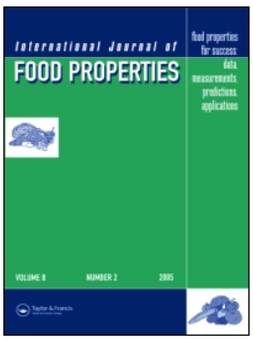

International Journal of Food Properties

ISSN: 1094-2912 (Print) 1532-2386 (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

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To cite this article: De-Jian Li, Yan-Fei Li, Hong-Jun Li, De-Cai Zhou, Chong-Xia Hu, Ren-You Gan, Harold Corke, Hua-Bin Li & Li Zhang (2020) An introduction to the "Li Spicy Unit" for the pungency degree of spicy foods, International Journal of Food Properties, 23:1, 108-115, DOI: 10.1080/10942912.2019.1711116

To link to this article: https://doi.org/10.1080/10942912.2019.1711116

Published online: 12 Jan 2020.

INTERNATIONAL JOURNAL OF FOOD PROPERTIES
2020, VOL. 23, NO. 1, 108–115
https://doi.org/10.1080/10942912.2019.1711116

An introduction to the "Li Spicy Unit" for the pungency degree of spicy foods

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ABSTRACT

Spicy foods are very popular in many countries of the world, but there is still a lack of a simple and intuitive unit to characterize the pungency degree of spicy foods. In this study, the pungency degrees of Spicy Hotpot were evaluated by a large-scale consumer testing, and six levels of pungency degree were graded and connected with contents of main capsaicinoids (CMC) in Spicy Hotpot, from which a new unit of pungency degree was established, the "Li Spicy Unit" (LSU), with the equation: LSU = 10.369 ln (CMC) + 65.264. In addition, it was found that LSU was also suitable for evaluating the pungency degree of several other spicy foods. Therefore, LSU can be a good indicator of the pungency degree of diverse spicy foods. The new LSU can provide an easy tool for public education to help consumers pick spicy foods with a suitable pungency degree.

ARTICLE HISTORY
Received 6 August 2019
Revised 26 December 2019
Accepted 26 December 2019

KEYWORDS
Hotpot; spicy foods; pungency degree; Li Spicy Unit; capsaicinoids; sensory evaluation

Introduction

Spicy Hotpot is a very popular food in China and many other countries in the world, mainly due to its pungent sensation. The pungent sensation of Spicy Hotpot is mainly due to the red peppers in its seasoning. Many studies have demonstrated that red pepper is rich in capsaicinoids, which mainly contribute to its pungent taste.[1] There are more than 20 capsaicinoids found in red peppers, with capsaicin and dihydrocapsaicin (Figure 1) as the predominant ones, accounting for 80–90% of the total present in red peppers.[2] These two compounds provide about twice the pungency degree to human sense compared to other minor capsaicinoids.[1] Therefore, capsaicin and dihydrocapsaicin are also the main pungent compounds in the Spicy Hotpot.

The pungency degree was initially measured by the Scoville Heat Unit (SHU), which indicates the number of times that the substance has to be diluted until the pungency cannot be perceived.[1] Although SHU has many current applications, it only has good accuracy in measuring relatively low pungency degrees, while its discrimination is poor over a wide range in measuring high pungency degrees, which limits its wider application.[3] In addition, the pungency degree is also evaluated by measuring the contents of capsaicinoids in peppers and related food products, mainly via high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) or fluorescent detector (FD), and gas chromatography.[1,4–6] Our previous study found that HPLC-FD was superior to HPLC-UV for the detection of capsaicin and dihydrocapsaicin in Spicy Hotpot seasonings, as the latter could be interfered with by other substances in the seasonings.[7] Besides, several rapid

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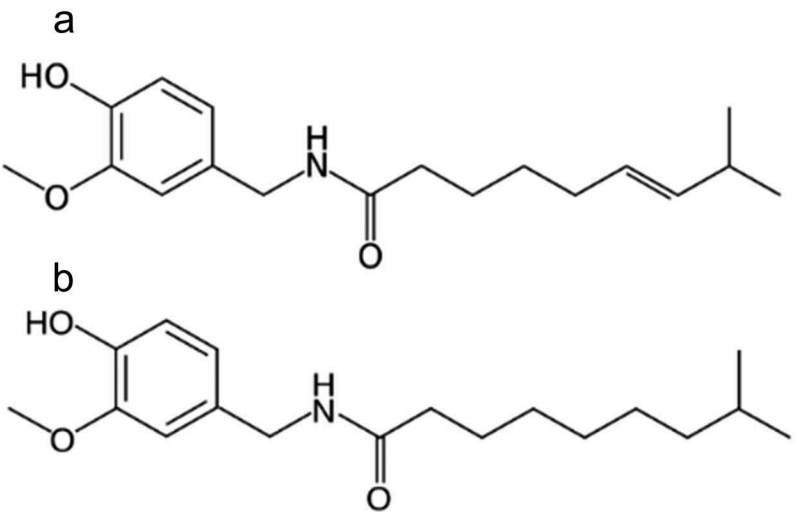

Figure 1. The chemical structure of capsaicin (a) and dihydrocapsaicin (b).

evaluation methods, such as the near-infrared spectrometry (NIRS), colorimetric tube-based method, test paper-based methods, enzyme-linked immunosorbent assay (ELISA), and electrochemical method, have been reported to evaluate the pungency degree of dry peppers.[3,8,9] However, these methods have not been widely used to evaluate the pungency degree of real spicy foods. Moreover, all existing evaluation methods are highly technical, and cannot be easily understood and accepted by the public to pick spicy food products with a proper pungency degree, such as the Spicy Hotpot seasonings. Therefore, it is essential to establish a new pungency degree unit that is easily understood by consumers. In this study, we made a large-scale sensory evaluation on the pungency degree of Spicy Hotpot, and also detected the contents of main capsaicinoids in the Spicy Hotpot seasonings, and these data were used to establish a new pungency degree unit, the "Li Spicy Unit" (LSU), which was also found suitable to evaluate the pungency degree of other spicy food products. We hope that the new LSU can provide an easy understanding of the pungency degree of spicy food products, and can be helpful for consumers in choosing proper spicy foods for their sensory preferences.

Materials and methods

Materials, chemicals, and reagents

The Spicy Hotpot and other spicy seasonings were provided by Chongqing Dezhuang Agricultural Products Development Co., Ltd. (Chongqing, China). Methanol and tetrahydrofuran (HPLC grade) were purchased from Kelong Chemical Co., Ltd. (Chengdu, China). Capsaicin and dihydrocapsaicin standards were purchased from Anpu Experimental Technology Co., Ltd. (Shanghai, China). Ultrapure water was used for all the experiments.

Consumer sensory evaluation

The pungency degrees of Spicy Hotpot and other spicy food products were investigated based on consumer sensory evaluation. The sensory evaluation of Spicy Hotpot was performed by more than 20,000 consumers at more than 600 Dezhuang Hotpot Chain Stores and some Hotpot Food Festivals in different cities of China between March and November 2017. Briefly, the hotpot was prepared by mixing commercial solid hotpot seasonings with water at the ratio of (1:3, m/v) by fixing the mass of hotpot seasonings and changing the mass of water, and was next boiled to cook foods. Consumers were required

to directly taste foods from the hotpot without adding/mixing other seasonings, and then to give a taste description of foods cooked in different Spicy Hotpot seasonings by a questionnaire. In addition, the sensory evaluation of other spicy food products was performed by 100 pre-trained staff in Chongqing Dezhuang Agricultural Products Development Co., Ltd. (Chongqing, China). Briefly, the bread was cut into small pieces (1 cm × 1 cm), which was evenly spread with 10 g of different spicy food products. Participants were required to taste different spicy food-covered bread pieces, and to make a pungency degree assessment of different spicy food products.

Extraction of capsaicinoids in Spicy Hotpot

The extraction of capsaicinoids in Spicy Hotpot was carried out based on a previous study.[10] Briefly, the commercial Spicy Hotpot seasoning was thawed by hot water and mixed well before extraction. Well-mixed hotpot seasoning sample (3.0 g) was mixed with 25 mL of methanol-tetrahydrofuran solution (1:1, v/v), and the mixture was sonicated in a water bath at 60°C for 30 min. The mixture was put at −20°C for 30 min to precipitate fat, and then filtered, and the filtrate was collected. The residue was extracted by 10 mL of methanol-tetrahydrofuran solution (1:1, v/v) under sonication twice more, and the filtrates were combined and adjusted to 50 mL using the methanol-tetrahydrofuran solution (1:1, v/v), and filtered with 0.45 μm nylon filter before HPLC analysis.

HPLC analysis

The contents of capsaicin and dihydrocapsaicin in Spicy Hotpot were determined by HPLC as previously reported.[10] Briefly, HPLC analysis was performed using a Shimadzu 20A HPLC System (Shimadzu, Tokyo, Japan), consisting of a binary pump and a fluorescent detector, and equipped with a Shimadzu VP-ODS column (5 μm, 250 × 4.6 mm) (Shimadzu, Tokyo, Japan) at 30°C. Mobile phase was 70% methanol in water, and the flow rate was fixed at 1.0 mL/min, and the injection volume was 10 μL. For fluorescent detection, the excitation wavelength was set at 229 nm, and the emission wavelength was set at 320 nm. Capsaicin and dihydrocapsaicin were identified primarily based on the comparison of retention time and fluorescent spectra with authentic standards, and quantified according to the peak areas under the detection wavelength. The stock solutions of capsaicin and dihydrocapsaicin standards (200 mg/L) were prepared in methanol, and the calibration standards (20–200 mg/L) were prepared from the stock solution by serial dilutions.

Statistical analysis

All the measurements were performed in triplicate and expressed as mean ± standard deviation (SD). LSU was established based on the regression analysis using Excel 2003 (Microsoft, Redmond, WA, USA) between the pungency degree and main capsaicinoid contents in Spicy Hotpot. The statistical analysis of the regression analysis was performed by SPSS 20.0. (IBM SPSS Statistics, IBM Corp., Somers, NY, USA), and p value < .05 was defined as statistical significance.

Results and discussion

Consumer sensory evaluation on Spicy Hotpot

Based on the initial big data analysis and the consumption habit of people for Spicy Hotpot, the pungency degree was divided into six levels. In addition, according to the results of taste questionnaires, we summarized the corresponding taste description of different pungency degrees of Spicy Hotpot by consumers as shown in Table 1. In order to check whether the corresponding pungency degree and the taste description are reasonable and supported by most consumers, we counted the number of consumers with the correct taste description with regard to corresponding

Table 1. The taste description of pungency degrees of Spicy Hotpot seasonings by consumers.

Pungency degrees	Symbol description	Taste description
Slight	+	A slight sense of pungency.
Low	++	An evident sense of pungency and can be endured without effort.
Medium	+++	An evident sense of pungency but not necessary to specially endure it.
High	++++	A strong sense of pungency, and it can barely be endured.
Super	+++++	A very strong sense of pungency, and it cannot be endured over time.
Extraordinary	++++++	A particularly strong sense of pungency, and it cannot be endured with immediate effect.

pungency degrees as shown in Table 2. There were totally 21,902 customers that took part in our survey, and the Spicy Hotpot with medium pungency degree seemed to have the most popular taste, with more than one-third of customers choosing it for taste, followed by the low, high, and super pungency degrees (Table 2). Spicy Hotpots with slight or extraordinary pungency degrees were only tasted by few customers compared to other pungency degrees, indicating that most people are not interested in hotpot without pungent feeling, and they also dare not challenge hotpot that is too pungent.

Based on the results of Table 2, most consumers could describe a correct feeling upon the six-level pungency degrees, with the correct percentage between 84.3% and 97.3%, suggesting that the six-level pungency degrees of Spicy Hotpot are distinguishable by most customers. However, the six-level pungency degrees are still abstract, and can not give people an intuitive impression. Therefore, it is necessary to establish a simple and intuitive pungency unit to characterize the pungency degrees of Spicy Hotpot, which can be helpful for consumers to easily pick proper Spicy Hotpot for their consumption.

Main capsaicinoids and their contents in Spicy Hotpot

The pungency degree of Spicy Hotpot is mainly associated with the capsaicinoids in red peppers; therefore, main capsaicinoids and their contents in Spicy Hotpot were further investigated. In order to quantify main capsaicinoids in Spicy Hotpot, 100 Spicy Hotpots for each pungency degree were randomly selected, which should be given a correct taste description of corresponding pungency degree by the consumer as shown in Table 1. The results found that capsaicin and dihydrocapsaicin were the main capsaicinoids in Spicy Hotpot, and the contents of main capsaicinoids (CMC) were quantified by HPLC-FD (Figure 2) in selected samples, and the data are presented in Table 3. Overall, six-level pungency degrees increased not evenly. Based on the mean value of CMC, the "Low to Slight," "Medium to Low," "High to Medium," "Super to High," and "Extraordinary to Super" degrees increased 7.2, 2.6, 2.3, 2.0, and 5.7-fold, respectively, indicating that there is a non-linear relationship between six-level pungency degree and CMC.

Although capsaicin and dihydrocapsaicin have been detected in red peppers in many previous studies,[2] few studies reported their contents in Spicy Hotpot. Our previous study found that several ingredients, such as the yeast extract, faba bean paste, ginger, and garlic, did not influence their contents, while ingredients, including Chinese prickly ash, amomum tsao-ko, star anise, and clove, could increase their contents in Spicy Hotpot, with the

Table 2. The statistics of the evaluation on the pungency degree of Spicy Hotpot seasonings by consumers.

Pungency degrees	No. of consumers who tasted	No. of correct consumers	Percentage correct (%)
Slight	958	932	97.3
Low	5841	5318	91.0
Medium	8546	7650	89.5
High	4013	3381	84.3
Super	1912	1645	86.0
Extraordinary	632	621	98.3

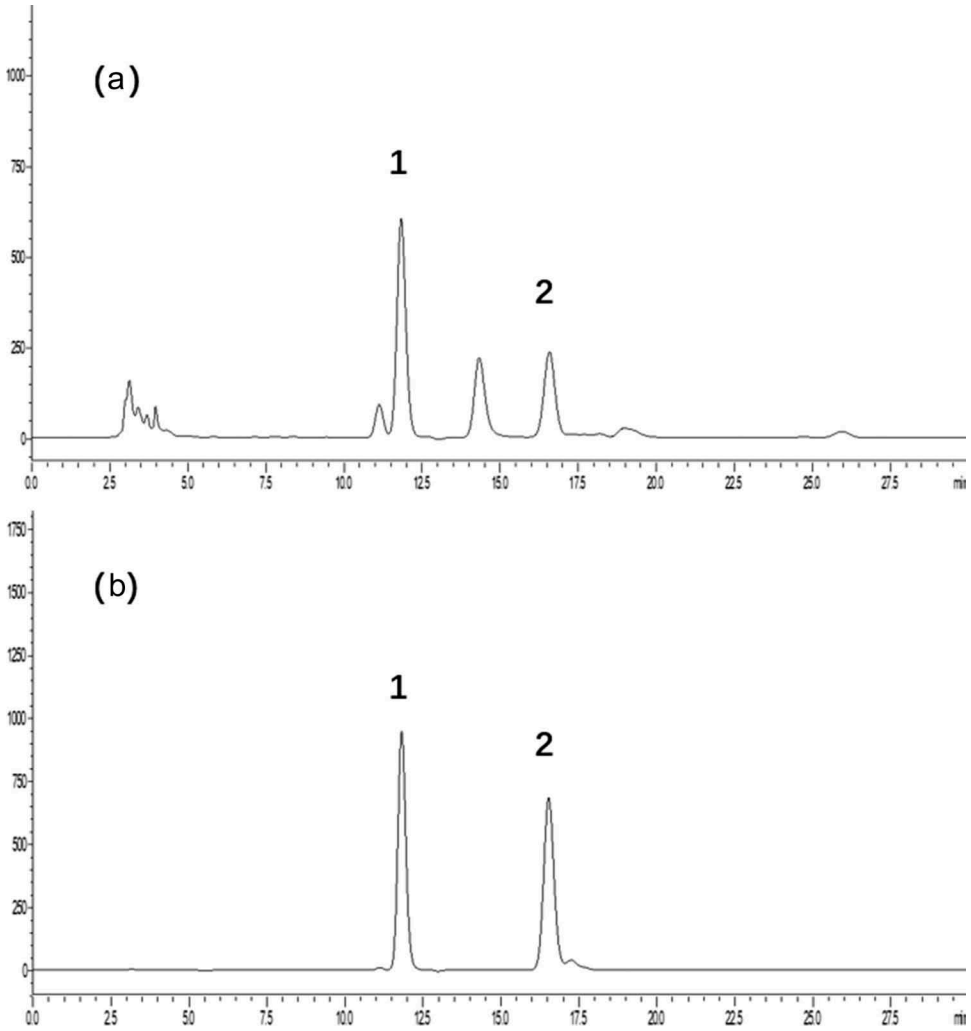

Figure 2. The chromatograms of HPLC-fluorescent detection. (a) Spicy Hotpot seasoning sample, (b) capsaicin (peak 1) and dihydrocapsaicin (peak 2) standards at 40 mg/L.

Table 3. The CMC in Spicy Hotpot seasonings with different pungency degrees.

Pungency degree	No. of samples	Representative LSU (°)	Range of CMC (mg/kg)	Mean value of CMC (mg/kg)
Slight	100	12	5.09–16.4	7.24
Low	100	36	37.0–96.2	52.1
Medium	100	45	94.0–189	133
High	100	52	201–789	304
Super	100	65	596–1351	623
Extraordinary	100	75	1895–5323	3551

mechanisms unclear.[10] In addition, it was also reported that the acid, salt, sugar, and oil could reduce the pungency degree in some spicy food products.[11] Overall, the influence factors of pungency degree in Spicy Hotpot were complex, and different ingredients might influence it.

Establishment of LSU for Spicy Hotpot

As is known that the content of ethanol in alcoholic drinks is expressed as degree (with the symbol "°") in China and many other countries in the world, and most people have an intuitive judgment on alcoholic drinks with 12°, 36°, 45°, 52°, 65°, and 75°, which can generally represent the degrees of main alcoholic drinks. For example, wine is commonly at 12°, and two Chinese spirits, Mao Tai and Wu Liangye, are at 52°. Therefore, the alcoholic degree is a good model with easy understanding and acceptance by the public.

In order to quantify the pungency degree of Spicy Hotpot, we, therefore, established a LSU with the unit "°" to represent the pungency degree of Spicy Hotpot, and to fix LSU between 12° and 75°, and used the 12°, 36°, 45°, 52°, 65°, and 75° to represent the representative degrees of slight, low, medium, high, super, and extraordinary pungency degrees, respectively. Next, in order to explore the relationship between LSU and CMC in Spicy Hotpot, we made a regression analysis between them and found that LSU and CMC in Spicy Hotpot did not exhibit a good linear relationship; however, they had a good logarithmic fit ($R^{[2]} = 0.983$, $p < .001$) (Figure 3), with the equation: LSU = 10.369 ln (CMC) + 65.264. Finally, based on the LSU equation and the comments of consumers, we finalized the ranges of LSU as shown in Table 4.

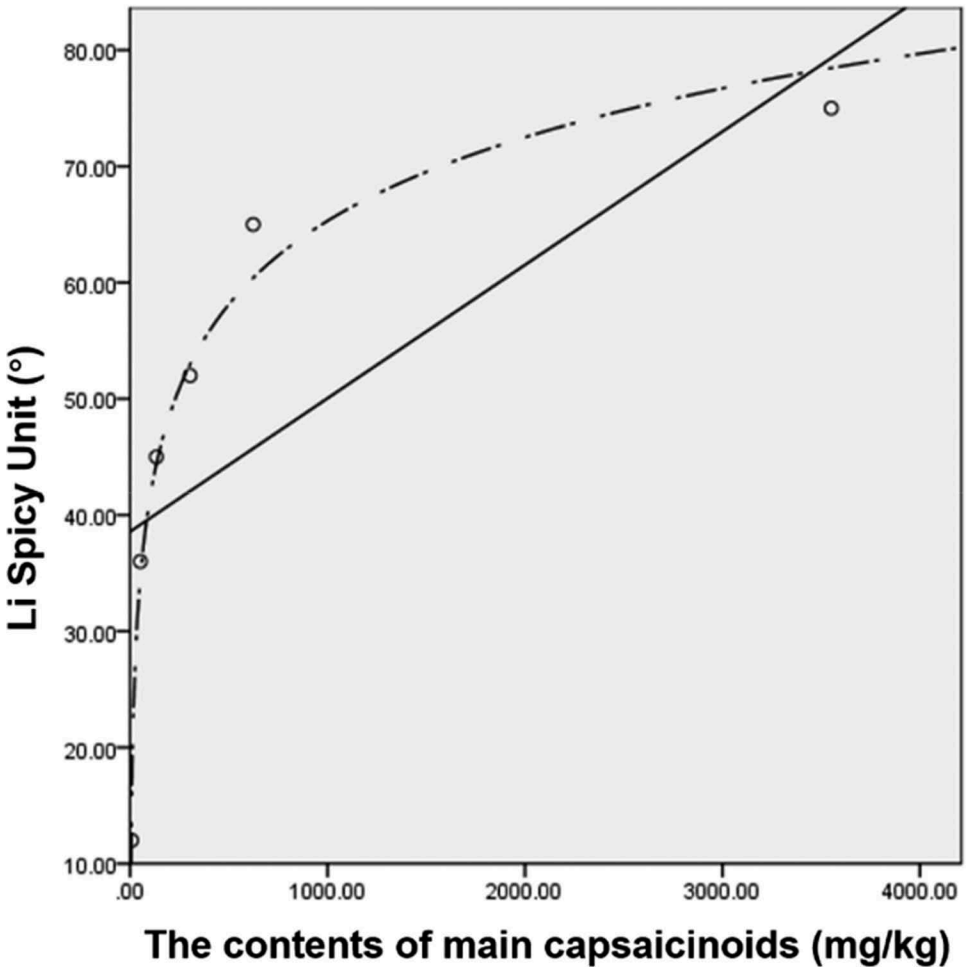

Figure 3. The regression analysis between LSU and the content of main capsaicinoids (CMC) in Spicy Hotpot seasonings. LSU and CMS exhibited a good logarithmic fit.

Table 4. The ranges of LSU for each pungency degree.

Pungency degree	Ranges of LSU (°)	Representative LSU (°)
Slight	9–29	12
Low	30–39	36
Medium	40–49	45
High	50–59	52
Super	60–69	65
Extraordinary	≥ 70	75

Table 5. Verification of LSU in other spicy food seasonings.

Spicy food seasonings	Pungency degree	CMC (mg/kg)	Ranges of LSU based on calculation (°)	Reference ranges of LSU (°)
Cumin chili powder	High	281–432	52–57	50–59
Natural chili powder	Super	714–899	62–64	60–69
Salad red oil	Slight	11.4–13.4	19–21	9–29
Chili oil	Medium	88.1–98.8	40–41	40–49
Lentinus edodes-chili sauce	Low	53.2–85.1	35–40	30–39
Yellow pepper sauce	Slight	20.7–23.7	25–26	9–29

Application of LSU in evaluating the pungency degree of other spicy foods

In order to check whether the LSU is suitable for evaluating the pungency degree of other spicy foods, we next performed another sensory evaluation, and also detected the CMC in respective spicy food, and calculated the LSU ranges based on the LSU equation as shown in Table 5. It was found that the calculated LSU (the fourth column in Table 5) was overall covered in the respective reference ranges of LSU (the fifth column in Table 5); therefore, the calculated LSU is consistent with the respective pungency degree (the second column in Table 5) described by the participants in the sensory evaluation. These results indicated that LSU might be suitable for evaluating the pungency degree of other spicy foods.

To our knowledge, LSU is the first unit established to grade the pungency degree of Spicy Hotpot. Currently, there have been several units to grade the pungency degree of red pepper and other spicy foods. SHU is the most commonly used unit to grade the pungency degree of peppers and related products. Li, Luo, Tu, Hu, Li, & Sun (2013) established a five-level pungency degree for red pepper and its products based on SHU, but the value of each level is too big and with a wide range.[12] Jia et al. (2015) also graded a five-level pungency degree for parts of spicy dishes, based on the content of capsaicin, and the five levels 1, 2, 3, 4, and 5 matched the content of capsaicin about <1.95, 1.95–19.5, 19.5–92.4, 92.4–291.8, and >291.8 mg/kg.[13] In addition, Zhang, Wang, Shi, Liu, Chen, & Zhao (2018) established a reference standard for evaluating the pungency intensity based on the commercial essential oil isolated from Sichuan pepper, and the calculated Weber fraction was stable at 0.15 and 0.20 for low and moderate pungency, respectively.[14] In general, these methods actually mainly characterize the low-to-medium pungency degrees and do not provide distinguishable high pungency degrees compared to our current study (Table 3). More importantly, these grading methods cannot provide a simple understanding; therefore, they cannot be easily remembered by the public or consumers. On the other hand, since the LSU is derived from the scale and unit of alcoholic drinks, which is familiar to the public, thus, LSU can be easily remembered and can provide an intuitive reference for the public to pick suitable spicy foods.

Conclusion

In conclusion, this study mainly investigated the pungency degrees of Spicy Hotpot based on the sensory evaluation by consumers and the measurement of capsaicinoids. Moreover, a new pungency degree unit, the LSU, was established to provide the consumer with an intuitive impression about the pungency degrees of Spicy Hotpot, and it was also found that the LSU was also suitable to evaluate the pungency degrees of certain other spicy foods. In the future, it is still necessary to further verify

the suitability of LSU in evaluating the pungency degree of more spicy food products. Overall, LSU is intuitive, and can be easily understood, remembered, and accepted by the public/consumers. Thus, it can be recommended to be applied in the food product packaging as a label to indicate the pungency degree of spicy food products, such as the Spicy Hotpot seasonings.

Acknowledgments

The authors acknowledge the funding support from Chongqing Dezhuang Agricultural Products Development Co., Ltd. The authors declared no conflict of interest.

Funding

This work was supported by the Chongqing Dezhuang Agricultural Products Development Co., Ltd.

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