

ANTIOXIDANT PROPERTIES OF ACETONE EXTRACTS FROM SELECTED FRESH AND DRIED SPICES

Beata Drużyńska, Magdalena Wojda

Division of Food Quality Control, Faculty of Food Technology, Warsaw University of Life Sciences, Warsaw

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The purpose of the study was to determine antioxidant properties of acetone extracts from selected fresh and dried spices. Three spices have been examined: garlic, oregano and rosemary. Preparations were obtained by the extraction of polyphenols with a 70% acetone solution. The contents of total polyphenols and total catechin were determined in the study. The ability of total polyphenols contained in the obtained extracts to chelate Fe(II) ions was examined, too. The antioxidant properties of the examined extracts were assayed with three methods: against stable, synthetic DPPH radicals, cation-radicals ABTS and against hydroxyl radicals.

On the basis of findings it turned out that condensed tannins are a dominant group of polyphenols in oregano, rosemary and fresh garlic. Granular garlic contained a higher level of catechins than tannins. All preparations of fresh spices displayed the ability to chelate iron ions. All extracts had antioxidant properties against synthetic radicals, with the exception of garlic preparation which did not inhibit hydroxyl radicals. Extracts of fresh spices demonstrated better antioxidant properties.

INTRODUCTION

In recent years, with an increasing importance of a safety problem of consumed food, approach to many components used as permitted additives to food products, including antioxidants, has changed.

Evidencing toxic activity of some synthetic antioxidants applied in the production and processing of food resulted in preferences of natural components among the consumers [Wang *et al.*, 2000]. The above mentioned phenomenon as well as justified necessity to apply antioxidants in food products, especially in edible fats, became the cause of deep examinations of plant raw materials aimed at searching for compounds that serve a function of natural antioxidants. Such compounds are polyphenols. Many research has confirmed their existence in plant materials (*i.e.* in tea, red wine and seeds of oil plants) and has shown that these compounds reveal activity effectiveness not less than synthetics [Drużyńska & Klepacka, 2004; Wang & Lin, 2000; Simonetti *et al.*, 1997].

This study examines polyphenols in selected spices due to several benefits resulting from their application in the food production. Polyphenols in spices prolong the shelf life of food products by inhibiting oxidation processes, and consumed with spiced products increase the antioxidative potential of a body, thus showing the health-promoting activity. It has been obvious for years that the addition of spices improves sensory traits of foodstuffs and prolongs their shelf life due to the compounds with bactericidal and bacteriostatic activity they contain.

MATERIAL AND METHODS

Research material were three types of spices: garlic (fresh and granulates) rosemary and oregano (fresh and dried). Spices were used for research in such a form in which they are directly available for a consumer.

A preparation with polyphenols has been made as follows: 10 g of spices (green previously ground spices) were added to 100 mL of 70% acetone [Sidhuraju & Becker, 2003]. Extractions have been performed for 2.5 h on a WL-1 shaker. The extract obtained was centrifuged at 6000 × g for 10 min. The liquid from the deposit was poured off to a conical flask with a cork and stored at freezing temperatures under conditions of limited access of air and light. Extracts were applied for two weeks at most.

Dry matter in fresh and dried spices and contents of total polyphenols were determined in the study with the method of Folin method (a result has been expressed in calculation into the gallic acid), [Singleton & Rossi, 1965] and condensed tannins with the method of Price (results converted into (+)catechin) [Price *et al.*, 1978]. The ability of polyphenols to chelate Fe(II) was tested in extracts of polyphenols with the addition of iron chloride (II) and ferrozine. The absorbance of a colour complex was measured after 10 min since the addition of ferrozines at the wave length of 562 nm in a Shimadzu UV-160A spectrophotometer [Lai *et al.*, 2001].

Antioxidant properties were examined towards hydroxyl, static and synthetic DPPH [Song *et al.*, 1999] radicals and cation-radicals ABTS [Re *et al.*, 1999]. Hydroxyl radicals were

TABLE 1. Content of total polyphenols and catechins in fresh spices extracts.

Kind o spice	Total polyphenols (g/100 g spice)	Total polyphenols (g/100 g d.m.)	Catechins (g/100 g spice)	Catechins (g/100 g d.m.)
Fresh garlic	0.11 (± 0.02)	0.28 (± 0.00)	0.20 (± 0.01)	0.50 (± 0.03)
Fresh rosemary	4.59 (± 0.05)	20.90 (± 0.03)	0.28 (± 0.01)	1.27(± 0.05)
Fresh oregano	10.53 (± 0.02)	46.15(± 0.04)	0.43 (± 0.01)	1.88(± 0.07)

TABLE 2. Content of total polyphenols and catechins in spices extracts after technological processes.

Kind o spice	Total polyphenols (g/100 g spice)	Total polyphenols (g/100 g d.m.)	Catechins (g/100 g spice)	Catechins (g/100 g d.m.)
Granulated garlic	0.22 (± 0.09)	0.23 (± 0.04)	0.17 (± 0.05)	0.18 (± 0.09)
Dried rosemary	0.71 (± 0.08)	0.77 (± 0.09)	0.35 (± 0.09)	0.38 (± 0.12)
Dried oregano	2.67 (± 0.09)	2.90 (± 0.10)	0.81 (± 0.11)	0.88 (± 0.05)

generated in a reaction mixture composed of copper sulfate and sodium benzoate, in the presence of DTET (dithioerythritol, added to accelerate the production of radicals). Samples were incubated at a temperature of 37°C. After incubation, the radical reaction was induced by H₂O₂. The amount of produced radicals was measured with a spectrofluorometer at the wave length of $\lambda_{\text{ext.}} = 308 \text{ nm}$, $\lambda_{\text{emission}} = 410 \text{ nm}$. Measurements were conducted after 10, 20 and 30 min of incubation [Hunt *et al.*, 1998].

The second method consisted in adding antiradical compounds to a methanol solution of DPPH which in a radical form shows the absorbance at 517 nm. The value of this absorbance drops after the addition of an antiradical compound.

Cation-radicals ABTS were generated as a result of ABTS oxidation by potassium persulfate. The addition of antioxidant resulted in the reduction of ABTS and drop of the color intensiveness of ABTS solution. The reduction level of ABTS was determined with the spectrophotometric method.

All determinations were carried out in at least three repetitions.

RESULTS AND DISCUSSION

The experimental extracts of spices were determined for total polyphenols and catechins. According to literature, catechins are the main compounds occurring in spices and herbs [Chun *et al.*, 2005]. Results have been presented in Tables 1 and 2.

The highest contents of total polyphenols were found in oregano. It refers both to fresh and dried oregano (respectively 46 and 3 g/100 d.m.). The contents of catechins were also the highest in this species. It shall be noted that, generally, the contents of polyphenols were observed to decrease significantly after the drying process. Garlic is an exclusion but trace amounts of these compounds were identified in this species. Garlic showed the least amount of both total polyphenols and catechins.

Binding properties of ions of transition metals have a significant influence on the antioxidant activity of polyphenols and their ability to chelate Fe(II) was determined in the study. Results were presented in Figure 1.

The highest ability to Fe(II) chelation had the extract of fresh oregano (74%). High capacity for Fe(II) chelation showed also the extract of fresh rosemary (56%). Perhaps, better chelating properties of rosemary and oregano preparations result from a higher content of polyphenol compounds in such preparations [Sanches-Moreno *et al.*, 1998]. Extracts of fresh and granulated garlic chelated at a very similar level (*ca.* 37%).

The conducted regression analysis showed a statistically significant correlation (at a significance level of $p=0.10$ in 90% within trust limits) between the contents of total polyphenols in fresh spices and the chelating ability of extracts of these species. The correlation ratio $r=0.97$ proves the existence of a very strong correlation between these variables.

In the case of dried rosemary and dried oregano extracts, there was probably an error of a method – due to precipitating deposit it was impossible to read out the absorbance.

The study examines the ability of acetone extracts of spices to deactivate OH radicals. Polyphenols in extracts from spices showed antioxidant activity to hydroxyl radicals both after 10, 20 and 30 min of incubation. Results were presented in Figures 2, 3, 4.

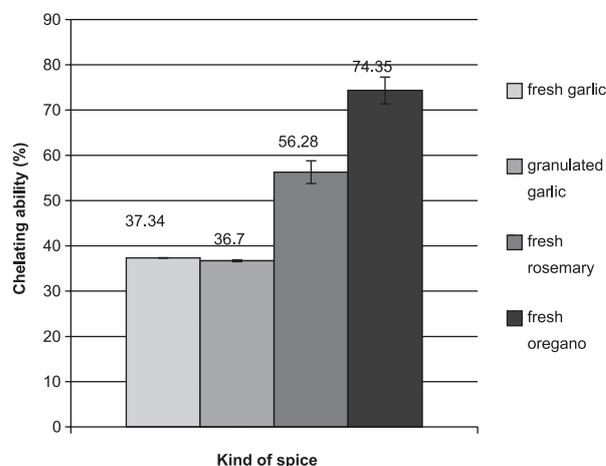


FIGURE 1. The ability to chelate Fe(II) by spice extracts.

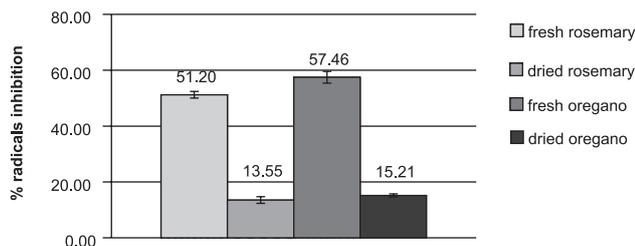


FIGURE 2. Antioxidant activity of rosemary and oregano extracts against hydroxyl radicals after 10-min incubation.

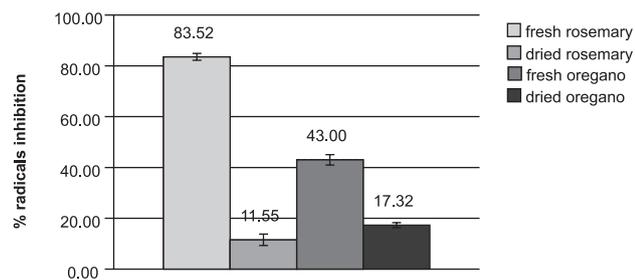


FIGURE 3. Antioxidant activity of rosemary and oregano extracts against hydroxyl radicals after 20-min. incubation.

After 10-min incubation the highest activity was observed for the extract of fresh oregano (radical scavenging 57.5%). A significant difference in the activity of extracts from fresh spices (a stronger activity) has been observed both for rosemary and oregano and extracts from dried spices (weaker activity).

After 20 min of incubation the extract from fresh rosemary exhibited the strongest activity (ca. 83.5%). The correlation between the activity of extracts from fresh spices (stronger activity) and these from dried spices (weaker activity) could be still observed. After 30 min of incubation the extract of fresh rosemary displayed the strongest activity (ca. 57.7%). The activity of extract from fresh oregano dropped significantly. The extracts from dried rosemary and fresh oregano showed the maximal activity after 10-min incubation, whereas those from fresh rosemary and dried oregano showed the maximal activity after 20-min incubation.

The authors stated that this ability depends upon the presence of especially (+)catechin and (-)epicatechin. The study does not demonstrate any statistically significant correlation between the contents of catechins in spices and their ability to scavenge hydroxyl radicals.

In literature, there is significantly few data on natural abilities of antioxidants to scavenge hydroxyl radicals. According to literature [Saint-Cric de Gaulejac *et al.*, 1999] while examining ethanol extracts of grapes also stated such a big ability to of degradation of these radicals. No antioxidant activity to OH radicals was revealed by polyphenols in garlic extracts. Antioxidant properties of extracts from spices have also been examined to synthetic, stable DPPH radicals. Results were presented in Figure 5.

All the examined spices had the ability to scavenge synthetic stable DPPH radicals. The strongest antioxidant properties towards DPPH radicals showed the extract from fresh oregano (81.5%), and that from fresh rosemary (55.6%).

Since these extracts are characterised by the highest contents of polyphenols in general, it can be stated that it is the reason why extracts from these spices exhibit the strongest inhibiting activity towards DPPH radicals.

The conducted regression analysis shows the existence of a very strong positive correlation between these variables at a significance level of $p=0.05$ in the 90% trust limits. A conclusion on the existence of a relation between the contents of polyphenols in spices and antioxidant activity of extracts from these spices can also be based upon literature data. Saint-Cricq de Gaullejac *et al.* [1999] found such a correlation in their examinations of red grapes.

The weakest antiradical properties were found for extracts from fresh and granulated garlic. According to Nuutila *et al.* [2003], the strongest antiradical activity towards DPPH radicals, among polyphenol compounds occurring in them, reveals quercetin. They confirmed the existence of a statistically significant correlation between the contents of quercetin in methanol extracts from garlic and their antiradical properties. According to their studies, quercetin occurs mainly in external parts of a garlic head. Thus, assuming that only internal parts of the head are used for the production of granulated garlic, we can state that differences in% inhibition of DPPH radicals result from different contents of quercetin in the examined extracts. Probably, the extract from fresh garlic contained more quercetin due to the fact that it has been produced from a shell and germs.

In the case of all examined extracts of spices, the percentage of scavenging radicals by extracts from fresh spices was higher than in the case of extracts from dried spices.

Antioxidant properties of polyphenols occurring in the extracts of spices to cation-radicals ABTS were presented in Table 3. The ABTS solution revealed the maximal absorbance at 734 nm, whereas the addition of the antiradical compound resulted in the drop of its absorbance.

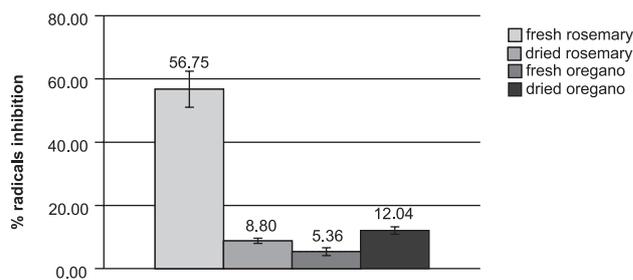


FIGURE 4. Antioxidant activity of rosemary and oregano extracts against hydroxyl radicals after 30-min incubation.

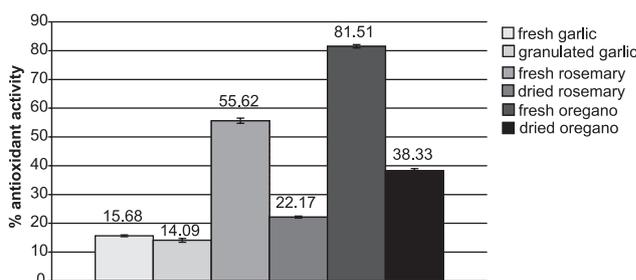


FIGURE 5. Antioxidant activity of extracts against DPPH radicals.

TABLE 3. Antioxidant activity of polyphenols in extracts against ABTS⁺ radicals.

Kind of spice	Antioxidant activity (%)	
Garlic	fresh	19.01 ± (0.45)
	granulated	11.32 ± (0.44)
Rosemary	fresh	41.72 ± (0.36)
	dried	11.39 ± (0.42)
Oregano	fresh	47.11 ± (0.55)
	dried	50.51 ± (1.00)

It has been stated that all spices examined have the ability to scavenge synthetic cation-radicals ABTS. The strongest antiradical properties, compared to ABTS radicals, revealed the extracts from dried and fresh oregano (respectively: 50.5% and 47%). According to Pannala *et al.* [2001], who examined the relation between the structure of a B ring of polyphenol antioxidants and their antioxidant activity expressed as ability to scavenge ABTS cation-radicals, a strong inhibiting activity to these radicals reveal catechins. The highest contents of catechins were stated in extracts from fresh and dried oregano with the best antiradical properties to ABTS (1.88g/100 g d.m. in fresh oregano and 0.88g/100 g d.m. in dried oregano). The conducted regression analysis showed the existence of a very strong correlation between the contents of catechins in spices extracts and their ability to inhibit cation-radicals ABTS⁺ at a significance level of $p=0.05$ at 95% trust limit.

Good antioxidant activity of oregano extracts towards ABTS may result from the fact that, according to literature, one of polyphenol compounds in oregano is quercetin treated as a very good scavenger of ABTS cation-radicals [Pannala *et al.*, 2001]. Quercetin is also one of the most important polyphenols occurring in garlic [Nuutila *et al.*, 2003], which is probably due to the fact that extracts from fresh and granulated garlic in which the contents of catechins are low (0.50 g/100g d.m. in fresh garlic and 0.18 g/100 g d.m. in granulated garlic), inhibit ABTS cation-radicals at a level similar to the extract from dried rosemary (where the content of catechins is higher and amounts to 0.38 g/100 g d.m.). The percentage of scavenging ABTS cation-radicals for fresh garlic extract amounts to 19%, for granulated garlic extract – to 11.3% and for dried rosemary – to 11.4%.

CONCLUSIONS

1. Higher contents of polyphenol compounds in the case of rosemary and oregano were found in fresh spices preparations. For garlic extracts, a diverse relation has been noted.

2. All extracts showed antioxidant properties to stable DPPH radicals and ABTS cation-radicals.

3. Rosemary and oregano extracts exhibited the antioxidant activity also to hydroxyl radicals.

4. Better antioxidant activity showed extracts from fresh spices.

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PRZECIWIUTLENIAJĄCE WŁAŚCIWOŚCI ACETONOWYCH EKSTRAKTÓW Z WYBRANYCH PRZYPRAW ŚWIEŻYCH I SUSZONYCH

Beata Drużyńska, Magdalena Wojda

Zakład Oceny Jakości Żywności, Wydział Technologii Żywności, Szkoła Główna Gospodarstwa Wiejskiego, Warszawa

Celem pracy było oznaczenie zawartości polifenoli i ich właściwości przeciwutleniających w wybranych przyprawach (czosnek, rozmaryn oregano). W acetonowych ekstraktach przypraw oznaczono zawartość polifenoli ogółem i tanin skondensowanych. Oznaczono zdolność polifenoli zawartych w ekstraktach do chelatowania jonów Fe(II). Właściwości przeciwutleniające zbadano metodami z wykorzystaniem rodników DPPH• i kationorodników ABTS•⁺ oraz metodą spektrofluorymetryczną wobec rodników hydroksylowych. Na podstawie otrzymanych wyników stwierdzono, że dominującą grupą polifenoli w oregano, rozmarynie i czosnku świeżym były taniny skondensowane. Preparaty z przypraw świeżych wykazywały zdolność do chelatowania jonów żelaza (II). Wszystkie ekstrakty wykazywały właściwości przeciwutleniające wobec syntetycznych rodników, z wyjątkiem preparatu z czosnku, który nie inhibował rodników hydroksylowych. Lepsze właściwości przeciwutleniające wykazywały ekstrakty z przypraw świeżych.