Exploring of the potential of secondary metabolites in the vegetative parts of berry plants during fermentation

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Abstract. This study examines the changes in the chemical composition of the vegetative parts of plants belonging to the Rosaceae and Grossulariaceae families. The subject of the analysis were stems, leaves, and buds of raspberries, currants, and blackberries. Fermentation can increase the content of certain polyphenols in raspberries and blackberries, precisely because of ellagic acid, but the content of total polyphenols in currant leaves decreases. The total flavonoid content increased during fermentation due to the breakdown of cell walls, resulting in improved aroma, taste, and beneficial properties. Plants contain a variety of flavonoids, including catechins and condensed tannins formed through the condensation of phenolic compounds. The formation of condensed tannins occurs both in the plant itself during biosynthesis and during fermentation. Phenolic acids, flavonoids, and tannins found in plants can be serve as natural preservatives and antioxidants in the food, cosmetic and pharmaceutical industries. The antioxidant activity of polyphenols helps protect cells from damage by free radicals and prevents the development of oxidative stress, which can lead to various diseases.

1 Introduction

Polyphenolic compounds are classified as secondary plant substances since they are not formed and are not consumed during the plant's primary metabolism. Their exact functions in the plant are still the subject of research, due to which they are synthesized by the plant as pest and disease defenses, growth regulators, and pigments. They largely determine the color, taste, and stability of tea and, ultimately, its quality [1-3].

Fermentation is a common cause of polyphenol conversion into other compounds during processing. Endogenous enzymes, primarily polyphenol oxidase, carry out these transformations. In our work, we use freezing-based fermentation. At low temperatures, the leaves crack and release juice, which changes the color and taste of the leaves. Reaction

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heat accelerates the fermentation process. Proper timing of fermentation cessation is a significant quality factor. A subsequent drying step completes the process [4, 5].

Not much research or publication has been done on the composition of samples of vegetative parts of plants. In the studied literature, there is no data on the transformation of flavonols, tannins, or catechins in raspberry, blackberry, and currant leaves. Therefore, this work was carried out to obtain information on possible transformations of these substances and changes in the chemical composition during fermentation. To do this, the vegetative parts of plants of the genus Rosaceae and Gooseberries (Grossulariaceae) were investigated and compared.

2 Materials and methods

Extraction is the first step in determining polyphenols. There are two standardized methods for preparing tea. Both use the same extraction: 70% (aqueous) methanol extract at 70°C. For most compounds, this is sufficient extraction. Additionally, compounds are more stable in 70% methanol than in water. Other extraction methods are not suitable for polyphenols; for example, boiling with a reflux condenser for one hour leads to a decrease in catechin yield [6, 7].

The total polyphenol content was determined using the Folin-Ciocalteu method. This method uses a reagent containing phosphotungstic acid as an oxidizing agent. The reagent reacts with a wide range of phenolic compounds, releasing a blue color (tungsten and molybdenum blue) by reducing easily oxidizable phenolic hydroxyl groups. It is possible to measure this blue color with a spectrophotometer because it has a wide maximum absorption wavelength of 765 nm. The standard deviation and coefficient of variation of the Folin-Ciocalteu method were 0.12 g/100 g and 0.71% on a dry weight basis, respectively [8].

Spectrophotometry determines total flavonoids. However, often, due to the possible contribution of other aromatic substances to the optical density of the analyzed solutions, it is necessary to resort to the purification of the sum of flavonoids (without chromatography) or a complex formation reaction. The most common method of analysis is to use an aluminium chloride solution. For example, flavones and flavonols, including rutin, have two absorption maxima - short-wave (260 nm) and long-wave (362 nm) - which can be analyzed not only for identification but also for quantitative evaluation, especially under differential spectrophotometry. In the presence of $AlCl_3$, a bathochromic shift of the long-wave band occurs, forming a maximum at 412 nm (analytical wavelength). This approach is one of the most commonly used in the analysis of flavonoids samples. This is due to the fact that it minimizes the contribution of accompanying substances to the optical density of the solutions under analysis [9].

The HPLC method for determining catechins was previously calibrated based on pure catechins. However, these standards are costly and often not pure enough for HPLC measurement. A relative response factor is therefore the basis of the method. Column chromatography uses silica gel or polyamide sorbents. Note that aluminium oxide is not suitable for the separation and purification of flavonoids since it forms irreversible complexes. Total catechins, expressed as a percentage by weight of the dry matter of the tea sample, was calculated by summing each of the five main catechins: Total catechins (total catechins = K + EC + EGK + EKG + EGCG, %). [10].

Using a titrometer, tannins in plant materials are determined. This is based on the oxidation of tannins with potassium permanganate in the presence of indigo carmine as an indicator [11, 12].

It was found that chromium, strontium, lead, arsenic, zinc, copper, nickel, and chromium were commonly found in raw materials, which were analysed with a MAX-

GF2E spectrophotometer. In direct determination, the sample material is ground, pressed, and placed in the spectrophotometer. No additional sample preparation is required for the analysis, as it is performed in accordance with the selected program [13, 14].

3 Results and Discussion

Scientific research shows that black and green tea possess anti-cancer, antioxidant, antiviral, and antimicrobial properties. The main ingredients are polyphenols, especially catechins. The overall level of polyphenols in green and black tea differs, but the levels in the studied samples are comparable. Results of determining total polyphenols show that green tea has the highest total polyphenol level. However, there were no significant differences in total phenolic content between different plant species of the Rosaceae and Grossulariaceae families.

Tea polyphenols, especially flavonoids, are of significant interest for their health-promoting properties. Fresh tea leaves contain up to 30% polyphenols dry. The most significant polyphenols are catechins, flavonol-O-glycosides, flavonol-C-glycosides, and proanthocyanidins.

Table 1 shows the range of variation in total phenolic content, as well as the total content of catechins and their ratio. The mean values show that black tea with an average value of 15.1% has a lower polyphenol content than green tea with 24.2%. The polyphenol content of blackberry, raspberry, and blackcurrant ranges from 15.8% to 18.9% on a dry weight basis, indicating a small range of variation. The values of the studied samples do not differ significantly depending on species.

Table 1. Content of total polyphenols, flavonoids and catechins.

	Green tea	**Black tea**	**Blackberry**	**Raspberries**	**Currant**
Extractive substances, %	**52.3**	**32.4**	**42.4**	**38.4**	**33.4**
C, %	0.4	0.6	0	0	0
EC, %	1.3	0.5	0.4	0.3	0.5
EGC, %	3.8	1.5	0.3	1.1	1.1
ECG, %	3.3	2.4	2.1	1.8	1.6
EGCG, %	6.3	3.1	1.5	1.3	1.8
Total catechins, %	14.1	8.1	4.3	4.5	5
Total polyphenols, %	24.2	15.1	18.9	17.3	15.8
Total flavonoids, %	17.8	18.4	5.7	4.8	7.4
Tannins, %	4.5	5.2	9.7	8.4	6.3

Scientific research has shown that black and green tea contain anti-cancer, antioxidant, antiviral, and antimicrobial properties due to their polyphenol content, particularly catechins. Green and black tea have comparable polyphenol levels, but green tea has a higher total polyphenol level. There were no significant differences in total phenolic content between different plant species in the Rosaceae and Grossulariaceae families. Fresh tea leaves contain up to 30% polyphenols on a dry weight basis. The most prominent polyphenols are catechins, flavonol-O-glycosides, flavonol-C-glycosides, and proanthocyanidins.

Table 1 shows the range of variation in total phenolic content, as well as the total content of catechins and their ratio. Black tea has a lower polyphenol content than green tea, with an average value of 15.1% compared to 24.2%. The polyphenol content of blackberry, raspberry, and blackcurrant ranges from 15.8% to 18.9% on a dry weight basis, with no significant variation between species. Catechins, such as epicatechin, epigallocatechin, gallate epicatechin, and gallate epigallocatechin, are the most potent flavonoids in tea leaves. Black tea is composed of mainly galloylated catechins (EGC) and epigallocatechin gallate (EGCG), as well as non-galloylated catechins and epicatechins. Green tea contains more EGCG and epicatechin gallate (ECG), as well as other catechins such as quercetin and kaempferol. Blackberry and raspberry leaves contain mostly epicatechins and epigallocatechins, while galloylated epigallocatechin is present in smaller amounts than in green tea. Raspberry and blackberry leaves contain galloylated epigallocatechin (EGCG) at 1-2%.

Tea also contains tannins and has antibacterial properties. They are high molecular weight compounds composed of many subunits (12-16 phenolic groups and 5-7 aromatic rings). Tannins are divided into two classes: hydrolysable tannins, formed by polyatomic alcohols such as glucose, with hydroxyl groups partially or completely esterified with gallic acid or related compounds; and condensed tannins, formed by the condensation of phenolic compounds such as catechins. Condensed tannins are formed in the plant during biosynthesis and during the fermentation process. Research shows that black tea leaves contain about 5-6% tannins, green tea about 4-5%, blackberry leaves about 10%, raspberry leaves about 8%, and blackcurrant leaves about 6%. However, tannin content can vary depending on factors such as variety, processing method, and plant origin.

Table 2 shows the total content of polyphenols, flavonoids, and catechins depending on fermentation. The average total polyphenol content after 24-hour fermentation in the studied samples was 17%, lower than black tea at 15.1%.

Table 2. Content of total polyphenols and catechins after fermentation.

	Green tea	**Black tea**	**Blackberry**	**Raspberries**	**Currant**
EC, %	**1.3**	**0.5**	**0.3**	**0.3**	**0.4**
EGC, %	3.8	1.5	0.3	1.1	0.9
ECG, %	3.3	2.4	1.7	0.8	1.6
EGCG, %	6.3	3.1	0.6	0.5	0.7
Extractive substances (24 h), %	-	-	42.4	38.4	33.4
Total catechins (24 h), %	-	-	2.9	2.7	3.6
Total polyphenols (24 h), %	-	-	19.5	18.1	13.8
Total flavonoids (24 h), %	-	-	5.7	4.8	7.4
Tannins (24 h), %	-	-	10.6	9.7	7.4

4 Conclusion

During fermentation, bonds between phenolic groups are destroyed. This leads to the formation of simpler compounds. Thus, fermentation leads to a change in the chemical composition of raspberry, currant and blackberry leaves and increases their antioxidant activity, bioavailability and potential health benefits. It can be hypothesized that fermentation may increase the content of certain polyphenols in raspberry and blackberry leaves, precisely because of ellagic acid. However, this may depend on many factors, such as fermentation time and temperature, as well as the variety of currant. In the leaves of raspberries, currants and blackberries, phenolic acids are also represented by gallic acid, ellagic acid and coumaric acid. They can be used as natural preservatives and antioxidants in the food industry as well as in the cosmetic and pharmaceutical industries. The amount of total flavonoids during fermentation increases due to the fact that fermentation contributes to the destruction of cell walls and the release of flavonoids from cells.

As a result, the amount of total flavonoids is increased, which leads to an improvement in its aroma, taste and beneficial properties.

The leaves of the studied plants contain a large group of flavonoids, including catechins and tannins, which are formed by the condensation of phenolic compounds such as catechins. Catechins are the most common flavonoids and have been found to improve heart health and lower cholesterol levels. Tannins can be divided into two classes: hydrolysable tannins, which are formed by multi-atom alcohols such as glucose, whose hydroxyl groups are partially or fully esterified with gallic acid or related compounds, and condensed tannins, which are formed by the condensation of phenolic compounds such as catechins. The formation of condensed tannins occurs both in the plant during biosynthesis and during fermentation. The analyzed samples were found to contain these compounds in the range of 7.4% to 10.6%, which is considered a positive factor. The antioxidant activity of polyphenols has been confirmed in various studies. They can help protect cells from damage by free radicals and prevent oxidative stress, which can lead to various diseases. Therefore, phenolic acids from raspberry leaves may represent a potential natural protection against various diseases and have a wide range of applications in the food, cosmetic, and pharmaceutical industries.

"It can be stated that the consumption of products with high levels of total polyphenols can make a positive contribution to the diversity of our diet," according to authors of articles in the European Journal of Clinical Nutrition [12, 13]. Plants of the Rose (Rosaceae) and Gooseberry (Grossulariaceae) families can be used not only as an addition to green tea but also as a separate food product. Polyphenols play a certain role in the prevention of several chronic diseases, and the total content of polyphenols in products produced by the food industry is underestimated. The relevance of enriching products with plant extracts lies in the absence of physiological caloric value, with established high concentrations of secondary substances [14-16].

References

1. D. Makris, G. Boskou, *Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts,* J of Food Composition and Analysis, **20**, 2, 132 (2007)
2. Y. Lin, Y. Tsai, *Factors affecting the levels of tea polyphenols and caffeine in tea leaves,* J of Agricultural and Food Chemistry, **51**, 7, 1864 (2003)
3. A. Esfandiari, A. Kelly, *The effects of tea polyphenolic compounds on hair loss among rodents,* J of the National Medical Association, **97**, **8**, 5 (2005)
4. H. Chung, K. Youn, *Effect of freezing treatment in tea preparation using Camellia sinensis leaves,* J Food Sci Technol, **57**, **11**, 4193-4200 (2020)
5. Z. Xiang, E. Wen, P. Yong, *The influence of fermentation and drying methods on the functional activities and sensory quality of Artemisia argyi H.Lév. & Vaniot herbal tea,* J of Applied Research on Medicinal and Aromatic Plants, **30** (2022)
6. B. Angshuman, B. Chiranjit, *Extraction of Polyphenols from Dried Tea Leaves,* Int J of Scientific & Engineering Research, **3**, 5, (2012)
7. M. Bindes, V. Cardoso, *Maximisation of the polyphenols extraction yield from green tea leaves and sequential clarification,* J of Food Engineering, **241**, 97-104, (2019)
8. A. Blainski, G. Lopes, J. Mello, Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L. Molecule, **18**, **6**, 65-6852 (2013)
9. A. Panche, A. Diwan, S. Chandra, *Flavonoids: an overview*, J Nutr Sci, **5**, 47, (2016)

10. A. Rana, E. Sharma, K. Rawat, R. Sharma, S. Verma, Y. Padwad, A. Gulati, *Screening and purification of catechins from underutilized tea plant parts and their bioactivity studies,* J Food Sci Technol, **53**, **11**, (2016)
11. L. Elba, E. João, J. Monteiro, *A Simple and Accurate Procedure for the Determination of Tannin and Flavonoid Levels and Some Applications in Ethnobotany and Ethnopharmacology,* Func Ecosyst and Com, Global Science Books, **1** (2008)
12. R. Aguilar-Sanchez, F. Ahuatl-Garcia, M. Davila-Jimenez, *Chromatographic and electrochemical determination of quercetin and kampferol in phytopharmaceuticals,* Journal of Pharma and Biomed Analys, **38**, 239-249 (2005)
13. K. Jurowski, M. Folta, B. Tatar, *The Toxicological Risk Assessment of Cu, Mn, and Zn in Herbal Medicinal Products,* J of Biolog Trace El Res, **200**, **4**, 1949-1955 (2022)
14. W. Wang, Y. Liu, F. Gu, Y. Tu, J. Yu, *Distribution Characteristics of Heavy Metals in Soils Affected by Different Land Use Types in a Superimposed Pollution Area with High Geological Background,* Journal of Huanjing Kexue, **43**, **4**, 2094-2103 (2022)
15. J. Azmir, M. Zaidul, *Techniques for extraction of bioactive compounds from plant materials*, J of Food Eng, **117**, **4**, 426-436 (2013)
16. M. Kahkonen, I. Hopia, *Antioxidant activity of plant extracts containing phenolic compounds*, J of Agricul and Food Chemistry, **47**, **10**, 3954-3962 (1999)