Improvement of antioxidant potential of wheat flours and breads by addition of medicinal plants

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Abstract

Introduction. The research aim – to determine the effect of two herbal mixtures on herb bread properties. The influence was established of the herbals on the total phenolic content and antioxidant activities of herbal mixtures, herbal-flour mixtures and herb breads.

Materials and methods. It was used two herbal mixtures (1 – thyme, oregano and lemon balm; 2 – thyme, oregano, lemon balm and fenugreek) with wheat flour for herb bread production. Total polyphenol content was determined following the *Folin-Ciocalteu* method. The antioxidant activities of sample extracts were evaluated by four methods: $ABTS^{\bullet+}$, CUPRAC, FRAP and DPPH assay.

Results and discussion. The highest total phenolic content from all investigated herbs showed oregano (30.43 mg GAE/g dw), herbal mixtures 1 and herbal mixtures 2 – 19.18 mg GAE/g dw and 17.47 mg GAE/g dw, respectively. In herbal-flour mixture and prepared breads the level of total phenolic content were in the range from 0.31mg GAE/g dw to 0.37 mg GAE/g dw. Therefore, the content of these bioactive compounds didn`t changed significantly during the baking process. The highest antioxidant activity of herbal mixtures, herbal-flour mixtures and breads were obtained by two of the used methods – ABTS and FRAP assay. The highest antioxidant potential was demonstrated by herbal mixture 1 consisted of 3 herbs – 16829.73 mM TE/100 g dw, followed by the herbal mixture 2 with 4 herbs – 14693.75 mM TE/100 g dw, respectively, both evaluated by the ABTS method. For the FRAP method, the antioxidant activity values were: 15997.65 mM TE/100 g dw for the herbal mixture 1 with 3 herbs and 14136.82 mM TE/100 g dw for the herbal mixture 2 of 4 herbs.

Conclusions. Herbs added to flour increased the total phenolics and antioxidant values of flour-mixtures and breads. Insignifiant differences in the antioxidant potentials were observed between breads with three and four herbs.

Introduction

One of the global problems among people is diseases caused by unbalanced eating. The current methods of solving these problems are to increase the nutritional and biological value of daily consumed food products, including bread. It is known that traditional types of bread that have a high energy value are characterized by an unbalanced amino acid composition, a low fiber content, vitamins and minerals. Therefore, an important task of bread-making is to strive for the production of bread enriched with physiological-functional ingredients [1].

The nutritional value of the bread depends on the type of flour used and on the variety of other ingredients added to the production. Bread is well absorbed by the body, as it has an elastic medium in which the proteins are optimally denatured, the starch is clustered and the sugars dissolved. They are thus available for the action of enzymes in the gastrointestinal tract [2].

To increase the nutritional and biological value of bread, we proposed to use medigal plants like thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.), lemon balm (*Melissa officinalis* L.), fenugreek (*Trigonella foenum-graecum* L.) how are rich sources of bioactive compound, especially essential oils, terpenes, phenolic acids and flavonoids with proven antioxidant activity.

Medicinal plants present abundant sources of natural antioxidants and find enormous application in human nutrition, not only as flavoring spices but also as natural remedy. Herbal fortification of white bread is a new trend to improve its nutritional value [3]. Herbs are rich in minerals, vitamins, flavouring agents and natural antioxidants. Roots, stems, leaves or seeds of herbal plants have long been used in cooking and in naturopathy all over the world. This supplementation of herbs add a spicy flavour, greatly improved taste and sensory properties, and enhanced the level of natural antioxidants. Bulgarian culinary herbs and medicinal plants find enourmous application in nutrition, because of their healthy effect due to the bioactive compounds as essential oils, phenolic acids and antioxidants [4].

The nutritional and biological value of traditional wild herbs and spices used in food preparation has been studied. According to this study, their chemical composition (% vc) includes: crude protein (from 4.6 to 22.1%), from 7.5 to 36.0% fat, from 34.6 to 71.9% carbohydrates and from 0.1 to 5.2% essential oils that determine the flavor of the herb-spice. For wild herbs and spices, high antioxidant activity has been detected due to their biologically active components [5]. Most herbs have been shown to exhibit an antioxidant, bactericidal, fungicidal, antiviral effect and lower blood sugar levels [6, 7].

Recently, herbs are included as ingredients of herbal food supplements to enrich them with biologically active substances useful for human health. In the ancient times, human used herbs as a spice in the bread. The introduction of herbs into the bread contributes to its enrichment with biologically active substances [8]. Dried herbs can be incorporated in a larger assortment of bread and bakery products, technologies have been developed in recent years to prepare ready-made flour mixtures enriched with ground herbal dried herbs [9]. It has been found that thyme, marjoram, lemon balm and fenugreek are proper for incorporation in ready-made wheat flour mixtures due to their pleasant aroma and their easier shredding [10].

Moreover, thyme possessed strong antioxidant effect that is mainly due to flavonoids, phenolic aids as rosmarinic acid and caffeic acid [11, 12, 13]. From the volatile aromatic substances contained in the thyme, the eugenol, thymol and carvacrol exhibit a strong antioxidant activity comparable to that of the known antioxidants such as α-tocopherol and butyl hydroxytoluene [14, 15]. In addition, oregano is also herb with strong radical-

scavenging activity, due to not only to presence of rosmarinic acid, but also of phenolic compounds as apigenin, luteolin and carnosic acid [11, 13, 16, 17].

Melissa officinalis L. (known as lemon balm) belongs to the family of Lamiaceae. Its therapeutic properties include sedative, carminative, antispasmodic, antibacterial, antiviral, anti-inflammatory and antioxidative activities [18]. Various in vitro studies indicated that the extract of lemon balm, oregano and thyme possessed antioxidant properties [19, 20, 21, 12, 13, 22]. Some authors Ando et al. [23]; Brown [24]; Kassaian et al. [25]; Naidu [26] have reported antioxidant properties of fenugreek, that makes it a valuable component of healthy nutrition.

The aim of the current study is to evaluate total phenolic content and in vitro antioxidant activity of herbal-flour mixtures and prepared herbal breads.

Materials and methods

Materials

All chemicals for chemical analysis were analytical grade. Medical plants used in the current study were as follows: thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.), lemon balm (*Melissa officinalis* L.) – leaves and *Trigonella foenum-graecum* L. areal parts. The plants were with Bulgarian origin and were purchased from drugstore (Yambol, Bulgaria). They were then heated to 40 °C for 1 h in a hot air oven and then ground to powder in a commercial kitchen grinder. Then they were sieved through 0.1 mm. Commercial bread-making wheat flour (type 1150 with moisture 13% and ash 11.5%), kindly delivered by AgroMel-Import Ltd (Saedinenie, Bulgaria), was used in bread preparation. Salt was purchased from the local stores of Yambol, Bulgaria. Dried baker's yeast („Pakmaya", Turkey) was used as the leavening agent. Drinking water used for bread preparation was with purity and proper for food purposes that was in accordance with the requirements of Bulgarian legislation.

Herbal mixtures (1– thyme, oregano and lemon balm) and (2 – thyme, oregano, lemon balm and fenugreek) were prepared by mixing, homogenizing and grinding of the medicinal plants in ratio 1:1:1 (herbal mixture 1) and in ratio 1:1:1:1 (herbal mixture 2).

Preparation of composite flour mixtures

Herbal-flour mixtures with 3 and 4 herbs were prepared as follow:

Herbal-flour mixture 1 contained wheat flour type 1150, 1.5% herbal mixture 1 (thyme, oregano and lemon balm), dried yeasts 1.4% and 1.5% salt.

Herbal-flour mixture 2 contained wheat flour 1150, 2% herbal mixture 2 (thyme, oregano, lemon balm and fenugreek), dried yeasts 1.4% and 1.5% salt.

Preparation of breads

The breads were prepared according to bread making methods in the Department of Technology of cereals, fodders, bread making and confectionary products (University of Food Technologies, Plovdiv). The bread formula contained wheat flour (100%), dried yeasts (1.4%), sodium chloride (1.5%), and deionized water (57%) and herbal-flour mixtures 1 and 2. Control bread sample was prepared without addition of herbal mixture, only wheat flour 1150, yeasts 1.4% and 1.5% salt. Bread dough was formed for 6 min at 29–31 ºC and fermented at 35 ºC for 34 min. Breads were baked at 230 – 240 ºC in a convection oven for 16 min. Breads were cooled to room temperature for 60 min [27, 28].

Sample extractions

Bread samples were sliced (3 cm width and 1 cm thickness) and air-dried for 24 h. The dried material was ground to obtain powdered bread samples. Powdered medicinal plants, herbal mixtures and breads (10 g) were extracted with 50 mL of 80% aqueous methanol for 24 h at 25 °C. Samples were then centrifuged at 3500 rpm for 15 min. The supernatant collected was used for further studies.

Determination of the total phenolic content (TPC)

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent according to Stintzing et al. [29] with slight modifications. Basically, 0.2 mL extract was mixed with 1 mL Folin–Ciocalteu`s reagent diluted five times and 0.8 mL 7.5% Na_2CO_3. The reaction was carried out 20 min at room temperature in darkness and the absorbance was measured at 765 nm against blank sample. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight.

Antioxidant activity

The antioxidant activities of sample extracts were evaluated by four methods: DPPH (1,1- diphenyl-2- picrylhydrazyl) radical and ABTS+ radical scavenging ability assay based on mixed hydrogen atom transfer (HAT) and both assay based only on single electron transfer mechanism FRAP (ferric reducing antioxidant power) and CUPRAC, respectively.

ABTS assay. ABTS radical was generated by mixing aliquot parts of water solution of 7.0 mM 2,2`-azinobis(3)ethylbenzthiazoline-6-sulfonic acid (ABTS) and 2.45 mM potassium persulfate. The generated ABTS radical was stable for several days. ABTS.+ solution (2.85 mL) was mixed with 0.15 mL extracts. After 15 min at 37 °C in darkness the absorbance was measured at 734 nm against methanol.

CUPRAC assay. The reaction was started by mixing of 1.0 mL $CuCl_2 \times 2H_2O$, 1.0 ml 7.5 mM Neocuproine (Sigma) in methanol, 1.0 mL 0.1 M ammonium acetate buffer (pH 7.0), 0.1 mL of investigated extract and 1.0 mL d. H_2O. Blank sample was prepared with methanol. The reaction time was 20 min at 50 °C in darkness. After cooling the absorbance was measured at 450 nm against blank [30].

Ferric reducing antioxidant power (FRAP) assay. Three mL freshly prepared FRAP reagent (10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3.6H_2O$ in d. H_2O) were mixed with 0.1 mL of investigated extract [31]. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against blank prepared with methanol [30].

DPPH radical-scavenging ability. Each extract (0.15 mL) was mixed with 2.85 mL freshly prepared 0.1mM solution of DPPH (2,2-diphenyl-1-picryl hydrazyl radical) in methanol. The sample was incubated for 15 min at 37 °C in darkness. The reduction of absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol [32].

The results from all methods for antioxidant activity were expressed as mM Trolox® equivalents (TE) per 100 g dry weight (dw).

Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean ± SD (standard deviation).Statistical analysis was performed using and Excel 2010.

Results and discussion

The total phenolic content of wheat flour, medicinal plants, herbal mixtures, herbal-flour mixtures and prepared breads were summerized in Table 1.

Polyphenols are compounds known to possess the ability to capture free radicals and inhibit lipid oxidation in vitro [33].

Table 1

Total phenolic content in herbs, herbal mixtures, herbal-flour mixtures and herb bread

Sample	Total phenolic content, mg GAE/g dw
Wheat flour Type 1150	-
Thyme	12.05±0.20
Oregano	30.43±0.20
Lemon balm	15.05±0.20
Fenugreek	12.34±0.05
Herbal mixture 1 (thyme, oregano and lemon balm)	19.18±0.05
Herbal mixture 2 (thyme, oregano, lemon balm and fenugreek)	17.47±0.07
Herbal-flour mixture 1	0.31±0.05
Herbal-flour mixture 2	0.36±0.05
Bread prepared with Herbal-flour mixture 1	0.32±0.02
Bread prepared with Herbal-flour mixture 2	0.37±0.05

The highest value of total phenolic content was found in oregano – 30.43 mg GAE/g dw, followed by Lemon balm – 15,05 mg GAE/g dw. The total polyphenol content (TPC) in lemon balm was close to reported values in previous research (18.17±0.04 mg GAE/g dw) mg GAE/g dw [18]. The content of total phenolic compounds in oregano was two time more in comparision with these ones in thyme, lemon balm and fenugreek. Among thyme, lemon and fenugreek herbs, no signifiant differences in the content of common phenolic substances were observed. In this study, the obtained values for the total phenolic content in thyme and fenugreek were close to a previous report for some trademarks of these herbs commercially available in Bulgaria – from 16 to 24 mg GAE/g dw [22].

The values for the total phenolic substances content in herbal mixtures 1 and 2 were in the range of 19.18 mg GAE/g dw for herbal mixture of 3 herbs and 17.47 mg GAE/g dw for the herbal mixture of 4 herbs, as the difference was negligible and due to the involvement of fenugreek in the mixture having a lower content of common phenolic substances – 12.34 mg GAE/g dw in the comparison with oregano and lemon balm.

The values for the content of common phenolic substances in herbal-flour mixture 1 and 2 and in the breads produced from them were in very close range – a total of 0.31 to 0.37 mg GAE/g dw. Compared to individual herbs and herbal mixtures, these values were significantly lower, due to the presence of flour in herbal mixtures, respectively in bread. Moreover, wheat flour present as the main raw material in the largest content. From the results presented, it can be seen that the produced breads have a higher content of polyphenols than the mixes used. Żmijewski et al. (2015) found the same [34].

Different methods were applied for the evaluation of antioxidant activities of herbs, herbal mixtures, herbal-flour mixtures and prepared breads. The results from antioxidant activity were presented (Figure 1, 2 and 3) evaluated by four methods, based on different mechanism (DPPH, ABTS, FRAP and CuPRAC).

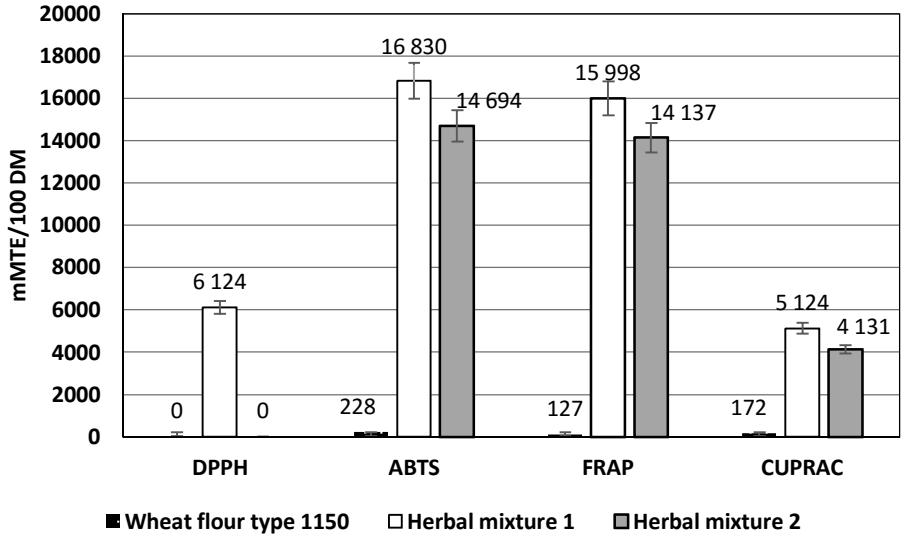

Figure 1. Antioxidant activity of flour and herbal mixtures

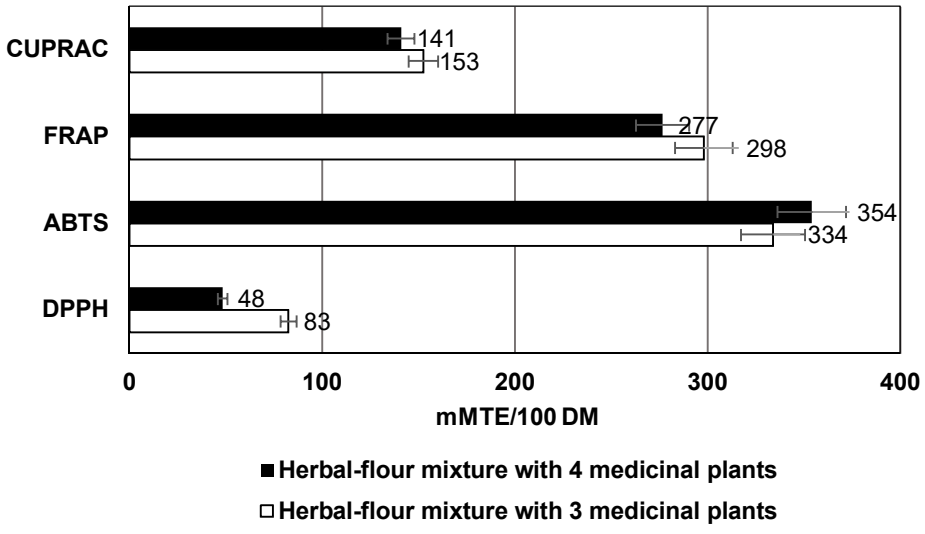

Figure 2. Antioxidant activity of herbal-flour mixtures

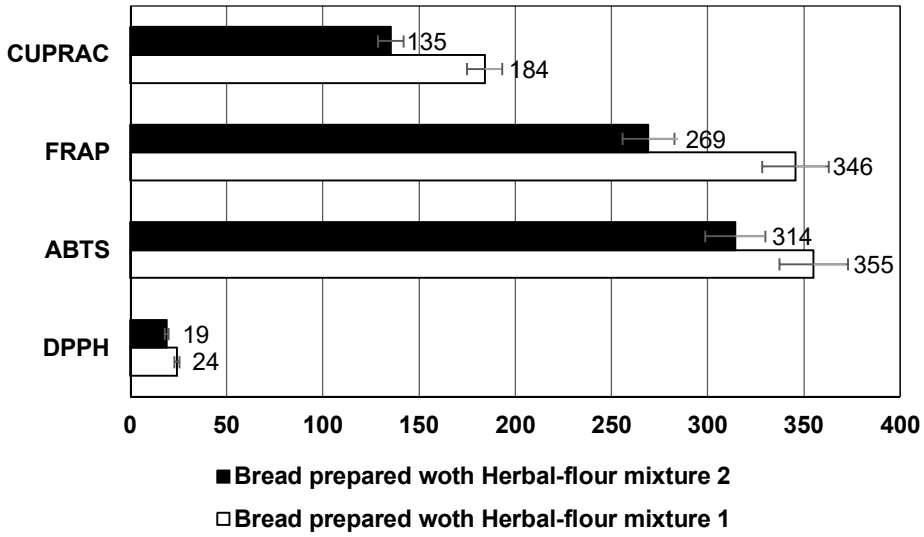

Figure 3. Antioxidant activity of herb breads

The bread production process can be divided into three leavening operations: mixing and forming the dough; fermentation; and backing. The duration of backing depends mainly on the type and mass of the bread. It has been established that the antioxidant potential of bakery products depends on the conditions of production and the raw materials used [35].

In their research, Han and Koh (2011) show that antioxidant activity and free phenolic acid content decrease when the raw materials are mixed and the dough is mixed, but they are restored after the fermentation and backing processes. This is explained by the fact that antioxidant linkages are hydrolysed during fermentation and antioxidants are released [36].

The highest antioxidant activity of herbal mixtures, herbal-flour mixtures and breads were obtained by two of the used methods - ABTS (the radical-scavanging activity against the ABTS radical - 2,2'-azino-bis) -ethylbenzothiazoline-6-sulfonic acid) and FRAP assay. The highest antioxidant potential was demonstrated by herbal mixture 1 consisted of 3 herbs - 16829.73 mM TE/100 g dw, followed by the herbal mixture 2 with 4 herbs - 14693.75 mM TE/100 g dw, respectively, both evaluated by the ABTS method. For the FRAP method, the antioxidant activity values were - 15997.65 mM TE/100 g dw for the herbal mixture 1 with 3 herbs and 14136.82 mM TE/100 g dw for the herbal mixture 2 of 4 herbs. High values of the antioxidant activity of herbal mixtures, herbal-flour mixtures and of breads were also reported in the CuPRAC method (reduction of copper ions). The lowest in the DPPH method (the radical capture activity against the DPPH radical - 2,2-difenyl- 1-picryl hydrazyl radical).

In herbal-flour mixtures and breads, the highest antioxidant activities were also evaluted by the ABTS and FRAP methods, followed by the CuPRAC method, as the insignificant differences in the different types of herbal mixtures and the breads were observed. Compared to herbal mixtures, the herbal-flour mixtures and breads demonstrated 5 times lower antioxidant activity, due to the high content of the wheat flour in herbal-flour mixtures and breads which it is the main raw material. The breads prepared with 3 herbs demonstrated slightly higher antioxidant potential compared to 4 herbal breads.

Higher values obtained from the ABTS method (conducted at neutral pH where the main mechanism is HAT) suggest that the antioxidant action of herbal mixtures is based mainly on hydrogen transfer [37]. The ability of herbs to neutralize reactive forms of oxygen through the involvement of various reaction mechanisms underlines its potential in the prevention of important diseases related with oxidative stress such as accelerated aging processes.

Some authors discussed that the heat treatment and baking process might damage or degrade the antioxidant compounds antioxidant activity that presented in different flours. The antioxidant potential in bakery products is strongly dependent on manufacturing, recipes dough mixing and kneading. Antioxidant activity of breads could be modified by active oxidative enzymes presented in ingredients of compounds used in breads production, or oxidized by atmospheric oxygen [38]. Antioxidant activity of flours was higher than in breads prepared with them (Figure 1 and Figure 3). However, the bread prepared with herbal mixtures showed good antioxidant activity evaluated by ABTS and FRAP methods (Figure 3). Antioxidant activity of breads evaluated by DPPH method showed lower results that was in accordance with tendency reported values for wheat and pseudo cereal breads [38, 39].

Many authors [34, 35, 36] are concerned with the enrichment of white bread with different ingredients containing functional components, but very few have studied the antioxidant characteristics of enriched bread. Seidel et al. (2007) have studied bread enriched with functional ingredients - green tea powder, herbs and tomato paste. The effects of bread produced on immunological and antioxidant parameters were compared with control sample of white bread.

In conclusion, the responses observed with the FRAP assay after intervention with enriched bread indicate a unique response in terms of the antioxidative potentials for this type of functional food [40].

The correlation between antioxidant activity of herbal-flour mixtures and prepared breads and addition of herbal mixtures with three and four herbs to the wheat flour was demonstrated (Table 2).

Table 2

Correlation coefficient (r^2) between antioxidant activities of herbal-flour mixtures and breads prepared with herbal-flour mixtures

Sample	Correlation coefficient (r^2)
Herbal-flour mixture 1 (with three herbs)	0.7499
Herbal-flour mixture 2 (with four herbs)	0.8071
Breads prepared with herbal-flour mixture 1	0.8067
Breads prepared with herbal-flour mixture 2	0.8186

There were significant positive correlations ranging from 0.7499 to 0.8186, which shows the significant influence of added herbal mixtures to basic wheat flour on the antioxidant activity of herbal-flour mixtures and bread made from them. Reported positive correlations showed that the antioxidant action of herbal-flour mixtures and bread could be regarded as a result of the addition of the herbal mixture to flour and enriching their effect on phenolic compounds.

Conclusion

It was found that herbs added to flours increased the total phenolics and antioxidant values of flour-mixtures and final breads. Herbal mixtures, herbal-flour mixtures and prepared breads demonstrated high antioxidant activity by radical-scavanging method ABTS and FRAP assay bease on reduction of ferric ions. The addition of herbs to the flours and breads improved the antioxidant activity of final wheat products. Therefore, the incorparation of herbal mixtures in breads enriched wheat breads of polyphenols and antioxidants, as improve the aroma, taste and healthy status of breads.

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