Evaluation of health promoting properties and quality of herbal teas obtained from fine-grained fraction of herbs

Ocena właściwości prozdrowotnych i jakości herbatek ziołowych otrzymanych z drobnoziarnistych frakcji ziół

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Abstract

The purpose of this paper was to determine the quality of herbal teas produced using the non-pressure agglomeration of fine-grained fractions of herbs, compared with their counterparts available on the market. In prepared infusions, the total polyphenol content was determined, using the Folin-Ciocalteu's reagent, as well as the antioxidative activity with the use of the DPPH radical and L* a* b* colour parameters, according to the CIELab colour scale, were identified. Moreover, the iron, lead and cadmium ion content in the raw materials used in the manufacture of herbal granules was determined. Herbal infusions presented high but diversified active compound content. Infusions prepared from herbal granules displayed lower antioxidative properties and higher polyphenol content than commercially available teas. Infusions obtained from herbal granules of the smallest size had the darkest colour. Results of the analyses show that infusions obtained from granulated herbal powder do not pose any health hazard, and bio-active compounds contained therein may improve consumers' health. Non-pressure agglomeration of fine-grained herbal fractions may be used as an alternative method for their management.

Keywords: agglomeration, antioxidative activity, colour, DPPH, heavy metal ions, infusions, polyphenols

Streszczenie

Celem pracy było określenie jakości ziołowych herbatek wytworzonych w procesie aglomeracji bezciśnieniowej drobnoziarnistych frakcji ziół w porównaniu z ich odpowiednikami dostępnymi na rynku. W sporządzonych naparach oznaczano zawartość polifenoli ogółem z użyciem odczynnika Folina-Ciocalteu`a, aktywność przeciwutleniającą z wykorzystaniem rodnika DPPH oraz parametry barwy L*, a*, b* w systemie CIE Lab. Ponadto określono zawartość żelaza, ołowiu i kadmu w surowcach użytych do produkcji ziołowych granulek. Ziołowe napary charakteryzowały się wysoką, ale zróżnicowaną zawartością związków aktywnych. Napary przygotowane z ziołowych granulek wykazywały niższą zdolność antyoksydacyjną i wyższą zawartość polifenoli niż herbatki dostępne na rynku. Napary otrzymane z najmniejszych granulek ziołowych charakteryzowały się najciemniejszą barwą. Wyniki przeprowadzonych analiz wskazują, że napary otrzymane z granulowanych pyłów ziołowych nie stanowią zagrożenia dla zdrowia, a ze względu na zawarte w nich związki bioaktywne mogą przyczynić się do poprawy zdrowia. Aglomeracja bezciśnieniowa drobnoziarnistych frakcji zielarskich może być wykorzystywana jako alternatywna metoda ich zagospodarowania.

Słowa kluczowe: aktywność przeciwutleniająca, aglomeracja, barwa, DPPH, jony metali ciężkich, napary, polifenole

Streszczenie szczegółówe

Ziołowe herbatki są bardzo chętnie spożywane ze względu na ich właściwości prozdrowotne i sensoryczne. Lecznicza wartość ziół wynika z obecności w nich określonych substancji biologicznie czynnych m.in. związków polifenolowych. Polifenole są doskonałymi antyoksydantami chroniącymi przed szkodliwymi dla organizmu wolnymi rodnikami, które reagując z cząsteczkami białek, lipidów i sacharydów powodują ich utlenianie, a w konsekwencji niszczenie struktur komórkowych, czym przyczyniają się do rozwoju wielu schorzeń.

Świeże zioła z powodu niskiej trwałości są najczęściej przetwarzane na susze bądź zamrażane. W czasie ich obróbki powstają liczne, pyliste odpady. Jedną z najpopularniejszych metod zagęszczania materiałów rozdrobnionych jest aglomeracja bezciśnieniowa polegająca na łączeniu drobnych frakcji materiałów sypkich w większe skupiska w wyniku mieszania surowca z dodatkiem cieczy wiążącej.

W niniejszej pracy oznaczono ogólną liczbę polifenoli, zdolność antyoksydacyjną oraz parametry barwy ziołowych herbatek wyprodukowanych z drobnoziarnistych frakcji ziół. Zbadano również zawartość wybranych metali ciężkich w surowcach poddanych aglomeracji bezciśnieniowej. Materiał badawczy stanowiły ziołowe napary sporządzone z trzech rodzajów suszonych ziół: melisy lekarskiej, rumianku pospolitego i szałwii lekarskiej o wymiarach frakcji 0.20 – 0.25 mm, które poddano aglomeracji bezciśnieniowej. Próbę kontrolną stanowiły komercyjne herbatki ziołowe.

Zawartość pierwiastków toksycznych (Pb i Cd) w badanych surowcach nie przekraczała ustalonej normy, a najwyższą zawartość żelaza stwierdzono w szałwii lekarskiej. Ziołowe napary charakteryzowały się wysoką, ale zróżnicowaną zawartością polifenoli oraz aktywnością przeciwutleniającą. Napary sporządzone z ziołowych granulek wykazywały znacznie niższe właściwości antyoksydacyjne i nieco wyższą zawartość polifenoli niż popularne herbatki dostępne na rynku. W naparach przygotowanych z granulowanych ziół oznaczono ciemniejszą barwą niż w popularnych herbatkach komercyjnych.

Introduction

Herbal teas have been extensively consumed because of their health promoting and sensory characteristics. The medicinal power of the herbs comes from specific active substances present in their tissues. Particularly great attention has been paid to studies on the content of polyphenol compounds in herbs and spices abounding in those compounds. Polyphenols are excellent antioxidants, ensuring protection against harmful free radicals, which, through reactions with proteins, lipids and saccharides, lead to their oxidation and consequently to the damaging of cellular structures, which contributes to the development of many diseases. Phenolic compounds have analgesic, anti-inflammatory, anti-bacterial, anti-viral and anti-allergic effects (Capecka et al., 2005; Hinneburg et al., 2006; Rusaczonek et al., 2007; Zych and Krzepiło, 2010). They also reduce the risk of cancer, diabetes, athero-sclerosis and nervous system and cardiovascular diseases. Products with high content of phenolic compounds, such as fruits and vegetables, green tea or some herbs, consumed as part of a daily sustainable diet, may produce health promoting effects (Scalbert et al., 2005; Grzegorczyk et al., 2013; Raza et al., 2013; Kozak at al., 2016).

Because of the short shelf life of fresh herbs, they have been usually dried or frozen. During their processing, large volumes of dusty waste are generated. The thickening of powdery materials may be performed in a variety of ways. One of the most popular methods is non-pressure agglomeration, that is the bonding of small particles of powdery materials into larger clusters as a result of blending the raw material with the binding liquid. This process consumes little energy, which allows the retention the original physico-chemical properties of the raw materials. The durability of these produced agglomerates depends on the properties of the raw material and the applied binding liquid, as well as the type of pelleting machine (Pietsch, 2002; Hejft and Obidziński, 2015).

The quality characteristics of food are a basic criteria for selecting foodstuffs by customers. While purchasing herbal teas, they focus in particular on the amount of healthy substances, product safety and sensory characteristics, i.a. colour of any particular tea (Kratchanova et al., 2010; Dmowski et al., 2014). What is more, the following pollutants are likely to affect the quality of herbal infusions: heavy metals, pesticide residues and native ingredients, such as oxalates producing a negative effect of the herbs on the human body. Tests for the microbial evaluation of herbal teas have shown that they may be significantly polluted with various types of bacterial micro-flora, depending on the content of plants included in dried herbal blends. It should be borne in mind, however, that levels of microbial populations permitted in herbal raw materials were specified in the guidelines to Pharmacopoeia, which ensures a high quality of products with herb content (Lasik et al., 2007; Almajano et al., 2008; Rusaczonek et al., 2010; Grzegorczyk et al., 2013).

The purpose of this paper was to determination of the total content of the polyphenols, antioxidant capacities, colour parameters of herbal teas produced from granulated powder and to examine if the herbal dust could be used for the preparation of valuable product. Also, the content of selected heavy metals in raw materials subject to non-pressure agglomeration process was determined.

Materials and methods

The research material consisted of herbal infusions prepared from granulates manufactured using the non-pressure agglomeration method. The dried herbs of three species were agglomerated: lemon balm (*Mellisa officinalis L. Lamiaceae*), chamomile (*Matricaria chamomilla L. Astraceae*) and sage (*Salvia officinalis L. Lamiaceae*) with particle sizes of 0.20 – 0.25 mm. The dried herbs used to granulation were derived from the polish producer of popular herbal teas, and they were constituted waste after production. The control was composed of commercially available instant herbal teas from the same producer what dried herbs (Table 1).

Table 1. Profiles of herbal teas analysed under this research (Producer`s data)

Tabela 1. Charakterystyka badanych herbat ziołowych (Dane producenta)

Product analysed	Country of origin	Form/Weight	Expiration date
Lemon balm	Poland	sachets leaves, 2 g	03/2018
Chamomile	Poland	sachets baskets, 2 g	03/2018
Sage	Poland	sachets leaves, 2 g	03/2018

Content of selected metals

In order to determine the quality of the raw materials, they were investigated for their content of selected metals, in accordance with the valid polish standard by atomic absorption spectrometry (AAS) after dry mineralization at 450 °C (PN-EN 14084, 2004).

Agglomeration and preparation of infusions

The plant material was agglomerated using a disk pelleting machine with a 40% aqueous starch solution as the moisturising liquid. After drying and hardening of the product, it was divided into three size-determined fractions: <1 mm; 1-2 mm and 2-3.15 mm.

In order to prepare the herbal teas, 2 g portions of pellets were weighed and closed in tea filters using a HENDI vacuum sealer. Infusions were prepared by pouring 200 ml portions of demineralised water (90 °C) over the bags filled with pellets, and brewed for three minutes, covered. The obtained infusions were subsequently cooled to an ambient temperature and filtered using the average absorbency filter paper. The control was infused for three and ten minutes (as suggested by the manufacturer).

Total content of polyphenols

To determine the total content of polyphenols in the infusions, a spectrophotometric method using the Folin-Ciocalteu's reagent was applied, with gallic acid as the standard (ISO 14502-1, 2005; Cybul and Nowak, 2008). The properly prepared herbal infusions were diluted with demineralised water at the ratio 1:5. Next, 1 cm^3 portions of the infusion were collected and placed into test-tubes, and 5 cm^3 portions of the Folin-Ciocalteu's reagent (POCH S.A.) were added. The obtained solutions were stirred and left for three minutes. After that time, 4 cm^3 portions of 20% aqueous sodium carbonate solution (POCH S.A.) were added. Then, the prepared samples were left in the dark for 60 minutes, and next their absorbance was measured at λ=765 nm relative to the distilled water. The total phenolic compound content was stated in gallic acid equivalents (mgGAE·cm^{-3} of the infusion).

Antioxidative activity

Antioxidative activity was determined according to the modified Zych and Krzepiło (2010) method using a stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (SIGMA). Absorbance of the solutions was measured at λ=517 nm. A 0.5 mM DPPH alcoholic solution was prepared by dissolving 19.71 mg of DPPH in 100 cm^3 of 95% ethanol. The obtained solution was diluted to achieve an absorbance of approximately 0.9 at λ=517 nm. Then, the prepared solution was stored in the dark. A spectrophotometer was calibrated using ethanol. The A_0 absorbance of the DPPH radical solution was measured by adding 0.04 cm^3 of etanol to 3 cm^3 of the DPPH alcoholic solution. The investigated sample contained 3 cm^3 of the DPPH solution and 0.4 cm^3 of a given herbal infusion; absorbance (A) was measured after 30 minutes of initiation of the reaction. For each measurement, three independent repetitions were made and the mean absorbance value (A_{avg}) was calculated for a given solution. Radical scavenging activity was stated as a per cent value of the inhibition of the investigated solution, using the following formula:

$$\%\ inhibition = 100\ (A_0 - A_{avg})/A_0$$

where: A_{avg} – mean absorbance of investigated infusion containing the anti-oxidant,

A_0 – absorbance of the DPPH radical solution.

Colour parameters

The colour parameters of herbal infusions were measured using a Lovibond CAM-System 500 tintometer. Glass measuring containers were filled with 50 cm^3 portions of prepared teas, placed on measuring heads and determined according to CIE Lab scale, where L* stands for lightness, a* stands for redness and greenness and b* yellowness and blueness (Dmowski et al., 2011; 2014).

Statistic methods

For all infusions, measurements were made in ten replications. The obtained results were subject to statistical analysis using STATISTICA 10.0 software, and the results were presented as the mean and standard deviation. Significant differences between the means (P) and F test values (ANOVA) were determined. In order to identify the relationships between the polyphenol content and the anti-oxidant properties of the investigated infusions, Pearson's correlation coefficient was calculated.

Results and discussion

The quality of herbal infusions is influenced by macro- and microelements contained in plants, as well as pollutants such as heavy metals or pesticide residues. The amount of substances present in herbs depends on the soil, water and air composition, technological processes used to process the raw material or the use of pesticide products (Sperkowska and Bazylak, 2010; Kozłowska and Ścibisz, 2011; Suliborska and Kaczmarek, 2011).

The Table 2 presents the results of selected heavy metal content analyses performed on the herbs used in the production of herbal teas. The largest iron level, mean 1,640 mg $Fe\cdot kg^{-1}$, was found in the sage. Significantly lower iron content was reported for lemon balm and chamomile, 1,070 and 1,100 $mg\cdot kg^{-1}$ respectively. Recorded results have shown that herbs may introduce more iron into the daily diet.

Other authors have also reported high variance of Fe ion in raw herbal materials. In various herb species, Olędzka and Szyszkowska (2000) found mean values ranging from 26.11 to 526.9 mg of iron per kg of the raw material. Ulewicz-Magulska et al. (2009) on the other hand, identified high iron content in thyme and marjoram, ranging respectively from 751.36 to 1,495.53 $mg\cdot kg^{-1}$ and from 1,073.08 to 1,316.08 $mg\cdot kg^{-1}$, depending on the place of origin.

Investigated raw materials displayed similar lead (Pb) content, with the highest content (1.96 $mg\cdot kg^{-1}$) reported for garden sage. The lead content in individual raw materials did not exceed 2 $mg\cdot kg^{-1}$ of dry matter, which complied with adopted recommendations (Rozporządzenie Ministra Zdrowia, 2003). The obtained results are close to the results recorded by Blicharska et al. (2008) who found from 0.12 to 2.23 mg $Pb\cdot kg^{-1}$ of the raw material, depending on the species and investigated plant organ.

Trace amounts of cadmium were found in lemon balm and sage. The highest concentration of this metal 0.211 $mg\cdot kg^{-1}$, was found in chamomile. Results of the performed analyses comply with studies of other authors who found that, depending of the species, plant organ and harvesting site, herbs are likely to contain trace or larger amounts of cadmium; however, never exceeding 0.3 mg·kg^{-1} of dry matter (Rozporządzenie Ministra Zdrowia, 2003; Blicharska et al., 2008; Grzegorczyk et al., 2013).

Table 2. Content of selected metal in dried herbs size fractions 0.20-0.25 mm

Tabela2. Zawartość wybranych metali w suszonych ziołach o wymiarach frakcji 0.20-0.25 mm

Raw material	Fe [mg·kg^{-1}]	Pb [mg·kg^{-1}]	Cd [mg·kg^{-1}]
Lemon balm	1,070±118	1.12±0.13	0.032±0.003
Chamomile	1,100±121	1.56±0.19	0.211±0.017
Sage	1,640±180	1.96±0.24	0.035±0.003

All the investigated herbal infusions showed, from the point of view of nutrition, a significant total polyphenol content, ranging from 323.84 to 752.84 mgGAE·cm^{-3} (Figure 1).

Lemon balm infusions were found to be the richest source of polyphenols. The smallest polyphenol content on the other hand, was found in sage-containing teas. It was observed that the polyphenol content in herbal infusions is determined by the brewing time and degree of herb fragmentation. The total polyphenol content in teas brewed for 3 minutes, constituting the control, ranged from 286.84 mgGAE·cm^{-3} for Sageto 717.98 mgGAE·cm^{-3} for Lemon balm. An extension of the brewing time up to 10 minutes (Figure 2) led to significant reduction of the polyphenol content from 257.75 (for Sage) to 494.66 (for Lemon balm) mgGAE·cm^{-3}. This might have been the effect of the delicate structure of herbs making polyphenols transfer quickly to the infusion and become oxidated over a longer brewing time, leading to a reduced content thereof. In the case of teas, longer brewing is recommended, as tea leaves have a different structure, they are thicker and harder, therefore polyphenols may need more time to be transferred to the infusion. Similar conclusions were drawn while investigating various jasmine teas (Młynarczyk et al., 2015).

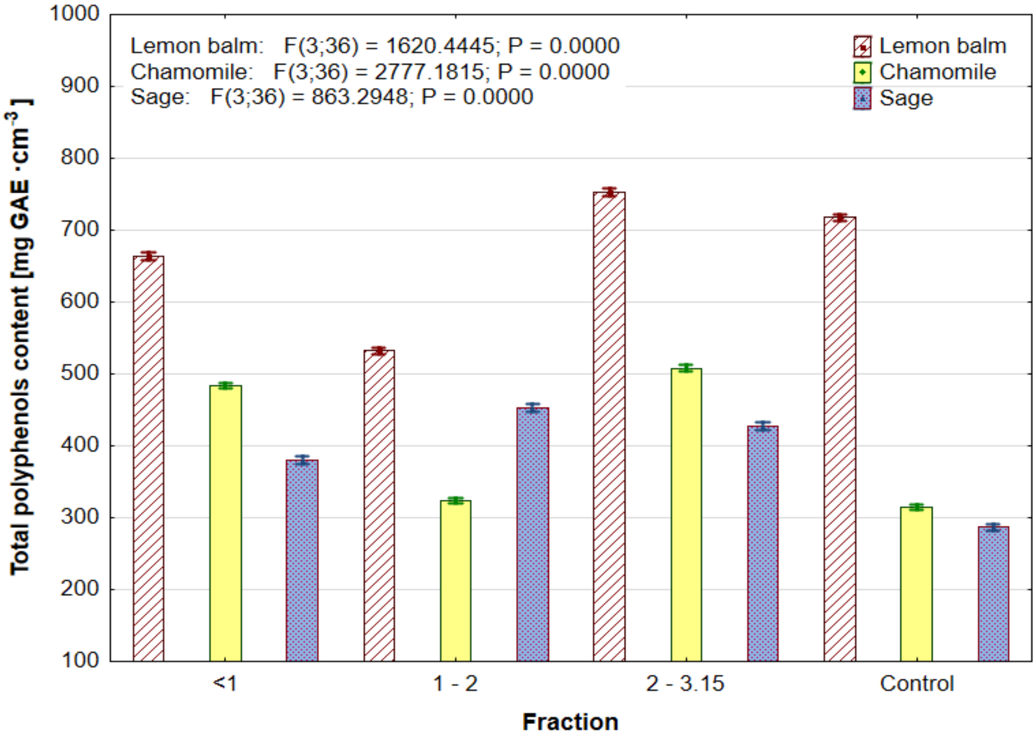

Figure 1. Content of total pholyphenols in infusions of herbs depending on dimensions of fraction

Rysunek 1. Całkowita zawartość polifenoli w naparach herbat w zależności od wymiarów frakcji

Infusions obtained from granules of a smaller size showed lower content of phenolic compounds, which may mean that the polyphenols are more easily extracted from fractions with finer grains; on the other hand, they need less time to become oxidised. A considerably lower polyphenol content was found in herbal teas being dietary supplements. Due to the presence of different ingredients in the products, they had varied a polyphenol content that ranged from 24.6 to 87.8 mgGAE·cm^{-3} (Sielicka et al., 2011). A high content of phenolic compounds was also found in white teas; the literature informs about the content ranging from 274 to 552 mg of catechin·dm^{-3} (Plust et al., 2011). In the case of the alcohol extracts of sage and thyme, the total polyphenol content was respectively, 140 and 210 mgGAE·g^{-1} of the extract (Kozłowska and Ścibisz, 2011). The method using the Folin-Ciocalteu's reagent is highly useful and simple; however, it is poorly specific. The determination of polyphenol content may be helpful in identifying the relationships between polyphenol content and antioxidant properties.

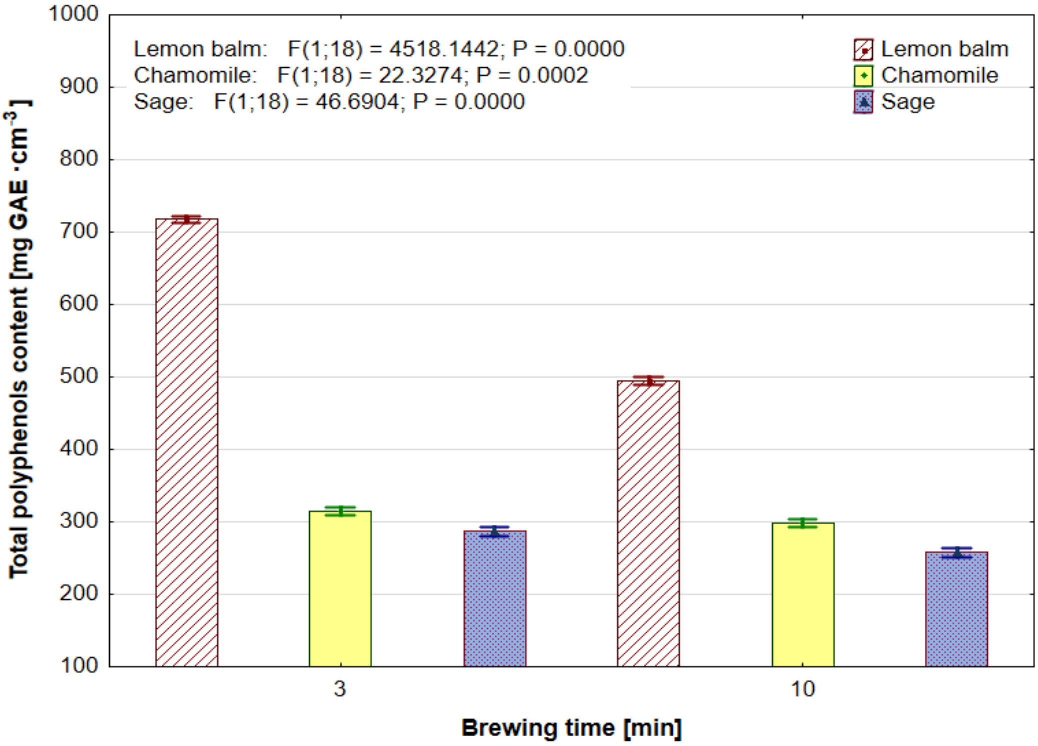

Figure 2. Content of total pholyphenols in the control infusions of herbs depending on brewing time

Rysunek 2. Całkowita zawartość polifenoli w naparach stanowiących próbę kontrolną w zależności od czasu ich parzenia

The investigated herbal teas showed a varied ability to react with the DPPH radical, as presented in Figure 3. In the described method, a high inhibition (per cent value) points to strong antioxidant properties and small residues of the unreacted DPPH radical in the sample. The highest antioxidative activity was found for lemon balm infusions, and the lowest for chamomile teas. The longer brewing time, in the case of popular herbal teas, led to a minor increase in their antioxidative activity, by 6.48% for lemon balm, 1.48% for chamomile and 1.41% for sage respectively (Figure 4). A similar trend was observed in the study on the antioxidant potential of black teas. Dmowski et al. (2014) found that an extended brewing time leads to the increased antioxidative activity of tea.

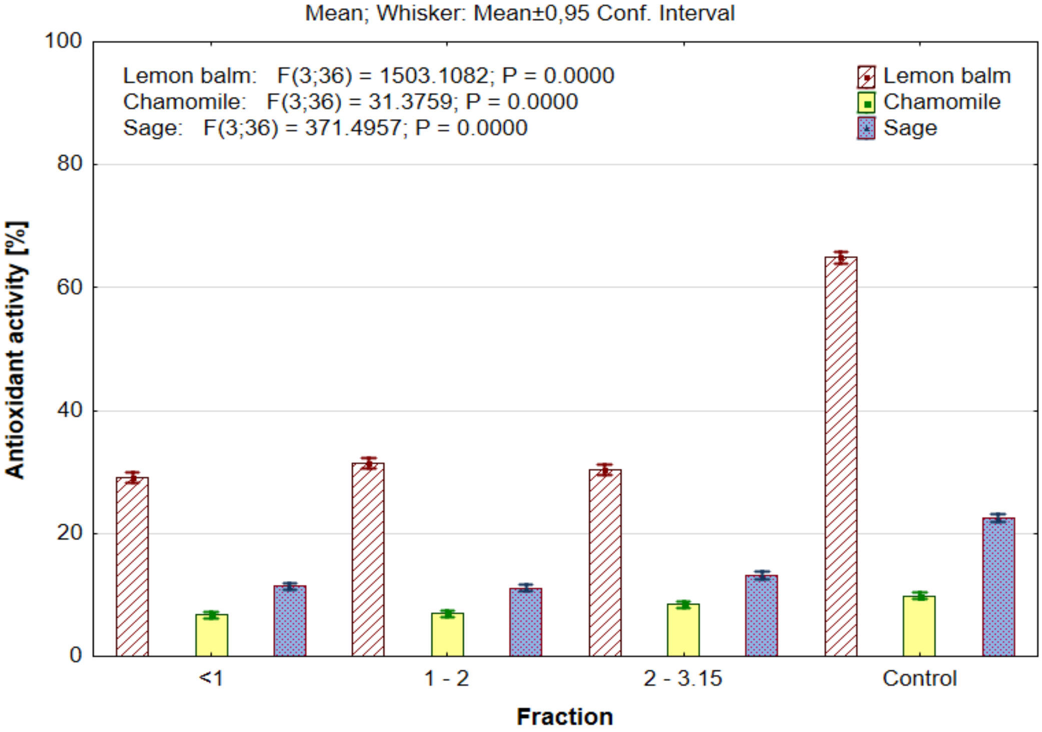

Figure 3. Antioxidant activities in infusions of herbs depending on dimennsions of fraction

Rysunek 3. Aktywność antyoksydacyjna naparów herbat w zależności od wymiarów frakcji

Infusions prepared from herbal granules showed significantly lower activity to react with the DPPH radical when compared to teas from the control; on average by 53% in the case of lemon balm, 42% - sage and 14% - chamomile. Infusions prepared from herbal granules of various sizes showed similar free radical scavenging abilities. The use of smaller granules in tea-making led to minor decreases in their antioxidative activities. It was found that the investigated lemon balm and chamomile infusions showed different DPPH radical scavenging abilities that were statistically significant. In the study, a positive correlation (r=0.691; P<0.05) between the antioxidative activity stated as per cent value of the scavenged DPPH radical and total polyphenol content was found, which complied with other findings (Zujko et al., 2011).

This relationship is related to the fact that the phenolic compounds may act as substances reducing or blocking free radicals. It was observed that in some cases, the antioxidant ability decreased irrespective of increasing the polyphenol content. This means that the antioxidative ability is also influenced by other active substances (Kozłowska and Ścibisz, 2011).

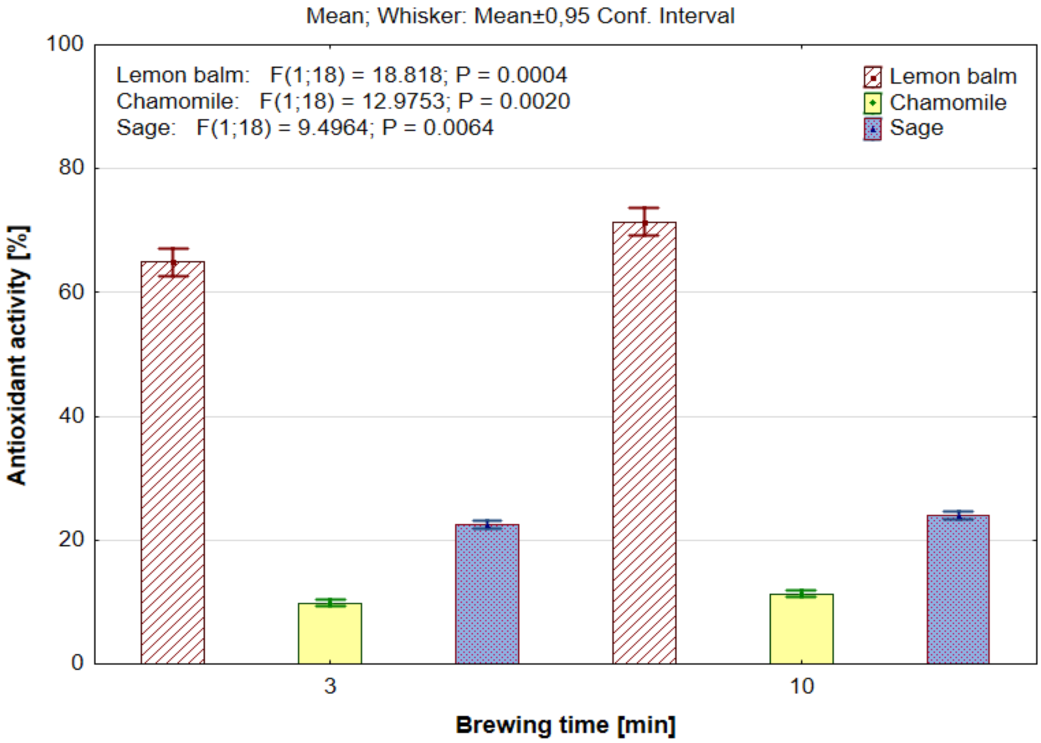

Figure 4. Antioxidant activities in the control infusions of herbs depending on brewing time

Rysunek 4. Aktywność antyoksydacyjna naparów stanowiących próbę kontrolną w zależności od czasu ich parzenia

In the study by Zych and Krzepiło (2010), considerably lower antioxidative activity of lemon balm and chamomile infusions was reported, as respectively 18.34% and 0.25%. For ethanol extracts of Lamiaceae herbs, an antioxidative capacity of 72–89 μmol of Trolox·g^{-1} of the extract was found. Green teas display significantly higher antioxidative activity, ranging from 69.4 to 94.1% (Kozłowska and Ścibisz, 2011).

It is very hard to compare the results of different studies, as antioxidative activity and the volume of identified polyphenols may differ depending on the type of applied extraction method, degree of fragmentation of the material as well as its place of origin. The phenolic compound content and free radical scavenging ability are also influenced by cultivation conditions, climatic conditions and the processing method used after harvesting (Shan et al., 2005; Modnicki and Balcerek, 2009; Pirbalouti et al., 2013).

Sensory quality is an important component of the general herbal tea quality. The factor determining whether herbal infusion is accepted or not is, besides the taste, the colour (Liang et al., 2003; Rusaczonek et al., 2010; Dmowski et al., 2011). Colour determination results for herbal teas are presented in Table 3. All the investigated infusions showed equal colour parameters specific for the given plant species. The brightest colour was found for teas from the control brewed for 3 minutes, for which L* was respectively: 56.1 for chamomile, 51.28 for sage and 47.76 for lemon balm.

An extension of the brewing time caused reduced lightness of herbal infusions. Chamomile and sage infusions prepared from herbal granules had a darker colour than popular teas brewed for 10 minutes. The highest values of this parameter was recorded for chamomile infusions (granules <1 mm). Negative values of a* and positive values of b* suggest that the produced infusions had green and yellow colour, which is characteristic for herbal teas.

Table 3. Herbal infusion colour parameters

Tabela 3. Parametry barwy badanych herbat

Product		L*	a*	b*
Lemon balm	Granules <1 mm	40.91±0.94	-2.27±0.51	45.42±0.94
	Granules 1-2 mm	40.44±0.85	-1.67±0.66	44.86±0.85
	Granules 2-3.15 mm	40.64±0.7	-2.72±0.68	45.02±0.84
	The control 3 min brewed	47.76±0.66	-6.54±0.34	47.77±0.89
	The control 10 min brewed	40.20±0.43	-1.04±0.51	44.30±0.53
Chamomile	Granules <1 mm	44.30±0.82	-6.46±0.39	49.08±0.59
	Granules 1-2 mm	41.04±0.51	-4.86±0.39	46.22±0.77
	Granules 2-3.15 mm	42.40±0.67	-5.74±0.34	47.47±0.8
	The control 3 min brewed	56.10±0.73	-11.08±0.41	23.42±0.41
	The control 10 min brewed	49.84±0.53	-3.90±0.81	44.54±0.8
Sage	Granules <1 mm	38.08±0.41	-1.59±1.18	41.51±0.65
	Granules 1-2 mm	39.32±0.82	-2.35±0.82	43.43±0.94
	Granules 2-3.15 mm	39.64±0.44	-4.14±0.51	44.35±0.89
	The control 3 min brewed	51.28±0.63	-7.18±0.41	30.12±0.7
	The control 10 min brewed	49.28±0.5	-7.34±0.34	34.42±0.56

Conclusions

1. The highest iron content was found in sage. The content of toxic elements (Pb and Cd) in the investigated raw materials did not exceed the fixed standard, which means that they fail to pose a risk to human health and may be used in production of herbal preparations.

2. Herbal infusions are characterised by high but diversified total polyphenol content and antioxidative activity. Infusions prepared from herbal granules showed significantly lower antioxidant properties and slightly higher polyphenol content when compared to popular commercially available teas.

3. A positive correlation between the content of active compounds – polyphenols and antioxidative activity was found in herbal infusions. Antioxidant activity depends not only on the amount of polyphenolic compounds contained in the herbs, but also on their activity resulting from the chemical structure.

4. Infusions prepared from herbal granules were determined to have a darker colour than popular teas brewed for 10 minutes. Teas prepared from sage granules of the smallest size were the darkest and the brightest were chamomile infusions (granules <1 mm).

5. The study found that the applied non-pressure agglomeration of fine-grained fraction of herbs is an alternative form of their use for food purposes and permits the obtaining of a new product – granulated herbal teas. In this chapter write conclusions. Conclusions should not contain references.

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