Investigation of the Antioxidant Capacity, Insecticidal Ability and Oxidation Stability of Chenopodium formosanum Seed Extract

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Abstract: To maximize the extraction of antioxidants from Chenopodium formosanum seeds, the process factors, such as the ethanol concentration (0–100%), extraction time (30–180 min) and temperature (30–70 °C), for the extraction of the bioactive contents as well as the antioxidant capacity are evaluated using response surface methodology (RSM). The experimental results fit well with quadratic models. The extract was identified by GC/MS, and it was found that some active compounds had antioxidant, repellency and insecticidal activities. Various concentrations of the extract were prepared for the evaluation of the insecticidal activity against Tribolium castaneum, and the toxicity test results indicated that the extract was toxic to Tribolium castaneum, with an LC50 value of 354.61 ppm. The oxidative stability of the olive oil determined according to the radical scavenging activity and p-anisidine test demonstrates that the extract obtained from the Chenopodium formosanum seeds can retard lipid oxidation.

Keywords: antioxidant; insecticidal; heat oxidation; Chenopodium formosanum; optimization

1. Introduction

Excessive amounts of reactive oxygen species (ROS), which are constantly generated in living organisms by aerobic organisms and exogenous sources, may cause biomolecular oxidation and generate oxidative stress that can cause diseases and disorders [1,2]. Environmental influences such as UV radiation, toxicants, pollutants and diet are also major factors in accelerating the human aging process. There is a high correlation between the aging process and oxidative stress in modern civilization. It has been recognized that the effects of oxidative stress can be reduced by some antioxidant compounds [3,4]. Antioxidant compounds play an important role in protecting the body from some diseases associated with aging [5]. In addition, natural bioactive compounds can act as alternatives to synthetic pesticides in the protection of plants against pests as natural insecticides for new insect control products and can be used to improve the oxidative stability of edible oils [6–8]. Both synthetic and natural antioxidants are widely used in skin care products, nutrient foods, controlling insect pests and preventing oxidative deterioration. However, synthetic antioxidants were not well accepted by customers and are restricted in many countries due to possible undesirable effects on human and animal health [9,10]. Herein, many natural plants and fruits have been investigated as sources of antioxidants.
Chenopodium formosanum, also known as djulis, is a native cereal plant of Taiwan that is a major source of colorants and antioxidants [11,12]. Chenopodium formosanum has colorful leaves, but its bright red seeds are most likely why it is called “Hung Li” in Chinese [13]. The solvent extraction process is relatively efficient and is usually applied to extract bioactive compounds from plants and fruits. Phenolic compounds are preferably extracted with ethanol, which is an environmentally friendly solvent with a high extraction efficiency and lower toxicity and cost [14]. To obtain the maximum extraction of antioxidants from Chenopodium formosanum seeds, the effect of the ethanol concentration, extraction time and temperature on the extraction of the bioactive contents as well as the antioxidant capacity is evaluated by using the response surface methodology (RSM). The extract of Chenopodium formosanum seeds based on the optimum extraction conditions was used to evaluate the insecticidal activity against Tribolium castaneum by toxicity bioassays as well as the oxidative stability of the olive oil by using the radical scavenging activity and the \( p \)-anisidine tests.

2. Results and Discussion

2.1. Model Fitting

The total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (FICA) experimental results of Chenopodium formosanum seed extracts based on the central composite design (CCD) of RSM are tabulated in Table 1. The TPC and TFC of Chenopodium formosanum seed extracts ranged from 3.051 to 11.861 mg GAE g\(^{-1}\) DS and from 1.265 to 3.522 mg QE g\(^{-1}\) DS, respectively. In addition, the results of the FICA assay were in the range of 0.02 to 12.092 mg EDTA g\(^{-1}\) DS.

Three commonly used models (linear, quadratic and cubic) were applied to represent the experimental data, and it was found that the quadratic model was the most suitable, with the \( p \) value less than 0.05, as shown in Table 2. The regression models in coded factors for TPC, TFC and FICA are obtained as follows:

\[
\text{TPC} = 11.47 - 1.32X_1 - 0.084X_2 + 0.11X_3 + 0.32X_1X_2 + 1.75 \times 10^{-3} X_1X_3 + 0.03X_2X_3 - 1.96X_1^2 - 0.14X_2^2 + 0.044X_3^2 \tag{1}
\]

\[
\text{TFC} = 3.31 - 0.28X_1 - 8.11 \times 10^{-3}X_2 + 0.12X_3 + 0.013X_1X_2 - 0.14X_1X_3 - 3.375 \times 10^{-3} X_2X_3 - 0.58X_1^2 + 0.013X_2^2 + 0.037X_3^2 \tag{2}
\]

\[
\text{FICA} = 11.23 - 2.9X_1 + 0.25X_2 + 0.61X_3 - 0.25X_1X_2 - 0.49X_1X_3 + 0.5X_2X_3 - 3.42X_1^2 - 0.45X_2^2 - 0.56X_3^2 \tag{3}
\]

The analysis of variance (ANOVA) was used to test the fitness of the quadratic models by using the least squares technique; the results are listed in Table 2. The linear and quadratic terms of the ethanol concentration were significant at the level of \( p < 0.05 \) for all of the response values. The linear term of the temperature and the interaction between the ethanol concentration and temperature for the TFC were also significant. The R-squared (\( R^2 \)), adjusted R-squared (Adj. \( R^2 \)), F test and lack of fit test were estimated and demonstrated the adequacy of the constructed models [15].

| Independent Variables | Coded Factor Levels |
|-----------------------|---------------------|
| Ethanol concentration (%) | \( X_1 \) | -1.68 | -1 | 0 | 1 | 1.68 |
| Extraction time (min) | \( X_2 \) | 0 | 20.3 | 50 | 79.7 | 100 |
| Extraction Temperature (°C) | \( X_3 \) | 30 | 60.4 | 105 | 149.6 | 180 |

Table 1. Original and coded values of the process factors and CCD design matrix.
### Table 1. Cont.

| Independent Variables | Coded Factor Levels | Experimental values |
|-----------------------|--------------------|---------------------|
| Run | $X_1$:E | $X_2$:t | $X_3$:T | TPC | TFC | FICA |
| 1 | 20.3 | 60.4 | 38.1 | 10.745 | 2.851 | 9.151 |
| 2 | 20.3 | 60.4 | 61.9 | 11.316 | 3.429 | 9.786 |
| 3 | 20.3 | 149.6 | 38.1 | 10.148 | 2.846 | 8.207 |
| 4 | 20.3 | 149.6 | 61.9 | 10.238 | 3.405 | 12.092 |
| 5 | 79.7 | 60.4 | 38.1 | 8.140 | 2.456 | 2.360 |
| 6 | 79.7 | 60.4 | 61.9 | 8.118 | 2.464 | 2.293 |
| 7 | 79.7 | 149.6 | 38.1 | 8.234 | 2.499 | 1.692 |
| 8 | 79.7 | 149.6 | 61.9 | 8.931 | 2.499 | 2.336 |
| 9 | 0 | 105 | 50 | 8.389 | 1.988 | 5.413 |
| 10 | 100 | 105 | 50 | 3.051 | 1.265 | 0.02 |
| 11 | 50 | 30 | 50 | 10.978 | 3.356 | 10.312 |
| 12 | 50 | 180 | 50 | 10.752 | 3.261 | 11.901 |
| 13 | 50 | 105 | 50 | 11.078 | 3.231 | 9.844 |
| 14 | 50 | 105 | 70 | 11.186 | 3.522 | 11.254 |
| 15 | 50 | 105 | 50 | 11.124 | 3.479 | 11.002 |
| 16 | 50 | 105 | 30 | 11.078 | 3.319 | 11.106 |
| 17 | 50 | 105 | 70 | 11.515 | 3.231 | 11.325 |
| 18 | 50 | 105 | 50 | 11.645 | 3.161 | 10.408 |

1 TPC were expressed in mg GAE g\(^{-1}\) DS. TFC were expressed in mg QE g\(^{-1}\) DS. FICA were expressed in mg EDTA g\(^{-1}\) DS.

### Table 2. ANOVA table for the effects of the ethanol concentration, extraction time and temperature on the TPC, TFC and antioxidant capacity of the *Chenopodium formosanum* seed extracts.

| Source | DF (Degree of Freedom) | SS (Sum of Squares) | F Value | p Value |
|--------|------------------------|---------------------|---------|---------|
| TPC    | Model                  | 9                   | 80.42   | 51.45   | <0.0001 |
|        | $X_1$                  | 1                   | 23.73   | 136.61  | <0.0001 |
|        | $X_2$                  | 1                   | 0.097   | 0.56    | 0.4732  |
|        | $X_3$                  | 1                   | 0.17    | 0.97    | 0.3477  |
|        | $X_1$$X_2$             | 1                   | 0.83    | 4.80    | 0.0533  |
|        | $X_1$$X_3$             | 1                   | $2.45 \times 10^{-5}$ | 1.41 $\times 10^{-4}$ | 0.9908 |
|        | $X_2$$X_3$             | 1                   | $7.08 \times 10^{-3}$ | 0.041 | 0.8440 |
|        | $X_1^2$                | 1                   | 55.23   | 318     | <0.0001 |
|        | $X_2^2$                | 1                   | 0.28    | 1.60    | 0.2351  |
|        | $X_3^2$                | 1                   | 0.028   | 0.16    | 0.6951  |
|        | Linear                 | 3                   | 23.99   | 2.20    | 0.1278  |
|        | Quadratic              | 3                   | 55.59   | 106.68  | <0.0001 |
|        | Cubic                  | 4                   | 0.94    | 1.77    | 0.2525  |
|        | Lack of fit            | 5                   | 1.40    | 4.23    | 0.0698  |
|        | $R^2 = 0.9789$         |                     |         |         |         |
|        | Adj $R^2 = 0.9598$     |                     |         |         |         |

| Source | DF (Degree of Freedom) | SS (Sum of Squares) | F Value | p Value |
|--------|------------------------|---------------------|---------|---------|
| TFC    | Model                  | 9                   | 6.49    | 51.65   | <0.0001 |
|        | $X_1$                  | 1                   | 1.07    | 76.87   | <0.0001 |
|        | $X_2$                  | 1                   | $8.99 \times 10^{-4}$ | 0.064 | 0.8049 |
|        | $X_3$                  | 1                   | 0.2     | 14.01   | 0.0038  |
|        | $X_1$$X_2$             | 1                   | $1.43 \times 10^{-3}$ | 0.1    | 0.7555  |
|        | $X_1$$X_3$             | 1                   | 0.16    | 11.41   | 0.0070  |
|        | $X_2$$X_3$             | 1                   | $9.11 \times 10^{-5}$ | $6.53 \times 10^{-3}$ | 0.9372 |
|        | $X_1^2$                | 1                   | 4.87    | 348.84  | <0.0001 |
|        | $X_2^2$                | 1                   | $2.54 \times 10^{-3}$ | 0.18   | 0.6790  |
|        | $X_3^2$                | 1                   | 0.02    | 1.44    | 0.2583  |
Table 2. Cont.

| Source       | DF (Degree of Freedom) | SS (Sum of Squares) | F Value | p Value |
|--------------|------------------------|---------------------|---------|---------|
| Linear       | 3                      | 1.27                | 1.26    | 0.3203  |
| Quadratic    | 3                      | 5.06                | 120.78  | <0.0001 |
| Cubic        | 4                      | 0.056               | 1.00    | 0.4750  |
| Lack of fit  | 5                      | 0.071               | 1.03    | 0.4876  |
| R² = 0.9789  |                        |                     |         |         |
| Adj R² = 0.9600 |                    |                     |         |         |

2.2. Analysis of the Response Surfaces

Figure 1 is the response surface plot showing the effects of the ethanol concentration/temperature on the TPC (Figure 1a), TFC (Figure 1b) and FICA (Figure 1c) of *Chenopodium formosanum* seed extracts. The ethanol concentration was the most significant process factor in regards to the response variables, and the result was consistent with the ANOVA. When the ethanol concentration increased to the medium region (33.3~48.6%), all of the response values increased to the maximum; however, they decreased when the ethanol concentration was higher than the optimal values. The polarity of the solvent plays a crucial role due to its ability to extract substances by solubilization [10]. The presence of water will lead to the polarity of the ethanol solution increasing and will also increase the contact surface area between the plant matrix and the solvent by the swelling of the plant material [16], as shown in Figure 2.

Temperature is also an important parameter, and the interaction between the ethanol concentration and temperature for the TFC was also significant (Figure 1b). This is because the plant tissues were softened, and mass transfer between the plant and solvent was accelerated to promote the diffusion of more active compounds into the solvent under higher temperature [17,18]. However, the heating process may degrade the active compounds by hydrolytic cleavage, decarboxylation or dehydrogenation [19]. Thus, the maximum temperature in the experimental design was set at 70 °C to prevent the degradation of the active components.
Figure 1. Response surface plots showing the effects of the ethanol concentration and temperature on the (a) TPC, (b) TFC, and (c) FICA.

Figure 2. Scanning electron micrographs of *Chenopodium formosanum* samples extracted with (a) no solvent, (b) 100% water, and (c) 100% ethanol.
According to the ANOVA results from Table 2, the extraction time did not significantly contribute to the release of active components from the *Chenopodium formosanum* seeds. Moreover, the interaction between the ethanol concentration and time for the response variables was found to have no effect, except for the TPC ($p = 0.0533$, non-significantly). In general, the quantity of analytes extracted increased by increasing the extraction time, although there is a risk that the degradation of phenolic compounds may occur [20]. Therefore, the response variables of the extraction process should be influenced by the extraction time, but this impact compared to the significant effect of the ethanol concentration could be ignored.

2.3. Determination of the Optimal Conditions and Model Verification

The optimal conditions of the extraction process were obtained by solving the regression Equations (1)–(3) using Design Expert software based on the CCD experimental results. The predicted TPC, TFC and FICA values under the optimal conditions were 11.813 mg GAE g$^{-1}$ DS, 3.802 mg QE g$^{-1}$ DS and 12.580 mg EDTA g$^{-1}$ DS, respectively. To verify the validity of the predicted values, the extraction under optimal conditions was conducted thrice for each assessment. Under the optimal conditions, the experimental values of the TPC, TFC and FICA were 12.233 ± 0.236 mg GAE g$^{-1}$ DS, 3.628 ± 0.101 mg QE g$^{-1}$ DS and 12.040 ± 0.495 mg EDTA g$^{-1}$ DS, respectively. By comparing the experimental and predicted values of the response variables, low relative error values (RE < 5%) were obtained, and they verified that the fitted quadratic models are well-suited.

2.4. GC-MS Analysis of the Extract

Phenolic compounds include phenolic acids, flavonoids, and other active components, which can produce antioxidant, anticarcinogenic, antimutagenic, and anti-inflammatory effects as well as being harmful to insects [21]. Additionally, the optimal conditions for the TPC, TFC and FICA are quite close each other. Therefore, the extraction of *Chenopodium formosanum* seeds was conducted under the optimal TPC conditions, and the extract was obtained for the further investigation of the insecticidal activity against *Tribolium castaneum* as well as the oxidative stability of the olive oil. The extract was analyzed by using GC-MS to characterize the chemical composition. According to the analysis results in Table 3, the main component of the extract was sucrose, which accounted for 25.81%. Guaiacol (9.10%), 1,2-benzenediol (pyrocatechol) (2.18%) and hydroxy methyl furfural (5-hydroxymethylfurfural) (2.04%) have been reported to have antioxidant properties [22–24].

Table 3. The major compounds of the extract of *Chenopodium formosanum* seeds.

| Retention Time | Composition                          | Peak Area (%) |
|----------------|--------------------------------------|---------------|
| 4.9            | 2-Hydroxy-2-cyclopenten-1-one        | 3.46          |
| 7.97           | 2-Heptanone                          | 4.57          |
| 9.7            | Guaiacol                             | 9.10          |
| 14.09          | 1,2-Benzenediol                      | 2.18          |
| 14.52          | 4-Methylbenzaldehyde                 | 3.67          |
| 15.03          | Hydroxy methyl furural               | 2.04          |
| 18.45          | 2-Methoxy-4-Vinyl phenol             | 3.17          |
| 26.25          | Sucrose                              | 25.81         |
| 31.33          | Ethyl D-glucoside                    | 5.50          |
| 48.94          | Methyl oleate                        | 8.87          |
| 49.16          | Octadecadienoic acid                 | 4.84          |
|                | Other                                | 26.79         |
| **Total**      |                                      | **100**       |
2.5. Toxicity Bioassays

Figure 3 displays the toxicity test results of Chenopodium formosanum seed extract against Tribolium castaneum. The results indicated that the extract of Chenopodium formosanum seeds is toxic to Tribolium castaneum with an LC\textsubscript{50} value of 354.61 ppm. The effective insecticide of the extract was due to the active compounds such as 1,2-benzenediol, hydroxyl methyl furfural (5-hydroxymethylfurfural) and 2-heptanone, which were investigated to have repellency or insecticidal activity. Some studies have shown that 1,2-benzenediol (pyrocatechol) and 2-heptanone have repellency activity [25,26]. Tunón et al. [25] evaluated that the phenolic compound 1,2-benzenediol has repellency activity and was harmful to insects due to the two hydroxyl groups in the ortho-position. The insecticidal effect of 5-hydroxymethylfurfural against D. melanogaster adults is obvious, with an LD\textsubscript{50} value (the lethal dose for 50% mortality) of 34.0 mg/adult [27]. The component methyl oleate accounted for 8.87% in Chenopodium formosanum seeds, although it is known to have oviposition-deterrent activity [13,28]. The effect of methyl oleate did not contribute directly to the toxicity test results of Chenopodium formosanum seed extract against Tribolium castaneum, and it resulted in the higher LC\textsubscript{50} value (354.61 ppm) than the Tagetes lemmonii leaf extract (26.28 ppm) [8].

![Figure 3. Toxicity of the extract against Tribolium castaneum.](image)

2.6. Oxidative Stability of Olive Oil

The DPPH radical scavenging activity and the p-anisidine test was used to evaluate the ability of the antioxidants in the olive oil to scavenge free radicals as well as the secondary lipid oxidation products produced during the decomposition of hydroperoxides, respectively. These experimental results are shown in Figure 4. Figure 4a shows that the radical scavenging activity results of olive oil without extracts (control sample) increase under accelerated storage. After 48 h, the radical scavenging activity decreased with the increase in the concentration of the additive, which indicates that some active components affect the stability of DPPH. The level of formation of secondary oxidation products in olive oil and the AV values of olive oil with various concentrations of extract are shown in Figure 4b. Initially, the AV values of all of the samples increased with the increase in the storage period. Obviously, the AV values of the olive oil with the extract were lower than for the control sample. A significant difference in the AV values was observed between the control and samples with the extract at 72 h. The presence of active components (guaiacol, 1,2-benzenediol and hydroxy methyl furural) showed that the antioxidant property of the extract can decrease the absorbance of DPPH. In addition, these active compounds can prevent the autoxidation of olive oil and retard lipid oxidation through the decay of lipid hydroperoxides [29,30].
was determined by the standard calibration equation. The content of total phenolics was expressed as milligrams of gallic acid equivalents per gram of dried seeds (mg GAE g$^{-1}$).

To determine the TFC of the extracts, 100 µL of the extract was mixed with 1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The absorbance was recorded at 415 nm.

The TPC of the extracts was assessed according to the method of Singleton and Rossi [31]. The diluted sample (0.5 mL) was separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The absorbance was recorded at 760 nm.

3. Materials and Methods

3.1. Materials

Acetic acid (>99%), methanol (>99%), ethanol (99.8%), sodium carbonate (Na$_2$CO$_3$) (99.5%) and Folin-Ciocalteu’s reagent (2.0 N) were purchased from Fisher Chemical (Fair Lawn, NJ, USA). Acetic acid potassium (99%), aluminum chloride hexahydrate (AlCl$_3$·6H$_2$O) (99%), EDTA (99%), ferrozine, gallic acid (98%), iron (II) chloride tetrahydrate (FeCl$_2$·4H$_2$O) and iron (III) chloride hexahydrate (FeCl$_3$·6H$_2$O) were supplied by Acros (Geel, Belgium). DPPH (2,2-diphenyl-1-picrylhydrazyl) (95%) and p-anisidine (99%) were purchased from Alfa Aesar (Haverhill, MA, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively.

3.2. Extraction of Chenopodium formosanum Seeds

Chenopodium formosanum seeds obtained locally in Tainan, Taiwan, were ground to a fine powder. Approximately 5 g of Chenopodium formosanum seeds was extracted with ethanol solution, and the influential parameters such as the ethanol concentration, extraction time and temperature on the phenolic content and antioxidant capacity of the extract were determined. The extract was filtered through Whatman No. 1 filter paper, separated by centrifugation (6000 rpm), evaporated in a rotary evaporator and stored at 4 °C for further utilization. The GC/MS equipped with a Trace™ 1300 gas chromatograph (Thermo, Waltham, MA, USA) was used to analyze the bioactive compounds of the extract. The operating parameters were described elsewhere [8].

3.3. Phenolic Content Analysis

The determination of TPC of the extracts followed the method of Singleton and Rossi [31] and was determined with Folin-Ciocalteu’s reagent. Fifty microliters of the diluted extract was mixed with 200 µL of Folin-Ciocalteu’s reagent and 2 mL of distilled water. After being agitated for 5 min to mix well, 15% sodium carbonate (1 mL) was added, vortexed and incubated at room temperature for 2 h in the dark. The absorbance (760 nm) was measured by a spectrophotometer, and the TPC content was determined by the standard calibration equation. The content of total phenolics was expressed as milligrams of gallic acid equivalents per gram of dried seeds (mg GAE g$^{-1}$ DS).

The TPC of the extracts was assessed according to the method of Chang et al. [32]. The diluted sample (0.5 mL) was separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The absorbance was recorded at 415 nm.

Figure 4. (a) DPPH radical scavenging activity and (b) AV values of olive oil supplemented with different amounts of the extract.

Chenopodium formosanum
by a spectrophotometer after 30 min. The content of the total flavonoids was expressed as milligrams of quercetin equivalents per gram of dried seeds (mg QE g\(^{-1}\) DS).

### 3.4. Antioxidant Capacity Analysis

The antioxidant capacity of the extract was evaluated by the ferrous ion chelating activity (FICA) based on the method of Dinis et al. [33] with minor changes. Briefly, 0.5 mL of the diluted extract was mixed with 1.9 mL of 99% methanol and 0.05 mL solution of FeCl\(_2\) (2 mM). After 30 s, 0.1 mL of ferrozine (5 mM) was added, and then, the mixture was vigorously shaken and left at room temperature. The absorbance of the mixture at 562 nm was measured after 10 min. The chelating activity of the extract for Fe\(^{2+}\) was expressed as milligrams of EDTA equivalents per gram of dried seeds (mg EDTA g\(^{-1}\) DS).

### 3.5. Insect Cultures and Toxicity Bioassays

*Chenopodium formosanum* seed extract was investigated for its insecticidal activity against *Tribolium castaneum*. The insects were reared using the method described by Bougherra et al. [34], and the conditions are presented in Ma et al. [8]. Adult *Tribolium castaneum* was used in the toxicity bioassays, which were based on the procedure described by Peixoto et al. [35]. Briefly, a 500 µL volume of sample extract was dropped onto a piece of filter paper, which was placed on the bottom of the Petri dish (8 cm diameter) with 20 adult insects for the toxicity test. To prevent the fast escape of the diluted extract, Parafilm was used to seal the Petri dish. In addition, some pinholes were created in the Parafilm to avoid the death of the insects from suffocation. The positive and negative controls were 0.5% of the insecticide permethrin diluted with ethanol and ethanol without permethrin, respectively. Each toxicity test was conducted in three replicates at 25 ± 1 °C and 60% relative humidity in the dark. The dead insects were recorded every 30 min, and the observed mortality (%) was evaluated after 3 h. Abbott’s formula [36] was used to correct the control mortality (%) to obtain the corrected mortality (%):

\[
\text{Corrected mortality (\%)} = \left( \frac{\text{observed mortality (\%)} - \text{control mortality (\%)}}{100 - \text{control mortality (\%)}} \right) \times 100
\]  

(4)

Various concentrations of the extract were prepared for the evaluation of the insecticidal activity against *Tribolium castaneum*, and the plot of the test sample concentrations versus the corrected mortality can be obtained to determine the lethal concentration of the sample to kill 50% of the insects (LC\(_{50}\) value).

### 3.6. Oxidative Stability of Olive Oil

Different concentrations (200–800 ppm) of *Chenopodium formosanum* seed extract (150 µL) were added to 50 mL of olive oil, and the samples were stored in a 100 °C oven for 3 h (pre-heated) and then cooled in a 4 °C refrigerator for 21 h. Both the heating and cooling processes were cycled for 48 h to accelerate the oxidation of the olive oil, which was based on the study of Yim et al. [37]. To investigate the oxidative rancidity of the oil, the DPPH scavenging activity was determined according to the method described by Martinez and Maestri [38], and the p-anisidine value (AV) was determined following the method described by Chong et al. [29] with some modifications. The detailed test procedures were explained in other literature [8].

### 3.7. Experimental Design and Statistical Analysis

The CCD with five levels and three process factors was applied to study the effects of the process factors on multiple responses, including the TPC, TFC and antioxidant capacity. Table 1 lists the original and coded values of the process factors, CCD design matrix and the corresponding response data. According to the experimental data, the fitting model was constructed using the Design Expert
program (https://www.statease.com/) (version 10.0). Based on Fisher’s F-test, the analysis of variance (ANOVA) for multiple responses was used to check the adequacy of the proposed model.

4. Conclusions

The response surface methodology was used to evaluate the effect of the ethanol concentration, extraction time and temperature on the extraction of bioactive contents from *Chenopodium formosanum* seeds. The ethanol concentration was the most significant process factor in the extraction process. Based on the CCD experimental results, the optimal conditions of the extraction process were obtained. The relative error values between the experimental and predicted values of the response variables are less than 5%, indicating that the fitted quadratic models are adequate to predict the TPC, TFC and antioxidant capacity of the extract from *Chenopodium formosanum* seeds. The contents of the extract were further analyzed by GC/MS, and it was found that the extract containing some active compounds had antioxidant, repellency and insecticidal activities. The toxicity test results indicated that the extract of *Chenopodium formosanum* seeds is toxic to *Tribolium castaneum* with an LC$_{50}$ value of 354.61 ppm. Additionally, the oxidative stability of olive oil during accelerated oxidation storage was enhanced due to the presence of active components in the extract. Therefore, the present study may reduce the utilization of synthetic antioxidants and pesticides as well as promote the application of natural plants and fruits.

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**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| RSM          | Response surface methodology |
| ROS          | Reactive oxygen species |
| TPC          | Total phenolic content |
| TFC          | Total flavonoid content |
| FICA         | Ferrous ion chelating activity |
| EDTA         | Ethylenediaminetetraacetic acid |

**References**

1. Chew, Y.L.; Lim, Y.Y.; Omar, M.; Khoo, K.S. Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT-Food Sci. Technol.* **2008**, *41*, 1067–1072. [CrossRef]
2. Gliszczynska-Swiglo, A. Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays. *Food Chem.* **2006**, *96*, 131–136. [CrossRef]
3. Chyau, C.C.; Chu, C.C.; Chen, S.Y.; Duh, P.D. Djulis (*Chenopodium formosanum*) and its bioactive compounds protect against oxidative stress in human HepG2 cells. *J. Funct. Foods* **2015**, *18*, 159–170. [CrossRef]
4. Gutteridge, J.M.C.; Halliwell, B. Antioxidants: Molecules, medicines, any myths. *Biochem. Biophys. Res. Commun.* **2010**, *393*, 561–564. [CrossRef] [PubMed]
5. Vattakandy, L.S.; Chaudhari, G.S. Estimation of total phenolic content and antioxidant activity of *Dendrobium herbaceum* Lindl. *Int. J. Pharm.* **2013**, *104*, 302–305.
6. Kyarimpa, C.M.; Bohmdorfer, S.; Wasswa, J.; Kiremire, B.T.; Ndiege, I.O.; Kabasa, J.D. Essential oil and composition of *Tagetes minuta* from Uganda. Larvicidal activity on *Anopheles gambiae*. *Ind. Crops Prod.* **2014**, *62*, 400–404. [CrossRef]
7. Su, S.W.; Lee, T.M.; Mok, H.K.; Chio, E.H. Insecticidal activity and insect repellency of four species of sea lily (comatulida: comatulidae) from Taiwan. *J. Insect Sci.* **2016**, *16*, 1–5. [CrossRef] [PubMed]
8. Ma, C.M.; Cheng, C.L.; Lee, S.C.; Hong, G.B. Antioxidant capacity, insecticidal ability and heat-oxidation stability of Tagetes lemmonii leaf extract. Ecotoxicol. Environ. Saf. 2018, 151, 68–75. [CrossRef] [PubMed]

9. Hrasia, A.R.; Hadolin, M.; Kenz, Z.; Bauman, D. Comparison of antioxidantive and synergistic effects of rosemary extract with a-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chem. 2000, 71, 229–233. [CrossRef]

10. Ilaiyaraja, N.; Likhith, K.R.; Sharath Babu, G.R.; Khanum, F. Optimisation of extraction of bioactive compounds from Feronia limonia (wood apple) fruit using response surface methodology (RSM). Food Chem. 2015, 173, 348–354. [CrossRef] [PubMed]

11. Narkprasom, K.; Su, W.L.; Cheng, M.Y.; Wang, S.P.; Hsiao, S.M.; Tsai, P.J. Relative effects of alcohol and pH on betacyanin stability in aqueous djulis extracts and their color qualities after organic acid addition. J. Food Qual. 2012, 35, 283–291. [CrossRef]

12. Yang, B.Y.; Cheng, M.H.; Ko, C.H.; Wang, Y.S.; Yang, Y.P.; Chen, H.T.; Chang, F.C. Potential bioethanol production from Taiwanese cherenpods (Chenopodium formosanum). Energy 2014, 76, 59–65. [CrossRef] [PubMed]

13. Chio, E.H.; Yang, E.C.; Huang, H.T.; Hsu, E.L.; Chen, C.R.; Huang, C.G.; Huang, R.N. Toxicity and repellence of Taiwanese indigenous djulis, Chenopodium formosanum, against Aedes albopictus (Diptera: Culicidae) and Forcipomyia taiwana (Diptera: Ceratopogonidae). J. Pest Sci. 2013, 86, 705–712. [CrossRef]

14. Amado, I.R.; Franco, D.; Sanchez, M.; Zapata, C.; Vazquez, J.A. Optimisation of antioxidant extraction from Solanum tuberosum potato peel waste by surface response methodology. Food Chem. 2014, 165, 290–299. [CrossRef] [PubMed]

15. Bagheri, A.R.; Ghaedi, M.; Asfaram, A.; Hajati, S.; Ghaedi, A.M.; Bazrafshan, A.; Rahimi, M.R. Modeling and optimization of simultaneous removal of ternary dyes onto copper sulfide nanoparticles loaded on activated carbon using second-derivative spectrophotometry. J. Taiwan Inst. Chem. Eng. 2016, 65, 212–224. [CrossRef]

16. Hemwimon, S.; Pavasant, P.; Shoipruck, A. Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia. Sep. Purif. Technol. 2007, 54, 44–50. [CrossRef] [PubMed]

17. Shi, J.; Yu, J.; Pohorly, J.; Young, C.; Bryan, M.; Wu, Y. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. J. Food Agric. Environ. 2003, 1, 42–47.

18. Tekin, K.; Akalin, M.K.; Seker, M.G. Ultrasound bath-assisted extraction of essential oils from clove using central composite design. Ind. Crops Prod. 2015, 77, 954–960. [CrossRef]

19. Azeredo, H.M.C. Betalains: Properties, sources, applications, and stability—a review. Int. J. Food Sci. Technol. 2009, 44, 2365–2376. [CrossRef]

20. Proestos, C.; Boziaris, I.S.; Nychas, G.J.E.; Komaitis, M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 2006, 95, 664–671. [CrossRef]

21. Dieng, H.; Zawawi, R.B.M.; Mohamed Yusof, N.I.S.B.; Ahmad, A.H.; Abang, F.; Ghani, I.A.; Satho, T.; Ahmad, H.; Zaharah, W.F.; Majid, A.H.A.; et al. Green tea and its waste attract workers of formicine ants and kill their workers—implications for pest management. Ind. Crops Prod. 2016, 89, 157–166. [CrossRef]

22. Azadfar, M.; Gao, A.H.; Bule, M.V.; Chen, S. Structural characterization of lignin: A potential source of antioxidants guaiacol and 4-vinylguaiacol. Int. J. Biol. Macromol. 2015, 75, 58–66. [CrossRef] [PubMed]

23. Kosobutskii, V.S. Pyrocatechol and its derivatives as antioxidants and prooxidants. Russ. J. Gen. Chem. 2014, 84, 746–749. [CrossRef]

24. Zhao, L.; Chen, J.; Su, J.; Li, L.; Hu, S.; Li, B.; Zhang, X.; Xu, Z.; Chen, T. In vitro antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. J. Agric. Food Chem. 2013, 61, 10604–10611. [CrossRef] [PubMed]

25. Tönös, H.; Thorsell, W.; Bohlin, L. Mosquito repelling activity of compounds occurring in Achillea millefolium L. (asteraceae). Econ. Bot. 1994, 48, 111–120. [CrossRef]

26. Rieth, J.P.; Wilson, W.T.; Levin, M.D. Repelling honeybees from insecticide-treated flowers with 2-heptanone. J. Apicult. Res. 1986, 25, 78–84. [CrossRef]

27. Miyazawa, M.; Anzai, J.; Fujikake, J.; Isikawa, Y. Insecticidal compounds against drosophila melanogaster from Curinus Officinalis Sieb. et Zucc. Nat. Prod. Res. 2003, 17, 337–339. [CrossRef] [PubMed]

28. Bird, T.G.; Hedin, P.A.; Burks, M.L. Feeding deterrent compounds to the boll weevil, Anthonomus grandis Boheman in rose-of-sharon, Hibiscus syriacus L. J. Chem. Ecol. 1987, 13, 1087–1097. [CrossRef] [PubMed]
29. Chong, Y.M.; Chang, S.K.; Sia, W.C.M.; Yim, H.S. Antioxidant efficacy of mangosteen (Garcinia mangostana Linn.) peel extracts in sunflower oil during accelerated storage. Food Biosci. 2015, 12, 18–25. [CrossRef]
30. Hashemi, S.M.B.; Khaneghah, A.M.; Koubaa, M.; Lopez-Cervantes, J.; Yousefabad, S.H.A.; Hosseini, S.F.; Karimi, M.; Motazedian, A.; Asadifard, S. Novel edible oil sources: Microwave heating and chemical properties. Food Res. Int. 2017, 92, 147–153. [CrossRef] [PubMed]
31. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am. J. Enol. Viticult. 1965, 16, 144–158.
32. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 2002, 10, 178–182.
33. Dinis, C.P.; Madeira, V.M.C.; Almeida, L.M. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 1994, 315, 161–169. [CrossRef] [PubMed]
34. Bougherra, H.H.; Bedini, S.; Flamini, G.; Cosci, F.; Belhamel, K.; Conti, B. Pistacia lentiscus essential oil has repellent effect against three major insect pests of pasta. Ind. Crops Prod. 2015, 63, 249–255. [CrossRef]
35. Peixoto, M.G.; Bacci, L.; Blank, A.F.; Araujo, A.P.A.; Alves, P.B.; Silva, J.H.S.; Santos, A.A.; Oliverira, A.P.; Costa, A.S.; Arrigon-Blank, M.F. Toxicity and repellency of essential oils of Lippia alba chemotypes and their major monoterpenes against stored grain insects. Ind. Crops Prod. 2015, 71, 31–36. [CrossRef]
36. Abbott, W.S. A method for computing the effectiveness of the insecticide. J. Am. Mosq. Control Assoc. 1987, 3, 302–303. [CrossRef] [PubMed]
37. Yim, H.S.; Chye, F.Y.; Liow, M.L.; Ho, C.W. Antioxidant potential of Pleurotus Porrignon extract and application in sunflower oil during accelerated storage. Chiang Mai J. Sci. 2013, 40, 34–48.
38. Martinez, M.; Maestri, D.M. Oil chemical variation in walnut (Juglans regia L.) genotypes grown in Argentina. Eur. J. Lipid Sci. Technol. 2008, 110, 1183–1189. [CrossRef]