Czech University of Life Sciences Prague

Faculty of Tropical AgriSciences

Phenotypic and metabolomic characterization of quinoa (*Chenopodium quinoa* Willd.): Identification of superior genetic resources

DISSERTATION THESIS

Author: Ing. Lucie Dostalíková
Supervisor: Ing. Iva Viehmannová, Ph.D.
Co-supervisors: Ing. Petra Hlásná Čepková, Ph.D.
RNDr. Michal Jágr, Ph.D.

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Declaration

I hereby declare that I have completed this thesis entitled ´Phenotypic and metabolomic characterization of quinoa (*Chenopodium quinoa* Willd.): Identification of superior genetic resources´ independently, except for the jointly authored publications that are included. In the case of such publications, my specific contributions have been clearly stated at the start of the relevant publication chapter. Furthermore, I confirm that proper acknowledgment has been provided within this thesis for any references made to the works of others, I also ensure that this work has not been, nor is it currently submitted, for any other degree, to this or any other university. All information sources have been quoted and acknowledged by means of complete references.

In Prague 31. 10. 2024

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Lucie Dostalíková

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Abstract

Environmental stresses and climatic changes present escalating challenges for global agriculture and food security. Quinoa (*Chenopodium quinoa* Willd.), known for its adaptability and nutritional richness, presents itself as an excellent genetic resource to overcome the challenges of the 21st century. This dissertation was conducted to comprehensively evaluate quinoa accessions with distinct genetic backgrounds and select the most promising genotypes. Employing a multifaceted approach encompassing field experiments and subsequent laboratory analysis, the thesis aimed to elucidate the interplay between genotype variations, environmental influences, and processing methods in shaping quinoa's overall nutritional profile. The assessment of quinoa seeds revealed significant variability in studied traits across genotypes and cultivation years; however, certain genotypes, including ´Mint Vanilla´, ´Cahuil A´, ´Cohamamba B´, ´Braunschweig B´, ´Apelawa A1´, ´Red Head B´, ´Isluga A´, and ´QQ87´ demonstrated stability in selected parameters. Six compounds (2-OH-cinnamic acid, homoorientin, luteolin, naringenin, N-feruloyl octopamine, and 4-OH-benzaldehyde) were identified and quantified in quinoa seeds for the first time. The nutritional profile of quinoa seeds is further influenced by heat-utilizing methods from which roasting and microwaving were identified as superior methods for enhancing polyphenol content, whereas flaking improved the protein content. Nonetheless, the degree of change in nutritional profile varied depending on the duration of heat treatment applied to the seeds. Germination also emerged as a promising strategy to boost the nutritional attributes, particularly increasing the content of specific bioactive metabolites, albeit with variation by genotype and germination duration. In the face of unfavorable environmental conditions that may impact seed harvest and quality, cultivating quinoa for its leaves emerges as an alternative approach, with ´Faro (Prague)´, ´Red Head A´, ´Isluga A´, and ´DE-1´ identified as genotypes with suitable nutritional content in their leaves. This dissertation revealed the conveniences of quinoa performance and underscored the significance of quinoa germplasm preservation as a crucial component of quinoa breeding initiatives and variety development. Quinoa seeds and leaves emerged as a rich source of nutrients and bioactive compounds that can be further enhanced or degraded through specific processing techniques.

Keywords: genetic resources, germination, protein, phenolics, thermal processing, quinoa

Abstrakt

Klimatické změny představují stále větší výzvy pro globální zemědělství a potravinovou bezpečnost. Quinoa (*Chenopodium quinoa* Willd.), neboli merlík čilský, je rostlina známá svou adaptibilitou a příznivým nutričním profilem. Z toho důvodu se také jeví jako slibný genetický zdroj pro překonání výzev 21. století. Tato disertační práce byla zaměřena na komplexní zhodnocení rozsáhlé kolekce genotypů quinoy za využití polních pokusů i laboratorních analýz, s cílem vybrat nejperspektivnější genotypy vhodné pro pěstování v klimatických podmínkách střední Evropy i pro šlechtění nových odrůd s požadovanými vlastnostmi. Důraz byl kladen na stanovení vlivu genotypu, prostředí a zpracování semen na formování celkového nutričního profilu quinoy. Hodnocením genotypů a ročníku byla potvrzena významná variabilita v hodnocených znacích. Nicméně některé genotypy, například ´Mint Vanilla´, ´Cahuil A´, ´Cohamamba B´, ´Braunschweig B´, ´Apelawa A1´, ´Red Head B´, ´Isluga A´ a ´QQ87´, vykazovaly stabilitu ve vybraných nutričních parametrech i v rámci hodnocených ročníků. V semenech quinoy bylo identifikováno a kvantifikováno šest nových fenolických sloučenin (2-hydroxyskořicová kyselina, homoorientin, luteolin, naringenin, N-feruloyl oktopamin a 4-OH-benzaldehyd). Obsah nutričních látek v semenech quinoy byl dále ovlivněn tepelným zpracováním, přičemž pražení a mikrovlnná příprava byly identifikovány jako nejvhodnější metody pro zvýšení obsahu polyfenolů, zatímco vločkování zlepšilo obsah bílkovin. Nicméně míra změn v nutričním profilu se lišila v závislosti na délce zpracování. Klíčení se ukázalo jako vhodná strategie pro zlepšení některých nutričních atributů, zejména pak pro zvýšení obsahu některých bioaktivních látek. Míra tohoto nárustu však závisela na genotypu a délce klíčení. Dále byla práce zaměřena na nutriční vlastnosti listů, které se jeví jako vhodný alternativní zdroj potravy v klimatických oblastech, které neumožňují pěstování quinoy pro semeno. Genotypy ´Faro (Prague)´, ´Red Head A´, ´Isluga A´ a ´DE-1´ byly identifikovány jako genotypy s vhodným obsahem analyzovaných nutričních látek. Tato disertační práce poukázala na benefity quinoy a zdůraznila význam uchování jejích genetických materiálů jako klíčové součásti iniciativ zaměřujících se na šlechtění a vývoj odrůd. Semena i listy quinoy se ukázaly jako bohatý zdroj živin a bioaktivních látek, které mohou být dále ovlivněny podmínkami prostředí a technikami zpracování.

Klíčová slova: genetické zdroje, klíčení, bílkoviny, fenolické látky, tepelné zpracování, quinoa

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1000-SW–weight of thousand seeds; 4BA–4-hydroxybenzaldehyde; AA–Amino Acid; ANOVA1–A one-way analysis of variance; ANOVA2–A two-way analysis of variance; AA–Antioxidant activity, BQ–Bolivian quinoa; C4B–4-hydroxybenzaldehyde; CFA–Caffeic acid; COA–*p*-coumaric acid; CP–Crude protein; DPPH–2,2-diphenyl-1-picrylhydrazyl, DW–Dry weight; EAA–Essential amino acids; ESI–Electrospray ionization; EMO–Emodin; G1D–1 day of germination; G2D–2 day of germination; G3D–3 days of germination; G4D–4 days of germination; G5D–5 days of germination; GA–Gallic acid; FA–Caffeic acid; FAO–Food and Agriculture Organization; FC–Folin-Ciocalteau; HI–Harvest index; IH–Isorhamnetin; IL–Inflorescence length; IQCE–Isoquercetin; ISR–Isorhamnetin; IVPD– *In vitro* protein digestibility; IV-PDCAAS– *In vitro* protein digestibility corrected amino acid scores; IQ–Isoquercetin; KMP–Kaempferol; MAU–Mauritianin; MIQ–Miquelianin; MUFA–Mono-unsaturated fatty acid; n.d.– not defined; MW–Molecular weight; NAR–Naringenin; NFO–N-feruloyloctopamine; NW–Northwestern; PC–Protein content; PC–Pinocembrin; PCB–Pinocembrin; PDCAAS–Protein digestibility corrected amino acid score; PH–Plant height; PHE–Sum of phenolic compounds; PL– Panicle length; PQ–Peruvian quinoa; PUFA–Poly-unsaturated fatty acids; Q3G–Quercetin 3-O-glucuronide; QBQ–Big black quinoa; QCE–Quercetin; QGQ– Sanjiang Gray, gray quinoa; QWQ–Qingli No.1, white quinoa; RUT–Rutin; SA–Salicylic acid; SAC–Salicylic acid; SDS-PAGE– SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis; SFA–Saturated fatty acids; SGQ–Aihua No.1, gray quinoa; SD–Stem diameter; SQ–Spanish quinoa; SWQ–Jiaqi Diamond No.1, white quinoa; SMP–Soil matric potential; SSP–Seed storage protein; TDF–Total dietary fiber; TFC–Total flavonoid content; TE–Trolox equivalent; TPC–Total phenolic content; UNU–United Nations University; WHO–World Health Organization; WTS–Weight of thousand seeds

1. General introduction and thesis framework

Quinoa (*Chenopodium quinoa* Willd.), once a staple crop confined to the Andean regions, has gained global attention due to its exceptional adaptability and nutritional richness. Quinoa has been emerging as a strategic crop for food security and economic growth, especially in Andean regions and potentially in Europe.

In the last decade, two milestones in quinoa research have significantly advanced our understanding and utilization of this crop. The year 2013 was particularly significant, as it was declared the "International Year of Quinoa" by the Food and Agriculture Organization (FAO et al., 2013b), resulting in the worldwide promotion of this pseudocereal. Later, in 2017, the complete genome sequence of quinoa was published, providing insights into quinoa evolutionary history and identification of mechanisms likely controlling the production of saponins, that will significantly help direct future breeding strategies (Jarvis et al., 2017).

Nutritional evaluation of quinoa has also seen substantial progress. Researchers have extensively profiled the macronutrient and micronutrient content of quinoa seeds, underscoring their irreplaceable role in a balanced human diet (Präger et al., 2018; Reguera et al., 2018; Craine and Murphy et al., 2020; Granado-Rodríguez et al., 2021b; Craine et al., 2023). Moreover, the various compounds with potential biological activities in quinoa have been identified (Tang et al., 2016b; Lin et al., 2019; Liu et al., 2020a; Tabatabei et al., 2022).

Despite these advancements, several gaps persist in quinoa research. One major limitation is the insufficient understanding of quinoa's adaptability and performance in non-native environments. While there have been efforts to introduce quinoa to various regions (Alandia et al., 2020), comprehensive studies evaluating its agronomic and nutritional performance across diverse climates are still insufficient. Further, those studies are often constrained by the evaluation of a limited number of analyzed samples, lack of long-term cultivation comparisons, and/or a limited number of attributes used for the evaluation of quinoa genetic resources.

Additionally, there is a need for in-depth and comprehensive research on the nutritional changes that quinoa undergoes during processing. Most studies focus on raw quinoa seeds, overlooking the fact that quinoa is typically consumed after processing, which can significantly alter its nutritional composition and bioavailability. Similarly, the potential of quinoa leaves as an alternative food source has been largely overlooked, in spite of their

considerable potential as a source of nutrients, especially in marginal environments (Gómez et al., 2024).

This dissertation made significant strides in addressing these research gaps, offering new insights and discoveries that advance the current understanding of quinoa. One of the key contributions of this dissertation is the complex nutritional and morphological evaluation of a wide spectrum of quinoa genotypes under climatic conditions of the Czech Republic for 4 years. This is a pioneering effort, as it represents the first extensive assessment of quinoa's performance in this region. Additionally, the dissertation thesis brings new and valuable information by identifying genotypes and traits with low responsiveness to changing weather conditions across cultivation years, highlighting their potential for stable production in diverse environmental scenarios. The findings provide invaluable data for future breeding programs aimed at developing varieties suited for specific climatic conditions of Central Europe.

Furthermore, the application of UHPLC-ESI-MS/MS instrumentation in this research represents a significant methodological advancement. The high precision and accuracy of this method enable comprehensive profiling of quinoa's metabolome, providing novel information on the biochemical changes under different cultivation conditions. By employing this methodology, the thesis further described so far little-known metabolomic dynamics during quinoa germination and thermal processing.

Although there are studies suggesting the implementation of quinoa leaves in the human diet, the research related to this area is yet scarce, often missing a larger number of studied samples. To ensure that the full range of nutritional variations is captured and to provide a better understanding of the species' nutritional potential, a diverse array of quinoa genetic materials was evaluated in terms of the content of protein, polyphenols, and antioxidant activity in their leaves. Specific samples with the potential to be used in future breeding were identified.

Concerning the research contextualization, it is essential to clarify the terminology employed throughout the study; in particular, the term "genotype", which occurs in Chapters 1, 3, 4, and 5. This term is utilized to refer to the quinoa samples under investigation, obtained from the U. S. National Plant Germplasm System; and from Gene Bank, Crop Research Institute (CRI). These samples are currently included in a so-called working collection in the CRI Gene Bank, meaning that plant material is tested in field conditions and further assessed in the laboratory, however, it is not yet included in the active

collections. Further, studied samples are not officially registered cultivars/varieties in the Czech Republic, hence they are called genotypes. The choice of this terminology was also aiming to provide clarity and consistency for the readers throughout the thesis. Conversely, plant material was referred to as variety, cultivar, landrace, and genotype predominantly in Chapter 2, following the nomenclature used by the respective authors of the referenced studies.

1.1. Aims of the thesis

The main goal of the dissertation was a comprehensive chemical and nutritional characterization of an extensive collection of quinoa accession in order to identify genotypes with superior and stable nutritional value which provide essential and complex information for quinoa breeding purposes. Additionally, another objective was to evaluate various food processing methods applied to quinoa seeds to identify techniques that significantly enhance the nutritional content of the final product.

The partial aims focused on the assessment and evaluation of:

- morphological traits of quinoa plants in the experimental field
- protein content in quinoa seeds and leaves;
- phenolic content, phenolic composition, and antioxidant activity in quinoa seeds;
- phenolic content and antioxidant activity in quinoa leaves;
- changes in nutritional content and composition in thermally processed quinoa seeds;
- changes in nutritional content and composition in germinated quinoa seeds;
- changes in nutritional content and composition in quinoa seeds cultivated in distinct weather conditions.

The following hypotheses were adopted:

I. The content of proteins, bioactive compounds, and antioxidant activity in quinoa seeds vary significantly among different genotypes;
II. The process of germination significantly alters nutritional content and composition (proteins, bioactive compounds, and antioxidant activity);
III. Changes in the nutritional profile during the germination process are genotype-dependent;

IV. Different thermal preparation methods applied to quinoa seeds lead to significant variations in their nutritional content and composition (protein content, bioactive compounds, and antioxidant activity);
V. Quinoa leaves exhibit higher protein content compared to quinoa seeds;
VI. Variations in weather conditions significantly impact the content and composition of nutritional parameters (proteins, bioactive compounds, and antioxidant activity) in quinoa seeds;
VII. Variations in weather conditions significantly impact the morphological traits of quinoa plants.

2. Nutritional value and variability of quinoa genetic resources in diverse environments

Adapted from: Hlásná Čepková. P., **Dostalíková, L.**, Viehmannová, I., Jágr, M., Janovská, D. (2022). Diversity of quinoa genetic resources for sustainable production: A survey on nutritive characteristics as influenced by environmental conditions. *Frontiers in Sustainable Food Systems* 6:960159. https://doi.org/10.3389/fsufs.2022.960159

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Abstract

Environmental extremes and climatic variability have enhanced the changes in numerous plant stressors. Researchers have been working to improve “major” crops for several decades to make them more adaptable and tolerant to environmental stresses. However, neglected and underutilized crop species that have the potential to ensure food and nutritional security for the ever-growing global population have received little or no research attention. Quinoa is one of these crops. This pseudocereal is considered a rich and balanced food resource due to its protein content and protein quality, high mineral content, and health benefits. This review provides currently available information on the genetic resources of quinoa and their quality in terms of variability of economically important traits such as yield, and the content and composition of a wide spectrum of nutritional parameters. The influence of variety and environmental conditions on selected traits is also discussed. The various types of nutrients present in the different varieties form the basis and they are the key element for future breeding efforts and efficient, healthy, and sustainable food production.

2.1. Introduction

Most staple foods comprise grain crops; therefore, feeding the ever-increasing global population means increasing the production of these crops (Bvenura and Kambizi, 2022). However, it is well known that climate change is rapidly degrading the conditions of crop production. Salinization and aridity are forecasted to increase in most parts of the world (Choukr-Allah et al., 2016). Moreover, globally, the food crisis is mainly triggered by shocks such as drought and escalated by trade restrictions leading to price rises as an impact of the COVID-19 pandemic and as a consequence of the current war in Ukraine (Rahut et al., 2022).

Therefore, new stress-tolerant or new alternative crops or species must be identified and used for future food security (Choukr-Allah et al., 2016). The present situation is that common wheat, rice, and maize as major crops seem to be near 80% of their potential. This shows the potential of many small-scale and marginal crops and wild plants that can be used as high-quality food sources. Since many of these species are well adapted to extreme environments, their role in the current scenario of climate change has become extremely important (Chrungoo and Chettry, 2021).

These crops have the potential to complement the major cereals and play a greater role in a safe household diet. A better understanding of these crops that feed the world and their potential role in nutrition will help secure their future and ensure food and nutrition security. *Chenopodium quinoa* Willd. was selected as one of the crops that will contribute to food security in the twenty-first century, because of its high resilience to extreme environmental conditions, its qualities as a functional food (Bvenura and Kambizi, 2022; Singh et al., 2022), and as a potential strategic crop that plays a vital role in food security and sovereignty (Rojas et al., 2015).

In addition, quinoa has gained importance in international consumer markets in the last decade, which provides economic opportunities for Andean producers (Anaya et al., 2022). On the other hand, quinoa could be used for crop diversification in Europe and other parts of the world, outside of its genetic origin, as an alternative for marginal agricultural land (Jacobsen, 2017).

In the present work, we attempt to summarize the available information about quinoa genetic resources for the whole world by highlighting the situation in the Czech Republic. We also explored the results of current research focused on nutraceutical properties, including carbohydrates, lipids, proteins, amino acids, secondary metabolites, vitamins, and minerals. This overview provides an insight into the enormous variability of morpho-phenological traits and

nutritive components that are possessed by quinoa germplasm cultivated in different global conditions and shows us how important it is to conserve and protect this richness.

2.2. Quinoa origin and ecotypes

Quinoa is taxonomically categorized as a pseudocereal within the genus *Chenopodium*, family Amaranthaceae (Royal Botanic Gardens Kew, n.d.). Although quinoa is currently cultivated across the globe in temperate, subtropical and tropical regions (Alandia et al., 2020), its primary place of origin is traced to Lake Titicaca, situated along the border of Peru and Bolivia, South America. Archaeological findings suggest that quinoa was domesticated in this region approximately 7,000 years ago. Through the agricultural practices of ancient Andean civilizations, quinoa gradually disseminated northward and southward from its epicenter (Bazile et al., 2013; Fuentes et al., 2012).

Over millennia of domestication, selection, and adaptation to diverse climatic and soil conditions, quinoa has reached considerable genetic diversity. Based on distinct sub-centers of diversity, quinoa is categorized into five ecotypes: Salares, Highlands (Altiplano), Inter-Andean Valley, Yungas, and Sea-Level (Coastal lowlands), each endowed with specific traits enabling adaptation to their respective habitats (Bazile et al., 2013).

According to Jacobsen (2017), Coastal lowland ecotypes exhibit promising potential for incorporation into the development of new varieties suitable for cultivation in northern latitudes of Europe, especially due to their insensitivity to day length variations, which is considered a major problem for the introduction of quinoa to North European conditions (Bendevis et al., 2014; Christiansen et al., 2010). Further, the Salares ecotype, thriving in environments of cold deserts with extremely low precipitation (Bazile et al., 2013), is considered one of the most drought-resistant ecotypes among others (Raney et al., 2014). On the other hand, Inter-Andean Valley accessions are commonly cultivated for their ample foliage, utilized as a leafy vegetable in their native habitats (Bazile et al., 2013) due to their low harvest index (Gomez-Pando, 2015).

2.3. Botanical description of quinoa

Quinoa is a herbaceous annual plant that exhibits a remarkable diversity in morphological traits. Quinoa plants can attain heights of up to 3 meters, featuring either branched or unbranched stems (Gomez-Pando, 2015). Stem

morphology typically manifests angular shape with green or colored striae (Manjarres-Hernández et al., 2021). Stem coloration ranges across a spectrum from green, yellow, and orange to pink, purple, black, or red (Biodiversity International et al., 2013) (Figure 2.1).

Figure 2.1 Variability in stem and striae coloration of quinoa accessions cultivated on the experimental fields of Crop Research Institute in Prague, Czech Republic. Noticeable striae are indicated by white arrows.

The leaves of quinoa also exhibit significant variation in shape, including lanceolate, rhomboidal, or triangular forms, as well as in the number of teeth along the leaf margins, ranging from 3 to 48. Generally, lower leaves tend to be larger with a higher number of teeth compared to the smaller upper leaves (Gomez-Pando & Eguiluz de la Barra, 2011; Rojas, 2003) (Figure 2.2). The coloration of leaves may transform to green, red, purple, or orange at various stages of quinoa development (Gomez-Pando & Eguiluz de la Barra, 2011).

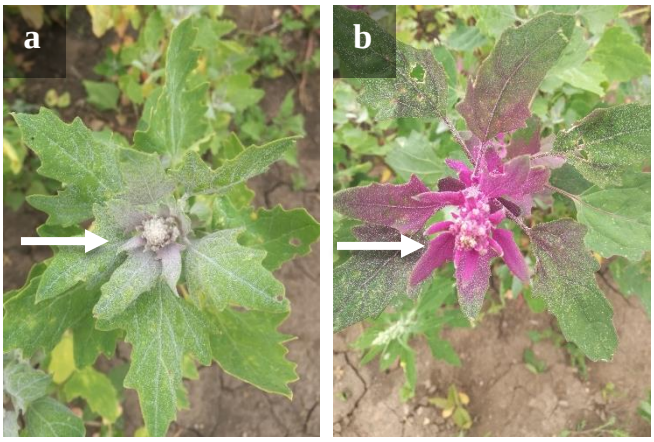

Figure 2.2 Variability in leaf morphology in different quinoa accessions grown in the experimental fields of Crop Research Institute in Prague, Czech Republic. Young leaves near inflorescence display creamy (a) and purple (b) coloration (indicated by arrows), low number of teeth and lanceolate shape. Older leaves have rhomboid-like shape and green coloration with increased number of teeth.

The inflorescence of quinoa has the form of a panicle, characterized by varying lengths of pedicels, which classify the panicle into loose, medium, or compact. Within quinoa, three distinct types of inflorescences are identified – glomerulate, amaranthiforme, and intermediate (transition type) (Figure 2.3) (Biodiversity International et al., 2013), exhibiting lengths spanning from 15 to 70 cm (Rojas, 2003).

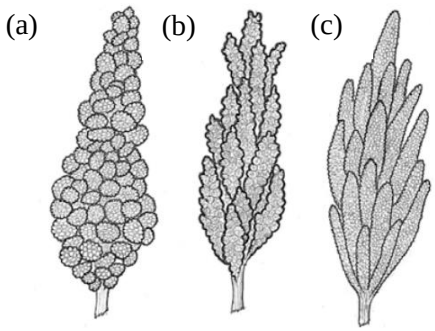

Figure 2.3 Three types of quinoa inflorescence (Biodiversity International et al., 2013)
(a) glomerulate inflorescence; (b) intermediate inflorescence; (c) amaranthiforme inflorescence

The coloration of inflorescence includes a diverse array of hues (Figure 2.4), such as green, yellow, orange, red, purple, brown, gray, or black, with pigmentation undergoing alterations across different developmental stages (Biodiversity International et al., 2013; Fuentes & Bhargava, 2011; Gomez-Pando & Eguiluz de la Barra, 2011). Quinoa is predominantly a self-pollinator; however, both intra- and interspecific outcrossing phenomena have been documented in the literature, ranging from 3.81% to 19.88% (Anchico-Jojoa et al., 2023).

Figure 2.4 Variability in inflorescence shape and color of different quinoa accessions grown in the experimental fields of Crop Research Institute in Prague, Czech Republic

The fruit of quinoa is categorized as an indehiscent achene, comprising a pericarp, thin endosperm followed by the embryo with two cotyledons radicula and perisperm – the storage tissue. The diameter of the fruit can range from 1.80 to 2.66 mm (Gomez-Pando & Eguiluz de la Barra, 2011; Rojas, 2003) exhibiting variable shapes, as represented in Figure 2.5.

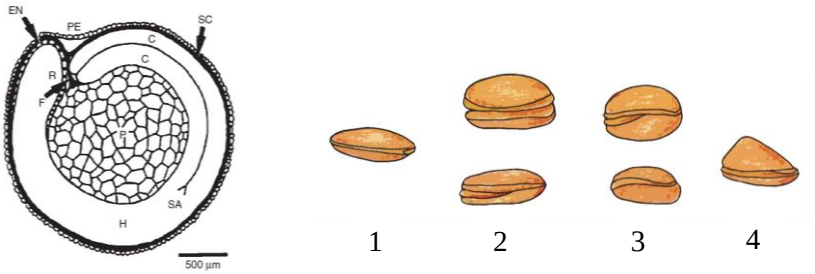

Figure 2.5 Median longitudinal section of quinoa achene (Prego et al., 1998) and achene shape variability (Schmidt et al., 2021)
C: Cotyledons; EN: Endosperm; F: Funicle; H: Hypocotyl radicle; P: Perisperm; PE: Pericarp; R: Radicle; SA: Shoot apex; SC: Seed coat
1: lenticular; 2: cylindrical; 3: ellipsoid and 4: conical shape

The coloration of the achene can vary across several shades, including black, pink, red, yellow, cream, or brown (Biodiversity International et al., 2013). Seeds exhibit rapid germination, occurring within hours after hydration, facilitated by the attachment of endosperm cells to the embryo, which are quickly consumed during growth (Vega-Gálvez et al., 2010).

2.4. Global production of quinoa

At present, quinoa is grown throughout North and South America, Europe, Asia, Africa, and Oceania (Hinojosa et al., 2021). Alongside South American countries, China, India, and some European countries cultivate quinoa (Bazile and Baudron, 2015; Mosyakin and Schwartau, 2015; Yang et al., 2019). However, the biggest world producers remain countries of the traditional region of quinoa cultivation: Peru, with the production of 100,115 t; Bolivia, with 70,170 t (Faostat, 2022); and Ecuador, with more than 4,500 t (Hinojosa et al., 2021), while the United States is the top importer (Bvenura and Kambizi, 2022). The global harvested area of quinoa almost doubled last decade from 95,979 ha in 2010 to 188,878 ha in 2020. Annual production in China was 20,000 t in 2018 and the harvested area reached nearly 12,000 ha (Yang et al., 2019). Globally, the average yield slightly increased from 0.83 t/ha in 2010 to 0.93 t/ha in 2020 (Faostat, 2022).

In the last decade, quinoa has evolved from being a neglected traditional food to an important export crop, promoted as a “superfood” throughout the Western world (Bazile and Baudron, 2015; Nuñez De Arco, 2015). Rising demand among Western consumers has created new economic opportunities for quinoa

farmers in Bolivia´s southern Altiplano. The negative aspect of the high interest in quinoa and the extreme increase in demand for quinoa seeds is that it has caused a spectacular increase in market price (Tschopp et al., 2018). However, this quinoa boom has brought environmental disaster in the traditional regions of quinoa cultivation in Bolivia (Jacobsen, 2011).

Similarly, in Peru, the area under quinoa cultivation has been expanded by 264% and its cultivation has spread to all regions of Peru (Bedoya-Perales et al., 2018) which had a strong negative impact on the environment – soil degradation, pests, and diseases occurrence; likewise on socio-economic links and relations in local communities (Jacobsen, 2011). In the context of the above-mentioned facts, countries of the Andean region have tried to make a great effort to establish a harmonious interaction between socio-economic and environmental demands (Bedoya-Perales et al., 2018) and apply strategies for saving quinoa diversity, established breeding and research priorities, built more transparent commercial chain policy, and ensure more efficient cooperation with local farmers and cooperatives to decrease the negative impact of quinoa growth expansion (Ruiz et al., 2014; Bazile and Baudron, 2015; Bazile et al., 2016b; Bedoya-Perales et al., 2018; Hinojosa et al., 2021).

2.5. Conservation of global quinoa genetic resources and history of research on quinoa in the Czech Republic

Quinoa plant genetic resources are essential for food and nutrition security and sovereignty of peoples, and they make a significant contribution to meeting the basic needs of humanity. They are part of ancestral and cultural heritage, especially for the countries of the Andean region. Their conservation and sustainable use are therefore the responsibility of society as a whole (Rojas et al., 2015). Quinoa is one of the underutilized crops with public breeding or evaluation programs in South American countries such as Peru, Ecuador, and Bolivia (Galluzzi and Noriega, 2014).

Quinoa seeds of different accessions are currently conserved in several gene banks around the world (*ex-situ* conservation). However, the conservation of agrobiodiversity means the conservation of the culture associated with indigenous farmers living in the Andean region (Bazile et al., 2016a; Jacobsen, 2017). Thus, although the importance of gene banks for biodiversity conservation is well known, the success of future conservation and breeding programs depends on the transfer of knowledge and associated practices that can help to adapt quinoa to new regions (Ruiz et al., 2014).

Quinoa germplasm and its wild relatives are estimated at 16,422 accessions worldwide and they are held in 59 institutions (universities, gene banks, research, and agricultural institutions) in 30 countries around the world. A total of 88% of accessions are conserved within the Andean region. The largest collections of quinoa and its wild relatives are held by institutions in Bolivia and Peru, with more than 6,000 accessions (Rojas et al., 2015). Compared to outdated information about quinoa accessions conserved in gene banks published by Jacobsen and Mujica in 2002, the collection, characterization, and evaluation of quinoa genetic resources have greatly improved in recent years.

According to available data, the genetic resources of quinoa conserved in collections outside the Andean region comprise a total of 2,137 accessions (Table 2.1). In the database, the biological status of 1,329 accessions is indicated as traditional cultivar/landrace, 552 accessions are listed as wild, 1,007 accessions are shown as advanced/improved cultivar, and 100 accessions as others (Genesys, 2022).

The provenance of accessions is mostly Peru, followed by the USA and Bolivia. In 1,329 accessions, the type of germplasm storage is not identified, 543 genetic resources are kept in long-term seed collection, 193 are conserved in seed collection, and 45 accessions are in the short-term collection. In total, 478 accessions have safety duplication in the Svalbard Global Seed Vault in Norway and 143 accessions in the National Seed Storage Laboratory, USDA-ARS in the USA. Most of the accessions (1,306) are conserved in the International Center for Biosaline Agriculture in the United Arab Emirates. In Europe, the largest collection (528 accessions) is held by the Genebank of Leibniz Institute of Plant Genetics and Crop Plant Research in Germany (Eurisco, 2022).

In the Czech Republic, research on quinoa genetic resources began in 1999 with Dr. Anna Michalová, who obtained 22 quinoa genotypes from South America. Subsequently, a working collection of quinoa genotypes was established in the gene bank of the Crop Research Institute in Prague. The quinoa accessions were evaluated under field conditions for selected agro-morphological traits (days to flowering, days to harvest, 1,000-seed weight, etc.), and selected nutritional components in the seeds (crude protein content) were also analyzed in the laboratory.

Evaluation of the quinoa working collection was then stopped until 2016 when Dr. Dagmar Janovská and Dr. Petra Hlásná Čepková resumed work on quinoa genetic resources cultivated under the conditions of the Czech Republic. Currently, the working collection of quinoa includes 70 genotypes. They are

being tested under field conditions using descriptors for quinoa and its wild relatives (Biodiversity International et al., 2013) while analyses are being conducted in the laboratory to determine the nutritional quality of the seeds of each genotype. The promising material will be used for future breeding purposes.

Table 2.1 Quinoa genetic resources in collections outside the South American region (Genesys, 2022)

Country	Holding Institute	Institute code	No. of accessions
United Arab Emirates	International Center for Biosaline Agriculture	ARE003	1,306
Germany	Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research	DEU146	528
United States	North Central Regional Plant Introduction Station, USDA-ARS, NCRPIS	USA020	162
United Kingdom	Genetic Resources Unit, Institute of Biological, Environmental & Rural Sciences, Aberystwyth University	GBR016	23
Hungary	Centre for Plant Diversity	HUN003	19
Slovakia	NAFC-Research Institute of Plant Production	SVK001	14
Australia	Australian Grains Genebank, Agriculture Victoria	AUS165	13
Ethiopia	International Livestock Research Institute	ETH013	11
Slovenia	Crops and Seed Production Department, Agricultural Institute of Slovenia	SVN019	5
Australia	Australian Pastures Genebank	AUS167	4
Others			20
Total			**2,105**

2.6. Quinoa´s adaptability to a diverse environment

In different countries around the world, farmers and researchers have been trying to find, test, and introduce nutritionally valuable seed crops that would be suitable for diverse growing conditions, achieve satisfactory yields, and offer versatile applications in food production and consumption (Gardner et al., 2019; Toderich et al., 2020; Habiyaremye et al., 2022).To fully exploit the potential of the crop for marginal environments, the identification of new and high-yielding quinoa genotypes with good local adaptation and high nutritional quality is crucial, which requires intensified screening and adaptation research (Choukr-Allah et al., 2016). Recently, the performance of different quinoa genotypes in different global environments with an emphasis on their adaptability and seed nutritional quality has been studied in several countries and regions (Table 2.2).

Table 2.2 Different quinoa genotypes performance in different environments

Plant material	Growing condition	Locality, country	Agro-morphological evaluation	Biochemical markers	Seed yields	Reference
468 genotypes	Drip irrigation	Dubai, the United Arab Emirates	11 morphological traits	400 seed metabolites	n.d.	Tabatabaei et al. (2022)
2 cultivar ICBA-Q5, Titicata	Field, supplemental irrigation	Rehamna region, Morocco	Physiological and morphological traits in plants, yield, and its components	n. d.	0.08-0.84 t/ha	Taaime et al. (2022)
9 novel quinoa genotypes and 1 commercial cultivar Regalona Baer	Field with full and reduced irrigation	Atacama Desert	Physiological and morphological traits, thermal infrared, and hyperspectral imaging	n. d.	2.45-3.24 t/ha	Dumschott et al. (2022)
15 quinoa varieties and 5 breeding lines	Eastern lowland region and Highland region, exp. fields	Rwanda	Emergency, Days to flowering, Days to maturity, PH, yield	n. d.	Min: 0.14 t/ha QuF9P1-20 Max: 3.00 t/ha NL-6	Habiyaremye et al. (2022)
30 quinoa accessions	Greenhouse	Tunja, Columbia	12 qualitative and 9 qualitative traits	n. d.	n.d.	Manjarres-Hernandez et al. (2021)
13 quinoa commercial or selected varieties	Field experiments	North-West European, Melle, Belgium	Seed characteristics	Chemical composition of seeds	Min: 0.47 t/ha Atlas, Pasto Max: 3.42 t/ha Vikinga,Titicaca	De Bock et al. (2021b)
Cultivars Regalona, Puno, titicaca, Vikinga, Q3, Q5	Field experiments under irrigation	Zamadueñas, Spain	SW, area, viability, color, and germination rate, grain yield	Saponin content, protein content, AA, mineral content, FRAP, TPC, TFC	Min: 0.70 t/ha Vikinga Max: 3.25 t/ha Q3 cultivar	Granado-Rodriguez et al. (2021a)

(Continued)

Table 2.2 (Continued)

Plant material	Growing condition	Locality, country	Agro-morphological evaluation	Biochemical markers	Seed yields	Reference
Jessie, Marisma, Roja, Duquesa, Pasto	Field experiments under irrigation	Southwestern Spain	Above-ground biomass, HI, seed yield, 1000-SW, nutrient uptake	Moisture, fat, total dietary fibre, PC, carbohydrate, mineral, ash contents	Min: 1.58 t/ha Roja Max: 3.04 t/ha Marisma	Matías et al. (2021)
Regalona, AG 2010, Cauhil, Morado	Field experiments under 5 irrigation treatments	Diguillín Province, Ñuble Region, Chile	Seed yield, seed yield efficiency	PC, globulin and albumin yield, and technical efficiency	Min: 0.41 t/ha Morado Max: 3.35 t/ha Cahuil	Pinto et al. (2021)
KVL-SRA2, Chipaya, Q-37	Field experiment	Cairo, Egypt	Plant growth performance, leaf pigment	PC, ash, fat, dietary fibre, total carbohydrate content, total saponins, and tannins, TPC, TFC	Min: 1.20 t/ha KVL-SRAZ Max: 2.40 t/ha Q-37	El-Serafy et al. (2021)
14 genotypes	Field experiments	Rabat, El Kbab, Meknes, Berrechid, Tinejdad, Morocco	Germination rate, seed size and yield, PH, stem diameter, dry matter, HI, 1000-SW, *Peronospora farinosa* sensitivity	n. d.	Min: 0.00 t/ha Amarilla de Marangani Max: 7.83 t/ha SW2	Thiam et al. (2021)
Q5 variety	Three levels of salinity, greenhause	Karakalpakstan, Uzbekistan	PH, shoot lengths, panicle weight, seed yield, 1,000-SW	PC, AA content, oil content, FA content, Element content	n.d.	Toderich et al. (2020)
6 quinoa accessions	Field experiments	Northern Israel	Biomass and seed characterization	Chemical composition of seeds and biomass	Min: 1.54 t/ha accession 5 Max: 6.36 t/ha accession 4E	Asher et al. (2020)

(Continued)

Table 2.2 (Continued)

Plant material	Growing condition	Locality, country	Agro-morphological evaluation	Biochemical markers	Seed yields	Reference
6 quinoa genotypes - Q18, Q21, Q22, Q29, AMES 13761, NSL 106398	Field experiments - salinity treatments, drip irrigation	Dubai, United Arab Emirates	Various morphological traits of plants, seed yield, yield stability, HI	Protein content	Min: 1.27 t/ha Q21 genotype Max: 2.30 t/ha Q18 genotype	Hussain et al. (2020)
Q5 variety	The circular drainable lysimeters	Semi-arid area with a warm climate, Bahgar, Iran	Crop evapotranspiration, grain yield, biomass, water productivity	n. d.	n.d.	Ahmadi et al. (2019)
Different varieties	Field	24 provinces of China	Grain yield	Protein content	Min: 1.48 t/ha Longli Max: 5.27 t/ha Qingli-1	Yang et al. (2019)
Cultivar Regalona, Salcedo-INIA, Titicaca	Rainfed field experiments	El Pobo, Teruel, Spain; Arequipa, Peru; Río Hurtado, Chile	Grain yield, SW per plant, HI, PH, SD, PL and diameter, plant weight, days to flowering and maturity	Mineral composition, phytate content, PC, AA, FRAP, fibre, and saponin content	Min: 1.53 t/ha Titicaca Max: 5.17 t/ha Salcedo	Reguera et al. (2018)
Jessie, Titicaca, Puno, Zeno	Field experiments	Southwestern Germany	Soil mineral content, grain yield, 1000-SW,	Total PC, lipid and saponin content, FA and AA profile	1.73–2.43 t/ha	Prager et al. (2018)
Commercial genotype Regalona and 1 quinoa accessions	Three thermal treatments (increased night temperatures), exp. fields	Valvidia, Chile	Physiological and morphological traits, grain yields, chlorophyll content, water-soluble carbohydrates, grain PC	n. d.	Min: 2.93 t/ha Accession Max: 6.00 t/ha Regalona	Lesjak and Calderini (2017)

(Continued)

Table 2.2 (Continued)

Plant material	Growing condition	Locality, country	Agro-morphological evaluation	Biochemical markers	Seed yields	Reference
$F_{2:5}$ population	Field experiments	Coastal environment, Rabat, Morocco	16 qualitative and 7 qualitative traits	n. d.	n. d.	Benlhabib et al. (2016)
10 landraces, 8 varieties under development,3 registered varieties	2-5 sites in nine countries	North Africa, the Near East, Asia	19 morphological and phenological traits	n.d.	Min: 0.20 t/ha Sajama Iranshar Max: 2.05 t/ha Titicaca	Bazile et al. (2016b)
10 quinoa cultivars	Field experiments under irrigation	Encalilla, North Western Argentina; Altiplano, Bolivia	Root, aerial, and seed biomass, plant height, grain yield	AA composition, total protein content	Min: 0.38 t/ha Samaranti Max: 3.86 t/ha Sayaňa	Gonzalez et al. (2012)
Titicaca	Field experiment under three salinity treatments and three irrigation regimes	Southern Italy	Seed yield, climatic conditions, soil water content, electrical conductivity	Saponin content, carbohydrate, protein, oil, ash, and dietary fibre content	Max: 2.3 t/ha Min: 2.7 t/ha	Pulvento et al. (2012)

* HI = harvest index
* PC = protein content
* PH = plant height
* PL = panicle length
* SD = stem diameter
* TPC = total phenolic content
* TFC = total flavonoid content
* 1000-SW = weight of thousand seeds

n. d. – not defined

The considerable variability in yield for different quinoa genotypes in the different environments was confirmed outside of the Andean region. The lower yields were observed at 0.08 t/ha in Morocco (Taaime et al., 2022) and the highest at 7.83 t/ha (Thiam et al., 2021), also in Morocco. The range of yield in experimental fields of the Czech Republic in 2018–2021 was estimated between 0.12 and 3.99 t/ha (unpublished data). Observed yield levels in Northern Europe were between 1–3 t/ha (Pulvento et al., 2012; Jacobsen, 2017; Prager et al., 2018; De Bock et al., 2021b; Granado-Rodriguez et al., 2021b; Matías et al., 2021).

As suggested previously, a range of factors may affect production, including the choice of cultivars, optimal sowing date, and nutrient availability (Choukr-Allah et al., 2016). Grain yield is further influenced by the cultivation conditions, therefore there is a need to evaluate the varietal grain yield stability across contrasting environments and even different growing seasons (Thiam et al., 2021). In testing of 20 quinoa genotypes in two different environments in Rwanda, it was observed that low water availability affected the growth and yield of quinoa (Habiyaremye et al., 2022). In contrast, the local landrace Cahuil cultivated in Chile had the best seed yield under water stress (Pinto et al., 2021). Similar results were reported by Pathan et al. (2023) for 10 accessions grown under different environments and locations in the USA.

Choukr-Allah et al., (2016) reported that the salinity may promote the growth of quinoa but up to a certain threshold, beyond which growth and productivity start to be negatively affected. For example, the genotype ′Titicaca′ (originating from the Andes) showed a good adaptation to the Mediterranean environment with tolerance to salinity and drought (Pulvento et al., 2012). On the other hand, high salinity can reduce the yield significantly and further change the nutritional composition of seeds (Hussain et al., 2020).

Rising temperatures are challenging for quinoa as well as for other crops. High temperatures during flowering and heat stress during the vegetative stage in certain quinoa varieties considerably lowered yield (Matías et al., 2021). In the growing conditions of Chile, the influence of increased night temperature on quinoa plants was evaluated (Lesjak and Calderini, 2017). Grain yields were reduced in the range of 12–31% by increased night temperatures. Similarly, the aboveground biomass was affected negatively.

As concluded by Taaime et al. (2022), optimal conditions contributing to better growth and the highest yield in quinoa include a temperature range between 10– 25°C, high and well-distributed precipitation, and short photoperiods. The susceptibility of quinoa to high temperatures (above 32°C) was reported due to

the flower closing during the day and limited pollination caused a reduction of the yield by up to 86% (Tovar et al., 2020). In some regions of southeast China, the combination of high temperatures and heavy rainfalls had negative effects on the growth of quinoa. Fortunately, quinoa germplasm collected from Taiwan showed resistance to high temperatures and heavy rainfalls (Yang et al., 2019).

Nonetheless, the establishment of quinoa in many agronomical areas outside South America is still, unfortunately, relatively limited. It could be considered that the quinoa cultivar selection process remains unfinished for new cultivation areas, including those located in southern Europe which are characterized by having intense precipitations at early growth stages and high temperatures at later stages of crop development (Granado-Rodriguez et al., 2021b). There is still very limited information regarding the stability of seed nutritional characteristics under changing environments (Granado-Rodriguez et al., 2021b).

As with any other new crop, one of the key factors for the successful introduction and establishment of quinoa under new climatic conditions will be the identification of appropriate planting material. Therefore, it is important to study the adaptation and yield of several potential quinoa genotypes from different provenances to select the most promising ones suitable for the local agro-climatic conditions (Choukr-Allah et al., 2016). Not only should adaptation of quinoa be discussed, but also sustainable establishment in a new environment.

2.7. Nutritional characteristics of quinoa seeds

Quinoa has outstanding nutritional value in all its edible parts – seeds and leaves, which were recognized even by ancient populations that considered quinoa a sacred food (Jacobsen et al., 2003). Quinoa seeds are a superior source of vitamins, minerals, dietary fiber, and lipids with the presence of health-beneficial polyunsaturated fatty acids (Repo-Carrasco et al., 2003). As reported by Schlick and Bubenheim (1996), quinoa is one of the single food sources that can supply all essential macro and micronutrients needed for balanced human nutrition.

2.7.1. Carbohydrates, starch, and total dietary fiber

Quinoa seeds contain a relatively variable amount of carbohydrates in their seeds. The lowest content was reported in the variety ´Roja´, reaching 41.52% in fresh weight (Gomez et al., 2021). Additionally, the lowest carbohydrate content expressed in dry weight was reported by Ferreira et al. (2015), reaching

43.64%. Conversely, the highest value (82.89% in DW) was found in accessions cultivated in Peru (Encina-Zelada et al., 2017). As summarized in Table 2.3, there are significant differences in carbohydrate content in various genotypes. For example, Miranda et al. (2012) detected higher carbohydrate content in Chilean highland ecotypes as opposed to southern ecotypes. Pereira et al. (2019) reported higher mean carbohydrate content in black and white varieties but lower in red varieties. In spite of that, many other variables modify total carbohydrate content, such as environmental conditions and sowing date. For example, in sea level genotypes and one cross genotype cultivated in Argentina, winter sowing at 18°C resulted in expanded seed weight, and therefore higher carbohydrate content in seeds (Curti et al., 2018).

In terms of environmental influence, increased carbohydrate content was reported for lowland/coastal quinoa genotypes ´Regalona Baer´ and ´Villarrica´ in arid conditions with lower soil organic matter content and a mean temperature of approximately 18°C during the growing season (Miranda et al., 2013). Experiments conducted with genotypes cultivated in Spain resulted in decreased carbohydrate content in a growing season with a mean temperature of approximately 25°C, in contrast to a growing season with a mean temperature lowered by 5°C (Matías et al., 2021). This was also supported by Garcia-Parra et al. (2022), indicating the highest carbohydrate content (65.5%) in cultivars grown in a cold climate. There were also significant differences in carbohydrate content reported in irrigated and drought conditions (Pathan et al. 2023). While high carbohydrate content could be beneficial for some food applications, it negatively affects the total protein content in quinoa seeds (Craine and Murphy, 2020; De Bock et al., 2021a, b).

The most prevailing fraction of quinoa carbohydrates is starch, situated primarily in the perisperm, in contrast to the cereals (Burrieza et al., 2014). The minimal value for starch content was 44%, found in genotype ´Cica´ cultivated in Argentina (Jimenez et al., 2019), whereas the most abundant starch content of 72.5% was described by De Bock et al, (2021b) in genotype ´Titicaca´ grown under North-West European field conditions. Nonetheless, the values for starch content varied between different years of field experiments in the mentioned study. Similarly, Grimberg et al. (2022) characterized the genotype ´Titicaca´ as one with the most prominent starch content. Aluwi et al. (2017) evaluated the maximal starch content in genotype ´CO 407D´ (64% in DW) and the lowest for ´UDEC-1´ (55%), both cultivated in the USA.

Table 2.3 Variability of the carbohydrate content in quinoa seeds divided according to the genotype name and seed color

	Sample genotype	Seed color	Production area	Carbohydrate content	Reference
Genotype name	Highland ecotypes: Ancovinto, Cancosa Central ecotypes: Cahuil, Faro Southern ecotypes: Regalona, Villarrica	n. d.	Chile	Min: 56.54 [1] Villarrica Max: 68.12 [1] Ancovinto	Miranda et al. (2012)
	n = 78 accessions	n. d.	Bolivia Brazil Peru	Min: 43.64 [1] Max: 76.37 [1]	Ferreira et al. (2015)
	n = 77 accessions	Beige Black Orange Yellow	Peru	Min: 78.48 [1] Max: 82.89 [1]	Encina-Zelada et al. (2017)
	Real	n. d.	Colombia	68.30 [1]	Contreras-Jimenez et al. (2019)
	Cica Kamiri Inga Pirca		Argentina	Min: 72.81 [2] Inga Pirca Max: 74.74 [2] Kamiri	Contreras-Jimenez et al. (2019)
	F5:F6 advanced breeding lines Cherry Vanilla CO407 Dave, Kaslaea	n. d.	USA	Min: 69.56 [2] Max: 74.00 [2]	Craine and Murphy (2020)
	Atlas, Jessie Marisma, Pasto Pot_4, Roja	n. d.	Spain	Min: 41.52 [3] Roja Max: 52.62 [3] Pasto	Gomez et al. (2021)

(Continued)

Table 2.3 (Continued)

	Sample genotype	Seed color	Production area	Carbohydrate content	Reference
Genotype name	Iniap Tunkahuan	n. d.	Ecuador	60.37 [1]	Villacres et al. (2022)
	Blanca real Nariño Pasankalla Soracá Puno Titicaca	n. d.	Colombia	Min: 56.00 [1] Puno Max: 70.66 [1] Pasankalla	Garcia-Parra et al. (2022)
Seed color	Commercial – unknown (n = 29) Blanca Kancolla Blanca Hualhuas Negra Collana Negra Pasankalla Pasankalla Roja Pasankalla Rosada de Huancayo Salcedo INIA	Black Red White	Peru Spain	Min: 75.3 [2] Red quinoa Max: 77.0 [2] White quinoa	Pereira et al. (2019)

[1] The results are expressed as %
[2] The results are expressed as g/100 g of dry weight
[3] The results are expressed as g/100 g of fresh weight
Max – maximum value; Min – minimum value; n. d. – not defined

Quinoa starch is rich in polysaccharide amylopectin, which represents 54–85% of DW (Dong et al., 2021; Kheto et al., 2022). Amylose content is, on the other hand, relatively low. It ranges from approximately 6% in ′Tianjing Tibet Quinoa′ (Li and Zhu, 2017) up to 20% in the Argentinian variety ′Jujuy′ (Nascimento et al., 2014). Specific starch and amylopectin structures give quinoa starch various functional properties that can be used in a wide range of food products (Li et al., 2016; Aluwi et al., 2017; Li and Zhu, 2017). Nevertheless, climatic conditions during the growing season may alter final functionality, even though starch biosynthesis is determined primarily by genetics (Garcia-Parra et al., 2021, 2022). Additionally, seed color seems to correlate with starch physiochemical properties, as reported by Peng et al. (2022), in opposition to Li et al. (2016), describing no correlation between the seed color and starch characteristics.

Total dietary fiber (TDF) content in quinoa is also highly heterogeneous, ranging from approximately 7% (De Bock et al., 2021a) up to 23% (Granado-Rodriguez et al., 2021b). The variation can be explained by the genotype effect (Curti et al., 2018), but also by growing conditions since fiber content can be enhanced under saline conditions (Pulvento et al., 2012) and high temperatures during the grain filling period (Matías et al., 2021). Negative correlations were found between TDF, carbohydrate, and fat content (Vidueiros et al., 2015). Overall, high amounts of TDF (over 18% TDF) were found in genotypes ′Rainbow′, ′Faro′, ′Baer′, and ′Colorado 407D′ cultivated in Poland (Sobota et al., 2020), ′Titicaca′ grown in Italy (Pulvento et al., 2012), and ′Roja′ and ′Duquesa′ grown in Spain (Matías et al., 2021). Less prominent amounts (below 14% TDF) were presented in ′Faro Red′, ′Puno′ (Sobota et al., 2020), ′Pasto′, (Matías et al., 2021), white Bolivian and Peruvian quinoas (Pellegrini et al., 2018), and genotypes ′Cica′, ′Kamiri′, and ′Inga Pirca′ (Jimenez et al., 2019).

Although the TDF values in quinoa may be comparable to that of cereal grains, the fiber composition of quinoa resembles that of leguminous seeds, fruits, or vegetables rather than typical cereals. As described by Lamothe et al. (2015), insoluble fiber comprises 78% of TDF and approximately 22% of TDF constitutes soluble fiber. TDF in quinoa is composed of pectic polysaccharides and xyloglucans in varying amounts and structures depending on the fiber fraction. The insoluble fraction of dietary fiber encompass homogalacturonans interspersed with rhamnogalacturonan-I stretches, branched xyloglucans, and cellulose, whereas the soluble fraction constitutes homogalacturonans and arabinans, with xylose present in smaller proportions. The composition of

quinoa TDF is primarily formed by galactose, arabinose, and galacturonic acid (Lamothe et al. 2015; Liu et al. 2020b). On the other hand, Pedrali et al. (2023) reported uronic acid, glucose and arabinose as three main components in quinoa TDF, however, their specific content significantly varied among different quinoa cultivars.

Despite extensive research on quinoa's nutritional profile, the specific composition of its sugars is often underexplored, with most sources documenting only general, common sugars (Pereira et al., 2019; Tan et al., 2021; Gómez et al., 2021). However, recent work by Song and Peng (2024) identified 33 distinct sugars across three quinoa varieties, with 25 reported for the first time. Notable sugars such as D-talose, levoglucosan, 6-deoxy-D-glucose, and gentiobiose showed significant variation among the cultivars.

2.7.2. Protein content and amino acid composition

Quinoa is primarily prized for its protein, with the content ranging between 7.47% in DW and 20.80% in DW (Graf et al. 2016; Gargiulo et al., 2019). The protein in quinoa seed is predominantly localized within the embryo in the amount of approximately 23.5%. Hence, a high correlation was detected between embryo weight ratio and protein content (Gargiulo et al., 2019). Additionally, a lesser proportion of protein is presented in the perisperm, estimated at 7.2% (Ando et al., 2002).

Variations in protein content were significant in several genotypes cultivated in distinctive agroecological conditions. For example, the cultivar ´Jessie´ originating in France was cultivated in Belgium and reached almost 19% protein content (De Bock et al., 2021b), whereas the same genotype cultivated in Germany reached a protein content of approximately 12% (Prager et al., 2018). Nevertheless, ´Jessie´ cultivated for two years in southwest Spain showed a steady mean protein content of 16.7% (Matías et al., 2021).

Table 2.4 Variability of protein content in quinoa seeds divided according to the genotype and seed color

	Sample genotype	Seed color	Production area	Protein content	Reference
Genotype name	Highland ecotypes: Ancovinto, Cancosa Central ecotypes: Cahuil, Faro Southern ecotypes: Regalona, Villarrica	n. d.	Chile	Min: 11.13 [1] Cahuil Max: 16.18 [1] Villarrica	Miranda et al. (2012)
	Breeding line AG2010 B080, Regalona	n. d.	Chile	Min: 17.40 [2] Max: 18.90 [2]	Escuredo et al. (2014)
	Jujuy Salta	n. d.	Portugal	Min: 12.20 [5] Jujuy Max: 16.30 [5] Salta	Mota et al. (2016)
	n = 12 accessions	Cream Grey Orange Yellow	Peru	Min: 13.58 [1] Quillahuaman INIA, cream Max: 17.83 [1] Pasankalla, grey	Apaza et al. (2015)
	n = 9 commercial varieties Ancovinto Blanco Ancovinto Roja, Cancosa Socaire, Cáhuil Faro, Regalona Villarrica	Black Red White	Bolivia Chile Ecuador USA	Min: 7.47 [2] Kalustyan´s Black, Peru Max: 15.73 [2] Wegman´s Red, Bolivia/Peru	Graf et al. (2016)
	n = 28 accessions	n. d.	USA	Min: 13.00 [1] CO 407D WMF Max: 15.8 [1] QuF9P39-64	Aluwi et al. (2017)
	n = 77 accessions	Beige Black Orange Yellow	Peru	Min: 8.33 [1] Max: 11.38 [1]	Encina-Zelada et al. (2017)

(Continued)

Table 2.4 (Continued)

	Sample genotype	Seed color	Production area	Protein content	Reference
Genotype name	Kvl-sra2 Kvl-sra3 Regalona Q37, Q52	n. d.	Egypt	Min: 12.03 [2] Kvl-sra3 Max: 19.03 [2] Kvl-sra2	Saad-Allah and Youssef (2018)
	Titicaca	n. d.	Ethiopia	13.57 [2]	Agza et al. (2018)
	Jessie Puno Titicaca Zeno	n. d.	Germany	Min: 16.10 [1] Zeno Max: ≈ 12 [1] Jessie	Prager et al. (2018)
	Regalona Salcedo-INIA Titicaca	n. d.	Chile Peru Spain	Min: ≈ 14 [1] Salcedo, Peru Max: ≈ 17 [1] Regalona, Chile	Reguera et al. (2018)
	Altiplano Pasankalla Regalona Titicaca	n. d.	n. d.	Min: 15.40 [1] Titicaca Max: 20.80 [1] Altiplano	Gargiulo et al. (2019)
	n = 25 accessions	n. d.	Poland	Min: 12.40 [2] Q629, USA Max: 15.98 [2] Faro, Argentina	Sobota et al. (2020)
	F5:F6 advanced breeding lines Cherry Vanilla CO407 Dave Kaslaea	n. d.	USA	Min: 10.04 [3] Max: 13.68 [3]	Craine and Murphy (2020)
	Puno Titicaca	n. d.	Morocco	Min: 13.41 [3] Puno Max: 13.43 [3] Titicaca	Mhada et al. (2020)

(Continued)

Table 2.4 (Continued)

	Sample genotype	Seed color	Production area	Protein content	Reference
Genotype name	Q5	n. d.	Uzbekistan	14.40 [3]	Toderich et al. (2020)
	n = 13 accessions	Dark White	Belgium	Min: 12.10 [2, a] Oro de Valle Max: 18.80 [2, a] Jessie	De Bock et al. (2021b)
	IC341709 IC329184 IC507733 IC107299 NIC22513 NIC22506	n. d.	India	Min: 14.10 [1] IC341709, IC507733 Max: 15.40 [1] IC329184, NIC22506	Ghumman et al. (2021)
	Puno Q3 Q3 Regalona Titicaca Vikinga	n. d.	Spain	Min: 13.80 [1] Max: 19.10 [1]	Granado-Rodriguez et al. (2021a)
	n = 14 accessions	Dark White	Spain	Min: ≈ 9 [1] A-SE-06, white Max: ≈ 16.50 [1] A-SE-15, dark	Granado-Rodriguez et al. (2021b)
	Gannan Geermu Haili	n. d.	China	Min: 11.60 [1] Geermu Max: 12.60 [1] Haili	Jiang et al. (2021)
	Duquesa Jessie Marisma Pasto Roja	n. d.	Spain	Min: 13.20 [1] Roja Max: 20.40 [1] Duquesa	Matías et al. (2021)

(Continued)

Table 2.4 (Continued)

	Sample genotype	Seed color	Production area	Protein content	Reference
Genotype name	Atlas, Jessie Marisma, Pasto Pot_4, Roja	n. d.	Spain	Min: 15.59 [4] Pasto Max: 18.73 [4] Atlas	Gomez et al. (2021)
	Blanca Real, Nariño Pasankalla, Soracá Puno, Titicaca	n. d.	Colombia	Min: 12.36 [1] Soracá Max: 16.56 [1] Titicaca	Garcia-Parra et al. (2021)
Seed color	Bolivian quinoa (BQ) Peruvian quinoa (PQ) Spanish quinoa (SQ)	Black Red White	Bolivia Peru Spain	Min: 11.62 [4] SQ, white Max: 13.66 [4] BQ, white	Pellegrini et al. (2018)
	Commercial – unknown (n=29) Blanca Kancolla Blanca Hualhuas Negra Collana Negra Pasankalla Pasankalla Roja Pasankalla Rosada de Huancayo Salcedo INIA	Black Red White	Peru Spain	Min: 14.4 [2] White quinoa Max: 15.6 [2] Red quinoa	Pereira et al. (2019)
	n. d.	Black Yellow	Peru	Min: 16.20 [1] Black quinoa Max: 18.70 [1] Yellow quinoa	Sanchez-Resendiz et al. (2019)

[1] The results are expressed as % of dry weight
[2] The results are expressed as g/100 g of dry weight
[3] The results are expressed as g/100 g of sample
[4] The results are expressed as g/100 g of fresh weight
[5] The results are expressed as g/100 g of the edible portion on a fresh weight basis
[a] The protein content per variety averaged over the different years of field trials
Max – maximum value; Min – minimum value; n. d. – not defined

The Danish-bred cultivar ´Titicaca´ was analyzed in at least 10 studies under distinctive environmental conditions. Despite that, this genotype reached analogous values (13–15%) in the cultivation conditions of Ethiopia (Agza et al., 2018), Morocco (Mhada et al., 2020), Belgium (De Bock et al., 2021b), USA (Aluwi et al., 2017), and Germany (Prager et al., 2018). Besides, slightly higher protein content (above 15%) was observed under cultivation in Poland (Sobota et al., 2020) and Colombia (Garcia-Parra et al., 2022). In addition, Reguera et al. (2018) reported higher protein content for ´Titicaca´ cultivated in Chile compared to Spain, which follows the results of Granado-Rodriguez et al. (2021a), reaching comparable values in mean protein content averaged for three cultivation years.

Genotype ´Regalona´, originating in southern regions of Chile, was described in at least eight studies. The values of protein content were quite inconsistent. Miranda et al. (2012), Graf et al. (2016), and Granado-Rodriguez et al. (2021a) detected protein content reaching approximately 13–15% for ´Regalona´ cultivated in Chile and Spain, whereas other authors achieved higher values of approximately 17% under field experiments in Chile and Egypt (Lesjak and Calderini, 2017; Reguera et al., 2018; Saad-Allah and Youssef, 2018). Even higher values were achieved by Gargiulo et al. (2019) (18.3%); however, the authors did not specify the cultivation location.

The protein content of the Danish cultivar ´Puno´ was described in at least seven studies. The majority of the results were quite consistent in diverse environments (USA, Germany, Poland, Belgium, Colombia), ranging between 13 and 15% (Aluwi et al., 2017; Sobota et al., 2020; De Bock et al., 2021b; Garcia-Parra et al., 2022). On the other hand, (Garcia-Parra et al., 2021) evaluated reduced protein content, reaching almost 12% in ´Puno´ cultivated in Colombia. Although the Peruvian genotype ´Pasankalla´ was tested in at least 4 studies, the referred values of protein content are quite distant. Apaza et al. (2015) and Gargiulo et al. (2019) discovered protein content of 18.73–20.60%, while Garcia-Parra et al. (2021) and Garcia-Parra et al. (2022) achieved lower values (14.5–15.5%, respectively) during experiments conducted in Colombia.

There are many factors affecting the resulting protein content. Besides the influence of genotype, the importance of soil matric potential (SMP) and nitrogen fertilization was indicated (Wang et al., 2020). High SMP values (over −55 kPa) cause significant water stress and may also limit nitrogen uptake, which concurs with other studies (Sun et al., 2014; Walters et al., 2016).

Therefore, to reach optimal protein content, irrigation is crucial for some genotypes cultivated in adverse soil-water conditions (Pathan et al. 2023),

although slight water stress may enhance protein content (Wang et al., 2020). The intense application of nitrogen from 80 to 240 kg/ha increased protein content by approximately 1.5%. The positive effect of nitrogen fertilization was also presented by Wu et al. (2016) and Jacobsen and Christiansen (2016).

In addition, protein content in quinoa rises under salinity treatment, which was reported for varieties ´CO407D´, ´UDEC-1´, ´Baer´, ´QQ065´ (Wu et al., 2016), and ´NSL106398´ (Hussain et al., 2020). In contrast, Ruiz et al. (2016) expressed a drop in protein content by 7–12% in coastal lowland Chilean landraces (´VI-1´, ´Villarrica´) and genotype ´R49´ (Salares ecotype). In terms of temperature influence, protein content under heat stress was outstanding in varieties ´Pasto´, ´Marisma´, ´Jessie´, ´Roja´, and ´Duquesa´ (Matías et al., 2021). Garcia-Parra et al. (2022) detected higher mean protein values for cultivation in the cold climate of Colombia, compared to temperate and warm conditions; but, as reported by the authors, protein content was not rapidly affected by elevated temperatures. The exception in this paper was the cultivar ´Pasankalla´, showing a decline in protein content in hotter conditions.

Probably even more important than overall protein content is the quality of protein, given by the composition of essential amino acids (EAA). Quinoa protein generally contains all EAAs and several authors throughout the literature have concluded that quinoa protein is complete due to the superior composition of amino acids (AA) (Nowak et al., 2016; Maradini et al., 2017; Schmidt et al., 2021).

Nonetheless, Craine and Murphy (2020) argued that many of those studies evaluated outdated daily requirements or considered AA requirement values only for adults, not for children, whose requirements for EAAs are greater, as estimated by WHO/FAO/UNU (2007). The authors further stated that the quinoa protein is only “nearly complete”. Regarding this statement, Boye et al. (2012) labeled valine and lysine as limiting AA for children up to the age of 10 years. In comparison, Gonzalez et al. (2012) suggested lysine, tyrosine, and tryptophan as limiting AA for the age group of 2–5 years. Craine and Murphy (2020) identified low leucine content, which does not achieve the recommended daily requirements for infants and children, therefore considering it as limiting AA. Recently, Craine et al., (2023) reported that 48.6% of studied genotypes (n = 360) met adult requirements, but only two samples (´Moroccan Yellow´ and ´Ames-13733´) met all EAA requirements for all age groups.

With regards to the previously mentioned limiting AAs, several genotypes accomplished the daily requirements for EAAs in infants and children.

Sufficient lysine content (over 5.7 g/100 g protein) was identified in genotypes ´Jessie´, ´Pasto´, and ´CICA´ (Table 2.5). Valine content (over 4.3 g/100 g protein) was satisfactory in genotypes ´Ancovito´, ´CICA´, ´Jessie´, ´Rouge Marie´, ´Zwarte´, and ´Roja´. Suitable leucine content (over 6.6 g/100 g protein) was found in genotypes ´Villarrica´, ´Rataqui´, ´Atlas´, and ´Jessie´. Tryptophan content (over 0.85 g/100 g protein) was met in genotypes ´Sajama´, ´B080´, ´Regalona´, ´Zeno´, ´Puno´, and in all genotypes analyzed by De Bock et al. (2021b) (Table 2.5).

Table 2.5 Minimum and maximum values of amino acid composition (g/100 g protein) in various quinoa genotypes and production areas

	Miranda et al. (2012)	**Gonzalez et al. (2012)** [a]	**Escuredo et al. (2014)** [a]	**Prager et al. (2018)**	**Wang et al. (2020)**	**De Bock et al. (2021b)** [a]	**Gomez et al. (2021)**
N. of accessions	6	10	3	4	6	12	6
Production area	Chile	Bolivia NW Argentina (A) Encalilla, Argentina (E)	Chile	Germany	China	Belgium	Spain
Growing seasons	2011	2007–2009	2010–2011	2015–2016	n. d.	2017–2019	2017
Histidine	2.70 Ancovinto, Cahuil 3.50 Villarrica	1.36 Sajama (E) 3.79 CICA (A)	1.71 Regalona 2.17 AG2010	1.33 Zeno (2015) 2.48 Puno (2016)	3.16 QWQ 3.70 QBQ	2.50 Bastille 3.20 Zwarte	3.67 Atlas 8.31 Roja
Isoleucine	2.90 Cahuil 3.80 Ancovinto	1.65 Chucapaca (E) 3.40 CICA (A)	0.75 Regalona 0.82 AG2010, B080	2.00 Zeno (2015) 3.19 Puno (2016)	2.80 QWQ 3.58 QBQ	3.90 Zwarte 4.80 Rouge Marie	3.75 Pot_4 4.61 Roja
Leucine	6.40 Cahuil 7.20 Villarrica	3.75 Sajama (E) 7.46 Ratuqui (E)	2.27 B080 2.52 Regalona	3.67 Zeno (2015) 5.55 Puno (2016)	5.07 QGQ 6.5 QBQ	7.00 Pasto 7.60 Atlas, Jessie	4.55 Pot_4 5.67 Pasto
Lysine	4.10 Cancosa, Cahuil 4.80 Villarrica	2.44 Sajama (E) 6.72 CICA (A)	2.35 AG2010 2.42 B080	2.77 Zeno (2015) 4.99 Puno (2016)	5.07 QWQ 6.02 SWQ	4.60 Rouge Marie 5.90 Pasto	5.40 Atlas 13.55 Jessie
Methionine	1.40 Ancovinto 1.90 Villarrica	0.73 Sajama (E) 1.87 CICA (A)	0.31 AG2010 0.69 Regalona	1.10 Zeno (2015) 1.80 Jessie, Puno (2016)	1.67 [b] QGQ 2.09 [b] SGQ, QBQ	2.00 Atlas 2.60 Puno	1.37 Pasto 1.64 Atlas

(Continued)

Table 2.5 (Continued)

	Miranda et al. (2012)	Gonzalez et al. (2012) [a]	Escuredo et al. (2014)[a]	Prager et al. (2018)	Wang et al. (2020)	De Bock et al. (2021b)[a]	Gomez et al. (2021)
N. of accessions	6	10	3	4	6	12	6
Production area	Chile	Bolivia NW Argentina (A) Encalilla, Argentina (E)	Chile	Germany	China	Belgium	Spain
Growing seasons	2011	2007–2009	2010–2011	2015–2016	n. d.	2017–2019	2017
Phenylalanine	3.90 Cancosa, Cahuil 4.50 Villarrica	2.26 Sajama (E) 4.55 CICA (A)	1.49 B080 1.54 AG2010	2.20 Zeno (2015) 3.55 Puno (2016)	2.62 [c] QGQ 3.70 [c] SWQ	3.60 Zwarte 4.50 Atlas	3.73 Atlas 4.81 Roja
Threonine	3.20 Cancosa 3.60 Faro	2.09 Sajama (E) 4.59 CICA (A)	5.53 B080 8.89 Regalona	2.13 Zeno (2015) 3.27 Puno (2015)	1.79 QGQ 2.15 SWQ	3.60 Atlas, Bastille, Rouge Marie 4.40 Zwarte	3.43 Atlas 7.82 Jessie
Tryptophan	n. d.	0.58 Chucapaca 1.05 Sajama	0.99 B080 1.07 Regalona	0.88 Zeno (2016) 1.11 Puno (2016)	n. d.	1.50 n = 5 accessions [b] 1.9 Bastille [b]	0.40 Pot_4 0.58 Atlas
Valilne	4.30 Regalona 4.90 Ancovinto	2.19 Chucapaca (E) 4.39 CICA (A)	1.83 AG2010 2.31 B080	3.80 Puno (2016) 5.67 Jessie (2016)	2.50 QWQ 3.58 QBQ	5.30 Bastille 6.40 Rouge Marie, Zwarte	3.76 Atlas 5.81 Roja

[a] Amino acid content per variety is averaged over the different years of field trials.
[b] Values are expressed for Methionine + cystine
[c] Values are expressed for Phenylalanine + tyrosine
n. d. – not defined; NW – Northwestern; QBQ – Big black quinoa; QGQ – Sanjiang Gray, gray quinoa; QWQ – Qingli No.1, white quinoa; SGQ – Aihua No.1, grey quinoa; SWQ – Jiaqi Diamond No.1, white quinoa

Overall, the remarkable variations in EAA composition might be caused by genotype, environment, and their interactions. According to De Bock et al. (2021b), the content of EAAs varied between growing seasons, but not between genotypes, in contrast to Prager et al. (2018), who noticed significant differences among genotypes and experimental years. Pathan et al. (2023) reported that there was no significant difference in all EAA except methionine and tryptophan, between the irrigated and drought environments.

In terms of cultivation area, Steffolani et al. (2016) pointed out that Bolivian varieties had higher EAA content than Peruvian varieties. Gonzalez et al. (2012) indicated dissimilarities in EAA content between two experimental sites with higher EAA content in the Bolivia/Argentina location, which authors then explain by adaptation of the genotypes to the conditions they were bred in. Reguera et al. (2018) noted that varieties grown in Chile did not exhibit inter-cultivar variations in AA content compared to the same varieties grown in Spain, except for cultivar ´Titicaca´ which had consistent AA content among varieties and locations.

Most of the EAAs were not negatively affected by salinity in ´Q5´, a new salt- and drought-tolerant line, except for tyrosine (Toderich et al., 2020). Aloisi et al. (2016) found variations in genotype response to saline conditions. EAAs remained constant or declined, except for increased methionine in genotype ´R49´, belonging to the group of Salares ecotype; and leucine in genotype ´Villarrica´ (coastal-lowland ecotype). A strong decline in EAAs under salinity treatment was detected in genotype VI-1 (coastal-lowland ecotypes).

An indispensable aspect of assessing protein is the digestibility of individual amino acids (FAO et al., 2013a). Nonetheless, the information about this parameter in quinoa is sparse and/or outdated in available scientific literature. Further, the available data are inherently non-comparable due to distinct methodological frameworks.

Shi et al. (2020) reported lower digestibility (measured by the *in-vitro* protein digestibility-corrected amino acid score – IV-PDCAAS) in cultivar ´NQ94PT´, compared to the commercial blend of cultivars ´Kankolla´ and ´Blanca Juli´. Further, Jimenez et al. (2019) reported quinoa *in-vitro* protein digestibility (using the AOAC 971.09 method) between ~61–63% in varieties ´Cica´, ´Kamiri´, and ´Inga Pirca´ obtained from Argentina. In addition, Craine and Murphy (2020) evaluated the protein digestibility corrected amino acid scores (PDCAAS) in varieties ´Colorado D407´ ranging from 0.74 to 0.90 and 0.78 to 0.95 for the 1–2 and 10-year-old children, respectively. To the authors' knowledge, there is no study applying the digestible indispensable amino acid

score (DIAAS), which is a recommended method for the measurement of protein digestibility by FAO et al. (2013a).

Since quinoa is not edible raw, it is essential to measure protein digestibility in processed samples rather than raw seeds. Previous studies have demonstrated that various heat processing methods (Rizzello et al., 2016; Lorusso et al., 2017; Dong et al., 2021; He et al., 2022) and germination (Jimenez et al., 2019) may improve the overall protein digestibility. On the other hand, digestibility is reduced by the presence of starch, fiber (Opazo-Navarrete et al., 2019), and various antinutritional compounds (Gilani et al., 2012).

2.7.3. Lipid content and composition

Lipid content in seeds is, among other factors, strongly affected by genotype (Curti et al., 2020; Garcia-Parra et al., 2022). Since the primary lipid storage is located in the embryo, embryo size may also correlate to overall seed lipid content (De Bock et al., 2021b). The highest lipid yield was described in the genotype ´Yellow Marangí´, cultivated in Peru, reaching almost 10% (Apaza et al., 2015), whereas the lowest lipid content reached nearly 3% in the quinoa variety ´QU5´, cultivated in Belgium (De Bock et al., 2021a) and commercial variety ´Gramolino´ from Ecuador (Graf et al., 2016) (Table 2.6). In addition, colored seed samples tend to exhibit higher lipid content than white seed samples (Pellegrini et al., 2018); yet Tang et al. (2015a) and Shen et al. (2022) obtained the opposite findings. Overall oil content was negatively correlated to protein content (Matías et al., 2021).

In terms of oil production, quinoa performed well in temperate climates since heat stress reduced average oil content by almost 30% (Garcia-Parra et al., 2022). Curti et al. (2018) found strong interactions between cultivar and sowing date, related to the various photo-thermal conditions during sowing. In a two-year experiment with cultivars ´Titicaca´ and ´Jessie´, stable results were achieved with a mean crude fat content of 7.5 and 7.3%, respectively (Prager et al., 2018). Unfortunately, there are only a small number of studies on quinoa oil production concerning meteorological conditions during the growing season and the adaptive response of the genotype. Nonetheless, the study of Pathan et al. (2023) indicates no statistical differences in crude fat content between irrigation and drought conditions.

Table 2.6 Variability of lipid content in quinoa seeds divided according to genotype name and seed color

	Sample genotype	Seed color	Production area	Lipid content	Reference
Genotype name	Highland ecotypes: Ancovinto, Cancosa Central ecotypes: Cahuil, Faro Southern ecotypes: Regalona, Villarrica	n. d.	Chile	Min: 5.57 [1] Villarrica Max: 7.06 [1] Cahuil	Miranda et al. (2012)
	n = 12 accessions	Cream Grey Orange Yellow	Peru	Min: 4.88 [1] Illpa Inia, cream Max: 9.78 [1] Yellow Maranganí, orange	Apaza et al. (2015)
	n = 9 commercial varieties Ancovinto Blanco Ancovinto Roja Cancosa, Socaire Cáhuil, Faro Regalona, Villarrica	Black Red White	Bolivia Chile Ecuador USA	Min: 2.93 [2] Gramolino, white, Ecuador Max: 5.62 [2] Ancovinto Roja, white, Chile	Graf et al. (2016)
	Ecologicos Quinoa Mum´s Original Heirloom Organic Quinoa Quinta Quinoa-BC12a Inca Gold Quinoa Vitabio Royal Quinoa Quinta Quinoa-BC12 Quinta Quinoa-BM12 Quinta Quinoa-Ch12 Quinta Quinoa-CVC12 GoGo Quinoa Red Organic Organic Garage Organic Red	Golden Red White	Bolivia Canada Unknown	Min: 6.03 [1] Mum´s Original Heirloom Organic Quinoa Max: 6.74 [1] GoGo Quinoa Red Organic Quinoa	Tang et al. (2016)

(Continued)

Table 2.6 (Continued)

	Sample genotype	Seed color	Production area	Lipid content	Reference
Genotype name	n = 28 accessions	n. d.	USA	Min: 5.08 [1] Blanca Max: 7.5 [1] Red Head	Aluwi et al. (2017)
	n = 77 accessions	Beige, Black Orange, Yellow	Peru	Min: 5.35 [1] Max: 7.78 [1]	Encina-Zelada et al. (2017)
	Kvl-sra2, Kvl-sra3 Regalona, Q37, Q52	n. d.	Egypt	Min: 6.20 [2] Q37 Max: 8.04 [2] Kvl-sra2	Saad-Allah and Youssef (2018)
	Jessie, Puno Titicaca, Zeno	n. d.	Germany	Min: 5.50 [1] Zeno Max: 7.50 [1] Titicaca	Prager et al. (2018)
	Titicaca	n. d.	Ethiopia	6.30 [2]	Agza et al. (2018)
	Cica, Kamiri Inga Pirca	n. d.	Argentina	Min: 6.53 [2] Kamiri Max: 7.48 [2] Cica	Jimenez et al. (2019)
	Amarilla de Maranganí Blanca de Juli, Roja Pasankalla Negra Collana	White Red Black	Peru	Min: 4.97 [1] Amarilla de Maranganí Max: 6.46 [1] Roja Pasankalla	Vera et al. (2019)
	F5:F6 advanced breeding lines Cherry Vanilla CO407 Dave, Kaslaea	n. d.	USA	Min: 4.56 [2] Max: 7.19 [2]	Craine and Murphy (2020)
	n = 13 accessions	Dark White	Belgium	Min: 5.42 [2, a] Pasto Max: 8.54 [2, a] Summer Red, dark	De Bock et al. (2021b)
	n = 7 commercial varieties	n. d.	Belgium Netherlands	Min: 2.74 [2] QU5 Max: 7.34 [2] n. d.	De Bock et al. (2021a)

(Continued)

Table 2.6 (Continued)

	Sample genotype	Seed color	Production area	Lipid content	Reference
Genotype name	IC341709, IC329184 IC507733, IC107299 NIC22513, NIC22506, IC415403	n. d.	India	Min: 7.50 [1] IC341709 Max: 8.70 [1] IC507733, IC107299	Ghumman et al. (2021)
	Gannan, Geermu, Haili	n. d.	China	Min: 4.00 [1] Haili Max: 5.21 [1] Gannan, Geermu	Jiang et al. (2021)
	Duquesa, Jessie Marisma, Pasto, Roja	n. d.	Spain	Min: 5.90 [1] Duquesa Max: 6.60 [1] Marisma	Matías et al. (2021)
	Atlas, Jessie, Marisma, Pasto Pot_4, Roja	n. d.	Spain	Min: 3.90 [3] Pot_4 Max: 5.21 [3] Marisma	Gomez et al. (2021)
	Blanca real, Nariño, Pasankalla, Soracá, Puno, Titicaca	n. d.	Colombia	Min: 5.77 Pasankalla Max: 7.50 Soracá	Garcia-Parra et al. (2022)
Seed color	n. d.	Black**,** Red White	South America	Min: 6.57 [1] Black quinoa Max: 7.17 [1] Red quinoa	Tang et al. (2015)
	Bolivian quinoa (BQ) Peruvian quinoa (PQ) Spanish quinoa (SQ)	Black, Red White	Bolivia Peru Spain	Min: 4.87 [3] BQ, white Max: 6.48 [3] BQ, red	Pellegrini et al. (2018)
	n = 29 commercial varieties Blanca Kancolla, Blanca Hualhuas Negra Collana, Negra Pasankalla Pasankalla, Roja Pasankalla Rosada de Huancayo Salcedo INIA	Black, Red White	Peru Spain	Min: 6.00 [2] White quinoa Max: 6.80 [2] Black quinoa	Pereira et al. (2019)

(Continued)

Table 2.6 (Continued)

	Sample genotype	Seed color	Production area	Lipid content	Reference
Seed color	n. d.	Black Red White	China Peru	Min: 5.68 [2] Black quinoa Max: 6.19 [2] White quinoa	Shen et al. (2022)
Production area	n. d.	n. d.	Argentina	6.31 [2]	Nascimento et al. (2014)
	n. d.	n. d.	Egypt	6.79 [1]	El-Sohaimy and Mehany (2015)
	n. d.	n. d.	China	Min: 5.61 [1] Max: 5.68 [1]	Wu et al. (2020)

[1] The results are expressed as %
[2] The results are expressed as g/100 g of dry weight
[3] The results are expressed as g/100 g of fresh weight
[a] The lipid content per variety averaged over the different years of field trials
Max – maximum value; Min – minimum value; n. d. – not defined

Quinoa lipid profile is composed predominantly of essential polyunsaturated ω-6 linoleic acid (C18:2). The minimum content of C18:2 reached 43% in accession ′CHEN414′ originating in dry valleys of North Argentina (Vidueiros et al., 2015), whereas the maximum content was measured in variety ′Temuko′ cultivated in the USA, reaching 63% (Chen et al., 2019). Quinoa oil also contains a relatively high volume of monounsaturated oleic acid (C18:1), reaching minimum values of 16% in the commercial variety ′Quinta Quinoa-BC12′ (Tang et al., 2016a) and maximum values of 33% in accession ′CHEN 465′ originating in the transition zone of Northwest Argentina (Vidueiros et al., 2015). Saturated palmitic acid (C16:0) was presented in 3.4–13% in genotype ′QuF9P39-73′ (Chen et al., 2019) and white quinoa genotypes (Tang et al., 2016a; Shen et al., 2022), respectively. A negative correlation was found between palmitic acid (C16:0) and oleic acid (C18:1), as reported by Chen et al. (2019).

Less abundant fatty acid in quinoa lipid profile is an essential ω-3 α-linolenic acid (C18:3), which reached 4–8% (Tang et al., 2016a; De Bock et al., 2021a,b; Shen et al., 2022); yet Vera et al. (2019) found values reaching up to 11% in yellow quinoa cultivar. Vidueiros et al. (2015) determined the range for a-linolenic acid as 3.2–9.4% for accessions ′CHEN 465′ and ′CHEN 60′, respectively. Quinoa oil also has several minor fatty acids, such as myristic acid (C14:0), stearic acid (C18:0), behenic acid (C22:0), gadoleic acid (C20:1), arachidonic acid (C20:4), and erucic acid (C22:1); however, those are presented only in negligible amounts (below 2%) (Tang et al., 2015a; De Bock et al., 2021b; Shen et al., 2022).

Several authors noticed variations in fatty acid profiles between varieties (Tang et al., 2016a; De Bock et al., 2021b; Shen et al., 2022), but Prager et al. (2018) did not report any significant alterations between varieties or years. Toderich et al. (2020) indicated changes in fatty acid composition in genotype ′Q5′ grown in saline soils. While the majority of fatty acids declined in medium salinity, the content of palmitoleic acid (C16:1) and arachidic acid (C20:0) was slightly raised. Besides that, the high mixed salinity of sodium chloride and sodium sulfate resulted in a significant increment of stearic acid (C:18:0). The authors also concluded that sulfate salinity affects the fatty acid composition more than the sodium chloride type of salinity.

Elevated temperature, together with cultivar-specific response, resulted in lower content of some fatty acids, especially oleic acid (C18:1), stearic acid (C18:0), gadoleic acid (C20:1), and behenic acid (C22:0) (Matías et al., 2021). In contrast, the content of linoleic acid (C18:2) increased or remained

unaffected in hot conditions in some cultivars (Curti et al., 2020; Matías et al., 2021). In terms of major fatty acid content, genotype ´Jessie´ with the shortest life cycle performed better in hot conditions compared to other genotypes. A very important role in quinoa oil quality is also played by optimal fertilization since correlations between some minerals and fatty acid content were observed by Matías et al. (2021).

Based on the available scientific literature, genotypes with black seeds tend to have higher polyunsaturated fatty acid (PUFA) content as opposed to genotypes with red or white seeds (Tang et al., 2015a; Pellegrini et al., 2018; Pereira et al., 2019; Shen et al., 2022). Moreover, the highest monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) content were present in red genotypes (Tang et al., 2015a; Pellegrini et al., 2018; Pereira et al., 2019; Vera et al., 2019), in contrast to Shen et al. (2022) who obtained opposed outcomes (Table 2.7).

The overall nutritional quality of oils is characterized by the ω-6/ ω-3 ratio, with an ideal composition of 1–4/1 in the human diet, as recommended by Simopoulos (2002). Nevertheless, the ω-6/ ω-3 ratio of quinoa did not meet the required values since it ranged from 4.7% in the variety ´Amarilla de Maranganí´ up to nearly 20% in the variety ´Negra Collana´ produced in Peru (Vera et al., 2019) (Table 2.7). Despite that, the fatty acid proportion and related nutritional quality are better than in amaranth with values reaching 33–69% (Tang et al., 2016a; Paucar-Menacho et al., 2018).

Table 2.7 Variability of lipid composition in quinoa seeds divided according to the genotype and seed color

	Sample genotype	Seed color	SFA (relative %)	MUFA (relative %)	PUFA (relative %)	ω-6/ω-3 (relative %)	Reference
Genotype name	Ecologicos Quinoa Mum´s Original Heirloom Organic Quinoa Quinta Quinoa-BC12a Inca Gold Quinoa Vitabio Royal Quinoa GoGo Red Organic Quinoa Organic Garage Red Quinoa Quinta Quinoa: BC12 BM12 Ch12 CVC12	Golden Red White	Min: ≈ 10 Ecologicos Quinoa Max: ≈ 12 Quinta Quinoa-BC12	Min: ≈ 20 Quinta Quinoa-BC12 Max: ≈ 33 GoGo Quinoa Red Organic Quinoa	Min: ≈ 52 Organic Garage Red Quinoa Max: ≈ 63 Quinta Quinoa-BC12	Min: 5.30 Quinta Quinoa-BM12 Max: 10.60 Mum´s Original Heirloom Organic Quinoa	Tang et al. (2016)
	n = 28 accessions	n. d.	Min: 3.30 CO 407 WMF Max: 9.10 QuF9P39-65	Min: 14.40 NL-7 Max: 28.30 UDEC2	Min: 36.70 NL-7 Max: 62.80 Temuko	n. d.	Chen et al. (2019)
	Amarilla de Maranganí Blanca de Juli Negra Collana Roja Pasankalla	Black Red White	n. d.	Min: ≈ 21 Amarilla de Maranganí Max: ≈ 34 Roja Pasankalla	Min: ≈ 55 Roja Pasankalla Max: ≈ 63 Amarilla de Maranganí	Min:4.68 Amarilla de Maranganí Max: 19.59 Negra Collana	Vera et al. (2019)

(Continued)

Table 2.7 (Continued)

	Sample genotype	Seed color	SFA (relative %)	MUFA (relative %)	PUFA (relative %)	ω-6/ω-3 (relative %)	Reference
Genotype name	n = 13 accessions	Dark White	Min: 10.20 Summer Red, dark Max: 13.40 Titicaca	Min: 18.10 Puno Max: 25.10 Vikinga	Min: 61.40 Vikinga Max: 70.60 Puno	Min: 6.70 Bastille Max: 12 Summer Red, dark	De Bock et al. (2021b)[a]
	Atlas, Jessie Marisma, Pasto Pot_4, Roja	n. d.	Min: 9.77 Jessie Max: 11.29 Pot_4	Min: 19.67 Marisma Max: 22.67 Roja	Min: 66.64 Pot_4 Max: 70.40 Jessie	Min: 7.03 Jessie Max: 8.92 Pasto	Gomez et al. (2021)
Seed color	n. d.	Black Red White	Min: 10.52 Black quinoa Max: 11.09 Red quinoa	Min: 29.88 Black quinoa Max: 33.29 Red quinoa	Min: 54.23 Red quinoa Max: 58.34 Black quinoa	Min: 5.62 White quinoa Max: 6.35 Red quinoa	Tang et al. (2015)
	Bolivian quinoa (BQ) Peruvian quinoa (PQ) Spanish quinoa (SQ)	Black Red White	Min: 10.66 BQ, black Max: 11.44 BQ, red	Min: 29.07 BQ, black Max: 33.28 BQ, red	Min: 55.28 BQ, red Max: 60.27 BQ, black	Min: 6.51 BQ, white Max: 11.42 PQ, white	Pellegrini et al. (2018)
	Unknown (n=29) Blanca Kancolla Blanca Hualhuas Pasankalla Roja Pasankalla Rosada de Huancayo Salcedo INIA Negra Collana Negra Pasankalla	Black Red White	Min: 27 Black, white quinoa Max: 29 Red quinoa	40 Black, red, white quinoa	Min: 31 Red quinoa Max: 33 Black, white quinoa	n. d.	Pereira et al. (2019)

(Continued)

Table 2.7 (Continued)

	Sample genotype	Seed color	SFA (relative %)	MUFA (relative %)	PUFA (relative %)	ω-6/ω-3 (relative %)	Reference
Seed color	n. d.	Black Red White	Min: 14.48 Black quinoa Max: 18.87 White quinoa	Min: 25.76 Red quinoa Max: 27.76 White quinoa	Min: 52.53 White quinoa Max: 56.87 Black quinoa	n. d.	Shen et al. (2022)

[a] Fatty acid composition per variety averaged over the different years of field trials
Max – maximum value; Min – minimum value; n. d. – not defined
MUFA – mono-unsaturated fatty acids
PUFA – poly-unsaturated fatty acids;
SFA – saturated fatty acids

2.7.4. Secondary metabolites and their biological effects

Quinoa exhibits a diverse array of secondary metabolites, categorized into five principal groups: phenolic acids, flavonoids, terpenoids, steroids, and nitrogen-containing metabolites (Lin et al., 2019). Nonetheless, the predominant compounds detected in quinoa belong to the group of phenolic acids and flavonoids (Tang & Tsao, 2017).

Phenolic acids represent a class of organic compounds distinguished by their characteristic benzene ring structure, which includes a carboxylic group and one or more hydroxyl and/or methoxyl groups. This class is further subdivided into two subgroups: hydroxybenzoic acids and hydroxycinnamic acids (Al Mamari, 2021). In quinoa, the most abundant representatives of the hydroxybenzoic group are benzoic acid, gallic acid, protocatechuic acid, vanillic acid, and syringic acid (Gawlik-Dziki et al., 2013; Tang et al., 2016b).

On the other hand, the hydroxycinnamic acid subgroup includes representatives such as caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, cinnamic acid, and sinapic acid in quinoa (Gawlik-Dziki et al., 2013; Paśko et al., 2008; Tang et al., 2016b). Metabolites from both groups were primarily detected in quinoa leaves and seeds, however, some were also isolated from quinoa sprouts (Lin et al., 2019) and they both exhibit diverse biological activities, including antimicrobial, antiviral, hepatoprotective, anti-inflammatory, anticancer, antioxidative, and anti-inflammatory effects (El-Hawary et al., 2016; Kiokias & Oreopoulou, 2021; Liu et al., 2020a).

As for flavonoids, they constitute a class of compounds characterized by a common structural motif consisting of two benzene rings linked by a pyrene ring (Caleja et al., 2017). Flavonoids are further classified into flavones, flavonols, flavanones, flavanols, and isoflavones based on variations in hydroxyl group positions, alkylation, and glycosylation patterns (Panche et al., 2016).

Flavones, including acacetin, isovitexin, orientin, and vitexin, have been predominantly identified in quinoa seeds, with the exceptions of isovitexin and vitexin, which were exclusively observed in sprouts (Paśko et al., 2008). Overall, 21 flavonols have been identified in quinoa, mainly in glycoside form, with representatives such as kaempferol, quercetin, rutin, and isorhamnetin (Gawlik-Dziki et al., 2013; Tang et al., 2015b). Flavanols, predominantly found in quinoa seeds, are represented by catechin, epicatechin, and epigallocatechin (Tang et al., 2015b; Tang et al., 2016b). Flavanones, such as hesperidin, neohesperidin, and naringin, are primarily located in quinoa seeds, with some detected in quinoa sprouts as well (Paśko et al., 2008; Tang et al., 2015b).

Isoflavones, including biochanin A, daidzein, and genistein, have been exclusively identified in quinoa seeds (Lutz et al., 2013; Tang et al., 2015b).

Flavonoids show strong antioxidative activity, especially the members of the flavones group (Panche et al., 2016). Additionally, they exhibit diverse biological activities, including anti-inflammatory, anti-cancer, anti-Alzheimer's disease, antibacterial, antituberculosis, and neuroprotective effects (Al-Khayri et al., 2022; Ayaz et al., 2019; Kopustinskiene et al., 2020; Rabaan et al., 2022; Shamsudin et al., 2022).

The total polyphenol content (TPC), total flavonoid content (TFC), and related antioxidant activity (AA) were evaluated in quinoa in several studies. Nonetheless, the values reported across the scientific literature were often extremely diverse and not very well comparable to each other due to the use of different solvents and extraction methods (Acosta-Estrada et al. 2014). Previous studies suggested a positive correlation between TPC, TFC, and AA (Pellegrini et al., 2018; Granado-Rodriguez et al., 2021). On the other hand, Antognoni et al., (2021) and Pedrali et al. (2023) argued that the total AA is more related to the specific compositions of compounds with antioxidant properties, rather than the total phenolic content. Similarly, Buitrago et al. (2019) did not observe any correlation between TFC and AA. These discrepancies could be, however, partially explained by distinct methodologies applied in the mentioned studies.

Higher TPC was observed in colored quinoas compared to white or yellow ones. Similarly, higher TFC and AA were evaluated in dark-colored and red samples (Tang et al., 2015a, b; Pellegrini et al., 2018; Liu et al., 2020a). Even the metabolite composition differs between white, red, and black genotypes. For example, protocatechuic acid, *p*-coumaric acid, betanin, and isobetanin (Tang et al., 2015b; Liu et al., 2020a) were exclusively found in colored quinoa.

The AA, TPC, and TFC of the sample were reported to be influenced by the genetic makeup of the plant (Fischer et al., 2017; Granado-Rodriguez et al. 2021b), whereas the cultivation location seemed to be an insignificant factor (Pedrali et al., 2023). This statement partially agrees with Reguera et al. (2018), who reported no differences in AA between three different locations in cultivars ´Titicaca´ and ´Salcedo-INIA´, but significant differences were displayed in ´Regalona´. As concluded by Antognoni et al. (2021), both ´Titicaca´ and ´Regalona´ did not show any relevant genotype-dependent fluctuations in studied parameters, probably because both were bred from the same gene pool. Nonetheless, the agroecological conditions can, to some extent, change the

biochemical content and composition, which agrees with the conclusions of Granado-Rodriguez et al. 2021a).

Considering the environmental influence, limited water supply resulted in a decrease of both TPC and TFC by 70% and 76%, respectively (Toubali et al., 2022). On the other hand, Fischer et al. (2017) reported that the water restriction increased the AA by 2-fold approximately, which could refer to the increased need of the plant to minimize oxidative damage during drought stress. When cultivated under salinity conditions, landrace ´R49´ displayed a strong increase in TPC and AA, whereas landrace Villarica had the most abundant increase in TFC under non-saline conditions (Aloisi et al., 2016).

2.7.5. Vitamin and minerals

Quinoa seeds generally contain minerals such as Ca, Fe, Mg, Na, P, K, and Zn in a sufficient amount to meet a balanced human diet (Repo-Carrasco et al., 2003; Granado-Rodriguez et al., 2021a, b). As indicated by several authors, quinoa seeds have an even higher content of many minerals than common cereals (Martin et al., 2014; Nascimento et al., 2014; Mhada et al., 2020; Hussain et al., 2021). The content of minerals fluctuates due to genotype, soil type, year, and fertilization (Miranda et al., 2013; Prado et al., 2014; Pellegrini et al., 2018; Granado-Rodriguez et al., 2021a; Bock et al., 2022).

According to Granado-Rodriguez et al. (2021b), the contents of P, Ca, and Fe remained consistent between varieties, unlike K, Mg, and Na. Matías et al. (2021) also found significant fluctuations in K and Mg. Granado-Rodriguez et al. (2021a) stated that Mg, Fe, and Zn content was not strongly influenced by cultivar x year interactions. Reguera et al. (2018) observed changes only in Zn between locations, while De Bock et al. (2021b) noted no variations in P and Ca over the years but found differences among varieties. Additionally, dark-colored varieties had higher P, which correlated with increased linoleic acid (C18:2) and lower MUFAs (Matías et al., 2021). This may explain the higher PUFA content in black-seeded varieties compared to red or white ones. Strong correlations were also found between P and protein content (Granado-Rodriguez et al., 2021b; Matías et al., 2022).

Significant contrasts in mineral concentration among cultivars were also analyzed between hot and cool years, which were probably caused due to little-understood heat-induced adaptation mechanisms and/or interactions among nutrients (Matías et al., 2021). Genotypes ´Pasto´, ´Dutchess´, ´Atlas´, and ´Summer Red´ cultivated in Belgium had the highest amount of minerals, in contrast to the other studied genotypes in the experiment of De Bock et al.

(2021b). Further, genotypes ′Marisma′ and ′Jessie′ grown in Spain were evaluated as genotypes with significantly high mineral content (Granado-Rodriguez et al., 2021b, Matías et al., 2021).

In terms of adaptability to adverse conditions, Toderich et al. (2020) referred to the genotype ′Q5′ as suitable for saline environments since there was a remarkable increment of Fe, Zn, and Ca content under salinity. Mineral concentration varied under contrasting irrigation treatments, except for Mn concentration, which was not significantly different (Walters et al., 2016). The authors also estimated that heterogeneity in concentrations might occur due to the dilution effect.

Although there is not enough current data on the overall vitamin content in quinoa, it was concluded in previous studies that quinoa has a satisfactory concentration of thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), folic acid, and vitamins A, C, and E (Koziol, 1992; Ruales and Nair, 1992). Vitamin E is a general term for tocopherols (α-, β-, γ-, and δ-) and tocotrienols (α-, β-, γ-, and δ-), also named vitamin E homologs. According to Fischer et al. (2013), vitamin E content in quinoa seeds ranged between 1.04–1.28 mg/100 g, and overall content was not altered by escalated moisture deficit in genotypes ′Regalona′, ′B080′, and ′AG2010′.

Tang et al. (2016) found significant variations in overall vitamin E content and the composition of vitamin E homologs. The most abundant vitamin E homolog in quinoa was g-tocopherol followed by a-tocopherol, and d-tocopherol, which is following the results of Pereira et al. (2019) and Granda et al. (2018). No tocotrienols were detected in any of the mentioned studies. Pereira et al. (2019) also determined higher content of β- and γ-tocopherols in the black genotype, but higher α-tocopherol content in the red genotype.

Miranda et al. (2013) uncovered significant alterations in vitamin B content caused by distinct environmental conditions in two studied localities with the highest concentration of B vitamins in the arid locality Vicuña in Chile. Granda et al. (2018) also observed a diverse content of vitamin B. While the content of B2 and B6 was relatively similar among varieties, diverse values were determined for B1. The highest concentration of B1 was found in non-pigmented varieties ′Tunkahuan′ and ′Titicaca′. Increased content of B2 appeared in colored varieties and the highest content of B6 was identified in the pigmented variety ′Pasankalla′. The vitamin C content also changes between distinctive locations with the highest content (49.30 mg/100 g DW) in genotype ′Villarrica′ cultivated in the area of Temuco with a cold temperate climate (Miranda et al., 2013).

2.8. Antinutritional factors

Despite its considerable nutritional attributes, quinoa also harbors various antinutritional substances that might be capable of diminishing nutrient absorption and thereby impacting the overall nutritional quality of quinoa-based foods (Filho et al., 2017). Among these, saponins represent the primary antinutritional factors. Other compounds, such as phytic acid, protease inhibitors, tannins, and oxalates are found in quinoa in lesser quantities (Zhou et al., 2023).

Saponins constitute a diverse family of chemical compounds characterized by the presence of a steroid or triterpenoid aglycone (sapogenin) connected to one or more oligosaccharide moieties, forming glycosides (Liener, 2003). Quinoa is known to contain approximately 40 different saponins, primarily isolated from flowers, seeds, and bran (El Hazzam et al., 2020), although some reports indicate their presence in leaves, stems, and roots as well (Lim et al., 2020; Stoleru et al., 2022a). Saponin content ranges from less than 0.1 mg/g to 7.9 mg/g in quinoa seeds and it is influenced by genotype and environmental conditions (De Bock et al., 2021b; El Hazzam et al., 2020).

While saponins contribute a bitter taste and decrease the bioavailability of some nutrients (Samtiya et al., 2020), they possess immense therapeutic potential demonstrating anti-inflammatory, antidiabetic, hepatoprotective, and anti-cancerous effects (Sharma et al., 2023). In plants, they serve as important plant defense mechanisms associated with anti-microbial, anti-fungal, and insecticidal effects (Zaynab et al., 2021).

Phytic acid, also known as inositol-6-phosphate or phytate, serves as the primary storage form of phosphorus in plant tissues and controls the uptake and homeostasis of zinc and inorganic phosphate (Belgaroui et al., 2022). While phytic acid is an essential element of plant growth and development (Pramitha et al., 2021), it is the undesired compound in the human diet since it forms complexes with nutrient cations (calcium, iron, and zinc) thereby reducing their absorption in the digestive tract (Lee et al., 2015; Silva et al., 2021). Despite its antinutritional properties, phytic acid exhibits anti-carcinogenic, anti-inflammatory, and anti-microbial activities (Hou et al., 2022; Masunaga et al., 2019; Nassar et al., 2021).

Protease inhibitors, together with tannins and oxalates may become a health risk factor for humans when consumed in elevated amounts (Kårlund et al., 2021; Salgado et al., 2023). Nonetheless, in quinoa, both compounds are presented generally in trace amounts, therefore they do not possess any

significant health concerns associated with their consumption (Saad-Allah & Youssef, 2018; Sobota et al., 2020; Villacrés et al., 2022).

2.9. Summary

This overview provides a summary focused on current research on different quinoa genetic resources in diverse growing conditions. Quinoa is considered a highly nutritive crop that is also resistant to drought and salt suitable for marginal regions. According to our findings, the different environmental conditions can have a strong impact on the nutritive compounds of quinoa seeds. Further, the adaptation of quinoa to adverse conditions has limitations in the case of elevated temperatures, high salinity levels, or a combination of weather extremes – heavy rainfall followed by temperatures over 30°C – together with cultivar response may negatively affect growth and productivity which can result in changed content of nutritive compounds. However, an insight into the enormous variability of nutritive components possessed by quinoa germplasm cultivated in the different conditions of the world shows us how important it is to conserve and protect this richness, and to select outstanding accessions suitable to different conditions. It gives us the potential and hope to develop new varieties of quinoa adapted to different environments and production systems.

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3. Nutritional and phenotypic evaluation of quinoa genetic resources grown in the climatic conditions of the Czech Republic

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Abstract

Quinoa displays huge genetic variability and adaptability to distinct climatic conditions. Quinoa seeds are a good source of nutrients; however, the overall nutritional composition and nutrient content are influenced by numerous factors. This study focused on the nutritional and morphologic evaluation of various quinoa genotypes grown in the Czech Republic. Significant differences between years were observed for morphological traits (plant height, inflorescence length, weight of thousand seeds). The weather conditions in the year 2018 were favorable for all the morphological traits. The protein content of quinoa accessions ranged between 13.44 and 20.01% and it was positively correlated to mauritianin. Total phenolic content varied greatly from year to year, while the antioxidant activity remained relatively stable. The most abundant phenolic compounds were the flavonoids miquelianin, rutin, and isoquercetin. Isoquercetin, quercetin, and N-feruloyl octopamine showed the highest stability under variable weather conditions in the analyzed years. A total of six compounds were detected and quantified in quinoa for the first time. Most varieties performed well under Central European conditions and can be considered a good source of nutrients and bioactive compounds. These data can be used as a source of information for plant breeders aiming to improve the quality traits of quinoa.

3.1. Introduction

Quinoa is a pseudocereal from the Amaranthaceae family, with its origin located around Lake Titicaca, lying on the border of Peru and Bolivia. Thanks to its long-term domestication and the various farming activities of ancient societies living in the Andean range [1], quinoa today displays a huge genetic variability. This allows quinoa to adapt to different abiotic stresses [2,3] and opens the possibility of cultivation in relatively distinct climatic conditions worldwide [4].

Thanks to its resilience, quinoa can be sustainably produced in marginal environments, which is a crucial trait, because salinization and aridity are predicted to increase in most parts of the world. It is estimated that climate change will negatively impact food safety in low-income countries relying primarily on agriculture and with limited inputs. Therefore, quinoa might be, together with other indigenous foods, a significant tool in fighting against hunger and malnutrition [5].

Quinoa seeds and leaves are consumed in the form of traditional and novel food products and beverages [6]. Thanks to the presence of valuable nutrients, quinoa can be used for the improvement of the nutritional profile of gluten-free products [7]. Quinoa contains a good amount of minerals and vitamins, together with a relatively high amount of nutritionally valuable oil, with a predominance of health-beneficial polyunsaturated fatty acids [8]. Thanks to its exceptional features and characteristics, quinoa starch has interesting physiochemical properties, allowing its potential use in a broad spectrum of food products. Quinoa is further prized for its relatively high seed protein content, with the presence of all essential amino acids [9,10]. In addition to the primary metabolites, quinoa contains numerous secondary metabolites, divided into five groups: phenolic acids, flavonoids, terpenoids, steroids, and nitrogen-containing metabolites. The majority of them are biologically active, possessing, for example, anticancer [11,12], immunoregulative [13,14], antimicrobial [15], and anti-inflammatory properties [16,17]

On the other hand, the reported nutritional composition and nutrient content of quinoa is highly variable throughout the literature. Besides the effect of genotype, the nutrient content and composition of quinoa were previously reported to be influenced by agroecological conditions [18–20], as well as the metabolomic and morphological characteristics of the plant itself [21].

It is necessary to broaden the current knowledge of quinoa, by analyzing and evaluating the wide range of quinoa genetic resources, which will serve as a great source of information about which quinoa genotypes have the potential to

be cultivated intensively and which should be improved. This study evaluated an extensive collection of 41 quinoa genotypes grown for 4 consecutive years (2018–2021) under the climatic conditions of the Czech Republic. The main aim was to characterize the chemical and nutritional compositions, together with the agro-morphological traits, of selected varieties with the best performance under Central European climatic conditions. The data obtained will provide necessary and detailed information for further quinoa breeding purposes.

3.2. Materials and methods

3.2.1. Plant material

A total of 41 quinoa accessions were subjected to analysis. All the accessions were provided by the U.S. National Plant Germplasm System operated by USDA. During consecutive years 2018–2021, the genotypes were sown on the experimental fields of the Crop Research Institute in Prague—Ruzyně, Czech Republic. All accessions were sown in two rows 1 m in length, 25 cm apart, and with 50 seeds per row. In each studied year, the original samples provided by the National Plant Germplasm System were sown. Sowing was conducted in alignment with the prevailing weather conditions specific to each year, typically occurring between the second half of May and the beginning of June.

No pesticide or fungal control was applied. The morphological characteristics of the plants were evaluated according to the descriptors for quinoa and wild relatives [31]. The plant height and inflorescence length measurements were performed in 5 randomly selected plants in each genotype. Seeds were harvested at full maturity. The seeds were dried, cleaned, and stored for further analysis.

3.2.2. Weather conditions

Figure 3.1 describes the weather conditions during four consecutive years 2018–2021. The meteorological data were gathered from the agrometeorological station at Crop Research Institute, Prague—Ruzyně, Czech Republic. In general, there were variable weather conditions during the analyzed years. The year 2018 showed extremely hot weather during the first half of the growing season; however, in the second half, the mean temperature was the lowest compared to all analyzed years and the 30-year average. This year was also the driest, because the precipitation rate was lower than the 30-year average (1981–2010) during all months, except for June. Extremely dry

conditions were observed during May and July 2018. The years 2019 and 2020 had relatively similar temperature patterns, except for June, when the temperature was significantly higher in 2019.

In terms of rainfall, the average precipitation rate was quite variable in both years. Relatively abundant rainfall occurred in June, August, and October 2020, whereas May and July were drier, with precipitation rates lower than the 30-year average. In 2019, there was relatively high precipitation during September, but the other months reached values that were comparable to or lower than the 30-year average. Overall, the year 2019 can be considered the warmest of all studied years and drier compared to 2020. In terms of mean temperature, the year 2021 was more or less comparable to what was seen in 2019 and 2020, except for June and August. In contrast, the precipitation rate showed several extremes in 2021. The most abundant rainfall occurred during May and September, whereas a low amount of rainfall was observed in August and October. The precipitation rate of the two resting months (June and July) was comparable to the 30-year average.

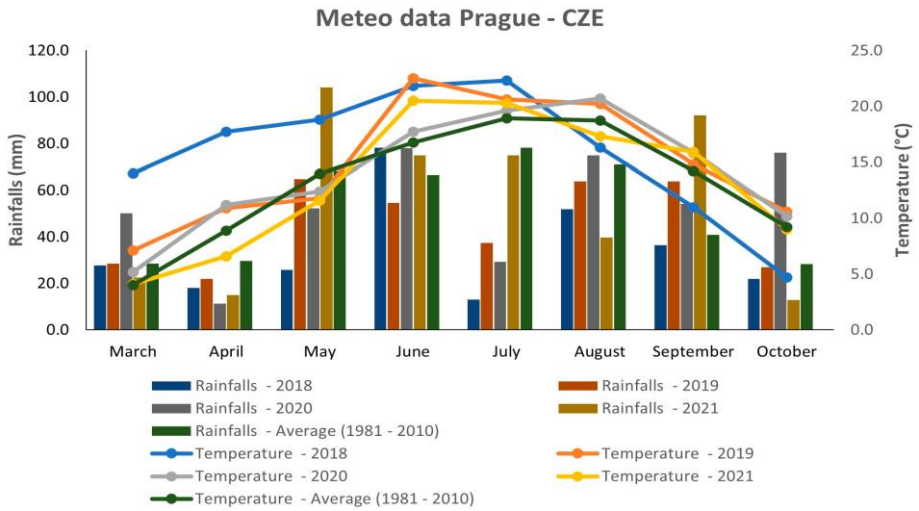

Figure 3.1 Weather conditions in 2018–2021 in Prague, Czech Republic

3.2.3. Chemicals

Standards of the phenolic compounds 2-OH cinnamic acid, 4-OH benzaldehyde, apigenin, caffeic acid, catechin, chlorogenic acid, emodin, epicatechin, gallic acid, genistein, glycitein, hesperidin, homoorientin, isoquercetin, isovitexin, isorhamnetin, kaempferol, luteolin, n-feruloyl octopamine, naringenin, neochlorogenic acid, mauritianin, miquelianin,

orientin, *p*-coumaric acid, pinocembrin, quercetin, quercitrin, rhamnetin, rutin, salicylic acid, taxifolin, umbelliferone, vitexin, and the internal standard probenecid and verapamil hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol (LC-MS grade, ≥99.9%) was obtained from Riedel de Haën (Seelze, Germany). Formic acid (LC-MS grade, 99%) was purchased from VWR (Leuven, Belgium). Pure water was attained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

3.2.4. Sample and standard preparation

To prepare reference stock solutions, reference standards of each phenolic compound were dissolved in methanol, to obtain stock solutions of 0.5 mg/mL. The reference stock solutions were stored at −18°C. The calibration curves for the phenolic compound quantification were prepared by dilution of stocks, with a methanol concentration range of 0.001–2.000 μg/mL. Furthermore, probenecid and verapamil were dissolved in methanol at 0.5 mg/mL, to prepare a stock solution of the internal standard. Internal standards were then added to the individual reference standard solutions or test samples, to a final concentration of 0.1 μg/mL.

The seeds of quinoa were milled with an IKA A11 basic mill (IKA-Werke, Staufen, Germany), and the flour was stored in a dark cold place (4°C) in well-sealed plastic bags. For the mass spectrometric analysis, the extraction of seed samples was based on the method described by Janovská et al. [22]. Briefly, 0.1 g of the whole meal flour was extracted twice with 1 mL of extraction solvent (80% methanol with probenecid and verapamil as internal standards at a concentration of 0.1 μg/mL) in Eppendorf tubes for 60 min at 45°C and using an ultrasonic bath. Samples were then centrifuged for 10 min at 13,500 rpm. Obtained supernatants from each sample were filtered through 0.2 μm nylon syringe filters (Thermo Scientific, Rockwood, TN, USA). Extracts were prepared a maximum of 2 days before the UHPLC-ESI-MS/MS analysis and stored at −18°C.

3.2.5. UHPLC-ESI-MS/MS instrumentation

The chromatographic system (Dionex UltiMate 3000 UHPLC system, Dionex Softron GmbH, Germering, Germany) consisted of a binary pump (HPG-3400RS), an autosampler (WPS-3000RS), a degasser (SRD-3400), and a column oven (TCC-3000RS). Detection was carried out on a quadrupole/orbital ion trap Q Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Analytes were separated on a reversed-phase Ascentis Express C18 column (2.1 mm × 100 mm, 2.7 μm) from Supelco (Bellefonte, PA, USA). The

LC-MS system was equipped with a heated electrospray ionization source (HESI-II) and Xcalibur software, version 4.0 (Thermo Fisher Scientific, San Jose, CA, USA).

3.2.6. UHPLC-ESI-MS/MS analysis

Chromatographic separation was carried out using gradient elution, with 0.2% formic acid (v/v) in water as solvent A and methanol with 0.2% formic acid (v/v) as solvent B. The LC gradient started with 99% of solvent A + 1% of solvent B; followed by gradient elution to 40% A + 60% B at 11 min. The column was eluted with 100% of solvent B for 2 min. Equilibration was achieved by washing the column with 99% A + 1% B for 2 min. The total analysis took 15 min. The column was maintained at 40 ◦C at a flow rate of 0.35 mL/min. The injection volume was 1 µL.

The mass spectrometer analysis was run in negative ESI mode. The spray voltage was maintained at −2.5 kV. The sheath gas flow rate was 49 arbitrary units, the auxiliary gas flow rate was 12 arbitrary units, and the sweep gas flow rate was 2 arbitrary units. The capillary temperature was 260°C. Nitrogen was used as the sheath, auxiliary, and sweep gas. The heater temperature was maintained at 419°C. The S-lens RF level was 30. The precursor ions in the inclusion list were isolated within the retention time window of ± 60 s, filtered in the quadrupole at the isolation window (target m/z ± 0.8 m/z), and fragmented in an HCD collision cell C-trap at a resolution of 17,500 FWHM (full width at half maximum) resolution, an AGC target value of 1 × 106, and a maximum injection time of 50 ms.

The normalized collision energy (NCE) was optimized for each compound. The precursor and daughter ions monitored, retention times, and NCE values are shown in Table S1. The precision and calibration of the Q Exactive Orbitrap LC/MS/MS instrument were examined using a reference standard mixture obtained from Thermo Fisher Scientific. The measurements were performed in three replicates. Data were evaluated with Quan/Qual Browser Xcalibur software, version 4.0.

3.2.7. Determination of the phenolic compound concentration in quinoa samples

Identification of phenolic compounds in quinoa samples was based on their retention times relative to the authentic standards and mass spectral data (accurate mass determination generating elemental composition and fragmentation patterns of a molecular ion) obtained through LC-MS/MS, most

were compared with those described in our previous studies [22]. Calibration curves were constructed by plotting the peak area (adjusted with probenecid and verapamil as internal standards) versus the concentration of the relevant reference standards.

3.2.8. Chemical analyses

The dry weight (DW) content of seed samples (5 g) was further dried in an electric hot-air drier at 105°C for 4 h, according to the standard method [23]. The content of crude protein from each sample was determined using the classic Kjeldahl mineralization method and calculated with a conversion factor of 6.25 [24]. The protein content measurements were performed in two replicates. The results were expressed as % in DW. Total phenolic content (TPC) was determined using the Folin–Ciocalteau reagent according to Holašová et al. [25] with slight modifications. The results of the TPC analysis were expressed in grams of gallic acid equivalent (GAE) per kilogram of sample dry weight (DW) (GAE g/kg DW). The antioxidant activity (AA) of the samples was determined using a DPPH assay [26]. The results of the DPPH assay were expressed in millimoles of Trolox equivalent (TE) per gram of sample dry weight (DW) (μmol TE/g DW).

3.2.9. Statistical analyses

Selected morphological descriptors for the whole collection of 41 genotypes were measured in 3 biological replicates. Statistical analysis was performed in the R program (R Development Core Team 2020) and Microsoft Office Excel v. 2016. A two-way analysis of variance (ANOVA2) was applied to the data, to test whether there was a significant effect of year and genotypes on the evaluated traits. To compare each accession concerning each descriptor, the means and the standard deviations for each descriptor were calculated separately for each accession and year of observation. Boxplots were also generated, to compare the distribution of values for a set of 22 descriptors between individual years of observation. Years with significantly different means were determined with a Tukey HSD test. Spearman´s rank correlation was also calculated for each pair of descriptors based on the mean values.

The correlation test function was applied to test whether the correlation coefficient was significantly different from zero. Furthermore, a heatmap was created for selected traits using the Complex Heatmap package, to display differences between genotypes. Each genotype was color-coded from max (red) to min (blue) based on the values of the respective descriptors in individual years, and a boxplot showing the distribution of values across individual years

and genotypes was plotted. Heatmaps were combined with a dendrogram based on the average linkage clustering of the Euclidean distance dissimilarity matrix of the values for the respective traits. Summarized data of the evaluated traits and nutritive compounds (means and standard deviation) for the tested genotypes in all years are presented in Table S2. To show the association among samples, data for a set of 19 descriptors were used for the principal component analysis (PCA). Before the PCA, the data were scaled, and missing values were imputed using the missMDA package. The quality of representation of the variables on the factor map was also calculated for the first two components with the largest variance.

3.3. Results and discussion

3.3.1. Weather conditions

The weather conditions during the four consecutive years 2018–2021 showed several extremes in temperature and precipitation, mostly during the years 2018 and 2021 (Figure 3.1). The years 2019 and 2020 had relatively similar characteristics; however, they both were different from the years 2018 and 2021. The effect of the environment on plant morphology and seed quality is undebatable. As described previously, the growing conditions during the year can significantly affect important quinoa traits, such as yield [27], fiber content [19], protein and amino acid content [10,28], as well as metabolomic composition [29,30].

3.3.2. Morphological evaluation

In this study, all genotypes were evaluated under field conditions using the descriptors for quinoa *Chenopodium quinoa* Willd. and wild relatives [31]. The selected descriptors were plant height (PH), inflorescence length (IL), and the weight of thousand seeds (WTS). The mean PH value was the highest in 2018 (127.65 ± 13.77 cm) and the lowest in 2021 (97.88 ± 20.63 cm). A statistically significant difference between the years was only noticed in the year 2018; other years had no significant differences. Statistical differences also existed among genotypes (Figure 3.2).

The height of a plant is, among other factors, strongly influenced by genotype [32]. This study detected maximum PH in the ´Mint Vanilla´ (167.67 ± 3.68 cm) in 2018 (Figure 3.3). This genotype steadily obtained top PH values in almost all studied years, except for 2019. A similar range of quinoa heights was found in the scientific literature. Thiam et al. [33] reported the range of studied

quinoa genotypes at 34.85–127.35 cm, while Tabatabei et al. [34] evaluated a broader range (17.20–145.25 cm).

A relatively high and stable PH values among three studied years (2018, 2019, and 2020) were noticed in genotype ´QQ57 A´, with the mean PH at 114.58 ± 25.83 cm. A very low variation in PH between years was described in genotypes ´Tallin B´ and ´Faro´. The height of the plant was positively correlated to WTS (0.25) (Figure 3.4). The PH is known to positively correlated to overall seed yield and seed size [35,36].

The heritability of plant height (PH) reached up to 73%, highlighting its significance as a trait for future selection of promising lines and yield improvement [37]. Controlling plant height is particularly crucial for preserving quinoa yield, as accessions with excessive height (>176.72 cm) and long panicles (>57.94 cm) often exhibit lower yields and smaller seed sizes [35]. Moreover, taller plants are more prone to lodging which leads to significant yield losses [38]. Damage to crops, including lodging and associated waterlogging, due to heavy rainfall and hailstorms has been identified as a risk factor for agriculture in the Czech Republic [100], hence it is highly relevant for this region to identify genotypes that can withstand such weather extremes.

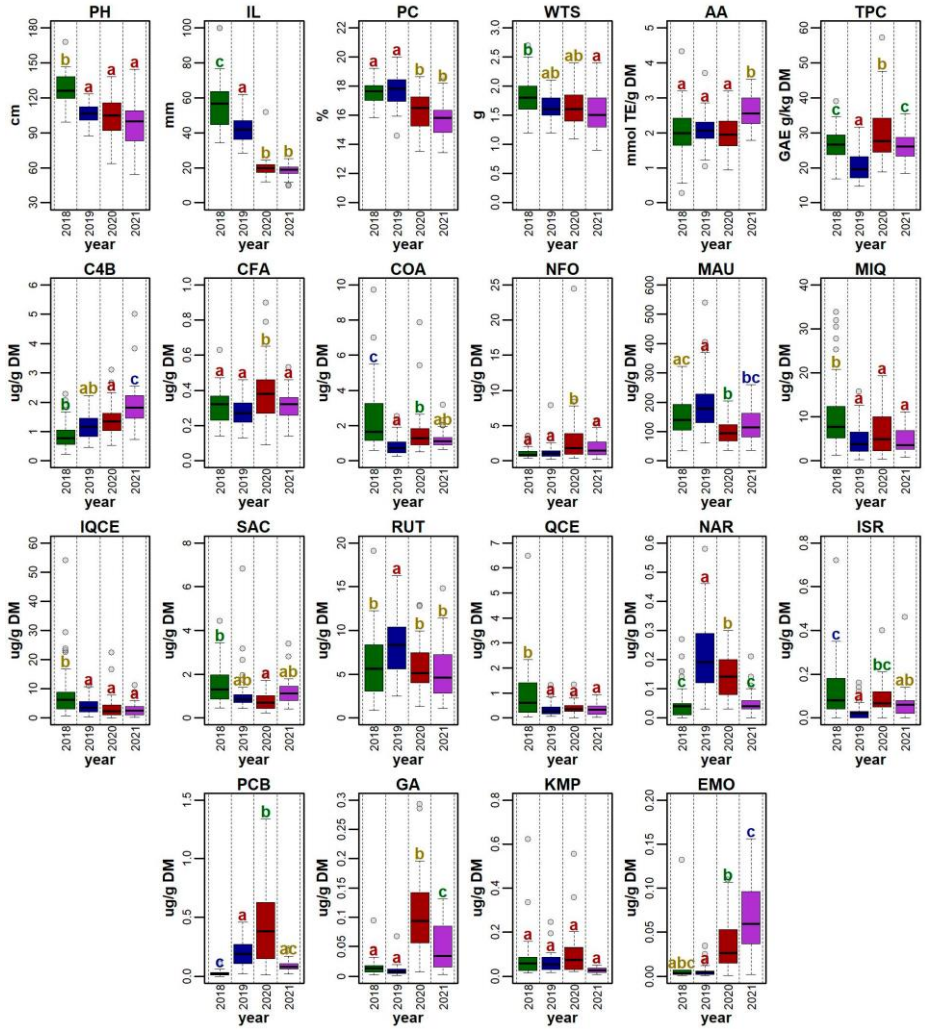

Figure 3.2 Distribution of values for a set of 22 morpho-phenological parameters and chemical compounds observed for 41 quinoa genotypes grown in the Czech Republic between 2018 and 2021

For each descriptor, the values recorded for each accession in a given year were used for the plot. Boxplots show the distribution of values, with grey-shaded points representing outlier values. Significant differences in means between years are denoted by the different letters (Tukey HSD) above each boxplot. The abbreviations for the selected descriptors are as follows: plant height (PH), inflorescence length (IL), protein content (PC), weight of thousand seeds (WTS), antioxidant activity (AA), total polyphenols (TPC), 4-hydroxybenzaldehyde (C4B), caffeic acid (CFA), *p*-coumaric acid (COA), N-feruloyl octopamine (NFO), mauritianin (MAU), miquelianin (MIQ), isoquercetin (IQCE), salicylic acid (SAC), rutin (RUT), quercetin (QCE), naringenin (NAR), isorhamnetin (ISR), pinocembrin (PCB), gallic acid (GA), kaempferol (KMP), and emodin (EMO).

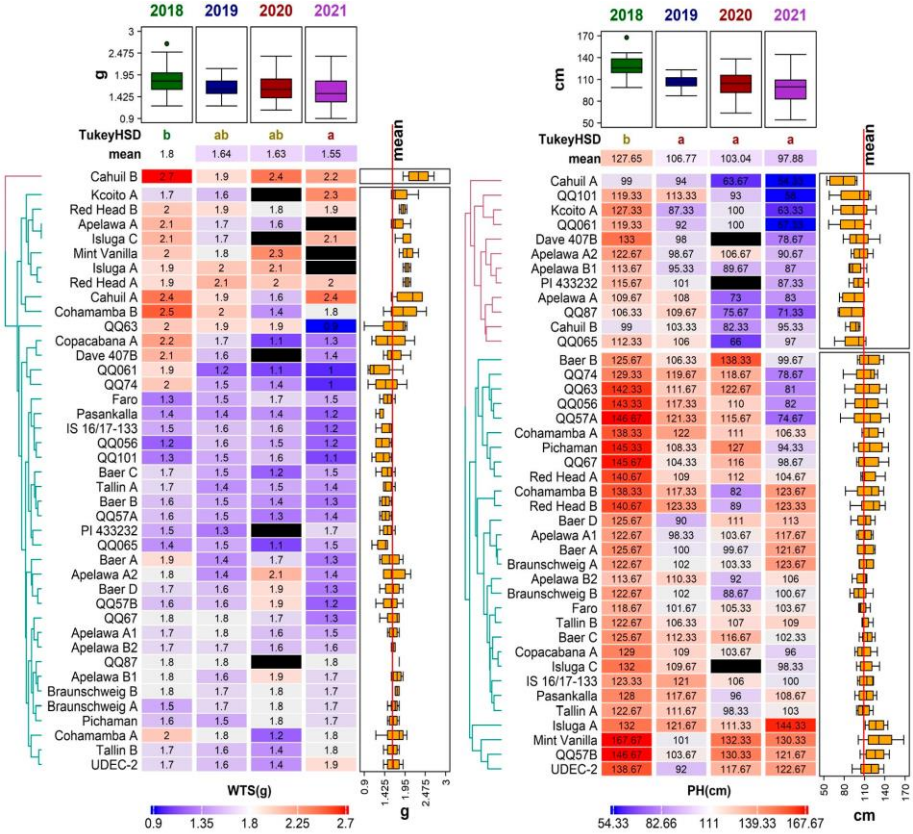

Figure 3.3 Diversity of 41 quinoa genotypes in terms of weight of thousand seeds (WTS, (left)) and plant height (PH, (right)) values, illustrated using a heatmap combined with a dendrogram based on average linkage clustering of the Euclidean distance dissimilarity matrix

Values for the respective traits are displayed on a scale from blue (min) to red (max), according to color key below each heatmap. Black rectangles indicate missing values for a given trait in a given genotype. Years with significantly different means are denoted by the different letters (Tukey HSD) above the individual columns of the respective heatmaps. Boxplots above each heatmap show the distribution of values across all accessions in individual years, while the boxplots next to each heatmap show the distribution across all years for individual accessions. The line crossing the side boxplots marks the mean of all values.

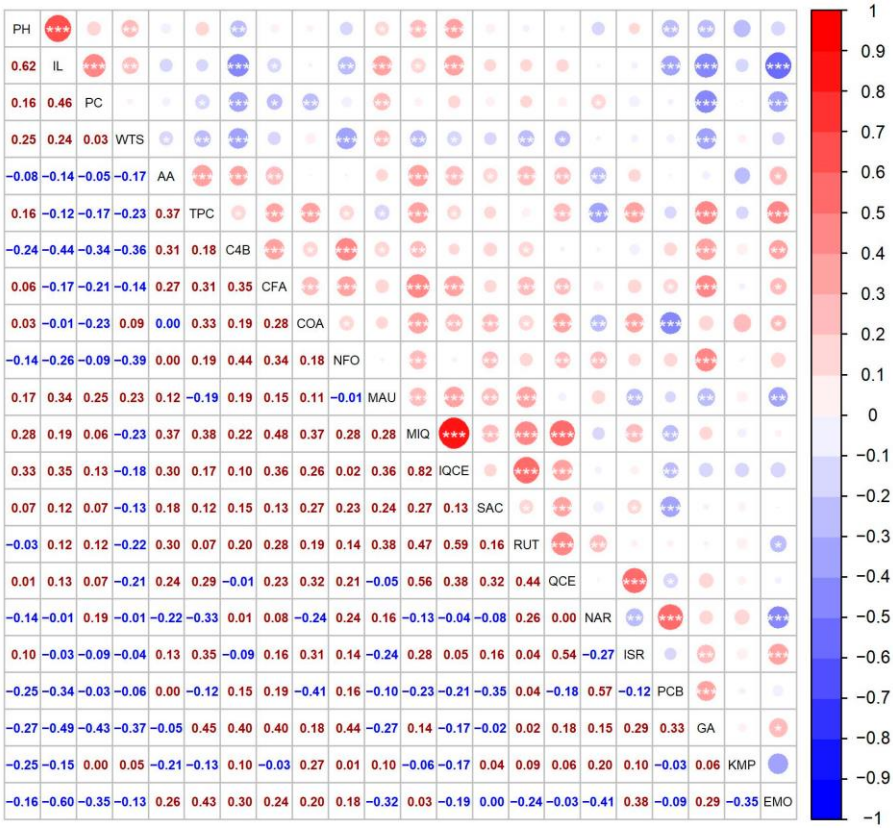

Figure 3.4 Spearman´s correlation between 22 descriptors for a collection of 41 quinoa genotypes

The circles above the diagonal indicate whether the correlation between the pair of descriptors was negative (red) or positive (blue), while their size represents the magnitude of the correlation, as indicated by the color key and the Spearman´s ρ values below the diagonal. Significant correlations are denoted by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$), respectively.

The abbreviations for the selected descriptors are as follows: plant height (PH), inflorescence length (IL), protein content (PC), weight of thousand seeds (WTS), antioxidant activity (AA), total polyphenols (TPC), 4-hydroxybenzaldehyde (C4B), caffeic acid (CFA), *p*-coumaric acid (COA), N-feruloyl octopamine (NFO), mauritianin (MAU), miquelianin (MIQ), isoquercetin (IQCE), salicylic acid (SAC), rutin (RUT), quercetin (QCE), naringenin (NAR), isorhamnetin (ISR), pinocembrin (PCB), gallic acid (GA), kaempferol (KMP), and emodin (EMO).

Mean IL was the highest in 2018 (56.11 ± 15.21 cm) and lowest in 2021 (18.51 ± 3.90 cm). The result of the Tukey HSD showed statistical differences between the years, but there was no statistical significance between 2020 and 2021 (Figure 3.2). The longest inflorescence was recorded in the genotype ´QQ57 A´ (99.67 ± 40.00 cm) in 2018; however, this genotype did not perform well in any other year. A relatively low variability in this trait was detected in the genotype

′Dave 407B′. Tabatabaei et al. [34] reported similar values, ranging between 7.05–71.75 cm, in 468 quinoa accessions. Different ranges of inflorescence length were observed in different environments: 36.90–120.70 cm [37] and 29.70–62.70 cm [39].

The year 2021 was not suitable for inflorescence development, since almost 50% of the cultivated genotypes had below-average values of panicle length (less than 18.51 cm). The correlation analysis showed a relatively strong positive association (0.62) with the height of the plant (Figure 3.4), which agrees with other authors [35,39,40].

Regarding all four studied years, the WTS ranged between 0.90 g (genotype ′QQ63′ in 2021) and 2.74 g (genotype ′Cahuil B′ in 2018). Significant differences were detected between 2018 and 2019 and between genotypes (Figure 3.3). Compared to several other experiments conducted in Europe, the WTS values in this study were relatively low. For example, the WTS reported in Poland, Belgium, Germany, Italy, and Spain ranged between 1.20 and 3.68 g [18,41]. The most favorable year for this trait was 2018 (mean WTS 1.80 ± 0.32 g) (Figure 3.3). On the other hand, most genotypes had relatively low WTS in 2021, except for ′Cahuil A′, ′Kcoito A′, ′PI 433232′, ′Pichaman′, ′Tallin B′, and ′UDEC-2′, which had a higher WTS in this year compared to the other three years.

Genotype ′Cahuil B′ showed above-average performance in WTS, with values ranging between 1.87 and 2.74 g in all studied years. Several genotypes in this paper showed relatively stable WTS values during all years of analysis (′Red Head A′, and ′Red Head B′); however, the lowest variability was observed in the genotype ′QQ87′ achieving approximately 1.80 g among all years of analysis. As previously reported, the WTS contributes to overall quinoa yield [33,41].

The overall genotype performance depends more or less on the genetic makeup, environment, and their interactions [32,33]. A proper understanding of quinoa germplasm and its adaptation to various environments is crucial for effective breeding programs and cultivar development [42]. The favorable performance of majority of quinoa genotypes in 2018 under conditions of high temperatures and lower-than-average precipitation suggests that quinoa could be a valuable alternative replacing drought-susceptible crops, since drought and heatwaves were projected as one of the major threads for agriculture in Czech Republic [100]. Quinoa might be also an ideal crop for drought-prone areas [43,44], offering farmers a means to diversify their crop portfolio and mitigate risks associated with water scarcity.

On the other hand, the generally negative impact on morphological traits during years 2020 and 2021 with excessive precipitation rate highlights challenges that agriculture in Czech Republic, and thus in Central Europe, may face, particularly in regions prone to heavy rain or poor soil drainage. Waterlogging stress and related nutrient deficiencies [45,46], together with increased incidence of fungal diseases in those wet years probably caused poor performance of many quinoa genotypes involved in this study.

However, it is noteworthy that some genotypes in the study demonstrated stable morphological traits across cultivation years and performed well even under rainy conditions. Hence, these genotypes with broad weather tolerance should be the focus of breeding objectives, aimed at developing quinoa cultivars suited to the characteristic conditions of Central Europe.

3.3.3. Crude protein content

Protein content (PC) fluctuated between 13.44 ± 0.12% in DW (genotype ´Pichaman´ in 2021) and 20.01 ± 0.17% in DW (genotype ´Baer C´ in 2019). According to the Tukey HSD results, there were no significant differences between the years 2018 and 2019, and between the years 2020 and 2021 (Figure 3.5). The values gathered in this study were similar to several other trials on quinoa grown in Europe, such as those reported in Belgium (12.10–18.80% in DW) [18] and Spain (13.20–20.40% in DW) [19,47], but higher than those reported in Poland (12.40–15.98 g/100 g in DW) [48] and Germany (11.90–16.10% in DW) [41].

The highest mean PC was reported for the year 2019 (17.69 ± 1.14%) (Figure 3.5). On the other hand, the lowest mean PC (15.79 ± 1.19%) was analyzed in 2021. Overall, 56% of genotypes achieved the highest PC in 2019 and almost 37% of genotypes reached the highest PC in 2018. In comparison, only two genotypes (´Cahuil B´, ´Cohamamba B´) had the highest PC in 2021 and one genotype in 2020 (´Isluga A´). Even though some genotypes had low mean PC values, the amount of crude protein was still higher than in most cereals, such as wheat (12%), oat (13%), and rice (7%) [49]. In addition to a balanced [50] or ´nearly balanced´ amino acid composition [10], quinoa is a great and valuable source of protein for human nutrition.

The observed variation in PC can be explained by environment and/or genotype-environment interactions. The year 2019 was characterized as the warmest of all analyzed years. The precipitation rate in this year was the second lowest of all studied years. Heat stress and slight water stress may enhance the protein content in seeds [19], however, significant water stress can cause a

decrease in the PC [51]. The high precipitation rate during 2020 and 2021 was more harmful in our case. The effect of heavy rainfall and potential waterlogging on protein content is not well documented in quinoa specifically; however, research carried out on winter wheat and red clover concluded that there was a decrease in protein content with high water levels [52–55].

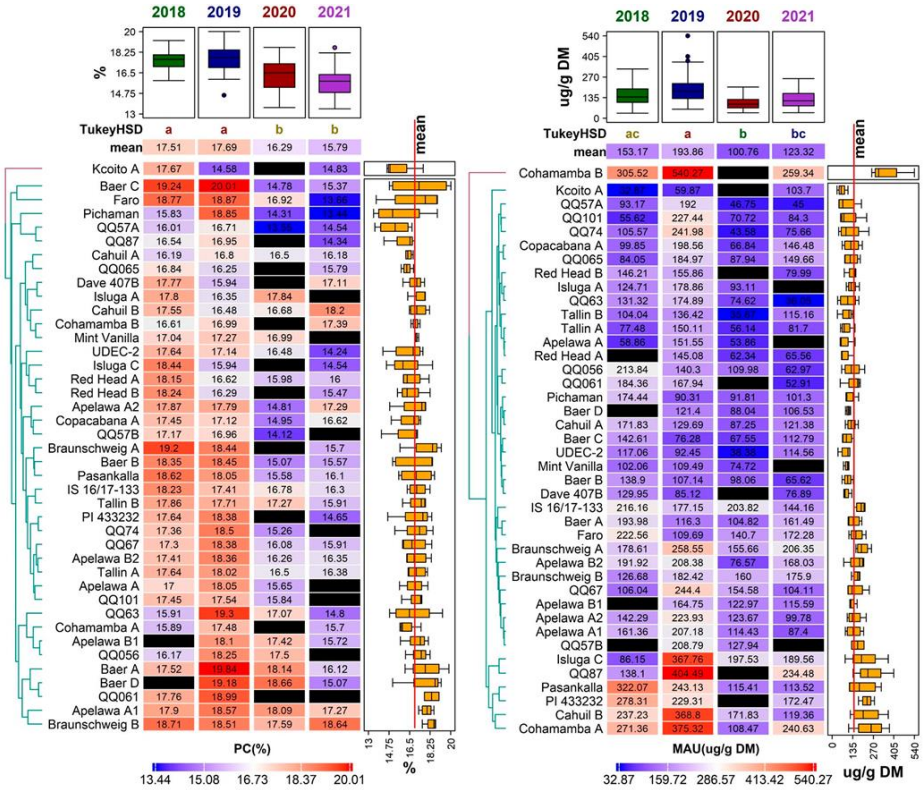

Figure 3.5 Diversity of the 41 quinoa genotypes in terms of protein content (PC, (left)) and mauritianin content (MAU, (right)) values, illustrated using a heatmap combined with a dendrogram based on average linkage clustering of Euclidean distance dissimilarity matrix values for the respective traits, displayed on a scale from blue (min) to red (max) according to the color key below each heatmap**.**

Black rectangles indicate missing values for a given trait in a given genotype. Years with significantly different means are denoted by the different letters (Tukey HSD) above the individual columns of the respective heatmaps. Boxplots above each heatmap show the distribution of values across all accessions in individual years, while the boxplots next to each heatmap show the distribution across all years for individual accessions. The quality of the representation of the variables is shown in the factor map.

Despite the influence of the environmental conditions, several genotypes exhibited consistently stable protein content. As with the morphological traits, genotypes that maintain stable nutrient levels under changing weather conditions are critical for breeding programs aimed at enhancing food security. Specifically, the genotypes 'Mint Vanilla', 'Cahuil A', 'Cohamamba B', 'Braunschweig B', and 'Apelawa A1' demonstrated high stability in protein content across the studied years (Figure 3.5).

A medium contribution to the amount of protein was noticed in IL (0.46) (Figure 3.4). Contrarily, Granado-Rodriguez et al. [47] reported a negative correlation between panicle size and protein content. Furthermore, protein content was positively associated with mauritianin content (0.25). A negative association was observed with emodin (−0.35) and gallic acid (−0.43) (Figure 3.4).

3.3.4. Total phenolic content

The TPC value ranged between 14.74 ± 0.34 GAE mg/g in DW (genotype ´QQ101´ in 2019) and 57.25 ± 2.87 GAE mg/g in DW (genotype ´Mint Vanilla´ in 2020). The Tukey HSD showed a significant difference between years; however, the years 2018 and 2021 were not statistically different (Figure 3.2). The analyzed range of TPC in this investigation was higher than that determined previously. Generally, the TPC fluctuated between approximately 2 and 15 GAE mg/g in the DW in quinoa samples [56–58].

The highest mean TPC was recorded in the year 2020 (30.56 ± 9.20 GAE g/kg DW). The majority of genotypes reached the highest TPC this year in comparison to what was measured in the other years (Figure 3.2). The lowest mean TPC was measured in the year 2019 (20.34 ± 4.06 GAE g/kg DW). The highest stability in TPC values was reported for the genotypes ´Red Head B´, ´Apelawa A´, ´Isluga C´, and ´PI 433232´. The variety and origin of the sample may significantly affect quinoa metabolomics and final polyphenol content [59]. The observed variations in TPC could have been caused by the reaction of the plant to abiotic stress [60,61]. As suggested by Toubali et al. [29], drought stress decreases the TPC by up to 76%. Nonetheless, this conclusion does not apply to our results, since the driest year was 2018, while the TPC for this year was the second highest.

In this study, several factors contributed to the overall TPC. Correlation analysis showed a weak or medium positive association between TPC and the majority of the metabolites. The strongest contributors to TPC were emodin

(0.43) and gallic acid (0.45) (Figure 3.4). TPC was also positively correlated with AA; however, the association was medium (0.37).

3.3.5. Antioxidant activity

The highest mean AA (2.59 ± 0.74 µmol TE/g DW) was measured in 2021 (Figure 3.2) and the lowest mean AA was measured in 2020 (1.95 ± 0.48 µmol TE/g DW). Among all the accessions, the highest AA value was determined for ´Faro´ (3.54 µmol TE/g DW) in 2021 and the lowest for ´Cahuil A´ (0.28 µmol TE/g DW) in 2018. The obtained results are difficult to compare with the current scientific literature since the authors used a different method (e.g., FRAP, ABTS, FIC) and/or expression of the measured values.

In terms of trait stability, very similar values throughout the years were obtained in genotypes ´QQ056´, ´QQ57B´, and ´Isluga A´; nonetheless, all the genotypes did not reach full maturity in 2021. The number of chemical components related to antioxidant properties varies under different cultivation areas and depends on the genotype-environment interactions [62,63]. In our case, the stress was probably caused by the extreme precipitation rate during 2021 and the higher incidence of fungal diseases. A weak or moderate positive association was determined between AA and the majority of the analyzed metabolites. The strongest contributor to AA was miquelianin (0.37) and 4-hydroxybenzaldehyde (0.31) (Figure 3.4).

3.3.6. Composition and content of phenolic compounds

A total of 34 metabolites were evaluated in this study. From this number, a total of 13 compounds were detected in all analyzed genotypes, and 15 compounds were detected in trace amounts and/or only in some genotypes. Six compounds were not detected in any of the studied genotypes. To our knowledge, a total of six compounds (2-OH-cinnamic acid, homoorientin, luteolin, naringenin, N-feruloyl octopamine, and 4-OH-benzaldehyde) had never been identified or quantified in quinoa before.

The chemical classes detected in this study were flavones (7 compounds), phenolic acids (7 compounds), flavonols (6 compounds), and flavanols (3 compounds). In addition, groups of hydroxybenzaldehydes, flavans, flavanones, anthraquinones, and methoxybenzaldehydes were detected in quinoa, each represented by one compound.

The results of quantification showed that the most dominant compounds throughout the analyzed years were mauritianin, miquelianin, rutin, and isoquercetin. This was not in agreement with other sources, which considered

quercetin and kaempferol as the two major flavonoids in quinoa [64–66]. The rest of the analyzed compounds had a mean concentration lower than 2 µg/g DW.

Mauritianin belongs to the group of flavonols. This compound has been well described in the genus *Astragalus* [67,68], but in quinoa, this compound has only been reported in two studies [69,70]. The potential health effects of this compound have not been well described. Mauritianin was confirmed as highly effective against *Candida albicans* [71]. Moreover, an antioxidative effect of mauritianin against DPPH was observed [72]; however, this value was low in comparison to other compounds in the study. The correlation analysis in this study showed that mauritianin is not a very strong contributor to the AA.

Mauritianin had the highest mean content in 2019 (193.86 ± 97.72 µg/g DW). In this year, several extremely high values for this metabolite were observed in the genotypes ´Cohamamba B´ (540.27 ± 52.78 µg/g DW), and ´QQ87´ (404.49 ± 11.68 µg/g DW); however, these extremes were not detected in any other year (Figure 3.4). In contrast, the lowest mean concentration of this compound was detected in 2020 (100.76 ± 43.42 µg/g DW) (Figure 3.2). The results of mauritianin content reported by Gomez-Caravaca et al. [70] are similar to those measured in the year 2020 in this study. The specific role of mauritianin in plants is not known; nevertheless, the results suggest that the weather conditions in 2019 induced the synthesis of this compound. The genotype ´Cohamamba B´ had an exceptionally high mauritianin content in all years, apart from 2020, where data were not obtained (Figure 3.5).

Another abundant flavonol detected in this study was isoquercetin (also referred to as isoquercitrin or quercetin 3-glucoside). The highest mean content of isoquercetin was measured in 2018, with 9.10 ± 10.23 µg/g DW (Figure 3.2). This year also showed notably high values in a total of five genotypes. The genotype ´QQ056´ had the best performance in this trait, attaining the highest mean isoquercetin content regarding all four years of analysis. The lowest mean isoquercetin content was measured in the year 2021 (2.93 ± 2.44 µg/g DW). In comparison to the available literature, the values measured in this study were considerably higher [20,56].

In contrast to mauritianin, the isoquercetin values showed a relatively low fluctuation throughout the analyzed years between the majority of the genotypes. This suggests that isoquercetin in quinoa is less dependent on the growing conditions in a given year. Nonetheless, geographical variability in the content of this compound was reported in *Cornus* species [73] and *Ceratonia siliqua* L. [74].

Rutin (quercetin-3-rutinoside) was the next most abundant flavonol detected in this study. In quinoa, it was observed to improve plant salinity tolerance through K+ and Na+ regulation in leaf mesophyll [75]. The content of rutin ranged between 0.88 ± 0.03 µg/g DW (genotype ´Isluga A´) and 19.07 ± 0.61 µg/g DW (genotype ´QQ056´), both measured in the year 2018. The lowest mean rutin content was measured in 2021 (5.40 ± 3.18 µg/g DW), but a very similar value was also measured in 2020. Accumulation of rutin is impacted by environmental conditions, especially by drought; however, this mechanism has been described in other species but not in quinoa [76–78]. In this case, the highest rutin content was observed in 2019 (8.21 ± 3.50 µg/g DW) and the lowest in 2021 (5.40 ± 3.18 µg/g DW). Unlike the results from Pellegrini et al. [56], the content of rutin in our quinoa accessions was lower. On the other hand, similar values to this paper were described in the study of Antognoni et al. [63].

The mean content of the flavonol quercetin ranged between 0.31 ± 0.24 µg/g DW in 2021 and 0.878 ± 1.16 µg/g DW in 2018 (Figure 3.2). An unusually high value occurred in 2018 in genotype ´Copacabana A´, reaching 6.48 ± 0.21 µg/g DW. This tendency was also recognized in other years, except for 2021, where this genotype had an average content of quercetin. The contents of quercetin determined in the available literature are quite variable, ranging between 5.27 and 14.30 µg/g DW [64,79,80]. In various plant species, the quercetin level increased due to drought [78], salt [81], and lead stress [82]. Several studies carried out on various plant species concluded that higher quercetin accumulation is a response to increased light exposure and UV-B radiation [83,84], which may partially explain the seasonal variations in the quercetin content observed in our study.

Another minor flavonol identified in this study was kaempferol. Only three genotypes, namely ´Cahuil A´, ´Cohamamba A´, and ´QQ74´, showed the presence of kaempferol in three out of four years of analysis. None of the genotypes showed the presence of kaempferol in all four years. Quercetin, together with kaempferol exhibited a content variability between samples with different geographical origins; therefore, they could be considered metabolic markers [59]. The year 2021 was the least favorable for kaempferol accumulation. Similarly to quercetin, kaempferol synthesis is impacted by light exposure and UV-B radiation [83]. Therefore, the abundant rainfall in 2021 probably decreased the amount of sunlight reaching the quinoa accession, causing a low content of kaempferol.

Lastly, quercitrin (also referred to as quercetin 3-rhamnoside or quercetin 3-O-rhamnoside) was identified in this study; however, trace amounts occurred in

only 17 genotypes grown in 2020 and in three genotypes grown in 2019. This compound was previously quantified in the study by Jiang et al. [79]; however, in contrast to our results, the authors indicated quercitrin, together with glycitein, as the major polyphenols in quinoa. In our study, no glycitein was found.

The next group of secondary metabolites detected in this study was the phenolic acids. The most abundant compound from this class was *p*-coumaric acid. The highest content was detected in genotypes ´Cohamamba B´ (9.72 ± 0.37 µg/g DW in 2018), and ´Cahuil B´ (7.87 ± 0.24 µg/g DW) in 2020. These genotypes, however, did not perform well in other years. Overall, the lowest content of *p*-coumaric acid throughout all four years was found in the genotypes ´Red Head A´ and ´Red Head B´. Different values among genotypes were observed [20,64]; therefore, the reported *p*-coumaric content in the available literature does not correspond to the data obtained in this study.

The year with the highest *p*-coumaric acid value was 2018 (Figure 3.2), which may suggest that the synthesis of this compound is upregulated by heat and increased exposure to sunlight, similar to what was reported in *Nicotiana langsdorffii* Weinmann [85] and hard fescue (*Festuca trachyphylla*) [86]). In comparison to other genotypes, ´Dave 407B´, ´Apelawa B1´, and ´Mint Vanilla´ showed relatively high stability in *p*-coumaric acid content during the studied years.

Salicylic acid was the next metabolite identified in our study. This important phytohormone regulates several metabolic processes, and the production of metabolites thereby protecting the plant against multiple abiotic stresses. For example, it serves as a protection against heat [86] or high contents of heavy metals in the soil [82]. In quinoa, salicylic acid improves salinity tolerance [87] and it increases under UV-B exposure in some genotypes [88]. An unusually high concentration of this metabolite was recognized in the genotypes ´Apelawa A´ (6.82 ± 0.67 µg/g DW) and ´Dave 407B´ (4.43 ± 0.25 µg/g DW) in 2019 and 2018, respectively.

Caffeic acid was only found in the quinoa accessions in relatively low quantities (0.09 ± 0.00–0.90 ± 0.07 µg/g DW). The highest amount of this compound was measured in the year 2020 (Figure 3.2). Galieni et al. [77] reported a higher synthesis of caffeic acid under drought stress. In the sum of precipitation, the year 2020 was not the driest; however, April and July of this year had extremely low rainfall, which could have contributed to the higher accumulation of this phenolic acid.

A very low amount of gallic acid was evaluated in all quinoa genotypes. Increased levels of this phenolic acid were observed in 2020, especially in the genotypes ′Baer D′ and ′Cohamamba A′. Furthermore, chlorogenic acid, neochlorogenic acid, and 2-OH- cinnamic acid were identified in quinoa accessions; nonetheless, they were not present in all genotypes, and/or they were found in trace amounts. In addition, neochlorogenic acid and 2-OH-cinnamic acid had not been identified in quinoa previously.

The group of flavones was primarily represented by isorhamnetin, with only a trace concentration. This compound was previously reported by Stikic et al. [80] with the content of 3.00 μg/g DW in the genotype ′Puno′, but with none in the genotype ′Titicaca′. Other minor compounds detected in this study were apigenin, vitexin, isovitexin, and orientin, which were previously found in other studies [20,89]. Furthermore, homoorientin and luteolin were also detected in minor concentrations; however, they were present only in the year 2021. These compounds had not been described in quinoa before. Nonetheless, all the minor compounds were detected only in a few genotypes. Lastly, rhamnetin was not indicated in any of the analyzed genotypes.

The most abundant compound from the flavanols groups was miquelianin, also named quercetin 3-O-glucuronide or quercetin glucuronide. The level of this compound ranged between 0.26 ± 0.02 μg/g DW (genotype ′Red Head B′ in 2019) and 33.86 ± 1.10 μg/g DW (genotype ′QQ056′ in 2018). Similar values were reported by Gomez-Carvaca et al. [90]. The year 2018 showed a total of five extremely high values, for the same genotypes as reported for isoquercetin. In addition, 2018 was also the year with the highest mean concentration of miquelianin (Figure 3.2). The result of the correlation study showed that miquelianin and isoquercetin had a strong positive association (Figure 3.4).

Furthermore, epicatechin and taxifolin were quantified only in some quinoa genotypes and during some years, with the highest mean content in 2021. Epicatechin had already been identified in quinoa [64]; however, taxifolin was described here for the first time. Catechin was not detected in this research in any genotype, but it was reported by Tang et al. [8]. Naringenin was the only flavanone detected in this study; however, its amount was negligible in comparison to the other compounds. This compound had not been detected in quinoa previously. Furthermore, hesperidin was also screened, but its presence was not confirmed, as opposed to in Jiang et al. [79].

The only flavan identified in this study was pinocembrin. This compound was highly accumulated during the year 2020, whereas the lowest mean content was reported in 2021. No pinocembrin was found in the year 2018, except for in the

genotypes ´Baer B´ and ´QQ57A´. Garcia-Parra et al. [20] observed similar values of pinocembrin content. The group of methoxybenzenes was represented by N-feruloyl octopamine (NFO).

This compound reached the highest mean concentration in 2020 (3.30 ± 4.32 μg/g DW) (Figure 3.2). This year showed extremely high values in the genotypes ´Cohamamba A´ and ´Tallin A´, which were also observed in 2021. This compound had not been detected or quantified in quinoa before. NFO was reported as an accelerator for cell apoptosis [91] and a promising treatment for hepatocellular carcinoma [92]; however, the role of this metabolite in plants has not been fully elucidated. The results of our research showed a relatively low variability in this compound throughout the analyzed years, which suggests that NFO is less affected by environmental conditions; however, further investigation is needed.

4-OH-benzaldehyde (4-hydroxybenzaldehyde) is the representative of the group of hydroxybenzaldehydes. 4-hydroxybenzaldehyde was previously reported to have antifungal, antiobesity, anti-inflammatory, antiangiogenic, and antinociceptive activities [93–95]. The concentration of 4-hydroxybenzaldehyde compound ranged between 0.21 ± 0.01 μg/g DW (genotype ´Kcoito A´ in 2018) and 5.01 ± 0.22 μg/g DW (genotype ´QQ87´ in 2021).

Emodin was classified as an anthraquinone. This compound possesses antifungal properties against *Candida albicans* [96]. Several studies also reported anticancer activity [97,98]. In this study, the content of this metabolite was very variable between the years and genotypes. In 2018, only five genotypes contained emodin; as opposed to 2021, in which all genotypes contained this metabolite. To our knowledge, emodin, and 4-hydroxybenzaldehyde had never been identified or quantified in quinoa previously. The highest synthesis of both compounds was observed in 2021, which suggests the potential role of these metabolites in quinoa protection against high water levels and/or possible fungal diseases; however, this area requires deeper investigation. Furthermore, genistein and umbelliferon were searched for in this study, but no content of these metabolites was found. The presence of genistein in quinoa was reported by Antognoni et al. [63]. In contrast, umbelliferon was not found in quinoa [99].

3.3.7. PCA analysis

A PCA representation of the data for the 19 selected descriptors further distinguished between the individual genotypes (Figure 3.6). In the diagram,

the large central group of genotypes of Chilean provenance is located in the lower right corner. Of greater interest are the several genotypes located in the outer parts of the plot. The separation of these genotypes suggests the uniqueness of their respective genotypes with respect to the analyzed samples. Although some accessions of the same origin are located close together in the plot, geographical provenance seems to have little to no effect on the spatial distribution of the accessions within the plot. Of the analyzed traits, the separation of genotypes along the first axis, explaining 16.39% of the total variance, is mostly affected by MIQ, TPC, RUT, AA, and IQCE (Supplementary Figure S1). On the other hand, the strongest influence on the distribution of genotypes along the second axis, explaining 11.21% of the total variance, was from MAU and PCB values.

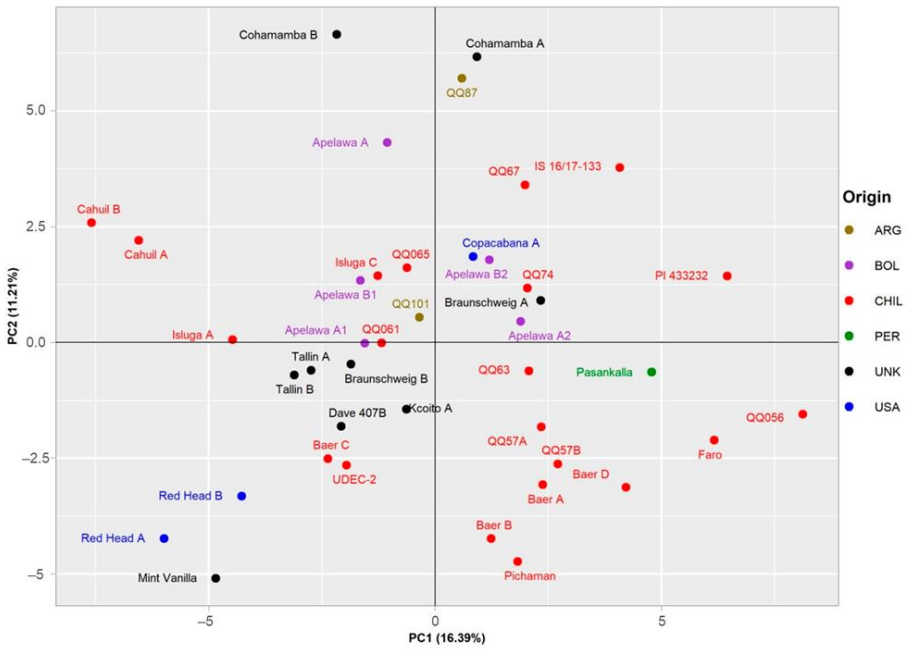

Figure 3.6 Principal component analysis based on a set of 19 descriptors for the set of 41 genotypes. Two main components explaining 16.39% and 11.21% of the total variability, respectively, are displayed. Individual accessions are labeled according to the country of origin, as illustrated in the legend on the right side of the plot.

3.4. Conclusion

For the first time, an extensive collection of 41 quinoa genotypes was evaluated over four years under the environmental conditions of the Czech Republic, and

Central Europe. The morphological traits of plant height, inflorescence length, and weight of a thousand seeds were determined. Most of the quinoa accessions had a better performance in the selected morphological traits in the year 2018, characterized as the driest and with high temperatures in the first half of the growing season.

The crude protein content of quinoa accessions was within the range previously reported for quinoa cultivated in Europe. The protein content was the highest in warm years, but high precipitation significantly affected the protein synthesis. A similar pattern was observed for the accumulation of phenolic compounds. Contrarily, the TPC and AA were enhanced by high rainfall.

A total of 28 metabolites were detected and quantified in quinoa. The most abundant flavonoids were mauritianin, miquelianin, rutin, and isoquercetin. The most abundant contributor to AA was miquelianin. The content of all phenolic compounds varied with the changing weather conditions in the analyzed years, except for isoquercetin, quercetin, and N-feruloyl octopamine, which remained relatively stable values throughout the years of analysis. To our knowledge, six compounds (2-OH-cinnamic acid, homoorientin, luteolin, naringenin, N-feruloyl octopamine, and 4-OH-benzaldehyde) have never previously been identified or quantified in quinoa.

A proper selection of appropriate genotypes to accomplish given production aims is needed. Furthermore, the determination of genotype-variable and genotype-stable traits is crucial. Over the four distinct growing periods, the tested genotypes showed variability in response to different environmental conditions. Nonetheless, the genotypes. ´Mint Vanilla´, ´Cahuil A´, ´Apelawa A1´, and ´Braunschweig B´ seemed to be less affected by weather conditions in a given year, since they reached relatively high and stable protein contents throughout all four years of analysis in the conditions of the Czech Republic. In addition, ´Red Head A´, together with ´QQ87´ and ´Isluga A´ performed best regarding their stability in the weight of a thousand seeds.

Altogether, our results confirmed the potential of quinoa as a promising source of nutrients and various bioactive compounds. Furthermore, several quinoa genotypes that are well suited to the climatic conditions of Czech Republic, were identified in this study. With its ability to perform stably or even benefit from periods of hot and drought stress, quinoa might be a potential solution for farmers threatened by the weather extremities caused by climatic change.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/foods12071440/s1, Table S1. Mass spectrometric data,

negative ionization. Table S2. Summarized data of evaluated traits and nutritive compounds (means and standard deviation) of the tested genotypes in all years. Figure S1. The quality of representation of the variables in the factor map. Squared coordinates are displayed. The color scale is proportional to the color key on the right side of the plot.

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4. The impact of germination and thermal treatments on bioactive compounds of quinoa seeds

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(*Original research paper*)

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Abstract

Quinoa is a highly nutritious crop with diverse applications in the food industry. The study assessed the impact of various processing techniques, including microwaving, boiling, roasting, steaming, flaking, and germination, on the CP, TPC, AA, and 12 phenolic compounds in quinoa. CP was significantly affected by the heat treatments. Boiled quinoa flakes exhibited the highest average CP while boiling and roasting were the lowest. Microwaving strongly enhanced the TPC and the content of six bioactive compounds (CFA, KMP, NAR, QCE, RUT, SA) while boiling and steaming had the most adverse effect. Germination improved the overall nutritional profile of quinoa. The most pronounced increase in the bioactive metabolites occurred between the third and fifth day of germination in a genotype-dependent manner. Six metabolites (NAR, SA, 4BA, IQ, PC, IH) were detected in germinated quinoa for the first time. The results emphasize the substantial influence of processing techniques and type of sample on quinoa nutritional quality and underscore the importance of proper consideration of those factors to obtain nutritionally optimal food products.

4.1. Introduction

Quinoa is a highly versatile crop with outstanding nutritional value, which was recognized even by ancient Andean populations, considering this pseudocereal a sacred food [1]. Although its cultivation has already spread worldwide, the biggest producers are still the countries of quinoa origin—Peru, Bolivia, and Ecuador [2]. Quinoa has been traditionally consumed in the form of grain or as an ingredient in many food products and dishes, such as soups, porridges, buns, and drinks [3]. Its growing popularity has led to the development of novel foods containing quinoa, in particular gluten-free, vegetarian, vegan, and dairy-free products [4, 5].

Although quinoa´s nutrient-rich profile has been the subject of extensive research, most of the studies were, however, realized on raw materials. Nonetheless, quinoa is usually not eaten raw but processed to decrease the content of anti-nutritional compounds, such as saponins and phytic acid [6, 7]. Studies have shown that commonly used processing methods, such as boiling, steaming, microwaving, and extrusion may alter the nutritional content and composition, as well as the overall bioavailability of nutrients. For example, microwaving and boiling under pressure have been reported as a suitable technique for the preservation of polyphenols. In comparison, boiling caused the major loss of phenolic compounds and minerals [6, 8]. Although the protein content is not significantly affected by the common heat-utilizing preparations [6], it has been described that some methods like microwaving and fermentation may increase the protein digestibility of the final quinoa product [9, 10].

Apart from heat-utilizing preparations, germination has emerged as an alternative and relatively cheap processing technique for improving the nutritional profile by promoting enzymatic activity and release of various bioactive chemicals and minerals [11, 12], while reducing the content of antinutritional factors like phytic acid and tannin [13, 14]. Germination may further improve the biological value of quinoa protein and its overall digestibility [15, 16].

Comprehensive studies are needed to explore the impact of different preparation methods on those traits and their implications for further food processing. Therefore, this paper aimed to evaluate a spectrum of quinoa seed preparation methods and evaluate their impact on the content of protein, antioxidant activity, total phenolic content, and 13 phenolic compounds. By providing an extensive analysis of these effects, this paper aims to raise awareness about quinoa nutritional quality and the selection of appropriate

processing techniques to preserve the high-quality nutritional profile of quinoa food products.

4.2. Materials and methods

4.2.1. Plant material

A total of three quinoa samples were subjected to analysis. The original seeds of two quinoa samples (genotypes ´Besancon´ and ´Faro´) were provided from the U.S. National Plant Germplasm System operated by USDA. The seeds of these two genotypes were multiplied to provide sufficient material for further experiments in the experimental field of the Crop Research Institute in Prague in the Czech Republic during the year 2021. One commercial quinoa sample (Probio) was kindly provided by PRO-BIO Ltd, Czech Republic.

4.2.2. Procedure of germination

Germination was carried out on commercial Probio samples and genotypes ´Besançon´ and ´Faro´. A total of 10 g of healthy and undamaged seeds from each sample was used for the experiment. In addition, seeds of genotypes ´Besançon´ and ´Faro´ were thoroughly rinsed in 30% (v/v) hydrogen peroxide for disinfection purposes to minimize microbiological contamination of the seed surface from the field condition. Then, the seeds of all three samples were washed several times in distilled water. All three samples were soaked in distilled water for 4 h, drained, and then placed in a sterile Petri dish lined with moist filter paper and covered with the lid. Hydrated quinoa seeds were allowed to germinate for 1 day (24 h), 2 days (48 h), 3 days (72 h), 4 days (96 h), and 5 days (120 h), respectively. The germination of the Probio sample is shown in Figure 4.1. Germination was performed at room temperature under a 16/8 day/night regime and seeds were regularly watered with distilled water. Sprouted seeds were collected each day of germination and lyophilized before the next use. Samples were stored in a cold and dark place for following laboratory analyses. The non-germinated samples were indicated as control samples.

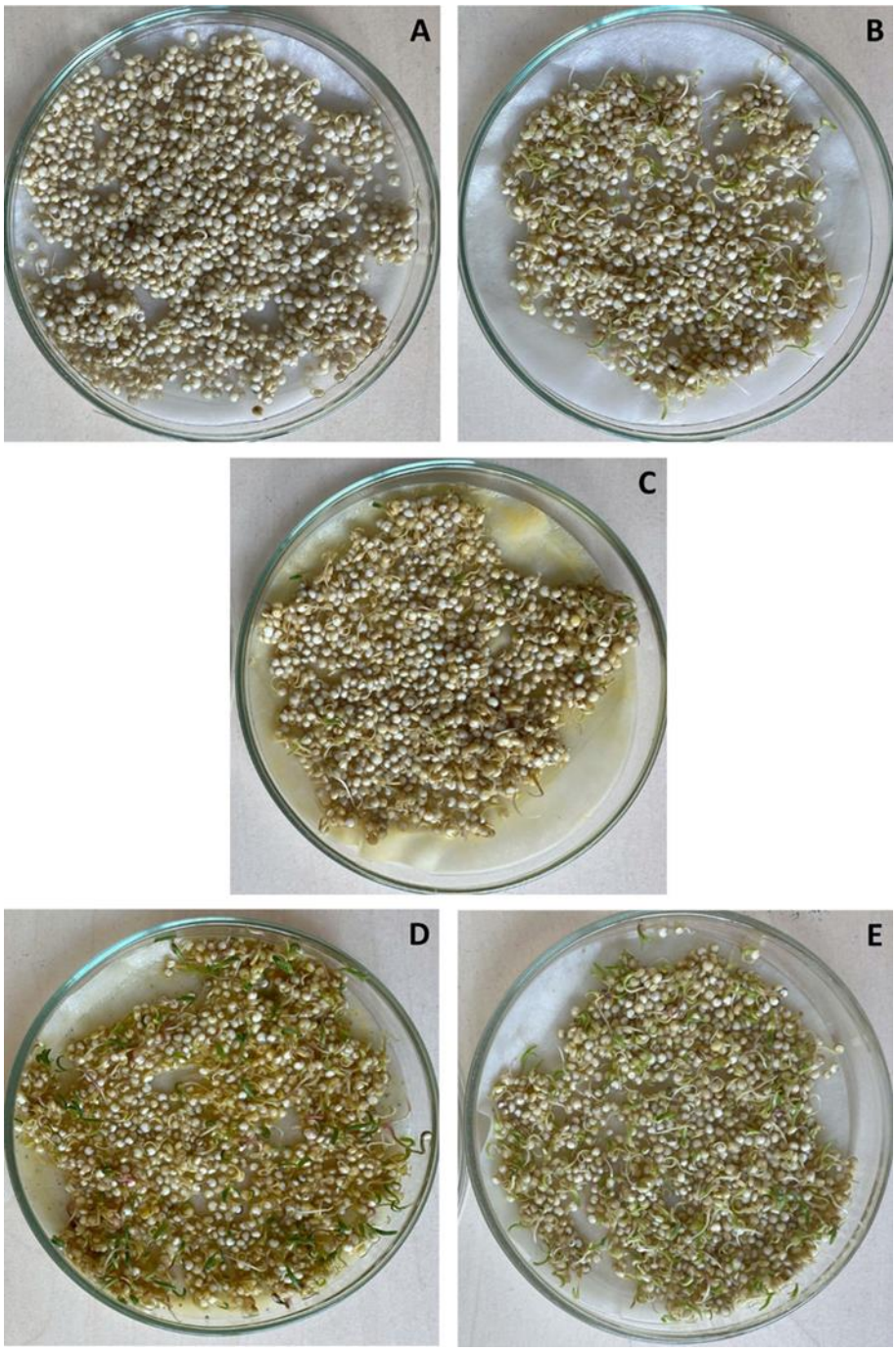

Figure 4.1 Germination of Probio sample for 1 day (24 h, A), 2 days (48 h B), 3 days (72 h, C), 4 days (96 h, D), and 5 days (120 h, E)

4.2.3. Processing techniques

The commercial sample Probio was subjected to several heat-utilizing processing techniques. All processing techniques were carried out under atmospheric pressure at room temperature. Before each thermal processing, the Probio sample was soaked in distilled water for 24 hours. The excess water was drained from the samples before the following treatments. For each treatment, the sampling intervals were established. After each sampling interval, quinoa seeds were immediately drained from any excess water, transferred to a sterile container, and labeled accordingly for subsequent analysis. After a cool-down, samples were lyophilized and stored in a cold and dark place for following laboratory analyses. The non-processed samples were indicated as control samples.

In the case of boiling in plain water, grains were boiled in distilled water in the ratio of 1:2 (w/v) for 5, 10, and 15 min. The boiling in NaCl used the same proceeding, salt was at the concertation of 10 g/L (w/v). Microwaving was realized in the microwave oven (ETA 2209 90,000, ETA a.s., Czech Republic) for 1, 2, and 3 min at the power of 1050 W. Another batch of samples was roasted on the pan for 5, 10, and 15 min at the temperature of 180 ± 20 °C. Lastly, steaming was carried out by placing the quinoa grains on a fine mesh sieve and set over boiling water, covered with a lid. The sample was steamed for 5, 10, and 15 min.

Further, raw Probio seeds were mechanically pressed to obtain flakes using a food processor (Jupiter Küchenmaschinen, System Drive Unit, Weimar, Germany) equipped with a flake roller. Raw quinoa flakes were boiled in distilled water in a ratio of 1:2 (w/v) for 1, 2, 3, 4, and 5 min following the same procedure as mentioned in the first paragraph. Boiled flakes were subjected to chemical analysis.

4.2.4. Chemicals

Polyphenolic compounds, including 4-OH benzaldehyde, caffeic acid, gallic acid, isoquercetin, isorhamnetin, kaempferol, naringenin, pinocembrin, quercetin, quercetin 3-O-glucuronide, rutin, and salicylic acid, along with the internal standard probenecid were procured from Sigma–Aldrich (St. Louis, MO, USA). Methanol of LC-MS grade (≥ 99.9%) was sourced from Riedel de Haën (Seelze, Germany), while formic acid of LC-MS grade (99%) was obtained from VWR (Leuven, Belgium). Pure water was acquired from a Milli-Q purification system manufactured by Millipore (Bedford, MA, USA).

4.2.5. Standard and sample preparation

The preparation of reference stock solutions involved dissolving the methanol-dissolved reference standards of each phenolic compound to create stock solutions at a concentration of 0.5 mg/mL. These reference stock solutions were subsequently stored at −18°C. To establish the calibration curves for quantifying the phenolic compounds, the stock solutions were diluted within a methanol concentration range of 0.001–2.000 µg/mL. In addition, probenecid was dissolved in methanol at a concentration of 0.5 mg/mL to generate a stock solution of the internal standard. The internal standard was then added to the individual reference standard solutions or test samples, resulting in a final concentration of 0.1 µg/mL.

For the analysis using mass spectrometry, the lyophilized samples were milled using an IKA A11 basic mill (IKAWerke, Staufen, Germany), and the resulting mixture was stored in well-sealed plastic bags in a dark, cold place at 4°C. The extraction of the sample followed the method described by Janovská et al. [17]. Briefly, 0.1 g of the milled mixture was extracted twice with 1 mL of extraction solvent (comprising 80% methanol with probenecid as internal standard at a concentration of 0.1 µg/mL) in Eppendorf tubes. The extraction was performed using an ultrasonic bath for 60 min at 45°C. After extraction, the samples were centrifuged for 10 min at 13,500 rpm. The obtained supernatants from each sample were then filtered through 0.2 µm nylon syringe filters for further analysis.

4.2.6. UHPLC-ESI–MS/MS instrumentation

The chromatographic analysis was conducted using the Dionex UltiMate 3000 UHPLC system (Dionex Softron GmbH, Germering, Germany), comprising a binary pump (HPG-3400RS), an autosampler (WPS-3000RS), a degasser (SRD-3400), and a column oven (TCC-3000RS). Detection of analytes was performed on the quadrupole/orbital ion trap Q Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The LC-MS system was equipped with a heated electrospray ionization source (HESI-II) and operated using Xcalibur software, version 4.0 (Thermo Fisher Scientific, San Jose, CA, USA).

4.2.7. UHPLC-ESI–MS/MS analysis

The analytes were separated on a reversed-phase C18 Ascentis Express column (2.1 × 100 mm, 2.7 µm) from Supelco (Bellefonte, PA, USA). The chromatographic separation was performed using a gradient elution method.

Solvent A consisted of 0.2% formic acid (v/v) in water, while solvent B comprised methanol with 0.2% formic acid (v/v). The LC gradient commenced with 99% of solvent A and 1% of solvent B at 0 min., followed by a linear gradient elution to 40% A and 60% B at 11 min. The column was then flushed with 100% solvent B for 2 min. Equilibration of the column was accomplished by washing with 99% A and 1% B for an additional 2 min. The total analysis time was 15 min. The column temperature was maintained at 40°C, and the flow rate was set to 0.35 mL/min. The injection volume was 1 µL.

The mass spectrometer analysis was conducted in negative electrospray ionization (ESI) mode. The spray voltage was set at −2.5 kV, and the sheath gas flow rate, auxiliary gas flow rate, and sweep gas flow rate were 49, 12, and 2 arbitrary units, respectively. The capillary temperature was 260°C, and nitrogen was used as the sheath, auxiliary, and sweep gas. The heater temperature was maintained at 419°C, and the S-lens RF level was set to 30. Precursor ions in the inclusion list were isolated within a retention time window of ± 60 s, filtered in the quadrupole at the isolation window (target m/z ± 0.8 m/z), and fragmented in an HCD collision cell C-trap at a resolution of 17,500 FWHM (full width at half maximum). The AGC target value was 1 × 106, and the maximum injection time was 50 ms.

The normalized collision energy (NCE) was optimized for each compound. Details of the precursor and daughter ions monitored, retention times, and NCE values can be found in Table S1. The precision and calibration of the Q Exactive Orbitrap LC/MS/MS instrument were assessed using a reference standard mixture provided by Thermo Fisher Scientific. The measurements were performed in triplicate, and the data were evaluated using Quan/Qual Browser Xcalibur software, version 4.0.

4.2.8. Determination of the phenolic compound concentration in quinoa samples

The identification of phenolic compounds in the quinoa samples relied on their retention times compared to authentic standards and the analysis of mass spectral data obtained through LC-MS/MS. Accurate mass determination was employed to generate elemental compositions and fragmentation patterns of the molecular ions. Quantification was done based on the transition from precursor ion [M + H]+ to the corresponding quantification ion (Table S1). Calibration curves were then established by plotting the peak area, adjusted with probenecid as an internal standard, against the concentration of the corresponding reference standards.

4.2.9. Chemical analyses

All three quinoa samples were also investigated for the effects of germination on the protein content (CP), total phenolic content (TPC), and antioxidant activity (AA). For analysis, lyophilized samples were used. The CP content of each sample was measured using the classical Kjeldahl mineralization method and calculated using a conversion factor of 6.25 [18]. For this method, 1 g of milled sample was utilized. The TPC was determined using Folin–Ciocalteau (FC) reagent with slight modifications based on the method [19]. The FC method employed 2 g of sample. The TPC results were expressed as grams of gallic acid equivalent (GAE) per kilogram of sample DW (GAE g/ kg DW). The AA of the samples was assessed using a DPPH assay [20], utilizing 1 g of milled sample in this study. The results of the DPPH assay were expressed as millimoles of Trolox equivalent (TE) per gram of sample DW (μmol TE/g DW). Two replicates were performed for each protein content, TPC, and AA measurement.

4.2.10. Statistical analyses

Three biological replicates were measured for descriptors of interest. Statistical analysis was conducted using the R program [21]. Means and standard deviations were calculated for each sample type and processing method in individual traits. One-way analysis of variance (ANOVA2) was performed to determine whether there was a significant effect of the preparation method or sample type on evaluated traits. For germination data, the method was also applied to confirm if there was a significant difference between the three evaluated cultivars. Tukey´s honestly significant difference (HSD) test was employed to identify processing methods and their variants with significantly different means. To explore the association among samples, a principal component analysis (PCA) was conducted using scaled data for a set of 14 descriptors. The quality of the representation of variables on the factor map was also assessed for the first two components with the largest variance. The routines within FactoMineR [22] and factoextra packages [23] were used for this task and to visualize PCA results.

4.3. Results and discussion

4.3.1. The effect of germination

The presented study investigated the effects of germination on the CP, TPC, AA, and the quantity of twelve metabolites in two quinoa genotypes ´Faro´ and ´Besançon´ and one commercial sample Probio (Figure 4.2). During germination, slight but statistically significant changes (at $p < 0.05$) in CP were observed across all samples, depending on the duration of germination. Probio displayed the highest increase on the fourth day, reaching 17.18 ± 0.01% DW compared to 15.47 ± 0.21% DW in control. 'Faro' and 'Besançon' peaked on the third day reaching CP of 13.93 ± 0.02% and 15.58 ± 0.15% DW, respectively. Such increases in protein content have also been documented in other Amaranthaceae species [14].

The elevated CP levels can be attributed to the enhanced enzymatic activity, particularly α-amylase, liberating proteins packed proteins in starch granules [24] or due to *de novo* synthesis [14]. Additionally, seed respiration during germination reduces dry weight, contributing to an apparent increase in CP percentage [14].

Germination also significantly improved AA, which assess it as the superior processing technique compared to others in this study. Although AA initially declined by 30% in 'Faro' and 'Besançon', it subsequently increased, peaking on the third day in 'Faro', fourth in 'Besançon', and fifth in Probio.

The rise in AA is likely due to elevated enzymatic activity and the synthesis of low-molecular-weight antioxidants, although germination conditions play a critical role in the magnitude of this increase [7].

Besides, our results indicated that differences in AA increment were related to the studied sample/genotype, confirming the earlier reported research carried out on white and red quinoa [25]. In our case, the most promising sample was Probio, since it did not show any remarkable drop in the beginning of germination, and it further reached the highest AA values on the fourth day of germination among other studied samples.

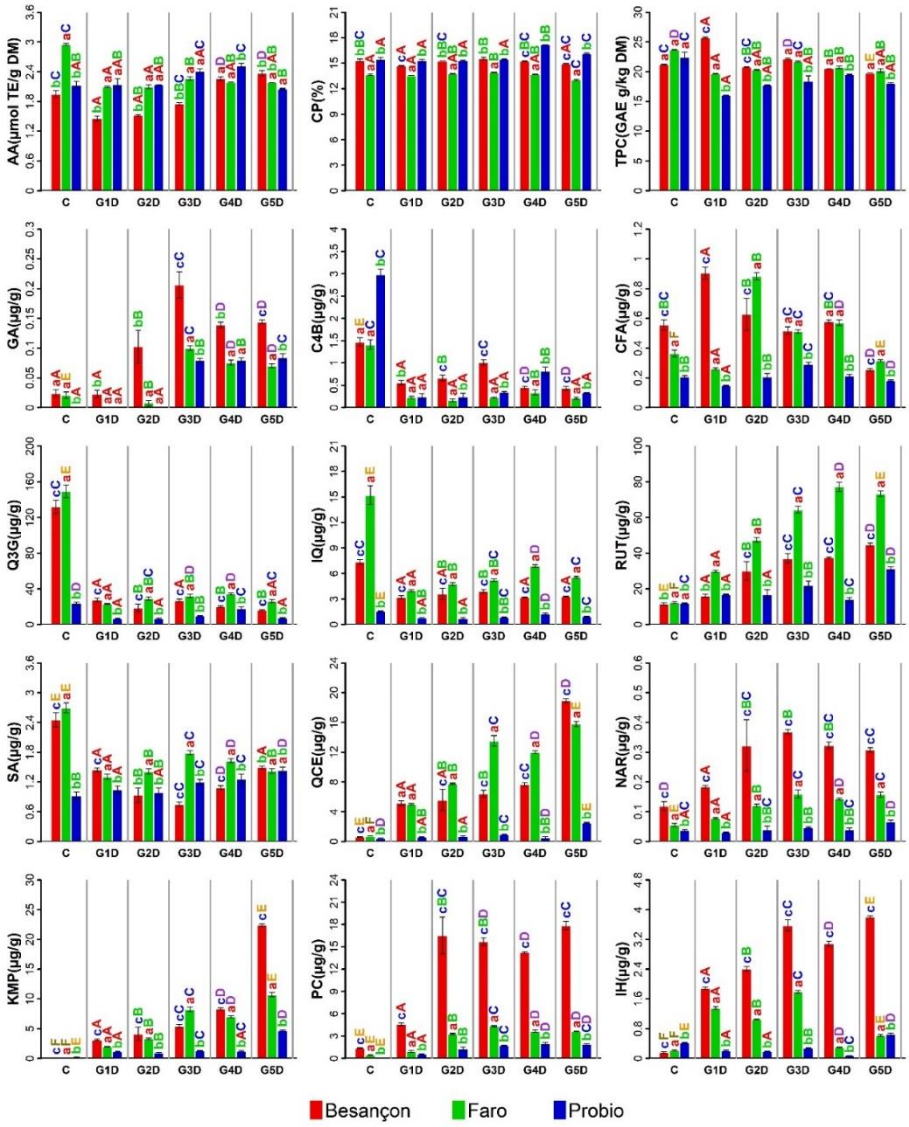

Figure 4.2 The effect of germination time on selected nutritional parameters of three quinoa samples.

Significant differences in means among control (C) and days of germination [1 day (G1D), 2 days (G2D), 3 days (G3D), 4 days (G4D), and 5 days (G5D)], are denoted by the different letters (Tukey HSD) above each column. Letters A-C indicate statistical differences within treatments, while letters A-E denote statistical differences among treatments for individual cultivars. The error bars displayed in the respective plots represent the standard deviations from the means. The abbreviations for the selected descriptors are as follows: gallic acid (GA), 4-hydroxybenzaldehyde (C4B), caffeic acid (CFA), quercetin-3-O-glucuronide (Q3G), isoquercetin (IQ), rutin (RUT),

salicylic acid (SA), quercetin (QCE), naringenin (NAR), kaempferol (KMP), pinocembrin (PC), isorhamnetin (IH), crude protein content (CP), antioxidant activity (AA), total phenolic content (TPC)

Significant variations ($p < 0.05$) in TPC were indicated among the quinoa samples and germination days. The highest TPC was recorded for the ´Besançon´ (25.77 ± 0.15 GAE g/kg DW) on the second day of germination, which aligns with the findings of Guardianelli et al. [25], but conflicts with Bhinder et al. [25], who recognized the peak values during the third and fourth day of germination. Detected contradictions may be attributed to the dynamic chemical changes during the germination including compound synthesis, release from bound form, or consumption [26]. In addition, specific germination conditions should be taken into consideration as factors influencing the TPC during germination [27]. As opposed to ´Besançon´, ´Faro´, and Probio samples showcased their highest TPC values in the non-germinated state (23.71 ± 0.08 and 22.46 ± 0.88 GAE g/kg DW, respectively). Different rates of polyphenol accumulation in two different quinoa samples were published formerly [25], indicating that selecting the optimal genotype is crucial for maximizing phenolic compound levels during germination.

The content of twelve studied metabolites determined by UHPLC-ESI–MS/MS analysis is given in Figure 4.2. The dominant compound in non-germinated quinoa sample was quercetin 3-O-glucuronide (Q3G), also known as miquelianin, whereas rutin (RUT) became the most abundant in germinated samples. RUT demonstrated an increasing accumulation with extended germination time, peaking between the fourth and fifth days, particularly in ‘Faro’. Similar findings were presented in the study of Al-Qabba et al. [28] and Bhinder et al. [28].

As mentioned in the beginning, Q3G was the most abundant metabolite in non-germinated quinoa seeds, which is in agreement with Dostalikova et al. [29]. This metabolite has been primarily detected in aerial plant parts in various plant species [30–32], but research quantifying the content of Q3G in seeds is insufficient. During the germination, Q3G showcased an opposite pattern as RUT with an 80% decline in the initial days of germination in ´Besançon´ and ´Faro´. Contradictory results were published by Pilco-Quesada et al. [16] demonstrating a significant growth in the content of Q3G after 72 h of quinoa germination.

The isoquercetin (IQ) followed the same trend as discussed here in the case of Q3G. The drop in values was also noticed for salicylic acid (SA) and 4-

hydroxybenzaldehyde (4BA) after the first day of germination. Gallic acid (GA), naringenin (NAR), and caffeic acid (CFA) were presented in quinoa samples in relatively trace concentrations, concerning other studied compounds. The germination process improved their content, especially during the first 3 days of germination. To the best of our knowledge, NAR, SA, 4BA, and IQ have not been quantified in germinated quinoa before.

A small amount of kaempferol (KMP), pinocembrin (PC), quercetin (QCE), and isorhamnetin (IH) was detected in non-germinated samples. These metabolites were rapidly synthesized during the fifth day of the germination process, but the degree of increment varied among the studied genotypes. Besides, the mean PC content was the highest in germinated quinoa contrasting to raw and heat-treated samples. While the increase in KMP and QCE concentrations during germination has been already published for quinoa [7, 28], it was not as prominent as observed in our study. To our knowledge, the presence of PC and IH in germinated quinoa has not been evaluated before, nonetheless, they have already been described in sprouted mung bean [33] and buckwheat [34].

Overall, the germination process led to the enhancement of several bioactive compounds, including GA, CFA, RUT, QCE, NAR, KMP, PC, and IH in comparison to the control sample, suggesting their potential role in the germination process. The most substantial increase in the content of these metabolites was reported between the third and fifth days of germination. This pattern further aligns with consumer trends favouring antioxidant-rich foods, known for their health benefits, particularly in reducing oxidative stress. The ability to enhance bioactive compounds through germination offers potential for food industry aiming to position quinoa as a functional food [57].

Conversely, germination initiated a decline in the levels of 4BA, Q3G, IQ, and SA. The alterations in metabolite quantity occurred in a genotype-dependent manner, with ´Besançon´ and ´Faro´ exhibiting the most intense synthesis of metabolites during germination. On the other hand, the changes in the chemical content of the Probio sample were less prominent.

It has been suggested previously that various metabolic and enzymatic events occurring during germination may synthesize or consume the phenolic compounds, thus elevating or decreasing their overall content. In addition, those compounds play a non-negligible role in protection against free radicals generated during the germination process [26]. However, other factors like genotype, agronomic conditions, maturity level at harvest, and postharvest

storage conditions may considerably contribute to the variations in the polyphenol content of germinated quinoa [26].

4.3.2. Seed soaking

Quinoa seeds are prized for their superior nutritional quality, especially their high content of proteins and bioactive chemicals [35]. While this area has been researched extensively, most of the studies examined only raw materials, which might not give a full picture of quinoa's potential and health benefits. Therefore, this paper evaluated the effect of various processing methods and processing time on the CP, TPC, and AA (Figure 4.3) and the content of selected bioactive compounds (Table 4.1) of the Probio sample. Soaking was proven to be effective in minimizing the content of anti-nutritional compounds [36]. However, our results indicated that soaking in water worsened the majority of the studied nutritional parameters. The exceptions were metabolites KMP, NAR, PC, and RUT where soaking led to a rise in their content. Presented alterations after soaking might be related to various factors.

The reduction in CP could be attributed to a leaching of quinoa seed storage proteins into soaking water [14]. Similarly, a softening of cell wall tissues could potentially facilitate the increased release of polyphenols into the soaking medium [37], thus possibly reducing the TPC and AA of soaked seeds. In addition, the variability in the metabolite content could be ascribed to the commencement of the seed germination processes, as discussed above.

4.3.3. Boiling in plain water and NaCl solution

When comparing two boiling solutions, boiling in plain water showed slightly better results in CP content and the AA, principally after 15 min of treatment, with respect to the boiling in NaCl. The values reported after 15 min of boiling in plain water were relatively similar to the control sample in both parameters. It was previously stated that no significant alterations in CP occurred after 15 min of boiling [6]. However, other studies have reported that a decrease in CP may occur due to leaching into the boiling solution [14], and this effect is more pronounced when washing is combined with boiling [36].

Boiling significantly decreased the TPC and AA in quinoa seeds. Several factors may affect the parameters, including sample variety, processing conditions, and analytical methods. A decrease observed in this study was likely due to the leaching of polyphenols into the boiling water, which reduced their concentration in the final product. Furthermore, thermal degradation may contribute to the overall decline in TPC and AA [38]. Conversely, the release

of polyphenols and the inactivation of phenol oxidase during boiling may enhance polyphenol availability, as previously observed in buckwheat [37].

Boiling in plain water was also not suited for the enhancement of 4BA, CFA, IQ, Q3G, and SA (Table 4.1), but it slightly improved the content of KMP and PC, compared to control. Boiling in NaCl was considered more beneficial in contrast to boiling in plain water since most of the studied metabolites reached higher mean values in their content. The presence of salt in the solution could increase the boiling point and therefore induce a higher degree of thermal dissociation of bound molecules, as proposed for pulses [39].

In the principal component analysis (PCA) plot (Figure 4.4), boiling in both solutions was grouped in the lower-left region, indicating that both methods were generally not very effective for nutrient preservation compared to other processing methods, possibly due to the nutrient leaching. Therefore, to minimize nutrient loss, boiling should be conducted with precise amount of water to avoid draining of the solution that may contain valuable nutrients leached from the seeds.

4.3.4. Flaking

Flakes from whole quinoa seeds demonstrated a noteworthy reduction in the required boiling time, reducing it to a mere 5 min, concerning the boiling of whole seeds. Therefore, further utilization of quinoa flakes could be potentially advantageous in mitigating the heat-induced degradation of thermally unstable compounds. It is noteworthy that research in this specific domain for quinoa remains scarce. The shorter cooking time of quinoa flakes enhanced the TPC and the content of IQ, NAR, and SA, in contrast to the boiled seeds, however, compared to other treatments, flaking was characterized by lower antioxidant activity, reduced phenolic content, and minimal association with key bioactive compounds, except for 4BA and Q3G (Figure 4.4). In addition, boiled flakes achieved the highest CP values when compared to all the other heat-utilizing methods investigated herein. While the precise impact of flaking and boiling of quinoa flakes on the final nutritional quality has not been studied yet, it was previously concluded that flaking of ancient cereals and legumes may increase or decrease the TPC and AA depending on the type of sample. Contrary, the protein content was not significantly affected by flaking [40].

Growing environmental awareness, concerns about animal welfare and a strong shift toward sustainable eating habits have driven an increased demand for plant-based protein sources [52]. Additionally with the increasing demand for protein-rich foods in human diets [53], rolled quinoa flakes may offer a

promising alternative used as enhancement of low-protein morning cereals and porridges [54]. Moreover, quinoa's high nutritional value makes it an ideal ingredient for the development of novel, protein-enriched food products.

4.3.5. Microwaving

Microwaving was a relatively suitable method for enhancing the protein content since the mean CP of microwaved samples was the second highest among other studied treatments (15.56 ± 0.10%). Furthermore, CP remained unaffected by varying microwave exposure times (Figure 4.3). There is a lack of comprehensive studies elucidating the impact of microwaving on quinoa CP, however, studies conducted on other species, such as legumes and buckwheat, indicated quite variable outcomes in this area [41, 42]. While the mean AA values were statistically comparable to roasting and boiling in NaCl, the TPC values for microwaving were outstanding, reaching the peak in the third minute (27.15 ± 0.82 GAE g/kg DW). In a parallel study, a similar reduction in AA with increasing time of processing was noticed, nevertheless, the highest TPC was detected after 5 min of microwaving [43].

Half of the studied metabolites, namely CFA, KMP, NAR, QCE, RUT, and SA, displayed the highest mean content during microwaving (Table 4.1), in comparison to other heat treatments and raw samples. This observation aligns with the PCA analysis results (Figure 4.4), where microwave-treated samples distinctly cluster along the first principal component axis, revealing a strong influence from the mentioned traits. Including all heat treatments, GA was only found in microwaved and roasted samples.

As concluded by Drulyte and Orlien [44], the heating effect of microwaving is more intense and faster than alternative cooking methods. This distinctive trait leads to a reduction in overall processing time and, notably, correlates positively with diminished losses of polyphenolic compounds [38]. In addition, microwaving generates heat that causes rapid expansion and pressure build-up within plant cells. This leads to the rupture of cell walls, facilitating the release of phenolic compounds that were previously bound within the cellular matrix or associated with cell wall components [51]. Our results confirmed the conclusions of other studies that microwaving yields the highest number of polyphenols among other heat treatments, thereby increasing the overall antioxidant capacity [39, 43, 45, 46].

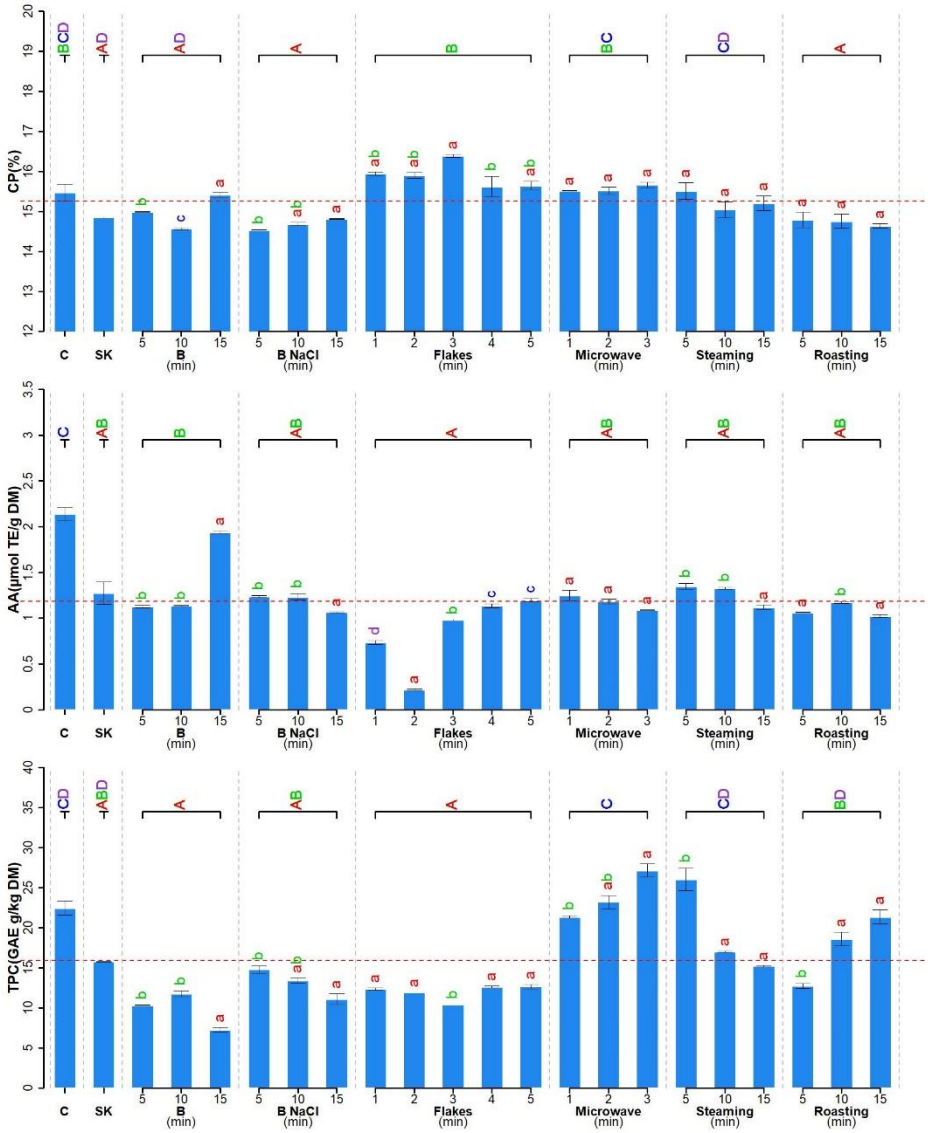

Figure 4.3 The effect of various processing methods and processing time on crude protein content (CP), antioxidant activity (AA), and total phenolic content (TPC) of commercial Probio sample.

Significant differences in means (Tukey HSD) within individual treatments are indicated by different letters (a-c) above each column. The letters (A-E) in the header of the plot show the difference among individual treatments. A dashed red line within each plot denotes the overall mean of data for the respective variable. The error bars displayed in the respective plots represent the standard deviations from the means. The abbreviations for the selected processing methods are as follows: control (C), SK (soaking), B (boiling), and B NaCl (boiling in NaCl) (min).

Table 4.1 The effect of various thermal processing on the content of selected quinoa metabolites*

	4BA	CFA	GA	IH	IQ	KMP
Control	3.104±0.119D	0.217±0.007F	0.001±0B	0.437±0.013BCD	1.593±0.044E	0.27±0.02BF
Soaking	0.326±0.01AC	0.147±0.003A	n.d.	0.48±0.012BCD	0.579±0.02AB	1.293±0.082AE
Boiling 5(min)	0.36±0.068bA	0.102±0.011bE	n.d.	0.114±0.004bBD	0.632±0.078aA	0.751±0.023bEF
Boiling 10(min)	0.269±0.028bA	0.09±0.004abE	n.d.	0.873±0.041cBD	0.536±0.02aA	0.691±0.027bEF
Boiling 15(min)	0.407±0.049aA	0.088±0.008aE	n.d.	0.575±0.011aBD	0.533±0.041aA	1.068±0.123aEF
Boiling NaCl 5(min)	0.27±0.028aA	0.116±0.004bA	n.d.	0.055±0.002bA	0.57±0.017aAB	1.311±0.024bA
Boiling NaCl 10(min)	0.338±0.053aA	0.129±0.004cA	n.d.	0.079±0.003cA	0.62±0.008bAB	1.687±0.061aA
Boiling NaCl 15(min)	0.314±0.028aA	0.105±0.002aA	n.d.	0.05±0.007aA	0.611±0.046aAB	1.649±0.063aA
Flakes 1(min)	0.931±0.034dB	0.022±0.001eB	n.d.	0.026±0.002eA	0.554±0.013dC	0.153±0.003eB
Flakes 2(min)	1.818±0.029aB	0.041±0.001aB	n.d.	0.091±0.006aA	1.031±0.029aC	0.412±0.015aB
Flakes 3(min)	1.674±0.047bB	0.076±0.003bB	n.d.	0.08±0.003bA	0.875±0.021bC	0.328±0.021bB
Flakes 4(min)	1.451±0.107cB	0.06±0.003cB	n.d.	0.052±0.008cA	0.655±0.025cC	0.218±0.032cB
Flakes 5(min)	1.366±0.063cB	0.071±0.002dB	n.d.	0.055±0.002dA	0.7±0.047cC	0.268±0.028dB
Microwave 1(min)	0.431±0.046aAC	0.276±0.005aC	0.002±0.001abA	0.325±0.008cB	0.869±0.023cD	4.396±0.132cC
Microwave 2(min)	0.412±0.05aAC	0.271±0.014aC	0.001±0aA	0.48±0.055aB	0.825±0.022aD	3.277±0.097aC
Microwave 3(min)	0.491±0.047bAC	0.361±0.014bC	0.003±0.001bA	0.63±0.031bB	1.007±0.023bD	5.876±0.115bC
Steaming 5(min)	0.458±0.016bC	0.11±0.003bA	n.d.	0.179±0.009bD	0.718±0.02bBC	1.145±0.051bA
Steaming 10(min)	0.312±0.029cC	0.091±0.004cA	n.d.	0.478±0.066cD	0.628±0.036cBC	1.928±0.119cA
Steaming 15(min)	0.696±0.037aC	0.181±0.005aA	n.d.	1.629±0.038aD	0.762±0.022aBC	2.401±0.05aA
Roasting 5(min)	0.357±0.026bC	0.145±0.003bD	0.001±0aB	0.026±0.002bAC	0.672±0.035bD	1.859±0.068bD
Roasting 10(min)	0.591±0.065cC	0.177±0.004cD	0.001±0.001aB	0.396±0.013cAC	0.797±0.048cD	2.339±0.17cD
Roasting 15(min)	0.716±0.041aC	0.215±0.008aD	0.001±0.001aB	0.157±0.033aAC	1.296±0.066aD	3.888±0.261aD

(Continued)

Table 4.1 (Continued)

	NAR	PC	Q3G	QCE	RUT	SA
Control	0.04±0.003E	0.098±0.002B	25.057±1.266D	0.487±0.028AB	12.09±0.217BF	0.999±0.077C
Soaking	0.097±0.007B	0.842±0.023E	7.137±0.155AC	0.745±0.03AB	30.118±0.863D	1.025±0.054BC
Boiling 5(min)	0.039±0.002aE	0.234±0.022bF	7.517±1.411bC	0.559±0.032abAB	11.188±1.124bA	0.574±0.035bD
Boiling 10(min)	0.019±0.003bE	0.22±0.025bF	4.983±0.207aC	0.523±0.034bAB	9.481±0.192abA	0.356±0.012aD
Boiling 15(min)	0.043±0.008aE	0.271±0.007aF	5.226±0.899aC	0.62±0.066aAB	9.04±0.61aA	0.368±0.01aD
Boiling NaCl 5(min)	0.076±0.006bA	0.375±0.014bA	7.673±0.531bA	0.954±0.032aA	11±0.346bAB	0.795±0.044bA
Boiling NaCl 10(min)	0.074±0.004bA	0.375±0.018bA	8.755±0.176cA	1.789±0.279bA	11.712±0.305cAB	0.727±0.026cA
Boiling NaCl 15(min)	0.062±0.003aA	0.347±0.011aA	7.866±0.088aA	1.166±0.118aA	10.381±0.63aAB	0.526±0.021aA
Flakes 1(min)	0.053±0.004dBC	0.143±0.013bB	11.364±0.311dB	0.06±0.007dB	5.347±0.154cC	0.776±0.03bA
Flakes 2(min)	0.095±0.006aBC	0.217±0.005aB	19.24±0.467aB	0.265±0.018aB	9.17±0.174aC	1.192±0.055aA
Flakes 3(min)	0.091±0.007aBC	0.132±0.005bB	17.799±0.373bB	0.282±0.02aB	7.268±0.086bC	0.728±0.027bA
Flakes 4(min)	0.076±0.002bBC	0.111±0.02cB	12.854±0.378cB	0.184±0.049bB	5.35±0.217cC	0.493±0.013cA
Flakes 5(min)	0.112±0.008cBC	0.079±0.004dB	13.17±0.65cB	0.197±0.007cB	5.458±0.261cC	0.507±0.026cA
Microwave 1(min)	0.141±0.006bD	0.517±0.018aC	13.011±0.346cB	5.512±0.2cC	30.746±1.129cD	1.138±0.032cB
Microwave 2(min)	0.133±0.007aD	0.526±0.018aC	10.654±0.659aB	3.898±0.107aC	27.672±0.841aD	1.046±0.018aB
Microwave 3(min)	0.139±0.003bD	0.503±0.013aC	18.169±0.334bB	8.292±0.297bC	36.635±1.001bD	1.351±0.076bB
Steaming 5(min)	0.064±0.006bAC	0.492±0.024bC	9.824±0.491aA	0.543±0.041bA	14.539±0.64aEF	1.101±0.039aBC
Steaming 10(min)	0.08±0.003cAC	0.609±0.017cC	8.828±0.958bA	0.574±0.028bA	14.254±0.677aEF	1.064±0.017aBC
Steaming 15(min)	0.092±0.005aAC	0.45±0.014aC	10.613±0.452aA	1.973±0.081aA	14.256±0.518aEF	1.092±0.035aBC
Roasting 5(min)	0.098±0.009aB	0.453±0.029bD	7.785±0.305bA	1.61±0.051bD	13.354±0.537bE	0.948±0.025bC
Roasting 10(min)	0.085±0.005aB	0.443±0.015abD	8.861±0.512cA	2.834±0.154cD	14.103±0.842bE	0.894±0.019cC
Roasting 15(min)	0.096±0.008aB	0.464±0.013aD	9.88±0.443aA	5.587±0.383aD	19.98±1.122aE	1.166±0.035aC

* Results are expressed as mean ± standard deviation. n.d. – not detected.
Different lowercase letters a-g indicate statistical differences by Tukey´s test ($p<0.05$) within different treatments.
Different uppercase letters A-F indicate statistical differences by Tukey´s test ($p<0.05$) among different treatments.
The abbreviations for the selected descriptors are as follows: 4-hydroxybenzaldehyde (4BA), caffeic acid (CFA), gallic acid (GA), isorhamnetin (IH), isoquercetin (IQ), kaempferol (KMP), naringenin (NAR), pinocembrin (PC), quercetin-3-O-glucoronide (Q3G), quercetin (QCE), rutin (RUT), salicylic acid (SA)

4.3.6. Steaming

Steaming is, together with boiling, a commonly employed method of quinoa preparation. Even though both processing methods generally led to a reduction in the content of biologically active compounds compared to raw samples [47], steaming is recommended as a more optimal method for better nutrient retention over boiling [6]. This preference stems from the fact that, during steaming, the quinoa seeds are not in direct contact with boiling water as opposed to boiling, thereby minimizing nutrient leaching into the water [8]. Our results confirmed this statement since the TPC in steamed sample was higher than in boiled samples. In addition, steaming did not significantly affect protein content in quinoa seeds, aligning with previously published research [6, 48] although in contrast with Motta et al. [49], who reported a significant decrease in CP in studied pseudocereals (Amaranthus sp., quinoa, and buckwheat). In terms of studied metabolites, their quantity was either comparable or lower than those observed in other heat treatments, except for IH, reaching the highest value in this study (1.60 ± 0.04 μg/g DW) after 15 min of steaming (Table 4.1).

4.3.7. Roasting

Roasted quinoa seed did not reach any outstanding values for the content of protein and AA since both parameters were statistically comparable to boiling in plain water and boiling in NaCl, respectively (Figure 4.3). Nevertheless, roasted seeds exhibited a great content of total polyphenols, reaching values comparable to the control after 15 min of roasting. The overall increment in TPC during roasting can be attributed to the release of bound chemicals due to heat and the formation of Maillard reaction products, but the yield of phenolics is also influenced by the roasting temperature and time used during processing [11]. This might explain the contradictory results of some studies, showing the TPC and AA of roasted seed with values even higher than the control sample [43, 46] and others with significantly reduced polyphenolic content [8].

In the case of metabolite content, roasting was associated with the enhancement of some metabolites, such as NAR, RUT, KMP, and QCE, especially after 15 min of roasting time (Figure 4.4). Similar metabolites were investigated previously in amaranth [50], nonetheless, the pattern of the changes during roasting was distinct from our results. For example, QCE and KMP significantly decreased after 15 min of roasting, whereas GA and CFA increased rapidly.

The roasting of seeds offers dual benefits by not only enhancing specific bioactive compounds but also improving sensory attributes such as flavor and

aroma [50, 56]. Consequently, roasting can be considered a potentially advantageous preparation method for consumers, contributing to both nutritional value and palatability.

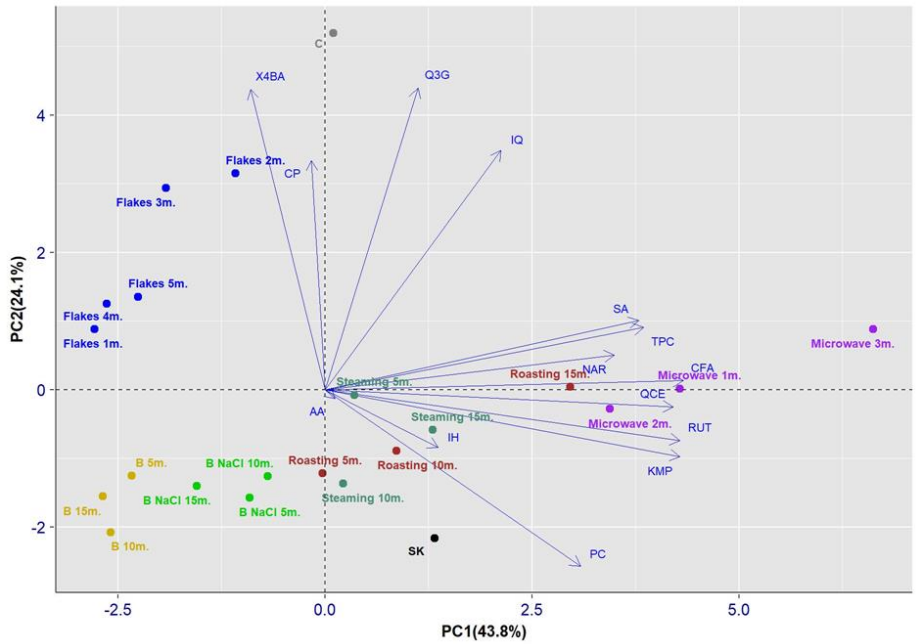

Figure 4.4 Principal component analysis biplot based on scaled data for the set of 14 descriptors and 22 different culinary treatments.

Two main components explaining 43.8 and 24.1% of total variance, respectively, are displayed. Individual points in the plot stand for individual culinary treatments, highlighted by different colors and variants of those treatments, representing treatment duration in minutes (m). The arrows within the plot show the quality of representation of individual descriptors on factor maps and their contribution to the first two axes. The abbreviations for the selected processing methods and descriptors are as follows: control (C), SK (soaking), B (boiling), B NaCl (boiling in NaCl), gallic acid (GA), 4-hydroxybenzaldehyde (4BA), caffeic acid (CFA), quercetin-3-O-glucoronide (Q3G), isoquercetin (IQ), rutin (RUT), salicylic acid (SA), quercetin (QCE), naringenin (NAR), kaempferol (KMP), pinocembrin (PC), isorhamnetin (IH), crude protein content (CP), antioxidant activity (AA), total phenolic content (TPC).

4.4. Conclusion

The present investigation was conducted to assess the impact of germination, soaking, boiling, flaking, microwaving, steaming, and roasting on the selected nutritional characteristics of quinoa. The quantitative analysis of 12 bioactive compounds was conducted in three distinct quinoa samples during a 5-day germination period. In all studied samples, GA, CFA, RUT, QCE, NAR, KMP, IH, and PC were enhanced compared to the control, but the level of increment was contingent upon the type of sample. This underscores the importance of proper selection of genotype for optimum content of biologically active compounds in germinated quinoa. The most substantial increase in bioactive compounds was noticed between the third and fifth day of germination with the highest accumulation of metabolites occurring in the genotypes ´Besançon´ and ´Faro´. Six compounds (NAR, SA, 4BA, IQ, PC, IH) were detected in germinated quinoa for the first time.

This study further examined a range of various heat-utilizing methods. Statistically significant differences were observed in CP among heat treatments. Boiled quinoa flakes exhibited the highest average protein content and proved to be a time-efficient preparation method due to reduced boiling time. The lowest mean values of CP were associated with roasting and boiling. Most of the heat treatments caused a decrease in TPC and AA in comparison to the raw sample. An exception to this trend was microwaving which strongly enhanced the overall TPC of the quinoa sample and the content of several metabolites (CFA, KMP, NAR, QCE, RUT, and SA).

It can be concluded that different processing methods influenced the nutritional content and composition of quinoa differently. The specific effects varied depending on the processing technique, duration of treatment, compound measured, and genotype. Nonetheless, further research is warranted to elucidate the underlying mechanisms driving these changes. The alterations observed in this study emphasize the importance of considering those variables in optimizing the processing standards used for quinoa to obtain the best nutritional profile of the final food product. Therefore, this knowledge contributes to the development of processing techniques that preserve or enhance the nutritional value of quinoa and promote its utilization as a source of health-promoting compounds in human diets.

Supplementary Information: The online version contains supplementary material available at https://doi.org/10.1007/s00217-024-04466-3.

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5. Quinoa leaves as a promising source of nutritional compounds

5.1. Introduction

Quinoa has long been celebrated for its nutritional prowess. While overshadowed by the prominence of quinoa seeds, the utilization of quinoa leaves persisted predominantly in regions of quinoa origin, where it is traditionally used as a vegetable in salads and soups (Angeli et al., 2020). However, recent nutritional analyses have highlighted the potential for integrating quinoa leaves into the modern human diet (Gómez et al., 2024; Villacrés et al., 2022).

Quinoa leaves contain a wealth of phytochemicals, which contribute to their nutritional and health-promoting properties. These phytochemicals include but are not limited to phenolic acids such as coumaric acid, ferulic acid, or gallic acid, and a variety of flavonoids, such as isorhamnetin, quercetin, or rutin (Lin et al., 2019). Additionally, there are approximately 15 monoterpenoids, more precisely monocyclic monoterpenoids, isolated exclusively from quinoa leaves (Dembitsky et al., 2008).

As reported by Gawlik-Dziki et al. (2015), extracts from quinoa leaves possess strong antioxidant capacity and high bioavailability of phenolic compounds, hence underlining its potential in cancer treatment. Additionally, quinoa leaves are rich in bioavailable minerals (Stoleru et al., 2022a), vitamin E, and carotenoids (Tang et al., 2014). They are also great sources of protein with a well-balanced composition of essential amino acids.

Although leaves of quinoa may lack sufficient content of carbohydrates and lipids (Pathan et al., 2019), their abundance in other nutrients makes them an appealing component in food production with the ability to effectively fortify common food products like wheat flour and bread (Gawlik-Dziki et al., 2015; Świeca et al., 2014) or replace other nutritionally poorer leafy vegetables such as spinach, lettuce and rucola (Gómez et al., 2024; Stoleru et al., 2022a; Vazquez-Luna et al., 2019). Additionally, quinoa leaves could be used as an alternative source for individuals seeking plant-based proteins or as a tool for reducing nutritional deficiencies in rural populations (Villacres et al., 2022; Gómez et al., 2024).

On the other hand, the commercialization of quinoa leaf products in the European Union may face regulatory limitations due to novel food legislation. While quinoa seeds are not classified as a novel food since they have been used for human consumption to a significant degree within the Union before 15 May 1997, quinoa leaves require authorization under the EU's novel food regulation (Food and Feed Information Portal Database, 2023). To obtain this status, it is necessary to conduct a thorough assessment of the leaves' safety, nutritional

profile, and potential allergenicity. Expanding our understanding of quinoa leaf cultivation under specific conditions could support their successful introduction as a novel food in Europe, in accordance with Regulation (EU) 2015/2283.

Although quinoa leaves may possess extraordinary nutritional quality, they might be, to some extent, affected by variables, such as sowing date, harvest date, and genotype (Adamczewska-Sowińska et al., 2021; Gómez et al., 2024; Stoleru et al., 2022b). Unluckily, the comprehensive research shedding light on this matter is still insufficient. Hence, this chapter aimed to explore 66 different quinoa genotypes and assess the protein content, antioxidant activity, and total phenolic content of their leaves. The obtained data can be further used for the proper selection of genotypes with preferred traits that will contribute to a diversified and balanced diet.

5.2. Materials and methods

5.2.1. Plant material

A total of 66 quinoa accessions were subjected to analysis. All the accessions were provided by the U.S. National Plant Germplasm System operated by USDA. During the year 2021, the genotypes were sown on the experimental fields of the Crop Research Institute in Prague – Ruzyně, Czech Republic. All accessions were sown in two rows of 1 m in length, 25 cm apart, and 50 seeds per row. In each studied year, the original samples provided by the National Plant Germplasm System were sown. No pesticide or fungal control was applied. The leaves were collected 9 weeks after the sowing at the beginning of the flowering stage. The collection was realized from randomly selected plants and multiple positions on each plant within each experimental plot. Samples were further dried and stored for further analysis.

5.2.2. Sample preparation and chemical analysis

Dried quinoa leaves were milled with an IKA A11 basic mill (IKA-Werke, Staufen, Germany), and the powdered samples were stored in a dark cold place (4°C) in well-sealed plastic bags. The dry weight (DW) content of leaf samples (5 g) was further dried in an electric hot-air drier at 105°C for 4 h, according to the standard method (American Association of Cereal Chemists 1999). The content of crude protein from each sample was determined using the classic Kjeldahl mineralization method and calculated with a conversion factor of 6.25 (ČNS EN ISO 20483 (46 1401), 2014). The protein content measurements were done in two replicates. The results were expressed as % in DW. Total phenolic

content (TPC) was determined using the Folin–Ciocalteau reagent according to Holasova et al., (2002) with slight modifications. The results of the TPC analysis were expressed in grams of gallic acid equivalent (GAE) per kilogram of sample DW (GAE g/kg DW). The antioxidant activity (AA) of the samples was determined using the DPPH assay (Şensoy et al., 2006). The results of the DPPH assay were expressed in millimoles of Trolox equivalent (TE) per gram of sample DW (μmol TE/g DW).

5.2.3. Statistical analysis

Statistical analysis was done in the GraphPad Prism software (GraphPad Software 2024) and Microsoft Office Excel v. 2016. A one-way analysis of variance (ANOVA1) was applied to the data to test whether there was a significant effect of genotypes in evaluated traits. To compare each accession concerning each descriptor, the means along with the standard deviations for each descriptor were calculated separately for each accession and year of observation. Boxplots were also generated to compare the distribution of values among individual genotypes. The performance of studied genotypes in selected traits (protein content, antioxidant activity, total phenolic content) was calculated using the Z-score normalization method. By employing this method, the data were fairly compared across studied parameters.

5.3. Results and discussion

5.3.1. Crude protein content

The investigation of crude protein content (PC) unveiled significant variations among the analyzed quinoa genotypes. Notably, the lowest value of PC was reported for genotype ´QQ57A´ (11.99 ± 0.03% in DW), while the highest PC was attained by genotypes ´DE-1´ (27.60 ± 0.24% in DW) and ´Rosa Junin´ (26.33 ± 0.17% in DW).

Despite the variability, the PC values in the genotypes evaluated in our investigation exceeded those reported for other commonly consumed leafy vegetables, such as spinach, kale, or amaranth, that reach PC values of around 3% (USDA, 2021). Furthermore, the protein content in quinoa leaves surpassed the highest values previously reported for quinoa seeds (20% in DW) (Dostalíková et al., 2023; Hlásná Čepková et al., 2022). Nearly 38% of the samples surpass the protein threshold of 20% in DW. Conversely, the remaining 62% of the samples exhibited protein content values below this threshold.

Previous studies have reported variable PC values in quinoa leaves. Pathan and Siddiqui (2022) documented notably high protein content ranging between 28.2% and 37.0% in DW, in leaves collected in 30–45 days after germination. Similarly, Rodríguez Gómez et al. (2024) reported comparable values of 36–37% in DW in leaves harvested approximately 20 days post-emergence. Aside from the genotype, the age of the leaves appears to be another critical factor influencing the overall PC, potentially elucidating the relatively lower values observed in our study, where leaf samples were approximately 63 days old.

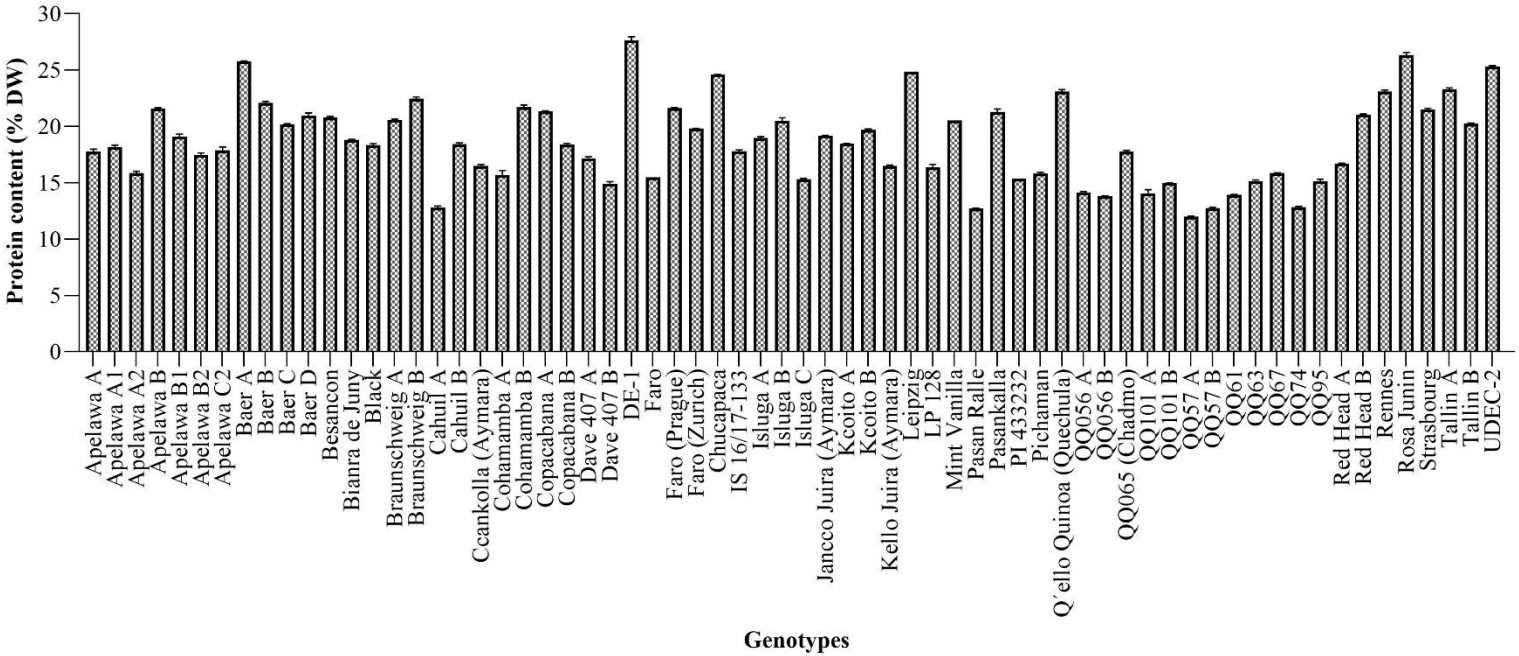

Figure 5.1 The distribution of protein content (% DW) observed in the leaves of 66 quinoa genotypes. Bars represent mean protein content for each genotype, with error bars indicating standard deviation (SD) based on replicate measurements.

5.3.2. Total phenolic content and antioxidant activity

Phenolic compounds, a diverse class of secondary metabolites ubiquitous in the plant kingdom, play a pivotal role in plant defense mechanisms and exert significant antioxidant properties due to their ability to scavenge free radicals (Lin et al., 2019). In the context of quinoa seeds, the accumulation of phenolic compounds is linked to several factors, including agro-environmental conditions, stress, and genetic makeup (Granado-Rodríguez et al., 2021; Reguera et al., 2018). While comprehensive elucidation of these processes in quinoa leaves is lacking, prior investigations have suggested cultivar variability and harvest period as influential determinants of phenolic content (Stoleru et al., 2022b).

Total phenolic content (TPC) and antioxidant activity (AA) in this study were both significantly affected by the studied genotype. The TPC ranged between 117.96 ± 3.60 (genotype ´Leipzig´) and 502.71 ± 3.35 GAE g/kg DW in genotype ´Isluga A´ (Figure 5.2). Notably, the TPC valued in quinoa leaves fall within the results reported by Pathan and Siddiqui (2022), but they are significantly lower as opposed to Gómez et al. (2024) and Villacres et al. (2022).

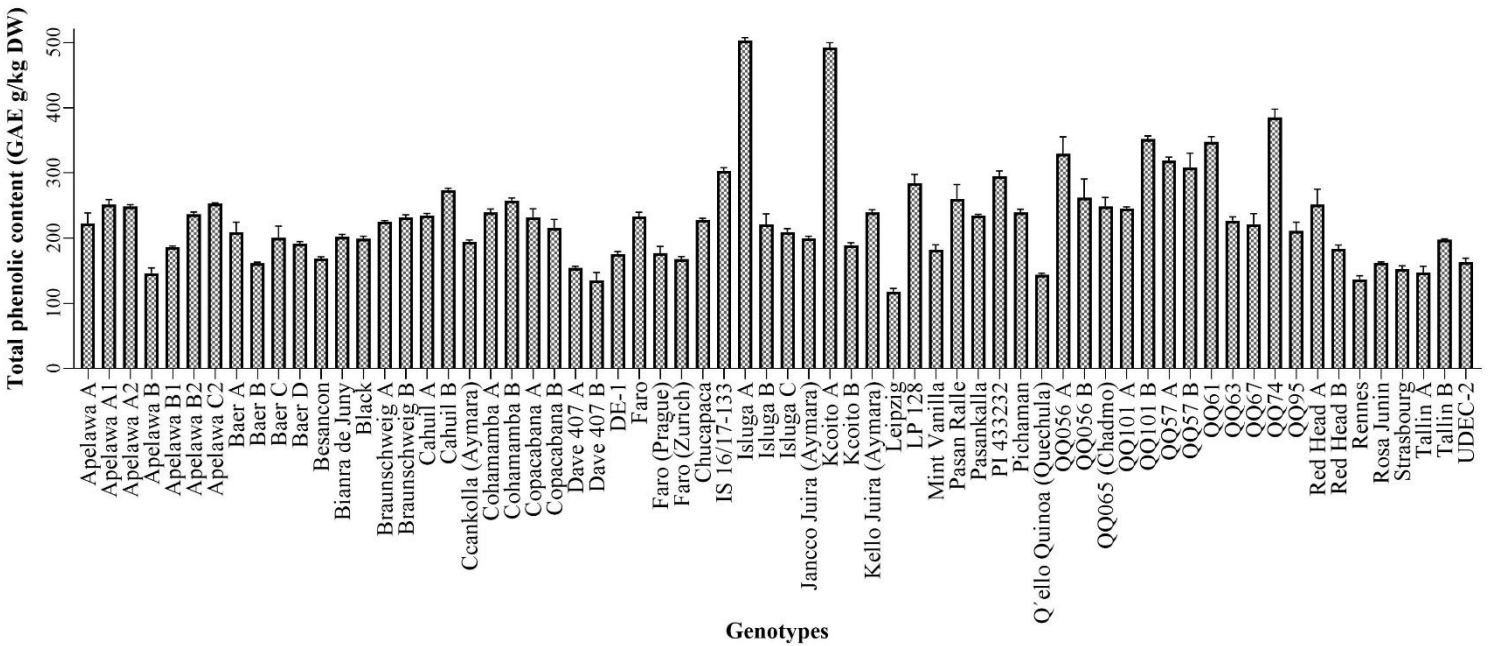

Figure 5.2 The distribution of total phenolic content (TPC) (g/kg GAE DW) observed in the leaves of 66 quinoa genotypes. Bars represent mean TPC content for each genotype, with error bars indicating standard deviation (SD) based on replicate measurements.

Regarding AA, the values ranged between 12.35 ± 0.68 (genotype ´Cohamamba B´) and 59.83 ± 6.18 μmol TE/g DW (genotype ´Red Head A´) (Figure 5.3). Relatively high levels of AA potentially correlate with the abundance and composition of bioactive compounds, such as ferulic acid, hydroxycinnamic acid, quercetin-3-rutinoside, and flavonoids that are presented in notable amounts within quinoa leave (Gómez et al., 2024).

Genotypes were further evaluated using the Z-score normalization method. The results revealed that genotypes ´Isluga A´, ´Kcoito A´, ´Faro (Prague)´ and ´Red Head A´ displayed above-average performance in all three studied parameters, thus making them promising candidates suitable for further cultivation and investigation. Oppositely, genotypes ´Dave 407´, ´Cahuil A´, ´Bianra de Juny´, and ´QQ97´ had the poorest performance among studied genotypes. Nevertheless, further research is required to elucidate the fluctuations of obtained values due to the interactions between genotype and environment.

Beyond their undisputed nutraceutical properties and potential use in food production and fortification, quinoa leaves may also play a significant role in sustainable agriculture. As reported previously, leafy vegetables can be cultivated under natural or artificial lighting, removing seasonal restrictions on their growth (Zhang et al., 2020) and they can thrive under various cultivation modes such as hydroponics, substrate, or soil (Fu et al., 2020; Zha et al., 2024). In the case of quinoa leaves specifically, harvesting can be realized in approximately one month after sowing (Wan et al., 2022).

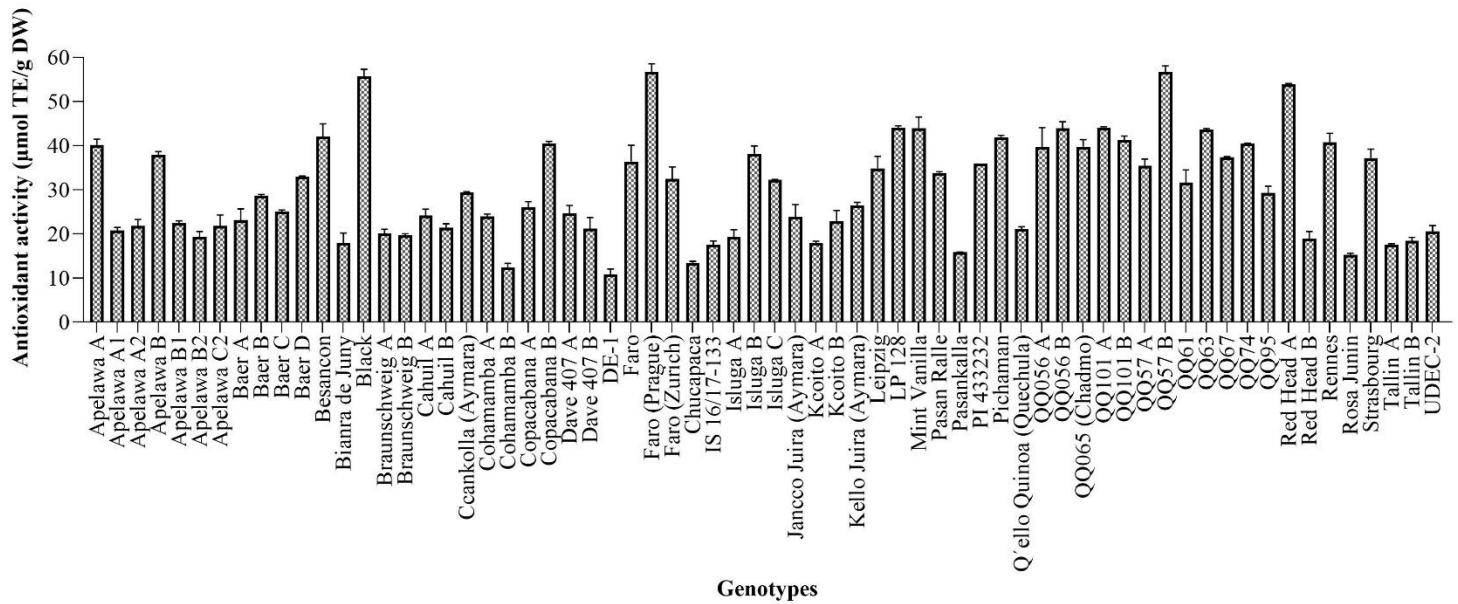

Figure 5.3 The distribution of antioxidant activity (AA) (µmol TE/g DW) observed in the leaves of 66 quinoa genotypes. Bars represent mean AA content for each genotype, with error bars indicating standard deviation (SD) based on replicate measurements.

5.4. Conclusion

Quinoa leaves recently emerged as an alternative food source with exceptional nutritive properties offering potential avenues for addressing dietary deficiencies and promoting sustainable food systems. Beyond their nutritional significance, quinoa leaves hold promises for future agriculture, offering flexibility in cultivation practices and seasonal independence.

The investigation into the crude protein content, total phenolic content, and antioxidant activity of quinoa leaves provided valuable insights into genotype-specific variations and performance in studied traits. Notably, certain genotypes (′Isluga A′, ′Kcoito A′, ′Faro (Prague)′ and ′Red Head A′) demonstrated exceptional performance across all parameters, suggesting their suitability for further cultivation and investigation. Conversely, genotypes with lower performance indicate areas for potential improvement and further research to understand the underlying factors influencing nutrient composition. In essence, our study underscored the benefits of quinoa leaves and their potential to contribute to a healthy diet. However, further research is warranted to ensure the optimization of quinoa leaf production for nutraceutical purposes.

6. Assessment of quinoa germplasm through seed storage protein profiling

6.1. Introduction

Storage seed proteins (SSP) are a class of proteins synthesized by plants and accumulated within the seeds during the maturation phase of seed development (Wakasa & Takaiwa, 2013). They play a vital role in a variety of plant functions. Primarily, they serve as readily available storage reserves of energy, nutrients, and building blocks for growth and development during germination and early stages of seedling establishment (Fujiwara et al., 2002). Additionally, SSPs are involved in plant defense mechanisms against pathogens and abiotic stresses (Jain, 2023).

Osborne´s classification divided SSP based on solubility into albumins, globulins, prolamins, and glutelins. Unlike cereals, albumins and globulins are the major protein fractions in quinoa seeds, occupying over 50% of the total protein in the seed (Tavano et al., 2022; Wang et al., 2020). Glutelins represent the third largest fraction, ranging from 22% to 34% of the total protein content. Lastly, the prolamin fraction is the least abundant, ranging around approximately 2%–7% (Sobota et al., 2020; Tavano et al., 2022).

The albumin fraction is notably rich in cysteine, arginine, histidine, and lysine, although it is relatively deficient in methionine. In contrast, the globulin fraction contains a higher level of methionine, along with glutamic acid, aspartic acid, arginine, serine, or leucine (Dakhili et al., 2019). The specific amino acid composition and secondary structure of globulins contribute to their superior digestibility compared to albumins (Ghumman et al., 2021).

Quinoa protein is a good precursor of bioactive peptides, which are primarily derived from albumin and globulin fractions. These peptides are released through processes such as gastrointestinal digestion, enzymatic hydrolysis, or fermentation (Guo et al., 2021). Studies have shown that quinoa-derived peptides exhibit various biological activities, including antioxidant, hepatoprotective, and anti-inflammatory effects (Tavano et al., 2022; Ren et al., 2023). Furthermore, due to its low prolamin content, quinoa is a valuable nutrient source for individuals with celiac disease or gluten intolerance (Peñas et al., 2014).

As interest in quinoa continues to grow, there is a need to evaluate its genetic diversity. The electrophoretic separation of SSP is a very effective, cheap, and easily accessible method (Polišenská et al., 2011) that is commonly used for genotype identification in cereals, legumes, and other crops (Kumar et al., 2018; Laze et al., 2019; Liu et al., 2010; Mukhlesur & Hirata, 2004). In the case of quinoa, several studies were conducted to describe its SSP profile (Elsohaimy et al., 2015; Ghumman et al., 2021; Peñas et al., 2014), however,

none of them compared a larger number of quinoa accessions of different cultivation years.

This chapter focused on investigating the SSP profiles of 32 quinoa genotypes cultivated over three consecutive years to elucidate the allelic variations in SSP across different genotypes and assess the impact of cultivation years on protein profile. Evaluation and conservation of quinoa genetic diversity is paramount for developing improved cultivars that are resilient to environmental stressors and meet the nutritional needs of diverse populations. Hence, these results hold implications for further quinoa breeding programs and preservation strategies of genetic resources.

6.2. Methodology

6.2.1. Plant material

A total of 32 quinoa samples were involved in the study, sourced from the U.S. National Plant Germplasm System operated by USDA. Samples were cultivated for three consecutive years, from 2018 to 2020, on the experimental fields of Crop Research Institute in Prague – Ruzyně, Czech Republic. Each accession was sown in two rows, each 1 meter in length and spaced 25 cm apart, with 50 seeds per row. No pesticide or fungal control measures were applied to the experimental plots.

6.2.2. Sample preparation and extraction of seed storage protein

Quinoa seeds underwent milling using an IKA A11 basic mill (IKA-Werke, Staufen, Germany) and were subsequently stored in sealed plastic bags in a dark, cold environment at 4°C. For the extraction of SSP, 0.02 g of quinoa flour was combined with 0.25 ml of a solvent comprising gel buffer 6.8 (0.25 M Tris + HCl at pH 6.8), 10% (w/v) SDS (sodium dodecyl sulfate), glycerol, 2-mercaptoethanol, and distilled water. All samples were allowed to be extracted at room temperature for 2 hours. Then, samples were placed into boiling water for 2 minutes. Afterward, they were centrifuged (Universal 32R Hettich Centrifugen, Germany) at 15,000 rpm for ten minutes, and the resulting supernatant was then transferred into new tubes. This process was conducted in duplicate for each genotype. Samples were then refrigerated before analysis.

6.2.3. Storage seed protein separation

SSP separation was carried out using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), following the method outlined by

Laemmli (1970) with minor adjustments. A polyacrylamide gel (180 x 160 x 0.75 mm) was prepared, consisting of a 12% (w/v) resolving gel at pH 6.8 and a 4% (w/v) stacking gel at pH 8.8 (Table 6.1).

Table 6.1 Chemical composition of the SDS-PAGE gel

Resolving gel 12%	**Stacking gel 4%**
29 ml Acrylamide and Bis solution (Bio-Rad, Germany)	4 ml Acrylamide and Bis solution (Bio-Rad, Germany)
63 ml Tris 8.8 (Sigma-Aldrich, USA)	13.4 ml Tris 6.8 (Sigma-Aldrich, USA)
0.72 ml 10% SDS (Sigma-Aldrich, USA)	0.26 ml 10% SDS (Sigma-Aldrich, USA)
7 ml Distilled water	8.8 ml Distilled water
0.72 ml 10% Ammonium persulfate	0.26 ml 10% Ammonium persulfate
42.6 µl TEMED (Sigma-Aldrich, USA)	10.6 µl TEMED (Sigma-Aldrich, USA)

Each well on the gel was loaded with 0.15 µl of extract, with two wells allocated per genotype. Commercially purchased protein ladders (Thermo Scientific™ PageRuler™ Unstained Broad Range Protein Ladder, Thermo Fischer Scientific Life Scientific, Czech Republic) served as molecular weight (MW) standard markers. One well per one gel was loaded with 0.1 µl of MW marker.

Electrophoresis was performed using the vertical electrophoresis Hoefer SE 600 (Hoefer, USA) at 50 mA for 30 minutes, followed by an increase to 60 mA for approximately three hours. Subsequently, gels were fixed in a 20% (w/v) trichloroacetic acid solution, stained with 0.05% (w/v) Coomassie Brilliant Blue (CBB) R250, and then bleached in distilled water to remove excess coloration before scanning into a computer.

6.3. Results and discussion

The electrophoretic separation of SSP in 32 distinct quinoa genotypes revealed 22 allelic positions. This contrasts with the findings of Drzewiecki et al. (2003), who identified a total of 41 bands in quinoa seed proteins yet align with the results reported by Wang et al. (2020) and Peñas et al. (2014). The bands on the electrophoretic gels spanned in the molecular weight (MW) range from approximately 5 to 100 kDa, similar to the report of Van de Vondel et al. (2020).

The majority of observed bands exhibited medium to high intensity, facilitating their clear visualization of the electrophoretic gel. The most prevalent protein bands across all varieties were situated within 5 to 35 kDa. Additionally, bands of lesser abundance were observed within the molecular weight range of 48 to

70 kDa. This range corresponds to MW of major quinoa protein fractions, namely albumins (less than 20 kDa) (Elsohaimy et al., 2015) and globulins (approximately 20–50 and 78 kDa) (Shen et al., 2021; Thanapornpoonpong et al., 2008).

Although the banding profiles exhibited a relatively low level of polymorphism among the studied genotypes, discernible differences in the presence/absence and intensity of bands were identified, particularly within the 30–38 kDa and 48–70 kDa ranges, corresponding to globulin subunits (Figure 6.1). Similar observations regarding varietal differences in banding patterns have been documented by Ghumann et al. (2021).

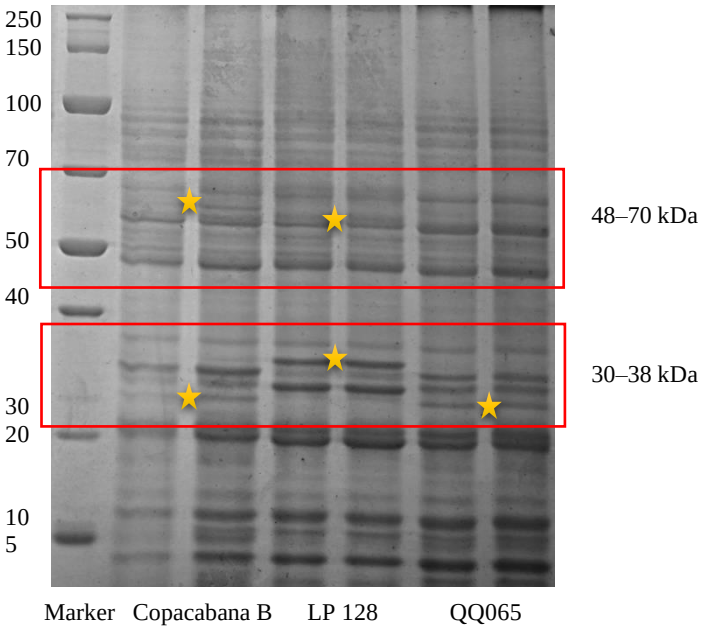

Figure 6.1 The example of distinct banding profile of three quinoa genotypes. The major differences in band positions and abundance for each genotype are marked by the yellow asterisk. Red rectangles highlight the location of the highest variability in allelic positions

As mentioned in the beginning, evaluated genotypes were cultivated in three consecutive years from 2018 to 2020. The weather conditions in each year are described comprehensively in Chapter 2, subchapter 3.2.2. The comparison of the banding profile showed that SSP was not significantly impacted by the weather conditions in a given year. Minor variations observed across cultivation years were primarily manifested as changes in the relative abundance of certain bands, rather than shifts in their positions. Hence, SSP

appears to be a reliable marker for the classification of quinoa germplasm over time.

While other studies exploring alterations in protein banding patterns across different cultivation years are scarce, Aloisi et al. (2016) investigated variations in protein patterns under varying saline conditions. The authors documented significant modifications in band positions and relative abundance, particularly in response to a 300 mM NaCl treatment. Another factor influencing the band pattern is germination. As noted by Jimenez et al. (2019), certain bands in germinated samples may exhibit reduced abundance compared to non-germinated samples due to enzymatic hydrolysis processes occurring during germination.

Conversely, while SDS-PAGE proved valuable in discerning between different quinoa genotypes based on their SSP profiles, it exhibited limitations in distinguishing between genotypes with phenotypic variations since distinct phenotypes within the same genotype yielded identical protein band patterns.

6.4. Conclusion

A total of 35 quinoa genotypes were evaluated using the electrophoretic separation of SSP. The banding profile of quinoa SSP had a relatively low degree of heterogeneity, but the differences in the presence, absence, and intensity of bands within distinct quinoa genotypes were observed, primarily in the globulin subunit. Banding patterns and band positions of quinoa SSP were reproducible over time since they were not significantly affected by the weather conditions of a cultivation year. Nonetheless, the method was not sensitive enough to distinguish phenotypic variations in genotypes. Overall, electrophoretic analysis of SSP is a helpful and reliable tool to discriminate quinoa genotypes as a first step in evaluating quinoa genetic resources.

7. General discussion

The escalating demand for staple foods, driven by climate change-induced environmental pressures, underscores the urgent need to explore alternative crops that may sustain agricultural productivity and ensure global sovereignty. Quinoa, with its remarkable adaptability and nutritional richness, presents a viable solution for addressing the challenges of the 21st century. As the Czech Republic and Central Europe experience the environmental impacts of climate change, the introduction of quinoa into these regions opens new pathways to diversify crops and strengthen agricultural resilience. However, successful establishment of quinoa in new climatic zones requires identifying and utilizing superior genetic resources adaptable to new regions.

Although several studies have already evaluated various aspects of quinoa across Europe, including its agro-morphological characteristics, yield potential, and seed nutritional content (Craine et al., 2023; Granado-Rodríguez et al., 2021; Matías et al., 2021, 2022; Präger et al., 2018; Reguera et al., 2018; Tabatabaei et al., 2022; Toderich et al., 2020), many have often been constrained by the use of a small number of genotypes and/or a lack of long-term cultivation comparisons. Additionally, there is an insufficient number of field experiments conducted under the environmental conditions of Central Europe, with none in the Czech Republic. Hence, the presented doctoral research addressed these gaps by assessing a broad range of quinoa germplasm sourced from diverse genetic backgrounds. The main goal was to identify and select the most promising genotypes suited for climatic conditions in the Czech Republic, that may hold substantial potential for quinoa breeding programs in the region.

The introduction of quinoa into Europe represents not only an opportunity, but also a challenge for plant breeding programs, particularly in regions like the Czech Republic where climate change and unpredictable weather conditions poses increasing threats for traditional crops. The assessment of stable performance therefore provides a foundation for developing locally adapted quinoa varieties that could further support the large-scale adoption of quinoa in this region. Additionally, the evaluation of nutritional profiles of quinoa genotypes further positions this crop as a strategic commodity in agricultural development programs, addressing nutritional deficiencies and enhancing nutritional value of the food supply in the Czech Republic.

Building upon the previous paragraph, plant-based nutrients, particularly proteins, are highly valuable, offering numerous health benefits, such as

reduced risks of cardiovascular disease, type 2 diabetes, and certain cancers (Naghshi et al., 2020). From a sustainability perspective, plant protein production has a lower environmental impact, requiring fewer resources and generating fewer greenhouse gases than animal protein production (Poore & Nemecek, 2018). In this study, quinoa showed the protein content between 13.44–20.01 % in DW, surpassing the values reported for cereals (USDA, 2021). Consistent and relatively high seed protein content over four years of cultivation was observed in genotypes ´Mint Vanilla´, ´Cahuil A´, ´Cohamamba B´, ´Braunschweig B´, and ´Apelawa A1´.

Quinoa seeds have been identified as a rich source of diverse secondary metabolites with distinct biological activities (Tang et al., 2016b; Lin et al., 2019; Capraro et al., 2020; Liu et al., 2020; Stikic et al., 2020; Tabatabei et al., 2022). The findings of this thesis not only confirmed this but also highlighted the relative under-researched nature of quinoa in this context. Notably, six phenolic compounds (2-OH-cinnamic acid, homoorientin, luteolin, naringenin, N-feruloyl octopamine, and 4-OH-benzaldehyde) were identified and quantified in quinoa seeds for the first time.

The identification of bioactive compounds such as isoquercetin, quercetin, and other phenolics, which showed low seasonal variability, adds another dimension to quinoa's potential as a nutritionally rich crop. Consequently, the genotypes ´Red Head B´ and ´Isluga A´ demonstrated comprehensive results in total phenolic content and antioxidant activity over four years of cultivation. Therefore, these findings may enhance the breeding efforts focusing on boosting quinoa's value in functional food markets.

Regarding quinoa morphological characteristics, the WTS – an important contributor to overall seed yield, was evaluated. Genotypes ´QQ87´, ´Isluga A´ and ´Red Head A´ displayed consistent values over multiple years of cultivation, making them promising candidates for breeding programs aimed at stable yield production.

While studies conducted on raw quinoa seeds provide valuable insights into their inherent nutritional profile, it is crucial to recognize that quinoa is primarily consumed in processed form. Such studies are essential for accurately assessing the health benefits and dietary contributions of quinoa, ensuring that recommendations for breeding programs are based on practical, real-world applications. Certain processing techniques may even compensate for the suboptimal nutritional profiles observed in quinoa cultivated under unfavorable conditions or in genotypes with less superior nutritional characteristics.

One promising technique addressing these issues is germination. Seed germination has gained popularity in human diets due to its nutritional and health benefits. It is considered eco-friendly and cost-effective method feasible for both, home and commercial production (Ebert 2022; Oliveira et al., 2022; Gunathunga et al., 2024). As it was assumed in the hypotheses, the results showed that germination strongly enhanced the content of specific bioactive metabolites in quinoa, such as rutin, quercetin, gallic acid, kaempferol, and isorhamnetin. Nonetheless, the extent of metabolite content enhancement or degradation occurred in a genotype-dependent manner and was further influenced by the duration of germination, underscoring the importance of optimizing both factors to achieve the best nutritional profile in quinoa.

While quinoa germination was found to be a beneficial preparation technique, heat-utilizing methods are more commonly used in practise. Especially boiling of whole quinoa seeds to make a porridge is a traditionally applied method in many households (FAO 2011). However, our results indicated that boiling whole seeds is not ideal for preserving protein and polyphenol content, although it does enhance antioxidant activity. In contrast, boiling rolled quinoa flakes proved to be a promising method for protein enhancement – a finding that has not been extensively documented in the scientific literature. Additionally, roasting and microwaving, although less traditional than boiling, were found to be superior methods for improving polyphenol content. These results support the hypothesis that different preparation methods result in varying nutritional content and composition, and thus, selecting the appropriate processing technique is essential to preserve key nutrients.

In situations where unfavorable environmental conditions may lead to a poor seed harvest, an alternative approach is to cultivate quinoa specifically for its leaves. This strategy could be particularly beneficial for farmers in Central Europe who may face challenging growing conditions during the cultivation period. Despite its potential, the use of quinoa leaves as a viable food source has been largely overlooked in the scientific literature, even though their use could increase the overall versatility of quinoa in culinary applications and in fortifying less nutritious food products (Świeca et al., 2014; Gawlik-Dziki et al. 2015; Hu et al., 2023). Furthermore, no breeding programs have yet focused on improving the nutritional quality of quinoa leaves, which presents a valuable opportunity for the development of new quinoa varieties specifically cultivated for their leaves.

Remarkably, 35% of the accessions studied in this thesis exhibited protein values in leaves exceeding 20% in DW, surpassing the highest protein content

observed in quinoa seeds, which aligns with the initial hypothesis. Also, the antioxidant activity and total content of polyphenols was multiple times higher in leaves, compared to seeds. Further evaluation of protein and polyphenol content, alongside antioxidant activity, identified the genotypes ´Isluga A´, ´Kcoito A´, ´Faro (Prague)´ and ´Red Head A´ exhibiting above-average values across all three parameters, making them strong candidates for future breeding efforts.

8. Conclusion and future recommendations

This dissertation has provided a comprehensive agro-morphological, nutritional, and biochemical assessment of quinoa germplasm cultivated under the climatic conditions of Czech Republic, with a particular focus on identifying genotypes most suitable for this region. The research confirmed the original hypotheses and contributed significantly to both academic understanding and practical applications for quinoa breeding and adoption in Central Europe. Furthermore, the detailed exploration of various processing methods provided a foundation for the food processing industry to develop quinoa-based products with enhanced health benefits.

With increasing focus on sustainable farming, crop diversification and food quality in European agricultural policies, quinoa seems particularly relevant for this region, offering both economic and nutritional benefits. Genotypes such as ´QQ87´, ´Mint Vanilla´, ´Cahuil A´, ´Isluga A´ and ´Red Head A´ showed stable performance across multiple years of cultivation among selected trait, even under the fluctuating weather patterns, that are characteristic for Czech Republic. Hence, strategic selection and cross-breeding of specific genotypes with desirable traits will help to expand quinoa production in this area.

In the light of above, future breeding efforts should focus on expansion and conservation of the quinoa genetic pool sourced from diverse ecological regions. This will ensure long-term sustainability and prevents the loss of valuable genetic materials. Additionally, breeding programs should prioritize the evaluation of genotypes' physiological and biochemical responses to identify those capable of retaining its nutritional quality and ability to cope with climatic extremities caused by global warming.

Long-term, multi-location field trials will be crucial for refining the selection of genotypes and confirming their adaptability on a larger scale. Specifically for the region of Central Europe, the breeding initiatives should focus on development of varieties resistant to increasingly unpredictable weather

conditions that may occur during the growing period. Furthermore, research should continue to explore the potential of quinoa leaves as a food source, an area that remains underexplored. Developing varieties specifically for leaf production could be a novel direction for breeding programs, offering farmers additional economic benefits and ensuring more comprehensive utilization of the crop. In addition, future research should investigate antinutritional compounds in quinoa leaves to ensure their safe consumption. Efforts should also be made to standardize processing techniques that maximize the nutritional benefits and sensory qualities of quinoa seeds and leaves, addressing both consumer health and market demand.

In summary, the strategic selection and breeding of quinoa genotypes with desirable traits will be essential for the successful introduction of quinoa in Central Europe. Government policies should support these efforts by providing incentives for farmers, promoting crop diversification, and fostering consumer awareness of quinoa's health benefits, thereby creating a favorable environment for its widespread adoption.

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Appendix

Curriculum vitae

Name: Lucie Dostalíková

Date of Birth: 07. 05. 1995

Email Address: dostalikova@ftz.czu.cz

Education

PhD in Tropical Agrobiology and Bioresources Management

Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague [31/08/2020 – Current]

Thesis: Phenotypic and metabolomic characterization of quinoa (*Chenopodium quinoa* Willd.): Identification of superior genetic resources

Master´s Degree in Tropical Crop Management and Ecology

Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague [30/09/2017 – 29/06/2020]

Thesis: Assessment of avenin polymorphism in selected oat varieties

Bachelor´s Degree in Agriculture in Tropics and Subtropics

Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague [30/09/2014 – 29/05/2017]

Thesis: Ethnobotanical inventory of medicinal plants in the province Imbabura, Ecuador

Training and Internship Experience

Summer School in Kyrgyzstan

American University of Central Asia, Bishkek, Kyrgyzstan [24/07/2023 – 10/08/2023]

Summer School in Indonesia

Satya Wacana Christian University, Salatiga, Indonesia [27/06/2023 – 23/07/2023]

Erasmus+ Internship – Determination of End-Use Quality in Cereals

University of Natural Resources and Life Sciences, Department of Crop Sciences, Tulln, Austria [31/01/2019 – 29/06/2019]

Erasmus+ Study Program

Ghent University, Faculty of Bioscience Engineering, Ghent, Belgium [31/01/2019 – 29/06/2019]

European League of Life Sciences Summer School – Genomics for Plant Breeding and Biotechnology

Warsaw University of Life Sciences, Warsaw, Poland [19/08/2018 – 25/08/2018]

Summer School in Cambodia – Scientific Work Step-by-Step

Royal University of Phnom Penh, Phnom Penh, Cambodia [10/07/2017 – 12/08/2017]

Bachelor´s thesis research – ethnobotanical research of medicinal plants in the province Imbabura, Ecuador [06/2016 – 08/2016]

Universidad Tecnica del Norte, Imbabura, Ecuador

Research Projects

Internal Grant Agency FTA 20242115 Biotechnological and proteomic methods: tools for crop improvement. Financial donor: Faculty of Tropical AgriSciences, CZU, Prague. Co-researcher.

Internal Grant Agency FTA 20233115 Biotechnological, proteomic, and molecular methods as a tool in plant breeding. Financial donor: Faculty of Tropical AgriSciences, CZU, Prague. Co-researcher.

Internal Grant Agency FTA 20223105 Biotechnological, proteomic, and molecular methods as a tool in plant breeding. Financial donor: Faculty of Tropical AgriSciences, CZU, Prague. Co-researcher.

Internal Grant Agency FTA 20213114 Biotechnological, proteomic, and molecular methods as a tool in plant breeding. Financial donor: Faculty of Tropical AgriSciences, CZU, Prague. Co-researcher.

Internal Grant Agency FTA 20205006 Biotechnological, proteomic, and molecular methods as a tool in plant breeding. Financial donor: Faculty of Tropical AgriSciences, CZU, Prague. Co-researcher.

Internal project of the Ministry of Agriculture of the Czech Republic (No. RO0418 and RO0423). National Programme for the Conservation and Use of Plant Genetic Resources and Agrobiodiversity (no. 62551834 2017 MZE 17253).

National Programme for the Conservation and Use of Plant Genetic Resources and Agrobiodiversity (no. 62551834 2017 MZE 17253)

Scientific Conferences and Seminars

Dostalíková, L., Hlásná Čepková, P. 2022. Evaluation of quinoa genetic resources cultivated in the climatic conditions of the Czech Republic. Report contribution at seminary "Využití genetických zdrojů rostlin ve šlechtění", Prague, Czech Republic, online.

Dostalíková, L., Hlásná Čepková, P., Viehmannová, I., Janovská, D., Dvořáček, V. Assessment and quantification of new metabolites in quinoa (*Chenopodium quinoa*) using UHPLC-Q-Orbitrap mass spectrometer. Poster presentation at Tropentag conference 2022, Prague, Czech Republic.

Dostalíková, L., Hlásná Čepková, P., Viehmannová, I., Janovská, D. Assessment of antioxidative activity and total polyphenol content in quinoa (*Chenopodium quinoa* Willd.) genotypes cultivated in the Czech Republic. Poster presentation at ICC conference 2022 in Vienna, Austria.

Dostalíková, L., Hlásná Čepková, P., Viehmannová, I., Janovská, D., Dvořáček, V. Assessment of morphological traits and seed protein content in quinoa (*Chenopodium quinoa* Willd.) genotypes cultivated in the Czech Republic. Poster presentation at Czech Food Chem conference 2021 in Prague, Czech Republic, online.

Dostalíková, L., Hlásná Čepková, P., Viehmannová, I., Janovská, D., Dvořáček, V. Seed Storage Proteins in *Chenopodium quinoa* Germplasm. Poster presentation at Tropentag conference 2021, Stuttgart, Germany, online.

Dostalíková, L., Hlásná Čepková, P., Viehmannová, I., Janovská, D., Dvořáček, V. Nontargeted metabolomic analysis of quinoa (*Chenopodium quinoa* Willd.) using UHPLC-Q-Orbitrap mass spectrometer. Poster presentation at Czech Mass Spectrometry Conference 2021 in Prague, Czech Republic.

Publications

Dostalíková, L., Hlásná Čepková, P., Janovská, D., Jágr, M., Svoboda, P., Dvořáček, V., Viehmannová, I. (2024). The impact of germination and thermal treatments on bioactive compounds of quinoa (*Chenopodium quinoa* Willd.) seeds. *European Food Research and Technology*, 250:1457–1471. https://doi.org/10.1007/s00217-024-04466-3

Dostalíková, L., Hlásná Čepková, P., Janovská, D., Svoboda, P., Jágr, M., Dvořáček, V., Viehmannová, I. (2023). Nutritional evaluation of quinoa genetic resources growing in the climatic conditions of Central Europe. *Foods*, 12:1440. https://doi.org/10.3390/foods12071440

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