Ultrasound-assisted extraction of functional compound from mulberry (Morus alba L.) leaf using response surface methodology and effect of microencapsulation by spray drying on quality of optimized extract

Supasit Insang, Isaya Kijpatanasilp, Saeid Jafari, Kitipong Assatarakul

Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

ARTICLE INFO

Keywords:
Mulberry leaf
Ultrasound-assisted extraction
Antioxidant activity
Response surface methodology
Microencapsulation
Spray drying

ABSTRACT

This study aimed to optimize the ultrasound-assisted extraction (UAE) condition of mulberry leaf extract (MLE) using response surface methodology and to microencapsulate MLE by spray drying using different coating materials and ratios of coating material and MLE. The extraction results showed that MLE from condition of 60 °C (X1, temperature), 30 min (X2, time) and 60% v/v (X3, ethanol concentration) exhibited the highest bioactive compound and antioxidant activity (DPPH and FRAP assay). Based on this optimal condition, MLE was further encapsulated by spray drying. It was found that MLE encapsulated with resistant maltodextrin at ratio of MLE and resistant maltodextrin 1:1 (w/w) showed the highest encapsulation yield (%) and encapsulation efficiency (%). Water solubility, moisture content and water activity were non-significant (p > 0.05) among the microcapsules. The scanning electron microscope (SEM) revealed that the types of coating material affected their microstructures and microcapsules prepared by resistant maltodextrin as coating material had a spherical shape, smooth surface and less shrinkage than microcapsules prepared by maltodextrin and gum arabic which had rough surfaces. The highest antioxidant activity was obtained from microcapsule prepared by gum arabic at ratio of MLE and gum arabic 1:2 (w/w). In conclusion, optimal condition from UAE and encapsulation by spray drying suggest the critical potential for production of functional food with improved bioactive compound stability and maximized antioxidant activity.

1. Introduction

Nowadays, the healthy food industry is quickly growing, particularly in the area of herbal food product. This is due to the presence of numerous bioactive components with antioxidant capabilities, such as phenolic compounds and flavonoids, which may help prevent chronic diseases such as heart disease, cancer, and other complications [1]. Mulberry (Morus alba L.) fruit possesses biological activities such as anticancer, antioxidant, anti-thrombotic, and anti-inflammatory activity, and its bark and root aid to minimize overheating in the human liver, asthma symptom, and boost diuretic activity, according to previous study [34]. Mulberry leaf is also used in Chinese traditional medicine to treat diabetes, reduce thirst, and as an expectorant and anti-cough agent, in addition to the fruit. Previous research has shown that mulberry leaf extract (MLE) had antibacterial and antioxidant properties [24]. These properties are mostly due to the presence of phenolic substances such as quercetin, kaempferol, rutin, isoquercetin, astragalin, myricetin derivatives, and other glucosides in MLE which has recently been described in the literatures [7].

Ultrasound-assisted extraction (UAE) has been extensively used for the extraction of phenolic compounds from various materials. The UAE disrupts the solid material’s cell wall, making it easier to penetrate the matrix, increase mass transfer, and increase extraction rate [29]. UAE is becoming increasingly popular for extracting important compound from a variety of natural matrices. The UAE will boost extraction rate and reduce processing time compared to traditional phenolic compound extraction methods since it is a faster, more effective, and solvent-saving procedure. Although UAE is effective extraction method to isolate bioactive compound from plant material, however, the effect of parameters such as temperature, extraction time, and solvent concentration, on the recovery of active components from MLE are still undetermined.

Microencapsulation is a useful method for improving bioactive component stability in food/beverage. Several microencapsulation
technologies for application in the food industry have been developed, and they showed promise for the creation of functional food. Furthermore, these technologies may help in the delivery of bioactive substance to the gastrointestinal tract [14]. Spray drying is the most used method for microencapsulation because it is cost-effective, easy to use, and it is reported to create high-quality particle [30].

Response surface methodology (RSM) is a statistical and mathematical methodology that uses a second-degree polynomial model to explore the correlations between one or more response variables and several independent factors using univariate or multivariate methodologies [35]. The conventional selection of the optimal extraction parameters can be done “one variable at a time,” but this technique is highly time consuming and does not account for possible interaction between variables and parameters. Many researchers have declared RSM as one of the most efficient optimization strategies for determining the interaction and correlation of experimental factors in a variety of processes for high yields and product quality approval [9,25]. As a result, when any procedure contains numerous parameters and interactions that may affect the recovery of bioactive chemicals from the extract, this technique can be deemed an appropriate option for optimization.

In the last decade, numerous papers have been published worldwide on the microencapsulation of bioactive compounds from variant sources by spray drying. However, to the best of our knowledge, there are paucity of information in the literature about the above mentioned from MLE using RSM. Therefore, the authors of the present work have put their best efforts to fill the current need by conducting a precise research to maximize extraction of bioactive compound with antioxidant activity from MLE using RSM based on Box-Behnken design and to investigate the effect of microencapsulation condition (coating material and ratio of MLE and coating material) on physical and chemical properties of MLE microcapsules.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All the chemicals, reagents, and solvents used in this experiment were analytical grade. The materials and chemicals used in this research were: mulberry leaf Buriram 60 variety (Kanchanaburi, Thailand), maltodextrin DE 10–12 (Zhucheng Dongxiao, China), fibersol-2, DE12 (Matsutani, Japan), gum arabic (Agrigram, UK), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Fluka, U.S.A), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Fluka, Denmark), aluminium chloride (Ajax Finechem, New Zealand), ethanol (EMSURE®, Germany), ferric chloride (Fisher Scientific, UK), Folin-Ciocalteu reagent (Carlo Erba, France), gallic acid (Fluka, Spain), glacial acetic acid (QR Chem, New Zealand), hydrochloric acid (Ajax Finechem, Australia), methanol (Fisher Scientific, UK), and quercetin (Sigma-Aldrich, Germany).

### 2.2. Mulberry leaf sample preparation

The leaves (two-month old) of mulberry (variety Buriram 60) were collected from a mulberry farm (Kanchanaburi, Thailand) and transported to Department of Food Technology, Faculty of Science, Chulalongkorn University (they were provided by Mulberrix Co., Ltd). In the lab, the leaves were rinsed under water to remove any dirt and dust, washed, and then dried in a hot air oven (Memmert, DO 6062, Germany) at 60 °C until moisture content < 5%. The dried mulberry leaves were powdered using a 50-mesh sieve, vacuum-packed in an aluminum-laminated foil bag and kept in a freezer at −20 °C for use in UAE experiment.

### 2.3. Ultrasound-assisted extraction (UAE)

For extraction of mulberry leaf, 3 g of sample was mixed with 120 mL of solvent in a 250 mL beaker. Then, the UAE was performed with an ultrasound processor (UP400S Ultrasonic processor, Hielsher, Germany) with a 400 W power, 0.7 s cycle, 55 % amplitude, 24 kHz and a titanium probe (H22D, 22 mm). Ice bath must be employed for controlling the temperature during the process. After extraction, the samples were centrifuged (Centrifuge Kubota, series 6000, Japan) at 6,000 rpm for 15 min at room temperature and the supernatant was filtered through a No.1 filter paper, then evaporated under vacuum condition at 40 °C with a rotary evaporator (Oilbath B-485, BÜCHI, Switzerland). The final sample volume was then adjusted to 10 mL with distilled water and sampled after the extraction of the above conditions in an amber vial at 4 °C before the further analysis.

2.3.1. Optimization of ultrasound-assisted extraction in mulberry leaf

Responses surface methodology (RSM) with Box-Behnken Design (BBD) was used to evaluate the optimum level of the three independent variables (X₁: temperature; X₂: extraction time; X₃: ethanol concentration) and three levels (-1, 0, 1) were used to determine the optimum combinations of four responses; total phenolic compound (TPC), total flavonoid content (TFC), antioxidant activity by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity assay and antioxidant activity by ferric reducing antioxidant power (FRAP) assay. The independent variable and their levels were shown in Table 1.

The variation of TPC, TFC and antioxidant activity by DPPH and FRAP assays related to the three variables X₁, X₂ and X₃ were evaluated using a second-order polynomial equation:

\[ Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \]  

where Y is response (total phenolic compound, total flavonoid content, antioxidant activity by DPPH and FRAP assays, respectively), \( \beta_0 \) is the constant coefficient of intercept, \( \beta_i \), \( \beta_{ii} \) and \( \beta_{ij} \) are linear, quadratic and interaction coefficients, respectively. While \( X_i \) and \( X_j \) are the levels of three independent variables (temperature, extraction time and ethanol concentration). Polynomial equations were employed to generate 3D surface plots to observe the correlation between the dependent variables and the values of each independent variable.

Box-Behnken design with 17 experimental runs (5 center points included) generated by RSM was applied to optimize the operating...
condition which including extraction time (X1: 10–30 min), temperature (X2: 40–80 °C), and ethanol concentration (X3: 60–100 % v/v) of ultrasound-assisted extraction in mulberry leaf (Table 2).

2.4. Preparation of mulberry leaf extract for determination of antioxidant properties

1 g of MLE was dissolved in 10 mL of distilled water, vortexed for 3 min and put in a hot shaking water bath (30 °C for 30 min), centrifuged at 4000 rpm for 20 min. Then, the supernatant was collected for further determination.

2.5. Determination of total phenolic compound content

Total phenolic compound content was determined following to Folin-Ciocalteu method previously described by Waterhouse [33]. In brief, 100 µL of supernatant (or MLE) was pipetted into a 10 mL volumetric flask, 7 mL of distilled water and 500 µL of Folin-Ciocalteu reagent were added and left for 5 min at room temperature. Thereafter, saturated sodium carbonate solution (1.5 mL) was added and adjusted the volume with distilled water to 10 mL, incubated for 2 h at room temperature in a dark place. Gallic acid (0–0.5 mg/mL) standard was prepared in the same manner. Then, the absorbance of mulberry leaf extract and gallic acid were read at 765 nm using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). Total phenolic compound was reported as mg gallic acid equivalents/100 g dry basis (mg GAE/100 g db) using the gallic acid standard curve equation (y = 0.0012x + 0.0054, R² = 0.99).

2.6. Determination of total flavonoid content

The analysis of total flavonoid content (TFC) was conducted by aluminum chloride colorimetric method according to the method of Ramakrishnan et al. [23]. Briefly, 250 mL of supernatant (or mulberry leaf extract) was vortex-mixed and left in the dark at room temperature for 30 min, absorbance of FRAP solution and mulberry leaf extract were measured at 593 nm. using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). Antioxidant activity by FRAP assay was calculated according to the following equation and the results were reported as µM trolox equivalents/g db.

\[
A_{\text{diff}} = A_{\text{final}} - A_{\text{initial}}
\]

where \( A_{\text{initial}} \) is absorbance of FRAP, \( A_{\text{final}} \) is absorbance of sample and \( A_{\text{diff}} \) is difference of the absorbance between FRAP and sample.

2.7. DPPH radical scavenging activity

The analysis of antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method was according to the method of Brand-Williams et al. [5]. Briefly, 250 mL of supernatant (or mulberry leaf extract) was mixed with 4.75 mL of DPPH methanol solution, vortex-mixed, and incubated in darkness at room temperature for 15 min. Absorbance of DPPH daily solution and mulberry leaf extract were measured at 515 nm using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). Antioxidant activity by DPPH assay was reported as µM trolox equivalents/g db using the following equation:

\[
A_{\text{diff}} = A_{\text{initial}} - A_{\text{final}}
\]

where \( A_{\text{diff}} \) is the difference of the absorbance between DPPH and sample, \( A_{\text{initial}} \) is absorbance of DPPH, and \( A_{\text{final}} \) is absorbance of sample.

2.8. Ferric reducing antioxidant power

Antioxidant activity by ferric reducing antioxidant power (FRAP) method was according to the method of Benzie and Strain [4]. Briefly, the FRAP solution was preheated at 37 °C for 5 min until the color was changed from brown to orange-brown. Then, 4.75 mL of FRAP solution and 250 µL of supernatant (or mulberry leaf extract) were vortex-mixed and incubated for 5 min at room temperature for 30 min, absorbance of FRAP solution and mulberry leaf extract were measured at 593 nm. using spectrophotometer (GENE-SYSTM 20 Visible, Thermo Fisher Scientific, USA). Antioxidant activity by FRAP assay was calculated according to the following equation and the results were reported as µM trolox equivalents/g db.

\[
A_{\text{diff}} = A_{\text{final}} - A_{\text{initial}}
\]

where \( A_{\text{initial}} \) is absorbance of DPPH, \( A_{\text{final}} \) is absorbance of sample and \( A_{\text{diff}} \) is difference of the absorbance between FRAP and sample.

2.9. Microencapsulation experiment

Microencapsulation with maltodextrin, resistant maltodextrin and gum arabic as coating material were prepared as follows: mulberry leaf extract was chosen according to the highest antioxidant activity and was further used in microencapsulation experiment.

2.10. Determination of microcapsules physiochemical properties

The analysis of moisture content (%) was according to AOAC [3] by drying at 105 °C for 4–5 h.

The water activity (a) was analyzed according to AOAC method (2000) by water activity analyzer (model MS1, Novasina, Switzerland) at 25 °C.

The analysis encapsulation yield (%) was based on the method of Ramakrishnan et al. [23] following this equation:

\[
\text{Encapsulation yield(%) } = \frac{\text{Weight of mulberry leaf extract microcapsule}}{\text{Total solid content in the sample prior to drying}} \times 100
\]

The analysis of encapsulation efficiency (%) was adapted from Saénz et al. [27].

The appearance analysis of microcapsules was analyzed by scanning electron microscope and energy dispersive X-ray spectrometer (JEOL, JSM-IT300 Oxford, X-Max N 20) at 30 kV magnification for 500 and 1000 x.

The water solubility analysis was conducted according to the method of Ahmed et al. [2] and calculated using the following equation:

\[
\text{Water solubility(%) } = \frac{\text{Solid content in supernatant}}{\text{Total solid content}} \times 100
\]

The analysis of glass transition temperature (Tg) followed the method of Ramakrishnan et al. [23].
Table 4
Experimental factors and measured values of responses. The extraction factors were temperature in °C (X1), time in min (X2), and ethanol concentration in % (X3). The responses were measured in triplicate as total phenol content (TPC) expressed as mg GAE/100 g db, total flavonoid content (TFC) expressed as mg QCE/100 g dry db, and antioxidant activity by DPPH and FRAP methods expressed as μM trolox equivalents/g db.

| Treatment | Independent Variables | Responses |
|-----------|-----------------------|-----------|
|           | Temperature (X1) | Time (X2) | Concentration (X3) | TPC | TFC | DPPH | FRAP |
| 1         | 40                   | 10        | 80                 | 896.89 | 0.32 | 324.2 | 612.53 |
| 2         | 80                   | 10        | 80                 | 1063.97 | 0.55 | 405.53 | 418.36 |
| 3         | 40                   | 30        | 80                 | 976.06 | 2.72 | 537.59 | 503.94 |
| 4         | 80                   | 30        | 80                 | 1236.19 | 3.91 | 576.1 | 884.43 |
| 5         | 40                   | 20        | 60                 | 980.14 | 1.54 | 503.32 | 884.45 |
| 6         | 80                   | 20        | 60                 | 1505.92 | 2.54 | 590.08 | 961.18 |
| 7         | 40                   | 20        | 100                | 955.22 | 1.51 | 469.95 | 432.65 |
| 8         | 80                   | 20        | 100                | 1019.25 | 2.15 | 561.87 | 454.88 |
| 9         | 60                   | 10        | 60                 | 1080.64 | 1.95 | 477.6 | 801.44 |
| 10        | 60                   | 30        | 60                 | 1395.78 | 5.29 | 643.03 | 983.45 |
| 11        | 60                   | 10        | 100                | 878.97 | 0.74 | 313.23 | 430.14 |
| 12        | 60                   | 30        | 100                | 950.22 | 4.11 | 583.4 | 628.33 |
| 13        | 60                   | 20        | 80                 | 1096.19 | 0.62 | 511.36 | 764.98 |
| 14        | 60                   | 20        | 80                 | 1051.75 | 0.88 | 565.08 | 751.7 |
| 15        | 60                   | 20        | 80                 | 1105.08 | 0.74 | 558.92 | 761.02 |
| 16        | 60                   | 20        | 80                 | 1143.56 | 0.91 | 543.53 | 717.01 |
| 17        | 60                   | 20        | 80                 | 1105.22 | 0.8 | 508.15 | 687.69 |

Encapsulation type and concentration (w/w)
- 40% Maltodextrin ratio (1: 1)
- 40% Maltodextrin ratio (1: 2)
- 40% Resistant maltodextrin ratio (1: 1)
- 40% Resistant maltodextrin ratio (1: 2)
- 20% Gum arabic ratio (1: 2)
- 20% Gum arabic ratio (1: 3)

The color value of the mulberry leaf extract microcapsules was measured at room temperature by CIE LAB system by chroma meter Minolta CR-400 color meter, which uses illuminant D65 displaying the color values in the CIE system (L*, a*, and b*). The L* value is the lightness ranging from 0 to 100 (where 0 is black and 100 is white), a* is the green value (+a*) through red (+a*), b* is the blue value (+b*) through the yellow (+b*).

2.11. Statistical analysis
The UAE experiment was optimized using the response surface methodology (RSM) with Box- Behnken Design and three-dimensional

Table 5
Analysis of variance (ANOVA) of responses for total phenolic and total flavonoid contents.

| Source                | DF | SS   | MS   | F-Value | P-Value | DF | SS   | MS   | F-Value | P-Value |
|-----------------------|----|------|------|---------|---------|----|------|------|---------|---------|
| Model                 | 9  | 430,400 | 47,822 | 27.50 | 0.000 | 9  | 33,0238 | 3,6693 | 38.95 | 0.000 |
| Linear                | 3  | 347,992 | 115,997 | 66.70 | 0.000 | 3  | 21,6010 | 7,2003 | 76.43 | 0.000 |
| X1                    | 1  | 129,293 | 129,293 | 74.34 | 0.000 | 1  | 1,1726 | 1,1726 | 12.45 | 0.010 |
| X2                    | 1  | 50,845 | 50,845 | 29.24 | 0.001 | 1  | 1,4730 | 1,4730 | 206.33 | 0.000 |
| X3                    | 1  | 167,854 | 167,854 | 96.51 | 0.000 | 1  | 0.9098 | 0.9098 | 10.52 | 0.014 |
| Square                | 3  | 12,069 | 4023 | 2.31 | 0.163 | 3  | 11,1576 | 3,7192 | 39.48 | 0.000 |
| X1*X2                 | 1  | 355 | 355 | 0.20 | 0.665 | 1  | 0.0000 | 0.0000 | 0.00 | 0.992 |
| X1*X3                 | 1  | 9663 | 9663 | 5.56 | 0.051 | 1  | 4,9658 | 4,9658 | 52.71 | 0.000 |
| X2*X3                 | 1  | 2415 | 2415 | 1.39 | 0.277 | 1  | 5,5451 | 5,5451 | 58.86 | 0.000 |
| 2-Way Interaction     | 3  | 70,339 | 23,446 | 13.48 | 0.003 | 3  | 0.2625 | 0.0884 | 0.94 | 0.472 |
| X1*X2*X3              | 1  | 2165 | 2165 | 1.24 | 0.301 | 1  | 0.2323 | 0.2323 | 2.47 | 0.160 |
| X1*X3*X4              | 1  | 53,303 | 53,303 | 30.65 | 0.001 | 1  | 0.0326 | 0.0326 | 0.35 | 0.575 |
| X2*X3*X4              | 1  | 14,870 | 14,870 | 8.55 | 0.022 | 1  | 0.0003 | 0.0003 | 0.00 | 0.995 |
| Error                 | 7  | 1274 | 1729 | 1.74 | 0.6594 | 7  | 0.0942 | 0.0942 | 1.74 | 0.160 |
| Lack-of-Fit            | 3  | 7882 | 2627 | 2.45 | 0.204 | 3  | 0.6304 | 0.2011 | 14.35 | 0.103 |
| Pure Error             | 4  | 4292 | 1073 | 2.45 | 0.0561 | 4  | 0.0140 | 0.0140 | 14.35 | 0.103 |
| Total                 | 16 | 442,574 | 33,6832 | 98.04% | 95.53% | 16 | 33,6832 | 33,6832 | 95.53% | 71.08% |

DF: degree of freedom; SS: Sum of squares; MD: mean squares; The term is significant at p < 0.05. X1 represents temperature (°C); X2 represents time (min); X3 represents ethanol concentration (%).
Phenolic compounds can be found in fruits, vegetables, and herbs such as mulberry leaf and its extract contains antioxidant properties [28]. Because of their significant antioxidant effects, phenolic compounds are useful in disease prevention and cancer control. Table 4 shows the lowest TPC value was identified for the extraction condition of 10 min at 60 °C and 100% ethanol concentration, while the highest TPC value was detected when the sample was treated for 20 min at 80 °C and 60% ethanol concentration. The linear effect on the TPC value of the temperature, time and ethanol concentration, and the interaction effect between temperature and ethanol concentration, applied to the MLE samples was found to be statistically significant (p ≤ 0.05). However, the interaction effect between temperature and time, time and ethanol concentration and the remaining quadratic effects had no impact on the TPC values (p > 0.05) (Table 5). The significant terms were thus included in the final model. The equilibrium of the second-order polynomial model which indicates the effect of temperature, time and ethanol concentration on the value of the TPC in the samples as a result of the response surface analysis, according to the experimental design was as follows:

\[
TPC = -1092 + 61.6X_1 + 42.2X_2 + 12.36X_3 - 0.092X_1^*X_1 - 0.479X_2^*X_2
+ 0.0599X_3^*X_1 + 0.233X_2^*X_2 - 0.577X_1^*X_1 - 0.305X_2^*X_2.
\]

This was likely due to the fact that the longer the ultrasound-assisted extraction time, the higher the TPC values. Furthermore, TPC values rose with increasing temperature. According to Cisowska et al. [7] temperature can affect sample extraction by affecting its diffusion coefficient and solubility in the solvent. As a result, raising the temperature raises the diffusion coefficient and hence the rate of diffusion, as well as the TPC values. Normally, high temperature with increased time could negatively influence on bioactive compound by degradation reaction; however, the effect of higher temperature on increasing mass transfer of bioactive compound overcomes the effect of higher temperature on bioactive compound degradation resulting in net effect of increasing yield [10]. Fig. 1 b depicts the effect of temperature and ethanol concentration on the TPC values of MLE samples. The surface plot illustrated that increasing the ethanol concentration from 60% to 100% (v/v) tended to slowly dropped the TPC values. Hence, the result showed that the TPC values of the MLE samples was maximum at 60 °C. Additionally, the effect of time and ethanol concentration on the TPC values of MLE samples is shown in Fig. 1 c which demonstrated that increased extraction time led to the higher extraction efficiency of TPC. Ultrasound-assisted extraction is a technique for extracting intracellular substances faster and in greater quantities [34]. As a result, the extraction of bioactive component from plant material, such as phenolic compounds, using ultrasound may be improved. In the extraction of phenolic compounds, the water to ethanol ratio was discovered to be a critical element as described by Rostagno, Palma, and Barros [26]. They also discovered that the phenolic compounds in the extract were lower when the solvent content was >60% (v/v). The use of ethanol to water at a concentration of 68.30% (v/v) for 20 min was found to be the best conditions for extracting phenolic compounds from mulberry leaf using RSM [20]. In line with previous studies, the greatest TPC value was found in the samples extracted for 20 min at 80 °C and 60% ethanol concentration (v/v) in the current investigation.

Flavonoids are polyphenolic compound found in plants exhibiting a diverse range of chemical and biological properties. Flavonoids have
been shown to lower the risk of cancer and cardiovascular diseases [1].

As interest in flavonoids from dietary sources grows, the importance of analyzing flavonoid sources in food and beverage is becoming more relevant. The results in Table 4 suggested that the lowest TFC value was found at 60°C and 60% ethanol concentration, while the highest TFC value was detected when the sample was treated for 30 min at 60°C and 60% ethanol concentration. The linear effect of temperature, time and ethanol concentration, followed by the two quadratic effects of temperature, time and ethanol concentration, was found to be statistically significant (p < 0.05) (Table 5). On the other hand, the interaction of all independent variable and the remaining quadratic effects of temperature did not show any effect on the TFC values of the MLE samples, and thus were excluded from the final model. According to the experimental design, the equilibrium of flavonoids from plants [31]. According to Radziejewska-Kubzdela et al. [21], factors impacting the efficacy of phenolic compound extraction had the same effect on flavonoid extraction ability, implying that mixing water and ethanol as a solvent can increase flavonoid extraction efficiency as well. Flavonoids are prevalent in the phenolic chemical group and are an important component of food, cosmetics, and pharmaceuticals. Studies on flavonoids’ anti-allergic, anti-bacterial, anti-cancer, and anti-inflammatory qualities have been widely documented since they were discovered to offer health advantages for human. The flavonoids’ most important biological function is antioxidant activity, which has been demonstrated in experiments to be stronger than vitamin C [31]. Flavonoid compounds have also been shown to lower the risk of coronary heart disease, reduce nervous system disruption, and aid in improving learning efficiency.

Antioxidants act against cancer and cardiovascular disease by inhibiting the oxidation of oxidizing substances [12]. The equilibrium of the second-order polynomial model indicating the effect of temperature, time and ethanol concentration on the antioxidant activity by DPPH assay of mulberry leaf extract samples was as follows:

\[
\text{TFC} = 20.78 + 0.027X_1 - 0.378X_2 - 0.4594X_1X_2 - 0.00002X_1X_2^2 + 0.01086X_2 + 0.000241X_2^2 - 0.000451X_1^2 + 0.00023X_1X_2 - 0.0064X_2X_1 + 0.00041X_1^2 + 0.000041X_2^2 + 0.00006X_1X_2 + 0.000041X_1^2 + 0.000041X_2^2. \tag{5}
\]

The lack of fit test was insignificant (p > 0.05) on TFC, indicating that model fitted well. The obtained coefficient of determination (R² = 98.04) and adjusted coefficient of determination (R²_adj = 95.53) revealed adequacy in predicting experimental results as well as a high degree of fit. TFC values increased as the extraction time increased (Fig. 2a). Fig. 2b demonstrated that TFC values slightly decreased when ethanol concentration was increased, however, after ethanol concentration was increased > 80%, the TFC values were slowly increased. It could be seen from Fig. 2c that TFC values were lowest at extraction time of 10 min then gently grow at the maximum 30 min. It was found that ethanol is a good polar solvent for extracting flavonoids from plants [31]. According to Radziejewska-Kubzdela et al. [21], factors impacting the efficacy of phenolic compound extraction had the same effect on flavonoid extraction ability, implying that mixing water and ethanol as a solvent can increase flavonoid extraction efficiency as well. Flavonoids are prevalent in the phenolic chemical group and are an important component of food, cosmetics, and pharmaceuticals. Studies on flavonoids’ anti-allergic, anti-bacterial, anti-cancer, and anti-inflammatory qualities have been widely documented since they were discovered to offer health advantages for human. The flavonoids’ most important biological function is antioxidant activity, which has been demonstrated in experiments to be stronger than vitamin C [31]. Flavonoid compounds have also been shown to lower the risk of coronary heart disease, reduce nervous system disruption, and aid in improving learning efficiency.

Antioxidants act against cancer and cardiovascular disease by inhibiting the oxidation of oxidizing substances [12]. The equilibrium of the second-order polynomial model indicating the effect of temperature, time and ethanol concentration on the antioxidant activity by DPPH assay of mulberry leaf extract samples was as follows:

\[
\text{DPPH} = -939 + 56.6X_1 + 16.3X_2 + 4.05X_1X_2 - 0.460X_1^2 - 0.305X_2^2 - 0.0064X_1^2 - 0.107X_2^2 - 0.206X_1X_2 + 0.1309X_1^2 + 0.1309X_2^2. \tag{6}
\]

As the result, the lowest DPPH value was identified for 10 min at
60 °C and 100% ethanol concentration, while the highest DPPH value was detected when the sample was treated for 30 min at 60 °C and 60% ethanol concentration (Table 4). According to analysis of variance (Table 6), the linear effect of the time and ethanol concentration, the interaction effect of temperature and ethanol concentration, followed by quadratic effect of time applied to MLE samples on DPPH values was statistically significant (p ≤ 0.05). Whereas the linear effect of the temperature, the interaction effect of temperature and time, and time and ethanol concentration, and the two remaining quadratic effect of time and ethanol concentration applied to MLE samples on DPPH values was not statistically significant (p > 0.05). The lack of fit test was insignificant (p > 0.05) on antioxidant activity by DPHH assay, indicating that the model fitted well. High R² values indicated that the quadratic model was highly efficient for fitting the data under the condition of experiment and adjusted R² values implied a good agreement between the predicted and experimental values of the model for the antioxidant activity by DPHH assay (Table 6). Consistently, Sungthong and Phadungkit [32] demonstrated that extracting of mulberry leaf at a concentration of 60% (v/v) for 25.5 min by using the ultrason wave had the highest antioxidant activity by DPHH method. Additionally, three-dimensional surface plot for the antioxidant activity by DPHH of MLE samples was shown in Fig. 3. The effect of extraction time and temperature on the antioxidant activity by DPHH assay of the MLE samples was shown in Fig. 3a. The TPC values increased with increasing time but decreased when the temperature was higher than 60 °C. Obviously, the effect of time had a larger effect on the antioxidant activity by DPHH assay than temperature. The effect of ethanol concentration and temperature on the antioxidant activity by DPHH of MLE samples was shown in Fig. 3b. At constant extraction time (20 min), increasing temperature resulted in a higher the antioxidant activity by DPHH assay. The use of various ethanol concentrations at 40 °C did not show the differences, while the temperature at 60–80 °C were applied; it resulted in differences on the antioxidant activity by DPHH assay. The effect of ethanol concentration and time was shown in Fig. 3c on the antioxidant activity by DPHH. The 3D surface plot demonstrated that when ethanol concentration was increased > 60% (v/v), the antioxidant activity by DPHH assay tended to decrease. This research was also consistent with Liyanapathirana et al. [11] who reported the use of ethanol at concentrations between 50 and 60% (v/v) gave the higher antioxidant activity by DPHH method than the same ethanol concentration but with the longer extraction time. It is important to notice that the extraction period must be long enough to prevent bioactive chemicals from degrading, resulting in lesser antioxidant activity [32].

Antioxidant activity analysis by FRAP assay is a method of antioxidant activity analysis based on the electron transfer of the ferric (Fe³⁺) to ferrous (Fe²⁺) ion reduction [4]. The results showed that the lowest FRAP value was identified for 10 min at 80 °C and 80% ethanol concentration, while the highest FRAP value was detected when the sample was treated for 30 min at 60 °C and 60% ethanol concentration as shown in Table 4. The linear effect of the time and ethanol concentration, the cross-interaction effects between temperature and time variables, and the two quadratic effects of temperature and time, applied to MLE samples on FRAP values was statistically significant (p ≤ 0.05) (Table 6). Furthermore, the linear effect of the temperature, the interaction effect of temperature and ethanol concentration, and time and ethanol concentration, followed by the remaining quadratic effect of ethanol concentration, applied to MLE samples on antioxidant activity by FRAP assay gave a statistically insignificant (p > 0.05) (Table 6). The surface plot in Fig. 4a-c illustrated the effect of temperature and time, temperature and ethanol concentration, and ethanol concentration and temperature, respectively. The surface plot of the effect of temperature and time (Fig. 4a) showed that raising the temperature from 40 °C to 80 °C led to a slow down on antioxidant activity analysis by antioxidant activity by FRAP assay. Fig. 4b showed that increasing the ethanol concentration resulted in decreasing of antioxidant activity analysis by FRAP assay. In addition, the surface plot of the effect between ethanol concentration and time demonstrated that the lowest antioxidant activity analysis by FRAP assay obtained when ethanol concentration at 100% (v/v) and time at 10 min were employed for the extraction of MLE samples. The equilibrium of the second-order polynomial model indicating the effect of temperature, time and ethanol concentration on the FRAP values of MLE samples was as follows:

$$ \text{FRAP} = 1121 + 46.7X_1 - 26.3X_2 - 19.77X_3 - 0.821X_1^2X_3 - 0.546X_2X_3X_1 + 0.0723X_2^2X_1 + 1.387X_1^2X_2 - 0.068X_2X_3 + 0.020X_3X_1. $$ (7)

The results showed that the lack of fit test was not significant (p > 0.05) for antioxidant activity by FRAP assay of the MLE samples, indicating that the model fitted well (Table 6). High R² values indicated that the quadratic model was highly efficient for fitting the data under the condition of experiment and the adjusted R² values implied strong agreement between the predicted and experimental values of model for antioxidant activity by FRAP assay.

According to the results obtained from second-order polynomial equation and response surface plot, it was obviously seen that the quadratic polynomial equation could be utilized to explain the 3D response surface plots and predict the TPC, TFC, and antioxidant activity (DPPH and FRAP assay) of the MLE samples (Figs. 1-4). Visually, the curvature of response surfaces may be observed, which represents the degree of effect of independent variables on the response value. As a consequence of the images in Figs. 1-4, one can conclude that temperature (X₁), time (X₂), and concentration (X₃) all had a major impact on the bioactive compounds and antioxidant activity of the MLE. Different shapes denote different interactions between the variables being studied. The interactions between the corresponding variables were highly important if the contour plot’s form was elliptical [35], a circular contour plot, on the other hand, indicated that there were no significant interactions between variables [9]. This study showed a major relationship between (temperature × concentration) and (time × concentration) for TPC, as well as (temperature × time) for antioxidant activity by FRAP assay and (temperature × concentration) for antioxidant activity by DPHH method.

![Fig. 4. Response surface plots (3D) of antioxidant activity (FRAP) as a function of significant interaction between factors; (a) temperature and time; (b) temperature and ethanol concentration; (c) time and ethanol concentration of mulberry leaf extract.](image-url)
3.2. Effects of microencapsulation on bioactive compound and physicochemical properties of MLE

Based on the highest antioxidant activity, ultrasound-assisted extraction condition of time 30 min, temperature 60 °C and 60% ethanol concentration was employed for microencapsulation study. TPC was found to be between 45.77 and 163.83 mg GAE/100 g db. (Table 3). The use of gum arabic at ratio of 1:2 (w/w) gave the highest TPC (p < 0.05). Rajabi et al. [22] reported that the use of gum arabic as an encapsulation agent in the production of honey powder, amla extract and basil leaf increased the phenolic content as well as the antioxidant activity. They also attributed their results to the high solubility, low viscosity and emulsion properties in gum arabic microcapsule. Our results as well as others in the literature were confirmed by Murali et al. [15] studying the effect of different microcapsules from saffron extract, and black carrot juice on the antioxidant activity of the product.

The effect of different wall materials and ratio of MLE and wall material on the production of MLE microcapsules by spray drying were studied on the antioxidant activity by DPPH method. The antioxidant activity by DPPH method was significantly affected by wall materials and ratio (p < 0.05). The MLE microcapsule had the highest antioxidant activity by DPPH assay in the range of 43.15 to 184.43 μM trolox equivalents/g db. The use of gum arabic at the ratio of MLE and coating material 1:2 (w/w) showed the highest DPPH value. In line with our study, Ferrari, Marconi Germer, Alvim, and de Aguirre [8] reported that using gum arabic as an encapsulation agent showed a higher antioxidant activity by DPPH assay than maltodextrin in the spray dried blackberry juice. These results could be because of the protein fraction in the gum arabic may play a role in Maillard and browning reaction during the drying process.

Pitahua et al. [18] studied the antioxidant activity of beetroot juice powder from spray drying using gum arabic and found that the protein content of gum arabic contributed to Maillard reaction, which resulted in the intermediate compounds causing the increase in the antioxidant activity of the product.

From the analysis of antioxidant activity by FRAP method of MLE microcapsules, it was found that the MLE microcapsules using gum arabic at the ratio of of MLE and coating material 1:2 (w/w) had the highest antioxidant activity by FRAP assay followed by gum arabic at the ratio of 1:3 (w/w), maltodextrin at the ratio 1:1 (w/w), resistant maltodextrin at the ratio 1:1 resistant maltodextrin at the ratio 1:2 (w/w) and maltodextrin at the ratio 1:2 (w/w), respectively. As discussed earlier, the MLE microcapsules using gum arabic had the highest TPC and TFC. Therefore, it was probable that the gum arabic microcapsules exhibited such a higher antioxidant activity. The other studies also reported that microcapsules using gum arabic as coating material in encapsulation had a higher antioxidant activity than that of maltodextrin [6,23].

3.3. Effects of encapsulation on quality of MLE

Microencapsulation by spray drying is a method of protecting essential substances from undesired decomposition or reaction during storage. The effect of type and the ratio of encapsulation materials on MLE physical properties was studied (Table 7). It was found that the encapsulation yield (%) obtained from the spray drying of MLE was almost between 38% and 74%. There was a statistically significant difference in encapsulation yield and encapsulation efficiency percentages among the treatments (p < 0.05). The resistant maltodextrin at a ratio of MLE and coating material 1:1 (w/w) had the highest encapsulation yield (73.99%) and efficiency (96.97 %), respectively while the gum arabic at the ratio of MLE and coating material 1:3 (w/w) as well as the maltodextrin at the ratio of MLE and coating material 1:2 (w/w) had the lowest encapsulation yield (37.87%) and efficiency (70.28%), respectively.

Our results were consistent with the Murugesan and Orsat [16] who showed that encapsulation of dried powder from elderberry juice (Sambucus nigra L.) by gum arabic gave the lowest yield (59.26%) compared with the use of maltodextrin as the coating material in
encapsulation. Pai et al. [17] demonstrated that by increasing the concentration of resistant maltodextrin as the encapsulation agent from 20% to 40% (w/w), the encapsulation efficiency of narinjin (grapefruit polyphenol) increased from 60% to 80% (w/w). It was also found that increasing the percentage of coating material resulted in the increase in the thickness of the microcapsules which protected the extract inside the core efficiently [6].

The glass transition temperature ($T_g$) is a critical property of the production of microcapsule by spray drying indicating the temperature at which the sample transition from glassy to rubbery state. Table 8 shows the glass transition temperature of the MLE microcapsule was between 52.8 and 68.1 °C meaning that the MLE microcapsules were in a glassy state if stored at room temperature.

Moisture content and water activity are important factors affecting the quality and shelf life of the microcapsules. There were no significant differences in terms of moisture content, water activity and solubility among the microcapsules (p > 0.05). It was found that the water activity value was related to the growth of microorganisms causing food spoilage specially the growth of microorganisms producing toxin which may harm consumer [23]. Consistent with our study, Padziuvelyte et al. [19] found that the increasing concentration of maltodextrin, resistant maltodextrin and gum arabic resulted in a decrease in the moisture content of the microcapsules from spray drying. In addition, the encapsulation of blackberry juice by spray drying using maltodextrin and gum arabic resulted in a lower moisture content [8]. The moisture content of all MLE microcapsules in this study was < 6%.

According to color values of the MLE from the spray drying (Table 8), it could be derived that the coating material type and the encapsulation ratio affected the color values (p ≤ 0.05). The MLE microcapsule using maltodextrin at the ratio of MLE and coating material 1:2 (w/w) and resistant maltodextrin at the ratio of MLE and coating material 1:1 (w/w) had the greatest and lowest L* value, respectively (p ≤ 0.05). The $a^*$ value in all samples was negative indicating the sample’s greenness. The MLE microcapsule using maltodextrin at the ratio of MLE and coating material 1:1 (w/w) and resistant maltodextrin at the ratio of MLE and coating material 1:1 (w/w) had the greatest and lowest L* value, respectively (p ≤ 0.05). The $a^*$ value in all samples was negative indicating the sample’s greenness. The MLE microcapsule using maltodextrin at the ratio of MLE and coating material 1:1 (w/w) and resistant maltodextrin at the ratio of MLE and coating material 1:1 (w/w) had the greatest and lowest L* value, respectively (p ≤ 0.05). The $a^*$ value in all samples was negative indicating the sample’s greenness. The MLE microcapsule using maltodextrin at the ratio of MLE and coating material 1:1 (w/w) and resistant maltodextrin at the ratio of MLE and coating material 1:1 (w/w) had the greatest and lowest L* value, respectively (p ≤ 0.05). The $a^*$ value in all samples was negative indicating the sample’s greenness. The MLE microcapsule using maltodextrin at the ratio of MLE and coating material 1:1 (w/w) and resistant maltodextrin at the ratio of MLE and coating material 1:1 (w/w) had the greatest and lowest L* value, respectively (p ≤ 0.05). The $a^*$ value in all samples was negative indicating the sample’s greenness.

### 3.4. Surface structure characterization of MLE microcapsules

The surface structure of the good microcapsules should have a smooth surface, spherical shape with no pleats or dents. Fig. 5 shows the results of the external contour study with the SEM at 500- and 1,000-times magnification of the MLE microcapsule. It was found that the

Fig. 5. Scanning electron microscopy at 500 × and 1000 × magnification of mulberry leaf extract microcapsules (left to right, respectively): maltodextrin encapsulation at the ratio 1:1 (a), Maltodextrin encapsulation at the ratio 1:2 (b), Resistant maltodextrin encapsulation at the ratio 1:1 (c), Resistant maltodextrin encapsulation at the ratio 1:2 (d), Gum arabic encapsulation at the ratio 1:2 (e), and Gum arabic encapsulation at the ratio 1:3 (f).
shape of the MLE using maltodextrin as coating material at the ratio of MLE and coating material 1:1 (w/w) had a spherical shape. The dents on the microcapsules were due to spray drying, which created a pressure of steam on the internal structure resulted in a rapid shrinkage of the surface from the loss of moisture [27]. By increasing the ratio of MLE and maltodextrin from 1:1 to 1:2 (w/w), it was found that the surface areas of the microcapsules were more spherical and smoother which were due to the increase in the thickness of the encapsulation layer, resulting in the shape of the microcapsules having a smoother surface and shape. When considering the use of gum arabic as the coating material, it was found that the microcapsules using gum arabic at the ratios of MLE and coating material 1:2 and 1:3 (w/w) were similar in shape, however, more dents and pleats were observed at the ratio of 1:2 (w/w). Consistently, Ramakrishnan et al. [23] demonstrated that increasing the ratio of the coating material with greater molecular mass can result in the microcapsules having a thicker layer of microcapsules and less dents and pleats which was observed in the current study. Another study and consistent with ours showed that encapsulation of blackberry juice powder with resistant maltodextrin as compared with gum arabic resulted in a smoother surface and a more spherical shape [8].

4. Conclusions

The customer’s demand for value-added and/or functional products to enhance health and/or prevent disease has increased dramatically. Microencapsulation appears to be a promising approach for achieving this goal. Mulberry leaf contains a high concentration of phenolic compounds and antioxidant activity which can be recovered by extraction. The most efficient conditions for extracting phenolic compound, flavonoid compound, and strong antioxidant activity (DPPH and FRAP assay) from MLE using UAE were 60 ºC, 30 min, and 60% ethanol concentration. The encapsulation of MLE by spray drying showed that MLE microcapsules with resistant maltodextrin at the ratio of MLE and coating material 1:1 (w/w) had the highest encapsulation yield (%) and encapsulation efficiency (%), however, the greatest antioxidant activities (DPPH and FRAP assay) were obtained from MLE microcapsules prepared by gum arabic as a coating material at the ratio of MLE and coating material 1:2 (w/w). The results of the current study showed that encapsulation with gum arabic at the ratio of MLE and coating material 1:2 (w/w) had a prospect in terms of functional properties. Moreover, the findings of this study could be used as a guideline for future development of food ingredient enhanced with antioxidant activity.

CRediT authorship contribution statement

Supasit Insang: Investigation, Formal analysis, Data curation, Writing – original draft. Isaya Kijpatanasilp: Investigation, Data curation, Writing – original draft. Saeid Jafari: Investigation, Formal analysis, Data curation, Writing – original draft. Kitipong Assatarakul: Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The Scholarship from the Graduate School, Chulalongkorn University to commemorate the 72nd anniversary of his Majesty King Bhumibol Adulyadej is gratefully acknowledged and also the 90th Anniversary Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) with number of GCUGR1125612076M. The authors would like to thank Second Century Fund (C2F), Chulalongkorn University for Dr. Saeid Jafari’s postdoctoral fellowship.

Authors contribution

Supasit Insang carried out experiments, analyzed collecting data and prepared manuscript. Isaya Kijpatanasilp analyzed collecting data and prepared manuscript. Saeid Jafari carried out experiments, interpreted of analyzed data and prepared manuscript. Kitipong Assatarakul, PI of this project, designed experiments, provided guidance on experimental techniques, interpreted of analyzed data and prepared manuscript.

References

[1] M. Achour, L. Bravo, B. Sarrir, M. Ben Fredj, M. Nouira, A. Mizroui, S. Saguem, R. Mateos, Bioavailability and nutraceuticals of rosemary tea phenolic compounds in humans, Food Res. Int. 139 (2021) 109815, https://doi.org/10.1016/j.foodres.2020.109815.
[2] M. Ahmed, M.S. Akter, J-B. Eun, Impact of α-amylase and maltodextrin on physicochemical, functional and antioxidant capacity of spray-dried purple sweet potato flour. J. Sci. Food Agr. 90 (3) (2010) 494-502. https://doi.org/10.1002/jsfa.3845.
[3] AOAC, Official Methods of Analysis of AOAC International, 17th ed., Gaithersburg, MD, USA, 2000.
[4] I.F. Benzie, J.J.A. Strain, b, The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay, Anal. Biochem. 239 (1) (1996) 70-76.
[5] W. Brand-Williams, M.-E. Cuvelier, CIL-FS & Technology, Use of a free radical method to evaluate antioxidant activity, LWT-Food Sci. Technol. 28 (1) (1995) 25–30.
[6] V.M. Buch, A. Pereyra-Gonzalez, N. Segatini, P.R. Santagapita, N.P. Ulhö, M. Puera, Propolis encapsulation by spray drying: Characterization and stability, LWT-Food Sci. Technol. 75 (2021) 227–235.
[7] J.K. Cisowska, O. Szczepaniak, D.S. Powałowska, J. Jęcchowska, P. Szulc, M.J. Czochanski, r, Antioxidant potential of various solvent extract from Morus alba fruits and its major polyphenols composition, Ciencia Rural 50 (1) (2020) 17.
[8] Cristhiane Caroline Ferrari, Silvia Pimentel Marconon Gerber, Izabela Dutra Alvim, Jose Mauricio de Aguirre, Storage stability of spray-dried blackberry powder produced with maltodextrin or gum arabic, Drying Technol. 31 (4) (2013) 470-478.
[9] S. Jiao, Y. Li, Z. Wang, D. Sun-Waterhouse, G.I. Waterhouse, C. Liu, Preservation and antioxidant activity of blackberry juice microencapsulated with protein and maltodextrin blends, Food Chem. 214 (2017) 612–620.
[10] Jiao Luo, Sasika le Cessie, Saskia le Cessie, Diana van Heemst, Raymond Noordam, Diet-derived carbohydrate and lipid intake and circulating antioxidants and risk of coronary heart disease: a mendelian randomization study, J. Am. Coll. Cardiol. 77 (1) (2021) 45-54.
[11] Kitipong Assatarakul, PI of this project, designed experiments, provided guidance on experimental techniques, interpreted of analyzed data and prepared manuscript.
[12] J. Luo, K. Okusaka, K. Yamanaka, H. Takeuchi, T. Fukui, H. Nakagawa, N. Sato, Effect of esterase on the stability of phenolic compounds in powdered BRS Violeta grape juice microencapsulated with protein and maltodextrin blends, Food Chem. 214 (2017) 308–318.
[13] M Surali, Abhijit Kar, Debabandya Mohapatra, Pritam Kalia, Encapsulation of black carrot juice using spray and freeze drying, Food Sci. Technol. Int. 21 (8) (2015) 604-612.
[14] R. Murugesan, V.J.D.T. Orsat, Spray drying of elderberry (Sambucus nigra L.) juice to maintain its phenolic content, Drying Technol. 29 (14) (2011) 1729–1740.
[15] D.A. Pai, V.R. Vangala, J.W. Ng, W.K. Ng, R.B.H. Tan, Resistant maltodextrin as a shell material for encapsulation of naringin: Production and physicochemical characterization, J. Food Eng. 161 (2015) 68-74.
[16] E. Pitalua, M. Jimenez, E. Vernon-Carter, C.J.F. Beristain, Antioxidative activity of mulberry leaves using Response Surface Methodology (RSM), Romanian Biotechnol. Lett. 17 (3) (2012) 7295–7308.
[21] E. Radziejewska-Kohzdelka, A. Olejnik, R.J.J. Bieganska-Marecik, Effect of pretreatment on bioactive compounds in wild rocket juice, J. Food Sci. Technol. 56 (12) (2019) 5234–5242.

[22] H. Rajabi, M. Ghorbani, S.M. Jafari, A.S. Mahoonak, G.J.F. Rajabzadeh, h, Retention of saffron bioactive components by spray drying encapsulation using malodextrin, gum Arabic and gelatin as wall materials, Food Hydrocolloids 51 (2015) 327–337.

[23] Y. Ramakrishnan, N.M. Adzahan, Y.A. Yusof, K.J.P. Muhammad, t, Effect of wall materials on the spray drying efficiency, powder properties and stability of bioactive compounds in tamarillo juice microencapsulation, Powder Technol. 328 (2018) 406–414.

[24] D.M. Riche, K.D. Riche, H.E. East, E.K. Barrett, W.L. May, Impact of mulberry leaf extract on type 2 diabetes (Mul-DM): a randomized, placebo-controlled pilot study, Complement. Therap. Med. 32 (2017) 105–108.

[25] Y. Riciputi, E. Diaz-de-Cerio, H. Akyol, E. Capanoglu, L. Cerretani, M.F. Caboni, V.J.F. Verardo, c, Establishment of ultrasound-assisted extraction of phenolic compounds from industrial potato by-products using response surface methodology, Food Chem. 269 (2018) 258–263.

[26] M.A. Rostagno, M. Palma, C.G. Barroso, Pressurized liquid extraction of isoflavones from soybeans, Anal. Chim. Acta 522 (2) (2004) 169–177.

[27] C. Sanchez, M. Nigen, V. Mejia Tamayo, T. Doco, P. Williams, C. Amine, D. Renard, Acacia gum: history of the future, Food Hydrocolloids 78 (2016) 140–160.

[28] P. Santos, A.C. Ayuiz, G.F. Barbero, C.A. Rezende, J.J.U. Martínez, s, Supercritical carbon dioxide extraction of capsaicinoids from Capsicum frutescens L. assisted by ultrasound, Ultrason. Sonochem. 22 (2015) 78–88.

[29] B. Speranza, L. Petruzzi, A. Bevilacqua, M. Gallo, D. Campanietto, M. Sinigaglia, Encapsulation of active compounds in fruit and vegetable juice processing: current state and perspectives, J. Food Sci. 82 (6) (2017) 1291–1301.

[30] Srivastava, N., & Bezwada, R. J. H. N. I. C. c. (2015). Flavonoids: The Health Boosters. White Paper. Hillsborough NJ: Indofine Chemical company.

[31] Bunleu Sungthong, Methin Phadungkit, Anti-tyrosinase and DPPH radical scavenging activities of selected Thai herbal extracts traditionally used as skin toner, Pharmacogn. J. 7 (2) (2015) 97–101.

[32] Waterhouse, A. L. J. C. p. i. f. a. c. (2002). Determination of total phenolics. Current protocols in food analytical chemistry, 6(1), I1-1.

[33] C. Wen, J. Zhang, H. Zhang, C.S. Dzah, M. Zandile, Y. Duan, X.J.U.S. Luo, Advances in ultrasound assisted extraction of bioactive compounds from cash crops-A review, Ultrason. Sonochem. 48 (2018) 538–549.

[34] S.J.F. Yıkmıs, Optimization of uruset apple vinegar production using response surface methodology for the enhanced extraction of bioactive substances, Foods 8 (3) (2019) 107.