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# A Functional End-Use of Hemp Seeds Waste: Technological, Qualitative, Nutritional and Sensorial Characterization of Fortified Bread

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**Abstract:** The edible portion of *Cannabis sativa* L. are the seeds. The seeds are generally used for the production of hemp oil, appreciated for the high percentage of α-linolenic acid. The waste obtained after seeds oil extraction, reduced in fine powder, is the subject of this study. Since hemp seeds are considered to be a source of many valuable bioactive compounds, the addition of hemp seed flour to durum wheat for making fortified bread could have an effect on the health of consumers. The aim of this study was to use variable percentages of hemp seed flour for the production of bread, and to determine the fortification impact on chemicals, texture, organoleptic characteristics, crumb color, changes in crumb texture, total polyphenols, free radical scavenging activity and amino acids contents. The rheological and chemical qualities of bread samples, obtained by using a durum wheat cultivar Ciclope fortified with defatted hemp seeds flours, were evaluated. The solid residue left after the extraction of the oil from seeds (generally thrown away as waste or added to feed) was shredded and sieved at 0.530 mm (Hemp 1) or 0.236 mm (Hemp 2). Samples of fortified breads were obtained by substitution of variable percentages of durum wheat flour with the two hemp flours (5%, 7.5%, and 10%). Fortified bread's total phenolic content was in a range of 0.73±0.017 and 1.73±0.029 mg GAE/g and anti-radical scavenging activity was included in the range from 1.17±0.099 and 3.18±0.071mmol TEAC/100g, according to the increasing fortification. Comparison between bread made with Ciclope semolina and hemp flour fortified breads, showed a large increase in amino acid content in fortified samples ones. In particular, bread fortified with 10% Hemp 2 flour highlights a greater content of glutamic acid, tyrosine, proline and essential amino acids such as leucine and isoleucine, compared to the other samples with same percentage of substitution. The amount of hemp seed flour influenced the color of the crumb by increasing the yellow index from 18.24 (100% Ciclope) to 21.33 (bread with Hemp 2 flour at 5%).

**Keywords:** hemp waste; food fortification; Ciclope; Futura 75; hemp flour; dough mixing; amino acids; fatty acids

## 1. Introduction

In the last years, a great amount of concern was paid to isolation of bioactive compounds from natural sources due to their antioxidant properties. In particular, the interest was directed to the examination and use of products obtained from the transformation processes of vegetable raw materials [1,2]. Cereal-based products, especially pasta and bread, are well suited for nutrient supply through fortification. Furthermore, the development of new functional products based on fortified breads and flours may have an impact on the influence of metabolism and other health conditions [3]. Unfortunately, fortification often affects the quality of cereal-based products, in relation to texture, color, cooking quality and sensory properties. One the main challenges faced by the food industry is to improve the healthfulness of foods without compromising their sensory attributes [4].

Bakery products, such as bread and pasta, are foods based on simple ingredients such as wheat flour, yeast, water and salt. Nevertheless, bread is one of the basic food products in the human diet, constituting a valuable source of many important nutrients necessary for the proper functioning of the body. Just because of its simpleness and its wide consumption, bread is appropriate to be enriched and fortified with healthy bioactive compounds [5,6].

Industrial *Cannabis sativa* L. plants, with a low level of d-9-tetrahydrocannabinol (THC, < 0.2%), are grown to obtain fiber, seeds and their derivatives, such as oil [7]. It is important agricultural commodity in Canada [6,8], USA [9] and China [10]. Hemp fiber is widely used in the modern production of paper and fabrics in these countries. The edible part of Cannabis sativa is the seeds, which have become an attractive by-product for the production of hemp seed food products that have become available to consumers. In China toasted hemp seeds are sold in the markets, although most seeds are exported (untoasted) as bird seeds. In Eastern European countries, hemp seeds oil has been used as a butter substitute, typically by those who could not afford dairy products [11].

Hemp seeds have a high nutritional value thanks to their high content of easily and quickly digestible proteins, essential amino acids, and a good ratio between omega-6 and omega-3 polyunsaturated fatty acids (PUFA) [12,13]. Prociuk et al. studied the effects of a hempseed enriched diet on cholesterol levels. They observed a reduction in plasma cholesterol probably related to increased levels of plasma $\gamma$-linolenic acid [14]. Other authors investigated the beneficial effects of hemp seeds to improve pain symptoms in patients with osteoarthritis [15].

As evidenced by previous analyses [16–18], from seeds is obtained an oil rich in fatty acids, mainly linoleic and $\alpha$-linolenic acids.

After oil extraction, seeds can be ground to obtain edible flours. Hemp seeds flour is an example of promising bio-sustainability waste raw material with a high nutritional value that can be obtained from industrial by-products.

Foods fortified with these "non-wheat flours" would have an additional supply of fiber, minerals, proteins, and polyphenols. Therefore, food fortification increases the nutritional and potential beneficial properties of the final product, and enhances by-products of agri-food industries, as new sources of bioactive components, from the perspective of a circular economy and a biorefinery approach both for environmental and economic reasons. In this regard, many authors have already investigated the impact of hemp seed flour enrichment on technological, nutritional, and sensorial characteristics of bakery products [19,20] and pasta [21–23].

One of the objectives of this study was to reuse hemp seed processing waste after oil extraction. In particular, the technological and nutritional characteristics of bread samples obtained with "Cyclops" durum wheat semolina were evaluated, compared to those of breads fortified with 5%, 7.5% and 10% hemp seed flour.

## 2. Materials and Methods

### *2.1. Raw materials*

The Ciclope durum wheat variety, registered in the National Register of Varieties in 2006, was established by researchers from the former Experimental Institute for Cereal crops in Catania, today the Cereal and Industrial Crops Research Center of CREA, in Acireale (Catania - Sicily). Its qualitative and technological properties, in particular the high gluten index, make the Ciclope variety particularly suitable for industrial transformation processes. In this work, the first transformation involved the grinding of the kernels performed with an experimental mill Bona Labormill 4RB. The semolina obtained had a granulometry of about 250 μm and the extraction yield stood at 55-60%. Instead, the hemp flours were obtained from the milling waste of the hemp seeds, for the purpose of oil extraction; the entire transformation process took place at the Molino Crisafulli Soc. Coop. RL, Caltagirone - Sicily. Based on the type of sieve used, two types of flour were obtained, differing in particle size: 530 μm, namely Hemp 1 and 236 μm, namely Hemp 2.

### *2.2. Bread Rheological Characteristics*

Moisture content was determined according to the AACC 08-01 methods (AACC, 2000) [24]. The farinograph indices were determined according to the AACC 54- 21 method (AACC, 2000) by a farinograph (Brabender instrument, Duisburg, Germany), equipped with the software Farinograph® (Brabender instrument, Duisburg, Germany). According to the standard procedure, the following farinograph indices were determined: water absorption needed to achieve the dough consistency of 500 ± 20 Brabender Units (B.U.) (WA), dough development time (DT) and dough stability (S) were measured. The alveographic test was used to analyze the effect of additions on the dough rheological behavior performed by Chopin alveograph (Chopin,Villeneuve La Garenne, France) according to the standard alveographic (UNI n° 10453 method) (a). Each sample was analyzed in five repetitions and deformation energy W (strength) and P/L (tenacity/extensibility ratio) were calculated. Wet and dry gluten and gluten index were determined using a Glutomatic System (Glutomatic 2200, Centrifuge 2015, Glutork 2020; Perten Instruments AB, Huddinge, Sweden), according to the UNI 10690 method (UNI, 1979).

### *2.3. Technological and qualitative analysis for fortified bread*

#### 2.3.1. Bread-Making Test

In order to determine the optimal development of the doughs, an experimental bread-making test was performed, using the AACC 10.10 method [24], modified for durum wheat. Three different percentages of hemp flour integration were considered (5%, 7.5% and 10% w/w), in addition to the Ciclope durum wheat semolina. Bread made from 100% Ciclope durum wheat semolina was used as a control (CTRL) (Table 1). The bread-making process was carried out in the laboratory, at a constant temperature (25 °C). The production of each fortified bread sample involved the use of 200 g of flour (xg of Ciclope durum wheat + xg of hemp flour), brewer's yeast (3%), sugar (6%), NaCl (2%), ascorbic acid (80 p.p.m) and shortening (3%) to distilled water. The quantity of water to be added to obtain the mixtures was calculated through farinographic analysis. The entire bread-making process involved four leavening phases, for a total time of 4.20 hours. The leavening process took place in a leavening chamber with controlled temperature and humidity, respectively at 29 ± 1.41 ◦C and 82.5% ± 3.54%. During the first phase, lasting 2.15 hours, the doughs were placed in the leavening compartment. The second phase, lasting 50 minutes, concerned the samples subjected to the first rolling. After the set time, the dough was rolled out a second time and then left to rise for another 25 minutes. The last leavening phase, which lasted 50 minutes, involved the dough rolled up manually and placed in individual metal molds. Then, the loaves were baked for 18 minutes at 215–220 ◦C., in a continuous oven (Pavailler Engineering S.r.l., Galliate, Italy). On the breads, the following properties were tested for each bread sample: volume (BV) (determined according to the rapeseed displacement in a loaf volume meter, AACC 10.05.01 method), height (BH) (DigiMaxTM digital caliper, Scienceware, N.J., USA), and weight (BW) were measured.

**Table 1.** Raw materials and fortified bread samples (5%, 7.5% and 10% of substitution with 0.530 mm (Hemp 1) and 0.236 mm (Hemp 2) flours.

| **Raw material** | | **Fortified hemp bread** | | | |
|---|---|---|---|---|---|
| | | 0 | 5% | 7.5% | 10% |
| Ciclope semolina | Ciclope | CTRL | | | |
| Hemp 1 flour | Hemp 1 | | Hemp 1_5 | Hemp 1_7.5 | Hemp 1_10 |
| Hemp 2 flour | Hemp 2 | | Hemp 2_5 | Hemp 2_7.5 | Hemp 2_10 |

2.3.2. Sensorial Analysis of Bread

Once the transformation process was completed, sensory analysis was carried out on the bread samples obtained. The different types of breads (CTRL and 5%, 7.5% and 10% of bread fortified with Hemp 1 and Hemp 2 flour) were evaluated by 10 previously trained tasters, aged between 25 and 62 years old. The loaves were cut into slices, one centimeter thick, and placed in containers previously labeled with numbers. The thickness of the crust, the elasticity, the hardness, the friability, the apparent softness were evaluated. Regarding the crumb, the elasticity, friability, cohesion and humidity were evaluated and the crumb porosity was estimated using the Mohs scale. In order to determine a general evaluation, a scale of values ranging from 1 to 10 was used, where 6 represents the minimum threshold of acceptability.

2.3.3. Bread Colour Estimate

The crust and crumb color data of the loaves were measured through the use of a Minolta CR-300 colorimeter (Osaka, Japan), in the L∗, a∗, b∗ color space with the illuminant D65. The Brown index was calculated as 100 − L∗ [25].

*2.4. Chemical characterization*

2.4.1. Chemicals and reagents

DPPH, 6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, heptylamine 99%, Folin–Ciocalteu reagent, methanol, chloroform, hydrochloric acid, sodium hydroxide, formic acid, potassium hydroxide, toluene, hexane were purchased from Sigma-Aldrich (Steinheim, Germany). Supelco 37 Component FAMEs Mix, a mix of 17 Amino acid standards containing L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine L-phenylalanine, L-proline, L-serine, L-threonine. L-tyrosine and L-valine (0.5 µmole ml-1 except for L-cystine at 0.25 µmole ml-1) (Supelco Bellefonte, PA, USA) were used. L-Tryptophan, L-asparagine and L-glutamine pure standards were acquired from Merck (Darmstadt. Germany). Purified water was obtained through a Milli-Q Integral 5 system (Millipore. Merck KGaA. Darmstadt. Germany).

2.4.2. Total Phenolic Content (TPC)

Folin–Ciocalteu method was used in order to determine total phenolic content (TPC) as previously described [23]. A calibration curve was obtained with gallic acid standard solutions [0.001 to 0.25 mg/mL] (y=10.955x+0.1405, R2 = 0.992). Results were expressed as mg gallic acid equivalents per g (mg GAE g−1) of sample. The method used a methanolic/water (80:20) extraction of samples. The TPC was measured four times for each sample.

2.4.3. Fatty Acid Composition

The fatty acid composition was performed according to previous procedure [25,26]. ISQ™ 9000 Quadrupole GC-MS System (Thermo Fisher Scientific, Waltham, MA, USA) gas chromatography-mass spectrometry (GC/MS) was used for the determination of fatty acid methyl esters (FAMEs) after methylation. Analyses were performed in triplicate and FAMEs were identified by comparing their retention times with the external standard mix solution (Supelco 37 Component FAME Mix). Amount of individual fatty acid methyl esters was expressed as relative percentage (%).

2.4.4. Amino Acids (AAs) quantification by HPLC-FLD method

As already reported [23], the procedure for quali-quantitative determination in dried bread samples involves the preventive acid hydrolysis at 110°C for 24 hours of the proteins. Following that, derivation of amino acids using FMOC-Cl (9 fluorenyl-methyl chloroformate) was required before the HPLC-FLD analysis. Analyses of the derivatized amino acids were performed using an Agilent

1100 series HPLC chromatography system, equipped with a fluorescence FLD detector. Derivatized amino acids were quantified using calibration curves of commercial AAs standard solutions in a range from 0.025 mM to 0.4 mM (Table S1). The results were expressed in grams of amino acids per 100 grams of sample.

2.4.5. Antiradical properties of bread

The antiradical activity of samples was measured by using the DPPH method previously reported [27–30]. The DPPH is an assay for the study of in vitro anti-radical activity and is commonly used for the evaluation of the scavenger activity towards free radicals. Scavenging activity can be monitored by spectrophotometric analysis of the absorbance at a wavelength of 517 nm using a UV-Vis spectrophotometer (Varian Cary® 50) and methanol as the blank. The results were also reported as TEAC (Trolox equivalent antioxidant activity) and expressed as mmol Trolox equivalent (TE)/100g of sample. As standard was used Trolox in a range of 5 to 400 µM ($y = 0.0037x + 0.1655$ and $R^2 = 0.987$). All experimental procedures were replicated three times.

### *2.5. Data analysis*

Data were submitted to Bartlett's test for the homogeneity of variance and then analyzed using one or two-way analysis of variance (ANOVA). Means were statistically separated on the basis of the Student-Newmann-Kewls test. when the 'F' test of ANOVA for treatment was significant at least at the 0.05 probability (CoHort Software, CoStat version 6.451).

## 3. Results

### *3.1. Bread quality*

3.1.1. Rheological Characteristics

As already highlighted in a previous work [25], the qualitative characteristics of gluten influence the technological properties of doughs. In fact, the two protein subunits that make up gluten, glutenins and gliadins, respectively give dough toughness and extensibility. In this study, the results of the rheological characteristics obtained on the doughs (Table 2) show that the addition of hemp flour positively influenced its qualitative and technological properties. In fact, the different percentages of substitution of the Ciclope durum wheat semolina with the two types of hemp flour led to significant differences in the doughs highlighted through the determination of the alveographic and farinographic indices. It is known that the alveographic indices W and P/L provide important indications regarding the gluten qualitative characteristics. In particular, the value of the P/L ratio shows the correspondence between toughness and extensibility. A P/L value close to unity gives indications about the bread-making aptitude of a flour [31]. Table 2 shows the significant variations that occurred in the dough following the integration of Ciclope semolina with hemp flour. These variations were highlighted by the rheological analyzes by determining the alveographic (W and P/L) and farinographic (WA, DT, S) parameters. Comparing the CTRL sample (100% Ciclope semolina) and those enriched with hemp flour, different behavior of the Hemp 1 and Hemp 2 flours was observed. As regards the alveographic parameter W, it is possible to detect a decrease in its value in all samples. In particular, in the replacement of Hemp 2_5 bread, the W value decreased by about 15%. In Hemp 1, a decrease in the value of W is observed up to a maximum of 10% in Hemp_1 10. As far as the P/L alvographic parameter is concerned, an increase in its value is observed in all samples, in particular up to 40% on the Hemp 1_10 sample. In Hemp 2, its value only increases up to 15% in Hemp_2 10. Table 2 shows that the value of the water absorption farinographic index decreases as the percentages of substitution increase, only in Hemp 1_7.5 and in Hemp 1_10. In Hemp 2 bread the values remain almost unchanged compared to the CTRL. Regarding the development time, all the added samples showed a decrease in values up to 40%. As regards the stability parameter, the value rises up to 50% in Hemp 1, but drops from 15% (Hemp 2_5) to 50% in Hemp 2_10.

**Table 2.** Alveographic and Farinographic indices of bread doughs.

| Doughs bread | Moisture (%) | Alveograph analysis | | Farinograph analysis | | |
|---|---|---|---|---|---|---|
| | | W ($10^{-4}$ J) | P/L | Water absorption % | Development time (min) | Stability (min) |
| CTRL | 13.8 | 201 | 1.04 | 60.4 | 5.5 | 4.7 |
| Hemp 1_5 | 13.5 | 200 | 1.12 | 60.0 | 3.8 | 7.8 |
| Hemp 1_7.5 | 13.5 | 186 | 1.36 | 58.7 | 4.0 | 6.6 |
| Hemp 1_10 | 133 | 180 | 1.52 | 58.7 | 3.3 | 7.1 |
| Hemp 2_5 | 13.6 | 171 | 1.17 | 60.5 | 3.0 | 4.0 |
| Hemp 2_7.5 | 13.3 | 182 | 1.08 | 60.2 | 3.3 | 4.5 |
| Hemp 2_10 | 13.2 | 173 | 1.19 | 60.1 | 3.3 | 4.9 |
| Mean | 13.4 | 182 | 1.24 | 59.7 | 3.45 | 5.82 |
| St. Dev. | ± 0.15 | ± 10,45 | ± 0.17 | ± 0.79 | ± 0.37 | ± 1.55 |

*3.2. Color, Form, and Organoleptic Characteristics of the Fortified Breads*

The first parameter of quality evaluated by consumers is the color of the food. The objective values of CIELAB, on crust and crumb, the height, and the volume of the bread samples are reported in Table 3. The color indices were affected by Hemp flour and levels of fortification. Except for Hemp 1 at 5% substitution, all samples have L* values lower than CTRL both for crust and crumb. The a* value represents the green-red spectrum and negative values go towards green. For the crust, the values ranged from 10.63 (Hemp 2_10) to 12.68 (Hemp 1_5), while for the crumb the values ranged from 2.04 (Hemp 2 _10) to 0.26 (Hemp 1_5). Concerning the blue-yellow spectrum (b*), for the crust, the bread sample Hemp 2 at 10% substitution, shows a decrease in the yellow index of 12,3%, while the bread sample Hemp_2 at 5% substitution gives darker crumb. The CTRL shows values of L* 43.06, a* 14.34, and b* 23.0 for the crust, while L* 73.12, a* −2 and b* 18.24 are recorded on the crumb. For the control bread sample, prepared with Ciclope semolina, the average volume of 427 $cm^3$ and the height of 8.1 cm represent a good bread-making quality. Regarding the bread samples fortified, the baking test results showed that the partial volumes were diminishing namely from 427 $cm^3$ for CTRL bread to 300 $cm^3$ in Hemp_1 10. The reason for this phenomenon can be seen in the change in the gluten protein content in this bread, after replacing part of the wheat flour with hemp flour, which does not have it, as well as in the increased content of fiber which reduced the ability to retain fermentation gases [32]. Regarding the height of the enriched bread samples, in comparison with the CTRL, the values showed a decrease of about 30%, in particular for Hemp 1_10. As regards the pitting of the crumb, no significant variations in the size of the pits were observed following the addition of hemp flour. (Figure 1).

**Table 3.** Baking test parameters and color of Ciclope bread (CTRL) and breads enriched with hemp flours. * Mohs scale, ranging from 1 to 8, for higher and lower porosity, respectively.

| Bread sample | Crust | | | Crumb | | | Volume | BH | BW | PO* |
|---|---|---|---|---|---|---|---|---|---|---|
| | L* | a* | b* | L* | a* | b* | ($cm^3$) | (cm) | (g) | |
| CTRL | 43.06 | 14.34 | 23 | 73.12 | -2 | 18.24 | 427 | 8.1 | 143 | 6 |
| Hemp 1_5 | 44.25 | 12.68 | 24.66 | 61.64 | -0.26 | 19.04 | 375 | 7.3 | 144 | 6 |
| Hemp 1_7.5 | 42.58 | 11.75 | 21.64 | 56.59 | 0.61 | 20.8 | 350 | 7 | 141 | 6 |
| Hemp 1_10 | 42.28 | 11.12 | 20.83 | 49.91 | 1.3 | 18.47 | 300 | 5.9 | 142 | 7 |
| Hemp 2_5 | 42.55 | 12.67 | 22.2 | 56.59 | 0.86 | 21.33 | 370 | 7.5 | 144 | 6 |
| Hemp 2 _7.5 | 42.47 | 12.23 | 22.56 | 51.48 | 1.51 | 21.02 | 392 | 7.7 | 143 | 6 |
| | 41.31 | 10.63 | 20.17 | 48.14 | 2.04 | 20.89 | 325 | 6.5 | 143 | 6 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean St. Dev. | 42.57 ± 0.95 | 11.85 ± 0.84 | 22.01 ± 1.57 | 54.06 ± 5.08 | 1.01 ± 0.80 | 20.26 ± 1.19 | 352 ± 34.32 | 6.98 ± 0.68 | 142.83 ± 1.17 | 6.17 ± 0.41 |

Sensory parameters were evaluated in bread two hours after baking and self-cooling.

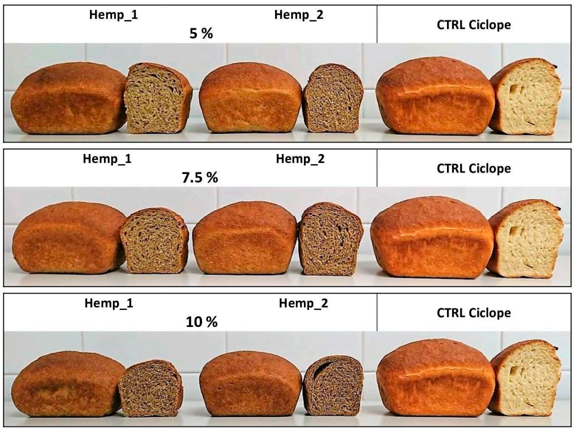


**Figure 1.** Bread obtained with the experimental bread-making test.

As shown in Table 4, the amount of fiber and protein present in the two types of hemp flour influenced the quality of the finished product, also in terms of a decrease in the elasticity values of both the crust and the crumb. Crust thickness decreased with respect to CTRL (5.0), but was still higher than 4.0 in all samples, except Hemp 2_10. The decrease in crust hardness values, as the integration percentages increase, is justified by the presence of the oil content in the hemp flour, which can improve the consistency of the finished product [20]. All bread samples had good elasticity, thickness, crispness and apparent softness with very little variation between the two hemp flours. The overall judgment has always been higher than 6 (threshold of acceptability).

**Table 4.** Sensory attributes of the crust and crumb of the Ciclope bread (CTRL) and fortified breads and their overall judgment.

| | | **Bread Hemp 1** | | | **Bread Hemp 2** | | |
|---|---|---|---|---|---|---|---|
| **Crust** | CTRL | 5% | 7.5% | 10% | 5% | 7.5% | 10% |
| Thickness a | 5.5 | 4.5 | 4.5 | 4.0 | 4.0 | 4.0 | 3.0 |
| Elasticity a | 5.5 | 5.0 | 50 | 4.5 | 5.3 | 5.0 | 4.8 |
| Hardness a | 5.0 | 4.8 | 4.6 | 4.5 | 4.9 | 4.8 | 4.6 |
| Friability a | 4.0 | 3.5 | 3.5 | 3.0 | 3.5 | 3.5 | 3.0 |
| Apparent softness a | 3.5 | 3.0 | 3.0 | 3.0 | 3.5 | 3.5 | 3.0 |
| **Crumb** | | | | | | | |
| Elasticity a | 5.2 | 4.8 | 4.5 | 4.0 | 5.0 | 4.9 | 4.5 |
| Apparent softness a | 3.3 | 3.5 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Friability a | 5.0 | 5.0 | 6.0 | 5.5 | 5.0 | 6.0 | 6.0 |
| Cohesiveness a | 5.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Humidity a | 3.8 | 5.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Average size of the alveoli a | 3.5 | 3.5 | 3.5 | 3.0 | 3.5 | 3.5 | 3.0 |
| Homogeneity of the alveoli a | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cohesiveness to the crust a | 7.5 | 7.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Bread overall judgment b | 8.0 | 8.0 | 7.0 | 6.5 | 8.0 | 7.0 | 7.0 |

a 1—good feeling and 10—bad feeling; b 1—extremely unpleasant, 10—extremely pleasant.

### *3.3. Chemical characterization of bread samples*

Chemical characterization of raw material was discussed in a published research [23] (Table S2). Antioxidant activity of the bread samples and the antiradical activity were measured; in particular, as shown in Table 5, a high total phenolic content (TPC) was mostly highlighted in bread Hemp 1_10 and in bread Hemp 2_10 samples (1.73±0.029-1.64±0.222 mgGAE/g, respectively). The analysis shown in these samples also high values of antiradical activity (3.18±0.071- 2.72 ± 0.018 mmol TE/100g, respectively) and % Scavenging (46.27 and 42.08). The lipid profile (Table 6) was evaluated by GC-MS analysis and linoleic acid, palmitic acid and oleic acid were mostly found in all samples. Table 7 highlights also the amino acids content in bread samples and a majority of tyrosine, glutamine, proline and isoleucine was identified, with increasing concentration in essential AAs according to fortification percentage.

**Table 5.** Total phenolic content and antiradical activity of Ciclope bread (CTRL) and hemp fortified bread.

| | CTRL | **Bread Hemp 1** | | | **Bread Hemp 2** | | |
|---|---|---|---|---|---|---|---|
| | | 5% | 7.5% | 10% | 5% | 7.5% | 10% |
| TPC (mgGAE/g) | 0.54±0.028 | 0.73±0.017 | 1.22±0.135 | 1.73±0.029 | 0.98±0.019 | 1.11±0.017 | 1.64±0.222 |
| | 0.32±0.018 | 1.17±0.099 | 2.34±0.124 | 3.18±0.071 | 0.61±0.062 | 1.87±0.053 | 2.72±0.018 |
| % Scavenging | 20.2 | 28.02 | 38.64 | 46.27 | 22.90 | 34.35 | 42.08 |

**Table 6.** Fatty acids content OF Ciclope bread (CTRL) and hemp fortified breads.

| | | | | **Bread** | | | |
|---|---|---|---|---|---|---|---|
| Fatty acid relative percentages (%) | **CTRL** | **Hemp 1_5** | **Hemp 1_7.5** | **Hemp 1_10** | **Hemp 2_5** | **Hemp 2_7.5** | **Hemp 2_10** |
| Palmitic acid | 22.85±4.6 | 15.58±0.09 | 14.7±0.25 | 14.03±0.11 | 14.98±0.15 | 13.96±0.075 | 13.23±0.2 |
| Stearic acid | 1.93±0.35 | 1.83±0.09 | 2.29±0.14 | 2.13±0.025 | 2.4±0.14 | 2.2±0.045 | 2.47±0.1 |
| Oleic acid | 15.3±0.14 | 14.87±0.075 | 14.84±0.29 | 14.8±0.075 | 15.23±0.12 | 14.98±0.25 | 14.6±0.3 |
| Linoleic acid | 59.6±0.50 | 59.4±0.4 | 59.36±0.44 | 59.18±0.5 | 58.6±1.26 | 58.84±0.47 | 58.9±1.6 |
| γ linolenic acid | 0.86±0.07 | 0.84±0.01 | 0.7±0.035 | 0.91±0.035 | 0.77±0.16 | 0.98±0.09 | 1.60±0.2 |
| α linolenic acid | 3.4±0.16 | 6.7±0.015 | 7.6±0.035 | 8.9±0.33 | 8.1±0.71 | 9.04±0.1 | 9.99±0.67 |
| $\Sigma\omega$ 6 | 60.46 | 60.24 | 60.06 | 60.09 | 59.37 | 59.82 | 60.5 |
| $\Sigma\omega$ 3 | 3.4 | 6.7 | 7.6 | 8.9 | 8.1 | 9.04 | 9.99 |

*Anova*

| Main effect | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid | γ linolenic acid | α linolenic acid |
|---|---|---|---|---|---|---|
| Hemp particle size | *** | ** | Ns | ** | *** | *** |
| % of substitution | *** | ** | *** | *** | *** | *** |
| Interaction | ns | ** | * | ns | *** | *** |

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**Table 7.** Amino acids content (g/100 g) in Ciclope bread (CTRL) and hemp fortified breads.

| Bread sample | Arg | Ser | Gln | Tyr | Ala | His | Pro | Thr | Leu | Met | Val | Phe | Ile | Lys | ∑ AA | ∑ EAA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CTRL | 0.12 | 0.21 | 0.19 | 0.2 | 0.1 | 0.04 | 0.62 | 1.1 | 0.01 | 0.05 | 0.05 | 0.1 | 0.37 | 0.03 | 3.19 | 1.71 |
| Hemp 1_5 | 0.14 | 0.26 | 0.29 | 0.26 | 0.1 | 0.07 | 0.74 | 1.3 | 0.01 | 0.07 | 0.06 | 0.12 | 0.48 | 0.26 | 4.16 | 2.3 |
| Hemp 1_7.5 | 0.16 | 0.32 | 0.33 | 0.31 | 0.11 | 0.11 | 0.85 | 1.4 | 0.05 | 0.1 | 0.08 | 0.15 | 0.59 | 0.57 | 5.13 | 2.94 |
| Hemp 1_10 | 0.18 | 0.37 | 0.36 | 0.38 | 0.13 | 0.18 | 0.98 | 1.83 | 0.08 | 0.12 | 0.11 | 0.21 | 0.79 | 0.95 | 6.67 | 4.09 |
| Hemp 2_5 | 0.15 | 0.35 | 0.31 | 0.35 | 0.11 | 0.12 | 0.94 | 0.69 | 0.04 | 0.08 | 0.06 | 0.15 | 0.05 | 0.95 | 4.35 | 2.02 |
| Hemp 2_7.5 | 0.17 | 0.43 | 0.36 | 0.52 | 0.14 | 0.15 | 1.14 | 0.73 | 0.07 | 0.1 | 0.09 | 0.2 | 0.14 | 0.98 | 5.22 | 2.31 |
| Hemp 2_10 | 0.21 | 0.55 | 0.41 | 0.69 | 0.18 | 0.22 | 1.26 | 1.08 | 0.12 | 0.14 | 0.14 | 0.25 | 0.34 | 1.61 | 7.2 | 3.68 |

## 4. Discussion

The study of the technological properties of the Ciclope durum wheat doughs enriched with hemp flour has provided important indications regarding the qualitative and functional characteristics of the breads obtained. The most significant difference found in the different types of dough bread concerned their decrease in terms of extensibility, as the integration percentages increased. In fact, it was possible to detect an increase in the values of the alveographic P/L, in all samples, up to 40% on the Hemp 1_10 sample. The farinographic stability index highlighted how the addition of the three different additions of hemp flour led to an increase in its values, especially in Hemp 1_5 and in Hemp 1_10 bread samples. In general, considering all the rheological and technological parameters, the inclusion of bioactive compounds in durum wheat semolina has certainly given the fortified bread good potential in relation to health benefits.

Chemical analyses showed that the bread fortification using hemp flour at different percentages increased the antiradical and antioxidant activity, and also improved amino acid and lipidic profiles and sensory and good cooking qualities. The Folin–Ciocalteau method was used for the evaluation of total phenolic content.

High values of TPC in bread samples fortified with a higher percentage of hemp flour (bread Hemp 1_10 and bread Hemp 2_10), and lower values in the CTRL bread sample (0.54±0.028 mg GAE/g) were highlighted. The supplementation of hemp flour in bread enhanced also the antiradical and antioxidant activity. The highest increase in antiradical activity was observed in bread samples containing 10% of Hemp 1 flour fortification (3.18±0.071 mmol TEAC/100g) while the lowest was recorded for the CTRL bread sample (0.32±0.018 mmol TEAC/100g).

This study also focused on the aminoacidic composition of studied samples. The contents of some amino acids considered essential in the human diet can be low in wheat proteins, especially lysine and threonine. Enriching the functional bread with different proportions of hemp flour has the potential to boost the content of essential amino acids. Aminoacidic analysis revealed an increase in the amino acid content of the fortified bread compared to CTRL (100% Ciclope flour). In particular, the analyzes showed higher content of lysine and threonine usually deficient in products based on cereals. The lysine content was found to be 0.03 g/100 g in the CTRL, a lower value than those found in bread samples added to 10% of hemp flour (0.95-1.61 g/100g in bread Hemp 1_10 and in bread Hemp 2_10 respectively); while higher values of threonine were found in bread Hemp 1_10 (1.83 g/100g) compared to the CTRL (1.1 g/100g). High levels of total essential amino acids were found in the samples of fortified bread, respectively 4.09 and 4.68 g/100 g in bread Hemp 1_10 and in Bread Hemp 2_10, compared to values found in durum wheat bread (1.71 g/100 g). This increase in amino acid values shows how much fortification can improve the protein characteristics of a fortified bakery product compared to one made only of durum wheat.

An increase in mono and polyunsaturated fatty acids was also observed in fortified bread samples respect to CTRL. The total ω 3 contents varied between 8.1 and 9.99% in bread Hemp 2 samples and between 6.7-8.9% in bread Hemp 1 samples. Linoleic, palmitic and oleic were the fatty acids present in the largest amount in bread Hemp 1_10 sample (59.18% - 14.03% - 14.8% respectively). The CTRL sample had a lower amount of total ω 3 (3.4%).

## 5. Conclusions

This study demonstrates that incorporating hemp seed flour into bread enhances its nutritional properties and aligns with the increasing demand for fortified products and the growing interest in utilizing agrifood waste in the food chain.

Through the fortification process, a notable increase in proteins and essential amino acids, lipids, unsaturated fatty acids, fiber, and minerals has been achieved, without significantly impacting the rheological effects of the final product. Despite consumers' growing interest in these fortified products, their development and design must balance the percentage of plants/extracts or byproducts and the sensory attributes to ensure consumer satisfaction. Enriching durum wheat flour with 10% hemp flour yielded favorable results, striking a balance to maintain excellent rheological characteristics in breads, while simultaneously increasing the omega-3 essential fatty acid content and enhancing antioxidant properties. Since traditional bread in Sicilian and Mediterranean cultures is primarily made from durum wheat, incorporating 10% hemp flour represents a valuable strategy to increase its nutritional properties, to the advantage of human health benefits.

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: Retention time (min.), coefficient of determination (R2) and linear regression model of external standards used for amino acids quantification; Table S2: Chemical characterization of raw materials

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