Summary

Background. Aloe contains numerous bioactive compounds which are attributed to have health advantages. Widely used in the cosmetic and pharmaceutical industries, also found its application in the food industry. The antioxidant compounds in aloe may increase the stability and nutritional value of food. The aim of the paper was to evaluate the results of the antioxidant properties of aqueous extract of aloe in model systems.

Material and methods. Analyses were conducted on true aloe (Aloe vera L.). Extraction was run using water at temperature 80–90°C. The level of phenolics was determined spectrophotometrically with the Folin-Ciocalteu reagent, using gallic acid as a standard. Antioxidant activity of extract was analysed in relation to linoleic acid, running incubation for 19 h, by scavenging of stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) and cation radical ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) and on the basis of metal chelating ability. Recorded results were compared with the activity of BHT (butylated hydroxytoluene).

Results. The total phenolic content of water extract of aloe was 17.85 mg/g d.m. The extract exhibited concentration-dependent DPPH and ABTS radical scavenging activity. The ability was in the range of 4.32–8.87 and 0.58–0.87 mg of trolox per 1 g d.m., respectively. A lower chelating ability of the aloe extract was 1.23 mg EDTA per 1 g d.m. at 20 mg/ml concentration and the higher was 8.76 mg EDTA per 1 g d.m. at 10 mg/ml concentration. The aloe extract reduced levels of the conjugated dienes in the emulsion system. The samples with additions of 0.1–0.5 mg/ml extract contain over 90% less of dienes than control sample. The addition of 0.05 mg/ml and 0.06 mg/ml results in significantly lower (40% and 57%, respectively) activities of the aloe extract.
Conclusions. The extract of aloe showed different antioxidant properties in various model systems. The available literature on the antioxidant potential of aloe is still rather scarce. Due to the complex composition and broad activity of aloe, raw material should be further studied.

Key words: aloe, antioxidant activity, model systems

Introduction

The reactions of lipid oxygenation taking place in food negatively influence the taste, smell, texture and nutritional value. Presently, the food processing industry shows a tendency to substitute synthetic antioxidants with natural oxygenation inhibitors in order to protect food against the processes of oxygenation. The basic antioxidants posing neither fears nor reservations in toxicological tests are polyphenols (Szajdek and Borowska, 2004; Szukalska, 2003). The results of the research carried out worldwide confirm the health advantages of consuming polyphenol-rich products and their significant effect on forming food sensory features (Budryn and Nebesny, 2006; Dai and Mumper, 2010; Kosiorek et al., 2013; Vauzour et al., 2010).

Aloe belongs to the group of raw materials rich in bioactive compounds showing health-promoting advantages. It contains e.g. polyphenols, anthranoids, and derivatives of pyron, saponins, steroids, fibre, salicylic acid, mineral components (calcium, chromium, iron, manganese, potassium, phosphorus, natrium, and zinc), as well as vitamins (C, E, β-carotene, B1, B2, B3, B6, choline, B12, folic acid) (Jasso de Rodriguez et al., 2005; Sahu et al., 2013). The content of polyphenolic compounds is slightly higher in the leaf skin than in the flower. Catechin prevails in the skin, while the flower contains more gentisic acid (López et al., 2013).

Owing to the rich chemical composition, as well as its antioxidative properties, aloe has become the focus of numerous phytochemical and pharmacological tests which deliver very interesting results (Cieślik and Turcza, 2015; Khanam and Sharma, 2015; López et al., 2013; Nowak and Starek, 2003). It has been proved it supports the treatment of gastrointestinal canal diseases, such as: inflammations, stomach, large intestine and duodenum ulcers. The bioactive compounds in aloe act anti-inflammatoryly and deliver the substances being a good medium for symbiotic bacteria. The extract supports the lipid-carbohydrate metabolism which positively affects the blood sugar and cholesterol level, as well as the right body mass. The exterior application of aloe is wide in the area of skin regeneration (Cieślik and Turcza, 2015; Nejatzadeh-Barandozi, 2013; Surjushe et al., 2008; Szaufer-Hajdrych et al., 2007). Widely used in the cosmetic and pharmaceutical industries, also found an application in the food industry. The forms of concentrate, gel, juice or powder can be applied as an addition to numerous food products, increasing their nutritional and health-promoting value, as well as sensory attractiveness.

Aloe can be added to jellies, jams, water, tea, or juices; candies, also those vitamin-enriched, chewing gums, fruit smoothies; alcohol-free beverages, laxatives, electrolyte-enriched beverages for sportmen, dietetic drinks, as well as yoghurts; cottage cheeses or ice-creams (Ahlawat and Khatkar, 2011). The most popular products approved by International Aloe Science Council (IASC) are aloe leaf flesh juices, which may addi-
tionally contain cranberry and apple, as well as peach juices (Kukulowicz and Steinka, 2010).

The aim of the carried out research was an evaluation of the antioxidative activity of the aloe extract in fat-free model systems, as well as those containing the emulsified fat.

**Material and methods**

The experimental material was aqueous extract of *Aloe vera* L. It was cultivated in controlled conditions of development and growth on sandy-clay foundation, all year long in the temperature above zero (in winter 10–16°C, in summer 20–25°C, moderate insolation), at the Department of Food Service and Catering of the Poznań University of Life Sciences. The collection of leaves for the extract preparation was carried out after ten years of aloe vegetation. Butylated hydroxytoluene, a synthetic antioxidant, was applied for comparative reasons in the analysis. It was purchased in Merck company. BHT performs a confirmed high antioxidative activity so it frequently serves as a reference point in evaluating the antioxidative activity properties of natural compounds (Estévez et al., 2007; McCarthy et al., 2001; Tepe et al., 2005). Since it is a synthetic compound and there are premises proving its supposed negative influence on the human organism, its application is strictly defined and limited. That is the reason why in the below analysis the accepted level of the concentration of the synthetic antioxidant is lower compared to aloe extract (Regulation of the Minister of Health, 2010 – Rozporządzenie..., 2010).

The extract was produced in the Research Station of the Department of Food Concentrates and Starch Products of the Institute of Agricultural and Food Biotechnology in Poznań. The material was extracted using water in a continual double-start worm extractor. It was proportioned to the charging hopper of the extractor, and next subjected to extraction at pH 5–7 and temperature 80–90°C with the efficiency of 10–15 kg/h. After passing the filtration screen the pomace was separated from the solids, and next directly transferred to the vacuum evaporator. The process of the extract setting took place in the vacuum evaporator, in the conditions of a lowered pressure, for 7.5–10 h, until 3–4% of dry mass was obtained. The obtained extract was subjected to drying in a pulverizing drier and next screened applying an oscillating granulator to get powder of a uniform granulation. Water was used as a solvent in phenolic compounds extraction, since it is the solvent most widely approved of by consumers. Other solvents will be taken into consideration in further research, e.g. ethanol, methanol, ethyl acetate and their mixtures.

The content of polyphenols was marked colorimetrically at wavelength of 750 nm, applying the Folin-Ciocalteu method (Horwitz et al., eds., 1970). The method is based on a colour reaction between polyphenols and the Folin-Ciocalteu (FC) reagent. Phenolic compounds, in the basic environment, occur in the phenol anion form, which reduces the FC reagent creating a blue dye. The results of the analysis were presented as an equivalent of the gallic acid concentration (GAE) in milligrams per 1 g of dry mass of extract.

The antioxidative properties of extract were evaluated on the basis of the capability of the extract to neutralize the DPPH radical (2,2-diphenyl-1-picrylhydrazyl), as well as cation radical ABTS (2,2’-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]), to chelate ring formation of metals, and also towards the linoleic acid.
The capability to neutralize the DPPH radical was defined basing on the colorimetrically evaluated DPPH radical concentration towards the blank determination (Mensor et al., 2001; Sanchez-Moreno et al., 1998). The measurement of absorbance was conducted at the wavelength of 517 nm after a 30-minute aloe solution incubation (5–10 mg/ml) and BHT (0.1–0.2 mg/ml) solution at room temperature, with no light access. The antiradical activity of the samples was expressed in equivalents of trolox (synthetic analogue of E vitamin), after the analytical curve had been developed. The results were presented in milligrams of trolox per 1 g of extract’s dry mass.

The capability of antioxidant compounds to reduce the cation radical ABTS was defined basing on the direct generating of ABTS$^{+\cdot}$ as a result of ABTS oxygenation by potassium persulfate ($K_2S_2O_8$) (Re et al., 1999). An addition of the antioxidant causes a reduction of ABTS$^{+\cdot}$ to ABTS and a decrease of the coloring intensity of the radicals solution. The degree of ABTS$^{+\cdot}$ reduction was defined spectrophotometrically for aloe samples (5–10 mg/ml), and BHT (0.1–0.2 mg/ml) at wavelength of 734 nm. The antiradical activity of the samples was expressed in milligrams of trolox per 1 g of d.m. of extract.

The determination of the chelating capacity of metals consisted in the colorimetric measurement of the decoloration degree by the extracts of iron chloride complexes (II) with ferrozine (Tang et al., 2002). The wavelength applied was 562 nm. The metal chelating activity of aloe extracts (10–20 mg/ml) and BHT (0.2–0.4 mg/ml) was expressed in EDTA equivalents (ethylenediaminetetraacetic acid, synthetic complexing compound). The results were presented in milligrams of EDTA per 1 g of d.m. of extract.

The capacity of inhibiting the process of auto-oxidation of linoleic acid was determined by Lingnert et al. (1979) method. The method consisted in the spectrophotometric notation of the conjugated dienes growth in 10 mM of linoleic acid emulsion at pH 7.2. The tested extracts were introduced into the prepared emulsion at the level 0.05–0.5 mg/ml, and next the absorbance was measured at the wavelength of 234 nm, both directly after the antioxidant had been added and after a 19-hour incubation at 37°C, with no light access. The content of dienes was calculated applying the molar coefficient of absorption for conjugated dienes in agreement with the Lambert-Beer law:

$$A = \varepsilon \cdot c \cdot l$$

where:

- $A$ – absorbance,
- $\varepsilon$ – molar coefficient of absorption ($\varepsilon = 2.56 \cdot 10^4 \text{M}^{-1}\text{cm}^{-1}$),
- $c$ – concentration of peroxides with conjugated dienes (M),
- $l$ – thickness of the absorption layer (cell/cuvette thickness = 1 cm).

The obtained results were subjected to a statistical analysis. The results discussed in the paper are an arithmetic mean of an independent series of measurements in three replications. The analysis of the least significant differences with an application of monofactorial variance was carried out in the Statistica 9 program. Statistical interference was conducted at the significance level of $p = 0.05$. 
Results and discussion

The content of total polyphenols in the tested aloe extract was defined at the level of 17.85 mg GAE per 1 g of d.m. The value is two times lower compared to the data provided by Ray et al. (2013). The authors determined the total content of phenolic compounds in the aloe gels methanol extracts at the level of 30.11–35.77 mg GAE per 1 g of extract. Then the ethanol extracts from the aloe leaf skin and aloe gels contained respectively 7.99 and 2.06 mg GAE per 1 g (Vidic et al., 2014). Kammoun et al. (2011) tested five aloe extracts in which they applied various solvents. The highest phenolic compounds content was found in the aloe extract obtained as a result of chloroform and ethanol mixture (40.5 mg GAE per 1 g) use, whereas the lowest one was observed in the aqueous extract (2.072 mg GAE per 1 g). Hexane and butanol showed a lower capacity of extracting the phenolic compounds compared to ethyl acetate. The content of polyphenols in the extracts prepared with the solvents was respectively 9.6, 16.9 and 23.8 mg GAE per 1 g. Zheng and Wang (2001) found that the content of polyphenolic compounds in the aloe extract was at the level of 0.23 mg GAE per 1 g of d.m. and it was the lowest value among the 39 tested plants of culinary and medicinal use (sage, oregano, thyme, rosemary, maidenhair tree, lavender, amaranth).

Figure 1 presents the scavenging ability of radical DPPH by aloe extracts (5 and 10 mg/ml) and the synthetic antioxidant BHT (0.1 and 0.2 mg/ml). The tested concentrations of the extract proved a capacity to deactivate radicals, respectively at the level of 8.87 and 4.32 mg of trolox per 1 g of d.m., whereas the synthetic antioxidant BHT at the level of 655.01 and 471.44 mg of trolox per 1 g of d.m. It was found out that the capacity to terminate the DPPH radicals decreases alongside with an increase in the polyphenols.

Fig. 1. DPPH scavenging effect of aloe extracts (A) and BHT (B). Aloe 1 – 5 mg/ml, Aloe 2 – 10 mg/ml, BHT 1 – 0.1 mg/ml, BHT 2 – 0.2 mg/ml. Different letters mean statistically significant differences at the level of p = 0.05

Rys. 1. Zdolność neutralizowania rodnika DPPH przez ekstrakty aloesu (A) i BHT (B). Aloes 1 – 5 mg/ml, Aloes 2 – 10 mg/ml, BHT 1 – 0.1 mg/ml, BHT 2 – 0.2 mg/ml. Różne litery oznaczają różnice statystycznie istotne na poziomie p = 0.05
addition. The research on the DPPH radicals scavenging effect by the extracts prepared from aloe at different growth stages found that the activity was dependent on the extract. In this case with increasing levels of additive (0.2–1.0 mg/ml) a higher antiradical activity (2–10%) was noted (Ray et al., 2013). The research results of Oomah and Mazza (1996) confirm that the relation between the polyphenols content and antioxidative activity is not always strong.

The paper also focused on determining the ability of the tested antioxidants to deactivate cation radicals ABTS (Fig. 2). It was found that an aloe vera extract addition at 5 and 10 mg/ml showed an ability to terminate the synthetic cation radicals ABTS at the level of 0.87 and 0.58 mg of trolox per 1 g of d.m., respectively. It is a significantly lower activity compared to BHT, whose obtained values were 53.27 and 33.94 mg of trolox per 1 g of d.m., respectively, for the concentration of 0.1 and 0.2 mg/ml. Khanam and Sharma (2015) report that aqueous aloe extracts are characterized by a meaningfully lower scavenging degree of ABTS” compared to methanol extracts.

Fig. 2. ABTS scavenging effect of aloe extracts (A) and BHT (B). Aloe 1 – 5 mg/ml, Aloe 2 – 10 mg/ml, BHT 1 – 0.1 mg/ml, BHT 2 – 0.2 mg/ml. Different letters mean statistically significant differences at the level of p = 0.05

Aloe extract showed a low ability of weakening the activities of catalysts (Fig. 3). The effect of the activity decrease was observed along with the addition level increase. The lowest chelating activity – of 1.23 mg EDTA per 1 g of d.m. – was noted in the extract at the level of 20 mg/ml. A significantly higher activity (p < 0.05) was observed in the extract with a lower concentration (10 mg/ml) and BHT (0.2 and 0.4 mg/ml). Chelating properties may depend on the participation of tanins condensed in the plant material. Testing the presence of phytochemical compounds in Aloe vera, Arunkumar and Muthuselvan (2009) detected tanins, saponins and flavonoids. According to Kumari and Jain
(2015) the content of tanins in aloe gel is 1.38 mg/g, but in comparison with other plant materials, e.g. some acacia species (106.24 mg/g), it is very low. Such a low content of tanins may influence the low ability of aloe extracts to chelate metals. According to Ray et al. (2013), the chelating activity of aloe aqueous extract is at the level of 2–48% and depends on the plant growth stage.

The results in Table 1 present the influence of aloe extract and BHT on the stability of linoleic acid in an emulsion. The conjugated dienes increase, assumed in the research, depended on the level of antioxidants addition. An addition of extracts with higher concentrations showed a stronger protective effect towards the linoleic acid than the extracts of a lower concentration. The samples with extract additions at the level of 0.1, 0.2 and 0.5 mg/ml contained 90% less dienes compared to the control sample. A lower antioxidant activity was characteristic for the aloe extracts with a lower concentration (0.05 and 0.06 mg/ml), which lowered the content of conjugated dienes in the incubated emulsion by respectively 40 and 57%, compared to the control sample. The synthetic BHT antioxidant, applied for comparative reasons, showed a similar activity in the emulsion system to the aloe extract.

The obtained results indicate a variety of activity mechanisms of the antioxidants contained in the researched material. The investigations proved a high antioxidant potential of the aqueous aloe extract in the emulsion system. The protective effect in relation to the linoleic acid was close to the synthetic BHT antioxidant. At the same time a low activity was found of the extract to bind iron ions, as well as in the system of both stable DPPH radicals and ABTS cation radicals. It may be connected with the type of the extraction solvent applied in the investigations. Polar organic solvents possess
Table 1. Effect of extract of aloe and BHT to change the content of conjugated dienes in an emulsion of linoleic acid

Tabela 1. Wpływ ekstraktu z aloesu i BHT na zmiany zawartości dienów sprzężonych w emulsji kwasu linolowego

<table>
<thead>
<tr>
<th>Sample – Próba</th>
<th>Content of conjugated dienes (10^{-5}) M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After preparation of the emulsion – Po przygotowaniu emulsji</td>
</tr>
<tr>
<td>Aloe 0.05 mg/ml – Aloes 0.05 mg/ml</td>
<td>0.179(^{ab}) ±0.012</td>
</tr>
<tr>
<td>Aloe 0.06 mg/ml – Aloes 0.06 mg/ml</td>
<td>0.192(^{a}) ±0.015</td>
</tr>
<tr>
<td>Aloe 0.1 mg/ml – Aloes 0.1 mg/ml</td>
<td>0.189(^{a}) ±0.026</td>
</tr>
<tr>
<td>Aloe 0.2 mg/ml – Aloes 0.2 mg/ml</td>
<td>0.218(^{ab}) ±0.049</td>
</tr>
<tr>
<td>Aloe 0.5 mg/ml – Aloes 0.5 mg/ml</td>
<td>0.205(^{a}) ±0.018</td>
</tr>
<tr>
<td>BHT 0.1 mg/ml – BHT 0.1 mg/ml</td>
<td>0.180(^{a}) ±0.027</td>
</tr>
<tr>
<td>BHT 0.2 mg/ml – BHT 0.2 mg/ml</td>
<td>0.313(^{bc}) ±0.041</td>
</tr>
<tr>
<td>Control – Kontrolna</td>
<td>0.250(^{ab}) ±0.069</td>
</tr>
<tr>
<td></td>
<td>After 19 h of incubation – Po 19 h inkubacji</td>
</tr>
<tr>
<td>Aloe 0.05 mg/ml – Aloes 0.05 mg/ml</td>
<td>3.340(^{c}) ±0.037</td>
</tr>
<tr>
<td>Aloe 0.06 mg/ml – Aloes 0.06 mg/ml</td>
<td>2.420(^{b}) ±0.162</td>
</tr>
<tr>
<td>Aloe 0.1 mg/ml – Aloes 0.1 mg/ml</td>
<td>0.682(^{a}) ±0.076</td>
</tr>
<tr>
<td>Aloe 0.2 mg/ml – Aloes 0.2 mg/ml</td>
<td>0.458(^{a}) ±0.017</td>
</tr>
<tr>
<td>Aloe 0.5 mg/ml – Aloes 0.5 mg/ml</td>
<td>0.448(^{a}) ±0.022</td>
</tr>
<tr>
<td>BHT 0.1 mg/ml – BHT 0.1 mg/ml</td>
<td>0.388(^{a}) ±0.016</td>
</tr>
<tr>
<td>BHT 0.2 mg/ml – BHT 0.2 mg/ml</td>
<td>0.505(^{a}) ±0.036</td>
</tr>
<tr>
<td>Control – Kontrolna</td>
<td>5.440(^{d}) ±0.069</td>
</tr>
</tbody>
</table>

Different letters mean statistically significant differences within the designation at the level of \(p = 0.05\).

Różne litery oznaczają różnice statystycznie istotne w obrębie oznaczenia na poziomie \(p = 0.05\).

A high extracting ability of phenolic compounds and, as a result, a high antioxidant activity of extracts (Przybylski et al., 1998). Inglett et al. (2011) report that applying the absolute alcohol to extraction makes it possible to reach a meaningfully higher content of bound phenolic compounds, and the 50% ethanol will extract more unbound phenolic compounds compared to water and absolute alcohol.

**Conclusions**

Despite the numerous health-promoting properties of aloe and a rich range of the bioactive compounds, the carried out research does not confirm a high antioxidant activity
of the aqueous aloe extract in the investigated models. A rich chemical position of the plant depends upon many factors, i.e. type and conditions of the cultivation, harvest time, climate, position of a leaf on the stem, species of aloe and the method of leaves collecting (Choi and Chung, 2003). The activity of aloe preparations may be a result of applying various methods of phenolic compounds extraction, the used solvent, and plant part from which a given sample has been taken (aloe leaf skin, aloe gel). The available literature is still insufficient in the research concerning the antioxidant aloe potential in model systems, especially in the emulgated fat systems, as well as fat in mass.

References


**Streszczenie**

**Wstęp.** Aloes jest źródłem wielu składników bioaktywnych, którym przypisuje się działanie prozdrowotne. Powszechnie używany w przemyśle kosmetycznym i farmaceutycznym, znalazł swoje zastosowanie również w przemyśle spożywczym. Obecne w aloesie substancje o działaniu przeciwnościerńcym mogą wpływać na zwiększenie trwałości i wartości odżywczej żywności. Celem pracy była ocena właściwości przeciwnościerńcych wodnego ekstraktu z aloesu w układach modelowych.

**Materiał i metody.** Badaniom poddano aloes zwyczajny (*Aloe vera* L.). Ekstrakcję prowadzono z użyciem wody w temperaturze 80–90°C. Poziom związków fenolowych oznaczono spektrofotometrycznie z odczynnikiem Folina-Ciocalteu, stosując jako wzór kwas galusowy. Aktywność antyoksydacyjną ekstraktu badano wobec kwasu linolowego, prowadząc inkubację przez 19 h, metodą neutralizowania rodnika DPPH (2,2-difenyl-1-pikrylohydrozyl) i kationorodnika ABTS (kwas 2,2'-azynobis-3-etylobenzotiazolino-6-sulfonowy) oraz na podstawie zdolności chelatowania metali. Uzyskane wyniki porównano z aktywnością BHT (butylohydroksytoluen).

**Wyniki.** Zawartość polifenoli ogółem w wodnym ekstrakcie aloesu oznaczono na poziomie 17,85 mg/g s.m. Stwierdzono, że badany ekstrakt w zależności od stężenia wykazywał zdolność dezaktywacji rodników DPPH oraz kationorodników ABTS, odpowiednio, w przedziałach: 4,32–8,87 i 0,58–0,87 mg troloksu na 1 g s.m. Mniejszą aktywność chelatującą – 1,23 mg EDTA na 1 g s.m. – wykazał ekstrakt wprowadzony na poziomie 20 mg/ml, a większą – 8,76 mg EDTA na 1 g s.m. – ekstrakt o słabszym stężeniu: 10 mg/ml. W układzie emulsyjnym próbki z dodatkami ekstraktu na poziomie 0,1–0,5 mg/ml zawierały ponad 90% mniej dienów sprzężonych niż próba kontrolna. Dodatek ekstraktu w ilości 0,05 mg/ml i 0,06 mg/ml ograniczył przyrost tych związków odpowiednio o 40 i 57%.

**Wnioski.** Ekstrakt otrzymany z aloesu, który może być wykorzystywany jako dodatek do żywności, cechował się bardzo zróżnicowanymi właściwościami przeciwnościerńczymi. Dostępna literatura jest jeszcze uboga w badania dotyczące potencjału przeciwnościerńczej aloesu, szczególnie w układach tłuszczu zemulgowanego i tłuszczu w masie, i dlatego, jak również ze względu na złożony skład oraz szerokie spektrum działania, surowiec ten powinien być nadal przedmiotem badań, których wyniki mogą się stać naukową podstawą jego zastosowania jako dodatku do żywności.

**Słowa kluczowe:** aloes, właściwości przeciwnościerńcze, układy modelowe

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