Microwave-assisted extraction performed in low temperature and in vacuo for the extraction of labile compounds in food samples

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In this study, low temperature vacuum microwave-assisted extraction, which simultaneously performed microwave-assisted extraction (MAE) in low temperature and in vacuum environment, was proposed. The influencing parameters including solid/liquid ratio, extraction temperature, extraction time, degree of vacuum and microwave power were discussed. The predominance of low temperature vacuum microwave-assisted extraction was investigated by comparing the extraction yields of vitamin C, β-carotene, aloin A and astaxanthin in different foods with that in MAE and solvent extraction, and 5.2–243% increments were obtained. On the other hand, the chemical kinetic for vitamin C and aloin A, which composed two different steps including the extraction step of analyte transferred from matrix into solvent and the decomposition step of analyte degraded in the extraction solvent, were proposed. All of the decomposition rates (Kc) for the selected analytes in low temperature, in vacuo and in nitrogen atmosphere decreased significantly comparing with that in conventional MAE, which are in agreement with that obtained from experiments. Consequently, the present method was successfully applied to extract labile compound from different food samples. These results showed that low temperature and/or in vacuo environment in microwave-assisted extraction system was especially important to prevent the degradation of labile components and have good potential on the extraction of labile compound in foods, pharmaceutical and natural products.

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1. Introduction

It is very important of extraction and enrichment for analytes prior to final analysis of food samples. Conventional extraction methods for food sample such as soxhlet extraction and solvent extraction usually required long extraction time and large volume of organic solvent, resulting in loss or degradation of active compounds with relative lower extraction efficiency [1,2]. Many innovative techniques including microwave-assisted extraction (MAE) [3] accelerated solvent extraction (ASE) [4] and supercritical fluid extraction (SFE) [5] can shorten the extraction time and reduce the solvent consumption, they were successfully applied on food analysis. MAE had attracted special interest and was widely used in different fields such as natural product [6], food and agricultural analysis [7,8], because it not only has less solvent consumption and shorter extraction time, but also it has equivalent or even higher extraction yield than conventional methods [9]. However, the operating temperature in MAE was commonly applied near to the boiling point of the solvent, some oxygen sensitive compounds were easily oxidized due to presence of air oxidation, and some thermo sensitive compounds were also degraded due to high temperature. The stability of phenolic compound under microwave irradiation was related to its chemical structure, the fewer substituent in the aromatic ring, the higher stability of phenolic compound [10]. Moreover, the sterols in phytosterol enriched milk [11] and carotenoids such as (all-β)–astaxanthin in marine animals and plants [12] also showed significant instability with the increasing both treatment time and microwave power in MAE.

Application of vacuum system during extraction process, inhibit the oxidation process and possibly favour to the extraction of oxygen sensitive components due to the absence of air along with the lower boiling point of solvent. MAE performed in vacuum system (VMAE) was successfully applied for the extraction of polyphenolic compounds and pigments from Chinese herbs. The extraction yields in VMAE for resveratrol, myricetin and safflomin A were 7.4%, 6.4% and 9.4% higher than that obtained from MAE, respectively, indicating the good potential of VMAE on the extraction of thermo sensitive compounds [13]. Moreover, for the extraction of typical antioxidants such as vitamin C and vitamin E, VMAE was also superior to MAE with higher extraction yields, in which the low oxygen atmosphere and subpressure in the extraction system were benefit to the extraction of antioxidants [14].
Performing MAE at low temperature and thin oxygen system would utilize the vastly accelerated extraction rate provided by microwave energy. It also can prevent the degradation of thermo sensitive compounds and the oxidation of oxygen sensitive compounds, which was benefit for the extraction of thermo and/or oxygen sensitive effective compounds from foods or plants. Moreover, in order to study the mass transfer process of solvent from sample matrix transfer to the extraction solvent, the kinetic during the MAE extraction of essential oil [15], thymol [16], etc. had been reported. However, literature data about optimization, modeling and simulation of the MAE process were still scarce. During the extraction process, the main driving force was attributed to the concentration gradient between the external and internal solid material, and the interactions among the fluxed and the tissue matrix, fewer literatures such as Farhat [17] and Fan [18] had developed kinetic models and studied the mechanism of MAE under closed vessel conditions according to the chemical kinetics and Fick’s second law.

Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables, it is also a main natural antioxidant occurring in feeds and sensitive to heat as well as prone to oxidation [19]. β-Carotene is the most effective vitamin A precursor and has been reported to protect humans against certain types of cancer. The isomerisation of cis-β-carotene to its trans-isomer was occurred at more than 90 °C, and high stabilities were found during heating of nitrogen-protected β-carotene [20]. Aloin A is the main component of aloe vera. It was reported that the amount of aloin A decreased with temperature and time, the higher temperature and the longer period of heat treatment may provide more effect on the instability of aloin A [21]. Astaxanthin is the main carotenoid pigment found in aquatic animals and its chemical structure is closely similar to β-carotene. It cannot be synthesized by animals and must be acquired from the diet. Free astaxanthin is particularly sensitive to oxidation and commonly used as a strong antioxidant [22].

The main aim of this work was to develop a simple and efficient method by performing microwave-assisted extraction in vacuo as well as in lower temperature for the extraction of labile compounds from food samples. The influencing parameters including solid/liquid ratio, extraction temperature, extraction time and degree of vacuum were investigated using β-carotene and aloin A as models. The extraction yields of vitamin C, β-carotene, aloin A and astaxanthin in different food matrix were compared with that in MAE and solvent extraction. To further study the mechanism of low temperature vacuum MAE, the extraction chemical kinetics was also investigated and compared with experimental results.

2. Experimental

2.1. Chemicals and materials

Methanol, ethanol, acetone, potassium dihydrogen phosphate and acetic acid were all of analytical reagent grade and purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Acetonitrile and methanol of HPLC grade were supplied by Merck (Darmstadt, Germany). Standards of vitamin C, β-carotene, aloin A and astaxanthin were obtained from Guangdong Institute for the Control of Pharmaceutical Product (Guangzhou, China). Ceston Pured Water (Shenzhen, China) was used throughout the experiments. Peppers, guava, aloe vera, shrimps, and carrot were purchased from a local market, all samples were cut or triturated to 20–40 mesh and stored in desiccators. The same batch of each sample was used here for the experiments.

2.2. Extraction procedures

The extraction conditions such as solid/liquid ratio, extraction temperature, extraction time and degree of vacuum for all of the samples were optimized and each extraction was performed in triplicate.

2.2.1. Determination of extraction yields

Vitamin C was extracted according to literature [14], 5.0 g of sample was weighed accurately and put into an extraction tank with 50 mL of 1.0 mol L⁻¹ acetic acid aqueous. The tank was placed in the microwave oven and connected with a condenser. The air in the vessel was pumped out till the degree of vacuum was reach to 0.05 MPa. Then, the extraction was performed as 25 °C for 10 min.

For β-carotene, 5.0 g of sample was extracted with 60 mL acetonitrile:ethanol (1:2, v/v) at 25 °C for 20 min, the degree of vacuum was set at 0.04 MPa. For aloin A, 5.0 g of aloe vera was extracted with pure water, the solid/liquid ratio was 1:20 (g/mL), the extraction temperature was 35 °C and the extraction time was 15 min, while the extraction was performed at air atmosphere (degree of vacuum = 0). For the extraction of astaxanthin from shrimps, the optimized extraction conditions were that extraction solvent was ethanol, the extraction temperature was 45 °C, the extraction time was 15 min and degree of vacuum was 0 MPa.

For solvent extraction, 5.0 g of different samples were put into a conical flask with 100 mL of solvent. Then the extraction was performed at room temperature, the extraction time was 20, 60, 45 and 30 min for vitamin C, β-carotene, aloin A and astaxanthin, respectively.

2.2.2. Monitoring of chemical kinetic

5.0 g sample and 100 mL extraction solvent were introduced into the extraction tank, then a magnetic agitation, a condenser and a vacuum pump were equipped and the operation of the batch experiments were performed under the optimum conditions. Liquid samples were taken out at different time and the analyte concentration was then measured by HPLC. As the analytical sample volume was 0.5 mL each time, the measurement was supposed to have no significant influence on the process.

2.3. HPLC analysis

The extracts were filtrated and the solvent was added until a final volume of 100 mL. A ten-time diluted solution was used for high performance liquid chromatography (HPLC) analysis after it was filtrated through a 0.45-μm microporous membrane. A
Shimadzu LC-2010 system (Shimadzu, Japan) and Diamonsil C18 column (250 mm × 4.6 mm ID, 5 μm, Dikma, China) or an Inertsil ODS-P column (150 mm × 4.6 mm ID, 5 μm, Dikma, China), attached a 7.5 mm C18 security guard column (Phenomenex, Torrance, CA, USA) were used.

Vitamin C was determined using a mobile phase consisting of 0.05 mol L\(^{-1}\) potassium dihydrogenphosphate in water and acetonitrile (97/3, v/v) at 254 nm. The conditions of β-carotene analysis were that a mobile phase consisting of methanol and acetonitrile mixture (90/10, v/v) was used and the elution was monitored at 450 nm. The mobile phase for aloin A was 0.2% acetic acid in water and acetonitrile (26/74, v/v) and the wavelength was set at 360 nm, while the mobile phase for astaxanthin was methanol and acetonitrile (85/15, v/v) and the wavelength was 476 nm. The entire flow rate during HPLC analysis was 1 mL min\(^{-1}\) and the temperature was room temperature.

The calibration graphs were constructed for six working aqueous standards ranged from 0.1 to 100 mg L\(^{-1}\), the limit of detection (LOD) varied between 0.02 mg L\(^{-1}\) and 0.54 mg L\(^{-1}\). The repeatability of the method, expressed as relative standard deviation (RSD), was calculated for six replicates of a working standard at a low concentration of the calibration curve, the values varying from 1.3% to 4.6% as summarized in Table 1. Fig. 1 shows the HPLC chromatograms of standard and extract from pepper (A), carrot (B), shrimp (C) and aloe vera (D).

Briefly, the extraction yield was defined as follows:

\[
\text{Extraction yield (mg/100g)} = \frac{\text{quantity of analyte in extraction solution (mg)}}{\text{quantity of original sample (100g)}}
\]

3. Results and discussion

3.1. Actualization of the low temperature vacuum MAE

The low temperature vacuum MAE was to exploit the rapid energy transfer property of microwave energy while maintaining a low bulk extraction temperature in vacuum, there was need for a compact device that carries out microwave-assisted extraction at low temperature and in vacuo simultaneously. The schematic
plot of the device is shown in Fig. 2. It consisted of a MAS-11 microwave oven (Sino, Shanghai, China), a homemade low temperature extraction tank containing an interior cooling tube, a DLSB-5L/25 low temperature cooling liquid circulating pump (Gongyi, Henan, China), and an AP-02B vacuum pump (Autoscience, Tianjin, China) connected with an outer condenser.

The homemade extraction tank was the principal part of the device which consisted of an extraction tank wall formed of microwave transparent material such as glass or PTFE, and an extraction chamber for extraction. In preferred embodiments the extraction chamber had a volume of about 200 mL. A low temperature cooling liquid circulating pump was used to help maintain lower temperature in the extraction tank, an interior cooling tube was placed in the extraction chamber and the fluid coolant such as alcohols at the desired low temperature, e.g. in the range of about −20 °C to 60 °C, was supplied and exited to the coolant reservoir. And then microwave irradiation was applied in the extraction tank while simultaneously circulating coolant in the extraction chamber. A vacuum pump was connected with the upper portion of the outer condenser to keep a certain degree of vacuum in the whole system in process of extraction.

The extraction temperature and the degree of vacuum were controlled and adjusted with the low temperature cooling liquid circulating pump and vacuum pump, and then, the device was easy to perform MAE, vacuum MAE (VMAE), and low temperature vacuum MAE, etc.

3.2. Study of the low temperature vacuum MAE method

Based on the interior circulating cooling tube in the extraction tank and the vacuum pump, a steady low temperature and in vacuo atmosphere was provided for the extraction of labile compounds. Some significantly factors such as solid/liquid ratio, extraction temperature, extraction time and degree of vacuum were investigated. β-Carotene is a typical thermo sensitive and oxygen sensitive vitamin A precursor, aloin A is a thermo sensitive effective compound in aloe vera, and then β-carotene and aloin A were selected as models to study the low temperature vacuum MAE method.

3.2.1. Extraction for thermo and oxygen sensitive β-carotene

The extraction solvents were firstly optimized according to the polarity of analyte, acetone:ethanol (1:2, v/v) showed better extraction efficiency and it were selected as the optimum solvent for β-carotene. And then the effect of solid/liquid ratio on the extraction yield of β-carotene was studied and the results are shown in Fig. 3A. A larger solvent volume of 24 mL at a solid/liquid ratio of 1:12 (g/mL) led to highest extraction yield. The reason was probably that a larger concentration gradient of analyte between matrix and solvent was obtained within a larger volume of solvent, which can accelerate the mass transfer process and improve the extraction of analyte. Moreover, the in vacuo atmosphere not only provides a less oxygen system and benefit for the extraction of oxygen sensitive compounds, but also it can provide a pressure difference between the inner and outer of the cell wall, which enhancing the extraction efficiency of analyte.

The effect of extraction temperature on the extraction yield of β-carotene was investigated and the results are shown in Fig. 3B. Although the extraction temperature can be performed at 5 °C or lower, the best extraction yield for β-carotene was obtained when it was extracted at nearly room temperature, which is 25 °C. Higher than 50 °C or lower than 20 °C were both disadvantageous, the extraction yield of β-carotene in 10 °C decreased than that in 25 °C with a decrement of 27.1%. The reason probably due to that higher extraction temperature can accelerate the diffusion of analytes between the matrix and solvent, which was beneficial for the extraction of analyte. For labile compounds, although the extraction process performed in low temperature could reduce the degradation of analyte, it also cut down the speed of mass transfer during extraction. And then, a suitable extraction temperature was significantly important.

Extraction time was also a significant parameter should be optimized in MAE, due to the dramatic thermo effect, longer extraction time was commonly not recommended. Fig. 3C shows the effect of extraction time on the extraction yield of β-carotene. When the extraction time increased from 5 to 20 min, the extraction yield of β-carotene was enhanced significantly. The probably reason was that in regular MAE the extraction temperature was usually fixed and the microwaves were stopstarted to guarantee a fixed temperature in the solvent, the microwaves were greatly limited because the extraction solvent was rapidly heated to the fixed temperature under microwave irradiation. While in low temperature vacuum MAE, an interior cooling tube is used to fix the extraction temperature, then, the microwaves should continue working in order to maintain the fixed temperature in the system, which was beneficial to the extraction of labile compounds and longer equilibrium time between the extraction of analytes from sample matrix and the degradation of analytes in extraction solvents was needed. So, longer equilibrium time was accepted in the vacuum and in low temperature environment.

Oxygen in the extraction system can also affect the extraction yield of labile compounds, especially for the oxygen sensitive analyte. Fig. 3D shows the effect of degree of vacuum (0.02–0.06 MPa) on the extraction yield of β-carotene. The pressure in the system had great effect on the extraction of β-carotene and its highest extraction yield occurred within 0.02 MPa. Performing MAE in vacuo can reduce the oxidation of oxygen sensitive compounds and be beneficial for the extraction of β-carotene. However, the extraction solvent of acetone:ethanol (1:2, v/v) was reduced greatly when the degree of vacuum was larger than 0.06 MPa, while higher degree of vacuum would also easily result in the loss of analyte and decrease its extraction yield.

Microwaves are recognized to enhance chemical reactions such as oxidation, hydrolysis, especially for the degradation of fat and oil [23]. For the extraction of oxygen sensitive compound such as β-carotene, the microwave power also shows obvious affect on the extraction yield (Fig. 3F). The extraction yield of β-carotene was increased when the microwave power increased from 300 W to 500 W, while it was decreased when the microwave power was continued to increase.

3.2.2. Extraction for thermo sensitive aloin A

For the thermo sensitive aloin A, the extraction solvent was also optimized and pure water was selected as the optimum solvent. Fig. 4A showed the effect of solid/liquid ratio on the extraction yield of aloin A. The highest extraction yield of aloin A was obtained when 40 mL of pure water at a solid/liquid ratio of 1:20 (g/mL) was used. The results confirmed that larger volume of extraction solvent was benefit to the extraction of thermo sensitive analyte in low temperature vacuum MAE method.

The effect of extraction temperature on the extraction yield of aloin A is shown in Fig. 4B. Similar to that for β-carotene, better extraction yield of aloin A was obtained when the extraction was performed near to the room temperature. Comparing to that extracted in 35 °C, not only is the extraction yield of aloin A decreased when extracted in 20 °C, but also it was decrease when the extraction temperature was higher than 45 °C. And then, extraction temperature was a significant factor for the extraction of both thermo and/or oxygen sensitive compounds.

Fig. 4C and D shows the effects of extraction time and degree of vacuum on the extraction yield of aloin A. When the extraction time increased from 5 to 15 min, the extraction yield of aloin A was significantly enhanced. But the extraction yield was
slightly decreased when the extraction time increased from 15 to 20 min. However, the subpressure in the system showed little effect on the extraction yield of aloin A, the extraction yield was slightly changed when the degree of vacuum increased from 0.02 to 0.04 MPa.

The effect of microwave power on the extraction yield of aloin A is shown in Fig. 4E and similar results as that for β-carotene was obtained. The best extraction yield of aloin A was obtained when the sample was irradiated at 500 W. Higher microwave power was probably induced to the degradation of labile compounds, resulting in the slightly decrease of extraction yields such as aloin A and β-carotene.

Overall, the extraction temperature and extraction time were significant factors for labile compounds, the degree of vacuum showed greater effect on the extraction yields of oxygen sensitive compound than that of thermo sensitive compound. Additionally, other commonly antioxidants such as vitamin C and astaxanthin were also extracted and their extraction conditions were optimized. The vitamin C was extracted with 50 mL of 1.0 mol L\(^{-1}\) acetic acid aqueous (1:10 g mL\(^{-1}\)) at 25 °C for 10 min and the degree of vacuum was 0.05 MPa. The optimum extraction conditions for astaxanthin were that extraction solvent was ethanol, the extraction temperature was 45 °C, the extraction time was 15 min and degree of vacuum was 0 MPa.

### 3.3. Evaluation of the low temperature vacuum MAE

#### 3.3.1. Comparison with conventional methods

To evaluate the extraction efficiency of the low temperature vacuum MAE method, the conditions of MAE and solvent extraction for all of the analytes except vitamin C were optimized in triplicate, and then, they were also applied for the extraction of vitamin C from green pepper, β-carotene from carrot, aloin A form Aloe vera and astaxanthin from Shrimp head under their optimum conditions. The comparison of extraction yields for all of compounds in different methods are shown in Fig. 5.

The extraction yield of vitamin C in low temperature vacuum MAE was 30.0% higher than that in MAE and 35.8% higher than that in solvent extraction, respectively, these results were in accordance with our previously study [14]. The extraction yield of β-carotene in low temperature vacuum MAE was also significantly enhanced, an increment of 51.7% compared to that obtained in MAE and 243% increment compared to that obtained in solvent extraction were obtained, respectively. For the thermo sensitive aloin A, the extraction yield in low temperature vacuum MAE was 5.2% higher than that in MAE and 30.0% higher than that in solvent extraction. The extraction yield of astaxanthin in the low temperature vacuum MAE was also slightly higher than that obtained in MAE and solvent extraction. These results indicated the low temperature vacuum

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**Fig. 3.** Effect of solid/liquid ratio (A), extraction temperature (B), extraction time (C), degree of vacuum (D) and microwave power (E) on the extraction of β-carotene from carrot.

**Fig. 4.** Effects of solid/liquid ratio (A), extraction temperature (B), extraction time (C), degree of vacuum (D) and microwave power (E) on the extraction of aloin A from aloe vera.
MAE was more suitable for the extraction of labile compounds than conventional methods.

3.3.2. Investigation on the effects of low temperature and in vacuo in the extraction system

To further discuss the effects of temperature, in vacuo and oxygen during the extraction process, a series of experiments were proposed and β-carotene and aloin A were selected as models. At first, 2.0 g carrot was extracted with 24 mL of acetone–ethanol solvent (1:2, v/v) at 500 W for 20 min, the extraction temperature was set at 25 °C and 40 °C, respectively. While for aloin A, 2.0 g sample was extracted with 40 mL of pure water for 15 min, the extraction temperature was 35 °C and 70 °C, respectively. The results are showed in Fig. 6A. About 13% increment of extraction yield for β-carotene in 25 °C was obtained comparing to that in 40 °C, while the extraction yield of aloin A in 35 °C were 28% higher than that obtained in 70 °C, which conformed that low temperature was benefit for the extraction of labile compounds.

Moreover, in order to investigate the effect of oxygen and in vacuo in the extraction process, MAE was performed in vacuo and in air pressure including nitrogen and air atmosphere, respectively, to extract β-carotene from carrot and aloin A from aloe vera, the results are shown in Fig. 6B. When the extractions were performed under air pressure, the extraction yield of β-carotene in the nitrogen atmosphere was significantly higher than that in air atmosphere, and 34.0% of increment was obtained. While for aloin A, which is insensitive to oxygen, similar extraction yields in both nitrogen and air atmosphere were obtained. These results indicated that the low oxygen atmosphere in the extraction process was greatly beneficial and suitable for the extraction of oxygen sensitive compounds.

The extraction yields of β-carotene and aloin A in vacuo compare to that in the air pressure of nitrogen atmosphere were also studied. About a significant increment of 10.1% for β-carotene was obtained, and 3.2% increment for aloin A was also achieved, showing the positive effect of in vacuo on the extraction process, especially for the extraction of oxygen sensitive compounds.

3.4. The chemical kinetic study of low temperature vacuum MAE

Performing at low temperature not only reduces the degradation of thermo sensitive compounds during the extraction process, the low concentration of oxygen in the system could also protect the oxygen sensitive compounds. In order to further discuss the effects of temperature, in vacuo and oxygen during the extraction process on the extraction of analytes in low temperature vacuum MAE, their chemical kinetics were proposed and investigated. Vitamin C, a simple and abundant antioxidant found in fruit and vegetables, along with aloin A were chosen as the models.

3.4.1. Chemical kinetic model

Because of the decomposition of analytes, there are two continuous steps, the extraction step of analyte from matrix (S) into the solvent (L) and the decomposition step of analyte in the extraction solvent (D), during extraction of labile compounds. The extraction yield measured with HPLC was influenced by both of the extraction and decomposition steps. The extraction process could be written as follows:

\[ S \xrightarrow{K_1} L \xrightarrow{K_2} D \]

where \( K_1 \) is the extraction rate of analyte transfer from the matrix into the solvent and \( K_2 \) is the decomposition rate of analyte in extraction solvent.

The extraction process inside a material particle is a mass transfer process of analyte in the solid–liquid phases, in which the rate controlling step is analyte diffused from the internal matrix towards the surface [24]. The first-order chemical kinetic equation can be used to describe this process in MAE [17] and the kinetic equation for the extraction step of labile compound is expressed as:

\[ \frac{dc}{dt} = K_1 c \quad (1) \]

\[ \frac{dc}{dt} = K_2 c \quad (2) \]

Fig. 5. Comparison of extraction yields in different extraction methods. LT-VMAE: low-temperature vacuum microwave-assisted extraction; MAE: microwave-assisted extraction; SE: solvent extraction.

Fig. 6. Comparison of the extraction yields of β-carotene from carrot and aloin A from aloe vera in MAE performed in low temperature, MAE performed in nitrogen atmosphere, MAE performed in vacuo and conventional MAE.
Since both extraction and decomposition steps were hypothesized to be first-order rate reaction, and then they could be described as follows:

$$\frac{dc}{dt} = K_1 c_i$$

(3)

$$\frac{dc_i}{dt} = K_1 c_i - K_2 c_i$$

(4)

where $c_i$, $c_i$ were the concentration or amount of analyte in the matrix and in the extraction solvent, respectively.

With initial values in (4), when $t = 0$, then $c_i = C_0$, $c_i = 0$.

The differential equation (4) can be solved such that

$$c_i = \frac{K_1 c_0}{K_2 - K_1} (e^{-K_1 t} - e^{-K_2 t})$$

(5)

where $c_i$, $c_0$, and $t$ were determined with experiments. Especially, $C_0$ was determined by analysis of all the extraction solutions with three repeated extractions of the same sample under the optimum conditions. Extraction ratio is expressed as a percentage of the concentration of analyte in extraction solution relative to its total amount in samples ($C_0$).

The parameters of kinetic model (constants $K_1$ and $K_2$) were determined from experimental data using non-linear regression. The concordance between experimental data and calculated value was established by the correlation coefficient ($r^2$) and the root mean squared deviation.

3.4.2. Simulation of the extraction process

For the thermo and oxygen sensitive vitamin C, it is easily lost during thermal processing. Gadiert [25] found that vitamin A and biotin are most sensitive to heat, whereas vitamins A, D and C are more prone to oxidation. When the extraction temperature was 25°C and microwave power was 500 W, batch experiments were done in order to investigate the effect of vacuum degree on the decomposition of standard vitamin C and the results are shown in Fig. 7A. It was observed that there was good linear relationship between $\ln(C_i/C_0)$ and $t$ (min) and the extraction time with correlation coefficient ($r^2$) more than 0.95, which confirmed that the decomposition of vitamin C to be a first-order rate reaction. Moreover, the decomposition rate ($k_2$) of vitamin C was 1.3 $\times$ 10$^{-3}$. $2.76 \times 10^{-2}$ and 2.76 $\times 10^{-2}$ min$^{-1}$ for different vacuum degree of 0, 0.018 and 0.035 MPa, respectively. A decrease in decomposition rate value was obtained with the increase of vacuum degree in the extraction system, showing good protection of vitamin C in vacuum.

Fig. 7B shows the influence of temperature on the extraction vitamin C. Experiments were conducted under the optimum low temperature vacuum MAE conditions. The decomposition rate ($k_2$) of vitamin C at 25°C was 1.3 $\times$ 10$^{-3}$ min$^{-1}$, which was similar to that in the nitrogen atmosphere at the same temperature. However, the $k_2$ value obtained at 40°C, that is 2.33 $\times$ 10$^{-1}$ min$^{-1}$, was almost 18 times increased than that obtained at 25°C. The result confirmed that extraction temperature can significantly influence the stability of vitamin C. Moreover, owing to the greater
decomposition of vitamin C at higher temperature as well as a relative extraction rate at 25 °C and 40 °C, the largest extraction yield occurred at 8 min when vitamin C was extracted at 25 °C, while only 3 min occurred the largest extraction yield at 40 °C.

The influence of oxygen was also studied by performing MAE in nitrogen and in air atmosphere. For vitamin C, the chemical kinetic equation (5) applied to the experimental results gave well fit and the correlation coefficient ($r^2$) was 0.943. The decomposition rate ($K_2$) of vitamin C was $1.4 \times 10^{-4}$ min$^{-1}$ in the nitrogen atmosphere, which was greatly lower than that obtained in air atmosphere, $-1.16 \times 10^{-1}$ min$^{-1}$, indicating that the decomposition of vitamin C was reduced in the nitrogen atmosphere.

For the thermo sensitive aloin A, Chang et al. [21] reported that the amount of aloin A decreased with temperature and time, the higher temperature and the longer period of heat treatment may provide more effect on the instability of aloin A. And then, the decomposition of standard aloin A at different temperature was also investigated. Fig. 8A shows the linear relationship between ln(C/C0) and the extraction time for 90 °C, 70 °C and 35 °C. The decomposition rate ($K_2$) of aloin A at different extraction temperature was $1.83 \times 10^{-4}$, $1.63 \times 10^{-4}$ and $1.13 \times 10^{-3}$ min$^{-1}$ with correlation coefficient ($r^2$) of 0.990, 0.992 and 0.864, respectively, indicating that the decomposition of aloin A was a first-order rate reaction and lower temperature was benefit to the extraction of aloin A.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Analytical results of food samples.</th>
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<tbody>
<tr>
<td>Samples</td>
<td>Compounds</td>
</tr>
<tr>
<td>Green pepper</td>
<td>28.7</td>
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<tr>
<td>Bell pepper</td>
<td>66.8</td>
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<tr>
<td>Red pepper</td>
<td>110.2</td>
</tr>
<tr>
<td>Guava</td>
<td>80.0</td>
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<tr>
<td>Carrot</td>
<td>9.86</td>
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<tr>
<td>Spinach</td>
<td>0.6</td>
</tr>
<tr>
<td>Aloe vera</td>
<td>24.5</td>
</tr>
<tr>
<td>Chinese aloe</td>
<td>6.9</td>
</tr>
<tr>
<td>Shrimp</td>
<td>3.4</td>
</tr>
<tr>
<td>Shrimp shell</td>
<td>18.2</td>
</tr>
<tr>
<td>Shrimp head</td>
<td>9.1</td>
</tr>
</tbody>
</table>
The influence of temperature on the extraction of aloin A is further shown in Fig. 8B. The decomposition rate (\(K_d\)) of aloin A at 70 °C was 1.15 × 10^{-4} \text{ min}^{-1}, which was 3 times than that obtained at 35 °C, 3.0 × 10^{-3} \text{ min}^{-1}, while the extraction rate (\(K_e\)) at 70 °C and 35 °C was 5.05 × 10^{-1} and 4.51 × 10^{-1} \text{ min}^{-1}, respectively. A slightly increase of \(K_1\) was got with the temperature increased from 35 °C to 70 °C, and then, the largest extraction yield of aloin A was occurred at 8 min for 70 °C and 15 min for 35 °C, respectively.

Moreover, the simulation results and the experimental data had also well fit with correlation coefficient (\(r^2\)) over 0.986 by performing MAE both in vacuo and in air pressure environment, the decomposition rate (\(K_d\)) in nitrogen, 7.5 × 10^{-3} \text{ min}^{-1}, was similar to that obtained from MAE performed in vacuo (1.15 × 10^{-2} \text{ min}^{-1}) and in air atmosphere (1.0 × 10^{-2} \text{ min}^{-1}). Oxygen in the extraction system had slightly effect on the extraction of aloin A.

Overall, a good fitting between experimental data and chemical kinetic was obtained. For the thermo sensitive and oxygen sensitive compound such as vitamin C, the decomposition rate (\(K_d\)) in vacuo with vacuum degree of 0.035 MPa (2.76 × 10^{-3} \text{ min}^{-1}) was lower than that in air atmosphere with vacuum degree of 0 MPa (1.16 × 10^{-1} \text{ min}^{-1}). Moreover, compare to that in air atmosphere, \(K_d\) in nitrogen atmosphere (1.4 × 10^{-2} \text{ min}^{-1}) and in low temperature (1.3 × 10^{-2} \text{ min}^{-1}) was also decreased significantly, indicating that both of the subpressure and low temperature environment in low temperature vacuum MAE could protect vitamin C from decomposition and oxidation. Moreover, the extraction rate (\(K_e\)) in vacuo (4.2 × 10^{-1} \text{ min}^{-1}) was also higher than that obtained in nitrogen atmosphere (2.0 × 10^{-1} \text{ min}^{-1}), showing the in vacuo environment was benefit to the extraction in low temperature vacuum MAE. For the thermo sensitive aloin A, although the decomposition rate (\(K_d\)) at low temperature (35 °C) was lower than that at higher temperature (70 °C and 90 °C), the \(K_d\) in nitrogen atmosphere was similar to that in vacuo and in low temperature environment. These results showed that low temperature and/or in vacuo in the extraction system were of benefit to the extraction, which were well agreed with the experimental data.

### 3.5. Application of the low temperature vacuum MAE

Under their optimum conditions, vitamin C in fruit and vegetables, \(\beta\)-carotene in carrot and spinach, aloin A in different aloe vera and astaxanthin in marine organisms of shrimp A were extracted with low temperature vacuum MAE and determined by HPLC. The analytical results for the food samples are summarized in Table 2. The recoveries were ranged from 102.1% to 116.7%, 95.1%-98.4%, 88.0%-94.0% and 85.0%-97.0% for vitamin C, \(\beta\)-carotene, aloin A and astaxanthin, respectively, the relative standard deviation (RSD, \(n = 3\)) were lower than 5%, indicating that the presented method was suitable for the extraction and analysis of thermo and/or oxygen sensitive compounds from different food samples.

### 4. Conclusion

In the present work, MAE performed in low temperature and in vacuo, which was the combination of microwave irradiation, low temperature and in vacuo, was proposed and applied to extract labile compounds from food samples. Better extraction efficiencies than that in MAE and in solvent extraction for vitamin C, \(\beta\)-carotene, aloin A and astaxanthin in different food matrix were obtained. Moreover, the chemical kinetics for the extraction process of vitamin C and aloin A were proposed. The results showed that low temperature and/or in vacuo in the extraction system were of benefit to the extraction, which were well agreed with the experimental data. This extraction method offers an opportunity to develop efficient procedure for the extraction of valuable thermo sensitive and/or oxygen sensitive components and have good potential on the extraction of components in many fields such as food, pharmaceutical and natural products.

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