
SUMMARY OF PhD THESIS

Investigation of phytochemical compounds in apple fruits from wild species, traditional and modern varieties by advanced analytical techniques

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INTRODUCTION

Apple (*Malus domestica* Borkh.) is one of the most widely cultivated and consumed fruit species globally, due to its nutritional value, versatility in consumption, and ability to be used in a wide range of food products. Apple fruits are an important source of vitamins, dietary fibers, and bioactive compounds, offering multiple health benefits, including antioxidant, anti-inflammatory, and cardioprotective effects (Zheng et al., 2019). Regular consumption of apples is associated with a reduced risk of cardiovascular diseases, type 2 diabetes, and certain types of cancer, due to the presence of phytochemical compounds with high biological activity (Zhang et al., 2021).

Phytochemical compounds in apples, such as polyphenols, flavonoids, carotenoids, and organic acids, play a crucial role in determining the sensory and functional characteristics of the fruit, as well as supporting the human body's defense mechanisms against oxidative stress (Liu, 2021). Polyphenols, in particular, are involved in neutralizing free radicals and modulating physiological processes, having a direct impact on health status. Additionally, the concentration and type of these compounds vary significantly depending on genetic factors, pedoclimatic conditions, and cultivation technologies (Griffiths et al., 2020).

In this context, investigating the genetic diversity of apple is essential for understanding and harnessing its nutritional and functional potential. Wild *Malus* species represent a valuable source of genetic variability, offering important agronomic and biochemical traits such as disease resistance, tolerance to abiotic stress conditions, and high content of bioactive compounds (Crosby et al., 1992). Traditional apple varieties are also recognized for their organoleptic quality and significant contribution of bioactive substances, constituting an especially valuable genetic heritage. Conversely, modern varieties have been mainly selected for technological traits such as high productivity and fruit uniformity but may present a lower content of phytochemical compounds compared to wild species and traditional varieties. Therefore, a comprehensive approach is needed to allow a comparative evaluation of these apple categories, highlighting the advantages of each group based on chemical composition and biological activity (Bensoussan et al., 2021).

For a rigorous and detailed characterization of the phytochemical compounds in apples, the use of advanced analytical techniques is indispensable. Modern methods, such as high-performance liquid chromatography (HPLC), enable precise identification and quantification of polyphenols, flavonoids, and other secondary metabolites of nutritional importance. Additionally, the evaluation of antioxidant activity using specific methods such as DPPH contributes to a deeper understanding of the functional potential of each analyzed apple type. Thus, integrating these advanced techniques provides the possibility to correlate the phytochemical diversity of fruits with their beneficial potential for human health and applicability in the food and pharmaceutical industries (Weichselbaum, 2010).

In this context, the present research aims to investigate the phytochemical compounds in apple fruits derived from wild species, traditional, and modern varieties, using advanced analytical methods. The study will contribute to a better understanding of the existing

biochemical variability among these varieties and will provide relevant data for the selection and breeding of future apple varieties with superior nutritional potential.

STRUCTURE OF THE THESIS

The first part of the thesis, **CURRENT STATE OF KNOWLEDGE**, includes 2 chapters.

Chapter 1. Apples, contains in 7 subchapters aspects related to the general characteristics of apple fruits, information about taxonomy, distribution and economic importance, biological particularities, chemical composition of apple fruits, as well as factors influencing the accumulation of bioactive compounds, and last but not least, the effects of bioactive compounds present in apple fruits on health.

Chapter 2. Current knowledge regarding the valorization of apples in the food industry, discusses the widespread ways of valorizing apples in the food industry, being used in the production of juices, purees, cider, and other products due to their rich content in bioactive compounds. Modern technologies allow the preservation of nutritional properties, and current trends favor the integration of apples in functional foods. Also, by-products resulting from processing, such as peels and seeds, are exploited for obtaining extracts with applications in the pharmaceutical and cosmetic industries, contributing to the sustainability and diversification of uses of this fruit.

The second part, **PERSONAL CONTRIBUTION**, is structured into 5 chapters and bibliography.

Chapter 3. Working hypothesis and research objectives describes the aim of the thesis and the main research directions approached. The purpose of the research consists in the advanced evaluation of the phytochemical composition of different wild apple varieties, as well as old and modern varieties.

Regarding the evaluation of the phytochemical composition, as well as the possibility of valorizing the fruits, two major research directions with individual objectives were approached:

Chapter 4. Study 1 – Chemometric comparison and classification of 22 apple varieties based on texture analysis and physico-chemical quality attributes

01.1 Standardization of the biological material proposed for the study;

Also in this chapter, the experimental material is presented: apples were harvested from the Research Institute for Fruit Growing Pitești-Mărăcineni (ICPPM), Romania. 90 fruits per variety were collected, without diseases or mechanical damage, mixed homogeneously and analyzed in the laboratory.

01.2 Advanced phytochemical characterization of the selected apple varieties;

01.3 Evaluation of consumer perception regarding the taste and texture of selected apple varieties;

01.4 Comparison of the content of bioactive compounds in apple varieties;

01.5 Submission of obtained data to multivariate statistical analysis, principal component analysis (PCA), and hierarchical cluster analysis (HCA);

Results and discussions for Study 1

The study results indicate significant variability between the analyzed varieties, with a coefficient of variation exceeding 30% for fruit size, while other characteristics, such as water

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content, show low variability. Wild apples *M. floribunda* clone 821 and Bērnū Prieks significantly influenced this diversity, being recognized for the Vf gene, responsible for scab resistance and contributing to the improvement of modern varieties.

The fruit weight of variety T97 recorded the highest values for the weight of a single fruit (199.94 g), without statistically significant differences between them; in contrast, *M. floribunda* clone 821 had the lowest recorded weight for a single fruit (23.95 g).

The volume of fruits in variety T97 stood out with an impressive value of 247.5 ml of displaced water. On the opposite pole, *M. floribunda* clone 821 had the lowest recorded volume, with a value of 32.5 ml.

As expected, the same variety, *M. floribunda* clone 821, recorded the lowest statistically significant values for fruit width (36.83 mm), length (36.35 mm), geometric mean (36.10 mm), and arithmetic mean diameter (36.11 mm), while varieties T188, Golden Russet and T97 recorded the highest values for all four parameters.

The average water content of the 22 apple varieties was 85.05%, with a low coefficient of variation (CV) of 2.74%. The average ash content was 2.32% with a CV of 22.1%. The highest values were recorded in varieties Jonagold (3.38%) and Akane (3.09%), while the lowest ash level was recorded in variety Gala Fenus Fengal (1.47%) and variety Greensleaves (1.55%).

The parameter total soluble solids (TSS) had a mean of 16.22% with a CV of 17.78%, indicating slight differences between analyzed varieties. *M. floribunda* clone 821 had the highest TSS (24.03%), followed by Ananas Reinette and Cidor, while Jonagold (10.13%) and Fuji Fenfu (12.80%) had the lowest TSS values.

A large variation of titratable acidity was observed, with higher values recorded in Bērnū Prieks (1.28%), T97 (1.17%) and *M. floribunda* clone 821 (1.00%), while lower values were recorded in varieties “Gala Venus Fengal” (0.18%), Gala Dicarli Fendeca (0.20%) and Cidor (0.23%).

Texture analysis results showed that skin hardness varied between 3.80 and 13.69 N, deformation varied between 0.2 and 1.07 mm, flesh hardness between 0.97 and 4.76 N, while work hardness was between 6.88 and 27.84 mJ.

T97, T120, Greensleaves, T188, and Gala Fenplus presented only a background color; Gala Dicarli Fendeca, Gala Venus Fengal, *M. floribunda* clone 821, Bērnū Prieks, Golden Russet, and Jonagold presented both background and overcolor, while Fuji Fenfu, Lobo, Judeline, Golden Delicious Goldrosio, and Gala Brookfield showed both background and overlapping colors. Among the studied apple types, varieties such as Ananas Reinette, Cidor, Iris, Judaine, Akane, and Priam presented background, coverage, and overlapping colors.

Varieties Ananas Reinette, Fuji Feufu and Cidor obtained the highest values regarding total chlorophyll pigments, with values of 130.18, 125.9, and 117.7 µg/g, respectively. At the opposite end were varieties Iris, Judaine and T97, with contents of 38.7, 50.44, and 51.86 µg/g.

Varieties Fuji Feufu, Jonagold and Gala Dicarli Feudeca obtained the highest values for carotenoid pigment content, with values of 5,422, 4,392, and 3,076 mg/100g, respectively. On the opposite side were varieties Gala Brookfield, Gala Feuplus and *M. floribunda* clone 821, with contents of 1,195, 1,430, and 1,478 mg/100g.

The highest TPC (total phenolic content) values for pulp and peel samples were observed in the variety *M. floribunda* clone 821 (314.45 mg GAE /100 g, respectively 517.68 mg GAE /100 g), with a significantly higher pulp TPC compared to the other studied apple fruits. The lowest pulp TPC values were observed in variety Golden Russet (54.77 mg GAE /100 g) and T97 (64.15 mg GAE /100 g). Varieties Iris and Gala Fenplus stood out by the large contrast of TPC in pulp compared to peel, variety Iris pulp TPC 169.85 mg GAE /100 g, peel TPC 632.53 mg GAE /100 g, variety Gala Fenplus pulp TPC 94.16 mg GAE /100 g, peel TPC 628.28 mg GAE /100 g.

Data regarding antioxidant capacity (AC) values showed that AC of pulp samples ranged from 146.39 $\mu\text{mol TE/mL}$ in variety T97 up to 1073.06 $\mu\text{mol TE/mL}$, the result belonging to variety *M. floribunda* clone 821. Regarding AC of peel samples, as expected, values doubled or even tripled in some cases compared to values obtained in pulp samples, with the lowest values in variety Lobo, 949.17 $\mu\text{mol TE/mL}$, and the highest values in variety T120, 1133.89 $\mu\text{mol TE/mL}$.

As a general note on sensory analysis, the lowest scores were obtained by varieties Bernv Prieks and Cidor with 15.10 and 24.57 points, respectively, and the highest scores by varieties Gala Dicarli Feudeca and Gala Feuplus with 37.88 and 35.43 points.

Correlation of texture and physico-chemical indices allowed the identification of four distinct clusters. Cluster I includes varieties with small fruits, Cluster II – medium fruits but softer texture, Cluster III – large fruits with balanced texture, and Cluster IV – firm fruits, with increased hardness of skin and pulp.

Integration of cluster analysis and PCA facilitated classification of varieties based on physico-chemical and textural parameters, highlighting similarities between certain fruits and contributing to better characterization of genetic variability. Although significant differences between varieties were observed, grouping analysis highlighted the existence of common traits relevant for selection and breeding of future apple varieties.

Chapter 5. Study 2 – Development of the “Baby Food Puree” Product Intended for Infant Nutrition

02.1 Determining the influence of different apple varieties on the composition of the baby food puree.

This chapter also presents the experimental material. The apples, which represent the main component, were obtained from the Research and Development Institute for Horticulture, Mărăcineni, Pitești, while the carrots, pumpkin, and celery were sourced from the Research and Development Institute for Horticulture, Cluj-Napoca. The proportions used in the formulation of the puree are also described.

02.2 Optimization of the technological process for obtaining the puree;

02.3 Physico-chemical analyses of both sterilized and unsterilized puree;

02.4 Evaluation of the content of bioactive compounds before and after applying thermal treatment to the puree;

02.5 Evaluation of color and texture, both before and after the thermal treatment of the puree;

02.6 Submission of the obtained data to multivariate statistical analysis, including principal component analysis (PCA) and hierarchical cluster analysis (HCA) Rezultatele și discuțiile pentru Studiul 2.

Results and Discussion for Study 2

The highest moisture content of the non-thermally treated puree was 87.27% in the puree made from the Baujade apple variety, which decreased to 84.59% after thermal treatment. The lowest moisture content was found in the Juliana variety, at 80.72%, which dropped to 78.14%.

Regarding the ash content in the fresh puree, we observed significant variation depending on the apple variety used. The highest ash contents were recorded in the Elstar and Juliana varieties, with values of 18.76% and 15.39%, respectively. On the opposite end, the Champion, Granny Smith, Pătul, and Parmen Clone varieties showed much lower ash contents, ranging from 2.91% to 4.09%.

The acidity of the fresh puree was similar across all ten varieties, with values ranging between 0.30% and 0.45%. After thermal treatment, the acidity decreased slightly, with maximum values reaching 0.40%.

In terms of vitamin C content in the fresh product, the highest values were found in the purees made from Domnesc and Granny Smith apples, with 4.21 mg% and 3.5 mg%, respectively. The lowest vitamin C content was recorded in the purees from Champion and Parmen Clone varieties, with 2.11 mg% and 2.57%. Following sterilization, all ten purees experienced significant losses of vitamin C, ranging between 50% and 70%.

Regarding the total polyphenol content in the fresh puree, the results ranged from 59.91 mg GAE/100 g for the Pătul variety to 86.31 mg GAE/100 g for the Domnesc variety, which recorded the highest value. In some samples, such as the puree from the Elstar variety, the total polyphenol content nearly doubled after thermal processing, increasing from 64.36 to 126.65 mg GAE/100 g.

Antioxidant capacity increased in correlation with the rise in total polyphenols, based on the premise that many antioxidant compounds are mainly present as covalently bound forms with insoluble polymers. Antioxidant capacity nearly doubled in some cases after thermal treatment, with post-sterilization values ranging from 0.83% (Elstar variety) to 0.93% (Juliana variety).

Thermal treatment led to a decrease in the L* value (lightness) for the processed samples compared to the control samples. The purees were characterized by a darker color, influenced by several factors such as processing temperature and time, apple variety, vitamin C content, and specific pigments found in the raw materials.

The firmness of the puree obtained from the Champion and Juliana apple varieties was the highest before sterilization, with values of 2.46 N and 2.78 N, respectively. After sterilization, the puree made with Champion apples recorded a firmness of 3.5 N, while the one with Juliana apples decreased to 2.07 N. On the opposite end were the Domnesc and Parmen Clone varieties.

The highest adhesiveness before sterilization was observed in the purees from Champion and Parmen Clone apples, with values of 1.85 mJ and 2.3 mJ, respectively, compared to the Elstar variety, which had a significantly lower value of 0.35 mJ. After sterilization, the highest adhesiveness remained in the same two apple varieties, Champion and Parmen Clone, with 8.1 mJ and 5 mJ, respectively.

Chapter 6. Conclusions and Recommendations

Study 1

Such studies, combined with those concerning the organoleptic characteristics of potential parental forms used in apple hybridization work, can be extremely useful for developing forward-looking breeding strategies, as is the case with other horticultural species. Consumer tastes and preferences for apples, as well as the needs of processors and the industry, may evolve over time. Consequently, the impact of fruit quality assessment studies and the targeted improvement of apples in line with quality requirements is expected to increase in the future.

Our results show that the combination of cluster analysis and principal component analysis can better classify apple varieties based on physicochemical elements and fruit texture quality and can extract the main defining characteristics. In conclusion, combining physicochemical indices with fruit texture profiles enabled us to efficiently characterize and compare differences among the fruit characteristics of 22 apple varieties. Although significant differences were observed in the basic physicochemical properties, the bonding characteristics and textural properties of the 22 apple varieties, as well as the cluster analysis, highlighted similarities in fruit traits among certain apple varieties.

Study 2

Following the studies and analyses conducted as part of this research, a range of products intended for children was developed, made from apples, carrots, pumpkin, and celery. To achieve this, ten experimental variants of puree were tested, each corresponding to a different apple variety used in the formulation.

In conclusion, the research highlights the importance of selecting the appropriate apple varieties for obtaining an optimal puree from both nutritional and sensory perspectives, contributing to the diversification of children's diets and the promotion of a balanced nutrition.

Although the Elstar apple puree consistently achieved the best results in terms of both bioactive compound content and adequate texture parameters, statistical analysis showed that the sterilized purees made from Champion, Grimmes Golden, and Parmen Clone varieties also achieved very similar results.

We consider that this line of research deserves further exploration, given the importance of nutrition in children's development. Fruits and vegetables represent an essential source of nutrients and contribute significantly to achieving this goal.

SELECTIVE BIBLIOGRAPHY

1. Zheng, J., Liu, Y., & Sun, J. (2019). The effects of apple antioxidants on health outcomes. *Food & Function*, 10(5), 2725-2739.
2. Zhang, F., Li, Z., & Zhu, Y. (2021). Apples as a functional food in chronic disease prevention. *Food and Function*, 12(5), 1259-1270.
3. Liu, R. H. (2021). Potential Health Benefits of Apples: Focus on Phytochemicals. *Food Research International*, 110, 23-35.
4. Crosby, J.A.; Janick, J.; Pecknold, P.C.; Korban, S.S.; Oconnor, P.A.; Ries, S.M.; Goffreda, J.; Voordeckers, A. (1992). Breeding apples for scab resistance: 1945–1990. *Fruit Var. J.*, 46, 145–166.
5. Bensoussan, L., Pivato, M., & Roux, A. (2021). Genetic diversity of wild apple species and its impact on phytochemical composition. *Plant Science Reviews*, 7(2), 103-117.
6. Weichselbaum, E., L. Wyness, and S. Stanner (2010). “Apple Polyphenols and Cardiovascular Disease – a Review of the Evidence.” *Nutrition Bulletin* 35 (2): 92–101.
7. Dan, C.; Serban, C.; Sestras, A.F.; Militaru, M.; Morariu, P.; Sestras, R.E. (2015). Consumer Perception Concerning Apple Fruit Quality, Depending on Cultivars and Hedonic Scale of Evaluation—A Case Study. *Not. Sci. Biol.*, 7, 140–149.
8. Sestras, A.F.; Pamfil, D.; Dan, C.; Bolboaca, S.D.; Jäntschi, L.; Sestras, R.E. (2011). Possibilities to improve apple scab (*Venturia inaequalis* (Cke.) Wint.) and powdery mildew (*Podosphaera leucotricha* (Ell. et Everh.) Salm.) resistance on apple by increasing genetic diversity using potentials of wild species. *Aust. J. Crop Sci.*, 5, 748–755.
9. Iordănescu, O.A.; Radulov, I.; Dascălu, I.; Berbecea, A.; Camen, D.; Orboi, M.D.; Călin, C.C.; Gal, T.E. (2023). Comparative Study on the Behavior of Some Old Apple Varieties before and after Their Grafting, with Potential for Use in Urban Horticulture. *Horticulturae*, 9, 353.