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Investigation on the changes of carotenoids and capsaicinoids in chili oil at different frying temperature by using ^{1}H NMR

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ABSTRACT

The color and pungency are important indicators for evaluating the quality of chili oil, which are mainly determined by the carotenoids and capsaicinoids, respectively. In this study, the effect of frying temperature on the changes of carotenoids and capsaicinoids in chili oil was qualitatively and quantitatively analyzed by ^{1}H NMR. The increasing frying temperature caused the thermal degradation of carotenoids to be intensified, and the degradation of red carotenoids was greater than that of yellow carotenoids. After 10 min of frying at 130, 150, 170 and 190 °C, the contents of capsanthin in chili oil were 40.3, 15.4, 9.6 and 6.2 mg/kg, respectively. Meanwhile, the contents of total carotenoids were 63.0, 25.5, 17.7 and 13.3 mg/kg, respectively. The observed change of R/Y values correlated well with the degradation of carotenoids. The contents of capsaicinoids were 14.8, 20.9, 19.4 and 7.4 mg/kg, respectively. The best frying temperature for the extraction of carotenoids was 130 °C, and over 90% of the carotenoids were dissolved in the frying oil at this frying condition. However, capsaicinoids were more stable than carotenoids, and the best frying temperature for capsaicinoids was 150–170 °C with over 90% extraction rate. Therefore, the temperature fried at 130–150 °C was suitable for the quality of chili oil, considering the higher extraction rates of both total carotenoids and capsaicinoids. This study is of great significance for the quality control of chili oil.

1. Introduction

Chili oil is a traditional Chinese seasoning oil, also known as red oil, which is mainly made of vegetable oil and dried chili powder in a certain proportion by deep-fat frying processing, with the characteristics of bright red in color, strong in spicy flavor. Chili oil is widely used in the catering industry and food industry, and it can play important roles in increasing color and flavor. The color of chili oil is an important quality indicator as the good impression conveyed by the bright red color will determine the purchasing willingness of consumers, and the flavor (mainly the spicy and pungent taste) of chili oil is favored by many consumers.

Carotenoids are fat-soluble colorants, and the carotenoids from chili are the respective sources of chili oil's red color. Carotenoids, including capsanthin, capsorubin, zeaxanthin, β-carotene and cryptoxanthin (Fig. 1) in chili are closely related to color, and they can be divided into red carotenoids and yellow carotenoids. Capsanthin and capsorubin are two red carotenoid compounds in chili, which account for 50–60% of chili carotenoids. The yellow carotenoids include zeaxanthin, β-carotene and cryptoxanthin, of which the content of zeaxanthin is the highest among the yellow carotenoids (Deli et al., 1996). The ratio of the red carotenoid fraction to the yellow carotenoid fraction can reflect the color change, and it can be monitored by R/Y values (red/yellow) (Hornero-Méndez and Mínguez-Mosquera, 2001).

The pungency of chili oil is proportional to the content of capsaicinoids. Capsaicinoids are alkaloids containing phenolic hydroxyl groups, which are the key chemical substances causing the spicy and pungent taste of chili. The main components of capsaicinoids are capsaicin and dihydrocapsaicin (Fig. 1), which account for about 90% of the total capsaicinoids in chili (Woodman and Negoescu, 2019). The contents of capsaicinoids can be converted to Scoville heat units (SHU). SHU is an objective and repeatable index for assessing the effects of spicy chili and its products (Sricharoen et al., 2017). This index gives five levels for pungency: non-pungent (0–700 SHU), low pungency

Abbreviations: CL-130, Chili oil for frying at 130 °C; CL-150, Chili oil for frying at 150 °C; CL-170, Chili oil for frying at 170 °C; CL-190, Chili oil for frying at 190 °C; IS, internal standard; SHU, Scoville heat units; NMR, nuclear magnetic resonance; TMS, tetramethylsilane.

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(700–3000 SHU), moderate pungency (3000–25000 SHU), high pungency (25000-70000 SHU) and very high pungency (>80000 SHU) (Al Othman, Ahmed, Habila and Ghafar, 2011).

Both carotenoids and capsaicinoids are fat-soluble compounds, so they can be easily extracted by frying oil. Higher frying temperatures can promote the dissolution of carotenoids and capsaicinoids from chili powder into chili oil. Meanwhile, as both carotenoids and capsaicin contain unsaturated structures, especially the highly unsaturated structure of carotenoids, they are prone to degradation during the frying processing, thus affecting the overall color and pungency of the chili oil (Zhang et al., 2021a,b). It would be expected that the contents of carotenoids and capsaicinoids in the chili oil would vary at different frying temperatures, causing the changes of bright red color, spicy and pungent taste of chili oil. Therefore, it is necessary to evaluate the effects of heat treatment on the color and taste of chili oil.

The color of chili oil is not only due to the contents of carotenoids, but also the Maillard reaction that occurs during frying. The Maillard reaction is a non-enzymatic browning caused by the reaction between carbonyl compounds and amino compounds, which is of great significance in food color (Hemmler et al., 2018). Melanoid is a class of brown nitrogen-containing compounds produced by the Maillard reaction, and its maximum absorption wavelength is 420 nm. The color of food changes with the molecular weight of the melanoidin, and the molecular weight of melanoidin is significantly dependent on thermal intensity (Nooshkam et al., 2019). Heating can lead to the formation of high molecular weight melanoidins, which darken the color of the food. For a better understanding of the color change of the chili oil, it is necessary to monitor the Maillard reaction, besides the contents of carotenoids in chili oil during the frying process.

For many years, high-performance liquid chromatography (Oliveira Junior et al., 2019; Ponder et al., 2021), gas chromatography (Peña-Alvarez et al., 2009), and spectrophotometry (Hornero-Méndez and Mínguez-Mosquera, 2001) and other technical methods have been used to detect the colorants and pungent compounds in chili. However, these methods are time-consuming and laborious, and cannot comprehensively analyze those compounds in foods quickly. Nuclear magnetic resonance (NMR) is a promising spectroscopic technique that can simultaneously provide detailed results of many compounds without complex preprocessing. The qualitative and quantitative capabilities of NMR undoubtedly empower the technique for versatile application. The qualification of known compounds can be achieved by their specific spectral signals. In an NMR spectrum, chemically distinct spins produce signals, whose area is proportional to the number of nuclei. This species independent direct proportionality is a key advantage of NMR over other techniques, that is, it does not need to use calibration curves for quantitative reference (Ben-Tal et al., 2022). Initially, NMR was used for clinical applications in drug research and metabolomics. Recently, this technique has been widely used in the field of food science, and it is used to directly evaluate the composition of food, such as cream (Ye et al., 2022), peel (Hitaka et al., 2013), capsicum (Florentino-Ramos et al., 2019), brown sugar (Liu et al., 2022), and so on. Therefore, the use of ^{1}H NMR for the qualification and quantification of key compounds in chili oil is very desirable.

This study hypothesizes that frying temperature has a great impact on the changes of carotenoids and capsaicinoids in chili oil, thus causing the changes in bright red color and pungent taste, and those changes would be detected by ^{1}H NMR. Although carotenoids and capsaicinoids in chili peppers have been extensively studied, to the best of our

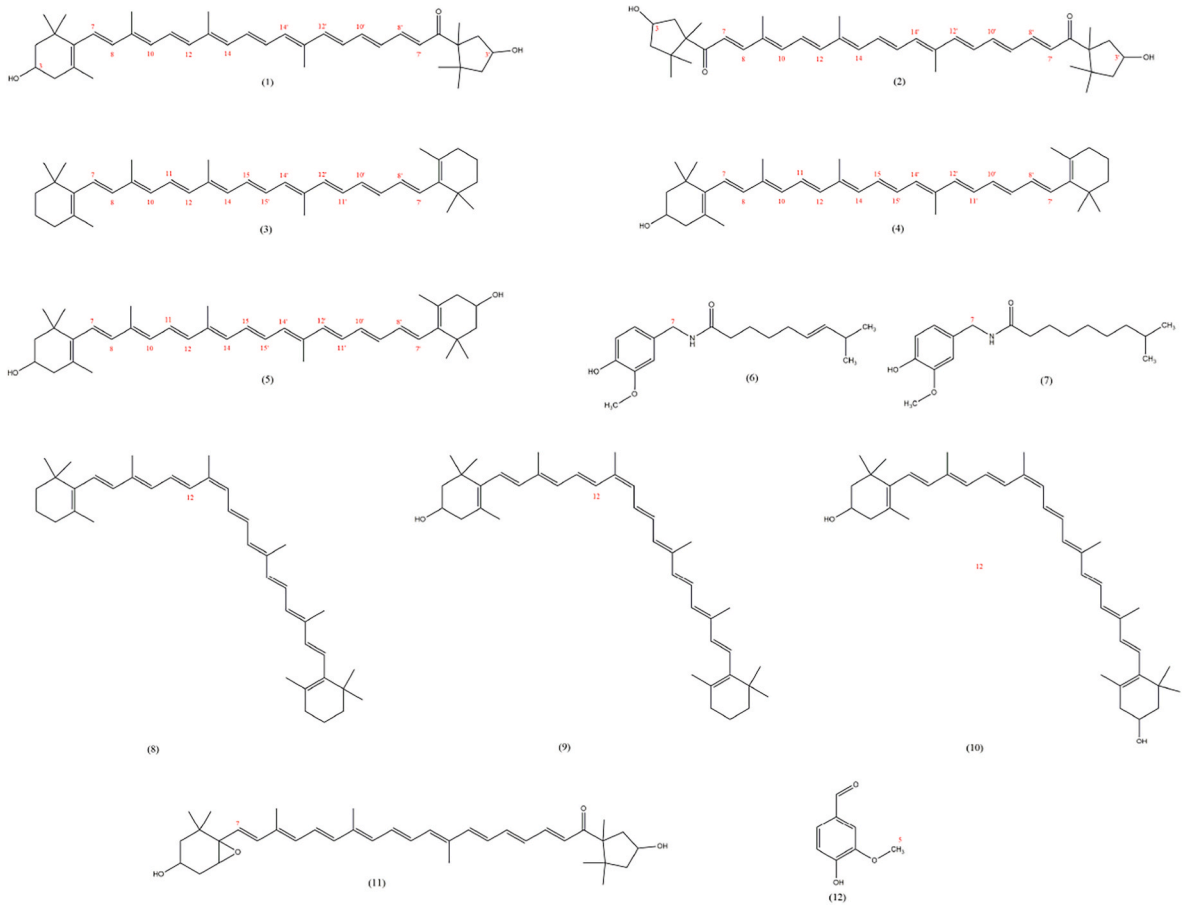

Fig. 1. Structures of carotenoids, capsaicinoids and their degradation productions (1) capsanthin, (2) capsorubin, (3) β-carotene, (4) cryptoxanthin, (5) zeaxanthin, (6) capsaicin, (7) dihydrocapsaicin, (8) (13-*Z*) β-carotene, (9) (13-*Z*) cryptoxanthin, (10) (13-*Z*) zeaxanthin, (11) capsanthin 5,6-epoxide, and (12) vanillin.

knowledge, no research has reported the effect of frying temperature on the color and pungency quality of chili oil. The objective of this study is to investigate the contents of carotenoids and capsaicinoids in chili powder and chili oil fried at different temperatures (130, 150, 170 and 190 °C) by using ^{1}H NMR, and characterize the color and pungency of various chili oil by assessing the changes of R/Y value and SHU. It is to study and analyze the color and spicy taste of chili oil, and provide a new method for the quality control of chili oil products.

2. Materials and methods

2.1. Regents and chemicals

Solvent for NMR analysis ($CDCl_3$) (99.8% + 0.03% V/V TMS (tetramethylsilane)) was purchased from Macklin Biochemical Co.Ltd. (Shanghai, China), sodium hydroxide, acetone, methanol, ethanol, hexane, acetonitrile, and C_{18} solid phase extraction column were purchased from Mclean Co. Ltd. (Shanghai, China), Quantitative filter paper (fast filtering, pore size 20 μm, ashless hardened, 90 mm diameter) was purchased from Hangzhou Double Circle filter paper Co. Ltd (Hangzhou, China).

2.2. Sample preparation

Dried chili powder and sunflower seed oil were purchased from the local retail market. Chili powder (5.0 g) was placed into 100 ml glass Duran bottles containing 20.0 g of sunflower seed oil, and deep-fried at 130, 150, 170 and 190 °C for 10 min, respectively.

2.3. Extraction of carotenoids and capsaicinoids from chili powder

Two grams of chili powder were mixed with 50 mL of acetone solution in a 100 mL beaker. The beaker was sealed with plastic wrap and the mixture was extracted in an ultrasonic cleaner for 15 min; the insoluble residue was filtered using a quantitative filter paper with a pore size of 20 μm (Hangzhou Double Circle filter paper Co. Ltd Hangzhou, China) and extracts were dried under vacuum at 35 °C in a rotary evaporator. The dried residue was redissolved in $CDCl_3$ (600 μL) and methanol (0.791 mg) was added as an internal standard (IS). The solution was transferred to 5 mm of NMR tube.

2.4. Extraction of carotenoids and capsaicinoids from the chili oil

The method of extracting carotenoids was based on the method described by Zhang et al. (2021) with some modifications. Four grams of chili oil were mixed with 42 ml of ethanol and saponified with 18 mL of 3% NaOH at 50 °C for 2 h. Following the addition of 60 mL of n-hexane, the mixture phase-separated into organic and water layers. The organic layer was concentrated to 3 mL by rotary evaporation. Dissolved 0.20 mL of concentrated extract in 400 μL $CDCl_3$ and the methanol was added as an internal standard. The solution was transferred to 5 mm of NMR tube.

Capsaicinoid was obtained from chili oil through the solid-phase extraction technique. One gram of chili oil was mixed with 1 mL of dichloromethane and 3 mL of 2% NaOH. Phase separation was obtained by centrifugation at 4000 rpm for 10 min and the water layer extract was collected. The organic layer was mixed with 3 mL of 2% NaOH and re-extracted as previously described. All water layer extracts were combined and adjusted to pH 2 with diluted sulfuric acid. The acidified water layer extracts were used for solid-phase extraction purification. A C_{18} solid phase extraction column was used for the partial purification of the capsaicinoids. The column was activated with 9 mL acetonitrile, followed by 6 mL of water. The acidified water layer extracts were added into the column and another 6 mL of water was applied to the column. The capsaicinoids were eluted with 6 mL of acetonitrile. The eluate was collected and dried under vacuum at 35 °C in a rotary evaporator. The dried residue was redissolved in $CDCl_3$ (600 μL) and methanol was added as an internal standard. The solution was transferred to 5 mm of NMR tube.

2.5. NMR analysis

The collection of ^{1}H NMR data was performed on the same day as sample preparation on an AVANCE III HD 500 MHz nuclear magnetic resonance instrument equipped with a 5 mm forward broadband liquid nitrogen ultra-low temperature probe (Bruker, Germany). ^{1}H NMR sampling parameters were as follows: spectral window: 19.9947 ppm; time-domain points: 16 k; acquisition time: 3.2768 s; relaxation delay: 2.0 s and number of scans: 128. Relaxation time (T1) determines the interscan delay required between acquisition cycles for accurate quantification. To ensure the complete relaxation of all protons of the samples, the inversion recovery sequence was used to determine T1 (Ben-Tal et al., 2022). The relaxation delay and acquisition time set in the experiment can ensure that the protons were completely relaxed, and the number of scans was sufficient to make the peaks of species with a small content obvious. The signal areas were proportional to the number of protons that produced them, making them useful for quantitative purposes (Martin-Rubio et al., 2018).

NMR spectral processing and peak picking were carried out by MestReNova software (version 14.4.4, Spain). The spectra were processed by applying exponential multiplication of the Free induction decays by a factor of 0.3 Hz, and Fourier transformation of 64 k points. Phase correction was manually performed, and the baseline correction was applied over the entire spectra range. The residual solvent peak at 7.26 ppm was used as the reference. The IS solution (79.1 mg/mL) was prepared by dissolving methanol in $CDCl_3$. $CDCl_3$ was chosen as the solvent for NMR analysis due to its ideal solubility of carotenoid and capsaicinoid components and its non-overlapping residual signal at 7.26 ppm. And methanol was chosen as the internal standard (IS) because of its high solubility under analytical conditions, with a well-defined clear signal at 3.45 ppm (s, 3H) that does not overlap with any other signal in the spectrum. The TMS contained in the $CDCl_3$ solvent produced a signal at 0.00 ppm, and it had the same concentration in all ^{1}H NMR experiments, so TMS can also be used as the IS for quantitative purposes. The compounds corresponding to the peaks were identified by ^{1}H NMR spectra using ChemBioDraw 12.0 and related references (Bora et al., 2021; Hitaka et al., 2013; Sobolev et al., 2005; Valverde and This, 2008).

The compounds were quantified using the SMA analysis tool integrated with the MestRenova package. The SMA analysis tool was employed to set a semi-automatic protocol for metabolite identification and quantification, building specific libraries for the matrices analyzed. This protocol employed the GSD (global spectrum deconvolution) algorithm: overlapping regions were deconvoluted and absolute quantification was possible for compounds with resonances in crowded spectral areas too. In complex reaction mixtures, it is common to encounter overlapping peaks, and errors can be introduced in the integration of these overlapped signals. Deconvolution of spectra by the mathematical fitting of peaks is the best method to separate overlapping resonances so that each overlapping signal can be integrated correctly. The deconvolution technique was successfully used to separate peaks in overlapping regions for the absolute quantification of compounds that resonate in crowded spectral regions (Ciaramelli et al., 2021; Liu et al., 2022; Ye et al., 2022).

The quantities of compounds were calculated by comparing the integral of a single signal with the IS signal. The equation that was used to calculate the concentration was (Malz and Jancke, 2005):

$$M_X = \left(\frac{A_X}{n_X}\right) \Big/ \left(\frac{A_{IS}}{n_{IS}}\right) \times M_{IS}$$

where M_X was the quantity of analyte, n_X and n_{IS} were the number of

protons contained in the quantitative peak of the analyte and IS, respectively, A_X and A_{IS} were the peak area of the analyte and IS, respectively, and M_{IS} was the added quantity of IS.

2.6. Color measurement

The color of chili oil was measured as described by Kim et al. (2009). The chili oil (100 mg) was quantitatively extracted with 10 mL acetone, and the mixed solution was stored at room temperature for 16 h. The absorbance of the supernatant was measured with a spectrophotometer at 420, 472, and 508 nm with acetone as blank, and calculated with the standard equation. Additionally, the overall color change was monitored using R/Y values (Hornero-Méndez and Mínguez-Mosquera, 2001):

$$R/Y = \frac{A_{508} \times 2144.0 - A_{472} \times 403.3}{A_{472} \times 1724.3 - A_{508} \times 2450.1}$$

where: R/Y was the ratio of the red carotenoid fraction to the yellow carotenoid fraction. A_{508} and A_{472} were the read absorbance at these wavelengths expressed in nm.

2.7. SHU and pungency degree calculation

Capsaicinoid content can be converted to Scoville heat units (SHU) by multiplying the capsaicinoid concentration in mg/kg by the coefficient of the heat value for each compound which is 16.1 for both capsaicin and dihydrocapsaicin and 9.3 for other capsaicinoids, as depicted in the equation:where 0.9 was the conversion factor of capsaicin and dihydrocapsaicin, and 0.1 was the conversion factor of other capsaicinoids (Sricharoen et al., 2017).

The SHU can be further converted to a pungency degree, and the conversion relationship between a pungency degree and SHU is: 1° equals 150 SHU.

2.8. Statistical analysis

The data of the four chili oil samples (130, 150, 170, and 190 °C) were analyzed using One-way ANOVA (SPSS 26, IBM), and means were compared using the least significant difference (LSD) test (homogeneity of variance) or Tamhane's T2 (Heterogeneity of variance) at $p = 0.05$.

3. Results and discussion

3.1. ^{1}H NMR quantitative determination of carotenoids and capsaicinoids

In the present study, the carotenoids and capsaicinoids in chili powder were extracted by acetone assisted with ultrasound, and ^{1}H nuclear magnetic resonance technology was applied for the determination of carotenoids and capsaicinoids. Fig. 2 shows the ^{1}H NMR spectra of the extracts of chili powder in $CDCl_3$. The compounds and groups in the main regions were characterized in Figs. 1 and 2 and Table 1. Since the chemical structures of the five carotenoids in chili are similar, most of their signals in ^{1}H NMR spectra are overlapped. The spin system at 6.14, 6.36 and 6.63 ppm was attributed to the fragment -(CH3)C═CH–CH═CH═C(CH3)- common to all carotenoids (Sobolev et al., 2005), and these signals correspond to total carotenoids. The protons of the two red carotenoids (capsanthin and capsorubin) were detected at 4.51, 6.45, 6.55, 6.58 and 7.35 ppm, due to their characteristic ketone groups. Whereas, for the yellow carotenoids (β-carotenoid, cryptoxanthin and zeaxanthin), no one-by-one characterization was performed due to overlapping signals. As the signals at 6.58 ppm and at 6.63 ppm were found to be sharp, intense, well-defined, and non-overlapping in chili (Bora et al., 2021). Therefore, the signals at 6.58 ppm and 6.63 ppm were selected for the quantification of red carotenoids and total carotenoids, respectively (Hitaka et al., 2013; Masetti et al., 2017; Sobolev et al., 2005; Tian et al., 2021; Valverde and This, 2008). Complete ^{1}H NMR assignments of carotenoids in chili are depicted in Table 1.

Capsaicinoids, including capsaicin and dihydrocapsaicin, are the key

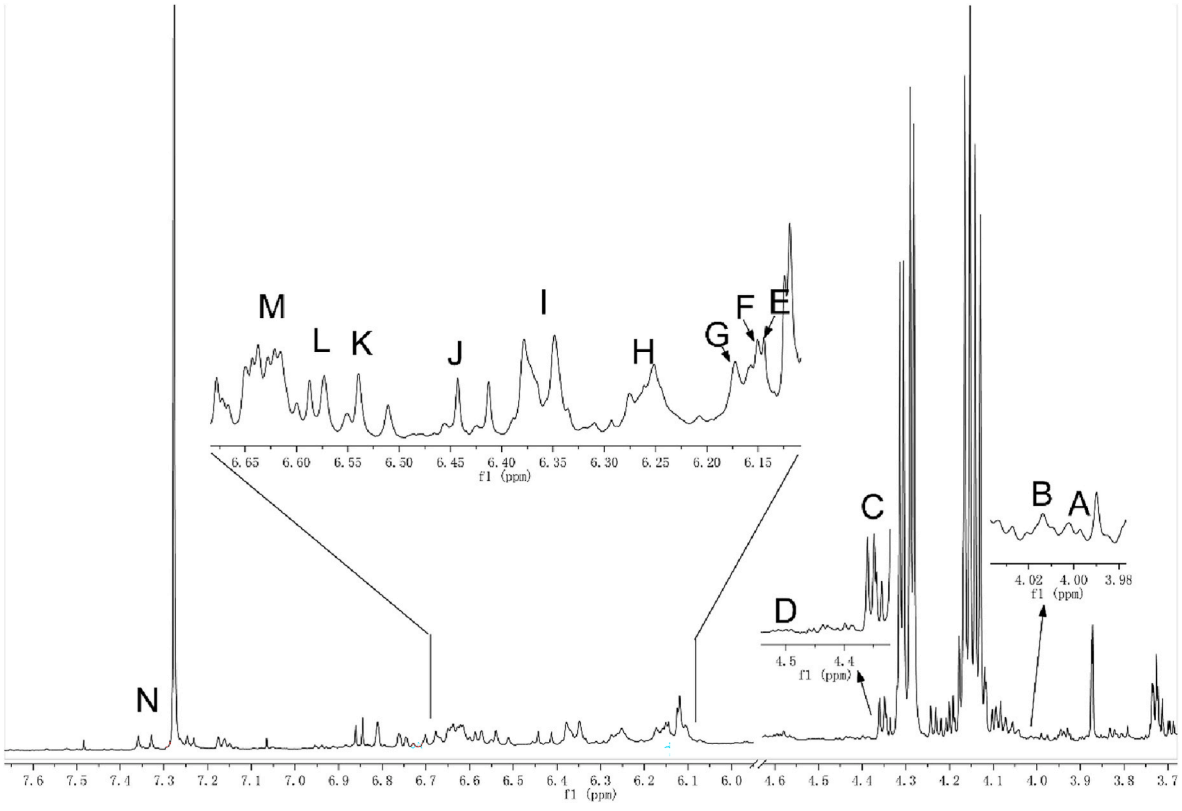

Fig. 2. Detail of δ 3.7–7.6 ppm region of ^{1}H NMR spectrum of chili powder extract.

Table 1
Chemical shift distribution of ^{1}H NMR signals of carotenoids and capsaicinoids in $CDCl_3$.

Signal	Chemical Shift/ppm	Peak Shape	Component
A	4.00	s	Capsanthin (CH-3)
B	4.01	s	β-cryptoxanthin (CH-3), Zeaxanthin (CH-3/3′), Capsanthin (CH-3)
C	4.35	d	Capsaicinoids (CH-7)
D	4.50–4.51	m	Capsanthin (CH-3′), Capsorubin (CH-3/3′)
E	6.14	s	Carotenoids (CH-10/10′)
F	6.15	s	Carotenoids (CH-7/7′)
G	6.17	s	Carotenoids (CH-8/8′)
H	6.22–6.26	m	Carotenoids (CH-14/14′)
I	6.31–6.39	d	Carotenoids (CH-12/12′)
J	6.45	d	Capsanthin (CH-7′), Capsorubin (CH-7/7′)
K	6.55	d	Capsanthin (CH-12′), Capsorubin (CH-12/12′)
L	6.58	d	Capsanthin (CH-10′), Capsorubin (CH-10/10′)
M	6.60–6.65	dd	Carotenoids (CH-11/11′, CH-15/15′)
N	7.35	d	Capsanthin (CH-8′), Capsorubin (CH-8/8′)

substances responsible for the pungency of chili. Capsaicin and dihydrocapsaicin can be differentiated in the ^{1}H NMR spectrum by using the signals at 0.95 ppm and 0.85 ppm (Valim et al., 2019), but this approach is not viable for the actual chili sample as the presence of other peaks in the spectrum that overlap these signals (Woodman and Negoescu, 2019). The signal at 4.35 ppm refers to the benzyl methylene protons which are commonly present in capsaicinoids, and it is related to the H7 protons in capsaicinoids. This signal is sharp and non-overlapping in chili (Bora et al., 2021), so the signal at 4.35 ppm was chosen for the quantification of total capsaicinoids (Table 1).

Both methanol and TMS were used as IS for the quantitative analysis of carotenoids and capsaicinoids in chili by ^{1}H NMR. The contents of carotenoids and capsaicinoids in samples were calculated from the peak area ratio of the selected signals of carotenoids or capsaicinoids versus that of IS, and their contents could be detected simultaneously. The signals at 6.58, 6.63 and 4.35 ppm are usually used to quantify capsanthin, total carotenoids and total capsaicinoids, and their contents in chili powder and chili oil are shown in Table 2. When methanol was used as the IS, the contents of capsanthin and total carotenoids in chili powder were 166.2 and 270.3 mg/kg, respectively, so capsanthin was the predominant carotenoid in chili powder which accounted for 61.5% of the total carotenoids. The content of total capsaicinoids in chili powder was 80.5 mg/kg. When TMS was used as the IS, the contents of capsanthin, total carotenoids and total capsaicinoids in chili powder were 162.9, 266.6 and 79.0 mg/kg, respectively. The results of the two calculation methods are in good agreement, and the errors between two calculation methods are less than 3%, so methanol could be used as the IS in this study.

Other signals, such as the sharp, non-overlapping signals at 6.55 and 6.36 ppm could also be used to quantify red carotenoids and total carotenoids, and the aromatic peak of capsaicinoids at 6.86 ppm can also be used to determine the content of capsaicinoids. The contents of capsanthin, total carotenoids and total capsaicinoids in chili powder and chili oil by these signals are shown in Supplementary Table 3. When methanol was used as the internal standard, the contents of capsanthin, total carotenoids and total capsaicinoids in chili powder were 166.9, 272.5 and 80.2 mg/kg, respectively. Meanwhile, when TMS was used as the internal standard, the contents of capsanthin, total carotenoids and total capsaicinoids in chili powder were 163.5, 272.5 and 78.6 mg/kg, respectively. The results showed that the content of the same substance measured by different signals was almost the same, which indicated that ^{1}H NMR is an ideal method for quantification.

Although the contents of carotenoids and capsaicinoids can give rise to the difference in harvesting times, varieties, processing and others (Kim et al., 2009), the quantitative analysis results and the relative content between capsanthin and total carotenoids in this study were consistent with the previous report (Jiménez et al., 2021).

3.2. Changes in carotenoids and color in chili oil

The contents of carotenoids in chili oil samples are presented in Table 2. After 10 min of frying at 130, 150, 170 and 190 °C, the contents of capsanthin in chili oil were 40.3, 15.4, 9.6 and 6.2 mg/kg, respectively (methanol as the IS). It seemed that the capsanthin content decreased with the increasing frying temperature. Meanwhile, the contents of total carotenoids in chili oil were 63.0, 25.5, 17.7 and 13.3 mg/kg, respectively, and it had a similar trend to that of the capsanthin. As the contents of capsanthin and total carotenoids in chili powder were 166.2 and 270.3 mg/kg, respectively, and 4 times the weight of oil was used for the extraction, it could be calculated that the extraction rate of capsanthin fried at 130, 150, 170, and 190 °C were 97.0%, 37.1%, 23.1%, 14.9%, respectively. The corresponding extraction rate of total carotenoids was 93.2%, 37.7%, 26.2%, and 19.7%, respectively. Therefore, it could be clearly found that frying at 130 °C for 10 min was the best condition for the extraction of carotenoids from chili powder, and over 90% of the colorants were dissolved in the frying oil.

Usually, higher temperatures can increase the properties of the solution and diffusion of carotenoids, prompting more carotenoids to be released into the frying oil (Yonekura and Nagao, 2007). However, the extraction rates decreased with the increasing frying temperature, probably because of the thermo-oxidative degradation of carotenoids at a higher temperature. As shown in Fig. 1, carotenoids have a 40-C skeleton with some conjugated double bonds, and this structural attribute makes carotenoids highly susceptible to oxidation and degradation by external agents, such as heat, dissolved oxygen, light exposure,

Table 2
The contents of carotenoids, capsaicinoids and their degradation productions in chili powder and chili oil by ^{1}H NMR.

Compounds	Chemical Shift/ppm	IS	Content (mg/kg)	Content in chili oil (mg/kg)			
				130 °C	150 °C	170 °C	190 °C
Capsanthin	6.58	Methanol	166.2 ± 8.6	40.3 ± 0.8^a	15.4 ± 0.9^b	9.6 ± 0.2^c	6.2 ± 0.5^d
		TMS	162.9 ± 8.6	40.0 ± 0.8^a	15.3 ± 1.1^b	9.5 ± 0.2^c	6.1 ± 0.5^d
Total carotenoids	6.63	Methanol	270.3 ± 10.1	63.0 ± 0.8^a	25.5 ± 0.6^b	17.7 ± 1.0^c	13.3 ± 1.1^d
		TMS	266.6 ± 13.1	62.5 ± 1.6^a	25.3 ± 0.6^b	17.6 ± 1.0^c	13.2 ± 1.1^d
Total capsaicinoids	4.35	Methanol	80.5 ± 6.3	14.8 ± 2.2^b	20.9 ± 0.7^a	19.4 ± 0.8^a	7.4 ± 0.5^c
		TMS	79.0 ± 6.3	14.6 ± 2.2^b	20.5 ± 0.7^a	19.0 ± 0.7^a	7.3 ± 0.4^c
Capsanthin 5,6-epoxide	5.88	Methanol	nd	4.0 ± 0.8^c	9.5 ± 0.4^b	10.0 ± 0.1^{ab}	10.6 ± 0.4^a
		TMS	nd	3.9 ± 0.8^c	9.4 ± 0.4^b	9.9 ± 0.1^{ab}	10.5 ± 0.4^a
(13-Z)-carotenoids	6.95	Methanol	nd	6.7 ± 0.9^d	17.2 ± 1.0^c	22.8 ± 2.7^b	26.2 ± 0.5^a
		TMS	nd	6.7 ± 0.7^d	17.1 ± 1.0^c	22.6 ± 2.7^b	26.0 ± 0.5^a
Vanillin	3.84	Methanol	nd	5.4 ± 0.4^d	7.6 ± 0.8^c	8.6 ± 0.4^b	10.8 ± 0.9^a
		TMS	nd	5.3 ± 0.4^d	7.5 ± 0.7^c	8.6 ± 0.4^b	10.6 ± 0.9^a

Values (mean ± SD, n = 3) with similar superscripts in a row do not differ significantly ($p \leq 0.05$) among heat treatments; nd = not detected.

transition metal, and interactions with radical species. (Boon et al., 2010; Sant'Anna, Gurak, Ferreira Marczak and Tessaro, 2013). During the frying processing, these external agents were widely present in the chili oil matrix, so the thermo-oxidative degradation of carotenoids was inevitable, and a higher frying temperature led to a higher loss of carotenoids. In the present study, the degradation was very low at a lower temperature (130 °C) as over 90% of carotenoids were extracted into the frying oil.

High thermal energy can induce significant isomerization of carotenoids, causing the all-trans carotenoids to be converted into cis-structure (Boon et al., 2010; Chen et al., 2009). NMR can distinguish different stereoisomers of carotenoids. In previous reports (Aman et al., 2005; Dachtler et al., 2001), protons produced different resonance frequencies on the NMR spectrum due to the abolition of the centrosymmetry of the *Z*-stereoisomerism. In addition, the protons in H-10, H-12, H-14, H-15 and H-15' in (13-*Z*)-zeaxanthin have different chemical shift values from (all-*E*)-zeaxanthin. These protons are derived from the fragment -(CH3)C═CH–CH═CH═C(CH3)- common to all carotenoids, which means that (13-*Z*) carotenoids could be characterized by the chemical shifts of these protons. During the thermal treatment, (13-*Z*) carotenoids were easily formed (Chen et al., 1996). In the present study, (13-*Z*) carotenoids were characterized by the above protons in the NMR spectrum, and a signal at 6.95 ppm was selected for the quantification of the (13-*Z*) carotenoids (Supplementary Table 1). After 10 min of frying at 130, 150, 170, and 190 °C, the contents of (13-*Z*) carotenoids in chili oil were 6.7, 17.2, 22.8 and 26.2 mg/kg, respectively (methanol as the IS). The measured values were similar when TMS was used as an internal standard. A higher temperature can accelerate the isomerization of carotenoids (Boon et al., 2010), causing a large amount of all-trans carotenoids to be converted into cis-structure with lower stability, thereby accelerating the degradation of carotenoids.

The main red pigments in chili (capsanthin and capsorubin) reacted with O^{2-} and •OH to generate epoxides and internal epoxides, mainly including capsorubin 7,8-epoxide, or capsanthin 5,6-and 5,8-epoxide (Boon et al., 2010; Nishino et al., 2016). In the present study, the signal at 5.88 ppm was selected for the quantification of the capsanthin 5, 6-epoxide, and the contents are shown in Table 2. After 10 min of frying at 130, 150, 170 and 190 °C, as expected, the contents of capsanthin 5,6-epoxide increased with the increasing frying temperature, and their corresponding contents were 3.9, 9.5, 10.0 and 10.6 mg/kg, respectively, and the contents of capsanthin in chili oil were 40.3, 15.4, 9.6 and 6.2 mg/kg, respectively. The increase in frying temperature led to the increases of (13-*Z*) carotenoids and capsanthin 5,6-epoxide, further demonstrating that higher temperature exacerbated the oxidative degradation of carotenoids.

The proportions of capsanthin content in the total carotenoids fried at 130, 150, 170 and 190 °C were 64.8%, 60.6%, 54.2% and 46.3%, respectively. Higher temperatures led to a reduction in the proportion of capsanthin in the total carotenoids, which meant that the degradation rate of red carotenoids was higher than that of yellow carotenoids. In the previous reports (Jarén-Galán et al., 1999; Pérez-Gálvez et al., 2000), there was an isokinetic temperature (83 °C) for the carotenoids in oleoresins, and the red carotenoids had higher stability below this isokinetic temperature, while the yellow carotenoids had higher stability at a higher temperature. In the present study, the frying temperatures were higher than the isokinetic temperature, so the yellow carotenoids were more stable. In another recent report (Zhang et al., 2021), carotenoids degraded when heating temperature and time were increased, and about 40% of capsanthin was degraded when fried in oil at 160 °C for 10 min. In addition, the stability of capsanthin was lower than those of β-carotene and zeaxanthin when heating at 120–180 °C, which quite agreed with our results.

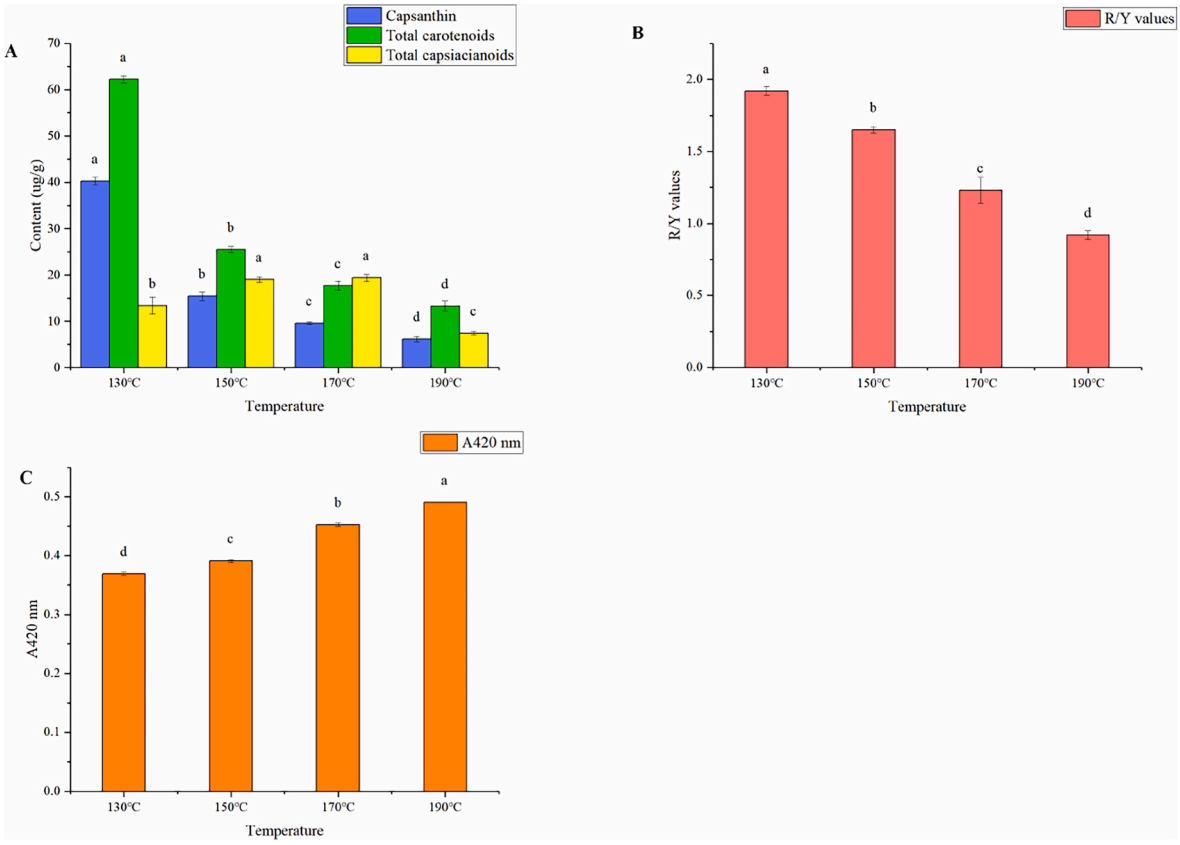

Fig. 3. (A) Change in contents of carotenoids and capsaicinoids at different frying temperature. (B) Change in R/Y value at different frying temperature. (C) Change in Abs 420 nm at different frying temperature. Each value is given as the mean ($n = 3$). Values with similar same superscripts do not differ significantly ($p \leq 0.05$) among the treatments.

Changes in the R/Y value of chili oil at different frying temperatures are shown in Fig. 3B (Supplementary Table 2). The chili oil heated for 10 min at 130, 150, 170 and 190 °C had R/Y values of 1.92, 1.66, 1.23 and 0.92, respectively. The R/Y values of chili oil decreased with the increase in frying temperature. This result was consistent with the results of NMR quantitative analysis and proved that the yellow carotenoids in chili oil were more stable than the red carotenoids. This result also suggested that the color of chili oil changed from red to yellow gradually when the frying temperature increased.

Chili oil samples were observed to gradually darken with the increase of frying temperature, reflected by increased A_{420} (Fig. 3C and Supplementary Table 2). The variation in the overall color of chili oil was not only due to the degradation and isomerization of carotenoids but also to the formation of melanoidins via the Maillard reaction. The Maillard reaction happened in food whenever carbonyl compounds (mainly reducing sugars) occur together with amino compounds (proteins, peptides, amino acids or amines), and they happened vigorously at higher temperatures and low water activity (Cuzzoni et al., 1988). The fried chili could satisfy all the conditions for the Maillard reaction, so the formation of melanoidins was inevitable. Melanoidins are a kind of brown colorant, and the absorbance value at 420 nm can be selected as the measurement mark for melanoidins. In this study, the absorbance values at 420 nm were increased with the increase of frying temperature, which meant that the Maillard reaction in chili oil became more intense, and more melanoidins were generated.

3.3. Changes in capsaicinoids and pungent taste in chili oil

The contents of capsaicinoids are shown in Table 2. After 10 min of frying at 130, 150, 170 and 190 °C, the contents of capsaicinoids were 14.8, 20.9, 19.4 and 7.4 mg/kg, respectively, and the corresponding extraction rates were 73.5%, 103.9%, 96.4% and 36.8%, respectively (methanol as the IS). Therefore, it seemed clear that frying at 150–170 °C for 10 min was the most efficient in the extraction of capsaicinoids from chili powder. A lower frying temperature (130 °C) could cause the release problem of capsaicinoids, while a higher frying temperature (190 °C) could cause serious loss of capsaicinoids. Capsaicin was not resistant to high temperatures, and the alkyl group attached to the amide was cleaved at higher temperatures (Henderson and Henderson, 1992). This cleavage and subsequent oxidation led to the formation of thermal decomposition products, and the main products of capsaicin degradation are vanillin, 8-methyl-6-nonenoamide and 8-methyl-6-nonenoic acid (Henderson and Henderson, 1992). In the present study, the signals at 3.84 and 6.98 ppm were selected for the quantification of the vanillin. The contents of vanillin are shown in Supplementary Table 3. After 10 min of frying at 130, 150, 170 and 190 °C, the contents of vanillin measured at 3.84 ppm were 5.4, 7.6, 8.6 and 10.8 mg/kg, respectively, while the contents of vanillin measured at 6.98 ppm were 5.4, 7.5, 8.8 and 10.6 mg/kg, respectively. The content of vanillin increased with the increasing frying temperature. The changing trend of vanillin content was not the same as that of capsaicinoids, which might be the content of capsaicinoids was affected by both the release and loss. The increase in vanillin content confirmed from the side that the increase in temperature promotes the degradation of capsaicin.

The content of capsaicinoids can be converted to the SHU, and the SHU can be further converted to the pungency degree (Al Othman et al., 2011). Both the SHU and pungency degree were positively correlated with the content of capsaicinoids. The results of SHU and pungency degree value are summarized in Supplementary Table 2. After 10 min of frying at 130, 150, 170 and 190 °C, the chili oil had SHU of 228, 321, 300 and 115, respectively. Through these conversions, the changes of spiciness in chili oil at different frying temperatures could be observed more intuitively. The results showed that the pungency of chili oil varied with frying temperature. As the SHU lower than 700 means non-pungent (Al Othman et al., 2011), and the maximum SHU was 321 in all the chili oil samples, therefore, all the samples were non-pungent because the chili powder used in this study was low pungency (the SHU of chili powder was 1241).

4. Conclusion

The quality of chili oil could be evaluated by the changes of carotenoids, capsaicinoids and their thermal degradation products in chili oil at different frying temperatures by using ^{1}H NMR. The increasing frying temperature caused the thermal degradation reaction to being intensified, resulting in the reduction of the contents of capsanthin, total carotenoids and capsaicinoids, and the increment of the contents of capsanthin 5,6-epoxide, (13-*Z*) carotenoids and vanillin. The best frying temperature for the extraction of carotenoids is 130 °C, and the corresponding extraction rate of total carotenoids was 93.2%. While extraction of capsaicinoids was 150–170 °C, and the corresponding extraction rate was higher than 90%. Therefore, the temperature fried at 130–150 °C was suitable for the quality of chili oil, considering the higher extraction rates of both total carotenoids and capsaicinoids. This study is of great significance for the quality control of chili oil.

CRediT authorship contribution statement

Xueying Bai: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Peng Wan:** Methodology, Software, Validation, Formal analysis, Investigation. **Jie Liu:** Software, Validation, Formal analysis. **Jingyu Yao:** Software, Formal analysis, Investigation. **De-Wei Chen:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.100411.

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