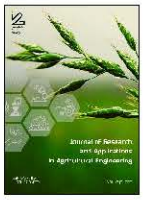
Article citation info:
Hęś M. 2022. Comparison of the Antioxidant Properties of Spices from Ecological and Conventional Cultivations. *Journal of Research and Applications in Agricultural Engineering* 67 (2): 5–12. https://doi.org/10.53502/QTTS5204

Łukasiewicz Poznań Institute of Technology

Journal of Research and Applications in Agricultural Engineering

Journal website: https://tech-rol.eu/



Comparison of the Antioxidant Properties of Spices from Ecological and Conventional Cultivations

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Article info
Received: 14 June 2022
Accepted: 5 October 2022
Published: 30 December 2022

Keywords
spices
thyme
rosemary
oregano
ecological/conventional cultivation
antioxidant activity

The aim of the study was to compare the overall total phenolic compounds content and antioxidant properties of spices (oregano, thyme and rosemary) from ecological and conventional cultivation. The antioxidant activity of the obtained spice extracts was estimated with the use of radical tests (DPPH and ABTS), binding and reduction of metal ions, accelerated Rancimat test and based on the spectrophotometric method. The obtained results indicate that spice extracts from ecological cultivation are characterized by a higher, overall total phenolic compound content compared to spice extracts grown in a conventional system. Ecological spice extracts showed better antioxidant properties than conventional spices in most tests. The results of the statistical analysis show a positive correlation between the total content of polyphenols and the antiradical activity of the extracts. The negative correlation was found between the content of polyphenols and the Rancimat test and the chelating activity. It seems that the usage of organic spice extracts in food production could be considered as natural antioxidants, reducing fat oxidation processes to a greater extent than with conventional cultivations.

DOI: https://doi.org/10.53502/QTTS5204

1. Introduction

Legal restrictions, doubts concerning the safety of using synthetic antioxidants and their negative perception by consumers, doctors and nutritionists contributed to the trend of replacing these compounds by substances of natural origin [1].

The current nutritional recommendations suggest an increase in the amount of plant-derived products in the diet, causing an increase in the content of polyphenols in the blood, which may significantly affect the biological properties of the cells [2–4]. Polyphenols are perceived as inhibitors of the action of free radicals, thus avoiding their negative effects both on fatty foods and indirectly on living organisms. The source of these compounds can be widely used in the food industry spice plants such as rosemary, thyme and oregano. These spices are found to be widely used in various industries, including in the food, pharmaceutical and cosmetic industries [5]. Currently, there is an observed increase in demand among consumers for food produced in a natural way, without the use of agricultural chemicals, resulting in a rapid development of organic farming [6]. Ecological farming (also known as biological, organic or biodynamic) is defined as an environmentally friendly system of sustainable

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management, both in ecological, economic and social terms. The essence of this system is the stimulation of natural processes that take place in ecosystems thanks to the use of ecological means of production. These measures ensure the durability of soil fertility and its further productivity, high biological quality of agricultural products, animal and plant health, and guarantee the health and safety of plant and animal products. Ecological farming is also based on a principle that rejects the use of fertilizers, preservatives and other additives in food production, as well as hormones, antibiotics and other veterinary drugs. Moreover, it is prohibited to irradiate food with ionizing rays and to use genetically modified organisms [7]. It stimulates plants to a higher synthesis of phenolic compounds, which perform the function of natural pesticides [8]. The results of many scientific studies on the quality of agricultural products from organic and conventional systems show a higher nutritional value and a higher content of biologically active compounds in organic raw materials [9–13]. At the same time, there are some results provided by other references which do not confirm such differences [14–17].

The purpose of this paper is to verify the hypothesis that spices from organic production contain more phenolic compounds and are characterized by a higher antioxidant activity than the conventional ones.

2. Material and methods

2.1. Material

The research material consisted of dried spices: rosemary, thyme and oregano. Conventional spices from the "KAMIS" company were purchased in the retail trade, while organic spices came from the "Dary Natury" farm in Koryciny, located in the south of Podlaskie Province (Poland), and were purchased online.

2.1.1. Preparation of plant extracts

Plant extracts were prepared by mixing 100 g of dried material with 250 cm^3 of 80% ethanol, and triple-macerating overnight at room temperature. The supernatants collected were filtered and ethanol was removed at 50°C on a rotary evaporator (Buchi, Switzerland). All samples were frozen at -20°C for 24 hours and then freeze-dried (Alpha 1–4 LSC Freeze dryer, Christ, Germany) and stored at 4°C in a dark place.

2.1.2. Preparation of linoleic acid emulsion

0.5 mL of Tween 20 was added to 5 mL of 1 M K_2HPO_4 and mixed for 5 min using a magnetic stirrer. Then, after 10 min, 0.5 mg of linoleic acid and 0.4 g of KOH were added and stirred for 2 min. In the next step, 11 mL of 1 M K_2HPO_4 were added and stirred for 10 min. After the addition of 150 mL of distilled water, the emulsion was stirred for 5 min and adjusted to pH of 7.2 [18].

2.2. Analytical methods

2.2.1. Determination of total phenolic compounds

The content of polyphenols was marked calorimetrically at wavelength of 750 nm, using the Folin-Ciocalteu method [19]. 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 in mg/g of extract's dry mass (mg GAE/g d.m.).

2.2.2. Scavenging of DPPH radicals

The ability to neutralize the radical DPPH was defined on the basis of the calorimetrically evaluated DPPH stable radical concentration towards the blank determination [20, 21]. The measurement of absorbance was conducted at the wavelength of 517 nm after a 30-minute spices and BHT (0.02%, m/v) solutions incubation 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 mg of Trolox/g of extract's dry mass (mg Trolox/g d.m.).

2.2.3. Scavenging of ABTS radicals

The capability of antioxidant compounds to reduce the cation radical ABTS was defined basing on the direct generating of $ABTS^{+\bullet}$ as a result of ABTS oxygenation by potassium persulfate ($K_2S_2O_8$) [22]. An addition of the antioxidant causes a reduction of $ABTS^{+\bullet}$ to ABTS and a decrease of the coloring intensity of the radicals solution. The degree of $ABTS^{+\bullet}$ reduction was defined spectrophotometrically for spices samples and BHT (0.02%, m/v) at wavelength of 734 nm. The antiradical activity of the samples was expressed in equivalents of Trolox (mg Trolox/g d.m.).

2.2.4. Metal chelating activity

The determination of the chelating capacity of metals consisted in the calorimetric measurement of the decoloration degree by the extracts of iron chloride complexes (II) with ferrozine [23]. The applied wavelength was 562 nm. The metal chelating activity of spices extracts and BHT (0.02%, m/v) was expressed in EDTA equivalents (ethylenediaminetetraacetic acid, synthetic complexing compound). The results were presented in mg EDTA/g of extract's dry mass (mg EDTA/ g d.m.).

2.2.5. Emulsion system

The capacity of inhibiting the process of auto-oxidation of linoleic acid was determined by Lingnert et al. method [18]. The method consisted of the spectrophotometric notation of the linked diens growth in 10 mM of linoleic acid emulsion at pH 7.2. The tested extracts were introduced into the prepared emulsion, 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 diens was calculated applying the molar coefficient of absorption for linked diens in agreement with the Lambert-Beer law.

$$A = \varepsilon \cdot c \cdot l \qquad (1)$$

where:
A – absorbance,
ε – molar coefficient of absorption ($\varepsilon = 2{,}56 \cdot 10^4\ M^{-1}\ cm^{-1}$),
c – concentration of peroxides with conjugated diens (M),
l – thickness of the absorption layer (cell/ cuvette thickness = 1 cm).

The protection coefficient (Pc) was expressed as the ratio of the increment in absorbance of the control sample and tested sample to the increase in absorbance of the control sample.

2.2.6. Rancimat test

The Rancimat test (Metrohm, Switzerland) is based on the conductometric measurement of volatile dissociation products formed during the lipid oxidation process. Lipid sample (2.5 g) was oxidized in a reaction vessel at 110°C; air flow (20 L/h). The end of the induction period (Ip) was characterized by the sudden increase of water conductivity, due to the dissociation of volatile carboxylic acids. Based on the received Ip reprints, the antioxidant effectivity of the spice extracts was marked. The protection coefficient (Pc) was determined as the relation of the induction period of the sample with antioxidant to the induction period of the control sample.

3. Chemicals

The following chemicals were used: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ethylenediaminetetraacetic acid (EDTA), Folin-Ciocalteu reagent (FCR), 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (Ferrozine), 2,4,6-tris(2-pyridyl)-s-triazine, Tween 20, linoleic acid, gallic acid (GAE), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich (Poznań, Poland); methanol, ethanol, hydrochloric acid, acetic acid, iron(III) chloride hexahydrate, sodium carbonate, potassium persulfate were purchased from POCh (Gliwice, Poland). Butylated hydroxytoluene (BHT) was purchased from Merck (Germany). All chemicals and solvents used in the tests were of analytical grade.

4. Statistical analysis

Results presented in the study constitute an arithmetic mean of independent series of measurements conducted in three replications. Least significant differences were analyzed using a one-way analysis of variance and the analysis of the linear correlation coefficient in the Statistica 9 software. Statistical inference was conducted at the significance level $p = 0.05$.

5. Results and discussion

The total content of phenolic compounds in the tested extracts ranged from 70 to 115 mg GAE/g d.m. Spices from the ecological cultivation were characterized by a significantly higher content of phenolic compounds as compared to those from conventional cultivation ($p<0.05$). Ecological Oregano contained 40.59 mg more phenolic compounds compared to conventional spices, in ecological rosemary and thyme this value was higher by 20.19 and 13.46 mg, respectively, compared to conventional spices (Tab. 1). Kazimierczak et al. [24] found that the method of cultivation affects the content of phenolic compounds in lemon balm, lovage, thyme and sage. They showed a higher mean content of the sum of phenolic acids in plants from ecological cultivation (33.49 mg/0.01 g f.w.) compared to plants from conventional cultivation (32.52 mg/0.01 g f.w.). Roghelia and Patel [25] compared the total content of phenolic compounds in cinnamon and cloves from ecological and conventional cultivation. They found a higher content of phenolic compounds in plants grown in accordance with the principles of organic farming compared to conventionally grown plants. The total content of phenolic compounds in ecological cloves was determined at the level of 50.39 mg and it was a value by over 20 mg higher compared to conventionally grown cloves. In the case of cinnamon, the total polyphenol content was 16.42 and 22.42 mg, respectively, in the organic and conventional spices.

Comparing the efficiency of scavenging the DPPH radical, it was found that ecological spices show significantly greater activity than those from conventional farming ($p<0.05$). The ecological oregano extract (412.32 mg Trolox/g d.m.) showed the highest activity. This activity was more than two times higher compared to the extract from traditional cultivation (194.81 mg Trolox/g d.m.), but by about 61% lower compared to the synthetic antioxidant BHT. The antiradical capacity of thyme and rosemary extracts was respectively 5.2% and 13.2% greater than that of conventional crops spice extracts. The same tendency in the activity of extracts from various crops was found in the case of the use of the radical ABTS. Correspondingly 44.6, 7.9 and 23.2% higher reduction potential had oregano, thyme and rosemary extracts from organic farming compared to conventional ones. Compared to BHT, only the organic oregano extract showed much higher activity. In the case of the remaining spices, the scavenging capacity was similar to that of the synthetic antioxidant. The conducted research showed that the $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ scavenging ability of extracts is positively correlated with the level of polyphenols present in them ($r = 0.972$ and $r = 0.970$, respectively). Knap et al. [26] verified if there are any differences in the antioxidant activity between selected Slovenian organic and conventional crops. Method of DPPH (2,2-diphenyl-1-picryhydrazyl) was used to determine the antioxidant activity of 16 samples from organic and conventional farms. The same varieties of crops were analysed. Estimated differences between interactions for the same crop and different farming practice were mostly not statistically significant except for the polar antioxidants for basil and beetroot. Higher statistically significant values were estimated for conventional crops. For the fraction in ethyl acetate soluble antioxidants in broccoli, cucumber, rocket and cherry statistically significant higher values were estimated for organic production.

All tried tests showed the ability to chelate metal ions in the reaction with ferrozine. Among the extracts, the strongest efficiency in binding iron ions was found in the case of thyme extract, and the weakest in the case of oregano. Ecological spices were characterized by a higher chelating activity compared to those grown in the conventional system. BHT was characterized by a higher chelating capacity compared to oregano extracts and ecological rosemary extract, while lower compared to thyme extracts and conventional rosemary extract. The results were statistically significantly different ($p<0.05$). The analysis of the correlation between the content of phenolic compounds in the tested spice extracts and the metal chelating activity shown by them turned out to be statistically insignificant ($p>0.05$). Phatak et al. [27] examined the antioxidant activity of various spices and found that nutmeg (65%) and caraway (52.6%) had the highest activity of chelating iron ions, while charlock (33.9%) had the lowest chelating capacity. Kong et al. [28] compared the chelating activity of spices with the synthetic antioxidant BHA. Studies have shown that the synthetic antioxidant exhibits the highest percentage iron chelating capacity (60.1%). Among natural antioxidants, the highest value was recorded for cardamom (40.1%) and rosemary (36.7%). A similar lack of correlation between the content of phenolic compounds in the tested basil extracts and the metal chelating activity was shown by Hinneburg et al. [29] who analyzed extracts of different herbs. In turn, in the study of Ebrahimzadeh et al. [30] a poor correlation between these variables was found.

In the FRAP method, all spices from the organic farm were characterized by a greater ability to reduce Fe^{3+} to Fe^{2+} ions than their conventional counterparts. The strongest reduction of iron ions was shown by organic oregano, while the weakest reducing properties were shown by rosemary from conventional cultivation. The average activity of the examined extracts was in the range of 21.2–35.2 mmol Fe^{2+}/L. Most of the natural antioxidants were characterized by more active than the synthetic BHT antioxidant. Their ability to reduce iron ions was about 10 times greater than that of BHT. The results were statistically significant ($p<0.05$). The regression and correlation analysis allows to state that the demonstrated ability to reduce in the FRAP test is related to the content of phenolic compounds in individual spice extracts. The correlation coefficient was 0.88. Haile [31] studied the antioxidant activity of 13 different spices. The highest ability to reduce iron (III) ions to iron (II) ions was found in the clove extracts (1646.7 mmol Fe^{2+}/g d.m.), cinnamon (605.9 mmol Fe^{2+}/g d.m.) and rosemary (274 mmol Fe^{2+}/g d.m.). All tested extracts showed very high antioxidant activity in the linoleic acid emulsion system. The increase of conjugated dienes in the samples with the addition of spice extracts was lower by 76.66–90.72% compared to the control sample. The obtained results (Tab. 2) show that the test with the addition of thyme extract from conventional cultivation showed the highest activity. After 19 hours of incubation, this test showed 76.23% fewer conjugated dienes compared to the control sample. This extract was characterized by a significantly higher activity of inhibiting the autoxidation of linoleic acid compared to the conventional extract ($Pc = 0.91$ and 0.79, respectively). No such effect was found for the remaining spices. The type of oregano and rosemary cultivation had no significant effect on the growth of conjugated dienes in the emulsion system. The synthetic antioxidant BHT (Tab. 2) showed the highest ability to inhibit the oxidation of the emulsified linoleic acid after 19 hours of incubation (95.1% less compared to the control sample, $Pc = 98$).

The results concerning the stability of pork lard with the addition of spice extracts in the Rancimat apparatus showed that rosemary extracts had the highest activity (Tab. 3). They extended the lard induction period by 3.65 and 4.30 times (from conventional and organic farming, respectively) compared to the control sample. The remaining extracts also showed very good stabilizing properties, expressed in the values of the coefficients 1.42–2.65. The weakest oxidative stability of the lard was found in the trials with the addition of oregano extracts, both from organic and conventional cultivation. The protection coefficients obtained for these trials were significantly different ($p<0.05$), and their mean value was 1.70 and 1.42, respectively. The 0.02% BHT addition turned out to be very effective, as in this test it showed a similar ability to extend the shelf life of lard to thyme extract. Statistical analysis showed ($p<0.05$) that the type of oregano and rosemary cultivation had an impact on the lard stabilizing activity, however, in the case of oregano, greater activity was found for the trials from conventional cultivation, while rosemary from organic cultivation. The results of the correlation studies between the content of phenolic compounds in spice extracts and their activity in the Rancimat test turned out to be statistically insignificant ($p>0.05$). According to Hęś et al. [32], the high temperature of accelerated tests and the aeration of the samples may cause different reactions in the fat tests than in the case of the test under normal conditions. For this reason, accelerated tests may give false results and it seems reasonable to confirm them by analysis under normal test conditions.

According to literature data, the organic crops presented higher antioxidant activity than the conventional ones [33–39]. This can be attributed to the fact that organic farming uses less phytopharmaceuticals and plant therefore develops several defence mechanisms [39, 40]. On the other hand, Lamperi et al. [41] and Cardoso et al. [42] argue that there is no difference in the antioxidant activity between products from different farming systems.

6. Summary

The obtained results allowed to indicate that the more favorable form of cultivation in shaping the quantitative composition of polyphenols and the antioxidant activity of individual spice extracts is the ecological method rather than the conventional one. The demonstrated antioxidant potential of ecological spices *in vitro* may suggest its beneficial pro-health properties *in vivo*. The anti-radical, reducing and chelating activity of organic spices, confirmed in research, can potentially enhance the action of the body's natural defence systems and prevent the occurrence of oxidative stress, and thus have a significant impact on the prevention and inhibition of the development of various diseases. In addition, the proven antioxidant properties of spice extracts, and in particular their high efficiency in inhibiting the growth of conjugated dienes in emulsified linoleic acid, may indicate the possible use of spices in extending the shelf life of food through oxidative stabilization of fats.

It should be emphasized that the assessment of the antioxidant potential in *in vitro* conditions, is an analysis difficult for an accurate and unambiguous interpretation. The action of antioxidants is based on various mechanisms, and therefore the methods of checking their activity are varied. Moreover, it is difficult to correctly analyze and compare the research with each other due to the lack of standardized procedures that will optimally determine the properties of the tested material. This problem results, among others, from the great diversity of plants' composition even within the species.

Tab. 1. Total phenolic contents (TPC) and total capacities for extracts of spices from ecological and conventional cultivations determined by using DPPH, ABTS, FRAP tests and metal chelating activity

Spices	TPC (mg GAE/g d.m)		DPPH (mg Trolox/g d.m.)		ABTS (mg Trolox/g d.m.)		FRAP (mmol Fe^{2+}/L)		Metal chelating (mg EDTA/g d.m.)	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Oregano	74.47^{b}	115.06^{e}	194.81^{ab}	412.31^{e}	14.58^{ab}	26.31^{e}	25.12^{b}	35.18^{c}	4.34^{a}	5.77^{b}
Thyme	70.94^{a}	84.40^{c}	189.46^{a}	199.73^{b}	15.74^{bc}	17.08^{cd}	24.76^{b}	25.52^{b}	13.36^{f}	14.51^{g}
Rosemary	70.30^{a}	90.49^{d}	253.71^{c}	292.13^{d}	12.92^{a}	16.82^{c}	21.73^{a}	25.65^{b}	7.13^{c}	10.46^{e}
BHT			595.53^{f}		18,54^{d}		25.38^{b}		7.97^{d}	

A – conventional cultivation
B – ecological cultivation
TPC: Total phenolic compounds; DPPH: Scavenging of DPPH radicals; ABTS: Scavenging of ABTS radicals; FRAP: Ferric reducing antioxidant power
Values with different letters for each test are significantly different at significance level $p = 0.05$.

Tab. 2. Effect on extracts of spices from ecological and conventional cultivations to change the content of conjugated dienes in an emulsion of linoleic acid

Spices	Conjugated dienes (10^{-5} M)				Protection coefficient (Pc)	
	After preparation of the emulsion		After 19 h of incubation of the emulsion			
	A	B	A	B	A	B
Oregano	0.547^{b}	0.517^{b}	0.997^{c}	1.146^{c}	0.83^{b}	0.77^{a}
Thyme	0.507^{b}	0.521^{b}	0.756^{d}	1.100^{e}	0.91^{c}	0.79^{a}
Rosemary	0.633^{c}	0.547^{b}	1.039^{e}	0.997^{e}	0.85^{b}	0.83^{b}
BHT	0.464^{a}		0.617^{c}		0.98^{d}	
Control	0.485^{ab}		3.180^{f}			

A – conventional cultivation
B – ecological cultivation
Values with different letters for each test are significantly different at significance level $p = 0.05$.

Tab. 3. Spices extracts from ecological and conventional cultivations influence on lard stability in Rancimat test

Spices	Induction period (h)		Protection coefficient (Pc)	
	Variant			
	A	B	A	B
Oregano	4.50^{c}	3.76^{b}	1.70^{c}	1.42^{b}
Thyme	7.00^{de}	6.37^{d}	2.65^{de}	2.41^{d}
Rosemary	9.65^{f}	11.36^{g}	3.65^{f}	4.30^{g}
BHT	7.11^{e}		2.69^{e}	
Control	2,64^{a}		1.00^{a}	

A – conventional cultivation
B – ecological cultivation
Values with different letters for each test are significantly different at significance level $p = 0.05$.

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