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The study of antioxidant and antimicrobial activity of extracts for meat marinades

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Abstract

In the present study the antioxidant activity, polyphenolic and flavonoid content, and antimicrobial activity of some ingredients commonly used in beef marinades were investigated. Reduction of DPPH radical formation and hydrogen peroxide scavenging ability showed variable evolution depending on marinade ingredients studied and type of extract (water or methanolic). The highest total phenolic (1333.68 ± 0.24 mg tannic acid/100g) and total flavonoid (661.26 ± 0.28 mg rutin/100g) contents were found in the *Majorana hortensis* methanolic extract. The most powerful antioxidant water extract mixture was that obtained from dry red wine, lime-tree honey, *Allium sativum, Thymus vulgaris and Armoracia rusticana* with the highest DPPH free radical scavenging activity and hydrogen peroxide scavenging activity being 87.18 ± 0.66% respectively 50.23 ± 0.62%. The statistical analysis of Plackett-Burman experimental design showed that the most important antimicrobial effect against *Bacillus subtilis* was found for the combination with the largest quantity of horseradish and marjoram extracts and the most important antimicrobial effect against *Bacillus cereus* was found for the combination with the largest quantity of horseradish, thyme and marjoram extracts. Using a larger number of ingredients rich in biologically active compounds will lead to marinades capable to increase the quality of beef meat.

Key words: antioxidant activity, antimicrobial activity, polyphenolic compounds, spices and beef marinades.

1. Introduction

Beef is a highly perishable food with a short shelf-life. Prolonging the shelf-life of fresh meat is important for both manufactures and consumers. The shelf-life of fresh meats can be extended by protecting them from discoloration, lipid oxidation and microbial growth (SALEEMI & al., SÁNCHEZ-ESCALANTE & al. [1, 2]). One of the most important meat quality aspects determining consumers' purchase choice is color. Meat discoloration is used by consumers as an indicator of freshness and wholesomeness (Mancini & Hunt [3]). Thus, improvement of color stability is important in the meat industry. Oxidation is one of the major causes of the chemical spoilage resulting in rancidity and/or deterioration of the nutritional quality, colour, flavour, texture and safety of foods (JUNG & al. [4]). This situation leads to significant economic losses for the meat industry. In order to reduce the sizable economic losses, the meat industry is looking for effective natural preservation methods providing the meat products with an extensive shelf life and fulfilling at the same time the consumers' demands for high quality, convenience and improved flavour (PATHANIA & al. [5]).

The marinating 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 flavour and tenderize the meat products (PATHANIA & al., BJORKROTH [5, 6]). 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 (KARGIOTOU & al. [7]). 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 (SUHAJ [8]). 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 (CALUCCI & al. [9]).

Being natural foodstuffs, the spices and herbs represent a viable alternative for many consumers who question the safety of synthetic food additives (SUHAJ [8]). Many studies have reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant and pharmaceutical properties (MENG & al. [10]). Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (MIGHRI & al. [11]).

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 studied the polyphenolic and flavonoid content and the antioxidant and antimicrobial 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).

3. Extracts preparation

The air-dried immature ground thyme and marjoram plants, ground and air-dried garlic bulbs and fresh horseradish were extracted with two different solvents, 80% methanol and distilled water, using ultrasounds bath (Transsonic T310, Elma, Singen, Germany) for 2h, at room temperature. The entire amount of samples (air-dried immature ground thyme and marjoram plants, ground and air-dried garlic bulbs and fresh horseradish) was divided into two groups. The first group of the samples was extracted with 80% methanol and the second group with distilled water. 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. Methanol and water phases obtained after extraction are used for flavonoids and polyphenols determination, thin-layer chromatography (TLC), antioxidant and antimicrobial 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 aluminium-backed TLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (Merck) compared to standards (quercetin, rutin, epicatechin, gallic acid, ferulic acid, chlorogenic acid). The mobile phase was ethyl acetate /formic acid/ acetic acid / H_2O (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 a versatile revealing solution consisting of 0.5 g thymol, 95 mL ethanol and 5 mL sulphuric acid. After immersion, the plates were dried at 110°C for few minutes, until the colourful spots appeared - depending on the type of compounds.

Analysis of the total phenolic content

The total polyphenol content (TPC) of the extracts was determined by spectrophotometry, using gallic and tannic acids as standards, according to the method described by the International Organization for Standardization (ISO) 14502-1 [12]. The TPC was expressed as gallic acid equivalents (GAE) in mg 100 g^{-1} material and tannic acid equivalents in mg 100 g^{-1} material.

Estimation of the total flavonoid content

The total flavonoid content in the investigated extracts was spectrophotometrically measured by using a method based on formation of complex flavonoid-aluminium having a maximum absorption at 430 nm. A quantity of 1 mL of samples was separately mixed with 1 ml solution of 2% $AlCl_3$; the absorbance was measured after 30 min incubation at room temperature. The flavonoids content was expressed as quercetin equivalents (QE) in mg 100 g^{-1} material and rutin equivalents (RE) in mg 100 g^{-1} material.

Antioxidant activity

The DPPH assay was performed as previously described by MIMICA-DUKIC & al. [13]. The RSC (radical scavenging capacity)- as expressed in percentage - was calculated by the following equation (1):

$$RSC\ (\%) = 100 \times (A_{blank} - A_{sample}/A_{blank}) \quad (1)$$

where A_{blank} is the absorbance of the control (methanol with DPPH), A_{sample} is the absorbance of the examined extracts and RSC is the radical scavenging capacity.

The hydrogen peroxide-scavenging ability of the examined extracts was determined according to RUCH & al. [14]. The percentage of H_2O_2 scavenging of examined extracts was calculated as % of scavenged $H_2O_2 = [(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control (phosphate buffer with H_2O_2) and A_1 is the absorbance of the examined extracts.

Antibacterial activity

The bacterial strains used were purchased from the American Type Culture Collection: *Bacillus subtilis* ATCC 19659 and *Bacillus cereus* ATCC 10876 preserved and multiplied on nutrient agar medium. The inoculums were prepared by transferring a loop of cells to 50 mL culture medium (Nutrient Broth) containing: casein peptone, (4.3 g/L), meat peptone (4.3 g/L) and sodium chloride (6.4 g/L), and grown at 37°C for 24 h. In order to test the antimicrobial activity of plant extracts, the inoculums were added to a mixture of Nutrient Broth medium

and plant extracts to a final volume of 6 ml, and then incubated at 37°C for 24h. Plant extracts were added based on Plackett-Burman design by varying the composition of chosen independent variables. The optical densities (OD_{600}) of all samples were recorded using a UV-VIS Spectrophotometer, Jenway, and then suppression percentages were calculated according to the following equation (2) adapted according to AL-AJLANI and HASMAIN [15]:

$$\text{Suppression } \% = [(OD_{600}\ \text{treatment} - OD_{600}\ \text{control})/\ OD_{600\ \text{control}}] \cdot 100. \quad (2)$$

where $OD_{600\ control}$ is the optical density of the control (Nutrient Broth with H_2O) and OD_{600} treatment is the optical density of the treated samples with the extracts.

Statistical analysis

All evaluations of total phenolic content, total flavonoid content, antioxidant and antibacterial activity were performed twice. Data were expressed as mean values ± standard deviation.

The statistical software package Design-Expert 8 (Stat-Ease, Minneapolis, MN) was used for the experimental design and data analysis. Variance analysis (ANOVA) was used to estimate the statistical parameters.

Plackett-Burman design represents an efficient and effective approach to systematically investigate and evaluate the effects of medium components (YUAN & al. [16]). In this study, a 22-run Plackett-Burman design was applied to evaluate six variable, and the antimicrobial activity (suppression, %) of extracts was selected as response. Each independent variable was tested at two levels, a high (+1) level – addition of 0.25 ml plant extract - and a low (−1) level – addition of 0.1 ml plant extract.

The model used by software for the tested experimental conditions can be generally described using the equation (3) for Response 1 (R1) and the equation (4) for Response 2 (R2).

$$R1 = \beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_4D+\beta_5E+\beta_6F+\beta_7AF+\beta_8BC+\beta_9CD+\beta_{10}DE \quad (3)$$

$$R2 = \beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_4D+\beta_5E+\beta_6F+\beta_7AC+\beta_8AE+\beta_9BE+\beta_{10}CD+\beta_{11}CE+\beta_{12}EF \quad (4)$$

where A-F are the independent variables studied (plant extracts codes: A- Horseradish extract; B- Thyme extract; C- Marjoram extract; D- Garlic extract; E-Dry red wine; F- Lime-tree honey) and β_0– β_{11} represent the constants for the overall process effect, the effects of each independent variable, and the interaction effects between variables on antibacterial activity, respectively.

Results and Discussion

Identification of flavonoid and polyphenolic compounds by TLC method

TLC separation of flavonoids and phenolic acids from water and methanolic extracts (Fig. 1) indicated the presence of a compound having R_F = 0.33 in all samples, a compound having a R_F = 0.21 in the *Majorana hortensis, Thymus vulgaris*, *Allium sativum, Armoracia rusticana* water extracts and dry red wine and *Majorana hortensis, Thymus vulgaris*, *Allium sativum* and *Armoracia rusticana* methanolic extracts and a compound having R_F = 0.55 only in dry red wine under the form of red spots. The red spots indicate the presence of polyphenolic compounds. Rutin (R_F = 0.64) was identified as yellow spots only in *Majorana hortensis* and *Thymus vulgaris* water and methanolic. Only in the methanolic extracts from *Majorana hortensis* and *Thymus vulgaris* was identified a compound (R_F = 0.70) as yellow spots

(chlorogenic acid). Epicatechin (R_F = 0.94) was identified as dark orange spot in the *Armoracia rusticana* methanolic extract and quercetin (R_F = 0.94) was identified as yellow spots in the *Majorana hortensis, Thymus vulgaris* water and methanolic 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 are considered potential active ingredients of water and methanolic extracts resulted from examined spices, seasoning plants, lime-tree honey and dry red wine. Eloff [17] and Cowan [18] found that methanol was more efficient than acetone in extracting phytochemicals from plant materials. Polyphenolic compounds such as flavones and most other reported bioactive compounds are generally soluble in polar solvents such as methanol.

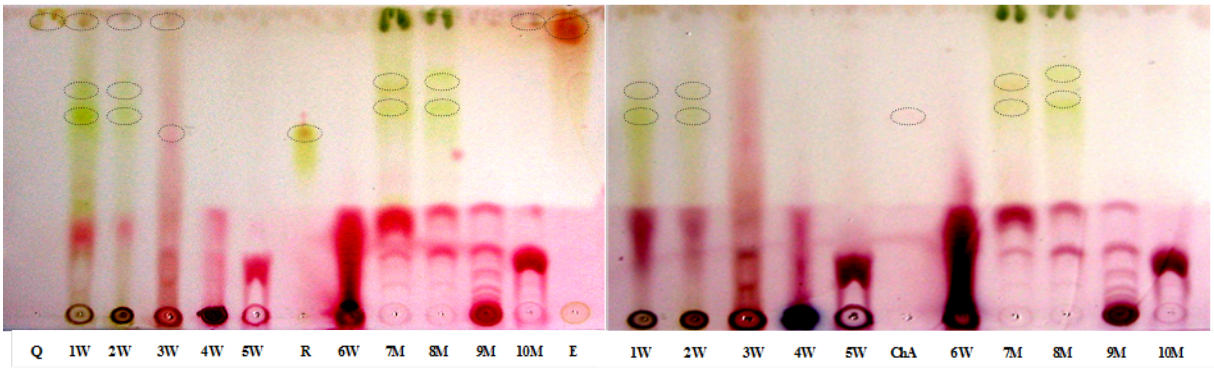

Fig. 1. TLC chromatogram of analyzed water and methanolic extracts and standards developed with ethyl acetate /formic acid/ acetic acid / H_2O 100: 11: 11: 20 (v/v/v/v) - revealing solution consisting of 0.5 g thymol, 95 mL ethanol and 5 mL sulfuric acid.
(Key to the spots: Q, quercetin, R, rutin, E, epicatechin, ChA, chlorogenic acid, 1W, *Majorana hortensis* water extract, 2W, *Thymus vulgaris* water extract, 3W, dry red wine, 4W, *Allium sativum* water extract, 5W, *Armoracia rusticana* water extract, 6W, lime-tree honey, 7M *Majorana hortensis* methanolic extract, 8M, *Thymus vulgaris* methanolic extract, 9M, *Allium sativum* methanolic extract, 10M, *Armoracia rusticana* methanolic extract).

Total phenolic and flavonoid contents

The results obtained showed that the total phenolic content varied greatly among the extracts, as indicated in Table 1. The lowest values for all the samples were determined in water extracts. 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 methanolic extracts of *Majorana hortensis* and *Thymus vulgaris* as being approximately 19 times higher than in case of *Armoracia rusticana* water extract. The total phenolic content average was similar with the one reported by SOCHA & al. [18] and SILICI & al. [19] for honey and RADOVANOVIC & al. [20] for red wine.

The total flavonoid content of the analyzed samples was the highest with the methanolic extracts by comparison with water extracts (Table 1). Therefore, from all analysed methanolic extracts, the highest values were recorded with *Majorana hortensis* and *Thymus vulgaris* extracts. These results were significantly higher than those recorded with water extracts. Thereby, in *Majorana hortensis* and *Thymus vulgaris* water extracts the results were approximately 8-times lower than with methanolic extracts. Dry red wine also contained a considerable amount of flavonoids. The total flavonoid content determined in this study was in accordance with the results reported by SOCHA et al. [18] for honey. The total flavonoid content for the red wine is lower compared with the values reported by the YANG et al, [21]

and for *Allium sativum* the total flavonoid content is significantly higher than the values reported by the BOZIN et al. [22] (5,78 µg QE/g). These differences can be explained by the different sources and also by the composition of raw materials used in the study. Although the determination of phenolics by using the Folin-Ciocalteu reagent and the determination of flavonoids by using aluminum chloride are based on different mechanisms of the reaction, the reactants exhibit different affinities to individual substrates.

Table 1. Total phenolics and flavonoid contents, DPPH free radical scavenging activity and hydrogen peroxide (H_2O_2) scavenging activity for the studied water extracts.

Extracts		Total phenolics		Total flavonoids		DPPH free radical scavenging activity (%)	H_2O_2 scavenging activity (%)
		mg GAE/100g	mg tannic acid/100g	mg QE/100g	mg rutin/100g		
Thymus vulgaris	W	468.30 ± 0.14	602.98 ± 0.03	49.72 ± 0.06	70.46 ± 0.20	75.86 ± 0.04	67.40 ± 0.04
	M	732.65 ± 0.14	1038.94 ± 0.52	379.75 ± 0.57	572.38 ± 0.42	86.26 ± 0.57	69.40 ± 0.42
Majorana hortensis	W	475.24 ± 0.08	682.63 ± 0.04	54.78 ± 0.23	78.50 ± 0.03	81.17 ± 0.03	73.34 ± 0.03
	M	928.53 ± 0.28	1333.68 ± 0.24	474.75 ± 0.20	661.26 ± 0.28	88.49 ± 0.42	75.74 ± 0.57
Allium sativum	W	57.26 ± 0.15	74.63 ± 0.06	25.73 ± 0.08	35.38 ± 0.16	25.77 ± 0.13	39.06 ± 0.04
	M	88.61 ± 0.14	129.86 ± 0.47	68.61 ± 0.18	75.19 ± 0.45	32.22 ± 0.43	39.61 ± 0.38
Armoracia rusticana	W	48.26 ± 0.09	69.36 ± 0.03	23.97 ± 0.17	33.66 ± 0.04	46.22 ± 0.03	27.12 ± 0.06
	M	68.73 ± 0.23	98.73 ± 0.42	59.47 ± 0.37	84.53 ± 0.28	49.41 ± 0.57	22.86 ± 0.47
Lime-tree honey	W	65.51 ± 0.12	78.67 ± 0.07	28.40 ± 0.04	36.93 ± 0.17	36.11 ± 0.04	26.76 ± 0.11
Dry red wine	W	365.2 ± 0.14	435.81 ± 0.04	39.47 ± 0.04	55.74 ± 0.03	57.13 ± 0.03	54.50 ± 0.11

The data are reported as mean ± standard deviation of twice replications.
W- water extract, M-methanolic extract

Due to the fact that this study analyzes the ingredients that are commonly used in the marinades for beef, it has been also carried-out a study on the composition of biologically active compounds in mixtures of water extracts of thyme (*Thymus vulgaris*), marjoram (*Majorana hortensis*), garlic (*Allium sativum),* horseradish (*Armoracia rusticana*), lime-tree honey and dry red wine. We considered that the wine, honey and garlic mixture represents the basis of the marinade where the remaining ingredients were added in different amounts and combinations (Table 2) in order to see how the total phenolic and flavonoid content and antioxidant activity are influenced. The total phenolic and flavonoid content of the analyzed water extracts combinations are shown in Table 2. The results obtained showed that the total phenolic content and the total flavonoid content varied greatly among the extracts combinations. The addition of different ingredients and the increase in the amount of extract resulted in a significant enhancement of the total flavonoid and phenolic values. In conclusion, use of a larger number of ingredients rich in biologically active compounds will lead to marinades capable to increase the quality of beef meat.

Table 2. Total phenolics and flavonoids contents, DPPH free radical scavenging activity and hydrogen peroxide (H_2O_2) scavenging activity for the mixture of water extracts.

Variantes of water extracts combination	Total phenolics		Total flavonoids		DPPH free radical scavenging activity(%)	H_2O_2 scavenging activity (%)
	mg GAE/100g	mg tannic acid/100g	mg QE/100g	mg rutin/100g		
1	769.28 ± 0.57	1092.37 ± 0.42	86.67 ± 0.17	123.93 ± 0.28	18.93 ± 0.25	12.05 ± 0.42
2	832.01 ± 0.42	1189.77 ± 0.17	89.23 ± 0.28	125.81 ± 0.21	24.27 ± 0.23	19.11 ± 0.38
3	855.23 ± 0.14	1214.42 ± 0.51	90.70 ± 0.13	127.86 ± 0.70	21.80 ± 0.04	16.71 ± 0.03
4	975.09 ± 0.28	1394.37 ± 0.43	111.50 ± 0.21	158.33 ± 0.55	26.00 ± 0.33	22.11 ± 0.45
5	853.06 ± 0.42	1228.40 ± 0.50	119.21 ± 0.28	169.27 ± 0.08	28.24 ± 0.61	39.01 ± 0.39
6	1040.12 ± 0.57	1487.20 ± 0.44	141.88 ± 0.27	202.88 ± 0.25	32.97 ± 0.20	42.69 ± 0.31
7	961.83 ± 0.40	1365.79 ± 0.39	123.17 ± 0.93	171.20 ± 0.54	37.83 ± 0.55	41.13 ± 0.45
8	1191.83 ± 0.56	1716.23 ± 0.29	149.05 ± 0.39	208.67 ± 0.48	45.94 ± 0.30	41.58 ± 0.50
9	1073.46 ± 0.42	1513.57 ± 0.25	160.85 ± 0.27	223.58 ± 0.51	43.24 ± 0.45	42.69 ± 0.42
10	1212.24 ± 0.30	1757.74 ± 0.72	173.24 ± 0.27	247.73 ± 0.17	50.18 ± 0.65	43.75 ± 0.40
11	912.24 ± 0.13	1286.25 ± 0.51	158.4 ± 0.48	226.51 ± 0.31	60.90 ± 0.19	44.05 ± 0.42
12	1065.30 ± 0.66	1502.07 ± 0.35	171.25 ± 0.57	243.17 ± 0.49	74.07 ± 0.37	49.21 ± 0.52
13	1040.81 ± 0.51	1477.95 ± 0.71	164.29 ± 0.61	239.86 ± 0.44	73.53 ± 0.41	47.34 ± 0.21
14	1285.71 ± 0.21	1838.56 ± 0.14	175.58 ± 0.28	249.32 ± 0.11	83.12 ± 0.78	49.33 ± 0.39
15	1302.04 ± 0.38	1822.85 ± 0.11	165.52 ± 0.42	254.90 ± 0.76	84.12 ± 0.28	49.82 ± 0.25
16	1589.75 ± 0.13	2225.65 ± 0.45	182.58 ± 0.13	279.34 ± 0.37	87.18 ± 0.66	50.23 ± 0.62

Legend of the variants of analyzed water extracts combination (mL): 1- Dry red wine: Lime-tree honey: *Allium sativum:*1:1:1; 2- Dry red wine: Lime-tree honey: *Allium sativum:*2:2:2; 3- Dry red wine: Lime-tree honey: *Allium sativum: Armoracia rusticana*: 1:1:1:1; 4- Dry red wine: Lime-tree honey: *Allium sativum: Armoracia rusticana*: 2:2:2:1; 5- Dry red wine: Lime-tree honey: *Allium sativum: Thymus vulgaris*: 1:1:1:1; 6- Dry red wine: Lime-tree honey: *Allium sativum: Thymus vulgaris*: 2:2:2:1; 7- Dry red wine: Lime-tree honey: *Allium sativum: Majorana hortensis*: 1:1:1:1; 8- Dry red wine: Lime-tree honey: *Allium sativum: Majorana hortensis*: 2:2:2:1; 9- Dry red wine: Lime-tree honey: *Allium sativum: Thymus vulgaris, Majorana hortensis*: 1:1:1:1:1; 10- Dry red wine: Lime-tree honey: *Allium sativum: Thymus vulgaris: Majorana hortensis*: 2:2:2:1:1; 11- Dry red wine: Lime-tree honey: *Allium sativum: Armoracia rusticana: Thymus vulgaris*: 1:1:1:1:1; 12- Dry red wine: Lime-tree honey: *Allium sativum: Armoracia rusticana: Thymus vulgaris*: 2:2:2:1:1; 13- Dry red wine: Lime-tree honey: *Allium sativum: Armoracia rusticana:*1:1:1:1; 14- Dry red wine: Lime-tree honey: *Allium sativum: Armoracia rusticana:*2:2:2:1; 15- Dry red wine: Lime-tree honey: *Allium sativum: Thymus vulgaris, Armoracia rusticana*: 1:1:1:1:1; 16- Dry red wine: Lime-tree honey: *Allium sativum: Thymus vulgaris, Armoracia rusticana*: 2:2:2:1:1.

The data are reported as mean ± standard deviation of twice replications.

Antioxidant activity

The results of DPPH radical scavenging activity are indicated in Table 1. The most powerful extracts were those obtained from *Majorana hortensis* and *Thymus vulgaris* with methanol 80%, 88.49 ± 0.42 %, respectively 86.26 ± 0.57 (Table 1). The methanolic extracts from *Armoracia rusticana* and *Allium sativum* expressed similar but significantly lower scavenging capacity than did those obtained from *Majorana hortensis* and *Thymus vulgaris.*

The ability of water and methanolic extracts to scavenge hydrogen peroxide is shown in Table 1. All extracts were able to neutralize the H_2O_2 proportionally with the dose used. Strong scavenging effects were observed, especially in the extracts obtained from *Majorana hortensis*, *Thymus vulgaris* and dry red wine. Relatively slight neutralization of hydrogen peroxide exhibited by the extracts from *Allium sativum, Armoracia rusticana* and lime-tree honey could be partially explained by the chemical composition and relatively low content of the total phenolics and flavonoids (Table 1).

The results of DPPH radical scavenging activity and the ability of the analyzed water extracts combinations to scavenge hydrogen peroxide are indicated in Table 2. The results obtained revealed that the scavenger effect expressed in DPPH free radical scavenging activity (%) and the ability to neutralize H_2O_2 varied greatly among the extracts combinations. Thus, like in the case of the total phenolic and flavonoid content determination, the addition of different ingredients and the increase in the added extract amount resulted in a significant increase of the radical scavenging activity.

Antibacterial activity

The test microorganism chosen for studying the antibacterial activity of the herbal extracts were *Bacillus subtilis* and *Bacillus cereus*, bacteria associated with meat and meat products. The presence on carcasses of *B. cereus* and other *Bacillus* spp. of soil origin, including *Bacillus subtilis* and *Bacillus licheniformis* is not unusual although their incidence is generally low. In raw meat products such as sausage, these organisms are both more numerous and more frequently present because of their introduction in cereal fillers and spices. The effect of each individual component is expressed in the Pareto chart and is ranked according to the greatest effect on the bacteria suppression % (Fig. 2 and Fig. 3). The experiment with the largest quantity of horseradish and marjoram extracts (Table 3) has produced the most important antimicrobial effect against *Bacillus subtilis*. The Pareto chart indicates that the order of effects for individual components that have a positive effect on the *Bacillus subtilis* suppression % are the thyme extract (B) > marjoram extract (C) > wine (E). The F-value (16.20) mean that the model is significant and the values of "Prob > F" lower than 0.0500 indicate that the model terms are significant. In this case, B, C, E, AF, BC, CD and DE are significant model terms. Values higher than 0.1000 indicate that the model terms are not significant. The R-Squared = 0.9364 indicated that the mathematical model chosen is adequate (Table 3).

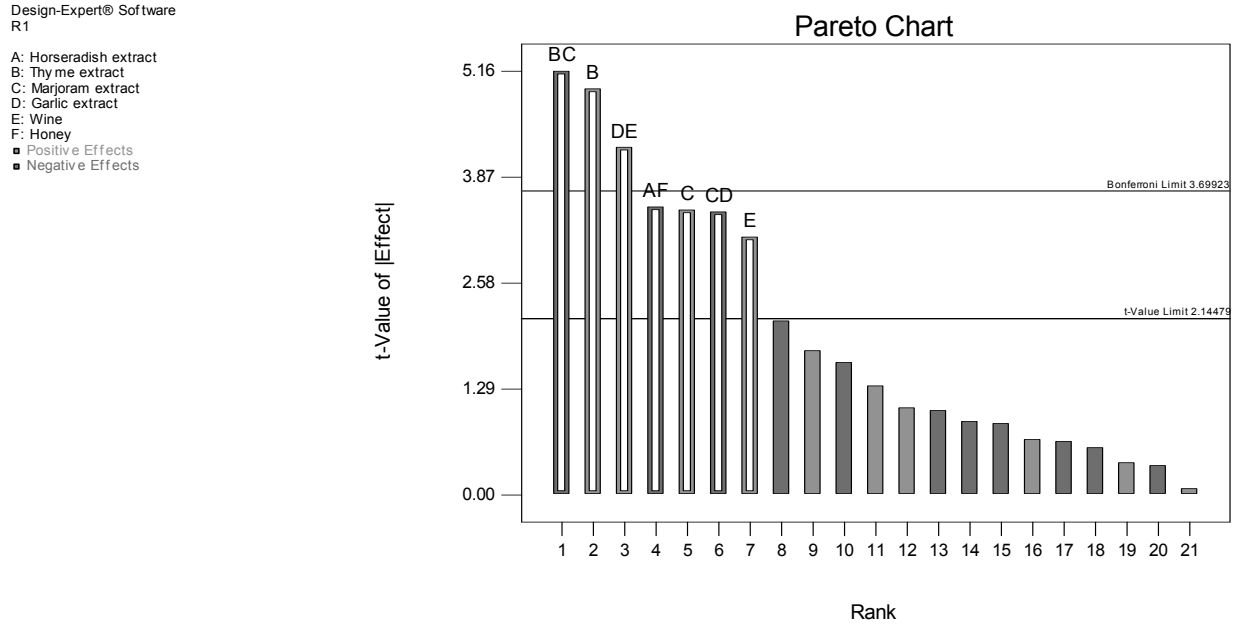

Fig. 2. Pareto chart showing the effects of plant extracts against *Bacillus subtilis.*

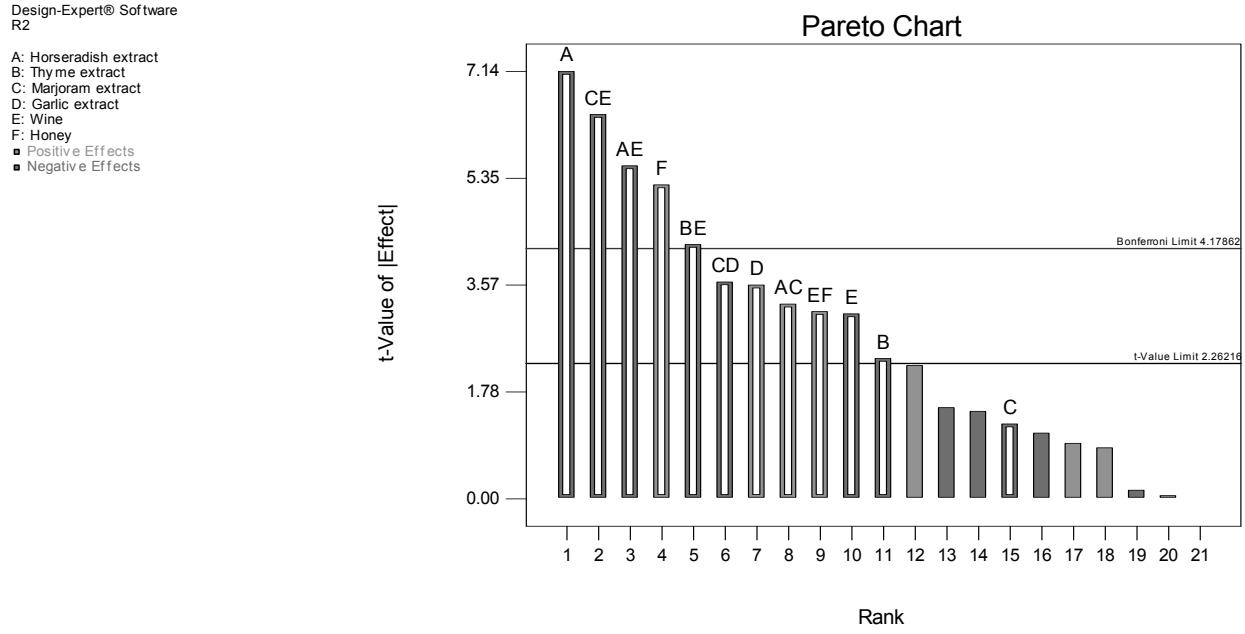

Fig. 3. Pareto chart showing the effects of plant extracts against *Bacillus cereus.*

Table 3. Statistical analysis of Plackett-Burman experimental design of each variable for *Bacillus subtilis* and *Bacillus cereus* suppression %.

Source	*Bacillus subtilis*			*Bacillus cereus*		
	Sum of Squares	F Value	Prob > F	Sum of Squares	F Value	Prob > F
Model	15689.47	16.20	< 0.0001	5015.34	12.56	0.0003
A - Horseradish	241.26	2.49	0.1428	1694.35	50.92	< 0.0001

extract						
B - Thyme extract	2312.31	23.87	0.0005	182.83	5.49	0.0437
C - Marjoram extract	1102.21	11.38	0.0062	51.98	1.56	0.2429
D - Garlic extract	26.71	0.28	0.6099	423.44	12.73	0.0060
E - Wine	969.38	10.01	0.0090	317.13	9.53	0.0130
F - Honey	14.58	0.15	0.7054	913.53	27.45	0.0005
AF	1064.90	10.99	0.0069	351.12	10.55	0.0100
BC	2634.15	27.19	0.0003	1027.74	30.89	0.0004
CD	990.49	10.22	0.0085	599.74	18.02	0.0022
DE	1456.23	15.03	0.0026	435.95	13.10	0.0056
	R-Squared = 0.9364			R-Squared = 0.9437		

The experiment with the largest quantity of horseradish, thyme and marjoram extracts (Table 3) has produced the most important antimicrobial effect against *Bacillus cereus*. Honey followed by garlic extracts were the only individual components having a positive effect on the *Bacillus cereus* suppression %. Antibacterial activity of honey has been attributed to its high osmotic effect, acidic nature, hydrogen peroxide concentration and its phytochemical nature, i.e. its content of tetracycline derivatives, peroxides, amylase, fatty acids, phenols, ascorbic acid, flavonides, streptomycin, sulfathiazole, trepens, benzyl alcohol and benzoic acids. The order of negative effects of components mixture was, as follows: (marjoram extract + wine) > (horseradish extract + wine) > (thyme extract + wine) (Fig. 3). The most negative effect for *Bacillus cereus* suppression % has been given by horseradish extract (A). A Model F-value of 12.56 involves a significant model. Values of "Prob > F" lower than 0.0500 indicate significant model terms. In this case, A, B, D, E, F, AC, AE, BE, CD, CE and EF are significant model terms. Values higher than 0.1000 indicate not significant model terms. The R-Squared = 0.9437 indicated that the mathematical model chosen is adequate (Table 4). For *Bacillus subtilis* suppression it can be notice that Thyme extract, Marjoram extract, Garlic extract and Wine are important factors. For *Bacillus cereus* suppression Garlic extract and Honey are most important parameters.

These significant factors identified by the Plackett-Burman design are to be considered in the next stage of the medium optimization using response surface optimization method for the fufurtherture study.

4. Conclusions

In the present study, it was established that all types of water and methanolic 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. The combinations of spices, seasoning plants, red wine and honey resulted in increased values both of total phenolic and flavonoid content and antioxidant activity The most important antimicrobial effect against *Bacillus subtilis* was found for the combination involving the highest quantity of horseradish and marjoram extracts and the most important antimicrobial effect against *Bacillus cereus* was found for the combination involving the largest quantity of horseradish, thyme and marjoram extracts.

These analyses performed for the first time demonstrated that analysed extracts will be useful in maintaining the meat quality, extending shelf-life and preventing economic losses.

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