Andrea VARGA-KÁNTOR[1], Loránd ALEXA[1], Emőke TOPA[1], Béla KOVÁCS[1], Nikolett CZIPA[1]

DOI: https://doi.org/10.52091/EVIK-2021/4-3-ENG

Received: May 2021 – Accepted: September 2021

Examination of breads enriched with dried basil and evaluation of the results

Keywords: bread, basil, total polyphenol content (TPC), flavonoid content, element content, functional food

1. SUMMARY

Both bread and spices play an important role in our daily diet. Basil is an extremely popular spice, the beneficial effects of which have long been known. This is why the enrichment of breads with commercially available dried basil was carried out. In the case of basil, its antioxidant and element contents were determined. With respect to these parameters, results indicating outstandingly advantageous properties were obtained. During the enrichment, 6 different concentrations were used and a control sample was prepared that did not contain basil. As the amount of spice was increased, the total polyphenol content (TPC), flavonoid and macronutrient contents of the breads also increased. There was no difference between the products in terms of their crude fat content. In the case of the protein content, a minimal increase was measured with increasing spice concentration.

[1] University of Debrecen, Institute of Food Science

Andrea VARGA-KÁNTOR	kantor.andrea@agr.unideb.hu	https://orcid.org/0000-0003-2296-3011
Loránd ALEXA	alexal@agr.unideb.hu	https://orcid.org/0000-0001-5281-2340
Emőke TOPA	papp-topa.emoke@agr.unideb.hu	https://orcid.org/0000-0002-0551-8058
Béla KOVÁCS	kovacsb@agr.unideb.hu	https://orcid.org/0000-0002-6439-4753
Nikolett CZIPA	czipa@agr.unideb.hu	https://orcid.org/0000-0001-6966-4380

2. Introduction

Basil is mostly grown in Mediterranean countries. Its leaves, fresh or dried, are used as a seasoning. It is also known as an herb, its use is recommended against headaches, coughs, diarrhea, constipation, warts, worms and kidney problems, among other things **[1]**. Rosmarinic acid is responsible for its antioxidant effect, as it binds free radicals. It cannot be used against fungi, but its antibacterial and antiviral activities are known **[2]**. It has outstanding values in terms of element content, which has been studied by Ghanjaoui et al. **[1]**, Özcan and Akbulut **[3]**, as well as Özcan **[4]**, among others.

Baking is one of the oldest human occupations related to food preparation. When prehistoric man settled down and switched to gathering and farming, cereals became the most important sources of food. With continuous learning and technological development, it has been possible to process the collected seeds into various products **[5]**. One such product was bread, which was very different from the product that is being consumed today. Nevertheless, many types of bread are still made today. In the Middle East, flat bread dominates, in China, steamed bread, while in America, corn-based product dominate. Bread is mostly made from wheat and some other commonly used cereals, as the proteins of these are best suited to make the right product **[6]**.

According to papers in the literature, there have been several attempts to enrich bread with different substances. Raba et al. **[7]** prepared and tested bread enriched with garlic and basil. Suleria et al. **[8]** used aqueous garlic extract, but breads enriched with yellow pepper flour **[9]**, ginger powder **[10]**, turmeric **[11]**, waste onion powder **[12]**, brown algae powder **[13]**, horseradish leaf powder [14], raspberry and strawberry oil cake **[15]**, as well as garlic and its preparations **[16]** have also been made.

Since, to the best of our knowledge, there have been few bread fortification experiments with spices and then few studies were carried out, we first used basil in our experiment and examined what measurable changes were caused in the baked product by the addition of the spice.

3. Materials and methods

3.1. Bread preparation

In our experiments, different parameters of 7 bread samples were examined. The ingredients were selected and the breads were prepared according to the method of Kántor et al. **[16]**. The ingredients used to make the products were purchased at retail stores. Basil was added to the bread dough before kneading in the amounts of 0.00 g; 4.25 g; 8.5 g; 12.75 g; 17.0 g; 21.25 g and 25.5 g ***(Table 1)***.

Table 1. The names of the breads tested and their basil content

Marking	The amount of spice used in the bread (g)
1	0
2	4.25
3	8.50
4	12.75
5	17.0
6	21.25
7	25.5

During the experiment, the total polyphenol (TPC), flavonoid and element content of basil was examined, and then the dry matter, total polyphenol (TPC), flavonoid, crude fat, cruse protein and macroelement contents of the breads were determined. In the case of the breads, the measured values were reported on a dry matter basis.

3.2. Determination of total polyphenol content (TPC)

For the analysis, the method of Singleton et al. **[17]** was used for both basil and the breads. The samples were soaked in methanol (Scharlab S. L., Spain): distilled water (80:20), then the mixture was filtered through 292 pleated filter-paper (Sartorius Stedim Biotech S.A., Gottingen, Germany). 1 ml of the resulting solution was pipetted into a test tube, to which 2.5 ml of Folin-Ciocalteu reagent (VWR International S.A.S., France) was added. After 5 minutes, 2 ml of 75 g/l sodium carbonate (Scharlab S. L., Spain) was added to give a colored compound, the absorbance of which was measured with a spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at 760 nm. For the determination of the total polyphenol content, calibration solutions were prepared from a gallic acid (Alfa Aesar GmbH&Co. KG, Karlsruhe, Germany) stock solution. The absorbance of the calibration solution series was also measured, from the results of which a calibration curve was constructed. The total polyphenol content of our sample solution was determined using this curve. Results are given in mg GAE/100 g.

3.3. Determination of flavonoid content

Flavonoid content analytical results for both the spice and the breads are expressed in mg catechin equivalent per 100 g (mg CE/100 g). As a result of the added reagents, the solutions turned pink. Absorbances were measured at 510 nm with a spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England). The following reagents were used for the analysis: catechin (Cayman Chemical Company, USA), aluminum chloride (Scharlab S.L., Spain), sodium nitrite (Scharlau Chemie S.A., Spain), sodium hydroxide (Sigma-Aldrich Chemie Gmbh, Germany) and methanol (Scharlab S.L., Spain) **[18]**.

3.4. Determination of element content

Sample preparation was carried out by the method of Kovács et al. **[19]** prior to measurement with ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer; Thermo Scientific iCAP 6300, Cambridge, UK). Samples were placed in digestion tubes and 10 ml of nitric acid (69% v/v, VWR International Ltd., Radnor, USA) was added to the tubes. The mixtures were allowed to stand overnight, and the next day pre-digestion was performed at 60 ºC for 30 minutes. After cooling, 3 ml of hydrogen peroxide (30% v/v, VWR International Ltd., Radnor, USA) was added to the samples and the main digestion was performed at 120 ºC for 90 minutes. After this, the cooled samples were diluted with Milli-Q distilled water (Millipore S.A.S., Molsheim, France) and filtered through 388 filter paper (Sartorius Stedim Biotech S.A., Gottingen, Germany). The following element contents were determined by ICP-OES in the resulting digestates:

- Ca 315.8 nm,
- K 769.8 nm,
- Mg 280.2 nm,
- Na 818.3 nm,
- P 185.9 nm,
- S 180.7 nm.

Measurement wavelengths are indicated after the chemical symbols of the elements.

3.5. Determination of dry matter, crude fat and crude protein contents

Breads were tested for these parameters according to standard MSZ 20501-1:2007 **[22]**.

3.6. Statistics

The experiments were performed in triplicate. SPSS statistical software (version 13; SPSS Inc. Chicago, Illinois, USA) was used to evaluate the results. This was also used to determine the mean and standard deviation. To determine statistically significant differences between the results, one-way analysis of variance (Tukey and Dunnett's T3 test; $P<0.05$) was used.

4. Results and evaluation

4.1. Results of the examination of basil

Average values of basil analyses are shown in ***Table 2***. This table shows the total polyphenol, flavonoid and element contents of the spice as determined by three replicate measurements.

Table 2. Basil measurement results

TPC mg GAE/100g	Flavonoid mg CE/100 g	Ca mg/kg	K mg/kg	Mg mg/kg	Na mg/kg	P mg/kg	S mg/kg
512±19	947±49	21.389±871	26.862±590	7.650±372	695±18	3.816±88	2.596±77

The polyphenol content values of basil were higher than the amounts (7.15-107 mg GAE/100 g) reported in the study of Moghaddam and Mehdizadeh **[20]**. However, in the dissertation of Kwee and Niemeyer **[21]**, in which a study of 15 basil varieties was reported, the total polyphenol content ranged from 347 to 1,758 mg GAE/100 g. Based on our results, it can be stated that the value measured by us was high. In the case of basil, outstanding flavonoid content values are to be expected.

Based on our measurements, the spice has primarily high calcium and potassium contents, the values of which were over 20,000 mg/kg. A similar potassium content was measured by Özcan **[4]** in the case of dried basil (24,811 mg/kg), but the calcium content was much lower than the concentrations determined by us (12,363 mg/kg). The magnesium content of the plant is not negligible either, as a value of almost 8,000 mg/kg was measured from the alkaline earth metal. This is much higher than the values obtained by Özcan **[4]** and Özcan and Akbulut **[3]** (5,738 mg/kg and 3,130 mg/kg, respectively).

The analytical results of phosphorus and sulfur were also in the order of thousands in the given sample. Higher P (4,960 mg/kg) and lower S (1,923 mg/kg) contents were measured by Özcan **[4]** in dried basil of Turkish origin. Of the macroelements, sodium was found to have the lowest value, compared to the values reported by Özcan and Akbulut **[3]** (2,895 mg/kg).

4.2. Results of bread analyses

The results of bread nutritional value measurements are shown in ***Table 3***.

Table 3. Bread nutritional value results on a dry matter basis

Sample no.	Dry matter content (%)	TPC mg GAE/100 g	Flavonoid mg CE/100 g	Crude fat (%)	Crude protein (%)
1	69.5±0.2	37.8±0.1	26.0±1.7	6.33±0.21	12.4±0.1
2	69.7±0.1	52.8±1.5	29.1±1.1	5.60±0.10	12.8±0.1
3	69.1±0.1	59.7±0.3	38.7±1.9	5.73±0.31	12.8±0.3
4	68.3±0.1	71.4±0.2	46.4±0.9	5.10±0.26	12.9±0.1
5	68.6±0.1	77.7±1.6	53.2±1.4	5.10±0.26	13.4±0.1
6	70.5±0.1	91.5±0.1	59.4±0.9	5.10±0.85	13.6±0.0
7	69.7±0.1	106±1	70.1±1.1	5.90±0.10	13.2±0.0

4.2.1. Results of dry matter content measurements

Dry matter contents of the bread samples were found to be similar. The dry matter content values of the samples ranged from 68.3% to 70.5%. Sample 4^{th} had the lowest value, while the highest value was measured for sample 6^{th}. Similar results were obtained for samples 1^{st}, 2^{nd} and 7^{th}. For these values, no statistically verifiable differences were found. Compared to the other samples, however, the results of the before mentioned breads were significantly different. The sample with the highest dry matter content, which was sample 6^{th}, differed significantly from all other samples. Nearly identical dry matter content values were measured for breads 4^{th} and 5^{th}, as well as for breads 3^{rd} and 5^{th}.

4.2.2. Results of total polyphenol content measurements

In terms of the total polyphenol content, it was found that even the control bread contained a certain amount of antioxidant compound, which was naturally the lowest of all samples. Kántor et al. **[16]** also found antioxidant compounds in their control bread. As the spices were added to the breads, the quantity of antioxidants increased steadily. The highest amount was measured in the case of sample 7^{th}. The analytical results of all samples showed significant differences when compared to each other.

4.2.3. Results of total flavonoid content measurements

The quantity of flavonoids increased in proportion to the amount of spice added to the bread dough, similar to the polyphenol content. The lowest value was measured in the control sample, from which the flavonoid content of sample 2^{nd} did not differ statistically, but in all other cases a significant difference was observed. Sample 7^{th}, with a spice content of 25.5 g, had the highest flavonoid content.

4.2.4. Results of crude fat content measurements

In terms of crude fat content, the results were mixed. Values between 5.10% and 6.33% were measured. The highest fat content was determined in the control sample, while the lowest values were found in samples 4^{th}, 5^{th} and 6^{th}. For breads 2^{nd} and 3^{rd}, the difference was only 0.1%. The fat content of bread sample 7^{th} was higher than the previous results, but not more than that of the control sample. None of these results differed from each other in a statistically verifiable way, so it was found that there were no statistically verifiable differences in the fat contents of the breads made by us.

4.2.5. Results of protein content measurements

In terms of protein content, values that differed from each other were measured. The highest value was measured in the case of sample 6^{th}, while the lowest was obtained in the case of the control sample. With the addition of basil, a minimal increase in the protein content was observed. There were no statistically verifiable differences in the case of the first 3 samples, however, the result of our control sample differed from the values of samples 4^{th}, 5^{th}, 6^{th} and 7^{th}. Samples with the highest protein content (5^{th} and 6^{th}) showed significant differences from samples 1^{st}, 2^{nd}, 3^{rd} and 4^{th}. The protein content of the bread enriched with the highest amount of spice differed only from the values obtained for samples 1^{st} and 3^{rd}, as in this case the protein content of the product was lower.

4.2.6. Results of macro element content measurements

Results of the macro element content measurements of the breads are summarized in ***Table 4***.

Table 4. Bread macroelement content results on a dry matter basis

Sample no.	Ca mg/kg	K mg/kg	Mg mg/kg	Na mg/kg	P mg/kg	S mg/kg
1	476±15	2.200±37	260±9	2.585±60	1.478±26	1.008±19
2	682±64	2.400±84	328±22	2.532±109	1.494±54	1.033±79
3	886±17	2.589±17	394±7	2.503±46	1.518±13	1.066±33
4	1.087±52	2.868±81	471±14	2.546±85	1.575±38	1.104±47
5	1.232±36	3.009±50	536±10	2.459±59	1.619±25	1.140±40
6	1.391±53	3.127±12	585±13	2.395±14	1.623±14	1.138±29
7	1.614±33	3.408±28	650±10	2.523±39	1.616±14	1.154±20

In the case of the control bread (sample 1st), our macro element content results were similar to those reported by Kántor et al. **[16]**, with the exception of sodium (Ca: 510 mg/kg; K: 2,418 mg/kg; Mg: 285 mg/kg; Na: 3,180 mg/kg; P: 1,512 mg/kg; S: 948 mg/kg).

Regarding the amount of macroelements that could be measured in the samples, it was found that their amount increased in each case with increasing spice concentration. Sodium and sulfur are exceptions to this, as although slightly different results were obtained, this difference could be verified statistically. The results of basil show that these two elements have the lowest amounts in the plant. While the amounts of calcium, potassium, magnesium, phosphorus and sulfur in the bread showed lower values than in the spice itself, the sodium content increased significantly due to the addition of the same amount of table salt to the samples.

The calcium content of the samples ranged from 476 to 1,614 mg/kg. With the addition of basil, the calcium content increased gradually, in most cases by 200 mg/kg between the individual concentrations. Significant difference could not be detected only between samples 4th and 5th.

When determining the potassium content of the bread, values higher than those obtained for calcium were measured. Compared to the control sample, which contained 2,200 mg/kg of potassium, even the product with the lowest amount of spice showed a significant difference. The highest element content was measured in the case of sample 7th, which contained more than 1,200 mg/kg more potassium than sample 1st. There were no statistically verifiable differences between samples 4th and 5th, or 5th and 6th, however, there were significant differences in all other cases.

The magnesium content also increased, as shown by the results. Once again, the control sample had the lowest value, while sample 7th had the highest value. In terms of macro element content, the lowest values were measured for this element, as even the bread with the highest basil content did not reach a value of 1,000 mg/kg. Based on our results, it can be stated that there were statistically verifiable differences between the measured values in all cases.

The phosphorus content in the samples analyzed was between 1,478 and 1,623 mg/kg, these results being measured in samples 1st and 6th. As the amount of spice increased, the phosphorus content also increased. There were statistically verifiable differences between samples 1st-5th, 1st-6th, 1st-7th, 2nd-5th, 2nd-6th, 2nd-7th and 3rd-6th. In the other cases there were no differences in the phosphorus content.

5. Conclusion

At the beginning of the experiment, basil itself was examined, and its antioxidant compounds and macro element content were determined. As the results show, the spice itself has a very high total polyphenol and flavonoid content. In addition to these parameters, its macro element content is also significant, as it has outstanding calcium and potassium contents, which is also supported by the studies mentioned in the literature. Magnesium, phosphorus and sulfur were also measured in non-negligible amounts.

During the preparation of the breads, all the ingredients were added in the same amount, except for the spice, so it was expected that there would be differences as the amount of basil increased.

We cannot draw a clear conclusion as to why the dry matter content has changed this way. The expected result was that as the amount of spice increases, the dry matter content of the bread increases as well. Differences may have been due to the nature of the convection oven.

Both the total polyphenol content and flavonoid content results were in line with our expectations, as the addition of basil, containing a large amount of antioxidant compounds, to the bread significantly increased the values of these parameters, despite the fact that most of these compounds are heat sensitive.

No difference was found in the cruse fat content of the samples analyzed, so enrichment does not affect this parameter.

However, differences were observed in the protein content, as an increase in protein content was achieved by the enrichment. Further research is needed to answer the question as to why this value has increased.

In the case of the macro element content, with the exception of sodium and sulfur, a significant increase was achieved, which may be due to the high element content of basil.

Based on our studies and results, it can be said that enrichment with basil had a positive effect on most of the measured parameters. Higher intakes of antioxidant compounds and macronutrients are also beneficial, because these compounds are required for the normal functioning of the human body.

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