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Chemical Composition, Phytochemical Profile, Antioxidant Activity of *Eruca sativa* Seeds, and Utilization of Defatted Seeds in the Production of Functional Biscuits

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ERUCA (*Eruca sativa*) seeds are valued as rich sources of macronutrients and phytochemicals, but their high erucic acid content may preclude their use in food applications, due to the health risks erucic acid. Therefore, this research aims to characterize the defatted Eruca seeds in terms of their chemical composition, phytochemical profile, antioxidant activity, functional properties, and the possibility of exploiting them in the production of functional biscuits with high phytochemical content and minimal erucic acid content. The results showed that defatted Eruca seeds flour (DESF) was extraordinarily rich in protein, carbohydrate, fiber, and ash contents with 38.78, 38.4, 9.13 and 5.33% (FW), respectively. The amino acids in Eruca protein were higher than those in the standard pattern except for threonine, methionine and tryptophan. Erucic acid content in Eruca oil was 39.16 g/100 fat and inDESF was 0.638 g/100 (FW). Whole Eruca seeds flour (WESF) extract showed greater antioxidant activity by about 1.24 and 1.53 times compared to DESF according to DPPH free radical-scavenging assay and FRAP assay, respectively. The most striking of phenolic compounds in both WESF and DESF is sinapic acid, which exceeds the amount of all other compounds that have been discovered. All studied functional properties were higher in DESF than in WESF. Finally, the results of the sensory evaluation indicate the possibility of replacing the DESF with up to 4% of wheat flour, with the improvement of most of the sensory acceptability parameters of the resulting biscuit, and the other sensory parameters not being negatively affected.

Keywards: Eruca seeds, Erucic, functional biscuits.

Introduction

One of the practical solutions to the global food crisis is to maximize the utilization ofagricultural by-products, especially in developing countries, while trying to reach non-traditional sources of food production.

Eruca sativa is a flowering plant belongingto the Brassicaceae family. It is alsocommonly referred to as Rocket plant, Taramira, or Argula. In Egypt and someArabic countries, it is locally known as Gargir or Jarjeer. It is an annual or biannual edible plantthat originated inWest Asia,the Mediterranean, Turkey, and northern India and is being nowadays extensively cultivated and consumed in various places, where its leaves are mostly used fresh as a salad for their hot pungent taste, aromatic, and spicy flavor (Rizwana et al., 2016; Montaut et al., 2017). In recent years, Erucaplants are evaluated as a good source of phytochemicals. Various researchers have reported Eruca leaves as a rich source of natural antioxidants because it has numerous antioxidant constituents including carotenoids, phenolics, glucosinolates, vitamin C and flavonoids (Barillari et al., 2005; Bennett et al., 2006; Keyata et al., 2021). These components, especially glucosinolates, have an important role in determining the characteristic spicy flavor of Eruca (Kim et al., 2004). Moreover, the plant leaves and seeds are used as antimicrobial, anticarcinogenic, diuretic, laxatives, to improve

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digestion, to improve kidney functions, astimulant in the treatment of stomach disorders, antiulcer, the treatment of nasal polyps and tumors, cure eye infection, as an aphrodisiac, and to stimulating the growth of testes and enhance the proliferation, maturation, and differentiation of spermatozoa (Barillari et al., 2005, Alqasoumi et al., 2009; Barlas et al., 2013; Abd El-Aziz et al., 2016;Rizwana et al., 2016).

Erucaseeds are used as curing teen's pimples and used to extract oil. They are groundand used for improving meat flavor. Moreover, Erucaseed extracthas antibacterial and antifungal effects (Khoobchandani et al., 2010; Gulfraz et al., 2011), Antiemetic activity, counteract neuroin flammation effect (Gugliandolo et al., 2018) and Antidiabetic activity in case of chemically induced diabetes mellitus in rats by reducing oxidative stress (Garg & Sharma, 2014). Feeding experimental rats with Erucaseed extract significantly protect against nephrotoxicity caused by mercuric chloride (Alam et al., 2007).

Recently, Erucaproduction in Egypt has witnessed a steady increase due to the increasing demand for volatile oils for use in pharmaceutical purposes (El-Fadaly et al., 2017), in addition to farmers' tendency to expand the production of herbs, spices, and non-traditional crops.

So, increased production and consumption of Erucain Egypt could provide cheap sources of phytochemicals and antioxidants which are important for thehealth of the consumer and can also be used for diet diversification. Moreover, Erucaseeds, as a rich source of protein, carbohydrates, and fiber, can represent a distinct addition to bakery products, in line with health recommendations to reduce sources of refined carbohydrates because they are associated with an increased risk of some diseases such as type-2 diabetes (Gross et al., 2004).

On the other hand, the results of several previous studies indicated that Eruca seeds are high in erucic acid. It is known that foods containing a large amount of erucic acid are not desirable forhuman consumption due to the association of this compoundclassified as a natural toxic substance with some heart diseases, especially the reduced contractility of myocardial in experimental animals, which led to the restriction of the permissible amount of this acid in many countries such as the European Union, New Zealand, and Australia (Wendlinger et al., 2014;Vetter et al., 2020).

Therefore, it is necessary to evaluate the chemical composition of the Eruca seeds to use in various food aims,while avoiding the potential dangers of ingesting erucic acid. Accordingly, this research aims to characterize the defatted Erucaseeds in terms of their chemical composition, phytochemical contents, antioxidant activity, and functional properties and the possibility of exploiting them in the production of functional biscuits with high phytochemical content and minimal erucic acid content.

Materials and Methods

Materials

Eruca seeds were purchased from the local Spice shopsin Damanhour City, Egypt.

The materials for biscuit preparation including wheat flour (72% extraction), shortening, sugar, fresh whole egg, powdered milk, salt, baking powder and vanilla were collected from the local market in Damanhour city, Egypt.

All chemicals and reagents of the analytical methods used in the present study were of high purity analytical grade. Organic solvents were obtained from El- Gamhouria Trading Chemicals and Drugs Company, Egypt. Pure standards were produced by Koch light Laboratories, Ltd, England.

Methods

Preparation of seeds flour and defatted seed flour

To prepare whole and defatted Eruca seed flour, the following procedure was adopted after several preliminary trials: Eruca seeds were manually cleaned to exclude foreign or uncolored grains, pebbles, straw, or any other visible impurities, and then sieved to remove any dust or fine impurities. Then, the seeds were crushed using a multi-speed electric grinder (Model No., MB-355, China) at speed 2 to allow the seeds flour to pass through a 50-meshsize sieve to obtain whole Eruca seed flour. To obtain the defatted flour, a certain weight of the whole flour was soaked for 12 h at 4ºC in hexane (1: 20w/v). After the time elapsed the extract was completely filtered through a sintered funnel (G4). The powder was dried toconstant weight by evaporating the solvent residue in a drying oven under a vacuum. The product was lightly ground to homogenize and preserved at 4°C in airtight polyethylene bags for use in the following analyzes and applications.

Chemical composition

Proximate chemical analysis of Eruca seeds including moisture content, crude protein

(N×6.25), crude fat using petroleum ether (b.p: 60-80°C) in a Soxhlet apparatus, crude fiber and ash were carried out according to the AOAC (2007) procedures. Total carbohydrates content was determined by difference (100 - moisture content + crude protein + ash + lipid content+ crude fiber content). The mineral elements: Na, K, Ca, Mg, Zn, P, Fe, Cu and Mn were determined according to the AOAC (2007).

Determination of amino acids

Amino acids of Eruca seeds powder were analyzed according to the method described in AOAC (2007), in National Research Center, Giza, Egypt, as follows: initially, the acid hydrolysis of the sample was performed using 6N HCl. The hydrolyzate was recovered by evaporating the acid using a rotary evaporator. The resulted amino acids were analyzed using an amino acid analyzer (LC 3000 amino acid analyzer, High-performance system, a product of LC biochrom Eppdrop, Germany), under flowing conditions: flow rate 0.2 ml/min, the pressure of buffer from 0 to 2 bars, thepressure of reagent to 0-150 bar and reaction temperature 123 ºC.

Tryptophan was determined chromatically according to Miller's method (Miller, 1967).

Chemical score of Eruca protein

The chemical score of Eruca seeds protein was calculated according to FAO/WHO/UNU (1985),by dividing the amino acid content (g) in 100 g of tested protein by the amino acid score in the FAO/WHO/UNU.The lowest result obtained refers to the first limiting amino acid in the tested protein while the second-lowest result refers to the second limiting amino acid.

Fatty acid analysis

The fatty acid profile for Eruca was determined as methyl ester by gas-liquid chromatography according to the method described by Radwan (1978) using Shimadzu gas chromatography (GC4-cm, PFE), column: 5% DEGS on 80/100 chromo Q, 180 ºC, detector: FID, 270 ºC, flow rate of N2, H2 and air:20, 75 and 0.5 mL/min, respectively.

Determination of total phenolics

The total phenols were spectrophotometrically determined according to the Folin-Ciocalteu procedure (Zilić et al., 2012). Briefly, 100 µL of the extract were transferred into a test tube and the volumewasadjusted to 3.5 ml with distilled water and oxidized with the addition of 250 µL of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous sodium carbonate (Na_2CO_3) solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank using Spectrophotometer (model T80 x UVNIS Spectrometer PG Instruments Ltd). The total phenolic content was determined using a calibration curve prepared with gallic acid and expressed as µg of gallic acid equivalent (mg GAE) per 100 g of sample.

Determination of total flavonoid content

Total flavonoid content was estimated using the aluminum chloride method (Woisky & Salatino, 1998) as follows: 1 mL sample extract was added to 2 mL of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solution. After 2.5 hr incubation at room temperature (24±1 ºC), the absorbance was measured at 440 nm using Spectrophotometer (model T80 x UVNIS Spectrometer PG Instruments Ltd). The total flavonoid content was expressed as mg rutin equivalent (RE)/g sample extract.

Determination of ascorbic acid content

The ascorbic acid content in Eruca sativa seeds was determined using 2.6-dichlorophenol indophenol dye as described by AOAC (2007).

Measuring the antioxidant activity

(a) DPPH Free Radical-Scavenging assay:

The method of Tehet al. (2014) was used to estimatethe DPPH free radical-scavenging assay with slight modification. 10µLfrom samples extract were mixed with 3.99 mL of 25 mM DPPH• methanolic solution. The product was mixed and hold at 24±1 ºC for 30 minutes in the dark. Then, the absorbance of the test mixture was read at 517 nm using a spectrophotometer (model T80 x UVNIS Spectrometer PG Instruments Ltd)against methanol only blank. All tests were performed in 5 replicates, and the resulting values were averaged.The percentage of inhibition of DPPH• was calculated as follows:

$$\% \text{ Inhibition} = 100 \times (A_{control} - A_{sample})/A_{control}$$

Where: $A_{control}$ and A_{sample}symbolize absorbance of the control and absorbance of tested extract solution, respectively.

*(b) Measurement of reducing activity with ferric reducing antioxidant power (FRAP) Assay*The reducing activity of Eruca seed extraction was measured with the method of Benzie and Strain (1996) with some modification. For FRAP reagent preparing, 12.5 mL acetate buffer (300 mM, pH 3.6) was mixed with 12.5 mL of methanol, then, 2.5 mL of 10 mmol/L

2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) in 0.04 mol/L HCl and 2.5 mL of 0.02 mol/L $FeCl_3.6H_2O$ were added to such a solution. The FRAP reagent was kept in a water bath at 37 °C before analysis.

For theestimate, 75 µL of thesample extract were mixed with 225 µL of 50% solution of methanol in water and added to the 2.25 mL of FRAP reagent and the reaction mixture was held at 37 °C for 4 min, then, it was measured spectrophotometrically at 593 nm against the blank. To prepare the blank: 40 µl of distilled water was added to 3 mL of FRAP reagent, the product was then incubated for 60 minutes at 37°C. To prepare the standard solution, ferrous sulfate heptahydrate ($FeSO_4 \cdot vH_2O$) was used in a different concentration (0.1, 0.2, 0.4, 0.6, 0.8, and 1 mM). Measurements were repeated 5 times. Theresults oftheFRAP Assay were expressed as µmol Fe^{+2}/g fresh weight.

Determination of phenolic compounds

The phenolic compounds were extracted as follows: 1 g each of whole and defatted Eruca seed flour were initially weighed, then put into a conical flask and 20 mL of 2M NaOH was added, the flask was rinsed with nitrogen gas, then the flask was shaken at 1200 rpm for 3 h at room temperature (24±1ºC), pH was adjusted to 2 with 6N HCl, then centrifuged at 5000 rpm for 10 min, thefiltrate was washed twice to obtain phenolic compounds with 50 mLof an equal mixture of ethyl acetate and ethyl ether. The product was then transferred to a separating funnel and the organic phase was separated and concentrated under vacuum at 45°C with a rotary evaporator and re-dissolved in 2 mL ethanol, filtered through a4.5 µmMillipore filter.

HPLC analysis was carried out according to Rozan (2017) using Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler and a diode-array detector. The analytical column was Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 60 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µLand peaks were monitored simultaneously at 280, 320 and 360 nm. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

Evaluation of functional properties

Functional properties of both whole Eruca seeds flour (WESF) and defatted Eruca seeds flour (DESF) including bulk density, water absorption capacity, oil absorption capacity, emulsifying capacity, emulsion stability, foam capacity and foam stability were estimated.

The bulk density of WESF and DESF were measured according to Siddiq et al. (2009) method with slight modification. About 50 g of sample were weighed and the weight was accurately recorded, then quantitatively transferred to a 100 mL graduated cylinder, the cylinder was placed on a laboratory table and tapped gently on its wall several times from various places until the volume was stable. The volume was recorded, and the bulk density was calculated as g/mL.

The water absorption capacity was estimated by following the method of Nguyen et al. (2015) with slight modification, where 2.5 g of defatted Eruca seed flour was weighed in pre-weighed 30 mL plastic centrifuge tubes. Then, 10 mL of distilled water were added and well mixed with the powder at room temperature (24±1ºC). The tubes were kept at the same temperature for 30 min and then centrifuged at 4000 rpm for 30 min (Hermle Labortechnik GmbH, Siemensstr, D-78564 Wehingen, Germany). Just after centrifugation, the supernatant was carefully poured, and the new weight of the sample was recorded. Water absorption capacity (WAC) as g water/g powder was calculated as:

WAC = final weight – sample weight/ sample weight

Oil absorption capacity was determined according to Nguyen et al. (2015) with slight modification. Two grams of defatted Eruca seed powder were weighed in apre-weighed 30 mL plastic centrifuge tube. Twenty mL of refined corn oil were added and well mixed with the sample using a Vortex mixer at the highest speed; the samples were subsequently allowed to stand at room temperature (24 ±1°C) for 30 min. The mixture was centrifuged at 4000 rpm for 30 min (Hermle Labortechnik GmbH, Siemensstr, D-78564 Wehingen, Germany). Just after centrifugation, the supernatant was carefully poured, and the new weight of the sample was recorded. Oil absorption capacity (OAC) as g oil/g powder was calculated as:

OAC = Oiled sample weight – initial sample weight/ initial sample weight

Emulsion capacity was estimated by placing 1 g of defatted Eruca seed flour in a conical flask, then 12 mL of distilled water was added to it, then refined corn oil was dripped using a graduated burette and blended until the emulsion breaking point (the separation of the mixture into two layers that can be seen). Emulsion capacity is calculated as the number of milliliters of oil consumed to reach the emulsion breaking point relative to 100 g of the sample.

To determine the stability emulsion stability, the emulsion is placed in 50 mL centrifuge tubes, heated in a water bath at 80 °C for 30 min, then cooled to room temperature (24±1 ºC), then centrifuged at 4000 rpm for 30 min. Emulsion stability is calculated by dividing the emulsion layer height by the aqueous layer as a percentage.

The method of Siddiq et al. (2009) with slight modification was used to estimate the capacity and stability of the foam of WESF and DESF. two grams of the sample was weighed and placed in a graduated cylinder with 100 ml of distilled water, then the mixture was mixed and shaken for five minutes at room temperature (24±1ºC). The foam capacity was calculated from the following equation:

% Foam capacity = (Volume of foam after whipping – Volume of foam before whipping)/ Volume of foam before whipping ×100.

The sample was held at room temperature (24±1 ºC) for 60 min, the foam volume was recorded, and the foam stability was calculated as a percentage of the foam volume after whipping using the following formula:

Foam stability = (Final foam volume after 60 min/ Foam volume after whipping) × 100

Biscuit's manufacturing

Biscuits were manufactured by partially replacing soft wheat flour (72% extraction) with 2%, 4% or8% Eruca seeds flour according to the modified recipe of the standard procedure for semi hard sweet biscuits produced by Senyorita, El-Sadat, Egypt, as shown in Table 1 (Rozan et al., 2022). An electric mixer (LFO60546, China) was used to obtainasmooth mixturefromsugar, fat, eggs, and powdered milk. Then, flour, baking powder, and salt were added to form soft dough. Then kneaded, rolled out into sheets, cut into the desired shape, and transferred to bake at 180 °C for 17 min.

Determination of physical properties of biscuits

The physical properties of the biscuits were determined following the procedure explained by Rozan et al. (2022). All estimates were made by taking an average of six measurements on six biscuits. The weight of biscuits was measured using laboratory weighing balance.Biscuit thickness and diameter were determined using digital Verniercallipers, the value reported in millimeters. Then, calculate the spread ratio (diameter/thickness). Hardness (N) expresses the maximum load (N) determined by using a Textile analyzer (Stable Micro Systems Serial No. 5014 England) TA-XT Plus.

TABLE 1. Recipe of biscuits prepared using different proportions of Eruca seeds flour as a substitute for wheat flour.

Ingredient (g)	Control	2-ESF	4- ESF	8- ESF
Wheat flour (72% extraction)	100	98	96	82
ESF	0	2	4	8
Shortening	25	25	25	25
Sugar	25	25	25	25
Fresh whole egg	8	8	8	8
powdered milk	2	2	2	2
baking powder	1	1	1	1
salt	0.5	0.5	0.5	0.5
Vanilla	0.3	0.3	0.3	0.3

Color measurements of biscuits

The method described by Tong et al. (2010) was used to measure color parameters (Lightness (L): L=0 for darkness, L=100 for lightness; a*: chromaticity on a scale of green (–) to red (+); b: chromaticity on ascale of blue (–) to yellow (+), 90˚= yellow; 180˚= bluish to green and 270˚= blue scale) in biscuit samples by Konica Minolta CR-410 Chroma meter (Konica Minolta, Sensing, INC., Japan). The following equation was used for calculating the total color difference (E):

$$\Delta E = (\Delta L^2+\Delta a^2+\Delta b^2)^{0.5}.$$

Where: $\Delta L = L_{sample} - L_{standard}$, $\Delta a = a_{sample} - a_{standard}$, and $\Delta b = b_{sample} - b_{standard.}$

Sensory evaluation of biscuits

Appearance (symmetry), color, aroma, taste, hardness, crunchiness, and overall acceptability of the coded biscuit samples were evaluated by a 36-member panel using a 9-point hedonic scale (1 indicates very poor, and 9 indicates excellent). Hardness is the force required to fully chew through the sample when put between molars.

Statistical analysis

The results are expressed by taking the average value for five replicates (M ± SD), using SPSS 16 software by one-way ANOVA. The results were considered significant at $p<0.05$.

Results and Discussion

Chemical composition of Eruca seeds

The chemical composition of whole Eruca seed flour (WESF) anddefatted Erucaseed flour (DESF) is shown in Table 2. The data revealed that WESFis rich in its content of protein and oil, as they recorded 26.19 and 25.43%, respectively. The ash content was 3.62%, while crude fiber and carbohydrate were 6.17 and 26%, respectively.

The oil Eruca seeds was extracted, so that the remaining oil after extraction was 1.63%, also, the DESF was partially dried during the disposal of solvent residue, so the moisture content of DESF was reduced to 6.73%, which led to a high percentage of the other components in defatted seeds as follows: 38.78% protein, 5.33% ash, 9.13% crude fiber and 38.4% carbohydrate. Eruca seeds flour is very rich in its protein content;it is about 42% on a dry weight basis, which means that it is a promising source for nutritional applications. Like that, it is rich in carbohydrates, fiber, and mineralswith values of 38.4, 9.13 and 5.33% (FW), respectively.

These values recorded for protein and oil contents are close to those found by Gulfraz et al. (2011), who found that *Eruca sativa* seeds contain 29.83% protein, 27.67% oil, and 2.6% ash, but they were far from the recorded values for carbohydrates, and fiber, which was3.09%, and 1.6%, respectively.

ELSadek (2014) found that *Eruca sativa*seeds had 10.77, 25.77, 26.11, 9.11, 3.12 and 25.12% of moisture, protein, oil, ash, fiber, and carbohydrate contents, respectively. Flanders & Abdulkarim (1985) reported that the Eruca seed had 4.1% moisture, 27.4% protein, 27.8% oil content, and 6.6% ash. These results are close to the values of some components and far from the values of others in the study of Kanya & Urs (1989) who found that the chemical composition of whole watercress seeds was as follows: 18.99% protein, 33.74% oil, 3.19% total ash, 7.05 crude fiber, 37.12% carbohydrate.

It makes sense to attribute these differences to genetic differences, soil type, climate, and pre- and post-harvest treatments.

TABLE 2. Chemical composition of whole and defatted Eruca seeds.

Component (%)	Whole seed	Defatted seed
Moisture	12.56±1.36	6.73±0.85
Protein	26.19±1.68	38.78±1.05
Ether extract	25.43±1.12	1.63±0.07
Ash	3.62±0.52	5.33±0.39
Crude fiber	6.17±0.98	9.13±0.47
Carbohydrate	26±0.85	38.4±1.24

Values: Mean ± standard deviation, five replicates

Mineral content

Minerals perform vital functions that keep the human body healthy. Minerals participate in building bones and maintaining the proper functioning of the heart, brain, and muscles. Moreover, minerals are of foremost importance in the work of enzymes and hormones. Table 3 shows the mineral content of whole and defatted E. sativa seeds. The most abundant minerals were Ca, K, Na, and Mg, and in comparatively smaller quantities: P and Cu, then Fe, Zn, and Mn.

ELSadek (2014) found that the values of Zn, Fe, K and Cu in E. Sativa seeds were 50.66, 58.56, 703.65 and 30.66 µg/g, respectively.

The mineral content of E. sativa seeds in our study was much higher than that reported by Keyata et al. (2021) in Girgir (*Eruca sativa*) leaves where they found thatleaves contain 67, 43, 26.75, 23.2,12, 5.2 mg/100g (DW) of Fe, Na, K, Ca, Zn, and P, respectively.

Amino acid profile of Eruca seeds protein

Amino acids are essential nutrients. It participates in building protein and contributes to the growth and development of immunity. The amino acid composition of *Eruca sativa* seeds is listed in Table 4. A total of ten essential and eight nonessential amino acids were identified, in total; glutamic acid was the largest amount (16.53%) followed by leucine (8.58%), aspartic (7.94%), arginine (7.45%), proline (7.03) and lysine (6.82%). Tryptophan was present in the lowest amount (0.8%).

Chemical score

Chemical score estimation is the first attempt to assess the ability of protein sources to meet the human body's need for essential amino acids. The purpose of this assessment is to compare the percentage of an amino acid in the tested protein with the percentage of the same acid in a standard protein, usually the egg protein. The lowest value is expressed in the first limiting amino acid which is the acid that represents the largest deficit in the assessed protein (Rozan et al., 2021).

Table 5 lists the value of theamino acid chemical score of Eruca seeds protein. The results show that all amino acids in the tested protein source were higher than those in the standard pattern (>100) except for threonine (97.06), methionine (92.27) which was the second limiting amino acid in the studied protein, and tryptophan (80) which was the first limiting amino acid in circulating protein. On the other hand, valine scored the highest chemical score (180), followed by histidine (166.32) and then isoleucine (157.14).

Fatty acid profile

The data presented in table (6) detailing the fatty acid composition of *Eruca sativa* seed oil. Ten fatty acids were detected under the conditions of analysis, including four saturated fatty acids, which are myristic (0.17%), palmitic (6.62%), stearic (1.94%) and behenic (1.19%), four monounsaturated fatty acids, which are palmitoleic (0.18%), oleic (13.86%), eicosenoic (1.19%) and erucic (39.16), and two polyunsaturated fatty acids, which are linoleic (11.15%) and linolenic (16.8%).These results are in agreement with the results of El Nagar and Mekawi (2014) who found that erucic constitutes the largest fatty acid present in the E. sativa seed oil as it reached 39.8%, and is also somewhat close to the results recorded by Chakrabarti & Ahmad (2009) who reported that erucic acid content was 40.8%, while it was smaller than that reported by Nazif et al. (2010) who found that erucic content of the E. sativa seed oil grown in Egypt was 44.1%.Likely, these differences in the relative abundance and absolute quantities of fatty acids present in Eruca sativa seed oil depend on genetic factors, pre-harvest factors, post-harvest treatments and extraction conditions (Bell &Wagstaff, 2019).

Using these data, the amount of fatty acids presented in 100 g of WESF and DESF was calculated, as the amount of erucic acid in them was 9.96 and 0.638 g/100g (FW), respectively. Note that the permissible limit values of erucic acid in oil sources in Australia and New Zealand and more recently in EU countries are the most stringent in the world, as it is stipulated that the percentage of erucic acid in the oil does not exceed 2% (Food Standards Australia New Zealand, 2003;EU (European Commission, 2019)

Total phenolic, total flavonoids and Ascorbic acid contents

Total phenolic compounds and total flavonoids and ascorbic acid content are primarily responsible for the antioxidant capacity (Mohdaly et al., 2010; Ji et al., 2011).

TABLE 3. Mineral composition of Eruca sativa seeds (mg/100 gDW).

Component	Whole seeds	Defatted seeds
Na	315.12±4.3	420.06±3.58
Ca	1223.56±5.23	1631.4±11.5
K	1145.11±2.43	1526.4±14.13
P	68.39±2.75	91.16±1.2
Mg	447.36±0.38	596.33±9.6
Fe	38.2±0.68	50.9±.82
Zn	6.21±0.45	8.28±0.14
Cu	58.36±7.48	77.79±1.38
Mn	3.44±0.09	4.59±0.12

Values: Mean ± standard deviation, five replicates

TABLE 4. Amino acid profile of Eruca seeds protein.

	Amino acids	(/100 protein)
Essential amino acids	Lysine	6.82
	Isoleucine	4.40
	Leucine	8.58
	Phenylalanine	5.20
	Tyrosine	1.82
	Histidine	3.16
	Valine	6.30
	Tryptophan	0.8
	Threonine	3.30
	Methionine	2.03
Non-essential amino acids	Aspartic	7.94
	Glutamic	16.53
	Cysteine	4.1
	Proline	7.03
	Serine	2.45
	Glycine	6.35
	Alanine	5.25
	Arginine	7.45

Values: Mean ± standard deviation, three replicates

TABLE 5.Chemical score of Eruca seeds protein compared with the amino acid scores pattern for the FAO/WHO/UNU (1985).

Amino acid	FAO/WHO/UNU	Eruca seeds protein	% Amino acid chemical score
Lysine	5.80	6.82	117.59
Isoleucine	2.80	4.40	157.14
Leucine	6.60	8.58	130
Phenylalanine + Tyrosine	6.30	7.02	111.43
Histidine	1.90	3.16	166.32
Valine	3.50	6.30	180
Tryptophan	1.00	0.80	80*
Threonine	3.40	3.30	97.06
Methionine	2.20	2.03	92.27**

Chemical score was calculated as a percentage of the FAO/WHO/UNU (1985). *First limiting amino acid.
**Second limiting amino acid.

TABLE 6. Fatty acid composition of Eruca seed oil.

Fatty acids	(/100 fat)*	g/100 g WESF(FW) **	g/100 g DESF(FW) ***
C14:0	0.17±0.03	0.04323	0.00277
C16:0	6.62±0.2	1.68347	0.10791
C16:1	0.18±0.03	0.04577	0.00293
C18:0	1.94±0.07	0.49334	0.03162
C18:1	13.86±0.42	3.5246	0.22592
C18:2	11.15±0.34	2.83545	0.18175
C18:3	16.80±0.39	4.27224	0.27384
C20:1	8.93±0.18	2.2709	0.14556
C22:0	1.19±0.09	0.30262	0.0194
C22:1	39.16±0.96	9.95839	0.63831

* All values are mean ± SD, three replicates. **The values in this column were calculated considering that the oil content in HESF is 25.43%.(FW) ***The values in this column were calculated considering that the oil content in DESF is 1.63% (FW).

The data presented in Table 7 show that total phenolics and total flavonoids contents in WESF were greater than that of DESF by about 1.47 and 1.71 times, respectively. This increase is due to the loss of these compounds during extraction and in the extracted oil. In previous studies, it was reported that hexane is the weakest solvent in extracting phenolic compounds due to low its polarity (Peschel et al., 2007;Teh et al., 2014), so it was used in our study to extract oil from Erucaseeds that the largest possible amount of phenolic compounds can be preserved. Most of the phenol compounds found in different plant parts are soluble in water. In the study of Toor & Savage (2005), researchers found that hydrophilic phenols represented about 78-87% of the total phenols found in tomato pulp.

Also, Table 7 shows that the content of ascorbic acid in DESF (57.37 mg/100 g seed DW) is significantly more ($p<0.05$) compared to its content in WESF (45.29 mg/100 g seed DW). This may be attributed to the fact that the oil extraction led to the concentration of the dissolved components in the water.

Antioxidant activity

Natural antioxidants include many heterogeneous compounds that have the same inhibitory or delaying effect on the oxidation in a living cell. The benefit of this lies in reducing the production or catching free radicals formed in some stages of lipid oxidation, which implicate in tissue damage and pathogenesis of various diseases such as cancer and diabetes (Barillari et al., 2005;Alqasoumi et al., 2009).

Studies indicate that some of the methods used to estimate antioxidant activity may not have a significant correlation with each other, so it is necessary not to limit one method to estimating antioxidant activity for the same sample or food (Moharram & Youssef, 2014). Therefore, our study used two methods to estimate the antioxidant activity of E. sativa seeds.

DPPH radical scavenging is used as a common and reliable measure to assess the antioxidant capacity of plant extracts. Also, the Ferric Reducing Antioxidant Power assay (FRAP) is a low-cost method that is useful in evaluating the antioxidant capacity and comparing the antioxidant efficiency of different compounds. This method is based on that once a colorless $Fe3^{+}$-TPTZ complex reacts with an antioxidant; it is reduced to a blue colored Fe^{2+}-TPTZ (Benzie &Strain, 1996).In vitro, antioxidant activityresults of WESF and DESF extracts are shown in Table 8. WESF extract showed greater antioxidant activity ($p<0.05$) byabout 1.24, 1.53 times compared to DESF according to DPPH free radical-scavenging assay and FRAP assay, respectively.

These results can be attributed to the higher content of compounds with antioxidant activity, especially phenolics and flavonoids, in WESF compared to DESF.

Eruca sativa seed extract contains significant concentrations of phenolics, which are natural antioxidant. Furthermore, Eruca seeds contain considerable concentrations of glucosinolates, especially glucoerucin, which have been reported to have antioxidant effects (Maia et al., 2015).

TABLE 7. Total phenolic, total flavonoids and ascorbic acid contents of Erucaseeds (DW).

	Total phenolic content mg GAE/g seed	Total flavonoids mg RE/g seed	Ascorbic acid mg/ 100 g seed
Whole seeds	57.40±2.74a	69.55±1.5a	45.29±2.11b
Defatted seeds	39.14±1.69b	40.58±1.54b	57.37±1.79a

Values: Mean ± standard deviation, five replicates. Values followed by different letters in columns are significantly different (LSD) at $p<0.05$.

TABLE 8. DPPH free radical-scavenging assay and Ferric reducing/antioxidant power (FRAP) assay of whole and defatted E. sativa seed extracts.

samples	% Inhibition of DPPH•	FRAP assay (µmol Fe^{+2}/g FW)
WESF	44.21±1.03a	17.69±0.54a
DESF	35.62±0.94b	11.54±0.47b

Values: Mean ± standard deviation, five replicates. Values followed by different letters in rows are significantly different (LSD) at $p<0.05$.

Phenolic compounds profile

Phenolics are small organic compounds containing one or more phenolic groups, which have a defensive response against cold injury, cellular stress, or ethylene exposureand have an important effect in protecting plants from ultraviolet rays and pathogens and have remarkable antioxidant activity (Strack 1997; Rozan 2017).Research and applied interest in phenolic compounds are increasing infood processing because it delays the oxidative rancidity of lipids, which improves food quality and acceptability. The presence of phenolic compounds in certain concentrations significantly inhibits the formation of conjugated diene (Aneta et al., 2007).

The phenolic compounds profile is shown in Table (9).Under the conditions of our study, it was possible to identify 8 phenolic compounds in each of WESF and DESF.

Sinapic acid, ferulic acid and vanillic acid were the most abundant phenolic compounds identified in bothWESF and DESF on our standards,and the most striking of these is sinapic acid, which exceeds the amount of all other compounds that have been discovered, its amount in WESF and DESF was 745 and 572 mg/100 g DW, respectively.

In the research literature, sinapic acid has been reported to be effective against various diseases such asinfections, oxidative stress, cancer, inflammation, diabetes, neurodegeneration, and anxiety (Chen, 2016).

All compounds identified were present in greater amounts in WESF than in DESF ($p<0.05$). These results show that the solvent used in oil extraction caused the loss of quantities of phenolic compounds greater than the concentration of those compounds by defatting.

Functional properties of Eruca seeds defatted flour

The bulk density of WESF (0.5 g/mL) was significantly different ($p<0.05$)than for DESF (0.59 g/mL), which means that DESF has a higher unit volume weight than WESF. This increase in the bulk density of DESF can be attributed to the lower density of the removed oil compared to the other flour components. The relatively high bulk density of DESF can lead to an increase in the weight of the DESF supplemented food without a volume change.

The ability to absorb water is an important determinant in supplements added to different foodstuff. This property determines the amount of water that can be present in processed foods under various conditions (Phillips & Sternberg, 1979).

The water absorption capacity of DESF (2.43 g/g) increased significantly ($p<0.05$)from that of the DESF (2.12 g/g). This difference could be attributed to higher protein content in DESF than in WESF.

TABLE 9. Phenolic acids content of Eruca seeds extract.

Compound (mg/100 g DW)	WESF	DESF
Gallic acid	5.15±0.17a	3.89±0.1b
Protocatechuic acid	3.67±0.08a	2.96±0.07b
Vanillic acid	14.32±0.2a	12.03±0.14b
Caffeic acid	5.12±0.1a	3.56±0.09b
p-Coumaric acid	6.38±0.13a	4.73±0.2b
Ferulic acid	41.08±1.05a	36.45±0.86b
Sinapic acid	745±11.35a	572±8.5b

Values: Mean ± standard deviation, five replicates. Values followed by different letters in rows are significantly different (LSD) at $p<0.05$.

The chemical composition, especially protein and starch, greatly affects the water absorption capacity, as it increases with the increase in the amount of starch and the increase in the number and quantity of hydrophilic amino acids that make up the protein (Bashir et al., 2016).

Oil absorption capacity also increased significantly ($P<0.05$) in DESF (2.21 g/g) compared to that of WESF (1.9 g/g). Oil absorption capacity is a very important property of many foods, as it can reduce cooking loss in processed meat products (Siddiq et al., 2009) and improve the flavor of food (Kanetro et al., 2021). This property is affected by the amount of protein in the substance and the number and amount of hydrophobic and hydrophilic amino acids (Bashir et al., 2016).

Emulsion capacity expresses the mass of oil that one gram of protein can emulsify. The table shows that the emulsion capacity of DESF is significantly greater than that of WESF ($P<0.05$). Also, the emulsion stability of DESF (51.59%) was significantly higher than that of WESF (42.37%). The emulsifying properties of seed proteins are related to the protein composition, protein denaturation and processing procedure to which the protein is exposed (Fidantsi & Doxastakis, 2001). These relatively high values of emulsifying capacity and stability make DESF a promising source for utilization in bakery products, processed meats, and colloidal foods.

The foam is a gaseous system incorporated in a continuous liquid phase. Protein can lower surface tension due to its ability to form a thin film at the aqueous interface capable of absorbing air incorporated during shaking or added by whisking (Kanetro et al., 2021). Three basic requirements make a protein a good foaming agent, namely: rapid adsorption at the water-air interface during foaming, the ability to rearrange at the interface, and eventually the formation of a viscous, cohesive rubber film. The first two norms are important for improving foaming ability, while the third is a necessary factor forfoam stability (Fidantsi & Doxastakis, 2001). The table shows that the foaming capacity of DESF (35.37%) is significantly ($p<0.05$) higher than that of WESF (28.39%). Similarly, foam stability after 60 min in the case of DESF (34.7%) was significantly greater than in the case of WESF (28.39%). This is consistent with the increase of protein content in DESF compared to that in WESF. This means that the film formed when DESF is added is viscous, elastic, and cohesive, making it a promising additive to foods where foam is a sought-after ingredient.

Physical properties of biscuits

The physical properties of bakery foods reflect the quality of the basic materials that make up the product. Table 11 summarizes the results related to the effect of incorporating graded levels of DESF on the physical properties of biscuits.The results show that the incorporation of DESF up to 8% did not significantly affect the spread ratio of the biscuits compared to the control, while the addition of DESF led to a significant decrease in the diameter, thickness and weight of biscuits compared to the control. The lack of influence on the spread ratiobyincorporating DESF may be attributed to the equal amounts of fat in the different biscuit formulas, where the percentage of fat is the most influential factor on the spread ratio (Grasso et al., 2019). In general,the higherspread ratio, the more palatable the biscuits (Chauhan et al., 2016).

TABLE 10. Functional properties of Eruca seeds defatted flour.

Property	WESF	DESF
Bulk density (g/mL)	0.5±0.07b	0.59±0.08a
Water absorption capacity (g/g)	2.12±0.18b	2.43±0.1a
Oil absorption capacity (g/g)	1.9±0.09b	2.21±0.15a
Emulsion capacity (ml oil/100 g)	47.68±0.89b	55.72±1.84a
Emulsion stability (%)	42.37±1.75b	51.59±1.69a
Foaming capacity (%) at zero time	28.39±0.28b	35.37±1.23a
Foaming stability (%) after 60 min	23.52±0. 2b	34.7±0.34a

Values: Mean ± standard deviation, five replicates. Values followed by different letters in rows are significantly different (LSD) at $p<0.05$.

A decreasein biscuit diameter and thus spread ratio with anincreasein level DESF incorporation may be attributed to the dilution of gluten or increase of fiber content (Srivastava et al., 2012 and Grasso et al., 2019).

These results are consistent with results from other studies that incorporated some other by-products with wheat flour to make biscuits (Srivastava et al., 2012; Grasso et al., 2019).

The results showed a direct significant increase in the hardness of the biscuit with the increase in the level of incorporation of DESF (Table 11). These results may be attributed to the increased protein content of the DESF, which may contribute to the increased hardness. Similar results have been reported when defatted soy flour and sunflower Seed Flourare added (Gandhi et al., 2001; Grasso et al., 2019).

Color attributes of biscuits

Table 12 shows color attributes of biscuits prepared from wheat flour blends with different levels of defatted E. sativa seed flour. The lightness (L*) of biscuits decreased significantly as the DESF addition level increased. The highest value of L* (81.43) was recorded in the control sample, whereas the lowest L* value (61.86) was measured inthe%8- DESF biscuit sample. This observation was expected because DESF is darker than wheat flour. On the contrary, the value of both a* and b* increased significantly with the increase in DESF proportion, which means that the intensity of yellow and red colors increased. As an expected result, increasing DESF proportion significantly increased the color difference (ΔE) between the samples.

Organoleptic properties of biscuits

The organoleptic properties of biscuits produced with different amounts of defatted Eruca seeds flour are shown in Table 13. Theincorporation of DESF at a level of 8% significantly improved the aroma in all biscuit samples. Biscuits made using 4% DESF get the highest score (7.54), while the control sample gets the lowest score (7.24). This result may be attributed to the presence of some volatile compounds that react with the odor-enhancing additives in DESF, which results in an improvement in consumer acceptance of the resulting biscuit aroma.

The appearance of the resulting biscuit by adding 2% was significantly more receptive than the arbitrators, as it obtained an average of 7.48 points, while the panelist' acceptance of each of the control samples and %4-DESF samples, then this acceptance decreased significantly with an increase in the level addition to 8% DESF.

A similar pattern was observed regarding the acceptability of the biscuit color, where the biscuit samples added with 2% DESF had the best significant acceptance from the judges (7.48 points), followed by both control samples and 4% DESF, and in the last 8%- DESF (7.15 points).

These results can be explained by the fact that the presence of a high percentage of protein in DESF with some sugars helped to increase the products of the Maillard reaction to the extent that improved the acceptance of the judges for the resulting biscuit, but with the increase of these products with an increase in the proportion of DESF, the color was dark, which negatively affected to accept the judges.

TABLE 11. Physical properties of biscuits produced with different rates of defatted Eruca seed flour.

	Control	2-DESF	4-DESF	8- DESF
(Diameter (mm	40.66±0.11a	40.45±0.08b	40.25±0.15c	39.13±0.12d
(Thickness (mm	6.6±0.05a	6.56±0.1a	6.53±0.1a	6.35±0.1b
Spread ratio	6.16±0.32a	6.17±0.72a	6.16±0.47a	6.16±0.25a
(Weight (g	13.65±0.14a	13.52±0.1b	13.41±0.12c	13.37±0.11c
(Hardness (N	97d	99c	99b	101a

The data are expressed as the mean ± standard deviation, five replicates. * DESF: defatted Eruca seeds flour, where 2, 4, and 8-DESF express the amount of defatted Eruca seeds flour replacement (W/W). Values followed by different letters in rows are significantly different (LSD) at $p<0.05$.

TABLE 12. Color attributes of biscuits prepared from wheat flour blends with defatted Eruca seeds flour.

samples	L*	a *	b *	ΔE
Control	81.43±0.63a	3.11±0.57d	32.97±1.23b	-
2-DESF	66.27±0.57b	6.67±0.24c	34.5±0.14a	15.65±0.18c
4-DESF	64.74±0.67c	8.86±0.34b	34.83±0.11a	17.75±0.09b
8- DESF	61.86±0.63d	10.14±0.4a	35.09±0.2a	20.9±0.21a

The data are expressed as the mean ± standard deviation (n = 5). * DESF: defatted Eruca seeds flour, where 2, 4, and 8-DESF express the amount of defatted Eruca seeds flour replacement (W/W). Values followed by different letters in columns are significantly different (LSD) at $p< 0.05$.Lightness (L): L=0 for darkness, L=100 for lightness; a*: chromaticity on a scale of green (–) to red (+); b: chromaticity on scale of blue (–) to yellow (+), 90˚= yellow; 180˚= bluish to green and 270˚= blue scale.

TABLE 13. Organoleptic properties of biscuits produced with different amounts of defatted Eruca seeds flour.

	Control	2-DESF	4-DESF	8- DESF
Appearance	7.19±0.21b	7.34±0.26a	7.18±0.17b	6.98±0.3c
Color	7.30±0.17b	7.48±0.31a	7.31±0.16b	7.15±0.14c
Aroma	7.24±0.11b	7.51±0.2a	7.54±0.31a	7.4±0.29a
Taste	7.25±0.2b	7.48±0.21a	7.49±0.29a	7.2±0.18b
Hardness	7.42±0.23c	7.51±0.25b	7.68±0.15a	7.39±0.14c
Crunchiness	7.58±0.18b	7.71±0.27a	7.73±0.11a	7.55±0.3b
Overall acceptability	7.36±0.37b	7.59±0.22a	7.57±0.14a	7.14±0.27c

The data are expressed as the mean ± standard deviation (n = 25). * DESF: defatted Eruca seeds flour, where 2, 4, and 8-DESF express the amount of defatted Eruca seeds flour replacement (W/W). Values followed by different letters in rows are significantly different (LSD) at $p<0.05$.

The taste of biscuits made with 2 or 4% DESF was significantly more acceptable compared to the control or %8 DESF samples. This improvement in the taste with the addition of relatively low amounts of DESF can be attributed to some phenolic compounds or flavonoids that interact with the components of the biscuit mixture, which is positively reflectedinthe taste of the resulting biscuit, but by increasing the addition, the amount of these compounds increases, causing the appearance of bitter, astringent, or sour taste.

Increasing the proportion of DESF to the biscuit mixture gradually increased the acceptability of the hardness to a level of 4% compared to the control and 8% DESF samples. These results agree with the results of automated measurement of biscuit hardness.

A relatively similar pattern was observedinCrunchiness, where samples with 2% or 4% DESF were more receptive than the control and 8% DESF samples. Perhaps the crunchiness is related to the hardness and the spread ratio.

Finally, the overall acceptability of the biscuits made with 2% or 4% DESF was significantly better than the control, and finally 8% DESF samples.

These results indicate the possibility of replacing the defatted E. sativa seed flour with up to 4% of wheat flour, with the improvement of most of the sensory acceptability parameters of the resulting biscuit, and the other sensory parameters not being negatively affected. But with an increase in the replacement ratio to 8%, the negative influence appears in most sensory receptivity parameters.

Conclusion

In our present work, DESF showed high contents of macronutrients, mineral elements, phytochemicals, and antioxidant activity, and its protein showed a distinct chemical score compared to the standard pattern, and it showed distinct functional properties that promise to be useful in many foods, as a source of protein, unrefined carbohydrates, dietary fibers, minerals, and phytochemicals, and can also contribute to nutritional programs directed at malnourished groups, especially while reducing the erucic acid content in Eruca seeds to a minimum. The researchersrecommend accumulating studies that employ DESF in food applications besides bakery products, such as meat products and pasta. The researchers also recommend intensifying plant breeding programs directed at developing varieties of *Eruca sativa* that are low in erucic acid content.

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