Antioxidant Properties of Fresh and Powdered Turmeric, Ginger, and Thyme

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Abstract

The antioxidant properties of commercial preparations of turmeric, ginger, and thyme were compared to those of the fresh spices. The composition of the spices was determined using chromatography, which allowed for comparison of the chemical composition of the fresh and powdered spices. ABTS and DPPH tests were performed to measure the antioxidant activity of the spice extracts. The results indicate that extracts prepared from fresh turmeric and ginger exhibit greater antioxidant properties, while thyme extract had higher antioxidant capacity if prepared from powdered material.

Keywords: antioxidants, turmeric, ginger, thyme, fresh spices, powdered spices

I. INTRODUCTION

Oxygen free radicals are called reactive oxygen species (ROS) due to their high chemical reactivity. They are formed mainly in the mitochondria, but also in response to the presence of certain chemical compounds, such as pesticides, substances found in cigarette smoke, medicines and some household chemicals[1]. ROS cause a range of negative effects on the body, including an increase in the risk of cancer and diabetes[2]. To decrease the adverse health impacts of ROS, it is advised to consume foods rich in antioxidants that neutralize ROS, such as herbs and spices[3]. The most commonly used products derived from the spices examined, turmeric, ginger, and thyme are powders, which are produced by curing, sun drying and grinding. Storage conditions of the powder, involving long exposure to varying conditions of temperature and humidity, may cause the antioxidant compounds in the spices to decompose, raising concerns regarding the level of antioxidants in the product. While there are some studies[4,5] that study how specific processes affect the antioxidant activity of plants, little research has been done to compare the antioxidant activity of the commercially prepared spice powder with the fresh counterpart[4,6]. Here, three of the most widely researched species: turmeric (*Curcuma longa L*), ginger (*Zingiber officinale Rosc*.), and thyme (*Thymus vulgaris* L.) were examined to evaluate their antioxidant properties in two forms: fresh and commercially prepared powders.

To examine the chemical composition of the extracts, Thin Layer Chromatography (TLC) was performed. This technique is employed to separate distinct chemical groups of compounds in a mixture. The mixture is placed on a TLC plate made of silica gel (stationary phase), which is then positioned in a container with polar (water-containing) or non-polar (organic) solvent system. The solvent system moves up on the plate, taking up substances that have greater affinity to the solvent than to the stationary phase. It is therefore possible to assess the relative polarities of the compounds and determine which antioxidant assay should be used.

Standard tests used to evaluate antioxidant capacity are ABTS and DPPH radicals scavenging tests. DPPH radical solution is non-polar and so it is used to examine non-polar substances, such as extracts containing organic compounds. ABTS radical solution is polar so is used for aqueous solutions. Both solutions are solutions of free radicals which change their colour when the unpaired electron is joined to an electron from an antioxidant molecule. The colour change is detected with spectrophotometry.

The effectiveness of radical scavenging by an antioxidant is evaluated by calculating the DPPH or ABTS percentage inhibition (I) using the formula

$$\mathrm{I}\,(\%) = \frac{\mathrm{A}_c - \mathrm{A}_s}{\mathrm{A}_c} \times 100, \qquad (1)$$

where A_c is absorbance of the radical solution (blank sample), and A_s is absorbance of the radical solution with an antioxidant added[7]. In the case of this particular investigation, both tests were performed, since both non-polar and polar compounds were detected on conducting TLC.

II. METHODS

Plant material preparation and extraction

Powdered spices were purchased from the spice company *KOTÁNYI Polonia Sp. z o.o.* and were not further processed. Fresh turmeric (originating from Peru), ginger (from China), and thyme (from Poland) were purchased at the local grocery store. The method of drying developed for each fresh spice was chosen so as to have the lowest effect on antioxidant compounds present in the spices. The turmeric and ginger roots were washed and cut into slices of 1 cm thickness without peeling. Next, they were placed in a vegetable dryer and dried at a temperature of 70°C for 24 hours. Most classes of compounds also present in the examined spices are typically stable under 80°C or higher[8], so the temperature was predicted not to cause their decomposition. The stems and leaves of thyme were washed and left on a paper for three days at 25°C. Next, the dried spices were ground using a coffee grinder for turmeric and ginger and a mortar and pestle for thyme. Extraction was carried out with the use of ethanol in the Soxhlet apparatus for 12 hours according to the procedure described by Redfern[9].

Thin Layer Chromatography

To examine both non-polar and polar compounds, two distinct systems of solvents were used, chloroform: methanol (93:7) and chloroform:methanol:water (61:32:7), respectively. Extracts were compared with four reference compounds: oleanoic acid, faradiol, alfa-amyrin and sitosterol, according to their increasing polarity. The plates were visualized after spraying with 50% sulphuric acid and heating. They were also viewed under the UV light.

DPPH radical scavenging test

Preparation of DPPH solution and measurements of absorbance were carried out according to the procedure described by Kedare[10] and percentage inhibition of DPPH was calculated. After preliminary measurements, the ethanolic extracts of turmeric (*Curcuma longa* L.) and thyme (*Thymus vulgaris* L.) were diluted by a factor of ten in order to obtain an absorbance value in the range 0-1. Ginger extract was not diluted, since due to its lower antioxidant activity[11], significant DPPH inhibition would not be observed. Fixed volumes of DPPH, ethanol and extracts were added together to obtain solutions of six different concentrations as shown in Table 1.

Volumes (µl)		Trial					
	0	1	2	3	4	5	6
EtOH	2000	1840	1680	1520	1040	400	0
Extract	0	160	320	480	960	1600	2000
DPPH	2000						
Total	4000						
C (%)	0	6	12	24	48	80	100

Table 1. Volumes and concentrations of the extracts used for the DPPH scavenging test

Plant material before extraction (after drying), as well as the final mass of the extract, were measured and the mass of the plant extract made from 1.00 g of dried plant material was calculated:

$$M_e = \frac{\text{mass of plant extract in solution (g)}}{\text{dry mass (g)}}. \qquad (2)$$

Such M_e was dissolved in 100.0 ml ethanol (EtOH) to obtain solutions which were assigned the concentration of 100%, and subsequent trial concentrations were calculated according to the percentage volume of the extract in extract and EtOH.

ABTS radical scavenging test

Preparation of ABTS solution and measurements of absorbance at 734 nm were carried out according to the procedure described by Kurrin[12]. For the ABTS scavenging test, only 2000 µl of extract were used since the spices exhibited much lower radical scavenging potential in the ABTS scavenging test due to low levels of polar substances present, which was confirmed by the TLC results. Percentage inhibition of ABTS was calculated using equation 1.

III. RESULTS AND DISCUSSION

TLC

The results of the TLC indicated whether the majority of the compounds present in the examined spices were polar or non-polar. Figure 1 shows the TLC plate resolved in a polar solvent system Figure 1a shows the TLC plates for the examined spices in a polar solvent system visualized with H_2SO_4 under normal light with oleanolic acid as a reference. Figure 1b shows the same plate viewed under UV light.

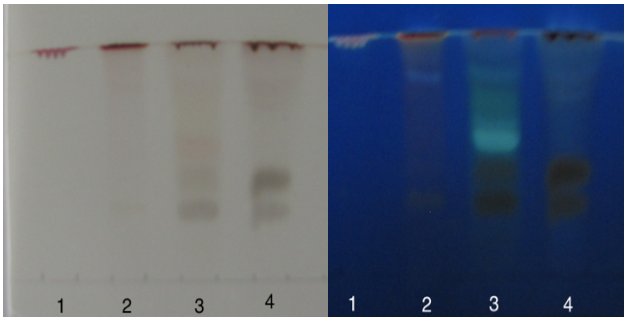

Figure 1a (left), 1b (right). Separation of compounds present in alcoholic extracts of turmeric (2), thyme (3), ginger (4) with reference to oleanolic acid (1) in the polar solvent system.

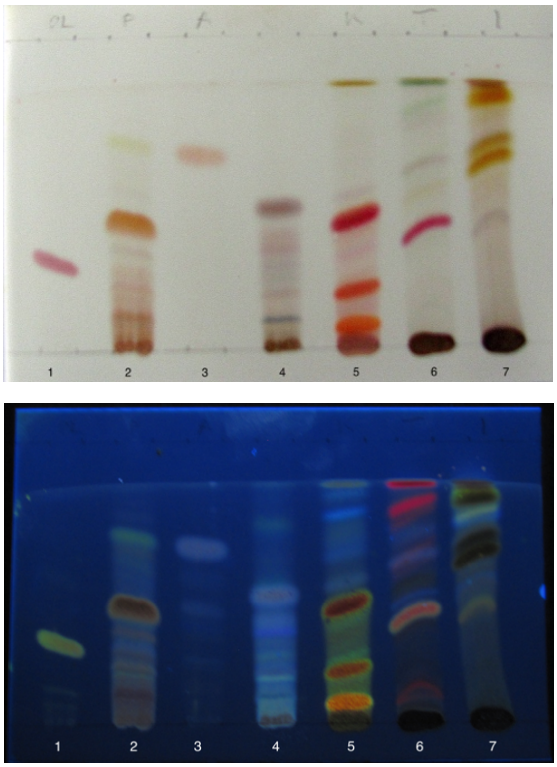

Figure 2a(top), 2b(bottom). Separation of compounds present in alcoholic extracts of turmeric (5), thyme (6), ginger (7) prepared from raw material compared to reference compounds: oleanolic acid (1), faradiol (2), alpha-amyrin (3), sitosterol (4) in the non-polar solvent system.

Figure 2a shows the TLC plate resolved in a non-polar solvent system visualized with H_2SO_4 under normal light with four reference compounds, oleanolic acid (1), faradiol (2), alpha-amyrin (3), and sitosterol (4), while Figure 2b shows the same plate viewed under UV light.

It can be seen that all of the spices are more abundant and contain greater variety of non-polar than polar compounds, which were detected only in thyme and ginger. All of the extracts possess similar groups of highly non-polar compounds, with compounds such as sterols and phenolic acids being observed. Compounds of structures similar to oleanoic acid (triterpenoids) were also identified. In turmeric, the main compound identified was curcumin. While the examined spices are abundant in non-polar compounds, there were fewer compounds that would exhibit polar properties[12,13], identified in ginger and thyme only.

Antioxidant properties: DPPH scavenging test

As seen in figures 3-5, fresh turmeric and ginger showed greater antioxidant levels, while dried thyme displayed higher levels of DPPH inhibition compared to the freshly purchased.

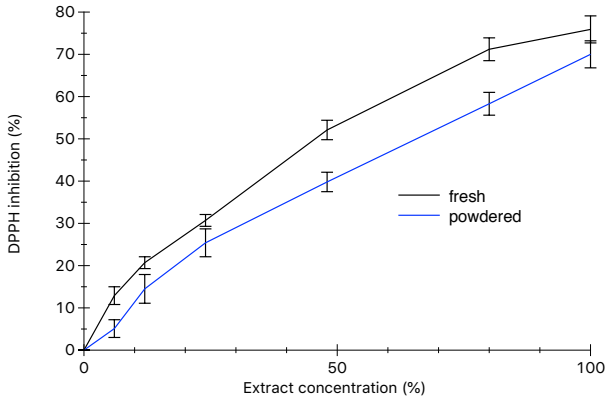

Figure 3. Comparison of % DPPH inhibition oxidation by fresh and powdered turmeric

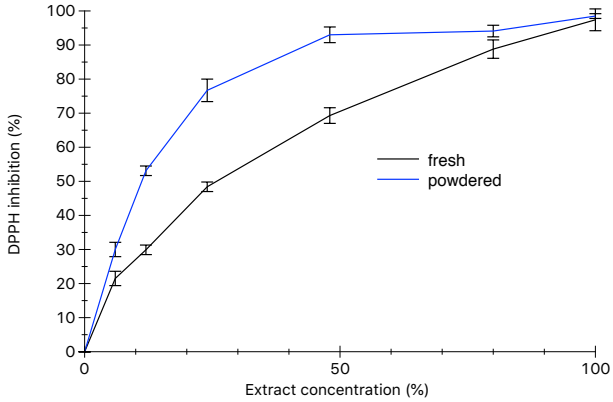

Figure 4. Comparison of % inhibition of DPPH oxidation by fresh and powdered thyme.

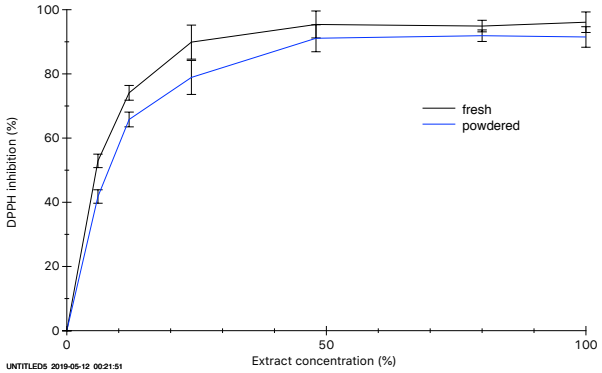

Figure 5. Comparison of % DPPH inhibition oxidation by fresh and powdered ginger.

In order to determine the statistical significance of the differences between the percent DPPH inhibition by fresh and dried plant material in the solutions of the highest extract concentrations, t-Test for unpaired samples was conducted. Due to limited number of samples, each of the results from the linear portion was converted to percentages and taken into account when calculating the P-value, to obtain more replicates. The level of significance was established at $\alpha = 0.05$.

Spice	Number of trials	Mean ± SD of the DPPH inhibition (%)		P-value
		Powdered	Fresh	
Turmeric	12	34.5 ± 1.9	43.9 ± 2.1	0.041
Ginger	12	62.2 ± 3.2	72.2 ± 3.8	0.221
Thyme	12	63.2 ± 2.2	55.6 ± 2.3	0.087

Table 2. Differences in the % DPPH inhibition by fresh and dried spices.

As seen in table 2, statistically significant differences were obtained in turmeric only. Several studies have suggested that subjecting certain organic compounds to high temperatures and additional safety processing, including irradiation and steam processing, leads to the decomposition of antioxidant compounds[14,15,16], which may explain the lower antioxidant capacity of turmeric powder. However, no significant difference was seen in other spices, therefore it is possible that the antioxidant compounds were affected in the fresh material as well. Interestingly, it was observed that dried, commercially available thyme displayed greater antioxidant capacity than extract prepared from fresh thyme, which suggests that the factors that contribute mostly to the antioxidant properties of thyme may be the conditions of cultivation and its quality. Despite no statistically proven difference it is possible that the fresh thyme tested here was of lower quality than the thyme used to produce the powdered spice.

Turmeric and thyme displayed significantly higher antioxidant activity than ginger, since comparable values of DPPH inhibition by chosen spices were achieved after turmeric and thyme extracts diluted by the factor of ten, while ginger extract was not diluted. This can be attributed to the higher content of antioxidant compounds in the case of these spices[13].

Antioxidant properties: ABTS scavenging test

In both ABTS and DPPH scavenging tests the trend of higher antioxidant activity of fresh turmeric and ginger and lower antioxidant activity of fresh thyme was maintained. However, the percentage inhibition of ABTS oxidation was highest in the case of ginger, which also displayed the lowest activity in the DPPH test. This may be attributed to the presence of more polar compounds in ginger than in turmeric, which was indicated in the TLC results.

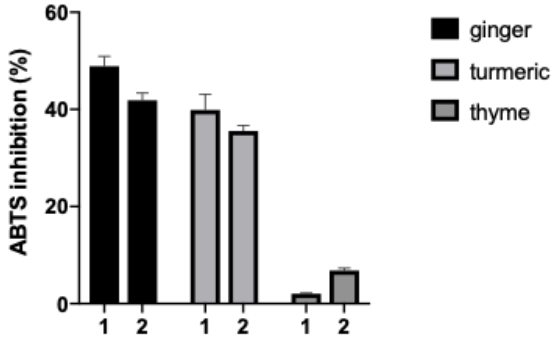

Figure 6. Comparison of the inhibition of ABTS absorbance by ginger, turmeric and thyme prepared from freshly purchased (1) and commercially available powdered form (2).

Antioxidant composition of spices depends greatly on the conditions of cultivation and the duration from harvesting to consumption, and the. As only one brand of powdered spices and one sample of fresh spices was tested, the results presented here must be considered to be preliminary. Also, it should be noted that turmeric and ginger are rhizomes, so the compounds present in them are likely to be more stable than the compounds in thyme leaves, which likely degrade quickly after harvesting.

It would be beneficial to examine more spice samples of different origin cultivated in distinct conditions in order to obtain more generalized results. Further exploration might determine the factors that enhance the antioxidant activity of these spices.

IV. CONCLUSION

The antioxidant components identified in all of the extracts were mainly non-polar and mostly belonged to the groups of phenolic acids and sterols. It was shown that extracts prepared from fresh turmeric and ginger exhibited higher antioxidant properties in both the DPPH and ABTS radical scavenging assays compared to commercially prepared powders, while dried thyme displayed greater antioxidant activity than the fresh herb. The results also indicate that turmeric and thyme exhibit significantly higher antioxidant properties than ginger, regardless of whether the plant material was commercially or freshly prepared. To maximize the health benefits from antioxidants, commercially prepared powders are preferred for thyme, which is difficult to maintain fresh for a long time, while turmeric and ginger, being rhizomes, should be used fresh.

V. REFERENCES

1. Phaniendra A., Jestadi D.B. (2015), Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases, Indian Journal of Clinical Biochemistry, 30(1): 11-26.

2. Davalli P., Mitic T., D'Arca D., Caporali A., Lauriola A. (2016), ROS, Cell Senescence, and Novel Molecular Mechanisms in Aging and Age-Related Diseases, Oxidative Medicine and Cellular

Longevity; vol. 2016, 3565127, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4877482/

3. Opara E., (2014), Culinary Herbs and Spices: Their Bioactive Properties, the Contribution of Polyphenols and the Challenges in Deducing Their True Health Benefits, International Journal of Molecular Sciences, 15(10): 19183-19202.

4. Hirun S., Utama-ang N., Roach P.D. (2014), Turmeric (*Curcuma longa* L.) drying: an optimization approach using microwave-vacuum drying, Journal of Food Science and Technology, 51(9): 2127-2133.

5. Vergara_Salinas JR, Perez Jimenes L., Torres J.L. (2012), Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (*Thymus vulgaris*), Journal of Agricultural and Food Chemistry.

6. Food and Agriculture Organization (FAO), Information on Post-harvest Operation (INPhO), Turmeric- Post-harvest operations, (2014), http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_Turmeric.pdf.

7. Esatbyoglu T., Ulbrich K., Rehberg C., Rohn S., Rimbach G. (2015), Thermal stability, antioxidant, and anti-inflammatory activity of curcumin and its degradation product 4-vinyl guaiacol, Food & Function, 2015(3), https://www.ncbi.nlm.nih.gov/pubmed/25619943, 25.04.2018

8. Bhattarai S., (2001), The stability of gingerol and shogaol in aqueous solution, Journal of Pharmaceutical Sciences, 2006, 90(10): 1658-1664.

9. Redfern J. et al (2014), Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties, Journal of Microbiology & Biology education, 15(1), 45-46.

10. Kedare SB (2011), Genesis and development of DPPH method of antioxidant assay, Journal of Food Science and Technology, 48(4), 412-422.

11. Kurin E. et al., (2012), *In vitro* antioxidant activities of three red wine polyphenols and their mixtures: An interaction study, Molecules (Basil, Switzerland), 17(12), 14336-14348, https://www.ncbi.nlm.nih.gov/pubmed/23208468, 05.11.2018.

12. Shadmani A., (2004) Kinetic studies on Zingiber officinale, Pakistani Journal of Pharmaceutical Sciences, 17(1), 47-54.

13. Lu Q., (2017), Prebiotic Potential and Chemical Composition of Seven Culinary Spice Extracts, Journal of Food Science, 82(8), 1807-1813.

14. Almela L. et.al (2002)., Microbial inactivation of paprika by a high-temperature short X-time treatment, Journal of Agricultural & Food Chemistry, 50(6), 1435-1440.

15. Jeong S.-M. et al. (2004), Effect of Heat Treatment on the Antioxidant Activity of Extracts from Citrus Peels, Journal of Agricultural & Food Chemistry, 52(11), 3389-3393.

16. Little CL., Omotoye R., Mitchell RT., (2003), The microbiological quality of ready-to-eat foods with added spices, International Journal of Environmental Health Resources, 13(1), 31-42.