

Evaluation of Polyphenols and Flavonoids in Marinades Used to Tenderize Beef Muscle

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Abstract

Marinating is an effective means of enhancing the quality of meats and a solution to flavor and tenderize meat products. Thus, in the present study identification of the flavonoid and polyphenolic content of some ingredients commonly used in beef marinades and the antioxidant activity were investigated. Antioxidant activity was evaluated as free radical-scavenging capacity (RCS) and hydrogen peroxide – scavenging activity. RCS was assessed by measuring the scavenging activity of water extracts of spices and seasoning plants studied (*Thymus vulgaris*, *Majorana hortensis*, *Allium sativum*, *Armoracia rusticana*), and water solutions of dry red wine and lime tree honey on 2,2-diphenyl-1-picrylhydrazyl (DPPH). Reduction of DPPH radical formation and hydrogen peroxide scavenging ability showed variable evolution depending on marinade ingredients studied. All types of water extracts from *Majorana hortensis*, *Thymus vulgaris*, *Allium sativum*, *Armoracia rusticana* and water solutions of dry red wine and lime-tree honey contain phenolic and flavonoids compounds and develop antioxidant activity. The total phenolic and flavonoid content and antioxidant activity varied greatly among different types of extracts and were found to be the highest in *Majorana hortensis*, *Thymus vulgaris* and dry red wine while *Allium sativum*, *Armoracia rusticana* and lime-tree honey showed low total phenolic and flavonoid content and consequently lower antioxidant activity

Keywords: beef marinades, antioxidant activity, polyphenolic compounds, flavonoids.

1. Introduction

The marination represents an effective method to enhance the quality and versatility of meats. Marination is the process of soaking or injecting meat with a solution containing ingredients such as vinegar, lemon juice, wine, soy sauce, brine, essential oils, salts, tenderizers, herbs, spices and organic acids to flavor and tenderize the meat products [1, 2]. Moreover, the shelf life of the meat may be positively affected by this process due to the acidic or alkaline nature of the solution, and the antimicrobial and antioxidant activity of some marinade ingredients [3]. At the present, there is recorded an increased interest - both in the industry and scientific research - for spices and aromatic herbs due to their strong antioxidant and antimicrobial properties exceeding many currently used natural and synthetic antioxidants [4].

These properties are induced by many substances including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. and render spices and some herbs or their antioxidant components as preservative agents in food [5].

Spices are natural plant products which have been used not only as flavoring and coloring agents but also as food preservatives and traditional medicines throughout the world for thousands of years. Being natural foodstuffs, the spices and herbs represent a viable alternative for many consumers who question the safety of synthetic food additives [4]. Many studies have reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant and pharmaceutical properties [6]. Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects [7].

The aim of this study was to characterize the biological active compounds present in the marinades used to improve the quality of the beef muscle including the appearance, flavour and tenderness. Thus, we have identified the flavonoids and polyphenols by thin layer chromatography method (TLC) and we have studied the polyphenolic and flavonoid content and the antioxidant activity of some ingredients commonly used in the Romanian beef marinades, namely *Thymus vulgaris*, *Majorana hortensis*, *Allium sativum*, *Armoracia rusticana*, dry red wine and lime-tree honey.

2. Materials and Methods

Plant material. Biological material analyzed in the present paper was represented by thyme (*Thymus vulgaris*), marjoram (*Majorana hortensis*), garlic (*Allium sativum*), horseradish (*Armoracia rusticana*), lime-tree honey and dry red wine. *Majorana hortensis* and *Allium sativum* have been purchased from Quatre épices Company (Bucharest, Romania), thyme from Research Institute Plantavorel (Piatra Neamt, Romania), *Armoracia rusticana* from a local supermarket, lime-tree honey from S.C. Apisalecom S.R.L. (Bacau, Romania) and dry red wine, minimum 12 % vol. alcohol content, from S.C. Viovin Prodserv S.R.L. (Odobesti, Romania).

Extracts preparation. The air-dried immature ground thyme and marjoram plants, ground and air-dried garlic bulbs and fresh horseradish were extracted with 80 % methanol or distilled water using ultrasounds for 2h, at room temperature. After the extraction, the extracts were collected and filtered. To remove the chlorophyll pigments, the methanol extracts of thyme and marjoram were subjected to repeated extraction with petroleum ether until disappearance of the green color. Methanol and water phases obtained after extraction were used for thin-layer chromatography (TLC) and water phases were used for flavonoids and polyphenols determination and antioxidant activity (the volume being adjusted to 100 mL with cold 80 % methanol and distilled water). For all determinations, the dry red wine sample was diluted with distilled water (1:20 v/v) and the lime-tree honey sample (5 g) was diluted with 50 mL with distilled water.

Identification of the flavonoid and polyphenolic compounds by thin layer chromatography method (TLC). The samples – the methanol and water plant extracts, dry red wine and lime-tree honey -were dripped to 10 cm × 14 cm aluminum-backed TLC plates coated with 0.2 mm layers of silica gel 60 F₂₅₄ (Merck) according to standards (quercetin, rutin, epicatechin, gallic acid, ferulic acid, chlorogenic acid). The mobile phase was ethyl acetate /formic acid/ acetic acid / H₂O (100: 11: 11: 20). The migration distance was 85 mm. The plates were dried in a flow of warm air for few minutes after development. The compounds were visualized by immersing the plates after drying into tow types of versatile revealing solutions consisting of 1 g de vanillin, 2 mL sulfuric acid and ethanol 95% to a volume of 100. After immersion, the plates were dried at 110 °C for few minutes, until the colorful spots appeared - depending on the type of compounds.

Analysis of the total phenolic content. The total polyphenol content was determined by a spectrophotometric method, using gallic acid as a standard, according to the method described by the International Organization for Standardization ISO14502-1 [8, 9, 10]. In a short description of the method, 0.1 mL of each extract was transferred into glass tubes, then 5.0 mL of Folin-Ciocalteu's reagent diluted 1/10 in distilled water, and 4.0 mL of a sodium carbonate solution (7.5% w/w) were added. All the experiments were run in duplicate, and allowed to stand at room temperature for one hour before the absorbance was determined at 765 nm against a blank containing distilled water. A standard curve of gallic acid (ranging from 10 to 50 µg gallic acid/mL and R² = 0.9988) was used to express the concentration of polyphenols in samples as gallic acid equivalents (GAE) in mg100 g⁻¹ plant material.

Estimation of the total flavonoid content. The total flavonoid content in the extracts was determined by a spectrophotometric method based on the formation of complex flavonoid-aluminium with an absorptivity maximum between 420-430 nm [11, 12]. Briefly, 1 mL of each extract was separately mixed with 1 mL of 2.5% AlCl₃·6H₂O, 2 mL of 10% sodium acetate solution, and 70% ethanol to a total volume of 10 mL. The experiments were run in duplicate, and after incubation at room temperature for 30 minutes, the absorbance of the reaction mixtures was measured at 420 nm against a water standard.

The flavonoid content values were determined from a standard curve prepared with quercetin (ranging from 10 to 50 µg/mL final volume and $R^2 = 0.9732$) and expressed as mg quercetin equivalents (QE) in mg100 g⁻¹ plant material.

Antioxidant activity. The DPPH assay was performed as previously described by Mimica-Dukic, Bozin, Sokovic, & Simin (2004) with some modifications [13]. The samples (ranging from 0.1 to 1 mL of stock solution) were mixed with 1 mL DPPH solution and made up with 80 % MeOH to a final volume of 4 mL. The absorbance of the resulting solutions and the blank (with same chemicals, except sample) was recorded after 1 h at room temperature. For each sample, three replicates were recorded. The disappearance of DPPH was measured spectrophotometrically at 515 nm. RSC, expressed as a percentage, was calculated by the following equation:

$$\text{RSC (\%)} = 100 \times (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}$$

where A_{blank} is the absorbance of control reaction (methanol with DPPH) and A_{sample} is the absorbance of the examined extracts.

The hydrogen peroxide-scavenging ability of examined extracts was determined according to the method of Ruch, Cheng, and Klaunig (1989) [14]. A solution of H₂O₂ (40 mM) was prepared in phosphate buffer (pH 7.4). Examined extracts in different concentrations (ranging from 10 to 50 µL/mL of stock solution) were added to 3.4 mL of phosphate buffer, together with 0.6 mL of H₂O₂ solution. The absorbance value of the reaction mixture was recorded at 230 nm. The blank solution contained only the phosphate buffer (4 mL). The percentage of H₂O₂ scavenging of examined extracts was calculated as

$$\% \text{ of scavenged H}_2\text{O}_2 = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control (phosphate buffer with H₂O₂) and A_1 is the absorbance of the examined extracts.

Statistical analysis. All evaluations of total phenolic content, total flavonoid content, antioxidant activity were performed twice. Data were expressed as mean values ± standard deviation using the Statistica 5.1. Programme for Windows.

The experimental results were analysed using Principal Component Analysis (PCA) with full cross-validation. PCA constitutes the most basic

statistical method of all multivariate data analyses, and involves decomposing one “data matrix” into a structural part (model) and a “noise” part (error). PCA was assessed using the Unscrambler X 10.1 software version from CAMO Software AS (Oslo, Norway).

3. Results and Discussions

The presence of flavonoids and polyphenolic compounds in water extracts of *Majorana hortensis*, *Thymus vulgaris*, *Allium sativum* and *Armoracia rusticana* and lime honey and dry red wine was determined by thin layer chromatography (TLC). TLC chromatograms were developed with ethyl acetate / formic acid / acetic acid / H₂O 100: 11: 11: 20 (w / w / w / w) compared with standard solutions: quercetin, rutin, epicatechin, chlorogenic acid, gallic acid and ferulic acid using a revealing solution composed of 1 g of vanillin, 2 mL 95% sulfuric acid and ethanol to a volume of 100 mL.

TLC separation of flavonoids and phenolic acids from water and methanolic extracts using the revealing solution composed of 1 g of vanillin, 2 mL 95% sulfuric acid and ethanol to a volume of 100 mL indicated the presence of a compound having $R_F = 0.31$ in all samples and a compound $R_F = 0.21$ in the *Majorana hortensis*, *Thymus vulgaris*, dry red wine and *Armoracia rusticana* water extracts and *Majorana hortensis*, *Thymus vulgaris*, *Allium sativum* and *Armoracia rusticana* methanolic extracts. The blue spots indicate the presence of polyphenolic compounds. Rutin ($R_F = 0.64$) was identified only in *Majorana hortensis* and *Thymus vulgaris* water extracts and *Majorana hortensis* and *Thymus vulgaris* methanolic extracts as yellow spots. Only in the samples *Majorana hortensis* and *Thymus vulgaris* methanolic extracts was identified a compound ($R_F = 0.70$) as yellow spots (chlorogenic acid). Epicatechin ($R_F = 0.88$) was identified as dark orange spot in the *Armoracia rusticana* methanolic extract and quercetin ($R_F = 0.95$) was identified as yellow spots in the *Majorana hortensis*, *Thymus vulgaris* water extracts.

The TLC of methanolic extracts has more spots due to a better solubility of the chemical compounds in methanol. The detected flavonoid compounds (quercetin, rutin and epicatechin) together with polyphenolic compounds confirms the potential active ingredients of water and methanolic extracts resulted from examined spices, seasoning plants, lime-tree honey and dry red wine.

The results obtained showed that the total phenolic content varied greatly among the extracts, as indicated in Table 1. Thus, from all analysed water extracts, the lowest values were recorded in *Armoracia rusticana* water extract, values increasing with lime-tree honey, *Allium sativum*, dry red wine, *Thymus vulgaris* and *Majorana hortensis*. The highest values were obtained with

water extracts of *Majorana hortensis* and *Thymus vulgaris* as being abt. 19 times higher than in case of *Armoracia rusticana* water extract. The total phenolic content average was similar with the one reported by Socha et al. (2009) and Silici et al. (2010) for honey and Radovanovic et al. (2009) for red wine [15, 16, 17].

Table 1. Total phenolics and flavonoid contents, DPPH free radical scavenging activity and hydrogen peroxide (H₂O₂) scavenging activity for the studied water extracts.

Extracts	Total phenolics	Total flavonoids	DPPH free radical scavenging activity (%)	H ₂ O ₂ scavenging activity (%)
	mg GAE/100g	mg QE/100g		
<i>Thymus vulgaris</i>	468.30 ± 0.14	49.72 ± 0,42	75.86 ± 0,62	67.40 ± 0,93
<i>Majorana hortensis</i>	475.24 ± 0.08	54.78 ± 0,14	81.17 ± 0,57	73.34 ± 0,86
<i>Allium sativum</i>	57.26 ± 0.15	25.73 ± 0,57	25.77 ± 0,85	39.06 ± 0,71
<i>Armoracia rusticana</i>	48.26 ± 0.09	23.97 ± 0,71	46.22 ± 0,99	27.12 ± 0,92
Lime-tree honey	65.51 ± 0.12	28.40 ± 0,48	36.11 ± 0,71	26.76 ± 1,03
Dry red wine	365.2 ± 0.14	39.47 ± 0,85	57.13 ± 0,41	54.50 ± 0,31

The data are displayed as having a mean ± standard deviation of twice replications.

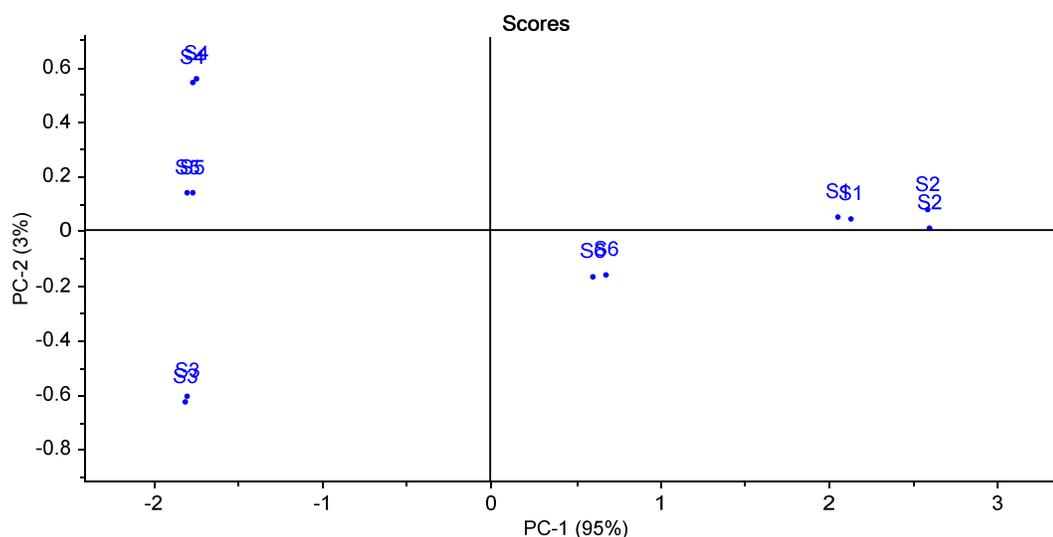


Figure 1. Score plot after PCA of the individuals in the plane defined by the two first PCs (S₁ - *Thymus vulgaris*, S₂ - *Majorana hortensis*, S₃ - *Allium sativum*, S₄ - *Armoracia Rusticana*, S₅ - Lime-tree honey, S₆ - Dry red wine).

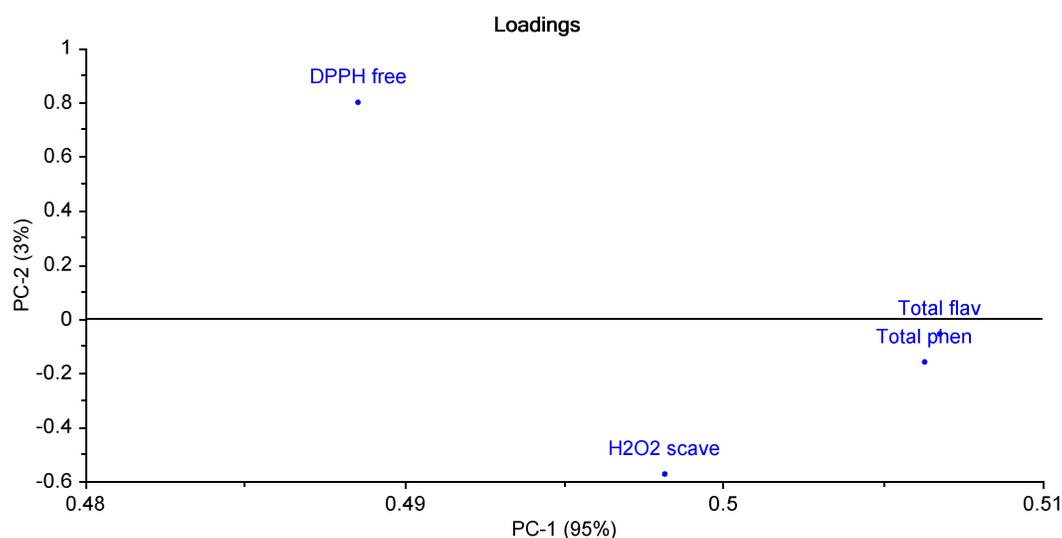


Figure 2. Loading plot after PCA with full cross-validation of the analysed samples

S₁, S₂ and S₆ are extracts correlated with each other, having the highest content in the bioactive compounds determined. In addition, these extracts are inversely correlated with the S₃, S₄ and S₅. S₁ and S₂ have the highest content in the total polyphenols and flavonoids and S₃, S₄ and S₅ present a low content in these compounds.

DPPH scavenging activity is correlated with the total polyphenols and flavonoids content in the samples; the bigger the amount of polyphenols and flavonoids is, the higher DPPH will be. Multivariate analysis of polyphenols, flavonoids and DPPH in analysed species pointed out that sample S₃ presents the lowest DPPH activity and the samples S₁ and S₂ present the highest DPPH. The H₂O₂ scavenging activity in PCA analysis is correlated with total polyphenols and flavonoids, samples S₁, S₂ and S₆ having the highest proportion of this activity.

4. Conclusion

In the present study, it was established that all types of water extracts from *Majorana hortensis*, *Thymus vulgaris*, *Allium sativum*, *Armoracia rusticana* and water solutions of dry red wine and lime-tree honey contain phenolic and flavonoids compounds and develop antioxidant activity. Chromatographic profile appears rich in secondary metabolites which give strong antioxidant activity to the analyzed plant extracts.

The total phenolic and flavonoid content and antioxidant activity varied greatly among different types of extracts and were found to be the highest in *Majorana hortensis*, *Thymus vulgaris* and dry red wine while *Allium sativum*, *Armoracia rusticana* and lime-tree honey showed low total phenolic and flavonoid content and consequently lower antioxidant activity. Thus, application of suitable natural ingredients developing both antioxidant and antimicrobial activities will be useful in maintaining the meat quality, extending shelf-life and preventing economic losses.

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Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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