Relationship between mustard pungency and allyl-isothiocyanate content: A comparison of sensory and chemical evaluations

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Abstract: The correlation of sensory and chemically evaluated pungency of mustard products was investigated via a time-intensity (TI) study and quantification of allyl isothiocyanate (AITC) contents using high-performance liquid chromatography (HPLC). Sweet, medium hot, hot, and extra hot commercial mustard products from different brands were examined. Notably, we found significant differences ($p < 0.05$) between the maximum perceived pungency intensity of various mustard products. The maximum perceived intensity ($I_{\text{max}}$), the duration of the decreasing phase ($DUR_{\text{Dec}}$), and the area under the curve (AUC) values increased proportionally to the increase in the sample AITC content and were also higher in products classified as hot than in sweet mustards. The AITC concentration varied greatly between products from different brands and also between different sensory evaluated pungency levels. Furthermore, sensory evaluations and analytical results were correlated using regression analysis. The best correlation (correlation coefficient 0.891) was observed between the AITC concentration and AUC, when compared to that between the AITC concentration and $DUR_{\text{Dec}}$ (correlation coefficient 0.856) or the $I_{\text{max}}$ value (correlation coefficient 0.803). The calculated regression model indicates that a higher AITC content induces an intensified trigeminal pungency sensation and that the sensory and chemical evaluations of mustard products were positively correlated. Therefore, by using this regression model, the sensory rating of mustard products may be predicted by chemical analysis of the AITC contents.

Keywords: Allyl isothiocyanate, Pungency, Mustard, Time Intensity analysis, HPLC analysis

Practical Application: This research paper provides a method to quantify the pungency inducing irritant allyl isothiocyanate in commercial mustard products and demonstrates a correlation between sensory and chemical data. Therefore, the amounts of sensory tests in product quality assurance can be reduced and replaced or at least supported by chemical quantification of pungent substances (especially AITC) in mustard products.

1. INTRODUCTION

Spicy foods are an increasingly popular aspect of culinary culture (Kalsec, 2020; Schlossareck & Ross, 2020) and can be divided into different groups, depending on the chemical stimulus inducing the pungency sensation (Schlossareck & Ross, 2019). One of those is a unique pungent, burning sensation, with additional lachrymatory effects, which are often caused by the presence of isothiocyanates (Nilius & Appendino, 2013; Ortner & Granvogl, 2018).

Wasabi, horseradish, and mustard are some of the condiments and vegetables that induce this pungent sensation upon consumption.

Mustard products are common in European cuisine (Bell, Oloyede, Lignou, Wagstaff, & Methven, 2018) and the burning sensation they induce is considered enjoyable to mustard consumers (Ghawi, Shen, Niranjani, & Methven, 2014). The “Code of Practice for Mustard” defines the compositional requirements and characteristic properties of mustard products produced in Europe. “Mustard” or “prepared mustard” are paste-like products made of mustard seeds or mustard flour and a liquid, in particular water, vinegar, grape juice, alcoholic beverages, and/or other liquids. Exclusively, seeds of various Brassicaceae species, namely Brassica nigra, Brassica juncea, and Sinapis alba, may be used for mustard production.

The pungency level of most commercially available mustard products is indicated as a marketing classification (sweet, mild, medium, hot) on the product label. According to the Code of Practice for Mustard, these classifications are optional and may only be used if they are in accordance with the characteristics of the mustard and if the pungency results directly from the mustard seeds and not from other added pungent ingredients, such as chili or black pepper (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015).

Aside from their usage as a condiment, unground mustard seeds are also used as ingredients in pickled foods and seasonings (Sindhu, Maya, & Indira, 2012; Vaughan & Hemingway, 1959).

Mustard is characterized by pungent and sharp aroma notes, as well as a lachrymatory effect, which is caused primarily by the presence of isothiocyanates (Clark, 1992; Gilbert & Nursten, 1972). Notably, two isothiocyanates, namely allyl isothiocyanate (AITC), which is derived from sinigrin, and 4-hydroxybenzyl isothiocyanate (4-HBITC), which is derived from sinablin, are responsible for the pungency and flavor of mustard products (Fenwick & Heaney, 1983; Ghawi et al., 2014). AITC has lachrymatory and pungent properties (Chin, Zeng, & Lindsay, 1996; Gilbert &
Nursten, 1972; Sindhu et al., 2012; Vaughan & Hemingway, 1959), while 4-HBITC causes the odor and intensive burning sensations associated with mustard products (Choubdar, Li, & Holley, 2010; Fahey, Zalcman, & Talalay, 2001; Ghawi et al., 2014; Heilmann & Hummel, 2004). Nevertheless, the pungency of 4-HBITC is described as mild when compared to that of AITC (Choubdar et al., 2010; Vaughan & Hemingway, 1959).

The sensory characteristics of food products are commonly assessed via sensory methods by untrained or specifically trained consumer or expert panels. Nevertheless, the evaluation of pungent products poses several challenges, as the assessment of products with intense sensory characteristics leads to the fatigue of the panelists (Paup, Barnett, Diako, & Ross, 2019; Rousseau, Rogeaux, & O’Mahony, 1999). Therefore, only a few products can be evaluated in each session and the inter-stimulus interval needs to be chosen depending on the chemical stimulus inducing the sensation in order to avoid desensitization (Brand, 2006; Brand & Jacquot, 2002).

Several studies showed that the perception of capsaicin pungency can be adequately described using the time-intensity (TI) method (Cliff & Heymann, 1993; Kostrya, Barylko-Pikielna, & Dabrowska, 2010; Schneider, Seuß–Baum, & Schlich, 2014). However, reducing the amount of necessary sensory assessments would be advantageous in industrial quality assurance processes and may be realized by establishing a correlation between sensory and chemical product evaluations, as demonstrated by numerous studies (Crowther et al., 2005; Rodrigues, Condino, Pinheiro, & Nunes, 2016; Schneider et al., 2014; Zhao, Tang, & Ding, 2007).

Crowther et al. (2005) compared pyruvate levels in onions, which were determined chemically using high-performance liquid chromatography (HPLC), while the sensory assessment was performed by a taste-panel in two steps. First, the marketing classification of the onions was reviewed by the taste panel and compared with the analyzed pyruvate content. Second, a new improved flavor classification based on pyruvate content was implemented, which was subsequently validated by the taste-panel.

Schneider et al. (2014) used a similar approach to compare the sensory and chemical characteristics of capsaicin-containing salsas. Moreover, Perkins et al. (2002) demonstrated that the pungency level labeled on commercially available salsa products did not correspond to the chemically determined capsaicin content. Therefore, the combined analysis of both the sensory perceived pungency and the concentration of the pungency inducing chemical irritant is considered a promising approach.

Several analytical methods describing the identification and quantification of isothiocyanates, particularly AITC, have been published. Among them are methods that use gas chromatography (Buttery, Guadagni, Ling, Seifert, & Lipton, 1976; Kuebler, 2010), reversed-phase HPLC (Herzallah & Holley, 2012; Ishikawa, Maruyama, Yamamoto, & Hara, 2014; Pelosi et al., 2014; Tsao, Yu, Potter, & Chiba, 2002; Wilson, Ennarah, Marchioni, Bergaatztlé, & Bindler, 2012), and HPLC–tandem mass spectrometry (MS/MS) (Franco et al., 2016).

The aim of this study was to determine if there is a correlation between the chemically determined AITC content and the sensory evaluation of AITC induced pungency in mustard products and if this correlation can be used to predict the results of the sensory evaluation of pungency. Thus, the sensory evaluation of mustard products was performed using the TI method and an HPLC method was used to determine the AITC concentration.

## 2. MATERIALS AND METHODS

### 2.1 Overview

The pungency of commercially available mustard products was evaluated in a sensory TI study by trained panelists. The AITC content of all tested products was determined by HPLC. The sensory pungency ratings and chemically determined AITC contents were correlated by regression analysis.

### 2.2 Sensory tests

TI tests were performed in individual test booths under red light conditions in a sensory laboratory, in accordance with DIN EN ISO 8589 (2010). The room temperature was regulated to 21 ± 2 °C. The TI test procedure used was in conformity with DIN 10970 (2002).

#### 2.2.1 Panelists.

The panel for the TI study consisted of 14 assessors (9 females, 5 males; age range: 19 to 54). All recruited panelists were experienced in sensory methods, familiar with the assessment of pungent products and students or employees of the Department of Food Technology at the University of Applied Sciences, Fulda. At least 11 panelists participated in each sensory evaluation session.

All subjects provided both written and verbal informed consent for the study procedure.

#### 2.2.2 Panelists training.

To generate reliable sensory data, the enrolled panelists were familiarized with the sensory software FIZZ (FIZZ software by Biosystèmes, Version 2.60, France) and specially trained to use the TI method. Therefore, panelists underwent three training sessions, as proposed by Peyvieux and Dijksterhuis (2001) and Schneider et al. (2014). The training started with a session to practice TI scoring with the sensory software using sugar solutions (15 g/L). Furthermore, they were trained to coordinate between scoring the intensity and the predefined method of mustard sample intake using a mustard carrier matrix with defined AITC concentrations (30 and 100 mg AITC/100 g mustard carrier). The mustard carrier samples were also used to familiarize the panelists with mustard pungency levels. After the first sensory training session, the panelists discussed the pungency perception induced by the two tested mustard samples among the group. The mustard carriers with different AITC concentrations were assigned equivalent sensory ratings on the 10-point pungency intensity scale (30 mg AITC/100 g mustard carrier was assigned as 1 and 100 mg AITC/100 g mustard carrier was assigned as 6). In the two following training sessions, sugar and mustard samples were tested again. This was done to familiarize the panelists with the TI method and to assess if the panelists were able to generate reproducible TI curves. The performance of the panelists was considered reproducible if the curves exhibited consistent TI parameters ($I_{max}$ and $DUR_{j,k}$) and had a similar shape (van Buuren, 1992). Additionally, all curves from the double determination of the mustard products were reviewed for good reproducibility after completion of the sensory evaluation sessions. Thus, the panelists could be excluded from the data in the case of deficient conformity of the curves.

#### 2.2.3 Samples.

Eight commercially available mustard products were evaluated in duplicate with the TI testing protocol. Table 1 lists the product characteristics such as pungency declaration, nutritional values, and moisture content. The moisture content was determined threefold using infrared moisture analyzer MA35 (Sartorius, Goettingen, Germany).

#### 2.2.4 TI test procedure.

TI tests were performed according to DIN 10970 (2002), in individual booths of a sensory laboratory
that conformed to DIN EN ISO 8589 (2010) standards. The TI data were recorded and analyzed using the FIZZ sensory software (Version 2.5.1, Biosystèmes, Couteurnon, France).

Mustard samples of 1.5 g were offered individually on teaspoons coded with a random three-digit number. Panelists were trained to incorporate the samples repeatable and, swallow the sample after 5 s as instructed by pop up messages on the screen. The perceived pungency intensity was rated for 300 s.

The intensity was rated by moving the mouse on a structured linear scale, labeled “0” and “not detectable” for the lowest intensity score and “10” and “extremely pungent” for the highest intensity score. Panelists initiated data acquisition themselves by clicking on the zero point of the scale.

Distilled water and toast coated with mascarpone were provided as neutralizers between samples. A countdown on the screen indicated the inter–stimulus interval of 90 s. Panelists were then asked to fill out a questionnaire assessing the location of the pungency sensation, descriptions of the sensation, and general remarks.

**2.2.5 Data processing and analysis.** Individual TI data were processed using the FIZZ sensory software. Significant differences in pungency perception (individual $I_{\text{max}}$ values) induced by mustard products were analyzed by two-way (matrix, replicates) analysis of variance (ANOVA) $(p < 0.05)$ followed by Tukey HSD post hoc tests using the IBM SPSS Statistics (24) software (IBM Deutschland GmbH, Ettlingen, Germany). For further analysis, the TI data were normalized using FIZZ to eliminate the individual influences of the panelists. Regression analysis was carried out using the IBM SPSS Statistics (24) software.

**2.3 HPLC analysis of mustard products**

**2.3.1 Chemicals and reagents.** HPLC grade (≥99.9%) acetonitrile (ACN) and water were used as a solvent and extraction eluent. The water used was supplied by a water purifier system (Sartorius AG, Göttingen, Germany). AITC (≥99.0% purity) and benzyl isothiocyanate (BITC; ≥98.0% purity), were purchased from Merck (Merck KGaA, Darmstadt, Germany). Commercial mustard products were either provided by producers or purchased from independent local distributors.

**2.3.2 Instrumentation.** The chromatographic analysis of AITC was performed on a liquid chromatography (LC) system (Jasco Labor-und Datentechnik GmbH Deutschland, Gross-Umstadt, Germany) with a multi-wavelength Photodiode-Array-UV detector using the ReproSil-Pur 120 C18-AQ, 5 μm (250 × 4.6 mm) column (Dr. A. Maisch GmbH, Ammerbuch-Entringen, Germany).

**2.3.3 HPLC method.** AITC, was quantified by HPLC using the internal standard BITC for multiple point internal standard calibration.

The mobile phase was HPLC grade water with 0.5% formic acid (component A) and ACN with 0.5% formic acid (component B), as described by Franco et al. (2016). The separation was achieved over a run time of 35 min using the following gradient program of component B: 10 min at 5%, 4 min at 24%, 4 min at 50%, 7 min at 80%, and 10 min at 5% at 1 mL/min flow rate. The column temperature was set to 30 °C and the temperature of the autosampler to 4 °C. The injected sample volume was 10 μL. The UV detection wavelength was set to 242 nm. The mean retention times were 20.19 ± 0.04 s for AITC and 22.31 ± 0.06 s for BITC. Peak identification was achieved by comparing the retention times of the samples with those of the standard substances as well as by spiking the samples.

**2.3.4 Standard preparations.** AITC was quantified based on a multiple point internal standard calibration, using BITC as an internal standard. Various standard solutions of AITC (0.1, 0.5, 1, 2, 4, and 6 g/L) and BITC (3 g/L) were prepared from a stock solution dissolved in 75% ACN and 25% HPLC-grade water (v/v).

**2.3.5 Extraction of AITC from commercially available mustard products.** The AITC extraction procedure was adapted from a protocol described by Pelosi et al. (2014). Briefly, 2 g of mustard were weighed in a 15 mL polypropylene tube. Then, 2.5 mL of LC-grade water and 7.5 mL of ACN were added. The mixtures were homogenized on an orbital shaker (VWR Mini Shaker, VWR, Darmstadt, Germany) at 450 rpm for 10 min and then sonicated for 30 min. The mixtures were subsequently centrifuged at 1,300 × g at 7 °C for 10 min. A 2 mL syringe was used to remove the supernatant and the extract was then filtered using a 0.45 μm RC membrane filter (Phenomenex, Aschaffenburg, Germany). Three replicates of each sample were analyzed.

### Table 1–Pungency classification, nutritional facts and moisture content of commercial mustard products.

| Mustard product | Pungency classification | Fat $^1$ (g) | Carbohydrates $^2$ (g) | Protein $^3$ (g) | Moisture content $^3$ [%M] |
|-----------------|-------------------------|-------------|------------------------|----------------|-------------------------|
| A               | Sweet                   | 2.8         | 39.0 (38.0 sugar)       | 7.0            | 50.1 (±0.1)             |
| B               | Sweet                   | 7.7         | 38.8 (38.7 sugar)       | 7.3            | 44.6 (±1.1)             |
| C               | Medium piquant          | 8.2         | 4.8 (4.5 sugar)         | 6.8            | 71.6 (±0.1)             |
| D               | Medium hot              | 6.7         | 2.2 (2.0 sugar)         | 6.1            | 77.0 (±0.4)             |
| E               | Medium hot              | 27.0        | 9.3 (4.5 sugar)         | 3.9            | 56.7 (±0.2)             |
| F               | Hot                     | 14.2        | 4.9 (2.9 sugar)         | 10.4           | 66.3 (±0.3)             |
| G               | Extra hot               | 12.6        | 1.5 (1.5 sugar)         | 8.9            | 66.6 (±0.1)             |
| H               | Not defined             | 11.0        | 3.5 (2.0 sugar)         | 7.0            | 70.3 (±0.1)             |

1 According to the voluntary pungency classification labeled on the products.
2 According to the nutritional facts labeled on the products in g/100 g.
3 Values in %M ± SD, threefold determination.

### 3 RESULTS AND DISCUSSION

**3.1 Sensory evaluation of the pungency perception of commercial mustard products.**

The results of the sensory evaluation of pungency perception of mustard products are presented in Table 2 and Figures 1–3. $I_{\text{max}}$ mean values were calculated based on the individual data provided by each panelist. Notably, we observed significant differences between the pungency of different mustard products. The mustard products A and B, B and C, as well as D, E, F, and H were not significantly different from each other. The composition of the products (as shown in Table 1) may explain these differences. First, products A and B are classified as sweet mustard products, which are characterized by a high carbohydrate content, especially...
Table 2—Pungency ratings ($I_{max}$, $DUR_{Dec}$, $AUC$) and AITC concentrations for commercial mustard products. $I_{max}$ (maximum perceived intensity) is calculated as mean values of individual ratings. Time related parameter $DUR_{Dec}$ (duration of decreasing phase) and calculated parameter $AUC$ (area under the curve) were obtained from normalized curves.

| Mustard product | $I_{max}$ | $DUR_{Dec}$ (s) | $AUC$ | AITC concentration (mg/100 g) | AITC concentration dry weight (mg/100 g) |
|-----------------|-----------|----------------|-------|--------------------------------|-----------------------------------------|
| A               | 0.5 a     | 12.8           | 8     | 43.9 (± 3.5)                   | 88.0                                    |
| B               | 0.9 a,b   | 13.1           | 13    | 28.8 (± 0.7)                   | 52.0                                    |
| C               | 2.3 b     | 18.2           | 32    | 21.3 (± 0.4)                   | 74.9                                    |
| D               | 5.0 c     | 30             | 109   | 38.1 (± 4.9)                   | 165.9                                   |
| E               | 4.5 c     | 32.2           | 117   | 99.7 (± 1.7)                   | 230.1                                   |
| F               | 5.9 c     | 48.6           | 185   | 135.6 (± 1.8)                  | 394.3                                   |
| G               | 9.0 d     | 56.2           | 307   | 183.1 (± 1.9)                  | 548.3                                   |
| H               | 5.0 c     | 35.9           | 123   | 34.0 (± 0.7)                   | 114.5                                   |

1Mustard product designation according to Table 1.
2a, b, c, d–$I_{max}$ mean values with different letters in columns are significantly different, $p < 0.05$.
3Values in mg/100 g ± SD of fresh weight; $n = 3$.
4Values in mg/100 g of dry weight; $n = 3$.

Figure 1—Sensory ratings ($I_{max}$) of commercial mustard products (scale 0–10; 0 = “not detectable”, 10 = “extremely pungent”).

Sugars, of approximately 40%. Kostyra et al. (2010) and Schneider et al. (2014) reported that starch content significantly influences the pungency perception of capsaicinoids. The reduced pungency intensity may be explained by the increase in both dry matter and viscosity of the products with high starch contents, which subsequently reduces the contact of irritant molecules with nociceptors (Hutchinson, Trantow, & Vickers, 1990). Furthermore, both mustard products have a grainy texture, as sweet mustard usually contains intact and partially ground mustard seeds. Isothiocyanates are hydrolyzed from their precursors, namely glucosinolates, when cell rupture of the intact seeds releases the endogenous myrosinase (thioglucoside glucohydrolase) (Oliviero, Verkerk, & Dekker, 2018). This may explain the low sensory pungency ratings of grainy mustard products, as probably only a low amount of sinigrin was enzymatically degraded to AITC during consumption.

Second, the fat content of the products ranges from 3 to 27 g/100 g. The majority of the products had a fat content of 10 ± 3 g/100 g. Products D and E were rated as having a medium pungency, with mean $I_{max}$ values of 5 and 4.5, respectively (Table 2), while the AITC concentration was 38.1 and 99.7 mg/100 g mustard, respectively. This indicates that even though the AITC content of product E was significantly higher, the sensory pungency rating of both products was similar. The different pungency sensation caused by AITC may be explained by the difference in fat content between the products, which was 7 and 27 g/100 g, respectively. Notably, previous studies have described the masking effects...
Sensory and chemical mustard pungency...

The spread of the $I_{\text{max}}$ data is illustrated with box plots in Figure 1. The large spread of data distribution may be explained by the individual differences between the pungency perception of the panelists (Smutzer & Devassy, 2016), even though the evaluation was carried out by a trained panel. Other TI studies (McGowan & Lee, 2006; Pionnier et al., 2004; Schneider et al., 2014) have shown a similar spread of the data. Therefore, McGowan and Lee (2006) grouped panelists with similar individual curve styles prior to TI data analysis and revealed that this method improves TI results when compared to the enhanced method developed by Liu and MacFie (1990). In order to eliminate the influence of individual differences in pungency perception on the data, the data was normalized using the algorithm integrated into the sensory software FIZZ. Thus, average curves were generated and the normalized parameters were calculated.

Furthermore, the average curves of the pungency rating (Figure 2) demonstrate that the mustard products tested display a broad range of pungency levels. Mustard products classified as sweet mustards (A and B) were rated as the least pungent, which is indicated by perception durations of approximately 20 s and maximum intensities ($I_{\text{max}}$) of approximately 1. The mustard product classified as extra hot (G) was rated to be the most pungent. The average duration of perception of product G was 68 s and the average $I_{\text{max}}$ was approximately 9. Among the products classified as medium hot (C–E), product C was significantly different when compared to the others. Product F, which was classified as hot, was rated with a pungency level in between those of the medium hot (C–E) and extra hot (G) products, having an $I_{\text{max}}$ value of 5.9 and a duration of perception of approximately 60 s. Therefore, regarding the sensory pungency ratings, commercial product classifications were appropriate for most products examined.

The panelists were asked to localize the pungency sensation at $I_{\text{max}}$ during the TI study for each tested mustard product. Depending on the product, the number of answers given and the localization varied, as shown in Figure 3. In particular, a pungency sensation in the nose was mentioned more frequently for products with a high AITC concentration. The high volatility and the lachrymatory character of AITC may explain these results (Terada, Masuda, & Watanabe, 2015). Furthermore, perception in the throat was found to increase proportionally with the increase in product pungency levels. Another study, which investigated AITC thresholds in water- and oil-based carrier matrices, reported similar findings (Eib et al., 2020). Moreover, Rentmeister-Bryant and Green (1997) reported that the localization of the perception of capsaicin and piperine pungency was influenced by the concentration and the time after the incorporation of the irritant.

3.2 AITC content (HPLC) of commercial mustard products

AITC quantification via HPLC was done by calculating the peak area and the peak area of the internal standard used. Both were identified by comparing the retention times of the samples with those of the standard analyte solutions. By using the
multiple point internal standard quantification method, the calibration curve \( y = 0.9929x - 0.0041 \) was calculated and used for subsequent AITC quantification in mustard products. The \( R^2 \) of the regression equation was 0.999. A liquid chromatogram of the standard substances AITC and BITC as well as a liquid chromatogram of the “extra hot” commercial mustard product G is exemplarily illustrated in Figure 4.

The AITC concentration in the analyzed mustard products was highly variable, as shown in Table 2 and Figure 5. Mustard products A–D and H were found to have AITC contents ranging from 27.3 to 43.9 mg/100 g, while the sensory rating of these products varied from 0.5 to 5.0 (\( I_{\text{max}} \), mean values). This variation in pungency perception may be explained by the differences in product composition (carbohydrate and fat content), as previously described (Kostyra et al., 2010; Schneider et al., 2014). Furthermore, 4-HBITC, which originates from yellow mustard seeds, is also able to induce a pungency sensation during the consumption of commercially available mustard products (Bhattacharya et al., 2010; Choubdar et al., 2010; Ekanayake, Zoutendam, Strife, Fu, & Jayatilake, 2012; Fahey et al., 2001; Fenwick & Heaney, 1983). The ratio between 4-HBITC and AITC depends on the mixture of mustard seeds from the varieties Brassica nigra, Brassica juncea, and Sinapis alba. In this study, 4-HBITC concentrations in mustard products were not presented due to a broad variation in the measurement data. This variation may be explained by the extraordinary instability of 4-HBITC (Choubdar et al., 2010; Kawakishi, 1966; Kjar, Rubinstein, Tjes, & Burreis, 1954). Nevertheless, other authors have described the pungency induced by 4-HBITC as a hot mouthfeel, while AITC induces a pungent aroma and sharp taste (Choubdar et al., 2010; Fahey et al., 2001).

Notably, seeds of the Brassicaceae species Brassica nigra, Brassica juncea, and Sinapis alba may be exclusively used for mustard production (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015). Depending on the variety, the seeds contain either the glucosinolate sinigrin (Brassica nigra, Brassica juncea) or sinalbin (Sinapis alba) (Hälväs, Hirvi, Mäkinen, & Honkanen, 1986; Velišek et al., 1995; Zrybko, Fukuda, & Rosen, 1997). Extra hot mustard products are produced from a high amount of mustard seeds that contain sinigrin. In general, few or no mustard seeds containing sinalbin are used in extra hot and hot mustard pastes (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015), resulting in a high ratio of AITC to 4-HBITC in the finished products. Thus, the amount of 4-HBITC in hot and extra hot mustard products is low and may not have a meaningful influence on the pungency classification of those products. The presence of both AITC and 4-HBITC may contribute to the discrepancies in the correlation of sensory and chemical data. In order to have a complete analysis of AITC containing mustard products, sweet mustard products were also evaluated in this study. The pungency induced by AITC was rated to be low in these products, as previously discussed.

Furthermore, storage and production processes may influence isothiocyanate concentrations (Stahl, Haider, Mersch-Sundermann, & Gminski, 2009). Depending on the temperature, pH value, and water content of the product, AITC may be degraded to various compounds such as N,N’-diallylthiourea, diallyldithiocarbamate, diallyl tetrasulfide, and diallyl pentasulfide (Chen & Ho, 1998; Hanschen, Lamy, Schreiner, & Rohn, 2001; Kawakishi & Namiki, 1969).
3.3 Correlation of sensory and chemical data in pungency evaluation

Furthermore, the sensory pungency evaluation and chemical analysis of AITC contents were compared to identify a possible correlation between these data.

Figure 5 shows that most products with a high AITC content were rated with high $I_{\text{max}}$ values (products F and G), while some products with moderate $I_{\text{max}}$ values had low AITC contents (products D and H).

To determine if the sensory pungency rating was influenced by the AITC content, the data were analyzed using regression analysis. A statistically significant positive correlation was found between the AITC concentration and $I_{\text{max}}$ (correlation coefficient 0.803), $AUC$ (correlation coefficient 0.891), and $DUR_{\text{Dec}}$ (correlation coefficient 0.856) values. The correlation coefficient describes the relationship of the two variables, in each case. Additionally, the regression models, predicting the value of the dependent variable, are illustrated in the Supporting Information using squared correlation coefficient $R^2$. Multiple regression analysis with the AITC concentration and the moisture content as independent variables showed that both significantly influenced sensory perception ($I_{\text{max}}$, $AUC$, and $DUR_{\text{Dec}}$) of the mustard.

Figure 4—Quantitative liquid chromatogram of (a) AITC (2 mg/10 mL) and BITC (3 mg/10 mL), and (b) mustard product with the pungency declaration "extra hot" (product G).
products. The correlation between the AITC concentration, the moisture content, and $I_{\text{max}}$ (correlation coefficient 0.944), $AUC$ (correlation coefficient 0.960), and $DUR_{D_{\text{O}2}}$ (correlation coefficient 0.954), values, respectively, was improved. Thus, results indicate that pungency sensation induced by the mustard products increased with increasing moisture content. This could be related to previous publications stating that AITC induced pungency was perceived more intense in water-based matrices than in oil-based matrices (Eib et al., 2020). Unpublished results of our group show that AITC pungency perception in mustard-based model matrices was lower than in water-based matrices. Furthermore, the AITC content and the influence of the food content of the mustard products (according to the nutritional values labeled on the product) on TI parameters was reviewed using multiple regression analysis. The fat content as an independent parameter in the regression analysis did not influence the correlation coefficient for all examined TI parameters ($I_{\text{max}}, AUC, DUR_{D_{\text{O}2}}$).

However, the data showed that the sensory pungency rating can be accurately correlated with the AITC concentration of mustard products.

The pungency intensities provided on the label of the tested mustard products were not always consistent with the sensory and chemically determined pungency, which can be seen especially in the data of product C. Similar findings were obtained in regards to the pungency of capsaicin in commercially available salsas. Perkins et al. (2002) reported that the capsaicinoid content of salsa products labeled with the same pungency category varied by up to three times. Additionally, Schneider et al. (2014) correlated the chemically determined capsaicinoid content of salsas with sensory ratings of pungency perception and indicated that pungency sensation caused by capsaicinoids depends on the capsaicinoid concentration, as well as the complexity and composition of the matrix.

4. CONCLUSION

This study demonstrates that commercial mustard products can be adequately categorized in pungency classifications based on their AITC content. However, perceived mustard pungency is also influenced by other factors, such as the 4-HBITC content and the composition of the mustard product (fat and carbohydrate content). Moreover, the declared pungency categorization is not always consistent with the sensory evaluation and the chemically determined AITC concentration. The HPLC method might be used to analyze AITC concentrations in mustard products as well as other AITC containing pastes, such as wasabi or horseradish. Thus, based on the AITC concentration determined by chemical analysis, sensory perceived pungency might be predicted by using the calculated model (Supporting Information).

ACKNOWLEDGMENTS

The authors are thankful to all panelists for their willingness to participate. We are grateful to Sarah Ramos Gajek and Leonard Mueller for helping to perform the HPLC measurements. This work was performed within the framework of the cooperative Ph.D. program “Food Economy and Technology” of the University of Kassel and the Fuku University of Applied Sciences and was financially supported by the Federal Hessian Ministry of Science and Art. The authors are very grateful for the financial support.

Open access funding enabled and organized by Projekt DEAL.

AUTHOR CONTRIBUTIONS

Eib, Schneider, and Seuß-Baum were associated with study concept and design. Eib performed the analysis and interpretation of data, drafted the manuscript, and performed statistical analysis. Eib, Schneider, Hensel, and Seuß-Baum were associated with the critical revision of the manuscript for important intellectual content. Eib and Seuß-Baum supervised the study.

CONFLICTS OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.