Food Chemistry 253 (2018) 13–21

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of lavender (*Lavandula angustifolia*) and melissa (*Melissa Officinalis*) waste on quality and shelf life of bread

Ivelina Vasileva[a], Rositsa Denkova[b], Rosen Chochkov[c], Desislava Teneva[d], Zapryana Denkova[d], Tzvetelin Dessev[c], Petko Denev[e], Anton Slavov[a,*]

[a] Department of Organic Chemistry, Technological Faculty, University of Food Technologies, 26 Maritsa Blvd., Plovdiv 4002, Bulgaria
[b] Department of Biochemistry and Molecular Biology, Technological Faculty, University of Food Technologies, 26 Maritsa Blvd., Plovdiv 4002, Bulgaria
[c] Department of Bread Technology, Technological Faculty, University of Food Technologies, 26 Maritza Blvd., Plovdiv 4002, Bulgaria
[d] Department of Microbiology, Technological Faculty, University of Food Technologies, 26 Maritza Blvd., Plovdiv 4002, Bulgaria
[e] Laboratory of Biologically Active Substances, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 139 Ruski Blvd, Plovdiv 4000, Bulgaria

ARTICLE INFO

Keywords:
Bread
Lavender
Melissa
Antimicrobial activity
Antioxidant activity
Waste valorization
Dietary fibers

ABSTRACT

The effect of lavender (*Lavandula angustifolia*) and melissa (*Melissa Officinalis*) waste on preparation, characteristics and shelf life of bread was investigated. It was found that lavender and melissa waste, generated yearly in large amounts, were rich on polyphenols (especially rosmarinic acid) and aroma compounds, and exhibited high antioxidant and antimicrobial activity. The bread with 2.5% lavender waste was characterized with the highest loaf volume and loaf specific volume. The total dietary fiber increased three times and the polyphenols and flavonoids increased more than four times for breads with added 5% lavender and melissa waste, compared to control sample. The breads with 2.5% and 5% added lavender waste had increased shelf life (up to 96 h) compared to control, and no fungal or bacterial spoilage was observed during storage at 22 °C, 30 °C and 37 °C for four days. The sensory evaluation demonstrated that the consumers preferred mainly bread with 2.5% lavender waste.

1. Introduction

Bread is an important source of nutrients in human diet, especially of carbohydrates, proteins and some important macro and microelements, such as: magnesium, potassium, selenium, phosphorus, manganese, iron, etc. The bread-making process dates back to the dawn of the mankind, approximately 12 000 years in the past. The nice and specific flavor, the brown melanoidins formed in the crust, and the white, stretchy crumb, giving pleasant mouthfeel, are among the characteristics that made the fresh bread very popular and indispensable (Mondal & Datta, 2008). The flour and water are compulsory ingredients, although nowadays various additives have been also used. Some of the substances improve and facilitate the technological processes, and others, such as bran, cellulose, plant extracts and wastes, herbs, spices, etc., functionalize the bread and extend its shelf life. The popularity of adding ingredients rich on dietary fibers in foods owes to the particular interest in formulating wholesome foods, and important food products, such as bread, are increasingly being used as source of dietary fibers (Gawlik-Dziki, Świeca, Dziki, Baraniac, Tomilo, & Czyż, 2013; Prokopov, Goranova, Baeva, Slavov, & Galanakis, 2015). The enhanced consumption of dietary fibers is associated with a numerous health benefits such as, increasing fecal bulk, stimulating colonic fermentation, and reducing pre-prandial cholesterol levels, and levels of postprandial blood glucose (Sullivan, O'Flaherty, Brunton, Arendt, & Gallagher, 2011). Products enriched with various fiber sources can be considered as functional foods (Prokopov et al., 2015). The application of dietary fibers in bread making is also beneficial by a number of technological properties they possess. For example, the dietary fiber water-holding capacity prevents bread from staling, which could extend bread shelf life (Kurek & Wyrwisz, 2015). A relatively new and underexplored approach for functionalized bread formulation is supplementation of wheat flour with 1–5% waste from agro, food and essential oil industry (Dziki, Różyło, Gawlik-Dziki, & Świeca, 2014).

The lavender (*Lavandula angustifolia*) is widely utilized crop in the essential oil industry. Bulgaria, France, UK, China, Ukraine, Spain, and Morocco are the biggest worldwide producers. Bulgaria has recently become the world's major producer with 100 tons lavender oil per year. The melissa (*Melissa Officinalis*), known also as lemon balm or common

* Corresponding author at: 26 Maritza Blvd., Plovdiv 4002, Bulgaria.
E-mail addresses: ivelinavas@abv.bg (I. Vasileva), rositsa_denkova@mail.bg (R. Denkova), rosen4o4kov@abv.bg (R. Chochkov), d_teneva@mail.bg (D. Teneva), zdenkova@abv.bg (Z. Denkova), dessev@mail.bg (T. Dessev), petkodenev@orgchm.bas.bg (P. Denev), antons@uni-plovdiv.net (A. Slavov).

https://doi.org/10.1016/j.foodchem.2018.01.131
Received 5 September 2017; Received in revised form 13 December 2017; Accepted 21 January 2018
0308-8146/ © 2018 Elsevier Ltd. All rights reserved.

balm, is a well-known medicinal herb that has been used by mankind for more than 2000 years (Shakeri, Sahebkar, & Javadi, 2016). The lower quantity of essential oil in the lavender (0.8–1.3% / fresh plant) and melissa (0.014% in the fresh herb) results in enormous quantities of solid residues generated yearly. These wastes commonly were discarded directly in the nearby locations, and this could lead to serious environmental issues.

Nevertheless, it has been demonstrated undoubtedly that the lavender and melissa waste contain exploitable natural biologically active compounds (Elguea-Culebras et al., 2016), and can be recovered and utilized for the production of a variety of foodstuffs. Chemically, these compounds are mainly polyphenols and aroma substances, remained in the waste. Phenolic compounds have been proven to exhibit numerous beneficial biological activities including antioxidant, antimicrobial, insecticidal, anti-inflammatory, anti-carcinogenic, etc. (Galanakis, 2012; Martins, Barros, Henriques, Silva, & Ferreira, 2015). Martins et al. (2015) highlighted also the potential antifungal activity of caffeic, chlorogenic, gallic and quinic acids against *Candida* species. The aroma compounds, such as terpenes, linalool, citronellal, camphor, terpineol, geraniol, lynalil acetate, α- and β-pinene, etc., were found to possess antibacterial, anti-fungal, and insecticidal properties (Blažeković, Vladimir-Knežević, Brantner, & Štefan, 2010; Guil-Guerrero et al., 2016).

The process of recovery of biologically active substances from agricultural and food waste is often complex and several aspects have to be taken into account: the safety of the applied processes, the problems related with changes of the functional properties of the targeted compounds, the cost of the overall methodology, the scalability of the approaches, market release permissions, etc. (Galanakis, 2012; Galanakis & Schieber, 2014). According to the "5-Stages Universal Recovery Strategy" presented recently at 8th World Congress on Polyphenols Applications (Galanakis, 2014) every step, including macroscopic pretreatment, macro- and micro-molecules separation, extraction, isolation and purification, and product formation, had to be well thought out and designed in order to successfully valorize wastes and recover valuable compounds. In the last years, in order to resolve and improve recovery processes, new emerging technologies for production of biologically active substances were introduced (Galanakis, 2013) which help override the technological and scale-up limits of the traditional technologies. Additionally, certain wastes could be added to food products in order to functionalized them without isolation and purification of important biologically active substances (Dziki et al., 2014; Gawlik-Dziki et al., 2013; Prokopov et al., 2015) but this strategy have to be carefully balanced between functionality and taste, appearance and consumer acceptance of the final product.

Bakery products usually have a very short shelf life. Microbial spoilage of bakery products is mainly due to fungal and bacterial growth. The main fungal species belong to the genera *Aspergillus*, *Fusarium* and *Penicillium*, and the main roping bacteria: *Bacillus* sp., especially *B. subtilis* and *B. licheniformis* (Mentes, Ercan, & Akcelik, 2007). Nowadays globalization, automatization of processes, large scale production and increased consumer demand for high quality food products and longer shelf life have expanded the necessity for functional food additives such as dietary fibers, antistaling agents, and natural antioxidants in bread to achieve the desired qualities (Martinez-Saez et al., 2017; Mondal & Datta, 2008).

The aim of the present work was to characterize the lavender (*Lavandula angustifolia*) and melissa (*Melissa officinalis*) waste, and to investigate the effect of addition of lavender and melissa waste on quality, shelf life and consumers' acceptance of bread.

2. Materials and methods

2.1. Materials

The lavender (*Lavandula angustifolia*) and melissa (*Melissa officinalis*) wastes were provided by Zelenikovo distillery (Zelenikovo, region of Plovdiv, Bulgaria – 2016 harvest). The waste materials were inspected for impurities, dried at 50 °C under vacuum, and kept at −18 °C before further treatment.

The Folin-Ciocalteu's reagent, 2,2′-azobis[2-methyl-propionamidine] dihydrochloride, fluorescein, $CoF_2{\cdot}4H_2O$, picolinic acid, hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma-Aldrich (Steinheim, Germany). The standard compounds, i.e. gallic acid, 3,4-dihydroxy benzoic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, ellagic acid, catechin, epicatechin, naringin, myricetin, quercetin, naringenin and kaempferol were purchased from Sigma-Aldrich (Steinheim, Germany). The DPPH [2,2-diphenyl-1- picrylhydrazyl] was from Merck (Germany) and hydrogen peroxide was from Fluka (Germany).

The LBG-agar with composition: tryptone (10 g/L), yeast extract (5 g/L), NaCl (10 g/L), glucose (10 g/L) and agar (15 g/L), and with pH 7.5 was obtained from Laboratorios Conda S.A. (Madrid, Spain). The LBG-agar and saline solution (5 g/L NaCl, Merck, Germany) were sterilized (20 min at 121 °C) prior to use.

The test microorganisms used were as follow: Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* NBIMCC 1390; Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 19433, *Listeria monocytogenes* ATCC 19111; Yeasts: *Candida utilis* ATCC 42402; Saprophytic microorganisms – *Bacillus subtilis* ATCC 19659, *Aspergillus niger* ATCC 1015, *Penicillium chrysogenum* ATCC 28089, and baker's yeasts: *Saccharomyces cerevisiae* ATCC 9763. All strains were deposited in the culture collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria.

A standard commercial grade wheat flour type 500 (moisture 10.6%), baker's yeast, and salt were used for bread formulation. All the reagents used were of analytical grade and were provided by the local distributors.

2.2. Methods

2.2.1. Preparative methods

2.2.1.1. Ultrasound-assisted extraction with 70% ethanol. Prior to extraction the dry wastes were ground and sieved (0.5 mm). Breads were sliced (about 1.5 cm thick), dried (40 °C, 24 h), ground in a mill, and sieved (0.5 mm sieve). Ethanol extracts from lavender and melissa waste, and bread were obtained with 70% ethanol (solid to liquid ratio 1:20) in an ultrasonic bath (VWR, Malaysia; 45 kHz, 30 W) at 45 °C for 15 min.

2.2.1.2. Bread preparation. After several preliminary tests using different quantities of lavender and melissa wastes, two suitable combination were chosen: with 2.5% lavender and melissa (combination L2.5 and M2.5, respectively) and with 5% lavender and melissa (combination L5.0 and M5.0, respectively) waste added. The control sample was prepared only with wheat flour. The baking recipe was: wheat flour 500 g (or combination of wheat flour and waste), 1.5% salt and 3.0% fresh yeast, as well as water 280 mL (as guided by the preliminary baking tests and the results of farinograph measurements). The batches of dough were prepared at 29–30 °C in a kneading machine (Labomix 1000, Labomix Bt., Hungary) for 15 min then allowed to rest and ferment (Tecnopast CRN 45–12, Novacel ROVIMPEX Novaledo, Italy) at 35 °C and 80–85% relative humidity for 20 min. The dough was taken out of the proofing chamber, divided into pieces of similar weight (230 g), and then each piece was shaped, put in a greased metal pan and placed in the fermenting chamber for additional 60 min under the same conditions. After the final fermentation, the pieces of dough were put into an electric oven (SALVA E-25, Salva, Spain) pre-heated to 230 °C. The baking time was 25 min. After baking, the bread was allowed to cool down for 4 h at room temperature.

2.2.2. Analytical methods

2.2.2.1. Determination of lavender and melissa waste composition. The quantity of protein in the lavender and melissa wastes was determined by the Kjeldahl method with automated nitrogen analyzer UDK152 (Velp Scientifica, Italia) using Nx6.25 for calculation of protein. Crude lipid content was determined by hexane extraction with a Soxhlet apparatus (5 g waste material extracted twice with 100 mL hexane for 4 h). The lignin content was determined according to Theander, Westerlund, and Aman (1993). Mineral content was determined by igniting 5 g sample in a muffle furnace at 605 °C until constant weight. The total carbohydrate quantity of wastes was calculated by difference after determination of protein, crude lipid, lignin, total polyphenols, and ash quantities. The total (TDF), soluble (SDF) and insoluble (IDF) dietary fibers were determined by the enzymatic-gravimetric method, using the total dietary fiber assay kit Bioquant 1.12979.0001 (Merck, Germany) and the instructions provided by the manufacturer. The individual phenolic compounds were determined by liquid chromatography as described in Slavov et al. (2017)

2.2.2.2. Total polyphenol and flavonoid content, and in vitro antioxidant activity determination. Total polyphenols were determined according to the method of Singleton and Rossi (1965) with Folin-Ciocalteu's reagent. Gallic acid was employed as calibration standard and the results were expressed as gallic acid equivalents (GAE) per gram waste. Total flavonoids were determined using $Al(NO_3)_3$ reagent and measuring the absorbance at 415 nm according to Kivrak et al. (2009). The results were expressed as mg equivalents quercetin (QE) per gram waste.

The antioxidant activities were evaluated by four methods as described in Slavov et al. (2017). The DPPH radical method is based on mixed hydrogen atom transfer and single electron transfer mechanisms. The FRAP (Ferric Reducing Antioxidant Power) method is based only on single electron transfer mechanism. The ORAC (Oxygen Radical Absorbance Capacity) assay measures the ability of the antioxidant to scavenge peroxyl radicals via hydrogen atom transfer. The HORAC (Hydroxyl Radical Averting Capacity) method measures the metal-chelating activity of antioxidants under the conditions of Fenton-like reactions. The results of ORAC, DPPH and FRAP analysis were expressed as μM Trolox equivalents (TE) per gram waste. The results of HORAC analysis were expressed in μmol GAE per gram waste.

2.2.2.3. Determination of aroma substances in the ethanolic extracts of the wastes. Determination of the individual volatile compounds by GC–MS was performed as follow: 1.0 mL ethanolic extract was extracted with 1.0 mL dichloromethane (triple). The combined organic layers were dried under vacuum at 30 °C. To the dry residue 100 μL dichloromethane was added. For analysis 1.0 μL from the solution was injected on gas chromatograph Agilent GC 7890 with mas-selective detector Agilent MD 5975 and column HP-5 ms. The temperature regimen was: initial temperature 40 °C and then increase to 300 °C with 5 °C/min (hold for 10 min); injector and detector temperatures – 250 °C, helium was used as carrier gas at 1.0 mL/min. The scanning range of mass-selective detector was m/z = 40–400 in splitless mode.

The individual compounds were identified comparing the retention times and the relative index (RI) with those of standard substances and mas-spectral data from libraries of The Golm Metabolome Database (http://csbdb.mpimp-golm.mpg.de/csbdb/gmd/gmd.html) and NIST'08 (National Institute of Standards and Technology, USA).

2.2.2.4. Determination of antimicrobial activity of lavender and melissa extracts against pathogenic and saprophytic microorganisms. The test pathogenic and saprophytic microorganisms were cultured on LBG-agar at 37 ± 1 °C for 24–48 h. Using sterile loop biomass of the developed test pathogenic or saprophytic microorganisms was suspended in sterile saline solution in order to obtain suspensions of the test microorganisms.

The antimicrobial activity was studied by disc-diffusion method. Sterile melted LBG-agar medium was poured in Petri dishes and after hardening of the agar, the dishes were spread plated with suspensions of the test pathogenic or saprophytic microorganisms. Decimal dilutions of the lavender and melissa extract in saline solution were prepared. The experiments were conducted with dilutions 1×, 10× and 100× in order to determine the Minimum Inhibitory Concentration (MIC). The used paper discs were 6 mm in diameter. 6 μL of the corresponding dilution were pipetted on the corresponding paper discs. Paper discs soaked in 70% ethanol were used as blanks. The results were recorded as diameters of the clear zones around the paper discs, in millimeters, after 24–48 h of incubation of the Petri dishes at optimal temperature for the growth of the corresponding test-microorganism (at 30 °C for the saprophytic microorganisms and at 37 °C for the pathogenic microorganisms) (Jirovetz et al., 2006). The MIC was defined as the lowest concentration of the extract at which the microorganism did not demonstrate visible growth (Randrianarivelo et al., 2009). The experiments were performed in quadruplicate. The MICs, in μg/ml, were calculated on the basis of the obtained results.

2.2.2.5. Determination of dough and bread properties. The effect of lavender and melissa waste on dough properties during kneading was determined by a Brabender Farinograph (Brabender, Duisburg, Germany). The parameters determined were: water absorption (%), consistency (Farinograph units, FU), dough development time (min), stability (min), and degree of softening (FU), according to ICC-Standard (1992).

The height and diameter of the bread samples were measured in 10 replicates (at different positions of the bread samples) each by using digital caliper S301 (Hedue GmbH, Mönchengladbach, Germany), and the mean values were determined. On the basis of the results the H/D index (the ratio of height to diameter) of bread was calculated. The loaf volume of bread samples was measured 120 min after the loaves were removed from the oven by the seed displacement method using bread volumeter (Sadkiewicz Instruments, Bydgoszcz, Poland). Specific loaf volume of bread was calculated as the ratio of loaf volume to weight. The moisture content was determined with Kern MLB 50-3 moisture analyzer (Kern & Sohn GmbH, Germany).

2.2.2.6. Image analysis of bread crumb. The gas pores in bread crumb were assessed using a procedure (Image Analysis) issued from mathematical morphology proposed to characterize visual texture in relation to particle size (Rouille, Della-Valle, Devaux, Marion, & Dubreil, 2005). Digital images were taken on color flatbed scanner "HP Scanjet G4010". The image resolution was 2056x1280p with pixel depth 300 dots per inch. One hour after baking, bread loaves were cut with electric slicer (Master 182 slicer - Graef, Arnsberg, Germany) transversely in order to obtain slices about 2 cm thick. Few slices were obtained per each loaf of bread, but just the middle ones were used for analysis. Special attention was paid to avoid cells damaging during this procedure. All images were processed and analyzed with Image J (U.S. National Institutes of Health, Bethesda, Maryland, USA). The thresholding technique was based on the Isodata algorithm. Twenty-five dilation and erosion steps were applied on the initially equalized image in order to generate statistical data for the gas pore size distribution.

2.2.2.7. Sensory evaluation of bread. Sensory evaluation of bread was performed by hedonic test according to ISO 13299:2016 with the following indicators: volume, odor, crust color, crumb color, mastication, porosity, taste, and aftertaste. Briefly, the samples were sliced (slices about 1.5 cm thick), coded and served to untrained consumers. The panel consisted of 20 consumers (22–43 years old), who evaluated the bread's acceptability. The degree of liking for different types of bread based on a nine-point hedonic scale (1: dislike extremely, 5: neither like nor dislike, 9: like extremely). Plain water was used for mouth rinsing before and after each sample testing.

2.2.2.8. Determination of bacterial and mold spoilage of bread. Four hours after preparation breads were wrapped with plastic bags (commercial ones, used for storage and selling of breads; with 6 holes, each 0.5 mm in diameter) and stored at 22 °C (room temperature, RT) and 37 °C for bacterial spoilage measurement, and at 22 °C (RT) and 30 °C for mold spoilage in a laboratory water-jacketed incubator TB150 (Robotika, Velingrad, Bulgaria) at 45% ± 1% relative humidity. The appearance of bacterial spoilage was performed by 10 judges in the production laboratory and was evaluated according to a scale of four degrees corresponding to the following descriptions:

I. barely noticeable (pleasant fruity odor);
II. weak (change in the odor – distinct);
III. medium (moisty, sticky crumb, sharp odor);
IV. strong (unpleasant odor, brown-yellow crumb).

The determination of the appearance of mold spoilage was performed in the production laboratory and was based on the appearance of single mold colony.

2.3. Statistical analysis

The values were expressed as mean ± SD. Statistical significance was detected by analysis of variance (ANOVA, Tukey's test; value of $p < .05$ indicated statistical difference). The homogeneity of variances assumption was checked by Levene's test.

3. Results and discussion

3.1. Characteristics of the lavender and melissa waste

The lavender and melissa waste, even though generated in large quantities, were commonly discarded or used for soil replenishment (Lesage-Meessen, Bou, Sigoillot, Faulds, & Lomascolo, 2015). Some studies for valorization of the lavender and melissa waste, and waste water were performed (Ashori, Hamzeh, & Amani, 2011; Zheljazkov & Astatkie, 2012), but generally these valuable by-products were under-explored. To date chemical composition of lavender (*Lavandula angustifolia* Mill.) and melissa (*Melissa Officinalis* L.) waste could not be found in the literature, although some information which is based on the composition of undistilled leaves, flowers and stems exists (Kaloustian, Pauli, & Pastor, 2000). Data on the composition of distilled straws of French fine lavender and lavandin (presented as personal communication) were mentioned by Lesage-Meessen et al. (2015), and according to them there were no significant influence of the distillation on the basic constituents. The antioxidant properties of distilled straws from spike lavender and lavandin have been investigated and clear correlation with their phenolic content was found (Parejo et al., 2002). Berasategi et al. (2011) investigated the antioxidant stabilizing effect of aqueous-ethanolic extracts of *Melissa officinalis* on new type bologna-sausages and found significant benefits on the inhibition of lipid oxidation – lower peroxides (6.11 meqO_2/kg) and thiobarbituric acid (0.27 mg malonaldehyde/kg) values, and high antioxidant capacity (lipophilic fraction ABTS: 0.37 meq Trolox/g product). Besides, the polyphenol compounds present in the waste, were found to possess antimicrobial activity against various harmful microorganisms (Guil-Guerrero et al., 2016). In regard of potential application of the wastes as additive in foodstuffs and having in mind the scarce information in the literature it was necessary to investigate the composition and antioxidant activity of the wastes (Table 1).

The results from the analysis suggested that the lavender and melissa wastes were rich source of dietary fibers – 625.86 ± 18.69 mg/g dry weight (DW) and 573.57 ± 16.94 mg/g DW TDF for lavender and melissa waste, respectively. The hexane soluble fraction (crude lipids) in both wastes was below 5%. The carbohydrates and lignin moieties were the most abundant ingredients. The lignin amount in lavender waste (215.66 ± 12.35 mg/g DW) was comparable with the amounts of lignin reported by Kaloustian et al. (2000) and Lesage-Meessen et al. (2015). The lignin in melissa waste was slightly lower to the reported 27.85% acid soluble and insoluble lignin content in melissa agro-residues by Ashori et al. (2011). The amount of protein in both wastes was around 10%, while the mineral content of melissa residues was twice higher than in lavender biomass.

Table 1
Composition and antioxidant activity of lavender and melissa waste.

Parameter	Lavender	Melissa
Protein, mg/g DW	88.01 ± 8.72[a]	102.50 ± 9.61[a]
Crude lipids, mg/g DW	45.15 ± 6.94[a]	37.18 ± 7.83[a]
Lignin content, mg/g DW	215.66 ± 12.35[a]	234.87 ± 16.82[a]
Total carbohydrates, mg/g DW	430.59 ± 14.54[a]	418.98 ± 21.05[a]
TDF, mg/g DW	625.86 ± 18.69[a]	573.57 ± 16.94[b]
SDF, mg/g DW	42.63 ± 5.71[a]	75.98 ± 6.32[b]
IDF, mg/g DW	549.77 ± 16.26[a]	506.67 ± 12.50[b]
Ash content, mg/g DW	56.03 ± 2.26[a]	137.11 ± 1.44[b]
Total polyphenols, mg/g DW	11.58 ± 0.24[a]	12.74 ± 0.36[b]
Total flavonoids, mg QE/g DW	6.89 ± 0.16[a]	5.96 ± 0.18[b]
Neochlorogenic acid, mg/g DW	0.59 ± 0.05[a]	0.49 ± 0.06[a]
Caffeic acid, mg/g DW	0.68 ± 0.06[a]	0.60 ± 0.08[a]
p-Coumaric acid, mg/g DW	1.36 ± 0.12[a]	0.02 ± 0.01[b]
Ferulic acid, mg/g DW	0.29 ± 0.05	–
3, 4-dihydroxy-benzoic acid, mg/g DW	0.71 ± 0.09[a]	0.48 ± 0.07[b]
Gallic acid, mg/g DW	–	1.33 ± 0.14
Rosmarinic acid, mg/g DW	2.56 ± 0.43[a]	4.40 ± 0.39[b]
Quercetin, mg/g DW	0.31 ± 0.04[a]	1.32 ± 0.13[b]
Quercetin-3-β-glucoside, mg/g DW	2.05 ± 0.32[a]	0.55 ± 0.05[b]
Myricetin, mg/g DW	0.61 ± 0.07[a]	0.25 ± 0.02[b]
Kaempferol, mg/g DW	0.24 ± 0.04[a]	0.27 ± 0.03[a]
Naringin, mg/g DW	0.91 ± 0.08[a]	0.63 ± 0.06[b]
Naringenin, mg/g DW	0.08 ± 0.01[a]	0.12 ± 0.02[a]
Catechin, mg/g DW	1.69 ± 0.14[a]	2.44 ± 0.26[b]
Epicatechin, mg/g DW	0.90 ± 0.09[a]	0.44 ± 0.08[b]
DPPH, μmol TE/g DW	788.45 ± 19.05[a]	848.61 ± 20.27[b]
FRAP, μmol TE/g DW	856.23 ± 14.12[a]	988.51 ± 17.15[b]
ORAC, μmol TE/g DW	13325.2 ± 119.9[a]	14688.4 ± 254.6[b]
HORAC, μmol GAE/g DW	4550.4 ± 74.8[a]	4781.1 ± 55.9[b]

TDF: total dietary fibers.
SDF: soluble dietary fibers.
IDF: insoluble dietary fibers.
DW: dry weight waste.
QE: quercetin.
DPPH: 1,1-diphenyl-2-picrylhydrazyl assay.
FRAP: ferric reducing antioxidant power assay.
ORAC: Oxygen Radical Absorbance Capacity assay.
HORAC: Hydroxyl Radical Averting Capacity assay.
TE: Trolox equivalents.
GAE: Gallic acid equivalents.
The results were expressed as mean ± SD (n = 3).
[a,b]Different letters mean statistical difference (Tuckey's HSD test, $p < .05$).

The ethanolic extracts of lavender and melissa wastes were rich in polyphenolic compounds – more than one third of the extracted substances (on dry weight basis) were polyphenols, and half of them were flavonoids (expressed as quercetin equivalents). The most abundant phenolic substance in both wastes was rosmarinic acid: 2.56 ± 0.43 and 4.40 ± 0.39 mg/g DW residue for lavender and melissa waste, respectively. The highest content in lavender waste had the flavonoids quercetin-3-β-glucoside (2.05 ± 0.32 mg/g DW) and catechin (1.69 ± 0.14 mg/g DW), and for melissa waste – catechin and quercetin (2.44 ± 0.26 and 1.32 ± 0.13 mg/g DW, respectively). The melissa waste showed higher antioxidant activity with all the methods used. The highest amount of polyphenols in the melissa extract than lavender suggested that the polyphenolic compounds were the main contributors of the observed antioxidant activity. Similar conclusion was made by Slavov et al. (2017) while investigating the antioxidant properties of extracts from *Rosa damascena* waste.

The steam distillation process extracts the majority of the essential

oil components but significant part remains in the distilled biomass (Tiliacos, Gaydou, Bessière, & Agnel, 2008; Zheljazkov & Astatkie, 2012). In subsequent experiments, in order to determine the composition of volatile substances, ethanolic extracts were subjected to GC–MS analysis (Supplementary Material 1).

Thirty components were identified in the melissa ethanol extract with the monoterpenes being prevalent: citronellal (17.61%), β-citronellol (9.83%), geraniol (14.77%), geranyl acetate (6.65%), the monoterpene aldehyde geranial (5.22%), and the sesquiterpene α-elemol (6.01%). Citronellal and geranial had insecticidal, antifungal, antioxidant and antimicrobial functions and they were widely applied in medicine and food industry (Singh et al., 2012). The composition of the lavender ethanol extract consisted of 32 compounds and the predominant components were the monoterpenes: linalool (34.09%), linalyl acetate (24.24%), the sesquiterpenes β-caryophyllene (5.56%) and (E)-β-farnesene (5.53%). Linalool and linalyl acetate were found to inhibit the growth of *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* (Jirovetz et al., 2006).

Based on the results for content of aroma substances and polyphenol compounds in the wastes, and the fact that most of these compounds possess antimicrobial properties, in the subsequent experiments the antimicrobial activity of waste extracts was evaluated (Table 2).

The lavender extract demonstrated antimicrobial activity against all tested pathogens. *Staphylococcus aureus* ATCC 25923 was the most sensitive towards the lavender extract (MIC 60 μg/ml), while for all other pathogens the MICs were above 600 μg/ml. The melissa ethanolic extract inhibited the growth of *Pseudomonas aeruginosa* NBIMCC 1390, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 19433 and *Candida utilis* ATCC 42402, with MICs above 600 μg/ml (Table 2).

Table 2
Antimicrobial activity of lavender and melissa extracts against pathogenic and saprophytic microorganisms.

Microorganism		Blank	Lavender extract	Melissa extract
Escherichia coli ATCC 25922, 1.3×10^9 cfu/cm^3	IZ, mm	–	9.18 ± 0.27	–
	MIC, μg/ml	–	< 600	–
Proteus vulgaris ATCC 6380, 5.2×10^9 cfu/cm^3	IZ, mm	–	9.17 ± 0.29	–
	MIC, μg/ml	–	< 600	–
Pseudomonas aeruginosa NBIMCC 1390, 2×10^{10} cfu/cm^3	IZ, mm	–	8.67 ± 0.58[a]	8.17 ± 0.29[a]
	MIC, μg/ml	–	> 600	> 600
Staphylococcus aureus ATCC 25923, 5×10^8 cfu/cm^3	IZ, mm	–	11.67 ± 1.53[a]	8.50 ± 0.50[b]
	MIC, μg/ml	–	60	> 600
Enterococcus faecalis ATCC 19433, 2.5×10^8 cfu/cm^3	IZ, mm	–	9.15 ± 0.25[a]	8.17 ± 0.27[a]
	MIC, μg/ml	–	< 600	> 600
Listeria monocytogenes ATCC 19111, 5×10^8 cfu/cm^3	IZ, mm	–	9.17 ± 0.29	–
	MIC, μg/ml	–	< 600	–
Candida utilis ATCC 42402, 2.6×10^7 cfu/cm^3	IZ, mm	–	9.19 ± 0.24[a]	8.67 ± 0.58[a]
	MIC, μg/ml	–	< 600	> 600
Aspergillus niger ATCC 1015, 1×10^5 cfu/cm^3	IZ, mm	–	–	–
	MIC, μg/ml	–	–	–
Penicillium chrysogenum ATCC 28089, 1×10^5 cfu/cm^3	IZ, mm	–	8.67 ± 0.58[a]	8.00 ± 0.20[a]
	MIC, μg/ml	–	> 600	> 600
Bacillus subtilis ATCC 19659, 1×10^5 cfu/cm^3	IZ, mm	–	12.00 ± 0.65[a]	9.17 ± 0.54[b]
	MIC, μg/ml	–	60	600
Saccharomyces cerevisiae ATCC 9763, 1×10^5 cfu/cm^3	IZ, mm	–	–	–
	MIC, μg/ml	–	–	–

IZ: inhibition zone.
MIC: minimal inhibition concentration.
Cfu: colony forming units.
The results were expressed as mean ± SD (n = 4).
[a,b]Different letters mean statistical difference (Tuckey's HSD test, $p < .05$).

The lavender and melissa ethanol extracts demonstrated antimicrobial activity against the fungi *Penicillium chrysogenum* ATCC 28089 and the bacteria *Bacillus subtilis* ATCC 19659. The MICs against *Penicillium chrysogenum* ATCC 28089 was above 600 μg/ml, while against *Bacillus subtilis* ATCC 19659 the MIC of the lavender ethanol extract was lower (60 μg/ml) in comparison with that of the melissa ethanol extract (600 μg/ml) (Table 2). Both extracts exhibited no inhibitory effect on baker's yeast *Saccharomyces cerevisiae* ATCC 9763.

The observed antimicrobial activity of the lavender and melissa ethanol extracts against pathogenic and saprophytic microorganisms was due to the presence of a number of components with proven antimicrobial activity against different pathogens. Limonene, α-terpineol, geraniol, geranyl acetate, β-caryophyllene and linalool were found in both extracts, and they have proven antimicrobial activity against *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Yersinia enterocolitica, Salmonella pullorum* (Dorman & Deans, 2000). α-terpineol exhibited antimicrobial activity against *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Yersinia enterocolitica, Salmonella pullorum* (Dorman & Deans, 2000). Geraniol demonstrated antimicrobial activity against *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella* sp. and *Candida albicans* (Jirovetz et al., 2006). Geranyl acetate was found to inhibit the growth of *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Yersinia enterocolitica, Salmonella pullorum* (Dorman & Deans, 2000). β-caryophyllene was shown to possess anti-inflammatory, antispasmodic and anti-colic action, and it was included in the composition of a wide range of drugs for symptomatic treatment (Park et al., 2011). Besides, it had antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Candida albicans* (Jirovetz et al., 2006). Linalool had insecticidal, antitumor, anti-inflammatory and antimicrobial action against *Staphylococcus aureus, Escherichia coli, Yersinia enterocolitica, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and it was used in medicine, food and plant protection (Jirovetz et al., 2006). These results suggested that the lavender and melissa wastes had a potent antimicrobial activity and could be used as an additive in foodstuffs. The observations that both extracts exhibited antimicrobial activity against *Bacillus subtilis* ATCC 19659 but did not influenced the development of the baker's yeast *Saccharomyces cerevisiae* ATCC 9763 implied that the wastes could be used for improvement of the shelf life of bakery products having in mind that the main roping bacteria are *Bacillus* sp., especially *B. subtilis* and *B. licheniformis* (Mentes et al., 2007).

3.2. Preparation and characterization of breads with lavender and melissa wastes

The demonstrated potent antimicrobial activity of the lavender and melissa waste allows their possible inclusion in the composition of a variety of food products in order to improve their microbiological safety and to extend their shelf life, thus acting as a biopreservative agents. Besides, both wastes were rich source of dietary fibers and polyphenols and could contribute to the functionality of the foodstuffs. In order to asses this possibility, breads with addition of lavender and melissa wastes were prepared. The addition of ingredients rich in dietary fibers is well known to affects the properties of dough, processing and final product characteristics (Kurek & Wyrwisz, 2015; Prokopov et al., 2015). For this reason the Farinograph characteristics of doughs with 2.5 and 5% of melissa and lavender waste were determined and compared with the control dough (made with white flour) (Table 3).

The water absorption for combinations M2.5 and M5.0 increased to 63.4 and 64.9%, respectively, compared to control: 62.8%. Addition of 2.5% and 5% lavender waste increased the water absorption to 63.9%

Table 3
Characteristics of dough and bread prepared with melissa and lavender waste.

Sample	Dough characteristics					Bread characteristics			
	Water absorption, %	Consistence, FU	Dough development time, min	Stability, min	Breakdown, FU	H/D index	Loaf volume, cm^3	Loaf specific volume, cm^3/g	Moisture content, %
C	62.8 ± 0.58[a]	490 ± 10[a]	1.5 ± 0.10[a]	10.5 ± 0.2[a]	120 ± 10[a]	0.49 ± 0.02[a]	925 ± 5[a]	4.63 ± 0.2[a]	44.33 ± 2.32[a]
L2.5	63.9 ± 0.61[a]	500 ± 11[a]	1.5 ± 0.12[a]	7.5 ± 0.3[b]	200 ± 6[b]	0.63 ± 0.06[b]	800 ± 10[b]	4.00 ± 0.3[a]	44.11 ± 2.15[a]
L5.0	65.1 ± 0.49[b]	500 ± 10[a]	1.5 ± 0.11[a]	4.0 ± 0.6[c]	320 ± 9[c]	0.59 ± 0.05[b]	750 ± 11[c]	3.75 ± 0.5[b]	43.25 ± 1.98[a]
M2.5	63.4 ± 0.54[a]	520 ± 9[b]	1.5 ± 0.09[a]	6.5 ± 0.8[b]	200 ± 9[b]	0.53 ± 0.07[a]	730 ± 12[c]	3.65 ± 0.6[b]	43.51 ± 1.85[a]
M5.0	64.9 ± 0.52[b]	520 ± 8[b]	1.5 ± 0.10[a]	6.5 ± 0.7[b]	190 ± 8[b]	0.52 ± 0.03[a]	630 ± 15[d]	3.15 ± 0.3[b]	43.34 ± 1.36[a]

FU: Farinograph units.
H/D index: the ratio of height to diameter of the bread.
The results were expressed as mean ± SD (n = 3).
[a,b,c,d]Different letters mean statistical difference compared to control sample (Tuckey's HSD test, $p < .05$).

and 65.1%, respectively. The dough breakdown was the lowest for the control sample: 120 Farinograph Units (FU). Addition of 2.5% and 5% lavender and melissa waste, as expected, increased the breakdown value and the highest value was observed for M5.0 combination: 320 FU. These results were expected since the gluten concentration and interactions in the doughs with lavender and melissa waste were reduced and the viscoelastic resistance of the doughs decreased. The same trend was observed for the stability of dough combinations: the highest value showed the control sample (10.5 min) and addition of lavender and melissa waste led to decreased stability. Sindhuja, Sudha, and Rahim (2005) reported similar results for farinograph characteristics of cookie dough incorporated with amaranth flour. Yousif, Nhepera, and Johnson (2012) investigated the effect of sorghum flour addition on flat bread and found that water absorption and stability were the highest for the control (100% flour) sample. Replacement of 30%, 40% and 50% of the wheat flour with sorghum flour reduced the dough water absorption and stability. Increasing the replacement amount of white flour resulted in a decrease in dough stability as previously reported by Elkhalifa and El-Tinay (2002). The dough development time was found similar (around 1.5 min) for all the combinations.

Based on the preliminary experiments and the data from rheological measurements of the dough, bread samples were prepared. The baked breads were examined for their loaf volume, bread weight, H/D index, loaf specific volume, and moisture content (Table 3). The addition of melissa or lavender waste, compared to the control sample (925 ± 5 cm^3) generally decreased the loaf volume. The closest value of loaf volume showed L2.5 combination: 800 ± 10 cm^3. The highest decrease in the loaf volume was observed for M5.0: 630 cm^3 which indicated a dense and compact bread crumb. Similar results for volume decrease of sponge cakes supplemented with 10% and 20% cabbage leaf powder were observed by Prokopov et al. (2015). The decrease in loaf volume may be attributed to lower gas retention of dough or to its toughening effect on gluten proteins. This may be also due to the stiff consistency of dough which is an indicator for a decrease in the water absorption of the samples with added waste. The moisture content was comparable for all breads and the results were statistically insignificant: from 43.51 ± 1.85% (M2.5) to 44.33 ± 2.32% (control sample).

A reduction in the loaf specific volume with the increase of the quantity of melissa or lavender waste added was observed. The highest loaf specific volume was observed for the control: 4.63 cm^3. The specific loaf volume of sample L2.5 was closest to that of the control: 4.00 cm^3. The difference between the values of the control sample C and sample M5.0 and L5.0 expressed as percentage were 31.96% and 19%, respectively. According to Sullivan et al. (2011), the effect of different additives on wheat flour consisted in decrease of the loaf volume and the specific loaf volume of bread. These observations were confirmed for the breads prepared with the addition of melissa or lavender wastes. The control bread had the lowest H/D index (0.49) while the L2.5 had the highest H/D index (0.63). With an increase of the melissa or lavender waste added, the H/D index decreased slightly, but nevertheless the results for all combinations were higher than those of the control bread.

The addition of lavender and melissa wastes improved the functionality of the breads (Table 4). The amount of total dietary fibers was two and three times higher for the M2.5 and L2.5, and M5.0 and L5.0, respectively, compared to the control bread. The same trend was observed for the quantities of soluble and insoluble dietary fibers, and polyphenols and flavonoids. The higher concentrations of polyphenolic compounds in the breads with addition of lavender and melissa wastes reflected also on their antioxidant properties. The observed antioxidant activity for the M2.5 and L2.5 was four and five times higher (by DPPH and FRAP method), respectively, compared to control bread. Increased antioxidant capacity of breads was also observed by Gawlik-Dziki et al. (2013) while investigating the quality and antioxidant properties of breads enriched with 2–3% dry onion skin powder.

Table 4
Total, soluble and insoluble dietary fibers, total polyphenols and antioxidant activity of breads prepared with lavender and melissa waste.

Bread sample	TDF, %	SDF, %	IDF, %	Total polyphenols, mg/g DW	Total flavonoids, mg quercetin/g DW	DPPH, μmol TE/g DW	FRAP, μmol TE/g DW
Control	3.72 ± 0.26[a]	0.69 ± 0.05[a]	2.89 ± 0.12[a]	0.67 ± 0.07[a]	0.19 ± 0.05[a]	0.49 ± 0.02[a]	11.10 ± 0.58[a]
L2.5	6.87 ± 0.29[b]	1.32 ± 0.08[b]	5.73 ± 0.28[b]	1.78 ± 0.08[b]	0.39 ± 0.04[b]	2.15 ± 0.08[b]	39.54 ± 0.98[b]
L5.0	10.35 ± 0.56[c]	2.57 ± 0.11[c]	7.94 ± 0.54[c]	3.59 ± 0.17[c]	0.75 ± 0.04[c]	4.27 ± 0.10[c]	88.14 ± 0.96[c]
M2.5	7.71 ± 0.32[b]	1.24 ± 0.09[b]	6.55 ± 0.31[b]	2.20 ± 0.11[d]	0.45 ± 0.06[b]	2.86 ± 0.09[d]	55.52 ± 1.02[d]
M5.0	9.89 ± 0.45[c]	2.38 ± 0.12[c]	7.31 ± 0.44[c]	4.18 ± 0.15[e]	0.89 ± 0.05[c]	5.47 ± 0.14[e]	118.68 ± 1.25[e]

TDF: total dietary fibers.
SDF: soluble dietary fibers.
IDF: insoluble dietary fibers.
DW: dry weight bread.
The results were expressed as mean ± SD (n = 3).
[a,b,c,d,e]Different letters mean statistical difference (Tuckey's HSD test, $p < .05$).

3.3. Image analysis of bread crumb

During baking the growth of the gas bubbles determines the expansion of the dough and therefore the ultimate volume and texture of the baked product. The limit of expansion is related to the rupture of the bubble walls caused by the loss in gas retention. Thus, the expansion depends on the dynamic equilibrium between internal pressure in the gas cells and visco-elastic properties of the continuous dough phase. As temperature rises, gases move to the air cells formed during mixing, enlarging them and the product increases in size and volume – the so called oven spring. However, crust formation is a factor limiting the bread expansion during baking. Crust formation restricts the expansion of the closed gas cells in the dough resulted in increased internal pressure, additionally stressing the cell membranes. If they are not able to resist on the increased pressure, the cell membranes will rupture on very early stage of baking and the cells will coalesce, resulting in low loaf volume and coarse crumb structure (He & Hoseney, 1991).

Gas pores were evaluated on two central slices (20 mm thickness) from each loaf. The gas pore area distribution (Supplementary Material 2A) is represented by means of six pre-selected dimensional classes based on the area occupied by each class (expressed as a percentage of the number of pores for each class compared to the total number of pores). In this study the percentage of gas cells having area $< 1\ mm^2$ was considered as a quantitative factor that can be used to define crumb fineness. As can be seen in Supplementary Material 2A in all analyzed samples more than 50% of the gas pores have area less than $1\ mm^2$. The lowest value of the mean gas pore area had M5.0 ($1.81 \pm 0.20\ mm^2$) bread, while the highest was calculated for L2.5: $2.53 \pm 0.37\ mm^2$. The results presented in Supplementary Material 2B were in reasonable agreement with those for the specific volume of the bread samples (Table 3).

3.4. Sensory evaluation of breads

Sensory evaluation of bread samples prepared with melissa and lavender waste, and control sample was conducted (Fig. 1 and Supplementary Material 3). The test panel preferred in all the degustations the breads with addition of 2.5% waste than these with 5%. The aftertaste of all bread samples was almost similar compared to control. The taste was found to differ slightly but in general the consumers did not find it unpleasant. The crumb color was less intense than that of the control bread sample especially for breads with added melissa wastes.

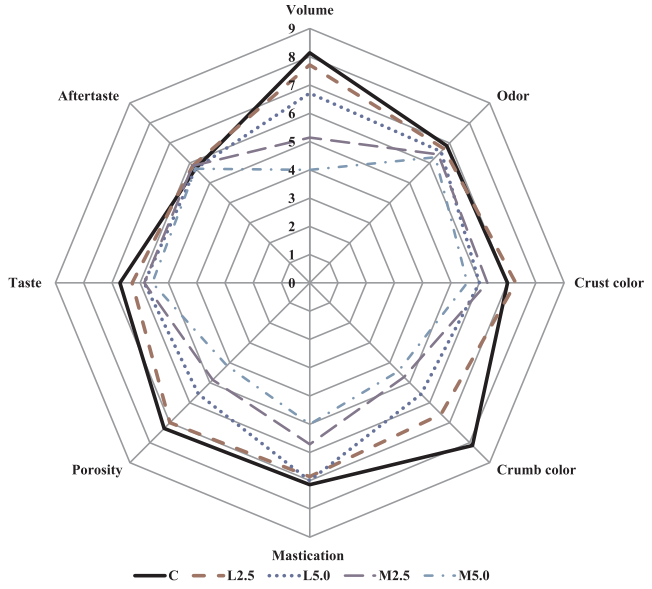


Fig. 1. Sensory profile of breads with lavender and melissa wastes.

The mastication of the lavender breads was found almost unchanged compared to control but for the melissa breads there were slightly negative sensations by the consumers. The porosity of the bread samples prepared with the inclusion of melissa or lavender waste slightly decreased compared to the control bread. The difference with the control sample was 3.8% and 19.6% for L2.5 and L5.0, respectively, and 33.4% and 43.2% for M2.5 and M5.0, respectively. The volume of the melissa breads decreased and was found quite unacceptable, especially for the bread with 5% waste. The volume of the breads with lavender wastes was close to the control bread, especially for the L2.5 sample. The overall acceptability (Supplementary Material 3) had the highest score for the control bread, although the values were not significantly different from the results for L2.5, L5.0 and M2.5. The L2.5 combination had closer value to the control bread and was the preferred by the test panel combination. The M5.0 had the lowest overall acceptability (values significantly different from the control bread) and is considered not suitable as variant for bread preparation.

3.5. Bacterial and fungal spoilage of the breads

The prepared breads were stored at 37 °C and at room temperature (RT, 22 °C) for determination of the appearance of bacterial spoilage, and at 30 °C and at RT for determination of the appearance of fungal spoilage (Table 5). It was observed that the earliest appearance of bacterial spoilage was in the control bread: on the 96th hour after taking the loaves out of the oven at 37 °C, while no bacterial spoilage was determined even at the 96th hour at room temperature. According to the standard requirements there should be no signs of bacterial decay up to the 48th hour. So, all bread samples met the standard requirements for microbiological purity of bakery products. Upon addition of 2.5% or 5% melissa waste there were signs of bacterial spoilage on the 96th hour at 37 °C, but the bread samples stored at room temperature showed no signs of roping on the 96th hour. No bacterial spoilage was established on the 96th hour in the bread samples with 2.5% and 5% lavender waste (Table 5). The observed delay in bacterial spoilage of

Table 5
Bacterial and fungal spoilage, during storage of the breads at 37 °C, 30 °C and at room temperature (RT, 22 °C).

Time, h	Spoilage	T, °C	Control	L2.5	L5.0	M2.5	M5.0
24	Bacterial	37	–	–	–	–	–
		RT	–	–	–	–	–
	Fungal	30	–	–	–	–	–
		RT	–	–	–	–	–
48	Bacterial	37	–	–	–	–	–
		RT	–	–	–	–	–
	Fungal	30	–	–	–	–	–
		RT	–	–	–	–	–
72	Bacterial	37	–	–	–	–	–
		RT	–	–	–	–	–
	Fungal	30	Yes	–	–	Yes	–
		RT	–	–	–	–	–
96	Bacterial	37	Yes-I	–	–	Yes-I	Yes-I
		RT	–	–	–	–	–
	Fungal	30	Yes	–	–	Yes	Yes
		RT	–	–	–	–	–
120	Bacterial	37	Yes-II	Yes-I	Yes-I	Yes-II	Yes-I
		RT	Yes-I	–	–	–	–
	Fungal	30	Yes	Yes	Yes	Yes	Yes
		RT	–	–	–	–	–

Degree of bacterial spoilage:
I. barely noticeable (pleasant fruity odor).
II. weak (change in the odor – distinct).
III. medium (moisty, sticky crumb, sharp odor).
IV. strong (unpleasant odor, brown-yellow crumb).
Fungal spoilage: the first appearance of single mold colony
The experiments were performed in quadruplicate.

the breads L2.5 and L5.0 were result of lavender waste antibacterial activity, demonstrated in the examination of the antimicrobial activity of the lavender ethanol extract and for the prevalence of linalool and linalyl acetate (Supplementary Material 1).

The earliest appearance of fungal spoilage was in the control bread: on the 72nd hour at 30 °C, but at RT no fungal spoilage was detected even on the 120th hour. Upon addition of 2.5% and 5% melissa waste the first signs of fungal spoilage became noticeable on the 96th hour at 30 °C, but at RT no fungal spoilage was detected even on the 120th hour. Upon addition of 2.5% and 5% lavender waste the first signs of fungal spoilage became noticeable on the 120th hour at 30 °C, but at RT no fungal spoilage was detected even on the 120th hour (Table 5). The observed delay in fungal spoilage of the bread with lavender waste could be a result of its antifungal activity, demonstrated in the examination of the antimicrobial activity of the lavender ethanol extract. Similar results were obtained by Park and Chung (2003) investigating the effect of rosemary, lemon balm and lavender on the fungal growth of bread at 30 °C. They have found that addition of 3% of the fresh herbs prevent fungal growth up to the 96 h. An advantage of the present study was utilization of wastes of essential oil industry (lavender and mellisa) for preparation of breads, and the results suggested that they could effectively delay fungal growth.

4. Conclusions

In this work the addition of 2.5% and 5% lavender and melissa waste, as a novel approach of bread preparation, was investigated. This approach allows functionalization of the breads, increase of their shelf life and valorization and utilization of cheap and abundant lavender and melissa wastes. A detailed analysis of the chemical composition and antioxidant activity of both wastes was performed and to the best of our knowledge such information for the melissa waste was missing in the scientific literature till now.

The addition of melissa and lavender waste decreased both the loaf volume and specific loaf volume of bread. The moisture content of all bread samples was comparable. Addition of melissa and lavender waste increased the H/D index, dietary fiber content and antioxidant activity of bread. The sensory evaluation showed that the addition of 5% melissa and lavender waste influenced slightly negatively consumers' perception and the test panel preferred mostly L2.5. The lavender and melissa ethanol extracts exhibited antimicrobial activity against pathogenic and saprophytic microorganisms due to the antimicrobial properties of the phenolic and aroma substances in the wastes. The shelf life of the breads prepared with the inclusion of lavender waste was extended due to the inhibition of saprophytic microorganisms. The demonstrated high antimicrobial activity of the lavender ethanol extract allows possible inclusion of lavender waste (or its extracts) in the composition of a variety of food products in order to improve their microbiological safety and to extend their shelf life acting as a biopreservative agents.

Acknowledgments

This research has been financially supported by the Scientific Fund (project № 01/17-H 2017) of the University of Food Technologies – Plovdiv, Bulgaria. The authors would like to thank the Zelenikovo distillery, (Zelenikovo, region of Plovdiv, Bulgaria) for providing the lavender and melissa waste. The authors declare no conflict of interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2018.01.131.

References

Ashori, A., Hamzeh, Y., & Amani, F. (2011). Lemon Balm (*Melissa officinalis*) stalk: Chemical composition and fiber morphology. *Journal of Polymer Environment, 19*, 297–300.

Berasategi, I., Legarra, S., García-Íñiguez de Ciriano, M., Rehecho, S., Calvo, M. I., Cavero, R. Y., et al. (2011). "High in omega-3 fatty acids" bologna-type sausages stabilized with an aqueous-ethanol extract of *Melissa officinalis*. *Meat Science, 88*, 705–711.

Blažeković, B., Vladimir-Knežević, S., Brantner, A., & Štefan, M. B. (2010). Evaluation of antioxidant potential of *Lavandula x intermedia* Emeric ex loisel. 'Budrovka': a comparative study with *L. Angustifolia* mill. *Molecules, 15*, 5971–5987.

Dorman, H., & Deans, S. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*, 308–316.

Dziki, D., Różyło, R., Gawlik-Dziki, U., & Świeca, M. (2014). Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. *Trends in Food Science & Technology, 40*, 48–61.

Elguea-Culebras, G. O., Sanchez-Vioque, R., Santana-Meridas, O., Herraiz-Penalver, D., Carmona, M., & Berruga, M. I. (2016). In vitro antifungal activity of residues from essential oil industry against *Penicillium verrucosum*, a common contaminant of ripening cheeses. *LWT – Food Science and Technology, 73*, 226–232.

Elkhalifa, A. E. O., & El-Tinay, A. H. (2002). Effect of cysteine on bakery products from wheat-sorghum blends. *Food Chemistry, 77*, 133–137.

Galanakis, C. M. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. *Trends in Food Science & Technology, 26*, 68–87.

Galanakis, C. M. (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: A viewpoint of opportunities and challenges (Short communication). *Food and Bioproducts Processing, 91*(4), 575–579.

Galanakis, C. M. (2014). Universal Strategy for the recovery of polyphenols: targeting industrial applications. In *8th World Congress on Polyphenols Applications. Book of Abstracts. International Society of Antioxidants in Nutrition and Health (ISANH), the French Society of Antioxidants (SFA), and the Japanese Society of Antioxidants (JSA), 6 June 2014*, (pp. 45). Lisbon, Portugal.

Galanakis, C. M., & Schieber, A. (2014). Editorial. *Food Research International, 65*, 299–300.

Gawlik-Dziki, U., Świeca, M., Dziki, D., Baraniac, B., Tomilo, J., & Czyż, J. (2013). Quality and antioxidant properties of breads enriched with dry onion (*Allium cepa* L.) skin. *Food Chemistry, 138*, 1621–1628.

Guil-Guerrero, J. L., Ramos, L., Moreno, C., Zúñiga-Paredes, J. C., Carlosama-Yepez, M., & Ruales, P. (2016). Antimicrobial activity of plant-food by-products: A review focusing on the tropics. *Livestock Science, 189*, 32–49.

He, H., & Hoseney, R. C. (1991). Gas retention of different cereal flours. *Cereal Chemistry, 68*, 334–336.

ICC-Standard No 115/1. (1992). Method for using the Brabender Farinograph.

Jirovetz, L., Eller, G., Buchbauer, G., Schmidt, E., Denkova, Z., Stoyanova, A., et al. (2006). Chemical composition, antimicrobial activities and odor description of some essential oils with characteristic floral-rosy scent and of their principal aroma compounds. *Recent Research Developvments in Agronomy & Horticulture, 2*, 1–12.

Kaloustian, J., Pauli, A. M., & Pastor, J. (2000). Chemical and thermal analysis of the biopolymers in the lavandin. *Journal of Applied Polymer Science, 77*, 1629–1641.

Kivrak, I., Duru, M., Öztürk, M., Mercan, N., Harmandar, M., & Topçu, G. (2009). Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. *Food Chemistry, 116*, 470–479.

Kurek, M., & Wyrwisz, J. (2015). The application of dietary fiber in bread products. *Journal of Food Processing and Technology, 6*, 1–4.

Lesage-Meessen, L., Bou, M., Sigoillot, J. C., Faulds, C. B., & Lomascolo, A. (2015). Essential oils and distilled straws of lavender and lavandin: a review of current use and potential application in white biotechnology. *Applied Microbiology and Biotechnology, 99*, 3375–3385.

Martinez-Saez, N., García, A. T., Pérez, I. D., Rebollo-Hernanz, M., Mesías, M., Morales, F. J., et al. (2017). Use of spent coffee grounds as food ingredient in bakery products. *Food Chemistry, 216*, 114–122.

Martins, N., Barros, L., Henriques, M., Silva, S., & Ferreira, I. (2015). Activity of phenolic compounds from plant origin against Candida species. *Industrial Crops and Products, 74*, 648–670.

Mentes, O., Ercan, R., & Akcelik, M. (2007). Inhibitor activities of two *Lactobacillus* strains, isolated from sourdough, against rope-forming *Bacillus* strains. *Food Control, 18*, 359–363.

Mondal, A., & Datta, A. K. (2008). Bread baking – A review. *Journal of Food Engineering, 86*, 465–474.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Flerlage, N., Burillo, J., & Codina, C. (2002). Comparison between the radical scavenging activity and antioxidant activity of six distilled and nondistilled Mediterranean herbs and aromatic plants. *Journal of Agricultural and Food Chemistry, 50*, 6882–6890.

Park, I.-D., & Chung, D.-O. (2003). Studies on the physiological and sensory properties of herbs bread. *Korean Journal of Food and Cookery Science, 19*, 539–545.

Park, K.-R., Nam, D., Yun, H.-M., Lee, S.-G., Jang, H.-J., Sethi, G., et al. (2011). B-Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. *Cancer Letters, 312*, 178–188.

Prokopov, T., Goranova, Z., Baeva, M., Slavov, A., & Galanakis, C. (2015). Effects of powder from white cabbage outer leaves on sponge cake quality. *International Agrophysics, 29*, 493–500.

Randrianarivelo, R., Sarter, S., Odoux, E., Brat, P., Lebrun, M., Romestand, B., et al. (2009). Composition and antimicrobial activity of essential oils of *Cinnamosma*

fragrans. *Food Chemistry, 114*, 680–684.
Rouille, J., Della-Valle, G., Devaux, M. F., Marion, D., & Dubreil, L. (2005). French bread loaf volume variations and digital image analysis of crumb grain changes induced by the minor components of wheat flour. *Cereal Chemistry, 82*, 20–27.
Sensory analysis (2016). Methodology – General guidance for establishing a sensory profile (ISO 13299:2016).
Shakeri, A., Sahebkar, A., & Javadi, B. (2016). Melissa officinalis L.-A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology, 188*, 204–228.
Sindhuja, A., Sudha, M. L., & Rahim, A. (2005). Effect of incorporation of amaranth flour on the quality of cookies. *European Food Research and Technology, 221*, 597–601.
Singh, H. P., Kaur, S., Negi, K., Kumari, S., Saini, V., Batish, D. R., & Kohli, R. K. (2012). Assessment of in vitro antioxidant activity of essential oil of *Eucalyptus citriodora* (lemon-scented Eucalypt; Myrtaceae) and its major constituents. *LWT–Food. Science and Technology, 48*, 237–241.
Singleton, V. L., & Rossi, J. A. J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.
Slavov, A., Denev, P., Panchev, I., Shikov, V., Nenov, N., Yantcheva, N., & Vasileva, I. (2017). Combined recovery of polysaccharides and polyphenols from *Rosa damascena* wastes. *Industrial Crops and Products, 100*, 85–94.
Sullivan, P., O'Flaherty, J., Brunton, N., Arendt, E., & Gallagher, E. (2011). The utilisation of barley middlings to add value and health benefits to white breads. *Journal of Food Engineering, 105*, 493–502.
Theander, O., Westerlund, E., & Aman, P. (1993). Structure and components of dietary fiber. *Journal of Cereal Foods World, 38*, 135–141.
Tiliacos, C., Gaydou, E. M., Bessière, J.-M., & Agnel, R. (2008). Distilled lavandin (*Lavandula intermedia* Emeric ex Loisel) wastes: a rich source of coumarin and herniarin. *Journal of Essential Oil Research, 20*, 412–413.
Yousif, A., Nhepera, D., & Johnson, S. (2012). Influence of sorghum flour addition on flat bread in vitro starch digestibility, antioxidant capacity and consumer acceptability. *Food Chemistry, 134*, 880–887.
Zheljazkov, V. D., & Astatkie, T. (2012). Distillation waste water can modify peppermint (*Mentha* ×*piperita* L.) oil composition. *Industrial Crops and Products, 36*, 420–426.