuszowi Ochmianowi**

*za opiekę naukową, okazaną życzliwość, wszechstronną pomoc oraz cenne wskazówki udzielone w trakcie realizacji pracy doktorskiej,*

**Marcelinie Krupie-Małkiewicz**

*za zaangażowanie w realizację pracy, dzielenie się wiedzą oraz za cierpliwość okazaną przez lata studiów doktoranckich.*

*Ponadto dziękuję*

**Gabrieli Wysockiej**

**Róży Stuart**

**Sylwii Czarneckiej**

**Kamili Pachnowskiej**

**Pawłowi Mazurowi**

**Monice Figiel-Kroczyńskiej**

*za wsparcie przy realizacji badań i miłą atmosferę pracy.*

## SPIS TREŚCI

# STRESZCZENIE

Wciąż rosnąca popularność wysokowartościowych produktów pochodzenia roślinnego (jako żywności funkcjonalnej) sprawia, że stanowią one interesujący temat badawczy. Jednym z takich produktów są jagody goji, które zawierają wiele bioaktywnych związków, w tym polifenole, polisacharydy, karotenoidy i wielonienasycone kwasy tłuszczowe.

Celem pracy doktorskiej było określenie wpływu cięcia krzewów kolcowoju chińskiego na plonowanie oraz jakość owoców oraz opracowanie metody dezynfekcji, procesu mikrorozmnażania i ukorzeniania dwóch odmian kolcowoju chińskiego w kulturach *in vitro*.

W ramach pracy przeprowadzono doświadczenie polowe, w którym oceniono wpływ cięcia roślin na jakość owoców. Badania polowe wykazały dobre przystosowanie roślin kolcowoju do warunków klimatycznych panujących w północno-zachodniej części Polski. Uprawiane krzewy goji nie wykazywały oznak uszkodzeń mrozowych, a kwiaty nie były uszkodzone przez wiosenne przymrozki. Największy wpływ na jakość owoców miało cięcie krzewów na 3 pędy. Owoce z krzewów ciętych były większe i ciemniejsze, o większej zawartości kwasu *L*-askorbinowego.

Wykonany cykl badań i otrzymane w ich trakcie wyniki pozwoliły na opracowanie kompletnego protokołu mikrorozmnażania dwóch odmian *Lycium chinense* ‘No 1’ i ‘New Big’. Podłoże MS uzupełnione *meta*-Topoliną w stężeniu 0,6 $mgL^{-1}$oraz podłoże WPM bez regulatorów wzrostu, wykazywały dobre wyniki w zakresie szybkiego namnażania i wzrostu pędów goji. Pożywki uzupełnione 20 ppm chitozanu okazały się bardzo efektywne na etapie ukorzeniania, gdyż zapewniły wysoki procent roślin ukorzenionych (70-80%) i dobrze rozwijające się sadzonki. Opracowanie protokołu może być przydatne do poprawy efektywności procesu mikropropagacji, ukorzeniania oraz aklimatyzacji sadzonekgoji.

**Słowa kluczowe:** cięcie roślin, dezynfekcja nasion, jagody goji, mikrorozmnażanie, polifenole, wielkość owoców

# ABSTRACT

Still gaining popularity of high quality plant-base products (as functional food) makes them an interesting research topic. One of such products are goji berries, which contain many bioactive compounds including polyphenols, polysaccharides, carotenoids and polyunsaturated fatty acids.

The aim of the doctoral dissertation was to determine the influence of pruning on yield and fruit quality and development of disinfection method, micropropagation and rooting of two cultivars of goji berries in *in vitro* cultures.

In this study, a field experiment was conducted to evaluate the effect of plant pruning on fruit quality. Field studies have shown that wolfberry plants are well adopted to the climatic conditions prevailing in the north-western part of Poland. The cultivated goji shrubs showed no signs of frost damage and the flowers were not damaged by spring frosts. The greatest influence on fruit quality had shrubs pruning on 3 shoots. Fruits from cut shrubs were bigger and darker, with a higher content of *L*-ascorbic acid.

The performed series of studies and the results obtained during them made it possible to develop a complete micropropagation protocol for two cultivars of *Lycium chinense* 'No 1'and 'New Big'. MS medium supplemented with *meta*-Topoline at a concentration of 0,6 mg $L^{-1}$ and WPM medium without growth regulators, showed good results for rapid multiplication and growth of goji shoots. The medium supplemented with 20 ppm chitosan proved to be very effective at the rooting stage as it provided a high percentage of rooted plants (70-80%) and well developed seedlings. Preparation of the protocol may be useful to improve the efficiency of micropropagation, rooting and acclimatisation of goji seedlings.

**Key words:** fruit size, goji berries, micropropagation, polyphenols, seeds disinfection, shrubs cutting

# DOROBEK NAUKOWY STANOWIĄCY ROZPRAWĘ DOKTORSKĄ

**Wpływ zabiegów agrotechnicznych na plonowanie oraz jakoś owoców kolcowoju chińskiego (*Lycium chinense*Mill.) oraz jego wzrost w kulturach *in vitro***

| Lp. | Tytuł publikacji: | Pkt. * | IF ** |
|---|---|---|---|
| **P1** | **Kruczek A**., Ochmian I. (2016). The influence of shurbs cutting method on yielding and quality of the goji berries (*Lycium barbarum* L.). Folia Pomeranae Universitatis Technologiae Stetinensis. Agricultura, Alimentaria, Piscaria et Zootechnica, 40(4 (330)). | 10 | - |
| **P2** | **Kruczek A**., Ochmian I., Krupa-Małkiewicz M., Lachowicz S. (2020). Comparison of morphological, antidiabetic and antioxidant properties of goji fruits. Acta Universitatis Cibiniensis. Series E: Food Technology, 24(1), 1-14. | 140 | - |
| **P3** | **Kruczek A**., Krupa-Małkiewicz M., Lachowicz S., Oszmiański J., Ochmian I. (2020). Health-Promoting Capacities of *In Vitro* and Cultivated Goji (*Lycium chinense* Mill.) Fruit and Leaves; Polyphenols, Antimicrobial Activity, Macro-and Microelements and Heavy Metals. Molecules, 25(22), 5314. | 100 | 4.41 |
| **P4** | **Kruczek A**., Krupa-Małkiewicz M., Ochmian I. (2017). The effectiveness of disinfection methods on germination of goji seeds (*Lycium barbarum* L.) in *in vitro* culture. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 336(43/3), 67-73. | 10 | - |
| **P5** | **Kruczek A**., Krupa-Małkiewicz M., Ochmian I. (2021). Micropropagation, rooting, and acclimatization of two cultivars of goji (*Lycium chinense*). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(2), 12271–12271. | 40 | 1.44 |
| **P6** | Krupa-Małkiewicz M., **Kruczek A**., Pelc J., Smolik B., Ochmian I. (2018). Alleviating effects of ascorbic acid on lead toxicity in goji (*Lycium barbarum* L.) *in vitro*. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 340(45/1), 55-64. | 10 | - |
| **Suma** | | 310 | 5.85 |

*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

Jagody goji jest to potoczna nazywa owoców pochodzących z krzewów *Lycium chinense* Mill., *Lycium barbarum* L. i *Lycium ruthenicum* Murr. (Chen et. al 2018). Według klasyfikacji botanicznej krzewy z rodzaju *Lycium* należą do rodziny *Solanaceae* i obejmują około 100 gatunków, które występują głównie w Azji Środkowej i Wschodniej. Są również gatunki występujące w Europie Południowej (*L. europaeum* L., *L. intricatum* Boiss.), Afryce Północnej (*L. europaeum* L.) oraz Ameryce Środkowej i Południowej (*L. chilense* Bert., *L horridum* Thunb., *L. pallidum* Miers.). Kolcowój, pod taką nazwą występuje w Polsce, jest krzewem liściastym, który dorasta do 3 metrów wysokości. Pędy tych krzewów są długie i cienkie, pokryte krótkimi cierniami (Llorent-Martinez et al. 2013; Marosz 2017). Owoce kolcowoju stanowią mięsiste, czerwono–pomarańczowe jagody o krągłym bądź owalnym kształcie, z dużą ilością nasion (Dănăilă-Guidea et al. 2015).

Blisko spokrewnione gatunki roślin mogą mieć podobne profile chemiczne. W przypadku goji, badania z ostatnich lat wykazały bliskie pokrewieństwo filogenetyczne między *L. barbarum* i *L. chinense*. W związku z tym, naukowcy próbują dowieźć, który gatunek powstał jako pierwszy lub czy nazwy te mogą być stosowane zamiennie dla owoców goji (Yao at el. 2021).

W ostatnich latach zarówno w Polsce, jak i w innych krajach europejskich nastąpił wzrost zainteresowania tymi owocami. Obecnie jagody goji polecane są jako środek o wielokierunkowym oddziaływaniu na organizm, m. in.: wspomaga funkcjonowanie układu odpornościowego, regenerację wątroby, opóźnia proces starzenia, poprawia ostrość widzenia (Niro et al. 2017; Pedro at al. 2019). Ze względu na bardzo korzystne właściwości odżywcze i prozdrowotne, nazwano je „super żywnością" lub „super owocem" (Kulczyński and Gramza-Michałkowska 2016; Yao et al. 2018). Nie tylko owoce, ale również liście oraz młode pędy rośliny kolcowoju chińskiego są szeroko wykorzystywane od ponad 2500 lat w Chinach w celach leczniczych (Burke et al. 2005; Chang and So 2008; Qian et al. 2017). W tradycyjnej medycynie chińskiej uważa się, że ich spożywanie może przedłużać życie i zapewniać energię mimo upływu lat – stąd ich nazwa „owoce długowieczności". W dostępnej literaturze można znaleźć doniesienia, które są poparciem dla tradycyjnych właściwości jagód. Współczesne badania wskazują, że ekstrakty z *Lycium chinense* posiadają przeciwutleniaczeo wysokim potencjałem antyoksydacyjnym. Wspomagają one funkcjonowanie układu immunologicznego, hamują wzrost komórek nowotworowych, obniżają poziom cholesterolu we krwi; w ten sposób włączenie jagód goji do regularnej diety może pomóc w zapobieganiu wielu chorobom związanym ze starzeniem się. Są również doskonałym źródłem makro- i mikroskładników(Chang and So 2008; Ma et al. 2019; Ilić et al. 2020). Najważniejszą grupą związków występujących w jagodach goji jest kompleks polisacharydowy LBP (*Lycium Barbarum Polysaccharides*), który jest rozpuszczalny w wodzie i stanowi 5-8% suszonych owoców. W skład LBP wchodzi sześć rodzajów monosacharydów tj. arabinoza, galaktoza, glukoza, ramnoza, mannoza, ksyloza i kwas galakturonowy (Ma et al. 2019). Ponadto w owocach występują również karotenoidy (zeaksantyna, *beta*-karoten, kryptoksantyna, neoksantyna), związki fenolowe (kwas kawowy, kwas kawoilochinowy, kwas chlorogenowy, kwas *p*-kumarowy, diglukozyd kwercetyny, kemferol, rutyna), witaminy (tiamina, ryboflawina, kwas askorbinowy) oraz składniki mineralne (Ca, Mg, P, Fe, Zn, Cu, Se) (Bunghez et. al 2012; Kulczyński et al. 2014; Paradowska et al. 2016; Kafkas et al. 2021). Jednak od pewnego czasu zaczęto kwestionować bezpieczeństwo tych roślin, zwłaszcza po wykryciu trzech alkaloidów tropanowych: atropina, skopolamina i hioscyjamina. Po szerszych badaniach różnych gatunków kolcowoju, wykazano, że w owocach, liściach, łodygach i korzeniach nie wykrywano żadnego toksycznego związku bądź ich zawartość wynosiła zaledwie około 3,0 ppb – znacznie poniżej dawki trującej. W związku z tym gatunek *Lycium chinense* Mill. jest prawdopodobnie nietoksyczny, a konsumenci mogą być pewni, że jego stosowanie jest bezpieczne (Qian et al. 2017).

Jagody goji są uprawiane głównie w Ningxia, Gansu, Qinghai, Xinjiang, Mongolii Wewnętrznej i Hebei w Chinach. Mogą rosnąć w strefach klimatycznych USDA 6-9. Pochodzenie geograficzne jest ważnym parametrem jakościowym dla jagód goji, ponieważ ich skład chemiczny zmienia się w zależności od klimatu, wody, gleby i metody uprawy. Duża wysokość nad poziomem morza, niska temperatura i długi dzień sprzyjają kumulowaniu się głównego składnika aktywnego. Region Zhongning w Ningxia, który charakteryzuje się alkaliczną glebą i dużą różnicą temperatur między dniem a nocą, jest oryginalnym obszarem produkcji wysokiej jakości jagód goji (Li et al. 2017).

Krzewy *Lycium* nie mają dużych wymagań co do warunków uprawy. Adaptują się do każdego rodzaju gleby, jednak najlepiej rosną na glebach lekko zasadowych, dobrze przepuszczalnych w miejscach nasłonecznionych. Zimą krzewy wytrzymują spadki temperatury nawet do –30°C (Marosz 2017; Szot et al. 2020). Rozległy system korzeniowy powoduje, że rośliny są odporne na suszę. Pierwsze owoce na krzewach można zaobserwować u roślin 2-3 letnich. Krzewy kolcowoju charakteryzują się silnym wzrostem i mocnym krzewieniem. Dlatego podstawowym zabiegiem agrotechnicznym stosowanym przy uprawie kolcowoju jest cięcie krzewów. Zabieg ten ma znaczący wpływ na plonowanie roślin oraz jakość owoców, ponadto zapewnia wystarczającą ilość światła oraz dobrą cyrkulację powietrza wewnątrz krzewów. Zabieg cięcia należy wykonać pod koniec sezonu wegetacyjnego bądź bardzo wczesną wiosną. Krzewy nieprzycinane łatwo gęstnieją, co utrudnia zbiór owoców oraz prowadzi do ich nierównomiernego dojrzewania i pogorszenia jakości (Rusnak 2012; Jiao and Liu 2020).

## KULTURY *IN VITRO*

Konwencjonalne rozmnażanie roślin przebiega powoli, a w przypadku wielu gatunków jest nieefektywne. Ich produkcja jest często niska i zależy od fazy fizjologicznej oraz rozwojowej rośliny. Krzewy kolcowoju rozmnażane z nasion pokazują szereg problemów związanych z rozmnażaniem generatywnym: słaba zdolność kiełkowania, brak jednorodności klonalnej (Silvestri at al. 2018). Mikrorozmnażanie jest lepszą alternatywą dla produkcji roślinnej z potencjałem do szybkiego rozmnażania roślin o dobrej jakości. W wyniku mikrorozmnażania z wyselekcjonowanych roślin w krótkim czasie można uzyskać dużą liczbę nowych, genetycznie jednorodnych mikrosadzonek. Taki wyrównany materiał roślinny może znaleźć zastosowanie przy zakładaniu mateczników i plantacji produkcyjnych (Wawrosz 2008; Mahta et. al 2012). Stąd też, mnożenie roślin w kulturach *in vitro* stanowi alternatywę, która pozwoliłaby uzyskać wysokiej jakości materiał roślinny. Mimo, że metodyka rozmnażania w kulturach *in vitro* jest bardzo podobna dla różnych roślin sadowniczych, wymagania jeżeli chodzi o regulatory wzrostu są bardzo różne. Dlatego też, zoptymalizowanie składu pożywki pozwalającej uzyskać wysoką efektywność regeneracji pędów przybyszowych oraz intensywną ryzogenezę jest niezwykle ważne i pozwoli na opracowanie całego procesu mikrorozmnażania dla każdego genotypu (odmiany) (Fan et al. 2017).

## UKORZENIANIE I AKLIMATYZACJA

Materiał roślinny uzyskany w drodze mikrorozmnażania ma nieprawidłowo wykształcone wiązki przewodzące, cienką kutikulę, nieuregulowane procesy transpiracji. Stąd, młode rośliny charakteryzują się duża wrażliwością na zasychanie. Aby temu zapobiec niezbędny jest etap aklimatyzacji, czyli etap przeniesienia sadzonek z kultur prowadzonych w szkle do warunków *ex vitro*. Adaptacja roślin oznacza całkowitą zmianę warunków funkcjonowania młodych roślin i wiąże się z trudnościami w przystosowaniu do nowego środowiska. Zmienia się tryb odżywiania roślin. W warunkach polowych rośliny uzyskują energię z procesu fotosyntezy, natomiast w warunkach *in vitro* rośliny wchodzą w tryb autotroficznego odżywiania, czyli pobierają pożywienie z pożywki (w skład pożywki wchodzi cukier złożony, sacharoza). Początkowy wzrost mikrosadzonek zależy od wielu czynników, między innymi od substancji, które oddziaływały na roślinę podczas procesu rozmnażania *in vitro* (Świstowska and Kozak 2004; Ruta et al. 2020). Z roku na rok wzrasta liczba gatunków roślin rozmnażanych metodą *in vitro* na skalę komercyjną. Stąd też, badania naukowe skoncentrowane są na czynnikach regulujących podstawowe procesy zachodzące na każdym etapie prowadzonych kultur (Ilczuk et al. 2013).

## ŁAGODZENIE STRESU

W naturalnych warunkach goji uprawiane są na obszarach, które od wieków nie są zanieczyszczane przez cywilizację i pestycydy, szczególnie w Chinach, Azji Południowo-Wschodniej, Europie i Ameryce Północnej (Kulczyński and Gramza-Michałkowska 2016). Ich uprawa możliwa jest na terenach suchych i półsuchych. Cechy fizjologiczne takie, jak odporność na suszę oraz zasolenie czynią z nich rośliny odpowiednie do zapobiegania pustynnienia ziemi i łagodzenia stopnia zasolenia gleby. Ma to bardzo duże znaczenie dla ekosystemu oraz rolnictwa w odległych rejonach (Dimitrova et al. 2016). Zanieczyszczenie gleby metalami ciężkimi może powodować zahamowanie wzrostu roślin i zmniejszenie plonów, a nawet może stanowić zagrożenie dla zdrowia ludzi poprzez łańcuch pokarmowy w wyniku akumulacji tych metali przez rośliny (Nagajyoti et al. 2010). Możliwą strategią przetrwania dla roślin w warunkach, gdzie występuje wysoka zawartość metali ciężkich jest zastosowanie pewnych związków, które mogą złagodzić efekt stresu. Wykorzystanie witamin jako antyoksydantów może pośredniczyć w tolerancji na metale ciężkie, a także poprawiać odporność roślin i ich adaptację do wielu abiotycznych czynników stresowych (Azooz et al. 2013).

## 2. **Cel pracy**

Celem prowadzonych badań wchodzących w skład rozprawy doktorskiej oznaczonych literą P oraz cyframi arabskimi zgodnie z wykazem publikacji było:

- określenie wpływu cięcia krzewów kolcowoju chińskiego na plonowanie oraz jakość owoców goji,
- opracowanie metody dezynfekcji, procesu mikrorozmnażania oraz ukorzeniania dwóch odmian kolcowoju chińskiego w kulturach *in vitro*.

**Cele badawcze składały się z:**

- porównanie wpływu cięcia na wzrost krzewów kolcowoju chińskiego odmiany No. 1 oraz określenie parametrów fizycznych owoców i ich skład chemiczny **(P1),**
- wytypowanie odmian, które charakteryzują się najbardziej wartościowymi owocami pod względem aktywności przeciwcukrzycowej i antyoksydacyjnej, o najwyższej zawartości polifenoli oraz o najlepszych właściwościach morfologicznych i fizykochemicznych **(P2),**
- porównanie właściwości fizyko-chemicznych liści i owoców goji uprawianych w sadzie oraz w kulturze *in vitro*, a także ocena właściwości przeciwdrobnoustrojowych liści goji, co może stanowić podstawę do wykorzystania tej rośliny jako żywności funkcjonalnej **(P3),**
- porównanie skuteczności różnych metod dezynfekcji nasion dwóch odmian goji 'A' i 'New Big' w kulturach *in vitro* **(P4),**
- opracowanie pożywki do rozmnażania pędów dwóch odmian goji w kulturach *in vitro* **(P5),**
- ocena proces ukorzeniania otrzymanych roślin *in vitro* i *ex vitro* **(P5),**
- określenie wpływu działania egzogennego 1 mM kwasu askorbinowego na wzrost i parametry biochemiczne *Lycium* w warunkach stresu wywołanego 1 mM $Pb(NO_3)_2$ w kulturach *in vitro* **(P6).**

# 3. Materiał i metody badań

## DOŚWIADCZENIE POLOWE:

Doświadczenie polowe przeprowadzono w Sadowniczej Stacji Badawczej Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. Stacja badawcza położona jest w podstrefie 7A w północno-zachodniej części Polski na Nizinie Szczecińskiej w odległości ok. 65 km od Morza Bałtyckiego (53°40′ N, 14°8′ E).

### Wpływ cięcia na wzrost roślin

Przedmiotem badań były krzewy *Lycium barbarum* L. odmiana No 1. W doświadczeniu porównano wzrost krzewów, plonowanie, parametry fizyczne owoców oraz ich skład chemiczny w zależności od metody cięcia krzewów (**P1**).

Krzewy były cięte według następującego schematu:

- krzewy cięte na 3 pędy główne (co roku jeden stary pęd zastępowano nowym, jednorocznym pędem, wyrastającym na korzeniach)
- krzewy cięte na 6 pędów głównych (co roku jeden stary pęd zastępowano nowym, jednorocznym pędem, wyrastającym na korzeniach)
- kontrolę stanowiły rośliny nie poddane zabiegowi cięcia

Po sezonie wegetacyjnym zmierzono i porównano: wysokość krzewów, długość pędów jednorocznych, liczbę oraz długość pędów odziomkowych, oznaczono barwę liści, indeks zazielenienia i współczynnik korelacji SPAD.

## ANALIZY LABORATORYJNE:

### Wpływ cięcia roślin na jakość plonu

Wpływ cięcia krzewów (cięcie z pozostawieniem 3 pędów głównych oraz 6 pędów głównych) na skład i właściwości antyoksydacyjne owoców, porównano z próbą kontrolną (rośliny nie poddane zabiegowi cięcia) (**P1**).

Badania obejmowały podstawowe parametry fizykochemiczne: masa 100 owoców, średnica i długość owców, jędrność, barwa (CIE L*a*b*), sucha masa, ekstrakt i kwasowość. Oceniono właściwości prozdrowotne przez określenie zawartości wybranych składników bioaktywnych, takich jak: prowitamina A, kwas *L*-askorbinowy oraz związki polifenolowe ogółem. Również w owocach oznaczono zawartość azotanów i azotynów. Owoce do przygotowania próby zbiorczej zebrano ze wszytskich krzewów objętych doświdczeniem.

**Porównanie jakość owoców 7 odmian goji**

Materiał badawczy obejmował owoce siedmiu odmian goji (No 1, New Big, Sweet Berry, Big Berry, Big Lifeberry), rosnących w stacji badawczej Zachodniopomorskiego Uniwersytetu w Szczecinie. Owoce zbierano ręcznie ze wszytskich krzewów objętych doświadczeniem. Pomiary parametrów fizykochemicznych wykonano na próbie zbiorczej (**P2**). Oznaczono: masę, długość i szerokość owoców, barwę (CIE L*a*b*), przebicie, jędrność, ekstrakt, kwasowość oraz zawartość azotanów, azotynów, prowitaminy A, kwasu *L*-askorbinowego, związków polifenolowych i związków fenolowych. Oceniono aktywność antyoksydacyjną (ABTS, FRAP) oraz działanie przeciwcukrzycowe. Przeprowadzono również badania pod kątem zawartości alkaloidów w owocach (skopolaminy i $\alpha$-solaniny).

**Porównanie różnic składu chemicznego pomiędzy odmianami hodowanymi w warunkach *in vitro* i uprawianymi na plantacji**

Do przeprowadzonych analiz chemicznych wykorzystano: owoce i liście zebrane z krzewów rosnących w sadzie oraz liście z pięciotygodniowych roślin rosnących w kulturach *in vitro* (**P3**). Właściwości fizykochemiczne oznaczone w surowcach: powierzchnia liścia, barwa zarówno liści jak i owoców metodą CIE L*a*b*, współczynnik NAI oraz NDVI, poziom polifenoli, w tym związków należących do antocyjanów, pojemność antyoksydacyjna (DPPH, FRAP), cukry rozpuszczalne, kwasy organiczne. Określono zawartość składników mineralnych (Ca,Cu, Fe, K, Mg, Mn, N, Na, P, Se, Zn) i metali ciężkich (Cd, Ni, Pb). Ocena działania bakteriostatycznego liści wobec szczepów bakterii Gram-dodatnich (*Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*) i Gram-ujemnych (*Escherichia coli*, *Proteus vulgaris*).

**DOŚWIADCZENIA W KULTURACH *IN VITRO***

Badania dotyczące procesu mikrorozmnażania przeprowadzono w Katedrze Genetyki, Hodowli i Biotechnologii Roślin Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. W doświadczeniach wykorzystano nasiona oraz pędy z krzewów kolcowoju chińskiego rosnących w sadzie doświadczalnym Katedry Ogrodnictwa, Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie.

**Dezynfekcja nasion**

Pierwszym etapem procesu mikrorozmnażania było założenie sterylnej kultury *in vitro* (**P4**). Jako materiał roślinny wykorzystano nasiona dwóch odmian krzewów goji: A i New Big. Nasiona pozyskano z dojrzałych jagód, po zbiorach owoców (listopad). Do dezynfekcji nasion wykorzystano: roztwór podchlorynu sodu (NaOCl) w stężeniach 7%; 10% i 15%, roztwór chlorku rtęci ($HgCl_2$) w stężeniu 0,2%, ozon ($O_3$) w stanie gazowym oraz ozon ($O_3$) rozpuszczony w wodzie.

Zdezynfekowane nasiona umieszczono w probówkach typu eppendorf zawierających 15 ml pożywki o podstawowym składzie MS (Murashige i Skoog, 1962) bez dodatku regulatorów wzrostu. Po 10 dniach określono procent zakażeń oraz procent nasion skiełkowanych.

**Namnażanie**

Zainicjowane pędy namnażano w warunkach sterylnych, wykładając 2 cm eksplantaty z jednym pąkiem aksylitowym na pożywki MS z dodatkiem *meta*-Topoliny w stężeniu 0,4, 0,6 i 0,8 $mg \cdot L^{-1}$, pożywki WPM (Woody Plant Media, Llyod and McCown 1980) oraz RA (Anderson Rhododendron Medium, 1984). Kontrolę stanowiła pożywka MS bez dodatku regulatorów wzrostu (**P5**).

Wszystkie kultury inkubowano w fitotronie w temperaturze 24 ± 2°C w 16-godzinnym fotoperiodzie z gęstością strumienia fotosyntezy (PPFD) 40 $\mu mol\ m^{-2}s^{-1}$ emitującą światło barwy chłodnej bieli. Po zakończeniu okresu doświadczalnego (pięć tygodni), eksplantaty wyciągnięto i przemyto wodą destylowaną, zmierzono długość pędów i korzeni, liczbę pędów na roślinie oraz oszacowano wskaźnik regeneracji pędów (%). Rośliny ważono w celu obliczenia świeżej masy roślin.

**Ukorzenianie i aklimatyzacja**

W kolejnym etapie doświadczenia namnożone pędy przenoszono na pożywkę ukorzeniającą (**P5**). W tym celu wykorzystano pożywki: MS, MS z dodatkiem chitozanu (CH) o masie cząsteczkowej 10 kDa w stężeniu 20 ppm, MS z dodatkiem auksyn NAA (kwas α-naftalenooctowy) i IAA (kwas 3-indolooctowy) w stężeniu 0,5 i 1,0 $mg \cdot L^{-1}$. Warunki hodowli były takie same jak w fazie namnażania.

Po 35 dniach inkubacji zmierzono długość pędów i korzeni, oceniono liczbę korzeni na roślinie, a także masę roślin.

Ukorzenione pędy przesadzono do doniczek z ziemią (mieszanina torfu i perlitu w stosunku 9:1) i umieszczono je pod tunelem foliowym. Tym samym rozpoczęto proces aklimatyzacji roślin z warunków *in vitro* do *ex vitro,* który trwał 2 tygodnie. Następnie rośliny przeniesiono do szklarni, gdzie po 3 miesiącach oceniono przeżywalność roślin (%).

**Reakcja roślin goji na stres związany z podwyższonym stężeniem metali ciężkich oraz mozliwości łagodzenia skutków tego stresu**

Jagody goji uprawiane w Polsce często spotykane są na terenach przydrożnych, gdzie stężenie metali ciężkich takich, jak ołów jest podwyższone. Stąd też, kolejnym etapem doświadczenia było zbadanie, czy kwas askorbinowy może łagodzić negatywne skutki stresu ołowiowego na eksplantaty roślin kolcowoju w kulturach *in vitro* (**P6**). Eksplantaty roślinne pochodziły z ustabilizowanej kultury *in vitro*. Pędy umieszczano na pożywce MS uzupełnionej 1 mM kwasu askorbinowego (ASA), 1mM $Pb(NO_3)_2$ oraz kombinacji 1 mM ASA z 1 mM $Pb(NO_3)_2$. Pożywka MS bez dodatku kwasu askorbinowego i roztworu soli $Pb(NO_3)_2$ stanowiła kontrolę.

Po 28 dniach przeprowadzono pomiary cech morfologicznych (długość pędów i korzeni, liczba korzeni z jednego eksplantatu, świeża i sucha masa) oraz cech biochemicznych (chlorofil a, chlorofil b, karotenoidy),wskaźniki stresu (MDA -dialdehyd malonowy i prolina), a także oznaczono zawartość wody w roślinach.

## ANALIZA STATYSTYCZNA

Wszystkie analizy statystyczne przeprowadzono przy użyciu programu Statistica (12.5; 13.0). Istotność statystyczną poszczególnych czynników określono testem jednorodności wariancji i normalności rozkładu, a następnie metodą ANOVA z testem post-hoc Tukeya. Istotność przyjęto na poziomie $p<0,05$. W celu określenia zależności między czynnikami, uzyskane wyniki poddano aglomeracyjnej analizie skupień i zaklasyfikowano do grup w układzie hierarchicznym metodą Warda.

## 4. Omówienie wyników badań

Rozprawa doktorska składa się z cyklu publikacji spójnych tematycznie, opublikowanych w czasopismach naukowych listy MNiSW. Artykuły opisują:

- metody cięcia krzewów oraz jego wpływ na wzrost roślin,
- właściwości fizykochemiczne owoców i liści,
- proces dezynfekci nasion, mikrorozmnażania i ukorzeniania roślin kolcowoju oraz ich adaptacja do warunków *ex vitro*.

**P1. The influence of shurbs cutting method on yielding and quality of the goji berries (*Lycium barbarum* L.).**

Cięcie krzewów jest jednym z podstawowych zabiegów agrotechnicznych. Ma ono istotny wpływ na plonowanie roślin i jakość uzyskanych plonów. Krzewy, które nie są poddawane zabiegowi cięcia silnie się rozrastają i gęstnieją, co prowadzi do nierównomiernego dojrzewania owoców oraz pogorszenia ich jakości.

Wielkość owoców jest czynnikiem, który w głównej mierze decyduje o ich atrakcyjności. Konsumenci poszukują owoców dużych, ładnie wybarwionych, charakteryzujących się właściwościami prozdrowotnymi. Cięcie krzewów spowodowało zmniejszenie plonu, przy jednoczesnej poprawie jakości owoców. Największy wpływ na jakość owoców miało cięcie krzewów na trzy pędy.

Największym plonem owoców (3,12 kg) charakteryzowały się krzewy, które nie zostały poddane zabiegowi cięcia (Tab. 1). Z krzewów prowadzonych na 3 pędy główne zebrano 1,94 kg owoców, które w porównaniu z owocami zebranymi z pozostałych roślin były największe - masa 100 owoców wynosiła 67 g, długość 14,8 mm, a średnica 9,9 mm.

Zróżnicowana ekspozycja owoców na promienie słoneczne miała wpływ na wybarwienie owoców. W przeprowadzonych badania jagody goji były ciemno pomarańczowo-czerwone, szczególnie te zebrane z krzewów ciętych na 3 pędy główne. Silne cięcie krzewów powodowało lepszy dostęp światła i szybsze dojrzewanie owoców.

Krzewy cięte, zwłaszcza na 3 pędy, wytworzyły większą liczbę krótszych pędów podstawowych, natomiast dłuższych pędów jednorocznych. Liście z krzewów pociętych na 3 i 6 pędów głównych charakteryzowały się wyższą wartością indeksu zieloności. Były również ciemniejsze i charakteryzowały się wyższą wartością parametru barwy a*.

**Tabela 1.** Plonowanie oraz jakość owoców goji w zależności od sposobów cięcia

| Badana cecha | | Ilość pędów | | |
|---|---|---|---|---|
| | | Kontrola | 6 pędów | 3 pędy |
| Plon (kg) | | 3,12c* | 2,65b | 1,94a |
| Masa 100 owoców (g) | | 52,2a | 59,1b | 67,0c |
| Średnica owoców (mm) | | 9,0a | 9,5ab | 9,9b |
| Długość owoców (mm) | | 13,3a | 14,4b | 14,8b |
| Jędrność ($G \cdot mm^{-1}$) | | 186a | 166ab | 142b |
| Barwa owoców (CIE) | L* | 35,3a | 33,4ab | 29,7b |
| | a* | 27,9a | 31,0ab | 33,7b |
| | b* | 41,3c | 36,2b | 27,9a |
| Sucha masa (%) | | 15,5b | 15,2b | 14,3a |
| Ekstrakt (%) | | 12,9ab | 12,3a | 13,6b |
| Kwasowość ($g \cdot 100\ g^{-1}$) | | 1,11b | 1,23b | 0,91a |
| Azotyny $NO_2$ ($mg \cdot 1000\ g^{-1}$) | | 0,71a | 0,84a | 0,97a |
| Azotany $NO_3$ ($mg \cdot 1000\ g^{-1}$) | | 47b | 40b | 28a |
| Prowitamina A ($mg \cdot 100\ g^{-1}$) | | 14,3ab | 11,6a | 16,9b |
| Kwas *L*-askorbinowy ($mg \cdot 100\ g^{-1}$) | | 24,5a | 29,6a | 40,1b |
| Suma polifenoli ($mg \cdot 100\ g^{-1}$) | | 43,7c | 34,9b | 29,4a |

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

**P2. Comparison of morphological, antidiabetic and antioxidant properties of goji fruits.**

Rośnie zainteresowanie przez konsumentów owocami określanymi mianem "superfood". Superżywność powinna charakteryzować się wysoką zawartością substancji bioaktywnych o pozytywnym wpływie na zdrowie człowieka. Warunki klimatyczne panujące w Polsce północno-zachodniej są odpowiednie do uprawy kolcowoju chińskiego.

Dotychczasowe badania nad roślinami jagód goji (*L. chinense*) uprawianych w polu w północnej części Europy są nieliczne. Porównano siedem odmian kolcowoju uprawianego w warunkach klimatycznych północno-zachodniej Polski.

Wyniki doświadczenia wykazały różnice w składzie biochemicznym i aktywności biologicznej siedmiu odmian jagód goji (Tab. 2). Krzewy te są bardzo dobrze przystosowane do warunków klimatycznych panujących w Polsce północno-zachodniej. Badane krzewy goji nie wykazywały oznak uszkodzeń mrozowych, a kwiaty nie były uszkodzone przez wiosenne przymrozki. Niezależnie od warunków pogodowych, krzewy plonowały corocznie.

Spośród wszystkich badanych w doświadczeniu odmian, dwie z nich: No. 1 i Big Lifeberry mogą być uznane za superżywność, ponieważ są bogate w polifenole, prowitaminę A oraz kwas *L*-askorbinowy, ponadto wykazują potencjalne działanie przeciwcukrzycowe i antyoksydacyjne.

W owocach nie wykryto obecności steroidów (*α*-solanina) oraz alkaloidów tropanowych (skopolamina), natomiast oznaczony poziom azotanów V i azotynów III był na znacznie niższym poziomie niż obowiązujące normy dla produktów ogólnego przeznaczenia – ***ponieważ nie ma oznaczonych norm dla owoców jagodowych*** (Rozporządzenie Ministra Zdrowia).

Owoce 'Big Lifeberry' są duże co może być bardzo atrakcyjne dla konsumentów. Owoce goji tej odmiany, uprawianej w warunkach klimatycznych północno-wschodniej Europy, mogą być brane pod uwagę jako jako ważne źródło naturalnych antyoksydantów w diecie.

**Tabela 2**. Parametry fizykochemiczne i jakość badanych owoców goji (*L. chinense*)

| | **Odmiana** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Compounds** | **No '1'** | **New Big** | **Sweet Berry** | **Big Berry** | **Big Lifeberry** | **Korean Big** | **Amber Sweet Goji** |
| Masa 100 owoców (g) | 62,4a* | 96,7c | 73,4ab | 112,6de | 104,5cd | 122,1e | 85,7b |
| Średnica owocu (mm) | 9,6ab | 8,4a | 9,8b | 11,8c | 11,2c | 10,7bc | 9,8b |
| Długość owocu (mm) | 14,2a | 24,2e | 15,0ab | 17,3b | 18,9cd | 21,0d | 16,1abc |
| Przebicie ($G \cdot mm^{-1}$) | 79,2de | 49,5a | 71,3cd | 88,0ef | 68,4bc | 95,1f | 59,3ab |
| Jędrność ($G \cdot mm^{-1}$) | 176b | 132a | 174b | 167b | 125a | 211c | 142a |
| Kwas *L*-askorbinowy ($mg \cdot 1000\ g^{-1}$) | 323e | 197c | 224c | 279d | 408f | 155b | 62a |
| Prowitamina A ($mg \cdot 1000\ g^{-1}$) | 153e | 122c | 94b | 137d | 190f | 118c | 35a |
| Azotyny III ($mg \cdot 1000\ g^{-1}$) | 1,42e | 0,68b | 1,12d | 0,94c | 0,62ab | 1,05d | 0,53a |
| Azotany V ($mg \cdot 1000\ g^{-1}$) | 42,4b | 33,8a | 49,3b | 74,1c | 29,5a | 106,6d | 42,4b |
| Ekstrakt (°Bx) | 11,4b | 16,4d | 18,4e | 12,5bc | 11,9b | 13,2c | 9,4a |
| Kwasowość ($g \cdot 1000\ g^{-1}$) | 11,1b | 12,4bc | 8,9a | 12,7c | 14,5d | 12,6bc | 13,0c |
| | | | | działanie przeciwcukrzycowe | | | |
| $\alpha$-amylaza $IC_{50}$ ($mg \cdot mL^{-1}$) | 38,5b | 48,6d | 43,1c | 50,8de | 33,4a | 52,7e | 73,4f |
| $\alpha$-glukozydaza $IC_{50}$ ($mg \cdot mL^{-1}$) | 9,9c | 9,2c | 6,5a | 13,2d | 9,9c | 7,8b | 5,7a |
| | | | | aktywność antyoksydacyjna | | | |
| ABTS·+ (mmol Trolox·$100g^{-1}$) | 4,11d | 3,45c | 3,88d | 2,29a | 6,21e | 2,95b | 1,89a |
| FRAP (mmol Trolox·$100g^{-1}$) | 4,28e | 3,01c | 3,46d | 1,84a | 5,58f | 2,61b | 1,66a |
| | | | | alkaloidy | | | |
| Skopolamina | NW** | | | | | | |
| $\alpha$-solanina | | | | | | | |
| | | | | owoce nie zawierały $\alpha$-solaniy i skopolaminy na wykrywalnym poziomie | | | |

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

**P3. Health-promoting capacities of *in vitro* and cultivated goji (*Lycium chinense* Mill.) fruit and leaves; polyphenols, antimicrobial activity, macro-and microelements and heavy metals.**

W tradycyjnej medycynie chińskiej wykorzystywane są zarówno owoce, jak i liście kolcowoju. Surowce roślinne pochodzące z krzewów goji charakteryzują się dużą wartością odżywczą. Rozkład kompozycji składników mineralnych, witamin i związków biologicznie czynnych wposzczególnych częściach rośliny (tj. w owocach lub liściach) zależny jest od skład gleby, miejsca uprawy i panujących tam warunków klimatycznych. Badanie składu chemicznego owoców uważanych za superfoods jest ważnez punktu widzenia żywieniowego i toksykologicznego.

Materiał roślinny (liście) przeanalizowano pod kątem zawartości mikro- i makroskładników, związków polifenolowych, aktywności przeciwbakteryjnej oraz wartości odżywczej. Stężenia pierwiastków zawartych w liściach obu odmian we wszystkich próbkach malały w następującej kolejności: N > K > Ca >P > Mg > Na (liście z sadu) oraz N > K > P > Ca > Mg > Na (liście z *in vitro*). Procentowy udział makro- i mikroelementów w owocach goji porównano z dziennym zapotrzebowaniem na składniki mineralne w diecie człowieka. Zalecane dzienne spożycie dla osoby dorosłej (RDA) zostało ustalone na poziomie 2 mg Cu, 18 mg Fe, 2 mg Mn, oraz 15 mg Zn. Spożycie 100 g świeżych jagód goji dziennie przyczynia się do pokrycia około 30% i 35% RDA dla Cu odpowiednio dla odmian No. 1 i New Big. Ponadto, świeże owoce goji są również bogatym źródłem żelaza. Sto gramów owoców stanowi 47% RDA dla tego pierwiastka.

Ekstrakty z liści w badanych stężeniach wykazywały aktywność hamującą (MIC) na wzrost analizowanych bakterii Gram-dodatnich, zwłaszcza na *S. aureus*. Natomiast wśród bakterii Gram-ujemnych, wrażliwy na jego działanie był tylko *P. vulgaris*. Zdecydowanie wyższym działaniem hamującym na wzrost bakterii charakteryzowały się ekstrakty z liści zebranych z krzewów rosnących w sadzie, zwłaszcza z odmiany New Big.

Uzyskane wyniki badań wzbogacają wiedzę na temat składu i wartości odżywczych świeżych owoców goji uprawianych w północno-wschodniej Europie. Badania własne potwierdziły, że zarówno owoce, jak i liśćie goji są ważnym źródłem związków prozdrowotnych, istotnych w diecie człowieka (takich jak: makro- i mikroelementy, antyoksydanty, cukry, kwasy organiczne i fenolowe). W badanych owocach dwóch odmian goji oznaczono następujące makroelementy: N, P, K, Na, Mg, Ca i mikroelementy: Fe, Cu, Zn, Mn, Se. Azot jest pierwiastkiem dominującym (24,32-29,85 g·kg) zarówno w owocach

'New Big' jak i 'No. 1'. Owoce odmiany New Big charakteryzowały się istotnie wyższą zawartością mikroelementów, za wyjątkiem selenu, którego było o 25% mniej niż w owocach odmiany No 1 (Tab. 3).

Należy wspomnieć, że w niniejszych badaniach wykryto również obecność pierwiastków toksycznych (tj. Pb i Ni). Ich stężenia były na niskim poziomie, poniżej dopuszczalnych poziomów granicznych/limitów. Zgodnie z Rozporządzeniem Komisji Europejskiej, wartości określone dla owoców ustawowo wynoszą: 0,20 mg/kg dla ołowiu, natomiast nie jest określona wartość dla nikielu (Rozporządzenie Parlamentu Europejskiego i Rady).

Uzyskana wiedzamoże być pomocna do określenia potencjału komercyjnego jagód goji w zastosowaniu nutraceutycznym oraz w produkcji żywności poprawiającej zdrowie człowieka.

**Tabela 3.** Średnie wartości mikroelementów znajdujących się w liściach i owocach dwóch odmian goji uprawianych w sadzie i w warunkach *in vitro*

| Compounds (mg·kg) | liście | | | | owoce | |
|---|---|---|---|---|---|---|
| | No 1 | | New Big | | No 1 | New Big |
| | sad | in vitro | sad | in vitro | sad | sad |
| Fe (40-60*) | 120,13c** | 71,00a | 97,81b | 77,44a | 66,03a | 79,44b |
| Zn (8-14) | 18,62b | 26,77c | 14,53a | 35,07d | 8,16a | 8,73b |
| Mn (70-260) | 50,70b | 176,89c | 42,07a | 244,64d | 7,04a | 7,74b |
| Cu (5-20) | 12,13d | 7,10a | 9,78c | 7,74b | 66,03a | 79,44b |
| Se | 0,089b | NW | 0,062a | NW | 0,012b | 0,009a |
| Pb | 0,034a | NW | 0,054b | NW | 0,017a | 0,029b |
| Cd | NW*** | NW | NW | NW | NW | NW |
| Ni | 0,019b | NW | 0,011a | NW | 0,007b | 0,003a |

*Optymalna zawartość skłądników dla liści wg Glonek i Komosa (2013)
**Średnie w kolumnach oznaczone tymi samymi literami alfabetu nie różnią się według testu Tukey'a na poziomie istotności $\alpha<0.05$
***NW-nie wykryto

**P4. The effectiveness of disinfection methods on germination of goji seeds (*Lycium barbarum* L.) in *in vitro* culture.**

Jednym z najważniejszych sposobów kontroli zanieczyszczeń w kulturach *in vitro* jest odpowiednia dezynfekcja materiału roślinnego. W kulturach *in vitro* zwykle stosuje się chemiczne środki dezynfekcyjne takie, jak podchloryn sodu, etanol lub tlenek rtęci. Jednakże, środki chemiczne stosowane w zbyt wysokich stężeniach mogą być toksyczne dla roślin i środowiska. Alternatywną metodą dezynfekcji w kulturach *in vitro* może być ozonowanie.

Skuteczność dezynfekcji nasion zależała od zastosowanego roztworu dezynfekującego i jego stężenia. Nasiona goji moczone w 15% roztworze NaOCl wykazały najniższą liczbę zakażeń, ale również najmniejszą zdolność kiełkowania. Natomiast przy zmniejszeniu stężenia roztworu NaOCl do 7%, procent wykiełkowanych nasion goji 'A' i 'New Big' był najwyższy (odpowiednio 62% i 78%).

Wyniki uzyskane w niniejszej pracy wykazały, że procent kontaminacji nasion goji przy różnych procedurach dezynfekcji wahał się od 0 do 40%.

Nasiona goji traktowane $O_3$ charakteryzowały się podobną skutecznością dezynfekcji i zdolnością kiełkowania, jak po zastosowaniu 15% roztworu NaOCl. Dlatego też, uznano ozonowanie jako alternatywną metodę dla powszechnie stosowanych środków dezynfekcyjnych (Tab. 4).

**Tabela 4.** Procent niezakażonych eksplantatów goji odmian ‘A’ i ‘New Big’, które skiełkowały, w zależności od zastosowanego środka dezynfekującego

| Metody dezynfekcji | | Odmiana | | |
|---|---|---|---|---|
| | | A | New Big | **Średnia** |
| | | % niezakażonych eksplantatów | | |
| Ozonowanie na sucho | 5 minut | 33 b* | 47 c | **40 cd** |
| | 15 minut | 40 bc | 7 a | **23 ab** |
| Ozonowanie w wodzie | 5 minut | 7 a | 13 a | **10 a** |
| | 15 minut | 47 c | 53 c | **50 d** |
| NaOCl | 7% | 62 d | 78 d | **70 e** |
| | 10% | 47 c | 47 c | **47 d** |
| | 15% | 38 b | 36 b | **37 c** |
| 0.2% $HgCl_2$ | | 37 b | 30 b | **33 bc** |

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

**P5. Micropropagation, rooting, and acclimatization of two cultivars of goji (*Lycium chinense*).**

Niska zdolność kiełkowania nasion goji oraz brak ekspancji klonów przyczyniły się do poszukiwania alternatywnych metod wspomagających rozmnażanie tych roślin. Wykorzystanie kultur *in vitro* pozwala uzyskać w krótkim czasie, na odpowiednio dobranych pożywkach, jednolity pod względem genetycznym oraz wolny od pategenów materiał. Spośród zastosowanych do mikrorozmnażania goji podłoży, pożywka MS uzupełniona *meta*-Topoliną w stężeniu 0,6 mg·L$^{-1}$ oraz WPM bez dodatku roślinnych regulatorów wzrostu pozwoliły uzyskać najwyższą intensywność wzrostu eksplantatów. W przypadku ukorzeniania goji pożywki MS z dodatkiem 20 ppm chitozanu pozytywnie wpłynęły na proces ryzoenezy, uzyskując do 80% roślin ukorzenionych (Tab. 5).

W procesie aklimatyzacji *ex vitro* mieszanina 90% torfu i 10% perlitu o wysokiej wilgotności (90%) była skutecznym podłożem dla odmian goji: No 1 i New Big. Uzyskane wyniki mogą być przydatne do poprawy efektywności mikrorozmnażania i ukorzeniania roślin kolcowoju.

**Tabela 5.** Wpływskładu pożywki na cechy morfologiczne i wskaźnik regeneracji w kulturach *in vitro* kolcowoju chińskiego ‘No 1’ i ‘New Big’

| Pożywka | Długość pędów(cm) | Liczba pędów na roślinie | Świeża masa(g) | Wskaźnik regeneracji(%) |
|---|---|---|---|---|
| | | ‘No 1’ | | |
| MS | 3,18 abc* | 1,17 ab | 0,445 ab | 71 |
| MS+0,4 mg·L$^{-1}$ *m*T | 2,89 ab | 1,25 abc | 0,576 cd | 92 |
| MS+0,6 mg·L$^{-1}$ *m*T | 3,61 bcd | 1,50 cb | 0,598 de | 96 |
| MS+0,8mg·L$^{-1}$ *m*T | 3,27 abc | 1,33 abc | 0,483 abc | 95 |
| WPM | 3,99 cd | 1,42 bc | 0,529 abcd | 97 |
| RA | 3,24 abc | 1,25 abc | 0,457 ab | 68 |
| | | ‘New Big’ | | |
| MS | 2,71 a | 1,00 a | 0,426 a | 68 |
| MS+0,4 mg·L$^{-1}$ *m*T | 2,83 ab | 1,33 abc | 0,493 abcd | 93 |
| MS+0,6 mg·L$^{-1}$ *m*T | 3,53 abcd | 1,42 bc | 0,581 cd | 95 |
| MS+0,8mg·L$^{-1}$ *m*T | 3,36 abcd | 1,33 abc | 0,694 e | 95 |
| WPM | 4,12 d | 1,58 c | 0,549 bcd | 97 |
| RA | 3,42 abcd | 1,25 abc | 0,444 ab | 72 |

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

**P6. Alleviating effects of ascorbic acid on lead toxicity in goji (*Lycium barbarum* L.) *in vitro*.**

Możliwą strategią przetrwania dla roślin w warunkach stresowych (w tym obecności na przykład metali ciężkich) jest zastosowanie niektórych związków, które mogłyby łagodzić efekt stresu. Zastosowanie keasu askorbinowego jako antyoksydantu pośredniczy w tolerancji na metale ciężkie, stanowiąc siłę napędową dla poprawy odporności i adaptacji do wielu abiotycznych czynników stresowych.

Dodatek do pożywki roztworu ołowiu miał negatywny wpływ na cechy morfologiczne goji takie, jak długość pędu i korzeni. Najwięcej zmian zaobserwowano w przypadku liczby korzeni. Korzenie obu odmian były krótkie i słabo rozwinięte. Stres spowodowany metalami ciężkimi spowodował zahamowanie ich wzrostu. Dodatkowo stres wywołany ołowiem wyraźnie zmniejszył zarówno świeżą, jak i suchą masę roślin. Dodanie do pożywki 1 mM ASA znacznie złagodziło wywołaną przez $Pb(NO_3)_2$ redukcję świeżej i suchej masy.

Ekspozycja eksplantatów goji na działanie 1 mM $Pb(NO_3)_2$ wyraźnie zmniejszyła zawartość chlorofilu a i b oraz karotenoidów, w porównaniu z próbą kontrolą. Dodatek do pożywki MS 1 mM ASA w warunkach stresu metalami ciężkimi wyraźnie zwiększył zawartość chlorofilu a i b oraz karotenoidów. Ponadto zaobserwowano, że aplikacja samego 1 mM ASA istotnie zwiększyła zawartość barwników fotosyntetycznych (takich jak chlorofil a oraz chlorofil b) i niefotosyntetycznych (karotenoidy). Ponadto, zaobserwowano, że zastosowaniu 1 mM ASA lub 1 mM $Pb(NO_3)_2$ zawartość proliny w siewkach goji była istotnie wyższa w porównaniu z próbą kontrolną (Tab. 6). Pomiar poziomu dialdehydu malonowego (MDA), jako wskaźnika peroksydacji lipidów, w warunkach stresowych wskazała na znacznyjego wzrost w liściach w porównaniu z próbą kontrolą. Dodatek do pożywki MS 1 mMPb$(NO_3)_2$ w połączeniu z 1mM ASA spowodowała zmniejszenie zawartość MDA o 4% w siewkach goji w porównaniu z dodatkiem do pożywki samego ołowiu.

**Tabela 6.** Wpływ 1 mM kwasu askorbinowego i 1 mM $Pb(NO_3)_2$ na zawartość proliny i MDA w liściach goji (*Lycium barbarum* L.) namnażanej w kulturach *in vitro*

| Pożywka | Prolina ($\mu mol \cdot g^{-1}$ ś.m.) | MDA ($nmol \cdot g^{-1}$ ś.m.) |
|---|---|---|
| MS | 1,88d* | 7,69d |
| MS + 1 mM ASA | 3,51c | 9,53c |
| MS + 1 mM $Pb(NO_3)_2$ | 4,56a | 11,22a |
| MS + 1 mM ASA + 1 mM $Pb(NO_3)_2$ | 3,7b | 10,8b |

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

## 6. Wnioski

Osiągnięto założone cele badawcze. W cyklu publikacji, który stanowi podstawę do ubiegania się o stopień naukowy doktora, wykazałam możliwość uprawy kolcowoju chińskiego (*Lycium chinense* Mill.) w naszych warunkach klimatycznych. Uzyskane owoce charakteryzowały się wysoką jakością oraz właściwościami prozdrowotnymi. Ponadto opracowałam metodykę mikrorozmanżania roślin.

1. Badane krzewy goji nie wykazywały oznak uszkodzeń mrozowych, a kwiaty nie były uszkodzone przez wiosenne przymrozki. Największy wpływ na wzrost krzewów i jakość owoców miało cięcie zimowe roślin na 3 pędy główne. Stwierdzono większą liczbę krótszych pędów odziomkowych i dłuższych pędów jednorocznych. Cięcie znacznie zmniejszyło plonowanie. Owoce były jednak większe i ciemniejsze, miały więcej kwas *L*-askorbinowego, mniejszą zawartość polifenoli i szkodliwych dla zdrowia azotanów.
2. W badanych owocach goji nie wykryto skopolaminy, a poziom azotanów oraz metali ciężkich był poniżej obowiązujących norm. Owoce są bogate w polifenole, prowitaminę A i kwas *L*-askorbinowy, wykazują działanie przeciwcukrzycowe i antyoksydacyjne.
3. Opracowano pełny cykl mikrorozmnażania goji od etapu inicjacji do adaptacji w warunkach *ex vitro*. Ozon, który jest mniej toksyczny dla środowiska, równie skutecznie dezynfekuje nasiona, jak powszechnie stosowany podchloryn sodu. Podłoże MS uzupełnione *meta*-Topoliną w stężeniu 0,6 $mg \cdot L^{-1}$ oraz podłoże WPM bez regulatorów wzrostu wykazują dobre wyniki w zakresie szybkiego namnażania i wzrostu pędów. Pożywka MS uzupełniona chitozanem lub IAA wykazała wysoką skuteczność ukorzeniania eksplantatów i ich adaptację do warunków *ex vitro* (60-80%).
4. Analiza składu mineralnego liści pobranych z roślin rosnących w warunkach *in vitro* i polowych może wskazywać, że rośliny te mają niskie wymagania pokarmowe, zwłaszcza w odniesieniu do N, K, Fe, Zn. Ponadto w liściach pobranych z roślin rosnących w warunkach *in vitro* stwierdzono niskie zawartości polifenoli.
5. Kwas *L*-askorbinowy dodany do pożywki MS + Pb wykazuje łagodzący wpływ na negatywne skutki wywołane tym czynnikiem stresowym.
6. Jagody goji, które wykazują dobre przystosowanie do warunków klimatycznych w Polsce oraz ze względu na szerokie spektrum aktywności biologicznej o korzystnym wpływie na zdrowie, mogą być uznane za superżywność lub żywność funkcjonalną. Wśród badanych odmian, owoce 'Big Lifeberry' mogły by przypaść do gustu konsumentom ze względu na wielkość, masę oraz skład chemiczny.

## 7. Literatura


1. Anderson W. C. (1984). A revised tissue culture medium for shoot multiplication of rhododendron. J. amer. Soc. hort. Sci., 109, 343-347.
2. Azooz M. M., Alzahrani A. M., Youssef M. M. (2013). The potential role of seed priming with ascorbic acid and nicotinamide and their interactions to enhance salt tolerance in broad bean ('Vicia faba'L.). Australian Journal of Crop Science, 7(13), 2091–2100.
3. Bungheza I. R., Marius A. S., Marian N., Georgeta R., Rodica-Mariana I. (2012). Obtaining of Carotenoid Extract FromLyciumChinenseand Characterization UsingSpectometrical Analysis. Digest Journal of Nanomaterials & Biostructures (DJNB), 7(2).
4. Burke D. S., Smidt C. R., Vuong L. T. (2005). Momordica cochinchinensis, Rosa roxburghii, wolfberry, and sea buckthorn-highly nutritional fruits supported by tradition and science. Current Topics in Nutraceutical Research, 3(4), 259.
5. Chang R. C. C., So K. F. (2008). Use of anti-aging herbal medicine, Lyciumbarbarum, against aging-associated diseases. What do we know so far? Cellular and Molecular Neurobiology, 28(5), 643-652.
6. Chen J., Chao C. T., Wei X. (2018). Gojiberry breeding: current status and future prospects. In Breeding and Health Benefits of Fruit and Nut Crops. IntechOpen. DOI:10.5772/intechopen.76388
7. Dănăilă-Guidea S. M., Dobrinoiu R. V., Vişan L., Toma, R. C. (2015). Protocol for efficient in vitro multiplication of *Lyciumbarbarum* L. (goji) by direct organogenesis. Sci. Bull. Ser. F Biotechnol, 19, 34-38.
8. Dimitrova V., Georgieva T.,Markovska Y. (2016). Influence of salt stress on some physiological characteristics of two *Lycium* varieties grown *ex vitro* in hydroponics. In Youth Scientific Conference Kliment’s Days. Sofia, 101(4), 141-148.
9. Glonek J., Komosa A. (2013). Fertigation of highbush blueberry (*Vaccinium corymbosum* L.). Part I. The effect on growth and yield. Acta Sci. Pol., Hortorum Cultus, 12(3), 47-57.
10. Ilczuk A., Jacygrad E., Jagiełło-Kubiec K., Pacholczak A. (2013). Rozmnażanie in vitro roślin drzewiastych–perspektywy i problemy. Ogrodnictwo ozdobne sektorem gospodarki narodowej, ed. J. Rabiza-Świder, E. Skutnik, SGGW, Warszawa, 41-48.

11. Ilić T., Dodevska M., Marčetić M., Božić D., Kodranov I., Vidović B. (2020). Chemical Characterization, Antioxidant and Antimicrobial Properties of Goji Berries Cultivated in Serbia. Foods, 9(11), 1614. DOI: 10.3390/foods9111614.
12. Jiao Y., Liu G. (2020). Goji Berry—a Novel Nutraceutical “Superfruit” for Florida Master Gardeners. EDIS,(5).
13. Kafkas N. E., Oğuz H. İ., Oğuz İ. (2021). Evaluation of fruit characteristics of various organically-grown goji berry (Lyciumbarbarum L., Lyciumchinense Miller) species during ripening stages. Journal of Food Composition and Analysis, 103846.
14. Kulczyński B., Groszczyk B., Cerba A., Gramza-Michałowska A. (2014).Goya (*Lyciumbarbarum*) fruits as bioactive components source in food – a literature review. Nauka Przyroda Technologie, 8(2), 19.
15. Kulczyński B., Gramza-Michałowska A. (2016).Goji berry (Lyciumbarbarum): composition and health effects–a review. Polish Journal of Food and Nutrition Sciences, 66(2), 67-76. DOI:10.1515/pjfns-2015-0040.
16. Li Q., Yu X., Xu L., Gao J. M. (2017). Novel method for the producing area identification of Zhongning Goji berries by electronic nose. Food Chemistry, 221, 1113-1119.
17. Llorent-Martinez E.J., Fernández-de Córdova M.L., Ortega-Barrales P., Ruiz-Medina A. (2013). Characterization and comparison of the chemical composition of exotic superfoods. Microchem. J., 110, 444-451.
18. Lloyd G., McCown B. (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture., 30, 421-427.
19. Ma Z.F., Zhang H., Teh S.S., Wang C.W., Zhang Y., Hayford F., Wang L., Ma T., Dong Z., Zhang Y., Zhu Y. (2019).Goji berries as a potential natural antioxidant medicine: An insight into their molecular mechanisms of action. Oxidative Medicine and Cellular Longevity. DOI: 10.1155/2019/2437397.
20. Mahta J., Naruka R., Sain M., Dwivedi A., Sharma D., Mirza J. (2012).An efficient protocol for clonal micropropagation of Mentha piperita L. (Pipperment). Asian Journal of Plant Science and Research 2(4): 518-523.
21. Marosz A. (2017). Fruits of goji berry (*Lyciumbarbarum* and *Lyciumchinense*) – new possibility for horticulture or risk for consumers?/ Owocejagody goji (*Lyciumbarbarum* i

*Lyciumchinense*) – nowemożliwościdlaogrodnictwaczyzagrożeniedlakonsumentów? Annales Horticulturae XXVII(1): 19–30.
22. Murashige T., Skoog F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologiaplantarum, 15(3), 473-497.
23. Nagajyoti P. C., Lee K. D., Sreekanth T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. Environmental chemistry letters, 8(3), 199–216.
24. Niro S., Fratianni A., Panfili G., Falasca L., Cinquanta I., Rizvi Alam M. D. (2017). Nutritional evaluation of fresh and dried goji berries cultivated in Italy. Ital. J. Food Sci., (29): 398-408.
25. Paradowska K., Czerniejewska M., Zielińska A., Sajkowska-Kozielewicz J. J. (2016).Antioxidative activity of dried *Lyciumbarbarum* goji fruit extracts. Żywność. Nauka. Technologia. Jakość, 4(107): 115-124.
26. Pedro A. C., Sanchez-Mata M. C., Perez-Rodriguez M. L., Camara M., Lopez-Colon J. L., Bach F., Bellettini M., Windson Ch., Haminiuk I. (2019). Qualitative and nutritional comparison of goji berry fruits produced in organic and conventional systems. ScientalHorticulturae, (257). (doi.org/10.1016/j.scienta.2019.108660)
27. Qian D., Zhao Y., Yang G., Huang L. (2017). Systematic review of chemical constituents in the genus Lycium (Solanaceae). Molecules, 22(6): 911.
28. Rozporządzenie Ministra Zdrowia z dnia 13 stycznia 2003 r. w sprawie maksymalnych poziomów zanieczyszczeń chemicznych i biologicznych, które mogą znajdować się w żywności, składnikach żywności, dozwolonych substancjach dodatkowych, substancjach pomagających w przetwarzaniu albo na powierzchni żywności. Dz. U.37, poz. 326 z późn. zm.
29. Rozporządzenie Parlamentu Europejskiego i Rady (UE) nr 1169/2011 z dnia 25 października 2011 r. w sprawie przekazywania konsumentom informacji na temat żywności. Dz. U. UE. 304: 18-63.
30. Rusnak J. (2012). Cięcie krzewów owocowych. Karniowice, Małopolski Ośrodek Doradztwa Rolniczego, 2-3.
31. Ruta C., De Mastro G., Ancona S., Tagarelli A., De Cillis F., Benelli C., Lambardi M. (2020). Large-Scale Plant Production of Lyciumbarbarum L. by Liquid Culture in Temporary Immersion System and Possible Application to the Synthesis of Bioactive Substance. Plants, 9(7), 844. DOI: 10.3390/plants9070844.

32. Silvestri C., Sabbatini G., Marangelli F., Rugini E., Cristofori V. (2018).Micropropagation and *Ex Vitro* Rooting of Wolfberry. Hort Science 53(10): 1494-1499.
33. Szot I., Zhurba M., Klymenko S. (2020). Pro-Health and Functional Properties of Goji Berry (*Lycium* Spp.). Agrobiodiversity for Improving Nutrition, Health and Life Quality, (4), 134-145.
34. Świstowska A., Kozak D. (2004). Wpływauksynna ukorzenianie mikrosadzonek i adaptacji roślin*Columneahirta*Klotzsch et Hanst. Cz. I. W kulturze*in vitro*. Acta Sci. Pol., HortorumCultus 3(2), 229-238.
35. Wawrosz C. (2008). Plant biotechnology for propagation, conservation and quality improvement of medicinal plants. ActaBiochimicaPolonica, 41-55.
36. Yao R., Heinrich M., Weckerle C. S. (2018).The genus Lycium as food and medicine: A botanical, ethnobotanical and historical review. Journal of Ethnopharmacology, 212, 50-66.
37. Yao R., Heinrich M., Zhao X., Wang Q., Wie J., Xiao P. (2021).What's the choice for goji: *Lyciumbarbarum* L. or *L. chinense* Mill.? Journal of Ethnopharmacology, JETHNO-D-20-01570.

## KOPIE ARTYKUŁÓW STANOWIĄCYCH JEDNOTEMATYCZNY CYKL PUBLIKACJI I OŚWIADCZENIA WSPÓŁAUTORÓW

# THE INFLUENCE OF SHURBS CUTTING METHOD ON YIELDING AND QUALITY OF THE GOJI BERRIES (LYCIUM BARBARUM L.)

**Article** · February 2017

DOI: 10.21005/AAPZ2016.40.4.14



Arleta Kruczek
West Pomeranian University of Technology, Szczecin


Ireneusz Ochmian
West Pomeranian University of Technology, Szczecin

DOI: 10.21005/AAPZ2016.40.4.14

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2016, 330(40)4, 131–138

*Arleta KRUCZEK, Ireneusz OCHMIAN*

# THE INFLUENCE OF SHURBS CUTTING METHOD ON YIELDING AND QUALITY OF THE GOJI BERRIES (*LYCIUM BARBARUM* L.)

# WPŁYW CIĘCIA KRZEWÓW NA PLONOWANIE I JAKOŚĆ OWOCÓW GOJI (*LYCIUM BARBARUM* L.)

Department of Horticulture, West Pomeranian University of Technology, Szczecin, Poland

**Streszczenie.** W doświadczeniu porównywano: wzrost krzewów, plonowanie, parametry fizyczne owoców oraz ich skład chemiczny w zależności od metody cięcia krzewów – liczby pędów głównych. Materiał stanowiły jagody goji rosnące w Sadowniczej Stacji Badawczej Katedry Ogrodnictwa Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. Jagody goji (*Lycium barbarum* L.) charakteryzują się właściwościami prozdrowotnymi i odżywczymi. Od wieków stanowią niezwykle ważny element tradycyjnej medycyny chińskiej. Są tam stosowane, jako bardzo istotny składnik zdrowej diety, m.in. ze względu na występowanie cennego kompleksu polisacharydowego LBP (Lycium Barbarum Polysaccharides). Cięcie pędów pobudziło wzrost krzewów. Na krzewach ciętych na 3 pędy wyrosło więcej krótszych pędów odziomkowych, natomiast pędy jednoroczne były najdłuższe. Liście z krzewów ciętych były ciemniejsze (L* = 41.2 i 39.8) oraz miały wyższy indeks zazielenienia (42,1 i 44,8), w porównaniu z krzewami, z których nie usuwano pędów (L* = 37,3; SPAD = 43,5). Stwierdzono również istotną zależność pomiędzy indeksem zazielenienia a wartością parametru a* (r = 0,92*). Cięcie krzewów, a zwłaszcza mocne – na 3 pędy, ograniczyło plonowanie, wpłynęło jednak na zwiększenie wielkości owoców.

**Key words:** chemical composition of fruits, goji, green index, fruit size, yielding.
**Słowa kluczowe:** goji, indeks zazielenienia, plonowanie, skład chemiczny owoców, wielkość owoców.

## INTRODUCTION

Goji berry (*Lycium barbarum* L.), also known as common wolfberry, belongs to the *Solanaceae* family (Jin et al. 2013). Commercial volumes of wolfberries grow in Japan, Korea, and Taiwan, but mostly in China where cultivation area exceeds 82 000 ha (China Daily Staff reporter 2004). Traditional food and medicine in East Asia have become increasingly popular in Europe and North America (Potterat 2010).

Goji berry bushes reaching a height of 3 meters, have arched overhanging twigs covered with spines. Fruits are bright red berries, characterized by an elongated shape and sensitivity (Bogacz 2009; Llorent-Martinez et al. 2013). To protect the fruits against any damage during harvest they should be shaked on soft mats, or plucked with the stulk. Two years old plants can be harvested with approximately 2 kg of fruit each. Goji berries are of high health and

Corresponding author – Autor do korespondencji: Ireneusz Ochmian, Department of Horticulture, West Pomeranian University of Technology, Szczecin, Juliusza Słowackiego 17, 71–434 Szczecin, Poland, e-mail: ireneusz.ochmian@zut.edu.pl

nutrition properties (Bogacz 2009; Amagase and Farnsworth 2011). The most valuable and most arousing the interest component of the goji berry is a polysaccharide complex LBP (Lycium Barbarum Polysaccharides), which is soluble in water (Luo et al. 2004). The presence of this component influences the bioactive therapeutic effectiveness of fruit goji. It either positively affects the function of the immune system (Gan et al. 2004; Amagase et al. 2009). Many studies indicate that the complex polysaccharide common Wolfberry can inhibit the growth of the tumor cells (He et al. 2012).

In fruits may be present an alkaloid with strong biological effect – atropine. A large amount have been detected in fruits originating in India (Harsh 1989). However, the amount of atropine which is supplied from the consumed amount of berries has no adverse impact on human health. The content is far below toxic levels (Adams et al. 2006). The lethal dose is 60–100 mg (Marquart and Schaefer 1997). Atropine is recommended in traditional Chinese medicine to treat arthritis (Lin and Chen 2002).

*Lycium barbarum* prefers well-drained slightly alkaline soils, however shrubs will grow in almost any type of soil (Peaceful Valley Farm Supply 2013) especially in sunny locations. Under such conditions, fruits will have the highest quality. Goji Berry plants will handle winter temperatures to –9°C and summer temperatures up to 38°C. Goji Berry plants have an extensive root system and are very drought tolerant once established. However first fruition is typically observed in 2–3 year-old plants. Shrubs cut are one of the basic agricultural treatments. It gives a significant impact on plant yielding and crop quality. It is a laborious procedure and out of a collection of fruits requires much labor effort. Uncut shrubs are easily thicken, which leads to uneven ripening of fruits and deterioration of their quality (Rusnak 2012). Fruits are formed on current year's wood. Pruning encourages new growth, increasing yields. Pruning is important to allow good light penetration and air circulation. Typically, pruning is not required during the first year. However, in the following years the cut is recommended to stimulate the shrubs to vigorous growth. After about 3 years usually start to grow up additional new shoots from the root system. Therefore it is important to reduce the number of these shoots. You can prune at the end of the growing season or in very early spring (Maughan and Black 2015, Planting instructions, http://isons.com/pdfs/Isons_Goji_Berry_planting_Instructions.pdf).

The aim of the study was to compare and determine the effect of cutting on the growth of shrubs of goji berries cultivar No. 1. It was also examined the quality of the fruit – their physical parameters and chemical composition.

## MATERIAL AND METHODS

The studies were carried out in the Laboratory of Orcharding at the Department of Horticulture, West Pomeranian University of Technology in Szczecin during years 2013–2015. The soil in the orchard was an agricultural soil with a natural profile, developed from silt-loam with a considerably lower density of 1.21 mg · $m^{-3}$, a pH 6.9 and a higher water capacity, it also contains much more organic matter − 33.1 g in kg of soil. Regardless of the site, the soils were characterized by similar low salinity EC 0.35−0.43 mS · $cm^{-1}$. The soil, in which the shrubs grew, regardless of the stand, in comparison to optimal mineral content of the soil by

Sadowski et al. (1990), was characterized by a high content of P (78 mg · $kg^{-1}$), K (164 mg · $kg^{-1}$) and Mg (52 mg · $kg^{-1}$). Every year in the spring nitrogen fertilization was used at a dose of 90 kg N and shrubs were watered in order to maintain optimum soil moisture.

The research station is located in the north-western part of Poland in the Szczecin Lowland. The majority of the West Pomeranian Province belongs to the zone 7A on the Heinz and Schreiber's "Map of zones of plant resistance to frost". However, in the area of Szczecin and in the nearby northern region, minimal temperatures range from –12°C to –15°C, which corresponds to values typical of zone 7B.

The *Lycium barbarum* L. shrubs, cultivar 'No 1' were planted at a spacing of 3.5 x 1.1 m in 2008 year. The experiment was carried a randomized sub–block design (3 replicates of 3 plants). In the year 2010 shoots were cut out from the shrubs according to the scheme.

**Scheme of the experiment.** Starting this year the control shrubs had been applied with sanitary cuttings only, while in other objects there had been removed all new basal shoots. Since 2013, year-by-year one old shoot was replaced with new one-year old shoots growing on roots: control – uncut bushes, bushes cut into 3 main shoots, bushes cut into 6 main shoots.

The following parameters were measured after the growing season: the height of the bushes, the length of one-year growths (without shoots growing on roots), the number of basal shoots.

During the first harvest of fruits, the index of leaf greenness (Chlorophyll Meter SPAD-502, Minolta, Japan) and color of leaves were measured. The color of leaves and fresh-cut berries (25 fruit from each shrub) was measured with a spectrophotocolorimeter Minolta CM-700 (Konica Minolta Sensing, Inc., Osaka, Japan). The CIE L* (lightness), CIE a* (green-red) and CIE b* (yellow-blue) were read using a D75 light source and the observer angle at 10° and consisting of a head with 3 mm diameter measuring area. There were measured leaves collected from the central part of the one year shoots –10 leaves from 3 shoots from each shrub.

Fruits were harvested manually with gently shaken, from all the shrubs covered by the experiment. They were used to prepare the aggregate sample on which were made measurements. Physical features of fruits (fruit size, firmness) were measured on fresh berries immediately after the harvest. The fruit weight was measured with RADWAG WPX 4500 electronic scales (0.01 g accuracy). Fruit diameter, firmness was measured with a FirmTech2 apparatus (BioWorks, USA) of 100 randomly selected berries from three replicate was expressed as a gram-force causing fruit surface to bend 1 mm.

Dry matter content was measured after drying at 105°C – 3 repetitions of 100 grams from each combination.

For juice extraction efficiency berry were homogenized with a blender and heated up to 50°C. with pectinase (Rapidaza Super) – 3 repetitions of 100 grams from each combination. Afterward (1 hour), the pulp was pressed a laboratory hydraulic press. Further, the acidity was determined by titration of a water extract of berry homogenate with 0.1 N NaOH to an end point of pH 8.1 (measured with an Elmetron pH meter). Titratable acidity was determined by potentiometric method and expressed as equivalents of citric acid 100 $g^{-1}$. Soluble solids content was determined with a digital refractometer PAL-1 (Atago, Japan). L-ascorbic acid, nitrates and nitrites content was measured with a RQflex 10 requantometer (Merck) and expressed as mg per 100 g berry juice.

The content of the pro-vitamin A was examined by HPLC with UV detection. Identification of phenolic compounds was checked by the UPLC-PDA/MS method. The fresh fruits were extracted with methanol acidified with 2.0% formic acid. The extraction was performed twice by incubation for 20 min each under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with occasional shaking. Next, the slurry was centrifuged at 19.000 × g for 10 min, and the supernatant was filtered through a Hydrophilic PTFE 0.20 µm membrane (Millex Samplicity Filter, Merck) and used for analysis. The content of polyphenols in individual extracts was determined by means of the ultra-performance liquid chromatographyphoto-diode array detector--mass spectrometry (LC-PDA-MS) method. All extractions were carried out in triplicate.

In order to determine the significance of differences, a one-factor analysis of variance was carried out, followed by the assessment of the significance of differences using the Tukey's test. The statistical analyses were performed using the Statistica 12.5 (StatSoft Polska).

## RESULTS AND DISCUSSION

Wolfberry is a shrub with thick, heavily ramose shoots. It can grow up to 2–3 meters high (Bogacz 2009). Based on our experiment, it was found that the heavy cutting of basal shoots foster the growth of shrubs. The plants carried out on 3 main shoots were lower than the bushes not cutted. They produced instead a large number of short basal shoots and long one-year shoots (Table 1). It also increased cross sectional area of left shoots.

Table 1. Characteristics of goji shrub *Lycium barbarum* L. depending on the method of cutting. The average of the years 2013–2015
Tabela 1. Charakterystyka krzewów goji *Lycium barbarum* L. w zależności od sposobu cięcia. Średnia z lat 2013–2015

| Measured feature – Badana cecha | | The number of shoots – Liczba pędów | | |
|---|---|---|---|---|
| | | control kontrola | 6 shoots 6 pędów | 3 shoots 3 pędy |
| Height shrubs – Wysokość roślin [cm] | | 246b | 238b | 217a |
| Shoot main cross-sectional area Powierzchnia przekroju poprzecznego pędu szkieletowego [cm$^2$] | | 4.52a | 7.05b | 12.37c |
| Total number of basal shoots Liczba pędów odziomkowych | | 2.7a | 6.5b | 9.2c |
| Average length of basal shoots Średnia długość pędów odziomkowych [cm] | | 36b | 117ab | 82a |
| Average length one-year shoots Średnia długość pędów jednorocznych [cm] | | 54a | 66ab | 79b |
| Green index − Indeks zazielenienia (SPAD) | | 37.3a | 42.1b | 44.8b |
| Leaves color − Barwa liści | L* | 43.5a | 41.2ab | 39.8b |
| | a* | –43.2a | –44.6a | –48.5b |
| | b* | 21.4b | 18.8ab | 14.6a |
| Correlation coefficient − Współczynnik korelacji SPAD/a* r = 0,92 | | | | |

Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to t-Tukey test.
Wartości średnie oznaczone tą samą literą nie różnią się statystycznie istotnie według testu Tukeya na poziomie istotności 0,05.
r – a significant correlation coefficient – współczynnik korelacji istotny na poziomie 0,05.

The leaves green index is highly correlated with the content of chlorophyll in leaves (Pacewicz and Gregorczyk 2009). On this basis we can conclude, among other things about condition of the plants. Leaves from the cut bushes had similar value of green index (44.8 SPAD – 3 shoots, 42.1 SPAD – 6 shoots), significantly higher than the leaves of not cut shrubs (37.3 SPAD). There was observed a significant correlation (r = 0.92*) between the green index, and the value of the parameter a*. The most green leaves were collected from shrubs cutted to 3 shoots. They were characterized by the highest value of green index (Table 1). A similar value of green index were characterized leaves of highbush blueberry (Ochmian 2012). Leaves measured on the cutted shrubs, especially cutted into 3 shoots were also darker, what is shown by the lower value of the parameter L*.

Cutting of shrubs resulted in a significant decrease of yield, while improved quality of the fruit (Table 2). The biggest fruits yield (3.12 kg from shrubs) characterized shrubs which have not undergone a cut. With shrubs carried out in 3 main shoots collected 1.94 kg of fruit. These fruits results in comparison to the collected from the remaining plants were highest - weight of 100 fruit was 67.0 g, the length of 14.8 mm and the diameter 9.9 mm. They were similar to the fruits of cultivar 'No. 1', but much smaller than the fruit of 'New Big' – 94.3 g (Poterańska and Ochmian 2015). Fruit size is a factor that mainly determines the attractiveness of the fruit. Consumers are looking for large fruit, nicely dyed, characterized by a pro-health properties.

Table 2. Yielding and quality of goji berries depending on methods of cutting. The average of the years 2013–2015
Tabela. 2. Plonowanie oraz jakość owoców goji w zależności od sposobów cięcia. Średnia z lat 2013–2015

| Measured feature – Badana cecha | | The number of shoots – Liczba pędów | | |
|---|---|---|---|---|
| | | control<br>kontrola | 6 shoots<br>6 pędów | 3 shoots<br>3 pędy |
| Yield − Plon [kg/shrubs] | | 3.12c | 2.65b | 1.94a |
| Weight of 100 fruits − Masa 100 owoców [g] | | 52.2a | 59.1b | 67.0c |
| Fruits diameter − Średnica owoców [mm] | | 9.0a | 9.5ab | 9.9b |
| Fruits lenght − Długość owoców [mm] | | 13.3a | 14.4b | 14.8b |
| Fruits firmness − Jędrność [G · $mm^{-1}$] | | 186a | 166ab | 142b |
| Fruits color − Barwa owoców (CIE) | L* | 35.3a | 33.4ab | 29.7b |
| | a* | 27.9a | 31.0ab | 33.7b |
| | b* | 41.3c | 36.2b | 27.9a |
| Dry matter − Sucha masa [%] | | 15.5b | 15.2b | 14.3a |
| Soluble solids − Ekstrakt [%] | | 12.9ab | 12.3a | 13.6b |
| Titratable acidity − Kwasowość [g · 100 $g^{-1}$] | | 1.11b | 1.23b | 0.91a |
| Nitrites − Azotyny $NO_2$ [mg · 1000 $g^{-1}$] | | 0.71a | 0.84a | 0.97a |
| Nitrates − Azotany $NO_3$ [mg · 1000 $g^{-1}$] | | 47b | 40b | 28a |
| Provitamin A − Prowitamina A [mg · 100 $g^{-1}$] | | 14.3ab | 11.6a | 16.9b |
| L-ascorbic acid − Kwas L-askorbinowy [mg · 100 $g^{-1}$] | | 24.5a | 29.6a | 40.1b |
| Total polyphenol − Suma polifenoli [mg · 100 $g^{-1}$] | | 43.7c | 34.9b | 29.4a |

Explanations see Table 1 – Objaśnienia jak w tab. 1.

Goji berries are very fragile fruits, with a low firmness, close to the delicate fruit of blue honeysuckle berries (Ochmian and Grajkowski 2007). To reduce the risk of damage the fruit should be harvested with the stalk (Cieślik and Gębusia 2012). Fruits harvested from the bushes not cut (control) were firmer (186 $G^{-1}$ mm) from the fruit obtained out of the bushes carried out in 3 shoots (142 G $mm^{-1}$). Firmness is a characteristic dependent on the method of cultivation of the species, in particular the size of the fruit. Larger fruits are usually characterized by lower firmness (Ochmian and Kozos 2014; Ochmian et al. 2014).

Coloration of fruit peel of cultivars *Lycium barbarum* L.: is variable, from the taint bright yellow-orange to dark orange-red (Poterańska and Ochmian 2015). Goji berries from our studies were dark orange-red, especially those collected from the the bushes cut into 3 main shoots. Strong cuttings resulted in better access of light and fruits ripen faster. It was a similar color to the fruit of other of cultivars goji berries (Poterańska and Ochmian 2015) and sea-buckthorn berries (L* 45.5, a* 20.2, b* 36.7), however, they were darker than them (George and Cenkowski 2007).

Goji berries in its composition contain many valuable nutrients, which are characterized by a very high biological activity. They are classified to the super-fruit (Kulczyński et al. 2014). They are the source of vitamin A, L-ascorbic acid, also contain polyphenols (Paszkiewicz et al. 2012). The richest in vitamin A (16.9 mg · 100 $g^{-1}$) and L-ascorbic acid (40.1 mg · 100 $g^{-1}$) have proved to be fruits obtained from the goji bushes cut into 3 shoots. However, this cutting method caused a decrease of polyphenol content in fruits to the level of 29.4 mg · 100 $g^{-1}$. The highest content of polyphenols was found in fruits harvested from control bushes 43.7 mg. In addition to fruit for medicinal purposes you can also use the leaves. They are characterized by similar polyphenol content as fruit. Larger quantities of polyphenols were found in the leaves of *Lycium chinensis* – 80.64 mg (Mocan et al. 2014). Compared to other berry plants, for example blackcurrant (614.0 mg) (Witkowska and Zujko 2009) goji contained a small amount of polyphenol compounds. The content of L-ascorbic acid is similar to blue honeysuckle berries (Szot et al. 2014). Goji berries do not have the ability to excessive accumulation of detrimental to health nitrates. Regardless of the number of main shoots of shrubs, nitrate levels in the fruit of Chinese wolfberry meets the most stringent requirements for the groups of plant foods intended for the infants and young children. According to the permissible nitrate content in vegetables meant for feeding babies and young children should not exceed 200 mg $NaNO_3$ $kg^{-1}$ (WE 1881). Berries harvested from bushes of 3 shoots contained the most nitrite (0.97 mg · 1000 $g^{-1}$). While the fruits from the control bushes have accumulated the smallest nitrite, but contained the greatest amount of nitrates. In the studies of Poterańska and Ochmian (2015), nitrates and nitrites in goji berries have occurred in similar quantities. The fruit flavor is determined mainly by the relationship between acids to extract. The highest acidity characterized fruits harvested from plants shoots cut into 6 (1.23 g · 100 $g^{-1}$) while they had the lowest overall content of the extract (12.3%). Fruit of the shrubs cut into 3 main shoots had the highest extract content (13.6%) and the lowest acidity (0.91 g · 100 $g^{-1}$). The fruit from the plant carried out in 3 main shoots were also specified by the smallest dry weight (14.3%).

## CONCLUSIONS

1. The cut shrubs, especially into 3 shoots, produced a higher number of shorter basal shoots, while the longer one-year shoots.
2. The leaves from the bushes cut into 3 and 6 main shoots were characterized by a higher value of the green index. They were also darker and characterized by higher value of a* color parameter.

3. There was found a significant correlation between green index, and the value of the parameter a*.
4. Cutting of bushes reduced yielding, and also had influence for the quality of the fruit. Fruits were bigger and darker but they had less content of polifenols. The bigest influence for the quality of the fruits had cutting of shrubs for three shoots.

## REFERENCES

**Adams M., Wiedenmann M., Tittel G.** 2006. HPLC-MS trace analysis of atropine in *Lycium barbarum* berries. Phytochem. Anal. 17, 279−283.

**Amagase H., Farnsworth N.R.** 2011. A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (Goji). Food. Res. Int. 44(7), 1702−1717.

**Amagase H., Sun B., Borek C.** 2009. *Lycium barbarum* (goji) juice improves *in vivo* antioxidant biomarkers in serum of healthy adults. Nutr. Res. 29, 19−25.

**Bogacz K.** 2009. Goji – owoc zdrowia i długowieczności [Goji − fruit of health and longevity]. PFiOW 9, 33−34. [in Polish]

**China Daily Staff reporter. Wolfberry festivaltobeheldin Ningxia, China Daily.** 2004, http://www.china--daily.com.cn/chinagate/doc/2004-07/19/content_349679.htm, access: May 2016.

**Cieślik E., Gębusia A.** 2012. Charakterystyka właściwości prozdrowotnych owoców roślin egzotycznych [Characteristic of healthy properties of exotic plants fruits]. Post. Fitot. 2, 93–100. [in Polish]

**Gan L., Hua Zhang S., Liang Yang X., Bi Xu H.** 2004. Immunomodulation and antitumor activity by a polysaccharide – protein complex from *Lycium barbarum*. Int. Immunopharmacol. 4, 563−569.

**George S., Cenkowski S.** 2007. Influence of harvest time on the quality of oil-based compounds in sea buckthorn (*Hippophae rhamnoides* L. ssp. sinensis) seed and fruit. J. Agric. Food Chem. *55*(20), 8054−8061.

**Harsh M.L.** 1989. Tropane alkaloids from *Lycium barbarum* Linn., in vivo and in vitro. Curr. Sci. 58, 817−818.

**He N., Yang X., Jiao Y., Tian L., Zhao Y.** 2012. Characterisation of antioxidant and antiproliferative acidic polysaccharides from Chinese wolfberry fruits. Food Chem. 133, 978−989.

**Jin M., Huang Q., Zhao K., Shang P.** 2013. Biological activities and potential health benefit effects of polysaccharides isolated from *Lycium barbarum* L. Int. J. Biol. Macromol. 54, 16−23.

**Kulczyński B., Groszczyk B., Cerba A., Gramza-Michałowska A.** 2014. Goya (*Lycium barbarum*) fruits as bioactive components source in food. Nauka Przyr. Technol. 8(2). [in Polish]

**Lin C.C., Chen J.C.** 2002. Medicinal herb Erycibe henri Prain (‘Ting Kung Teng’) resulting in acute cholinergic syndrome. J. Toxicol. Clin. Toxicol. 40, 185−187.

**Llorent-Martinez E.J., Fernández-de Córdova M.L., Ortega-Barrales P., Ruiz-Medina A.** 2013. Characterization and comparison of the chemical composition of exotic superfoods. Microchem. J. 110, 444−451.

**Luo Q., Cai Y., Yan J., Sun M., Corke H.** 2004. Hypoglycemic and hypolipidemiceffects and antioxidantactivity of fruit extracts from *Lycium barbarum*. Life Sci. 76, 137−149.

**Marquart M., Schaefer S.G.** 1997. Lehrbuch der Toxikologie. Heidelberg, Spektrum Akademischer Verlag, 673.

**Maughan T., Black B.** 2015. Goji in the Garden, http://www.Horticulture/Fruit/2015-05pr, access: May 2016.

**Mocan A., Vlase L., Vodnar D.C., Bischin C., Hanganu D., Gheldiu A.M., Oprean R., Silaghi--Dumitrescu R., Crisan G.** 2014. Polyphenolic content, antioxidant and antimicrobial activities of *Lycium barbarum* L. and *Lycium chinense* Mill. Leaves. Molecules. 19, 10056–10073.

**Ochmian I.** 2012. The impact of foliar application of calcium fertilizers on the quality of highbush blueberry fruits belonging to the ‘Duke’ cultivar. Not. Bot. Hort. Agrobot. 40(2), 163−169.

**Ochmian I., Dobrowolska A., Chełpiński P.** 2014. Physical parameters and chemical composition of fourteen blackcurrant cultivars (*Ribes nigrum* L.). Not. Bot. Hort. Agrobot. 42(1), 160−167.

**Ochmian I., Grajkowski J.** 2007. Wzrost i plonowanie trzech odmian jagody kamczackiej (*Lonicera caerulea*) na Pomorzu Zachodnim w pierwszych latach po posadzeniu [Growth and yielding of blue honeysuckle (*Lonicera caerulea*) three cultivars in Western Pomerania in first years after planting]. Rocz. AR. Pozn. 383(41), 351−355. [in Polish]

**Ochmian I., Kozos K.** 2014. Fruit quality of highbush blueberry (*Vaccinium corymbosum* L.) cv.'Duke' depending on the method of cultivation. Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 31, 117–126.

**Pacewicz K., Gregorczyk A.** 2009. Porównanie ocen zawartości chlorofilu chlorofilometrami Spad-502 i N-tester [Comparision values of chlorophyll content by chlorophyll meter Spad-502 and N-tester]. Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 9, 41–46.

**Paszkiewicz M., Budzyńska A., Różalska B., Sadowska B.** 2012. Immunomodulacyjna rola polifenoli roślinnych [The immunomodulatory role of plant polyphenols]. Post. Hig. Med. Dośw. 66, 637–646. [in Polish]

**Peaceful Valley Farm Supply. Goji (Wolf) Berries. Planting and growing guide.** 2013, http://www.grow-organic.com/media/pdfs/goji-l.pdf, access: May 2016.

**Planting instructions. Goji berry planting instructions,** http://isons.com/pdfs/Isons_Goji_Berry_planting__Instructions.pdf, access: May 2016.

**Poterańska N., Ochmian I.** 2015. Porównanie jakości owoców dwóch odmian goji (*Lycium barbarum*) [The comparison of fruits quality of two goji cultivars (*Lycium barbarum*)]. Bad. Roz. Młod. Nauk. Pol., Nauki Przyrod. 3(1), 2, 125–131. [in Polish]

**Potterat O.** 2010. Goji (*Lycium barbarum* and *Lycium chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med. 76, 7–19.

**Rusnak J.** 2012. Cięcie krzewów owocowych. Karniowice, Małopolski Ośrodek Doradztwa Rolniczego, 2−3.

**Sadowski A., Nurzyski J., Pacholak E., Smolarz K.** 1990. Określenie potrzeb nawożenia roślin sadowniczych. Cz. II. Zasady, liczby graniczne i dawki nawożenia. Instrukcja upowszechnieniowa nr 3. Warszawa, SGGW, 25. [in Polish]

**Szot I., Lipa T., Sosnowska B.** 2014. Jagoda kamczacka – właściwości prozdrowotne owoców i możliwości ich zastosowania [Blue honeysuckle – healthful properties of fruits and possibilities of their applications]. Żywn. Nauka Technol. Jakość 4(95), 18–29. [in Polish]

**Rozporządzenie Komisji WE 1881 z dnia 19 grudnia 2006 r. ustalające najwyższe dopuszczalne poziomy niektórych zanieczyszczeń w środkach spożywczych.** DzUrz. UE L 364/5 z 20.12.2006. [in Polish]

**Witkowska A., Zujko M.E.** 2009. Aktywność antyoksydacyjna owoców leśnych [Antioxidant activity of wild berries]. Bromat. Chem. Toksykol. 3, 900–903. [in Polish.]

**Abstract.** In the experiment were compared: the growth of shrubs, yielding, the physical parameters of fruits and their chemical composition depending on the method of cutting bushes – the amount of main shoots. Research material consisted of goji berries grown in Department of Horticulture Research Station of the West Pomeranian University of Technology in Szczecin. Goji berries (*Lycium barbarum* L.) are characterized by high potential of health promoting properties and nutritious. They have been extremely important part of traditional Chinese medicine for ages. They are used there as a very important part of a healthy diet, due to the appearance of the complex polysaccharide valuable LBP (Lycium Barbarum Polysaccharides). Cutting of shoots stimulated growth of shrubs. Bushes cut into 3 shoots grew with larger number of shorter basal shoots, while the one-year shoots were the longest. The leaves of the cut bush were darker (L* = 39.8 and 41.2) and had a higher green index (42.1 and 44.8), in comparison to uncut bushes (L* = 37.3, SPAD = 43.5). It was also found that there is a significant correlation between the green index and the value of the parameter a* (r* = 0.92). Cutting of shrubs, especially into 3 shoots, reduced yielding however influenced increasing the size of the fruit.

# COMPARISON OF MORPHOLOGICAL, ANTIDIABETIC AND ANTIOXIDANT PROPERTIES OF GOJI FRUITS -Research paper

**Article** *in* Acta Universitatis Cibiniensis. Series E: Food Technology · June 2020

DOI: 10.2478/aucft-2020-0001



4 authors:

Arleta Kruczek
West Pomeranian University of Technology, Szczecin


Ireneusz Ochmian
West Pomeranian University of Technology, Szczecin


Krupa-Małkiewicz Marcelina
West Pomeranian University of Technology, Szczecin


Sabina Lachowicz
Wrocław University of Environmental and Life Sciences

sciendo

10.2478/aucft-2020-0001

ACTA UNIVERSITATIS CIBINIENSIS Series E: Food technology

# COMPARISON OF MORPHOLOGICAL, ANTIDIABETIC AND ANTIOXIDANT PROPERTIES OF GOJI FRUITS

– Research paper –

Arleta KRUCZEK[*], Ireneusz OCHMIAN[*], Marcelina KRUPA-MAŁKIEWICZ[**][1], Sabina LACHOWICZ[***]

*Department of Horticulture, West Pomeranian University of Technology Szczecin, Słowackiego 17 Street, 71-434 Szczecin, Poland; iochmian@zut.edu.pl, ORCID 0000-0002-3606-1927
**Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology Szczecin, Słowackiego 17 Street, Szczecin, Poland; ORCID 0000-0002-4333-9122
***Department of Fermentation and Cereals Technology, Wrocław University of Environmental and Life Sciences, Chełmońskiego 37 Street, 51-630 Wrocław, Poland; sabina.lachowicz@upwr.edu.pl, ORCID 0000-0001-6182-0211

**Abstract:** There is a growing public interest in fruits labeled as „superfood" (functional food). A "superfood" should have a high content of bioactive substances with a positive impact on human health. Seven different cultivars of goji berry fruits (*Lycium chinense* Mill.) grown in north-western Poland were evaluated for its physicochemical parameters, antidiabetic and antioxidant activity, and polyphenol content. The length of 1-year-old shoots ranged from 36 cm ('Big Lifeberry') to 82 cm ('Korean Big'). Cultivars from the group of Big were characterized by the biggest fruits (17.3-24.2 mm) with the greatest weight of 100 fruits (96.7-122.1 g). 'Big Lifeberry' contained high amounts of *L*-ascorbic acid (408 mg 1000/g) and provitamin A (190 mg 1000/g) and showed high antidiabetic ($\alpha$-amylase $IC_{50}$=33.4 mg/mL; $\alpha$-glucosidase $IC_{50}$=9.9 mg/mL) and antioxidant activity (ABTS·+ 6.21 and FRAP 5.58 mmol T/100 g). 'Big Lifeberry' was also characterized by a high total content of polyphenols (43.64 mg 100/g). Furthermore, the nitrite content in all the cultivars tested was at a relatively low level. Among the examined cultivars, the most attractive one concerning the consumers' point of view of the size, weight and high content of health-promoting compounds is 'Big Lifeberry'.

**Keywords:** *Lycium chinense* Mill., polyphenols, biological activity, antidiabetic activity, harmful substances

## INTRODUCTION

An increase in the frequency of the occurrence and the need to prevent civilization diseases has led to intensified research on the use of compounds naturally occurring in plants. To date, many studies have demonstrated that lifestyle changes, exercise, and a well-balanced diet can significantly reduce the risk of human diseases. Therefore, it is good to include fruits with a high content of mineral and bioactive compounds in the diet (Kulczyński and Gramza-Michałowska, 2016).

*Lycium chinense* Mill. fruit (goji) in recent years has become more popular due to its public acceptance as a "superfood" with highly beneficial nutritive and antioxidant properties. It is a *Solanaceous* defoliated shrubbery, and its fruit is a famous traditional medicine, growing in the Tibetan valleys and in the Himalayas where the areas have not been polluted by civilization or by pesticides for centuries (Kulczyński and Gramza-Michałkowska, 2016). The goji fruits are called "red diamonds" because of their valuable health-promoting properties. The Latin name *Lycium* means "school"- Chinese masters of herbalism believed that goji fruit is, as a school, a source of knowledge. It has been widely used in China for medicinal purposes and as a functional food for more than 2500 years (Burke et al., 2005; Chang and So, 2008). In traditional Chinese medicine, it is believed that their consumption can prolong life and provide energy despite the passage of years-hence their name "fruits of longevity". To confirm such traditional properties, modern research indicates that *L. chinense* extracts have a number of biologic activities, including antioxidant properties (Chang and So, 2008). The extracts also exhibit antiaging

*Received: 10.01.2020*
*Accepted in revised form: 11.04.2020*

[1] Corresponding author. E-Mail address: mkrupa@zut.edu.pl

effects, neuroprotection, promotion of endurance, increased metabolism, improved control of glucose and other diabetic symptoms, antiglaucoma effect, immunomodulation, antitumor activity, and cytoprotection (Burke et al., 2005; Wojdyło et al., 2018).

Currently, the annual cultivation of goji fruit reaches 95,000 tones and covers an area of about 82,000 ha. They are mainly used in the production of dried fruit and also in the preparation of juices, liqueurs, wines, soup additives, meat, and vegetarian dishes, and tea. Besides, fruit extracts are used as natural, non-toxic dyes for beverages and in cosmetics (Bogacz, 2009).

To date, studies on goji berry plants (*L. chinense*) grown in the field in the northern part of Europe are scarce. Therefore, we have undertaken to compare seven cultivars of goji fruits grown in the climatic conditions of north-western Poland. This research will provide evidence to select shrubs, which are characterized by the most valuable fruit in terms of antidiabetic and antioxidant activity, with the highest content of polyphenols, as well as with the best morphological and physicochemical properties.

## MATERIALS AND METHODS

*Characteristic of the area of research*

The studies were carried out in the Laboratory of Orchard at the Department of Horticulture, West Pomeranian University of Technology in Szczecin in 2015-2018. The research station is located in the north-west part of Poland. According to Heinz and Schreiber's Map of zones, the minimum temperature in this region range from –12 ºC to –15 ºC and in the growing season (April-October) between 1951 and 2012 was 13.7 ºC and rainfall 391 mm (Mijowska et al., 2017, Ochmian et al., 2019). The wheatear during the experimental period significantly differed from the average temperature and rainfall for many years (Table 1). During the experiment, a higher average temperature was found during the growing season, especially in 2018, compared to many years. At the same time, much less rainfall was observed.

The soil in the orchard was an agricultural soil with a natural profile, developed from silt-loam with a considerably lower density of 1.21 mg/m$^3$, pH 6.9 and the higher water capacity. It also contains much more organic matter – 33.1 g/kg soil. Regardless of the stand, the soils were characterized by low salinity – EC 0.35-0.43 mS/cm, high content of P 78 mg/kg (optimum 20–40 mg/kg), K 164 mg/kg (optimum 50–80 mg/kg) and magnesium 52 mg/kg (optimum 25–40 mg/kg), in comparison to the optimal mineral content of the soil by Sadowski et al. (1990). Every spring, nitrogen fertilization was applied at a rate of 90 kg N per hectare.

The plantation was irrigated annually using a permanently-installed T-Tape drip irrigation line with the emitters performance of 1.5 L per 1 hour (3 L of water on a section of 1 linear meter of the installation). Soil moisture was maintained in the PF 1.8–2.1 range and was determined by contact tensiometers.

*Plant material*

The fruits of seven cultivars of *Lycium chinense* Mill. where used in this study. The research material contained cultivars: No '1', New Big, Sweet Berry, Big Berry, Big Lifeberry, Korean Big, Amber Sweet Goji. Shrubs were planted in the spring of 2010 in the orchard of Department of Horticulture in Szczecin, Poland.

Table 1. Weather conditions during the vegetative season (April-October) in the years 2015-2018 regarding the average growing season during the multi-year period (1951-2012)

| | Month | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **IV** | **V** | **VI** | **VII** | **VIII** | **IX** | **X** | |
| **year** | **average temperature (ºC)** | | | | | | | **Mean** |
| 1951-2012 | **8.0** | **13.0** | **16.4** | **18.2** | **17.6** | **13.8** | **9.2** | **13.7** |
| 2015 | 8.7 | 12.5 | 15.6 | 18.6 | 21.1 | 14.1 | 8.5 | 14.2 |
| 2016 | 8.8 | 15.7 | 18.5 | 19.0 | 17.8 | 16.8 | 8.6 | 15.0 |
| 2017 | 6.8 | 13.5 | 16.8 | 17.2 | 17.9 | 13.3 | 11.0 | 13.8 |
| 2018 | 12.9 | 17.5 | 18.1 | 20.1 | 19.7 | 15.2 | 10.3 | 16.3 |
| | **rainfall (mm)** | | | | | | | Total |
| 1951-2012 | **39.7** | **62.9** | **48.2** | **69.6** | **74.2** | **58.7** | **37.3** | **391** |
| 2015 | 29.0 | 48.0 | 32.8 | 62.0 | 14.7 | 34.4 | 22.1 | 243 |
| 2016 | 20.2 | 18.9 | 69.0 | 50.1 | 47.8 | 18.3 | 55.3 | 280 |
| 2017 | 42.3 | 99.2 | 118.1 | 182.4 | 45.4 | 31.6 | 95.1 | 614 |
| 2018 | 24.0 | 25.4 | 11.6 | 92.8 | 21.4 | 16.3 | 20.2 | 212 |

*Determination of physical parameters*

The one-year shoot's length (cm) was measured in autumn on all shrubs covered by the experiment.

*Foliage area of one leaf ($cm^2$)*

The foliage area of one leaf was measured using the Delta Image Analysis System (Delta-T Devices LTD, England ) scanner connected to the computer.

*Determination of Leaf greenness index and colour*

Prior to the first harvest of fruits, the leaf greenness index was determined using Chlorophyll Meter SPAD-502 (Minolta, Japan).

The pigment measurement (colour) of the leaf and fruits of the *Lycium chinense* Mill. was analyzed in a transmitted mode evaluated by photocolorimetric method tested in CIE L**a***b** system, as described by Krupa-Małkiewicz et al. (2019). The *a** value showed the place of appearing in the colour gamut, in the range from green to red (+*a** means redness; −*a** means greenness) on the surface of dried fruits of analyzed genotypes. The *b** parameter described the colour in the range from yellow to blue (+*b** means yellow; −*b** means blue) on the surface of dried fruits of tested genotypes. The value of L* parameter ranges from 0 to 100, black to white, respectively.

*Determination of Normalized Difference Vegetation Index and Anthocyanins Index*

Relative changes in concentration of chlorophyll and anthocyanins on the goji fruits was measured non-destructively spectrometer PA 1101 (Control in Applied Physiology, Berlin-Falkensee, Germany).

The head of the instrument contains five light-emitting diodes (LEDs) in the range of 400–1100 nm remitted from the fruit peel. Relative changes in chlorophyll concentration are expressed as normalized differential vegetation index (NDVI) = (I780-I660)/(I780+I660) and anthocyanins as normalized anthocyanins index (NAI) = (I780-I570)/(I780+I570). Where I is the intensity of the reflected light at a given wavelength (Ochmian et al., 2016).

Fruits from four trees of every cultivar and treatment were used from the lower part of the tree crown for an optimum composite sample, resulting in 120 determinations on each sampling date.

*General fruits parameters*

The fruit weight was measured with RADWAG WPX 4500 electronic scales (0.01 g accuracy). Diameter and firmness of fruits were measured on fresh berries immediately after the harvest with a FirmTech2 apparatus (BioWorks, USA) of 100 randomly selected berries from three replicate was expressed as a gram-force causing fruit surface to bend 1 mm.

*Determination of chemical parameters*

*Basic measurements*

The fruit was collected manually, gently shaking from all the shrubs included in the experiment and measurements were taken on a prepared bulk sample. For juice extraction efficiency berry were homogenized with a blender with pectinase (Rapidaza Super – 0.025 ml/250 g) at 40$^{O}$C - 3 repetitions of 250 g from each combination. Afterwards, after 60 minutes, the pulp was pressed a laboratory hydraulic press. *L*-ascorbic acid, nitrates V and nitrates III contents were measured with a RQflex 10 requantometer (Merck) and expressed as mg per 100 g berry juice (Piwowarczyk et al. 2020). The soluble solids content was determined with a digital refractometer PAL-1 (Atago, Japan). The acidity was determined by titration of an extract of berry homogenate with 0.1 N NaOH to an end point of pH 8.1 (measured with an Elmetron pH meter) (Mijowska et. al. 2016).

*Antidiabetic activity (α-amylase, α-glucosidase)*

Extraction mixed fruits were done with 70% ethanol at room temperature for 60 min with constant stirring. After centrifuging at 4000 rpm for 10 min, and filtration, the supernatants were concentrated at 40°C (vacuum evaporator) to remove the ethanol and the aqueous phase was diluted with water. For further analytical and biological activity assays, a gradient of concentrations was prepared via serial dilution of the fruit extracts in pure water.

The activity of the parasite and host extracts was assayed according to the procedure described previously by Podsedek et al. (2014) (*α*-glucosidase) and Nickavar and Yousefian (2011) (*α*-amylase). All samples were assayed in triplicate and the result was expressed as $IC_{50}$. The amount of the inhibitor (expressed as mg 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. All samples were assayed in triplicate.

*Antioxidant activity (ABTS·+, FRAP)*

ABTS•+ (2,2'-azobis(3-etylobenzotiazolino-6-sulfonian) compound was used to observe the kinetics of enzyme reactions assay (Arnao et al., 2001). The FRAP (Ferric-Reducing Antioxidant Power) assay was done according to Benzie and Strain (1996). The antioxidant capacity is expressed as Trolox equivalent. Measurements by means of ABTS•+ and FRAP assay involved UV-2401 PC spectrophotometer.

*Scopolamine and α-solanine*
The scopolamine and α-solanine were identified by comparing band colors after derivatization with Dragendorff and Carr-Price reagents (Kokotkiewicz et al., 2017).

*Provitamins A*
The content of provitamin A in fruits was determined by high-performance liquid chromatography with UV and fluorescence detection (Knauer K-1001 pump and Knauer K-2001 UV detector; Beckman ODS column (5 µm), dimensions 150×4.6 mm, column temperature 25°C) (EC Commission Regulation 2009).

*Polyphenol content*
Three replicates of 100 g randomly chosen goji fruits were kept frozen at −65°C until analysis, then prepared according to the methodology of Oszmiański et al. (2018). The content of polyphenols in individual extracts was determined by means of the ultra-performance liquid chromatographyphoto-diode array detector-mass spectrometry (LC-PDA-MS) method. All extractions were carried out in triplicate. Identification of polyphenols of *Lycium chinense* Mill. fruits extracts were carried out using an ACQUITY Ultra Performance LC system equipped with a photodiode array detector with a binary solvent manager (Waters Corporation, Milford, MA) with a mass detector G2 Q-TOF micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionisation (ESI) source operating in negative and positive modes. The PDA spectra were measured over the wavelength range of 200-600 nm in steps of 2 nm. The retention times and spectra were compared to those of the authentic standards.

*Statistical analysis*
All statistical analyses were performed with Statistica 12.5 (StatSoft Polska, Cracow, Poland). Statistical significance of the differences between means was established by testing homogeneity of variance and normality of distribution followed by ANOVA with Tukey's *post hoc* test, significance was set at $p<0.05$. The results are expressed as means ± SD. To determine the relationship between the cultivars and bioactive compounds the results obtained were subjected to agglomerative cluster analysis and classified into groups in a hierarchical order by means of the Ward's method.

## RESULTS AND DISCUSSION

*Basic physical and chemical properties of goji fruits and shrub*
The growth characteristics of the examined cultivars are presented in Table 2. The differences between tested goji genotypes with regard to the height of the one-year-old shoots are significant. The average height of the one-year-old shoots varied between 36 cm and 82 cm. Among all tested cultivars, the 'Korean Big' had taller plants, while the 'Big Lifeberry' was the shortest. It indicates that goji plants had very different growth rates and the ability to adapt. By comparing cultivar Ningqi 6 with the standard Ningqi 1, Wang et al. (2011) observed that cultivar Ningqi 6 is superior in terms of height vigor and productivity. Mencinicopschi and Bălan (2013) and Dzhugalov et al. (2015) also reported the differences in the vigor of goji cultivar. According to Kiełbasa et al. (2005) the size of the leaf blade, which is crucial in photosynthesis, can also indicate the intensity of growth. On analyzing the leaves, it was found that the largest leaf had a cultivar of Amber Sweet Goji with the size varying from 5.38 $cm^2$ (the biggest) to 2.33 $cm^2$ (the smallest) – No '1'(Table 2). SPAD chlorophyll meter is used to measure the leaves' green index, which is strongly correlated with the chlorophyll content of the leaves (Pacewicz and Gregorczyk, 2009). Based on these findings, we can assess the condition of the plants (Antal et al., 2013). The highest SPAD value (52.4) was observed in cultivar Sweet Berry, while the lowest (31.2) was in Big Berry (Table 2). The leaves' green index was significantly different but it correlated with the value of the parameter $a*$ (Table 2). The results of colour determination of the leaves and fruits were also analyzed in the transmitted mode with the photocolorimetric method in the CIE $L^*a*b*$ system. The leaves of *Lycium chinense* Mill. were also characterized by a similar value of the green index (Kruczek and Ochmian, 2016).
The value of $a*$ parameter (colour ranging from green to red) determined on the surface of leaf ranges from -32.9 ('Sweet Berry') to -59.4 ('Big Berry'). The leaves of cultivar Sweet Berry were also darker, which is shown by the lower value of the parameter $L^*$ (41.8). The value of parameter $L^*$ (reaching from 0 to 100, black to white, respectively) is usually used for tracking the colour changes (Ochmian et al., 2019). However, goji leaves of cultivar Amber Sweet Goji are 39% brighter compared to the Sweet Berry leaves (Table 2).

Table 2. Differences in the shrubs' growth and colour of the tested goji fruits (*L. chinense*)

| | | **Cultivar** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Compounds** | | **No '1'** | **New Big** | **Sweet Berry** | **Big Berry** | **Big Lifeberry** | **Korean Big** | **Amber Sweet Goji** |
| One-year shoots length (cm) | | 61±21bcd | 74±17de | 47±19ab | 68±15cde | 36±13a | 82±24e | 58±15bc |
| One leaf area ($cm^2$) | | 2.33±0.5a | 4.05±0.6b | 4.73±0.6cd | 5.14±0.5de | 3.77±0.4b | 4.26±0.6bc | 5.38±0.7e |
| Greening index SPAD | | 38.7±2.7c | 35.4±2.3bc | 31.2±2.5a | 52.4±3.0e | 42.5±1.9d | 44.1±2.9d | 33.7±1.3ab |
| Color parameters CIE leaves | L* | 46.3±2.5c | 43.2±3.4a | 41.8±3.5a | 51.0±2.9d | 45.4±2.5b | 44.7±2.8abc | 58.1±1.8e |
| | *a** | -38.5±4.5b | -33.1±3.8a | -32.9±4.1a | -59.4±2.7d | -35.3±4.4ab | -35.9±4.8ab | -44.7±2.2c |
| | *b** | 29.7±2.7c | 17.5±3.8a | 25.8±2.9b | 35.3±2.1d | 23.8±2.5b | 24.8±1.9b | 42.0±1.2e |
| Color parameters CIE fruit | L* | 34.7±3.7a | 49.1±3.4d | 32.6±2.8a | 45.8±3.3cd | 39.5±4.1b | 42.2±2.9bc | 77.3±1.9e |
| | *a** | 22.5±2.7ab | 34.8±2.4d | 31.3±2.5cd | 29.7±3.0c | 23.4±1.9b | 41.2±3.3e | 19.8±2.1a |
| | *b** | 25.9±2.9a | 44.7±2.5cd | 36.8±3.1b | 47.9±3.3de | 42.3±3.0c | 52.0±4.2e | 63.4±3.9f |
| NAI fruit | | 0.74±0.05bc | 0.77±0.04c | 0.75±0.05bc | 0.81±0.03cd | 0.69±0.03b | 0.84±0.04d | 0.34±0.02a |
| NDVI fruit | | -0.52±0.03ab | -0.53±0.03b | -0.57±0.02b | -0.50±0.04a | -0.64±0.03c | -0.47±0.02a | -0.68±0.03c |

*Means followed by the same letter do not differ significantly at P=0.05 according to Tukey multiple range; values are means ± standard deviation

The colour of the leaf surface is determined by parameter *b** (from yellow to blue colour) which ranged from 17.5 ('New Big') to 42.0 ('Amber Sweet Goji'). A similar value of the parameter *a** was observed for the leaves of *L. chinense* (Kruczek and Ochmian, 2016), while the value of the parameters $L^*$ and *b** was slightly lower in comparison to the goji leaves tested in this study. The appearance and colour of the fruit largely influence the choice of consumers (Chełpiński et al., 2019). The colour of the fruit may encourage consumption by influencing suggestions of certain flavors and/or discourage consumption by warning of their rottenness (Oszmiański and Wojdylo, 2005; Lachowicz et al., 2019). The color of food products may change during processing (Yusufe et al., 2017). The coloration of the fruit peel of *L. chinense* is variable, from the taint and bright yellow-orange to dark orange-red (Kruczek and Ochmian, 2016). Regarding the tested fruit colour, the value of *a** parameter measured was significantly different, which ranged from 19.8 ('Amber Sweet Goji') to 41.2 ('Korean Big') but correlated with the Normalized Anthocyanin Index (NAI) (meaning -1 and +1, redness and red, respectively). Anthocyanin dyes are responsible for the colour of the goji berry fruits. The NAI showed that the highest anthocyanin content was recorded in the fruit of cultivar Korean Big (0.84) and the lowest one was in cultivar Amber Sweet Goji (0.34). According to Kuckenberga et al. (2008) estimating the maturity and quality parameters in 'Golden Delicious' apple fruit can also be a useful Normalized Difference Vegetation Index (NDVI). In the current study, the value of NDVI index in goji fruits was between -0.68 ('Amber Sweet Goji') and -0.47 ('Korean Big'). According to Rutkowski et al. (2008), the different values of NDVI indicate the various stages of the fruit: value higher than -0.4 indicates immature fruit (pre-climacteric), the value between -0.4 and -0.6 characterizes fruits with the onset of ethylene production, and value below -0.8 is representative for fruits with more advanced ripening stage (ripe fruits).

The colour of the surface of the tested goji fruits determined by parameter *b** ranged from 25.9 ('No '1'') to 63.4 ('Amber Sweet Goji'). It was shown that cultivars Amber Sweet Goji and Korean Big contained the highest quantity of red-couloring substances and a smaller quantity of yellow-couloring ones. The value of $L^*$ parameter ranged from 32.6 ('Sweet Berry') to 77.3 ('Amber Sweet Goji'). Similar results and observations were obtained in the case of saskatoon berry where parameter $L^*$ ranged from 32.71 to 49.31 (Lachowicz et al., 2019). However, fruits examined by Kruczek and Ochmian (2016) were darker than in our own research; the value of the $L^*$ parameter ranged from 29.7 to 35.3. Also for highbush blueberry, the $L^*$ parameter was lower (19.2 - 22.9) than in the tested goji fruit (Ochmian et al., 2015).

*Firmness*

The size, firmness, and resistance of the fruit to mechanical damage are factors that determine the quality of the fruit. They allow the assessment of the suitability of a given species and cultivar, among others, for mechanical fruit harvesting, transport, and the way they are used. In the case of goji berries, the size of the fruit is not an important parameter, because they are intended mainly for processing (Ochmian et al., 2019). Goji berries are very fragile fruits with a low firmness, which is close to the delicate fruit of blue honeysuckle berries. To reduce the risk of damage, the fruit should be harvested with the stalk (Kruczek and Ochmian, 2016). Among the cultivars tested, Korean Big has the highest weight 122.1 g (100 fruits) with length, firmness and puncture being 21.0 mm, 211 G/mm and 95.1 G/mm, respectively. Because of these characteristics, the fruit of this cultivar was distinguished as 'Korean Big' (Table 3). The physical parameters of the fruit tested in this study were within normal limits or even higher than those reported by Kruczek and Ochmian (2016). This could probably occur due to different environmental conditions and genotypes. On the other hand, Kamchatka berry fruit is characterized by firmness at the level of 165–201 G/mm (Ochmian and Grajkowski, 2007) and cornelian cherry 129–211 G/mm (Ochmian et al., 2019).

*Biological activity*

Goji berries contain many valuable nutrients, which are characterized by a very high biological activity. They are classified as super-fruits (Mikulic-Petkovsek et al., 2012; Kulczyński et al., 2014; Kruczek and Ochmian, 2016). The content of *L*-ascorbic acid of 'Big Lifeberry' goji varied between 62 mg/1000 g and 408 mg/1000 g (Table 3).

Table 3. Physicochemicals parameters and quality of tested goji fruits (*L. chinense*)

| | Cultivar | | | | | | |
|---|---|---|---|---|---|---|---|
| **Compounds** | **No '1'** | **New Big** | **Sweet Berry** | **Big Berry** | **Big Lifeberry** | **Korean Big** | **Amber Sweet Goji** |
| Weight of 100 fruits (g) | 62.4±7.3a | 96.7±8.3c | 73.4±6.5ab | 112.6±8.5de | 104.5±9.0cd | 122.1±7.7e | 85.7±6.2b |
| Fruit diameter (mm) | 9.6±1.2ab | 8.4±1.1a | 9.8±1.3b | 11.8±1.0c | 11.2±1.5c | 10.7±1.3bc | 9.8±1.7b |
| Fruit length (mm) | 14.2±2.2a | 24.2±2.7e | 15.0±1.9ab | 17.3±2.1b | 18.9±2.3cd | 21.0±3.0d | 16.1±2.9abc |
| Puncture (G/mm) | 79.2±14.7de | 49.5±10.1a | 71.3±14.9cd | 88.0±11.7ef | 68.4±19.3bc | 95.1±13.3f | 59.3±16.2ab |
| Firmness (G/mm) | 176±21.3b | 132±17.3a | 174±18.6b | 167±20.3b | 125±12.2a | 211±17.3c | 142±18.5a |
| *L*-ascorbic acid (mg/1000 g) | 323±23e | 197±27c | 224±18c | 279±25d | 408±31f | 155±19b | 62±10a |
| Provitamin A (mg/1000 g) | 153±9e | 122±6c | 94±4b | 137±5d | 190±5f | 118±4c | 35±1a |
| Nitrates III (mg/1000 g) | 1.42±0.11e | 0.68±0.07b | 1.12±0.09d | 0.94±0.10c | 0.62±0.06ab | 1.05±0.09d | 0.53±0.05a |
| Nitrates V (mg/1000 g) | 42.4±5.2b | 33.8±3.3a | 49.3±5.1b | 74.1±3.2c | 29.5±2.4a | 106.6±3.8d | 42.4±2.5b |
| Extracts (°Bx) | 11.4±1.2b | 16.4±1.1d | 18.4±1.3e | 12.5±0.9bc | 11.9±0.6b | 13.2±0.9c | 9.4±0.6a |
| Total acidity (g/1000 g) | 11.1±1.0b | 12.4±0.7bc | 8.9±0.6a | 12.7±1.1c | 14.5±1.2d | 12.6±1.1bc | 13.0±1.3c |
| antidiabetic activity | | | | | | | |
| $\alpha$-amylase $IC_{50}$ (mg/mL) | 38.5±2.1b | 48.6±1.7d | 43.1±1.5c | 50.8±2.2de | 33.4±1.9a | 52.7±1.3e | 73.4±1.1f |
| $\alpha$-glucosidase $IC_{50}$ (mg/mL) | 9.9±0.9c | 9.2±0.8c | 6.5±0.7a | 13.2±0.6d | 9.9±0.5c | 7.8±0.6b | 5.7±0.5a |
| antioxidant activity | | | | | | | |
| ABTS·+ (mmol Trolox/$100g^{-1}$) | 4.11±0.29d | 3.45±0.32c | 3.88±0.25d | 2.29±0.17a | 6.21±0.35e | 2.95±0.15b | 1.89±0.13a |
| FRAP (mmol Trolox/$100g^{-1}$) | 4.28±0.28e | 3.01±0.19c | 3.46±0.42d | 1.84±1.0a | 5.58±0.38f | 2.61±0.13b | 1.66±0.16a |
| alkaloids | | | | | | | |
| Scopolamine | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| $\alpha$-solanine | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Fruits did not contain steroids ($\alpha$-solanie) and tylakoides (scopolamine) at the detected level | | | | | | | |

Goji berries of the present study showed similar ascorbic acid content compared to other fruits, such as raspberry (31.0–40.0 mg/100 g FW) (Pantelidis et al., 2007), strawberries (46 mg/g FW) (Roberts and Gordon, 2003), oranges (31 mg/g FW) (Roberts and Gordon, 2003), kiwi fruits (29–80 mg/g FW) (Nishiyama et al., 2004), and cornelian cherry (41.7 mg/1000 g FW) (Ochmian et al., 2019). Donno et al. (2015) reported that vitamin C content in goji berries reached 42 mg/100 g, which is typical of citrus fruits. In contrast, the content of provitamin A in the tested goji fruit was 35 mg/1000 g ('Amber Sweet Goji') and 190 mg/1000 g ('Big Lifeberry') (Table 3) and is much higher than the content of provitamin A in those described by Kruczek and Ochmian (2016). However, these values are lower than those for goji varieties described by Wojdyło et al. (2018). Huge variations in provitamin A content are concurrent with other reports (Dong et al., 2012, Wojdyło et al., 2018), suggesting variation in carotenoid content depending on plant origin, subspecies and growth condition. Moreover, environmental factors significantly affect the authenticity and the main active components of *Lycium chinense* Mill. fruits. According to Majchrzak et al. (2000) and Vuong et al. (2002) provitamin A carotenoids, particularly $\beta$-carotene, provide vitamin A after enzymatic cleavage, and play a key role in human nutrition. $\beta$-carotene was the dominant provitamin A carotenoid present in the *gac* fruit (175 µg/g edible portion), papaya (12.1 µg/g), mandarin orange (4.65 µg/g) and banana (2.90 µg/g) (Vuong et al., 2002).

The interaction between acid extracts determines the flavor of the fruit. The organic acid content is therefore essential for the taste of goji fruits, as increased sugar and lower acid levels make the fruit taste sweeter (Ochmian et al., 2014). The highest acidity characterized fruits of cultivar Big Lifeberry (14.5 g/1000 g) while they had low overall content of the extract (11.9%) (Table 3). The goji berries from cultivar Sweet Berry had the highest extract content (18.4%) and the lowest acidity (8.9 g 1000/g). Similar results were observed in *L. chinense* 'No 1' (Kruczek and Ochmian, 2016) and in cornelian cherry (Ochmian et al., 2019).

Nitrates III ($NO_2$) and nitrates V ($NO_3$) belong to the group of compounds adversely affecting the human body. Nitrates V belong to low-toxic compounds that do not directly threaten health. In contrast, nitrates III are six to ten times more toxic. The current regulations do not specify nitrate content in fruits, but only in a few selected vegetables. According to the EC Commission Regulation of 2006, the daily consumption of nitrates V should not exceed 3.65 mg per kg body weight. However, according to current regulations, these plants can also be considered safe for the consumer. Nitrate V content limits are set only in leafy vegetables in EU legislation. Fresh lettuce may contain up to 5000 mg/1000 g, and in processed foods for feeding infants and young children, it should not exceed 200 mg/1000 g (EU legislation, 2006). In contrast, nitrate III levels should not exceed 0.07 mg per kg of body weight per day. The nitrate III content in all the cultivars tested was at a relatively low level (Table 3). The highest nitrates III content (1.42 mg/1000 g) was obtained in the cultivar No '1', and nitrates V in the Korean Big cultivar (106.6 mg/1000 g). These values were higher in persimmon fruit (Ochmian et al., 2016) cornelian cherry (Ochmian et al., 2019) and highbush blueberry (Ochmian, 2012) while in comparison to grapevine fruit in which the nitrates III content was lower (Ochmian et al., 2013).

Some doubt has arisen about the atropine content of the fruits. In 1989, a sample of fruits harvested in India recorded 0.95% of atropine (Harsh, 1989). This finding has no evidence and is in clear contradiction with the widespread consumption of these fruits. Studies did not show the presence of steroids ($\alpha$-solanine) and tropane alkaloids (scopolamine) at detectable levels in goji fruit.

*Antidiabetic activity of goji fruits*

The inhibition of $\alpha$-amylase in the analyzed goji fruit ranged from 33.4 ('Big Lifeberry') to 73.4 mg/mL $IC_{50}$ ('Amber Sweet Goji'), while the inhibition of $\alpha$-glucosidase in these fruits was between 5.7 ('Amber Sweet Goji') and 13.2 mg/mL $IC_{50}$ ('Big Berry'). The extract obtained from 'Big Lifeberry' goji fruit showed the highest inhibitory activity of $\alpha$-amylase and $\alpha$-glucosidase (Table 3). However, the inhibitory effect obtained was for $\alpha$-amylase $IC_{50}$ 33.4 and $\alpha$-glucosidase $IC_{50}$ 9.9 mg/mL. The inhibition of these enzymes may be efficient in regulating type 2 diabetes by monitoring glucose absorption (Podsedek et al., 2014).

Both $\alpha$-amylase and $\alpha$-glucosidase inhibitory activity in fruits such as pomegranate, strawberry, raspberry, pear, kiwi, plum, lingonberry, black currant, and blueberry extracts has been confirmed in other studies (McDougall et al., 2005; Podsedek et al., 2014; Wojdyło et al., 2018; Ochmian et al., 2019; Lachowicz et al., 2019). Inhibition of these enzymes is particularly helpful in the treatment of non-insulin-dependent diabetes, as it slows down the release of glucose into the bloodstream (Wojdyło et al., 2018).

*Antioxidant activity of goji fruits*

In order to quantify the antioxidant activity of food samples, the term total antioxidant potential (TAP) is used to describe the ability to neutralize radicals of a mixture of antioxidants (Paradowska et. al., 2016). According to many authors (Wojdyło et al., 2018; Ochmian et al., 2019; Lachowicz et al., 2019), the antioxidant activity of various fruits was determined mainly by genotype (cultivar), fruit maturity, growing conditions, geographical location, climatic conditions, and collection methods. Fruits with high antioxidant activity are of great interest to a potential consumer. Total antioxidant activity in goji berries ranged from 1.89 to 6.21 mmol Trolox/100 g and from 1.66 to 5.58 mmol Trolox/100 g for ABTS·+ and FRAP assays, respectively (Table 3). Cultivar Big Lifeberry showed the lowest and Amber Sweet Goji the highest antioxidant activity. Our data agreed with those reported by Wojdyło et al. (2018) who showed a similar value of antioxidant activity in studied cultivars of goji. According to Donno et al. (2015), goji fruits are rich in a significant amount of phytochemicals and indeed unique health-promoting compounds. This study showed that the analyzed quality and nutraceutical parameters of the fruits of this species are comparable with those of other common fruit species, such as *Ribes nigrum*, *Actinidia deliciosa*, and *Citrus sinensis*.

A cluster analysis conducted using Ward's method (Figure 1) permitted the isolation of three groups having a similar influence on the physical and chemical properties and antidiabetic and antioxidant activities of the goji fruits. Opposing groups (a and c) formed dark-colored fruits of No '1' cultivar and the bright Amber Sweet Goji cultivar..

*Polyphenolic identification and compounds of goji fruits*

The largest group of phenolic compounds included flavonols, phenolic acid, and the smallest gallotannins (Table 4). Six flavonols identified in goji cultivars were quercetin derivatives, and one isorhamnetin.

Identification of polyphenolic compounds was performed using UPLC-PDA-ESI-MS/MS in negative ion mode. The presence of 14 polyphenolic compounds in goji fruits, including 5 phenolic acids, 1 hydrolyzed tannin and 8 flavonols, was tentatively determined. Compound 1 was initially suggested for the presence of tetragalloyl glucose with a characteristic base fragment at *m/z* 169 which was in line with previous reports for *Lycium barbarum* L. (Pires et al., 2018). Identification of compounds from 2 to 6 indicated the presence of caffeoylquinic acids derivatives with a typical base ion at *m/z* 191. Therefore, compounds 2, 3, 5 and 6 were tentatively referred to as di-*O*-caffeoylquinic acids due to their characteristic main peak at *m/z* 515 (Francescato et al., 2013). Compound 4 was then given as 5-*O*-caffeoylquinic acid and its presence confirmed with an available standard. Of the 8 flavonols, 7 quercetins and 1 isorhamnetins were tentatively described, which were characterized by the presence of base ions at *m/z* 301 and 315, respectively. These compounds were previously identified in *Lycium barbarum* L. as quercetin-3-Soph-7-glucoside (*m/z* 787), quercetin-3-*O*-rutinoside-hexoside (*m/z* 771), quercetin-3-*O*-glucuronide (*m/z* 477), quercetin dihexoside (*m/z* 625), quercetin-3-*O*-rutinoside (*m/z* 609), quercetin-3-*O*-robinobioside (*m/z* 609), oraz isorhamnetin-3-*O*-rutinoside (*m/z* 623) (Francescato et al., 2013; Pires et al., 2018; Tripodo et al., 2018).

As is known the antioxidative potential depends on the amount of bioactive components especially polyphenolics. Examined goji cultivars contained on average total phenolic compounds from 18.94 mg/100 g ('Amber Sweet Goji') to 55.46 mg/100 g ('No '1'') (Table 5).

Compounds belonging to gallotannins are essential for human health. The highest content of Tetragalloyl glucose was found in fruits of No '1' cultivar (9.27 mg/100 g). On the other hand, yellow-colored fruits had only 0.34 mg of this compound in their composition. Similar relationships were found in Phenolic acid and Flavonols. In the group of compounds classified as phenolic acid in all cultivars the dominant compounds were Di-O-caffeoylquinic acid and 5-Caffeoylquinic acid. In total they comprised 83% of all phenolic acids. In the flavonols group Quercetin-3-O-robinobioside dominated in most cultivars. According to Wojdyło et al. (2018), the average content of phenols in all goji cultivars grown in Poland was 97.23 mg/100 g, with considerable inter-cultivar differences. In comparison to other fruits, for example, kiwifruit and apple contained small quantities of polyphenolic compounds (70.23–83.40 mg GAE/100 g FW), while a significantly higher polyphenolic content was observed in strawberry (323.39 mg GAE/100 g FW) and blackcurrant (434.43 mg GAE/100 g FW). According to Donno et al. (2015), goji (268.5 mg GAE/100 g FW) was in an intermediate position between orange (158.70 mg GAE/100 g FW) and guava (310.10 mg GAE/100 g FW). The higher the total polyphenol content, the greater is the antioxidant activity (Hernández-Alcántara et al., 2016). Similar to the works of Wang et al. (2011), Donno et al. (2015), and Wojdyło et al. (2018), a dominant phenolic acid obtained in this study was 5-caffeoylquinic acid. Phenolic acid, especially chlorogenic acid, is a precursor of flavor in fruits and vegetables, and it shows anticarcinogenic, antimutagenic, and antioxidant properties *in vitro*. Chlorogenic acid is poorly absorbed in the human body, and is therefore metabolized mainly by colonic microflora (Sato et al., 2011).

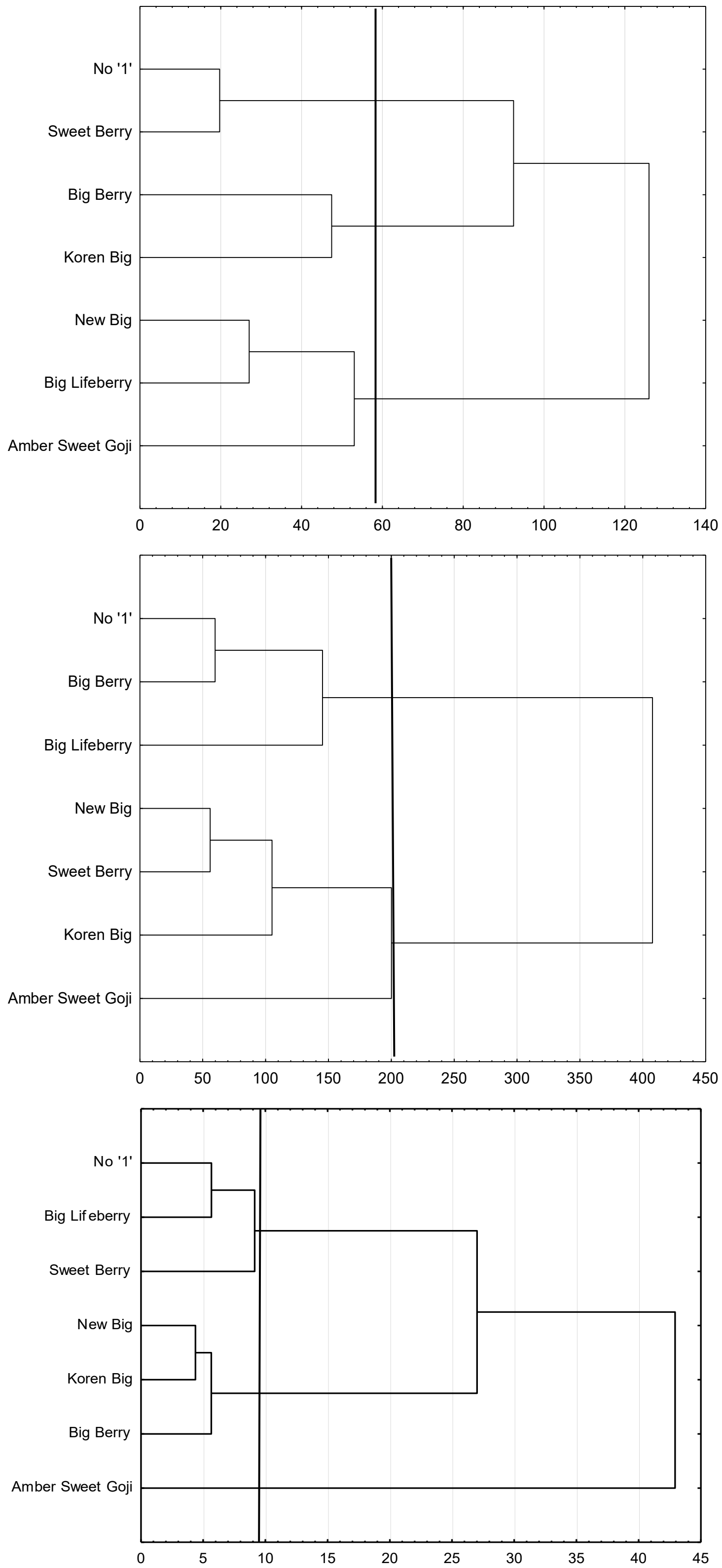



Figure 1. Dendrogram of cluster analysis for the physical composition, chemical composition and bioactive composition of tested goji fruits (*L. chinense*). The vertical line (linkage distance 57, 200, 9) indicate the cut-off used to form the groups

Table 4. Identification of polyphenolic compounds in the tested goji fruits (*L. chinense*)

| **Tentative compouns** | **MS [m/z]** | **Tr [min]** |
|---|---|---|
| Tetragalloyl glucose | 787/169 | 2.82 |
| Di-*O*-caffeoylquinic acid | 515/191 | 2.53 |
| Di-*O*-caffeoylquinic acid | 515/191 | 3.20 |
| 5-caffeoylquinic acid (Chlorogenic acid) | 353/191 | 3.45 |
| Di-*O*-caffeoylquinic acid | 515/191 | 3.89 |
| Di-*O*-caffeoylquinic acid | 515/191 | 4.37 |
| Quercetin-3-Soph-7-glucoside | 787/301 | 2.86 |
| Quercetin-3-*O*-rutinoside-hexoside | 771/609/301 | 3.69 |
| Quercetin-3-*O*-glucuronide | 477/301 | 5.18 |
| Quercetin dihexoside | 625/301 | 5.24 |
| Quercetin-3-*O*-rutinoside | 609/301 | 5.92 |
| Quercetin-3-*O*-robinobioside | 609/301 | 5.97 |
| Quercetin dihexoside | 625/301 | 6.17 |
| Isorhamnetin-3-*O*-rutinoside | 623/315 | 6.86 |

Table 5. Content of phenolic compounds (mg/100 g) of tested goji fruits (*L. chinense*)

| | **No '1'** | **New Big** | **Sweet Berry** | **Big Berry** | **Big Lifeberry** | **Korean Big** | **Amber Sweet Goji** |
|---|---|---|---|---|---|---|---|
| **Gallotannins** | | | | mg/100 g | | | |
| Tetragalloyl glucose | 9.27±0.24e | 4.96±0.32c | 2.86±0.17b | 7.09±0.33d | 6.99±0.35d | 5.65±0.17c | 0.34±0.02a |
| **Phenolic acid** | | | | | | | |
| Di-*O*-caffeoylquinic acid | 13.88±0.22e | 7.24±0.17c | 4.15±0.29a | 10.37±0.30d | 10.23±0.29d | 8.25±0.22c | 5.33±0.19b |
| Di-*O*-caffeoylquinic acid | 2.79±0.09c | 1.62±0.10b | 0.68±0.05a | 2.58±0.17c | 2.54±0.13c | 1.93±0.12b | 0.32±0.04a |
| 5-Caffeoylquinic acid (Chlorogenic acid) | 9.46±0.35e | 6.48±0.27c | 1.86±0.19a | 11.15±0.42f | 10.99±0.27f | 8.00±0.22d | 3.24±0.18b |
| Di-*O*-caffeoylquinic acid | 0.39±0.03b | 0.44±0.02c | 0.59±0.03d | 0.30±0.02b | 0.30±0.02b | 0.400.03bc | 0.11±0.01a |
| Di-*O*-caffeoylquinic acid | 1.37±0.13e | 1.00±0.09b | 0.80±0.05a | 1.20±0.09d | 1.18±0.11d | 1.06±0.08bc | 1.12±0.09cd |
| sum | 27.89f | 16.78c | 8.08a | 25.60e | 25.24e | 19.64d | 10.12b |
| **Flavonols** | | | | | | | |
| Quercetin-3-Soph-7-glucoside | 3.34±0.15d | 2.36±0.11c | 3.13±0.17d | 1.61±0.12b | 1.59±0.09b | 2.11±0.15c | 0.57±0.04a |
| Quercetin-3-*O*-rutinoside-hexoside | 0.28±0.02b | 0.24±0.03a | 0.25±0.02a | 0.24±0.02a | 0.23±0.01a | 0.24±0.01a | 0.64±0.03c |
| Quercetin-3-*O*-glucuronide | 0.60±0.04c | 0.30±0.02b | 0.02±0.00a | 0.57±0.03c | 0.56±0.03c | 0.39±0.01b | 0.13±0.00a |
| Quercetin dihexoside | 3.82±0.09c | 3.17±0.11b | 4.46±0.12d | 1.91±0.05a | 1.88±0.04a | 2.75±0.07b | 3.98±0.10c |
| Quercetin-3-*O*-rutinoside | 0.27±0.01b | 0.41±0.02d | 0.66±0.02e | 0.17±0.01a | 0.17±0.01a | 0.33±0.02c | 0.98±0.03f |
| Quercetin-3-*O*-robinobioside | 9.60±0.25e | 3.66±0.11b | 0.61±0.02a | 6.73±0.15d | 6.64±0.13d | 4.66±0.09c | 1.13±0.05a |
| Quercetin dihexoside | 0.10±0.00ab | 0.12±0.01bc | 0.17±0.01c | 0.07±0.00a | 0.07±0.00a | 0.10±0.00ab | 0.34±0.02d |
| Isorhamnetin-3-*O*-rutinoside | 0.29±0.01c | 0.22±0.01ab | 0.17±0.01a | 0.26±0.02bc | 0.26±0.02bc | 0.23±0.02ab | 0.71±0.03d |
| sum | 18.01d | 10.26b | 9.30ab | 11.30c | 11.14c | 10.58bc | 7.77a |
| **Total** | **55.46D** | **32.22B** | **20.42A** | **44.25C** | **43.64C** | **36.10B** | **18.94A** |

* For explanation, see Table 3.

## CONCLUSION

Differences in biochemical composition and biological activity of seven goji berry cultivars grown in north-western Poland were shown. These shrubs are highly adapted to the region's climatic conditions. The examined goji shrubs did not show signs of frost damage and the flowers were not damaged by spring frost. Regardless of the weather conditions, the shrubs yielded annually.

Goji berries have a high biological activity spectrum with beneficial effects on health. The cultivars rich in total polyphenols ('No '1'', 'Big Berry', 'Big Lifeberry'), provitamin A, and *L*-ascorbic acid ('Big Lifeberry', 'No '1''), and those that exhibit antidiabetic and antioxidant activity ('Big Lifeberry', 'No '1''), may be considered as a superfood or functional food. In addition, no scopolamine were detected in the tested goji, and the levels of nitrates V and nitrates III were at well below the current standards. Among the tested cultivars, 'Big Lifeberry' fruits for the consumer due to the size, weight, and health-promoting effects were most attractive. The goji fruits of this cultivar, cultivated under the climatic conditions of north-eastern Europe, could be taken into consideration as an important dietary source of natural antioxidants.

## REFERENCES


1. Antal, T., Sikolya, L., & Kerekes, B. (2013). Assessment of freezing pre-treatments for the freeze dried of apple slices. *Acta Univ. Cibiniensis, Ser. E: Food Technol.*, *17*(2), 3–14.
2. Arnao, M.B., Cano, A., Alcolea, J.F., & Acosta, M. (2001). Estimation of free radical-quenching activity of leaf. *Biochem. Techniq., 12*(2), 138-143.DOI: 10.1002/pca.571.
3. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem*., *239*(1), 70-76.
4. Bogacz, K. (2009). Goji – owoc zdrowia i długowieczności [Goji − fruit of health and longevity]. *PFiOW* 9, 33−34. [in Polish].
5. Burke, D.S., Smidt, C.R., & Vuong, L.T. (2005). *Momordica cochinchinensis*, *Rosa roxburghii*, wolfberry, and sea buckthorn-highly nutritional fruits supported by tradition and science. *Curr. Top. Nutraceutical Res., 3*(4), 259.
6. Chang, R.C.C., & So, K.F. (2008). Use of anti-aging herbal medicine, *Lycium barbarum*, against aging-associated diseases. What do we know so far? *Cell. Mol. Neurobiol.*, *28*(5), 643-652. DOI 10.1007/s10571-007-9181-x.
7. Chełpiński P., Ochmian I., & Forczmański P. (2019). Sweet cherry skin colour measurement as an non-destructive indicato of fruit maturity. *Acta Univ. Cibiniensis, Ser. E: Food Technol*, *23*(2), 157-166. DOI: 10.2478/aucft-2019-0019.
8. Donno, D., Beccaro, G. L., Mellano, M. G., Cerutti, A. K., & Bounous, G. (2015). Goji berry fruit (*Lycium* spp.): antioxidant compound fingerprint and bioactivity evaluation. *J. Funct. Foods*, 18, 1070-1085.DOI 10.1016/j.jff.2014.05.020.
9. Dong, J.Z., Wang, S.H., Zhu, L., & Wang, Y. (2012). Analysis on the main active components of *Lycium barbarum* fruits and related environmental factors. *J. Med. Plants Res*., *6*(12), 2276-2283. DOI: 10.5897/JMPR10.780.
10. Dzhugalov, H., Lichev, V., Yordanov, A., Kaymakanov, P., Dimitrova, V., & Kutoranov, G. (2015). First results of testing Goji berry (*Lycium barbarum* L.) in Plovdiv region, Bulgaria. *Sci Pap Ser B: Hortic.*, 59, 47-50.
11. Francescato, L. N., Debenedetti, S. L., Schwanz, T. G., Bassani, V. L., & Henriques, A. T. (2013). Identification of phenolic compounds in Equisetum giganteum by LC–ESI-MS/MS and a new approach to total flavonoid quantification. *Talanta*, *105*, 192-203.
12. Harsh, M. L. (1989). Tropane alkaloids from *Lycium barbarum* Linn., i*n vivo* and *in vitro*. *Curr. Sci*., 58, 817-818.
13. Hernández-Alcántara, A. M., Totosaus, A., & Pérez-Chabela, M. L. (2016). Evaluation of agro-industrial co-products as source of bioactive compounds: fiber, antioxidants and prebiotic. *Acta Univ. Cibiniensis, Ser. E: Food Technol, 20*(2), 3-16. DOI: 10.1515/aucft-2016-0011.
14. Kiełbasa, P., & Juliszewski, T. (2005). Pomiar powierzchni liści wybranych roślin metodą video-komputerową. [Measurement of the leaf surface for the selected plants using video-computer method]. *Inżynieria Rolnicza*, 14, 169-175. (In Polish).

15. Kokotkiewicz, A., Migas, P., Stefanowicz, J., Luczkiewicz, M., & Krauze-Baranowska, M. (2017). Densitometric TLC analysis for the control of tropane and steroidal alkaloids in *Lycium barbarum*. *Food Chem*., 221, 535-540.
16. Kruczek, A., & Ochmian, I. (2016). The influence of shrubs cutting method on yielding and quality of the goji berries (*Lycium barbarum* L.). *Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc. Zootech.*, *40*(4 (330)), 131-138. DOI: 10.21005/AAPZ2016.40.4.14.
17. Krupa-Małkiewicz, M., Oszmiański, J., Lachowicz, S., Szczepanek, M., Jaśkiewicz, B., Pachnowska, K., & Ochmian, I. (2019). Effect of nanosilver (nAg) on disinfection, growth and chemical composition of young barley leaves under *in vitro*. *J. Integr. Agr.* *18*(8), 1871-1881. DOI:10.1016/S2095-3119(18=621146-X.
18. Kuckenberg, J., Tartachnyk, I., & Noga, G. (2008). Evaluation of fluorescence and remission techniques for monitoring changes in peel chlorophyll and internal fruit characteristics in sunlit and shaded sides of apple fruit during shelf-life. *Postharvest Biol. Technol., 48*(2), 231-241. DOI:10.1016/j.postharvbio.2007.10.013.
19. Kulczyński, B., Groszczyk, B., Cerba, A., & Gramza-Michałowska, A. (2014). Owoce goi (*Lycium barbarum*) jako źródło związków bioaktywnych w żywności–przegląd literatury. [Goya (*Lycium barbarum*) fruits as bioactive components source in food – a literature review]. Nauka Przyroda Technologie, *8*(2), 19. (In Polish).
20. Kulczyński, B., & Gramza-Michałowska, A. (2016). Goji berry (*Lycium barbarum*): composition and health effects–a review. *Pol. J Food Nutr. Sci., 66*(2), 67-76. DOI:10.1515/pjfns-2015-0040.
21. Lachowicz, S., Wiśniewski, R., Ochmian, I., Drzymała, K., & Pluta, S. (2019). Anti-microbiological, anti-hyperglycemic and anti-obesity potency of natural antioxidants in fruit fractions of saskatoon berry. *Antioxidants, 8*(9), 397. DOI: 10.3390/antiox8090397.
22. Majchrzak, D., Frank, U., & Elmadfa, I. (2000). Carotenoid profile and retinol content of baby food products. *Eur. Food Res. Technol*., *210*(6), 407-413.
23. McDougall, G.J., Shapiro, E., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Different polyphenolic components of soft fruits inhibit *α*-amylase and *α*-glucosidase. *J Agric. Food Chem*., 53, 2760-2766.
24. Mencinicopschi, I. C., & Bălan, V. (2013). Growth and development characteristics of plant individuals from two *Lycium barbarum* L. varieties. *Sci. Pap. Ser. A: Agron.*, 56, 490-7.
25. Mijowska, K., Ochmian, I., & Oszmiański, J. (2016). Impact of cluster zone leaf removal on grapes cv. Regent polyphenol content by the UPLC-PDA/MS method. *Molecules*, *21*(12), 1688. DOI: 10.3390/molecules21121688
26. Mijowska, K., Ochmian, I., & Oszmiański, J. (2017). Rootstock effects on polyphenol content in grapes of 'Regent'cultivated under cool climate condition. *J. Appl. Bot. Food Qual*, 90, 159-164.
27. Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Stampar, F., & Veberic, R. (2012). Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. *J. Food Sci., 77*(10), C1064-C1070. DOI:10.1111/j.1750-3841.2012.02896.x
28. Nickavar, B., & Yousefian, N. (2011). Evaluation of *α*-amylase inhibitory activities of selected antidiabetic medicinal plants. *J. Verbr. Lebensm*., *6*(2), 191-195. DOI 10.1007/s00003-010-0627-6.
29. Nishiyama, I., Yamashita, Y., Yamanaka, M., Shimohashi, A., Fukuda, T., & Oota, T. (2004). Varietal difference in vitamin C content in the fruit of kiwifruit and other *Actinidia* species. *J. Agric. Food Chem*., *52*(17), 5472-5475. DOI: 10.1021/jf049398z
30. Ochmian, I. (2012). The impact of foliar application of calcium fertilizers on the quality of highbush blueberry fruits belonging to the Duke cultivar. *Not. Bot. Horti. Agrobot. Cluj Napoca, 40*(2), 163-169.
31. Ochmian, I., Angelov, L., Chełpiński, P., Stalev, B., Rozwarski, R., & Dobrowolska, A. (2013). The characteristics of fruits morphology, chemical composition and colour changes in must during maceration of three grapevine cultivars. *J. Hortic.Res*, *21*(1), 71-78.
32. Ochmian, I., & Grajkowski, J. (2007). Wzrost i plonowanie trzech odmian jagody kamczackiej (*Lonicera caerulea*) na Pomorzu zachodnim w pierwszych latach po posadzeniu. *Roczniki Akademii Rolniczej w Poznaniu* CCCLXXXIII, 41, 351-355. (In Polish).
33. Ochmian, I., Dobrowolska, A., & Chełpiński, P. (2014). Physical parameters and chemical composition of fourteen blackcurrant cultivars (*Ribes nigrum* L.). *Not. Bot. Horti. Agrobo., 42*(1), 160-167.
34. Ochmian, I., Kozos, K., & Mijowska, K. (2015). Influence of storage conditions on changes in physical parameters and chemical composition of highbush blueberry (*Vaccinium corymbosum* L.) fruit during storage. *Bulg. J. Agric. Sci*, *21*(1), 178-183.

35. Ochmian, I., Yordanov, A., Mijowska, K., & Chelpinski, P. (2016). Wpływ przechowywania owoców persymony (*Diospyros kaki*) w warunkach shelf life na wybrane cechy fizyczne i skład chemiczny. *Żywność Nauka Technologia Jakość*, *23*(1). DOI: 10.15193/zntj/2016/104/109. (In Polish).
36. Ochmian, I., Oszmiański, J., Lachowicz, S., & Krupa-Małkiewicz, M. (2019). Rootstock effect on physico-chemical properties and content of bioactive compounds of four cultivars Cornelian cherry fruits. *Sci. Hortic*., 256, 108588. DOI:10.1016/j.scienta.2019.108588.
37. Oszmiański, J., & Wojdylo, A. (2005). Aronia melanocarpa phenolics and their antioxidant activity. *Eur. Food Res. Technol*., *221*(6), 809-813. DOI:10.1007/s00217-005-0002-5.
38. Oszmiański, J., Lachowicz, S., Gławdel, E., Cebulak, T., Ochmian, I., 2018. Determination of photochemical composition and antioxidant capacity of 22 old apple cultivars grown in Poland. Eur. Food Res. Technol. 244 (4), 647–662. https://doi.org/10.1007/s00217-017-2989-9.
39. Pacewicz, K., & Gregorczyk, A. (2009). Porównanie ocen zawartości chlorofilu chlorofilometrami SPAD-502 i N-Tester. *Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc. Zootech.*, (269)*9*, 41-46. (In Polish).
40. Pantelidis, G. E., Vasilakakis, M., Manganaris, G. A., & Diamantidis, G. R. (2007). Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem., 102*(3), 777-783. DOI:10.1016/j.foodchem.2006.06.021.
41. Paradowska, K., Czerniejewska, M., Zielińska, A., & Sajkowska-Kozielewicz, J.J. (2016). Aktywność przeciwutleniająca ekstraktów z suszonych owoców goji. *Żywność. Nauka. Technologia. Jakość*, *4*(107), 115-124. (In Polish).
42. Pires, T. C., Dias, M. I., Barros, L., Calhelha, R. C., Alves, M. J., Santos-Buelga, C., & Ferreira, I. C. (2018). Phenolic compounds profile, nutritional compounds and bioactive properties of *Lycium barbarum* L.: A comparative study with stems and fruits. *Industrial crops and products*, *122*, 574-581.
43. Piwowarczyk, R., Ochmian, I., Lachowicz, S., Kapusta, I., Sotek, Z., & Błaszak, M. (2020). Phytochemical parasite-host relations and interactions: A Cistanche armena case study. *Sci. Total Environ.*, *716*, 137071. https://doi.org/10.1016/j.scitotenv.2020.137071
44. Podsędek, A., Majewska, I., Redzynia, M., Sosnowska, D., & Koziołkiewicz, M. (2014). *In vitro* inhibitory effect on digestive enzymes and antioxidant potential of commonly consumed fruits. *J Agric. Food Chem*., *62*(20), 4610-4617. DOI: 10.1021/jf5008264.
45. Roberts, W. G., & Gordon, M. H. (2003). Determination of the total antioxidant activity of fruits and vegetables by a liposome assay. *J. Agric. Food Chem*., *51*(5), 1486-1493. DOI: 10.1021/jf025983t
46. European Commission (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF.
47. Rutkowski, K. P., Michalczuk, B., & Konopacki, P. (2008). Nondestructive determination of 'Golden Delicious' apple quality and harvest maturity. *J. Fruit Ornam. Plant Res*., *16*(1), 39-52.
48. Sadowski, A., Nurzyński, J., Pacholak, E., & Smolarz, K. 1990. Określanie potrzeb nawożenia roślin sadowniczych. II. Zasady, liczby graniczne i dawki nawożenia. *Instr. Upow. Nauka–sadownictwu. SGGW-AR Warszawa*, pp. 1–25. (In Polish).
49. Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., Sugawara, M.; Iseki, K. (2011). *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid. *Int. J. Pharm.*, *403*(1), 136-138.
50. Tripodo, G., Ibáñez, E., Cifuentes, A., Gilbert-López, B., & Fanali, C. (2018). Optimization of pressurized liquid extraction by response surface methodology of Goji berry (*Lycium barbarum* L.) phenolic bioactive compounds. *Electrophoresis*, *39*(13), 1673-1682.
51. Vuong, L. T., Dueker, S. R., & Murphy, S. P. (2002). Plasma β-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit *Momordica cochinchinensis* (gac). *Am. J. Clin. Nutr*., *75*(5), 872-879. DOI: 10.1093/ajcn/75.5.872
52. Wang, J.X., Wang, Y.L., Chang, H.Yu., Xiong, X., & Tian, Y. (2011). Report on superior characteristics of ningxin woolberry new variety Ningqi No 6. *Modern Agricultural Science and Technology*, 23, 150-155.
53. Wojdyło, A., Nowicka, P., & Bąbelewski, P. (2018). Phenolic and carotenoid profile of new goji cultivars and their anti-hyperglycemic, anti-aging and antioxidant properties. *J. Funct. Foods*, *48*, 632-642. doi.org/10.1016/j.jff.2018.07.061.

54. Yusufe, M., Mohammed, A., & Satheesh, N. (2017). Effect of duration and drying temperature on characteristics of dried tomato (*Lycopersicon esculentum* L.) Cochoro variety. *Acta Univ. Cibiniensis, Ser. E: Food Technol*, *21*(1), 41-50.

*molecules*

Article

# Health-Promoting Capacities of In Vitro and Cultivated Goji (*Lycium chinense* Mill.) Fruit and Leaves; Polyphenols, Antimicrobial Activity, Macro- and Microelements and Heavy Metals

**Arleta Kruczek [1], Marcelina Krupa-Małkiewicz [2], Sabina Lachowicz [3], Jan Oszmiański [4] and Ireneusz Ochmian [1,*]**

1. Department of Horticulture, West Pomeranian University of Technology Szczecin, Słowackiego 17 Street, 71-434 Szczecin, Poland; ka21499@zut.edu.pl
2. Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology Szczecin, Słowackiego 17 Street, 71-434 Szczecin, Poland; Marcelina.Krupa-Malkiewicz@zut.edu.pl
3. Department of Fermentation and Cereals Technology, Wrocław University of Environmental and Life Science, 37, Chełmońskiego Street, 51-630 Wroclaw, Poland; sabina.lachowicz@upwr.edu.pl
4. Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Science, 37, Chełmońskiego Street, 51-630 Wroclaw, Poland; jan.oszmianski@upwr.edu.pl

* Correspondence: iochmian@zut.edu.pl; Tel.: 0048-91-449-6163

Academic Editor: Francesco Cacciola
Received: 26 October 2020; Accepted: 12 November 2020; Published: 14 November 2020

**Abstract:** There is a growing interest among the public in fruit with a positive impact on human health. Two goji berry cultivars ('No. 1' and 'New Big') were propagated *in vitro*, grown in an orchard and then evaluated for macro- and microelements and harmful heavy metals (i.e., Pb, Ni, and Cd). The leaves and fruit were also assessed for nutritional value, polyphenols and the antimicrobial activity of the leaves. 'New Big' was characterized by a higher content of macro elements in the leaves (*in vitro* and orchard) and a higher content of microelements in the fruit. The harmful substances content was below the minimum value. Furthermore, neither the fruit nor the leaves contained cadmium. This study also indicated that leaves had a higher content of polyphenols compared to the fruit. The fruits were characterized by their health-promoting capacities, while the leaves were characterized by their antibacterial activity. Among the Gram-positive bacteria, the most sensitive strain was *Bacillus subtilis*, and among the Gram-negative bacteria, it was *Proteus vulgaris*. Taking into consideration the Recommended Daily Allowance (RDA) for minerals, goji berries can be declared to be a source of Cu, Fe, Mn, Zn and P.

**Keywords:** antioxidant; antibacterial activity; mineral elements; *in vitro*; fruit; leaves

## 1. Introduction

The consumption of red berries has increased considerably in recent years. Werewolf berries (*Lycium chinense* Mill, *Solanaceae*), traditional food and medicines in East Asia, have become progressively more popular in Europe and North America [1–3] since the beginning of the 21st century. Two closely related species, *L. barbarum* and *L. chinenese*, are well known around the world, and both function as food and medicinal plants in China and other Asian countries. *L. chinense* Mill. berries contain multiple mineral and organic compounds (i.e., vitamins $B_1$, $B_6$, A, C, E), with potential to repair epidermal damage, and showing excellent effects on cardiovascular and cholesterol levels [3,4]. Goji leaves are also a rich source of bioactive compounds that can be used as additive in health-promoting preparation [5,6]. In support of such traditional properties, modern studies indicate that extracts from goji berries possess a range of biologic activities, including antioxidant properties [7]. According to Mocan et al. [5], goji fruits have an effect on ageing, neuroprotection, glucose control in diabetics, antioxidant properties, immunomodulation, and antitumor activity, as well as general well-being, fatigue, metabolism, energy expenditure, and cytoprotection. In addition, no harmful substances, such as $NO_2$ and $NO_3$, or the presence of steroids (α-solanine) and tropane alkaloids (skopolamin) have been reported in goji fruit [6]. According to Kulczyński and Gamza-Michałkowska [8], a well-balanced diet and a change in lifestyle have significant impacts in terms of reducing the frequency of diseases in society. Thus, natural antioxidants, particularly in fruits and vegetables, have gained interest among consumers and the scientific community.

Goji shrubs are propagated vegetatively, especially by semi-wooden cuttings and with great success *in vitro* [9]. According to Dzhugalov et al. [10], the optimum quality fruit can be obtained under hot summer conditions. Rain causes fruit cracking during ripening. Thus, there is a relationship between environmental conditions and the harvest. Optimal growing conditions allow for a production of almost 95,000 tons of fruit annually from August to October. Goji fruit is usually dried, but both fruit and young leaves, which are a valuable source of mineral compounds, can also be eaten raw [1,11]. In addition, fruit extracts are used as natural, non-toxic colorants for drinks or cosmetics [12]. In the literature, the main focus has been on the properties of goji fruit. However, the leaves of *L. chinense*, called "tianjingcao" (vitality and vigor of nature) have rarely been studied, despite their use in traditional Chinese medicine in alleviating mineral deficiency, combating heat distress, quenching thirst, dispelling wind, and enhancing eyesight. Moreover, *L. chinense* leaves have also been widely used as tea, medicinal vegetables, and herbal drugs in China [5]. Goji leaves contain high amounts of specific flavonoids and phenolic acids, such as chlorogenic acid, quercetin, and rutin. Additionally, goji leaves contain polysaccharides that exert high superoxide and DPPH scavenging abilities, thereby having high antioxidative activity [13]. Information regarding goji leaves, including their chemical composition and bioactive features, is not comprehensive and quite limited. However, recently, *L. chinense* leaves have been described as a sustainable source of antioxidants and antibacterial compounds.

Considering the excellent health properties and wide use of goji fruit and leaves, we compared the physical-chemical properties of goji leaves and fruit grown in orchard and *in vitro* culture. In addition, the particular aim of this study was to assess the antimicrobial properties of goji leaves as a scientific basis for the further use of this plant as a functional food/ingredient.

## 2. Results and Discussion

### 2.1. Mineral Compound of Leaves and Goji Fruits

N, P, K, Ca, Mg and Na (i.e., macroelements) are important for several physiological functions. Each of the ingredients plays a specific role in a plant's metabolism. The most important macroelement is nitrogen, a component of amino acids and proteins, DNA and RNA nucleic acids, enzymes, hormones, and energy carriers. Sodium, for example, is a macroelement responsible for membrane depolarization and, water control, and is a physiological cotransporter. Magnesium is required for nucleic acids in protein synthesis. The sodium and magnesium values in dried fruits correspond to 1/5 of the daily human need [14].

The contents (g/kg) of six macroelements in the leaves of two goji (*L. chinense* Mill.) cultivars in the samples analyzed are shown in Table 1. The distribution of the compositions of minerals in the different parts of the plant (i.e., fruit or leaves) was determined by the cultivation conditions. In general, the elemental concentrations in all samples decreased in the following order: N > K > Ca > P > Mg > Na and N > K > P > Ca > Mg > Na in the leaves of both cultivars, respectively, from the orchard and *in vitro*. Among the analyzed macroelements, the highest amount of nitrogen was in the leaves. This high level was found in both varieties grown in the orchard, as well as under the in vitro conditions (Table 1). However, the contents of macroelements such as N, P, and K were significantly higher in plants propagated *in vitro* compared to plants from the orchard cultivation, despite the high content of these components in the soil. Conversely, the contents of Ca, Mg, and Na were significantly lower in both cultivars *in vitro* compared to orchard conditions. The leaves from the New Big cultivar accumulated significantly higher concentrations of N, P, Ca, Mg, and Na. The exception was potassium, which in cultivar No. 1 was higher by 50% in the orchard and 9% *in vitro*. The mineral content of the leaves is also an indicator of a plant's nutrition and condition. There is no information available in the literature about the optimal nutrient content in goji leaves. In this experiment, the recommended values for goji were compared to berry shrubs [15,16]. It was found that in the leaves, regardless of growth conditions, there were very low level of P and Ca. The remaining macroelements were in the optimal range or even exceeded it.

**Table 1.** Average values of macroelements in leaves and fruit of two cultivars of goji cultivated in orchard and *in vitro* condition.

| Compounds (g/kg) | Leaves | | | | Fruit | |
|---|---|---|---|---|---|---|
| | No. 1 | | New Big | | No. 1 | New Big |
| | Orchard | *In Vitro* | Orchard | *In Vitro* | Orchard | |
| N (22–32 [2]) | 42.08 ± 1.71a [1] | 57.14 ± 2.37b | 43.50 ± 1.98a | 77.92 ± 3.05c | 29.85 ± 1.30B | 24.32 ± 0.92A |
| P (19–30) | 7.85 ± 0.42a | 11.93 ± 0.56c | 8.52 ± 0.47b | 13.78 ± 0.51b | 5.38 ± 0.19B | 4.85 ± 0.15A |
| K (12–20) | 29.57 ± 1.17b | 57.06 ± 1.98d | 14.73 ± 0.52a | 52.06 ± 1.50c | 4.30 ± 0.13A | 3.98 ± 0.11 A |
| Ca (4–8) | 11.00 ± 0.52b | 3.87 ± 0.18a | 11.39 ± 0.48b | 4.00 ± 0.21a | 0.91 ± 0.04B | 0.75 ± 0.03A |
| Mg (2–4.4) | 5.89 ± 0.17b | 2.87 ± 0.09a | 7.50 ± 0.27c | 3.04 ± 0.11a | 1.11 ± 0.05A | 1.02 ± 0.05A |
| Na (no data) | 4.22 ± 0.23c | 2.17 ± 0.19b | 4.60 ± 0.25d | 1.82 ± 0.13a | 4.03 ± 0.19B | 3.82 ± 0.15A |

[1] Means followed by the same letter in lines do not differ significantly at P = 0.05 according to Tukey multiple range/small letters for leaves, capital–fruit. [2] Optimal content for leaves according to Glonek and Komosa [15].

Fe, Zn, Mn, Cu and Se (i.e., microelements) perform an important function in many biochemical reactions. They are cofactors in enzymatic reactions, such as glucose tolerance factor, and metallo-enzymes in several endogenous reactions, including insulin storage, the immune system, and hormone activators [14,17]. The values obtained for five microelements (i.e., Fe, Zn, Mn, Cu, Se) in the leaves of the two goji cultivars are shown in Table 2. In general, the concentrations of the microelements in all samples decreased in the following order: Fe > Mn > Zn > Cu > Se > Pb > Ni in the orchard, and Mn > Fe > Zn > Cu *in vitro*. It was observed that in leaves of the No. 1 cultivar, these values were higher in comparison to in those of the New Big cultivar. This was opposite to the plants propagated *in vitro.* Moreover, no heavy metals such as Pb, Ni, and Cd were found in the leaves of the plants propagated *in vitro*. This was due to the absence of these elements in the MS medium. Additionally, in the leaves of goji from the orchard, no Cd was found, although its content in the soil was 0.296 mg/kg. Moreover, 'New Big' in the orchard variety accumulated 38% more lead in the leaves, while the 'No. 1' accumulated 72% more nickel in the leaves. This condition can be explained as being due to the mineral variations in the soil and medium, as well as a lower bioaccumulation of these minerals in the leaves. The growing conditions were not the same. Similar to the macroelements, the optimal content of the microelements was compared with the standards for berry shrubs [15,16]. Therefore, it was found that in the leaves collected in the orchard, only Mn was at a low level.

The leaves of both tested cultivars of goji contained greater amounts of macro- and microelements and metals than the fruit. Although the leaves were contaminated with more than twice the amount of lead and nickel than the fruit, this was attributed to the orchard being situated close to an industrial site, and therefore potentially being subject to heavy air pollution. Lead is more specific to air pollution than cadmium, and we did not detect it in neither the leaves or the fruit. However, the fruit of the No. 1 cultivar contained 41% less lead and 33% more nickel. These values were higher than those obtained by Sá et al. [14] in goji fruit grown in South America and those of Kulaitienė et al. [18] in goji fruit grown in Lithuania. Jeszka-Skowron et al. [19] showed that the presence of Cd was 0.046 mg/kg, Pb was 0.109 mg/kg, and Ni was 2.61 mg/kg in goji fruit. Llorent-Martínez et al. [20] established that Cd, Pb, and Ni in goji fruit was 0.035–0.090 mg/kg, 0.035–0.095 g/kg, and 0.33–0.90 mg/kg, respectively.

According to the Regulation of the European Commission [21], the values defined for fruit by law are 0.05 and 0.20 mg/kg for Cd and Pb, respectively. In the present study, the concentrations of toxigenic elements (i.e., Pb and Ni) were low and below the permissible limit levels.

The contents of macroelements in the goji fruit obtained in this study differed from those reported by other authors [2,14,18,22]. We did not expect the contents of the studied macroelements to be identical. It was quite difficult to find data for comparison of the same type of leaves and fruit that were investigated in this study. The major macroelements were as follows: N > P > K > Na > Mg > Ca for both cultivars (Table 1). Nitrogen is a predominant element (24.32–29.85 g/kg) for the goji fruit of cvs. New Big and No. 1. It was observed that cv. No. 1 had a significantly higher content of macroelements compared to cv. New Big. Significant differences were found between the cultivars in their concentrations of N, P, Ca, and Na. An opposite relationship was observed in the case of the concentrations of microelements in the studied goji fruit (Table 2), in which the major microelements were as follows: Fe = Cu > Zn > Mn > Se. Ferrum (i.e., iron) was found in the highest concentration in the goji fruit (66.03–79.44 g/kg, with the highest levels in 'No. 1' followed by 'New Big'). The New Big cultivar was characterized by a significantly higher content of microelements in its fruit, except for selenium, which was 25% lower than in the fruit of the No. 1 cultivar.

**Table 2.** Average values of microelements in leaves and fruit of two cultivars of goji cultivated in orchard and *in vitro* conditions.

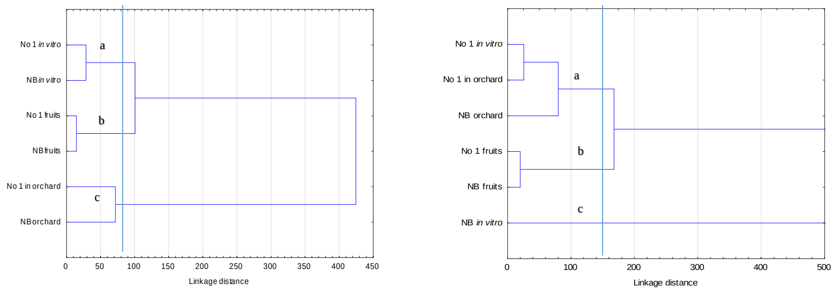
| Compounds (g/kg) | Leaves | | | | Fruit | |
|---|---|---|---|---|---|---|
| | No. 1 | | New Big | | No. 1 | New Big |
| | orchard | *In vitro* | Orchard | *In vitro* | Orchard | |
| Fe (40–60 [2]) | 120.13 ± 7.20c [1] | 71.00 ± 3.82a | 97.81 ± 4.07b | 77.44 ± 3.55a | 66.03 ± 2.04A | 79.44 ± 2.63B |
| Zn (8–14) | 18.62 ± 0.35b | 26.77 ± 0.47c | 14.53 ± 0.27a | 35.07 ± 0.31d | 8.16 ± 0.24A | 8.73 ± 0.19B |
| Mn (70–260) | 50.70 ± 1.04b | 176.89 ± 3.57c | 42.07 ± 0.88a | 244.64 ± 5.03d | 7.04 ± 0.12A | 7.74 ± 0.10B |
| Cu (5–20) | 8.42 ± 0.19d | 3.39 ± 0.16a | 6.06 ± 0.14c | 3.90 ± 0.11b | 4.72 ± 0.98A | 5.64 ± 1.25B |
| Se | 0.089 ± 0.005b | n.d. [3] | 0.062 ± 0.004a | n.d. | 0.012 ± 0.001B | 0.009 ± 0.001A |
| Pb | 0.034 ± 0.03a | n.d. | 0.054 ± 0.05b | n.d. | 0.017 ± 0.001A | 0.029 ± 0.002B |
| Cd | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Ni | 0.019 ± 0.002b | n.d. | 0.011 ± 0.002a | n.d. | 0.007 ± 0.001B | 0.003 ± 0.000A |

[1] Designation according to Table 1. [2] Optimal content for leaves according to Glonek and Komosa [15]. [3] n.d.—not detected.

Our results differ from those obtained by Sá et al. [14], where the concentration of macroelements in goji fruit was within the limits of 3.124–590.7 mg/kg. Additionally, Nascimento et al. [2], Llorent-Martínez et al. [20], and Niro et al. [22] reported slightly different results. As in other plant food, the mineral content of berries reflects the soil in which they are grown. It should be highlighted that the content of required and undesirable elements in the fruit depends largely on the properties of the soil, plant physiology, source and chemical composition of water, fertilizers, pesticides, insecticides, and fungicides used on the plantation. Each species has individual requirements and different tolerance levels to the absorbed and accumulated chemical compounds from the medium [22].

The study of the chemical composition of fruits that are considered to be superfoods is important from nutritional and toxicological perspectives. The percentages of macro- and microelements in the goji fruit were compared to the daily mineral requirements of the human diet [21]. Recommended Daily Allowances (RDAs) have been established, namely, 2 mg of Cu, 18 mg of Fe, 400 mg of Mg, 2 mg of Mn, 1000 mg of P, and 15 mg of Zn [2]. Niro et al. [22] reported that 100 g of fresh goji berries can be declared on the label as a source of Cu. The consumption of 100 g of fresh goji per day contributes to approximately 25% of the RDA of Cu [22]. In our study, we obtained similar results, where 100 g of fresh goji fruit constitutes 30% and 35% of the demand for this element, respectively, for the No. 1 and New Big cultivars (Table 2). Moreover, fresh goji fruit is also a rich source of ferrum. One hundred grams constitutes 47% of the RDA for this element; this was also confirmed by our own research. Copper is involved in maintaining cardiovascular health, and glucose and cholesterol metabolism [23]. Iron is an essential micronutrient due to the fact of its high functionality, while also being an important functional food for patients with iron deficiency. In contrast to the results obtained for goji fruit by Niro et al. [22], the fruit we studied were also a small source of selenium. In fact, 100 g of fresh berries contributed to approximately 13% and 17% of the RDA, respectively, for the New Big and No. 1 cultivars. In connection with the use of goji fruit in disease-preventing diets, the results of this study are interesting and very helpful and important. Knowledge of minerals and trace elements in natural products is extremely important. Goji fruit, called functional foods may be a main external source of these nutrients for humans and animals alike.

The analysis carried out using the Ward's method (Figure 1a) identified three groups with similar micro and macroelements in goji. This may indicate that the content of mineral components in leaves and fruits was more dependent on the place of cultivation (*in vitro*-group a, or orchard-group b). A completely separate group, connected with leaves in a very weak way, was the fruit-group c.




(**a**) (**b**)

**Figure 1.** Dendrogram of cluster analysis of micro- and microelements (**a**) and polyphenols (**b**) in two cultivars of goji fruit and leaves (cut off—85 and 150).

### *2.2. Leaf and Fruit Color*

According to Kiełbasa and Juliszewski [24], by measuring the area of one leaf, we can determine the intensity of growth and the related intensity of the photosynthesis process. Based on these findings, we can assess the condition of the plants [25]. In this study, the leaf surfaces of the two goji cultivars grown in the orchard and *in vitro* were compared (Table 3). Upon analyzing the leaves, it was found that the New Big cultivar had the largest leaf area, with a size varying from 4.18 $cm^2$ (orchard) to 2.78 $cm^2$ (*in vitro*) in comparison to the No. 1 cultivar. The results obtained were similar to those described by Kruczek et al. [6]. The leaves of the plants grown *in vitro*, despite the fact that they had more mineral components in their composition, that is, they were better nourished, which was also indicated by their more intense color; they were, however, much smaller.

The results of the color determination of the leaves and fruit were also analyzed in the transmitted mode using the photocolorimetric method in the CIE L**a*b** system. The leaves of *L. chinense* Mill. were also characterized by a similar value of the green index [3,6]. The value of the *a** parameter (color ranging from green to red) determined on the surface of leaf ranged from −33.84 to −43.56 (Table 4). The 'New Big' goji leaves under *in vitro* conditions were 22% greener compared to the goji leaves grown in the orchard. This could have been influenced by the much higher content of most macro- and microelements in these leaves (Tables 1 and 2). However, the value of the *a** parameter in the case of the No. 1 cultivar was at a similar level regardless of the cultivation conditions. The leaves of the New Big cultivar were also darker, which is shown by the lower value of the parameter L* (34.57 and 42.50, *in vitro* and orchard, respectively). The value of parameter L* (reaching from 0 to 100, black to white, respectively) is usually used for tracking color changes [6,26]. However, the goji leaves of cultivar No. 1 were 23% (*in vitro*) and 13% (orchard) brighter compared to the 'New Big' leaves. The color of the leaf surface was determined by parameter *b** (from a yellow to a blue color) which ranged from 23.71 ('New Big' *in vitro*) to 30.63 ('No. 1' orchard). These results are consistent with the Normalized Anthocyanin Index (NAI) (−1 and +1, lack of redness and red, respectively). Leaves that were intensely green had the lowest NAI index and ranged from -0.69 ('No. 1' *in vitro*) to −0.82 ('New Big' orchard).

On the other hand, the Normalized Difference Vegetation Index (NDVI), which indicates the vegetative potential of plants, was nearly 10% higher in the goji leaves propagated *in vitro* (Table 3). These results obtained are comparable to those described by Kruczek and Ochmian [3], and Kruczek et al. [6].

**Table 3.** The leaf area, color measurement and NAI and NDVI value in leaves and fruit of two cultivars of goji cultivated in orchard and *in vitro* condition.

| Compounds | | Cultivar | | | | | |
|---|---|---|---|---|---|---|---|
| | | No. 1 | | New Big | | No. 1 | New Big |
| | | Leaves | | | | Fruit | |
| | | Orchard | *In Vitro* | Orchard | *In Vitro* | Orchard | |
| Leaf area (cm$^2$) | | 3.53 ± 0.31c [1] | 2.11 ± 0.17a | 4.18 ± 0.39d | 2.78 ± 0.15b | - | - |
| Color parameters | L* | 47.82 ± 2.55a | 42.36 ± 1.53b | 42.50 ± 2.71b | 34.57 ± 1.70c | 35.88 ± 1.45B | 44.72 ± 2.04A |
| | *a** | −36.27 ± 3.08ab | −38.94 ± 1.98b | −33.84 ± 2.55a | −43.56 ± 2.12c | 25.63 ± 3.24A | 33.34 ± 3.42B |
| | *b** | 30.63 ± 2.50c | 27.73 ± 1.45bc | 23.71 ± 2.26a | 25.25 ± 1.63ab | 27.05 ± 2.44A | 41.40 ± 2.79B |
| NAI | | −0.76 ± 0.10b | −0.69 ± 0.06a | -0.82 ± 0.07c | −0.74 ± 0.05b | 0.62 ± 0.05A | 0.71 ± 0.05B |
| NDVI | | 0.69 ± 0.04a | 0.76 ± 0.05b | 0.84 ± 0.05c | 0.91 ± 0.03d | −0.46 ± 0.04B | −0.38 ± 0.03A |

[1] Designation according to Table 1.

The color of fruit is of crucial importance in consumers' choices. An intensive color may encourage purchases, and/or discourage consumption by warning of their rottenness [6,27,28]. According to Ochmian et al. [26], the color of fruit depends on the place of cultivation and the climatic conditions prevailing there. The color of food products may also change during processing [29]. Regarding the testing of the fruit color, the values of the *a** parameter measured were significantly different. The No. 1 cultivar fruit had more than a 23% red-coloring compared to the 'New Big' fruit (Table 3). Parameter *a** was corelated with the NAI [6,26]. The content of anthocyanin pigments determined by the NAI value was responsible for the color of the goji fruit. The NAI showed that the highest anthocyanin contents were recorded in the fruit of the New Big cultivar. When estimating the ripeness and quality of the fruit, the NDVI value was also determined, which in the tested goji fruit, was −0.38 in 'New Big', indicating immature fruit (pre-climacteric). In cultivar No. 1 the value of the NDVI index was −0.46 and characterized fruit with the onset of ethylene production. The obtained results were higher than those described by Kruczek et al. [6].

The color of the surface of the goji fruit skin was described by parameter *b**. The New Big cultivar contained the highest quantity of red-colored substances (41.40) and a smaller quantity of yellow-colored ones, and these values were in accordance with the values obtained by Kruczek et al. [6]. The value of the L * parameter was 20% higher in the New Big cultivar in comparison to the No. 1 cultivar. The results obtained in this study were compared to the results of Lachowicz et al. [28] in saskatoon berries (L* from −32.71 to 49.31) and Kruczek et al. [6] in goji berries (L* from −32.6 to 72.3). However, the goji fruit examined by Kruczek and Ochmian [3] and the highbush blueberries examined by Ochmian et al. [30] were darker than in our own research.

## *2.3. Polyphenolic Compounds and Health Promoting Capacities*

Polyphenols are secondary metabolites of plants and are generally involved in defense against biotic and abiotic stresses. The content of polyphenols in plants is influenced by many factors, including the degree of maturity at harvest, environmental factors, processing, and storage. In the last decade, there has been great interest in the potential health benefits of dietary plant polyphenols as antioxidants, identifying 10 flavonols, two flavan-3-ols, two tannins, and 10 phenolic acids (Table 4). The two cultivars of goji grown *in vitro* provided similar values in terms of total phenolic content (102.64 mg/100 g DW and 103.92 mg/100 g DW, 'No.'1 and 'New Big', respectively), while orchard-grown goji had a significantly higher amount (124.46 mg/100 g DW and 167.79 mg/100 g DW, 'No. 1' and

'New Big', respectively). This finding indicates that goji species are a significant source of phenolics. According to Wojdyło et al. [31], the average content of phenol in all goji cultivars grown in Poland was 97.23 mg/100 g, with considerable inter-cultivar differences. The content of total polyphenolic compounds was statistically different among the different species. Kiwifruit and apple contained small quantities of polyphenolic compounds (70.23–83.40 mg GAE/100 g FW), while a significantly higher polyphenolic content was observed in strawberry (323.39 mg GAE/100 g FW), and blackcurrant (434.43 mg GAE/100 g FW). The polyphenol content in the tested cultivars of goji was between that of orange (158.70 mg GAE/100 g FW) and guava (310.10 mg GAE/100 g FW). In this study, the dominant phenolic acid was 5-*O*-caffeoylquinic acid.

**Table 4.** Content of polyphenolic compounds in leaves and fruit of two cultivars of *L. chinensis*.

| Compounds (mg/100 g DW) | Leaves | | | | Fruits | |
|---|---|---|---|---|---|---|
| | No. 1 | | New Big | | No. 1 | New Big |
| | *In Vitro* | Orchard | *In Vitro* | Orchard | | |
| Quercetin-3-*O*-Gal | n.d. | n.d. | n.d. | n.d. | 0.80A [1] | 2.98B |
| Kaempferol-3-*O*-Glc-7-*O*-Soph | n.d. | n.d. | 11.47a | 14.25b | n.d. | n.d. |
| Quercetin-3-*O*-Rut-7-*O*-Glu | n.d. | n.d. | n.d. | n.d. | 2.94B | 2.07A |
| Quercetin-3-*O*-Soph-7-*O*-Rha | 12.73b | 14.65b | 4.42a | 24.03c | 0.24A | 2.31B |
| Kaempferol-3-*O*-Rut-7-*O*-Glu | n.d. | n.d. | n.d. | n.d. | 6.38A | 17.22B |
| Quercetin-3-*O*-Glu | n.d. | n.d. | 2.37b | 0.72a | 3.05A | 3.50A |
| Quercetin-3-*O*-Rut | 12.58a | 23.81b | 31.42c | 33.89c | 11.29B | 7.57A |
| Quercetin-3-*O*-Glu-7-*O*-Rha | 0.22a | 0.48b | 1.04c | 0.58b | n.d. | n.d. |
| Kaempferol-3-*O*-Rhu | 0.77a | 0.95b | n.d. | n.d. | 0.18 | n.d. |
| Kaempferol-3-*O*-Glu-7-*O*-Rha | 0.68a | 0.75a | 1.21c | 0.94b | n.d. | n.d. |
| *Total flavonols* | *26.98a* | *40.64b* | *51.93c* | *74.41d* | *24.88A* | *35.65B* |
| Procyanidin B dimer | 0.99b | 3.04c | 0.49a | 8.01d | 0.62A | 0.51A |
| (+)-Catechin | n.d. | n.d. | 7.38a | 21.41b | 18.44B | 11.04A |
| *Total flavan-3-ols* | *0.99a* | *3.04b* | *7.87c* | *29.42d* | *19.06B* | *11.55A* |
| Tetragalloyl-glucose | 0.23a | 0.29a | 4.22c | 3.81b | n.d. | n.d. |
| Galloylquinic acid | 0.17a | 0.21a | 2.55c | 2.09b | n.d. | n.d. |
| Total hydrolyzable tannins | 0.40a | 0.50a | 6.77c | 5.9b | | |
| 5-*O*-Ferruloylquinic acid | 0.41b | 0.30a | 1.77 | 1.60c | 0.56B | 0.21A |
| p-Coumaric acid | 1.66 | 1.52 | 2.15d | 1.06 | 8.29B | 6.35A |
| Caffeic acid | 0.64b | 0.82c | 0.37a | 0.34a | n.d. | n.d. |
| Caftaric acid | n.d. | n.d. | n.d. | n.d. | 0.74A | 5.06B |
| p-Coumaroyl acid dihexoside | n.d. | n.d. | n.d. | n.d. | 4.22B | 3.50A |
| 3-*O*-Caffeoylquinic acid (neochlorogenic acid) | 0.51c | 0.56c | 0.38b | 0.27a | 4.11A | 11.04B |
| 3-*O*-Caffeoylquinic acid derivative | 1.33d | 1.12c | 0.17a | 0.33b | 10.24B | 8.22A |
| 4-*O*-Caffeoylquinic acid (cryptochlorogenic acid) | 0.44b | 0.58c | 0.20a | 1.87d | n.d. | n.d. |
| 5-*O*-Caffeoylquinic acid (chlorogenic acid) | 66.47c | 73.05d | 31.04a | 52.15b | n.d. | n.d. |
| 5-*O*-Caffeoylquinic acid isomer | 2.81d | 2.33c | 1.27b | 0.44a | 2.52A | 4.21B |
| *Total phenolic acids* | *71.56c* | *77.64c* | *33.06a* | *55.06b* | *21.09A* | *26.97A* |
| TOTAL | 102.64A | 124.46B | 103.92A | 167.79C | 74.62A | 85.79B |

[1] Designation according to Table 1.

According to Sato et al. [32] and Kruczek et al. [6], chlorogenic acid has a significant influence on the flavor of fruit and vegetables. Moreover, it shows anticarcinogenic, antimutagenic, and antioxidant properties *in vitro*. In the human body, chlorogenic acid is poorly absorbed and metabolized by colonic microflora [32]. In this study, a significantly higher concentration of chlorogenic acid was detected in the leaves grown in the orchard than *in vitro* (Table 4). This is in accordance with the results obtained by Chen et al. [13], who also confirmed that in goji fruit the concentration of chlorogenic acid is very low in comparison to the leaves. This demonstrates that goji leaves are a valuable source of chlorogenic acid

Flavonoids, belonging to polyphenolic compounds, are also commonly found in plants, especially in fruit and vegetables [33]. The total amount of flavonoids in the flavonoid fraction was higher in the goji leaves than in the fruit. Moreover, quercetin-3-*O*-Gal and quercetin-3-*O*-Rut-7-*O*-Glu, which were present in the fruit, were not identified in the leaves of both cultivars. On the contrary, kaempferol-3-*O*-Glc-7-*O*-Soph, quercetin-3-*O*-Rut-7-*O*-Rha, and kaempferol-3-*O*-Glc-7-*O*-Rha were only identified in the leaves of both cultivars (Table 5).

For human health, gallotannins are essential [6]. The highest content of tetragalloyl-glucose, regardless of the cultivation method, was found in 'New Big' (4.22 and 3.81 mg/g DW, *in vitro* and orchard, respectively). In 'No. 1', these values were 95% and 92% lower *in vitro* and in orchard, respectively.

According to many authors [6,33,34], the higher the content of total polyphenols, the higher the antioxidant activity. Pandey and Rizvi [34] suggested that the long-term use of diets rich in plant polyphenols provides some protection against the development of cancer, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases.

### 2.4. Antioxidant Activity

The antioxidant activity was evaluated using DPPH scavenging activity and ferric-reducing antioxidant power (FRAP). Free radicals are known to be a major factor in biological damage. The DPPH radical-scavenging assay is a widely used method to evaluate the ability of plant extracts to scavenge free radicals generated from the DPPH reagent. The DPPH free radical scavenging activity of the two tested goji cultivars is presented in Table 5. Relatively higher DPPH scavenging abilities were recorded in goji fruit (7.61 mmol Trolox/100 g and 5.33 mmol Trolox/100 g, 'No. 1' and 'New Big', respectively), while the lowest DPPH scavenging abilities were found in the leaves grown in the orchard (3.26 mmol Trolox/100 g, 2.48 mmol Trolox/100 g, 'No. 1' and 'New Big', respectively).

The analysis carried out using the Ward method (Figure 1b) showed that the polyphenol content was divided into three groups with similar micro- and macroelements in goji. The fruits formed a separate group (b), and similar for the leaves of New Big cultivar (c).

**Table 5.** Health-promoting capacities of leaves end fruit of two cultivars *L. chinensis*.

| **Nutritional Value** | | **Leaves** | | | | **Fruit** | |
|---|---|---|---|---|---|---|---|
| | | **No. 1** | | **New Big** | | **No. 1** | **New Big** |
| | | ***In Vitro*** | **Orchard** | ***In Vitro*** | **Orchard** | **Orchard** | |
| DPPH (mmol Trolox/100g) | | 3.88c[1] | 3.26b | 4.25d | 2.48a | 7.61B | 5.33A |
| FRAP (mmol Trolox/100g) | | 4.02c | 2.54a | 5.89d | 3.48b | 2.89A | 3.93B |
| $\alpha$-amylase IC50 (mg/mL) | | 112.6b | 75.1a | 172.9d | 134.0c | 33.45A | 37.01A |
| $\alpha$-glucosidase IC50 (mg/mL) | | 25.41a | 22.05a | 42.28b | 37.06b | 8.36A | 7.44A |
| Soluble sugars (g/100 g DW) | fructose | 1.88c | 0.89a | 2.51d | 1.33b | 9.67A | 11.83B |
| | glucose | 1.55c | 0.51a | 1.88d | 1.04b | 10.11A | 13.06B |
| | sucrose | 0.20b | 0.24c | 0.18b | 0.12a | 0.51A | 0.77B |
| Organic acid (g/100 g DW) | oxalic acid | 0.021a | 0.024a | 0.073b | 0.080b | n.d. | 0.362 |
| | citric acid | 0.115b | 0.292c | 0.083a | 0.314d | 0.951A | 1.485B |
| | succinic acid | 0.027ab | 0.035bc | 0.021a | 0.042c | 0.547B | 0.322A |
| | fumaric acid | 0.019bc | 0.023c | 0.016ab | 0.014a | 0.078B | 0.066A |

[1] Designation according to Table 1.

Regarding the values of the total antioxidant capacity, expressed as the FRAP assay, the results showed large statistical variations among the tested cultivars. The highest radical scavenging activity (FRAP) was obtained in the goji leaves grown *in vitro* (4.02 mmol Trolox/100 g and 5.89 mmol Trolox/100 g, 'No. 1' and 'New Big', respectively); meanwhile, the FRAP values obtained for the leaves grown in the orchard and for fruit were at a similar level (Table 5). Our data agree with that reported by Kruczek et al. [6] and Wojdyło et al. [31], who demonstrated similar values of antioxidant activity in their studies. According to many authors [6,26,31], the antioxidant activity of various plant species (fruit or leaves) may be determined by growing conditions, geographical location, climatic conditions, genotype, fruit maturity, or even the collection methods. Many authors have highlighted that fruit rich in significant amounts of phytochemicals are of great interest to potential consumers [6,13].

### 2.5. Antidiabetic Activity of Goji Fruits

The inhibition of $\alpha$-amylase in the analyzed leaves ranged from 75.1 ('No. 1' in the orchard) to 172.9 mg/mL $IC_{50}$ ('New Big' *in vitro*), and in the fruits ranged from 33.45 mg/mL $IC_{50}$ in 'No. 1' to 37.01 mg/mL $IC_{50}$ in 'New Big'. While the inhibition of $\alpha$-glucosidase in leaves samples was between 22.05 ('No. 1') and 42.28 mg/mL $IC_{50}$ ('New Big'). Goji leaf extracts collected *in vitro* were more effective in the activity of α-amylase and α-glucosidase inhibitors (Table 5). However, there were no statistical differences in the activity of α-amylase and α-glucosidase in fruits. The inhibition of these enzymes may be effective for regulating type 2 diabetes by controlling the absorption of glucose [35]. Both α-amylase and α-glucosidase inhibitory activity in fruits such as pomegranate, strawberry, raspberry, pear, kiwi, plum, lingonberry, black currant, and blueberry extracts has been confirmed in other studies [16,28,31,35]. Inhibition of these enzymes is specifically useful in the treatment of non-insulin-dependent diabetes, as it slows down glucose release into the bloodstream [31].

### *2.6. Soluble Sugars and Organic Acid*

The organoleptic properties of the fruit are influenced by many ingredients, including sugars and organic acids [31]. The sugar content in the goji samples examined in this study is presented in Table 5. The main sugars identified in the analyzed leaves and fruit of the two cultivars of goji were fructose, glucose, and sucrose. The largest amounts of total sugars were fructose and glucose, both in the leaves (0.89–2.51 g/100 g DW and 0.51–1.88 g/100 g DW, fructose and glucose, respectively) and the fruit (9.67–11.83 g/100 g DW and 10.11–13.06 g/100 g DW, fructose and glucose, respectively). The cultivar with the highest sugar content in both its leaves and fruit was 'New Big'. Moreover, the leaves from goji grown *in vitro* had from 80% to a 120% higher total sugar amount than in the leaves grown in the orchard. This may be due to the addition of 3% sucrose to the MS medium.

Our results show significant differences ($p < 0.05$) in the content of organic acids among the tested cultivars (Table 5). The total organic acid content ranged from 0.182 to 0.450 g/100 g DW in the leaves and from 1.576 g/100 g DW in 'No. 1' fruit to 2.235 g/100 g DW in 'New Big' fruit. The New Big cultivar had the highest amount of organic acid, and it was also observed that leaves from goji grown *in vitro* were the lowest. Oxalic, citric, succinic, and fumaric acids were the main organic acids in the leaves and fruit. However, in the fruit from 'No. 1', oxalic acid was not detected. The highest amount of total organic acid was found to be citric acid, both in the leaves and the fruit. Other organic acids, (i.e., oxalic, succinic, and fumaric) were present in low concentrations. The types and amounts of sugars and organic acids were the same as reported by Montensano et al. [36] and Wojdyło et al. [31]. According to Potterat [1], the content of polysaccharides and total sugars in *L. chinensis* fruit is a major medicinal aspect of goji berries. The sugar/acidity ratio in fruit is an important choice for consumers.

PCA for minerals and phytochemical content of leaves and fruits explained (70.94) the total variance, where PC1 represented 45.30% and PC2 25.64% (Figure 2). The statistical method pointed out three major parts. Our results show that there were many correlations (positive, negative, weak) between antioxidant capacity and mineral extractability. The first group included polyphenols, which showed high dependence with antioxidant activity, and inhibitors activities. It is known that the antioxidant activity of fruits is influenced by their phenolic composition [37]. There was a positive correlation between the DPPH values and polyphenolic compounds content, meaning that the concentration of phenolic compounds may be a good indicator of the reducing capacity in the fruits. Polyphenolic compounds such as phenolic acids, flavonoids, anthocyanidins, and tannins, produced as secondary metabolites by plants, possess remarkable antioxidant and immunomodulatory activities [38]. A high correlation between polyphenols and NDVI index was also found. It was also found that macroelements (especially K, P, and N) were negatively correlated with microelements. This may be due to synergism and antagonism between minerals. It is well known, for example, that high phosphorus content may limit the uptake of microelements. A high content of these elements was found in the soil (Table 7) and leaves (Tables 1 and 2) of the studied goji cultivars. There is also antagonism between Cu and Zn, and potassium strongly limits the uptake of Na. In contrast, potassium shows strong synergy with nitrogen. There may also be an overaccumulation of some components in the soil (e.g., phosphorus and potassium) and depletion of others (magnesium and microelements).

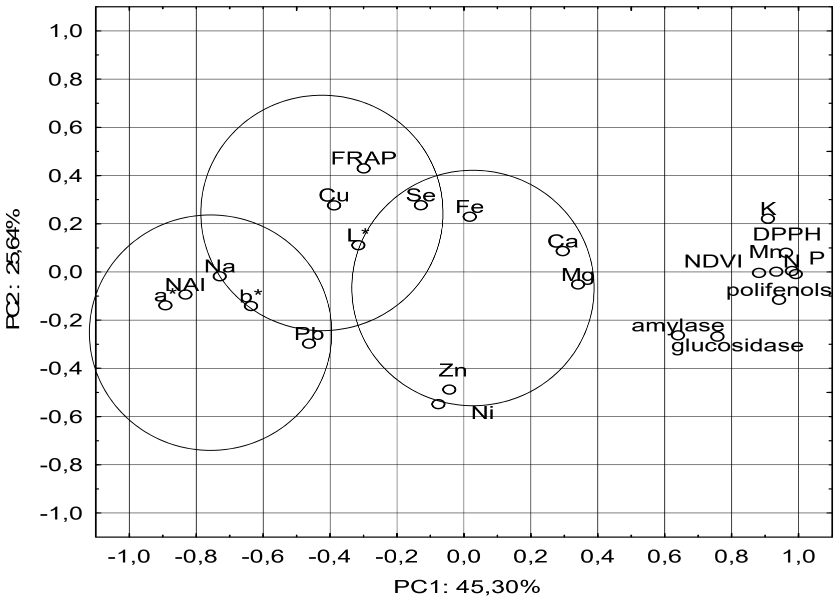



**Figure 2.** The principal component analysis (PCA) for mineral compositions and phytochemical content of goji leaves and fruit depending on growth conditions.

In the second group, a correlation between FRAP and some microelements. Generally, positive correlations were observed between FRAP and Fe, Cu, and Sn content in our research. These results demonstrate that FRAP in fruits has a significant impact on enhancing the extractability of Cu bound with the ability to form chelated metal ions by FRAP and to retain them (37). Antioxidants that react in the FRAP assay are those that can reduce, under the reaction conditions used, the $Fe^{3+}$-TPTZ salt to Fe2+-TPTZ form. These include polyphenolic compounds such as catechins and other flavonoids in plant-based foods [39].

In the third group, the dependency between NAI index and color parameters *a** and *b** was visible.

No anthocyanins were found in the polyphenolic profile of goji, as evidenced by the negative value of the NAI index (also a color indicator—Table 3). This is confirmed by high dependence on other parameters of color a * and b * and Mg, the basic component of chlorophyll. This correlation may indicate that the presence or absence of one metal has no effect on the other.

### 2.7. Antimicrobial Activity

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. *In vitro* antimicrobial activity should be determined first [40]. The leaf extracts in the tested concentrations showed inhibitory activity (MIC) on the growth of the analyzed Gram-positive bacteria, especially on *S. aureus*. However, among the Gram-negative bacteria, only *P. vulgaris* was sensitive to its effects (Table 6). A definitely higher inhibitory effect characterized extracts from the leaves collected from the shrubs that grew in the orchard, especially from the New Big cultivar. The antimicrobial activity of the leaf extracts has been confirmed in studies conducted with the use of the disc diffusion method (Table 6). Extracts from the leaves harvested in the orchard of both studied cultivars had a higher inhibition zone (9.0–16.7 mm) compared to the *in vitro* leaves (8.6–14.2 mm). The smallest inhibition diameter was determined for Gram-negative *E. coli* (8.6 mm) and the largest (16.7 mm) for Gram-positive bacteria such as *B. subtilis*. However, these inhibition capacities are lower than those observed by Mocan et al. [5] and Dahech et al. [41].

**Table 6.** Antibacterial activity and Minimal Inhibitory Concentration of leaves extracts and antibiotics against bacterial species tested by disc diffusion assay.

| Bacterial Strains | Inhibition Zone—IZ (mm) and Minimal Inhibitory Concentration—MIC (μg/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. 1 | | | | New Big | | | | Standard Antibiotic (Gentamicin) |
| | Orchard | | *In Vitro* | | Orchard | | *In Vitro* | | |
| | IZ | MIC | IZ | MIC | IZ | MIC | IZ | MIC | |
| *E. coli* | 9.6 ± 0.9 | >100 | 8.6 ± 0.6 | >100 | 10.5 ± 0.8 | 100 | 9.8 ± 0.8 | >100 | 12.5 |
| *P. vulgaris* | 12.2 ± 0.8 | 75 | 11.3 ± 0.9 | 75 | 14.3 ± 0.8 | 50 | 13.5 ± 0.9 | 100 | 17.0 |
| *B. subtilis* | 14.7 ± 0.8 | 100 | 13.2 ± 1.0 | 100 | 16.7 ± 1.1 | 100 | 14.2 ± 0.8 | >100 | 21.4 |
| *S. aureus* | 9.0 ± 0.6 | 75 | 8.8 ± 0.9 | 100 | 9.6 ± 0.8 | 75 | 9.1 ± 0.7 | >100 | 14.6 |
| *L. monocytogenes* | 10.3 ± 0.8 | 100 | 10.2 ± 0.7 | >100 | 10.9 ± 0.9 | 75 | 10.5 ± 0.4 | >100 | 18.6 |

## 3. Materials and Methods

### *3.1. Characteristics of the Research Area*

#### 3.1.1. Orchard Experiment

The study was carried out in the Department of Horticulture and the Department of Plant Genetics, Breeding and Biotechnology, of the West Pomeranian University of Technology in Szczecin. The research station is located in subzone 7A in the North-Western part of Poland in the Szczecin Lowland at a distance of approximately 65 km from the Baltic Sea (53°40′ N, 14°88′ E). The research was conducted at a production plantation specializing in the cultivation of highbush blueberry, located in the Szczecin's Lowland. In this area, there are numerous hills of 40–60 m a.s.l., the remnants of the frontal moraine. The climate of this area is also significantly affected by the presence of big water basins (Szczecin Lagoon, Dąbie Lake, the Odra River), which provide additional moisture in the period of plant vegetation. The average growing season (April–October) temperature from 1951 was 13.7 °C and rainfall 391 mm [42].

#### 3.1.2. *In Vitro* Experiment

The second part of the experiment was carried out in the Laboratory of *In vitro* Cultures, where all cultures were incubated in a growth room at a temperature of 24 ± 2 °C under a 16 h photoperiod with a photosynthetic photon flux density (PPFD) of 40 μmol/m$^2$/s provided by Narva (Germany) emitting daylight cool white.

### *3.2. Characteristics of the Plant Material*

#### 3.2.1. Orchard Experiment

The soil (pH 6.75) in the orchard was an agricultural soil with a natural profile, developed from silt loam (sand 42.7%, silt 52.9%, clay 4.4%) with a considerably lower density of 1.23 Mg/m$^3$. The groundwater level was 140–160 cm, and a higher water capacity of 46.2% ww. It also contained much more organic matter—32.4 g/kg of soil. Regardless of the site, the soil was characterized by similarly low salinity EC 0.33–0.42 mS/cm. The mineral content of the soil is shown in Table 7. The soil, in which the shrubs grew, regardless of the stand, in comparison to the optimal mineral content of the soil by Sadowski et al. [43], was characterized by a high content of P, K, and Mg. Every spring nitrogen fertilization was used at a dose of 45 kg N. Irrigation of the plantation was carried out annually using a permanently installed T-Tape drip irrigation line with the emitter's performance of 1.5 L/h (3 L of water on a section of 1 linear meter of the installation). The moisture content of the soil was maintained in the PF 1.8–2.1 range and was determined using contact tensiometers.

**Table 7.** Mineral composition of soil in which the two goji cultivars grew in orchard.

| N | P | K | Mg | Ca | Na | Fe | Mn | Zn | Cu | Cd | Pb | Ni | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| g/kg | | | | | | | mg/kg | | | | | | |
| 17.33 | 123.3 | 284.7 | 72.7 | 452 | 11.3 | 83.4 | 57.3 | 37.0 | 7.52 | 0.296 | 31.3 | 4.67 | 0.031 |

#### 3.2.2. *In Vitro* Experiment

The research material consisted of 15–20 mm stem nodes with an axillary bud of goji obtained from a sterile stabilized *in vitro* culture. The explants were transferred to MS medium according to Murashige and Skoog's [44] composition of vitamins, and macro- and microelements. All media contained 30 g/dm$^3$ of sucrose (Chempur, Poland) and 100 mg/dm$^3$ of myoinositol (Duchefa, The Netherlands) and were solidified with 8 g/dm$^3$ of agar (Biocorp, Poland). The pH of the media was adjusted to 5.7. The media were heated and then 30 mL was poured into 450 mL flasks, which were autoclaved at 121 °C (0.1 MPa) during the time required according to the volume of medium in the vessels. After the end of the experimental period (five weeks), the explants were removed and washed with deionized distilled water.

### 3.3. *Analysis of the Chemical Compounds*

For the chemical analyses, leaves were taken from the orchard at the beginning of August (during harvesting of the fruit); 100 pieces were taken from each combination. Typical, healthy leaves from the middle part of the annual shoots were collected. Leaves were taken from the multiplied 5-week shoot cultures of goji plants, to be used for chemical analyses. From each harvest, we also took samples of fruits that were then frozen. After finishing the harvest from each period, we prepared a collective sample that was dried (and 65 °C) and ground.

The content of elements in leaves and fruits were determined after mineralization: N, P, K, Ca, and Na were measured after wet mineralization in $H_2SO_4$ (96%) and $HClO_4$ (70%). The content of Cu, Zn, Mn, Fe, Se were determined after mineralization in $HNO_3$ (65%) and $HClO_4$ (70%) in a ratio soil 1:1 [45], leaves and fruits 3:1 [46]. The total N concentration in plants was determined by the Kjeldahl distillation method [47]. The content of K was measured with the atomic emission spectrometry, Mg, Ca, Na, Cu, Zn, Mn, Se, Fe Pb, Ni, and Cd content with the flame atomic absorption spectroscopy. P was assessed by the colorimetric method [16]. All tests were performed each year in three replications.

### 3.4. *Nutritional Value and Polyphenols of Leaves and Fruits*

Samples (leaves and fruit) were freeze-dried before the analysis, and then powdered and subjected to the extraction process according to the methodology of Oszmiański et al. [48]. Nutritional value was determined in the dehydrated and leaves and fruit. Soluble sugars and organic acids were determined according to the procedure described by Dias et al. [49]. Soluble sugars and organic acids were determined by UFLC-PDA.

The FRAP (Ferric-Reducing Antioxidant Power) assay was done according to Benzie and Strain [50] and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) was done according to Shimada et al. [51]. The antioxidant capacity is expressed as Trolox equivalent. The absorbance at 517 nm was determined by spectrophotometer UV-2401 PC. In goji leaves and fruit extracts, polyphenol identification in UPLC-PDA-ESI-MS/MS (ultra-performance liquid chromatography with photodiode array and electrospray ionization tandem mass spectrometry detection) was executed 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 ionization (ESI) source operating in both negative and positive modes [52].

### 3.5. Antidiabetic Activity (α-Amylase, α-Glucosidase)

The activity of the parasite and host extracts was assayed according to the procedure described previously by Podsedek et al. [35] (α-glucosidase) and Nickavar and Yousefian [53] (α-amylase). All samples were assayed in triplicate and the result was expressed as IC50. The amount of the inhibitor (expressed as mg per 1 mL of the reaction mixture under assay conditions) required to inhibit 50% of the enzyme activity is defined as the IC50 value. All samples were assayed in triplicate.

### 3.6. Antimicrobial Activity Assay

The antimicrobial activity of the extracts was evaluated using the agar well diffusion method [54]. Ready-made sterile Petri dishes (Ø 90 mm) with a Mueller–Hinton medium were used. The cell suspensions (100 µL) were evenly distributed on the Petri dishes. Six-millimeter wells were punched into the agar with a sterile Pasteur pipette, in which 60 µL of the extracts was applied. Gentamycin was used as a control for bacteria. The dishes were incubated at 37 °C for 24 h. The antimicrobial activity was evaluated by measuring the diameter of the circular inhibition zones around the well.

For the evaluation of the antimicrobial activity, the following were used: Gram-positive bacteria *Staphylococcus aureus* (ATCC-25923), *Bacillus subtilis* (ATCC-12228), and *Listeria monocytogenes* (ATCC-19115), and Gram-negative: *Escherichia coli* (ATCC-25922) and *Proteus vulgaris* 458. The MIC (Minimal Inhibitory Concentration) of the solutions was determined for each strain. The MIC value determines the lowest oil concentration (i.e., 10, 25, 50, 75, and 100 µg/mL), at which no growth (turbidity) of the tested bacterial strain is visually observed, and at the same time precedes the concentration at which growth is visible. For the above determinations, methodology consistent with Inouye et al. [55] was used.

### 3.7. Area of one Leaf ($cm^2$)

In autumn, the foliage area (taken from the central part of annual shoots) was measured using the Delta Image Analysis System (Delta-T Devices LTD, England) scanner connected to the computer.

### 3.8. Color and Pigment Parameters

The color parameters assessed were L* (L* = 100 means white; L* = 0 means black), *a** (+*a** means redness; −*a** means greenness), *b** (+*b** means yellow; −*b** means blue). Measurements were obtained with an aperture diameter of 3 mm; color was measured in glass cuvettes, through a 10° observer type and D65 illuminant. CIE L**a***b** (Color Measurement Committee of the Society of Dyers and Colorists) was measured using a spectrophotometer (Konica Minolta CM-700d) [56,57]. The pigment contents are displayed on the screen as normalized difference vegetation index (NDVI) and normalized anthocyanin index (NAI) [58]. The dried plant material was powdered in a laboratory mill in triplicate. About 3 g of ground plants were poured into the glass cuvette, and 50 measurements were made in triplicate. The samples were mixed before each measurement.

### 3.9. 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 had been previously established. The 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 $p < 0.05$. To determine the relationship between the cultivars and macro- and microelements, the results obtained were subjected to agglomerative cluster analysis and classified into groups in a hierarchical order by means of the Ward's method. Multivariate analysis was performed by applying principal component analysis (PCA). The data were auto-scaled during pre-processing.

## 4. Conclusions

The obtained results enrich the knowledge of the composition and nutritional values of fresh goji fruit grown in northeast Europe and will help to verify the information given on the packaging. Differences in the composition of the macro- and microelements of the two goji cultivars No. 1 and New Big, grown in an orchard and under *in vitro* conditions were shown. These differences resulted mainly from the growing conditions of these shrubs and the composition of the soil. Goji berries cultivated *in vitro* were confirmed as an important source of healthy compounds, providing a significant contribution to the diet through both its fruit and leaves. The cultivars were rich in macro- and microelements and low in levels of toxigenic elements (i.e., Pb and Ni)

The color of the leaves and the NDVI indicated that the plants had optimal nutrient content, so it can be concluded that the assigned norms of the macro- and microelements can be a good indicator of their nutrition.

The results presented in this study provide information that goji berries grown in Poland are an interesting fruit in terms of their important health-promoting contents such as macro- and microelements, antioxidants, and anti-microbiological properties, sugars, organic acid, and phenolic acids.

Among the Gram-positive bacteria, *B. subtilis* proved to be the most sensitive to the extracts, and among the Gram-negative bacteria, it was *P. vulgaris*. The strongest inhibitory and bactericidal effect (i.e., the lowest MIC values) in relation to the majority of the examined bacteria was found in the extract from the 'New Big' leaves collected in the orchard. Therefore, it can be concluded that the New Big cultivar is a source of active substances that inhibit the growth and development of selected types of bacteria.

The knowledge obtained from this study will help determine the commercial potential of goji berries used for nutraceutical applications and of the incorporation in food preparation that improves human health. Taking into consideration the RDAs for minerals established by the EU, fresh goji fruit can be a source of Cu, Fe, Mn, Zn, P, and Se.

**Author Contributions:** Material, A.K., I.O., M.K.-M., conceptualization, A.K., I.O., M.K.-M., methodology, S.L., I.O., M.K.-M., J.O., formal analysis, I.O., A.K., S.L.; writing—original draft preparation, A.K., I.O., M.K.-M., S.L., writing—review and editing, I.O., M.K.-M., J.O., visualization, I.O. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the Polish Ministry of Science and Higher Education Grant no. NN 310205737 and West Pomeranian University of Technology Grant no. 518-07-014-3171-03/18.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## References

1. Potterat, O. Goji (*Lycium barbarum* and *Lycium chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. *Planta Med.* **2010**, *76*, 7–19. [CrossRef] [PubMed]
2. Nascimento, A.N.; Silvestre, D.M.; de Oliveira Leme, F.; Nomura, C.S.; Naozuka, J. Elemental analysis of goji berries using axially and radially viewed inductively coupled plasma-opical emissin spectometry. *Spectometry* **2015**, *30*, 36–41.
3. Kruczek, A.; Ochmian, I. The influence of shurbs cutting method on yielding and quality of the goji berries (*Lycium barbarum* L.). *Folia Pomeranae Univ. Technol. Stetin. Agric. Aliment. Piscaria Zootech.* **2016**, *330*, 131–138. [CrossRef]
4. Dănăilă-Guidea, A.M.; Dobrinoiu, R.-V.; Vişan, L.; Toma, R.C. Protocol for efficient *in vitro* multiplication of *Lycium barbarum* L. (Goji) by direct organogenesis. *Sci. Bull. Ser. F Biotechnol.* **2015**, *19*, 34–38.

5. Mocan, A.; Zengin, G.; Simirgiotis, M.; Schafberg, M.; Mollica, A.; Vodnar, D.C.; Rohn, S. Functional constituents of wild and cultivated Goji (*L. barbarum* L.) leaves: Phytochemical characterization, biological profile, and computational studies. *J. Enzym. Inhib. Med. Chem.* **2017**, *32*, 153–168. [CrossRef] [PubMed]
6. Kruczek, A.; Ochmian, I.; Krupa-Małkiewicz, M.; Lachowicz, S. Comparison of morphological, antidiabetic and antioxidant properties of goji fruits. *Acta Univ. Cibiniensis Ser. E Food Technol.* **2020**, *24*, 1–14. [CrossRef]
7. Chang, R.C.C.; So, K.F. Use of anti-aging herbal medicine, *Lycium barbarum*, against aging-associated diseases. What do we know so far? *Cell. Mol. Neurobiol.* **2008**, *28*, 643–652. [CrossRef]
8. Kulczyński, B.; Gramza-Michałowska, A. Goji berry (*Lycium barbarum*): Composition and health effects–a review. *Pol. J. Food Nutr. Sci.* **2016**, *66*, 67–76. [CrossRef]
9. Osman, N.I.; Awal, A.; Sidik, N.J.; Abdullah, S. *In vitro* regeneration and antioxidant properties of *Lycium barbarum* L. (goji). *J. Teknol.* **2013**, *62*, 35–38.
10. Dzhugalov, H.; Lichev, V.; Yordanov, A.; Kaymakanov, P.; Dimitrova, V.; Kutoranov, G. First results of testing Goji berry (*Lycium barbarum* L.) in Plovdiv region, Bulgaria. *Sci. Pap. Ser. B Hortic.* **2015**, *59*, 47–50.
11. Kruczek, A.; Krupa-Małkiewicz, M.; Ochmian, I. The influence of shrubs cutting method on yielding and quality of the goji berries (*Lycium barbarum* L.). *Folia Pomeranae Univ. Technol. Stetin. Agric. Aliment. Piscaria Zootech.* **2017**, *336*, 67–74. [CrossRef]
12. Bogacz, K. Goji—Fruit of health and longevity. *PFiOW* **2009**, *9*, 33–34.
13. Chen, P.Y.; Shih, T.H.; Chang, K.C.; Wang, J.S.; Yang, C.M.; Chang, Y.S. Potential of galled leaves of Goji (*Lycium chinense*) as functional food. *BMC Nutrition.* **2020**, *6*, 1–10. [CrossRef] [PubMed]
14. Sá, R.R.; da Cruz Caldas, J.; de Andrade Santana, D.; Lopes, M.V.; dos Santos, W.N.L.; Korn, M.G.A.; Júnior, A.D.F.S. Multielementar/centesimal composition and determination of bioactive phenolics in dried fruits and capsules containing Goji berries (*Lycium barbarum* L.). *Food Chem.* **2019**, *273*, 15–23.
15. Glonek, J.; Komosa, A. Fertigation of highbush blueberry (*Vaccinium corymbosum* L.). Part I. The effect on growth and yield. *Acta Sci. Pol. Hortorum Cultus* **2013**, *12*, 47–57.
16. Ochmian, I.; Malinowski, R.; Kubus, M.; Malinowska, K.; Sotek, Z.; Racek, M. The feasibility of growing highbush blueberry (*V. corymbosum* L.) on loamy calcic soil with the use of organic substrates. *Sci. Hort.* **2019**, *257*, 108690. [CrossRef]
17. Maret, W. The metals in the biological periodic system of the elements: Concepts and conjectures. *Int. J. Mol. Sci.* **2016**, *17*, 66. [CrossRef]
18. Kulaitienė, J.; Vaitkevičienė, N.; Jarienė, E.; Černiauskienė, J.; Jeznach, M.; Paulauskienė, A. Concentrations of minerals, soluble solids, vitamin C, carotenoids and toxigenic elements in organic goji berries (*Lycium barbarum* L.) cultivated in Lithuania. *Biol. Agric. Hortic.* **2020**, *36*, 130–140. [CrossRef]
19. Jeszka-Skowron, M.; Zgoła-Grześkowiak, A.; Stanisz, E.; Waśkiewicz, A. Potential health benefits and quality of dried fruits: Goji fruits, cranberries and raisins. *Food Chem.* **2017**, *221*, 228–236. [CrossRef]
20. Llorent-Martínez, E.J.; Córdova, M.L.F.-D.; Ortega-Barrales, P.; Ruiz-Medina, A. Characterization and comparison of the chemical composition of exotic superfoods. *Microchem. J.* **2013**, *110*, 444–451. [CrossRef]
21. European Commission. Regulation EU. No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Off. J. Eur. Union* **2011**, *304*, 18–63.
22. Niro, S.; Fratianni, A.; Panfili, G.; Falasca, L.; Cinquanta, L.; Alam, M.R. Nutritional evaluation of fresh and dried goji berries cultivated in Italy. *Ital. J. Food Sci.* **2017**, *29*. [CrossRef]
23. Dougnon, T.V.; Bankolé, H.S.; Johnson, R.C.; Klotoé, J.R.; Dougnon, G.; Gbaguidi, F.; Rhin, B.H. Phytochemical screening, nutritional and toxicological analyses of leaves and fruits of *Solanum macrocarpon* Linn (Solanaceae) in *Cotonou* (Benin). *Food Nutr. Sci.* **2012**, *3*, 1595–1603. [CrossRef]
24. Kiełbasa, P.; Juliszewski, T. Pomiar powierzchni liści wybranych roślin metodą video-komputerową. [Measurement of the leaf surface for the selected plants using video-computer method]. *Inżynieria Rol.* **2005**, *14*, 169–175.
25. Antal, T.; Sikolya, L.; Kerekes, B. Assessment of freezing pre-treatments for the freeze dried of apple slices. *Acta Univ. Cibiniensis Ser. E Food Technol.* **2013**, *17*, 3–14. [CrossRef]
26. Ochmian, I.; Oszmiański, J.; Lachowicz, S.; Krupa-Małkiewicz, M. Rootstock effect on physico-chemical properties and content of bioactive compounds of four cultivars Cornelian cherry fruits. *Sci. Hortic.* **2019**, *256*, 108588. [CrossRef]

27. Oszmiański, J.; Wojdylo, A. Aronia melanocarpa phenolics and their antioxidant activity. *Eur. Food Res. Technol.* **2005**, *221*, 809–813. [CrossRef]
28. Lachowicz, S.; Wiśniewski, R.; Ochmian, I.; Drzymała, K.; Pluta, S. Anti-microbiological, anti-hyperglycemic and anti-obesity potency of natural antioxidants in fruit fractions of saskatoon berry. *Antioxidants* **2019**, *8*, 397. [CrossRef]
29. Yusufe, M.; Mohammed, A.; Satheesh, N. Effect of duration and drying temperature on characteristics of dried tomato (*Lycopersicon esculentum* L.) Cochoro variety. *Acta Univ. Cibiniensis Ser. E Food Technol.* **2017**, *21*, 41–50. [CrossRef]
30. Ochmian, I.; Kozos, K.; Mijowska, K. Influence of storage conditions on changes in physical parameters and chemical composition of highbush blueberry (*Vaccinium corymbosum* L.) fruit during storage. *Bulg. J. Agric. Sci.* **2015**, *21*, 178–183.
31. Wojdyło, A.; Nowicka, P.; Bąbelewski, P. Phenolic and carotenoid profile of new goji cultivars and their anti-hyperglycemic, anti-aging and antioxidant properties. *J. Funct. Foods.* **2018**, *48*, 632–642. [CrossRef]
32. Sato, Y.; Itagaki, S.; Kurokawa, T.; Ogura, J.; Kobayashi, M.; Hirano, T.; Sugawara, M.; Iseki, K. *In vitro* and in vivo antioxidant properties of chlorogenic acid and caffeic acid. *Int. J. Pharm.* **2011**, *403*, 136–138. [CrossRef] [PubMed]
33. Wang, C.C.; Chang, S.C.; Inbaraj, B.S.; Chen, B.H. Isolation of carotenoids, flavonoids and polysaccharides from *Lycium barbarum* L. and evaluation of antioxidant activity. *Food Chem.* **2010**, *120*, 184–192. [CrossRef]
34. Pandey, K.B.; Rizvi, S.I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* **2009**, *5*, 270–278. [CrossRef] [PubMed]
35. Podsedek, A.; Majewska, I.; Redzynia, M.; Sosnowska, D.; Koziołkiewicz, M. *In vitro* inhibitory effect on digestive enzymes and antioxidant potential of commonly consumed fruits. *J. Agric. Food Chem.* **2014**, *62*, 4610–4617. [CrossRef]
36. Montesano, D.; Rocchetti, G.; Cossignani, L.; Lucini, L.; Simonetti, M.S.; Blasia, F. Italian Lycium barbarum L. berry: Chemical characterization and nutraceutical value. *Nat. Prod. Commun.* **2018**, *13*, 1934578X1801300913. [CrossRef]
37. Bratu, M.M.; Birghila, S.; Popescu, A.; Negreanu-Pirjol, B.S.; Negreanu-Pirjol, T. Correlation of antioxidant activity of dried berry infusions with the polyphenols and selected microelements contents. *Bull. Chem. Soc. Ethiop.* **2018**, *32*, 1–12. [CrossRef]
38. Kumar, D.; Arya, V.; Kaur, R.; Bhat, Z.A.; Gupta, V.K.; Kumar, V. A review of immunomodulators in the Indian traditional health care system. *J. Microbiol. Immunol. Infect.* **2012**, *45*, 165–184. [CrossRef]
39. Benzie, I.F.; Devaki, M. The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: Concepts, procedures, limitations and applications. In *Measurement of Antioxidant Activity & Capacity*; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2017; pp. 77–106.
40. Varadarajan, P.; Rathinaswamy, G.; Asirvatahm, D. Antimicrobial properties and phytochemical constituents of *Rheo discolor* Hance. *Ethnobot. Leafl.* **2008**, *12*, 841–845.
41. Dahech, I.; Farah, W.; Trigui, M.; Hssouna, A.B.; Belghith, H.; Belghith, K.S.; Abdallah, F.B. Antioxidant and antimicrobial activities of *Lycium shawii* fruits extract. *Int. J. Biol. Macromol.* **2013**, *60*, 328–333. [CrossRef]
42. Mijowska, K.; Ochmian, I.; Oszmiański, J. Rootstock effects on polyphenol content in grapes of 'Regent'cultivated under cool climate condition. *J. Appl. Bot. Food Qual.* **2017**, *90*, 159–164.
43. Sadowski, A.; Nurzyński, J.; Pacholak, E.; Smolarz, K. *Określanie Potrzeb nawożenia Roślin Sadowniczych. II. Zasady, Liczby Graniczne i Dawki Nawożenia. Instrukcja Upowszechnieniowa nr 3*; SGGW: Warszawa, Poland, 1990; pp. 1–25.
44. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant.* **1962**, *15*, 473–497. [CrossRef]
45. Polish Committee for Standardization. *PN-R-04016-21. Chemical and Agricultural Analysis of Soil. Determination of the Content of Available Zinc, Copper, Manganese, Iron*; Polish Committee for Standardization: Warszav, Poland, 1992.
46. IUNG (Institute of Soil Science and Plant Cultivation). *Methods of Laboratory Tests in Chemical Laboratories. Part II. The Study of Plant Material*; IUNG: Puławy, Poland, 1972; pp. 25–83.
47. Lityński, T.; Jurkowska, H.; Gorlach, E. *Chemical Analysis of Soil*; PWN: Warszawa, Poland, 1976; pp. 135–143.

48. Oszmiański, J.; Lachowicz, S.; Gławdel, E.; Cebulak, T.; Ochmian, I. Determination of photochemical composition and antioxidant capacity of 22 old apple cultivars grown in Poland. *Eur. Food Res. Technol.* **2018**, *244*, 647–662. [CrossRef]
49. Dias, M.I.; Barros, L.; Morales, P.; Sánchez-Mata, M.C.; Oliveira, M.B.P.P.; Ferreira, I.C.F. Nutritional parameters of infusions and decoctions obtained from *Fragaria vesca* L. roots and vegetative parts. *LWT Food Sci. Technol.* **2015**, *62*, 32–38. [CrossRef]
50. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef]
51. Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* **1992**, *40*, 945–948. [CrossRef]
52. Lachowicz, S.; Oszmiański, J.; Rapak, A.; Ochmian, I. Profile and content of phenolic compounds in leaves, flowers, roots, and stalks of *Sanguisorba officinalis* L. determined with the LC-DAD-ESI-QTOF-MS/MS analysis and their *in vitro* antioxidant, antidiabetic, antiproliferative potency. *Pharmaceuticals* **2020**, *13*, 191. [CrossRef]
53. Nickavar, B.; Yousefian, N. Evaluation of $\alpha$-amylase inhibitory activities of selected antidiabetic medicinal plants. *J. Verbr. Lebensm.* **2011**, *6*, 191–195. [CrossRef]
54. Bauer, A.W.; Kirby, W.M.; Sherris, J.C.; Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **1966**, *45*, 493–496. [CrossRef]
55. Inouye, S.; Yamaguchi, H.; Takizawa, T. Screening of the antibacterial effects of a variety of essentials oils on respiratory tract pathogens, using the modified dilution assay method. *J. Inf. Chemother.* **2001**, *7*, 251–254. [CrossRef]
56. Ochmian, I.; Kozos, K.; Chelpinski, P.; Szczepanek, M. Comparison of berry quality in highbush blueberry cultivars grown according to conventional and organic methods. *Turk. J. Agric. Forest.* **2015**, *39*, 174–181. [CrossRef]
57. Krupa-Małkiewicz, M.; Kosatka, A.; Smolik, B.; Sędzik, M. Induced mutations through EMS treatment and *in vitro* screening for salt tolerance plant of *Petunia* × *atkinsiana* D. Don. *Not. Bot. Horti Agrobot.* **2017**, *45*, 190–196. [CrossRef]
58. Piwowarczyk, R.; Ochmian, I.; Lachowicz, S.; Kapusta, I.; Sotek, Z.; Błaszak, M. Phytochemical parasite-host relations and interactions: A *Cistanche armena* case study. *Sci. Total Environ.* **2020**, *716*, 137071. [CrossRef] [PubMed]


**Sample Availability:** Samples of the compounds are not available from the authors.

DOI: 10.21005/AAPZ2017.43.3.08

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2017, 336(43)3, 67–74

*Arleta KRUCZEK, Marcelina KRUPA-MAŁKIEWICZ[1], Ireneusz OCHMIAN*

# THE EFFECTIVENESS OF DISINFECTION METHODS ON GERMINATION OF GOJI SEEDS (*Lycium barbarum* L.) IN *IN VITRO* CULTURE

# WPŁYW ZASTOSOWANYCH METOD DEZYNFEKCJI NA ZDOLNOŚĆ KIEŁKOWANIA NASION JAGODY GOJI (*Lycium barbarum* L.) W KULTURACH *IN VITRO*

Department of Horticulture, West Pomeranian University of Technology, Szczecin, Poland
[1]Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin

**Streszczenie.** Do skutecznego usuwania patogenów i mikroorganizmów z powierzchni materiału roślinnego w kulturach *in vitro* stosuje się odpowiednio dobrane metody dezynfekcji. Alternatywnym rozwiązaniem do powszechnie stosowanych środków chemicznych jest ozonowanie. Porównano efektywność dezynfekcji nasion dwóch odmian goi ‘A’ i ‘New Big’ powszechnie stosowanymi środkami chemicznymi: NaOCl (7%, 10% i 15%) oraz $HgCl_2$ (0,2%) z metodą ozonowania na sucho i mokro w czasie 5 i 15 min. Najwyższy procent skiełkowanych nasion odmian ‘A’ i ‘New Big’ (odpowiednio 62% i 78%), przy najmniejszej liczbie zakażeń (odpowiednio 8% i 14%), uzyskano po zastosowaniu 7% NaOCl. Mniej skuteczny był roztwór 0,2% $HgCl_2$, po zastosowaniu którego procent skiełkowanych nasion odmian ‘A’ i ‘New Big’ był niższy (odpowiednio 37% i 30%). Natomiast po zastosowaniu ozonowania eksplantatów ‘A’ i ‘New Big’ na mokro przez 15 min uzyskano najwyższy procent skiełkowanych nasion (w przypadku odmiany ‘A’ – 47%, a w przypadku odmiany ‘New Big’ – 53%); sterylność kultur u odmiany ‘A’ wynosiła 100%. Ponadto ozonowanie nasion wykazało podobną efektywność dezynfekcji jak zastosowanie 15-procentowego roztworu NaOCl. W związku z tym stosowanie ozonowania na sucho może stanowić alternatywną metodę dezynfekcji.

**Key words:** goji berry, $HgCl_2$, *Lycium barabarum* L.*,* NaOCl, ozone, tissue culture.
**Słowa kluczowe:** $HgCl_2$, jagoda goji, kultury tkankowe, *Lycium barbarum* L., NaOCl, ozonowanie.

## INTRODUCTION

*Lycium barbarum* L. is commonly known as goji berry. It is a spiked, perennial shrub belonging to the family of *Solanaceae*. It comes from the north-western parts of China (Osman et al. 2013). Fruits of goji berry are round or oval, red or orange with a large number of seeds. *L. barbarum* L. berries contain multiple mineral and organic compounds (vitamins $B_1$, $B_6$, A, C, E), with the potential of repairing epidermal damage and showing excellent effects on the cardiovascular and cholesterol levels (Dănăilă-Guidea et al. 2015).

Corresponding author – Adres do korespondencji: Marcelina Krupa-Małkiewicz, Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Juliusza Słowackiego 17, 71-434 Szczecin, Poland, e-mail: mkrupa@zut.edu.pl

The tissue culture system allows the propagation of selected genotypes with high multiplication rate in an aseptic, temperature-controlled environment (Osman et al. 2013; Tarinejad 2013; Teixeira da Silva et al. 2016). Numerous scientific articles have reported using tissue culture techniques, with many researches successfully applying these to the goji shrub (Kairong et al. 1999; Hu et al. 2001, 2008; Osman et al. 2013; Dănăilă-Guidea et al. 2015). In these studies were used different sources of goji berry explants: shoot tips, nodal segments of leaves, stem axillary buds and roots. In most cases, shoot proliferation was achieved by axillary bud obtained from nodal explants. However, most of these works have no information regarding to the effectiveness of disinfection of the initial material. According to Amiri et al. (2013), Zeng (2014) and Teixeira da Silva et al. (2016) the establishment of an effective tissue culture from plant material derived from greenhouse or field up to the disinfection process. Well-chosen disinfectant allows to maintain a balance between highly effective in disinfecting explants and its high ability for survival and regeneration. In *in vitro* culture usually chemical disinfectants such as sodium hypochlorite, ethanol, calcium or mercuric chloride, hydrogen peroxide and silver nanoparticles or antibiotics have been used (Amiri et al. 2013; Tarinejad 2013). However, the chemicals used in too high concentrations may be toxic to the plant and to the environment. There is required a specialized utilization of these compounds. An alternative method of disinfection might be the ozonation. Ozone has relatively high solubility in water with a high redox potential and is recently declared Generally Recognized As Safe (GRAS) by an expert panel for use in food processing (Graham 1997). However, to date, it has been used in disinfection of potable water, processing, as well as in stored food (seeds, vegetables, fruits, meat). The aim of the study was to compare the effectiveness of seeds disinfection of two goji berries cultivars, ‘A’ and ‘New Big’ using different solution with disinfection methods used.

## MATERIAL AND METHODS

### Plant material

The experiment was conducted at tissue culture laboratory of the Department of Genetics, Plant Breeding and Biotechnology of West Pomeranian University of Technology in Szczecin.

Seeds of two cultivars ‘A’ and ‘New Big’ of goji berry (*Lycium barbarum* L.) were used as plant material. Goji fruits were obtained after harvest (November) from the experimental orchard of the Pomology Department of West Pomeranian University of Technology in Szczecin.

### Disinfestations methods

Seeds were submerged in 70% ethanol for 30 s and after twice washing with sterile deionized water, they were submerged in different disinfecting treatments:

- sodium hypochlorite (NaOCl) in 7%, 10% and 15% for 15 min;
- 0.2% mercury chloride ($HgCl_2$) for 15 min;
- ozone gas in two times of exposure (5 and 15 min);
- ozone in water in two times of exposure (5 and 15 minutes).

Ozon was produced by a discharge generator (ZYH 135) for 3.5 g $\cdot$ $h^{-1}$ capacity with the efficiency of the pump guaranteeing a flow of 15 liters of air within 1 minute.

After the above treatments, seeds were rinse three times with sterile and deionized water for 1, 2 and 5 minutes, respectively under a sterile laminar flow hood.

### Medium and culture condition

Aseptic, sterilized seeds were placed individually in glass tube with capacity of 35 × 110 mm containing 15 mL of MS medium (Murashige and Skoog 1962) without plant growth regulators. Each combination included one hundred seeds (four replicates of 25 seeds).

Culture medium were supplemented with 8.0 g · $dm^{-3}$ agar (Biocorp, Poland), 30 g · $dm^{-3}$ sucrose and 100 mg · $dm^{-3}$ myo-inositol, pH was adjusted to 5.7 by adding 0.1M of NaOH or HCl and autoclaved at 121ºC (0.1 MPa) for 19 minutes. The cultures were maintained in a growth room at a temperature of 24 ± 1ºC under 16h photoperiod under a fluorescent lamp (photosynthetic photon flux density 40 μmol · $m^{-2}$ · $s^{-1}$). Contamination and germination rate were analyzed after 10 days for each combination.

### Statistical analysis

The data were subjected to one-factor variance analysis (ANOVA). Mean comparisons were performed using Tukey's least significant difference (LSD) test; significance was set at $p < 0.05$. The percentage data of disinfection methods were transformed before analysis using the Bliss function $y = \arcsin\sqrt{x}$. To determine the relation between the disinfection method the results were subjected to an agglomerative cluster analysis and classified into groups in a hierarchical order by means of the Ward's method. The statistical analyses were performed using the Statistica 12.5 software (StatSoft, Polska).

## RESULTS AND DISCUSSION

Adequately disinfection of components is one of most important ways of controlling contamination in *in vitro* culture (Amiri et al. 2013). Different explants require different types of compounds, concentrations and exposure periods for the disinfection process to be optimized (Teixeira da Silva et al. 2016). NaOCl is considered a very effective microbe killer and has been frequently used for surface sterilization of plants for *in vitro* culture (Bakhsh et al. 2016). While mercuric chloride ($HgCl_2$) is extremely toxic to plants and humans and must be carefully disposed (Talei et al. 2011). Ozone is reported to have 1.5 times the oxidizing potential of chlorine and 3000 times the potential of hypochlorus acid (HClO) – Suslow (1998).

In the current study the effectiveness of seeds sterilization depend on the disinfection solution used and its concentration. It was observed that goji seeds submerged in 15% NaOCl solution indicated the lowest number of contamination as well as germination ability (Table 1). However, when 7% solution of NaOCl was used for disinfection, the percentage of germinated goji 'A' and 'New Big' seeds was the highest (62% and 78%, respectively) (Table 2). As compared to NaOCl disinfection of seeds with 0.2% $HgCl_2$ was less effective mainly to 'New Big'. The percent of infected goji seeds of 'A' and 'New Big' was higher (17% and 10%, respectively) (Table 1). Furthermore, $HgCl_2$ solution was more toxic and inhibited the germination of goji seeds, which was 37% for cultivar 'A' and 30% for 'New Big' cultivars (Table 2). It was observed that $O_3$ in water and $O_3$ gas affected seeds ability to germination (Table 3). The highest number (87%) of uncontaminated explants that did not developed shoots was noticed after disinfection of goji 'New Big' explants using $O_3$ in water for 5 minutes and $O_3$ gas for 15 minutes. Comparing ozonation method for disinfection of goji seeds, it was observed that the best results were obtained when $O_3$ in water for 15 minutes was used when the percentage of germination was the highest ('A' – 47% and 'New Big' – 53%) (Table 2), and the efficiency of sterilization of goji seeds in 'A' cultivar was 100% (Table1).

Table 1. The percentage of contaminated goji 'A' and 'New Big' explants according to disinfection method used

Tabela 1. Procent zakażonych eksplantatów goji odmian 'A' i 'New Big' w zależności od zastosowanego środka dezynfekującego

| Disinfection method<br>Metody dezynfekcji | | Cultivar – Odmiana<br>'A' | 'New Big' | Mean<br>Średnia |
|---|---|---|---|---|
| Ozone gas<br>Ozonowanie<br>na sucho | 5 min | 7 b | 40 d | 23.5 c |
| | 15 min | 7 b | 0 a | 3.5 a |
| Ozon in water<br>Ozonowanie<br>w wodzie | 5 min | 20 c | 0 a | 10 ab |
| | 15 min | 0 a | 7 b | 3.5 a |
| NaOCl | 7% | 8 b | 14 c | 11 b |
| | 10% | 7 b | 3 a | 5 a |
| | 15% | 4 ab | 2 a | 3 a |
| 0.2% $HgCl_2$ | | 17 c | 10 b | 13.5 b |

Means in the same column followed by the same letter are not significantly different at $\alpha < 0.05$ according to Tukey test – Średnie w kolumnach oznaczone tymi samymi literami alfabetu nie różnią się według testu Tukeya na poziomie istotności $\alpha < 0,05$.

Table 2. The percentage of uncontaminated goji 'A' and 'New Big' explants that germinated according to disinfection method used

Tabela 2. Procent niezakażonych eksplantatów goji odmian 'A' i 'New Big', które skiełkowały, w zależności od zastosowanego środka dezynfekującego

| Disinfection method<br>Metody dezynfekcji | | Cultivar – Odmiana<br>'A' | 'New Big' | Mean<br>Średnia |
|---|---|---|---|---|
| Ozone gas<br>Ozonowanie<br>na sucho | 5 min | 33 b | 47 c | 40 cd |
| | 15 min | 40 bc | 7 a | 23.5 ab |
| Ozon in water<br>Ozonowanie<br>w wodzie | 5 min | 7 a | 13 a | 10 a |
| | 15 min | 47 c | 53 c | 50 d |
| NaOCl | 7% | 62 d | 78 d | 70 e |
| | 10% | 47 c | 47 c | 47 d |
| | 15% | 38 b | 36 b | 37 c |
| 0.2% $HgCl_2$ | | 37 b | 30 b | 33.5 bc |

Explanations see Table 1 – Objaśnienia zob. tab. 1.

Table 3. The percentage of uncontaminated goji 'A' and 'New Big' explants that did not germinated according to disinfection method used

Tabela 3. Procent niezakażonych eksplantatów goji odmian 'A' i 'New Big', które nie skiełkowały, w zależności od zastosowanego środka dezynfekującego

| Disinfection method<br>Metody dezynfekcji | | Cultivar – Odmiana<br>'A' | 'New Big' | Mean<br>Średnia |
|---|---|---|---|---|
| Ozone gas<br>Ozonowanie<br>na sucho | 5 min | 53 b | 0 a | 26.5 a |
| | 15 min | 53 b | 87 e | 70 e |
| Ozon in water<br>Ozonowanie<br>w wodzie | 5 min | 67 d | 87 e | 77 e |
| | 15 min | 53 bc | 33 cd | 43 cd |
| NaOCl | 7% | 30 a | 44 d | 37 bc |
| | 10% | 47 b | 17 b | 32 b |
| | 15% | 60 cd | 44 d | 52 d |
| 0.2% $HgCl_2$ | | 47 b | 30 c | 38.5 bc |

Explanations see Table 1 – Objaśnienia zob. tab. 1.

According to many authors (Teixeira et al. 2006; Tiwari et al. 2012; Amiri et al. 2013; Teixeira da Silva et al. 2016) disinfection solution of sodium hypochlorite and mercury chloride played an important role in the contamination control. Hu et al. (2001) applied 0.1% $HgCl_2$ for 6 minutes followed by three washes with sterile water for disinfection of *L. barbarum*. Then seeds were imbibed in sterile water for 6 h at room temperature and next were sterilized a second time for 8 minutes in 0.1% $HgCl_2$. While, seeds of *L. barbarum* cultivar 'Ningji No.1' were surface-sterilize in 70% ethanol (30–40 s) and after that they were submerged in 0.1% $HgCl_2$ for 8–10 minutes, followed by 5 rinses with sterile distilled water (Hu et al. 2008). Another disinfection method of goji seeds applied Dănăilă-Guidea et al. (2015) using commercial bleach (ACE) containing 4.85% sodium hypochlorite for 10 minutes and a dilute bleach product concentration trading 4.50% sodium hypochlorite for 20 minutes, followed by three washes with sterile distilled water. Similarly, Osman et al. (2013) for disinfection of goji seeds, obtained from dried fruits, applied sodium hypochlorite (no report of concentration) with two drops of Tween 20, followed by subsequent three time rinsing procedures with sterile distilled water. However, these work do not describe the effectiveness of disinfection method on the germination rate of goji seeds.

Alternative techniques have been appreciated because all of these disinfectants may be toxic for plant tissue and environment. One of an alternative method might be disinfection with ozone. However, there are a low number of reports about the effect of ozone disinfection in *in vitro* culture. Much more likely is described ozone to be used in food processing and for storage of vegetables and fruits. Tiwari et al. (2010) demonstrated that ozone which is natural agent, may offer unique advantage for grain processing along with addressing growing concerns over the use of harmful pesticides. Nowakowicz-Dębek et al. (2013) treated wheat grains with ozone produced by an ozone generator of 100 mg $\cdot$ $h^{-1}$ capacity for 0, 0.5, 1, 3, 6 and 9 h. They observed that the exposure to ozone over longer period of time caused higher mold fungal reducibility. Krupa-Małkiewicz et al. (in press) compare the effectiveness of 7% and 10% NaOCl with ozone gas and ozone in water for disinfection of different seeds in *in vitro* culture. These have fund that ozone treatment of seeds gives good results, as in the case of using 7% NaOCl.

In the present study, goji seed contaminations under the different sterilization procedures varied from 0 to 40%. Analysis of the percentage of germinated seeds and their infections for both goji cultivars, carried out according to Ward's method, divided into four groups according to the similarity of their actions: A – 7% and 10% NaOCl, B – 0.2% $HgCl_2$, C – 15% NaOCl, 5 minutes $O_3$ gas and 15 minutes $O_3$ in water, D – 5 minutes $O_3$ in water and 15 minutes $O_3$ gas (Fig. 1). The results obtained in this study, demonstrated that the seeds cultured after $O_3$ treatment are characterized by a similar effectiveness of disinfection and germination as when applying 15% NaOCl. Therefore, ozone gas makes an alternative for commonly used disinfectants. Ozone rapidly attacks bacterial cell walls and is more effective against the thick-walled spores of plant pathogens than chlorine, at practical and safe concentrations (Suslow 1998).

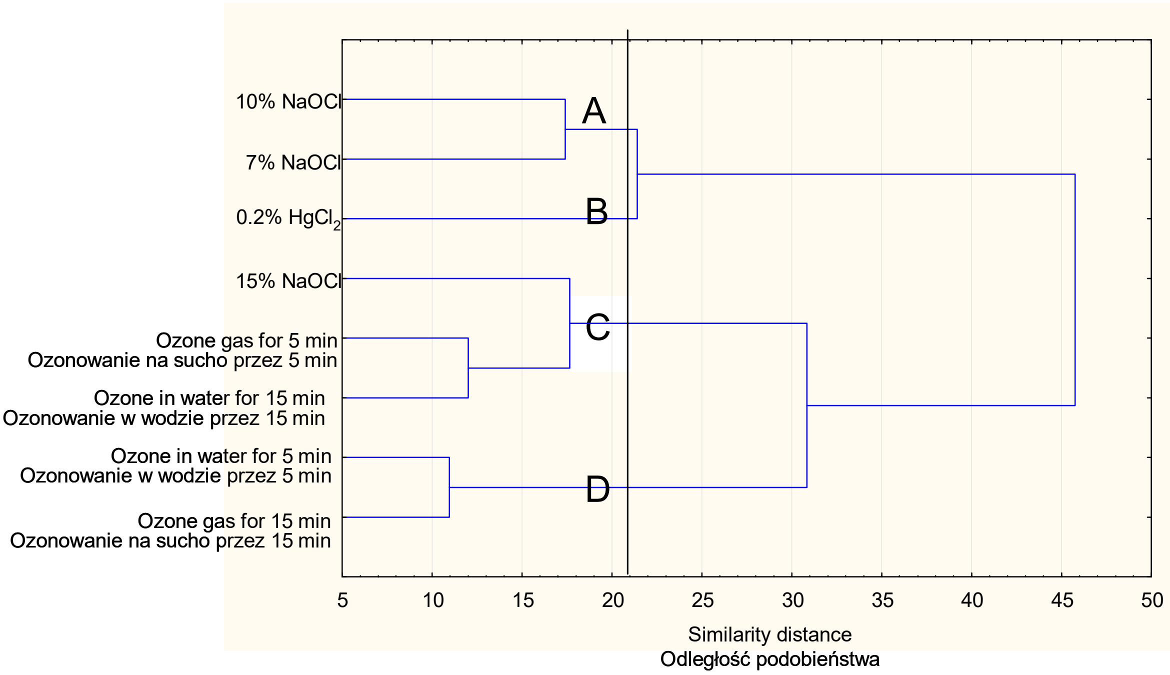



Fig. 1. Similarity distance between disinfection method use
Ryc. 1. Odległość podobieństwa pomiędzy zastosowanymi metodami dezynfekcji

## CONCLUSIONS

The effectiveness of disinfection of explants in *in vitro* culture depended on many factors. One of them is properly chosen disinfection method. Ozone gas ($O_3$) makes an alternative for commonly used disinfectants. The efficacy of ozone was similar to sodium hypochlorite which it is less toxic to the plants and the environment. However, it requires further researches to evaluate the optimum conditions for disinfections of explants in *in vitro* culture. Therefore, use of ozone gas as a natural disinfectant is considered a very effective microbe killer and is used frequently for surface disinfection.

## REFERENCES

**Amiri S., Ashtari S., Babaiy A.H., Nazari S.A., Khodadadi E., Khodadadi E., Sabzi M.** 2013. Control of contamination during micropropagation process of Rootstocks Mariana (*Prunus mariana*). Ann. Biol. Res. 4(3), 149–151.

**Bakhsh A., Anayol E., Sancak C., Özcan S.** 2016. An efficient and cost effective sterilizing method with least microbial contamination and maximum germination ratio for *in vitro* cotton (*Gossypium hirsutum* L.) culture. J . Anim. Plant Sci. 26(3), 868–873.

**Dănăilă-Guidea A.M., Dobrinoiu R-V., Vişan L., Toma R.C.** 2015. Protocol for efficient *in vitro* multiplication of *Lycium barbarum* L. (Goji) by direct organogenesis. Sci. Bull., Ser. F Biotechnologies 19, 34–38.

**Graham D.M.** 1997. Use of ozone for food-processing. Food Techn. 51, 72–75.

**Hu Z., Guo G.Q., Zhao D.L., Li L.H., Zheng G.C.** 2001. Shoot regeneration from leaf explant of *Lycium barbarum* and *Agrobacterium*-mediated genetic transformation. Russ. J. Plant Pysiol. 48, 453–458.

**Hu Z., Hu Y., Gao H.H., Guan X.Q., Zhuang D.H.** 2008. Callus production, somatic emryogenesis and plant regeneration of *Lycium barbarum* root explants. Biol. Plant. 52(1), 93–96.

**Kairong C., Gengsheng X., Xinmin L., Gengmei X., Yafu W.** 1999. Effect of hydrogen peroxide on somatic embryogenesis of *Lycium barbarum* L. Plant Sci. 146, 9–16.

**Krupa-Małkiewicz M., Kruczek A., Ochmian I.** 2017. Comparison of efficacy of NaOCl and ozonation in the process of disinfection of seed in *in vitro* cultures. [in press]

**Murashige T.M., Skoog F.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15, 473–497.

**Nowakowicz-Dębek B., Bojarczyk M., Krukowski H., Misztal-Majewska B., Wlazło Ł., Trawińska B.** 2013. Ozone disinfection of feed wheat. Ann. UMCS Lub. 31(1), 43–48.

**Osman N.I., Awal A., Sidik N.J., Abdullah S.** 2013. Callus induction and somatic embryogenesis from leaf and nodal explants of *Lycium barbarum* L. (Goji). Biotechnology 12(1), 36–45.

**Suslow T.** 1998. Basics of ozone applications for postharvest treatment of fruits and vegetables. Perish. Hand. Quart. 94, 9–11.

**Talei D**., **Saad M.S., Yusop M.K., Mihdzar A.K., Valdiani A.** 2011. Effect of different surface sterilizers on seed germination and contamination of King of Bitters (*Andrographis paniculata* Nees.) Am. Euras. J. Agric. Environ. Sci. 10, 639–643.

**Tarinejad A.** 2013. Effects of disinfectants and antibiotics on contamination during propagation of walnut (*Juglans regia* L.). Res. Crops. 14(1), 219–225.

**Teixeira S.L., Ribeiro J.M., Teixeira M.T.** 2006. Influence of NaClO on nutrient medium sterilization and on pineapple (*Ananas comosus* cv Smooth cayenne) behavior. Plant Cell. Tiss. Organ Cult. 86, 375–378.

**Teixeira da Silva J.A., Winarto B., Dobránszki J., Cardoso J.C., Zeng S.** 2016. Tissue disinfection for preparation of *Dendrobium in vitro* culture. Folia Hort. 28(1), 57–75.

**Tiwari B.K., Brennan C.S., Curran T., Gallagher E., Cullen P.J., O'Donnell C.P.** 2010. Application of ozone in grain processing. J. Cereal Sci. 51, 248–255.

**Tiwari A.K., Tripathi S., Lal M., Mishra S.** 2012. Screening of some chemical disinfectants for media sterilization during *in vitro* micropropagation of sugarcane. Sugar Tech. 14(4), 364–369.

**Zeng Y.** 2014. Optimization of the method to obtain effective sterile explants in tissue culture of *Ardisia mamillata* Hence. Agricult. Sci. Tech. 15(6), 917–936.

**Abstract.** Using disinfectant components is one of main ways to control of fungal and bacterial contaminations in the *in vit*ro culture. Alternative to commonly used chemicals is ozonation. The present study was focused on the efficiency of seeds disinfection of two goji cultivars 'A' and 'New Big' using eight different treatments, 7%, 10% and 15% NaOCl and 0.2% $HgCl_2$ for 15 minutes each, $O_3$ gas and $O_3$ in water for 5 and 15 minutes. The highest percentage of germinated seeds of 'A' and 'New Big' (respectively 62% and 78%) with the lowest number of infections (respectively 8% and 14%) was obtained after using 7% NaOCl solution. Less effective was 0.2% $HgCl_2$ solution, where the percentage of germinated seeds of 'A' and 'New Big' was lower (respectively 37% and 30%). However, after $O_3$ in water treatment of goji 'A' and 'New Big' for 15 minutes the percentage of germinated seeds was the highest (respectively 47%, and 53%) and sterility of the cultures of goji 'A' was 100%. In addition to, the ozonation of the seeds showed similar disinfection efficiency, as with 15% NaOCl solution. Therefore, ozone gas makes an alternative for commonly used disinfectants.

Kruczek A *et al.* (2021)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 49, Issue 2, Article number 12271
DOI:10.15835/nbha49212271
Research Article

# Micropropagation, rooting, and acclimatization of two cultivars of goji (*Lycium chinense*)

Arleta KRUCZEK[1], Marcelina KRUPA-MAŁKIEWICZ[2]*, Ireneusz OCHMIAN[1]

[1] *West Pomeranian University of Technology Szczecin, Department of Horticulture, Słowackiego 17 Street, 71-434 Szczecin, Poland; ka21499@zut.edu.pl; iochmian@zut.edu.pl*
[2] *West Pomeranian University of Technology Szczecin, Department of Plant Genetics, Breeding and Biotechnology, Słowackiego 17 Street, Szczecin, Poland; mkrupa@zut.edu.pl (*corresponding author)*

## Abstract

In recent years, *Lycium chinense* (goji) has become increasing popular because of its public acceptance as a "superfood". Hence, the present study aimed to develop a rapid production technology by using *in vitro* culture to produce plants with high health value, throughout year and in desired quantities. A micropropagation protocol for growing *L. chinense* 'No 1' and 'New Big' cultivars was developed. The explants were grown on MS medium supplemented with different concentrations of *meta*-Topolin (0.4-0.8 mg $L^{-1}$), and WPM and RA without plant hormones. Among the tested combinations, the maximum regeneration rate (95-97%) with the mean shoot length of 3.53-4.12 cm and mean shoot number of 1.42–1.58 ('No 1' and 'New Big', respectively) was recorded for plants grown on MS with 0.6 mg $L^{-1}$ *m*T and WPM. For *in vitro* rooting, healthy roots (4.71-4.91 cm) were obtained on MS with the addition of 20 ppm chitosan. A maximum of 70–80% plantlets ('No 1' and 'New Big', respectively) regenerated on the medium with chitosan were successfully acclimatized and established in the mixture of 90% peat and 10% perlite under field conditions.

***Keywords:*** acclimatization; chitosan; *in vitro* and *ex vitro* rooting; *meta*-Topolin; superfood wolfberry

## Introduction

In recent years, there has been a growing tendency to use products of plant origin in medicinal, cosmetic, and therapeutic products. This is related to the increasing public awareness of long-term consequences of the use of chemical compounds on health and environment. Hence, more attention is being focused on the use of certain fruits and plant compounds that have been traditionally used in folk medicine for many years. One of such plants is goji.

*Lycium chinense* (*Solanaceae*), commonly known as goji berries or wolfberries, is considered one of the healthiest foods in the world because of its highly beneficial nutritive and antioxidant properties (Kruczek *et al.*, 2020a). Goji fruits contain various nutrient, such as polysaccharides, organic acids, phenolic compounds, and antioxidants with high biological activity (Wojdyło *et al.*, 2018; Sá *et al.*, 2019; Kruczek *et al.*, 2020a). Hence, it is frequently called as "red diamonds". Goji is the most powerful antioxidant of all the existing foods

*Received: 15 Feb 2021. Received in revised form: 10 Jun 2021. Accepted: 10 Jun 2021. Published online: 23 Jun 2021.*
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.

in the world and contains more carotene than any other food known to date (Tabăra, 2017). These berries have been used in herbal medicine and as a health food for thousands of years. Goji is grown in areas that have not been polluted by civilization or pesticides for centuries, particularly in China, Southeast Asia, Europe, and North America (Kulczyński and Gramza-Michałkowska, 2016; Sá *et al.*, 2019; Kruczek *et al.*, 2020a).

Because of its high nutraceutical and pharmaceutical value (Dănăilă-Guidea *et al.*, 2015; Kruczek and Ochmian, 2016; Kruczek *et al.*, 2020a), the production of goji has been rapidly increasing. Wolfberry plants are traditionally propagated trough their seeds and usually encounter problems related to sexual propagation, especially low germination, lack of clonal expansion, and irregular agronomical performance (Silvestri *et al.*, 2018). Clonal micropropagation by *in vitro* culture is the best alternative to overcome these barriers and has great potential for rapid multiplication and production of high-quality plant material. Hence, it is crucial to develop and optimize the technology of rapid production by *in vitro* culture to produce plants with high economic and medicinal value, such as goji berries, throughout the year and in desired quantities. Regeneration capacity depends on genotype, composition of growth media, plant growth regulators (PGRs), and other organic substances (Karakas, 2020). Several studies have tested various culture media for goji (Fira *et al.*, 2016; Tabăra, 2017; Karakas, 2020; Kruczek *et al.*, 2020b). Although *in vitro* culture methods and conditions are similar for different goji genotypes, their requirements of growth regulators in culture media are different. Hence, it is important to develop a highly efficient plant regeneration system for each genotype.

This study aimed to develop a reliable protocol for *in vitro* shoot culture of goji by using different culture media (MS - Murashige and Skoog, 1968; WPM - Woody Plant Media Llyod and McCown, 1981; RA - Anderson Rhododendron Medium; Anderson, 1984) with the addition of *meta*-Topolin ((6-(3-hydroxybenzyloamino)-purine) for *in vitro* shoot multiplication of two cultivars of goji, namely 'No 1' and 'New Big'. This study also evaluated *in vitro* and *ex vitro* rooting. Optimal medium, auxin requirement, and chitosan treatment were identified for rooting of the two goji cultivars.

## Materials and Methods

### *Plant material*

Two cultivars of goji, 'No 1' and 'New Big', which were taken from the orchard of the Department of Horticulture West Pomeranian University of Technology Szczecin, Poland, and used as biological material. The orchard is located in subzone 7A in the North-Western part of Poland in the Szczecin Lowland at a distance of approximately 65 km from the Baltic Sea (53° 400′ N, 14° 880′ E). The research was conducted at a production plantation specializing in the cultivation of highbush blueberry. Goji cultivar 'New Big' is distinguished by large fruit with a length of 2 cm and width of 1 cm. In 2013, in Poland was selected in the first variation of the sweet fruit without seeds ('No 1').

### *Multiplication and culture conditions*

Shoots of about 2 cm of two *Lycium chinense*: 'No 1' and 'New Big' were taken in May 2019, as primary explants from 8-year shrubs cultivated in the orchard. An axillaries bud of goji was taken from sterile stabilized *in vitro* culture. The shoot explants were transferred to MS medium with the addition of *meta*-Topolin in a concentration of 0.0, 0.4, 0.6, and 0.8 mg $L^{-1}$; WPM and RA medium without addition of *meta*-Topoiln (*m*T). Each combination included 48 shoots (6 shoots per flask in eight replication). 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 the media was adjusted to 5.7. The media were heated and 30 ml were poured into a 450 ml flask and next they were autoclaved at 121 °C (0.1 MPa) during the time required according to the volume of medium in the vessel. All cultures were incubated in a growth room at a temperature of 24 ± 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. After the end of the experimental period (five weeks), explants were removed and washed with deionized distilled water, and the lengths of the shoots and roots, the number of shoots per plant were measured, and shoot regeneration rate (%) was estimated. The plants were weighed for calculated of plant fresh mass.

*In vitro and ex vitro rooting*

Shoots of 'No 1' and 'New Big', multiplied for four subcultures on MS containing 0.6 mg $L^{-1}$ *m*T or WPM, were transferred to a rooting inducing medium MS, MS with the addition of chitosan (CH) at molecular weight 10 kDa at a concentration of 20 ppm (Bartkowiak, 2001), MS with auxins NAA (*α*-Naphthalene acetic acid) and IAA (3-Indoleacetic acid) at concentration 0.5 and 1.0 mg $L^{-1}$. The culture condition was the same as at the multiplication stage. The length of the shoots and roots, and the number of roots per plant, as well as the mass of the plants, were calculated 35 days after the transferring to the rooting media.

Rooting shoots were transferred under the plastic tunnel to a mixture of 90% peat and 10% perlite with 90% of humidity for 2 weeks (pF 1.7-2.1). Then, plants were transferred to the greenhouse. Survival rate (%) was evaluated 3 months after the beginning of the acclimatization.

*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 $p<0.05$. To determine the relationship between the *in vitro* propagation and rooting for morphological traits, the results obtained were subjected to agglomerative cluster analysis and classified in a hierarchical order using Ward's method.

## Results and Discussion

There are no reports describing the application of *meta*-Topolin in *in vitro* shoot multiplication of goji plants. According to Bairu *et al.* (2007), naturally occurring cytokinins such as *m*T play an important role in retarding plant aging, increasing photosynthetic pigments, modulating antioxidant enzyme activity, and thereby improving root and shoot development. In our study, among the combinations of growth medium tested to induce shoot regeneration, WPM medium and MS with *m*T, yielded the best shoot regeneration rate from 92% to 97% in both goji 'No 1' and 'New Big' (Table 1). No significant differences were observed between the regeneration rate obtained for goji explants propagated on MS and RA medium, which was 68% to 72% for the two tested cultivars.

In both cases, the regeneration rate was higher than that obtained by Fira *et al.* (2016) for *Lycium barbarum* 'Ningxia N1', and by Tabăra (2017) for *L. barbarum*. Our results showed that WPM was the best medium for stimulating shoot length and the development of adventitious buds of goji. Compared to other culture media used, goji grown on WPM showed higher length of shoots (3.99 and 4.12 cm, for 'No 1' and 'New Big', respectively) (Table 1). The addition of *m*T to MS medium increased the average shoot length as compared to that obtained MS and RA medium. When MS was supplemented with 0.6 mg $L^{-1}$ *m*T, the maximum shoot length for 'No 1' and 'New Big' was 3.61 and 3.53 cm, respectively. Moreover, shoot culture of goji 'No 1' on MS with the addition of 0.6 mg $L^{-1}$ *m*T resulted in higher number of shoot formation (1.50 and 1.42 shoot/plant, 'No 1' and 'New Big', respectively). It was observed that plants of both cultivars grown on WPM and MS media with *m*T supplementation showed an increase in fresh weight from 7% to 33% as compared to that noted for the other culture media combinations used (MS and RA). To summarize, *m*T and WPM medium positively stimulated the growth and development of adventitious shoots of goji. These findings agree with Bairu *et al.* (2007) and Gentile *et al.* (2014) who obtained better results for micropropagation of

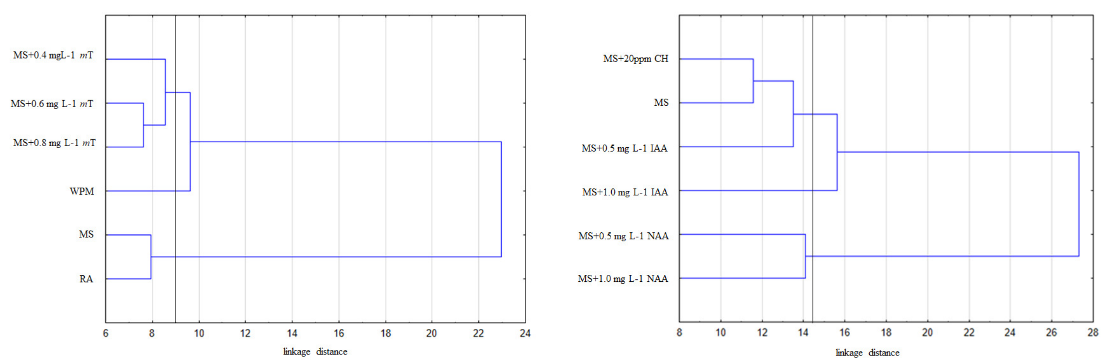
*Aloe polyphylla* and *Prunus*, respectively, in culture medium supplemented with *m*T relative to BA. According to Naaz *et al.* (2019), an increase in growth parameters of explants treated with *m*T may be due to positive signalling in dormant meristematic cells to form new shoots by maintaining juvenility in plant tissues.

A cluster analysis conducted using Ward's method (Figure 1a) showed three separate groups with a similar influence on the multiplication of goji 'No 1' and 'New Big'. The analysis showed that the shoots of both cultivars collected from WPM and MS with supplemented with *m*T had a similar regeneration rate.

**Table 1.** The influence of various medium on the morphological traits and regeneration rate *in vitro* of *L. chinense* 'No 1' and 'New Big'

| Treatments | Shoots length [cm] | No of shoots per explant | Fresh weight [g] | Shoot regeneration rate [%] |
|---|---|---|---|---|
| | | 'No 1' | | |
| MS | 3.18 abc* | 1.17 ab | 0.445 ab | 71 |
| MS+0.4 mg $L^{-1}$ *m*T | 2.89 ab | 1.25 abc | 0.576 cd | 92 |
| MS+0.6 mg $L^{-1}$ *m*T | 3.61 bcd | 1.50 cb | 0.598 de | 96 |
| MS+0.8 mg $L^{-1}$ *m*T | 3.27 abc | 1.33 abc | 0.483 abc | 95 |
| WPM | 3.99 cd | 1.42 bc | 0.529 abcd | 97 |
| RA | 3.24 abc | 1.25 abc | 0.457 ab | 68 |
| | | 'New Big' | | |
| MS | 2.71 a | 1.00 a | 0.426 a | 68 |
| MS+0.4 mg $L^{-1}$ *m*T | 2.83 ab | 1.33 abc | 0.493 abcd | 93 |
| MS+0.6 mg $L^{-1}$ *m*T | 3.53 abcd | 1.42 bc | 0.581 cd | 95 |
| MS+0.8 mg $L^{-1}$ *m*T | 3.36 abcd | 1.33 abc | 0.694 e | 95 |
| WPM | 4.12 d | 1.58 c | 0.549 bcd | 97 |
| RA | 3.42 abcd | 1.25 abc | 0.444 ab | 72 |

*Means followed by the same letter do not differ significantly at $P$=0.05 according to Tukey multiple range




(a) (b)

**Figure 1.** Dendrogram of cluster analysis for propagation (a) and rooting (b) of two goji cultivars 'No 1' and 'New Big' under *in vitro* culture. The vertical line (linkage distance 9 and 14.5, respectively) indicates the cut-off used to form the groups

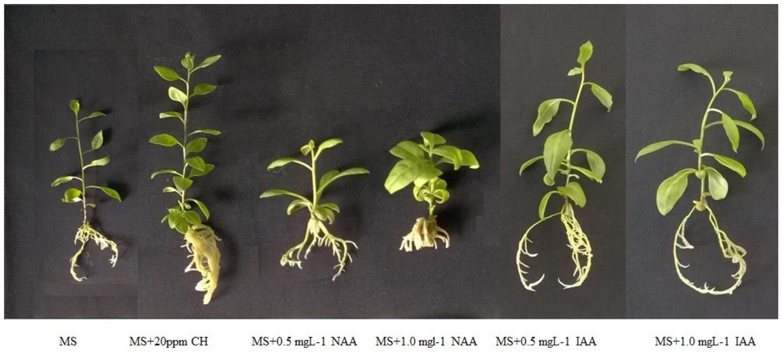
The effect of chitosan on the rooting of goji plants remains unelucidated, hence, we conducted an experiment to compare the efficacy of chitosan and the auxins IAA and NAA in *in vitro* rooting. Chitosan is an eco-friendly biopolymer, it is derived from chitin and shows good biodegradability, bioactivity, and biocompatibility (Krupa-Małkiewicz and Fornal, 2018). In the current study, NAA, IAA, and chitosan showed quite different effects on the two goji cultivars tested in *in vitro* shoot rooting (Table 2, Figure 2a, b). It was observed, that MS medium supplemented with 20 ppm chitosan was optimal for the initiation of rhizogenesis of goji plantlets, with the longest shoots (7.25 and 6.87 cm for 'No 1' and 'New Big', respectively), and roots (4.71 and 4.91 cm for 'No 1' and 'New Big', respectively) and the highest number of roots per plant (2.58). Moreover, the plants were more robust and better rooted, thus reducing labor requirements. No significant differences were observed when plantlets were rooted on MS supplemented with IAA, independent of its concentration. Moreover, plantlets rooted on MS with chitosan and MS with IAA had higher fresh mass (from 186% to 287%) than the control plantlets (0.222g). However, plantlets obtained from treatment with NAA had smaller roots and lower number of roots per plant, which was similar to the control plants (MS) of both the tested cultivars (Table 2, Figure 2a, b).

**Table 2.** *In vitro* rooting capacity and *ex vitro* rooting rate of *L. chinense* 'No 1' and 'New Big'

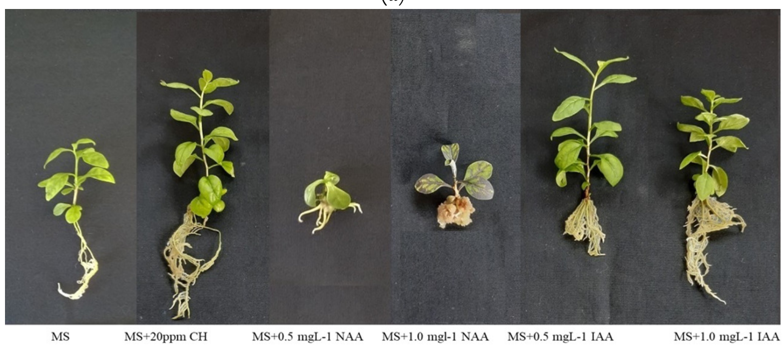
| Treatments | Shoot lenght [cm] | Root lenght [cm] | No of roots per explant | Fresh weight [g] | *Ex vitro* rooting rate [%] |
|---|---|---|---|---|---|
| 'No 1' | | | | | |
| MS | 2.98 bc* | 2.25 d | 1.33 b | 0.222 a | 20 |
| MS+ 20 ppm CH | 7.25 d | 4.71 e | 2.58 c | 0.637 cde | 80 |
| MS+0.5 mg L-1 NAA | 2.99 bc | 2.32 d | 2.50 c | 0.547 cd | 20 |
| MS+1.0 mg L-1 NAA | 3.13 bc | 2.03 cd | 2.25 bc | 0.490 bc | 20 |
| MS+0.5 mg L-1 IAA | 4.27 c | 4.37 e | 2.83 c | 0.706 cde | 75 |
| MS+1.0 mg L-1 IAA | 3.88 c | 4.39 e | 2.25 bc | 0.700 cde | 70 |
| 'New Big' | | | | | |
| MS | 3.76 c | 2.58 d | 1.50 b | 0.239 a | 20 |
| MS+ 20 ppm CH | 6.87 d | 4.91 e | 2.58 c | 0.677 cde | 70 |
| MS+0.5 mg L-1 NAA | 2.02 ab | 1.13 bc | 1.50 b | 0.732 de | 0 |
| MS+1.0 mg L-1 NAA | 1.33 a | 0.52 ab | 0.25 a | 0.542 cd | 0 |
| MS+0.5 mg L-1 IAA | 4.10 c | 4.41 e | 2.08 bc | 0.746 de | 60 |
| MS+1.0 mg L-1 IAA | 3.79 c | 4.37 e | 2.25 bc | 0.859 e | 60 |

Previous studies on this topic have shown that chitosan addition to the medium has varied influence on the morphology of different plant species. Ait Barka *et al.* (2004), used chitogel to stimulate the growth of grapevine *in vitro*, and showed that concentrations of over 2% (v/v) chitogel had a negative effect on plant growth, based on shoot-length measurements. Sopalun *et al.* (2010) suggested that chitosan promoted *in vitro* shoot formation of *Grammatophyllum speciosum* but not rooting. In contrast, Krupa-Małkiewicz and Fornal (2017) showed the stimulating effect of chitosan on the morphology and rooting of *Petunia × atkinsana* propagated *in vitro*. Our results on *in vitro* rooting of *L. chinense* on MS medium supplemented with chitosan are novel in the field of goji rooting.

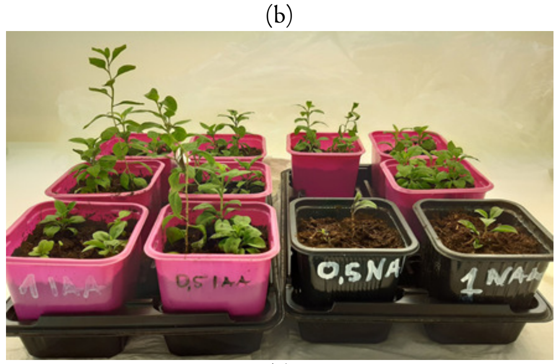
Direct *ex vitro* rooting of goji 'No 1' and 'New Big' shoots from MS + 20 ppm chitosan and MS + IAA in the greenhouse was 60–80% efficient (Table 2, Figure 2c, d). The rooting efficiency of goji 'No 1' shoots on MS supplemented with NAA was the lowest (20%) and similar to that for the control plants (Table 2). Explants of 'New Big' shoots rooted on MS supplemented with NAA did not survive. Rooting treatment with chitosan yielded good results for all the parameters studied; the plantlets were more robust and better rooted, thus reducing labour requirements.




(a)




(b)




(c)

(d)

**Figure 2.** *In vitro* rooting of goji 'No 1' (a) and 'New Big' (b) on different rooting medium. Plants acclimatized after 35 days after the transferring to the greenhouse 'No 1' – (c) and 'New Big' – (d)

A cluster analysis conducted using Ward's method (Figure 1b) showed three separate groups (a–c) with a similar influence of medium composition on the *in vitro* rooting of goji 'No 1' and 'New Big'.

Silvestri *et al.* (2018) showed that the percentage of rooted shoots of *L. barbarum* 'Nixia 1' was higher (87-95%) in ½ MS with 1% sucrose and indole butyric acid (IBA) with or without putrescine and the acclimatization rate of the plants in soil range from 88.7% to 95.1%. Tabăra (2017) rooted *L. barbarum* (boxthorn) with a higher success rate (90-95%) of explants transferred to a solid substrate of peat and sand. Fira *et al.* (2016) recommended floatation hydroculture for *ex vitro* acclimatization of *L. barbarum* cultivar Ningxia N1 on the basis of the high survival percentage obtained (90%).

## Conclusions

In conclusion, we developed a complete micropropagation protocol for *L. chinense* cultivars 'No 1' and 'New Big'. MS medium supplemented with *meta*-Topolin in the concentration of 0.6 mg $L^{-1}$ and WPM medium without plant growth regulators show good results in terms of rapid multiplication and growth of goji shoots. Media supplemented with 20 ppm of chitosan also proved to be very effective, as they provided high rooting rates (70-80%) and well-developing plantlets. For *ex vitro* acclimatization, the mixture of 90% peat and 10% perlite with high humidity (90%) was effective for goji 'No 1' and 'New Big' cultivars. The results obtained may be useful to improve the efficiency of micropropagation and rooting of goji.

## Authors' Contributions

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

## Acknowledgements

This work was supported by the West Pomeranian University of Technology, Szczecin, grant number 518-07-014-3171-03/18.

## Conflict of Interests

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

## References

Ait Barka E, Eullaffroy P, Clément C, Vernet G (2004). Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. Plant Cell Reports 22:608-614. *https://doi.org/10.1007/s00299-003-0733-3*

Anderson WC (1984). A revised tissue culture medium for shoot multiplication of rhododendron. Journal of the American Society for Horticultural Science 109(3):343-347.

Bairu MW, Stirk WA, Dolezal K, Van Staden J (2007). Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: can *meta*-topolin and its derivatives serve as replacement for benzyladenine and zeatin?. Plant Cell, Tissue and Organ Culture 90(1):15-23. *https://doi.org/10.1007/s11240-007-9233-4*

Bartkowiak A (2001). Binary polyelectrolyte microcapsules based on natural polysaccharides. Edt. PS Szczecin.

Dănăilă-Guidea SM, Dobrinoiu RV, Vişan L, Toma RC (2015). Protocol for efficient *in vitro* multiplication of *Lycium barbarum* L.(goji) by direct organogenesis. Scientific Bulletin. Series F. Biotechnologies 19:34-38.

Fira A, Joshee N, Cristea V, Simu M, Hârţa M, Pamfil D, Clapa D (2016). Optimization of micropropagation protocol for goji berry (*Lycium barbarum* L.). Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Horticulture 73(2). *https://doi.org/10.15835/buasvmcn-hort:12177*

Gentile A, Gutiérrez MJ, Martinez J, Frattarelli A, Nota P, Caboni E (2014). Effect of *meta*-Topolin on micropropagation and adventitious shoot regeneration in *Prunus rootstocks*. Plant Cell, Tissue and Organ Culture (PCTOC) 118(3):373-381. *https://doi.org/10.1007/s11240-014-0489-1*

Karakas FP (2020). Efficient plant regeneration and callus induction from nodal and hypocotyl explants of goji berry (*Lycium barbarum* L.) and comparison of phenolic profiles in calli formed under different combinations of plant growth regulators. Plant Physiology and Biochemistry 146:384-391. *https://doi.org/10.1016/j.plaphy.2019.11.009*

Kruczek A, Ochmian I, Krupa-Małkiewicz M, Lachowicz S (2020a). Comparison of morphological, antidiabetic and antioxidant properties of goji fruits. Acta Universitatis Cibiniensis. Series E: Food Technology 24(1):1-14. *https://doi.org/10.2478/aucft-2020-0001*

Kruczek A, Ochmian I (2016). The influence of shrubs cutting method on yielding and quality of the goji berries (*Lycium barbarum* L.). Folia Pomeranae Universitatis Technologiae Stetinensis. Agricultura, Alimentaria, Piscaria et Zootechnica 40(4 (330)):131-138. *https://doi.org/10.2478/aucft-2020-0001*

Kruczek A, Krupa-Małkiewicz M, Lachowicz S, Oszmiański J, Ochmian I (2020b). Health-promoting capacities of *in vitro* and cultivated goji (*Lycium chinense* Mill.) fruit and leaves; polyphenols, antimicrobial activity, macro-and microelements and heavy metals. Molecules 25(22):5314. *https://doi.org/10.3390/molecules25225314*

Krupa-Małkiewicz M, Fornal N (2018). Application of chitosan in vitro to minimize the adverse effects of salinity in *Petunia* × *atkinsiana* D. don. Journal of Ecological Engineering 19(1). *https://doi.org/10.12911/22998993/79410*

Kulczyński B, Gramza-Michałowska A (2016). Goji berry (*Lycium barbarum*): composition and health effects–a review. Polish Journal of Food and Nutrition Sciences 66(2):67-76. *https://doi.org/10.1515/pjfns-2015-0040*

Lloyd G, McCown B (1980). Commercially feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. Combined Proceedings of International Plant Propagators' Society 30:421-427.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum 15:473-497.

Sá RR, da Cruz Caldas J, de Andrade Santana D, Lopes MV, Dos Santos WNL, Korn MGA, Júnior ADFS (2019). Multielementar/centesimal composition and determination of bioactive phenolics in dried fruits and capsules containing Goji berries (*Lycium barbarum* L.). Food Chemistry 273:15-23. *https://doi.org/10.1016/j.foodchem.2018.05.124*

Silvestri C, Sabbatini G, Marangelli F, Rugini E, Cristofori V (2018). Micropropagation and *ex vitro* rooting of Wolfberry. HortScience 53(10):1494-1499. *https://doi.org/10.21273/HORTSCI13423-18*

Sopalun K, Thammasiri K, Ishikawa K (2010). Effects of chitosan as the growth stimulator for *Grammatophyllum* speciosum *in vitro* culture. International Journal of Innovative Research in Science Engineering Technology 4(11):828-830.

Tabăra GM (2017). Aspects of the *in vitro* organogenesis of the species *Lycium barbarum* L. (Goji). Revista Botanică 14(1):29-34.

Naaz A, Hussain SA, Anis M, Alatar AA (2019). *Meta*-topolin improved micropropagation in *Syzygium cumini* and acclimatization to *ex vitro* conditions. Biologia Plantarum 63(1):174-182. *https://doi.org/10.32615/bp.2019.020*

Wojdyło A, Nowicka P, Bąbelewski P (2018). Phenolic and carotenoid profile of new goji cultivars and their anti-hyperglycemic, anti-aging and antioxidant properties. Journal of Functional Foods 48:632-642. *https://doi.org/10.1016/j.jff.2018.07.061*

# ALLEVIATING EFFECTS OF ASCORBIC ACID ON LEAD TOXICITY IN GOJI (Lycium barbarum L.) IN VITRO

**Article** · April 2018

DOI: 10.21005/AAPZ2018.45.1.06



Krupa-Małkiewicz Marcelina
West Pomeranian University of Technology, Szczecin


Arleta Kruczek
West Pomeranian University of Technology, Szczecin


Justyna Pelc
West Pomeranian University of Technology, Szczecin


Ireneusz Ochmian
West Pomeranian University of Technology, Szczecin

DOI: 10.21005/AAPZ2018.45.1.06

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2018, 340(45)1, 55–64

*Marcelina KRUPA-MAŁKIEWICZ, Arleta KRUCZEK[1], Justyna PELC[2], Beata SMOLIK[2], Ireneusz OCHMIAN[1]*

# ALLEVIATING EFFECTS OF ASCORBIC ACID ON LEAD TOXICITY IN GOJI (*Lycium barbarum* L.) *IN VITRO*

# ŁAGODZENIE SKUTKÓW TOKSYCZNEGO DZIAŁANIA OŁOWIU POPRZEZ ZASTOSOWANIE DODATKU KWASU ASKORBINOWEGO W KULTURACH *IN VITRO* GOJI (*Lycium barbarum* L.)

Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Poland
[1]Department of Horticulture, West Pomeranian University of Technology, Szczecin, Poland
[2]Department of Plant Physiology and Biochemistry, West Pomeranian University of Technology, Szczecin, Poland

**Streszczenie.** Ołów jest jednym z najczęściej występujących metali ciężkich w środowisku. Celem badań było określenie wpływu działania egzogennego 1 mM kwasu askorbinowego na wzrost i parametry biochemiczne *Lycium barbarum* w warunkach stresu wywołanego 1 mM $Pb(NO_3)_2$ w kulturach *in vitro*. Eksplantaty stanowiły fragmenty pędów z pakami kątowymi. Na podstawie otrzymanych wyników badań stwierdzono, że ołów miał negatywny wpływ na cechy morfologiczne, takie jak długość pędu i korzeni eksplantatów goji. Dodatek do pożywki soli $Pb(NO_3)_2$ spowodował spadek zawartości chlorofili i karotenoidu, peroksydację lipidów, a także znacznie wpłynął na akumulację proliny w liściach goji. Dodatek do pożywki MS 1 mM kwasu askorbinowego łagodził skutki działania soli ołowiu na wzrost i rozwój eksplantatów goji, zawartość MDA i proliny. Obecność kwasu askorbinowego w podłożu, w warunkach stresu wywołanego przez $Pb(NO_3)_2$, miała pozytywny wpływ na świeżą i suchą masę roślin, ale nie wpływała istotnie na zawartość wody w roślinie.

**Key words:** abiotic stress, goji, heavy metal stress, micropropagation, $Pb(NO_3)_2$.
**Słowa kluczowe:** goji, stres metali ciężkich, mikrorozmnażanie, $Pb(NO_3)_2$, stres abiotyczny.

## INTRODUCTION

Heavy metal contamination in soil could result in inhibition of plant growth and yield reduction, and even pose a great to human health via food chain through the accumulation by plants (Nagajyoti et al. 2010; Lamhamdi et al. 2011; Qiao et al. 2015; Sędzik et al. 2015; Nowakowska et al. 2017). Lead (Pb) is one of the dangerous heavy metal pollutants of the environment that originates from various sources. Its contamination results from mining and smelting activities, lead containing paints, paper and pulp, gasoline and explosives as well as from the disposal of municipal sewage sludge enriched with lead (Boroumand Jazi et al. 2011; Lamhamdi et al. 2013; Qiao et al. 2015). As many of the Pb pollutants are indispensable for

Corresponding author – Adres do korespondencji: Marcelina Krupa-Małkiewicz, Department of Plant Genetics, Breeding and Biotechnology, West Pomerania University of Technology, Szczecin, Juliusza Słowackiego 17, 71-434 Szczecin, Poland, e-mail: mkrupa@zut.edu.pl

modern human life, soil contamination with Pb is not likely to decrease in the near future (Sharma and Dubney 2005). Lead is taken up by plants mainly through the root system and partly in minor amounts through the leaves (Boroumand Jazi et al. 2011). The effect of lead depends on the concentration, type of salt, soil properties and plant species (Lamhamdi et al. 2011). According to Ruley et al. (2004) and Boroumand Jazi et al. (2011) a concentration of Pb higher than 30 ppm in plant tissue is toxic for most of species. Lead can cause a broad range of physiological and biochemical dysfunctions (Lamhamdi et al. 2013). The steadily increasing levels of this metal in the environment causes yellowing of young leaves, reduction in absorption of essential elements such as iron and reduction in the rate of photosynthesis (Sharma and Dubey 2005). High lead concentration affected seed germination, seedlings growth, tolerance index, dry mass of roots and shoots. Although photosynthesis is usually limited, chlorophyll and carotenoid contents, photosynthetic rate and $CO_2$ assimilation are strongly decreased (Yang et al. 2011; Qiao et al. 2015). Increase of lead in plant tissue causing production of reactive oxygen species (ROS), and changes in lipid membrane structure and permeability (Sharma and Dubney 2005; Najeeb et al 2017).

A possible survival strategy for plants under heavy metal conditions is to use some compounds that could alleviate the $Pb(NO_3)_2$ stress effect. The use of vitamins as antioxidants mediated heavy metal tolerance as a selection factor as well as a driving force for improving resistance and adaptation to many abiotic stress factors (Azooz et al. 2013). Vitamins are required in trace amount to maintain normal growth and proper development of all organisms. In addition, vitamins are cofactors of many metabolic reactions (Abdelhamid et al. 2013). Vitamin supplements are known to enhance the plant activities and did not have toxic or mutagenic action (Hassanein et al. 2009; Azooz et al. 2013). Ascorbic acid is an organic acid with an antioxidant properties. The protective role of ascorbic acid in plant cells from the adverse effects of salinity stress was described by Younis et al. (2010) in *Vicia faba* seedlings, Bybordi (2012) in canola, Krupa-Małkiewicz et al. (2015) in tomato seedlings. The role of vitamins in modifying the environmental stresses induced changes in osmoprotectant contents was also investigated by Sadak et al. (2010) and Abdelhamid et al. (2013). According to Krupa-Małkiewicz et al. (2015) ascorbic acid may be of value within biotechnology for the production of valuable substances as well as plant protection.

*Lycium barbarum* L. is commonly known as goji berry. These perennial shrubs inhabiting arid and semiarid regions of Asia, America and Africa. Their special physiological characteristics of drought-resistance and salt-resistance make them a suitable plant to prevent land desertification and alleviating the degree of soil salinity, which is very important for an ecosystem and agriculture in the remote areas (Zheng et al. 2011; Dimitrova et al. 2016).

The objective of this work was to investigate whether ascorbic acid could be a protectant to ameliorate the influence of lead stress on goji explants in *in vitro* culture.

## MATERIAL AND METHODS

**Culture condition and treatments.** The plants material consisted of 15–20 mm shoots with auxiliary buds of goji ‘A’ (*Lycium barbarum* L.) obtained from sterile stabilized *in vitro* culture. The explants were multiplied on the MS medium according to Murashige and Skoog (1962) composition of vitamins, macro- and microelements, 3% (w/v) sucrose (Chempur, Poland), 0.8% (w/v) agar (Biocorp, Poland) and 100 mg · $dm^{-3}$ myo-inositol

(Duchefa Biochemie, Netherlands). After 35 days, shoots cuttings were placed on MS medium supplemented with 1mM ascorbic acid (ASA), 1 mM $Pb(NO_3)_2$ or 1 mM ASA with 1 mM $Pb(NO_3)_2$. MS medium without addition of ascorbic acid and $Pb(NO_3)_2$ salt solution was the control. After 28 days, morphological (shoot and root length, number of root per one explant, fresh and dry mass) and biochemical parameters (MDA, proline, Chl *a*, Chl *b*, Car), as well as plant water content were measured. Dry mass of explants was determined after drying in the hot-air oven at 70°C for 24 h. Plant water content (PWC%) was determined following Equations 1:

$$\text{PWC (\%)} = (\text{fresh mass} - \text{dry mass/fresh mass}) \times 100 \quad (1)$$

The pH of all the medium was adjusted to 5.8. Culture jars (300 ml) with the medium (30 ml) were autoclaved for 20 minutes at 121°C and 0.1 MPa. Sample size was 4 explants per culture vessel with eight replicates per treatment. All cultures were incubated in growth room at a temperature of 25°C under 16 hours photoperiod with a photosynthetic photon flux density (PPFD) of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

**Determination of malondialdehyde.** The level of peroxidation was measured in terms of malondialdehyde (MDA) (a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) according to Sudhakar i in. (2001). Plant tissue was homogenized in 5 $cm^3$ of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged for 15 min and 4.0 $cm^3$ of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 min and then quickly cooled on ice bath. The concentration of MDA was calculated from the absorbance at 600, 532 and 450 nm, and MDA contents were estimated using the following Equations 2:

$$\text{MDA } (\mu mol \cdot g^{-1}\, fm) = [6.45 \times (A_{532} - A_{600}) - 0.56\, A_{450}] \times V/fm \quad (2)$$

where:

$V$ – volume of the sample,

$A$ – absorbance,

$fm$ – fresh mass.

**Determination of proline.** Proline contents was measured according to the method described by Bates et al. (1973). Fresh seedlings (0.5 g) were ground in 3% (v/v) aqueous sulphosalicylic acid and proline were estimated by ninhydrin reagent. The absorbance of the fraction with toluene aspired from the liquid phase was read at 520 nm. The proline concentration was expressed in $\mu mol \cdot g^{-1}$ fresh mass.

**Determination of pigments content.** The levels of Chlorophyll *a*, *b* and carotenoid (Car) were measured in 80% (v/v) acetone extracts. Chlorophyll *a*, *b* and carotenoid content was determined spectrophotometrically at 663, 645 and 440 nm. The concentration of Chl *a* and Chl *b* were calculated according to Arnon et al. (1956) in modification to Lichtenthaler and Wellburn (1983) from Equations 3 and 4, respectively derived by Hendry and Grime (1993).

$$\text{chlorophyll } a \text{ } (mg \cdot g^{-1}\, fm) = [(12.7\, A_{663} - 2.69\, A_{645}) / 1{,}000 \times fm] \times V \quad (3)$$

$$\text{chlorophyll } b \text{ } (mg \cdot g^{-1}\, fm) = [(22.9\, A_{645} - 4.68\, A_{663}) / 1{,}000 \times fm] \times V \quad (4)$$

Carotenoid content was determined by the Equation 5 of Price and Henry (1991):

carotenoid (mg · $g^{-1}$ *fm*) = [($A_{480}$ + 0.114 $A_{663}$) – (0.638 $A_{663}$) × *V*/112.5 × *fm*] (5)

where:

*V* – volume of the sample,

*A* – absorbance,

*fm* – fresh mass.

**Statistical analysis.** Results obtained in *in vitro* cultures were statistically analysed using the Statistica v. 12 software. The significance of differences was determined by means of variance analysis (ANOVA) and Tukey's test, at the level of significance of $\alpha < 0.05$. Proline, MDA, Chl *a*, Chl *b* and Car were measured in triplicates for each experimental combination.

## RESULTS AND DISCUSSION

**Effect of lead on plant growth.** In this work, we analysed the possible role of exogenous nicotinamide treatment to alleviate the negative influence of $Pb(NO_3)_2$ stress factor. Pb stress induced plant growth inhibition has been well describe by many researchers (Ruley et al. 2004; Boroumand Jazi et al. 2011; Yang et al. 2011; Lamhamdi et al. 2013; Sędzik et al. 2015; Qiao et al. 2015). In the current study, shoot and root length, number of roots per one goji explant were significantly decreased at 1 mM $Pb(NO_3)_2$ treatment compared to control (Table 1, Fig. 1). Addition to MS medium 1 mM ascorbic acid with 1 mM $Pb(NO_3)_2$ increased the goji shoot and root length by 31% and 74.5%, respectively, compare to lead treatment explants. The most significant changes were observed in case of number of roots. It was noticed that heavy metal stress significantly decreased the number of roots and addition to MS medium 1mM ascorbic acid with or without lead had positive effect (Table 1).

Table 1. The influence of 1 mM ascorbic acid and 1 mM $Pb(NO_3)_2$ on shoot and root length and number of roots of goji (*Lycium barbarum* L.) *in vitro*

Tabela 1. Wpływ 1 mM kwasu askorbinowego (ASA) i 1 mM $Pb(NO_3)_2$ na długość pędu i korzeni oraz liczbę korzeni goji (*Lycium barbarum* L.) w kulturach *in vitro*

| Medium<br>Pożywka | Morphological traits<br>Cechy morfologiczne | | |
|---|---|---|---|
| | shoot length<br>długość pędu<br>[cm] | root length<br>długość korzenia<br>[cm] | number of roots per one explant<br>liczba korzeni na jednym eksplantacie |
| MS | 5.45 a | 3.27 a | 1.36 bc |
| MS + 1 mM ASA | 5.25 a | 4.07 a | 1.72 ab |
| MS + 1 mM $Pb(NO_3)_2$ | 3.06 c | 2.00 b | 1.27 c |
| MS + 1 mM ASA + 1 mM $Pb(NO_3)_2$ | 4.00 b | 3.49 a | 2.0 a |
| Mean – Średnia | 4.44 | 3.2 | 1.58 |

MS – Murashige and Skoog (1962) medium – pożywka wg składu Murashige and Skoog (1962), ASA – ascorbic acid – kwas askorbinowy.

Letters (a–c) indicate significant differences between medium. Means in the same column followed by the same letter are not significantly different ($\alpha < 0.05$) – Litery (a–c) oznaczają istotne różnice między rodzajami pożywek. Średnie oznaczone tymi samymi literami alfabetu nie różnią się istotnie ($\alpha < 0.05$).


Fig. 1. Influence of 1 mM ascorbic acid with or without 1 mM $Pb(NO_3)_2$ on micropropagation of goji (*Lycium barbarum* L.)
Ryc. 1. Wpływ 1 mM kwasu askorbinowego z 1 mM $Pb(NO_3)_2$ lub bez tego dodatku na mikrorozmnażanie goji (*Lycium barbarum* L.)

Moreover, goji grown in the MS medium supplemented with 1 mM ASA and 1 mM $Pb(NO_3)_2$ developed the highest number of roots per one explants (2.0). Similar reduction in growth performance were found in wheat in response to 3 mM $Pb(NO_3)_2$ (Lamhamdi et al. 2011). Also Boroumand Jazi et al. (2001) showed that increased concentration of $Pb(NO_3)_2$ in the medium from 0 to 2 mM decreased length of root and stem of *Brassica napus* var. Okapi as compared to the control. According to Nagajyoti et al. (2010) the degree to which root elongation is inhibited depends upon the concentration of lead and ionic composition and pH of the medium. Inhibitory effects of lead on growth and biomass production may possibly derived from its inhibitory effect on cell division or cell expansion in the elongation zone or both of them reduce root length (Nagajyoti et al. 2010; Boroumand Jazi et al. 2011).

**Effect of lead on fresh and dry mass and PWC.** Exposure of the goji explants to 1 mM $Pb(NO_3)_2$ markedly reduced fresh and dry mass by 53% and 52%, respectively, compared with control (Table 2).

Table 2. The influence of 1 mM ascorbic acid and 1 mM $Pb(NO_3)_2$ on fresh and dry mass and plant water content in goji (*Lycium barbarum* L.) in *in vitro* culture
Tabela 2. Wpływ 1 mM kwasu askorbinowego (ASA) i 1 mM $Pb(NO_3)_2$ na świeżą i suchą masę oraz zawartość wody w roślinie goji (*Lycium barbarum* L.) namnażanej w kulturach *in vitro*

| Medium<br>Pożywka | Fresh mass<br>Świeża masa<br>[g] | | Dry mass<br>Sucha masa<br>[g] | | Plant Water Content<br>Zawartość wody<br>w roślinie<br>[%] |
|---|---|---|---|---|---|
| MS | 0.248 | a* | 0.025 | b | 89.92 |
| MS + 1 mM ASA | 0.288 | a | 0.033 | a | 88.54 |
| MS + 1 mM $Pb(NO_3)_2$ | 0.117 | b | 0.012 | c | 89.74 |
| MS + 1 mM ASA + 1 mM $Pb(NO_3)_2$ | 0.250 | a | 0.024 | b | 90.40 |

Explanations see Table 1 – Objaśnienia zob. tab. 1.

Addition to MS medium 1 mM ASA with 1 mM $Pb(NO_3)_2$ greatly relieved Pb-induced reduction in fresh and dry mass and the values returned close to control. Furthermore, the use of ascorbic acid under non-stress condition caused increased in fresh and dry mass and the value were even higher than control by 16% and 32%, respectively. No effect was detected in ASA with or without $Pb(NO_3)_2$ treatment on plant water content of goji explants.

According to many authors (Munns 2005; Tuna et al. 2008; Piwowarczyk et al. 2016), one of the assessment method to determine the influence of stress factor on plants should be evaluation of plants dry mass. Therefore, it is believed that increased tolerance to stress factor can be associated with increase or unchanged dry mass content in plants from stressed and control conditions. A similar negative effect of Pb on the reduction of fresh and dry mass by 28% and 29%, respectively, at 15 mM Pb was observed by Lamhamdi et al. (2013) in spinach. According to mentioned authors these symptoms can be essentially attributed to a deficiency of macroelements, which results from an inhibition of their uptake under stress factor. Similar results about the inhibitory effects of salt stress in the plant *Lycium barbarum* and *Lycium chinense* have been reported (Dimitrova et al. 2016). Boroumand Jazi et al. (2011) showed that the fresh and dry mass of *Brassica napus* roots was significantly decrease as plants received Pb in the nutrient solution. However, 10 μM salicylic acid significantly increased the fresh mass of root and shoot as compare with lead treatment. Inhibition in plant fresh and dry mass under cadmium stress was significant alleviated by exogenous application of biologically active substances such as glutathione (GSH), glycinebetaine (GB), brassinosteroids (BRs), salicylic acid (SA) treatment in *Oryza sativa* (Cao et al. 2013).

**Effect of $Pb(NO_3)_2$ on chlorophyll *a* and *b* and carotenoid content.** Measurement of chlorophylls contents, which has been shown to correlate negatively with mineral uptake, is commonly used method to monitor oxidative stress in plants (Ruley et al. 2004). The inhibition of chlorophyll synthesis by heavy metals is often manifesting as chlorosis. The change in chlorophyll structure may indicate that absorption of Pb was higher than essential mineral ions, especially magnesium (Akinci et al. 2010). In the present study, exposure of the goji explants to 1 mM $Pb(NO_3)_2$ markedly reduced Chl *a* and *b* and Car contents by 21%, 51% and 54%, respectively, compared with control (Table 3).

Table 3. The influence of 1 mM ascorbic acid and 1 mM $Pb(NO_3)_2$ on chlorophyll *a* (Chl *a*) and *b* (Chl *b*) and carotenoid (Car) content in leaves of goji (*Lycium barbarum* L.) in *in vitro* culture
Tabela 3.Wpływ 1 mM kwasu askorbinowego i 1 mM $Pb(NO_3)_2$ na zawartość chlorofilu *a* (Chl *a*) i *b* (Chl *b*) oraz karotenoidu (Car) w liściach goji (*Lycium barbarum* L.) namnażanej w kulturach *in vitro*

| Medium<br>Pożywka | Chl *a*<br>[mg · $g^{-1}$ fw] | Chl *b*<br>[mg · $g^{-1}$ fw] | Car<br>[mg · $g^{-1}$ fw] |
|---|---|---|---|
| MS | 51.45 b* | 29.96 b | 18.73 b |
| MS + 1 mM ASA | 54.12 a | 30.59 a | 21.01 a |
| MS + 1 Mm $Pb(NO_3)_2$ | 40.63 d | 14.67 d | 8.64 d |
| MS + 1 mM ASA + 1 mM $Pb(NO_3)_2$ | 45.95 c | 21.94 c | 14.40 c |
| Mean – Średnia | 48.03 | 24.29 | 15.69 |

Explanations see Table 1 – Objaśnienia zob. tab. 1.

Addition to MS medium 1 mM ASA under heavy metal stress markedly increased Chl *a* and Chl *b* and Car content by 13% (Chl a), 49% (Chl b) and 67% Car) compared with Pb alone treatment. Moreover, it was observed that application of 1 mM ASA alone significantly increased photosynthetic (Chl *a* and Chl *b*) and nonphotosynthetic (Car) pigments contents compared with those in the control (Table 3). A negative influence of heavy metal stress on chlorophylls concentration in wheat and spinach plants was observed by Lamhamdi et al. (2013). Authors showed that concentrations of chlorophylls *a* and *b* were already significantly lower at 1.5 mM Pb, and this effect was more pronounced at 3 and 15 mM Pb. Similar result was obtained by Akinci et al. (2010) in tomato who found that Chl *a* and *b* and *a+b* was significantly affected by increasing lead concentration (0–300 mg · $dm^{-3}$).

According to many authors (Agami 2014; Hussein and Alva 2014; Krupa-Małkiewicz et al. 2015) the exogenous application of biologically active substances such as ascorbic acid is effective in mitigating the adverse effects of salt stress on growth of many plants. In addition, Cao et al. (2013) showed that Cd-induced chlorophyll synthesis inhibition was markedly reverted and the content was even more than control when rice seedlings were pre-treated with GSH, GB or SA.

**Effects of lead on proline and MDA content.** In opinion of many authors (Lamhamdi et al. 2011; Yang et al. 2011; Cao et al. 2013; Krupa-Małkiewicz et al. 2015) elevated proline and MDA levels in plant tissue are quite good indicators of the negative effects of various stress factors on a plant. Excessive proline accumulation occurs as a strong reaction of environmental stress, which results from its uncontrolled biosynthesis, limited oxidation, inhibition of its incorporation into proteins and even release from proteins due to proteolysis (Girija et al. 2002). Measurement of malondialdehyde (MDA) level is routinely used as an index of lipid peroxidation under stressful conditions (Yang et al. 2011; Cao et al. 2013).

In this study, the contents of proline in goji seedlings was significantly increased (3.51 μmol · $g^{-1}$ fm and 4.56 μmol · $g^{-1}$ fm, respectively) when 1.0 mM ASA or 1.0 mM $Pb(NO_3)_2$ were used in comparison to the control (1.88 μmol · $g^{-1}$ fm) – Table 4.

Table 4. The influence of 1 mM ascorbic acid and 1 mM $Pb(NO_3)_2$ on proline and MDA in leaves of goji (*Lycium barbarum* L.) in *in vitro* culture
Tabela 4. Wpływ 1 mM kwasu askorbinowego i 1 mM $Pb(NO_3)_2$ na zawartość proliny i MDA w liściach goji (*Lycium barbarum* L.) namnażanej w kulturach *in vitro*

| Medium<br>Pożywka | Proline<br>[μml · $g^{-1}$ fw] | | MDA<br>[nmol · $g^{-1}$ fw] | |
|---|---|---|---|---|
| MS | 1.88 | d* | 7.69 | d |
| MS + 1 mM ASA | 3.51 | c | 9.53 | c |
| MS + 1 Mm $Pb(NO_3)_2$ | 4.56 | a | 11.22 | a |
| MS + 1 mM ASA + 1 mM $Pb(NO_3)_2$ | 3.7 | b | 10.8 | b |
| Mean – Średnia | 5.91 | | 9.81 | |

Explanations see Table 1 – Objaśnienia zob. tab. 1.

However, addition to MS medium 1 mM ASA under heavy metal stress decreased proline content by 19%, compared with Pb alone treatment. These results confirm the finding of Azooz et. al (2013) who reported that most of the vitamins tend to increase the proline content under stress factor. Similar response to Pb treatment was previously noticed in bean (Zengin and Munzuroglu 2005), wheat (Yang et al. 2011), and in various plants species (Sędzik et. al 2015). Lamhamdi et al. (2011) observed that proline concentrations increase with those of lead in the growth medium, and this increase is more relevant in roots than in coleoptiles.

In the present study, Pb stress induced oxidative stress characterized significant increase in MDA content by 46% compare with control (Table 4). It may be suggest that more MDA accumulation could account for presence of the poisoning reactive oxygen species (ROS). Addition to MS medium 1 mM ASA with 1 mM $Pb(NO_3)_2$ decreased MDA content by 4% in goji seedlings compared with Pb alone treatment. However, in comparison with the control, 1 mM ASA alone treatment induced 24% more MDA accumulation in goji seedlings, over the control. Najeeb et al. (2017) reported that since lipid peroxidation is a biochemical marker for stress-induced damage in plants, elevated MDA level under lead indicated that Pb toxicity induced oxidative damage to *Juncus effusus* L. Lead induced lipid peroxidation has already been reported in wheat (Lamhamdi et al. 2011; Yang et al. 2011). In turn, Qiao et al. (2015) observed that significant accumulation of Pb resulted in oxidative stress in *Potamogeton crispus* L. but it was efficiency controlled. The role of biologically active substances in modifying the environmental stresses induced changes in osmoprotectant contents was also investigated by Boroumand Jazi et al. (2011), Cao et al. (2013) and Krupa-Małkiewicz et al. (2015). According to Krupa-Małkiewicz et al. (2015) ascorbic acid may be of value within biotechnology for the production of valuable substances as well as plant protection. This vitamin might act as activators of protein synthesis through modulating the activity of enzymes involved in the metabolism of proteins or sugars.

## CONCLUSION

It is obvious from our results that lead treatment even at low concentration (1 mM) induced large disturbances in plant growth, especially shoot and root length. Heavy metal stress had negative influence on biochemical parameters such as proline, MDA, chlorophylls and carotenoid. However, application of 1 mM ascorbic acid as an antioxidant compound increased apical growth as well as development and biochemical parameters of *Lycium barbarum* L. in *in vitro* culture.

## REFERENCES

**Abdelhamid M.T., Sadak M.S.H., Schmidhalter U.R.S., El-Saady M.** 2013. Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of faba bean plant. Acta Biol. Colomb. 18(3), 499−510.

**Akinci I.E., Akinci S., Yilmaz K.** 2010. Response of tomato (*Solanum lycopersicum* L.) to lead toxicity: growth, element uptake, chlorophyll and water content. Afr. J. Agric. Res. 5, 416−423.

**Agami R.A.** 2014. Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. Biol. Plant. 58(2), 341−347.

**Arnon D.J., Allen M.B., Whatley F.** 1956. Photosynthesis by isolated chloroplast. Biochim. Biophys. Acta 20, 449−461.

**Azooz M.M., Alzahrani A.M. Youssef M.M.** 2013. The potential role of seed priming with ascorbic acid and nicotinamide and their interactions to enhance salt tolerance in broad bean (*Vicia faba* L.). Aust. J. Crop. Sci. 7, 2091−2100.

**Bates L.S., Waldren R., Teare I.** 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39(1), 205−207.

**Boroumand Jazi Sh., Lari Yazdi H., Ranjbar M.** 2011. Effects of salicylic acid on some plant growth parameters under lead stress in *Brassica napus* var. Okapi. Iranian J. Plant Physiol. 1(3), 177−185.

**Bybordi A.** 2012. Effect of ascorbic acid and silicium on photosynthesis, antioxidant enzyme activity, and fatty acid contents in canola exposure to salt stress. J. Integr. Agric. 11, 1610−1620.

**Cao F., Liu L., Ibrahim W., Cai Y., Wu F.** 2013. Alleviating effects of exogenous glutathione, glycinebetaine, brassinosteroids and salicylic acid on cadmium toxicity in rice seedlings (*Oryza sativa*). Agrotechnol. 2, 107. DOI:10.4172/2168-9881.1000107.

**Dimitrova V., Georgieva T., Markovska Y.** 2016. Influence of salt stress on some physiological characteristics of two *Lycium* varieties grown *ex vitro* in hydroponics. Youth Scientific Conference Kliment's Days. Sofia 101(4), 141−148.

**Girija C., Smith B.N., Swamy P.M.** 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). Environ. Exp. Bot. 47, 1−10.

**Hassanein R.A., Bassuony F.M., Baraka D.M., Khalil R.R.** 2009. Physiological effects of nicotinamide and ascrobic acid on *Zea mays* plant grown under salinity stress. 1-changes in growth, some relevant metabolic activities and oxidative defense systems. Res. J. Agric. Biol. Sci. 5(1), 72−81.

**Hendry G.A.F., Grime J.P.** 1993. Methods in comparative plant ecology. New York, Marcel Dekker Inc.

**Hussein M.M., Alva A.K.** 2014. Effects of zinc and ascorbic acid application on the growth and photosynthetic pigments of millet plants grown under different salinity. Agric. Sci. 5, 1253−1260.

**Krupa-Małkiewicz M., Smolik B., Ostojski D., Sędzik M.** 2015. Effect of ascorbic acid on morphological and biochemical parameters in tomato seedling exposure to salt stress. Environ. Protect. Nat. Res. 24(2), 25−27.

**Lamhamdi M., Bakrim A., Aarab A., Lafont R., Sayah F.** 2011. Lead phytotoxicity on wheat (*Triticum aestivum* L.) seed germination and seedlings growth. C. R. Biol. 334, 118−126.

**Lamhamdi M., El Galiou O., Bakrim A., Nóvoa-Muñoz J.C., Arias-Estévez M., Aarab A., Lichtenthaler H.K, Wellburn A.R.** 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591−592.

**Munns R.** 2005. Genes and salt tolerance: bringing them together. New Phytol. 167, 645–663.

**Murashige T., Skoog F.** 1962. A revised medium, for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15(3), 473−479.

**Nagajyoti P.C., Lee K.D., Sreekanth T.V.M.** 2010. Heavy metals, occurrence and toxicity for plants: a review. Environ. Chem. Lett. 8, 199−216.

**Najeeb U., Ahmad W., Zia M.H., Malik Z., Zhou W.** 2017. Enhancing the lead phytostabilization in wetland plant *Juncus effuses* L. through somaclonal manipulation and EDTA enrichment. Arabian J. Chem. 10, 3310−3317.

**Nowakowska M., Ochmian I., Mijowska K.** 2017. The influence of street conditions on sea buckthorn fruit quality and content of micro- and macronutrients in berries and in soil. J. Elem. 22(1), 235−244

**Piwowarczyk B., Tokarz K., Kamińska I.** 2016. Responses of grass pea seedlings to salinity stress in in vitro culture conditions. Plant Cell. Tiss. Organ Cult. 124(2), 227−240.

**Price A.H., Hendry G.A.** 1991. Ion-catalyzed oxygen radical formation and its possible contribution to drought damages in nine native grasses and three cereals. Plant Cell Environ. 14(5), 477−484.

**Qiao X., Zheng Z., Zhang L., Wang J., Shi G., Xu X.** 2015. Lead tolerance mechanism in sterilized seedlings of *Potamogeton crispus* L.: Subcellular distribution, polyamines and proline. Chemosphere 120, 179−187.

**Ruley A.T., Sharma N.C., Sahi S.V.** 2004. Antioxidant defense in lead accumulating plant, *Sesbania drummondii*. Plant Physiol. Biochem. 42, 899−906.

**Sadak M.S.H., Abdelhamid M.T., El-Saady M.** 2010. Physiological responses of faba bean plant to ascorbic acid grown under salinity stress. Egyptian J. Agronom. 32(1), 89−106.

**Sędzik M., Smolik B., Krupa-Małkiewicz M.** 2015. Effect of lead on germination and some morphological and physiological parameters of 10-day-old seedlings of various plant species. Environ. Protect. Natur. Res. 26(3), 22−27.

**Sharma P., Dubey R.** 2005. Lead toxicity in plants. Brazilian J. Plant Physiol. 17, 35−52.

**Sudhakar C., Lakshim A., Giridarakumar S.** 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci. 161(3), 613−619.

**Tuna A.L., Kaya C., Dikilitas M., Higgs D.E.B.** 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environ. Exp. Bot. 62(1), 1−9.

**Yang Y., Zhang Y., Wei X., You J., Wang W., Lu J., Shi R.** 2011. Comparative antioxidative responses and proline metabolism in two wheat cultivars under short term lead stress. Ecotox. Environ. Saf. 74, 733−740.

**Younis M.E., Hasaneen M.N.A., Kazamel A.S.** 2010. Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Vicia faba* seedlings. Protoplasma 239(1), 39−48.

**Zengin F.K., Munzuroglu O.** 2005. Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. Acta Biol. Cracov. Series Botanica 47(2), 157−164

**Zheng J., Ding C.X., Wang L.S., Li G.L., Shi J.Y., Li H., Wang H.L., Suo Y.R.** 2011. Anthocyanins composition and antioxidant activity of wild *Lycium ruthenicum* Murr. from Qinghai-Tiber plateau. Food Chem. 126, 859−865.

**Abstract.** Lead (Pb) is the most common heavy metal pollutant in the environment. The objective of the presented study was to investigate the ameliorative effect of exogenous 1 mM ASA on key growth and biochemical parameters in *Lycium barbarum* seedlings under heavy metal ($Pb(NO_3)_2$) stress *in vitro*. Nodal cutting with an axillaries bud were used as an explants. The results showed that lead accumulation in goji explants had negative influence on morphological parameters of plant growth, such as shoot and root length. Lead caused a significant reduction in chlorophylls and carotenoid content, increased lipid peroxidation and induced significant accumulation of proline in goji leaves. Addition to MS medium 1 mM ASA greatly alleviated Pb-induced growth inhibition and Pb-induced MDA and proline accumulation. Presence of ASA in the MS medium under heavy metal stress increased plant fresh and dry mass with no significant effect on plant water content.

**OŚWIADCZENIA**

**o wkładzie autora w publikację**

**<u>Publikacja 1:</u> Kruczek Arleta**, Ochmian Ireneusz (2016) The influence of shurbs cutting method on yielding and quality of the goji berries (*Lycium barbarum* L.). Folia Pomeranae Universitatis Technologiae Stetinensis. Agricultura, Alimentaria, Piscaria et Zootechnica, 40(4 (330)).

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, wyborze metodyki badań, przeprowadzeniu doświadczeń polowych oraz analiz laboratoryjnych. Ponadto zbieranie danych, opracowanie i interpretacja wyników, pisanie publikacji oraz jej korekta.

Wkład mojej pracy w w/w publikację oceniam na 70%.

**Wkład pozostałych współautorów:**
Ireneusz Ochmian – 30%.

**<u>Publikacja 2:</u> Kruczek Arleta**, Ochmian Ireneusz, Krupa-Małkiewicz Marcelina, Lachowicz Sabina (2020) Comparison of morphological, antidiabetic and antioxidant properties of goji fruits. Acta Universitatis Cibiniensis. Series E: Food Technology, 24(1), 1-14.

Oświadczam, że w ramach badań współtworzyłam koncepcję pracy, wybrałam metody badań, przeprowadziłam część analiz laboratoryjnych, zebrałam dane, opracowałam i zinterpretowałam wyniki oraz pisałam publikację.

Wkład mojej pracy w w/w publikację oceniam na 50%.

**Wkład pozostałych współautorów:**
Ireneusz Ochmian – 20%
Marcelina Krupa-Małkiewicz – 20%
Sabina Lachowicz – 10%

**<u>Publikacja 3:</u> Kruczek Arleta**, Krupa-Małkiewicz Marcelina, Lachowicz Sabina, Oszmiański Jan, Ochmian Ireneusz (2020) Health-Promoting Capacities of *In Vitro* and Cultivated Goji (*Lycium chinense* Mill.) Fruit and Leaves; Polyphenols, Antimicrobial Activity, Macro-and Microelements and Heavy Metals. Molecules, 25(22), 5314.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji pracy, postawieniu hipotez badawczych, wyborze metodyki badań, przeprowadzeniu doświadczeń polowych, wykonaniu analiz laboratoryjnych, opracowaniu wyników i pisaniu manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 60%.

**Wkład pozostałych współautorów:**
Marcelina Krupa-Małkiewicz – 10%
Sabina Lachowicz – 10 %
Jan Oszmiański – 10%
Ireneusz Ochmian – 10%

**<u>Publikacja 4:</u> Kruczek Arleta**, Krupa-Małkiewicz Marcelina, Ochmian Ireneusz (2017) The effectiveness of disinfection methods on germination of goji seeds (*Lycium barbarum* L.) in *in vitro* culture. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 336(43/3), 67-73.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji pracy, postawieniu hipotez badawczych, wyborze metodyki badań, przeprowadzeniu analiz laboratoryjnych. Ponadto zbieranie danych, opracowanie i interpretacja wyników, obliczenia statystyczne, pisanie publikacji oraz jej korekta.

Wkład mojej pracy w w/w publikację oceniam na 70%.

**Wkład pozostałych współautorów:**
Marcelina Krupa-Małkiewicz – 15%
Ireneusz Ochmian – 15%

Kruczek

**<u>Publikacja 5:</u> Kruczek Arleta**, Krupa-Małkiewicz Marcelina, Ochmian Ireneusz (2021) Micropropagation, rooting, and acclimatization of two cultivars of goji (*Lycium chinense*). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(2), 12271–12271.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji pracy, postawieniu hipotez badawczych, wyborze metodyki badań, przeprowadzeniu analiz laboratoryjnych. Ponadto zbieranie danych, opracowanie i interpretacja wyników, obliczenia statystyczne, pisanie publikacji oraz jej korekta.

Wkład mojej pracy w w/w publikację oceniam na 70%.

**Wkład pozostałych współautorów:**
Marcelina Krupa-Małkiewicz – 15%
Ireneusz Ochmian – 15%

Kruczek

**<u>Publikacja 6:</u>** Krupa-Małkiewicz Marcelina, **Kruczek Arleta**, Pelc Justyna, Smolik Beata, Ochmian Ireneusz (2018) Alleviating effects of ascorbic acid on lead toxicity in goji (*Lycium barbarum* L.) *in vitro*. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 340(45/1), 55-64.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji pracy, postawieniu hipotez badawczych, wykonaniu analiz laboratoryjnych, opracowaniu wyników i pisaniu manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 30%.

**Wkład pozostałych współautorów:**
Marcelina Krupa-Małkiewicz - 40%
Justyna Pelc – 10%
Beata Smolik – 10%
Ireneusz Ochmian – 10%

Kruczek

**OŚWIADCZENIA**

**o wkładzie autora w pubikację**

**<u>Publikacja 1:</u>** Kruczek Arleta, **Ochmian Ireneusz** (2016) The influence of shurbs cutting method on yielding and quality of the goji berries (*Lycium barbarum* L.). Folia Pomeranae Universitatis Technologiae Stetinensis. Agricultura, Alimentaria, Piscaria et Zootechnica, 40(4 (330)).

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, wyborze metodyki badań, pisaniu publikacji oraz jej korekta.

Wkład mojej pracy w w/w publikację oceniam na 30%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 70%.

**<u>Publikacja 2:</u>** Kruczek Arleta, **Ochmian Ireneusz**, Krupa-Małkiewicz Marcelina, Lachowicz Sabina (2020) Comparison of morphological, antidiabetic and antioxidant properties of goji fruits. Acta Universitatis Cibiniensis. Series E: Food Technology, 24(1), 1-14.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, wyborze metod badań oraz korekta manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 20%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 50%
Marcelina Krupa-Małkiewicz – 20%
Sabina Lachowicz – 10%

**<u>Publikacja 3:</u>** Kruczek Arleta, Krupa-Małkiewicz Marcelina, Lachowicz Sabina, Oszmiański Jan, **Ochmian Ireneusz** (2020) Health-Promoting Capacities of *In Vitro* and Cultivated Goji (*Lycium chinense* Mill.) Fruit and Leaves; Polyphenols, Antimicrobial Activity, Macro-and Microelements and Heavy Metals. Molecules, 25(22), 5314.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, korekta manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 10%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 60%
Marcelina Krupa-Małkiewicz – 10%
Sabina Lachowicz – 10 %
Jan Oszmiański – 10%

**<u>Publikacja 4:</u>** Kruczek Arleta, Krupa-Małkiewicz Marcelina, **Ochmian Ireneusz** (2017) The effectiveness of disinfection methods on germination of goji seeds (*Lycium barbarum* L.) in *in vitro* culture. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 336(43/3), 67-73.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji pracy, obliczeniach statystycznych oraz pisanie i korekta manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 15%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 70%
Marcelina Krupa-Małkiewicz – 15%

**<u>Publikacja 5:</u>** Kruczek Arleta, Krupa-Małkiewicz Marcelina, **Ochmian Ireneusz** (2021) Micropropagation, rooting, and acclimatization of two cultivars of goji (*Lycium chinense*). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(2), 12271–12271.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, pisaniu muanuskyptu oraz jego korekta.

Wkład mojej pracy w w/w publikację oceniam na 15%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 70%
Marcelina Krupa-Małkiewicz – 15%

**<u>Publikacja 6:</u>** Krupa-Małkiewicz Marcelina, Kruczek Arleta, Pelc Justyna, Smolik Beata, **Ochmian Ireneusz** (2018) Alleviating effects of ascorbic acid on lead toxicity in goji (*Lycium barbarum* L.) *in vitro*. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 340(45/1), 55-64.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, przeprowadzeniu analiz laboratoryjnych i korekcie manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 10%.

**Wkład pozostałych współautorów:**
Marcelina Krupa-Małkiewicz - 40%
Arleta Kruczek – 30%
Justyna Pelc – 10%
Beata Smolik – 10%

**OŚWIADCZENIA**

**o wkładzie autora w pubikację**

**<u>Publikacja 2:</u>** Kruczek Arleta, Ochmian Ireneusz, **Krupa-Małkiewicz Marcelina**, Lachowicz Sabina (2020) Comparison of morphological, antidiabetic and antioxidant properties of goji fruits. Acta Universitatis Cibiniensis. Series E: Food Technology, 24(1), 1-14.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, wyborze metod badań oraz pisanie manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 20%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 50%
Ireneusz Ochmian – 20%
Sabina Lachowicz – 10%

**<u>Publikacja 3:</u>** Kruczek Arleta, **Krupa-Małkiewicz Marcelina**, Lachowicz Sabina, Oszmiański Jan, Ochmian Ireneusz (2020) Health-Promoting Capacities of *In Vitro* and Cultivated Goji (*Lycium chinense* Mill.) Fruit and Leaves; Polyphenols, Antimicrobial Activity, Macro-and Microelements and Heavy Metals. Molecules, 25(22), 5314.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji i korekta manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 10%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 60%
Sabina Lachowicz – 10 %
Jan Oszmiański – 10%
Ireneusz Ochmian – 10%

**<u>Publikacja 4:</u>** Kruczek Arleta, **Krupa-Małkiewicz Marcelina**, Ochmian Ireneusz (2017) The effectiveness of disinfection methods on germination of goji seeds (*Lycium barbarum* L.) in *in vitro* culture. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 336(43/3), 67-73.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji pracy, obliczeniach statystycznych i korekta manuskryptu.

Wkład mojej pracy w w/w publikację oceniam na 15%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 70%
Ireneusz Ochmian – 15%

**<u>Publikacja 5:</u>** Kruczek Arleta, **Krupa-Małkiewicz Marcelina**, Ochmian Ireneusz (2021) Micropropagation, rooting, and acclimatization of two cultivars of goji (*Lycium chinense*). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(2), 12271–12271.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, pisaniu muanuskyptu oraz jego korekta.

Wkład mojej pracy w w/w publikację oceniam na 15%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 70%
Ireneusz Ochmian – 15%

**<u>Publikacja 6:</u>** **Krupa-Małkiewicz Marcelina**, Kruczek Arleta, Pelc Justyna, Smolik Beata, Ochmian Ireneusz (2018) Alleviating effects of ascorbic acid on lead toxicity in goji (*Lycium barbarum* L.) *in vitro*. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 340(45/1), 55-64.

Oświadczam, że mój udział w pracy polegał na współtworzeniu koncepcji, postawieniu hipotez badawczych, przeprowadzeniu analiz laboratoryjnych, pisaniu manuskryptu i jego korekta.

Wkład mojej pracy w w/w publikację oceniam na 40%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 30%
Justyna Pelc – 10%
Beata Smolik – 10%
Ireneusz Ochmian – 10%

**OŚWIADCZENIA**

**o wkładzie autora w pubikację**

**<u>Publikacja 2:</u>** Kruczek Arleta, Ochmian Ireneusz, Krupa-Małkiewicz Marcelina, **Lachowicz Sabina** (2020) Comparison of morphological, antidiabetic and antioxidant properties of goji fruits. Acta Universitatis Cibiniensis. Series E: Food Technology, 24(1), 1-14.

Oświadczam, że w ramach badań wybrałam metody badań oraz przeprowadziłam analizy laboratoryjne (zawartość polifenoli w próbach roślinnych przygotowanych przez Arletę Kruczek).

Wkład mojej pracy w w/w publikację oceniam na 10%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 50%
Ireneusz Ochmian – 20%
Marcelina Krupa-Małkiewicz – 20%

**<u>Publikacja 3:</u>** Kruczek Arleta, Krupa-Małkiewicz Marcelina, **Lachowicz Sabina**, Oszmiański Jan, Ochmian Ireneusz (2020) Health-Promoting Capacities of *In Vitro* and Cultivated Goji (*Lycium chinense* Mill.) Fruit and Leaves; Polyphenols, Antimicrobial Activity, Macro-and Microelements and Heavy Metals. Molecules, 25(22), 5314.

Oświadczam, że mój udział w pracy polegał na wykonaniu analiz laboratoryjnych (zawartość polifenoli w próbach roślinnych przygotowanych przez Arletę Kruczek).

Wkład mojej pracy w w/w publikację oceniam na 10%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 60%
Marcelina Krupa-Małkiewicz – 10%
Jan Oszmiański – 10%
Ireneusz Ochmian – 10%

**OŚWIADCZENIE**

**o wkładzie autora w pubikację**

**<u>Publikacja 3:</u>** Kruczek Arleta, Krupa-Małkiewicz Marcelina, Lachowicz Sabina, **Oszmiański Jan**, Ochmian Ireneusz (2020) Health-Promoting Capacities of *In Vitro* and Cultivated Goji (*Lycium chinense* Mill.) Fruit and Leaves; Polyphenols, Antimicrobial Activity, Macro-and Microelements and Heavy Metals. Molecules, 25(22), 5314.

Oświadczam, że mój udział w pracy polegał na wykonaniu analiz laboratoryjnych w próbach roślinnych przygotowanych przez Arletę Kruczek (zawartość polifenoli).

Wkład mojej pracy w w/w publikację oceniam na 10%.

**Wkład pozostałych współautorów:**
Arleta Kruczek – 60%
Marcelina Krupa-Małkiewicz – 10%
Sabina Lachowicz – 10%
Ireneusz Ochmian – 10%

Oszmiański

**OŚWIADCZENIE**

**o wkładzie autora w pubikację**

<u>**Publikacja 6:**</u> Krupa-Małkiewicz Marcelina, Kruczek Arleta, **Pelc Justyna**, Smolik Beata, Ochmian Ireneusz (2018) Alleviating effects of ascorbic acid on lead toxicity in goji (*Lycium barbarum* L.) *in vitro*. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 340(45/1), 55-64.

Oświadczam, że mój udział w pracy polegał na przeprowadzeniu analiz biochemicznych przygotowanych przez Arletę Kruczek próbach roślinnych (zawartość chlorofili i karotenoidów).

Wkład mojej pracy w w/w publikację oceniam na 10%.

**Wkład pozostałych współautorów:**
Marcelina Krupa-Małkiewicz – 40%
Arleta Kruczek – 30%
Beata Smolik – 10%
Ireneusz Ochmian – 10%

**OŚWIADCZENIE**

**o wkładzie autora w pubikację**

**Publikacja 6:** Krupa-Małkiewicz Marcelina, Kruczek Arleta, Pelc Justyna, **Smolik Beata**, Ochmian Ireneusz (2018) Alleviating effects of ascorbic acid on lead toxicity in goji (*Lycium barbarum* L.) *in vitro*. Folia Pomeranae Universitatis Technologiae Stetinensis, Agricultura, Alimentaria, Piscaria et Zootechnica, 340(45/1), 55-64.

Oświadczam, że mój udział w pracy polegał na przeprowadzeniu analiz biochemicznych w przygotowanych przez Arletę Kruczek próbach roślinnych (zawartość proliny oraz MDA).

Wkład mojej pracy w w/w publikację oceniam na 10%.

**Wkład pozostałych współautorów:**
Marcelina Krupa-Małkiewicz – 40%
Arleta Kruczek – 30%
Justyna Pelc – 10%
Ireneusz Ochmian – 10%

Beata Smolik