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Uwarunkowania technologiczne w kształtowaniu jakości słodów specjalnych z nasion roślin strączkowych i potencjał ich wykorzystania w przemyśle spożywczym

Technological conditions in shaping the quality of special malts from legume seeds and the potential of their use in the food industry

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SPIS TREŚCI

STRESZCZENIE

Nasiona roślin strączkowych są jedną z kluczowych grup produktów roślinnych przeznaczonych do żywienia zarówno człowieka, jak i zwierząt hodowlanych. Nasiona te są jednym z najistotniejszych źródeł białka oraz energii w diecie człowieka. Niestety, nasiona te charakteryzują się wieloma wadami, które utrudniają wykorzystanie ich w różnych procesach wytwarzania żywności. Z tego względu coraz częściej testowane są różne metody przetwarzania nasion roślin strączkowych, mające na celu obniżenie w nich zawartości składników antyodżywczych, poprawę strawności, smaku i zapachu oraz ułatwienie obróbki mechanicznej i termicznej. Proces słodowania w przemyśle spożywczym jest aktualnie wykorzystywany głównie do przetwarzania nasion zbóż, przede wszystkim jęczmienia. W procesie słodowania wykorzystywany jest proces kiełkowania nasion, powodujący szereg zmian, zatem metoda ta może potencjalnie zostać wykorzystana również do modyfikacji nasion roślin strączkowych, co wskazuje na zasadność przeprowadzonych badań.

Celem badań było poddanie nasion roślin strączkowych procesowi słodowania i ocena technologiczna wytworzonych słodów, a także zbadanie możliwości wykorzystania słodów z nasion roślin strączkowych w technologii piwowarskiej. Wyniki badań zostały zaprezentowane w formie spójnego tematycznie cyklu publikacji naukowych, na który składa się pięć prac badawczych. W efekcie badań wykazano jakie zmiany powoduje w nasionach roślin strączkowych proces słodowania oraz jaka jest przydatność słodów z nasion roślin strączkowych w technologii piwowarskiej. Opracowano technologię wytwarzania słodu z soczewicy zielonej oraz sposób wytwarzania brzeczki z tego słodu, który umożliwia wyprodukowanie bezglutenowego piwa.

Na podstawie uzyskanych wyników określono, że słodowanie nasion roślin strączkowych takich jak: ciecierzyca, fasola, groch, groszek siewny, łubin, soczewica, soja i wyka w procesie zbliżonym do procesu produkcji słodu jęczmiennego typu pilzneńskiego nie pozwala na wytworzenie słodów o akceptowalnych parametrach technologicznych do wytworzenia brzeczki. Zastosowanie dodatku preparatów enzymatycznych zawierających amyloglukozydazę, proteazę, α-amylazę, β-glukanazę, celulazę, endopeptydazę, hemicelulazę i ksylanazę w niezadawalającym stopniu usprawniło proces wytwarzania brzeczki.

Wykazano jednak, że istnieje możliwość pozyskiwania brzeczek piwowarskich ze słodu pilzneńskiego z 30% dodatkiem słodów pozyskanych z nasion roślin strączkowych poddanych procesowi kleikowania przed zacieraniem.

Kolejna część badań obejmowała modyfikację procesu słodowania trzech odmian soczewicy oraz dwóch odmian fasoli oraz analizę parametrów technologicznych

wytworzonych słodów na podstawie zacierania kongresowego. Ponadto, w słodach i nasionach zbadano: kruchość, zawartość białka, błonnika, kwasu fitynowego oraz skrobi. Wykazano, że modyfikacja procesu słodowania w znacznym stopniu poprawia parametry technologiczne słodu z soczewicy zielonej oraz brązowej, takie jak czas filtracji i zawartość ekstraktu w brzeczce. Słody pozyskane z nasion roślin strączkowych charakteryzowały się mniejszą zawartością skrobi oraz kwasu fitynowego niż nasiona niesłodowane, natomiast zawartość białka w słodach była większa niż w nasionach przed procesem słodowania. Wszystkie słody cechowały się znacznie większą kruchością niż nasiona niesłodowane.

W kolejnym etapie badano, jak proces słodowania nasion oraz zacierania słodów wpływa na zawartość oligosacharydów z grupy rafinozy w słodach z soczewicy i fasoli oraz w uzyskanych brzeczkach. Słody wytworzone z nasion soczewicy oraz fasoli charakteryzowały się zmniejszoną zawartością rafinozy oraz stachiozy. Słodowanie zmniejszyło zawartość rafinozy w nasionach soczewicy o 80-96%, natomiast w nasionach fasoli o 68-78%. Zawartość stachiozy uległa redukcji w zbliżonym stopniu, o 79-95% w słodach z soczewicy i 63-79% słodach z fasoli.

W następnej publikacji opisano, w jaki sposób proces słodowania wpływa na zawartość i rodzaj związków lotnych w nasionach soczewicy. Analiza związków lotnych wykazała, że zawartość aldehydów w słodach z soczewicy jest większa niż w niesłodowanych nasionach, a wydłużanie czasu kiełkowania nasion ma wpływ na zwiększenie zawartości tych substancji. Proces słodowania redukował ilość alkoholi w nasionach soczewicy. Badania wykazały ponadto, że wyłącznie nasiona soczewicy zielonej charakteryzowały się znacznym udziałem terpenów (jak, na przykład, karen czy limonen) w ogólnej zawartości związków lotnych, a proces słodowania miał wpływ na redukcję ilości tych składników.

W badaniach przedstawionych w publikacji 5 przedstawiono usprawniony sposób słodowania wybranego surowca (soczewicy zielonej) oraz zmodyfikowano proces zacierania słodu, co umożliwiło wytworzenie bezglutenowego piwa o obniżonej zawartości alkoholu ze słodu z soczewicy zielonej. Opracowany procesu słodowania i zacierania umożliwił hydrolizę skrobi zawartej w słodzie i uzyskanie nowatorskiego, bezglutenowego piwa o obniżonej zawartości alkoholu ze słodu z soczewicy.

W artykułach stanowiących jednotematyczny cykl publikacji przedstawiono proces słodowania nasion roślin strączkowych i jego wpływ na nasiona roślin strączkowych, różne parametry fizykochemiczne i podstawowe cechy technologiczne uzyskanych słodów. Wskazano także na możliwość zastosowania słodu z soczewicy zielonej w technologii piwowarskiej. Wyniki uzyskane w trakcie prac eksperymentalnych wskazują również, że

wykorzystanie słodu z nasion roślin strączkowych może przynieść interesujące efekty dotyczące produkcji nowych rodzajów żywności o zmniejszonej zawartości składników antyodżywczych oraz zmodyfikowanym aromacie, co wskazuje na zasadność kontynuowania badań nad słodami z nasion roślin strączkowych w przyszłości.

ABSTRACT

Legume seeds are one of the key groups of plant products intended for feeding both humans and farm animals. These seeds are one of the most important sources of protein and calories in the human diet. Unfortunately, these seeds have many disadvantages that make them difficult to use in various food production processes. For this reason, various methods of processing legume seeds are increasingly being tested to reduce their content of anti-nutritional ingredients, improve their digestibility, taste and smell, and facilitate mechanical and thermal processing. The malting process in the food industry is currently used mainly for processing cereal seeds, primarily barley. The malting process uses the seed germination process, so it can potentially also be used to modify legume seeds, which indicates the validity of the research. The aim of the research was to subject legume seeds to the malting process and technological assessment of the produced malts, as well as to examine the possibility of using malts from legume seeds in brewing technology, as beer production is the most important sector of the food industry that uses malt. The research results were presented in the form of a thematically coherent series of scientific publications, which consists of five research papers. As a result, the research showed what changes the malting process causes in legume seeds and the usefulness of legume seed malts in brewing technology. A technology for producing malt from green lentils and a method for producing wort from this malt were developed, which allows the production of gluten-free beer. Based on the results obtained, it was determined that malting of legume seeds such as: chickpeas, beans, peas, grass pea, lupin, lentils, soybeans, and vetch in a process similar to the process of producing Pilsner-type barley malt does not allow for the production of malts with acceptable technological parameters to produce wort. The use of the addition of enzyme preparations containing amyloglucosidase, protease, α-amylase, β-glucanase, cellulase, endopeptidase, hemicellulase and xylanase did not satisfactorily improve the wort production process. However, it has been shown that it is possible to obtain brewing worts from Pilsner malt with a 30% addition of malt obtained from legume seeds subjected to the gelatinization process before mashing. The next part of the research included modification of the malting process of three varieties of lentils and two varieties of beans and analysis of technological parameters of the produced malts based on congress mashing. In addition, malt and seeds were tested for tenderness, protein, fiber, phytic acid, and starch content. It has been shown that modifying the malting process significantly improves the technological parameters of green and brown lentil malt, such as filtration time and extract content in the wort. Malts obtained from legume seeds had a lower starch and phytic acid content than unmalted seeds, while the protein content in malts

was higher than in seeds before the malting process. All malts were much more brittle than unmalted seeds. Subsequent studies analyzed how the process of seed malting and malt mashing affects the content of raffinose oligosaccharides in lentil and bean malts and in the obtained worts. Malts made from lentil and bean seeds were characterized by a reduced content of raffinose and stachyose. Malting reduced the raffinose content in lentil seeds by 80-96% and in bean seeds by 68-78%. The content of stachyose was reduced to a similar extent, by 79-95% in lentil malts and 63-79% in bean malts. The next publication describes how the malting process affects the content and type of volatile compounds in lentil seeds. The analysis of volatile compounds showed that the aldehyde content in lentil malts is higher than in unmalted seeds, and extending the seed germination time increases the content of these substances. The malting process reduced the amount of alcohol in lentil seeds. The research also showed that only green lentil seeds were characterized by a significant share of terpenes (such as carene or limonene) in the content of volatile compounds, and the malting process had an impact on the reduction of the content of these ingredients.

In fifth publication, an improved method of malting and modified mashing process, which enabled the production of gluten-free beer with a reduced alcohol content from green lentil malt was presented. The developed malting and mashing process allowed to hydrolyze the starch contained in the malt and obtain an innovative, gluten-free beer with a reduced alcohol content from lentil malt.

The articles constituting a single-topic series of publications present the process of malting legume seeds and the impact of this process on legume seeds, various physicochemical parameters, and basic technological features of the obtained malts. The possibility of using green lentil malt in brewing technology was also indicated. The results obtained during experimental work also indicate that the use of malt from legume seeds may bring interesting results in the production of new types of food with reduced nutritional content and modified aroma, which indicates the validity of continuing research on malt from legume seeds in the future.

1. WPROWADZENIE

Słodowanie to sposób modyfikacji nasion roślin, polegający na namaczaniu nasion, po którym następuje wielodniowy proces kiełkowania, który zatrzymywany jest poprzez suszenie wstępnie podkiełkowanych nasion (Kunze, 2019). Głównymi celami procesu słodowania jest: zwiększenie aktywności enzymatycznej (przede wszystkim amylolitycznej oraz proteolitycznej), zmiana struktury nasion (zwiększenie kruchości, ułatwiające rozdrabnianie bielma ziarna) oraz, w mniejszym stopniu, modyfikacja smaku, zapachu oraz barwy nasion (Palmer, 2017). Słód jest wykorzystywany głównie do wytwarzania brzeczki, niezbędnej do produkcji jednego z najbardziej popularnych napojów na świecie, jakim jest piwo (Kunze, 2019). Niemniej jednak słód może być wykorzystywany przez inne gałęzie przemysłu do wytwarzania ekstraktów słodowych oraz do produkcji whisky czy do wytwarzania mieszanek do wypieku pieczywa, słodyczy i podłoży mikrobiologicznych (Yang, 2021; Tricase i in., 2018; Mallet, 2014; Schwarz & Li, 2011, Briggs, 1998).

Przemysł słodowniczy wytwarza bardzo wiele rodzajów słodów, jednak głównym substratem do wytwarzania słodu są ziarna zbóż (nasiona roślin wiechlinowanych), od wielu tysiącleci wykorzystywane przez ludność zamieszkującą Europę, Afrykę czy Azję do produkcji piwa (Briggs, 1998). Najbardziej popularnym substratem do produkcji słodu są nasiona gatunków jęczmienia (*Hordeum vulgare* oraz *Hordeum distichon*), które od czasów neolitycznych selekcjonowane są przede wszystkim, aby zwiększyć ich przydatność dla przemysłu słodowniczego i piwowarskiego (Meussdoerffer, 2009). Słody z ziaren zbóż innych niż jęczmień albo nasion pseudozbóż, takich jak: pszenica, żyto, sorgo, owies, ryż czy gryka, najczęściej stanowią tylko dodatek do zasypu, składającego się w większości ze słodu jęczmiennego, wykorzystywanego w produkcji piwa, mający zmienić smak i zapach finalnego produktu (Yang i Gao, 2022; Kunze, 2019; Cioch-Skoneczny i in., 2019 Bogdan i Kordialik-Bogacka, 2017; Briggs, 1998). Piwa produkowane z dodatkiem słodów innych niż jęczmienne stanowią charakterystyczne produkty regionalne, takie jak piwo pszeniczne *Hefeweizen* z Niemiec, *Oatmeal stout* z Wielkiej Brytanii, czy żytnio-pszeniczne piwo *Schöps* z Wrocławia (Webb i Beaumont, 2016; Meussdoerffer i Zarnkow, 2014; Patterson i Hoalst-Pullen, 2014).

Słody można klasyfikować na różne rodzaje, jednak jednym z najbardziej podstawowych jest podział słodów na tak zwane „słody podstawowe" i „słody specjalne" (Kunze, 2019, Briggs, 1998). Słody podstawowe, takie jak na przykład słód pilzneński czy słód monachijski, mają wystarczającą aktywność amylolityczną, aby w procesie zacierania skrobia w nich zawarta uległa hydrolizie. Słody specjalne, takie jak słód karmelowy albo słód

czekoladowy, charakteryzują się zbyt niską aktywnością enzymatyczną i mogą być stosowane tylko jako dodatek do produkcji piwa, w połączeniu ze słodami podstawowymi, które zapewnią odpowiednią aktywność enzymatyczną niezbędną do hydrolizy skrobi w zacierze zawierającym mieszaninę słodów podstawowych i słodów specjalnych (Prado i in., 2021). Słody specjalne są najczęściej wykorzystywane jako dodatek mający zmodyfikować smak, zapach oraz barwę brzeczki, z której wytwarzane jest piwo (Castro i in., 2021).

Różnorodność dostępnych słodów wynika nie tylko ze zróżnicowania nasion poddawanych procesowi słodowania, ale również ze względu na modyfikacje parametrów procesu wytwarzania słodu (Briggs, 1998). Za najbardziej tradycyjny, wzorcowy schemat słodowania przyjmuje się proces produkcji najbardziej rozpowszechnionego na świecie słodu, czyli słodu typu pilzneńskiego (wytwarzanego z nasion jęczmienia) (Kunze, 2019). Proces namaczania niezbędny jest, aby zwiększyć zawartość wody w nasionach i wzbudzić w nich metaboliczne procesy życiowe, niezbędne do rozpoczęcia procesu kiełkowania (Montanuci i in., 2017). Z punktu widzenia przemysłu słodowniczego, najbardziej korzystnym ekonomicznie byłby proces namaczania, trwający jak najkrócej i wykorzystujący jak najmniejszą ilość wody. Niemniej jednak nasiona potrzebują określonej ilości wody i posiadają ograniczone tempo jej poboru, ponadto do wzbudzenia procesów życiowych potrzebują również tlenu (proces oddychania) (Briggs, 1998). Z tego względu często wykorzystuje się procesy namaczania wodno-powietrznego, podczas których to nasiona są na przemian zanurzane w wodzie oraz przetrzymywane w wilgotnej atmosferze, z której mogą pobierać tlen. Zbyt długie przechowywanie nasion całkowicie zanurzonych w wodzie może spowodować śmierć zarodka spowodowaną brakiem tlenu (Palmer, 2017). Kluczowe dla wytworzenia słodu jest również podniesienie zawartości wody do odpowiedniego poziomu (Cu i in., 2016; Holopainen i in., 2014). W pierwszym etapie namaczania zawartość wody wzrasta przede wszystkim w zarodku nasiona, co powoduje wydzielenia giberelin i auksyn, fitohormonów niezbędnych do rozpoczęcia procesu kiełkowania. Jednakże uwodnienie zarodka nie gwarantuje, że pozostałe tkanki ziarna (na przykład komórki bielma), osiągnęły wystarczającą wilgotność, która będzie niezbędna do zmodyfikowania ziarna przez enzymy w celu uzyskania słodu o oczekiwanej jakości. (Kunze, 2019; Cozzolino i in., 2013; Briggs, 1998). Zbyt niska wilgotność wytwarzanego słodu może sprawić, że część bielma w wyprodukowanym słodzie nie ulegnie przemianom biochemicznym w stopniu niezbędnym do wytworzenia słodu o właściwych parametrach.

Z drugiej strony, nadmierne wydłużenie procesu namaczania nasion może mieć wpływ na zbyt daleko posunięte modyfikacje składników słodu, co może skutkować uzyskaniem

słodu o, na przykład, zbyt ciemnej barwie. Nadmiernie szybki pobór wody może powodować poważne uszkodzenia tkanek kiełkującego nasiona z powodu tak zwanego „*imbibition damage*", czyli zbyt szybkiego uwodnienia i zmiany rozmiaru niektórych komórek, co może mieć wpływ na pękanie połączeń pomiędzy ścianami komórkowymi i uniemożliwić prawidłowy proces kiełkowania lub spowodować śmierć zarodka (Doria i in., 2019; De Ron, 2015). Ponadto, większa zawartość wody w namaczanych nasionach wydłuża proces suszenia słodu. Proces namaczania nasion w słodowniach, zależnie od rodzaju surowca oraz rodzaju produkowanego słodu, trwa typowo do 12-48 godzin (Briggs, 1998).

Po procesie namaczania, wilgotne nasiona przenoszone są do zbiorników (np. skrzyń Saladina czy bębnów Gallanda), w których przeprowadzany jest proces kiełkowania. Podczas procesu kiełkowania wytwarzane i uaktywaniane są enzymy celulolityczne, proteolityczne oraz amylolityczne, które umożliwiają rozłożenie składników zapasowych zgromadzonych, w przypadku zbóż, w bielmie i wykorzystanie wytworzonych cukrów oraz aminokwasów jako źródła energii i składników niezbędnych do wytworzenia liścieni oraz korzeni (Palmer, 2017; Briggs, 1998). Ponadto, prowadząc proces kiełkowania, należy mieć na uwadze, że nasiona zużywają część z substancji zapasowych zawartych w nasionach (na przykład skrobi), a ich zawartość jest istotna dla większości rodzajów słodu i przekłada się właściwości technologiczne produktu gotowego (Farzenah i in., 2017; Briggs, 1998). Głównymi parametrami, które można modyfikować podczas procesu kiełkowania w słodownictwie jest czas oraz temperatura. Wydłużenie czasu kiełkowania zwiększa straty suchej substancji w gotowym słodzie (ze względu na wydłużenie procesów metabolicznych i dłuższy okres wykorzystywania substancji zapasowych). Podobny efekt przynosi zwiększanie temperatury podczas procesu kiełkowania nasion, gdyż w wyższej temperaturze przyspiesza metabolizm organizmów żywych i zużywają one więcej energii (Tura i in., 2020). Z drugiej strony, zwiększenie temperatury procesu kiełkowania ma wpływ na rozluźnienie struktury bielma w nasionach zbóż, co może przekładać się na uzyskanie słodu lepszej jakości (Quek i in., 2019; Briggs, 1998). Zbyt wysoka temperatura jest niekorzystna ze względu na przyspieszenie rozwoju drobnoustrojów (grzybów czy bakterii), które mogą być obecne na kiełkujących nasionach. Może to skutkować uzyskaniem produktu niezdatnego do spożycia na przykład, z powodu nagromadzenia mykotoksyn), o niewłaściwych parametrach technologicznych czy nieodpowiednim smaku i aromacie (Justé i in. 2011). Zbyt niska temperatura natomiast wydłuża lub całkowicie zatrzymuje proces kiełkowania nasion, dlatego też istotne jest, aby w procesie słodowania dobierać odpowiednią temperaturę oraz czas kiełkowania niezbędny do wytworzenia słodu o oczekiwanej jakości (Kunze, 2019; Hosseini

i in., 2017). Podczas wytwarzania najpopularniejszego słodu, słodu pilzneńskiego, nasiona jęczmienia kiełkuje się przez ok. 120h (5 dni) w temperaturze ok. 15°C (Kunze, 2019; Briggs, 1998).

Po procesie kiełkowania, zawartość wody w nasionach musi zostać obniżona w celu zakończenia procesu słodowania. Nasiona mogą być poddane suszeniu lub prażeniu, w zależności od rodzaju słodu, który ma zostać wytworzony. W przypadku wytwarzania słodów podstawowych, istotne jest, aby proces suszenia zachodził w temperaturze, która nie będzie w istotny sposób zmniejszać aktywności enzymów obecnych w kiełkujących nasionach (Kunze, 2019, Briggs, 1998). Ponadto, wiele słodów podstawowych cechuje się jasnym kolorem, zatem temperatura procesu suszenia, szczególnie w pierwszym jego stadium, (kiedy zawartość wody w nasionach jest największa) powinna być odpowiednio niska, aby nie nasilać reakcji Maillarda i karmelizacji (Wang i in., 2023; Hellwig & Henle, 2020). Obniżenie temperatury wydłuża proces suszenia, który nie może być zbyt długi, gdyż większość funkcjonujących słodowni jest wyposażona jeden piec suszarniczy, zatem suszenie danej partii słodu powinno zakończyć szybciej niż w przeciągu 24h. Jest to niezbędne do zachowania ciągłej pracy słodowni, które typowo operują przez 365 dni w roku, przez 24h godziny na dobę (Kunze, 2019; Mallet, 2014; Cook, 2013; Briggs, 1998). Słody karmelowe oraz czekoladowe są suszone w inny sposób. Kiełkujące nasiona przenoszone są do prażarek rotacyjnych (często posiadających zamknięty obieg wody oraz dopływ wody z zewnątrz) i poddawane są procesowi prażenia (Yang, 2023; Mallet, 2014). Typowo, na początku prażenia, kiedy zawartość wody w nasionach jest duża, dobierana jest temperatura, która umożliwia działanie enzymom amylolitycznym i proteolitycznym, dzięki którym uwalniane są cukry proste oraz aminokwasy. Następnie temperatura w prażarce jest podnoszona, co umożliwia i przyspiesza zachodzenie reakcji Maillarda i karmelizacji. Procesy te w znaczącym stopniu modyfikują barwę, smak oraz zapach wytworzonego słodu (Parr i in., 2021; Yahya i in., 2014; Mäkinen i Arendt, 2012). Po procesie suszenia słód poddawany jest procesowi odkiełkowywania oraz pakowany w worki (Mallet, 2014; Briggs, 1998).

Opisany proces słodowania stosowany jest przede wszystkim przy produkcji słodu z ziaren zbóż, a w szczególności jęczmienia, jednak wszystkie przedstawione procesy technologiczne jak i biologiczne (metaboliczne) są uniwersalne nie tylko dla nasion roślin z rodziny wiechlinowatych (*Poaceae*), ale również dla innych rodzin (Kunze, 2019; Mohr i Schopfer, 2012; Briggs, 1998).

Rośliny strączkowe (bobowate, motylkowate, *Fabacea*) to bardzo zróżnicowana rodzina roślin, uprawianych na całym świecie w celu wykorzystania nasion w przemyśle

spożywczym oraz paszowym. Rośliny te charakteryzują się wieloma zaletami w uprawie. Dzięki bakteriom nitryfikacyjnym żyjącym z nimi w symbiozie w trakcie ich uprawy wiązany jest azot atomsferyczny, co może zmniejszać wykorzystanie nawozów sztucznych przez rolników (Foyer i in., 2019; Stagnari i in., 2017). Ponadto, wiele z gatunków roślin strączkowych cechuje się dużą odpornością na choroby wywoływane przez grzyby, bakterie i wirusy porażające rośliny uprawne, co może przekładać się na zmniejszenie wykorzystania pestycydów w uprawach (Kankanala i in., 2019). Wiele z tych roślin (soja, soczewica, fasola, ciecierzyca, groch) stanowi istotny element tradycyjnych produktów spożywczych produkowanych oraz spożywanych na niemalże każdym kontynencie (Szczebyło i in., 2019; De Ron, 2015). Nasiona te charakteryzują się dużą wartością energetyczną, dużą zawartością białka, a niektóre z nich cechują się znaczną zawartością tłuszczu (w tym wielonienasyconych kwasów tłuszczowych) (Caprioli i in., 2016).

Niestety, wykorzystanie nasion roślin strączkowych w technologii żywności może sprawiać wiele problemów. Ze względu na dość grubą łupinę nasienną są trudne do mielenia i wymagają namaczania przed długim procesem gotowania (Fabbri i Cosby, 2016; De Ron, 2015; Polak i in., 2015). Ponadto, w nasionach roślin strączkowych znajduje się wiele substancji antyodżywczych, takich jak: lektyny, kwas fitynowy, skondensowane taniny, saponiny, alkaloidy, oligosacharydy z grupy rafinozy, inhibitory enzymów trawiennych czy inne (Sharma, 2021). Wielu konsumentom nie odpowiada również smak i zapach nasion roślin strączkowych oraz różnych produktów z nich wytwarzanych (Senanayake i in., 2023; Chigwedere i in., 2022; Summo i in., 2016). Słodowanie modyfikuje w znaczący sposób smak i zapach nasion zbóż, zatem najprawdopodobniej smak i zapach słodowanych nasion roślin strączkowych również uległby zmianie po procesie słodowania, jednak w literaturze brak jest dostępnych danych na ten temat (Almaguer i in., 2023a; Almaguer i in., 2023b; Gasiński i in., 2022). Wiadomo, że w trakcie słodowania nasion zbóż, wskutek naturalnych procesów metabolicznych zmniejszeniu ulega ilość niektórych substancji antyodżywczych, takich jak, na przykład, kwas fitynowy (Ojha i in., 2018). W związku z tym słodowanie może mieć pozytywny wpływ na zmniejszenie zawartości składników antyodżywczych również w nasionach roślin strączkowych (Baranwal i in. 2014).

Zarówno spożycie roślin strączkowych jak i technologia słodowania są rozpowszechnione na całym świecie. Ponadto w dostępnej literaturze brakuje opracowań dotyczących wytwarzania słodu z nasion roślin strączkowych i potencjalnego zastosowania tych słodów w produkcji żywności. Ocena zmian zachodzących w nasionach roślin strączkowych podczas słodowania jest zatem interesująca zarówno z naukowego jak

i aplikacyjnego punktu widzenia. Nie jest również znane potencjalne wykorzystanie słodów z nasion roślin strączkowych w żadnej z gałęzi przemysłu spożywczego. Branża piwowarska, wykorzystująca większość słodu produkowanego na świecie, nie wytwarza obecnie żadnych napojów fermentowanych wytwarzanych z słodów z nasion roślin strączkowych. Badanie składu słodów z nasion roślin strączkowych oraz wpływu technologii słodowania może spowodować rozszerzenie wiedzy dotyczącej procesów zachodzących w nasionach oraz wpłynąć na zwiększenie asortymentu dostępnych produktów spożywczych, co wskazuje na fakt, że temat pracy stanowi ciekawy i innowacyjny kierunek badawczy (Carbas i in., 2023; Sonta & Rekiel, 2020).

2. CEL PRACY I HIPOTEZY BADAWCZE

Celem pracy było zbadanie możliwości słodowania nasion roślin strączkowych i analiza składu oraz parametrów technologicznych uzyskanych słodów oraz opracowanie technologii wykorzystania słodów z nasion roślin strączkowych w technologii piwowarskiej.

Hipotezy badawcze

1. Możliwe jest uzyskanie słodu z nasion roślin strączkowych w warunkach zbliżonych do parametrów procesu produkcji słodu jęczmiennego typu pilzneńskiego i wykorzystanie ich do pozyskiwania brzeczki w procesie zacierania kongresowego.
2. Dodatek preparatów enzymatycznych podczas zacierania kongresowego słodów z nasion roślin strączkowych usprawnia proces pozyskiwania brzeczki.
3. Zmiana parametrów procesu słodowania nasion roślin strączkowych ma wpływ na poprawę parametrów technologicznych wytworzonych z nich brzeczek.
4. Proces słodowania ma wpływ na zmniejszenie zawartości składników antyodżywczych takich jak kwas fitynowy czy oligosacharydy z grupy rafinozy w słodach z nasion roślin strączkowych.
5. Proces słodowania ma wpływ na modyfikację rodzaju i zawartości związków lotnych w słodach z nasion roślin strączkowych.
6. Możliwe jest wytworzenie bezglutenowego piwa ze słodu z nasion roślin strączkowych wykorzystując infuzyjną metodę produkcji brzeczki piwowarskiej.

3. POSTĘPOWANIE EKSPERYMENTALNE

Założenia badawcze pracy zweryfikowano w oparciu o analizę i interpretację wyników, które zostały uzyskane na podstawie przeprowadzonych eksperymentów laboratoryjnych. Wyniki badań opublikowano w jednotematycznym, powiązanym cyklu pięciu publikacji naukowych wydanych w czasopismach z listy Journal Citation Reports (JCR). Cykl badań został przedstawiony na schemacie nr 1.

Eksperyment 1:
Słodowanie wybranych nasion roślin strączkowych w technologii produkcji słodu typu pilzneńskiego

Eksperyment 2:
Słodowanie nasion fasoli i soczewicy w technologii dostosowanej do fizjologii nasion roślin strączkowych

Eksperyment 3:
Analiza zawartości oligosacharydów z grupy rafinozy w nasionach i słodach z fasoli i soczewicy

Eksperyment 4:
Analiza zmian w zawartości związków lotnych w nasionach soczewicy pod wpływem słodowania

Eksperyment 5:
Słodowanie nasion soczewicy zielonej wykorzystujące kiełkowanie w obniżającej się temperaturze oraz wytwarzanie piwa ze słodu z soczewicy zielonej

Rysunek 1. Przebieg eksperymentów.

3.1. Materiał badawczy

Materiał roślinny:

- Nasiona soczewicy jadalnej (*Lens culinaris*) odmiany Eston o zielonej łupinie nasiennej.
- Nasiona soczewicy jadalnej (*Lens culinaris*) odmiany Firat 87 o brązowej łupinie nasiennej.
- Nasiona soczewicy jadalnej (*Lens culinaris*) odmiany Beluga o czarnej łupinie nasiennej.
- Nasiona wyki siewnej (*Vicia* sativa) odmiany Hanka.
- Nasiona fasoli zwykłej (*Phaseolus vulgaris*) odmiany Kreacja o czerwonej łupinie nasiennej.
- Nasiona fasoli zwykłej (*Phaseolus vulgaris*) odmiany Biały Jaś o białej łupinie nasiennej.
- Nasiona grochu zwyczajnego (*Pisum sativum*) odmiany Ambrosia o żółtej łupinie nasiennej.
- Nasiona ciecierzycy pospolitej (*Cicer arietinum*) odmiany Kabuli.
- Nasiona soi warzywnej (*Glycine max*) odmiany Aurelina.
- Nasiona łubinu wąskolistnego (*Lupinus angustifolius*) odmiany Agat.
- Nasiona groszku siewnego (*Lathyrus sativus*) odmiany Astronaute
- Słód jęczmienny typu pilzneńskiego wyprodukowany przez firmę Viking Malt (Strzegom, Polska).
- Granulat chmielowy typu T90 wytworzony z odmiany chmielu (*Humulus lupulus*) odmiany Marynka.

Materiał biologiczny:

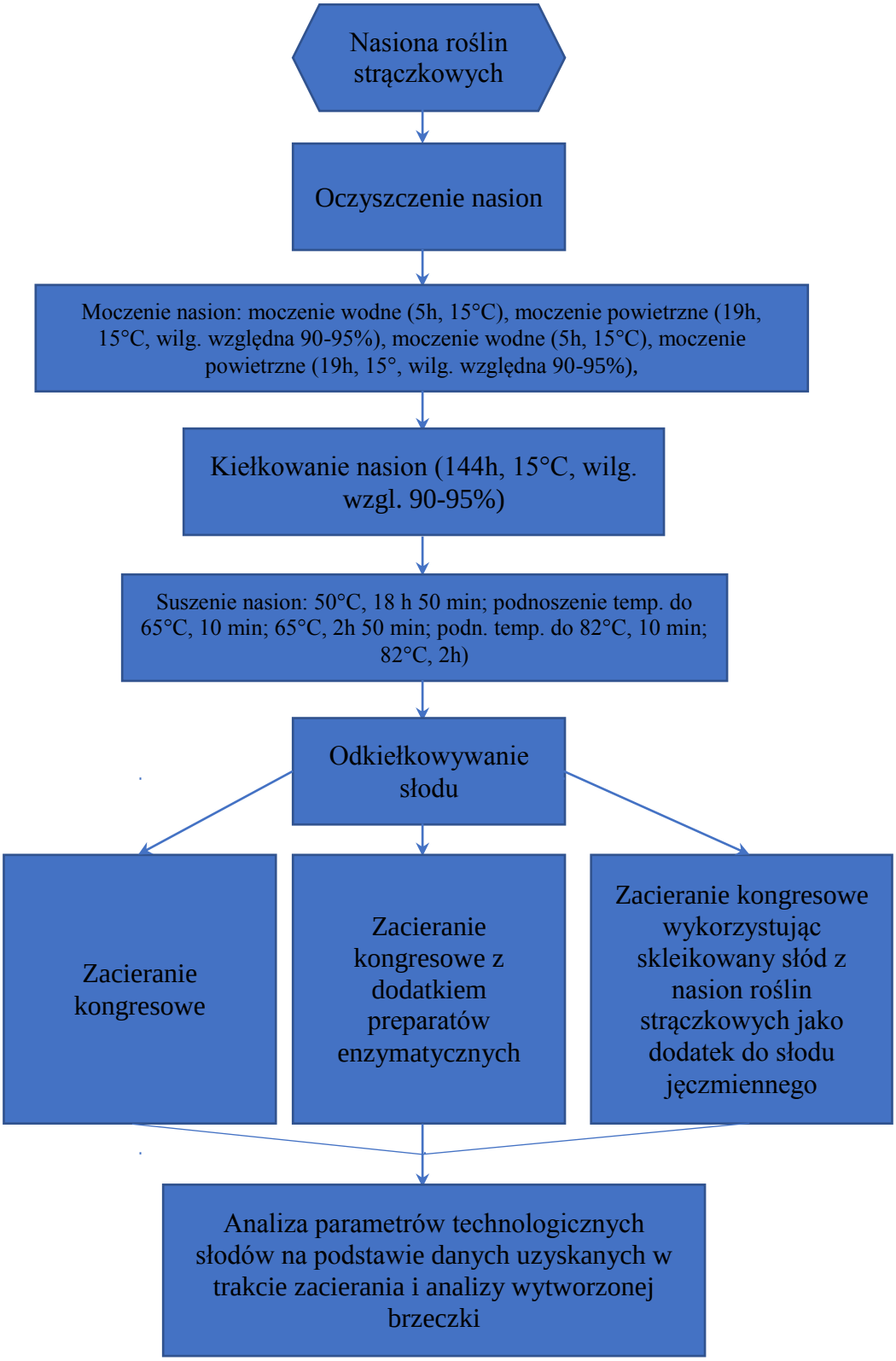
- drożdże *Saccharomyces cereviasie* - S-04, Fermentis (Lesaffre, Francja) - sugerowana przez producenta temperatura fermentacji w zakresie 18-26°C, stopień odfermentowania 74-82%, zalecane dawkowanie w ilości 50-80g na hektolitr brzeczki.

Preparaty enzymatyczne:

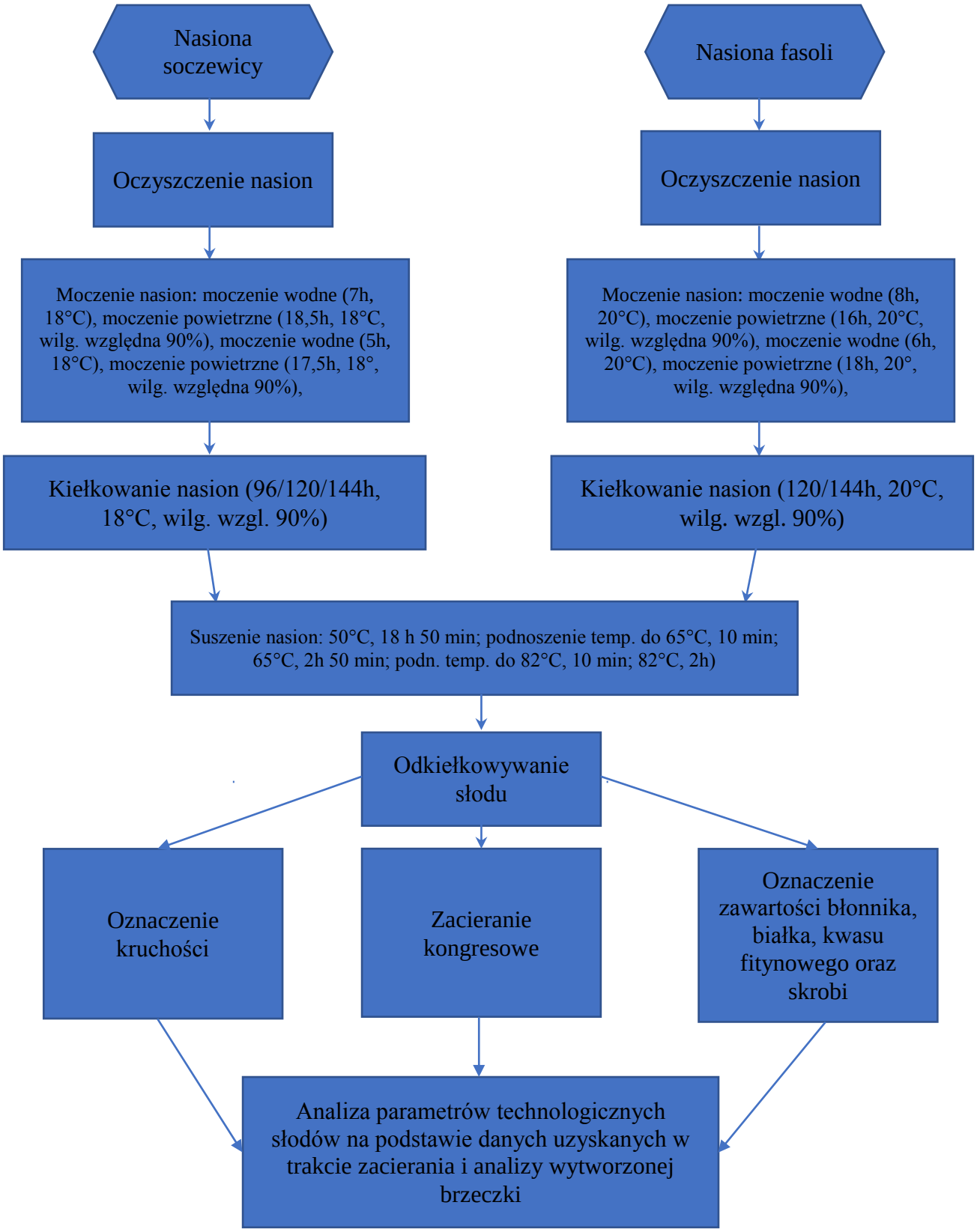
- Amigase Mega (amyloglukozydaza) (DSM Food Specialties) - sugerowane przez producenta dawkowanie: 1,2 cm^3 preparatu na 1 kg słodu.
- Filtrase BR-X (kompleks beta-glukanaz i hemicellulaz) (DSM Food Specialties) - sugerowane przez producenta dawkowanie: 200 mg preparatu na 1 kg słodu.

- Filtrase NL (kompleks beta-glukanaz i ksylanaz) (DSM Food Specialties) - sugerowane przez producenta dawkowanie: 600 mg preparatu na 1 kg słodu.
- Maxazyme NNP DS (proteaza) (DSM Food Specialties) - sugerowane przez producenta dawkowanie: 1 g preparatu na 1 kg słodu.
- Mats L Classic (alfa-amylaza) (DSM Food Specialties) - sugerowane przez producenta dawkowanie: 275 mg preparatu na 1 kg słodu.
- Mycolase LV (alfa-amylaza) (DSM Food Specialties) - sugerowane przez producenta dawkowanie: 40 mg preparatu na 1 kg słodu.
- Brewers Compass (alfa-amylaza, beta-glukanaza, cellulaza oraz eptopeptydaza) (DSM Food Specialties) sugerowane przez producenta dawkowanie: 4 g preparatu na 1 kg słodu.
- Termamyl 300 L (Sigma-Aldrich) - sugerowane przez producenta dawkowanie: 100-200 mm^3 preparatu na 1 kg słodu.

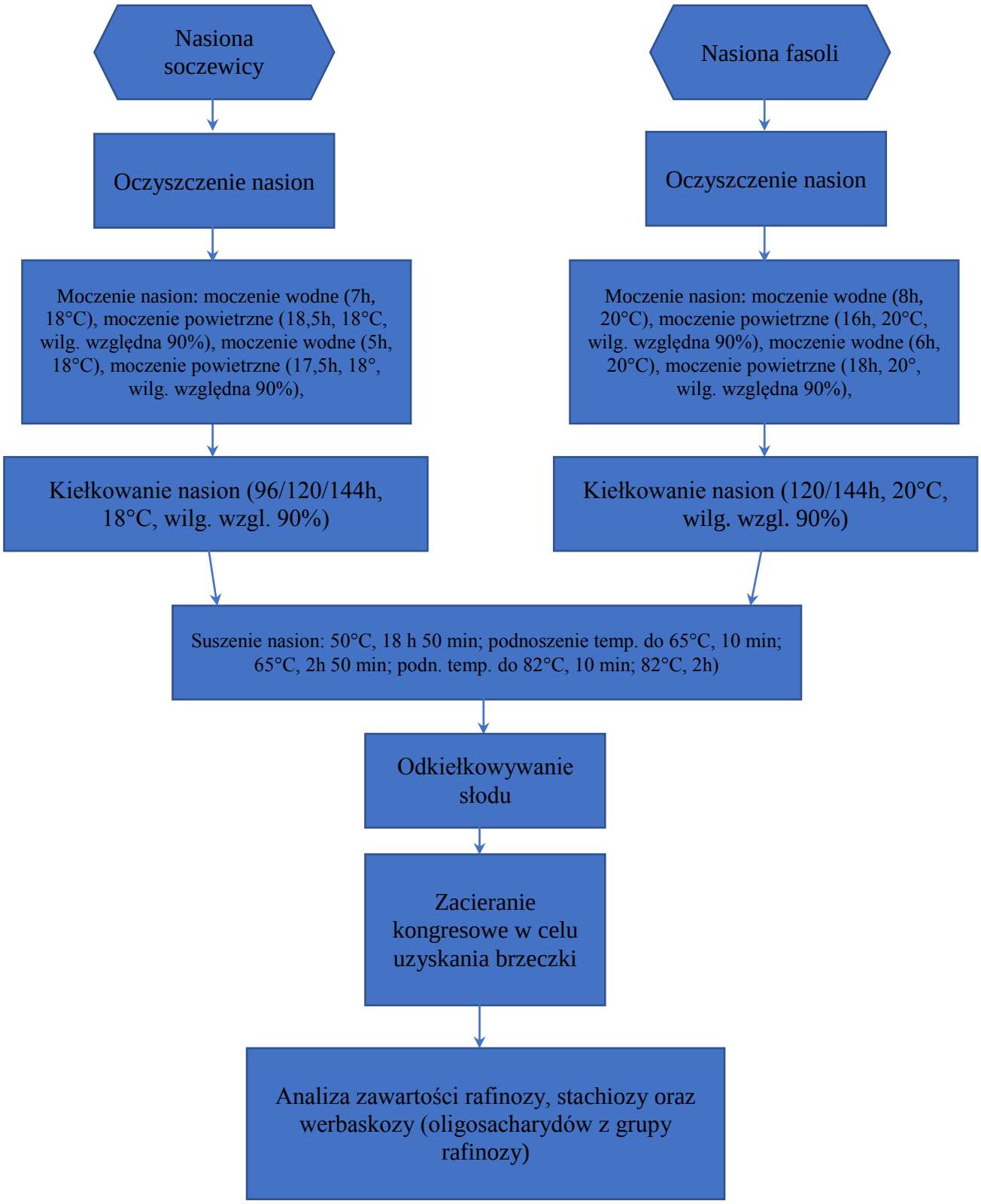
3.2. Modele eksperymentalne



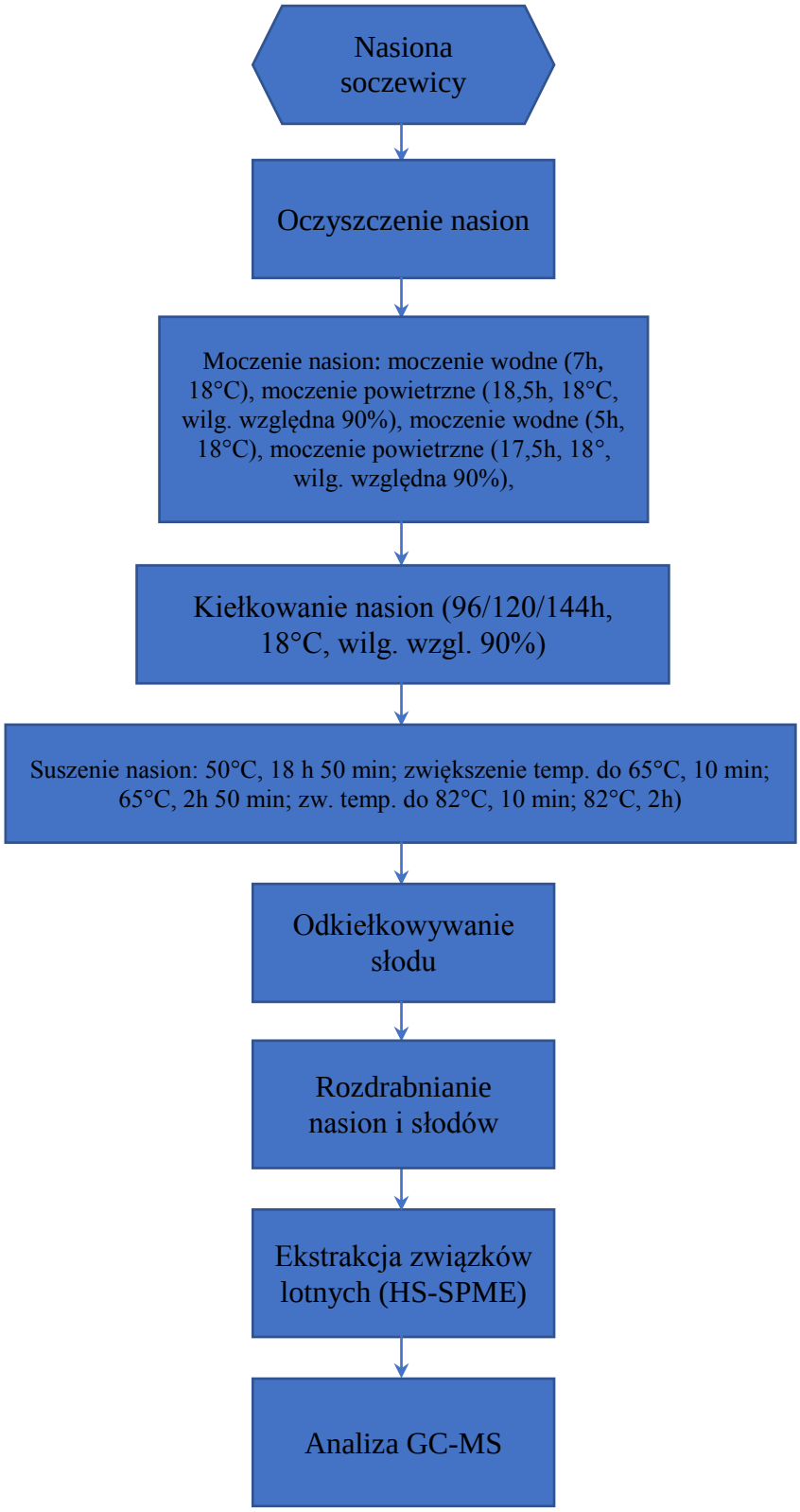
Rysunek 2. Model eksperymentalny badań przeprowadzonych w ramach eksperymentu nr 1



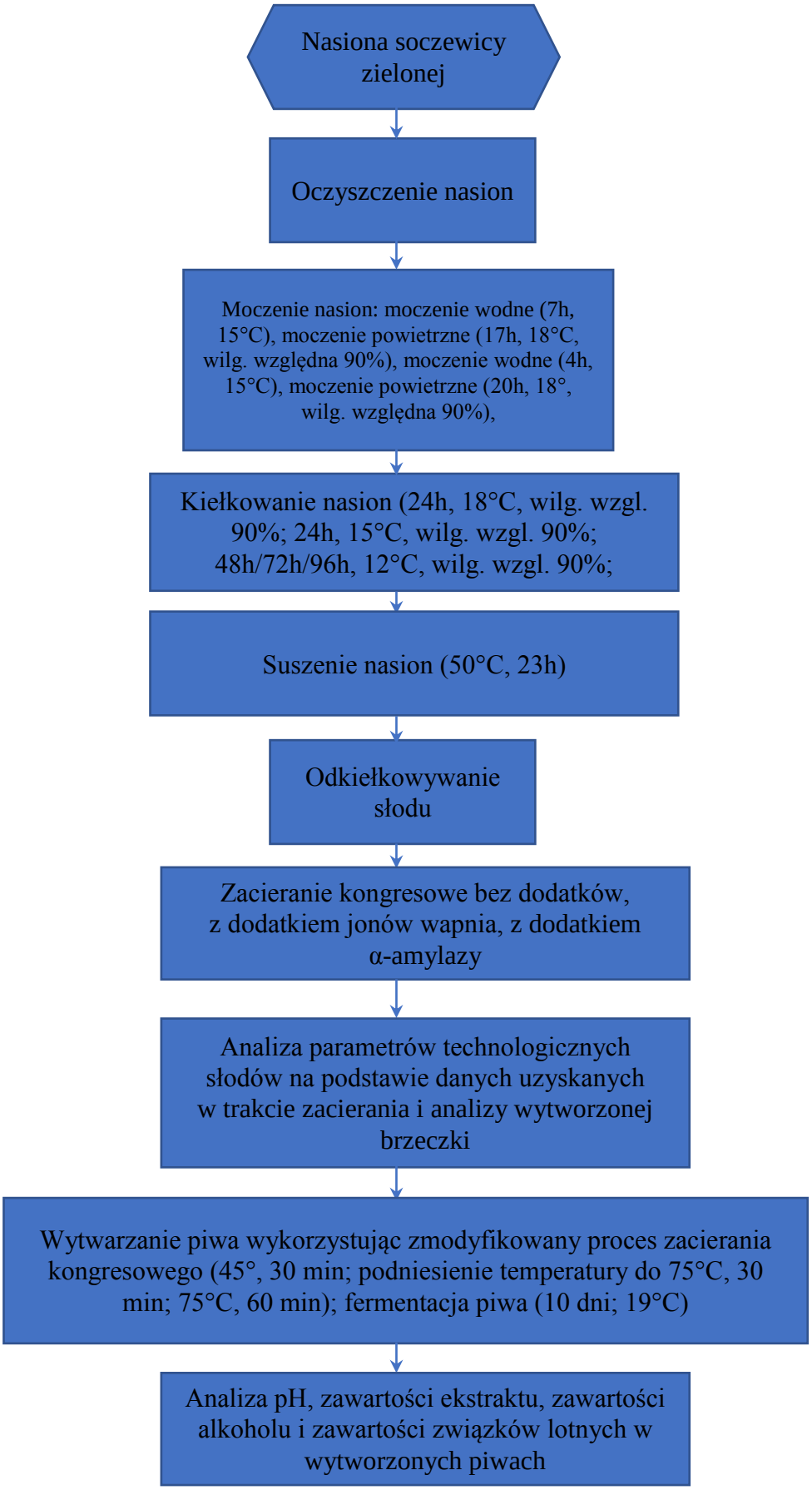
Rysunek 3. Model eksperymentalny badań przeprowadzonych w ramach eksperymentu nr 2



Rysunek 4. Model eksperymentalny badań przeprowadzonych w ramach eksperymentu nr 3



Rysunek 5. Model eksperymentalny badań przeprowadzonych w ramach eksperymentu nr 4



Rysunek 6. Model eksperymentalny badań przeprowadzonych w ramach eksperymentu nr 5

3.3. Metody badawcze oraz wykorzystywana aparatura laboratoryjna

W Tabeli 1 zaprezentowano stosowaną metodykę oraz wykonywane w trakcie eksperymentów opisanych w publikacji 1-5 analizy oraz wymieniono aparaturę, która została wykorzystana do przeprowadzania analiz.

Tabela 1. Metody badawcze, badane parametry oraz aparatura wykorzystywana do przeprowadzenia analiz w ramach eksperymentów 1-5.

Analiza laboratoryjna / badany parametr	Metoda	Aparatura
Analiza parametrów technologicznych słodów na podstawie zacierania kongresowego	Analytica EBC 4.5.1.: Zacieranie kongresowe	Zaciernica laboratoryjna Lochner Labor+Technik LB 12
Wartość pH	Potencjometryczna metoda pomiaru pH	pHmetr Mettler Toledo MP220
Zawartość ekstraktu w brzeczce	Oscylacyjne określenie gęstości roztworów	Densymetr oscylacyjny DMA 35 Anton Paar
Lepkość brzeczki	Analytica EBC 8.4.: Określenie lepkości brzeczki	Wiskozymetr KF 10 (Rheotec)
Kruchość słodu	Analytica EBC 4.15.: Kruchość, ilość ziaren szklistych i niezmodyfikowanych.	Friabilimetr Pfeuffer
Zawartość białka	Metoda Kjejdahla	Foss Tecator Kjeltec 2400
Zawartość skrobi	Metoda polarymetryczna	Polarymetr AA-55 (Optical Activity LTD)
Zawartość błonnika	Metoda grawimetryczna	Suszarka UF110 Plus oraz waga analityczna Sartorius
Zawartość kwasu fitynowego	Metoda spektrofotometryczna z wykorzystaniem	Czytnik mikropłytek Biotek Synergy H1

	odczynnika Wade'a	
Zawartość oligosacharydów z grupy rafinozy	Wysokosprawna chromatografia cieczowa powiązana z detektorem refraktometrycznym	Chromatograf cieczowy Hi-Plex H (Agilent) oraz detektor refraktometryczny (RID Agilent)
Zawartość związków lotnych w nasionach i słodach	Chromatografia gazowa sprzężona ze spektrometrią mas	Chromatograf gazowy GC-2010 Plus oraz spektrometr mas GCMS-QP2010 SE (Shimadzu)
Analiza parametrów fizykochemicznych piwa (gęstość, stopień odfermentowania, kaloryczność, barwa EBC)	Oscylacyjne określenie gęstości roztworów sprzężone ze spektroskopią w zakresie bliskiej podczerwieni (NIR)	Gęstościomierz oscylacyjny Anton Paar DMA 4500 M
Zawartość związków lotnych w piwie	Chromatografia gazowa sprzężona z detektorem płomieniowo-jonizacyjnym	Chromatograf gazowy GC-2010 Plus oraz detektor płomieniowo-jonizacyjny FID-2010 (Shimadzu)

3.4. Procesy technologiczne i wykorzystywana aparatura laboratoryjna

W Tabeli 2 zaprezentowano pozostałe procesy technologiczne wykonywane w trakcie eksperymentów opisanych w publikacjach nr 1-5 oraz wymieniono aparaturę, która była wykorzystana do przeprowadzenia tych procesów.

Tabela 2. Procesy technologiczne przeprowadzone w eksperymentach 1-5 oraz wykorzystywana do ich przeprowadzenia aparatura.

Proces technologiczny	Aparatura
Kiełkowanie namoczonych nasion	Komora fitotronowa KK 240 Smart Pro
Suszenie nasion po procesie kiełkowania	Suszarka Memmert UF110 Plus
Rozdrabnianie słodu	Młynek Bühler Miag DLFU
Rozdrabnianie nasion	Młynek IKA A10 Basic

4. WYKAZ PRAC TWORZĄCYCH JEDNOTEMATYCZNY CYKL PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

1. Gasiński, A., Błażewicz, J., Kawa-Rygielska, J., Śniegowska, J., & Zarzecki, M. (2021). Analysis of physicochemical parameters of Congress worts prepared from special legume seed malts, acquired with and without use of enzyme preparations. *Foods*, *10*(2), 304.

2. Gasiński, A., & Kawa-Rygielska, J. (2022). Mashing quality and nutritional content of lentil and bean malts. *LWT*, *169*, 113927.

3. Gasiński, A., Kawa-Rygielska, J., Mikulski, D., & Kłosowski, G. (2022). Changes in the raffinose family oligosaccharides content in the lentil and common bean seeds during malting and mashing processes. *Scientific Reports*, *12*(1), 17911.

4. Gasiński, A., & Kawa-Rygielska, J. (2023). Malting—A method for modifying volatile composition of black, brown and green lentil seeds. *Plos one*, *18*(9), e0290616.

5. Gasiński, A., & Kawa-Rygielska, J. (2024). Assessment of green lentil malt as a substrate for gluten-free beer brewing. *Scientific Reports*, *14*(1), 504.

Sumaryczne punkty MNiSW publikacji: **620**

Sumaryczny IF publikacji: **24,822**

5. OMÓWIENIE PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

Tematyka prac badawczych opublikowanych w ramach niniejszej rozprawy doktorskiej dotyczy procesu słodowania nasion roślin strączkowych. Wstępnie (publikacja nr 1) słodowano nasiona roślin strączkowych (takie jak fasola, soja, łubin, wyka siewna, groszek siewny soczewica, groch i ciecierzyca) w warunkach typowych dla produkcji jęczmiennego słodu typu pilzneńskiego, w celu selekcji nasion, które mogą stanowić najbardziej obiecujący surowiec do wytwarzania słodu. W publikacji 2 poddano słodowaniu nasiona soczewicy oraz fasoli w zmodyfikowanym procesie słodowania charakteryzującym się kiełkowaniem nasion o większej zawartości wody oraz wyższej temperaturze kiełkowania. Jako materiał badawczy zastosowano odmiany soczewicy zielonej, brązowej i czarnej oraz fasoli białej i czerwonej. W uzyskanych słodach z soczewicy oraz fasoli zbadano kruchość słodu, zawartość skrobi, białka, błonnika oraz kwasu fitynowego, a także oznaczono parametry technologiczne słodów na podstawie zacierania kongresowego. Uzyskane wyniki wskazały na zasadność przeprowadzenia szczegółowych analiz składu wytworzonych słodów. W publikacji 3 analizowano, jak proces słodowania nasion oraz zacierania słodów wpływa na zawartość oligosacharydów z grupy rafinozy w słodach z soczewicy i fasoli oraz w uzyskanych brzeczkach. W publikacji 4 przedstawiono wyniki dotyczące zawartości i rodzaju związków lotnych w nasionach oraz słodach z soczewicy. W badaniach przedstawionych w publikacji 5 przedstawiono usprawniony sposób słodowania wybranego surowca (soczewicy zielonej) oraz zmodyfikowano proces zacierania słodu, co umożliwiło wytworzenie bezglutenowego piwa o obniżonej zawartości alkoholu ze słodu z soczewicy zielonej.

W artykułach stanowiących jednotematyczny cykl publikacji przedstawiono proces słodowania nasion roślin strączkowych i wpływ tego procesu na nasiona roślin strączkowych, różne parametry fizykochemiczne i podstawowe cechy technologiczne uzyskanych słodów, a także wskazano na możliwość zastosowania słodu z soczewicy zielonej w technologii piwowarskiej.

Publikacja nr 1 wymieniona w rozdziale 5, wchodząca w skład jednotematycznego cyklu publikacji

W badaniach opisanych w pierwszej publikacji procesowi słodowania zostały poddane nasiona soczewicy jadalnej, ciecierzycy pospolitej, fasoli zwykłej, grochu zwyczajnego, wyki

siewnej, groszku siewnego, soi warzywnej oraz łubinu wąskolistnego. W celu oceny możliwości uzyskania słodów z nasion roślin strączkowych w procesie słodowania zastosowano technologię słodowania zbliżoną do metody wytwarzania najpopularniejszego słodu - słodu pilzneńskiego. Wykorzystano proces, w którym po namaczaniu zawartość wody w nasionach wynosiła 45%, kiełkowanie trwało 144 godzin (6 dni) w temperaturze 15°C, natomiast suszenie rozpoczęło się w temp. 50°C, zakończyło się w temp. 82°C i trwał krócej niż 24h (Palmer, 2017; Briggs, 1998). Ocena parametrów technologicznych wytworzonych słodów została wykonana w oparciu o metodę zacierania kongresowego zgodnie z metodyką Analytica EBC, umożliwiającą analizę takich parametrów jak: czas scukrzania, czas filtracji brzeczki, zawartość ekstraktu w brzeczce, objętość oraz pH brzeczki (Analytica EBC, 2010). Ponadto zastosowano dodatek siedmiu preparatów enzymatycznych takich jak Amigase Mega, Filtrase BR-X, Filtrase NL, Maxazyme NNP DS, Mats L Classic, Mycolase LV oraz Brewers Compass, zawierających grupy enzymów, takie jak: amyloglukozydazy, alfa-amylazy, beta-glukanazy, hemicellulazy, proteazy, czy endopeptydazy. Następnie wykorzystano zmodyfikowany proces zacierania kongresowego, aby określić, czy jest możliwe zastosowanie wybranych słodów po procesie kleikowania w ilości 30% jako dodatku do słodu pilzneńskiego w celu uzyskania brzeczki.

Analiza brzeczek kongresowych wykazała, że słody z nasion roślin strączkowych cechują się pogorszonymi parametrami w stosunku do typowego słodu pilzneńskiego. W trakcie zacierania słodów strączkowych nie uzyskano całkowitej hydrolizy skrobi (badanej na podstawie próby jodowej). Uzyskane brzeczki charakteryzowały się małą zawartością ekstraktu (1,59-5,51°Plato), małą objętością (35-150 mL) oraz długim czasem filtracji (wynoszącym 120 min dla wszystkich brzeczek) (Ribeiro-Filho i in., 2023; Kunze, 2019). Brzeczki wytworzone ze słodów z soi, fasoli, łubinu i groszku siewnego nie przefiltrowały się, co uniemożliwiło ich analizę. Kolejnym etapem badań było przeprowadzenie procesu zacierania słodów z nasion roślin strączkowych z dodatkiem preparatów enzymatycznych, gdyż jest to jedna z podstawowych metod na poprawę wielu parametrów uzyskanych brzeczek (Kunze, 2019; Gomaa, 2018; Sharma i in., 2016). Niestety, pomimo zastosowania dodatku preparatów enzymatycznych, filtracja brzeczek ze słodów z soi, fasoli, łubinu i groszku siewnego nie uległa poprawie, uniemożliwiając analizę brzeczek z tych surowców.

Zastosowanie preparatów enzymatycznych w trakcie zacierania słodów z soczewicy, grochu, ciecierzycy oraz wyki siewnej wpływało w większości przypadków na zwiększenie objętości brzeczki oraz zwiększenie ekstraktu brzeczki, jednakże nie spowodowało skrócenia czasu filtracji brzeczki. Preparaty Mats L Classic oraz Mycolase LV, zawierające alfa-

amylazę umożliwiły hydrolizę skrobi zawartej w słodzie z soczewicy. Preparat Mats L Classic (zawierający alfa-amylazę) oraz Amigase Mega (zawierający amyloglukozydazę) umożliwiły hydrolizę skrobi zawartej w słodzie z grochu, natomiast zastosowanie preparatów Maxazyme, Mats L Classic, Brewers Compass i Amigase Mega umożliwiło hydrolizę skrobi w procesie zacierania słodu z nasion wyki siewnej. Wszystkie preparaty enzymatyczne, z wyjątkiem Mycolase LV, miały wpływ na uzyskanie większej objętości brzeczki (o 15-32,5 mL) ze słodu z ciecierzycy niż w przypadku słodu z ciecierzycy zacieranego bez dodatku preparatów enzymatycznych (80 mL). Zastosowanie pięciu preparatów (Filtrase NL, Maxazyme NNP DS, Mats L Classic, Mycolase LV) podczas zacierania słodu z soczewicy umożliwiło uzyskanie większej objętości brzeczki (o 25-40 mL) w stosunku do brzeczki wytworzonej ze słodu z soczewicy zacieranego bez dodatku preparatów enzymatycznych (100 mL). Dodatek każdego z zastosowanych preparatów enzymatycznych do procesu zacierania słodu z grochu umożliwił uzyskanie większej o 20-40 mL ilości brzeczki w porównaniu do brzeczki uzyskanej ze słodu z grochu bez dodatku preparatów enzymatycznych (110 mL). W przypadku słodu z wyki siewnej, wyłącznie jeden z zastosowanych preparatów (Mycolase LV, zawierający alfa-amylazę), umożliwił uzyskanie większej ilości brzeczki (o 10 mL) niż podczas zacierania słodu z wyki bez dodatku preparatów enzymatycznych (115 mL). Natomiast zastosowanie czterech preparatów enzymatycznych (Filtrase BR-X, Filtrase NL, Maxaxyzme NNP DS, Mats L Classic) skutkowało uzyskaniem mniejszej ilości brzeczki (70-100 mL), niż w przypadku zacierania słodu z wyki bez dodatku preparatów enzymatycznych.

Zastosowanie dodatku preparatów enzymatycznych do procesu zacierania miało wpływ również na zmianę zawartości ekstraktu w brzeczkach uzyskanych ze słodów z nasion roślin strączkowych. Trzy z zastosowanych preparatów (Filtrase BR-X, Maxazyme NNP DS. oraz Mycolase LV) umożliwiły uzyskanie brzeczek charakteryzujących się zawartością ekstraktu większą o 0,21-3,31°Plato w stosunku do brzeczki ze słodu z ciecierzycy wytworzonej bez dodatku preparatów enzymatycznych (2,39°Plato). Każdy z wykorzystanych preparatów enzymatycznych umożliwił uzyskanie brzeczek ze słodu z soczewicy o zawartości ekstraktu większej o 0,74-1,81°Plato w stosunku do brzeczki uzyskanej bez dodatku preparatów enzymatycznych (1,59°Plato). Odmienną sytuację zaobserwowano w przypadku zacierania słodów z grochu. Dodatek jednego preparatu (Filtrase BR-X) umożliwił uzyskanie brzeczki o większej zawartości ekstraktu (o 0,3°Plato) w stosunku do brzeczki wytworzonej ze słodu z grochu bez dodatku preparatów (2,80°Plato), natomiast dodatek preparatu Mats L Classic skutkował uzyskaniem brzeczki charakteryzującej się mniejszą zawartością

ekstraktu (o 0,70°Plato) w stosunku do brzeczki wytworzonej bez dodatku preparatów enzymatycznych. Wykorzystanie pozostałych pięciu preparatów nie miało wpływu na modyfikację zawartości ekstraktu w stosunku do zawartości ekstraktu w brzeczce wytworzonej bez dodatku preparatów enzymatycznych. Sześć z zastosowanych preparatów enzymatycznych wykorzystanych w procesie zacierania słodu z wyki siewnej skutkowało uzyskaniem brzeczki charakteryzującej się mniejszą o 0,61-0,82°Plato zawartością ekstraktu od brzeczki wytworzonej bez dodatku preparatów enzymatycznych (2,40°Plato). Wyłącznie preparat Mats L Classic umożliwił uzyskanie brzeczki charakteryzującej się większą (o 0,40°Plato) zawartością ekstraktu.

Pomimo względnie niewielkich zmian w zawartości ekstraktu w brzeczkach uzyskanych podczas procesu zacierania z zastosowaniem dodatku preparatów enzymatycznych, część z wykorzystanych preparatów umożliwiła hydrolizę skrobi zawartej w słodach. Preparat Mats L Classic (zawierający alfa-amylazę) umożliwił hydrolizę skrobi w słodzie z soczewicy w ciągu 10 min, skrobi w słodzie z grochu w ciągu 25 min oraz skrobi w słodzie z wyki w ciągu 25 min zacierania w temperaturze 70°C. Preparat Mycolase LV (zawierający alfa-amylazę) umożliwił hydrolizę skrobi w słodzie z soczewicy w ciągu 10 min podczas procesu zacierania w temperaturze 70°C. Zastosowanie preparatu Amigase Mega (również zawierający alfa-amylazę) skutkowało hydrolizą skrobi w słodzie z grochu w ciągu 40 min oraz skrobi w słodzie z wyki w ciągu 30 min zacierania w temperaturze 70°C. Preparat Maxazyme NNP DS (zawierający proteazę) umożliwił hydrolizę skrobi w słodzie z wyki w ciągu 10 min zacierania w temperaturze 70°C, natomiast preparat Brewers Compass (zawierający alfa-amylazę, beta-glukanazę, cellulazę oraz endopeptydazę) umożliwił hydrolizę skrobi w słodzie z wyki w ciągu 20 min zacierania w temperaturze 70°C.

W kolejnym etapie badań dotyczących słodów z nasion roślin strączkowych zastosowano modyfikację procesu zacierania kongresowego. Przeanalizowano, czy wybrane słody z nasion roślin strączkowych (słód z ciecierzycy, słód z soczewicy oraz słód z wyki) mogą zostać wykorzystane po procesie kleikowania w temperaturze 90°C jako dodatek w ilości 30% wagowych do procesu zacierania słodu jęczmiennego typu pilzneńskiego. W tym etapie badań wykorzystano również preparat enzymatyczny Maxazyme NNP DS, zawierający proteazę, który okazał się mieć najlepszy wpływ na poprawę zawartości ekstraktu brzeczki z ciecierzycy. Nie zastosowano preparatów zawierających enzymy amylolityczne, gdyż zasyp do procesu zacierania, zawierający 70% wagowych słodu typu pilzneńskiego charakteryzuje się aktywnością amylolityczną niezbędną do rozłożenia skrobi zawartej w 30% wagowych surowca o niewielkiej aktywności enzymatycznej (Kunze, 2019; Szwed

i in., 2013; Zembold-Guła & Błażewicz, 2007). Brzeczki wytworzone z dodatkiem 30% skleikowanego słodu z nasion roślin strączkowych cechowały się tylko nieznacznie pogorszonymi parametrami w porównaniu do brzeczki wytworzonej wyłącznie ze słodu typu pilzneńskiego. Oznaczony czas scukrzania dla wszystkich prób wynosił 10 minut. Brzeczki wytworzone z udziałem 30% wagowych słodu z nasion roślin strączkowych charakteryzowały się nieznacznie mniejszą zawartością ekstraktu, wynoszącą 6,58-6,98°Plato, od brzeczki wytworzonej ze słodu jęczmiennego, w której zawartość ekstraktu wynosiła 7,08°Plato. Objętość brzeczek wytworzonych z wykorzystaniem skleikowanego słodu z nasion roślin strączkowych była jednak znacznie mniejsza niż objętość brzeczki wytworzonej ze słodu jęczmiennego. Próba zawierająca 30% wagowych skleikowanego słodu z soczewicy umożliwiła uzyskanie 215 mL brzeczki podczas zacierania bez dodatku preparatu enzymatycznego Maxazyme NNP DS oraz uzyskanie 205 mL brzeczki podczas zacierania z dodatkiem preparatu Maxazyme NNP DS, podczas gdy objętość brzeczki uzyskanej wyłącznie z słodu pilzneńskiego (próby kontrolnej) wynosiła 325 mL. Objętość brzeczki wytworzonej z zasypu zawierającego 30% dodatek wagowy skleikowanego słodu z ciecierzycy zacieranego bez dodatku preparatów enzymatycznych wynosiła 270 mL, podczas gdy w próbie z dodatkiem skleikowanego słodu z ciecierzycy oraz preparatu Maxazyme NNP DS uzyskano 250 mL brzeczki. Natomiast objętość brzeczki wytworzonej z zasypu zawierającego 30% dodatek wagowy skleikowanego słodu z wyki zacieranego bez dodatku preparatów enzymatycznych wynosiła 270 mL, podczas gdy w próbie z dodatkiem skleikowanego słodu z wyki oraz preparatu Maxazyme NNP DS uzyskano 280 mL brzeczki. Mniejsza objętość brzeczek uzyskanych w próbach, w których zastosowano skleikowany słód z nasion roślin strączkowych jest najprawdopodobniej spowodowana przez większą niż u ziarniaków zbóż wodochłonność nasion roślin strączkowych, którą, ponadto, może zwiększać proces słodowania (Stantiall i in., 2018; Shafaei i in., 2016; Ayodele & Beatrice, 2015; Miano i in., 2015; Rozbicki i in., 2015; Briggs, 1998).

W pracy wykazano, że słody z nasion roślin strączkowych takich jak: soczewica jadalna, ciecierzyca pospolita, fasola zwykła, groch zwyczajny, wyka siewna, groszek siewny, soja warzywna oraz łubin wąskolistny uzyskane w procesie słodowania charakterystycznego dla wytwarzania słodu pilzneńskiego cechują się niewłaściwymi parametrami technologicznymi do otrzymywania brzeczki.

W eksperymencie opisanym w publikacji nr 1 wykazano, że możliwe jest uzyskanie brzeczki o parametrach zbliżonych do brzeczki wytworzonej wyłącznie ze słodu

jęczmiennego stosując dodatek 30% wagowych skleikowanego słodu z soczewicy, ciecierzycy albo wyki siewnej do zasypu zawierającego 70% wagowych słodu jęczmiennego.

Następnie podjęto dalsze badania dotyczące zmodyfikowanego procesu słodowania mającego na celu poprawić parametry technologiczne słodów z wybranych nasion roślin strączkowych.

Publikacja nr 2 wymieniona w rozdziale 5, wchodząca w skład jednotematycznego cyklu publikacji

W publikacji drugiej opisano badania dotyczące słodowania nasion wybranych roślin strączkowych. Jako materiał badawczy wykorzystano nasiona dwóch gatunków roślin strączkowych, które zostały poddane procesowi słodowania w pierwszym etapie badań (publikacja nr 1). Wykorzystano nasiona jednego z gatunków (soczewicy jadalnej), spośród których udało się wytworzyć słody umożliwiające uzyskanie brzeczki w eksperymencie opisanym w publikacji nr 1 oraz nasiona jednego z gatunków (fasoli zwykłej), spośród których nie udało się wytworzyć słodów zdatnych umożliwiających uzyskanie brzeczki uzyskania brzeczki.

Ponadto, zróżnicowano próby wykorzystując odmiany o zróżnicowanej barwie łupiny nasiennej. Do procesu słodowania wybrano trzy odmiany soczewicy o łupinie nasiennej barwy brązowej, zielonej oraz czarnej i dwie odmiany fasoli o łupinie barwy czerwonej i białej. Soczewicę oraz fasolę wykorzystano ze względu na fakt, że nasiona tych gatunków charakteryzują się dużą zawartością skrobi, małą zawartością tłuszczów oraz są roślinami strączkowymi popularnie uprawianymi na wielu kontynentach, zatem potencjalnie technologia słodowania tych surowców może znaleźć zastosowanie na całym świecie (Kaale i in., 2023; Hughes i in., 2022; De Ron, 2015; Bellucci i in., 2014).

Modyfikacji poddany został proces słodowania wybranych nasion. Przyjęto takie parametry namaczania oraz kiełkowania nasion, aby były one bardziej zbliżone do optymalnych warunków kiełkowania nasion soczewicy oraz fasoli niż typowe parametry wykorzystywane podczas namaczania i kiełkowania jęczmienia wykorzystywane do produkcji słodu pilzneńskiego (Maqueira-Lopez i in., 2021; De Ron, 2015; Al-Quraan i in., 2014; Briggs, 1998).

Nasiona namoczono do zawartości wody wynoszącej 57-59% dla soczewicy oraz 58-60% dla fasoli, a następnie poddano procesowi kiełkowania w temperaturze 18°C (soczewica) oraz 20°C (fasola). Zróżnicowano również czas kiełkowania, mający wpływ na jakość słodu (Briggs, 1998). Planowano wytworzyć słody z nasion kiełkowanych 96h, 120h oraz 144h, jednak, ze względu na powolny wzrost kiełków liścieniowych i korzeni nasion fasoli, czas

kiełkowania tych nasion wynosił 120h i 144h. W rezultacie otrzymano dziewięć różnych słodów z nasion soczewicy i cztery słody z nasion fasoli.

Słody poddano analizie kruchości, analizie zawartości podstawowych składników odżywczych, takich jak skrobia, białko i błonnik. Słody wykorzystano również w procesie zacierania kongresowego, aby określić możliwość zastosowania ich w technologii piwowarskiej. Określono również zawartość związku antyżywieniowego obecnego w wielu produktach roślinnych, jakim jest kwas fitynowy.

Przeprowadzenie procesu zacierania kongresowego wykazało, że wiele parametrów technologicznych słodów z soczewicy uległo poprawie w porównaniu do słodu z soczewicy wytworzonego i opisanego w publikacji nr 1. Ponownie nie udało się uzyskać brzeczki ze słodów z fasoli ze względu na utrudniony proces filtracji. Podczas zacierania, skrobia w żadnym z zacieranych słodów nie uległa całkowitej hydrolizie. Mimo tego, brzeczki ze słodów z soczewicy zielonej (4,80-5,10% w/w), brązowej (3,40-3,60% w/w) i czarnej (4,75-5,00% w/w) cechowały się dużo większą zawartością ekstraktu niż brzeczka wytworzona ze słodu z soczewicy opisanego w publikacji nr 1 (1,59% w/w). Wskazuje to na fakt, że zastosowana modyfikacja procesu słodowania soczewicy miała wpływ na zwiększenie zawartości ekstraktu w pozyskiwanej brzeczce o 300%. Wyraźną różnicę zauważono również w czasie filtracji brzeczek ze słodów otrzymanych z nasion soczewicy zielonej oraz soczewicy brązowej w odniesieniu do czasu filtracji brzeczek wytworzonych z dowolnego słodu badanego w eksperymencie nr 1, który wynosił 120 minut (maksymalny dopuszczalny czas filtracji brzeczki kongresowej). Brzeczki z soczewicy zielonej przefiltrowały się o 20-45 min szybciej (w ciągu 75-100 minut), natomiast brzeczki z soczewicy brązowej przefiltrowały się w ciągu 43-50 minut, czyli w okresie zbliżonym do czasu filtracji brzeczki kongresowej ze słodu pilzneńskiego (Kunze, 2019). Objętość uzyskanej brzeczki ze słodów otrzymanych w badaniach opisanych w publikacji 2 nie uległa znacznemu zwiększeniu w porównaniu do wyników z publikacji 1 w przypadku słodów z soczewicy brązowej oraz słodów z soczewicy zielonej, jednak w przypadku soczewicy czarnej objętość uzyskanej brzeczki była znacznie mniejsza (zaledwie 22,5-32,5 cm^3). Jest to efektem bardzo powolnego procesu filtracji i prawdopodobnie wskazuje na obecność w słodach z soczewicy czarnej składników, które mogą nadmiernie wiązać wodę i uniemożliwiać swobodny spływ brzeczki, co wyklucza jej potencjalne zastosowanie w procesach zacierania wykorzystujących grawitacyjną metodę filtracji (Levent i in., 2023; Paranavitana i in., 2021; Piergiovanni, 2021). Lepkość uzyskanych brzeczek była mniejsza niż lepkość brzeczek ze słodu jęczmiennego, jednak jest to najprawdopodobniej skutek mniejszej zawartości ekstraktu w brzeczkach z soczewicy,

bowiem zawartość ekstraktu jest kluczowym czynnikiem mającym wpływ na lepkość brzeczki (Kunze, 2019).

Za pomocą friabilimetru oznaczono kruchość słodów z fasoli i soczewicy (Analytica EBC). Wykonano także analizę kruchości nasion niesłodowanych, pomimo tego, że nie jest to metoda zalecana przez żadną z instytucji podejmującej się analiz prób słodowniczych czy piwowarskich, takich jak MEBAK (Mitteleuropäische Brautechnische Analysenkomission), EBC (European Brewery Convention), ASBC (American Society of Brewing Chemists) czy VLB (Versuchs- und Lehranstalt für Brauerei). Badanie te wykonano by wykazać jak znaczące zmiany w surowcu powoduje proces słodowania. Kruchość soczewicy zielonej na wskutek procesu słodowania zmieniła się z 15,93% (niesłodowane nasiona) do 94,67-97,67% (słody). Podobne wyniki uzyskano dla słodów z soczewicy brązowej i czarnej. Pod wpływem słodowania zaobserwowano zmianę kruchości z 15,07% dla nasion niesłodowanych do 91,90-95,43% w słodach (soczewica brązowa) oraz z 16,53% do 90,83-95,17% (soczewica czarna). Znaczące różnice pomiędzy kruchością nasion a słodów odnotowano także w przypadku fasoli. Słodowanie miało wpływ na modyfikację kruchości fasoli białej z 2,38% (nasiona niesłodowane) do 20,63-24,10% (słody), natomiast kruchość fasoli czerwonej zmieniła się z 2,43% (nasiona niesłodowane) do 14,07-16,00 % (słody).

Wykonano także analizę zmiany zawartości składników, takich jak białko, skrobia czy błonnik, aby określić, czy słody z nasion roślin strączkowych mogą być bardziej przydatne niż nasiona niesłodowane w procesach produkcji żywności wysokobiałkowej albo o obniżonej ilości składników antyodżywczych (Sarkhel & Roy, 2022; Hídvégi & Lásztity, 2002). Podczas procesu słodowania w nasionach następują znaczne zmiany zawartości poszczególnych składników odżywczych (Palmer, 2017; Mallet, 2014; Briggs, 1998). Podczas kiełkowania, nasiona zużywają część zawartej w nich skrobi w celu wytworzenia glukozy, a następnie energii, zachowując jak największą ilość białek, co sprawia, że w 100 g suchej masy słodu jest więcej białka i mniej skrobi niż w 100 g suchej masy nasion przed procesem kiełkowania (Hernández-Becerra i in., 2020; Oseguera-Toleda i in., 2020; Gupta i in. 2010). Podczas kiełkowania w nasionach zawartość błonnika zwiększa się, ze względu na wytwarzanie nowych komórek, z których każda jest otoczona ścianą komórkową, jednak w procesie odkiełkowywania odrzucane są struktury zawierające duże ilości celulozy (m. in. korzonki), w związku z tym zawartość błonnika w słodzie nie różni się znacznie od zawartości błonnika w nasionach niesłodowanych (Olivares-Galván i in., 2022; Abdullah Badahdah i in., 2019; Hingade i in., 2019). Procesy namaczania oraz kiełkowania mają również wpływ na obniżenie zawartości kwasu fitynowego w nasionach, zarówno typowo

słodowanych zbóż, jak i nasion roślin strączkowych. Nadmierne spożycie kwasu fitynowego może przyczyniać się do ograniczenia wchłaniania istotnych składników mineralnych (takich jak wapń, magnez, żelazo, cynk) oraz powodować inhibicję działania enzymów trawiennych (amylazy, trypsyna, pepsyna), zatem zmniejszenie zawartości kwasu fitynowego w nasionach roślin strączkowych może być postrzegane jako istotna zaleta (Lemmens i in., 2019; De Ron, 2015, Bewley i in., 2013).

Nasiona poddane słodowaniu, w badaniach własnych, charakteryzowały się mniejszą zawartością skrobi w porównaniu do surowca niesłodowanego. W nasionach soczewicy zawartość skrobi uległa redukcji pod wpływem słodowania w większym stopniu niż w nasionach fasoli. Wydłużanie czasu kiełkowania miało wpływ na zmniejszenie zawartości tego węglowodanu. Zawartość skrobi w nasionach soczewicy zielonej wynosiła 57,6% (w przeliczeniu na suchą masę), podczas gdy w słodach z soczewicy zielonej, zawartość skrobi wynosiła 39,1-40,2%. Soczewica brązowa zawierała mniej skrobi (50,7%) niż soczewica zielona, a słody z soczewicy brązowej charakteryzowały się zawartością skrobi wynoszącą 38,5-41%. W przypadku soczewicy czarnej scharakteryzowano zmianę zawartości skrobi z 48,9% w nasionach do 39,4-41,3% w słodach. Słodowanie miało mniejszy wpływ na ubytek zawartości skrobi w przypadku fasoli niż soczewicy. Fasola biała charakteryzowała się zawartością skrobi wynoszącą 42,2%, a słody z niej wytworzone zawierały 36,4-40,2%. Zawartość skrobi w fasoli czerwonej wynosiła natomiast 38,2%, podczas gdy słody z fasoli czerwonej charakteryzowały się zawartością skrobi w zakresie 35,3-37,4%. Zmniejszenie zawartości skrobi podczas procesu słodowania o więcej niż 5-10% wagowych jest uznawana za znaczące i proces ten powinien zostać zoptymalizowany w celu zwiększenia opłacalności słodowania w warunkach komercyjnych (Mallet, 2014; Briggs, 1998).

Zawartość białka w słodach z soczewicy była większa niż w niesłodowanych nasionach. Należy jednak zwrócić uwagę na to, że kiełkujące nasiona nie mają możliwości pozyskiwania azotu niezbędnego do wytwarzania białek, a zatem zwiększenie zawartości białka wynika z utraty pozostałych składników podczas procesu kiełkowania (przede wszystkim skrobi), przez co udział białka w masie słodu jest większy niż w nasionach niesłodowanych (Bewley i in., 2013; Mallet, 2014; Briggs, 1998).

Zawartość białka w nasionach soczewicy zielonej wynosiła 24,6% (w przeliczeniu na suchą masę), podczas gdy w słodach z soczewicy zielonej, zawartość białka wynosiła 32,3-32,5%. Soczewica brązowa zawierała więcej białka (30,47%) niż soczewica zielona, a słody z soczewicy brązowej charakteryzowały się zawartością białka wynoszącą 32,4-32,8%. W przypadku soczewicy czarnej zaobserwowano zmianę zawartości białka z 26%

w nasionach do 28,4-29,07% w słodach. Fasola biała charakteryzowała się zawartością białka wynoszącą 24,9%, a słody z niej wytworzone zawierały 26,2-27,2% białka, natomiast zawartość skrobi w fasoli czerwonej wynosiła 38,2%. Słody z fasoli czerwonej były jedynymi z prób, w których zawartość białka była mniejsza, niż w niesłodowanych nasionach. Nasiona fasoli czerwonej zawierały 28,17% białka, podczas gdy słody z fasoli czerwonej zawierały 26,2-27,3% białka.

Wielu autorów uważa, że zazwyczaj zawartość błonnika nieznacznie wzrasta w przypadku słodowanych nasion i efekt ten można zauważyć w przypadku słodów wytworzonych z fasoli (Kunze, 2019; Palmer, 2017; Mallet, 2014, Briggs, 1998). Zawartość błonnika w nasionach fasoli białej wynosiła 24,53% (w przeliczeniu na suchą masę), natomiast słody z fasoli białej zawierały 25,93-27,01% błonnika. Nasiona fasoli czerwonej zawierały 24,39% błonnika, a słody z nich wytworzone charakteryzowały się zawartością tego składnika wynoszącą 25,28-29,07%. Słodowanie miało jednak odmienny wpływ na zawartość błonnika w słodach wytworzonych z nasion soczewicy. Niesłodowane nasiona soczewicy zielonej charakteryzowały się zawartością błonnika wynoszącą 18,56%, która uległa redukcji po procesie słodowania do zawartości 14-15,27%. Nasiona soczewicy brązowej charakteryzowały się zawartością błonnika wynoszącą 20,56%, podczas gdy słody wytworzone z tych nasion zawierały 14,56-14,93%. Soczewica czarna charakteryzowała się największą zawartością błonnika spośród przeanalizowanych nasion (26,84%), a słody z niej wytworzone zawierały 18,08-19,39% błonnika. Tak znaczące zmiany w zawartości błonnika w nasionach soczewicy po procesie słodowania mogą wskazywać na redukcję innych niż celuloza składników stanowiących frakcję błonnika pokarmowego. Podjęto dalsze badania dotyczące tego zagadnienia i opublikowano je w postaci publikacji nr 3.

Proces słodowania miał również wpływ na zawartość kwasu fitynowego w analizowanych nasionach. Każdy ze słodów cechował się mniejszą zawartością tego składnika, stanowiącego tzw. związek antyodżywczy niż nasiona niesłodowane, a wydłużenie czasu kiełkowania miało wpływ na zmniejszenie zawartości tego związku w niewielkim stopniu. Zawartość kwasu fitynowego w nasionach soczewicy zielonej wynosiła 1,24% (w przeliczeniu na suchą masę), podczas gdy w słodach z soczewicy zielonej zawartość tego składnika wynosiła 0,82-0,90%. W niesłodowanej soczewicy brązowej zawartość kwasu fitynowego wynosiła 1,36%, a proces słodowania zmniejszył zawartość tej substancji do 0,83-0,86%. Soczewica czarna charakteryzowała się najmniejszą zawartością kwasu fitynowego spośród niesłodowanych nasion (0,88%), a słody wytworzone z tych nasion zawierały 0,83-0,87% tego kwasu. W fasoli białej oznaczono zawartość kwasu fitynowego wynoszącą

1,80%, a słody wytworzone z tych nasion charakteryzowały się zawartością wynoszącą 1,42-1,46%. Zawartość kwasu fitynowego w nasionach fasoli czerwonej była największa spośród analizowanych prób (1,98%), a proces słodowania spowodował redukcję tego składnika do poziomu 1,91-1,94%. Niewielkie różnice w zawartości kwasu fitynowego w próbach kiełkowanych przez 4, 5 albo 6 dni sugerują, że najprawdopodobniej większość strat kwasu fitynowego w procesie słodowania następuje podczas etapu moczenia nasion, podczas którego kwas fitynowy przenika do wody. Ten efekt potwierdzają badania dotyczące moczenia nasion roślin strączkowych i zbóż, opisane przez Feizollahi i in. (2021).

Dane dotyczące zmiany składu roślin strączkowych wskutek procesu słodowania wykazują, że słodowane nasiona soczewicy i fasoli mogą stanowić substrat do produkcji różnych rodzajów żywności wysokobiałkowej o obniżonej zawartości składników antyodżywczych i węglowodanów.

Podsumowując ten etap badań, słód z nasion soczewicy brązowej i zielonej wytworzony w procesie o wyższej temperaturze kiełkowania i większej wilgotności kiełkujących nasion niż typowo stosowana w produkcji słodu pilzneńskiego charakteryzuje się poprawionymi parametrami technologicznymi (takimi jak czas filtracji i zawartość ekstraktu brzeczek) określonymi na podstawie zacierania kongresowego.

W pracy wykazano również, że proces słodowania wpływa na zmianę zawartości składników takich jak skrobia, białko, błonnik czy kwas fitynowy w nasionach soczewicy oraz fasoli.

Publikacja nr 3 wymieniona w rozdziale 5, wchodząca w skład jednotematycznego cyklu publikacji

Celem badań opisanych w publikacji nr 3 było zweryfikowanie przyczyny zmniejszenia zawartości błonnika w słodach opisanych w publikacji nr 2. Składnikami, które zaliczane są do grupy związków wchodzących w skład błonnika pokarmowego, występują w nasionach roślin strączkowych i nie są poddawane hydrolizie przez enzymy wykorzystywane w metodzie oznaczenia zawartości błonnika są oligosacharydy (Serna Saldívar & Hernández, 2020; Mussatto, & Mancilha, 2007). Istnieje wiele doniesień wskazujących na fakt występowania w nasionach roślin strączkowych oligosacharydów z grupy rafinozy (‘*raffinose family oligosaccharides*’, w skrócie RFO) (Sanyal i in., 2023; Tosh & Yada, 2008). Zalicza się do nich rafinozę, stachiozę oraz werbaskozę. Są to oligosacharydy składające się z cząsteczki glukozy, fruktozy i galaktozy (rafinoza); dwóch cząsteczek glukozy, jednej cząsteczki fruktozy i galaktozy (stachioza) oraz trzech cząsteczek

glukozy, jednej cząsteczki fruktozy i galaktozy (werbaskoza), (Yan i in., 2022). W układzie pokarmowym człowieka nie jest wytwarzana α-galaktozydaza, enzym niezbędny do rozłożenia tych węglowodanów na cukry proste, które mogą zostać wchłonięte do układu krwionośnego (Elango et al., 2022). Niestety, w układzie pokarmowym człowieka istnieją mikroorganizmy, które są w stanie wykorzystywać te oligosacharydy, wytwarzając jako produkt uboczny dwutlenek węgla, wodór czy metan. Związki te mogą przyczyniać się do problemów gastrycznych związanych z nadmiernym nagromadzeniem gazów w jelitach (Mao et al., 2018).

W eksperymencie opisanym w publikacji nr 3 wykonano analizę zawartości rafinozy, stachiozy oraz werbaskozy. Analizom zostały poddane nasiona trzech odmian soczewicy o różnych kolorach łupiny nasiennej (brązowej, czarnej i zielonej), dwie odmiany fasoli o dwóch kolorach łupiny nasiennej (czerwonej i białej), a także wykonane z nich słody (dziewięć słodów wytworzonych z nasion soczewicy oraz cztery słody wytworzone z fasoli) opisane w publikacji nr 2. Ponadto, badaniom poddano brzeczki ze słodów z dziewięciu rodzajów soczewicy uzyskane w trakcie zacierania kongresowego.

W żadnym z rodzajów nasion, słodów oraz brzeczek nie wykryto zawartości werbaskozy, pentasacharydu z grupy RFO, jednak w większości prób obecna była rafinoza i stachioza. Biorąc pod uwagę dane uzyskane w publikacji nr 2 dotyczące zawartości błonnika w nasionach soczewicy można zauważyć, że RFO stanowią znaczną część frakcji błonnika dostępnego w nasionach analizowanych odmian soczewicy.

Proces słodowania w przypadku każdego z wytworzonych słodów wykazał zasadniczą redukcję zawartości RFO w nasionach zarówno soczewicy, jak i fasoli. Zawartość rafinozy w soczewicy została zredukowana z wartości 6,77% w suchej masie surowca (soczewica brązowa), 7,82% (soczewica zielona) i 12,98% (soczewica czarna) do wartości 1,35% (słód z soczewicy brązowej kiełkowany 6 dni), 0,61% (słód z soczewicy zielonej kiełkowany 6 dni) oraz 0,46% (słód z soczewicy czarnej kiełkowany 6 dni). W nasionach fasoli zawartość tego związku była niższa niż nasionach soczewicy, dodatkowo stwierdzono, że proces słodowania również miał wpływ na obniżenie zawartości tego oligosacharydu. Zawartość rafinozy w nasionach fasoli białej została zredukowana z 3,03% do 0,66%, natomiast w fasoli czerwonej z 2,20% do 0,69%. Wyniki te wskazują, że proces słodowania może być wykorzystany do znaczącego obniżenia zawartości rafinozy w nasionach roślin strączkowych.

Oceniając zawartość stachiozy w nasionach fasoli oraz soczewicy stwierdzono istotną redukcję zawartości tego oligosacharydu. W soczewicy czarnej wykazano, że jego ilość zmniejszyła się z 7,32% do 0,36%; w przypadku soczewicy brązowej z zawartości 2,88% do

0,60%; w przypadku soczewicy zielonej z 5,66% do 0,28%. W nasionach fasoli zawartość stachiozy została zredukowana z 2,15% (fasola biała) i 2,45% (fasola czerwona) do, odpowiednio, 0,45% i 0,90%. Te rezultaty wskazują, że proces słodowania może być skutecznie wykorzystywany w celu obniżenia zawartości stachiozy w nasionach soczewicy i fasoli.

Analizom zawartości oligosacharydów zostały poddane również brzeczki z soczewicy. Brzeczki ze słodów z soczewicy charakteryzowały się bardzo małą zawartością rafinozy i stachiozy, wynoszącą maksymalnie 0,70 g rafinozy na dm^3 brzeczki w brzeczce uzyskanej ze słodu z zielonej soczewicy kiełkowanego 4 dni. Podobnie jak w nasionach i słodzie stężenie stachiozy było mniejsze niż stężenie rafinozy. Wyniki analizy zawartości RFO w brzeczkach mogą sugerować, że w słodach z nasion roślin strączkowych obecna jest α-galaktozydaza, która jest aktywna w którymś z etapów procesu zacierania kongresowego i rozkłada obecne w słodzie RFO (Avezum i in., 2024; Escobedo i in., 2019; Njoumi i in., 2019). Wskazuje to na możliwość zastosowania procesu zacierania słodów z nasion roślin strączkowych w celu dalszej redukcji RFO.

Najważniejszym osiągnięciem badań przedstawionych w pracy nr 3 było wykazanie, że słodowanie nasion soczewicy brązowej, czarnej i zielonej oraz nasion fasoli białej i czerwonej powoduję redukcję zawartości RFO takich jak rafinoza i stachioza.

Publikacja nr 4 wymieniona w rozdziale 5, wchodząca w skład jednotematycznego cyklu publikacji

Słodowanie jest procesem, który w znaczącym stopniu modyfikuje zapach przetwarzanych nasion, natomiast zapach nasion roślin strączkowych i produktów z nich wytwarzanych jest mało akceptowany przez część populacji (Trindler i in., 2022; Chigwedere i in., 2022; Luydmila i in., 2020; Kunze, 2019; Prado i in., 2019). Podczas procesu słodowania, w przetwarzanych nasionach zachodzi wiele przemian, m. in. enzymatycznych, które w dużym stopniu zmieniają zawartość różnych składników obecnych w nasionach, również związków lotnych (Gasiński i in., 2022; Kunze, 2019; Briggs, 1998). Kluczowym etapem mającym wpływ na zawartość związków lotnych jest proces suszenia (Huang i in., 2016). Podczas pierwszych godzin suszenia, zawartość wody w nasionach jest na tyle duża, że możliwe jest działanie enzymów hydrolitycznych, takich jak proteazy, lipazy, amylazy czy lipooksygenazy (Filipowska i in., 2021; Prado i in., 2019; Huang i in., 2016). Na skutek działania tych enzymów, tłuszcze rozkładane są do kwasów tłuszczowych, kwasy tłuszczowe są przekształcane w aldehydy, skrobia w dekstryny, maltotriozę, maltozę oraz glukozę,

natomiast z białek uzyskiwane są peptydy oraz pojedyncze aminokwasy (Kunze, 2019; Briggs, 1998). Z powstałych w ten sposób aminokwasów oraz cukrów podczas reakcji Maillarda powstają w różne związki, barwne oraz lotne (Wang i in., 2023; Hellwig & Henle, 2020). Ponadto, pewna ilość związków lotnych naturalnie występujących w kiełkowanych nasionach może ulec zmniejszeniu, gdyż proces suszenia zazwyczaj trwa wiele godzin i substancje te odparować z suszonego słodu (Kunze, 2019; Tong i in., 2019).

W publikacji 4 przedstawiono wyniki badań dotyczących zawartości związków lotnych zawartych w nasionach soczewicy brązowej, czarnej i zielonej oraz w słodach z nich uzyskanych po cztero-, pięcio- i sześciodniowym procesie kiełkowania. Wstępnie zakładano analizowanie zawartości związków lotnych również w nasionach i słodach z fasoli, jednak w analizie chromatograficznej słodów z fasoli zidentyfikowano tylko pięć związków lotnych, z czego zawartość trzech z nich była poniżej poziomu kwantyfikacji (LOQ). W związku z tym uznano, że materiał ten nie prezentuje wartości, która może zostać opublikowana i postanowiono przygotować tylko manuskrypt zawierający dane dotyczące soczewicy.

Z wykorzystaniem techniki mikroekstrakcji do fazy stałej (SPME) oraz analizy za pomocą chromatografii gazowej sprzężonej ze spektrometrią masową zidentyfikowano i skwantyfikowano 50 związków lotnych z takich grup chemicznych jak aldehydy, węglowodory, terpeny, ketony, estry, furany, pirazyny czy związki siarkowe. Największymi grupami, jeśli chodzi o ilość poszczególnych zidentyfikowanych związków były aldehydy (18 związków), węglowodory (9 związków) oraz alkohole (7 związków).

Aldehydy, najliczniejsza grupa związków, miały największy udział procentowy spośród związków lotnych skwantyfikowanych w nasionach soczewicy czarnej i brązowej i słodach z soczewicy brązowej, czarnej i zielonej. Stanowiły one od 32,24% do aż 58,75% całkowitej zawartości związków lotnych w tych nasionach/słodach. Zauważalny był wzrost udziału aldehydów po słodowaniu oraz w miarę wydłużania czasu słodowania, to znaczy, słody kiełkowane cztery dni charakteryzowały się mniejszym udziałem aldehydów w całkowitej zawartości związków lotnych niż słody kiełkowane pięć albo sześć dni. Główną przyczyną tego zjawiska jest prawdopodobnie działanie lipooksygenaz w kiełkujących nasionach, które podczas 120h albo 144h kiełkowania oddziałują na kwasy tłuszczowe obecne w soczewicy przez dłuższy czas, uwalniając większą ilość substancji, które mogą zostać przekształcone w aldehydy (Filipowska i in., 2021; Prado i in., 2019; Huang i in., 2016).

Alkohole stanowiły drugą największą grupę (pod względem udziału procentowego) związków lotnych w nasionach i słodach z soczewicy. Łączny udział alkoholi w nasionach

i słodach z soczewicy wynosił 20,96-53,77%. Proces słodowania miał wpływ na redukcję zawartości tych substancji w wytworzonych słodach. Głównymi związkami z tej grupy, stanowiącymi większościowy udział w całkowitej zawartości alkoholi, były alkohole takie jak 1-okten-3-ol oraz 1-nonanol, charakteryzujące się zapachami opisywanymi w literaturze jako „grzybowy", „ziemisty" (*„earthy"*) lub „groszkowy" (*„pea-like"*) (Matsui i in., 2018).

Analiza związków lotnych przyniosła interesujące dane dotyczące ilości terpenów w słodach i nasionach z soczewicy. Niesłodowana soczewica czarna oraz niesłodowana soczewica brązowa cechowały się niską zawartością związków z tej grupy (0.78 ppb oraz 1.19 ppb), podczas gdy w soczewicy zielonej skwantyfikowano aż 38.95 ppb terpenów (głównie limonenu oraz eukalyptolu). Proces słodowania przyczynił się do nieznacznego wzrostu zawartości terpenów w słodach z soczewicy czarnej oraz brązowej niż w nasionach przed słodowaniem. Słody z soczewicy zielonej cechowały się natomiast znacznie mniejszą zawartością terpenów (od 14.43 ppb do 0.56 ppb). Ten efekt może być spowodowany tendencją limonenu oraz eukalyptolu do degradacji podczas przebywania w warunkach, w których obecna jest duża ilość utleniaczy. Związki te są również wytwarzane podczas procesów metabolicznych nasion (Mahanta i in., 2021; Kern i in., 2014).

Najważniejszym osiągnięciem tego etapu badań było wykazanie, że proces słodowania w zasadniczym stopniu modyfikuje nie tylko ilość związków lotnych w nasionach soczewicy, ale także udział poszczególnych grup związków w całkowitej zawartości związków lotnych zawartych w nasionach soczewicy.

Wyniki badań nad zawartością związków lotnych w nasionach soczewicy oraz w słodach z soczewicy wykazały, że słodowanie może być skuteczną metodą na modyfikowanie zawartości związków lotnych w nasionach soczewicy, a co za tym idzie, zmianą ich zapachu. Wskazuje to na fakt, że słód z nasion soczewicy mógłby być potencjalnie wykorzystany do wytwarzania różnorodnych wysokobiałkowych produktów spożywczych, które nie cechowałyby się charakterystycznym dla roślin strączkowych zapachem.

W kolejnych badaniach, zaprojektowano proces słodowania nasion soczewicy zielonej (wybranej jako najbardziej obiecujący surowiec) i wytwarzania piwa z uzyskanych słodów.

Publikacja nr 5 wymieniona w rozdziale 5, wchodząca w skład jednotematycznego cyklu publikacji

W kolejnym etapie badań do modyfikacji procesu słodowania wybrano, na podstawie wyników przedstawionych w publikacji nr 2, nasiona soczewicy zielonej jako najlepszy substrat do wytworzenia brzeczki spośród przetestowanych wariantów. Niemniej jednak,

przed podjęciem próby wyprodukowania piwa ze słodu z soczewicy zielonej, dokonano kolejnej modyfikacji procesu słodowania, mającej na celu poprawę parametrów technologicznych słodu z soczewicy zielonej.

W procesie słodowania soczewicy zielonej zachowano poprzednio ustalone parametry dotyczące zawartości wody w nasionach niezbędnej do rozpoczęcia procesu kiełkowania, zmodyfikowana została natomiast temperatura kiełkowania. Wykorzystano nietypową metodę z obniżaniem temperatury podczas tego procesu. Metoda ta jest stosowana do wytwarzania słodów o wysokiej aktywności enzymatycznej, przeznaczonych do produkcji zacierów wykorzystywanych do produkcji destylatów (Briggs, 1998).

Obniżanie temperatury kiełkującego ziarna powoduje zmniejszenie aktywności enzymów hydrolitycznych uwalniających cukry czy aminokwasy z substancji zapasowych. Pomimo tego, metabolizm kiełkującego nasiona postępuje w sposób niewspółmierny do redukcji aktywności enzymów (Shewry i in., 2023; Rajjou i in., 2012). W takich warunkach ilość enzymów wytworzonych przez kiełkujące nasiona jest niewystarczająca do uwolnienia cukrów niezbędnych do zapewnienia wystarczającej ilości energii. W związku z tym, zarodek wytwarza większą ilość enzymów (Bewley i in., 2012; Mohr i Schopfer, 2012). Efekt ten może potencjalnie przyczynić się do poprawy aktywności enzymatycznej słodu, co może mieć istotny wpływ na poprawę parametrów technologicznych wytworzonego słodu (Mallet, 2014, Briggs, 1998).

Wytworzono trzy rodzaje słodów różniące się długością trwania procesu kiełkowania (96h, 120h oraz 144h). Na początku procesu kiełkowania zastosowano temperaturę 18°C utrzymywaną przez pierwsze 24h, po czym temperaturę obniżono do 15°C i utrzymywano przez kolejne 24h. Następnie obniżono temperaturę do 12°C i w tej temperaturze kiełkowano nasiona do końca trwania procesu, po czym zostały wysuszone oraz odkiełkowane.

Wysuszone, odkiełkowane i rozdrobnione słody zostały poddane zacieraniu kongresowemu, w celu określenia ich parametrów technologicznych. W celu usprawnienia procesu zacierania wprowadzono do zacieru jony wapnia albo preparat enzymatyczny zawierający alfa-amylazę. Próbę kontrolną stanowiła próba bez dodatków.

W próbach zacieranych bez dodatków poprawie uległa, w stosunku do słodów zacieranych w badaniach opisanych w publikacji nr 2, zawartość ekstraktu w uzyskanej brzeczce, jednak czas filtracji uległ pogorszeniu. Wprowadzenie jonów wapnia nie wpłynęło znacząco na parametry technologiczne słodu z wyjątkiem poprawy czasu filtracji w przypadku słodu kiełkowanego 120h.

Zastosowanie dodatku preparatu enzymatycznego z alfa-amylazą spowodowało poprawę dwóch kluczowych parametrów dotyczących wytwarzania brzeczki, czyli objętości oraz czasu filtracji brzeczki. Czas filtracji brzeczek wytworzonych ze słodu zacieranego z dodatkiem alfa-amylazy był sześciokrotnie krótszy niż w przypadku słodów zacieranych bez dodatków, natomiast objętość uzyskanych brzeczek była większa o 35-65 cm^3, co stanowiło poprawę o nawet 33%. Ponadto, lepkość brzeczek wytworzonych podczas procesu zacierania z dodatkiem alfa-amylazy była mniejsza, co potencjalnie ułatwiłoby wykorzystanie tych brzeczek w przemyśle (skrócenie czasu przepompowywania brzeczki, zmniejszenie obciążenia wykorzystywanych pomp).

Niemniej jednak wykorzystanie preparatu zawierającego alfa-amylazę podczas zacierania nie spowodowało całkowitej hydrolizy skrobi zawartej w słodzie z soczewicy. Podczas procesu produkcji brzeczki, skrobia zawarta w słodzie powinna być poddana całkowitej hydrolizie w celu uzyskania odpowiedniego stopnia odfermentowania i klarowności w produkcie gotowym: piwie (Kunze, 2019). Z tego względu zmodyfikowano proces zacierania słodu z soczewicy zielonej, aby wytworzyć brzeczkę, która mogłaby być wykorzystana do uzyskania nowatorskiego, bezglutenowego piwa wyprodukowanego bez użycia surowców zbożowych.

Piwo wytworzono z trzech rodzajów słodów z soczewicy zielonej: z nasion kiełkowanych 96h, 120h i 144h. Ponadto, wytworzono również piwo ze słodu jęczmiennego typu pilzneńskiego, które stanowiło próbę kontrolną. Do wytworzenia piwa ze słodu z soczewicy wykorzystano zmodyfikowany program zacierania kongresowego oraz zastosowano dodatek preparatu enzymatycznego zawierającego alfa-amylazę. Słód z soczewicy był zacierany w następującym programie temperaturowym: 30 min w temperaturze 45°C, zwiększenie temperatury zacieru do 75°C w ciągu 30 min, a następnie zacieranie w temperaturze 75°C przez 60 min. Uzyskane brzeczki gotowano z dodatkiem granulatu chmielowego typu T90 Próba kontrolna została wytworzona w zbliżonych warunkach, nie zastosowano jednak dodatku preparatu enzymatycznego zawierającego alfa-amylazę.

Brzeczki ze słodu z soczewicy charakteryzowały się mniejszą zawartością ekstraktu o 2,1-2,3% (w/w) niż brzeczka wytworzona ze słodu jęczmiennego (pilzneńskiego), która zawierała 9,21% (w/w) ekstraktu. Z tego względu przewidywano uzyskanie mniejszej zawartości alkoholu w produkcie gotowym. Zawartość alkoholu w piwach wytworzonych z soczewicy była znacznie mniejsza niż mogłaby sugerować różnica w ekstrakcie brzeczki z soczewicy i jęczmienia. Piwa z soczewicy zawierały od 2,07 do 2,12% objętościowych

alkoholu, podczas gdy piwo ze słodu jęczmiennego zawierało 3,97% alkoholu. Piwa również różniły się znacząco stopniem odfermentowania. W piwach z soczewicy wynosił on od 46,2% do 48,16%, podczas gdy piwo ze słodu jęczmiennego charakteryzował stopień odfermentowania wynoszący 67,79%. Różnica w zawartości ekstraktu w brzeczce oraz alkoholu w piwie miała znaczący wpływ na różnice w kaloryczności wyprodukowanych piw. Piwa z soczewicy zawierały 24,47-25,13 kcal/100 cm^3, natomiast piwo jęczmienne 32,72 kcal/100 cm^3. Ponadto, niski stopień odfermentowania sprawił, że mimo większej zawartości ekstraktu w brzeczce z słodu pilzneńskiego, próba kontrolna charakteryzowała się mniejszą zawartością ekstraktu (3,07% w/w) niż piwa z soczewicy (3,68-3,88% w/w).

Mniejsza zawartość alkoholu w piwach ze słodu z soczewicy może stanowić zaletę, gdyż aktualne trendy dotyczące piwowarstwa wskazują, że istnieje bardzo duża ilość konsumentów, którzy są zainteresowani piwami bezalkoholowymi albo o obniżonej zawartości alkoholu (Kozłowski i in., 2021; Callejo i in., 2019).

W przeprowadzonych badaniach analizowano również zawartość związków lotnych w uzyskanych piwach, ponieważ mają one istotny wpływ na zapach produktu gotowego. Piwa z soczewicy różniły się od piwa ze słodu jęczmiennego również zawartością związków lotnych. Większość związków lotnych w piwie jest wytwarzana przez drożdże, które wydzielają je jako produkt uboczny procesu fermentacji (Kucharczyk i in., 2020; Holt i in., 2019). Nie jest zatem zaskakujące, że zawartość większości substancji wytwarzanych przez drożdże podczas procesu fermentacji, takich jak octan etylu, estry etylowe czy metylobutanole była mniejsza w piwach wytworzonych ze słodu z soczewicy niż w piwie ze słodu jęczmiennego. Interesujące jest jednak, że zawartość poszczególnych związków, takich jak izobutanol, alkohol fenyloetylowy czy acetaldehyd w piwach z soczewicy była równa albo większa niż w próbie kontrolnej. Wymienione związki są wytwarzane przez drożdże na wskutek przemiany różnorodnych składników stanowiących ekstrakt brzeczki. Jednakże całkowita ilość substancji rozpuszczonych nie stanowi jedynego czynnika mającego wpływ na zawartość związków lotnych w piwie. Kluczowym aspektem jest udział poszczególnych składników, które mogą stanowić substrat do wytworzenia różnorodnych związków lotnych. Jedną z grup tych związków są, na przykład, aminokwasy (Lin i in., 2022; Holt i in., 2019). Nasiona jęczmienia różnią się ilością białka, jak i udziałem poszczególnych aminokwasów od nasion soczewicy. Ponadto, aktywność enzymów proteolitycznych w słodach z soczewicy i w słodach z jęczmienia może być różna, co również może przekładać się na różną zawartość aminokwasów w brzeczce, nawet gdyby zawartość białka i skład aminokwasowy słodu były takie same (Rani & Bhardwaj; 2021; Kunze, 2019; Akhtaruzzaman in., 2012; Ward, 2011).

Wyniki te wskazują, że słód z soczewicy zielonej umożliwia uzyskanie piwa o mniejszej zawartości alkoholu i mniejszej zawartości związków lotnych w porównaniu do tradycyjnych piw jęczmiennych, co może znaleźć zastosowanie w produkcji napojów fermentowanych dla populacji zainteresowanych spożyciem bezglutenowych napojów o obniżonej zawartości alkoholu.

W badaniach dokonano również analizy sensorycznej piwa według metodyki Analytica EBC (Analytica EBC). Piwo ze słodu jęczmiennego charakteryzowało się bardziej odczuwalnym zapachem „owocowym", „słodowym" oraz „zbożowym", natomiast w piwach ze słodu z soczewicy wyraźniej odczuwalne były zapachy „chmielowe". Piwo z soczewicy charakteryzowało się bardziej gorzkim smakiem w porównaniu do próby kontrolnej. W piwach z soczewicy w mniejszym stopniu wyczuwalne były posmaki „słodowe". Mniej wyczuwalny aromat zapachów „owocowych" jest najprawdopodobniej spowodowany mniejszym stężeniem estrów etylowych w piwie z soczewicy, natomiast mocniej wyczuwalne zapachy „chmielowe" mogą być spowodowane mniejszą ilością etanolu oraz alkoholi wyższych, które mają często wpływ na redukcję odczuwalnych aromatów podchodzących od chmielu w piwie (Holt i in., 2019; Rettberg i in., 2018).

Najważniejszym osiągnięciem na typ etapie badań było opracowanie procesu słodowania i zacierania, które umożliwiły hydrolizę skrobi zawartej w słodzie i uzyskanie nowatorskiego, bezglutenowego piwa z soczewicy o obniżonej zawartości alkoholu.

Wykazano, że istnieje możliwość wytwarzania słodów przydatnych w technologii piwowarskiej z nasion innych roślin niż zboża czy pseudozboża tradycyjnie wykorzystywanych w technologii słodowniczej oraz że słody te mogą znaleźć zastosowanie w produkcji napojów fermentowanych o nowych cechach sensorycznych, o obniżonej zawartości alkoholu, a także nie zawierających glutenu. Opracowano technologię wytwarzania słodu z nasion soczewicy zielonej, który może znaleźć zastosowanie w technologii piwowarskiej jako substrat do produkcji brzeczki i bezglutenowego piwa o zmniejszonej zawartości alkoholu.

Ponadto, efekty pracy zostały zgłoszone do **zastrzeżenia patentowego** w formie dwóch zgłoszeń, pod tytułem: „**Sposób wytwarzania słodu z nasion soczewicy zielonej**" i numerem zgłoszenia „**P.443732**" oraz pod tytułem: „**Sposób wytwarzania piwa bezglutenowego**" i numerem „**P.443994**".

6. PODSUMOWANIE

W ramach badań zaprezentowanych w jednotematycznym cyklu publikacji pod tytułem „Uwarunkowania technologiczne w kształtowaniu jakości słodów specjalnych z nasion roślin strączkowych i potencjał ich wykorzystania w przemyśle spożywczym" przeprowadzono badania, które miały na celu przeprowadzenie procesu słodowania nasion wybranych gatunków roślin strączkowych. Ponadto, badania umożliwiły określenie parametrów technologicznych brzeczek z uzyskanych słodów. Opracowano również proces słodowania nasion soczewicy zielonej w celu wytworzenia słodu umożliwiającego wykorzystanie go w technologii piwowarskiej w celu wytworzenia brzeczki i piwa. Zbadano również zawartość wybranych składników w nasionach i słodach w celu ustalenia zmian zachodzących na wskutek procesu słodowania.

W badaniach zaprezentowanych w **publikacji nr 1** wykazano brak przydatności słodów z nasion roślin strączkowych, wytworzonych w procesie słodowania o parametrach zbliżonych do parametrów produkcji słodu jęczmiennego typu pilzneńskiego, do wytwarzania brzeczki o odpowiednich parametrach technologicznych metodą zacierania kongresowego. Określono, że zastosowanie dodatku preparatów enzymatycznych do zacierania kongresowego słodów z nasion roślin strączkowych ma nieznaczny wpływ na poprawę procesu wytwarzania brzeczki. Ponadto, wykazano, że wybrane słody mogą stanowić 30% dodatek do słodu pilzneńskiego wykorzystywanego w procesie zacierania do uzyskania brzeczki o odpowiednich parametrach technologicznych.

W badaniach opisanych w **publikacji nr 2** zaprojektowano proces słodowania wybranych odmian soczewicy oraz fasoli o zróżnicowanych kolorach łupiny nasiennej, podczas którego wykorzystano zmodyfikowany proces namaczania nasion oraz zastosowano wyższą temperaturę kiełkowania nasion. W pracy wykazano, że słodowanie ma wpływ na zmniejszenie zawartości skrobi oraz kwasu fitynowego w słodach z nasion roślin strączkowych, a także na modyfikację zawartości białka i błonnika.

Najważniejszym osiągnięciem eksperymentów opisanych w **publikacji nr 3** było wykazanie, że proces słodowania wpłynął na redukcję zawartości rafinozy i stachiozy w słodach i brzeczkach z soczewicy i fasoli.

W badaniach opisanych w **publikacji nr 4** wykazano, że proces słodowania nasion soczewicy ma istotny wpływ na modyfikację ilości oraz rodzaju związków lotnych zawartych w słodach. Wykazano, że słodowanie w znaczącym stopniu zwiększa udział aldehydów w całkowitej zawartości związków lotnych, a ponadto zmniejsza udział alkoholi oraz terpenów. W badaniach udowodniono również, że czas kiełkowania oraz odmiana soczewicy

wybranej do słodowania ma znaczący wpływ na zawartość poszczególnych związków lotnych w słodach z soczewicy.

W eksperymentach opisanych w **publikacji nr 5** osiągnięciem naukowym było opracowanie technologii słodowania nasion soczewicy zielonej oraz wytwarzania piwa z uzyskanej brzeczki. Jest to pierwsza opisana metoda na wytwarzanie piwa wyłącznie ze słodu wyprodukowanego z nasion roślin strączkowych.

Opisane w serii publikacji wyniki wykazały, że słody z nasion roślin strączkowych mogą zostać wytworzone po odpowiedniej modyfikacji tradycyjnego procesu słodowania wykorzystywanego do produkcji słodów zbożowych. Brzeczki ze słodów z nasion roślin strączkowych charakteryzują się gorszymi parametrami technologicznymi niż brzeczki ze słodu jęczmiennego, takimi jak czas filtracji, zawartość ekstraktu czy czas scukrzania. Słodowanie ma pozytywny wpływ na zwiększenie kruchości słodów z nasion roślin strączkowych, obniżenie zawartości składników antyodżywczych takich jak kwas fitynowy czy oligosacharydy z grupy rafinozy, a także na modyfikację ilości oraz rodzaju związków lotnych zawartych w wybranych słodach.

Ponadto, podejmowane aktualnie w jednostce badania, wskazują na możliwość zastosowania słodów różnego rodzaju z nasion roślin strączkowych jako substratu do wytwarzania innych niż brzeczka czy piwo produktów spożywczych. Wstępne badania dotyczące słodów z nasion soi wykazały możliwość zastosowania tego surowca w produkcji napojów roślinnych. Wykorzystano także słody z soczewicy oraz fasoli w produkcji pieczywa oraz pieczywa cukierniczego, natomiast słody z ciecierzycy zostały wykorzystane w produkcji humusu.

Dane te wskazują na fakt, że słodowanie nasion roślin strączkowych może w przyszłości przyczynić się do wytworzenia nowych surowców, które potencjalnie mogą zostać wykorzystane w różnorodnych gałęziach przemysłu spożywczego.

7. WNIOSKI

1. Brzeczki otrzymane w procesie zacierania kongresowego, wytworzone ze słodów z nasion roślin strączkowych (ciecierzyca, fasola, groch, groszek siewny, łubin wąskolistny, soczewica, soja), poddanych słodowaniu w warunkach stosowanych w produkcji jęczmiennego słodu typu pilzneńskiego cechowały się nieodpowiednimi parametrami technologicznymi takimi jak: czas scukrzania, czas filtracji, zawartość ekstraktu.
2. Dodatek preparatów enzymatycznych (zawierających amylazy, proteazy, ksylanazy, beta-glukanazy, celulazy) do procesu zacierania w nieznacznym stopniu poprawił parametry technologiczne brzeczek ze słodów z nasion roślin strączkowych wytworzonych w technologii klasycznie wykorzystywanej do produkcji słodu jęczmiennego typu pilzneńskiego.
3. Słody z fasoli i soczewicy wyprodukowane w zmodyfikowanym procesie słodowania (wyższa temperatura kiełkowania, większa zawartość wody w nasionach) charakteryzowały się mniejszą zawartością skrobi i kwasu fitynowego oraz większą kruchością niż niesłodowane nasiona.
4. Zmodyfikowany proces słodowania nasion (wyższa temperatura kiełkowania, większa zawartość wody w nasionach) miał wpływ na poprawę parametrów technologicznych brzeczek otrzymanych ze słodów z soczewicy zielonej oraz soczewicy brązowej.
5. Słodowanie spowodowało znaczne zmniejszenie zawartości rafinozy i stachiozy w słodach z soczewicy oraz fasoli.
6. Słodowanie w znacznym stopniu modyfikowało rodzaj i ilość związków lotnych, zwiększając udział aldehydów i zmniejszając udział alkoholi oraz terpenów w całkowitej zawartości związków lotnych w słodach z soczewicy.
7. Modyfikacja warunków słodowania soczewicy zielonej (malejąca temperatura kiełkowania) oraz warunków zacierania uzyskanych słodów (dodatek α-amylazy, zacieranie w temperaturze 75°C) umożliwiła wytworzenie brzeczek piwowarskich o dobrych parametrach technologicznych oraz bezglutenowego piwa o obniżonej zawartości alkoholu.

8. LITERATURA

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9. PUBLIKACJE BĘDĄCE PRZEDMIOTEM ROZPRAWY DOKTORSKIEJ

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Analysis of Physicochemical Parameters of Congress Worts Prepared from Special Legume Seed Malts, Acquired with and without Use of Enzyme Preparations

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Abstract: This study was conducted to produce malt from legume seeds (chickpea, lentil, pea, and vetch) and test whether malting with parameters, typically barley grain, will result in well-modified legume seed malt. Analysis of malt was performed by producing congress worts from legume seed malts. Concentration of phenolic compounds, as well as antioxidant activity of legume seed malts was analysed. Acquired worts were characterised with poor technological characteristics (wort extract, wort volume, saccharification time, brewhouse efficiency); however, the malting process increased concentration of phenolic compounds and antioxidant activity of the plant material. Subsequent mashing tests with addition of different external enzymes and/or gelatinisation of legume seed malt were performed. Use of external enzymes improved saccharification time, extract content, wort volume, as well brewhouse efficiency in the case of some legume seed malts. The best brewhouse efficiencies and highest extract values were acquired by the samples prepared with 30% of gelatinised vetch malt or chickpea malt mixed with 70% of Pilsner malt. The study shows that there is possibility of creating legume seed malts, but malting and mashing characteristics need to be customised for these special malts.

Keywords: malt; congress mash; legumes; vetch; pea; chickpea; lentil; antioxidant activity; brewhouse efficiency

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1. Introduction

Legumes are a part of the *Leguminosae* family, which consists of 650 genera with 18,000 species. It is the third largest angiosperm family in the world. Seeds of legumes, sometimes called pulses, are the second most important source of nutrients in the world, just after cereals [1]. They are rich in carbohydrates, fats, calcium, iron, thiamine, riboflavin, and fibre, but the most important quality of legumes is their very high protein content, which can range from 16% to 50% [2]. There is increasing interest in consuming legumes, as well as other plant-based products, primarily due to three different factors: (1) awareness of climate change; (2) nutritional benefits of legume consumption; as well as (3) concerns about welfare of farm animals [3]. Unfortunately, transitioning to higher legume consumption is not as straightforward as a simple increase in legume production. Legumes are simple crops that do not need application of chemical fertilizers to maintain yield on optimal levels; moreover, growing legumes lowers the cost of production, and improves quality of the soil, due to nitrogen assimilation and carbon sequestration [4,5]. Legumes, despite their high nutritional value, are not utilised to their fullest potential because they possess few flaws, which mitigate their advantages. Legume grains are hard-to-cook, their proteins are hard to digest, the bioavailability of many minerals and vitamins present in seeds is low, and many of legumes possess antinutritional substances, such as phytic acid,

tannins, trypsin inhibitors, and α-galactosides [6,7]. If it was possible to remove some or all of these substances and improve digestibility of legume protein, then legumes could possibly play a larger role in the nutrition of humankind [8]. In this research, we attempted to use the process of malting to change characteristics of seeds of some legumes grown commercially in Europe, such as vetch (*Vicia sativa*), yellow pea (*Pisum sativum*), green lentils (*Lens culinaris*), and chickpea (*Cicer arietinum*) [9]. Malting is a technological process used to modify mainly grains of barley (*Hordeum vulgare*) as well as other cereal grains, and its usefulness in modification of legume seeds is mostly unknown [10]. It is a process in which grain moisture is increased by alternating the process of submerging it in water and storing grain in humid air (in a process called "steeping"), then grains with increased moisture (up to 42–45%) are germinated and dried in ovens in a process called "kilning". Conditions of kilning, steeping, and germination can be highly modified by maltsters (to acquire malt with different characteristics). The process of malting is used to modify the physical structure of the grains and allows for activation of many metabolic pathways in which grains change their composition, produce enzymes, and create various phytochemicals [11]. Germination of legume seeds, which is a part of the malting process, has been assessed by few research teams; studies show improvements in antioxidant ability, bioavailability of vitamins and minerals, higher concentration of polyphenols, and bioactive peptides in the germinated legume seeds [12–14]. These results show a possibility of using the malting process to improve the nutritional quality of legume seeds. The main advantages of malting over the sole germination process are the positive changes in the organoleptic characteristics of the finished product, as well as improved stability of malt over the germinated seed [15]. Legume malts could be used to produce gluten-free beer worts or malt extracts with high protein content, as well as to produce fermented food, such as tempeh, but with a reduced amount of antinutritional substances and an increased amount of antioxidants [2,3,8,10–12]. The aim of this study is to determine advantages and disadvantages of congress worts obtained from legume seed malts (chickpea, yellow pea, common vetch, and green lentil) produced in analogous malting and mashing conditions, such as congress worts made from typical Pilsen malt.

2. Materials and Methods

2.1. Reagents and Standards

Reagents used in this study were: acetic acid (99.5%), diammonium salt of 2,2′-azobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS+•) (98%), 1,1-diphenyl-2-picrylhydrazil radical (DPPH•) (95%), ferrous sulphate (98%), Folin–Ciocalteu reagent (2 M), gallic acid (98%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (97%), iron (III) oxide (99%), Lugol's iodine (iodine and potassium iodide solution in water) (5%), methanol (99%), sodium acetate (99%), sodium carbonate (99%), and 2,4,6-tripyridyl-S-thiazine (TPTZ) (98%).

2.2. Legume Seeds

Legume seeds used in this study were: common vetch—VS (*Vicia sativa*), green lentil—LS (*Lens culinaris*), chickpea—CS (*Cicer arietinum*), and yellow pea—PS (*Pisum sativum*) harvested in 2019, acquired from a company supplying the food industry with seeds of various plants.

2.3. Malt

Malt used in this study, as a control sample, were: Pilsen malt (BM) produced by Viking Malt Company (Strzegom, Poland) from barley harvested in 2019.

2.4. Enzyme Preparations

Enzyme preparations used in this study were produced by DSM Food Specialties (Heerlen, The Netherlands). They are enzyme preparations with different enzymatic activities: Amigase Mega (AM: fungal amyloglucosidase, standardised activity ≥36,000 AGI/g of

the preparation, batch number: 4192201011); Filtrase BR-X (FBR: thermostable β-glucanases and hemicellulases, standardised activity ≥6000 BGF/g of the preparation, batch number: 4191222011); Filtrase NL (FNL: thermostable fungal β-glucanases and xylanases, standardised activity ≥10,500 BGF/g of the preparation, batch number: 4191809011); Maxazyme NNP DS (MAX: bacterial protease, standardised activity ≥180,000 PC/g of the preparation, batch number: 418160001); Mats L Classic (MLC: bacterial thermostable α-amylase, standardised activity ≥7400 TAU/g of the preparation, batch number: 18001050), Mycolase LV (MLV: fungal α-amylase, standardised activity ≥2250 FAU/g of the preparation, batch number: 8192120011) and Brewers Compass (BC: bacterial α-amylase, bacterial β-glucanase, fungal cellulase and bacterial endopeptidase, standardised activity ≥7500 RAU/g of the preparation, batch number: 4192072011). Enzyme preparations were added to the studied mashes at the start of congress mashing at the highest dosage recommended by the producer, which equalled 200 mg of FBR for 1 kg of malt; 600 mg of FNL for 1 kg of malt; 1 g of MAX for 1 kg of malt; 275 mg of MLC for 1 kg of malt; 40 mg of MLV for 1 L of wort; 4 g of BC for 1 kg of malt; and 1.2 mL of AM for 1 kg of malt. Methods of assessment of the enzyme activity, as well as enzyme activity units are described in the Supplementary Materials.

2.5. Malting Procedure

2.5.1. Steeping and Germination

Water content in legume seeds was analysed by the Brabender MT moisture analyser (Brabender GmbH & Co, Duisburg, Germany). A total of 50 g portions of seeds were weighed and measured into stainless steel malting containers from the Automatic Micro-Malting System (Phoenix Systems, Adelaide, Australia) (Figure 1). Weight of the malting kit (container and grain) were weighed. The changes in moisture content of the legume seed samples were assessed by the changing weight of the malting kit. Steeping was executed by submerging containers in tap water (15 °C) for 5 h, removing containers from water and keeping them in humid air in a refrigerated malting cabinet (90–95% relative humidity, 15 °C) for 19 h, submerging containers in fresh tap water (15 °C) for 5 h and performing the last air rest in humid air (90–95% relative humidity, 15 °C). After 48 h of steeping, seeds acquired moisture content over 45%. After removing all unabsorbed water from seeds, the process of germination began. Grain was kept in the same malting containers in a refrigerated malting cabinet (90–95% relative humidity, 15 °C) and germinated for 144 h. During the germination process, germinated grains were mixed once every 24 h to avoid rootlets entanglement. Deficiencies in the mass of the malting sets, resulting mainly from water evaporation, were supplemented by adding distilled water, in order to maintain constant humidity of seeds throughout germination.

2.5.2. Kilning and Grinding

Kilning (23 h) was performed immediately after 144 h of germination. Malting containers were loaded into a UF110 Plus dryer (Memmert GmbH + Co, Schwabach, Germany) and kilned in the following conditions: 50 °C (18 h and 50 min), ramp up to 65 °C (10 min), 65 °C (2 h and 50 min), ramp up to 82 °C (10 min), 82 °C (2 h). After kilning, malt was transferred into tightly closed containers, which prevented moisture absorption during the cooling period. After temperature of malt dropped to 25 °C, rootlets of the malt were manually removed and malt was grinded on the Bühler Miag disc mill DLFU (Bühler, Uzwil, Switzerland), according to the Analytica EBC (European Brewery Concention) 1.1 method [16]. Gradation tests were not performed, because it was assumed that differences in legume malt grinding (compared to typical malt grinding) should be a subject for another study.

Figure 1. Malting containers with unmalted legume seeds: soy, chickpea, and lentil (from the left to right).

2.6. Mashing—Congress Wort Production

2.6.1. Congress Wort Production from 100% Legume Malt

Congress worts were produced in the automated laboratory mashing machine (LB Electronic, Lochner Labor + Technik, Berching, Germany) according to the Analytica EBC method 4.5.1, with modifications, due to the lower weight of grain samples [17]. Filtered wort was collected for analyses. Legume malt congress worts were prepared in duplicate. Mashing procedure is shown in the Figure 2. Mashes were prepared from chickpea seed malt, lentil seed malt, pea seed malt, and vetch seed malt, with and without external enzymes (FBR, FNL, MAX, MLC, MLV, BC, and AM). Wort prepared from Pilsen malt (M) was the control sample.

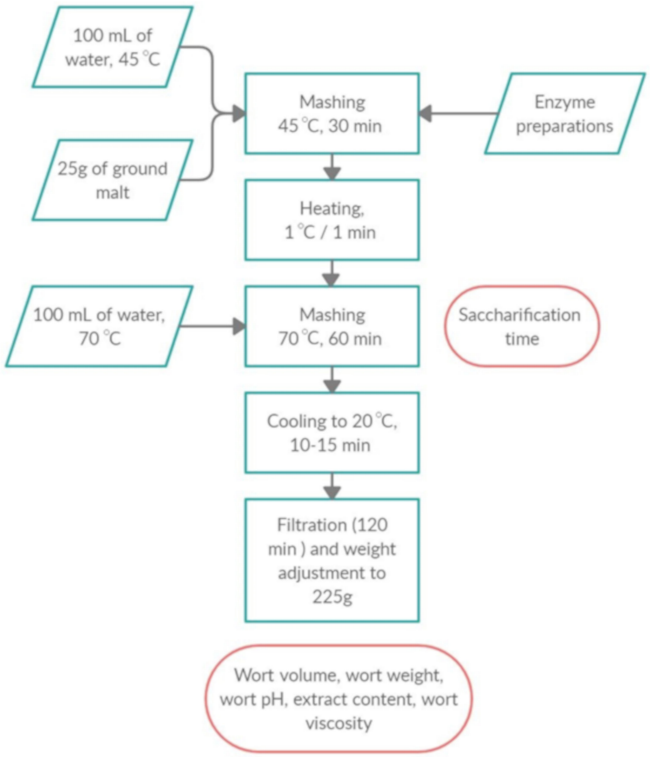

Figure 2. Mashing procedure for 100% legume seed malts.

2.6.2. Congress Wort Production with Addition of 30% Gelatinised Legume Malt

Analysis has been carried according to the Analytica EBC 4.5.1 method with modifications and is shown in the Figure 3 [17]. After the filtration process, filtered wort has been collected for analyses. Congress worts made with addition of 30% gelatinised legume malt have been prepared in duplicate. Mashes have been prepared from chickpea malt, lentil malt, and vetch malt with and without use of external enzymes (MAX). Control sample has been M2, where 30% of gelatinised legume seed malt has been substituted with 30% of Pilsen malt that also underwent gelatinisation. Congress wort with 30% of gelatinised pea malt has not been produced due to the lack of available plant material.

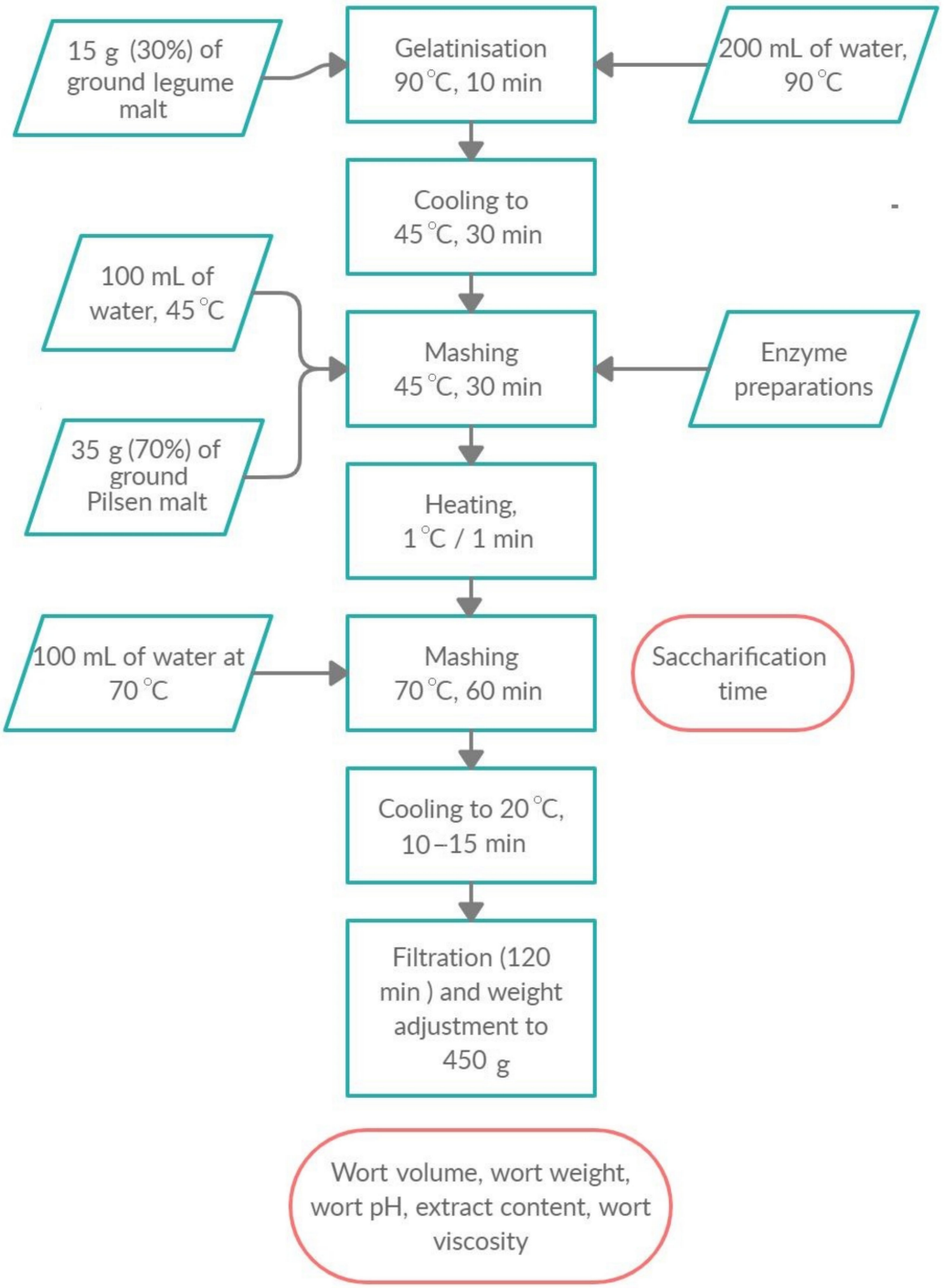

Figure 3. Mashing procedure for mashes with 30% gelatinised legume seed malts.

2.7. Analyses of the Acquired Worts

All of the worts were assessed by the same methods.

2.7.1. Saccharification Time

Saccharification time was assessed by the Analytica EBC 4.5.1 method. After adding water at a temperature of 70 °C to the mashes, measurement of saccharification time started, and was performed in 5 min intervals. If the iodine solution did not turn the mash blue, it meant that full saccharification of the starch occurred, and value of saccharification time was noted. The last test was performed after 60 min of mashing at a temperature of 70 °C.

2.7.2. Wort pH

Wort pH was assessed using pH-meter (MP220, Mettler Toledo, Columbus, OH, USA) in worts collected after the filtration process. Temperature of the tested sample was equal to 20 °C. Measurement was performed in duplicate for each wort sample.

2.7.3. Wort Extract Content

Extract content of the worts was assessed with the use of densimeter (DMA 35, Anton Paar, Graz, Austria) in filtered congress wort with temperature adjusted to 20 °C. Measurements were performed in duplicate for each wort sample.

2.7.4. Wort Volume

Wort volume was recorded from the scale of the graduated cylinder after the filtration process.

2.7.5. Wort Viscosity

Wort viscosity was assessed according to the Analytica EBC 8.4 method with the use of falling ball KF 10 viscometer (Rheotec Company, Schorisse, Belgium) [18]. Temperature of the wort was adjusted to 20 °C prior to analysis. In the cases of some wort samples, the acquired volume of the wort was too low to perform a viscosity test. Measurement was performed in duplicate for each tested wort sample.

2.7.6. Simplified Brewhouse Efficiency

Brewhouse efficiency is a parameter, which tells how much of the substances present in malt were transferred into the wort as a result of mashing [19]. Simplified brewhouse efficiency of the worts was calculated based on the wort volume, density of the wort, extract content in the wort, and mass of the malt, according to the formula below [20]:

$$BE = E \cdot 10 \cdot (V_k / V_{max}) \quad (1)$$

BE—simplified brewhouse efficiency (%);
E—extract content of the wort (°Plato);
V_k—final volume of the wort (mL);
V_k—maximal volume of the wort—for 2.6.1 set at 200 mL, for 2.6.2 set at 400 mL.

2.8. Analyses of Phenolic Components and Antioxidant Activity of Legume Seed Malts and Legume Seed Malt Worts

2.8.1. Preparation of the Worts

Worts, prior to the analysis of the concentration of phenolic compounds, as well as their antioxidant activity, were centrifuged (10 min, 5000 rpm) in the laboratory centrifuge MPW-351R (Warsaw, Poland) and filtered through the paper filter. Only chickpea malt wort (C), lentil malt wort (L), pea malt wort (P), vetch malt wort (V), Pilsen malt wort (M), gelatinised chickpea wort (GC30), gelatinised lentil wort (GL30), and gelatinised vetch malt wort (GV30), due to the lack of stored samples, were assessed.

2.8.2. Preparation of the Methanol Extracts from Legume Seeds, Legume Seed Malts, and Barley Malt

To assess concentration of phenolic compounds and antioxidant activity of the legume seed malts, as well as control Pilsen malt, methanol extracts were produced, according to the modified method of Nowak [21]. A total of 20 g of Pilsen malt, legume seed malt, and legume seed samples were finely ground in a laboratory mill WZ-1 (Bydgoszcz, Poland). A total of 2.5 g of ground malt/seed samples were weighed and transferred to 50 mL polypropylene falcon tubes. A total of 40 mL of 80% (v/v) methanol was added to the falcon tubes. Samples were sonicated in a XUB5 ultrasonic bath XUB5 (Shepreth, Great Britain) for 15 min and left in a fridge at 6 °C for 12 h. After 12 h, falcon tubes were sonicated again for 15 min and centrifuged at 6000 rpm for 10 min. Only the top, clear part of the extract, without any sediment particles, was analysed.

2.8.3. Concentration of Phenolic Compounds

Total content of polyphenol compounds in worts was determined using spectrophotometric Folin–Ciocalteu (F–C) method [22]. A total of 0.1 mL of wort/methanol extracts, followed by 0.2 mL of F–C reagent were pipetted into polystyrene cuvettes. After 3 min, 1 mL of 20% (v/v) sodium carbonate solution in water and 2 mL of distilled water were added into cuvettes, which were then stored in a dark place. After 1 h, absorbance of the prepared samples was analysed using A560 spectrophotometer (AOE instruments, Shanghai, China) with the wavelength set at 765 nm. Distilled water was used as a blind sample. Results were presented as an average value from three measurements for worts and as an average from nine measurements for legume seed malts/legume seed extracts. Results were expressed as mg of gallic acid equivalents per 100 mL in the case of worts, and as mg of gallic acid equivalent per 100 g of seed or malt in the case of legume seeds and legume seed malts. Calibration curve in the range of 10–200 mg gallic acid equivalent (GAE)/100 mL was used to read the results.

2.8.4. ABTS•+ Assay

Antioxidative ability of the tested samples was assessed by means of the ABTS•+ assay [23]. Samples of wort or methanol extracts (0.03 mL) were mixed in a polystyrene cuvette with 3 mL of ABTS+• water solution. Absorbance of the ABTS+• solution equalled 0.700 at the wavelength of 734 nm. After 6 min, the absorbance of the tested samples was measured. Nine measurements were performed for methanol extracts and three for the worts. Results were expressed as µmol Trolox equivalent (TE) of antioxidative capacity per 1 mL of the wort (µmol TE/mL) or µmol Trolox equivalent (TE) of antioxidative capacity per 1 g of legume seed/legume seed malt. Distilled water was used as a blank sample.

2.8.5. DPPH• Assay

Another method to assess antioxidative abilities of malts and worts was the DPPH• assay [24]. Samples of wort or methanol extracts (0.1 mL) were mixed with 2 mL of 0.04 mmol/L DPPH• solution in ethanol and 0.4 mL of distilled water in a polystyrene cuvette. After 10 min of incubation at room temperature, the absorbance was measured with a spectrophotometer at the wavelength of 517 nm. The data were expressed as Trolox equivalent (TE) of antioxidative capacity per 1 mL of the wort or 1 g of legume seed/legume seed malt (mmol TE/mL or mmol TE/g). All measurements were performed in triplicate for worts and in nine repetitions for seed/malt extracts. Ethanol was used as a blank sample.

2.8.6. FRAP Assay

In the FRAP assay, capacity of the methanol extracts or worts to reduce iron from ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine (Fe (III)-TPTZ) was assessed [25]. Reagent was prepared by mixing 10 mmol 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ)/L reagent with 20 mmol/L ferric (III) chloride in acetate buffer (pH 3.6). 0.1 mL of wort/methanol extract was mixed

in polystyrene cuvette with 0.9 mL distilled water and 3 mL of ferric complex. Change in absorbance was measured after 10 min. Quantitative analyses were performed by the external standard method using ferrous (II) sulphate (0.2 mmol/L) as the reference standard. Absorbance measured at wavelength of 593 nm was correlated with the concentration of the ferrous (II) sulphate. Three measurements were performed for each of the analysed worts and nine measurements were performed in the case of methanol extracts.

2.9. Statistical Analysis

The results acquired in this work were statistically analysed using Statistica 13.5 (StatSoft, Tulsa, OK, USA) using one-way ANOVA ($\alpha = 0.05$). Duncan's test was used to calculate differences and assess homogenous groups between the means.

3. Results

3.1. Analyses of Physicochemical Parameters of the Congress Worts Produced from Legume Seed Malts

Results acquired from the analyses of the physicochemical parameters of the worts are shown in Tables 1–5.

Table 1. Physicochemical parameters of chickpea malt worts with and without addition of enzyme preparations.

Sample [1]	Saccharification Time [2]	pH	Extract (°Plato)	Wort Volume (mL)	Wort Viscosity (mPa·s)	Brewhouse Efficiency (%)
C	X	5.44 ± 0.03 ^{cd}	2.39 ± 0.11 ^{ef}	80 ± 5 ^{d}	n.d.	9.53 ± 0.16 ^{d}
C-FBR	X	6.15 ± 0.07 ^{a}	2.60 ± 0.05 ^{d}	115 ± 5 ^{b}	1.39 ± 0.06 ^{b}	14.97 ± 0.94 ^{c}
C-FNL	X	5.55 ± 0.07 ^{bc}	2.46 ± 0.04 ^{def}	122.5 ± 2.5 ^{ab}	1.35 ± 0.07 ^{b}	15.07 ± 0.07 ^{c}
C-MAX	X	5.50 ± 0.04 ^{bc}	5.51 ± 0.03 ^{b}	95 ± 5 ^{c}	n.d.	26.18 ± 1.52 ^{b}
C-MLC	X	5.51 ± 0.02 ^{bc}	2.30 ± 0.06 ^{f}	95 ± 5 ^{c}	1.39 ± 0.03 ^{b}	10.94 ± 0.86 ^{d}
C-MLV	X	5.63 ± 0.02 ^{b}	2.80 ± 0.02 ^{c}	35 ^{e}	n.d.	4.91 ± 0.04 ^{e}
C-BC	X	5.51 ± 0.03 ^{bc}	2.54 ± 0.03 ^{de}	110 ± 5 ^{b}	1.37 ± 0.02 ^{b}	13.98 ± 0.80 ^{c}
C-AM	X	5.51 ± 0.01 ^{bc}	2.53 ± 0.02 ^{de}	110 ± 5 ^{b}	1.37 ± 0.03 ^{b}	13.95 ± 0.75 ^{c}
M	10	5.31 ± 0.05 ^{d}	6.84 ± 0.07 ^{a}	130 ^{a}	1.75 ± 0.03 ^{a}	44.47 ± 0.46 ^{a}

[1] C—wort from chickpea seed malt, C-FBR—wort from chickpea malt mashed with addition of Filtrase BR-X, C-FNL—wort from chickpea malt mashed with addition of Filtrase NL, C-MAX—wort from chickpea malt mashed with addition of Maxazyme NNP DS, C-MLC—wort from chickpea malt mashed with addition of Mats L Classic, C-MLV—wort from chickpea malt mashed with addition of Mycolase LV, C-BC—wort from chickpea malt mashed with addition of Brewers Compass, C-FNL—wort from chickpea malt mashed with addition of Amigase Mega, M—wort from Pilsen barley malt. Values are expressed as mean ($n = 2$) ± standard deviation in case of saccharification time, wort volume and brewhouse efficiency and as a mean ($n = 4$) ± standard deviation in case of pH, extract and viscosity. Mean values with different letters (a, b, . . . , f) within the same column are statistically different (p-value < 0.05). [2] "X" means that complete saccharification of the sample had not been acquired; n.d. stands for "no data" in the samples in which viscosity could not be assessed.

Table 2. Physicochemical parameters of lentil malt worts with and without addition of enzyme preparations.

Sample [1]	Saccharification Time [2]	pH	Extract (°Plato)	Wort Volume (mL)	Wort Viscosity (mPa·s)	Brewhouse Efficiency (%)
L	X	5.51 ± 0.05 ^{d}	1.59 ± 0.04 ^{f}	100 ^{c}	n.d.	7.95 ± 0.20 ^{f}
L-FBR	X	6.12 ± 0.03 ^{a}	2.60 ± 0.04 ^{c}	100 ^{c}	1.39 ± 0.03 ^{c}	13.00 ± 0.20 ^{de}
L-FNL	X	5.57 ± 0.02 ^{cd}	2.41 ± 0.03 ^{de}	130 ± 5 ^{ab}	1.32 ± 0.01 ^{cd}	15.66 ± 0.41 ^{c}
L-MAX	X	5.64 ± 0.03 ^{bc}	2.33 ± 0.03 ^{e}	125 ± 5 ^{b}	1.39 ± 0.01 ^{c}	14.57 ± 0.77 ^{cd}
L-MLC	25	5.25 ± 0.04 ^{e}	3.4 ± 0.02 ^{b}	140 ± 5 ^{a}	1.51 ± 0.03 ^{b}	23.80 ± 0.71 ^{b}
L-MLV	10	5.6 ± 0.03 ^{bcd}	2.39 ± 0.04 ^{e}	130 ± 5 ^{ab}	n.d.	15.55 ± 0.86 ^{c}
L-BC	X	5.55 ± 0.02 ^{cd}	2.4 ± 0.07 ^{de}	130 ^{ab}	1.37 ± 0.03 ^{c}	15.61 ± 0.46 ^{c}
L-AM	X	5.69 ± 0.03 ^{b}	2.55 ± 0.04 ^{cd}	100 ^{c}	1.29 ± 0.03 ^{d}	12.75 ± 0.2 ^{e}
M	10	5.31 ± 0.05 ^{e}	6.84 ± 0.07 ^{a}	130 ^{ab}	1.75 ± 0.03 ^{a}	44.47 ± 0.46 ^{a}

[1] L—wort from lentil malt, L-FBR—wort from lentil malt mashed with addition of Filtrase BR-X, L-FNL—wort from lentil malt mashed with addition of Filtrase NL, L-MAX—wort from lentil malt mashed with addition of Maxazyme NNP DS, L-MLC—wort from lentil malt mashed with addition of Mats L Classic, L-MLV—wort from lentil malt mashed with addition of Mycolase LV, L-BC—wort from lentil malt mashed with addition of Brewers Compass, L-FNL—wort from lentil malt mashed with addition of Amigase Mega, M—wort from Pilsen barley malt. Values are expressed as mean ($n = 2$) ± standard deviation in case of saccharification time, wort volume and brewhouse efficiency and as a mean ($n = 4$) ± standard deviation in case of pH, extract and viscosity. Mean values with different letters (a, b, . . . , f) within the same column are statistically different (p-value < 0.05). [2] "X" means that complete saccharification of the sample had not been acquired; n.d. stands for "no data" in the samples in which viscosity could not be assessed.

Table 3. Physicochemical parameters of pea malt worts with and without addition of enzyme preparations.

Sample [1]	Saccharification Time [2]	pH	Extract (°Plato)	Wort Volume (mL)	Wort Viscosity (mPa·s)	Brewhouse Efficiency (%)
P	X	5.7 ± 0.06 [b]	2.80 ± 0.08 [c]	110 ± 10 [b]	1.50 ± 0.05 [b]	15.44 ± 1.84 [c]
P-FBR	X	6.37 ± 0.06 [a]	3.10 ± 0.08 [b]	130 ± 15 [ab]	1.46 ± 0.03 [b]	20.10 ± 1.81 [b]
P-FNL	X	5.75 ± 0.1 [b]	2.74 ± 0.03 [c]	145 ± 5 [a]	1.33 ± 0.04 [cd]	19.86 ± 0.47 [b]
P-MAX	X	5.86 ± 0.03 [b]	2.76 ± 0.04 [c]	130 ± 5 [ab]	1.47 ± 0.03 [b]	17.95 ± 0.95 [bc]
P-MLC	25	5.34 ± 0.03 [c]	2.10 ± 0.05 [d]	150 ± 10 [a]	1.45 ± 0.04 [b]	15.78 ± 1.43 [c]
P-MLV	X	5.76 ± 0.01 [b]	2.77 ± 0.02 [c]	135 ± 5 [ab]	1.42 ± 0.01 [bc]	18.7 ± 0.56 [bc]
P-BC	X	5.77 ± 0.01 [b]	2.92 ± 0.04 [c]	130 [ab]	1.43 ± 0.02 [bc]	18.98 ± 0.26 [bc]
P-AM	40	5.87 ± 0.02 [b]	2.81 ± 0.03 [c]	135 ± 5 [ab]	1.31 ± 0.01 [d]	18.98 ± 0.91 [bc]
M	10	5.31 ± 0.05 [c]	6.84 ± 0.07 [a]	130 [ab]	1.75 ± 0.03 [a]	44.47 ± 0.46 [a]

[1] P—wort from pea malt, P-FBR—wort from pea malt mashed with addition of Filtrase BR-X, P-FNL—wort from pea malt mashed with addition of Filtrase NL, P-MAX—wort from pea malt mashed with addition of Maxazyme NNP DS, P-MLC—wort from pea malt mashed with addition of Mats L Classic, P-MLV—wort from pea malt mashed with addition of Mycolase LV, P-BC—wort from pea malt mashed with addition of Brewers Compass, P-FNL—wort from pea malt mashed with addition of Amigase Mega, M—wort from Pilsen barley malt. Values are expressed as mean (n = 2) ± standard deviation in case of saccharification time, wort volume and brewhouse efficiency and as a mean (n = 4) ± standard deviation in case of pH, extract and viscosity. Mean values with different letters (a, b, . . . , d) within the same column are statistically different (p-value < 0.05). [2] "X" means that complete saccharification of the sample had not been acquired.

Table 4. Physicochemical parameters of vetch malt worts with and without addition of enzyme preparations.

Sample [1]	Saccharification Time [2]	pH	Extract (°Plato)	Wort Volume (mL)	Wort Viscosity (mPa·s)	Brewhouse Efficiency (%)
V	20	5.53 ± 0.04 [bc]	2.40 ± 0.05 [c]	115 ± 5 [bc]	1.63 ± 0.03 [b]	13.82 ± 0.89 [b]
V-FBR	X	6.08 ± 0.07 [a]	1.80 ± 0.06 [d]	90 ± 5 [e]	n.d.	8.12 ± 0.72 [c]
V-FNL	X	5.40 ± 0.04 [cd]	1.66 ± 0.03 [de]	70 [f]	n.d.	5.82 ± 0.11 [d]
V-MAX	10	5.55 ± 0.04 [bc]	1.71 ± 0.03 [de]	100 [de]	n.d.	8.56 ± 0.15 [c]
V-MLC	25	5.11 ± 0.06 [e]	2.80 ± 0.08 [b]	100 ± 5 [de]	1.09 ± 0.05 [e]	14.02 ± 1.10 [b]
V-MLV	X	5.57 ± 0.03 [b]	1.60 ± 0.06 [e]	125 ± 5 [ab]	1.48 ± 0.03 [c]	10.02 ± 0.78 [c]
V-BC	20	5.47 ± 0.02 [bc]	1.58 ± 0.03 [e]	105 ± 5 [cd]	1.28 ± 0.04 [d]	8.29 ± 0.24 [c]
V-AM	30	5.58 ± 0.02 [b]	1.79 ± 0.01 [d]	115 ± 5 [bc]	1.28 ± 0.03 [d]	10.30 ± 0.51 [c]
M	10	5.31 ± 0.05 [d]	6.84 ± 0.07 [a]	130 [a]	1.75 ± 0.03 [a]	44.47 ± 0.46 [a]

[1] V—wort from vetch malt, V-FBR—wort from vetch malt mashed with addition of Filtrase BR-X, V-FNL—wort from vetch malt mashed with addition of Filtrase NL, V-MAX—wort from vetch malt mashed with addition of Maxazyme NNP DS, V-MLC—wort from vetch malt mashed with addition of Mats L Classic, V-MLV—wort from vetch malt mashed with addition of Mycolase LV, V-BC—wort from vetch malt mashed with addition of Brewers Compass, V-FNL—wort from vetch malt mashed with addition of Amigase Mega, M—wort from Pilsen barley malt. Values are expressed as mean (n = 2) ± standard deviation in case of saccharification time, wort volume and brewhouse efficiency and as a mean (n = 4) ± standard deviation in case of pH, extract and viscosity. Mean values with different letters (a, b, . . . , d) within the same column are statistically different (p-value < 0.05). [2] "X" means that complete saccharification of the sample had not been acquired; n.d. stands for "no data" in the samples in which viscosity could not be assessed.

3.1.1. Saccharification Time of the Worts

Mash prepared from Pilsen malt (M) saccharified in the first 10 min of mashing at temperature of 70 °C. Most of the mashes prepared from 100% legume seed malts were characterised with worse saccharification time. Only V-MAX and L-MLV saccharified in the same time, as M. From the legume seed malt mashes, vetch malt was saccharified in most of the mashing trials (V, V-MAX, V-MLC, V-BC, V-AM), while V-MAX acquired the fastest saccharification time (10 min) and V-AM the slowest (30 min). Chickpea malt mashes were not saccharified in samples with and without enzyme preparations. Lentil malts were not saccharified in a test without addition of external enzymes, although the addition of two of the enzyme preparations resulted in a saccharified lentil mash. Fastest saccharification of lentil mash was found out for L-MLV (10 min) and slowest for L-MLC (25 min). Mashes made out of pea malt also saccharified only in two samples with addition of enzymes. Slowest saccharification time was found out for P-AM (40 min) and the fastest for P-MLC (25 min). All mashes prepared with the addition of 30% gelatinised legume seed malt saccharified in the first 10 min, with and without the addition of Maxazyme enzyme preparation.

Table 5. Physicochemical parameters of the worts prepared with addition of gelatinised legume malt with and without addition of enzyme preparations.

Sample [1]	Saccharification Time	pH	Extract (°Plato)	Wort Volume (mL)	Wort Viscosity (mPa·s)	Brewhouse Efficiency (%)
GC30	10	5.42 ± 0.04 ^{a}	6.98 ± 0.07 ^{a}	270 ± 10 ^{bc}	1.62 ± 0.03 ^{b}	47.14 ± 2.22 ^{b}
GL30	10	5.34 ± 0.03 ^{ab}	6.69 ± 0.05 ^{bc}	215 ± 5 ^{d}	1.58 ± 0.03 ^{b}	35.96 ± 0.57 ^{d}
GV30	10	5.38 ± 0.02 ^{ab}	6.71 ± 0.04 ^{bc}	270 ± 5 ^{bc}	1.62 ± 0.02 ^{b}	45.30 ± 1.11 ^{bc}
GC30-MAX	10	5.37 ± 0.02 ^{ab}	6.76 ± 0.04 ^{b}	250 ± 5 ^{c}	1.61 ± 0.01 ^{b}	42.26 ± 1.10 ^{c}
GL30-MAX	10	5.32 ± 0.01 ^{b}	6.64 ± 0.04 ^{bc}	205 ± 5 ^{d}	1.54 ± 0.02 ^{b}	34.03 ± 0.63 ^{d}
GV30-MAX	10	5.37 ± 0.03 ^{ab}	6.58 ± 0.04 ^{c}	280 ± 10 ^{b}	1.55 ± 0.03 ^{b}	46.08 ± 1.93 ^{bc}
M2	10	5.32 ± 0.03 ^{b}	7.08 ± 0.04 ^{a}	325 ± 5 ^{a}	1.77 ± 0.03 ^{a}	57.49 ± 1.17 ^{a}

[1] GC30—wort from 30% gelatinised chickpea malt and 70% barley malt, GL30—wort from 30% gelatinised lentil malt and 70% barley malt, GV30—wort from 30% gelatinised vetch malt and 70% barley malt, GC30-MAX—wort from 30% gelatinised chickpea malt and 70% barley malt mashed with addition of Maxazyme NNP DS, GL30-MAX—wort from 30% gelatinised lentil malt and 70% barley malt mashed with addition of Maxazyme NNP DS, GV30-MAX—wort from 30% gelatinised vetch malt and 70% barley malt mashed with addition of Maxazyme NNP DS, M2—wort from 30% gelatinised Pilsen barley malt and 70% barley malt. Values are expressed as mean (n = 2) ± standard deviation in case of saccharification time, wort volume and brewhouse efficiency and as a mean (n = 4) ± standard deviation in case of pH, extract and viscosity. Mean values with different letters (a, b, c) within the same column are statistically different (p-value < 0.05).

3.1.2. pH of the Worts

pH of the worts prepared from legume seed malts was higher than pH for M (5.33). The type of the legume seed malts had significant impact on pH of acquired worts. P had highest pH value (5.7), V and L were characterised by similar pH (5.53 and 5.51) and pH of C was lowest (5.44). The addition of FBR increased pH of all the worts. Similar effects could be noted for worts prepared with the addition of MAX, MLV, and AM. FNL lowered pH of the V-FNL sample (from 5.53 to 5.4). The addition of MLC lowered pH of V-MLC (5.11), P-MLC (5.34), and L-MLC (5.25), but slightly increased it in the case of C-MLC (5.51). BC had little influence on the pH of the legume seed malt worts, increasing it by 0.04–0.07 for P-BC, L-BC, and C-BC and lowering it by 0.06 for V-BC. The type of the legume seed malt used in the samples with 30% gelatinised legume seed malt had a small impact on the wort pH and the addition of MAX did not change pH of the wort significantly.

3.1.3. Extract of the Worts

In comparison with the samples prepared from the legume seed malts, M was always characterised with the highest extract content. Extract content in L was the lowest from all of the tested samples (1.59 °Plato). In case of lentil malt worts, increase of the extract content due to the activity of enzyme preparations ranged from 0.74° to 1.59 °Plato (2.33° for L-MAX to 3.4 °Plato for L-MLC). The highest extract content for the legume seed malts prepared without the addition of enzyme preparations was found for P (2.8 °Plato). Most of the used enzyme preparations did not have a significant impact on the extract content of pea malt worts. Only P-MLC was characterised with lower extract content (2.1 °Plato) than P, and P-FBR acquired higher concentration of soluble solids (3.1 °Plato). Almost all vetch malt samples with the addition of enzyme preparations were characterised with lower extract content (1.58–1.8 °Plato) than V (2.27 °Plato), with the exception of V-MLC (2.8 °Plato). Most of the enzyme preparations used on the samples made from chickpea malt slightly increased extract content in the worts (from 0.07 °Plato in C-FNL to 0.41 °Plato in C-MLV). Only in case of C-MLC (2.3 °Plato) was the extract content lower than in C (2.39 °Plato). However, there was one exceptional difference concerning the chickpea malt worts. In the sample prepared with the use of MAX, the extract content of C-MAX was highest of all from the samples prepared from 100% legume seed malts and equalled 5.51 °Plato. Gelatinisation of 30% of legume seed malt mashed with Pilsen malt resulted in mashes with extract content similar—or only slightly lower—than M2. Highest extract content was noted for GC30 (6.98 °Plato) and the lowest for GV30-MAX (6.58 °Plato).

3.1.4. Volume of the Worts

Out of the mashes prepared from the legume seed malts without addition of external enzymes, the volume of the V was highest (115 mL), but it still was not as high as in M (130 mL). C acquired the lowest volume of the wort (80 mL). The addition of the FBR increased the wort volume in the case of C-FBR (115 mL) and P-FBR (130 mL). FNL also improved volume in most of the cases it was used. Volume of the chickpea, lentil, and pea mashes increased by 30–43 mL to 123 mL (C-FNL), 130 mL (L-FNL), and 145 mL (P-FNL). Both Filtrase enzymes reduced volume of vetch malt mashes to 90 mL (V-FBR) and 70 mL (V-FNL). The addition of MAX increased volume of the pea, lentil, and chickpea seed mashes by 15–25 mL to 130 mL (P-MAX), 125 mL (L-MAX), and 95 mL (C-MAX). Similar results were found for mashes prepared with the addition of MLC, which also allowed for acquiring higher wort volumes for P-MLC (150 mL), L-MLC (140 mL), and C-MLC (95 mL). The addition of MLV improved wort volume for P-MLV (135 mL), V-MLV (125 mL), and L-MLV (130 mL), but drastically reduced volume of C-MLV (35 mL). BC, a composition of many enzymes, increased volume of all the worts. AM increased volume of the P-AM (135 mL) and C-AM (110 mL), but did not affect volume of the worts from vetch or lentil malts. Volume of the worts prepared with the gelatinised legume seed malts should not be compared to the legume seed malt samples, because they were prepared from the greater amount of malt (50 g) and water (400 mL). Nevertheless, it is interesting to note that the volume of the worts acquired from GL30 and GL30-MAX (215 and 205 mL) were not much higher than the volume of the P-MLC (150 mL), which was made from 25 g of malt and 200 mL of water. The highest volume of wort from samples prepared with gelatinised legume seed malts was acquired from GV30-MAX, GC30, and GV30 (280, 270 and 270 mL, respectively). Interestingly, the addition of MAX did not significantly improve wort volume in the samples prepared with 30% of legume seed malt.

3.1.5. Viscosity of the Worts

Wort viscosity could not be assessed in the case of many samples, because volume of some collected worts was not high enough to perform analysis in the KF 10 Viscometer. In the samples prepared from legume malts, control sample M was characterised with the highest viscosity (1.75 mPa·s). The addition of enzyme preparations (FBR, FNL, MAX, MLC, MLV, BC) reduced viscosity of the worts prepared from legume seed malts. P acquired the highest viscosity (1.5 mPa·s), which was reduced in the greatest amount in P-AM to 1.31 mPa·s. Viscosity of V equalled 1.63 mPa·s and was significantly lowered to 1.09 mPa·s for V-MLC. Viscosity of L and C was not analysed, although tests could be performed for some of the samples produced from these malts with the use of external enzymes. The lowest viscosity in the case of lentil malt was found for L-AM (1.29 mPa·s) and the highest for L-MLC (1.51 mPa·s). Viscosity of chickpea seed malt worts could be analysed only in four samples, ranging from 1.35 mPa·s (C-FNL) to 1.39 mPa·s (C-FBR). Viscosity was tested in all of the samples prepared with the addition of 30% gelatinised legume malts and ranged from 1.54 mPa·s for GL30-MAX to 1.62 mPa·s for GV30 and GC30.

3.1.6. Simplified Brewhouse Efficiency

Brewhouse efficiency is a parameter that can more precisely tell how malt is modified compared to the sole analysis of wort extract or volume of the acquired wort [19]. Worts made out of legume malts without the addition of enzyme preparations were characterised by a poor brewhouse efficiency. The lowest was acquired by L (6.4%) and the highest by P (12.49%). The addition of external enzymes improved brewhouse efficiency in the case of pea, lentil, and chickpea malts. In the case of samples prepared with pea malts, the lowest increase of efficiency was noted for P-MLC (12.73%) and the highest for P-FBR (16.27%). Brewhouse efficiency was three times higher for L-MLC (19.29%) than for L, and was one of the highest efficiencies recorded for pure legume seed malt samples in this study. Worts made with the use of 30% gelatinised legume seed malt were characterised by far better brewhouse efficiency than pure legume seed malt worts. The highest efficiency

was acquired by GC30 (77.52%), as well as by GV30 and GV30-MAX (74.42% and 75.65%). Interestingly, the addition of MAX did not result in improved brewhouse efficiency in these samples.

3.2. Analyses of the Phenolic Components and Antioxidant Activity of Legume Seed Malts and Legume Seed Malt Worts

3.2.1. Concentration of Phenolic Components and Antioxidant Activity of the Legume Seeds, Legume Seed Malts, and in Barley Malt

Results of the F–C analysis and $ABTS^{+\bullet}$, $DPPH^{\bullet}$, and FRAP assays performed on the legume seeds prior to the malting process, legume seed malts, and barley malt, are shown in Table 6.

Table 6. Concentration of phenolic compounds and antioxidant activity of legume seeds, legume seed malts and barley malt.

Sample [1]	Concentration of Phenolic Compounds (mg GAE [2]/100 g)	$ABTS^{+\bullet}$ Assay (µmol TE [3]/g)	$DPPH^{\bullet}$ Assay (µmol TE/g)	FRAP Assay (µmol TE/g)
CS	44.09 ± 0.60^{f}	4.40 ± 0.04^{g}	1.05 ± 0.03^{f}	3.46 ± 0.02^{g}
CSM	112.14 ± 1.29^{b}	7.09 ± 0.06^{d}	1.22 ± 0.03^{e}	4.84 ± 0.02^{e}
LS	46.50 ± 0.98^{f}	5.66 ± 0.08^{f}	1.61 ± 0.03^{d}	4.77 ± 0.04^{e}
LSM	84.81 ± 1.53^{c}	6.06 ± 0.13^{e}	2.12 ± 0.05^{b}	5.78 ± 0.04^{b}
PS	21.21 ± 1.42^{g}	5.46 ± 0.14^{f}	0.86 ± 0.03^{g}	3.96 ± 0.03^{f}
PSM	85.59 ± 1.24^{c}	6.83 ± 0.09^{d}	1.66 ± 0.05^{d}	5.19 ± 0.02^{c}
VS	50.33 ± 0.68^{e}	9.85 ± 0.11^{a}	1.87 ± 0.02^{c}	4.77 ± 0.04^{e}
VSM	61.04 ± 0.64^{d}	8.43 ± 0.11^{b}	1.81 ± 0.02^{c}	4.97 ± 0.04^{d}
BM	128.62 ± 1.27^{a}	7.94 ± 0.10^{c}	4.28 ± 0.04^{a}	7.26 ± 0.04^{a}

[1] CS—chickpea seeds, CSM—chickpea seed malt, LS—lentil seeds, LSM—lentil seed malt, PS—pea seeds, PSM—pea seed malts, VS—vetch seeds, VSM—vetch seed malt, BM—Pilsen barley malt. Values are expressed as mean ($n = 9$) ± standard. Mean values with different letters (a, b, . . . , g) within the same column are statistically different (p-value < 0.05). [2] GAE— Gallic acid equivalent. [3] TE - Trolox equivalent.

Malting increased concentration of phenolic compounds in all of the legume seeds tested in the study. The greatest, with an almost 3-fold increase, was noted for chickpea seeds (CS) and chickpea seed malt (CSM) (from 44.09 mg GAE/100 g to 112.14 mg GAE/100 g). The lowest increase in concentration of phenolic compounds was found out for VS and VSM (from 50.33 mg GAE/100 g to 61.04 mg GAE/100 g). It is worth noting that VS was characterised by the highest concentration of phenolic compounds for unmalted seeds and by the lowest concentration for malted seeds. Barley malt was characterised with greater concentration of phenolic compounds than all the legume seed malts.

3.2.2. Concentration of the Phenolic Components and Antioxidant Activity in Legume Seed Malt Worts and Worts Prepared from Gelatinised Legume Seed Malts

F–C analysis of the phenolic compounds, as well as the $ABTS^{+\bullet}$, $DPPH^{\bullet}$, and FRAP assays were performed only for selected worts due to the lack of research material. Results of these analyses are shown in Table 7.

M was characterised by the highest antioxidant activity from the tested worts, however, in three tested worts (V, GC30, GV30), greater concentration of phenolic compounds was detected. V was characterised by the highest concentration of phenolic compounds (38.48 mg/100 mL) out of the worts prepared with the use of legume seed malts and acquired the highest antioxidant activity out of the four legume seed malt wort samples. In L, concentration of phenolic compounds was the lowest and equalled 33% of the amount detected in V, similar as C (which possessed slightly higher concentration of phenolic compounds than L, 36% of these detected in V). It is interesting to note that, in the GC30, GL30 and GV30 these differences were not as exceptional as in 100% legume seed malts. Further attention should be focused on the concentration of phenolic compounds in GC30, which, as mentioned before, was higher than M, despite C possessing lower concentration of phenolic compounds than M.

Table 7. Concentration of phenolic compounds and antioxidant activity of legume seed malt worts.

Sample [1]	Concentration of Phenolic Compounds (mg GAE [2]/100 mL)	ABTS$^{+\bullet}$ Assay (μmol TE [3]/mL)	DPPH$^{\bullet}$ Assay (μmol TE/mL)	FRAP Assay (μmol TE/mL)
C	13.90 ± 0.17 ^{f}	0.47 ± 0.01 ^{e}	0.26 ± 0.01 ^{f}	0.23 ± 0.01 ^{h}
L	12.70 ± 0.10 ^{g}	0.58 ± 0.01 ^{d}	0.37 ± 0.02 ^{d}	0.35 ± 0.01 ^{f}
P	16.59 ± 0.38 ^{e}	0.59 ± 0.01 ^{d}	0.27 ± 0.01 ^{f}	0.30 ± 0.01 ^{g}
V	38.48 ± 0.30 ^{a}	1.21 ± 0.03 ^{b}	0.92 ± 0.02 ^{b}	0.71 ± 0.03 ^{b}
GC30	20.82 ± 0.24 ^{c}	0.61 ± 0.02 ^{d}	0.42 ± 0.01 ^{c}	0.43 ± 0.01 ^{d}
GL30	16.31 ± 0.12 ^{e}	0.62 ± 0.01 ^{d}	0.31 ± 0.02 ^{e}	0.39 ± 0.01 ^{e}
GV30	23.01 ± 0.24 ^{b}	0.74 ± 0.02 ^{c}	0.38 ± 0.01 ^{d}	0.52 ± 0.02 ^{c}
M	19.01 ± 0.14 ^{d}	1.44 ± 0.03 ^{a}	1.09 ± 0.03 ^{a}	1.08 ± 0.02 ^{a}

[1] C—wort from chickpea seed malt, GC30—wort from 30% gelatinised chickpea malt and 70% barley malt, GL30—wort from 30% gelatinised lentil malt and 70% barley malt, GV30—wort from 30% gelatinised vetch malt and 70% barley malt,. Values are expressed as mean (n = 9) ± standard. Mean values with different letters (a, b, . . . , h) within the same column are statistically different (p-value < 0.05). [2] GAE— Gallic acid equivalent. [3] TE - Trolox equivalent.

4. Discussion

4.1. Analyses of Physicochemical Parameters of the Congress Worts Produced from Legume Seed Malts

4.1.1. Saccharification Time

Saccharification of the mashes is a result of hydrolysis of starch by the amylolytic enzymes present in the mash. For the full saccharification of the mash to happen, firstly, starch in the plant material must undergo a process of gelatinisation. Only then can it be hydrolysed by the amylolytic enzymes, such as α-amylase or β-amylase. Unfortunately, starch present in the legume seeds has higher gelatinisation temperature than starches present in the typically malted grains, such as barley or wheat [26]. However, the malting process can reduce gelatinisation temperature of starches even by 20 °C, depending on the malting conditions [27]. Currently there is no knowledge about characteristics of starch granules present in the legume seeds malts and about optimal temperature for legume seed malt enzymes to complete saccharification of starch present in legume seed malt mashes. There are many factors that can hinder starch hydrolysis, such as lack of amylolytic enzymes [28], non-catalytic binding of enzymes on non-substrate polymers, or physical barriers preventing access to the starch [6]. The conducted study shows that in the case of lentil malt, the main reason seems to be a lack of amylolytic enzymes, because samples L-MLC and L-MLV, prepared with external α-amylases, saccharified fully. Interestingly, L-AM (mash with addition of amyloglucosidase) did not undergo full saccharification. However, P-AM and P-MLC saccharified fully, but hydrolysis of starch was not complete in the case of P-MLV. Mashes prepared from chickpea malt were not saccharified, despite the addition of different compositions of external enzymes, which might show that the lack of internal enzymes is not the main problem in processing chickpea malt. The addition of proteolytic enzymes, as well as cellulases and β-glucanases did not improve saccharification of chickpea malt, so the possibility of starch being blocked by proteins, cellulose, or β-glucanase might also be excluded in the case of chickpea malt. Analysis of vetch malt mashes is one of the most interesting instances in this study, because V saccharified fully, but the addition of external enzymes mostly hindered saccharification of this malt. It is possible that used enzymes released some substances from vetch malt, which prevented proper activity of amylases, but the conducted study did not possess adequate means to confirm this assumption. In the case of the mashes prepared with the addition of gelatinised legume seed malts, all of the samples with 30% of legume malt saccharified fully. It might show, especially in the case of the chickpea malt, which gelatinisation temperature of the starch present in the legume malts is higher than 70 °C, and 70% of Pilsen malt addition possess a sufficient amount of enzymes to hydrolyse starch in the 30% of legume malt.

4.1.2. Wort pH

Analysis of the worts prepared by the congress mashing shows how much pH of the congress worts made from legume seed malts deviates from optimal pH for enzymes present in the barley malt (5.6–5.8 pH for α-amylase, 5.4–5.5 pH for β-amylase) [29]. These parameters might be optimal for the enzymes present in the legume seed malts; however, optimal pH of legume seed malt enzymes is currently unknown. In the prepared mashes, pH was not modified, so the wort pH is a result of all the substances, which were extracted from the malts and introduced to the worts. pH is an important parameter in the wort production, because it is one of the factors regulating activity of the external and internal enzymes present in the mash [29]. Most of the legume seed malt worts acquired lower pH value than non-typical worts produced from 100% oat malt in a study conducted by Klose et al., in which pH of the oat worts ranged from 5.9 to 5.99 [30]. pH of most of the legume malt worts was also lower than pH of rice malt wort acquired during congress mashing in the study conducted by Mayer et al. [31]. Beer with the addition of unmalted wheat and corn grist, produced and analysed by Vinko Krstanović et al., resulted in wort of pH higher (5.73) than most of the legume seed malt worts [32]. Wort pH is, usually, in the traditional congress mash analysis, a useful predictor for the extract of the acquired wort, as lower pH is known to improve saccharification, extract content, as well as filtering time [33]. However, as was said earlier, there is no available research concerning optimal pH values for activity of enzymes present in the legume seed malts. Correlation between wort pH and saccharification time could not be found in the case of legume malts used in this study and certain conclusions about the impact of the legume seed malts on the wort pH could not be acquired from the performed study. In the samples without the addition of external enzymes, samples prepared from legume seed malts were characterised with higher pH. This is confirmed by the samples prepared with the use of gelatinised legume seed malts: all samples with 70% of Pilsen malt acquired slightly higher pH value than M2. Without knowledge about optimal pH of the legume malt enzymes, it is hard to assess whether change in pH is positive or negative. The only certain result acquired from the wort pH analysis is that, with the increase in the amount of legume seed malt added to mash prepared with Pilsen malt, the activity of barley enzymes will decrease; this could create difficulties in acquiring wort with acceptable qualities.

4.1.3. Wort Extract

Extract of the collected worts is one of the parameters, which, combined with the volume of the wort, can tell how much of the substances can be transferred from the malt (brewhouse efficiency). In congress wort analysis, the best results are obtained when large volume of wort with high extract content is acquired; however, acquiring wort with perfect parameters is rarely the case in samples prepared from special, non-typical malts [11]. The suboptimal worts, either characterised with low extract content and high volume, or with high extract content and low volume, possess some flaws. Usually, with rise in the wort extract, wort viscosity increases, which is a hindrance in many of the processes [29]. Worts with low extract content are typically characterised by lower viscosity; however, for use in many branches of industry, such as production of malt extract or dietary supplements, beer brewing, baking industry, or production of nutrients for microorganisms, a product with high concentration of dissolved solids is preferred [34–36]. Production of the concentrated malt products from worts with low extract content require more water to be removed. Water is a liquid with very high thermal capacity, so water evaporation needs a great amount of energy to be utilised; thus, increasing costs of the technological process [37]. The extract content of worts prepared from the legume seed malts in most samples was far lower than extract content in M or M2. In nearly all of the samples in which enzyme preparations were used, worts made from vetch malt were characterised with the lowest extract content, which might explain the presence of the hard-to-digest seed cover characteristic for vetch, as well as the higher content of non-nutritional factors than in other legumes [38,39]. Extract content in the worts prepared only from the 30% gelatinised legume seed malt reached

similar levels as the control samples, prepared from Pilsen malt. The most promising results were found for the C-MAX sample, which, for 100% legume seed malt wort, acquired astonishingly high extract concentration, which might indicate that proteins are the main substances present in the chickpea malt, which hinder its extractability. Similar factors could not be noted for any of the other 100% legume seed malt samples, although analyses of brewhouse efficiency, discussed later, can also help in identifying critical impediments in the proper extraction processes.

4.1.4. Wort Volume

Low wort volume combined with low extract of collected wort show that the malt sample has very poor extractability. Disadvantages of worts with low extract content and high volume, as well as worts with high extract content and low volume, were discussed in Section 4.1.3. Filterability is an important parameter in the wort production, because high concentration of substances, which can hinder filtration, will lengthen the process of wort production. Factors affecting wort filtration are concentration of soluble substances in the wort, wort viscosity, concentration of phenols, content of insoluble polysaccharides, and characteristics of the grain/seed bed, through which the wort is filtered [29,40]. The worts prepared from the legume seed malts were characterised with lower wort volume than worts acquired from barley malt. The addition of the external enzymes to the prepared legume worts often improved wort filtration, which, due to the specificity of enzymes, might help in characterisation, where substances present in the special malts may cause difficulties. Volume of the worts acquired from the gelatinised legume seed malt samples was acceptable in the case of GV30, GC30, GV30-MAX, and GC30-MAX.

4.1.5. Wort Viscosity

According to Kunze, viscosity of the wort acquired from typical Pilsen malt should fall into the range of 1.5 mPa·s to 1.6 mPa·s [29]. In the conducted study, only three of the worts (GL30, GV30-MAX, GL30-MAX) were characterised by these parameters. Almost all of the legume malt worts in which wort viscosity could be assessed (with the exception of V) were characterised by lower viscosity, which was probably a result of poor malt extractability, because viscosity of the wort usually increases with the increase in the extract content [41]. This is confirmed by the results acquired in this study: most of the worts with high extract concentration acquired highest values for wort viscosity. Only in one of the samples, V, was wort with high viscosity characterised with low wort extract. This might indicate a possibility of extracting some substances, which, despite being present only in small concentrations in the wort, substantially increase wort viscosity.

4.1.6. Brewhouse Efficiency

Brewhouse efficiency is a parameter that takes into consideration both wort volume as the extract content [19]. The goal of the typical mashing process is to transfer most of the substances present in the mash to the filtered wort [42]. Brewhouse efficiency shows that legume malts, created by the malting features used typically for barley, are poorly modified. Nevertheless, samples prepared with the addition of 30% gelatinised vetch and chickpea malt show rather good efficiency, which means that they could be possibly used in the brewing technology in a way presented in this paper. However, more studies need to be performed on the quality and composition of the acquired legume seed malt wort, because legumes are rich in anti-nutritional factors, such as lectins, phytic acid, enzyme inhibitors, saponins, and haemagglutinins. Currently, it is not known whether they transfer easily to the wort acquired from legume seed malt [43]. However, it is known that some of the processes used in creating malt and wort, such as soaking, germination, thermal processing, and milling, reduce concentration of many of these harmful substances [44–46]. Future studies might show that low extractability of the legume seed malt will we be its advantage, because it might leave anti-nutritional substances in the spent legume seeds. Of course, the opposite might also be true—wort might be full of anti-nutritional factors,

which would make spent legume seeds a far more interesting product, reduced by its disadvantageous substances.

4.2. Analyses of the Phenolic Components and Antioxidant Activity of Legume Seed Malts and Legume Seed Malt Worts

Germination of seeds and grains, which is one of the main steps in the production of malt, has been analysed by many researchers in the past and it is commonly known that sprouted seeds possess high nutritional and antioxidative properties, and are eaten as a health food all around the world [47,48]. Many researchers also confirm that malt created from the grain possesses higher concentration of phenolic compounds, as well as higher antioxidant activity than unmodified grain [11,29]. It is therefore not surprising to see that legume seed malts contain higher concentrations of phenolic components and higher antioxidant activities than their unmalted counterparts. The most interesting fact about the results acquired in this study is the surprisingly high antioxidant activity of the VS and VSM, which possessed far smaller concentration of phenolic components than BM. However, it might be explained by the fact that legume seeds, especially these possessing dark, hard cover, as *Vicia sativa*, contain flavonoids and condensed tannins, which may increase antioxidant activity in a greater extent than phenolic compounds present in the BM [49]. It is also important to note that, in the case of legume seed malts, high concentration of phenolic compounds in malt did not always result in high concentration of phenolic compounds in the wort produced from this malt. From legume seed malts, VSM possessed the lowest concentration of these chemicals, but V acquired concentration of phenolic components, as well as antioxidant activity, second only to the M. Antioxidant activity of legume seed malts also did not have a straightforward impact on the properties of the legume seed malt worts. CSM was characterised with higher antioxidant activity ($ABTS^{+\bullet}$ assay) than LSM and similar antioxidant activity as PSM, but the same parameter of L and S was greater than of C. This results show that extraction of bioactive substances from legume seed malts is more difficult that extraction of the substances from the typical barley malt, so novel malting or mashing procedures need to be applied to the legume seeds and malts produced from them. Without detailed studies about composition of phenolic compounds in legume seed malts and worts prepared from these malts, it is hard to draw conclusions solely from the Folin–Ciocalteu, $ABTS^{+\bullet}$, $DPPH^{\bullet}$, or FRAP analysis. It is only certain that substitution of barley malt with gelatinised legume seed malts might increase concentration of phenolic compounds in the wort (in the case of GC30 and GV30); however, it decreases its antioxidant properties. In the study conducted by Gąsior et al., it was shown that use of traditional beer brewing adjuncts, such as chocolate malts or roasted, unmalted barley, increased concentration of phenolic compounds as well as antioxidative properties of the worts to a greater extent than the addition of gelatinised legume seed malts conducted in this research [15].

5. Conclusions

The conducted study shows that production of malts from legume seeds is possible, but technological properties of acquired malts are inferior to typical barley malts. Legume seed malt worts produced in this study by congress mashing were characterised with lower extract content, lower wort volume, and lower brewing efficiency than typical worts produced from barley malt. However, the addition of enzyme preparations to the mashes improved some of the characteristics of legume seed malt worts. Wort samples created from 30% addition of gelatinised vetch malt or chickpea malt were characterised with sufficiently good properties, which shows that legume malts might be used in the future as a substitute of unmalted adjuncts. The study also showed that malting increased concentration of phenolic compounds and antioxidant activity of the legume seeds; however, traditional mashing conditions only allowed producing legume seed malt worts with lower antioxidant activity than wort produced from barley malt. More research is needed on the composition of worts made from legume seed malts and on the influence of different malting conditions on the properties of legume seed malt worts.

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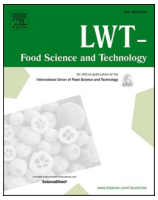

Mashing quality and nutritional content of lentil and bean malts

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ABSTRACT

Malting is a steeping, germination, and kilning technique used to produce barley malt, which is used in brewing technology. However, basic aspects of malting can be applied to a wide range of plant seeds. The goal of this study was to determine whether malting under appropriate physiological conditions could be applied to legume seeds (lentils and beans) and to determine the properties of acquired malts. The study found that, despite their poor mashing quality, legume seed malts have increased friability, higher protein content, lower starch content, and lower phytic acid concentration.

1. Introduction

Legume seeds are one of the world's most important sources of protein, fat, and energy (Caprioli et al., 2016). However, legume seeds such as chickpea, lentil, beans, peas, and soybeans are difficult to process because they require a long boiling time to prepare and are resistant to mechanical damage, making them difficult to grind (Pirhayati et al., 2011). Another disadvantage of legumes is that their seeds contain high levels of anti-nutritional factors such as phytic acid, tannins, and enzyme inhibitors (Gebrelibanoset, Tesfaye, Raghavendra & Sintayeyu, 2013). Malting is a food processing method that is commonly used in the industry to modify cereal grains, primarily barley, but this process is based on basic metabolical mechanisms found in the seeds of all plants (Kunze, 2019; Mohr & Schopfer, 2012). Steeping (hydration of the seeds), germination, and kilning (drying) are the three main stages of malting (Kunze, 2019). The main goal of the malting industry is to produce and activate enzymes that allow malt to be used during the mashing process. Malts are used in mashing to produce wort, which is high in fermentable sugars that microorganisms can use later (mainly *Sacharomyces cerevisiae* yeast). Malts have several advantages over unmodified seeds, including the activation and production of enzymes. Malted seeds have a higher concentration of various vitamins, a lower concentration of anti-nutritional compounds, and an improved aroma (Briggs, 1998). Malting reduces the calorie content of grains (by reducing the content of starch and other sugars) while increasing their health-benefitting potential and improving their taste (Kunze, 2019; Briggs, 1998). The aim of this study was to determine whether malts with adequate technological properties could be obtained from popular legume seeds such as black, brown, and green lentil, as well as red and white bean, and then used to produce novel fermented beverages such as legume-based gluten-free beer. Currently, only a preliminary study on malting legume seeds has been conducted by Gasiński et al. (2021), in which various legume seeds were malted in malting barley conditions that are not optimal for the physiology of various legumes (Kigel et al., 2015). Another goal of this study was to assess the nutritional characteristics of the acquired malts in order to determine whether malted lentils or beans could be used as a substrate to produce various legume-based food products with improved nutrients composition in future.

2. Materials and methods

2.1. Raw material

Seeds of three lentil (*Lens culinaris*) and two common bean (*Phaseolus vulgaris*) varieties were used as raw material, including black lentil Beluga (BL), brown lentil Firat 87 (BR), green lentil Eston (GR), white bean Piękny Jas (WB), and red bean Kreacja (RB). Seeds were acquired from BioPlanet Company (Leszno, Poland).

2.2. Malting procedure

Malting for lentil seeds and common bean seeds was done under different conditions and included seed steeping, seed germination, and seed drying.

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2.2.1. Seed cleaning and weighing

The lentils and beans were manually assessed before being weighed. Damaged seeds and seeds with visible discolouration were discarded. After cleaning and sorting, the moisture content of the seeds was determined using the automatic NIR analyser, Infratec 1241 (Foss, Hilleroed, Denmark).70 g of lentils and 70 g of beans were weighed into stainless steel perforated containers (12 containers for each seed type). The weights of the empty containers, seeds, and seed-filled containers were recorded. These measurements were used to determine how the moisture content of the seeds changed over time. The perforated stainless steel container containing the seeds will now be referred to as the 'malting kit'.

2.2.2. Seed steeping

Steeping was done in a water-air cycle with a 50 dm^3 stainless steel chamber and a KK240 Smart-Pro germination chamber (Pol-Eko Aparatura, Wodzisław Śląski, Poland). Malting kits containing lentil seeds were immersed in tap water for 7 h (water temperature equaled 18 °C), then placed in a germination chamber (18 °C, relative humidity 90%) for 18.5 h. After 18.5 h, lentil seeds were immersed again for 5 h (water temperature 18 °C) and kept in the germination chamber for 17.5 h (18 °C, relative humidity 90%). Malting kits with beans were immersed in tap water for 8 h (water temperature was equal to 20 °C), then placed in a germination chamber (20 °C, relative humidity 90%) for 16 h. After 16 h, the malting kits with beans were immersed again for 6 h (water temperature 20 °C) and kept in the germination chamber for 18 h (20 °C, relative humidity 90%). After each step of the seed steeping process, the malting kits were weighed and the moisture content of the seeds was calculated. It was assumed that the weight increase was proportional to the amount of water absorbed by the seeds. After steeping, the malting kits were weighed and kept in the germination chamber for seed germination. At the end of the steeping process, the moisture content of the bean seeds was in the range of 57–59%, while the moisture content of the lentil seeds was in the range of 58–60%.

2.2.3. Seed germination

Lentil seeds germinated at 18 °C while bean seeds germinated at 20 °C. The malting kits were weighed every day, and any weight loss was compensated for by spraying the seeds with sterile, distilled water. The seeds were visually assessed every day to determine the level of acrospire growth. After 96 h of germination, four malting kits with each lentil type were removed from the germination chamber and kilned (Section **2.2.5.**). After 120 h of germination, 4 malting kits with each lentil type and 6 malting kits with each bean type were removed from the germination chamber and kilned. After 144 h of germination, the remaining 4 malting kits with each lentil type and 6 malting kits with each bean type were removed from the germination chamber and kilned. The beans were dried only after the 120 h and 144 h, because acrospire growth was unsatisfactory after 96 h.

2.2.4. Seed drying

Following germination, each batch of malting kits was dried in the UF110 Plus dryer at the following conditions: 50 °C (17 h and 50min), ramp up to 70 °C (10min), 70 °C (2 h and 50min), ramp up to 80 °C (10min), and 80 °C (2 h). Following the drying process, malts from various malting kits were mixed and transferred to tightly closed containers. The germination and drying processes enabled the production of 15 distinct malt varieties:

- black lentil malt germinated for 96 h (4 days), 120 h (5 days) and 144 h (6 days) (BLL4, BLL5, BLL6);
- brown lentil malt germinated for 96 h (4 days), 120 h (5 days), and 144 h (6 days) (BRL4, BRL5, BRL6);
- green lentil malt germinated for 96 h (4 days), 120 h (5 days), and 144 h (6 days) (GRL4, GRL5, GRL6);
- red bean malt germinated for 120h (5 days); 144h (6 days) (RB5, RB6)
- white bean malt germinated for 120 h (5 days); 144 h (6 days) (WB5, WB6)

Before the analyses (Sections 2.3 and 2.4), malts and unmalted seeds were ground using the Bühler Miag disc mill DLFU (Bühler, Uzwil, Switzerland), according to the Analytica EBC 4.5.1 method (Analytica EBC, 2010).

2.3. Analysis of the technological parameters of legume malts

Congress worts were produced using the automated mashing machine (LB Electronic, Berching, Germany) according to the Analytica EBC method 4.5.1. The mashing cup was filled with 200 cm^3 of distilled water and heated to 45 °C. 50 g of ground malts were added to the mashing cups followed by stirring (100 rpm) of the mash. The 45 °C temperature was maintained for 30 min after which the temperature of the water bath in the mashing machine was increased to 70 °C at the rate of 1 °C per min. 100 cm^3 of distilled water at 70 °C was added to the mashing cups and kept at that temperature for 60 min. After this time, the contents of the mashing cups were cooled to 20 °C. The mash weight was adjusted to 450 g using distilled water (50 g of malt and 400 cm^3 of distilled water). Mashes were transferred to laboratory funnels (20 cm in diameter) fitted with Macherey-Nagel MN 614 ¼ filters (320 mm in diameter) (Macherey-Nagel GmbH & Co, Düren, Germany). To rinse the remaining malt, 100 cm^3 of the filtered wort was reversed to the mashing cup and poured back into the funnel. After reversing the first 100 cm^3 of wort, the filtering of the congress worts lasted up to 120 min. After filtration, wort was collected for analyses. The worts were prepared in duplicate. Congress worts could not be produced from bean malts because the first 100 cm^3 of wort, which had to be reversed into the funnel to begin the filtration process, could not be obtained.

2.3.1. Wort saccharification time

Saccharification time was calculated using the Analytica EBC 4.5.1 method (Analytica EBC).

2.3.2. Wort pH

A pH-meter (MP220, Mettler Toledo, Columbus, OH, USA) was used to measure the pH of worts collected after the filtration process (at 20 °C). For each wort sample, measurements were performed in triplicate (six measurements per type of malt).

2.3.3. Wort extract content

The extract content of the worts was determined using a densimeter (DMA 35, Anton Paar, Graz, Austria). For each wort sample, measurements were performed in triplicate (six measurements per type of malt).

2.3.4. Wort filtration time

The Analytica EBC 4.5.1. method was used to analyse the wort filtration time, yielding two readings for each type of malt (Analytica EBC).

2.3.5. Wort volume

After 120 min of filtration, the wort volume was measured using the graduated cylinder's scale, yielding two readings for each type of malt.

2.3.6. Wort viscosity

The Analytica EBC 8.4 method with the KF10 Viscometer was used to perform the wort viscosity test (Messtechnik GmbH, Ottendorf-Okrilla, Germany). Three readings were performed for each of the congress worts. Analyses could not be performed for the BLL4, BLL5, and BLL6, due to the small volume of acquired worts.

2.3.7. Malt friability

Malt and unmalted seed friability (3 repetitions) was analysed using Pfeuffer Friabilimeter (Pfeuffer GmbH, Kitzingen, Germany), according to the Analytica EBC 4.15 method (Analytica EBC).

2.4. Nutritional properties of legume seeds and legume malts

2.4.1. Preparation of the methanol extracts from malts and seeds

Methanol extracts were required to assess the total concentration of phenolic compounds and antioxidant activity. 2.5 g of ground malt/seed sample was transferred to 50 cm^3 polypropylene falcon tubes. 40 cm^3 of 80% (v/v) methanol was added to the tube, and samples were sonicated in a XUB5 ultrasonic bath (Grant Instruments, Shepreth, Great Britain) for 15min before being stored at 6 °C. After 12 h, samples were sonicated for 15min and centrifuged for 10min at 5500 rpm in a MPW-350 centrifuge (MPW Medical Instruments, Warsaw, Poland). Supernatant obtained after centrifugation was used in the analyses. The extracts were prepared in triplicate. Three analyses were performed on each extract for each assay, resulting in nine repetitions per type of seed or malt.

2.4.2. Analysis of total phenolic compounds content in the seeds and malts

The total content of phenolic compounds in the seeds and malts was determined using the spectrophotometric Folin–Ciocalteu (F–C) method on methanol extracts (Gasiński et al., 2021). Results were expressed as mg of gallic acid equivalent per 100 g of seed or malt.

2.4.3. Analysis of antioxidant activity of the seeds and malts by the ABTS•+ assay

Antioxidative ability of the tested samples was determined using the ABTS•+ assay (Gasiński et al., 2021). Results were expressed as μmol Trolox equivalent (TE) of antioxidative capacity of 1 g of seed/malt sample. Distilled water was used as a blank sample.

2.4.4. Analysis of antioxidant activity of the seeds and malts by the DPPH• assay

The DPPH• assay was used as the second method to determine the antioxidant activity of seeds and malts (Gasiński et al., 2021). Data were expressed as antioxidative capacity of Trolox (TE - Trolox equivalent) per 1 g of the seed/malt sample (μmol TE/g).

2.4.5. Analysis of antioxidant activity of the seeds and malts by the FRAP assay

In the FRAP assay, the ability of methanol extracts to reduce iron from ferric 2,4,6-tris(2-pyridyl)-1,3,5-triazine (Fe (III)-TPTZ) was tested (Gasiński et al., 2021). The external standard method was used for quantitative analyses, with ferrous (II) sulphate (0.2 mmol/dm^3) as the reference standard. The concentration of the ferrous (II) sulphate was correlated with absorbance measured at wavelength of 593 nm.

2.4.6. Analysis of the protein content of the seeds and malts

Protein content in malts and unmalted seeds was determined using the Kjeldahl method (Analytica EBC) with a conversion factor suitable for the analysis of legumes equal to 5.4 rather than the commonly used 6.25 (Mariotti et al., 2008). The analyses were carried out in triplicate for each sample using a Foss Tecator Kjeltec 2400 analyzer (Foss, Hilleroed, Denmark).

2.4.7. Analysis of the starch content of the seeds and malts

Starch content in the malts and unmalted seeds was analysed polarimetrically according to the ISO Ewers method (ISO, 2002), using AA-55 automatic polarimeter (Optical Activity LTD, Huntington, Great Britain). Analyses were performed in triplicate for each of the samples.

2.4.8. Analysis of the dietary fiber content of the seeds and malts

Total dietary fiber content in the seeds and malts was analysed with the AOAC Method 985.29 using total dietary fiber assay kits TDF-100 A-1 KT and TDF-C10 (Sigma-Aldrich, Saint Louis, Missouri, USA) (AOAC, 2012).

2.4.9. Analysis of the phytic acid content of the seeds and malts

The phytic acid content was determined using the method developed by Mikulski and Kłosowski (2017) and analysed using a Biotek Synergy H1 Microplate Reader (Biotek, Vinoski, Vermont, USA). 0.2 g of ground legume seed/legume malt sample was weighed into a 15 cm^3 falcon tube. 0.9 cm^3 of 60% (v/v) methanol was added to the tube and kept for 5min in a heated water bath with stirring at 80 °C. After 5min, 6 cm^3 of distilled water was added to the falcon tubes, which were then kept in the water bath with stirring for an additional 20min. After 20min, 3 cm^3 of 10% HCl (v/v) in 60% (v/v) ethanol solution was added to the falcon tubes, which were then transferred to a water bath with stirring at 20 °C and shaken for 5min. The falcon tubes then were centrifuged for 15min (5000 rpm). 0.02 cm^3 of the supernatant was added to the wells of the 96-well plate, which was then inserted into the microplate reader. 0.3 cm^3 of Wade reagent (0.27 g $FeCl_3$ x $6H_2O$ and 2.54 g × $2H_2O$ of sulfosalycylic acid dissolved in 1 dm^3 of distilled water) was added to the plates, which were then shaken for 15s. Absorbance was read at the wavelength of 510 nm. The blank sample was a mixture of solvents (methanol, water and hydrochloric acid in ethanol) without addition of malt or seed samples.

2.5. Data analysis

Data were analysed using the SPSS Statistics 26 program from IBM (Armonk, NY, USA) with two-way (seed variety and germination time) ANOVA ($\alpha = 0.05$). One-way ANOVA was performed for unmalted seeds (with the differentiating factor: seed variety). Bonferroni test was used to examine differences between means ($p < 0.05$) and establish homogenous groups. Data were expressed as means ± standard deviation.

3. Results and discussion

3.1. Technological parameters of the legume malts

Table 1 shows an analysis of the technological parameters of the malt based mainly on the results of congress mashing (Kunze, 2012; Analytica, 2010; Briggs, 1998).

3.1.1. Malt saccharification time

Saccharification time is an important parameter in the malting and brewing industry, because the main goal of the mashing procedure is quick and complete saccharification of the starch present in the mash. Saccharification of starch is the result of starch gelatinisation and hydrolysation of gelatinised starch granules. The saccharification time indicates how quickly starch in malt can be hydrolysed by amylolytic enzymes in the mash. incomplete saccharification of the starch present in the mash can be caused by a variety of factors, including incomplete starch gelatinisation, a lack of amylolytic enzymes, enzymes binding to non-substrate molecules, or the occurrence of physical barriers preventing access to the starch (such as cell walls or various protein matrices) (Kunze, 2019; Salimi et al., 2019; Dhital et al., 2016). Unfortunately, Congress mashing does not allow researchers to pinpoint which of the aforementioned factors prevents the starch from being completely saccharified. However, previous research on the malting of legumes in conjunction with the findings of this study may highlight some problems. Gasiński et al. (2021) malted legume seeds, including lentil seeds, in conditions typical for barley grains (43–45% moisture content, 15 °C germination temperature), which are not optimal for the physiology of lentil seeds (Kigel et al., 2015). However, when external amylases were added to the mash, lentil malts were fully saccharified, albeit at a very low efficiency (congress wort extract equal to 2.4–3.4% w/w). This demonstrates that the starch present in the lentils is gelatinised during the congress mashing temperature range. However,

Table 1
Technological parameters of the malts based on the Congress mashing.

Sample[1]	Saccharification time[a]	Wort pH	Wort extract [% w/w]	Filtration time [min]	Wort volume [cm^3]	Wort viscosity [mPa/s]
GL4	X	5.75 ± 0.04 b, A	4.80a, C	87.50 ± 2.50b, B	212.5 ± 2.5a, B	1.40 ± 0.04a, A
GL5	X	5.75 ± 0.02 b, A	4.90a, B	75 ± 1.00b, C	220a, C	1.38 ± 0.03a, B
GL6	X	5.74 ± 0.11, b A,	5.10a, A	100b, A	217.5 ± 2.5a, A	1.36 ± 0.04a, C
BRL4	X	5.71 ± 0.07 c, A	3.40b, C	43.00 ± 1.00c, B	225a, B	1.46 ± 0.04a, A
BRL5	X	5.73 ± 0.07 c, A	3.50b, B	50.00c, C	205a, C	1.42 ± 0.03a, B
BRL6	X	5.73 ± 0.07 c, A	3.60b, A	50c, A	225a, A	1.27 ± 0.03a, C
BLL4	X	5.81 ± 0.04a, A	4.75 ± 0.07a, C	120a, B	30 ± 5b, B	n.d.
BLL5	X	5.81 ± 0.02a, A	4.95 ± 0.02a, B	120a, C	22.5 ± 2.5b, C	n.d.
BLL6	X	5.83 ± 0.02a, A	5.00a, A	120a, A	32.5 ± 2.5b, A	n.d.
WB5	X	n.d.	n.d.	n.d.	n.d.	n.d.
WB6	X	n.d.	n.d.	n.d.	n.d.	n.d.
RB5	X	n.d.	n.d.	n.d.	n.d.	n.d.
RB6	X	n.d.	n.d.	n.d.	n.d.	n.d.

[1]Abbreviations are as follows: GL4 - malt from green lentil germinated 4 days, GL5 - malt from green lentil germinated 5 days, GL6 - malt from green lentil germinated 6 days, BRL4 - malt from brown lentil germinated 4 days, BRL5 - malt from brown lentil germinated 5 days, BRL6 - malt from brown lentil germinated 6 days, BLL4 - malt from black lentil germinated 4 days, BLL5 - malt from black lentil germinated 5 days, BLL6 - malt from black lentil germinated 6 days, WB5 – malt from white bean germinated 5 days, WB6 – malt from white bean germinated 6 days, RB5 – malt from red bean germinated 5 days, WB6 – malt from red bean germinated 6 days. Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups according to the variable 'days of germination'(Bonferroni test, $\alpha = 0.05$).

[a] X - sample did not saccharify completely.

incomplete saccharification results obtained during this study show that lentil, even when germinated in improved conditions, lacks adequate concentrations of amylolytic enzymes or essential cofactors required for endogenous enzymes to function efficiently. Similar conclusions cannot be drawn for bean malt because the beans in the previous study were not malted, despite the fact that many researchers have assessed the gelatinisation temperature of starch in various beans and found that the gelatinisation temperature onset is lower than 70 °C, but the temperature peak of gelatinisation is in the range of 71.7–82.1 °C, and the temperature of gelatinisation conclusion ranges from 79 °C to 91.3 °C (Du, Jiang, Ai, & Jane, 2014; Chung et al., 2008). As a result, even if the bean malt contains an adequate amount of amylolytic enzymes, the congress mashing procedure would not allow for complete saccharification of the congress mash.

3.1.2. Wort pH

The pH of the wort is an important parameter in the brewing process because it influences enzyme activity, wort stability, taste, and flavour (Kunze, 2019). During mashing in the brewing process using barley malt, the pH of the wort should be in the range of 5.4–5.6, as these pH values are optimal for the activity of α-amylases and β-amylases from barley. However, typical mash has a higher pH value, ranging from 5.6 to 5.9 (Kunze, 2019). As a result, wort produced from lentil malts is distinguished by the typical values expected by brewers and maltsters. Unfortunately, this cannot be described as a distinct advantage of lentil malts because the optimal pH values for the enzymes found in lentil malts can be lower or higher. These parameters must be examined in future studies.

3.1.3. Wort extract

Wort extract is one of two parameters used to determine malt extractivity. The most extractive malts have a high extract content in wort and a large wort volume. Typical barley wort produced in the congress mashing procedure should contain 8.0–9.0% (w/w) of extract (Kunze, 2019), indicating that all worts produced from the legume malt analysed contained far less extract than expected. This result, however, is not discouraging because barley has been cultivated for centuries for its mashing properties, and obtaining barley malt-like results in novel types of special malts is extremely unlikely. Many authors in recent years have produced new types of malts from cereal or pseudocereal grains (such as tritordeum, buckwheat or rice) that are more similar to the barley kernel than legume seeds (Zdaniewicz et al., 2020; Mayer et al., 2016; Wijngaard & Arendt, 2006), and all of these malts were characterised with lower extractivity. However, in the most recent study on legume seed malts, worts produced from each malt (vetch, chickpea, pea, and green lentil) possessed lower extract (1.59%–2.80% w/w). More importantly, the lentil malt wort produced in the previous study was characterised with the lowest (1.59% w/w) extract content (Gasiński et al., 2021). A simple change in seed germination temperature and moisture content was sufficient to increase extract content by 210–320%. It is also important to note that raising the germination temperature would benefit not only the malt's extractivity, but would also lower the cost of malting because germinating lentils would not have to be chilled as much as they are in traditional malthouses (Kunze, 2019; Briggs, 1998).

3.1.4. Wort filtration time

During the brewing process, it is critical that the mash filters as quickly as possible, reducing the overall time of the process and allowing the wort to come in contact with oxygen and cool before the boiling process. Many substances and properties, such as high concentration of beta-glucans, high-molecular proteins, and arabinoxylans, can obstruct wort filtration (Kunze, 2019). Typically, grains and seeds that have not been thoroughly cytolytic and proteolytically modified are to blame for filtration difficulties. The high protein content and properties of legume proteins may be the most important factors influencing the filterability of legume malt worts (Li et al., 2021). Furthermore, high water absorption of legume seeds can also result in long filtration time, due to the binding of water molecules to the components of ground malt (Du, Jiang, Yu, & Jane, 2014). During the Congress mashing, filtration time begins after the first 100 cm^3 of filtrate is reversed to the funnel with the mashed malts. Filtration that ends within the first 60 min is described as 'normal', while filtration that ends between 60 and 120 min is described as 'poor'. After 120 min, the filtering process is complete. Bean malts had the poorest filtration properties because the first 100 cm^3 of filtrate could not be obtained. The filtration time of the black lentil malts was very poor, because black lentil worts did not filter during the 120 min filtration time. This study found that black lentil malts contain significantly more compounds that impede filtration than brown or green lentil malts, which filtered in 43–50 min for brown lentils and 75–100 min for green lentils. Another factor that could have influenced the filtration time differences between different lentil varieties is the testa (legume hull) to endosperm ratio. In the case of cereals, the majority of the compounds which hinder the filtration process tend to reside in the cell walls and the grain hull (Kunze, 2019; Briggs, 1998). The black lentil seeds were the smallest of the malted lentil varieties, which may have

contributed to their poor filtration properties. However, it is worth noting that green and brown lentil malts had better filtration time than the entire legume malts malted in previous study (Gasiński et al., 2021).

3.1.5. Wort volume

The second indicator of malt extractivity is worth volume. The low volume of black lentil worts is due to poor filterability. The volume of the worts prepared from brown and green malts was less than that of typical Congress worts prepared from barley malt (Kunze, 2019). The main reason for this result is possibly high water capacity of legumes, which can retain 4–6 g of water per 1 g of legume flour (Du, Jiang, Yu, & Jane, 2014). The volume of the worts prepared from malted lentil seeds in previous study (Gasiński et al., 2021) was slightly lower (by 10–20%) than the volume obtained in this study, but the higher extract content of the GL4, GL5 and GL6 shows that the brewhouse efficiency is more than three times higher.

3.1.6. Wort viscosity

It is difficult to compare the viscosity of the worts obtained in this study with cereal malt parameters because typical worts are diluted to obtain an extract volume of 8.6% (Gastl et al., 2020) and should not exceed 1.56 mPa/s. High wort viscosity is problematic in the brewing process because it increases the time required for all of the procedures needed for the production of beer and wort (Gastl et al., 2020; Kunze, 2019). Despite an increase in wort extract, the wort viscosity of lentil malt worts (green and brown) shows a small decrease with the lengthening of the germination process. This finding suggests that increasing the germination time may aid in the hydrolysis of compounds responsible for wort viscosity.

3.1.7. Malt friability

Friability (shown in Table 2) is the ability of the material to easily crumble, which is one of the benefits of malt over unmalted grain (Kunze, 2019). Legume seeds are typically very rigid seeds that are difficult to process in food technology, which is why improving the legume seeds structure could be very beneficial for legume seed processing technology (Vishwakarma et al., 2018). The friability analysis performed in this study revealed that malting significantly improved the properties of the malts over unmalted seeds. Friability of lentil malts produced in this study was greater than 90%. When compared to unmalted lentils, the friability of lentil malts increased sixfold on average. Bean malts did not have the same friability as lentil malts, but unmalted bean seeds were almost non-friable, with a friability of 2.28–2.43%. As a result, the friability of the bean malts was increased up to tenfold. These findings indicate that legume seed malts do not have to be used in brewing technology, but could be applied to other technologies where legume seed hardness is an issue.

Table 2
Friability of the legume seeds and legume malts.

Sample[1]	Friability [%]
GL	15.93 ± 0.24***
GL4	94.67 ± 0.32a, C
GL5	96.00 ± 0.64a, B
GL6	97.67 ± 0.18a, A
BRL	15.07 ± 0.49**
BRL4	91.90 ± 0.32b, C
BRL5	93.57 ± 0.84b, B
BRL6	95.43 ± 0.38b, A
BLL	16.53 ± 0.66****
BLL4	90.83 ± 0.20b, C
BLL5	93.83 ± 0.33b, B
BLL6	95.17 ± 0.35b, A
WB	2.38 ± 0.38*
WB5	20.63 ± 0.26c, B
WB6	24.10 ± 0.29c, A
RB	2.43 ± 0.18*
RB5	14.07 ± 0.35d, B
RB6	16.00 ± 0.52d, A

[1]Abbreviations are as follows: GL4 - malt from green lentil germinated 4 days, GL5 - malt from green lentil germinated 5 days, GL6 - malt from green lentil germinated 6 days, BRL4 - malt from brown lentil germinated 4 days, BRL5 - malt from brown lentil germinated 5 days, BRL6 - malt from brown lentil germinated 6 days, BLL4 - malt from black lentil germinated 4 days, BLL5 - malt from black lentil germinated 5 days, BLL6 - malt from black lentil germinated 6 days, WB5 – malt from white bean germinated 5 days, WB6 – malt from white bean germinated 6 days, RB5 – malt from red bean germinated 5 days, WB6 – malt from red bean germinated 6 days. Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups according to the variable 'days of germination', various number of asterisks indicate homogenous groups between unmalted seeds according to the variable 'variety' (Bonferroni test, $\alpha = 0.05$).

3.2. Nutritional properties of legume malts

Malting not only alters the technological parameters of malted seeds, but it can also significantly alter the composition of various nutrients present in the seeds as a result of the significant metabolic processes that occur during germination (Table 3).

3.2.1. Protein content

Protein content in malts was higher than in unmalted seeds, with the exception of RB5 and RB6. Protein content decreases in germinating seeds are uncommon, as the seed embryo requires all of the stored proteins, as the metabolic apparatus of cells is made up of various peptides and proteins. Germination is a process that requires a significant amount of energy from the embryo, and until the plant produces its first leaves, the only source of energy must come from the substances found in the seeds. Carbohydrates and fats are the primary sources of energy from the germinating embryo, not proteins. The concentration of proteins in the seed increases as the concentration of fats and carbohydrates decreases (Taiz et al., 2015). This effect can be easily observed in the lentil malts: as the starch content decreases, the protein content increases. It is also worth noting that the highest apparent increase in protein content (up to 30%) can be observed in the case of green lentil malts, which were distinguished by the highest wort extract content as well as the highest friability, implying the strongest decomposition of the starch-protein matrix (Briggs, 1998). However, there are mechanisms by which germinating seeds can reduce their protein content. During the hydrolysis of proteins, amino acids are released, which are far more soluble in water than proteins and can be removed with steeping water (Briggs, 1998). If the germinating seed did not lose as much dry mass in the form of starch, the loss of protein content would be exacerbated. This could explain why red bean malts have a lower protein concentration.

3.2.2. Starch content

Some of the mechanisms involved in the decrease of the starch content of germinating seeds were discussed in paragraph **3.2.1.** When compared to the unmalted seeds, all of the malts had a lower concentration of starch. It is also worth noting that malts that performed the best during the mashing process lost the most starch. This finding indicates that seed germination and biochemical mechanisms such as production of enzymes, degradation of cell wall, starch granules, or starch-protein matrix require a significant amount of energy, which must be provided by carbohydrates. Similar mechanisms can be observed during traditional malting processes using barley seeds, though barley kernels lose less starch and perform better in brewing

Table 3
Concentration of protein, starch, phytic acid and fiber in legume seeds and legume malts.

Sample[1]	Protein content [% d.m.]	Starch content [% d.m.]	Phytic acid content [% d.m.]	Fiber content [% d.m.]
GL	24.60 ± 0.12*	57.6 ± 0.69****	1.24 ± 0.03**	18.56 ± 0.30*
GL4	32.30 ± 0.17a, C	40.2 ± 0.40a, A	0.90 ± 0.05c, A	15.27 ± 0.07d, C
GL5	32.50 ± 0.23a, B	40.2 ± 0.29a, B	0.86 ± 0.05c, B	14.00 ± 0.22d, B
GL6	32.50 ± 0.12a, A	39.1 ± 0.23a, C	0.82 ± 0.05c, C	14.30 ± 0.59d, A
BRL	30.47 ± 0.33****	50.7 ± 0.52***	1.36 ± 0.07***	20.56 ± 0.89**
BRL4	32.40 ± 0.40a, C	41.0 ± 0.35a, A	0.86 ± 0.04c, A	14.93 ± 0.15d, C
BRL5	32.70 ± 0.35a, B	39.5 ± 0.23a, B	0.84 ± 0.05c, B	14.58 ± 0.11d, B
BRL6	32.80 ± 0.29a, A	38.5 ± 0.29a, C	0.83 ± 0.05c, C	14.56 ± 0.15d, A
BLL	26.00 ± 0.17**	48.9 ± 0.52***	0.88 ± 0.08*	26.84 ± 0.13****
BLL4	28.40 ± 0.23b, C	41.3 ± 0.35a, A	0.87 ± 0.04c, A	18.08 ± 0.05c, C
BLL5	28.50 ± 0.17b, B	40.1 ± 0.29a, B	0.83 ± 0.04c, B	19.00 ± 0.18c, B
BLL6	29.07 ± 0.15b, A	39.4 ± 0.4a, C	0.82 ± 0.06c, C	19.39 ± 0.24c, A
WB	24.90 ± 0.17*	42.2 ± 0.64**	1.80 ± 0.09****	24.53 ± 0.36***
WB5	27.20 ± 0.23c, B	40.2 ± 0.4b, B	1.46 ± 0.05b, B	25.93 ± 0.13b, B
WB6	26.20 ± 0.17c, A	36.4 ± 0.17b, C	1.42 ± 0.05b, C	27.01 ± 0.16b, A
RB	28.17 ± 0.09***	38.2 ± 0.4*	1.98 ± 0.05*****	24.39 ± 0.28***
RB5	27.30 ± 0.12c, B	37.4 ± 0.23c, B	1.94 ± 0.10a, B	25.28 ± 0.13a, B
RB6	26.20 ± 0.17c, A	35.3 ± 0.46c, C	1.91 ± 0.05a, C	29.07 ± 0.14a, A

[1]Abbreviations are as follows: GL4 - malt from green lentil germinated 4 days, GL5 - malt from green lentil germinated 5 days, GL6 - malt from green lentil germinated 6 days, BRL4 - malt from brown lentil germinated 4 days, BRL5 - malt from brown lentil germinated 5 days, BRL6 - malt from brown lentil germinated 6 days, BLL4 - malt from black lentil germinated 4 days, BLL5 - malt from black lentil germinated 5 days, BLL6 - malt from black lentil germinated 6 days, WB5 – malt from white bean germinated 5 days, WB6 – malt from white bean germinated 6 days, RB5 – malt from red bean germinated 5 days, WB6 – malt from red bean germinated 6 days. Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups according to the variable 'days of germination', various number of asterisks indicate homogenous groups between unmalted seeds according to the variable 'variety' (Bonferroni test, $\alpha = 0.05$).

(Kunze, 2012). However, barley grown today is the result of centuries of breeding, primarily for malting and mashing (Briggs, 1998).

3.2.3. Phytic acid content

Phytic acid is an anti-nutritional factor found in most legume and cereal seeds (Taiz et al., 2017). It is the primary storage molecule for phospohorus and has a high chelatic power, allowing it to easily bind to proteins and various metals. Interactions between phytic acid and cations significantly reduce bioavailability of various elements, which is why low concentration of phytic acid in food products is advantageous (Ali et al., 2010). Phytases, enzymes capable of hydrolyzing phytic acid, are produced by seeds during germination. Malting reduced the concentration of phytic acid in the majority of the seeds. The greatest reduction was observed between GL and GL6 (34%), as well as between BRL and BRL6 (39%). These findings represent a significant improvement in the nutritional properties of lentil seeds, especially when compared to research breeding programs conducted between 2000 and 2019. During these programs, new varieties of various food crops with reduced phytic acid content were produced, and the decrease was comparable to the 20-39% decrease in phytic acid obtained in this study due to the simple malting procedure (Raboy, 2020).

3.2.4. Fiber content

Dietary fiber is a class of chemical compounds that cannot be digested or absorbed by the human digestive system (Turner & Lupton, 2011). The fiber content of malts increases during the traditional malting process using barley. It is critical for the seed to produce rootlets and sprout during germination because the plant cannot mature and produce new seeds without them. Cell walls in newly formed cells are made primarily of fiber (cellulose and various hemicelluloses) and proteins (Mohr & Schopfer, 2012). Similar results can be found in malts made from bean seeds, though the increase in fiber content in RB5, RB6, WB5 and WB6 is not as significant, and the fiber increase may be due solely to the loss of carbohydrates (starch) during the germination period. Malts made from lentil seeds exhibit a variety of characteristics. Every lentil malt is distinguished by a low fiber content. This could be due to the seed embryo using carbohydrates that are not digestible by humans (for example, raffinose family oligosaccharides) as a source of energy rather than just starch. Previous studies on germinating lentils (Oskaybaş-Emlek et al., 2021) found similar results.

3.2.5. Total phenolic content and antioxidant activity

In the case of green lentils, white beans, BLL6 and RB6, malting increased the concentration of phenolic acids (Table 4). BLL4 and RB6 showed a slight decrease. The greatest increase was observed between WB and WB5, with an increase in the range of more than 600%. The effect of increase in phenolic compounds could be attributed to the release of phenolic compounds from cell walls, the production of the phenolic compounds by the germinating seed, and the release of various nitrogenous compounds, such as amino acids tryptophan, tyrosine, or cysteine (Everette et al., 2010). It is important to note, however, that in food products, an increase in phenolic compounds correlates with an increase in antioxidant activity (Lamuela-Raventós, 2018). During the malting process, antioxidant activity in lentil malts decreased (with the exception of GL5 and GL6 analysed with FRAP and ABTS assays). Similar findings could be noted for RB5 and RB6 (with the exception of ABTS assay for RB6). This shows that phenolic compounds (and changes in their concentration) are not the main factor influencing fluctuations in antioxidant activity. It is also worth noting that malts with the shortest germination time (5 days for beans, 4 days for lentils) have the lowest antioxidant activity, which then increases or remains constant. There are two possible factors that play a significant role in this phenomenon. First, the water-soluble components with strong antioxidant potential are removed during the steeping process with waste water, and the germinating seed produces newer, additional components, which may increase antioxidative potential. To confirm this hypothesis, further research into the specific components of lentil/bean seeds, as well as a study of the wastewater, would be required in the future. The main, clear result of analysis of phenolic compounds and antioxidative analysis is that white bean malts have significantly improved pro-health properties.

4. Conclusions

Lentil and bean seeds cannot be malted as easily as typical cereals. Bean and lentil malts do not saccharify fully during the congress mashing regime, and bean worts do not filtrate. Although wort can be obtained from brown and green lentil malts, it has lower extract content and lower volume than worts produced from barley malt. The most significant advancements in legume seed technology are not related to mashing technology. All of the malts showed a significant improvement in friability. All the malted seeds had an increase in protein content and

Table 4
Total concentration of phenolic compounds and antioxidant activity in legume seeds and malts.

Sample[1]	Concentration of phenolic compounds [mg GA/100g]	DPPH assay [μmol/g]	FRAP assay [μmol/g]	ABTS assay [μmol/g]
GL	50.27 ± 4.71**	4.53 ± 0.06**	6.28 ± 0.21**	8.32 ± 0.33**
GL4	60.91 ± 2.12b, C	3.56 ± 0.11a, B	5.90 ± 0.10a, C	8.03 ± 0.21c, C
GL5	66.19 ± 5.91b, B	3.54 ± 0.18a, B	6.20 ± 0.14a, B	8.26 ± 0.17c, B
GL6	70.96 ± 6.61b, A	3.91 ± 0.13a, A	6.45 ± 0.16a, A	8.71 ± 0.27c, A
BRL	48.38 ± 3.21**	4.45 ± 0.16**	6.35 ± 0.10**	8.24 ± 0.23**
BRL4	45.88 ± 2.77d, C	2.41 ± 0.21e, B	5.04 ± 0.16e, C	6.58 ± 0.13e, C
BRL5	46.87 ± 5.85d, B	2.61 ± 0.15e, B	5.17 ± 0.20e, B	6.63 ± 0.39e, B
BRL6	48.31 ± 4.10d, A	2.95 ± 0.08e, A	5.25 ± 0.11e, A	6.98 ± 0.17e, A
BLL	59.73 ± 3.97***	5.51 ± 0.27***	7.34 ± 0.39****	9.21 ± 0.76***
BLL4	54.91 ± 2.87c, C	3.36 ± 0.22b, B	5.52 ± 0.17c, C	7.78 ± 0.32d, C
BLL5	56.91 ± 3.70c, B	3.39 ± 0.10b, B	5.61 ± 0.21c, B	7.99 ± 0.25d, B
BLL6	64.81 ± 5.36c, A	3.36 ± 0.15b, A	5.57 ± 0.18c, A	7.87 ± 0.37d, A
WB	9.51 ± 0.85*	1.07 ± 0.09*	3.53 ± 0.11*	3.23 ± 0.29*
WB5	72.63 ± 4.05a, B	2.73 ± 0.11d, B	5.24 ± 0.05d, B	11.16 ± 0.33a, B
WB6	84.93 ± 4.03a, A	3.23 ± 0.15d, A	5.61 ± 0.10d, A	12.89 ± 0.23a, A
RB	59.76 ± 4.72***	4.30 ± 0.33**	6.64 ± 0.31***	8.35 ± 0.71**
RB5	52.68 ± 3.43b, B	3.00 ± 0.12c, B	5.73 ± 0.11b, B	7.49 ± 0.28b, B
RB6	83.67 ± 4.18b, A	3.32 ± 0.24c, A	6.06 ± 0.34b, A	9.99 ± 0.37b, A

[1]Abbreviations are as follows: GL4 - malt from green lentil germinated 4 days, GL5 - malt from green lentil germinated 5 days, GL6 - malt from green lentil germinated 6 days, BRL4 - malt from brown lentil germinated 4 days, BRL5 - malt from brown lentil germinated 5 days, BRL6 - malt from brown lentil germinated 6 days, BLL4 - malt from black lentil germinated 4 days, BLL5 - malt from black lentil germinated 5 days, BLL6 - malt from black lentil germinated 6 days, WB5 – malt from white bean germinated 5 days, WB6 – malt from white bean germinated 6 days, RB5 – malt from red bean germinated 5 days, WB6 – malt from red bean germinated 6 days. Various small letters (a, b, c, d, e) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups according to the variable 'days of germination', various number of asterisks indicate homogenous groups between unmalted seeds according to the variable 'variety' (Bonferroni test, $\alpha = 0.05$).

a decrease in starch content. Malting reduced the amount of phytic acid in green, brown, and bean seeds. These findings suggest that malting could be a viable tool for producing various legume-based food products with improved quality and pro-health properties.

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CRediT authorship contribution statement

Alan Gasinski: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Joanna Kawa-Rygielska:** Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Changes in the raffinose family oligosaccharides content in the lentil and common bean seeds during malting and mashing processes

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Raffinose family oligosaccharides (RFOs) are sugars, which are considered anti-nutritional substances, which are not digestible by human gastric enzymes and can lead to flatulence. Legume seeds are often rich in these compounds, which can be cumbersome for many people, such as vegetarians or the population of developing countries, whose diets consists of large amounts of these food products. In this study, simple procedures used around the world in the brewing industry (malting and mashing) were used to determine, whether these processes could be applied to popular legume seeds (lentil and bean) to reduce the RFOs content. Acquired malts and worts were characterised by radically decreased concentration (up to 90%) of most ubiquitous RFOs, such as raffinose and stachyose.

Seeds of beans and lentils are important food product consumed worldwide. However, despite possessing high concentration of starch and proteins, the seeds possess vast array of so called anti-nutritional factors, such as phytic acid, trypsin inhibitors, tannins which diminish nutritive quality of these food products[1,2]. One of very interesting groups of anti-nutritional substances, present in the legume seeds, are so called raffinose-family oligosaccharides (RFOs)[3]. Raffinose, stachyose and verbascose are main carbohydrates from this group. The human digestive system does not produce α-galactosidase, the enzyme necessary to digest these sugars, so they are not hydrolysed and absorbed. However, in human gut microbiome there are various microorganisms capable of digesting RFOs. Unfortunately, the fermentation of RFOs can result in production of carbon dioxide, hydrogen and methane, which are components causing flatulence[4]. There are many strategies used for the improvement of nutritional quality of lentils and beans, which have proven to be successful in decreasing concentration of RFOs, such as dehulling, germination, soaking (in alcohol or water), various heat-treatments (boiling, autoclaving, microwave cooking, extrusion), enzymatic treatment, irradiation or fermentation[5,6]. However, in the scientific literature there is no mention of using malting technology to lower concentration of RFOs in legume seeds. Malting is a traditional process of seed washing, soaking, germination and drying, typically used to modify seeds of cereals, primarily barley[7]. However, as the malting procedure comprises of basic metabolic pathways and uses simple technological processes, it could be, in theory, applied basically to almost any kind of seed. Legumes can be malted, albeit with poor technological properties, as presented in the last study by Gasiński, Kawa-Rygielska, Błażewicz, Śniegowska & Zarzecki (2021)[8]. In this work, various common beans and lentils of different testa (seed coat) colour were malted and mashed, to determine, whether these procedures can be used to reduce concentration of RFOs in popularly eaten legume seeds. If malted beans and lentils would possess lower concentration of these sugars than their unmalted counterpart, they could prove as a useful substrate for production of various novel food products, such as legume-based beer, cereals, cookies or high-protein vegetarian meat substitutes or milk alternatives. Additionally, as the proteins from various legumes can be consumed by people suffering from celiac disease, non-celiac gluten sensitivity and other disorders, legume malts could be used to produce food products available for the consumption for population suffering from above-mentioned diseases[9].

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Sample	Raffinose concentration [% d.w.]	Stachyose concentration [% d.w.]
BL	12.98 ± 0.66*	7.32 ± 0.27*
BL4	0.61 ± 0.11c, A	0.43 ± 0.07b, A
BL5	0.61 ± 0.06c, A	0.37 ± 0.04b, B
BL6	0.46 ± 0.04c, B	0.36 ± 0.05b, C
BR	6.77 ± 0.08***	2.88 ± 0.26***
BR4	1.63 ± 0.05a, A	0.73 ± 0.01a, A
BR5	1.62 ± 0.03a, A	0.72 ± 0.08a, B
BR6	1.35 ± 0.06a, B	0.60 ± 0.02a, C
GR	7.82 ± 0.69**	5.66 ± 0.92**
GR4	0.72 ± 0.05b, A	0.33 ± 0.03c, A
GR5	0.63 ± 0.05b, A	0.31 ± 0.02c, B
GR6	0.61 ± 0.02b, B	0.28 ± 0.03c, C

Table 1. Concentration of stachyose and raffinose in lentil seeds and lentil malts. Data are shown as means ± standard deviation (n = 4). Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups between malts according to the variable 'days of germination', various number of asterisks indicate homogenous groups between unmalted seeds according to the variable 'variety' (Bonferroni test, α = 0.05). *BL* black lentil, *BL4* malt from black lentil germinated 4 days, *BL5* malt from black lentil germinated 5 days, *BL6* malt from black lentil germinated 6 days, *BR* brown lentil, *BR4* malt from brown lentil germinated 4 days, *BR5* malt from brown lentil germinated 5 days, *BRL6* malt from brown lentil germinated 6 days, *GR* green lentil, *GR4* malt from green lentil germinated 4 days, *GR5* malt from green lentil germinated 5 days, *GR6* malt from green lentil germinated 6 days.

Sample	Raffinose concentration [% d.w.]	Stachyose concentration [% d.w.]
WB	3.03 ± 0.33*	2.15 ± 0.20*
WB5	0.79 ± 0.03b, A	0.64 ± 0.03b, A
WB6	0.66 ± 0.05b, B	0.45 ± 0.02b, B
RB	2.20 ± 0.03**	2.45 ± 0.02**
RB5	1.45 ± 0.03a, A	1.60 ± 0.08a, A
RB6	0.69 ± 0.12a, B	0.90 ± 0.10a, B

Table 2. Concentration of stachyose and raffinose in bean seeds and bean malts. Data are shown as means ± standard deviation (n = 4). Various small letters (a, b) indicate homogenous groups according to the variable 'variety', various capital (A, B) letters indicate homogenous groups between malts according to the variable 'days of germination', various number of asterisks indicate homogenous groups between unmalted seeds according to the variable 'variety' (Bonferroni test, α = 0.05). *WB* white beans, *WB5* malt from white bean germinated 5 days, *WB6* malt from white bean germinated 6 days, *RB* red beans, *RB5* malt from red bean germinated 5 days, *RB6* malt from red bean germinated 6 days.

Results

Concentration of RFOs in legume seeds and malts. The concentration of raffinose and stachyose was assessed in the seeds of three lentil varieties and two bean varieties with different testa colours (black, brown and green lentil varieties and white and red bean varieties as well as in malts prepared from these seeds (germinated by 4, 5 and 6 day period in the case of lentil and germinated by 5 and 6 day period for the bean varieties). Results are shown in Tables 1 and 2.

Verbascose was not detected in any of the analysed types of seeds, however, this result is not very surprising, as this sugar is often found in faba beans, lupins, mung beans but not as abundant in common beans and lentils[10]. Stachyose and raffinose was detected in all the legume seeds and legume malt samples, but concentration of these sugars varied significantly. Raffinose was detected in greater quantity than stachyose in all unmalted seeds samples, with the exception of RB, and its content was in the range from 2.20% (d.w.) (RB) to 12.98% (d.w.) (BL). The lowest stachyose concentration for unmalted seeds was detected in WB (2.15% d.w.) and the greatest in BL (7.32% d.w.). Bean samples were characterised with lower concentration of raffinose, stachyose as well as total RFO content than lentil seeds.

Concentration of RFOs in worts produced from the lentil malts. Concentration of raffinose and stachyose in wort samples acquired during the congress mashing of the lentil malts is shown in Table 3. Worts were acquired from malts produced from three different lentil varieties with various testa colours (black, brown

Sample	Extract content [% w/w]	Raffinose concentration [g/dm^3]	Stachyose concentration [g/dm^3]
BL4W	4.75 ± 0.07a, C	0.18 ± 0.02c, A	n.d
BL5W	4.95 ± 0.02a, B	0.16 ± 0.02c, B	n.d
BL6W	5.00a, A	0.12 ± 0.01c, B	n.d
BR4W	3.40b, C	0.60 ± 0.16b, A	0.23 ± 0.03b, A
BR5W	3.50b, B	0.48 ± 0.10b, B	0.13 ± 0.02b, B
BR6W	3.60b, A	0.47 ± 0.16b, B	0.14 ± 0.04b, B
GR4W	4.80a, C	0.70 ± 0.04a, A	0.32 ± 0.03a, A
GR5W	4.90a, B	0.64 ± 0.04a, B	0.29 ± 0.03a, B
GR6W	5.00a, A	0.60 ± 0.02a, B	0.31 ± 0.02a, B

Table 3. Extract content and concentration of stachyose and raffinose in worts produced from lentil malts. Data are shown as means ± standard deviation (n = 6). Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B) letters indicate homogenous groups between malts according to the variable 'days of germination' (Bonferroni test, α = 0.05). N.d. stands for "not detected". *BL4W* wort from black lentil malt germinated 4 days, *BL5W* wort from black lentil malt germinated 5 days, *BL6W* wort from black lentil malt germinated 6 days, *BR4W* wort from brown lentil malt germinated 4 days, *BR5W* wort from brown lentil malt germinated 5 days, *BRW6* wort from brown lentil malt germinated 6 days, *GR4W* wort from green lentil malt germinated 4 days, *GR5W* wort from green lentil malt germinated 5 days, *GR6W* wort from green lentil malt germinated 6 days.

and green), which were germinated by different time periods (4, 5 and 6 days). Worts could not have been acquired from the bean malts due to the filtration problems during congress mashing procedure.

Acquired lentil worts were characterised with lower concentration of stachyose than raffinose. Average concentration of stachyose in lentil worts was equal to 0.16 g per dm^3 of wort and average concentration of raffinose in lentil worts was almost three times higher, equal to 0.44 g per dm^3 of wort. Worts acquired from black lentil malts were characterised with the lowest concentration of raffinose (0.15 g/dm^3) and stachyose (stachyose was absent in these worts). Average concentration of stachyose in worts from brown lentil malts was equal to 0.17 g/dm^3 and in worts from green lentil malt it was almost two times higher (0.31 g/dm^3). Lower difference can be noted for the content of raffinose in these worts: brown lentil worts were characterised with raffinose concentration of 0.52 g/dm^3 and green lentil worts with raffinose concentration of 0.65 g/dm^3.

Discussion

Concentration of RFOs in legume seeds and malts. Analysis of the acquired data shows, that different varieties of the same species have significantly different concentration of the raffinose and stachyose. Malts prepared from the legume seeds were characterised with drastically lower concentration of raffinose and stachyose than their unmalted counterparts. RFOs are abundant in the plant kingdom synthesised and utilised many plants[10]. They are accumulated in various parts of the plants, such as seeds or leaves in many different species, such as various legumes, grains sich as maize or rice and in tissues of various other plants, as, for example, cucumbers or coleuses[11–15]. Previous study by Gangl & Tenhaken (2016)[16] conducted on model plant seeds (*Arabidopsis thaliana*) have shown, that raffinose was required for the fast germination of the seeds in the dark. The drastic decrease of the raffinose content in lentil seeds would suggest that *Lens culinaris* and *Phaseolus vulgaris* also uses raffinose and stachyose during the germination. However, soybean seed research results[17] suggested, that these sugars are not required for the germination, albeit, when present, they are used by the germinating seed embryo. Data acquired in this study have shown that malting procedure (combination of seed hydration, germination and then kilning) has significant influence on the RFO content of legume seeds. Average concentration of stachyose in black lentil malts was 20 times lower thanthe stachyose content of BL and average concentration of raffinose in black lentil malts was 23 times lower than raffinose content of BL. Similar decrease could be noted for the stachyose content in the green lentil malt (decrease in malts equal to the 5.4% of the stachyose content in GR), but a lower change was found for the raffinose concentration in green lentil malts (raffinose content in green lentil malts was equal to 8.4% the content in GR). Brown lentil malts were characterised with the highest concentration of raffinose and stachyose. Additionally, malting had the least significant impact on the concentration of raffinose and stachyose in the BR4, BR5 and BR6. It might indicate that seeds which accumulate higher concentrations of RFOs are more capable of their utilisation, which is logical, reasonable and found in many instances in plant kingdom[18,19]. Malting decreased average concentration of raffinose in brown lentil malts to 22.6% of the starting value and decreased stachyose concentration to 23.7% of the concentration found in BR. Analysed beans were characterised with lower concentration of RFOs than lentils, but malting procedure performed on this seeds resulted in lower decrease of raffinose and stachyose. Malting decreased stachyose concentration in white bean malts to 25.3% of the starting value and reduced stachyose concentration in red bean malts to 51% of the amount present in the RB sample. Decrease in the raffinose concentration was similar. Malting of the white beans resulted in the decrease of raffinose to 23.9% of the concentration in WB and, in the case of red beans, to 48.6% of the concentration present in RB. Not only a seed species and variety should be mentioned as a factor influencing total concentration of RFOs. The length of the germination during the malting period also significantly affected content of RFOs in legume malts. Typical barley malts are germinated for

5 days, but some malthouses possess capacity to malt seeds for additional day (however, it increases losses for the malthouse). Malting for four days is also a viable option, if the maltsters want to speed up the production process, but the produced malt is of inferior quality compared to the malt germinated for the full length of the process. Malts germinated 1–3 days (called 'short' or 'chit' malts) are characterised with not adequate technological parameters and are not typically produced in the larger scale[7,20]. These are the reason why the most viable period of germination time was tested. Analysis of malts show, that 6-day germination time during malting had greatest influence on the concentration of raffinose and stachyose in lentil malts as well as bean malts. It decreased, on the average, concentration of stachyose present in the unmalted seeds by 90% and concentration of raffinose by 89.5%, in comparison to 4-day germination time which decreased concentration of stachyose present in the unmalted seeds by 87.5% and concentration of raffinose by 87.3%. Similar phenomenon can be seen in the bean malts, but the discrepancies between 5-day germination time and 6-day germination time were greater. 5 days of germination in the malting period resulted in the loss of 54% of raffinose and 52.4% loss of stachyose, while 6-day germination time resulted in the loss of 73.5% of raffinose and 71% of stachyose. This data shows, that if the goal of malting would be to produce substrate low in RFOs, in the case of lentil the germination time could be as short as 4 days, but to acquire acceptable results in reduction of RFOs in beans, 6 days of germination or more would be more recommended.

Concentration of RFOs in worts produced from the lentil malts. Mashing in the brewing process is a procedure, which is used to hydrolyse various substances present in the malts with the use of endogenous enzymes. Main goal of the mashing is complete hydrolysation of starch and adequate hydrolysation of proteins, which is why most of the research analysing malt enzymatic potential concentrates mainly on amylolytic enzymes (α-amylases, β-amylases and glucoamylases) and various proteases[21,22]. Additionally, typically malted seeds do not possess high concentration of RFOs. This is why in the literature there is not much data about activity of enzymes capable of hydrolysing RFOs in various malts (such as legume α-galactosidases), as well as no data about concentration of various RFOs in wort samples[23]. Different germination time during the malting process also had an impact on the amount of raffinose and stachyose present in the worts. Worts produced from malts germinated 4 days were characterised with higher concentration of raffinose (0.49 g/dm^3) and stachyose (0.18 g/dm^3) than malts germinated 5 and 6 days, in which raffinose concentration ranged from 0.43 to 0.40 g/dm^3 and stachyose concentration ranged from 0.15 to 0.14 g/dm^3. However, it can be seen that differences are not as crucial as could be seen in malts prior to the mashing, therefore, if the goal of the technological procedure would be to produce worts from lentil malts with low concentration of RFOs, 4-day germination time might prove adequate, decreasing production time and costs.

Materials and methods

Raw material. Raw material used in this study were seeds of three lentil (*Lens culinaris*) varieties and two common bean (*Phaseolus vulgaris*) varieties. Each variety was of different colour: black lentil of Beluga variety (BL), brown lentil of Firat 87 variety (BR), green lentil of variety Eston (GR), white bean of variety Piękny Jaś (WB) and red bean of variety Kreacja (RB). These are varieties (with the exception of Beluga lentil) commonly grown in the region. Black lentil of Beluga variety is the only one black lentil variety grown commercially in the area. Lentil and bean seeds were acquired from BioPlanet Company (Leszno near Warsaw, Poland). All procedures concerning use of the plant seeds were conducted in accordance to the institutional, national and international guidelines and legislation.

Chemicals and analytical standards. Analytical standards used for this study were raffinose, verbascose and stachyose, HPLC grade (99% purity) (Sigma Aldrich, Saint Louis, MO, USA). Additional reagents used in this study were sulphuric acid (H_2SO_4, 99.999% purity, Sigma Aldrich, Saint Louis, MO, USA) and glucoamylase: Saczyme Plus enzyme preparation from Novozymes (Denmark) with the activity of 750 AGU/g (one unit of amyloglucosidase activity is defined as the amount of enzyme required to release one µmole of D-glucose reducing-sugar equivalents per minute from soluble starch at pH 4.5 and 40 °C).

Malting procedure. Malting was performed in different conditions for lentil seeds and common bean seeds, because there are significant physiological differences between these species. Malting procedure consisted of seed steeping (hydration), seed germination and seed kilning (drying).

Seeds cleaning and weighing. Before seeds were weighed, the lentils and beans were manually assessed and damaged seeds as well as seeds with visible discolouration were discarded. After cleaning and sorting out, moisture of the seeds was assessed using automatic NIR grain analyser Infratec 1241 (Foss, Hilleroed, Denmark) equipped with program for analysis of legume seeds to determine the starting moisture content of seeds. Seventy gram portions of lentils and seventy gram portions of beans were weighed to the corresponding stainless steel perforated containers (12 containers for each seeds type). The weight of the empty containers, weighed seeds and containers with seeds were carefully noted. These measurements were used in the future to determine the changing moisture content of the steeped seeds. The perforated stainless steel container with the seeds inside will from now on be mentioned as the 'malting kit'.

Seeds steeping. Steeping was conducted in the water–air cycle, using 50dm^3 stainless steel chamber and KK 240 Smart Pro germination chamber (Pol-Eko Aparatura, Wodzisław Śląski, Poland). Malting kits with lentil seeds were submerged in the tap water for 7 h (water temperature was equal to 18 °C), then kept in germination

chamber (18 °C, relative humidity 90%) for 18.5 h. After 18.5 h, lentil seeds were submerged another time for 5 h (water temperature 18 °C) and then kept in the germination chamber for 17.5 h (18 °C, relative humidity 90%). Malting kits with beans were submerged in the tap water for 8 h (water temperature was equal to 20 °C), then kept in germination chamber (20 °C, relative humidity 90%) for 16 h. After 16 h, malting kits with beans were submerged another time for 6 h (water temperature 20 °C) and then kept in the germination chamber for 18 h (20 °C, relative humidity 90%). After each step of the seed steeping (after first submerging, after first air rest in the humid atmosphere, after second submerging and after the second air rest in the humid atmosphere), the malting kits were weighed and the moisture content of the seeds were calculated based on the changed weight of the malting kit. It was assumed that weight increase was equal to the amount of water absorbed by the seeds. After the steeping process, malting kits were weighed kept in the germination chamber for the seed germination. The moisture content of the bean seeds at the end of the steeping process was in the range of 57–59%, while the moisture content of lentil seeds was in the range of 58–60%.

Seeds germination. Lentil seeds were germinated at 18 °C and bean seeds were germinated at 20 °C. Each day, the malting kits were weighed and the weight loss was supplemented by spraying the seeds with sterile, distilled water. Each day the seeds were visually assessed to determine the level of acrospire growth and were manually mixed to avoid root entanglement. After 96 h of germination, 4 malting kits with each lentil type (black, brown and green) were removed from the germination chamber and kilned (as described in the Sect. "Seeds drying"). After 120 h of germination, four malting kits with each lentil type and six malting kits of each bean type (red and white) were removed from germination chamber and kilned. After 144 h of germination, last four malting kits with each lentil type and last six malting kits with each bean type were removed from germination chamber and kilned. The beans were dried only after the 120 h and 144 h, because the acrospire growth after 96 h was unsatisfactory.

Seeds drying. After the germination process, each batch of malting kits was dried in the UF110 Plus dryer (Memmert GmbH + Co, Schwabach, Germany) at the following conditions: 50 °C (17 h and 50 min), ramp up to 65 °C (10 min), 65 °C (2 h and 50 min), ramp up to 82 °C (10 min), 82 °C (2 h). The malts, after the drying process, malts of one type from different malting kits were mixed together and transferred to tightly closed containers, to prevent moisture absorption during cooling period. The germination and drying processes allowed for the production of 15 different malt types:

- black lentil malt germinated for 96 h (BL4);
- black lentil malt germinated for 120 h (BL5);
- black lentil malt germinated for 144 h (BL6);
- brown lentil malt germinated for 96 h (BR4);
- brown lentil malt germinated for 120 h (BR5);
- brown lentil malt germinated for 144 h (BR6);
- green lentil malt germinated for 96 h (GR4);
- green lentil malt germinated for 120 h (GR5);
- green lentil malt germinated for 144 h (GR6);
- red bean malt germinated for 120 h (RB5);
- red bean malt germinated for 144 h (RB6);
- white bean malt germinated for 120 h (WB5);
- white bean malt germinated for 144 h (WB6);

Before the mashing procedure (Sect. "Congress mashing of the legume malts") malts were ground with the use of Bühler Miag disc mill DLFU (Bühler, Uzwil, Switzerland), according to the Analytica EBC 4.5.1 method[24]. Malts and unmalted seeds before analysis of RFOs concentration were ground using IKA A10 basic mill (Staufen, Germany).

Congress mashing of the legume malts. Congress worts were produced in the automated laboratory mashing machine (LB Electronic, Lochner Labor and Technik, Berching, Germany) according to the Analytica EBC method 4.5.1. Wort, after filtration, was collected for analyses. Congress worts from legume malts were prepared in duplicate. It was not possible to produce congress worts from the bean malts, because the first 100cm^3 of the wort, which had to be reversed into the funnel to start the filtration process could not have been acquired. Congress mashing, therefore, allowed for production of 9 congress wort samples:

- wort from black lentil malt germinated for 96 h (BL4W);
- wort from black lentil malt germinated for 120 h (BL5W);
- wort from black lentil malt germinated for 144 h (BL6W);
- wort from brown lentil malt germinated for 96 h (BR4W);
- wort from brown lentil malt germinated for 120 h (BR5W);
- wort from brown lentil malt germinated for 144 h (BR6W);
- wort from green lentil malt germinated for 96 h (GR4W);
- wort from green lentil malt germinated for 120 h (GR5W);
- wort from green lentil malt germinated for 144 h (GR6W);

Extract content of the legume malt worts was analysed with the use of densimeter (DMA 35, Anton Paar, Graz, Austria). For each wort sample, measurement was performed in triplicate (which equals six extract measurements per one type of malt).

Analysis of the RFO content in the legume seeds, legume malts and worts produced from legume malts. *Extraction and enzymatic hydrolysis of maltotetraose, maltotriose and maltose in the legume seeds and malts.* To carry out necessary identification and quantification of raffinose, stachyose and verbascose, various disaccharides, trisaccharides and tetrasaccharides (products of starch hydrolysis, such as maltose, matotriose and maltotetraose) had to be hydrolysed, because otherwise these sugars would have prevented optimal identification and quantification of RFOs. Two grams of ground seed or malt sample and 20cm^3 H_2SO_4 (5 mmol concentration) were added to the Erlenmeyer flask. The flasks were shaken for 3 h at the room temperature. After 3 h, the sulphuric acid solution was filtered through syringe filter (pore size 0.45 µm). 1cm^3 of the filtered solution was added to test tube. Glucoamylase preparation (10mm^3) was added to the test tube, which was then closed with cap and incubated for 1 h at temperature of 60 °C. After the incubation, the solution was centrifuged (10,000 rpm, 5 min) and supernatant was added to the chromatographic vial and analysed.

Preparation of worts produced out of legume malts for the chromatographic analysis. The wort (1cm^3), after the filtration through the paper funnel (in accordance to the Analytica 4.5.1 method) was pippeted to the test tube and diluted 10 times using H_2SO_4 (5 mmol). Glucoamylase preparation (10mm^3) was added to the test tube, which was then closed with cap and incubated for 1 h at temperature of 60 °C. After incubation, worts were centrifuged and supernatant was added to the chromatographic vials and analysed (Supplementary Fig. 1).

Chromatographic analysis of the RFOs. Concentration of RFOs (raffinose, stachyose and verbascose) was assessed using high performance liquid chromatography (HPLC). Chromatographic analyses were performed using Agilent Technologies model 1220 (Agilent Technologies, Santa Clara, CA, USA) apparatus equipped with a thermostat and refractometric detector (RID). The separation was performed using a Hi-Plex H (Agilent Technologies®, Santa Clara, CA, USA) column. The mobile phase was 5 mmol H_2SO_4 flowing at 0.6cm^3/min under isocratic conditions. The working temperature of the column was 60 °C. The working temperature of the refractometric detector was 50 °C. External standards (ESTD) and the appropriate calibration curves were used to determine the concentration of the analysed compounds. The separation parameters were in accordance with the recommendations of the chromatography column manufacturer. Limit of detection (LOD) for raffinose was equal to 0.011 g per dm^3 of the solution (extract or wort); limit of quantitation for raffinose was equal to 0.032 g per dm^3 of the solution. Limit of detection (LOD) for stachyose was equal to 0.005 g per dm^3 of the solution; limit of quantitation for stachyose was equal to 0.015 g per dm^3 of the solution. Limit of detection (LOD) for verbascose was equal to 0.021 g per dm^3 of the solution; limit of quantitation for stachyose was equal to 0.064 g per dm^3 of the solution. Linearity equations were as follows: for raffinose $y = 76810x - 817.4$ ($R^2 = 0.9997$); for stachyose $y = 208{,}455.0x + 193.34$ ($R^2 = 0.9999$); for verbascose $y = 331911x + 2520.9$ ($R^2 = 0.9987$). Sample chromatograms of RFOs content in lentil and bean malts and unmalted seeds are provided in the Supplementary Material (Supplementary Fig.1–5).

Data analysis. The results of the analysis of oligosaccharide content of seeds and malts were statistically analysed using the SPSS Statistics 26 program from IBM (Armonk, NY, USA). Two-way ANOVA was used for the determination of differences in concentration of RFOs between lentil malts as well as bean malts (with the variables: length of germination and seed variety). One-way ANOVA was used for the determination of differences between malted seeds and unmalted seeds (with the variable of seed variety). Two-way ANOVA for the analyses of lentil worts (with the variables: days of germination, lentil variety). Bonferroni test was used as a post-hoc test with $\alpha < 0.05$. Extraction and enzymatic hydrolysis was performed in duplicate for each of the malts and seeds and each of the extracts was analysed chromatographically in two repetitions, which resulted in four readings per type of seed/malt. Worts were prepared in duplicate during the congress mashing procedure. Each hydrolysed wort was analysed chromatographically in three repetitions, which resulted in six reading per type of wort. Extract content of the worts was analysed in triplicate, which resulted in six readings per type of wort.

Conclusions

Lentil seeds and bean seeds are rich in raffinose and stachyose, but conducted study shows, that malting procedure can be successfully used as a tool to decrease concentration of these sugars. Malting with 6-day germination time radically decreased raffinose and stachyose content in lentil seeds. The reduction in the RFO content was smaller in the case of bean malts, but these seeds were characterised with lower concentration of RFOs than lentil seeds. Mashing process allowed producing lentil worts with very low concentration of stachyose (ranging from 0.13 g/dm^3 to 0.32 g/dm^3) and raffinose (ranging from 0.40 g/dm^3 to 0.49 g/dm^3). These results show, that malting and mashing procedures can be used to modify legume seeds and produce novel raw material adequate for production of various food products with reduced RFOs content.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

A.G. conceptualized the experiment. A.G., J.K.-R. and D.M. prepared methodology used during the investigation. A.G. and D.M. performed the experiments. A.G. and D.M. performed formal analysis and validated results acquired in the study. A.G. wrote the main manuscript text. All authors reviewed and edited the manuscript text. J.K.-R. and G.K. supervised and administered the study.

Competing interests

The authors declare no competing interests.

Additional information

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RESEARCH ARTICLE

Malting—A method for modifying volatile composition of black, brown and green lentil seeds

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Abstract

Technique of malting legume seeds is not currently widespread among scientists as well as industrial maltsters. However, this method of seed modification is successfully used by humankind for millennia to improve technological parameters, as well as change taste and aroma of various food products. Three lentil cultivars (black, brown and green) were malted (steeped, germinated for three various time periods and then kilned) to produce nine lentil malts. Malting had significant influence on the volatile composition of lentil seeds. Total concentration of volatiles in the green lentils increased and decreased in the case of black and brown lentils after malting procedure. However, most importantly, in every lentil cultivar the contribution of various groups of compounds (such as aldehydes, alcohols, terpenes or ketones) to the overall volatilome was changed due to the malting procedure.

1. Introduction

Legumes are one of the oldest cultivated crops and play an important part in the plant-based diet. Legumes are still currently one of the most important sources of protein and energy in the diet of people, as well as farm animals around the world [1,2]. Lentils are a high-protein legumes, which are drought resistant and can be grown in various climates, as they are able to thrive in the warm and cool environments [3]. Furthermore, lentils are present in the most of the cuisines throughout the world and can be used to prepare various dishes, therefore improvement of lentil properties could benefit large quantities of people [4]. Furthermore, improvement of the lentil's nutritional value, organoleptic characteristics and technological properties might have an influence on the origin of many novel, plant-based food products. It would be of great importance, because even raw, unmodified lentils possess vast arrays of bioactive phytochemicals, such as phenolics, phytosterols, phytic acid, saponins, tocopherols and carotenoids, which are beneficial in the prevention of many non-communicable diseases [5–7]. Malting is a process which is used primarily to modify grains and the main reason of malting is increasing its enzymatic activity due to the generation and activation of various enzymes [8]. However, during the malting procedure (which consists of the seed steeping, germination and then kilning) many different processes occur, one of which is substantial change of the

dataset/Whole_data_volatiles_lentil_malts_xlsx/24013944 https://doi.org/10.6084/m9.figshare.24013944.v1.

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volatile composition of the malted seed material. Changes in the volatile composition occur due to the physiological changes in the germinating grain and to thethermal process of drying at the end of the malting procedure. Most malted grains acquire aroma which is described as cookie-like, bread-like, toffee-like, nutty and even caramel, chocolate-like, roasted, or coffee-like, depending on the time and temperatures of the kilning process [9]. As the aroma of legumes (raw, boiled or canned) is often not recognized as 'pleasant' or 'tasty' by most consumers, it seems that the procedures which can improve aroma of the legumes (such as malting, typically used for the modification of the barley grains) might be of interest for the farmers and food producers alike [10]. In the previous studies about malting legumes, tin was determined, that soybean, lentil, vetch, peas and chickpeas can be malted in the conditions typical for malting barley, but produced malts were characterised with unadequate technological properties. Mashes produced from these malts had not saccharified, filtered very slowly and yielded low volume of the wort [11]. However, consequent study about malting lentils and beans have shown that, despite poor brewhouse efficiency, malting procedure carefully tailored for the legume seeds might improve their friability as well as reduce concentration of various anti-nutritonal components, such as phytic acid or raffinose-family oligosaccharides [12,13]. In this study, a malting process was applied to three different varieties of *Lens culinaris*, to determine, whether such a simple procedure, which might be implemented into food production process virtually anywhere in the world might significantly change volatile compositions of lentil seeds.

2. Materials and methods

2.1. Materials

2.1.1. Raw material. Plant material used in this study were seeds of three lentil (*Lens culinaris*) varieties, each with different colour: black lentil of Beluga variety (BL) with green cotyledon colour, brown lentil of variety Firat 87 (BR) with orange cotyledon colour and green lentil of variety Eston (GR) with green cotyledon colour. Lentil seeds were acquired from BioPlanet company (Leszno near Warsaw, Poland). Lentils after harvest, prior to the study were stored in the silo for nine months. Lentils seeds, prior to the malting procedure and analyses, were manually sifted to discard damaged seeds and seeds with visible discolouration. Moisture content of the seeds, before and after malting process, was analysed with the use of MT Moisture Analyser (Brabender, Duisburg, Germany).

2.1.2 Reagents and standards. Reagents used in this study were 2-undecanone (99%, suitable for GC analyses) purchased from the Sigma-Aldrich company (Saint Louis, MO, USA), cyclohexane (99%), sodium chloride (99.8%) and sodium hypochlorite (15%) (Chempur, Piekary Śląskie, Poland). 2-undecanone was mixed with the cyclohexane to produce internal standard with the concentration of one mg of 2-undecanone per one dm^3 of cyclohexane. Standards used for identification of volatiles were: 3-octen-2-one (≥98% purity, Supelco); trans-β-ionone (≥97%, Sigma Aldrich); 1-octen-3-ol (≥98%, Sigma Aldrich); 2-ethyl-1-hexanol (≥99%, Sigma Aldrich); 1-octanol (≥99%, Sigma Aldrich); 3,5-dimethylcyclohexanol (≥97.0%, Sigma Aldrich); 1-nonanol (≥98%, Sigma Aldrich); 1,7-octanediol, 3,7-dimethyl (≥98.0%, Lluch Essence, Barcelona, Spain); 1-dodecanol (≥98%, Sigma Aldrich); 3-carene (≥95%, Supelco), (R)-(+)-limonene (≥97%, Sigma Aldrich), eucalyptol (≥99%, Sigma Aldrich); D-carvone (≥96%, Sigma Aldrich); undecane (≥99%, Sigma Aldrich); dodecane (≥99%, Sigma Aldrich); tridecane (≥99%, Sigma Aldrich); tetradecane (≥99%, Sigma Aldrich); pentadecane (≥99%, Sigma Aldrich); hexadecane (≥99%, Sigma Aldrich); octadecane (≥99%, Sigma Aldrich); 4,6-dimethyldodecane (≥98%, SimSon Pharma Limited, Maharashtra, India), 2-pentylfuran (≥98%, Sigma Aldrich); benzothiazole (≥96%, Sigma Aldrich);

2-ethyl-3,5-dimethyl-2-pyrazine (≥95%, Sigma Aldrich); propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester (≥95%, Toronto Research Chemicals; Toronto, Canada); dodecanoic acid, 1-methylethyl ester (≥98%, Sigma Aldrich).

2.2. Methods

2.2.1. Malting procedure. Simplified diagram of the malting procedure is shown in the Fig 1.

Eighty gram portions of black, brown and green lentil were weighed and transferred to perforated, stainless steel malting containers (24 containers for each of the lentil varieties), which were previously disinfected by drying in the UF110 Plus dryer (Memmert GmbH + Co, Schwabach, Germany) for two h at 200˚C and then cooled to room temperature. Containers filled with known mass of lentil were then weighed (the container filled with lentil seeds will be from now on mentioned as the 'malting kit'). Changes in the moisture content of the seeds during the first step of the malting process (steeping) were calculated based on the changing weight of the malting kit, assuming, that the increase in weight of the kit is equal to the quantity of water adsorbed by the seeds. Steeping was executed in the water-air steeping cycle. At the start of the

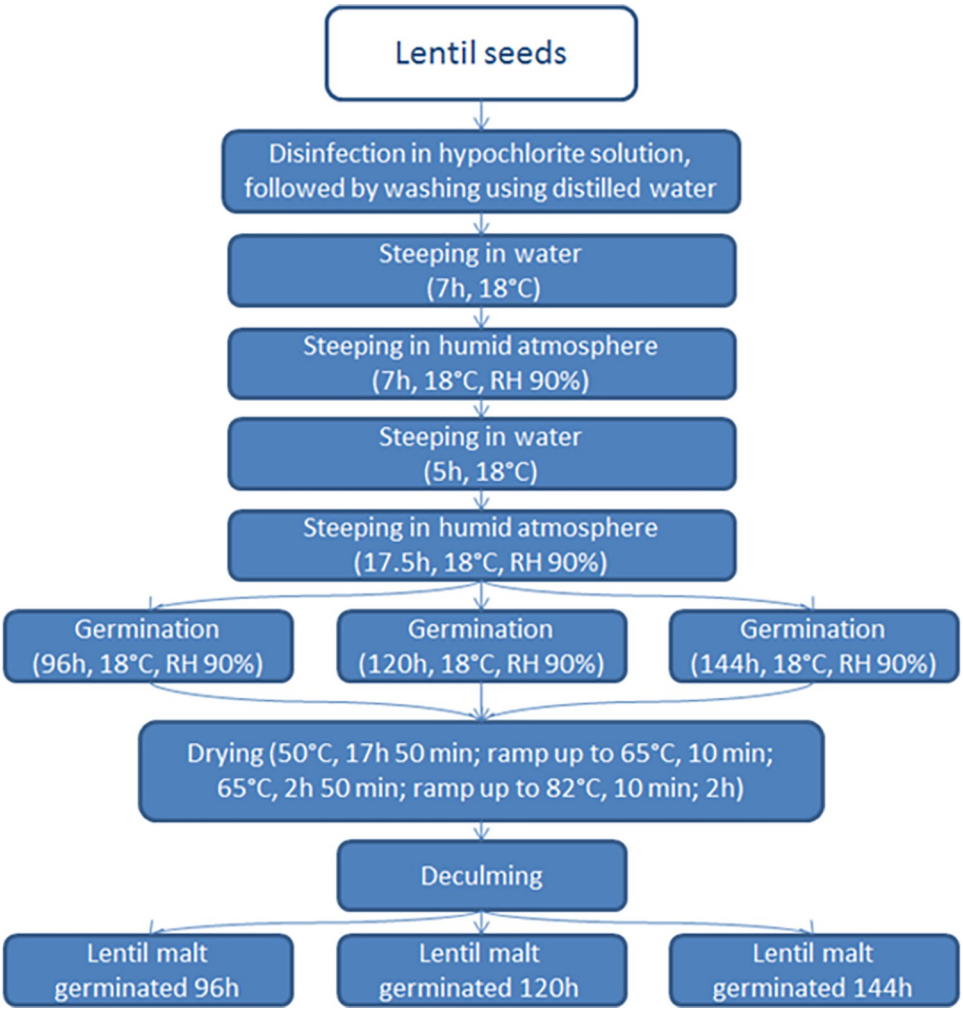

Fig 1. Lentil malting procedure.

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process, malting kits were submerged in the 1.5% sodium hypochlorite solution for 10 min to surface sterilise the seeds. Malting kits were then removed from the sodium hypochlorite solution and immediately washed three times with distilled water. After this process, malting kits were submerged in tap water (disinfected previously by boiling and cooled) at temperature of 18˚C for seven h, then transferred to the KK 240 Smart Pro germination chamber (with humidity set at 90% relative humidity and temperature set at 18˚C) for 18.5 h; then submerged another time in fresh, disinfected tap water at a temperature of 18˚C for five h; then transferred to the germination chamber (temp. 18˚C, relative humidity 90%) for 17.5 h. After each step, malting kits were weighed to determine changing moisture content of the lentil seeds. At the end of the steeping process, the moisture content of the lentil seeds was equal to 53–55%. Lentils were germinated in the germination chamber with the temperature set at 18˚C and relative humidity 90%. Eight of each of the lentil varieties samples (total of 24 malting kits) were germinated for four days; eight of each of the lentil varieties samples (total of 24 malting kits) were germinated for five days; eight of each of the lentil varieties samples (total of 24 malting kits) were germinated for six days. The germination time used in this study was selected using standard germination time used for production of grain malts [8,14]. After the germination process, each batch of malting kits was dried in the UF110 Plus dryer at the following conditions: 50˚C (17 h and 50 min), ramp up to 65˚C (10 min), 65˚C (two h and 50 min), ramp up to 82˚C (10 min), 82˚C (two h). The malting procedure resulted in production of 9 different lentil malt samples:

- black lentil malt germinated 96 h (four days) (BL4)
- black lentil malt germinated 120 h (five days) (BL5)
- black lentil malt germinated 144 h (six days) (BL6)
- brown lentil malt germinated 96 h (four days) (BR4)
- brown lentil malt germinated 120 h (five days) (BR5)
- brown lentil malt germinated 144 h (six days) (BR6)
- green lentil malt germinated 96 h (four days) (GR4)
- green lentil malt germinated 120 h (five days) (GR5)
- black lentil malt germinated 144 h (six days) (GR6)

The malts, after the drying process, malts of one type from different malting kits were mixed together and transferred to tightly closed containers, to prevent moisture absorption during cooling period. Malts, as well as unmalted lentils, were ground with the use of Bühler Miag disc mill DLFU (Bühler, Uzwil, Switzerland), according to the Analytica EBC 4.5.1 method for the subsequent analysis [15].

2.2.2. Adsorption of volatile compounds to the solid-phase microextraction fiber. To perform chromatographic analysis of volatiles present in the lentil seeds and malts, the volatiles had to be adsorbed on the solid phase microextraction fiber (SPME) [16]. Ground malt or seed sample (2.5 g) was transferred to the 20 cm^3 headspace vial, followed by the addition of 0.5 g sodium chloride and four cm^3 of distilled water. Twenty ng of internal standard (2-undecanone, in the form of 20 mm^3 of 2-undecanone in hexane solution) was added to the vial, which was then closed with a magnetic screw-top cap with a septum. SPME holder needle, equipped DVB/CAR/PDMS fiber (50/30μm) (Supelco, Bellefonte, PA, USA) was used to pierce the septum. Vial was positioned on the heatplate set at 80˚C. After 5 min of temperature equilibration, the fiber was extended from the holder needle, to allow adsorption of the volatiles on the fiber surface for 45 min. After adsorption of the volatiles, fiber was retracted into the holder.

2.2.3. Gas chromatography and mass spectrometry. Gas chromatography and mass spectrometry of the volatiles was performed using GC-2010 Plus chromatograph coupled with GCMS-QP2010 SE mass spectrometer (Shimadzu, Kyoto, Japan) equipped with ZB-5 column (Phenomenex, Torrance, CA, USA) (30 m length x 0.25 mm internal diameter x 0.25 μm film thickness). Injection port temperature was held at 195˚C. Analyses were carried out with the use of helium as a carrier gas with a flow rate of 1.78 cm^3/min and a starting pressure set at 100 kPa. Following program was used for the oven temperature: 40˚C at the beginning; hold for one min, ramp up at a rate of eight˚C/min to 195˚C; hold for five min. Ion source temperature was maintained at 250˚C, while interface temperature was at 195˚C. Scanning was carried out in the 35–350 m/z range using 70 mV electron ionisation with the event time equal to 0.3 s (scan speed equal to 1111). Adsorption of volatiles and gas chromatography was performed in triplicate for each of the lentil samples.

2.3. Data analysis

Volatile compounds separated from the lentil seeds and lentil malts were identified by mass spectral analysis based on NIST17 chemical standard libraries and spectra of authentic chemicals, whenever possible, comparison of retention time with retention time of authentic chemicals and by confirmation by comparison of retention indices with Kovats standards. If the authentic chemical standard was not available, the similarity search based on the NIST libraries had to be at least 95% to determine the compound as identified. If the compound was identified with the use of authentic chemicals, the retention time of the authentic chemical could not deviate by more than 0.05 min from the retention time of the authentic chemical sampled on the same column, with the same temperature program. Kovats indices (KI) were used to confirm the identification: if the KI of the identified peak deviated by more than 10 from average KI for the compound declared in the NIST Webbook, then the compound was classified as unidentified and not recorded. Chromatographic peaks were integrated with the use of Shimadzu PostRun Analysis program (Shimadzu, Kyoto, Japan). The results of the analysis of antioxidative activity and phenolic content of lentil seeds and lentil malts were statistically analysed in the Statistica 12.5 program from Statsoft (Tulsa, OK, USA) using two-way ANOVA with variables: variety of lentil and length of germination (unmalted samples were described as having zero days of germination) with $\alpha = 0.05$ using Tukey test.

3. Results and discussion

3.1. Concentration of volatile compounds in the lentil seeds and lentil malts

Gas chromatography and mass spectrometry allowed for identification and quantification of 50 volatile compounds. The largest group of compounds were aldehydes (18 compounds) and hydrocarbons (nine compounds). Smaller groups of compounds were alcohols (seven compounds), terpenes (sex compounds), ketones (four compounds) and other minor constituents, such as esters, furans, pyrazines and sulphur compounds (total of six compounds). Concentration of total identified volatiles was in the range of 33.10–114.58 ppb, with the lowest for BL and the highest for GR. Malting resulted in a decrease of total concentration of volatiles for green lentil, with minor decrease (by 4.6%) for GR4 and major reduction of volatiles for GR5 and GR6 (by 34.5% and 58.5%, consecutively). However, malting procedure increased the total concentration of volatiles in the samples produced from black and brown lentil. The highest increase of the volatile compounds content for the black lentil was noted between sample BL and BL4, where it increased from 33.10 ppb to 44.11 ppb (increase of 32.8%). Extension of the

germination time resulted in decrease of the total volatile content in the malts from black lentil (37.53 ppb for BL5 and 35.64 ppb for BL6). Different result can be seen in the malts produced from the brown lentil. Sample BR4 was characterised with the lowest concentration of volatiles (35.74 ppb), which increased with the malting, albeit the highest concentration of volatiles was determined in the sample BR5 (50.71 ppb). The main difference between various impact of the malting on the total concentration of volatiles in malts produced from the lentils of different colour seems to be vastly different content of volatiles in the particular type of lentil seeds. The main compounds in the GR, which are absent or in far lower concentration than in BR or BL are eucalyptol, limonene, 3-carene and 1-octen-3-ol. Changes of these constituents are more broadly discussed in the sections 3.1.2 and 3.1.4. Additionally, malting changes the contribution of various groups of volatiles (such as aldehydes, alcohols, esters, ketones, terpenes, furans and pyrazines) in the volatilome of the lentil malts and these results are shown on the Figs 2–4.

3.1.1. Concentration of aldehydes in the lentil seeds and lentil malts. Most of the volatiles present in the lentil and lentil malt samples were aldehydes (Table 1). Aldehydes were most abundant group of volatiles in eight out of nine malted lentil samples (with the exception of GR5), where they constituted from 32.24% to 58.75% of all identified volatiles. Malting resulted in the increase of concentration of aldehydes in most of the samples, from 28% to 96%, compared to the unmalted lentil, with the exception of GR6, where the total amount of aldehydes was reduced only by small margin of 0.3 ppb (1.3%). All of the aldehydes present in the lentils and lentil malts have been previously identified in the lentil seeds or lentil flours by various researchers in the previous years [17–19]. Many differences could be seen between various lentil cultivars analysed in this study. Benzaldehyde, compound characterised with almond-like aroma, produced by the Strecker degradation of phenylalanine amino acid was present only in the malted brown lentil samples [20]. This result suggests, that proteases in the brown lentil allowed for the release of phenylalanine during the malting procedure. Benzene

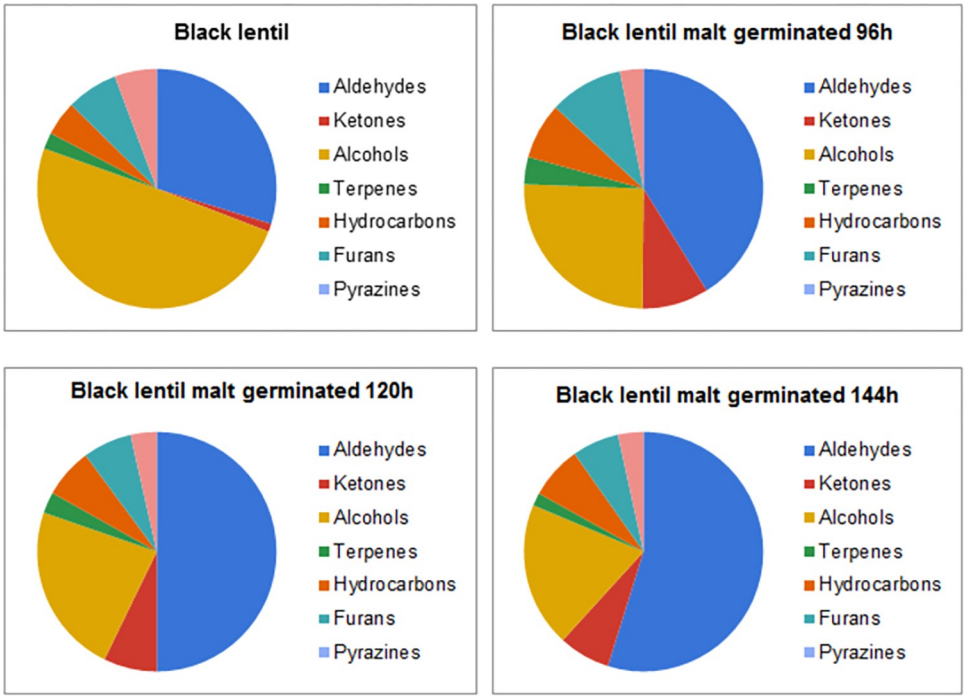

Fig 2. Contribution of various chemical groups in the volatilome of black lentil and black lentil malts.

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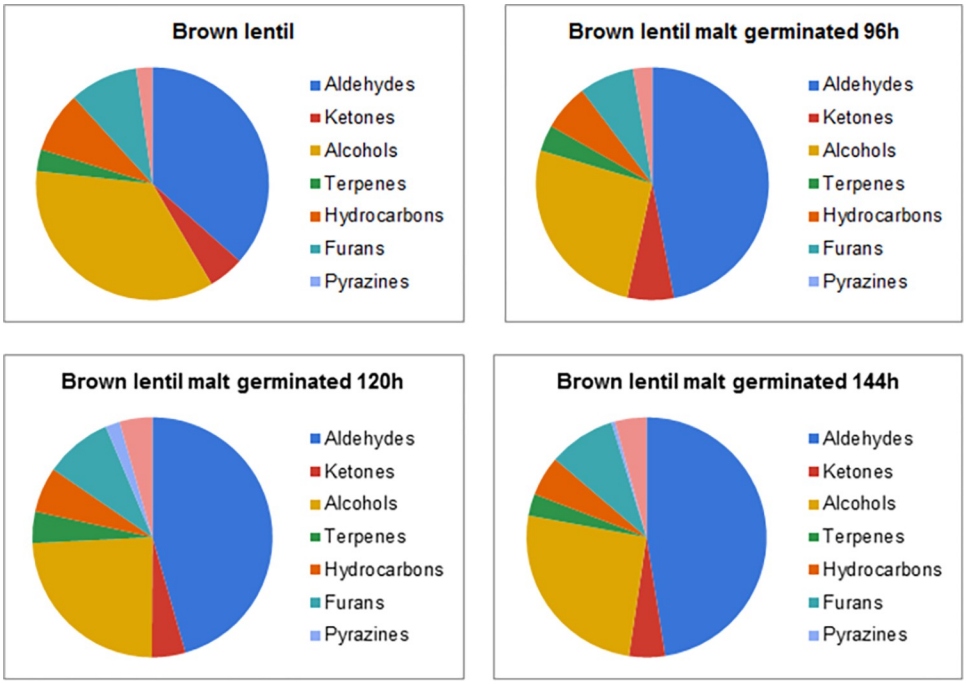

Fig 3. Contribution of various chemical groups in the volatilome of brown lentil and brown lentil malts.

https://doi.org/10.1371/journal.pone.0290616.g003

acetaldehyde (known also as phenylacetaldehyde), characterised with floral, sweet and honey-like flavour, is a compound, which was not found in the unmalted BR, BL and GR samples, but was present in all the lentil malts. Benzene acetaldehyde was similarly not detected in raw legume products by other scientists such as Wang et al. [21] and Murat et al. [22], but was

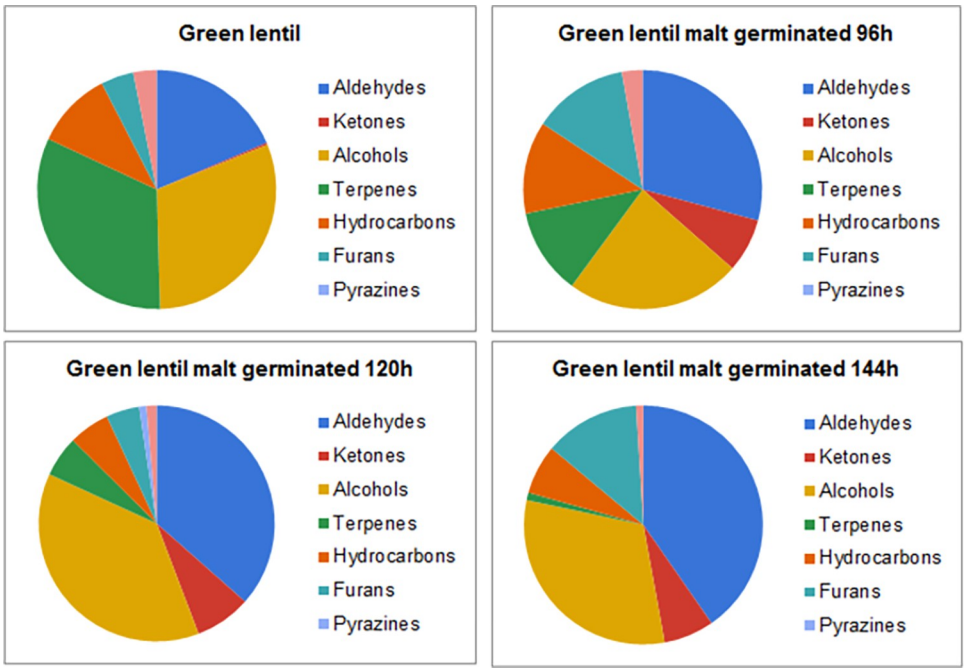

Fig 4. Contribution of various chemical groups in the volatilome of green lentil and green lentil malts.

https://doi.org/10.1371/journal.pone.0290616.g004

Table 1. Concentration of aldehydes in lentils and malted lentils.

	Compound[1]	BL	BL4	BL5	BL6	BR	BR4	BR5	BR6	GR	GR4	GR5	GR6
		ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
1	Benzaldehyde	0.00 b, B	0.00 b, A	0.00 b, A	0.00 b, A	0.00 a, B	7.48 ± 0.92 a, A	7.34 ± 0.88 a, A	7.96 ± 1.05 a, A	0.00 b, B	0.00 b, A	0.00 b, A	0.00 b, A
2	Octanal	0.00 c, B	0.00 c, C	0.00 c, D	0.00 c, A	0.00 b, B	0.42 ± 0.09 b, C	0.19 ± 0.08 b, D	0.76 ± 0.21 b, A	0.87 ± 0.17 a, B	0.00 a, C	0.00 a, D	1.03 ± 0.25 a, A
3	Benzeneacetaldehyde	0.00 b, D	0.69 ± 0.18 b, C	0.87 ± 0.25 b, A	1.23 ± 0.21 b, B	0.00 b, D	0.89 ± 0.18 b, C	1.09 ± 0.24 b, A	0.53 ± 0.13 b, B	0.00 a, D	0.92 ± 0.18 a, C	2.52 ± 0.54 a, A	0.94 ± 0.31 a, B
4	2-Octenal, (E)-	0.61 ± 0.18 b, C	0.72 ± 0.22 b, B	0.90 ± 0.24 b, B	1.17 ± 0.29 b, A	0.91 ± 0.28 a, C	0.44 ± 0.16 a, B	1.09 ± 0.21 a, B	1.21 ± 0.43 a, A	0.00 c, C	1.50 ± 0.18 c, B	0.59 ± 0.12 c, B	0.68 ± 0.21 c, A
5	Nonanal	2.70 ± 0.41 c, B	5.15 ± 0.98 c, A	4.40 ± 1.03 c, C	4.21 ± 0.77 c, D	5.48 ± 1.12 b, A	4.81 ± 0.68 b, B	4.84 ± 0.83 b, C	4.44 ± 0.91 b, D	11.75 ± 2.18 a, B	15.28 ± 2.84 a, A	7.61 ± 1.96 a, C	5.91 ± 1.04 a, D
6	2-Nonenal, (Z)-	0.00 b, C	0.14 ± 0.07 b, A	0.00 b, C	0.00 b, B	0.00 c, C	0.00 c, A	0.00 c, C	0.00 c, B	0.00 a, C	1.25 ± 0.38 a, A	0.00 a, C	0.19 ± 0.08 a, B
7	Trans-2-nonenal	3.22 ± 0.72 b, D	8.94 ± 1.15 b, A	8.27 ± 0.98 b, B	7.58 ± 1.15 b, C	3.17 ± 0.61 c, D	6.41 ± 1.12 c, A	6.23 ± 1.24 c, B	6.24 ± 0.93 c, C	1.72 ± 0.41 a, D	11.57 ± 2.81 a, A	10.41 ± 1.54 a, B	8.39 ± 0.99 a, C
8	Decanal	1.81 ± 0.44 b, A	1.75 ± 0.31 b, D	2.26 ± 0.52 b, C	2.50 ± 0.72 b, B	2.65 ± 0.81 b, A	1.48 ± 0.46 b	1.51 ± 0.37 b, C	1.98 ± 0.48 b, B	4.41 ± 0.86 a, A	2.55 ± 0.61 a, D	2.53 ± 0.84 a, C	2.20 ± 0.68 a, B
9	2,4-Nonadienal, (E,E)-	0.00 b, C	0.15 ± 0.07 b, B	0.00 b, A	0.00 b, C	0.00 a, C	0.11 ± 0.06 a, B	0.16 ± 0.09 a, A	0.00 a, C	0.00 a, C	0.00 a, C	0.26 ± 0.12 a, A	0.00 a, C
10	2-Decenal, (E)-	0.35 ± 0.11 a, C	0.61 ± 0.15 a, B	0.54 ± 0.18 a, A	0.49 ± 0.16 a, A	0.39 ± 0.12 a, C	0.39 ± 0.14 a, B	0.56 ± 0.21 a, A	0.50 ± 0.19 a, A	0.00 b, C	0.48 ± 0.21 b, B	0.53 ± 0.18 b, A	0.55 ± 0.21 b, A
11	Benzeneacetaldehyde,.alpha.-ethylidene-	0.00 b, D	0.19 ± 0.08 b, A	0.00 b, B	0.13 ± 0.06 b, C	0.00 b, D	0.12 ± 0.04 b, A	0.14 ± 0.05 b, B	0.00 b, C	0.00 a, D	0.53 ± 0.18 a, A	0.63 ± 0.21 a, B	0.42 ± 0.18 a, C
12	Undecanal	0.63 ± 0.21 a, C	0.37 ± 0.11 a, D	0.41 ± 0.15 a, B	0.50 ± 0.18 a, A	0.12 ± 0.03 b, C	0.00 b, D	0.39 ± 0.12 b, B	0.42 ± 0.14 b, A	0.00 c, C	0.00 c, D	0.17 ± 0.05 c, B	0.17 ± 0.09 c, A
13	2-Octenal, 2-butyl-	0.00 b, C	0.00 b, C	0.00 b, C	0.00 b, C	0.00 b, C	0.00 b, C	0.00 b, C	0.00 b, C	0.00 a, C	0.00 a, C	0.94 ± 0.31 a, A	0.54 ± 0.17 a, B
14	Dodecanal	0.00 a, B	0.00 a, B	0.21 ± 0.09 a, A	0.62 ± 0.21 a, A	0.00 b, B	0.00 b, B	0.38 ± 0.11 b, A	0.00 b, A	0.00 c, B	0.00 c, B	0.00 c, A	0.00 c, A
15	5-Methyl-2-phenyl-2-hexenal	0.00 b, A	0.00 b,A	0.00 b, C	0.00 b, B	0.00 b, A	0.00 b, A	0.00 b, C	0.00 b, B	0.38 ± 0.12 a, A	0.33 ± 0.14 a, A	0.00 a, C	0.08 ± 0.05 a, B
16	Tridecanal	0.45 ± 0.19 a, A	0.50 ± 0.16 a, C	0.74 ± 0.28 a, A	0.86 ± 0.29 a, B	0.64 ± 0.18 b, A	0.45 ± 0.18 b, C	0.50 ± 0.21 b, A	0.52 ± 0.25 b, B	0.89 ± 0.32 a, A	0.60 ± 0.16 a, C	0.82 ± 0.25 a, A	0.29 ± 0.08 a, B
17	Tetradecanal	0.22 ± 0.12 a, D	0.24 ± 0.10 a, C	0.51 ± 0.22 a, A	0.52 ± 0.19 a, B	0.26 ± 0.09 b, D	0.24 ± 0.09 b, C	0.29 ± 0.11 b, A	0.18 ± 0.08 b, B	0.00 c, D	0.17 ± 0.08 c, C	0.40 ± 0.21 c, A	0.16 ± 0.08 c, B
18	Pentadecanal	0.67 ± 0.28 b, A	0.76 ± 0.27 b, C	1.02 ± 0.34 b, B	1.13 ± 0.39 b, D	0.89 ± 0.32 c, A	0.85 ± 0.25 c, C	0.67 ± 0.21 c, B	0.60 ± 0.19 c, D	2.36 ± 0.81 a, A	0.97 ± 0.33 a, C	1.24 ± 0.38 a, B	0.52 ± 0.16 a, D
	Total aldehydes	**10.67**	**20.21**	**20.12**	**20.94**	**14.52**	**24.09**	**25.38**	**25.33**	**22.38**	**36.14**	**28.67**	**22.08**
	% of all volatiles	**32.24%**	**45.81%**	**53.62%**	**58.75%**	**40.62%**	**50.96%**	**50.04%**	**52.76%**	**19.53%**	**33.10%**	**38.28%**	**46.54%**

[1] Abbreviations are as follows: GL4—malt from green lentil germinated 4 days, GL5—malt from green lentil germinated 5 days, GL6—malt from green lentil germinated 6 days, BRL4—malt from brown lentil germinated 4 days, BRL5—malt from brown lentil germinated 5 days, BRL6—malt from brown lentil germinated 6 days, BLL4—malt from black lentil germinated 4 days, BLL5—malt from black lentil germinated 5 days, BLL6—malt from black lentil germinated 6 days. Values are expressed as means (n = 3) ± standard deviation. Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C, D) letters indicate homogenous groups according to the variable 'days of germination'(Tukey test, $\alpha = 0.05$).

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identified in the processed legume products, such as flours and protein isolates. Malting increased the concentration of trans-2-nonenal, which is produced by the oxidation of free fatty acids, in all the lentil varieties. In the malting industry, this compound is viewed negatively, as the aroma of trans-2-nonenal is characterised with the stale beer (possessing so-called 'cardboard' flavour) [23], albeit study performed by the Shi et al. [24] about beverage produced from the legume seeds (soymilk) showed, that experts in sensory analysis preferred legume products with higher concentration of trans-2-nonenal. This result might suggest that legume malts are not the best substrate for the production of wort and beer, albeit might find their uses in the production of the milk substitutes.

3.1.2. Concentration of alcohols in the lentil seeds and lentil malts. Alcohols were the second largest group of volatiles in the lentil malts, in which the volatile composition consisted from 20.96% (BL6) to 39.45% (GR5) of alcohols (Table 2). Alcohols were main volatile components in the sample BL (53.77%), as well as constituted second largest groups of compounds in the BR (39.15%) and GR (32.19%) sample. Malting had almost no impact on the total concentration of alcohols in the malts prepared from the brown lentil (13.99 ppb in BR, 13.40–13.64 ppb in the malted samples). The concentration of alcohols in black and green lentil, however, was decreased by malting. Total reduction of alcohols in the black lentil was lowest for BL4, in which the decrease by 5.29 ppb was noted (reduction by 29.7% of total alcohols) and the highest for BL6, in which total concentration of alcohols was reduced by 10.33 ppb (reduction by 58%). Similar results can be seen in the green lentil malts: the lowest decrease, by 7.61 ppb (20.6%), for GR4, and the highest, by 19.8 ppb (53.7%), for GR6. However, close attention ought to be put to the concentration of two important alcohols, which were identified in all the analysed lentil and lentil malt samples. 1-octen-3-ol is a compound with characteristic, unpleasant mushroom aroma, which is present in various legumes, such as soybeans and legume-based food products [25,26]. Malted black and green lentils were characterised with lower concentration of this compound, especially samples BL6 and GR6, in which concentration of 1-octen-3-ol was reduced by more than a 50%. Second alcohol, characterised with earthy, pea-like note, which was reduced in the course of malting, was 1-nonanol. Malting allowed for the reduction of this compound in unmalted lentils by 2.04 ppb (79.4%) in case of GL6; by 0.76 ppb (65.5%) in the of BL6 and by 0.29 ppb (36.7%) in case of BR6. These results suggest that lentil malts can be used to produce lentil-based products for the groups of population which try to avoid legume-based products, because of their aroma.

3.1.3. Concentration of hydrocarbons in the lentil seeds and lentil malts. Volatilome of the lentils seeds and lentil malts contained vast array of hydrocarbons, albeit most of the compounds from this chemical group were present only in small amounts (below one ppb) (Table 3). Sample BL was characterised with lowest concentration of hydrocarbons (1.68 ppb) and malting seemed to increase total concentration of hydrocarbons, as well as their share in the volatilome of black malts, with the highest increase (by 121.4% of terpenes) noted for BL4. In contrast, malting did not change significantly concentration of hydrocarbons in BR4 and BR5 and decreased total concentration of hydrocarbons in the sample BR6 by 0.5 ppb (by 14.7% of all terpenes in the sample). The greatest change of the concentration of hydrocarbons in the course of malting could be seen in the malts prepared from the green lentil. Sample GL4 possessed 2.92 ppb of the hydrocarbons more than GL (increase of 23.2%), but increasing germination time to five or six days resulted in far lower concentration of hydrocarbons in GL5 (lower by 8.17 ppb, decrease by 65%) and GL6 (lower by 9.91 ppb, decrease by 70.8%) than in GL. In the previous years, scientists such as Rajhi et al. [17] and Bi et al. [27] have detected small amounts of hydrocarbons in the volatilome of lentils and various, different legume seeds. It was speculated, on the basis of the research conducted by the Oomah et al. [28] about volatilome of beans, that most hydrocarbons (specifically n-alkanes) in the volatile composition of

Table 2. Concentration of alcohols in lentils and malted lentils.

		BL	BL4	BL5	BL6	BR	BR4	BR5	BR6	GR	GR4	GR5	GR6
		ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
1	1-Octen-3-ol	14.22 ± 2.31 c, A	9.96 ± 2.08 c, B	8.20 ± 1.14 c, C	6.25 ± 0.98 c, D	11.28 ± 2.98 b, A	12.17 ± 2.08 b, B	11.26 ± 1.58 b, C	10.87 ± 1.85 b, D	28.73 ± 3.11 a, A	26.26 ± 2.51 a, B	24.89 ± 2.08 a, C	14.20 ± 1.98 a, A
2	1-Hexanol, 2-ethyl-	0.00 b, A	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, A	0.00 b, B	0.00 b, B	0.00 b, B	1.09 ± 0.41 a, A	0.00 a, B	0.00 a, B	0.00 a, B
3	1-Octanol	1.57 ± 0.39 c, A	0.00 c, D	0.00 c, C	0.30 ± 0.12 c, B	1.24 ± 0.42 b, A	0.00 b, D	0.64 ± 0.28 b, C	1.33 ± 0.51 b, B	3.14 ± 0.92 a, A	0.00 a, D	1.82 ± 0.43 a, C	1.43 ± 0.28 a, B
4	Cyclohexanol, 3,5-dimethyl-	0.00 c, D	1.27 ± 0.32 c, A	0.00 c, B	0.00 c, C	0.41 ± 0.11 b, D	0.46 ± 0.18 b, A	0.67 ± 0.21 b, B	0.48 ± 0.15 b, C	0.00 a, D	0.72 ± 0.30 a, A	1.22 ± 0.22 a, B	0.59 ± 0.18 a, C
5	1-Nonanol	1.16 ± 0.43 b, A	0.65 ± 0.28 b, B	0.52 ± 0.21 b, C	0.40 ± 0.09 b, D	0.79 ± 0.19 c, A	0.40 ± 0.17 c, B	0.43 ± 0.18 c, C	0.50 ± 0.22 c, D	2.57 ± 0.94 a, A	1.47 ± 0.76 a, B	0.86 ± 0.25 a, C	0.53 ± 0.21 a, D
6	1,7-octanediol, 3,7-dimethyl	0.42 ± 0.18 b, A	0.23 ± 0.05 b, B	0.20 ± 0.08 b, C	0.18 ± 0.09 b, D	0.09 ± 0.06 b, A	0.28 ± 0.17 b, B	0.30 ± 0.12 b, C	0.31 ± 0.11 b, D	0.99 ± 0.23 a, A	0.82 ± 0.24 a, B	0.39 ± 0.15 a, C	0.20 ± 0.08 a, D
7	1-Dodecanol	0.42 ± 0.18 a, A	0.39 ± 0.15 a, D	0.38 ± 0.17 a, B	0.34 ± 0.21 a, C	0.18 ± 0.06 c, A	0.09 ± 0.05 c, D	0.14 ± 0.06 c, B	0.15 ± 0.06 c, C	0.36 ± 0.11 b, A	0.00 b, D	0.39 ± 0.17 b, B	0.12 ± 0.04 b, C
	Total alcohols	**17.80**	**12.51**	**9.29**	**7.47**	**13.99**	**13.40**	**13.44**	**13.64**	**36.88**	**29.27**	**29.55**	**17.08**
	% of all volatiles	**53.77%**	**28.35%**	**24.77%**	**20.96%**	**39.15%**	**28.34%**	**26.50%**	**28.41%**	**32.19%**	**26.81%**	**39.45%**	**36.01%**

[1] Abbreviations are as follows: GL4—malt from green lentil germinated 4 days, GL5—malt from green lentil germinated 5 days, GL6—malt from green lentil germinated 6 days, BRL4—malt from brown lentil germinated 4 days, BRL5—malt from brown lentil germinated 5 days, BRL6—malt from brown lentil germinated 6 days, BLL4—malt from black lentil germinated 4 days, BLL5—malt from black lentil germinated 5 days, BLL6—malt from black lentil germinated 6 days. Values are expressed as means (n = 3) ± standard deviation. Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups according to the variable 'days of germination'(Tukey test, $\alpha = 0.05$).

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Table 3. Concentration of terpenes and ketones in lentils and malted lentils.

	BL	BL4	BL5	BL6	BR	BR4	BR5	BR6	GR	GR4	GR5	GR6
	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
3-Carene	0.00 b, A	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, A	0.00 b, B	0.00 b, B	0.00 b, B	3.24 ± 0.84 a, A	0.00 a, A	0.00 a, A	0.00 a, A
Limonene	0.00 c, B	0.97 ± 0.31 c, A	0.45 ± 0.12 c, C	0.00 c, D	0.00 b, B	0.71 ± 0.28 b, A	1.08 ± 0.33 b, C	0.68 ± 0.21 b, D	10.49 ± 1.16 a, B	12.81 ± 2.08 a, A	3.17 ± 0.69 a, C	0.00 a, D
Eucalyptol	0.00 b, A	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, A	0.00 b, B	0.00 b, B	0.00 b, B	23.63 ± 2.49 a, A	0.00 a, B	0.00 a, B	0.00 a, B
D-Carvone	0.50 ± 0.11 a, A	0.13 ± 0.06 a, B	0.00 a, B	0.00 a, B	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, B
Gamma-Muurolene	0.28 ± 0.15 c, C	0.44 ± 0.18 c, A	0.46 ± 0.21 c, B	0.42 ± 0.18 c, D	0.76 ± 0.28 b, C	0.76 ± 0.25 b, A	0.89 ± 0.31 b, B	0.69 ± 0.17 b, D	0.88 ± 0.18 a, C	1.61 ± 0.34 a, A	0.75 ± 0.25 a, B	0.41 ± 0.17 a, D
.tau.-Cadinol	0.00 b, A	0.26 ± 0.13 b, B	0.23 ± 0.11 b, A	0.24 ± 0.14 b, B	0.42 ± 0.15 a, A	0.37 ± 0.12 a, B	0.39 ± 0.14 a, A	0.20 ± 0.09 a, B	0.71 ± 0.25 a, A	0.00 a, B	0.47 ± 0.22 a, A	0.14 ± 0.05 a, B
Total terpenes	**0.78**	**1.80**	**1.14**	**0.66**	**1.19**	**1.84**	**2.36**	**1.56**	**38.95**	**14.43**	**4.39**	**0.56**
% of all volatiles	**2.37%**	**4.08%**	**3.04%**	**1.84%**	**3.32%**	**3.90%**	**4.66%**	**3.26%**	**33.99%**	**13.21%**	**5.86%**	**1.18%**
3-Octen-2-one	0.00 c, D	0.52 ± 0.21 c, B	0.16 ± 0.05 c, A	0.00 c, C	0.00 b, D	0.55 ± 0.23 b, B	0.23 ± 0.12 b, A	0.43 ± 0.18 b, C	0.00 a, D	0.84 ± 0.23 a, B	1.81 ± 0.54 a, A	0.84 ± 0.33 a, C
3,5-Octadien-2-one, (E,E)-	0.00 a, A	1.61 ± 0.46 a, A	1.11 ± 0.27 a, B	0.63 ± 0.18 a, C	1.62 ± 0.42 b, A	0.00 b, A	0.00 b, B	0.00 b, C	0.00 c, A	0.00 c, B	0.00 c, C	0.00 c, C
2-(1-Hydroxybut-2-enylidene) cyclohexanone	0.00 c, D	2.06 ± 0.54 c, A	1.42 ± 0.43 c, B	1.68 ± 0.38 c, C	0.08 ± 0.05 b, D	2.50 ± 0.92 b, A	2.08 ± 0.71 b, B	1.54 ± 0.38 b, C	0.00 a, D	7.64 ± 2.05 a, A	3.83 ± 1.04 a, B	2.61 ± 1.15 a, C
Trans-.beta.-Ionone	0.12 ± 0.05 a, A	0.00 a, B	0.00 a, B	0.03 ± 0.02 a, B	0.08 ± 0.05 a, A	0.00 a, B	0.00 a, B	0.00 a, B	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, B
Total ketones	**0.12**	**4.19**	**2.69**	**2.34**	**1.78**	**3.05**	**2.31**	**1.97**	**0.00**	**8.48**	**5.64**	**3.44**
% of all volailes	**0.36%**	**9.49%**	**7.16%**	**6.56%**	**4.97%**	**6.46%**	**4.55%**	**4.11%**	**0.00%**	**7.77%**	**7.53%**	**7.26%**

[1] Abbreviations are as follows: GL4—malt from green lentil germinated 4 days, GL5—malt from green lentil germinated 5 days, GL6—malt from green lentil germinated 6 days, BRL4—malt from brown lentil germinated 4 days, BRL5—malt from brown lentil germinated 5 days, BRL6—malt from brown lentil germinated 6 days, BLL4—malt from black lentil germinated 4 days, BLL5—malt from black lentil germinated 5 days, BLL6—malt from black lentil germinated 6 days. Values are expressed as means (n = 3) ± standard deviation. Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups according to the variable 'days of germination'(Tukey test, $\alpha = 0.05$).

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legumes are a product of lipid oxidation. Different concentrations of hydrocarbons in the malts of different varieties of lentils might indicate that the composition of lipids, as well activity of lipases in these lentils is very different. Further study concentrating on the changes in the lipid fraction of lentils during germination and drying are needed to confirm and clarify these hypotheses.

3.1.4. Concentration of terpenes in the lentil seeds and lentil malts. Terpenes were mostly present only in the GL and green lentil malts (Table 4). Terpenes constituted only 2.37% of all volatiles in BL and 3.32% of BR. Malting had not changed significantly the contribution of terpenes to the total volatilome of the malt in the case of black and brown lentil malts. 1.82% to 4.06% of the black lentil malt volatilome consisted of terpenes and, similarly, volatilome of brown lentil malts constituted from 3.22% to 4.64%. In contrast, volatile composition of GL contained 33.99% (38.95 ppb) of terpenes. It is worth pointing out, that concentration of terpenes in sample GL is higher than concentration of all volatiles in the samples BL and BR (33.10 and 35.74 ppb). It is worth noting, that terpenes (monoterpenes and sesquiterpenes alike) are not very abundant in the legume seeds, but limonene seems to be most dominant compound of this chemical family and previous researches have detected it in the flours prepared from green and red lentil [29,30]. The effect of malting on the concentration of terpenes, especially 3-carene, limonene and eucalyptol is striking: sixday malting reduced concentration of terpenes in GL6 by 98.6%. Eucalyptol and 3-carene were absent in GL4, GL5 and GL6. However, 4-day malting increased concentration of limonene by 2.32 ppb (22.1%) and of gamma-muurolene by 0.86 ppb (83%). However, five day and sixday malting period resulted in significant decrease of these two terpenes, despite their increase by fourday malting period. This result might be due to the tendency of limonene and other terpenes to degrade under prolonged oxidative conditions [31].

3.1.5. Concentration of ketones in the lentil seeds and lentil malts. Malting increased total concentration of ketones in all the analysed lentil varieties (Table 4). Ketones were absent or almost absent in unmalted samples BL and GR (0.12 ppb and 0.34 ppb, consecutively). The most significant increase could be noted for the malted green lentil samples, from zero to 3.44–8.48 ppb (with the highest for GR4 and lowest for GR6). Similar phenomenon could be seen in the malts produced from the other lentil varieties. Malts germinated for fourdays malts were characterised with the highest concentration of ketones, however, difference between malts germinated for four, five or six days from black or brown malts was not as considerable, as in green lentil malts. One of the most interesting aspects of malting on the concentration of ketones in the lentils malts is the presence of 2-(1-hydroxybut-2-enylidene)cyclohexanone. This compound is not widely described in the scientific literature; only mention could be found in the work of Moldoveanu [32], as one of the products of thermal degradation of RuBisCO (ribulose-1,5-bisphosphate carboxylase-oxygenase); a plant enzyme which is involved in the carbon fixation process [33]. As the enzymatic activity and metabolic pathways connected to the photosynthesis increase during the steeping and germination of the seeds, this reason of the increased concentration of 2-(1-hydroxybut-2-enylidene)cyclohexanone seems most plausible, but to confirm this, a study concentrating on this particular problem would be needed in the future [34].

3.1.6. Concentration of minor volatile constituents (furans, pyrazines, sulphur compounds and esters) in the lentil seeds and lentil malts. Concentration of constituents from four different groups of chemical compounds (furans, pyrazines, sulphur compounds and esters) constituted minority of the total volatilome of lentil seeds and lentil malts (Table 5). Benzothiazole, a sulphur compound, was present only in the malted lentil samples. The main formation pathway of this compound in the food products is related to the non-enzymatic browning reactions between reducing sugars and amino acids occurring in the presence of

Table 4. Concentration of hydrocarbons in lentils and malted lentils.

		BL	BL4	BL5	BL6	BR	BR4	BR5	BR6	GR	GR4	GR5	GR6
		ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
1	Undecane	0.00 c, B	0.00 c, A	0.00 c, D	0.00 c, C	0.00 b, B	0.50 ± 0.21 b, A	0.29 ± 0.08 b, D	0.42 ± 0.18 b, C	4.06 ± 0.92 a, B	4.36 ± 1.14 a, A	0.00 a, D	0.92 ± 0.34 a, C
2	Dodecane	0.00 b, B	2.04 ± 0.85 b, A	1.02 ± 0.26 b, C	0.58 ± 0.13 b, D	1.18 ± 0.34 b, B	1.00 ± 0.25 b, A	0.80 ± 0.28 b, C	0.57 ± 0.18 b, D	4.67 ± 0.88 a, B	4.77 ± 1.05 a, A	1.92 ± 0.64 a, C	1.09 ± 0.29 a, D
3	4-methyldodecane	0.21 ± 0.09 c, A	0.00 c, B	0.00 c, B	0.00 c, B	0.46 ± 0.12 b, A	0.00 b, B	0.00 b, B	0.00 b, B	1.19 ± 0.32 a, A	0.00 a, B	0.00 a, B	0.00 a, B
4	Dodecane, 4,6-dimethyl-	0.17 ± 0.06 c, D	0.00 c, A	0.06 ± 0.04 c, C	0.31 ± 0.15 c, B	0.32 ± 0.17 b, D	0.18 ± 0.08 b, A	0.25 ± 0.11 b, C	0.26 ± 0.14 b, B	0.00 a, D	1.45 ± 0.49 a, A	0.31 ± 0.13 a, C	0.32 ± 0.15 a, B
5	Tridecane	0.15 ± 0.09 c, C	0.16 ± 0.08 c, A	0.11 ± 0.05 c, B	0.25 ± 0.14 c, B	0.19 ± 0.08 b, C	0.27 ± 0.11 b, A	0.28 ± 0.14 b, B	0.22 ± 0.15 b, B	0.45 ± 0.28 a, C	1.34 ± 0.49 a, A	0.44 ± 0.18 a, B	0.38 ± 0.15 a, B
6	Tetradecane	0.49 ± 0.11 b, B	0.90 ± 0.23 b, A	0.74 ± 0.28 b, C	0.67 ± 0.18 b, D	0.56 ± 0.23 c, B	0.70 ± 0.31 c, A	0.67 ± 0.28 c, C	0.52 ± 0.18 c, D	1.45 ± 0.42 a, B	1.81 ± 0.49 a, A	0.96 ± 0.33 a, C	0.53 ± 0.15 a, D
7	Pentadecane	0.32 ± 0.15 c, C	0.31 ± 0.13 c, A	0.39 ± 0.16 c, B	0.46 ± 0.21 c, C	0.47 ± 0.24 b, C	0.46 ± 0.27 b, A	0.55 ± 0.28 b, B	0.41 ± 0.21 b, C	0.40 ± 0.16 a, C	1.39 ± 0.42 a, A	0.58 ± 0.18 a, B	0.33 ± 0.12 a, C
8	Hexadecane	0.22 ± 0.07 a, B	0.21 ± 0.11 a, A	0.27 ± 0.06 a, C	0.35 ± 0.14 a, D	0.19 ± 0.09 b, B	0.22 ± 0.10 b, A	0.23 ± 0.08 b, C	0.19 ± 0.05 b, D	0.35 ± 0.17 a, B	0.36 ± 0.11 a, A	0.20 ± 0.09 a, C	0.09 ± 0.06 a, D
9	Octadecane	0.11 ± 0.06 b, C	0.10 ± 0.04 b, C	0.11 ± 0.07 b, A	0.11 ± 0.06 b, B	0.00 a, C	0.00 a, C	0.34 ± 0.15 a, A	0.28 ± 0.16 a, B	0.00 c, C	0.00 c, C	0.00 c, A	0.00 c, B
	Total hydrocarbons	**1.68**	**3.72**	**2.71**	**2.73**	**3.38**	**3.32**	**3.40**	**2.88**	**12.58**	**15.50**	**4.41**	**3.67**
	% of all volatiles	**5.07%**	**8.43%**	**7.21%**	**7.66%**	**9.45%**	**7.03%**	**6.70%**	**6.00%**	**10.97%**	**14.19%**	**5.89%**	**7.73%**

[1] Abbreviations are as follows: GL4—malt from green lentil germinated 4 days, GL5—malt from green lentil germinated 5 days, GL6—malt from green lentil germinated 6 days, BRL4—malt from brown lentil germinated 4 days, BRL5—malt from brown lentil germinated 5 days, BRL6—malt from brown lentil germinated 6 days, BLL4—malt from black lentil germinated 4 days, BLL5—malt from black lentil germinated 5 days, BLL6—malt from black lentil germinated 6 days. Values are expressed as means (n = 3) ± standard deviation. Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups according to the variable 'days of germination'(Tukey test, $\alpha = 0.05$).

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Table 5. Concentration of minor constituents (furans, pyrazines, sulphur compounds and esters) in lentils and malted lentils.

	BL	BL4	BL5	BL6	BR	BR4	BR5	BR6	GR	GR4	GR5	GR6
	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
Furan, 2-pentyl-	0.00 b, B	0.00 b, A	0.00 b, B	0.00 b, B	0.00 b, B	0.00 b, A	0.00 b, B	0.00 b, B	0.00 a, B	1.88 ± 0.54 a, A	0.00 a, B	0.00 a, B
Total furans	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**1.88**	**0.00**	**0.00**
% of all volatiles	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**1.72%**	**0.00%**	**0.00%**
Pyrazine, 2-ethyl-3,5-dimethyl-	0.00 c, C	0.00 c, C	0.00 c, A	0.00 c, B	0.00 a, C	0.00 a, C	1.07 ± 0.24 a, A	0.25 ± 0.12 a, B	0.00 b, C	0.00 b, C	0.79 ± 0.28 b, A	0.00 b, B
Total pyrazines	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**1.07**	**0.25**	**0.00**	**0.00**	**0.79**	**0.00**
% of all volatiles	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**2.11%**	**0.52%**	**0.00%**	**0.00%**	**1.06%**	**0.00%**
Benzothiazole	0.00 b, D	0.13 ± 0.05 b, C	0.15 ± 0.08 b, A	0.19 ± 0.10 b, B	0.00 a, D	0.19 ± 0.09 a, C	0.29 ± 0.14 a, A	0.12 ± 0.07 a, B	0.00 b, D	0.00 b, C	0.36 ± 0.14 b, A	0.07 ± 0.05 b, B
Total sulfur compounds	**0.00**	**0.13**	**0.15**	**0.19**	**0.00**	**0.19**	**0.29**	**0.12**	**0.00**	**0.00**	**0.36**	**0.07**
% of all volatiles	**0.00%**	**0.29%**	**0.41%**	**0.54%**	**0.00%**	**0.40%**	**0.57%**	**0.24%**	**0.00%**	**0.00%**	**0.48%**	**0.15%**
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	0.78 ± 0.25 c, A	0.68 ± 0.23 c, B	0.62 ± 0.31 c, C	0.56 ± 0.28 c, D	0.80 ± 0.33 a, A	0.65 ± 0.23 a, B	1.06 ± 0.34 a, C	0.78 ± 0.31 a, D	1.70 ± 0.54 b, A	1.36 ± 0.28 b, B	0.00 b, C	0.00 b, D
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	1.05 ± 0.28 c, B	0.73 ± 0.31 c, A	0.51 ± 0.17 c, C	0.54 ± 0.19 c, D	0.09 ± 0.05 b, B	0.63 ± 0.24 b, A	1.26 ± 0.34 b, C	1.12 ± 0.48 b, D	2.10 ± 0.58 a, B	2.11 ± 0.63 a, A	1.09 ± 0.27 a, C	0.54 ± 0.21 a, D
Dodecanoic acid, 1-methylethyl ester	0.22 ± 0.12 a, D	0.15 ± 0.08 a, C	0.30 ± 0.12 a, B	0.21 ± 0.13 a, A	0.00 b, D	0.10 ± 0.07 b, C	0.14 ± 0.06 b, B	0.36 ± 0.14 b, A	0.00 c, D	0.00 c, C	0.00 c, B	0.00 c, A
Total esters	**2.05**	**1.56**	**1.42**	**1.32**	**0.89**	**1.38**	**2.47**	**2.26**	**3.80**	**3.47**	**1.09**	**0.54**
% of all volatiles	**6.19%**	**3.54%**	**3.80%**	**3.69%**	**2.49%**	**2.91%**	**4.87%**	**4.71%**	**3.32%**	**3.18%**	**1.45%**	**1.14%**
Total minor constituents	**2.05**	**1.69**	**1.58**	**1.51**	**0.89**	**1.57**	**3.83**	**2.63**	**3.80**	**5.35**	**2.24**	**0.61**
% of all volatiles	**6.19%**	**3.83%**	**4.21%**	**4.23%**	**2.49%**	**3.31%**	**7.55%**	**5.47%**	**3.32%**	**4.90%**	**2.99%**	**1.29%**

[1] Abbreviations are as follows: GL4—malt from green lentil germinated 4 days, GL5—malt from green lentil germinated 5 days, GL6—malt from green lentil germinated 6 days, BRL4—malt from brown lentil germinated 4 days, BRL5—malt from brown lentil germinated 5 days, BRL6—malt from brown lentil germinated 6 days, BLL4—malt from black lentil germinated 4 days, BLL5—malt from black lentil germinated 5 days, BLL6—malt from black lentil germinated 6 days. Values are expressed as means (n = 3) ± standard deviation. Various small letters (a, b, c) indicate homogenous groups according to the variable 'variety', various capital (A, B, C) letters indicate homogenous groups according to the variable 'days of germination'(Tukey test, $\alpha = 0.05$).

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hydrogen sulphide originating from the degradation of sulphur containing amino acids [35]. During the germination, the activity of proteases and amylases increases [14], resulting in the increased concentration of reducing sugars and amino acids in the germinated lentil, which can lead to the formation of benzothiazole during the drying processes. Malting decreased concentration of propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester and pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester in the case of black and green lentils and increased in the case of brown malts. These compounds are not thoroughly examined in the scientific literature, albeit Zhao et al. [36] have shown, that these compounds are potential indicators of aroma deterioration.

4. Conclusions

This study shows that malting can be a straightforward and simple way of changing volatile composition of lentil seeds. Malting resulted in the increase of total volatiles in the black lentil malts as well as brown lentil malts and in the decrease of volatiles in the green malts. The contribution of particular chemical groups of chemical compounds to the volatilome of malts was also changed by applying malting procedure. All lentil malts were characterised with greater contribution of aldehydes in the volatilome. Malting significantly reduced the concentration and contribution of terpenes in the volatilome of green lentil malts. However, precise mechanisms, which influence the changes in the composition of volatiles in the lentil seeds during the course of malting need to be investigated further in the upcoming studies, to understand the particular factors which have an impact on the creation of the volatilome of the lentil malts.

Author Contributions

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Assessment of green lentil malt as a substrate for gluten-free beer brewing

Alan Gasiński ✉ & Joanna Kawa-Rygielska

The aim of this study was to analyze whether it is possible to brew beer without using cereals so that the produced beverage could be easily accessible for the population suffering from celiac disease and other gluten-related disorders. Green lentil seeds were malted and then mashed using a congress mashing procedure to assess their advantages and disadvantages in the brewing process. Based on the congress mashing procedure, the mashing process needed to produce beer was developed, and beers were produced from the lentil malts germinated during malting for 96 h, 120 h and 144 h. It was possible to produce beers from the lentil malts; however, they were characterized by a lower alcohol content, lower degree of attenuation and some discrepancies between the concentrations of various volatiles (such as acetaldehyde, ethyl acetate, and 1-propanol) compared to the control beer produced from barley malt.

Lentil is a popularly grown high-protein legume that is drought resistant and can be grown in various climates, as it is able to thrive in warm and cool environments[1]. Lentil seeds are used in many cuisines to prepare a vast array of different dishes, which might also mean that various populations could be interested in novel lentil-based food products[2]. Beer, however, is a beverage not typically produced from lentils but from 4 main ingredients: water, malt, hops and yeast. Malting is a process that is used primarily to modify barley grains, and the main reason for malting is to increase the enzymatic activity of the modified seeds, primarily amylases[3]. On a smaller scale, malts are also produced from various cereals other than barley, such as wheat, rye, and oats, or from various pseudocereals, such as buckwheat. These malts are used in the production of various specialty beers, which are characterized by different organoleptic properties than the typical barley malt beer. Legumes are not used in beer brewing technology; however, in recent years, few trials on malting various legume seeds and analyzing the technological properties of these malts have been conducted. Gasiński et al.[4] malted various legume seeds (such as lentil, common vetch, chickpea and peas) in conditions typical for the production of Pilsner-type barley malt, but the acquired malts were characterized by insufficient technological parameters for beer brewing: they had not saccharified during mashing, were filtered for a long time and wort produced from these malts possessed very low extract content. Even the addition of various commercial brewing enzyme preparations during mashing did not allow for the production of acceptable wort. In a subsequent study, where lentil steeping was performed under conditions that allowed lentil to acquire up to 65% water content and then germinate at a higher temperature than is typical for Pilsner-type barley malt, wort from green lentil malt prepared in this way was characterized by improved parameters compared to the last study[5]. However, lentil malts still had not fully saccharified during mashing, and acquired worts were characterized by suboptimal extract content. Data acquired in these two studies have shown that changing simple malting characteristics resulted in significantly improved properties of this malt type. Additionally, Trummer et al.[6] showed that lentil malt can be used as an additive to beer grain bill in amounts of up to 20%. However, these data still do not explain why there is an interest in the production of lentil malt. Malts do not always have to be used in the production of wort for beer production but can function as a substrate for various other food products. Typically, malting changes the concentration of vitamins, improves the bioavailability of minerals, reduces the concentration of various antinutritional substances, improves the digestibility of carbohydrates and proteins and changes the organoleptic properties of malted seeds[5,7–9]. Additionally, due to the increasing temperatures in many of the world regions due to climate change, barley might not be a suitable brewing material in the future because the starch gelatinization temperature of cereals, barley included, increases when plants are cultivated in warm environments[10,11]. This might prove very problematic for brewers, which is why the assessment of alternative substrates for beer production is reasonable. Additionally,

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consumers are currently looking for new flavors and fragrances in beer, and as lentil is a rich source of proteins and yeast produces various flavor-active volatile components from the amino acids present in the wort, lentil malts could possibly be used as a means to produce novel-tasting beer styles[12,13]. Furthermore, various cereals and cereal-based products cannot be consumed by people suffering from celiac disease, nonceliac gluten sensitivity, dermatitis herpetiformis, gluten ataxia and other disorders, which are diagnosed in a far larger percent of the world's population than in the years before[14,15]. In this study, green lentil seeds were malted in a malting procedure used sometimes to produce diastatic malts and then mashed with various adjuncts to determine whether simple adjustments to the composition of the mash can result in full saccharification of the starch of the lentil malt[9]. In the second part of the study, green lentil malt was used in a mashing regime designed for the parameters of this malt to produce novel lentil-based beers.

Materials and methods

Materials

Raw material

The plant materials used in this study were seeds of green lentil (*Lens culinaris*) of the Eston variety. Lentil seeds were acquired from BioPlanet company (Leszno near Warsaw, Poland). Lentil seeds, prior to the malting procedure and analyses, were manually sifted to discard damaged seeds and seeds with visible discoloration. The moisture content of the seeds before the malting process was analyzed with an MT moisture analyzer (Brabender, Duisburg, Germany). All techniques and analyses used on the raw material were carried out in accordance with relevant institutional, international and national guidelines and legislation.

Biological material

The biological material used in this study was S-04 *Saccharomyces cerevisiae* yeast (Fermentis, Paris, France), added to the wort in the amount recommended by the producer (0.5 g per 1dm^3 of wort). Yeast (8.75 g) was rehydrated in 100 cm^3 of sterile, distilled water at 20 °C 20 min prior to inoculation. All techniques and analyses used on the biological material were carried out in accordance with relevant institutional, international and national guidelines and legislation.

Reagents and standards

The standards used in this study were acetaldehyde (2,3-butanodione), ethyl acetate, 1-propanol, 2-butanol, 2-methylbutanol, 3-methylbutanol, ethyl hexanoate, ethyl octanoate, ethyl decanoate, furfural, isobutanol (2-methylpropan-1-ol), isopentyl acetate (isoamyl acetate) and phenylethyl alcohol (purity of the standards was equal to or higher than 99%, suitable for GC analyses) purchased from Merck (Merck KGaA, Darmstadt, Germany). Reagents used in this study were calcium carbonate (99%), sodium hypochlorite (15%), aqueous iodine (2% *w/v* of iodine), diatomaceous earth (Chempur, Piekary Śląskie, Poland) and α-amylase from *Bacillus* sp. solution (amylolytic activity equal to 28,345 units per cm^3 of solution, where 1 unit will liberate 1 mg of maltose from starch in 3 min. at pH 6.9 at 20 °C) (Merck KGaA, Darmstadt, Germany).

Methods

Malting procedure

Eighty grams of green lentil was weighed and transferred to perforated, stainless steel malting containers (total of 24 containers), which were previously disinfected by drying in a UF110 Plus dryer (Memmert GmbH + Co, Schwabach, Germany) for 2 h at 200 °C and then cooled to room temperature. Containers filled with a known mass of lentil were then weighed (the container filled with lentil seeds will be referred to as the 'malting kit'). Changes in the moisture content of the seeds during the first step of the malting process (steeping) were calculated based on the changing weight of the malting kit, assuming that the increase in weight of the kit is equal to the quantity of water adsorbed by the seeds. Steeping was executed in the water—air steeping cycle. At the start of the process, malting kits were submerged in 1.5% sodium hypochlorite solution for 10 min to surface sterilize the seeds. Malting kits were then removed from the sodium hypochlorite solution and washed three times with distilled water. After this process, malting kits were submerged in sterile tap water (disinfected previously by boiling and cooled) at a temperature of 15 °C for 7 h, transferred to the KK 240 Smart Pro germination chamber (with humidity set at 90% relative humidity and temperature set at 18 °C) for 17 h, submerged another time in fresh, disinfected tap water at a temperature of 15 °C for 4 h, and then transferred to the germination chamber (temp. 18 °C, relative humidity 90%) for 20 h. After each step, malting kits were weighed to determine the changing moisture content of the lentil seeds. At the end of the steeping process, the moisture content of the lentil seeds was equal to 62–62.5%. Lentils were germinated in the germination chamber with the relative humidity set at 90%. Lentils were divided into three different batches of eight containers, which were germinated for different amounts of time. One batch was germinated for 96 h, one batch for 120 h and one batch germinated for 144 h. The temperature during germination was programmed as follows: 18 °C for the first 24 h; 15 °C for the second 24 h and 12 °C for the remainder of the germination time (48 h, 72 h or 96 h, depending on the batch of malt produced). After the germination process, each batch of malting kits was dried in a UF110 Plus dryer at 50 °C for 23 h[4,5]. The malting procedure resulted in the production of 3 different lentil malt samples:

- Green lentil malt germinated for 96 h (4 days) (GR4)
- Green lentil malt germinated for 120 h (5 days) (GR5)
- Green lentil malt germinated 144 h (6 days) (GR6)

Malts of one type from different containers, after the drying process, were mixed together and transferred to tightly closed containers to prevent moisture absorption during the cooling period. Malts, as well as unmalted lentils, were ground with the use of a Bühler Miag disc mill DLFU (Bühler, Uzwil, Switzerland), according to the Analytica EBC 4.5.1 method for the subsequent analysis[16].

Analysis of the technological properties of the malts on the basis of the congress mashing procedure. Technological properties of the green lentil malts, such as saccharification time, wort filtration time, wort volume, wort extract and wort pH, were assessed on the basis of the Analytica EBC 4.5.1 congress mashing procedure[16]. Additional factors, such as the influence of calcium ions in the mash or the addition of α-amylase solution on the mashing performance, were also analysed. Mashing was performed in the twelve-cup automated mashing apparatus LB-12 (LB Electronic, Berching, Germany). The basic procedure used to analyze solely lentil malts was as follows: the mashing cup was filled with 200 cm^3 of distilled water and heated to 45 °C. Fifty grams of ground malt was added to the mashing cups followed by stirring (100 rpm) of the mash. The 45 °C temperature was maintained for 30 min, after which the temperature of the water bath in the mashing machine was increased to 70 °C at a rate of 1 °C per min. One hundred cm^3 of distilled water at 70 °C was added to the mashing cups. Ten minutes after the addition of 100 cm^3 of water, the saccharification time measurement was started. Saccharification of the mashes was assessed via the iodine test in 5 min intervals. A temperature of 70 °C was maintained for 60 min, after which the contents of the mashing cups were cooled to 20 °C. The mash weight was adjusted to 450 g using distilled water. Mashes were transferred to laboratory funnels (20 cm in diameter) fitted with Macherey–Nagel MN 614 ¼ filters (320 mm in diameter) (Macherey–Nagel GmbH & Co, Düren, Germany). To rinse the remaining malt, 100 cm^3 of the filtered wort was reversed to the mashing cup and poured back into the funnel. After reversing the first 100 cm^3 of wort, the filtering of the congress worts lasted up to 120 min. After filtration, wort was collected for analyses. The worts were prepared in duplicate. The volume of the wort was read from the scale of the cylinder. The extracted content of the worts, cooled to 20 °C, was assessed using a DMA 35 densimeter (Anton Paar, Graz Austria) in triplicate. Wort pH was assessed using an MP220 pH meter (Mettler Toledo, Columbus, OH, USA) in triplicate for each wort sample[4,16,17].

To analyze the influence of the calcium ions on the mashing performance, 100 cm^3 of distilled water in the mashing cup (at the temp. of 45 °C) was substituted by 100 cm^3 calcium carbonate solution (with the Ca^{2+} ion concentration equal to 50 mg/dm^3). To analyze the influence of alpha-amylase solution, 0.01 cm^3 of alpha-amylase solution was added to the mash at 70 °C after the addition of 100 cm^3 of water. This mashing regime allowed the analysis of the mashing properties of the lentil malts in 9 different variants:

- Lentil malt germinated for 96 h and mashed under a congress mashing regime (4D)
- Lentil malt germinated for 96 h and mashed under a congress mashing regime (4D-Ca) with the addition of calcium ions
- Lentil malt germinated for 96 h and mashed under a congress mashing regime (4D-Am) with the addition of alpha-amylase solution
- Lentil malt germinated for 120 h and mashed under a congress mashing regime (5D)
- Lentil malt germinated for 120 h and mashed under a congress mashing regime (5D-Ca) with the addition of calcium ions
- Lentil malt germinated for 120 h and mashed under a congress mashing regime (5D-Am) with the addition of alpha-amylase solution
- Lentil malt germinated for 144 h and mashed under a congress mashing regime (6D)
- Lentil malt germinated for 144 h and mashed under a congress mashing regime (6D-Ca) with the addition of calcium ions
- Lentil malt germinated for 144 h and mashed under a congress mashing regime (6D-Am) with the addition of alpha-amylase solution.

Lentil beer brewing
Mashing of the lentil malts for the lentil beer brewing was performed in the LB-12 mashing apparatus. Based on the results acquired during the congress mashing procedure, it was decided that the addition of alpha-amylase and an increase in the temperature during the final stage of the mashing is necessary. All lentil malts were used to produce beer, and Pilsener malt was used as the control sample. Each of the malts was mashed in duplicate. Mashing cups were filled with 200 cm^3 of distilled water and heated to 45 °C. Fifty grams of ground malt samples were added to the mashing cups, and stirring (100 rpm) was started. After 30 min, the temperature was increased to 75 °C at a rate of 1 °C per min. After the temperature of the mash reached 75 °C, 100 cm^3 of distilled water (75 °C) was added, followed by the addition of 0.01 cm^3 of alpha-amylase solution (with the exception of Pilsener malt, to which alpha-amylase solution was not added). A temperature of 75 °C was maintained for 60 min. After 10 min, an iodine test was performed on all the mashes, and it turned out to be negative. After 60 min, the mashing cups were weighed, and the mass of the mashes was adjusted to 450 g using distilled water at 75 °C. Worts were then filtered, without cooling, through Macherey–Nagel paper filters. A total of 250 cm^3 of clear wort was transferred to clean mashing cups, which were then inserted into the mashing machine and heated to 100 °C. Hop pellets were added to each of the cups (0.25 g) at the start of the boil. Wort was boiled for 60 min and then filtered through Macherey–Nagel paper filters into previously autoclaved 300 cm^3 Erlenmeyer flasks, which were then cooled to 19 °C in an ice bath. Worts were inoculated with 1 cm^3 *Saccharomyces cerevisiae* yeast solution and fermented in a cooled cabinet at 19 °C for 10 days. After fermentation, the beer was decanted from the yeast, degassed, mixed with diatomaceous earth (1 g per 100 cm^3), filtered through a paper filter and collected for analyses. This beer brewing regime allowed us to obtain four different beer samples:

- Beer produced from the Pilsner malt sample (C)
- Beer produced from green lentil malt germinated for 96 h (4 days) (B4)
- Beer produced from green lentil malt germinated for 120 h (5 days) (B5)
- Beer produced from green lentil malt germinated for 144 h (6 days) (B6).

Analysis of the physicochemical parameters of lentil beers
Analysis of basic physicochemical parameters of the beer (alcohol content, extract content, density, real degree of attenuation, calorie content, beer color, and extract content of the wort) was conducted using a DMA 4500 Beer Analyzer (Anton Paar, Graz, Austria). Each beer was analyzed in duplicate, resulting in four readings per type of malt used.

Analysis of the volatile compounds present in lentil beers
The volatile profile of the beers was analyzed using the GC-FID method using a GC2010 Plus apparatus equipped with an FID-2010 detector and HS-20 headspace autosampler (Shimadzu Corporation, Kyoto, Japan). Volatiles were separated on a CP-WAX 57 CB capillary column (50 m × 0.32 mm ID × 0.2 µm) (Agilent Technologies, Santa Clara, CA, USA). Ten cm3 of degassed and filtered beer was transferred to 20 cm^3 headspace vials. Each vial, before analysis, was conditioned and shaken in the headspace autosampler oven at 40 °C for 20 min. One cm3 of volatiles was withdrawn from the vial and transferred to the HS-20 headspace loop connected and injected onto the capillary column. The following oven temperature program was used for the separation of volatiles on the column: starting temp. : 40 °C (hold 3 min); temp. increase (5 °C/min) to 80 °C; hold for 3 min; temp. increase (10 °C/min) to 140 °C; hold for 9 min; temp. increase (20 °C/min) to 160 °C; hold for 4 min. Helium was used as the carrier gas, and the initial pressure of the gas was 100 kPa; the initial column flow was equal to 0.33 cm^3/min; the initial linear velocity was equal to 11.8 cm/s; and the purge flow was set at 3 cm^3/min. The FID was operated at 280 °C with a sampling rate of 40 ms. The flow of hydrogen gas to the FID was at a rate of 50 cm3/min, helium gas at a rate of 30 cm3/min and synthetic air at a rate of 400 cm3/min. Each produced beer was analyzed in triplicate (resulting in six repetitions per type of malt used)[18,19].

Organoleptic analyses of the beer
Sensory analysis was performed by 5 trained panellists (4 men, 1 women, aged 28–34 years old). Beers were assessed by 10 point scale where 0 meant that the analysed attribute was absent and 9 indicated that the attribute was extremely strong. Parameters such as aroma (using 12 descriptors: fruity, alcoholic, citrusy, hoppy, DMS, grainy/cereal-like, malty, caramel, burnt/cooked, sulphuric, oxidised/aged, sweet) and taste (using 17 descriptors: fruity, alcoholic, citrusy, hoppy, DMS, grainy/cereal-like, malty, caramel, burnt/cooked, sulphuric, oxidised/aged, sweet, bitter, acidic, astringent, body, lingering) of the beverage were analysed[20]. Beers samples (10–15 cm^3) were served in plastic clear cups in the sensory analysis rooms with red lights overhanging each of the panellist's booths, which hid sample colour and transparency. Temperature of the served beer was 8 °C. The samples were coded with random three digit numbers. Panellists were not acquainted with the details of the tested samples.The organoleptic analyses and all methods connected with the sensory evaluation of the beverages produced in this study were carried out in accordance with relevant guidelines and regulations obligatory in the European Union, Poland and Wrocław University of Environmental and Life Sciences. All experimental protocols connected with the sensory evaluation were approved by The Polish Committee for Standardization and Bioethics Committee. Participants who performed sensory analysis, presented in the Supplementary Data, gave informed consent via the statement 'I am aware that my responses are confidential, and I agree to participate in this survey' where an affirmative reply was required to enter the survey. Panellists were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

Data analysis
Chromatographic data were integrated and quantified in LabSolutions software (Shimadzu Corporation, Kyoto, Japan). Identification of compounds was performed using analytical standards. Quantification was performed using external standards (described in Section "Reagents and standards".) based on a standard curve with five calibration points (coefficient of determination R2 was greater than or equal to 0.999). The results of the assessment of the technological parameters of the malts, physico-chemical parameters of the beers and composition of the volatiles in the beers were statistically analyzed in the Statistica 13 program from StatSoft (Tulsa, OK, USA) with one-way ANOVA ($\alpha = 0.05$) using Tukey's test. The results (as the means) of the organoleptic analysis are shown on the radar charts, constructed using Microsoft Excel 2010 (Microsoft, Redmont, WA, United States of America) software.

Results and discussion
Technological properties of the green lentil malts
The main goal of analyzing the technological properties of the malts is to assess whether the analyzed malt is characterized with parameters that would allow it to be used in the brewery to create wort. The best malts used in traditional beer brewing should yield a high volume of wort, characterized by a high extract content, quick saccharification time, short filtration time and pH in the range of 5.6 to 5.8[3]. The technological parameters of malts produced in this study are presented in Table 1 and were far from optimal but nevertheless better than those of all legume malts produced in previous studies[4,5].

The main problem, which was recognized in malts produced in this study and was also seen in previous research, is the saccharification time of the malts. Typically, the addition of amylolytic enzymes should mitigate

Sample[1]	Saccharification time (min)	Filtration time (min)	Wort volume (cm^3)	Wort Extract (% w/w)	Wort pH	Wort viscosity (mPa/s)
4D	X	120 ± 0 a	175 ± 5 d	6.01 ± 0.02 a	6.01 ± 0.02 a	1.589 ± 0.035 a
4D-Ca	X	120 ± 0 a	180 ± 5 d	5.99 ± 0.02 ab	5.99 ± 0.01 ab	1.514 ± 0.024 ab
4D-Am	X	18 ± 2 c	240 ± 0 a	5.94 ± 0.03 bc	5.95 ± 0.01 c	1.484 ± 0.026 ab
5D	X	120 ± 0 a	185 ± 5 d	5.90 ± 0.04 d	6.00 ± 0.01 a	1.564 ± 0.004 a
5D-Ca	X	90 ± 1 b	212.5 ± 2.5 b	5.88 ± 0.03 d	5.89 ± 0.02 d	1.552 ± 0.033 a
5D-Am	X	17 ± 1 c	235 ± 5 a	5.95 ± 0.03 bc	5.95 ± 0.02 c	1.482 ± 0.019 ab
6D	X	120 ± 0 a	200 ± 0 c	5.95 ± 0.02 bc	5.95 ± 0.01 c	1.569 ± 0.011 a
6D-Ca	X	120 ± 0 a	200 ± 5 c	5.92 ± 0.03 cd	5.93 ± 0.02 cd	1.542 ± 0.029 ab
6D-Am	X	18 ± 1 c	235 ± 5 a	5.96 ± 0.03 abc	5.96 ± 0.01 bc	1.432 ± 0.014 b

Table 1. Technological parameters of the lentil malts. [1]Values are expressed as the mean (n = 6 or n = 2 in the case of saccharification time and wort volume) ± standard deviation. Mean values with different letters (a, b, c, d) within the same column are significantly different (α = 0.05) according to Tukey's test. Abbreviations are as follows: 4D—malt from green lentil germinated for 96 h, 4D-Ca—malt from green lentil germinated for 96 h mashed with the addition of calcium ions, (…), 6D-Am malt from green lentil germinated for 144 h mashed with the addition of alpha-amylase solution. 'X' stands for 'no saccharification' during mashing.

problems with slow saccharification time or the absence of saccharification, but all analyzed lentil malts had not saccharified during the congress mashing regime, despite the addition of amylolytic enzymes or calcium ions[21,22]. However, it is important to note that despite the lack of complete saccharification, the addition of calcium ions or amylolytic enzymes improved various parameters of the malt. Wort, after the congress mashing procedure, ought to filter during first 60 min to be described as 'normal' or 'optimal'. Worts 4D, 5D, 6D, 4D-Ca and 6D-Ca were not filtered fully, and filtration was stopped after 120 min, according to the congress mashing procedure. Each malt type, when it was mashed with the addition of amylolytic enzymes (4D-Am, 5D-Am, 6D-Am), not only filtered fully but also filtered in a very short time, in the range of 17–18 min, over six times quicker than malts without the addition of enzymes. These data show that one of the most crucial factors impending lentil wort filtration is the presence of starch and various products of incomplete starch hydrolysis, such as dextrins, which have been previously proven to hinder wort and beer filtration[3,23]. The volume of the acquired wort was also the highest (equal to 235–240 cm^3) in the samples with the addition of amylolytic enzymes, higher by 17.5–42.9% than the volume of the wort produced solely from the lentil malts. However, the addition of the amylolytic enzymes or calcium ions did not have a very significant effect on the extract content of the worts, decreasing it slightly (by 0.07% w/w) in sample 4D-Am or increasing it by 0.05% (w/w) in sample 5D-Am. The addition of calcium ions or amylolytic enzymes also resulted in worts with lower pH, but the decrease was in the range of 0.01–0.07 pH, and the resulting pH was closer to the recommended wort pH level in the 5.6–5.8[3]. The viscosities of the worts were similar, and the main difference could be seen in sample 6D-Am, which had the lowest viscosity of 1.432 mPa/s. The results acquired in this analysis not only show that green lentil malts lack an adequate amount of amylolytic enzymes to completely hydrolyze starch but also suggest that a temperature of 70 °C is too low to fully gelatinize all of the starch, which is why a higher temperature was used in the process of lentil beer brewing, and the data discussed in Section "Physicochemical parameters of the lentil beers" confirm this statement. In previous studies on malted lentils and other legumes, green lentil malts produced under the conditions typical for the production of Pilsner barley malt (45% moisture content of the germinating seed, five days of germination, temperature of germination equal to 15 °C) were characterized by very poor parameters in comparison to barley malt and all of the lentil malt samples produced in this study[4]. Worts acquired from the lentil malts in the 2021 study were characterized with extract content equal to 1.59% (w/w), which increased to 2.39–3.40% when they were mashed with the use of external amylolytic enzymes. These results show that significant improvement in the technological properties of lentil malt can be achieved by introducing simple changes to the steeping and germinating regime of the malting process and can possibly be improved to a greater extent in the future.

Physicochemical parameters of the lentil beers

Beers brewed from the lentil malts were characterized with different basic physicochemical properties than the control sample, and the results of this analysis are shown in Table 2.

The starting extract content of the wort of the lentil beers was lower (6.92–7.09% w/w) than that in the C (9.21%), which most certainly influenced other parameters of the lentil beers, such as alcohol content, density and energy content. A higher extract content of the wort typically results in the production of beers with higher alcohol content because *Saccharomyces cerevisiae* yeast has more of the sugars to convert to ethanol[3]. This is certainly true in the case of the lentil beers brewed in this study, as the alcohol content of the B4, B5 and B6 samples is in the range of 2.07–2.12% v/v, compared to 3.97% in C. It is, however, important to note that the wort extract content of samples B4, B5 and B6 is lower by 30% than that in C, while the alcohol content of the lentil beers is lower by 48%. This shows that during the mashing processes, more of the extract transferred to the lentil wort comprised substances that cannot be fermented by the yeast. This statement is supported further by the degree of attenuation, which was lower by 20% in the lentil beers. This is not surprising, as the typical protein content of barley malt should not be higher than 11.5% of the dry weight, and lentil malts are characterized by a far higher protein content, up to 32.8%[3,5]. An experiment performed by Trummer et al.[6] where 10% or 20% of

Sample	Alcohol content (%v/v)	Beer extract content (%w/w)	Wort extract content (%w/w)	Density (g/cm^3)	Degree of attenuation (%)	Energy content (kcal/100 cm^3)	Beer color (EBC)
C	3.97 ± 0.01 a	3.07 ± 0.03 b	9.21 ± 0.01 a	1.00442 ± 0.00008 b	67.79 ± 0.04 a	32.72 ± 0.02 a	4.56 ± 0.07 c
B4	2.12 ± 0.09 b	3.70 ± 0.15 a	7.01 ± 0.13 b	1.00952 ± 0.00043 a	48.16 ± 1.23 b	24.82 ± 0.48 b	13.57 ± 0.10 a
B5	2.07 ± 0.03 b	3.88 ± 0.05 a	7.09 ± 0.04 b	1.01031 ± 0.00022 a	46.20 ± 0.19 b	25.13 ± 0.13 b	12.35 ± 0.57 b
B6	2.08 ± 0.04 b	3.68 ± 0.09 a	6.92 ± 0.05 b	1.00950 ± 0.00014 a	47.72 ± 0.92 b	24.47 ± 0.21 b	11.63 ± 0.45 b

Table 2. Physicochemical parameters of the lentil beers. [1]Values are expressed as the mean (n = 4) ± standard deviation. Mean values with different letters (a, b, c) within the same column are significantly different (α = 0.05) according to Tukey's test. Abbreviations are as follows: B4—beer brewed from green lentil malt germinated for 96 h, B5—beer brewed from green lentil malt germinated for 120 h, B6—beer brewed from green lentil malt germinated for 144 h, C—beer brewed from standard Pilsner barley malt.

barley malt was substituted by lentil malt, also resulted in worts with lower extract content. Differences in the density of the beers are because, first, B4, B5 and B6 are characterized by higher extract content in the finished beverage, and beer C is characterized by higher alcohol content, which, due to its low density, naturally decreases the density of the beer[3]. Various energy content of the analyzed beers is also rather simply explained, but it is not as straightforward to describe whether this trait is an advantage or disadvantage. The lower calorie content of the finished product is typically viewed as an advantage because obesity and obesity-related diseases are still increasing in developed countries[24]. This parameter could promote lentil beer over regular beer for various people who want to reduce their calorie intake, which could pave the road for the formulation of various novel beverages with reduced alcohol and energy contents. The last parameter, also differentiating lentil beers from the control sample, is the beer color. Beers produced from lentil malts were darker, characterized with color in the range of 11.63–13.57 on the EBC scale, with B6 and B5 being the lightest and B4 the darkest. Most of the compounds that influence the darker color of the malt are the result of the Maillard reaction[9]. In a recent study, Gasiński & Kawa-Rygielska[5] showed that increasing the germination time for lentil malts reduces the concentration of starch, while the concentration of proteins remains similar. This would mean that there is lower amount of substrates in malts germinated longer (such as used for the brewing of B5 and B6) for the formation of Maillard products and results acquired in this study would confirm that statement. Analysis of the physicochemical parameters of the lentil beers has shown that there is a possibility of utilizing this type of material in the industry to produce fermented beverages; however, creating lentil beers characterized by the same properties as beers produced from barley malt can be problematic.

Concentration of volatile compounds in the lentil beers

Currently, in the scientific literature, there are no data about the possible volatile composition of the beverage produced from fermented lentil malt wort. The sole scientific report about this topic can be found in the work by Trummer et al.[6] where the aroma of the beer brewed with the addition of 10% or 20% lentil malt in the grain bill was described as 'normal', i.e., without 'any unwanted odors'. GC-FID analysis allowed for the identification and quantification of 12 volatile compounds in the brewed beers (11 for sample C), as shown in Table 3, which are typically detected in fermented beverages[13,25].

Beers brewed from the lentil malts were characterized by higher concentrations of acetaldehyde, in the range of 3.574 ppm for B4 to 7.675 ppm for B6. Acetaldehyde is a volatile that is produced by the *Saccharomyces cerevisiae* yeast as a side-product of ethanol fermentation. In small concentrations, this compound can give beverages the aroma of green apples, but a greater concentration of this aldehyde significantly worsens flavor (etheric, pungent aroma)[26]. The concentration of acetaldehyde was below the threshold level in all the tested beers, but potentially, with the increase in the extract content (and, therefore, increase in pyruvate concentration, which is a substrate for the production of acetaldehyde) during the brewing of beers with increased extract content in the future, an increased concentration of acetaldehyde in the beverage might be troublesome for the production of lentil beers characterized by acceptable quality[13]. However, lentil beers were characterized by lower concentrations of various other components, which can create unwanted aromas in the volatilome of alcoholic beverages. One group of such components was higher alcohols, also called 'fusel alcohols'. These volatiles have a pungent aroma described often as "solvent-like" and are produced by *Saccharomyces* yeast from amino acids via the so-called Ehrlich pathway[27]. During the production of alcoholic beverages, the presence of higher alcohols can be viewed as an advantage or disadvantage, depending on the desire of the producer. Very high concentrations of higher alcohols can be viewed as a defect in the production of spirits and lager beers, while high concentrations of particular alcohols (such as phenylethyl alcohol) are typical for wheat beers. However, higher alcohols play a crucial part in the formation of various esters, which are one of the largest and most important groups of compounds forming beer aroma, and lentil beers were characterized by significantly lower concentrations of these components[28]. Similarly, the ethyl acetate concentration, which imparts a fruity aroma to the beverages, was over two times higher in C than in B4, B5 or B6. This component was determined at a level below or on the verge of the detection limit in the lentil beers. The concentrations of ethyl hexanoate and ethyl octanoate were 2–4 times lower than those in C, although these components are characterized by very low odor threshold limits and were present in samples B4, B5 and B6 at detectable levels[29]. The concentration of ethyl decanoate was 5 times lower in B4 than in C, over 6.5 times lower in B5 and 11 times lower in B6. Formation of C6-C10 esters by the *Saccharomyces cerevisiae* yeast occurs through condensation reaction of ethanol and corresponding fatty

Compound	C[1]	B4	B5	B6
	(mg/dm^3)			
Acetaldehyde	1.515 ± 0.356 d	3.574 ± 0.437 c	5.017 ± 0.580 b	7.675 ± 0.854 a
Ethyl acetate	7.332 ± 0.496 a	3.063 ± 0.714 b	3.272 ± 0.269 b	3.256 ± 0.224 b
2-butanol	n.d	0.377 ± 0.008 b	0.397 ± 0.009 a	0.395 ± 0.016 a
1-propanol	4.973 ± 0.375 b	2.816 ± 0.299 c	3.016 ± 0.048 c	13.959 ± 1.751 a
Isobutanol	6.343 ± 0.493 c	7.683 ± 0.168 b	8.438 ± 0.086 a	8.089 ± 0.261 a
Isopentyl acetate	0.489 ± 0.019 a	0.436 ± 0.002 b	0.437 ± 0.018 b	0.437 ± 0.014 b
2-methylbutanol	2.779 ± 0.222 a	1.532 ± 0.201 bc	1.505 ± 0.078 c	1.675 ± 0.123 b
3-methylbutanol	8.625 ± 0.700 a	5.559 ± 0.762 b	5.546 ± 0.450 b	6.368 ± 0.227 b
Ethyl hexanoate	0.061 ± 0.015 a	0.030 ± 0.003 b	0.026 ± 0.003 b	0.015 ± 0.001 c
Ethyl octanoate	1.374 ± 0.159 a	0.454 ± 0.011 b	0.484 ± 0.031 b	0.478 ± 0.021 b
Ethyl decanoate	2.534 ± 0.221 a	0.569 ± 0.346 b	0.379 ± 0.081 b	0.230 ± 0.193 b
Phenylethyl alcohol	3.446 ± 2.041 a	3.234 ± 1.208 a	3.628 ± 1.982 a	2.982 ± 0.996 a

Table 3. Volatile compounds in lentil beers. [1]Values are expressed as the mean (n = 6) ± standard deviation. Mean values with different letters (a, b, c) within the same column are significantly different (α = 0.05) according to Tukey's test. Abbreviations are as follows: B4—beer brewed from green lentil malt germinated for 96 h, B5—beer brewed from green lentil malt germinated for 120 h, B6—beer brewed from green lentil malt germinated for 144 h, C—beer brewed from standard Pilsner barley malt. N.d. stands for 'not detected'.

acyl-CoA[30]. These results might indicate that the extraction of fatty acids from lentil malt is not as efficient as that from barley malt and that lentil reduces its concentration of C10 fatty acids during prolonged germination, but a more detailed analysis concentrating on the C6-C10 fatty acid content in lentil seeds/malts would be needed to confirm these hypotheses. Analysis of the volatile compounds in the lentil beers shows that the aroma of the produced beverage is not the same as that of the regular beer produced from barley malt but could possibly be adjusted by the brewers using various yeasts, fermentation temperatures and additives to acquire cereal-free beer with the typical, traditional aroma expected of this beverage.

Aroma of the lentil beers

Results of the sensory analysis of 'aroma' parameter of the lentil beers are shown on Fig. 1.

Aroma of the beers produced from the lentil malt differed significantly from beer C in few aspects. C was characterised with aroma described as 'fruity' and possessed rather substantial 'malty' and 'grainy' notes, which

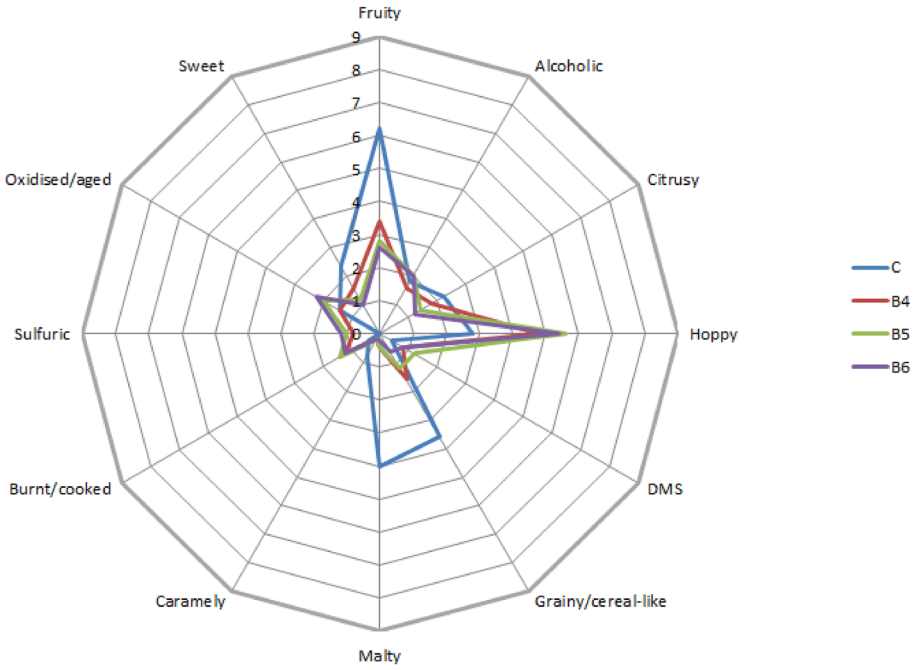

Figure 1. Radar chart showcasing results of the sensory analysis of the 'aroma' parameter of the lentil beers.

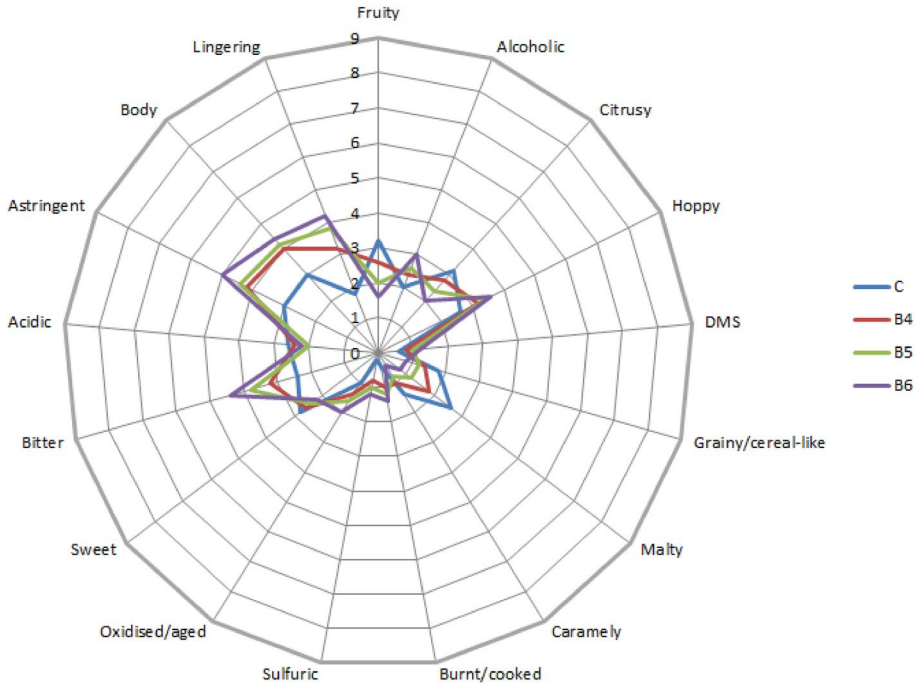

Figure 2. Radar chart showcasing results of the sensory analysis of the 'taste' parameter of the lentil beers.

were almost non-existent in the lentil beers. However, aroma of the lentil beers was characterised with more pronounced 'hoppy' notes. It is important to pinpoint that 'citrusy' aroma, which typically is more pronounced in highly-hopped beers was noted at very low level, in the C and B4, B5 and B6 alike[31,32]. It is furthermore important to note that lentil beers were not characterised with sulphur-like off-flavour, which is often associated with the legumes[33]. These results show that lentil malts could be used to produce gluten-free beers which would be characterised with rather neutral aroma.

Taste of the lentil beers

Results of the sensory analysis of 'taste' parameter of the lentil beers are shown on Fig. 2.

Lentil beer taste was rated by panellists more negatively than the beer aroma. Lentil beers were characterised with more pronounced 'lingering' and 'astringent' taste than C. However, C was rated lower in the 'body' parameter, which is probably a result of the higher content of extract in the B4, B5 and B6. Panellists rated lentil beers as having slightly more pronounced 'alcoholic' taste than beer C. Additionally, as with the aroma, 'hoppy' taste was stronger in the lentil beers. This result might also be connected with the higher perceived bitterness of the lentil beers[34]. Taste of the C was rated higher only in parameters such as 'fruity', 'citrusy', 'malty' and by a very small discrepancy in the 'sweet' parameter, which corresponds well with the results of the 'aroma' analysis. It is however interesting to see that the C was not rated as less 'sweet' than the lentil beers, which were characterised with higher extract content.

Conclusion

Data acquired in this study show that there is a possibility of producing cereal-free beers using only slightly changed methods used in the malting and brewing industry. Lentil malt produced by the method described in this manuscript can be successfully mashed with the use of only one type of external enzyme (α-amylase). However, the technology and methods of cereal-free beer brewing, using legume malts as substrates, are still very novel techniques, and beers produced in this way are characterized by some technological shortcomings. Beers produced from lentil malt are characterized by a lower degree of attenuation (which could be an advantage in the production of low-alcoholic beers) and lower calorie content than beers produced solely from barley malt. Additionally, various volatile components in the beer brewed from lentil malts are present in lentil beers in higher concentrations than in the beer brewed from barley malt, which could lead to various off-flavors in the perceived aroma. Results of the sensory analysis show, that the beers produced from the green lentil malt are not characterised with aroma or taste which is very distinct of the aroma of typical beer produced from barley malt. These results might indicate that there is a possibility of compiling recipes for the production of cereal-free beers adequate for people suffering from celiac disease or other gluten-related illnesses, which could be indistinguishable from typical beers popularly consumed throughout the world.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

A.G. designed the experiment, prepared investigated material, performed analyses, applied statistics and wrote main manuscript text. All authors reviewed the manuscript. J.K.-R. supervised and administered the project.

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Competing interests
The authors declare no competing interests.

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10. ZAŁĄCZNIKI

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Alan Gasiński

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OŚWIADCZENIE

Oświadczam, że w pracy

Gasiński, A., Błażewicz, J., Kawa-Rygielska, J., Śniegowska, J., & Zarzecki, M. (2021). Analysis of physicochemical parameters of Congress worts prepared from special legume seed malts, acquired with and without use of enzyme preparations. Foods, 10(2), 304

mój udział polegał na wytworzeniu słodów z nasion roślin strączkowych, analizie brzeczek uzyskanych w procesie zacierania kongresowego, analizie statystycznej wyników oraz napisaniu manuskryptu złożonego do czasopisma.

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Gasiński, A., & Kawa-Rygielska, J. (2022). Mashing quality and nutritional content of lentil and bean malts. LWT, 169, 113927

mój udział polegał na wytworzeniu słodów z nasion roślin strączkowych, przeprowadzeniu zacierania kongresowego, analizie brzeczek uzyskanych w procesie zacierania kongresowego, analizie kruchości wytworzonych słodów oraz analizie statystycznej wyników i napisaniu manuskryptu złożonego do czasopisma.

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Gasiński, A., Kawa-Rygielska, J., Mikulski, D., & Kłosowski, G. (2022). Changes in the raffinose family oligosaccharides content in the lentil and common bean seeds during malting and mashing processes. Scientific Reports, 12(1), 17911

mój udział polegał na wytworzeniu słodów z nasion roślin strączkowych, przeprowadzeniu zacierania kongresowego, analizie statystycznej wyników i napisaniu manuskryptu złożonego do czasopisma.

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Gasiński, A., & Kawa-Rygielska, J. (2023). Malting—A method for modifying volatile composition of black, brown and green lentil seeds. Plos one, 18(9), e0290616

mój udział polegał na wytworzeniu słodów z nasion roślin strączkowych, analizie zawartości związków lotnych w słodach i nasionach oraz analizie statystycznej wyników i napisaniu manuskryptu złożonego do czasopisma.

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Gasiński, A., & Kawa-Rygielska, J. (2024). Assessment of green lentil malt as a substrate for gluten-free beer brewing. *Scientific Reports, 14*(1), 504

mój udział polegał na wytworzeniu słodów z nasion roślin strączkowych, zacieraniu kongresowego słodów, wytworzeniu piwa z nasion roślin strączkowych oraz analizie wyprodukowanego piwa oraz analizie statystycznej wyników i napisaniu manuskryptu złożonego do czasopisma.

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Potwierdzam treść oświadczenia.

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data i podpis promotora