Analysis of key pungent compounds in mustard products: A comparison of sensory and chemical–analytical determinations

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Abstract

The consumption of mustard, horseradish and wasabi is characterized by a pungent sensation and lachrymatory effect primarily induced by allyl isothiocyanate (AITC). This study aimed to check the possible correlation between sensory-perceived pungency and the quality-determining pungency-inducing ingredients of mustard.

Primarily, the detection thresholds of sinigrin in a water-based matrix and AITC in water- and oil-based matrices were determined in a prestudy (10 panelists) and a main study (64 panelists) using a three-alternative forced choice test (3-AFC). In the prestudy, the resulting value of the group best-estimate detection threshold (BET) for sinigrin in a water-based matrix was 10.9 mg/100 mL. The BET value for AITC in a water-based matrix was 0.1 mg/100 mL both in the pre and main studies, which differed significantly from that in oil-based matrices with 0.5 and 0.6 mg/100 mL in the pre and main studies, respectively.

Further, a time-intensity (TI) study identified the AITC concentration and carrier matrix composition as significant influencing factors for the intensity and time course of pungency perception. An increase in the AITC concentration increased the maximum perceived pungency intensity (I_{max}) and extended the duration of pungency sensation (end time of pungency perception T_{End}; duration of decreasing phase DUR_{Dec}). Time- and intensity-related sensory parameters increased depending on the carrier matrix in the order oil-based matrix < mustard-based matrix < water-based matrix at equivalent AITC concentrations.

Additionally, an adapted HPLC method for the quantification of AITC in commercial mustard products was applied. Regression analysis revealed positive correlations between the AITC content and sensory-determined TI parameters of commercial mustard products. With an increasing AITC content, an increase in the I_{max}, DUR_{Dec} and *AUC* (area under the curve) values was observed. Despite the fact that mustard products classified by the producer as pungent had a higher AITC content than products classified as sweet, great variations between products and sensory-determined pungency levels were identified. Nevertheless, correlation coefficients of 0.944 (I_{max}), 0.954 ($\mathrm{DUR}_{\mathrm{Dec}}$), and 0.960 (*AUC*) indicated a very strong correlation between the sensory pungency perception, the AITC concentration and the moisture content of the mustard products.

Moreover, an increasing AITC content provoked a shift from a pungency sensation only on the tongue (at low AITC concentrations: <10 mg AITC/100 mL, <50 mg AITC/100 mL, and <50 mg AITC/100 g in the water-based, oil-based, and mustard-based matrices, respectively) to an additional and a more intense pungency sensation in the throat and nose region (at high AITC concentrations: >50 mg AITC/100 mL, >100 mg AITC/100 mL, and >100 mg AITC/100 g in the water-based, oil-based, and mustard-based matrices, respectively).

In summary, the established regression model highlights the relationship between predominantly AITC induced pungency perception and the AITC content of mustard products and may facilitate the reduction of necessary sensory evaluations in quality assurance and product development of pungent products.

Kurzzusammenfassung

Der Verzehr von Senf, Meerrettich und Wasabi ist durch einen scharfen sensorischen Sinneseindruck und einen tränentreibenden Effekt charakterisiert, der primär durch Allylisothiocyanat (AITC) induziert wird. Das Ziel dieser Arbeit war es, am Beispiel von Senf eine Korrelation zwischen sensorisch wahrgenommener Schärfe und qualitätsbestimmenden Inhaltsstoffen von Senf, die einen Schärfereiz auslösen können, zu prüfen.

Zunächst wurden die Reizschwelle für Sinigrin (wässrige Matrix) und AITC (wässrige bzw. ölige Matrix) in einer Vorstudie (10 Panelisten) und einer Hauptstudie (64 Panelisten) mit einem three-alternative forced choice Test (3-AFC) ermittelt. Der in der Vorstudie ermittelte Gruppenreizschwelle (BET) für Sinigrin in wässriger Matrix ergab einen Wert von 10,9 mg/100 mL. Die BET-Werte für AITC in wässriger Matrix lagen in der Vor- und Hauptstudie jeweils bei 0,1 mg/100 mL und haben sich signifikant von den BET-Werten von AITC in öliger Matrix mit 0,5 mg/100 mL in der Vorstudie und 0,6 mg/100 mL in der Hauptstudie unterschieden.

In einer Zeit-Intensitäts-(TI) Studie wurde nachgewiesen, dass die AITC-Konzentration und die Zusammensetzung der Trägermatrix einen signifikanten Einfluss auf die Intensität und den Zeitverlauf der Schärfewahrnehmung zeigen. Mit zunehmender AITC-Konzentration stieg die maximal wahrgenommene Schärfeintensität (I_{max}) an und die Dauer der Schärfewahrnehmung (Endpunkt der Schärfewahrnehmung T_{End}; Dauer der Schärfeabnahme DUR_{Dec}) nahm zu. Zeit- und intensitätsbezogene sensorische Parameter stiegen bei gleicher AITC-Konzentration in Abhängigkeit von der Matrix, in der Reihenfolge ölige Matrix < Senf-basierte Matrix < wässrige Matrix an.

Eine adaptierte HPLC-Methode wurde zur Quantifizierung von AITC in kommerziellen Senfprodukten eingesetzt. Bei einer Regressionsanalyse konnte eine positive Korrelation zwischen der AITC-Konzentration und den sensorisch ermittelten TI Parametern von marktgängigen Senfprodukten aufgezeigt werden. Mit steigender AITC-Konzentration wurde eine Erhöhung der Parameter I_{max}, DUR_{Dec} und AUC (Fläche unter der Kurve) beobachtet. Wenngleich Senfprodukte, die vom Produzenten als scharf eingestuft sind, höhere AITC-Konzentrationen aufwiesen als Produkte, die als süß eingestuft sind, wurde eine große Variation zwischen den Handelsprodukten und den sensorisch bestimmten Schärfe-Leveln identifiziert. Dennoch weisen die Korrelationskoeffizienten 0,944 (I_{max}), 0,954 (DUR_{Dec}) und 0,960 (AUC) auf eine sehr gute Korrelation zwischen der sensorisch wahrgenommenen Schärfeintensität, der AITC-Konzentration und dem Feuchtegehalt des Produktes hin.

Es wurde auch beobachtet, dass mit steigender AITC-Konzentration eine Verlagerung der Schärfewahrnehmung von der Zunge (bei niedrigen AITC-Konzentrationen; in wässriger Matrix <10 mg AITC/100 mL; in öliger Matrix <50 mg AITC/100 mL; in Senf-basierter Matrix <50 mg AITC/100 g) zu

einer zusätzlichen und intensiveren Schärfewahrnehmung im Rachen- und Nasenraum (bei hohen AITC-Konzentrationen; in wässriger Matrix >50 mg AITC/100 mL; in öliger Matrix >100 mg AITC/100 mL; in Senf-basierter Matrix >100 mg AITC/100 g) stattfindet.

Zusammenfassend kann festgestellt werden, dass das aufgestellte Regressionsmodell die postulierte Beziehung zwischen der vorwiegend durch AITC induzierten Schärfewahrnehmung und dem AITC-Gehalt von Senfprodukten unterstreicht und entsprechende Berechnungen dazu beitragen können, die Anzahl an notwendigen sensorischen Untersuchungen bei Qualitätssicherung und Produktentwicklung von scharfen Produkten zu reduzieren.

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Abbreviations

2-PEITC	2-phenylethyl isothiocyanate
4-HBITC	4-hydroxybenzyl isothiocyanate
3-AFC	three-alternative forced choice
ACN	acetonitrile
AITC	allyl isothiocyanate
ANOVA	analysis of variance
AUC	total area under the curve, from the first to the last acquisition point
AUC_{Dec}	area under the curve, decreasing phase
BET	best-estimate threshold
BITC	benzyl isothiocyanate
DUR_{Dec}	duration of the decreasing phase
ESM	epithiospecifier modifier protein
ESP	epithiospecifier protein
GC	gas chromatography
GC-MS	gas chromatography mass spectrometry
GSL	glucosinolates
HPLC	high performance liquid chromatography
I_{max}	maximum intensity
ITC	isothiocyanate
LC	liquid chromatography
LC-MS	liquid chromatography mass spectrometry
MS/MS	tandem mass spectrometry
n	size of a statistical sample
NC	nose clip
NSP	nitrile-specifier proteins
p	level of significance
ppm	parts per million
R	variable side chain
SD	standard deviation
TAS2R	TASTE 2 receptor
T_{End}	end time of pungency perception
TFP	thiocyanate-forming protein
TI	time-intensity

T_{Imax}	time to reach maximum intensity of pungency
TRP	transient receptor potential
TRPA	transient receptor potential ankyrin
TRPA1	transient receptor potential ankyrin 1
TRPC	transient receptor potential canonical
TRPM	transient receptor potential melastatin
TRPM8	transient receptor potential melastatin 8
TRPML	transient receptor potential mucolipin
TRPP	transient receptor potential polycystin
TRPV	transient receptor potential vanilloid
TRPV1	transient receptor potential vanilloid 1
w	with
w/o	without

1 Introduction

The consumption of products or dishes that induce a pungent sensation is widespread, popular and anchored in many cultures (Spence, 2018). These products include mustard-, wasabi-, cinnamon- and chili-containing foods. Pungent sensations are induced by chemical stimuli such as capsaicin, allyl isothiocyanate (AITC) and piperine. These stimulating compounds vary with pungent-sensation-inducing products.

This work focuses mainly on the pungency of mustard products. Mustard products are popular condiments in European cuisine. In 2019, the per head consumption of mustard in Germany was 879 g with a market share of 11.1 % of the total quantity of condiments and sauces (Kulinaria Deutschland e.V., 2019). Specialized mustard stores sell numerous different products with ingredients such as whiskey, beer and gin.

Yellow and black mustard seeds are the most common mustard seeds in Europe and were spread abroad to China and India by explorers (Webb et al., 1988). Mustard crops form bitter and pungent secondary plant substances to reduce insect proliferation and protect against herbivores (Agrawal & Kurashige, 2003). The production of mustard pastes dates back to 3000 BC (Hemingway, 2003). Crushed mustard seeds were mixed with the sweet must of wine to form a paste called “hot must” or “mustum ardens” leading to the term “mustard” (Vaughan & Hemingway, 1959). Although a variety of mustard products are now available, their basic ingredients are mustard seeds, water and vinegar, wine or grape juice (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015).

Moreover, mustard is used in many other food products such as sauces and dressings (Patterson, 2016), fermented vegetables (Fang et al., 2008) and edible oils (Hemingway, 2003; Mustakas et al., 1962). The early use of mustard in many products may be explained by its antimicrobial and antifungal properties (Bahmid et al., 2020; Spence, 2018; Torrijos et al., 2019). Thus, mustard became traditionally rooted in many cultures before the development and distribution of refrigeration (Spence, 2018).

Why is the consumption of spicy foods so widespread and popular?

The most widely used spice is hot pepper, containing the pungent substance capsaicin (Govindarajan & Sathyanarayana, 1991). Reportedly, 25% of the people on earth consume chilis daily (Cordell & Araujo, 1993). Nevertheless, the consumption of pungent ingredients leads to diverse reactions from aversion to strong liking (Prescott & Stevenson, 1995; Tepper et al., 2003; Törnwall et al., 2012). It is generally assumed that the first capsaicin intake provokes aversive reactions (McDonald et al., 2016).

Thus, individuals may learn to like the pungent sensation induced by chili, mustard, wasabi and other spices (Lawless et al., 1985; Stevenson & Prescott, 1994). Other examples confirm the possibility of learning to like foods that are initially disliked, such as coffee, tobacco, and alcohol (Rozin & Schiller, 1980). These food products are consumed because of social or post-ingestive effects and preference is acquired over time (Rozin & Schiller, 1980). Historically, the use of spices, such as cinnamon, cloves and nutmeg, might have expressed a high social status (Spence, 2018). Nowadays, several hypotheses that address the consumption and liking of spicy food products are being discussed. Especially male consumers test their abilities to resist extremely pungent foods. This aspect of consumption is based on masochistic thrill-seeking behavior. (Logue & Smith, 1986; Rozin & Schiller, 1980) Food culture is another aspect affecting individual preference for pungency (McDonald et al., 2016). Various pungent spices are enrooted in traditional cuisines globally. Furthermore, inter-individual biological differences lead to variability in the response to pungent food (Hayes et al., 2013).

Pungent sensations induced by different chemical stimuli vary. In this context, the most discussed sensory attribute is the pungency intensity of products. There have always been attempts to create comparable pungency intensity ratings for consumers. For example, the well-established Scoville test, developed by Wilbur L. Scoville in 1912, provides a scale for the pungency assessment of capsaicin-containing products (Scoville, 1912). Several authors have subsequently focused on the applicability of the Scoville scale and discussed further methods to rate the pungency intensity of these products (Korel et al., 2002; Schneider et al., 2014a; Todd et al., 1977). The importance of the rating of pungency intensities is related to the intention to make pungency a measurable quantity, thus generating comparability between products. The specification of the pungency intensity on a product label represents a sensory claim, which needs to be scientifically proven and reproducible. Providing information about a product's pungency classification is common for many food products. Salsas, wasabi, horseradish, mustard, and other products may be labeled with pungency specifications such as "sweet," "medium," "hot," or "extra hot."

The perception of pungency has been a research topic for many years; however, most studies have focused on capsaicin-induced pungency sensations. There is a dearth of knowledge of the perception of mustard pungency, particularly about the pungent substance AITC, which is primarily responsible for mustard pungency. Therefore, this thesis focuses on the evaluation of the sensory perception of the quality-determining pungency-inducing ingredients of mustard. Additionally, the concentration of pungency-inducing substances in mustard products, the relationship between sensory-perceived mustard pungency and the content of quality-determining pungency-inducing ingredients, and a model for the classification of mustard products based on pungency are determined, reviewed, and presented, respectively, herein.

2 State of knowledge

Pungency level is a quality-determining attribute for mustard products; thus, product labels usually indicate the pungency level using terms such as mild, medium, hot, pungent, and strong (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015). In the mustard product quality evaluation, organized by the Deutsche Landwirtschafts-Gesellschaft (DLG) e.V., assessments addressing the pungency level indicated on product labels were discussed. The assessors and consumers' individual pungency perceptions and expectations may deviate from the pungency declaration defined by the producer. The perception of pungency is a "chemestetic" sensation mediated by the trigeminal system (Green, 1996; Slack, 2016). Chemical compounds such as AITC induce a pungent sensation via the direct stimulation of the transient receptor potential ion channels belonging to the sense of pain (Everaerts et al., 2011; Jordt et al., 2004). Factors influencing pungency perception are multifaceted. These factors include chemical stimulus (Haley & McDonald, 2016), carrier matrix composition and viscosity (Buettner et al., 2002; Kostyra et al., 2010; Schneider et al., 2014a), product temperature (Bandell et al., 2004), individual preferences (Nilius & Appendino, 2011), saliva flow rate, and saliva composition (Horne et al., 2002; Mosca & Chen, 2017).

In recent years, AITC has attracted attention owing to its antimicrobial and antifungal properties. Thus, AITC or ground mustard has been applied in product or packaging development as an active ingredient to extend the shelf life of various food products (Bahmid et al., 2020; Gao et al., 2018; Lopes et al., 2018; Milani, 2013; Olaimat & Holley, 2016; Otoni et al., 2014; Torrijos et al., 2019) by preventing microbial spoilage of these products. The application of AITC or ground mustard for this particular purpose also needs to consider the sensory characteristics of the pungent substance.

2.1 Perception of trigeminal chemical stimuli

The perception of pungency is a "chemesthetic" quality (Green, 1996) and was described as being distinct from taste and olfaction for the first time by Parker (1912) using the term "common chemical sense." Chemesthesis describes sensations initiated by chemical stimuli that activate somatosensory nerves (Green, 1991, 2012; McDonald et al., 2016) and receptors assigned to pain, touch, temperature and texture senses (Nilius & Appendino, 2011). Chemically induced pungency sensation varies in quality, time course and area of perception (S. M. Bennett & Hayes, 2012; Bryant & Mezine, 1999; Cliff & Heymann, 1992, 1993; Lawless, 1984; McDonald et al., 2016). These sensations range from the cooling induced by menthol (Green, 1985, 1986) to the slowly increasing and long-lasting burns of capsaicinoids (Cliff & Heymann, 1992; Lawless et al., 1985; Reinbach et al., 2007; Schneider et al., 2014a). Pungency sensations elicited by mustard and horseradish products are characterized by a rapid onset and offset (McDonald et al., 2016), burning sensation (Ghawi et al., 2014), stinging (Brand & Jacquot, 2002; Jacquot et al., 2005), and a lachrymatory effect (Frijters et al., 1981; Gilbert & Nursten, 1972). The somatosensory nerves, especially the trigeminal, vagus, and glossopharyngeal, respond to these oral chemesthetic agents. The trigeminal nerve responds to chemical irritants in the nasal cavity, tongue, palate, nostrils, and nasopharyngeal mucosa. In the posterior oral cavity, the vagal and glossopharyngeal nerves are stimulated by chemical irritants (Simons & Carstens, 2008).

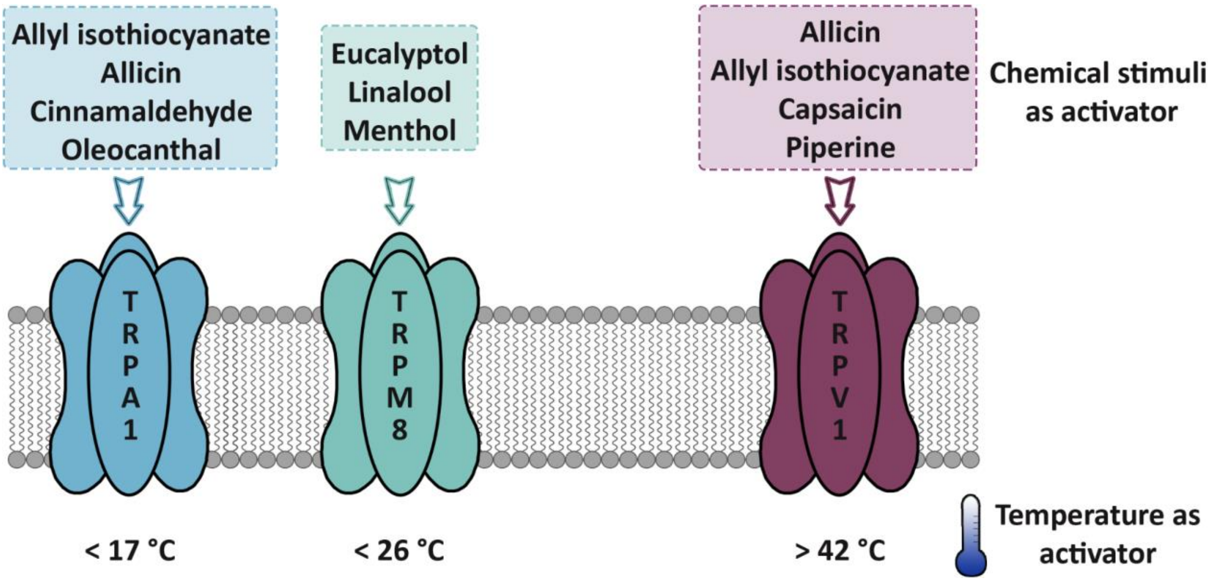

Figure 2.1 Activation of pain-related transient receptor potential ion channels by chemical stimuli and temperatures

Figure 2.1 illustrates the activation of the transient receptor potential (TRP) ion channels in general. TRP ion channels are nonselective Ca^{2+}-permeable cation channels that respond to various chemical stimuli and mediate thermosensation (Bandell et al., 2004; Mickle et al., 2015; Venkatachalam & Montell, 2007). The TRP family consists of 28 ion channels grouped into six subfamilies: ankyrin (TRPA), canonical (TRPC), melastatin (TRPM), mucolipin (TRPML), polycystin (TRPP), and vanilloid (TRPV) (Mickle et al., 2015). TRPA, TRPM, and TRPV are known to be involved in the sensory perception of pain and nociception (Mickle et al., 2015; Venkatachalam & Montell, 2007).

TRPA1 activation can be induced by several chemical stimuli including acetoxychavicol acetate (galangal, blue ginger) (Narukawa et al., 2010), allicin (garlic) (Macpherson et al., 2005), AITC (mustard) (Bandell et al., 2004; Iwasaki et al., 2008), cinnamaldehyde (cinnamon) (Iwasaki et al., 2008), and oleocanthal (virgin olive oil). However, the structural mechanism of activation has not yet been fully clarified (Mickle et al., 2015).

Moreover, TRPV1 can be activated by various pungent compounds such as allicin (Macpherson et al., 2005), AITC (Alpizar, Gees, et al., 2014; Gees et al., 2013), capsaicin (chili pepper) (Caterina et al., 1997; Oh et al., 1996), and piperine (black pepper) (McNamara et al., 2005). Binding of an irritant molecule to the TRPV1 receptor activates ion channel opening, which leads to the influx of Ca^{2+} and Na^{+} (Chung et al., 2008).

TRPM8 is activated by eucalyptol (eucalyptus) (Frasnelli et al., 2011), linalool (cinnamon) (Behrendt et al., 2004) and menthol (mint) (Bautista et al., 2007).

Additionally, the receptors TRPA1, TRPV1, and TRPM8 are temperature sensitive; TRPA1 is activated by cold temperatures (<17 °C), (Bandell et al., 2004; Moparthi et al., 2014), TRPM8 is sensitive to temperatures <26 °C (Bautista et al., 2007), whereas TRPV1 is heat sensitive (Bandell et al., 2004; Tominaga et al., 1998). For TRPV1, two previous studies reported differing findings. According to Bandell et al. (2004) it responds to temperatures >42 °C, whereas according to Tominaga et al. (1998), to temperatures >46 °C.

Furthermore, the TRPA1 and TRPV1 ion channels respond to weak acids (Caterina et al., 1997; Jordt et al., 2000; Wang et al., 2011).

While consuming mustard and mustard food products, the sensory perception of pungency is predominantly induced by the chemical stimulus generated by AITC (Bandell et al., 2004; G. S. Clark, 1992; Iwasaki et al., 2008; Velíšek et al., 1995), which activates the ion channels TRPA1 (Iwasaki et al., 2008; Jordt et al., 2004) and TRPV1 (Alpizar, Boonen, et al., 2014; Everaerts et al., 2011; Ohta et al., 2007).

The chemesthetic sensations elicited by the interaction of chemical irritants with receptors of the TRP ion channel family vary between individuals owing to biological and social differences, as well as personal preferences (McDonald et al., 2016). In general, the consumption of food products that provoke oral irritation, especially pungency, has been studied, largely focusing on the stimulus induced by capsaicin- or capsaicinoid-containing products. Factors influencing the perception of pungency are diverse and controversial. Differences in the oral anatomy, such as in the density of fungiform papillae, may influence sensitivity to chemical stimuli (Tepper & Nurse, 1998). The amount, flow rate, and composition of saliva (Dinnella et al., 2009; Heinzerling et al., 2011; Horne et al., 2002; Mosca & Chen, 2017), as well as polymorphisms in the TRPV1 ion channel (Park et al., 2007), may influence oral irritation. Furthermore, owing to cultural influences and personality characteristics, some individuals appreciate spicy food products, whereas others are averse to their consumption (Nilius & Appendino, 2011; Tepper et al., 2003). Several aspects may affect preferences for pungent food. According to Ludy and Mattes (2012), a liking for spiciness correlates with prior experience. Stevenson and Prescott (1994) demonstrated that repeated intake of capsaicin solutions resulted in a reduction in the perceived pungency intensity. Other studies reported that users regularly consuming spicy foods were less sensitive to capsaicin-induced pungency than nonusers (Lawless et al., 1985; Prescott & Stevenson, 1995; Stevenson & Prescott, 1994). Nevertheless, further studies have reported contradictory findings (Byrnes & Hayes, 2013; Nasrawi & Pangborn, 1989; Orellana-Escobedo et al., 2012; Schlossareck &

Ross, 2019). Moreover, personality characteristics (Byrnes & Hayes, 2013; Rozin & Schiller, 1980; Spinelli et al., 2018) and cultural background (Gutierreza & Simona, 2016; Rozin & Schiller, 1980) may be related to the variability in pungency perception. One of the most reported aspects the sensation-seeking behavior and herewith gender (Kish & Donnenwerth, 1972; Spinelli et al., 2018). Thrill-seekers tend to experience feelings of danger, and this behavior is positively correlated with the preference for spicy food products (Logue & Smith, 1986; Rozin & Schiller, 1980). Males tend to enjoy the pungency induced by capsaicin more than females (Byrnes & Hayes, 2015; Logue & Smith, 1986).

Pungency is an important attribute in the sensory description of various food products. Therefore, pungency-imparting irritant compounds are quality-determining ingredients in special food products. Consequently, understanding the mechanisms of pungency perception is essential in food science.

2.2 Quality-determining ingredients of mustard

2.2.1 Definition and characteristics of mustard

The use of mustard as a spice and therapeutic agent has been on record in Sumerian and Sanskrit writings since the epoch 3000 BC (Hemingway, 2003; Patterson, 2016). Seeds were crushed and mixed with the "must" of wine to produce a paste known as "mustum ardens" or "hot must." This could explain the origin of the term "mustard." (Hemingway, 2003)

In 2018, the global production of mustard seeds was 710,349 t, harvested on an area of 930,278 ha. The main producing countries (average production 1994–2018) are Canada and Nepal, with production amounts of 191,304 and 138,538 t, respectively. Germany is among the top 10 mustard seed-producing countries (average production 1994–2018) with a production amount of 8,697 t. (FAOSTAT, 2020)

The composition of mustard seed is displayed in Table 2.1.

Table 2.1 Composition of mustard seed (Adapted from Hemingway [2003])

Ingredient	Percentage of seed (dry weight)
Neutral oil	24-45 (*Sinapis alba* 25-30, *Brassica juncea* 35-45)
Polar lipids	6-12
Protein	20-30
Carbohydrates	12-18
Glycoside	1-3
Phytins	2-3
Water[1]	8-12

[1] Normal commercial mustard seed as traded on international basis

“Mustard” or “prepared mustard” food products are paste-like products made of mustard seed or mustard flour and liquid, such as water, vinegar, grape juice, and alcoholic beverages (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015). Mustard paste may contain additional ingredients such as spices, herbs, salt, sugar, and cereal flour. The seeds of *Brassicaceae* species *Brassica nigra*, *Brassica juncea*, and *Sinapis alba* may be used for mustard production (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015). Many types of mustard products are commercially available, and their possible use with/in other foods, such as meat products, mayonnaise, or salad sauce, is diverse. Along with classic mustard products, various pastes containing spices, such as paprika, ginger, and garlic, or herbs, including marjoram and thyme, are sold (Newerli-guz, 2014). Mustard products may be classified and labeled as “mild,” ”sweet,” “strong,” “hot,” or “pungent” (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015). The Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe (2015) has defined guidelines for several types of mustard products. “Dijon mustard” and “Düsseldorfer Senf” are both characterized by their patented production process and are solely produced with black (*B. nigra*) and/or brown (*B. juncea*) mustard seeds (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015; Düsseldorfer Senf - Geographisches-Herkunfts-Gewähr-Zeichen, RAL-RG 0140, 2015). Both mustards are characterized by an intense pungency sensation, especially in the nose (Stahl et al., 2009). Medium hot mustard, often referred to as “table mustard,” is produced from yellow (*S. alba*) mustard seeds, sometimes combined with small amounts of black or brown mustard seeds, and is characterized by a mild pungency. “Sweet mustard” consists of both brown or black and yellow mustard seeds. The “sweet” characteristic results from caramelized sugar. The mixture and grinding degree of mustard seeds and the variation of additional ingredients facilitate the production of a variety of mustard pastes (Stahl et al., 2009). The general composition of mustard pastes is displayed in Table 2.2.

Table 2.2 General composition of mustard pastes (Adapted from FrymaKoruma AG [2020])

Ingredient	Percentage by weight
Mustard seed	15-35
Salt	1-5
Acid (as 100% acetic acid)	1-5
Water	50-80

In Germany, 88,569 t mustard paste was produced in 2019, accounting for 11.1% of the total quantity of condiments and sauces produced in the same year (799,533 t); the consumption per head was 879 g (Kulinaria Deutschland e.V., 2019).

Aside from their usage as condiments, mustard seeds are used as ingredients in pickled foods and seasonings (Sindhu et al., 2012; Vaughan & Hemingway, 1959). More mustard-based food products are listed in Table 2.3.

Table 2.3 Use of mustard in food products

Product	Mustard Varieties and Processing	References
Condiment	*Sinapis alba*, *Brassica nigra*, and *Brassica juncea* are used as pungency-imparting condiment in food products. Whole, toasted and/or ground mustard seeds are used as ingredient in pickled foods, sauces, snacks, curries and other recipes.	Chavasit & Photi (2018); Cools & Terry (2018); Hemingway (2003); Mustakas, Kirk, & Griffin (1962); Sindhu et al. (2012); Vaughan & Hemingway (1959)
Sauce/Dressing	Mustard flour (*S. alba* and/or *B. juncea*) is used in sauces and dressings as a natural flavoring agent and to enhance stability for oil/water emulsions.	Patterson (2016)
Vegetable/Salad	Mustard leaves and stems of *B. juncea* are used as salad greens. The so-called potherb mustard (*B. juncea*) is used as a fermented vegetable in traditional Chinese cuisine.	Fang, Hu, Liu, Chen, & Ye (2008); Houbein (2008); Zhao, Tang, & Ding (2007)
Edible oil	*B. juncea* seeds are used for the production of edible oil for human consumption especially in India.	Hemingway (2003); Mustakas et al. (1962); Warwick (2011)

Several isothiocyanates and glucosinolates (GSLs), the precursors of isothiocyanates, contribute to the characteristic flavor of *Brassica* vegetables in general and mustard in particular (Possenti et al., 2017). Mustard is characterized by pungent and sharp aroma notes, as well as a lachrymatory effect that is caused primarily by isothiocyanates (G. S. Clark, 1992; Gilbert & Nursten, 1972). Most notably, two isothiocyanates including AITC derived from sinigrin and 4-HBITC derived from sinalbin, are responsible for mustard pungency and flavor (Figure 2.2) (Fenwick & Heaney, 1983; Ghawi et al., 2014).

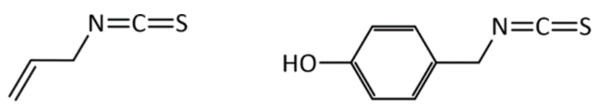

Figure 2.2 Allyl isothiocyanate (left) and 4-hydroxybenzyl isothiocyanate (right)

AITC has lachrymatory and pungent properties (Chin et al., 1996; Gilbert & Nursten, 1972; Sindhu et al., 2012; Vaughan & Hemingway, 1959). Additionally, sulfurous, garlic-like and mustard-like aroma notes are often perceived during AITC consumption (Chin et al., 1996; G. S. Clark, 1992; Gilbert & Nursten, 1972; Hanschen et al., 2014; Marcinkowska & Jeleń, 2020). 4-HBITC is responsible for the mustard odor and the intense burning sensation (Choubdar et al., 2010; Fahey et al., 2001; Ghawi et al., 2014), though the pungency of 4-HBITC has been described as mild in comparison to that of AITC (Choubdar et al., 2010; Vaughan & Hemingway, 1959). However, both substances are quality-determining ingredients of mustard.

2.2.2 Glucosinolates

GSLs are sulfur-rich non-nutritive bioactive phytochemicals that occur primarily in vegetables of the *Brassicaceae* family (Clarke, 2010; Fahey et al., 2001). In 2011, a total of 132 documented and natural GSLs were reported (Agerbirk & Olsen, 2012). Other authors have described additional GSL structures (Bellostas et al., 2008; R. N. Bennett et al., 2004; Fahey et al., 2001). The number of known GSLs is approximately 200 (Clarke, 2010), and structurally, they are characterized by a ß-D-thioglucose group, a sulfonated oxime group, and a variable side chain (Figure 2.3) (Fahey et al., 2001; Haller, Grune, & Rimbach, 2013). Based on structural similarities of their side chain, GSLs are classified as aliphatic, indole and aromatic (Fahey et al., 2001; Hanschen et al., 2014). These phytochemicals are very stable and hydrophilic owing to the large amount of polar groups in their structures (Fahey et al., 2001; Johnson et al., 2016).

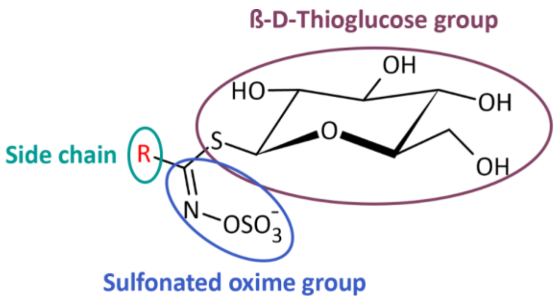

Figure 2.3 General structure of glucosinolates

In plants, GSL and some degradation products participate in plant defense mechanisms against herbivores and pathogens (Grubb & Abel, 2006; Lambrix et al., 2001; Tierens et al., 2001). Thus, the GSL concentration in different plant compartments (roots, leaves, stems, and seeds) varies (Brown et al., 2003; Clossais-Besnard & Larher, 1991; Fahey et al., 2001; Haller et al., 2013; Zrybko et al., 1997) and depends on the time of harvest and the environment (Cartea et al., 2008; Coogan et al., 2001; Farnham et al., 2004; Haller et al., 2013). Despite the fact that a great variety of GSLs has been described so far, each plant species contains a limited number, ranging from 2 to 12, of specific GSLs (Fahey et al., 2001; Zrybko et al., 1997). In mustard seeds, the main GSL in the varieties *B. nigra* and *B. juncea* is sinigrin and in the variety *S. alba* sinalbin (Hälvä et al., 1986; Velíšek et al., 1995; Zrybko et al., 1997). The sinigrin content of brown mustard seed is approximately 8.8±2.0 mg/g (*B. juncea*) (Zrybko et al., 1997) and of black mustard seed 16.6 to 61.2 mg/g (*B. juncea* and *B. nigra*) (Velíšek et al., 1995). The sinalbin content in yellow mustard seed (*S. alba)* ranges from 21.0 to 60.0 mg/g (Hälvä et al., 1986; Velíšek et al., 1995; Zrybko et al., 1997).

Commonly, GSLs in *Brassica* vegetables are associated with a bitter taste (Cartea et al., 2008; Engel et al., 2002; Fenwick et al., 1983). Several studies have focused on the reduction of bitterness through a breeding (Ishida et al., 2014; Z. Liu et al., 2012) or debittering process in food preparation (Fenwick et al., 1983; Sindhu et al., 2012). Further, GSLs have been shown to have health-promoting effects (Traka & Mithen, 2009). Hence, Hennig et al. (2014) presented an approach to improve GSL retention during food processing through specific breeding to enhance the nutritional quality of food products.

2.2.3 Isothiocyanates

Isothiocyanates are unstable, lipophilic, and electrophilic compounds originating from the enzymatic degradation of GSLs (Bones & Rossiter, 1996; Grob & Matile, 1979; Johnson et al., 2016). Cell rupture, in the presence of moisture, leads to the interaction of GSL and the enzyme myrosinase, a thioglucoside glucohydrolase (Björkman & Janson, 1972; Björkman & Lönnerdal, 1973). Under normal conditions, both are stored in different compartments of cells (Bones & Rossiter, 1996; Grob & Matile, 1979). Myrosinase hydrolyzes GSL into glucose and an unstable aglycone. Depending on hydrolysis conditions and the structure, the aglycone decomposes into a variety of products such as nitriles, thiocyanates, isothiocyanates, or indoles (Figure 2.4) (Hanschen et al., 2014; Van Eylen et al., 2006, 2008). Hydrolysis-influencing factors include pH, the presence of specific proteins, and metal ions (Bones & Rossiter, 2006; Hanschen et al., 2014).

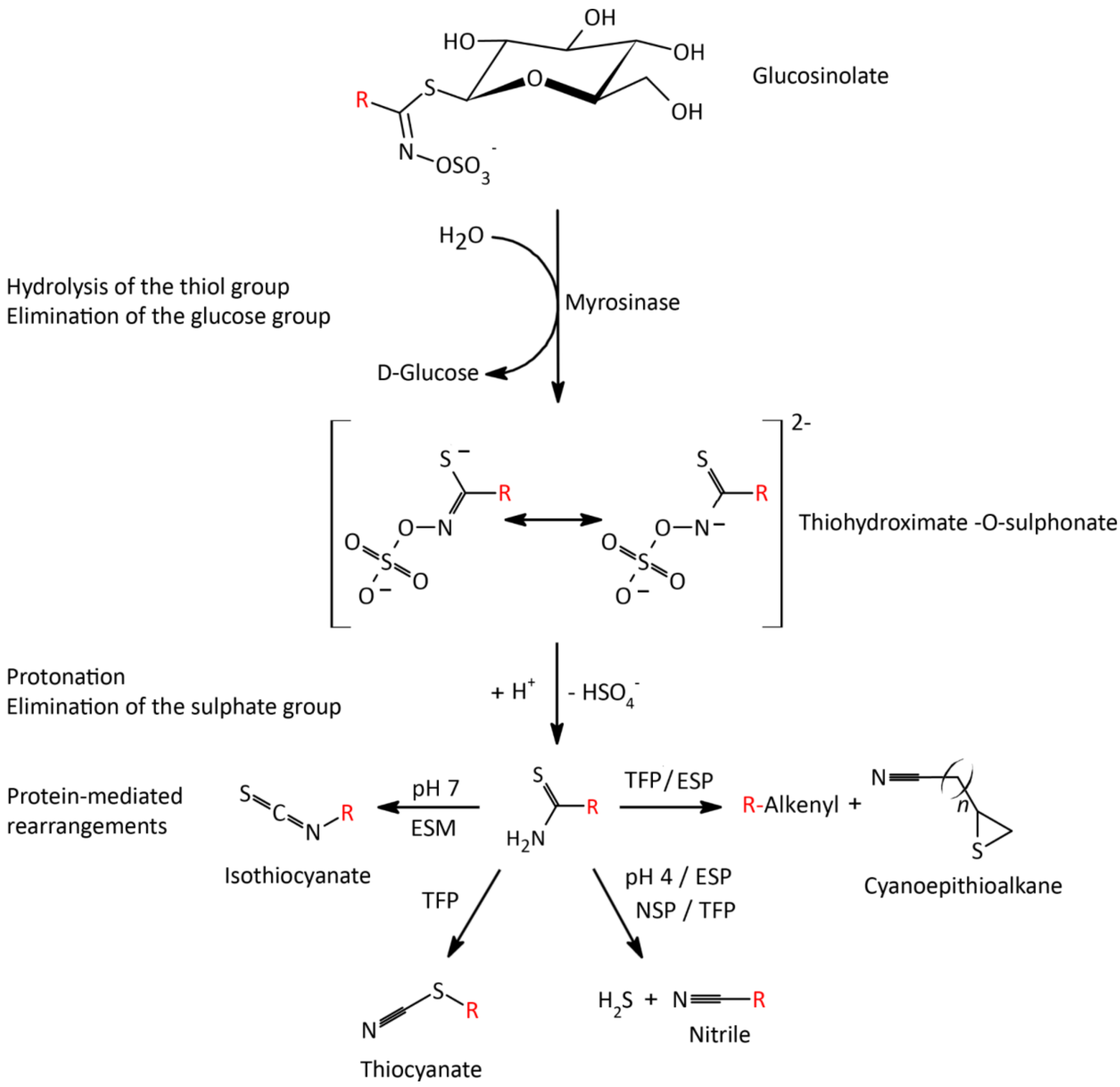

Figure 2.4 Enzymatic degradation of glucosinolates into glucosinolate breakdown products. ESM: epithiospecifier modifier protein; TFP: thiocyanate-forming protein; ESP: epithiospecifier protein; NSP: nitrile-specifier proteins (Adapted from Hanschen et al. [2014])

After the enzymatic degradation of sinalbin, the resulting 4-HBITC decomposes to 4-(hydrocymethyl) phenol owing to its instability (Bones & Rossiter, 2006; Borek & Morra, 2005; Ekanayake et al., 2012; Paunović et al., 2012), whereas AITC forms several different degradation products (diallyldithiocarbamate, diallyl tetrasulfide, diallyl pentasulfide, sulfur, and *N*,*N'*-1,3-diallylthiourea) under aqueous conditions (Figure 2.5) (Kawakishi & Namiki, 1969).

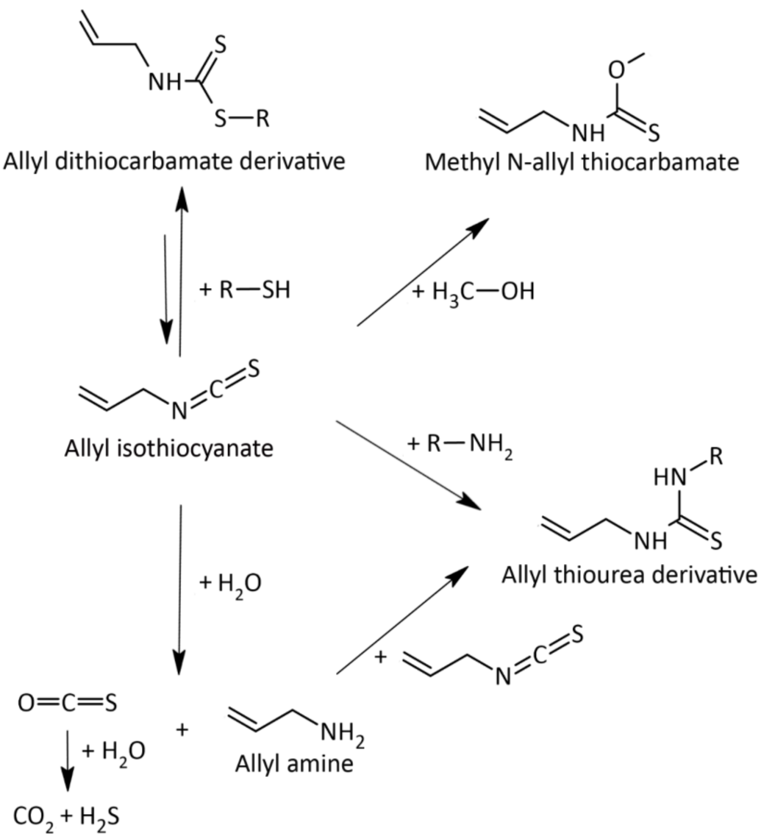

Figure 2.5 Allyl isothiocyanate reaction pathways under aqueous conditions (Adapted from Hanschen et al. [2012])

An increase in temperature (Figure 2.6) or pH increases the degradation of AITC (C. W. Chen & Ho, 1998; Hanschen et al., 2012). The AITC content in autolyzed mustard seeds ranges from 4 to 18 g/kg in *B. nigra* and 3.3 to 4.4 g/kg in *B. jucea.* In *S. alba* seeds, the 4-HBITC content varies between 15 and 38 g/kg when enzymatically cleaved from sinalbin (Shankaranarayaba et al., 1972). However, the molecular mass of AITC (99.15 g/mol) is lower than that of 4-HBITC (165.21 g/mol).

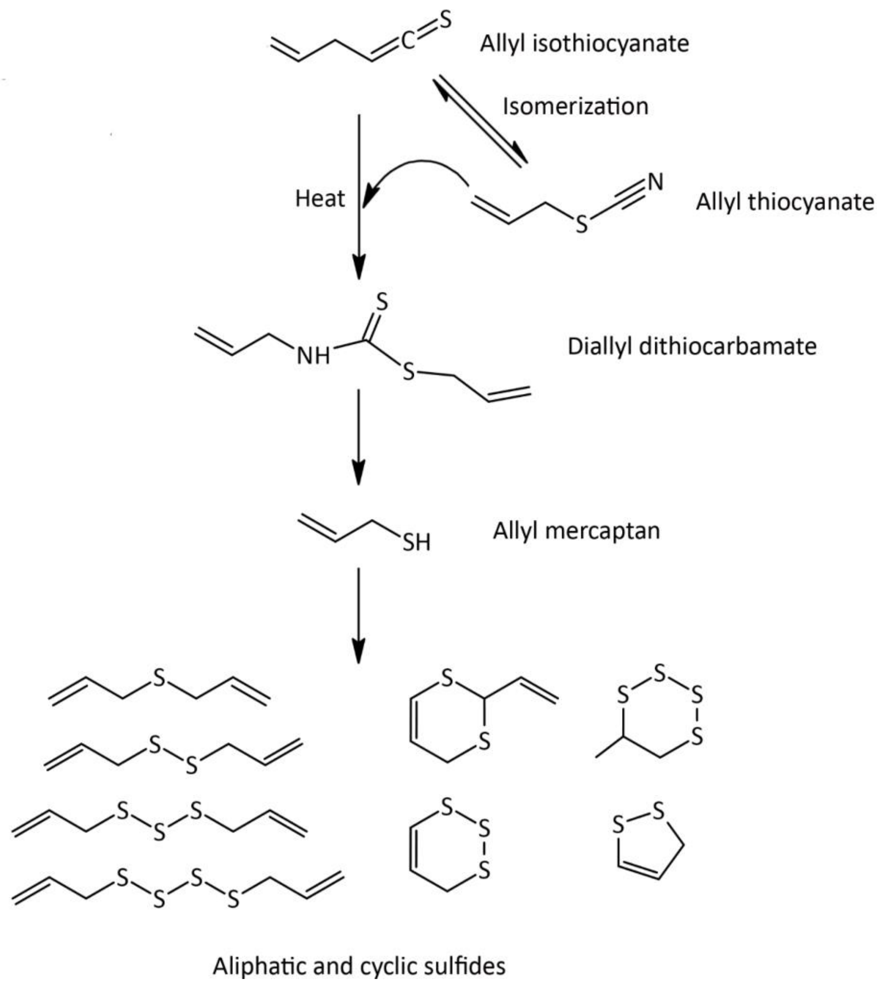

Figure 2.6 Thermal degradation of allyl isothiocyanate (Adapted from Weerawatanakorn et al. [2015])

2.3 Sensory analysis of quality-determining ingredients of mustard

In industrial processes, the sensory quality of mustard products is verified by sensory tests within the scope of quality assurance. One of the most important aspects is to review the pungency level of these products. The pungency classification is part of the product development process in which recipe, often historically grown, composition, especially the selection of mustard seeds and other ingredients, and the production process are defined. Both ingredients and production process influence sensory characteristics, especially the pungency level of the final product.

The sensory assessment of pungent products is limited to a few products per session (Busch-Stockfisch, 2020). Further limitations of the sensory tests result from the test procedure. Both sample properties and the testing method may lead to the fatigue of the panelists and a reduction in the sensitivity and differentiability (Hanrieder, 2012).

The effects of sensitization and desensitization influence the sensory evaluation of pungent products, and the nature of chemical stimulus influences the intensity of the trigeminal sensation. The chemical irritation induced by AITC may result in different responses to repetitive stimulation. This implies that the inter-stimulus interval, meaning the time between the intake of samples, influences the perception of AITC (Brand & Jacquot, 2002; Cain, 1990). Therefore, sensitization is defined as an increase in the rated intensity upon repeated stimulation in short intervals. Desensitization describes a decreased intensity rating upon repeated stimulation in long intervals. (Brand & Jacquot, 2002)

With regard to sensory tests with pungent products, sensitization means that in repeated tests of samples with the same AITC concentration, the second sample is rated as being more intense than the first. Another effect is that consumers who regularly consume pungent products rate spiciness as being less intense. The effects of desensitization could be responsible for this. In this context, Engel et al. (2006) reported the higher sensitivities of cauliflower non-consumers to AITC compared to those of medium and high consumers. Further, Brand and Jacquot (2002) reported that AITC induced sensitization upon repeated nasal stimulation at 30- or 90-s intervals and desensitization after a 3-min 30-s recreation period. Despite the fact that these observations have been described in several studies (Lawless et al., 1985; Prescott & Stevenson, 1995), others have reported contrary findings for capsaicinoid-containing samples (Nasrawi & Pangborn, 1989; Orellana-Escobedo et al., 2012; Schlossareck & Ross, 2019; Schneider et al., 2014b).

These reported effects are dependent on the concentration of the stimulus applied, the time frame between stimulations, and the frequency of application (Dowell et al., 2005). Furthermore, individual differences in pungency perception may affect sensitization and desensitization (Prescott, 1999; Smutzer & Devassy, 2016).

For industrial processes, having regard for temporal effects on the perception of chemical stimuli and testing only a small number of samples in one session are aspects that are difficult to realize. Thus, even tests conducted by a trained panel might be influenced by sensitization and desensitization effects. Repeated stimulation of the trigeminal receptors in short intervals might lead to inaccurate results. Therefore, recreation periods between samples should be defined and followed constantly in spite of economic and organizational conditions.

Most studies dealing with the sensory analysis of quality-determining ingredients of mustard have focused on the compounds sinigrin and AITC. Studies assessing the sensory quality of mustard products or using mustard pastes in methodical sensory studies are rare. Newerli-guz (2014) evaluated the sensory attributes of seven polish mustard products using paired comparison tests. Differences in taste between the mustard products were observed but not specified. Rousseau et al. (1999) exemplarily used Dijon mustard because of its fatiguing nature to assess consumers' discrimination abilities in the same-different testing method.

Further, in 1983, Fenwick et al. determined the detection threshold of sinigrin via a 3-fold assessment of several sinigrin concentrations and calculated the 50% detection level. The detection threshold was 10.6 mg/100 mL, which ranged from 8.1 to 13.0 mg/100 mL. Additionally, panelists associated sinigrin with bitterness. This sensory characteristic is common for GSLs (Drewnowski & Gomez-Carneros, 2000; Engel et al., 2002; Schonhof et al., 2004; Van Doorn et al., 1998; Wieczorek et al., 2018).

Because of the degradation of GSLs to isothiocyanates and other degradation products during the mustard production process (Frijters et al., 1981; Hanschen et al., 2014; Sindhu et al., 2012), their effect on the sensory quality of mustard pastes is low.

The sensory attributes of the quality-determining isothiocyanates, AITC and 4-HBITC in mustard have been described repeatedly in published literature (Frasnelli et al., 2011; Ghawi et al., 2014; Gilbert & Nursten, 1972). However, only a few studies have determined sensory parameters such as the detection threshold or focused on the temporal aspects of perception. In a study by Stone et al. (1967), the olfactory detection threshold of AITC in air was $2.7–24 \times 10^{-8}$ g/L, whereas a detection threshold of $0.09 \pm 0.22 \times 10^{-3}$ g/L was reported for AITC in aqueous solution (Marcinkowska & Jeleń, 2020).

AITC-induced sensations have been described as pungent, stinging, and sharp with lachrymatory properties (Chin et al., 1996; Frasnelli et al., 2011; Gilbert & Nursten, 1972). Additionally, yellow mustard seeds containing 4-HBITC have been described as pungent in taste and odor (Ghawi et al., 2014).

2.4 Chemical analysis of quality-determining ingredients of mustard

Several methods for the analysis and quantification of the quality-determining ingredients of *Brassica* vegetables have been published. These studies generally focused on several GSLs and isothiocyanates, while a few particularly focused on sinigrin and AITC. Among these methods are gas chromatography (GC), reversed-phase high performance liquid chromatography (HPLC) and HPLC tandem mass spectrometry (MS/MS). Buttery et al. (1976) characterized volatile oils in the *Brassica* vegetables cauliflower (*Brassica oleracea L.,* Botrytis group), cabbage (*B. oleracea L.*, Capitata group) and broccoli (*B. oleracea L.*, Italica group). The compounds characterized were sulfides, aromatic compounds, and isothiocyanates such as AITC and 2-phenylethyl isothiocyanate (2-PEITC). Characterization was performed using capillary gas chromatography and mass spectrometry (GC-MS). Additionally, Paunović et al. (2012) used solvent extraction and the GC-MS technique to compare sinalbin degradation products in yellow mustard seeds and mild yellow mustard paste. In mustard seeds and paste, 2-(4-hydroxyphenyl) acetonitrile and 4-(hydroxymethyl) phenol were the main sinalbin-degradation products, respectively. Despite the fact that sinalbin is degraded into pungency-inducing 4-HBITC by enzymatic hydrolysis, this isothiocyanate was not directly detected. AITC, benzyl isothiocyanate (BITC) and 2-PEITC have been quantified using GC-MS in sweet, medium hot, and hot mustard products with a simple sample preparation method, achieving tolerable selectivity and sensitivity (Paunović et al., 2012; Stahl et al., 2009).

Another method used for GSL and isothiocyanate analysis is the HPLC method. The HPLC approach developed by Pelosi et al. (2014) was used to quantify AITC in commercial mustard solutions. Further, Herzallah and Holley (2012) focused on the determination of sinigrin, sinalbin, AITC, and BITC in mustard powder extracts in which the analysis procedure varied for GSL and isothiocyanates. A direct and simultaneous HPLC quantification method for sinigrin and AITC in mustard seeds, bran and ground has been implemented by Tsao et al. (2002).

2.5 Correlation of sensory studies and chemical-analytically or physically determined parameters

The correlation of chemically or physically determined parameters, such as the content of aroma components and sensory evaluations in a human panel, can provide valuable insight into the perception of examined food products. Therefore, the amount of necessary sensory evaluations in industrial production processes may be reduced and partially replaced by instrumental measurements predicting sensory properties. Additionally, these methods are cost-saving, fast, reproducible, and an objective tool for food quality control. (Chen, 2014)

In general, food products induce a complex sensation upon consumption, and each individual's ability to distinguish between multiple attributes is different (Prescott & Monteleone, 2014). Therefore, correlations have to be interpreted with care as not all relevant flavors and tastes, as well as synergetic effects, might have been included (Bell et al., 2018). Furthermore, sensory testing with pure isolates is necessary to analyze their contribution to the overall sensory impression (Bell et al., 2018).

For instance, regarding mustard products with chili, it is not possible to distinguish the magnitude of the pungency sensation resulting from isothiocyanates or capsaicinoids individually (Simons et al., 2003). Using chemical–analytical methods, the amounts of isothiocyanates and capsaicinoids could be quantified; however, the possible additive sensory effects of these compounds remain unidentified.

This approach has been applied by many researchers for several classes of food products (Chen, 2014). For example, Crowther et al. (2005) correlated the pyruvate concentrations of onions with the sensory assessment of a taste panel to verify established flavor classifications (sweet, mild, and strong) of onions and concluded that pyruvate quantification is suitable as a quality control method for onions.

In 2018, Bell et al. reviewed studies relating the sensory and instrumental evaluations of GSLs and isothiocyanates and indicated a lack of studies correlating the sensory attributes with GSL hydrolysis products such as isothiocyanates. Several studies have focused on the quantification of GSLs and their correlation with the bitterness of *Brassica* vegetables. Groenbaek et al. (2019) evaluated the influence of cultivar, life cycle, and seasonal effects on the GSL content and bitterness of baby leaf rapeseed (*Brassica napus* var. *oleifera*). The sensory profile and bitterness intensity were affected by the amount of bitter-tasting GSL, which varied owing to seasonal effects and throughout the product life cycle (Groenbaek et al., 2019). Fenwick et al. (1983) reported that the sinigrin concentrations of fresh Brussels sprouts (*B. oleracea L.* var. *gernrnifera*) could be positively correlated with the sensory-determined bitterness of the cooked sprouts rated on a 6-level intensity scale. The combined effect of several GSLs enhanced the correlation between their concentration and bitterness (Fenwick et al., 1983).

Other studies have related the sensory attribute pungency to the content of several chemical stimuli using correlation analysis. Sugai et al. (2014) reported that the content of α-sanshool compounds affected the sensory-determined degree of pungency in Japanese pepper (*Zanthoxylum piperitum DC.*). Schneider et al., (2014a) also quantified capsaicinoids in commercially available salsa products and related the results with the parameters of the temporal assessment of the products. Their results indicated that the capsaicinoid-induced pungency of salsas might be predicted using instrumental analysis.

Further, in 2019, Schlossareck and Ross used a new device, the electronic tongue, to evaluate the sensory properties of spicy paneer cheese. This potentiometric method is based on sensors with different selectivities for ions and compounds related to the perception of the five basic tastes, spicy, and metallic. The electronic tongue showed a high ability to distinguish between paneer samples along the whole capsaicin concentration range tested; however, panelists were not able to distinguish the samples at the lowest and highest concentrations. The comparison of instrumental and human sensory evaluations illustrated that the electronic tongue may be used as a rapid and reproducible tool for pungency evaluation in addition to human sensory testing. (Schlossareck & Ross, 2019)

2.6 Related studies on glucosinolates and isothiocyanates

Many recently published studies have focused on GSLs and isothiocyanates, addressing the potential characteristics of these substances. This shows the current relevance of the work presented here. To provide insight into the possible applications of GSLs and isothiocyanates, some studies are referenced below.

One main research focus is the possible health-promoting effect of GSLs and isothiocyanates, respectively. Because of the volatile character of isothiocyanates, their reported health benefits depend, among others, on the amount of *Brassica* vegetable intake, GSL and isothiocyanate types and concentrations, preparation process, as well as individual metabolism (Ramirez et al., 2020). In particular, the application of isothiocyanates for the chemoprevention of cancer has been reviewed by many authors (Gründemann & Huber, 2018; Ramirez et al., 2020; Stahl et al., 2009). Additionally, studies related to the prevention of diabetes (G.-C. Chen et al., 2018; Jia et al., 2016; Patel et al., 2018) and cardiovascular diseases (Citi et al., 2020; Martelli et al., 2014; Testai et al., 2016) reported the beneficial properties of cruciferous vegetables and isothiocyanates, respectively. However, other studies evaluating health benefits correlated with GSL or isothiocyanate intake reported contrary findings.

Therefore, the use of *Brassica* vegetables in manufactured foods to improve their nutritional quality, as well as in nutraceuticals as ingredients, has been reported to be favorable (Ramirez et al., 2020). For example, Carvalho et al., (2018) developed a functional milk chocolate enriched with kale (*B. olereacea* var. *acephala*) to enhance the content of dietary fibers and minerals. The addition of kale altered the sensory properties of the milk chocolate; however, no change in purchase intention of the panelists was observed. Additionally, Ghawi et al. (2014) added mustard seeds (*S. alba*) to cooked broccoli (*B. oleracea* var. *italica*) to increase bioactivity, expecting that the seeds would provide the active enzyme myrosinase to ensure the hydrolysis of the contained GSLs. The majority of consumers disliked the resulting change in the sensory attributes. Further, García-Saldaña et al. (2016) published the development of an isothiocyanate (sulforaphane, from broccoli seed extracts)-containing nutraceutical. They succeeded in producing a fine powder, sulforaphane in a gelatin/pectin complex using microencapsulation technology.

Because of their antimicrobial and antifungal properties, AITC, mustard powder, or mustard seeds may well be used in food packaging or for the preservation of the food product itself. Bahmid et al. (2020) developed an active microbial packaging in which AITC is released from ground mustard seeds into the headspace of the packaging. Therefore, their research focused on the mechanisms of AITC release from ground mustard seeds of differing grinding size and fat content, the partitioning between headspace and mustard seeds, and hydrolysis from sinigrin. To achieve a preserving effect, the AITC concentration in the headspace is relevant and limited by the sinigrin concentration of the mustard seeds. (Bahmid et al., 2020) Other researchers used AITC vapor for food preservation either by applying AITC vapor as modified atmosphere packaging or by adding a sachet releasing AITC continuously into the headspace of the packaging (Gao et al., 2018; Isshiki et al., 1992; Lopes et al., 2018; Otoni et al., 2014).

Furthermore, several authors have focused on food product development using AITC (Olaimat & Holley, 2016), mustard flour (Li, 2012; Milani, 2013; Torrijos et al., 2019), mustard extract (Olaimat & Holley, 2016), and other *Brassica* species-based supplements (Biegańska-Marecik et al., 2017; Shen et al., 2018) in the recipe to inhibit microbial activity. For example, Torrijos et al. (2019) used the AITC approach in the headspace of food packaging to avoid fungal contamination of pita bread. In their study, the development of a bioactive sauce with mustard flour was presented. By the packaging of both pita bread and mustard sauce in an active packaging system, a shelf-life improvement of 3 days for the bread was achieved because AITC volatilized from the sauce in the headspace of the packaging system. Another example is the use of an AITC or mustard extract-based food product coating to inhibit bacterial growth (here: *Listeria monocytogenes* and aerobic bacteria) on refrigerated, vacuum-packed, and cooked cured chicken slices (Olaimat & Holley, 2016).

The application of synthetic additives in food products to preserve food quality by inhibiting microbial and fungal spoilage is widespread (Tajkarimi et al., 2010). In recent years, the demand for additives from natural sources has gained importance (Bhavaniramya et al., 2019; Davidson & Harrison, 2002). These studies demonstrated that AITC, ground mustard, or mustard flour may function as a natural preserving agent.

The amount of AITC used is crucial to ensure antimicrobial and antifungal effects. Concurrently, the AITC concentration is the decisive factor to be considered so as not alter the sensory properties of packed food products (Isshiki et al., 1992; Lopes et al., 2018).

Independent of the application of GSLs and isothiocyanates in general or mustard seeds, ground mustard, or AITC, in particular, in nutraceuticals or food packaging, sensory product characteristics need to be considered and tested. As already pointed out by others, products with bitter or pungent sensory attributes may lead to product rejection and diminution of the purchase intention (Drewnowski & Gomez-Carneros, 2000; Engel et al., 2002; Ghawi et al., 2014; Verkerk et al., 2009).

3 Aim of this work and hypotheses

This work aimed to classify mustard products into pungency categories based on the sensory and quantitative chemical analysis of quality-determining, particularly pungency-determining, ingredients.

The determination of sensory detection thresholds of sinigrin in water-based and AITC in water- and oil-based carrier matrices is expected to provide information about concentration ranges for subsequent sensory studies. Additionally, information about differences in the perception of AITC in water- and oil-based matrices will be gained. The threshold study intends to determine whether AITC and its precursor sinigrin are perceived in different concentration ranges and if sinigrin and AITC both induce pungency sensations.

Within the framework of the sensory studies, the temporal perception and localization of AITC-induced pungency in water-, oil-, and mustard-based carrier matrices will be tested with a broad AITC concentration range and compared with the results of commercially available mustard products to review transferability.

Furthermore, an AITC quantification method (HPLC) will be adapted, and the AITC content of commercially produced products will be measured. A correlation analysis will be used to assess whether the sensory-determined perception parameters and AITC content of the products are related and if the developed regression model can be used to predict the induced pungency intensity via quantification of the AITC content.

By the combination of human sensory and instrumental measurements, mathematical models can be deduced. In this case, the model aims to predict the pungency level of AITC-containing pastes, especially mustard products. The establishment of mathematical models may reduce the amount of necessary human sensory tests in industrial processes. The use of instrumental analyses is time-saving and generates reliable, objective, and reproducible results.

Nevertheless, instrumental analyses cannot completely replace the necessity of human sensory tests.

To achieve these objectives, hypotheses and the associated experimental approaches are proposed hereinafter.

1st Hypothesis

The sensory detection thresholds of AITC and sinigrin differ significantly (α = 5%) in the threshold concentration level.

Experimental approach:
The detection thresholds of sinigrin in water- and AITC in water- and oil-based carrier matrices are determined using the 3-Alternative-Forced-Choice (3-AFC) test according to ASTM E-679-04 and ISO 13301. Differences in the thresholds are then tested for significance by performing analysis of variance (ANOVA).

The first hypothesis is reviewed in Chapter 4.

2nd Hypothesis

The sensory detection thresholds of AITC of mustard users and nonusers differ significantly (α = 5%).

Experimental approach:
The sensory panel is divided into mustard users and nonusers according to their individual mustard consumption habits. Therefore, the preferences for mustard products in particular and spicy foods in general, as well as the consumption behavior of panelists, are assessed using a questionnaire adapted from Reinbach et al. (2007). Sensory detection thresholds of AITC in water- and oil-based carrier matrices are then compared between user groups.

The second hypothesis is reviewed in Chapter 4.

3rd Hypothesis

AITC is perceived less pungent in oil- than in water-based matrices because of its lipophilic character.

Experimental approach:
AITC detection thresholds are determined in water- and oil-based carrier matrices using the 3-AFC method (ASTM International, 2011; ISO 13301, 2018). Significance of differences in both thresholds are then evaluated using ANOVA. Furthermore, a sensory time-intensity (TI) study according to DIN 10970 is conducted to evaluate the perception of AITC pungency over a defined period of time in water- and oil-based carrier matrices. The TI study is expected to deliver temporal information about AITC-induced pungency sensation, and additional parameters such as maximum perceived intensity (I_{max}), duration of decreasing phase (DUR_{Dec}), and area under the curve (AUC) are calculated using the sensory software FIZZ.

The third hypothesis is evaluated in Chapters 4 and 5.

4th Hypothesis

Sensory pungency evaluation, involving parameters such as maximum perceived pungency intensity (I_{max}), area under the curve (AUC), and duration of decreasing phase (DUR_{Dec}), depends on both the AITC concentration and the composition of the carrier matrix.

Experimental approach:
In a sensory TI study, various concentrations of AITC in water-, oil-, and mustard-based carrier matrices are tested by a trained (according to Peyvieux & Dijksterhuis, 2001; Schneider et al., 2014a) panel. Curve parameters such as I_{max}, DUR_{Dec}, and *AUC* are then extracted from the generated data, reviewed, and tested for significant differences.

The fourth hypothesis is reviewed in Chapter 5.

5th Hypothesis

The values of the sensory TI parameters of commercial mustard products do not differ significantly (α = 5%) from those of the mustard-based carrier matrix with a comparable AITC concentration.

Experimental approach:
Sensory TI studies with samples based on water-, oil and mustard model carriers and with commercially produced mustard products are carried out. To represent a broad concentration range of AITC, several AITC concentrations are adjusted in a model carrier matrix. A production process, on a laboratory scale, would be developed to prepare a mustard carrier matrix that does not induce a pungency sensation upon consumption. The AITC concentrations of commercial mustard products are subsequently determined via HPLC analysis. The sensory parameters of the commercial and model products are reviewed for transferability.

The fifth hypothesis is evaluated in Chapters 5 and 6 and discussed in Chapter 7.1.

6th Hypothesis

Localization of the AITC pungency perception in the mouth, throat and nose depends on the AITC concentration.

Experimental approach:
In all sensory tests, panelists are asked to localize the pungency sensation after consumption. Therefore, a questionnaire would be completed for each sample localizing a pungency sensation in the mouth (palate, tongue, and lips), throat and nose.

The sixth hypothesis is evaluated in Chapters 4, 5, and 6.

7th Hypothesis

The sensory pungency evaluation (TI parameter) of mustard products can be correlated with the quantified AITC content of these products.

Experimental approach:

Sensory parameters such as I_{max}, DUR_{Dec}, and *AUC,* are determined in a TI study for various commercially available mustard products. The AITC concentrations of these products are determined via HPLC analysis. Therefore, an HPLC method for the quantification of AITC would be developed, validated, and implemented. Subsequently, the correlation between TI parameters and the AITC concentration will be reviewed using regression analysis.

The seventh hypothesis is reviewed in Chapter 6 and discussed in Chapter 7.1.

4 Determination of sensory thresholds for sinigrin in water-based matrices and allyl isothiocyanate in water- and oil-based matrices

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Abstract

In the present study, sensory detection thresholds for sinigrin in a water-based matrix and allyl isothiocyanate (AITC) in water- and oil-based matrices were determined using a 3-alternative forced choice test with ascending concentrations. The study consisted of a prestudy with 10 assessors (3 mustard "users"; 7 mustard "nonusers") and a main study with 64 assessors (10 mustard "users"; 54 mustard "nonusers"). In addition, panelists were asked to locate (throat, tongue, throat and tongue) the predominant sensation (sinigrin: bitterness; AITC: pungency). The group threshold for sinigrin in the water-based matrix in the prestudy was 10.928 mg/100 mL. Moreover, the group thresholds for AITC in the water-based matrix in the prestudy and main study were 0.135 mg/100 mL and 0.121 mg/100 mL, respectively. The group thresholds for AITC in the oil-based matrix in the prestudy and main studies were 0.497 and 0.582 mg/100 mL, respectively. However, mustard "users" and mustard "nonusers" showed no significant differences in thresholds. Sinigrin and AITC were both prevalently perceived on the tongue.

Practical applications

Threshold determination for the primary pungent substance allyl isothiocyanate (AITC), and the corresponding precursor sinigrin in cruciferous plants such as mustard, horseradish and wasabi is important for product development and quality assurance, especially for new products. These parameters will help to estimate whether pungency is a relevant characteristic of the product. In particular, differentiation of the matrix (water- or oil-based matrix) helps to evaluate whether the particular AITC concentration is noticeable in food products depending on the food composition. Further studies should be conducted to determine whether AITC is perceived at the same levels in different food systems. Sinigrin causes the unpleasant bitterness of brassica vegetables. Nevertheless, sinigrin is a desirable substance in food products owing to its potential health-promoting effects. Therefore, knowledge about the detection threshold of sinigrin will facilitate the breeding of sinigrin-containing products without unpleasant bitterness and may increase consumer acceptance.

4.1 Introduction

Spicy foods are part of diverse food cultures around the globe, and their popularity in western cuisine is increasing (Orellana-Escobedo et al., 2012). The most common pungent substance is capsaicin, which is known from hot pepper (Spence, 2018, 2019). The perception of capsaicin has been well investigated in sensory studies. Another class of chemical irritants are isothiocyanates (ITCs), which occur in cruciferous plants. ITCs have unique properties causing a hot sensation, a burning taste and pungent odor (Ortner & Granvogl, 2018).

Mustard (Velíšek et al., 1995), horseradish (Kroener & Buettner, 2017), broccoli (Saha et al., 2012), and wasabi (Sultana, Savage, et al., 2003; Terada et al., 2015) are some of the condiments and vegetables containing glucosinolates. Glucosinolates are the precursors of ITCs. Cell rupture leads to the release of endogenous myrosinase (thioglucoside glucohydrolase), which hydrolyses glucosinolates mainly to ITCs (Oliviero et al., 2018). Allyl isothiocyanate (AITC), derived from the glucosinolate sinigrin (MacLeod & MacLeod, 1970), is associated with lachrymatory and pungent properties (Gilbert & Nursten, 1972). Chin et al., 1996 described AITC as presenting a characteristic sharp aroma with black-mustard-like notes. Glucosinolates in general (Cartea et al., 2008), and sinigrin, in particular, are associated with bitterness (Beck et al., 2014; Bell et al., 2018; Fenwick et al., 1983; Van Doorn et al., 1998). Sinigrin, the precursor of AITC, is present in cabbage tissue (Beck et al., 2014; Chin et al., 1996), kale (Cartea et al., 2008), horseradish (Gilbert & Nursten, 1972), wasabi (Kumagai et al., 1994; Sultana, Savage, et al., 2003), and black (Brassica nigra) and brown (Brassica juncea) mustard seeds (Herzallah & Holley, 2012; Popova & Morra, 2014; Velíšek et al., 1995).

In 1912, the sense of irritation caused by chemical stimuli was described as differing from taste and olfaction by Parker (1912) using the term "common chemical sense". These chemesthetic qualities are initiated by chemical irritants stimulating the trigeminal nerves (Green, 1996). The irritation of trigeminal nerves in oral and nasal cavities leads to a multitude of sensations such as burning, tingling, heat and coolness, depending on the chemical stimulus (Alimohammadi & Silver, 2002).

Many studies on pungency thresholds have been conducted in recent decades. Most of these studies have examined the pungency of capsaicinoids (Lawless et al., 2000; Orellana-Escobedo et al., 2012; Schneider et al., 2014b). Schneider et al. (2014b) used a 3-alternative forced choice (3-AFC) sensory test with ascending concentrations to evaluate the chemical senses thresholds of capsaicin and dihydrocapsaicin. In 2000, Lawless et al. used a 3-AFC test to compare capsaicin thresholds in different matrices (oil and water).

Nevertheless, only limited data on the chemical sense thresholds of sinigrin and AITC have been published. Engel et al. (2006) designed a rapid test for estimating individual thresholds and reported that nonconsumers of cauliflower present lower olfactory thresholds for AITC and lower gustatory thresholds for sinigrin in water than intermediate and high consumers. Fenwick et al. (1983) reported sinigrin in aqueous solution to be bitter with a detection threshold of 10.6 mg/100 mL.

The aim of the present study was to determine the chemical sense thresholds of sinigrin in water-based matrices and AITC in water- and oil-based matrices in a 3-AFC sensory test with ascending concentrations. As the results of our prestudy with sinigrin in water-based matrices (10.9 mg/100 mL) were consistent with those of Fenwick et al. (1983) (10.6 mg/100 mL), no further tests with sinigrin were conducted. Moreover, the influence of mustard consumption habits on threshold levels was evaluated. In addition, the study aimed to localize the main sensation of either sinigrin (bitter) or AITC (pungent) in the oral cavity and throat during threshold determination.

4.2 Materials and methods

4.2.1 Subjects

Ten subjects (n = 10, age range 24–37) participated in the prestudy. Sixty-four subjects (n = 64, age range 19–38) participated in the main study. They were all members of the Department of Food Technology at the University of Applied Sciences Fulda. Participant selection was based on willingness, availability and basic knowledge of sensory methods. The assessors were compensated for their participation with sweets.

Panelists were asked to provide information on their consumption habits and their preference for spicy food in general as well as mustard products in particular. Therefore, a questionnaire modified from Reinbach et al. (2007) was completed during the first test session.

Based on the questionnaire, the assessors were grouped into mustard “users” and mustard “nonusers,” depending on their preferred pungency intensity (11-point scale: 0 = not pungent to 10 = extremely pungent) and frequency of mustard consumption (7-point scale: more than once a day, daily, two or three times a week, once a week, once a month, less than once a month, once a year or less), as adapted from Schneider et al. (2014b). Pungency was defined as biting, tingling and sharp aroma in the nose, oral cavity, and on the tongue and palate, as well as a lachrymatory effect. According to Reinbach et al. (2007) pungency intensities were rated using panelists’ former experiences with pungent products. Panelists who preferred pungency intensities of five or higher and consumed mustard products at least once a week were categorized as “mustard users”, panelists who preferred pungency intensities of less than five and consumed mustard products less than once a week, and those who preferred pungency intensities of five and higher but who consumed mustard products less than once a week were categorized as mustard “nonusers”. In the prestudy, three assessors were categorized as mustard “users”, and seven assessors were categorized as mustard “nonusers”. In the main study, 10 assessors were categorized as mustard “users” and 54 assessors as mustard “nonusers”.

The study was performed in accordance with the Declaration of Helsinki, and the procedure was approved by the University of Applied Sciences Fulda Ethics Review Board. The informed consent of all participants was obtained, both in writing and verbally.

4.2.2 Stimulus/sample preparation

The main stimuli used in this study were sinigrin (PhytoLab, ≥95% purity, potassium salt), as the main glucosinolate, and AITC (Sigma Aldrich, Munich, Germany ≥95% purity), as the main irritant ITC.

A singrin stock solution (320 mg/100 mL, 3200 ppm) was prepared by mixing 320 mg of sinigrin with 100 mL of deionized water. Sinigrin test samples were prepared by diluting (dilution factor of two) the stock solution with deionized water to concentrations of 1, 2, 4, 8 and 16 mg sinigrin/100 mL.

AITC is not hydrophilic; thus, polysorbate 80 (Carl Roth GmbH, Karlsruhe, Germany) was used as a food-grade emulsifier to dissolve AITC in water. AITC stock solutions (500 mg/100 mL, 5000 ppm) were prepared by mixing 500 mg AITC with either 100 mL safflower oil or deionized water containing polysorbate 80 (180 mg/100 mL, 1800 ppm). The water-based AITC mixtures were homogenized using a T 25 digital Ultra Turrax® homogenizer (IKA®-Werke GmbH & Co. KG, Staufen, Germany) until the AITC was completely dissolved.

Test samples were prepared by diluting the stock solutions with safflower oil or deionized water. In the prestudy involving AITC samples in oil-based matrices, a dilution factor of three was used, and for AITC samples in water-based matrices, a dilution factor of two was chosen. The prestudy was conducted with oil-based AITC samples at concentrations of 0.04, 0.12, 0.36 and 1.08 mg AITC/100 mL (factor of three) and water-based AITC samples at concentrations of 0.01, 0.02, 0.04, 0.08, 0.16, 0.32 mg AITC/100 mL (factor of two). The sample concentrations were chosen based on experience from preliminary tests. For the main study, the number of tested concentrations was reduced to three: 0.12, 0.36, and 1.08 mg AITC/100 mL (factor of three) for oil-based matrices and 0.02, 0.08, and 0.32 mg AITC/100 mL (factor of four) for water-based matrices.

For the tests with AITC-containing samples, the reference samples were adjusted to the identical polysorbate 80 concentration to ensure comparability. A polysorbate 80 stock solution (6 g/L, 6000 ppm) was prepared by mixing 6 g of polysorbate 80 and 1000 mL of deionized water. The polysorbate 80 stock solution was placed in a Transsonic T 700/H ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) at room temperature until the polysorbate 80 was completely solved. A dilution series of polysorbate 80 concentrations was prepared for the reference samples according to the polysorbate 80 concentration of the corresponding test samples.

4.2.3 Sensory tests

Within this study, the threshold values of sinigrin in water-based matrices and AITC in water-based and safflower oil-based matrices were evaluated using the 3-AFC test according to the provided guidelines (ASTM International, 2011; ISO 13301, 2018).

Prior to the assessment of threshold values, all panelists were trained in the 3-AFC method using sugar solutions.

The study was separated into a prestudy and a main study. The prestudy focused on the threshold value determination of sinigrin in water-based matrices and AITC in water- and oil-based matrices by a small panel consisting of ten assessors. The prestudy was conducted to identify threshold differences of sinigrin and the hydrolysis product AITC. Furthermore, the prestudy aimed to optimize the concentration range used. Based on the results of the prestudy, the number of test samples was reduced in the main study to minimize exertion of panelists. The main study intended to identify difference in AITC perception in water- and oil-based matrices by a larger panel. The threshold values of AITC in water- and oil-based matrices were evaluated by a panel consisting of 64 subjects.

All tests were performed in a sensory lab conform to DIN EN ISO 8589 (2010). Test booths were lighted with fluorescent lights equivalent to daytime illumination. The booth room temperature was set to 21 °C. The samples were presented at room temperature in laboratory glasses (5 mL) marked with a random three-digit code. For each concentration, three samples, one test sample and two reference samples, were presented in a row. The rows were presented in an order with ascending concentrations of the test sample. Three presentation orders (AAB, ABA, BAA) were randomly distributed across all samples handed to the panelists to avoid positional bias. The assessors were instructed to test from left to right, taking the sample in their mouth and swirling it before swallowing it.

According to the forced-choice principle, the panelists were asked to identify one sample as differing even if they could not clearly detect the deviating sample. For each row, they were asked to identify the location of the sensation (throat, tongue, throat, and tongue) if discernable. Preliminary tests showed that a nasal sensation was only elicited at higher concentrations than those used in the threshold determinations. Therefore, no reference to nasal perception was made during the prestudy or the main study. The panelists had to neutralize their mouths between samples using deionized water and Matzen Bread (Aerzener Brot und Kuchen GmbH, Aerzen, Germany). The test samples were split into several test sessions on different days to prevent sensitization or desensitization and carry-over effects. Each session consisted of a single threshold determination of either sinigrin in water-based matrices or AITC in water- or oil-based matrices.

4.2.4 Data analysis/statistical analysis

Individual best-estimate thresholds (BETs) were calculated as the geometric mean of the last missed concentration and the next higher concentration at which a correct response was given. If the panelist failed to identify the test sample at the highest presented concentration, the hypothetically next higher concentration was used for the individual BET calculations. If the panelist identified the correct test sample from the first presented concentration, the hypothetically next lower concentration was used for individual BET calculation. Group BETs were calculated as the geometric mean of the individual BETs of the considered panelists for each defined group (ASTM International, 2011; ISO 13301, 2018).

The statistical significance of the differences (α = 5%) was evaluated with two-way (product/matrix and panelist) analysis of variance (ANOVA). For the prestudy results, ANOVA was performed to evaluate the differences in the thresholds for sinigrin in water-based matrices and AITC in water- and oil-based matrices. For the main study results, two-way ANOVA was performed to evaluate the differences in the thresholds for AITC in water-based and oil-based matrices. The thresholds values of the prestudy and main study were verified to be at the same level. The differences in the thresholds for mustard “nonusers” and “users” were evaluated using one-way ANOVA. Because of the unequal sample sizes, homogeneity of variances was verified using Leven’s test.

The localization of the sensations was determined by counting the number of answers of all panelists who correctly identified the test sample.

4.3 Results

The group thresholds of the prestudy and main study are illustrated in Fig. 4.1. For sinigrin in the water-based matrix, the group BET was 10.928 (standard deviation of $\log_{10}$ BET ± 0.384) mg/100 mL. The group BETs for AITC in the water-based matrix in the prestudy and main study were 0.135 (standard deviation of $\log_{10}$ BET ± 0.617) mg/100 mL and 0.121 (standard deviation $\log_{10}$ BET ± 0.627) mg/100 mL, respectively. The group BETs for AITC in the oil-based matrix in the prestudy and main study were 0.497 (standard deviation of $\log_{10}$ BET± 0.599) mg/100 mL and 0.582 (standard deviation of $\log_{10}$ BET ± 0.509) mg/100 mL, respectively.

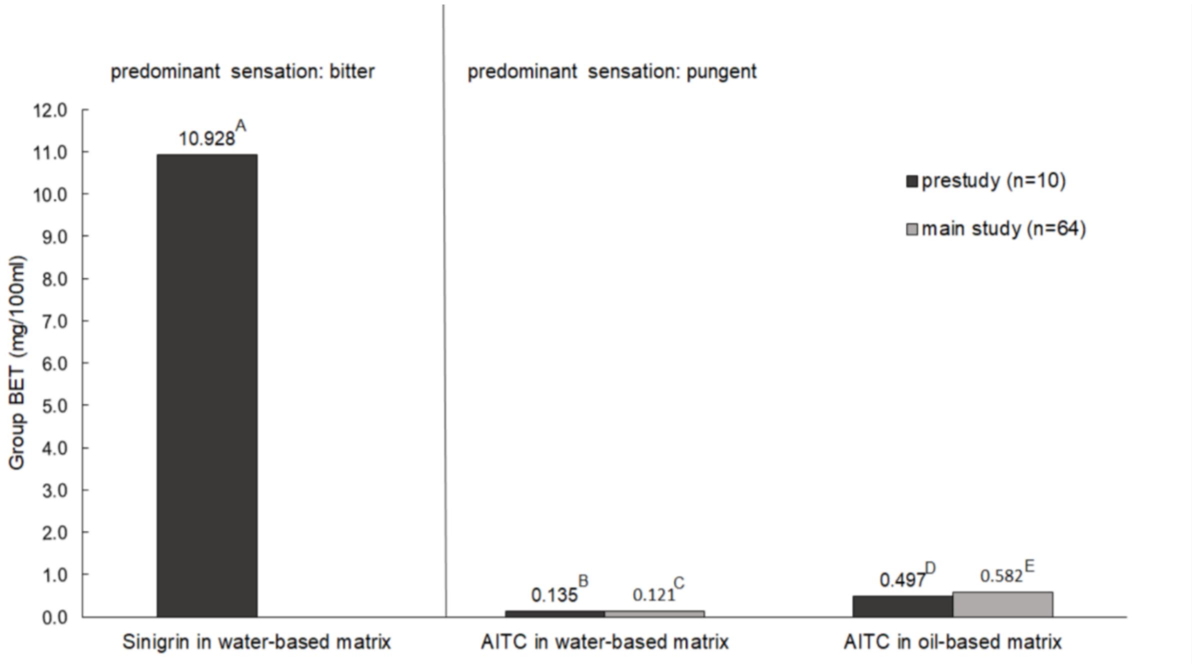

Figure 4.1 Group BET (geometric mean in mg/100 mL) for sinigrin in water-based matrix and allyl isothiocyanate (AITC) in water- and oil-based matrix. A, 10.928 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.384); B, 0.135 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.617); C, 0.121 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.627); D, 0.497 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.599); E, 0.582 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.509); according to ISO 13301 (2018) and ASTM International (2011)

The statistical analysis using two-way ANOVA (product/matrix and panelist) indicated a significant difference in the thresholds of sinigrin and AITC in the water-based matrix ($p < 0.001$). Furthermore, a significant difference was found between the thresholds of AITC in the water- and oil-based matrices in the prestudy ($p \leq 0.01$) and the main study ($p < 0.001$). The statistical analysis showed no significant impact of the panelists in all evaluations. The thresholds of the prestudy and main study of AITC in the water- and oil-based matrix tended to be identical.

Mustard "users" presented a tendency toward slightly lower thresholds than "nonusers" in all tests (Fig. 4.2). However, no significant differences in their thresholds were observed for sinigrin in the water-based matrix ($p = 0.530$) or AITC in the water-based matrix ($p = 0.910$, prestudy; $p = 0.520$, main study) or oil-based matrix ($p = 0.132$, prestudy; $p = 0.984$, main study).

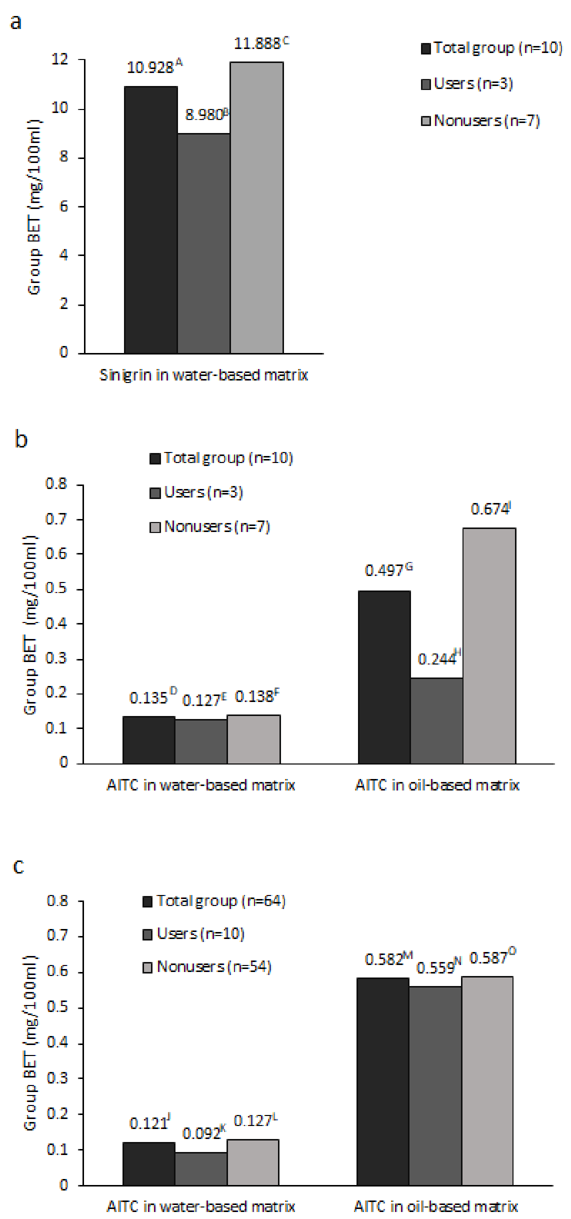

Figure 4.2 Threshold (group BET, geometric mean in mg/100 mL) comparison divided in "total group", mustard "user" and "nonuser" of (a) Sinigrin in water-based matrix, pre-study; (b) Allyl isothiocyanate (AITC) in water- and oil-based matrix, pre-study; (c) Allyl isothiocyanate (AITC) in water- and oil-based matrix, main-study. A, 10.928 mg/100 mL (*SD* of log_{10} BET ± 0.384); B, 8.980 mg/100 mL (*SD* of log_{10} BET ± 0.411); C, 11.888 mg/100 mL (*SD* of log_{10} BET ± 0.382); D, 0.135 mg/100 mL (*SD* of log_{10} BET ± 0.617); E, 0.127 mg/100 mL (*SD* of log_{10} BET ± 0.643); F, 0.138 mg/100 mL (*SD* of log_{10} BET ± 0.630); G, 0.497 mg/100 mL (*SD* of log_{10} BET ± 0.599); H, 0.244 mg/100 mL (*SD* of log_{10} BET ± 0.721); I, 0.674 mg/100 mL (*SD* of log_{10} BET ± 0.511); J, 0.121 mg/100 mL (*SD* of log_{10} BET ± 0.627); K, 0.092 mg/100 mL (*SD* of log_{10} BET ± 0.647); L, 0.127 mg/100 mL (*SD* of log_{10} BET ± 0.627); M, 0.582 mg/100 mL (*SD* of log_{10} BET ± 0.509); N, 0.559 mg/100 mL (*SD* of log_{10} BET ± 0.474); O, 0.587 mg/100 mL (*SD* of log_{10} BET ± 0.519); according to ISO 13301 (2018) and ASTM International (2011)

The panelists localized the area of sensation in both the prestudy and main study (Fig. 4.3). The bitter sensation of sinigrin was predominantly perceived on the tongue by most panelists. The pungent sensation of AITC in the water- and oil-based matrices was prevalently perceived on the tongue during the prestudy and main study.

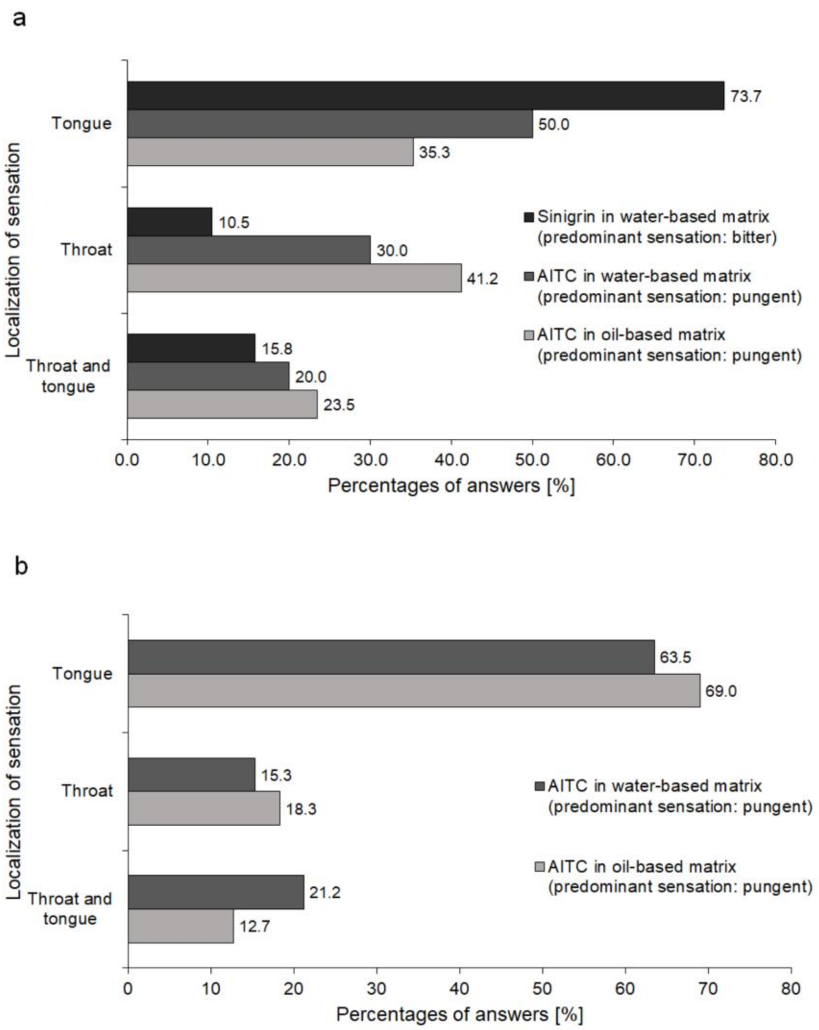

Figure 4.3 Localization of sensation. (a) Sinigrin in water-based matrix, Allyl isothiocyanate (AITC) in water- and oil-based matrix, pre-study. (b) AITC in water- and oil-based matrix, main-study

4.4 Discussion

The BET for sinigrin in a water-based matrix (10.9 mg/100 mL) was consistent with the results of Fenwick et al. (1983), who reported a group BET of 10.6 mg/100 mL. Therefore, no further study with a larger number of panelists was necessary.

Buttery et al. (1976) estimated an olfactory threshold of AITC in an aqueous matrix of 37.5 mg/100 mL. Nevertheless, this olfactory threshold cannot be compared to the oral threshold in this study. Inhaled irritant molecules such as AITC are perceived via stimulation of the receptors located in the rear of the nasal cavity, whereas in this study, AITC was released in the mouth under dynamic conditions affected by, e.g., the matrix, temperature and salvia (Burdach & Doty, 1987; Diaz, 2004; Heilmann & Hummel, 2004).

Significant differences in the thresholds of sinigrin and AITC may be explained by differing perception mechanisms. Sinigrin is associated with bitterness; this is a basic taste, which is predominantly perceived via the TASTE 2 Receptor (TAS2R) family on the tongue (Bell et al., 2018). In particular, sinigrin binds preferably to TAS2R38 (Meyerhof et al., 2010). The hydrolysis product AITC induces pungency (Fenwick et al., 1983), through interaction with pain receptors of the trigeminal system by stimulating free nerve endings in the eyes, mouth, and nose (Jordt et al., 2004).

As no directly comparable AITC threshold studies are available, the results of this study will be compared with those of capsaicinoid studies. Both AITC and capsaicinoids are chemical irritants that stimulate trigeminal nociceptors of the transient receptor potential (TRP) channel family (Everaerts et al., 2011; Jordt et al., 2004). AITC shows an effect on the cation channel ankyrin 1 (TRPA1) (Bautista et al., 2006; Jordt et al., 2004) as well as the “capsaicin receptor” TRP vanilloid 1 (TRPV1) (Alpizar, Gees, et al., 2014; Gees et al., 2013). Gees et al. (2013) suggested that AITC activates TRPV1 by direct and reversible interaction with the capsaicin-binding site. Therefore, the results of this study and capsaicinoid studies may be figuratively comparable.

The BET of AITC was lower in the water-based matrix than in the oil-based matrix. Schneider et al. (2013) and Lawless et al. (2000) reported similar findings for the threshold of capsaicin. This can be explained by two effects. First, fat may form a barrier between chemical irritants and trigeminal receptors in the oral cavity (Lynch et al., 1993). Because AITCs exhibit high solubility in oil, the interaction of the stimulus with the trigeminal receptor is reduced, resulting in reduced pungency perception (Baron & Penfield, 1996; Lawless et al., 2000). Second, the oil-based matrix has a higher viscosity and increased complexity than the water-based matrix, which may lead to a suppression effect on pungency perception (Kostyra et al., 2010; Schneider et al., 2013).

Different individual thresholds may be explained by differences in the sensitivities or fatigue of the panelists (ASTM International, 2011). Additionally, the repeated application of AITC may lead to transient desensitization effects in the oral (Simons et al., 2003) and nasal (Brand & Jacquot, 2002) mucosa. Simons et al. (2004) suggested that low AITC concentrations are excitatory and that the effect of desensitization does not overcome the excitatory component. With increasing AITC concentrations, the excitatory component is quickly exceeded by desensitizing mechanisms.

Our results showed no influence of mustard consumption frequency on AITC thresholds. The classification of the panel in mustard “users” and “nonusers” is based on the subjective classification of the panelists. The panelists were asked to rate their preferred pungency intensity according to prior experience with pungent products. This may have led to inconsistent usage of the pungency scale and therefore uncertainty of classification into the user groups according to Reinbach et al. (2007). Some authors reported lower sensitiveness of frequent chili consumers to capsaicinoids (Lawless et al., 1985; Prescott & Stevenson, 1995). Nevertheless, Nasrawi and Pangborn (1989); Orellana-Escobedo et al. (2012); Schlossareck and Ross (2019) and Schneider et al. (2014b) reported contrary results. Their studies did not confirm that regular chili consumption resulted in lower responsiveness to capsaicinoids.

The localization of the perception of AITC was predominantly specified by the panelists as being on the tongue. This is in contrast to the location of the burning sensation caused by capsaicin and dihydrocapsaicin, which is predominately perceived in the throat (Schneider et al., 2014b). The different localizations of AITC and capsaicin perception may be explained by the receptors responsible for trigeminal sensation. AITC stimulates both TRPA1 and TRPV1 receptors (Everaerts et al., 2011; Gees et al., 2013), whereas capsaicin solely activates TRPV1 (Caterina et al., 1997).

For all samples of AITC in a water-based matrix, polysorbate 80 was used as an emulsifier. In 2000, Lawless et al. reported no impact of polysorbate 80 as a dissolving agent in threshold studies. Schneider et al. (2014b) and Orellana-Escobedo et al. (2012) likewise chose polysorbate 80 as an emulsifier in sensory capsaicinoid threshold determinations.

4.5 Conclusion

This study showed statistically significantly different thresholds for sinigrin in water-based and AITC in water- and oil-based matrices using the 3-alternative forced choice method. The BET for sinigrin was 10.928 mg/100 mL. This result is consistent with the literature. For AITC in the water-based matrix in the prestudy and main study, the values were 0.135 mg/100 mL and 0.121 mg/100 mL, and those in the oil-based matrix were 0.497 mg/100 mL and 0.582 mg/100 mL, respectively. Thus, it was possible to show that the carrier matrix has an influence on AITC thresholds. No significant differences were observed between mustard “nonusers” and mustard “users”. The localization of perception was predominantly specified by the panelists as occurring on the tongue.

4.6 Acknowledgments

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5 Evaluation of trigeminal pungency perception of allyl isothiocyanate – A time intensity (TI) study

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Abstract

Allyl isothiocyanate, the primary pungent substance within mustard, horseradish, and wasabi, causes a burning sensation and a lachrymatory effect during consumption. In this time intensity study, the sensory perception of the chemical stimulus allyl isothiocyanate was evaluated in water-based, oil-based, and mustard recipe-based carrier matrices. The results indicate that perceived pungency intensity and time course of perception were both strongly dependent upon allyl isothiocyanate concentration and the composition of the carrier matrices. Increasing allyl isothiocyanate concentrations led to a significant ($p < 0.05$) increase in maximum intensity of pungency (I_{max}) in all tested carrier matrices. The intensity of pungency perception for allyl isothiocyanate decreased depending upon the specific matrix used. Specifically, this decrease occurred in the order oil-based carrier < mustard-based carrier < water-based carrier at similar allyl isothiocyanate concentrations. The values for end time of pungency perception (T_{End}) and duration of decreasing phase (DUR_{Dec}) indicate that increasing allyl isothiocyanate concentrations prolong the duration of pungency perception. Allyl isothiocyanate was perceived longer in water than in oil- and mustard-based carrier matrices.

5.1 Introduction

Time intensity (TI) studies are used to evaluate the dynamic changes and temporal aspects of perception. Many food products undergo changes in texture, taste, or smell during consumption (Salles & Benjamin, 2017). TI curves visualize the perception of sensory stimuli by illustrating the maximum intensity (I_{max}), rise, inclination (T_{Imax}), and duration of perception (T_{End}).

In the first TI studies, simple methods were used to record temporal information regarding sensory perception. Assessors marked the perceived intensities of perception on a scorecard at defined time intervals (Jellinek, 1964; Sjöström, 1954). In 1978, Larson-Powers and Pangborn implemented a moving chart recorder to allow for continuous data collection, and, in 1984, Lawless applied the TI method to analyze sensory irritation of the chemical stimuli capsicum oleoresin, vanillyl nonamide, piperine, and ginger oleoresin.

Since 1984, several studies have used the TI method to evaluate pungency perception. Schneider et al. (2014a) tested the effect of food ingredients such as starch, fat, and sugar on pungency perception of capsaicin in model food matrices possessing increasing complexity. In 2010, Kostyra et al. evaluated the effect of capsaicin concentration and carrier matrix complexity on TI parameters, flavor, and taste attributes such as bitterness, saltiness, and acidity. Reinbach et al. (2007) used TI to evaluate the influence of different textures and chili products on the meat flavor and pungency intensity of pork patties. Pungency evoked by mustard products has not yet been studied using the TI method.

The pungent and lachrymatory properties of some *Brassicaceae* vegetables are primarily caused by allyl isothiocyanate (Chin et al., 1996; Gilbert & Nursten, 1972). Mustard, broccoli, kale, horseradish, and cabbage are *Brassica* vegetables that contain glucosinolates, the precursors of isothiocyanates (Fenwick et al., 1983; Kroener & Buettner, 2017), which are formed during enzymatic degradation by myrosinase, a thioglucoside glucohydrolase. Glucosinolates and myrosinase are enclosed within different cell compartments. During processing and/or consumption of *Brassicaceae* plants, the cells are disrupted, which causes the contact of glucosinolates with myrosinase and the start of enzymatic degradation (Bones & Rossiter, 2006; Grob & Matile, 1979; Oliviero et al., 2018). Depending on various factors (pH, presence of specific proteins and/or metal ions, structure of the glucosinolate side chain), different breakdown products of the glucosinolates are formed (Hanschen et al., 2014). Hanschen et al. (2014) demonstrated that glucosinolates are enzymatically split into glucose and an unstable aglucone that is further converted into thiocyanates, isothiocyanates, nitriles, or epithionitriles. Glucosinolates and isothiocyanates have, however, been reported to be potentially beneficial to human health (Bhattacharya et al., 2010; Gründemann & Huber, 2018; Saha et al., 2012).

Mustard is commonly used in the form of seeds or paste in many food cultures as a food ingredient or as seasoning. Allyl isothiocyanate, which originates from the glucosinolate sinigrin (2-propenyl-glucosinolate), is primarily responsible for the characteristic sharp aroma (Chin et al., 1996) and biting and bitter taste (Sindhu et al., 2012) of mustard. Mustard pastes are categorized as sweet, mild, or hot products depending on their degree of pungency. In general, mustard paste consists of 50 to 80% water, 15 to 35% mustard seeds (more or less grounded), 1 to 5% salt, and 1 to 5% vinegar. Overall, mustard seeds consist of 38 to 44% fats and possess a protein content of approximately 25% (Sindhu et al., 2012). The sinigrin concentration within brown mustard seeds is between 8.8 ± 2.0 mg/g (*Brassica juncea*) (Zrybko et al., 1997) and 16.6 to 61.2 mg/g (*Brassica juncea* and *Brassica nigra*) (Velíšek et al., 1995). *Brassica nigra* possesses an allyl isothiocyanate content of 4 to 18 mg/g. *Brassica juncea* exhibits lower allyl isothiocyanate concentrations that range from 3.3 to 4.4 mg/g when enzymatically degraded from sinigrin (Shankaranarayaba et al., 1972). The degree of pungency of mustard products increases with increasing allyl isothiocyanate concentrations and varies among different brands, even when they are labeled with the same pungency category. Kuebler (2010) reported allyl isothiocyanate concentrations of 4.0 mg/100 g for sweet, 28.1 to 118 mg/100 g for hot, and 212 mg/100 g for extra hot mustard products that were analyzed by gas chromatography.

Two receptors of the transient receptor potential (TRP) family of ion channels are stimulated by allyl isothiocyanate. Macpherson et al. (2007) observed that transient receptor potential ankyrin 1 (TRPA1) is activated by covalent binding of allyl isothiocyanate to cysteine residues. Additionally, the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1) is activated by interaction of allyl isothiocyanate with the capsaicin binding site (Gees et al., 2013) and contributes to the allyl isothiocyanate-induced pungency perception (Alpizar, Gees, et al., 2014; Everaerts et al., 2011; Gees et al., 2013).

In 2018, Bell et al. reviewed the literature on perception of glucosinolates, isothiocyanates, and related volatile organic compounds focusing on studies based on correlating sensory and analytical data. Even though several authors focus on evaluation of glucosinolates (Baik et al., 2003; Schonhof et al., 2004; Van Doorn et al., 1998), studies focusing on sensory analytical correlations of glucosinolate hydrolysis products such as isothiocyanates were reported lacking (Bell et al., 2018). Additionally, studies on detection threshold, sensory attributes in general and the impact of these compounds on sensory traits within their own respective food matrices are reported to be of scientific interest (Bell et al., 2018).

The aim of this study was to evaluate the pungency perception of allyl isothiocyanate in water-, oil-, and mustard-based carrier matrices with different allyl isothiocyanate concentrations using the TI method. The location of pungency sensation within the mouth (throat, palate, tongue, lip) and nose at several stages of TI recording was also determined.

Within this study, samples were tested with and without the use of a nose clip to determine the influence of volatiles on the trigeminal pungency perception of allyl isothiocyanate.

5.2 Materials and methods

5.2.1 Overview

Pungency intensities of allyl isothiocyanate in water-, oil-, and mustard-based carrier matrices were evaluated by trained panelists using a TI testing protocol. The study was divided into two parts. In the first part of the study, allyl isothiocyanate samples with oil- and water-based carrier matrices were tested with and without the use of a nose clip. The second study part focused on the temporal perception of allyl isothiocyanate in a given mustard carrier matrix. The allyl isothiocyanate concentrations that were tested varied depending on the carrier matrix due to the different perceptions within the matrices that were determined according to pre-tests.

5.2.2 Panelists

The two study parts were performed using different sensory panels. Some of the assessors participated in both panels. The panel that tested allyl isothiocyanate in oil- and water-based carrier matrices consisted of ten assessors (mean age 26.4; age range 20-37; 9 females and 1 male). Mustard-based carrier samples were tested by 19 participants (mean age 25.2; age range 20-34; 15 females and 4 males). All recruited panelists were trained in sensory assessment according to DIN EN ISO 8586 (2014) and were familiar with the assessment of pungency from previous studies; however, they were not all familiar with the TI methodology. Panelists provided information regarding consumption behavior of spicy food in general as well as mustard products in particular by completing a questionnaire inspired by Reinbach et al. (2007). Assessors were compensated for their participation with sweets. The study was performed in accordance with the Declaration of Helsinki, and the procedure was approved by the University of Applied Sciences Fulda Ethics Review Board. The informed consent of all participants was obtained written and verbally.

5.2.3 Panelist training

The panelists were trained in the computer-aided TI methodology using a procedure adapted from Peyvieux and Dijksterhuis (2001) and Schneider et al. (2014a). To accomplish this, three training sessions using FIZZ (FIZZ software by Biosystèmes, Version 2.60, France) software were conducted. The first step of the training was to familiarize the assessors with the computer software used for this analysis and to coordinate training to facilitate and practice parallel sample intake and intensity scoring over time. Sugar solutions (15 g/L) were presented and rated according to TI testing. The second step was to familiarize the panelists with the pungent stimuli induced by allyl isothiocyanate. TI training using oil- and water-based test samples was performed using three different allyl isothiocyanate concentrations (0.1, 5 and 100 mg/100 mL) in water-based carrier matrices. The concentration 0.1 mg/100 mL was chosen to assess the ability of panelists to perceive allyl isothiocyanate at levels near the detection threshold (0.121 (SD $\log_{10}$ ± 0.627) mg/100 mL) concentration (Eib, Ramos Gajek, et al., 2020).

Assessors testing mustard carrier-based samples were trained using samples representing mild mustard with an allyl isothiocyanate concentration of 50 mg allyl isothiocyanate/100 g and an extra hot mustard containing 200 mg allyl isothiocyanate/100 g carrier matrix.

Training samples were tested in duplicate to verify reproducibility of each participant. In both study parts, panelists were asked to rate perceived pungency intensity using their former experience with pungent products (Reinbach et al., 2007). Subsequent to the first training session, pungency intensity and overall perception characteristics were discussed among the group until consensus of pungency intensity rating for each tested sample concentration was achieved. Pungency of allyl isothiocyanate was defined as tingling and biting as well as the retronasal pungency perceived as occurring in the mouth, nose and throat and the common lachrymatory effect.

Based on the reproducibility of the assessments in the training sessions, 9 panelists from the first study part and 18 panelists from the second study part were selected to participate in the main studies. Therefore, curve shapes of each panelist were compared for each tested concentration (Van Buuren, 1992). If the curves of the double determination exhibit the same shape and approximately the same I_{max} and T_{End} curves they are considered as acceptable replicates. Tolerable variation of I_{max} values was defined as +/- 1 intensity units (scale 0-10) and tolerable variation of T_{End} was defined as 25%. After completion of all sessions, all double determined curves produced by the panelists were assessed for good reproducibility. In case of unacceptable deviations, panelists could be subsequently excluded from the data. In both panels one panelist each was removed from the data because of unacceptable deviations in the double determined curves.

5.2.4 Sample preparation

The primary pungent component of mustard was the irritant compound allyl isothiocyanate (Sigma Aldrich, ≥95% purity).

5.2.4.1 Oil- and water-based carrier samples

Allyl isothiocyanate is not hydrophilic, and thus, polysorbate 80 (Carl Roth GmbH) was used as a food-grade emulsifier to disperse allyl isothiocyanate in water. Allyl isothiocyanate stock solutions (500 mg/100 mL respectively 5000 ppm) were prepared by mixing 500 mg allyl isothiocyanate with either 100 mL safflower oil or deionized water containing polysorbate 80 (180 mg/100 mL respectively 1800 ppm). The water-based allyl isothiocyanate mixtures were homogenized using a T25 digital

Ultraturrax® (IKA®-Werke GmbH & Co. KG) until allyl isothiocyanate was completely dispersed. Test samples were prepared by diluting the stock solutions with safflower oil or deionized water to the concentrations 5, 10, 50, 100, and 200 mg allyl isothiocyanate/100 mL. The allyl isothiocyanate concentration of 200 mg/100 mL was individually tested as an oil-based sample, while the concentration 5 mg/100 mL was only tested as a water based sample. Sample concentrations were chosen based on observations from preliminary tests.

5.2.4.2 Mustard-based carrier samples

To achieve defined allyl isothiocyanate concentrations in the mustard based carrier matrix, a method to produce mustard without perceivable pungency was developed. The mustard-based carrier matrix was prepared as follows (formulation stated as percentage of the total amount of mustard-based carrier matrix produced):

1. Milling of yellow mustard seeds (20.0%) (Blanks GmbH & Co. KG, Uplengen, Germany) for 60 s at 10,200 rpm in a food processor Thermomix TM31 (Vorwerk Elektrowerke GmbH & Co. KG, Wuppertal, Germany).
2. Addition of water (69.8%).
3. Mashing for 15 min at 20 ± 2 °C.
4. Addition of white wine vinegar (6.5%) (Hengstenberg GmbH & Co. KG, Esslingen, Germany, 6.0% acidity), salt (3.0%), and sugar (0.7%)
5. Mixing for 6 min at 2000 rpm in a Thermomix TM31.
6. Heating up to 60 °C at 500 rpm in a Thermomix TM31, holding time of 35 min at 60 °C.
7. Heating up to 80 °C at 500 rpm in a Thermomix TM31, holding time of 5 min at 80 °C.
8. Maturation of the mustard-based carrier matrix for 7 days at 3 °C.

Reproducibility of the mustard based carrier production was validated by sensory analysis, pH, and dry weight determination. To ensure that the mustard carrier matrix does not induce a perceivable pungency sensation, a 2-alternative forced choice study consisting of 73 participants was performed (results not shown here).

Mustard-based carrier matrices were produced freshly seven days prior to each TI test session. Test samples were prepared approximately one hour before the session by weighing allyl isothiocyanate into a closable glass container and then subsequently mixing allyl isothiocyanate with a small amount of mustard carrier to better blend both. Test samples were transferred into larger sealable cans, and additional mustard carrier matrix was added to the concentrations of 50, 100, and 200 mg allyl isothiocyanate/100 g mustard-based carrier matrix. Concentrations were chosen according to common allyl isothiocyanate concentrations found in commercial mustard pastes (Kuebler, 2010).

5.2.5 TI tests

TI tests were performed according to DIN 10970 (2002) in a sensory laboratory in accordance with DIN EN ISO 8589 (2010). The test booth room temperature was set to 21 °C. Booths were illuminated with lights that were equivalent to daylight. Data were collected and analyzed using FIZZ sensory software (Version 2.51, Biosystems, Couternon, France).

5.2.5.1 Oil- and water-based carrier samples

Samples (5 mL each) were offered individually in laboratory glasses, covered with watch dishes, and coded with random three-digit numbers. To reduce possible sensitization and to limit simple carry-over, samples were tested with increasing allyl isothiocyanate concentrations using two different sample sets (Set A: 10 mg/100 mL in water; 10 mg/100 mL in oil, 100 mg/100 mL in water, 100 mg/100 mL in oil; Set B: 5 mg/100 mL in water, 50 mg/100 mL in water, 50 mg/100 mL in oil, 200 mg/100 mL in oil) inspired by Nolden and Hayes (2017). A maximum of four samples was assessed per session. Tests were performed in duplicate over eight sessions.

5.2.5.2 Mustard-based carrier samples

Test samples (1 g each) were presented separately on spoons marked with a three-digit code in ascending concentrations. Because of the intense taste of mustard products, only two mustard carrier-based samples in varying sample sets (Set A: 50 and 100 mg/100 g mustard carrier matrix; Set B: 0 and 200 mg/100 g mustard carrier matrix; Set C: 0 and 100 mg/100 g mustard carrier matrix; Set D: 50 and 200 mg/100 g mustard carrier matrix) were tested in one session. A two-fold determination was performed for all test samples distributed among the four sessions.

5.2.5.3 TI test procedure

Panelists were asked to rate the pungency intensity by moving the mouse on a structured linear scale. The lowest score of the scale was marked “0” and “not detectable,” and the highest intensity score was marked “10” and “extremely pungent.” Data acquisition was initiated by clicking on the zero point of the intensity scale. Instructions for sample incorporation were provided using pop-up messages on the screen. Assessors were trained to use the following procedure: pinching nostrils with a clip (depending on test), placing the sample into the mouth, swallow the sample after 5 s as instructed on screen, and begin rating the perceived pungency intensity for 300 s. Data acquisition is not stopped by returning the mouse to zero of the intensity scale. Mascarpone toast and distilled water were served as neutralizers, and assessors were asked to neutralize prior to each test and between samples. The inter-stimulus interval was set to 90 s and indicated by a countdown appearing on the screen. Panelists recorded details on their individual pungency perception using the questionnaire displayed in Table 5.1. The perceived intensity of burn was evaluated using previous experienced oral and trigeminal burn as comparisons (Reinbach et al., 2007).

Table 5.1 Questionnaire on pungency perception of allyl isothiocyanate in oil and allyl isothiocyanate in water (Adapted from Reinbach et al. [2007])

I	How hot do you perceive this sample? Not hot ○ ○ ○ ○ ○ ○ Extremely hot 0 1 2 3 4 5
II	Where in throat and oral cavity did you sense the burn at first? ○ palate ○ tongue ○ lip ○ throat ○ nose
III	Where in throat and oral cavity did you sense the burn at maximum pungency intensity? ○ palate ○ tongue ○ lip ○ throat ○ nose
IV	How will you describe the burn if you sensed any?
V	Did the burn change during the assessment? If yes, please describe the change.
VI	How pungent is the sample compared to the hotness of the spicy food, you normally eat. Much milder ○ ○ ○ ○ ○ ○ ○ ○ Much hotter 0 1 2 3 4 5 6 7

5.2.6 Data processing and analysis

The TI curves for each judge were registered and extracted using FIZZ sensory software. The individual influence of each panelist on TI data was eliminated by normalization of the curves using the normalization algorithm integrated in FIZZ. For statistical evaluation, the TI parameter I_{max}, T_{Imax}, T_{End}, DUR_{Dec} (duration of the decreasing phase), *AUC* (total area under the curve, from the first to the last acquisition point), and AUC_{Dec} (area under the decreasing phase) were extracted from non-normalized data. The significant differences in pungency perception were assessed by three-way and four-way analysis of variance (ANOVA) ($p < 0.05$) of I_{max}, and incorporating irritant concentration, panelist, and if applicable, type of carrier matrix and nose clip condition as variability factors followed by Tukey HSD post hoc tests using IBM SPSS Statistics software (24) (IBM Deutschland GmbH Ettlingen, Germany).

5.3 Results

The main findings from the TI evaluation are shown in Table 5.2 and Figure 5.1. Oil- and water-based samples were tested with and without the use of a nose clip. Mustard-based carrier samples were not tested with a nose clip, as mustard products are not commonly consumed using a nose clip.

Table 5.2 Mean values of I_{max} (maximum intensity) for different concentrations of allyl isothiocyanate in various matrices and nose clip conditions. I_{max} mean values are based on individual results of panelists

Matrix / Nose clip condition[A]	I_{max} Allyl isothiocyanate concentration [mg/100 mL] or [mg/100 g]					
	0	5	10	50	100	200
Water w/o NC	-	3.4^{a}	3.0^{b}	8.0^{a}	8.7^{a}	-
Water w NC	-	3.1^{a}	4.7^{a}	8.3^{a}	8.7^{a}	-
Oil w/o NC	-	-	0.4^{d}	2.9^{b}	2.1^{c}	5.4^{b}
Oil w NC	-	-	0.7^{c}	$2.1^{b\,c}$	$2.3^{b\,c}$	5.2^{b}
Mustard carrier w/o NC	1.0	-	-	1.7^{c}	3.2^{b}	7.1^{a}

$^{a,\,b,\,c}$ – I_{max} mean values with different letters in columns are significantly different, p≤ 0.05

[A] Nose clip condition – with nose clip (w NC), without nose clip (w/o NC)

Table 5.2 shows the I_{max} of allyl isothiocyanate pungency perception under different test conditions (allyl isothiocyanate concentration, carrier matrix, and nose clip condition). The results indicate that pungency perception depended strongly both upon allyl isothiocyanate concentrations and allyl isothiocyanate carrier matrices. Under increasing allyl isothiocyanate concentrations, a distinct increase in I_{max} was observed ($p < 0.05$; in all test conditions (combination of carrier matrix and nose clip condition)). The use of a nose clip did not significantly influence pungency perception. The I_{max} for oil- and water-based carrier samples tested in the presence of a nose clip was significantly higher than that observed without a nose clip for samples of 10 mg allyl isothiocyanate/100 mL carrier. For all other allyl isothiocyanate concentrations, no significant influence of the nose clip was observed. I_{max} mean values of mustard-based carrier samples were compared to the I_{max} mean values of oil- and water-based carrier samples at comparable allyl isothiocyanate concentrations (50, 100, and 200 mg allyl isothiocyanate/100 g mustard carrier matrix). For mustard-based carrier samples with no added allyl isothiocyanate (0 mg allyl isothiocyanate/100 mg), an I_{max} mean value of 1.0 was recorded.

Time-related and calculated parameters from normalized TI curves are listed in Table 5.3 and 5.4. The parameter T_{Imax} was higher in oil- and mustard-based carrier samples compared to that of water-based carrier samples at similar allyl isothiocyanate concentrations. The higher the allyl isothiocyanate concentration in oil-based samples, the later the T_{Imax} was reached, with the exception of the 50 mg allyl isothiocyanate/100 g oil-based carrier tested with a nose clip. T_{End} and DUR_{Dec} indicated that the total duration of pungency perception and the duration of decreasing phase were prolonged with increasing allyl isothiocyanate concentration and that pungency perception tended to be longer in water-based matrices compared to that in oil- and mustard-based carrier matrices. Significant differences in the parameters T_{End} and DUR_{Dec} were found for each tested combination of carrier matrix and nose clip condition ($p < 0.05$). The *AUC*, and AUC_{Dec} values were also dependent upon the carrier matrix and the allyl isothiocyanate concentration ($p < 0.05$; in all test conditions (combination of carrier matrix and nose clip condition)). All parameters based on the calculation of the area under the curve (*AUC*, AUC_{Dec}) were, at comparable concentrations, higher in water-based carrier matrices compared to those in oil- and mostly in mustard-based carrier matrices. For all evaluated parameters, no explicit difference between mustard- and oil-based samples was observed.

Table 5.3 Time-related parameters of allyl isothiocyanate pungency perception. The parameters T_{Imax} (time to reach maximum intensity of pungency), T_{End} (end time of pungency perception), DUR_{Dec} (duration of the decreasing phase) were obtained from individual curves for each tested combination of allyl isothiocyanate / Matrix / Nose clip condition

Matrix / Nose clip condition[A]	T_{Imax} [s] Allyl isothiocyanate concentration [mg/100 mL] or [mg/100 g]						T_{End} [s] Allyl isothiocyanate concentration [mg/100 mL] or [mg/100 g]						DUR_{Dec} [s] Allyl isothiocyanate concentration [mg/100 mL] or [mg/100 g]					
	0	**5**	**10**	**50**	**100**	**200**	**0**	**5**	**10**	**50**	**100**	**200**	**0**	**5**	**10**	**50**	**100**	**200**
Water w/o NC	-	3.8^{a}	2.7^{a}	$3.2^{a\,b}$	2.4^{b}	-	-	43^{a}	51^{a}	92^{a}	$74^{a\,b\,c}$	-	-	35^{a}	47^{a}	87^{a}	$69^{a\,b}$	-
Water w NC	-	1.7^{b}	1.4^{a}	1.1^{b}	2.0^{b}	-	-	26^{b}	40^{a}	89^{a}	88^{b}	-	-	23^{b}	34^{b}	84^{a}	81^{a}	-
Oil w/o NC	-	-	0.7^{a}	5.8^{a}	8.6^{a}	$11{,}2^{a}$	-	-	3^{b}	33^{b}	$65^{a\,c}$	85^{a}	-	-	2^{c}	$25^{b\,c}$	54^{b}	$69^{a\,b}$
Oil w NC	-	-	2.1^{a}	0.9^{b}	7.4^{a}	$8.1^{a\,b}$	-	-	6^{b}	6^{c}	$51^{d\,c}$	99^{a}	-	-	4^{c}	4^{c}	22^{c}	88^{a}
Mustard carrier w/o NC	2.1	-	-	4.5^{a}	6.1^{a}	4.8^{b}	11	-	-	33^{b}	41^{d}	65^{b}	7	-	-	25^{b}	30^{c}	57^{b}

[a, b, c] T_{Imax}, T_{End}, and DUR_{Dec} mean values with different letters in columns are significantly different, $p \leq 0.05$

[A] Nose clip condition – with nose clip (w NC), without nose clip (w/o NC)

Table 5.4 Calculated parameters of allyl isothiocyanate pungency perception. The parameters *AUC* (total area under the curve, from the first to the last acquisition point), *AUC$_{Dec}$* (area under the decreasing phase) were obtained from individual curves for each tested combination of allyl isothiocyanate / Matrix / Nose clip condition

Matrix / Nose clip condition[A]	*AUC* Allyl isothiocyanate concentration [mg/100 mL] or [mg/100 g]						*AUC$_{Dec}$* Allyl isothiocyanate concentration [mg/100 mL] or [mg/100 g]					
	0	**5**	**10**	**50**	**100**	**200**	**0**	**5**	**10**	**50**	**100**	**200**
Water w/o NC	-	73^a	100^a	297^a	255^a	-	-	54^a	82^a	261^a	218^a	-
Water w NC	-	49^b	93^a	295^a	280^a	-	-	40^a	77^a	280^a	253^a	-
Oil w/o NC	-	-	2^b	36^b	86^b	218^a	-	-	1^b	28^b	65^b	161^a
Oil w NC	-	-	4^b	5^b	76^b	219^a	-	-	2^b	3^b	55^b	170^a
Mustard carrier w/o NC	11	-	-	50^b	87^b	202^a	7	-	-	35^b	56^b	149^a

a, b, c – *AUC, and AUC$_{Dec}$* mean values with different letters in columns are significantly different, $p \leq 0.05$

A Nose clip condition – with nose clip (w NC), without nose clip (w/o NC)

All recorded TI data were normalized and are shown as average curves in Figure 5.1 a-e. Normalization was performed to eliminate the individual influence of each assessor. TI average curves illustrate the impact of the allyl isothiocyanate concentration on pungency perception. The intensity of pungency perception was higher for tested allyl isothiocyanate concentrations in water-based matrices compared to those in mustard- and oil-based carrier matrices. Average curves of water-based samples indicated that the intensity maximum was reached almost directly after intake, while the intensity maximum of allyl isothiocyanate samples in mustard- and oil-based carrier matrices was reached 5 to 10 seconds later. Taken together, the results of the normalized average curves (Figure 5.1 a-e) are in accordance with the calculated TI parameters (Tables 5.2-5.4) and illustrate the time course of allyl isothiocyanate pungency perception.

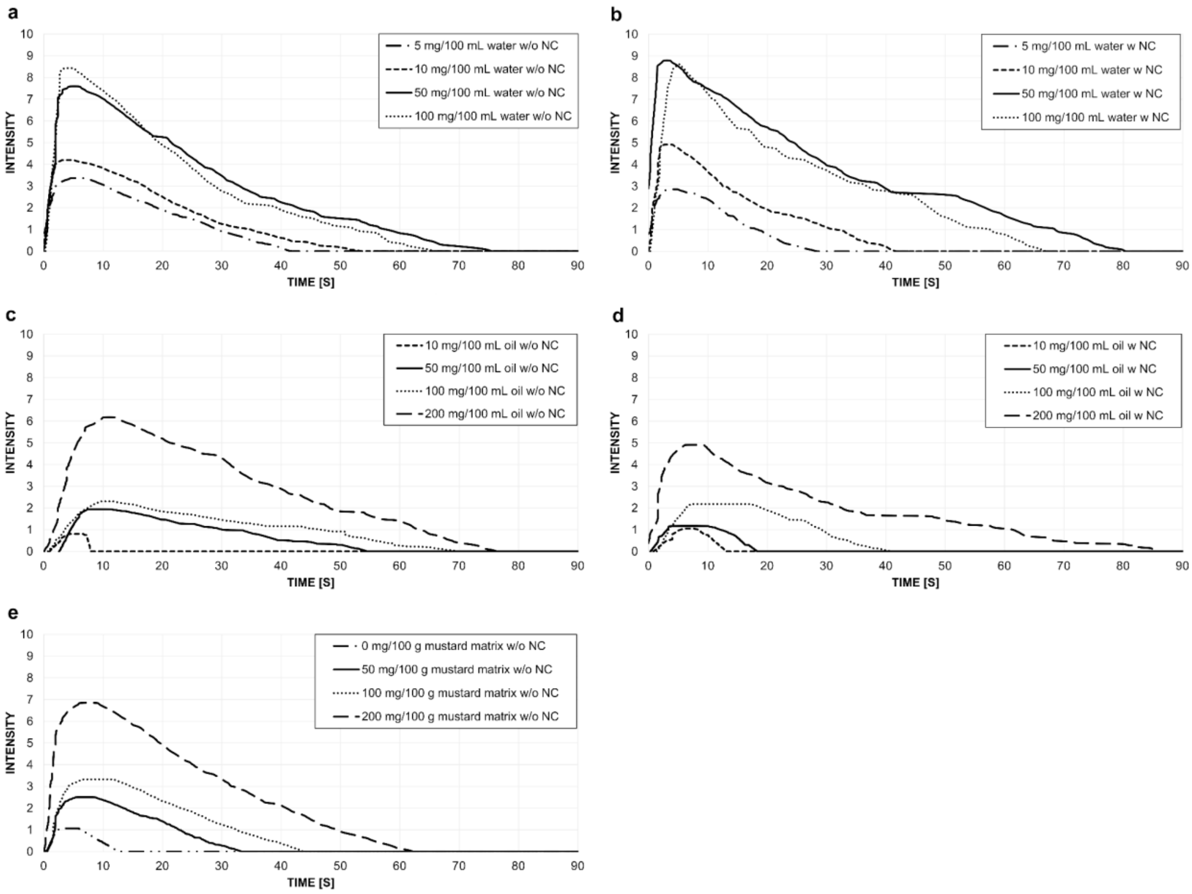

Figure 5.1 Time intensity average curves of allyl isothiocyanate (AITC) in water- and oil-based carrier matrices tested with or without a nose clip (NC) and in mustard-based carrier matrix. These curves were calculated as the mean values of the normalized TI data (a): AITC in water-based carrier matrix tested without nose clip (w/o NC) (b): AITC in water-based carrier matrix tested with nose clip (w NC) (c): AITC in oil-based carrier matrix tested without nose clip (w/o NC) (d): AITC in oil-based carrier matrix tested with nose clip (w NC) (e): AITC in mustard-based carrier matrix tested without nose clip (w/o NC)

In addition to the TI test recorded using FIZZ, all assessors were asked to describe and locate the pungency sensation using a questionnaire (Table 5.1). Independent from the carrier matrix, most assessors reported the first burning sensation to occur on the tongue. Results of the localization of pungency sensation at I_{max} are shown in Figure 5.2 a-e. At maximum pungency sensation, panelists reported burning at multiple localizations within the oral cavity (tongue, palate, and throat) and nose depending on the allyl isothiocyanate concentration of the sample. In addition to the localization of pungency sensation at I_{max}, several assessors described an increase in pungency intensity after swallowing and a change in localization from burning in the mouth to the throat and the nose. Furthermore, some assessors reported that the sensation in the mouth and nose diverted quickly while the sensation in the throat was longer-lasting. A small number of panelists reported garlic-like aroma notes that were perceived particularly in water-based carrier samples.

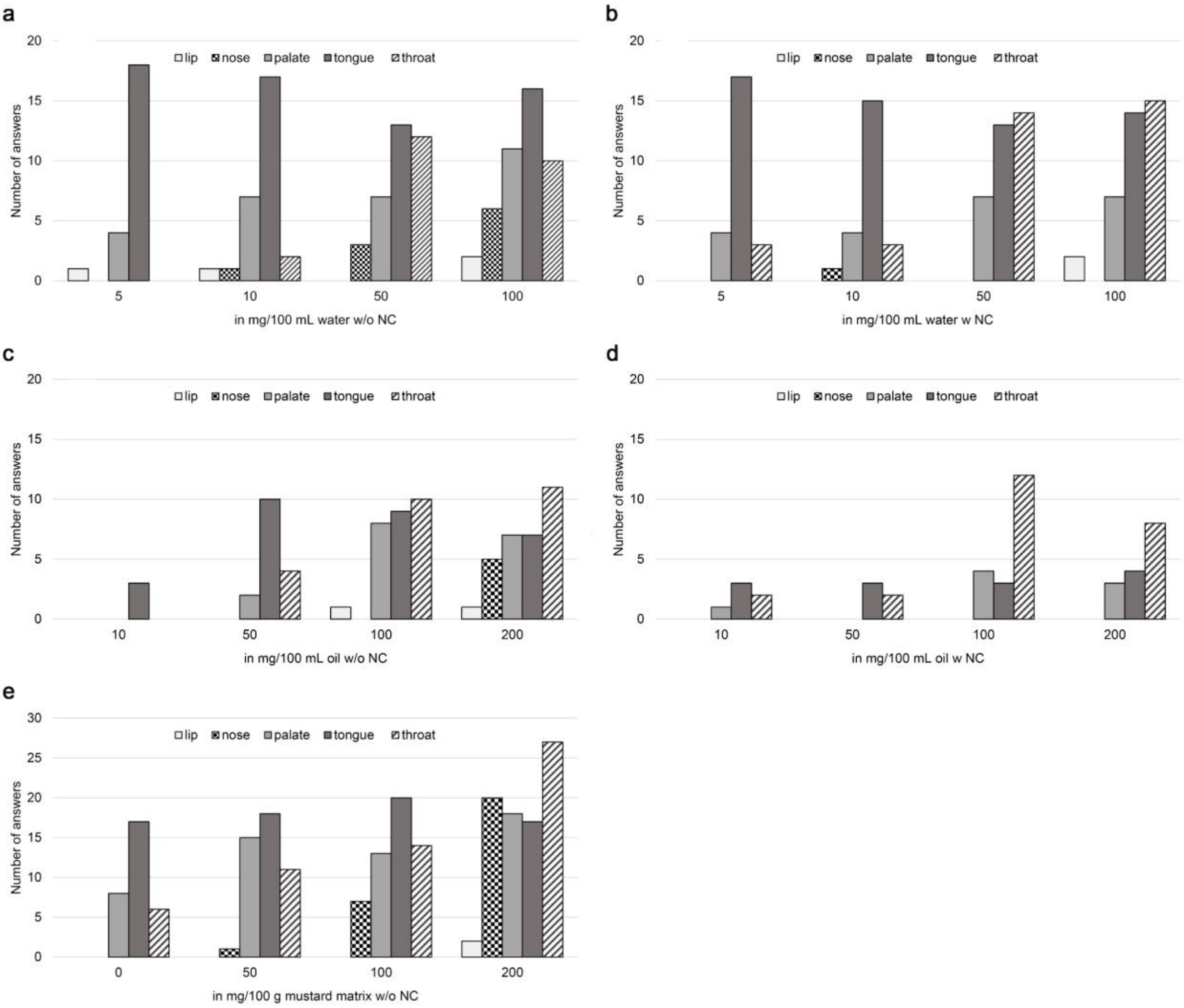

Figure 5.2 Localization of pungency sensation at maximum intensity of pungency (number of answers) (a): AITC in water-based carrier matrix tested without nose clip (w/o NC) (b): AITC in water-based carrier matrix tested with nose clip (w NC) (c): AITC in oil-based carrier matrix tested without nose clip (w/o NC) (d): AITC in oil-based carrier matrix tested with nose clip (w NC) (e): AITC in mustard-based carrier matrix tested without nose clip (w/o NC)

5.4 Discussion

In this study water, oil- and mustard-based food carrier matrices were used to evaluate the pungent sensation caused by allyl isothiocyanate. This enabled us to compare pungency perception in simple and complex carriers, as the pungent sensation caused by allyl isothiocyanate is commonly evoked by consumption of processed food products such as mustard, wasabi, or horseradish pastes. As suggested by Green (1996), more research examining the stimuli added to mixtures is needed to allow for a greater understanding of chemesthesis. In his opinion, stinging and burning are sensations that add multi-dimensionality to an existing flavor, ultimately turning pungency into pleasant piquancy.

Pungency evoked by allyl isothiocyanate was significantly affected by the type of carrier matrix. Identical allyl isothiocyanate concentrations in varying matrices evoked different irritations as indicated by the TI parameters, especially I_{max}, DUR_{Dec}, and *AUC*. The influence of the matrix (water/oil/mustard) on pungency perception may be explained by two effects. First, a suppression effect on pungency perception may be caused by the higher viscosities of oil- and mustard-based carrier matrices (Kostyra et al., 2010; Schneider et al., 2014a). Second, fat has the ability to form a barrier that inhibits the interaction of the chemical stimulus with the trigeminal receptor (Lynch et al., 1993). The high solubility of allyl isothiocyanate in oil results in a weaker interaction of the irritant with the trigeminal receptor within the oral cavity. This may explain reduced pungency perception of chemical stimuli in oil-based carrier matrices (Baron & Penfield, 1996; Lawless et al., 2000).

Other studies investigated the influence of carrier matrices on pungency perception of capsaicin. Schneider et al. (2014a) reported lower pungency scores for higher fat contents in 3-component-based model matrices (starch, sugar and cream (0.3 or 30% fat)). Additionally, Carden et al. (1999) reported the same effect of fat content on pungency intensity in cheese sauces with capsaicin.

For mustard-based carrier matrices, the panelists assessed a sample without addition of allyl isothiocyanate (0 mg allyl isothiocyanate/100 g mustard based carrier matrix). Although this sample should not have evoked a pungency sensation, a maximum perceived intensity of 1.0 and sensation duration of approx. 12.6 seconds was recorded. This may be explained by residual allyl isothiocyanate contents within the mustard-based carrier matrices that were not volatilized during production process. Another explanation may be a misinterpretation of acidity. Pre-tests during mustard carrier development indicated that the acidity of mustard-based carrier matrices lacking added allyl isothiocyanate was more intense than that observed after addition of allyl isothiocyanate. The receptor TRPV1 can be activated by protons (Tominaga et al., 1998) and as a consequence, Simons et al. (2004) found that mustard oil could desensitize acid-evoked responses. Regardless, in another TI study, Carden et al. (1999) recorded low pungency perception of samples with capsaicin concentrations of 0.0 ppm in cheese sauces.

During food consumption, several environmental factors such as temperature, dilution, and surface of exchange, and individual physiological characteristics of the panelists influence the release of volatile aroma compounds (Cayot et al., 2008). Reviewing the process of sample incorporation and swallowing, various effects influence the perception of allyl isothiocyanate induced pungency. First of all, the swallowing process influences the temporal perception profile as release of an aroma compound can be grouped in three main stages (Buettner et al., 2002). In the first stage, the sample is introduced in the oral cavity without exchange of aroma compounds with other compartments. Due to dilution with saliva, the product volume increases. Additionally, the concentration of the aroma compound in the air of the oral cavity increases due to the volatility of the compound. (Doyennette et al., 2011) The second step is the act of swallowing, at which the product and saliva mixture passes the oro-pharynx and is further transported (Buettner et al., 2002). Depending on the viscosity of the product, a layer of product and saliva mixture is formed on the pharynx (Doyennette et al., 2011). Last, the air mixed with the volatilized aroma compound is distributed in the upper airways and pharynx (Doyennette et al., 2011), referred to as the “swallow-breath” (Buettner et al., 2002). This consumption process influences the temporal perception of allyl isothiocyanate as interaction with the respective receptors and herewith the subsequent transduction of sensory signals is depending on the distribution of allyl isothiocyanate in the respective compartments (Buettner et al., 2002). Regarding the used food-matrices, differences in the diffusion coefficient may have led to differences in the release of AITC from the product matrix into the gas phase as well as differences in dispersion (mass transfer) within the product and saliva mixture (Cayot et al., 2008). Comparing the allyl isothiocyanate carrier matrices used, presumably, the water-based carrier has the best diffusivity (Cayot et al., 2008) and the remaining product and saliva layer covering the oral and pharyngeal mucosa after swallowing is low (Doyennette et al., 2011). Furthermore, Marin et al. (1999) showed high mass transfer coefficients for flavor compounds in water, therefore concentration of ally isothiocyanate in the gas phase increases fast resulting in fast and intense perception of allyl isothiocyanate induced pungency. The oil-based carrier matrix, has the lowest diffusion and mass transfer coefficients which may be the explanation for the less intense pungency sensation induced by allyl isothiocyanate. Another aspect is the partitioning of AITC between oil, saliva and gas phase. A model published by McNulty and Karel (1973) predicts an increasing release of volatile molecules with increasing dilution of the emulsion with saliva and with increasing partition coefficient of an oil/water system. The oil/water partition coefficient of volatile aroma compounds is dependent on the temperature, the concentration and nature of the oil phase in the emulsion and the characteristics of the volatile compounds (Carey et al., 2002; Zhang et al., 2010). Partition coefficients of AITC in oil/water and oil/air systems with olive and canola oil were determined by Zhang et al. (2010). They reported that partitioning of AITC into the oil phase was stronger than into the water or air phase. The partition coefficient of AITC between canola oil and water is 93.2 and between canola oil and air 869.7, respectively (Zhang et al., 2010). These partition coefficients indicate that the AITC concentration available to induce a pungency sensation is reduced by the partitioning of AITC into the oil phase. According to McNulty and Karel (1973) flavor compounds have to be dissolved in the aqueous phase to induce a sensation.

In general, fat is able to suppress responses to a stimulus and decrease intensity by the formation of a mouthcoating interfering with the access of the tastant to the receptors (Lynch et al., 1993). During the stay of the oil-based carrier sample in the oral cavity, it is diluted with saliva into an emulsion with decreasing fat content over time. The mustard-based carrier matrix consists of a lipid and a water phase. Allyl isothiocyanate has a low water solubility. With increasing fat content of the matrix less allyl isothiocyanate partitions into saliva followed by oral tissue and the gas phase and to the trigeminal receptors (Lawless et al., 2000; Mcclements, 2019). Others reported that increasing the fat content of the carrier matrix resulted in decreasing release of unpolar aroma compounds (Arancibia et al., 2011; González-Tomás et al., 2007).

Furthermore, for incorporation of mustard-based carrier samples a spoon was used. According to Buettner et al. (2002) the usage of a spoon enables a retronasal aroma impression in the moment of sample intake by opening of the velum-tongue border.

Another possible reason could be the power of suggestion (Kirsch, 1997, 2017; Michael et al., 2012). The results show that some panelists were susceptible to the suggestion while others rated the perceived pungency as zero throughout the recording time. Therefore, the mean value of the maximum perceived intensity may be influenced by panelists anticipated the sample to be pungent and automatically rated to perceive a pungent sensation.

Mustard pastes consist of approximately 4% fat, 4 to 6% carbohydrates, and 6% protein (Wahrburg & Egert, 2009). In comparison to the water- and oil-based matrices, the mustard-based carrier is a complex model food system. The I_{max} of the mustard-based carrier samples was significantly lower than I_{max} of water based samples (50, 100 mg/100 mL) tested with and without the use of a nose clip at all comparable concentrations and was significantly higher than the I_{max} of oil-based samples (50, 100, 200 mg/100 mL) tested without the use of a nose clip. Compared to the concentrations of 50 and 100 mg allyl isothiocyanate/100 mL of oil-based carrier tested with the use of a nose clip, the 50 and 100 mg allyl isothiocyanate/100 g of mustard-based carrier exhibited no significant differences in pungency. The I_{max} of the oil-based samples tested with the use of a nose clip was significantly lower than the I_{max} of the mustard-based carrier samples at a concentration of 200 mg allyl isothiocyanate/100 mL and 200 mg allyl isothiocyanate/100 g. The complexity of the mustard-based carrier may explain the lower I_{max} of allyl isothiocyanate in the mustard-based carrier compared to that of the water-based carrier. Kostyra et al. (2010) and Schneider et al. (2014a) demonstrated that pungency perception of capsaicin is influenced by the complexity (number of components) of the carrier. Increasing complexity resulted in decreased ratings of pungency intensities. Additionally, both studies indicated that pungency perception is impacted more strongly by starch content and complexity of the carrier than by fat content. (Kostyra et al., 2010; Schneider et al., 2014a). Regardless, the influence of the higher complexity of the mustard-based carrier did not affect I_{max} as much as the oil content of the carrier in this study. For all carrier matrices tested, the evoked maximum pungency intensity increased significantly with increasing concentrations of the chemical stimulus allyl isothiocyanate. This result is consistent with those from studies investigating the influence of increasing capsaicin concentrations on pungency perception (Baron & Penfield, 1996; Kostyra et al., 2010; Lawless, 1984; Schneider et al., 2014a).

Allyl isothiocyanate is a volatile substance and exerts a lachrymatory effect (Terada et al., 2015). To test the effect of retronasal pungency perception, oil- and water-based carrier samples were tested with and without a nose clip. No clear influence of the use of a nose clip was observed with regard to pungency perception. As shown by Pionnier et al. (2004) in regard to the salty and sour attributes in model cheese food systems, we expected pungency perception to be higher in samples tested without the use of a nose clip. Our results may be explained by changes in perception modalities such as respiratory flow and frequency due to the use of a nose clip. Another explanation for our findings may be that long sample preparation times or sample handling could cause volatilization of allyl isothiocyanate prior to sample intake.

For water-based carrier samples the emulsifying agent polysorbate 80 was used to disperse allyl isothiocyanate. Polysorbate has been used to disperse irritant molecules in several other studies evaluating pungency perception. Lawless et al. (2000) used polysorbate in a capsaicin threshold study and stated that small amounts of polysorbate, used to disperse irritant molecules, have little or no sensory impact. Furthermore, polysorbate was used as emulsifier in TI studies evaluating capsaicin induced pungency perception (Carden et al., 1999; Schneider et al., 2014a).

Furthermore, results may be influenced by the dumping effect (C. C. Clark & Lawless, 1994). Participants rated a single attribute, the overall pungency intensity, and no response alternatives were given. This effect may have increased the pungency intensity ratings of the panelists. Results of the mustard carrier matrix may be influenced by a greater extent than results of water- and oil-bases carrier matrices, because of the higher complexity of sensations evoked by the mustard carrier matrix. Even though panelists underwent a short training, a more intense training could have influenced the size of the dumping effect (C. C. Clark & Lawless, 1994). As discussed above, the perception of acidity of the mustard carrier matrix may have affected rating of the pungency. Considering the dumping effect, the acidity of samples may have increased the pungency rating.

A significant influence of the panelists on I_{max} was also observed for all samples tested. This can be caused by individual differences in pungency perception.

Evaluation of the questionnaires completed for each sample revealed a garlic-like aroma of allyl isothiocyanate samples. This observation is in accordance with other studies that reported a garlic-like aroma for allyl isothiocyanate (G. S. Clark, 1992; Gilbert & Nursten, 1972; Hanschen et al., 2014).

The localization of pungency perception was influenced by concentration of the stimulus allyl isothiocyanate as well as the time of rating as previously reported (Rentmeister-Bryant & Green, 1997) for capsaicin and piperine. Allyl isothiocyanate was predominantly perceived on the tongue and in the throat. At low concentrations, panelists reported the burn to be prevalent on the tongue, while at higher concentrations, pungency perception in the throat increased and was reported most frequently with the exception of samples tested in water without the use of a nose clip. Allison et al. (1999) reported that capsaicin burn was significantly higher on the tongue than in the throat and oral cavity; however, others reported the tongue was less responsive to capsaicin than was the throat (Rentmeister-Bryant & Green, 1997; Schneider et al., 2014a). In this study, samples were swirled in the mouth for 5 s prior to being swallowed. Alterations in the time setting of sample intake may have changed the localization of pungency sensation at I_{max}.

5.5 Conclusion

This study reports sensory results on the temporal perception of the sinigrin hydrolysis product allyl isothiocyanate. Until now, sensory studies focusing on the allyl isothiocyanate induced pungency, especially in related food matrices, are not available. Herewith, further insights in the sensations induced by this chemical irritant were gained and can now be used as reference for others. The results demonstrate that perceived pungency intensity induced by allyl isothiocyanate concentration was affected by the type of carrier matrix used. The same allyl isothiocyanate concentration in water-, oil-, or mustard-based carrier matrix evoked different irritation effects in regard to pungency intensity and time course of perception.

Thus, it was possible to determine the influence of concentrations and carrier matrices on the trigeminal pungency of allyl isothiocyanate. Further studies should be performed to investigate the pungency perception of allyl isothiocyanate in commercial products.

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6 Relationship between mustard pungency and allyl isothiocyanate content – A comparison of sensory and chemical evaluations

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Supporting information

The supporting information of this publication can be found in Chapter 10.

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_{max}), the duration of the decreasing phase (DUR_{Dec}), and the area under the curve (*AUC*) values increased proportionally to the increase in the sample AITC content and was 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_{Dec} (correlation coefficient 0.856) or the I_{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.

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.

6.1 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 which induce this pungent sensation upon consumption.

Mustard products are common in European cuisine (Bell et al., 2018) and the burning sensation they induce is considered enjoyable to mustard consumers (Ghawi et al., 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 et al., 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 (G. S. 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 sinalbin, are responsible for the pungency and flavor of mustard products (Fenwick & Heaney, 1983; Ghawi et al., 2014). AITC has lachrymatory and pungent properties (Chin et al., 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 et al., 2010; Fahey et al., 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 et al., 2019; Rousseau et al., 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; Kostyra et al., 2010; Schneider et al., 2014a). 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 et al., 2016; Schneider et al., 2014a; Zhao et al., 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. Secondly, a new improved flavor classification based on pyruvate content was implemented, which was subsequently validated by the taste-panel.

Schneider et al. (2014a) 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 which use gas chromatography (Buttery et al., 1976; Kuebler, 2010), reversed-phase HPLC (Herzallah & Holley, 2012; Ishikawa et al., 2014; Pelosi et al., 2014; Tsao et al., 2002; Wilson et al., 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.

6.2 Materials and methods

6.2.1 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.

6.2.2 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).

6.2.2.1 Panelists

The panel for the TI study consisted of 14 assessors (9 females, 5 males; age range: 19-54). 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.

The panelists were compensated for their participation with sweets.

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

6.2.2.2 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. (2014a). 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_{Dec}) 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.

6.2.2.3 Samples

Eight commercially available mustard products were evaluated in duplicate with the TI testing protocol. Table 6.1 lists the product characteristics such as pungency declaration, nutritional values and moisture content. The moisture content was determined 3-fold using infrared moisture analyzer MA35 (Sartorius, Göttingen, Germany).

Table 6.1 Pungency classification, nutritional facts and moisture content of commercial mustard products

Mustard product	Pungency classification[1]	Fat[2] (g)	Carbohydrates[2] (g)	Protein[2] (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*, 3-fold determination

6.2.2.4 TI test procedure

TI tests were performed according to DIN 10970 (2002), in individual booths of a sensory laboratory which conformed to DIN EN ISO 8589 (2010) standards. The TI data were recorded and analyzed using the FIZZ sensory software (Version 2.51, Biosystèmes, Couternon, 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.

6.2.2.5 Data processing and analysis

Individual TI data were processed using the FIZZ sensory software. Significant differences in pungency perception (individual I_{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.

6.2.3 HPLC analysis of mustard products

6.2.3.1 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.

6.2.3.2 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 multiwavelength Photodiode-Array-UV detector using the ReproSil-Pur 120 C18-AQ, 5 µm (250 x 4.6 mm) column (Dr. A. Maisch GmbH, Ammerbuch-Entringen, Germany).

6.2.3.3 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.

6.2.3.4 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).

6.2.3.5 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 1300 *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.

6.3 Results and discussion

6.3.1 Sensory evaluation of the pungency perception of commercial mustard products

The results of the sensory evaluation of pungency perception of mustard products are displayed in Table 6.2 and Fig. 6.1-6.3. I_{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 6.1) may explain these differences. Firstly, products A and B are classified as sweet mustard products, which are characterized by a high carbohydrate content, especially sugars, of approximately 40%. Kostyra et al. (2010) and Schneider et al. (2014a) 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 et al., 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 et al., 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.

Secondly, 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 6.2), 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 of fat in studies analyzing capsaicin pungency perception (Baron & Penfield, 1996; Carden et al., 1999; Kostyra et al., 2010; Schneider et al., 2014a). Fat inhibits the interaction between the chemical irritant and the trigeminal receptor via two mechanisms, namely by forming a barrier (Lynch et al., 1993) and by trapping the irritant, due to the high solubility of AITC in oil. Furthermore, the higher viscosity of fat-based matrices may have suppressive effects on pungency perception (Eib, Ramos Gajek, et al., 2020; Kostyra et al., 2010; Schneider et al., 2014a).

Table 6.2 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[1]	I_{max}[2]	Dur_{Dec} (s)	*AUC*	AITC concentration fresh weight[3] (mg/100 g)	AITC concentration dry weight[4] (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

[1] Mustard product designation according to Table 1

[2] a, b, c, d –I_{max} mean values with different letters in columns are significantly different, $p < 0.05$

[3] Values in mg/100 g ± *SD* of fresh weight; *n* = 3

[4] Values in mg/100 of dry weight; *n* = 3

The spread of the I_{max} data is illustrated with box plots in Figure 6.1. 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., 2014a) 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 calculated.

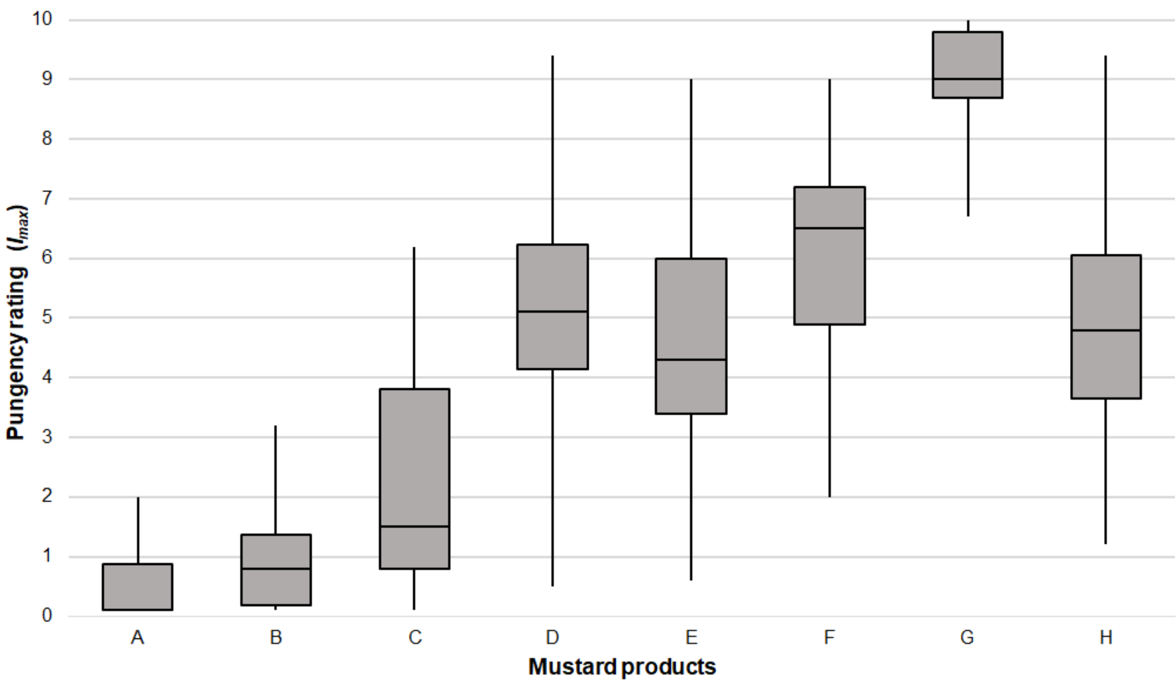

Figure 6.1 Sensory ratings (I_{max}) of commercial mustard products (scale 0–10; 0 = "not detectable", 10 = "extremely pungent")

Furthermore, the average curves of the pungency rating (Figure 6.2) 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_{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_{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_{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.

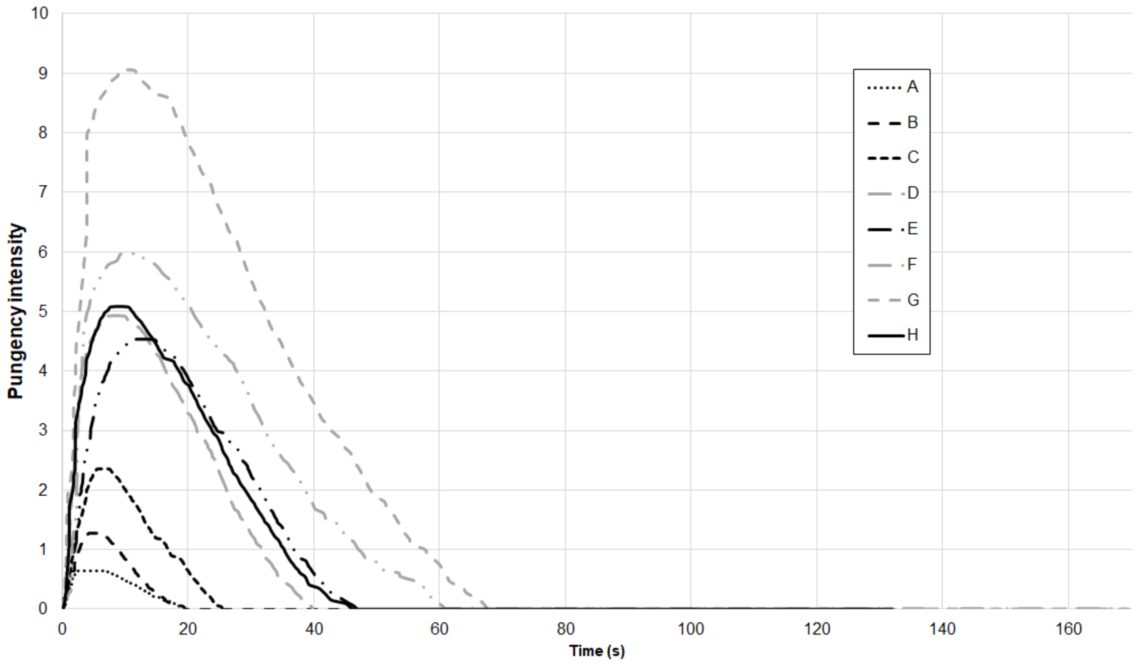

Figure 6.2 Time intensity (TI) average curves of mustard products (A–H designation according to Table 1). These curves were calculated as the mean values of the normalized TI data (scale 0–10; 0 = "not detectable", 10 = "extremely pungent")

The panelists were asked to localize the pungency sensation at I_{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 6.3. 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 et al., 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, Ramos Gajek, 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.

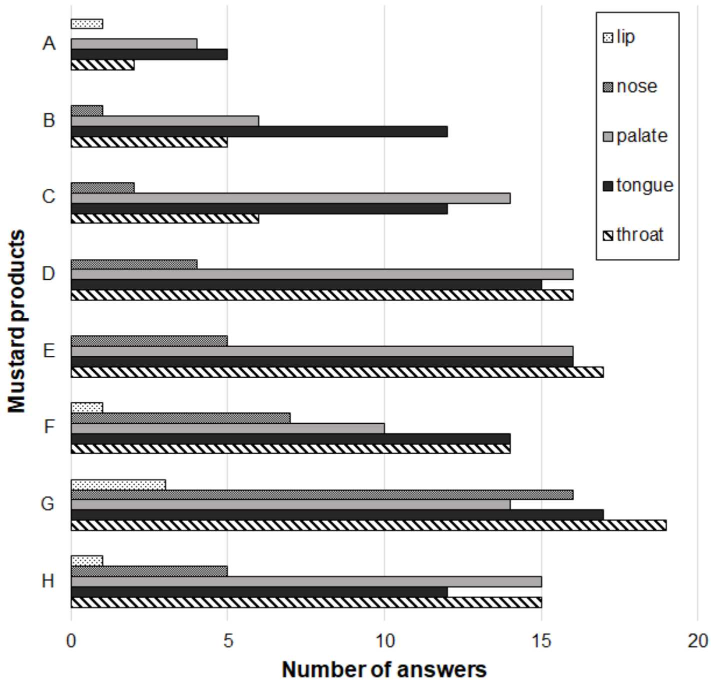

Figure 6.3 Localization of pungency sensation at maximum pungency intensity (I_{max})

6.3.2 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 6.4.

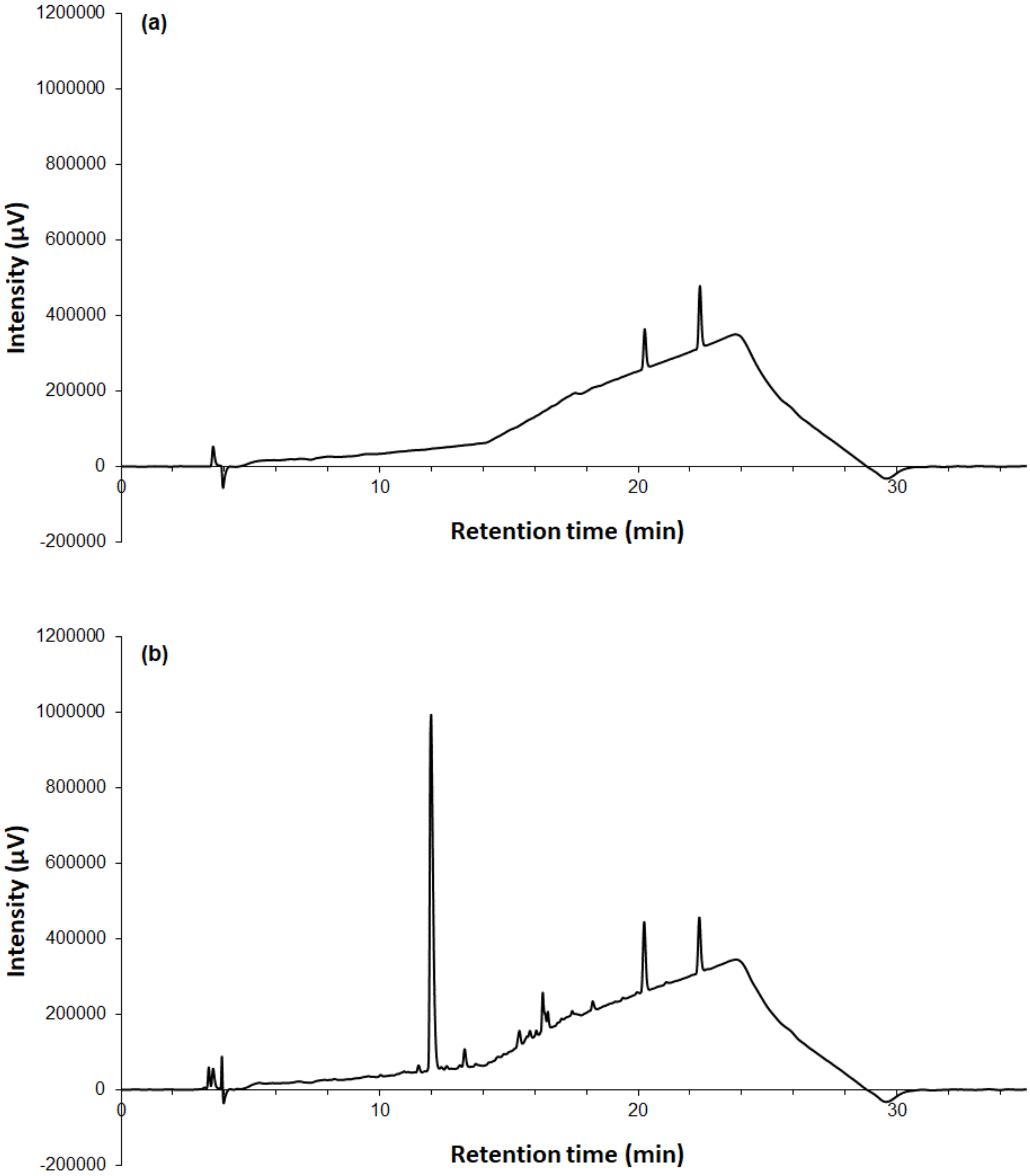

Figure 6.4 Quantitative liquid chromatogram of (a) AITC (2 mg/10 mL) and BITC (3 mg/10 mL), and (b) mustard product with the pungency declaration "extra hot" (product G)

The AITC concentration in the analyzed mustard products was highly variable, as shown in Table 6.2 and Figure 6.5. 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_{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., 2014a). 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 et al., 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; Kjær et al., 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 alb*a 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*) (Hälvä et al., 1986; Velíšek et al., 1995; Zrybko et al., 1997). Extra hot mustard products are produced from a high amount of mustard seeds which 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.

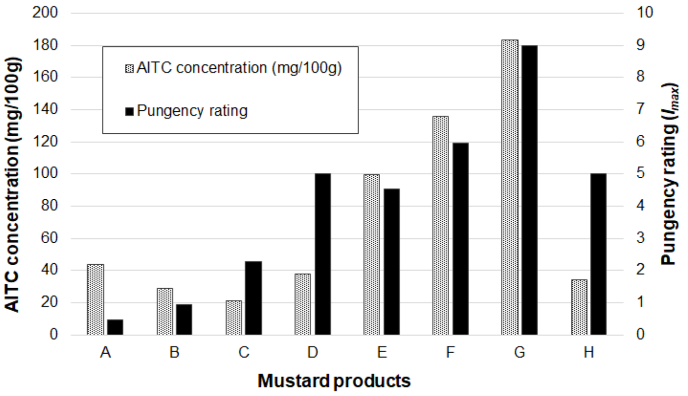

Figure 6.5 Sensory pungency rating maximum pungency intensity (I_{max}), mean value; scale 0–10; 0 = "not detectable", 10 = "extremely pungent") and analytically determined AITC concentration of commercial mustard products

Furthermore, storage and production processes may influence isothiocyanate concentrations (Stahl et al., 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 (C. W. Chen & Ho, 1998; Hanschen et al., 2014; Kawakishi & Namiki, 1969).

6.3.3 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 6.5 shows that most products with a high AITC content were rated with high I_{max} values (products F and G), while some products with moderate I_{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_{max} (correlation coefficient 0.803), *AUC* (correlation coefficient 0.891), and DUR_{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 supplemental material 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_{max}, *AUC*, and DUR_{Dec}) of the mustard products. The correlation between the AITC concentration, the moisture content and I_{max} (correlation coefficient 0.944), *AUC* (correlation coefficient 0.960), and DUR_{Dec} (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, Ramos Gajek, 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 fat 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_{max}, *AUC*, DUR_{Dec}).

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 3 times. Additionally, Schneider et al. (2014a) 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.

6.4 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 (supplemental material).

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6.6 Author contributions

Study concept and design: Eib, Schneider, and Seuß-Baum. Analysis and interpretation of data: Eib. Drafting of the manuscript: Eib. Critical revision of the manuscript for important intellectual content: Eib, Schneider, Hensel, and Seuß-Baum. Statistical analysis: Eib. Study supervision: Eib and Seuß-Baum.

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7 Overall discussion

Hereinafter, the results are summarized and discussed on the basis of the defined hypotheses and relevant literature.

7.1 Discussion of hypotheses

To review the hypotheses tested in this work, an overview of the verified, partially verified, and falsified hypotheses is presented in Table 7.1. Additionally, a detailed discussion of the verification is presented below.

Table 7.1 Overview of tested hypotheses

1st Hypothesis	*The sensory detection thresholds of AITC and sinigrin differ significantly in the threshold concentration level.*	verified
2nd Hypothesis	*The sensory detection thresholds of AITC of mustard users and nonusers differ significantly.*	falsified
3rd Hypothesis	*AITC is perceived less pungent in oil- than in water-based matrices because of its lipophilic character.*	verified
4th Hypothesis	*Sensory pungency evaluation involving parameters such as maximum perceived pungency intensity (I_{max}), area under the curve (AUC), and duration of decreasing phase (DUR_{Dec}) depends on both the AITC concentration and composition of the carrier matrix.*	partially verified
5th Hypothesis	*The values of the sensory TI parameters of commercial mustard products do not differ significantly (α = 5%) from those of the mustard-based carrier matrix with comparable AITC concentration.*	partially verified
6th Hypothesis	*Localization of the AITC pungency perception in the mouth, throat, and nose depends on the AITC concentration.*	verified
7th Hypothesis	*The sensory pungency evaluation (TI parameter) of mustard products can be related with the quantified AITC content of these products.*	verified

1st Hypothesis

The sensory detection thresholds of AITC and sinigrin differ significantly (α = 5%) in the threshold concentration level.

The sensory detection threshold of AITC and its precursor sinigrin differed significantly ($p < 0.5$) in water-based matrices. The detection threshold of AITC was 0.134 mg/100 mL, whereas that of sinigrin was 10.928 mg/100 mL. Consequently, the 1st hypothesis was verified.

The determined group detection threshold for sinigrin is in accordance with the group threshold of 10.6 mg/100 mL (Fenwick et al., 1983). The olfactory threshold of AITC in air has been reported to be

2.7–24.0 × 10^{-6} mg/100 mL (Stone et al., 1967). In 1976, an olfactory threshold of 37.5 mg/100 mL for AITC in an aqueous carrier was determined by Buttery et al. (1976). Further, in 2020, Marcinkowska and Jeleń reported that the olfactory detection threshold of AITC was 0.009 ± 0.022 mg/100 mL. The estimated olfactory threshold values quoted cannot be directly compared to the oral threshold determined in this study. The published detection thresholds are inconsistent, which illustrates the considerable influence of the experimental method, purity of the standards used, panel composition, and size (Marcinkowska & Jeleń, 2020). The olfactory threshold describes the sensation induced by inhaled chemical irritant molecules stimulating receptors located in the nasal cavity (Buettner, 2017; Diaz, 2004), whereas the oral threshold considers the dynamic release of chemical stimuli in the mouth, which is influenced by matrix composition, temperature, and saliva (Burdach & Doty, 1987; Diaz, 2004; Heilmann & Hummel, 2004).

The GSL sinigrin is the precursor of the isothiocyanate AITC (MacLeod & MacLeod, 1970); however, their threshold levels have been found to be significantly different (Eib, Ramos Gajek, et al., 2020). Additionally, both induce different sensory impressions. Sinigrin is associated with the perception of the basic taste bitter (Bell et al., 2018), whereas AITC induces a trigeminal pungency sensation through an interaction with the free nerve endings of the pain receptors in the mouth, nose, and eyes (Fenwick et al., 1983; Jordt et al., 2004).

2nd Hypothesis

The sensory detection thresholds of AITC of mustard users and nonusers differ significantly (α = 5%).

The sensory thresholds of mustard users and nonusers did not differ significantly in all evaluations. Therefore, no impact of the mustard consumption frequency of the panelists could be confirmed. The influence of the consumption frequency and/or liking of pungent products on their stimulus threshold level or sensory perception in general is discussed, especially for capsaicin and capsaicin-containing products. Several factors affect the evaluation independent of the chemical stimulus studied. First, the criteria and questionnaire used for classification essentially influence group composition. In this work, panelist classification resulted from the subjective completion of a questionnaire based on previous experience with pungent and mustard products. Classification criteria and questionnaire were adapted from Reinbach et al. (2007). Among other questions, panelists rated their preferred pungency intensity on a scale, which might have been interpreted differently by the panelists. This differing interpretation of questions might have influenced the classification into user groups (Eib, Ramos Gajek, et al., 2020). Furthermore, panel composition and size might not have been adequate to determine the influence of mustard consumption frequency on the threshold level. In the main study, the mustard user group consisted of 10 panelists (5 females and 5 males), whereas the nonuser group consisted of 54 panelists (40 females and 14 males). Furthermore, the age range of the panelists was 19-38 years (Eib, Ramos Gajek, et al., 2020). To enhance the robustness of the results, a larger panel, with an even distribution of users and nonusers, and a wider age range would have been beneficial. Additionally, future panels should consist of equal numbers of male and female subjects as gender-dependent differences in pungency preferences have been previously reported (Nolden & Hayes, 2017; Schlossareck & Ross, 2020).

Moreover, Lawless et al. (1985) and Prescott and Stevenson (1995) confirmed that the consumption frequency influenced panelists' responsiveness to capsaicinoids. In their studies, regular chili users were less sensitive to capsaicinoids than nonusers (Lawless et al., 1985; Prescott & Stevenson, 1995). However, other researchers did not confirm this hypothesis (Nasrawi & Pangborn, 1989; Orellana-Escobedo et al., 2012; Schlossareck & Ross, 2019; Schneider et al., 2014b).

3rd Hypothesis

AITC is perceived less pungent in oil- than in water-based matrices because of its lipophilic character.

The research confirmed the hypothesis that the sensory-perceived pungency of AITC in oil-based matrices is less intense than that in water-based matrices. The hypothesis was reviewed using the results of the detection threshold and TI studies, which were both conducted with AITC in water- and oil-based matrices. Several factors might be responsible for the reported significantly lower responsiveness of the panelists to AITC in oil- than in water-based matrices, as discussed in Chapter 5.4.

First, because of their lipophilic character, AITCs are highly soluble in oil-based matrices. Thus, the interaction of AITC in oil-based matrices with the trigeminal receptor is weaker than that in water-based matrices, and the induced pungency sensation is consequentially reduced. (Baron & Penfield, 1996; Lawless et al., 2000) This effect might be intensified by the ability of oil to form a mouthcoating, preventing the stimuli from accessing the receptors (Lynch et al., 1993).

Furthermore, the mechanisms of AITC release from the matrix influence sensory perception. In this context, the process of food consumption and environmental factors including temperature, surface of exchange, and individual characteristics such as saliva flow might be of major influence (Cayot et al., 2008). Additionally, the partition coefficients of AITC indicate the partitioning of AITC into the oil phase in fat-containing matrices (Zhang et al., 2010). Thus, the amount of AITC in the aqueous phase is reduced. Consequently, as only flavor compounds in the aqueous phase may stimulate the dedicated receptors, the pungency sensation is also affected (McNulty & Karel, 1973).

4th Hypothesis

Sensory pungency evaluation involving the parameters such as maximum perceived pungency intensity (I_{max}), area under the curve (AUC), and duration of decreasing phase (DUR_{Dec}) depends on both the AITC concentration and the composition of the carrier matrix.

The sensory pungency perception of AITC depends significantly on the AITC concentration and the composition of the carrier matrix. This hypothesis was partially confirmed by evaluating the sensory TI parameters I_{max}, *AUC* and DUR_{Dec} for various AITC concentrations in water-, oil-, and mustard-based carrier matrices as illustrated in Figure 7.1.

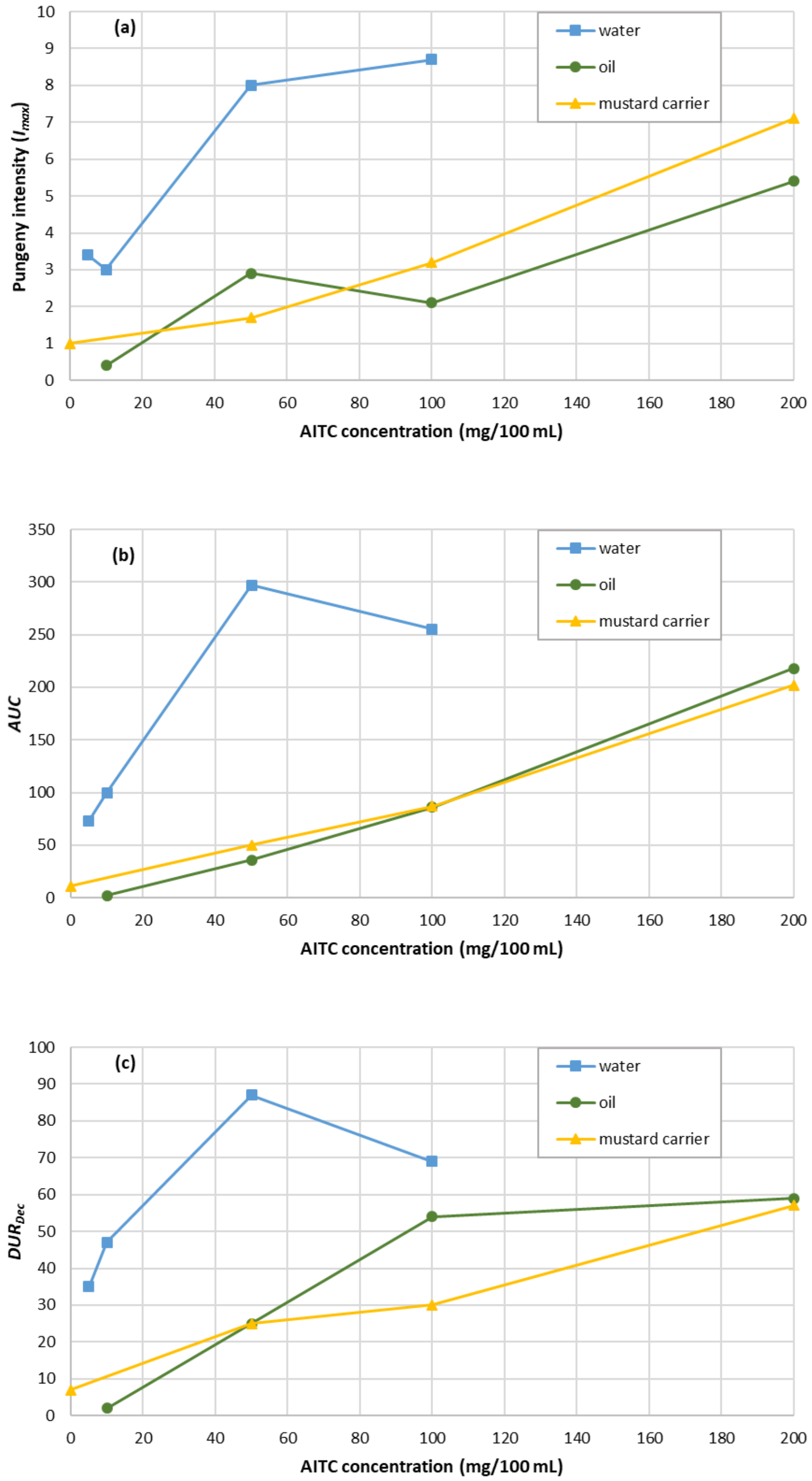

Figure 7.1 Comparison of the (a) maximum perceived pungency intensity rating (I_{max}), (b) area under the curve (*AUC*) and (c) duration of decreasing phase (DUR_{Dec}) in water-, oil-, and mustard carrier-based matrices for various AITC concentrations

With increasing AITC concentrations, the sensory parameters I_{max}, *AUC* and DUR_{Dec} increased, with a few exceptions. The results show that between 50 and 100 mg AITC/100 mL, the water-based matrix *AUC* and DUR_{Dec} decreased. The *AUC* values of 50 and 100 mg AITC/100 mL in the water-based matrix were not significantly different, whereas the DUR_{Dec} values under the same conditions differed significantly. In the oil-based matrix, I_{max} (calculated for 100 mg AITC/100 mL) was lower than that for 50 mg AITC/100 mL; however, both were not significantly different from each other. One explanation might be the greater variation in-between the panel than between the AITC concentrations (50 and 100 mg/100 mL) of the water- and oil-based matrices.

The influence of the water- and oil-based AITC carrier matrices on pungency perception is discussed in detail in Chapter 5.4 and in the 3rd hypothesis. Additionally, the influence of the mustard model carrier is reviewed in the 5th hypothesis. Therefore, no further evaluation of the matrices is stated in this context.

5th Hypothesis

The values of the sensory TI parameters of commercial mustard products do not differ significantly (α = 5%) from those TI results of the mustard-based carrier matrix with comparable AITC concentration.

Sensory studies point out that their results might only be partially applicable to commercial mustard products. The TI study demonstrated that the induced pungency sensation of AITC in the mustard carrier matrix was in-between that in the oil-based and water-based carrier matrices. Despite the fact that the sensory perception of added AITC in the mustard carrier-based matrix seems to be comparable to that of the commercially available mustard products, statistical evaluation identified significant differences, as shown in Table 7.2.

Table 7.2 Comparison of maximum perceived pungency intensity ratings (I_{max}) of commercial mustard products and mustard carrier samples with similar AITC concentrations

Commercial mustard products				Mustard carrier for comparison		
Product designation[1]	**Pungency classification[2]**	**AITC concentration commercial product[3]**	I_{max}[4]	**AITC concentration mustard carrier[5]**	I_{max}[4]	**Statistic ANOVA (α = 0.05)**
A	Sweet	43.9 (± 3.5)	0.5	50	1.7	p <0.05
D	Medium hot	38.1 (± 4.9)	5.0	50	1.7	p <0.05
E	Medium hot	99.7 (± 1.7)	4.5	100	3.2	p <0.05
F	Hot	135.6 (± 1.8)	5.9	100	3.2	p <0.05
F	Hot	135.6 (± 1.8)	5.9	200	7.1	p <0.05
G	Extra hot	183.1 (± 1.9)	9.0	200	7.1	p <0.05

[1] Mustard product designation according to Eib, Schneider, et al. (2020b)

[2] According to the voluntary pungency classification labeled on the products

[3] Values in mg/100 g ± *SD* of fresh weight; *n* = 3

[4] I_{max} mean values based on individual curves

[5] Values in mg/100 g ± *SD* of fresh weight; added AITC concentration

The I_{max} values of the commercial mustard products were significantly different from those of the mustard carriers with comparable AITC concentrations. These differences may be explained by the diversity of mustard products compositions, respectively ingredients. As indicated by the results of the detection threshold and the TI studies, the fat content of a product influences the sensory perception of pungency (Eib, Ramos Gajek, et al., 2020; Eib, Schneider, et al., 2020a). This observation is consistent with the results reported by Carden et al. (1999) and Schneider et al. (2014a). Additionally, Carden et al. (1999) discovered that increasing the fat content of cheese sauces resulted in a decrease in the capsaicin-induced pungency intensity. Schneider et al. (2014a) reported that capsaicin pungency scores decreased as the fat level of the cream-based model food matrices increased.

The results indicate that sugar affects pungency perception, as sweet mustards (products A and B) were rated least pungent. The I_{max} values of both mustard products were significantly different from that of the product with a comparable AITC concentration (product D). Other researchers have previously reported a relationship between the sugar content, sweetness, and capsaicin-induced pungency (Nasrawi & Pangborn, 1989; Prescott et al., 1993). A study by Lee and Kim (2013) indicated that sucrose reinforced the capsaicin pungency-reducing effect of fat. One possible explanation for the pungency-reducing effect of sweetness is the production of enjoyable emotions (Steiner et al., 2001). Sucrose has the ability to reduce pain and raise the pain threshold (Blass & Shah, 1995; Ramenghi et al., 1996). This effect is mediated by endogenous opioids or the stimulation of the “pleasure center” in the central nervous system (Blass & Shah, 1995; Nasrawi & Pangborn, 1989), which could explain the low pungency scores of sweet mustard products.

Another influencing factor may be the acidity of the product, as protons can activate the TRPV1 receptor (Tominaga et al., 1998).

Furthermore, product viscosity influences the retention time of pungent stimuli on the oral and pharyngeal mucosa owing to the formation of a coating layer (Doyennette et al., 2011). A long retention time of pungency-inducing stimuli may result in a high overall pungency evaluation (De Loubens et al., 2011). Moreover, Hutchinson et al. (1990) reported that products with a high dry matter contents, such as rice, most effectively reduced capsaicin-induced pungency. The dry matter content of the mustard model carrier was approximately 23.4%, whereas those of the commercial products ranged from 23.0 to 55.4%. According to McNulty and Karel (2007), only stimuli dissolved in an aqueous phase can induce a sensation. Thus, increasing dry matter content lowers the share of the aqueous phase in which pungency-inducing isothiocyanates could have partitioned. Additionally, the sweet mustard products tested were grainy; thus, the differing tactile sensation might have influenced pungency intensity (Hutchinson et al., 1990).

For more detailed insight into the reason the mustard-based carrier matrix was significantly different from commercial mustard products with comparable AITC concentrations, further research and a differing approach will be necessary. In addition to the sensory tests of AITC in water- and oil-based carrier matrices, further matrices with increasing complexity could have been tested. This approach would have enabled a conclusion regarding the influence of particular mustard ingredients and combinations of ingredients. In this context, (Kostyra et al. (2010) used carriers of different complexities to evaluate their influence on capsaicin-induced pungency. A more precise method was previously applied by Schneider et al. (2014a) who evaluated the influence of four basic matrices and combinations of those with increasing complexities on pungency. Both studies indicated that evoked pungency sensations were affected by carrier type and complexity.

The development of a sensorial non-pungent mustard carrier matrix was successfully realized by the systematic adaption of the mustard paste production process at the laboratory scale. A sensory comparison test, pH value measurements, and dry weight determination confirmed the reproducibility of the production process. The absence of 4-HBITC and AITC was confirmed via HPLC analysis.

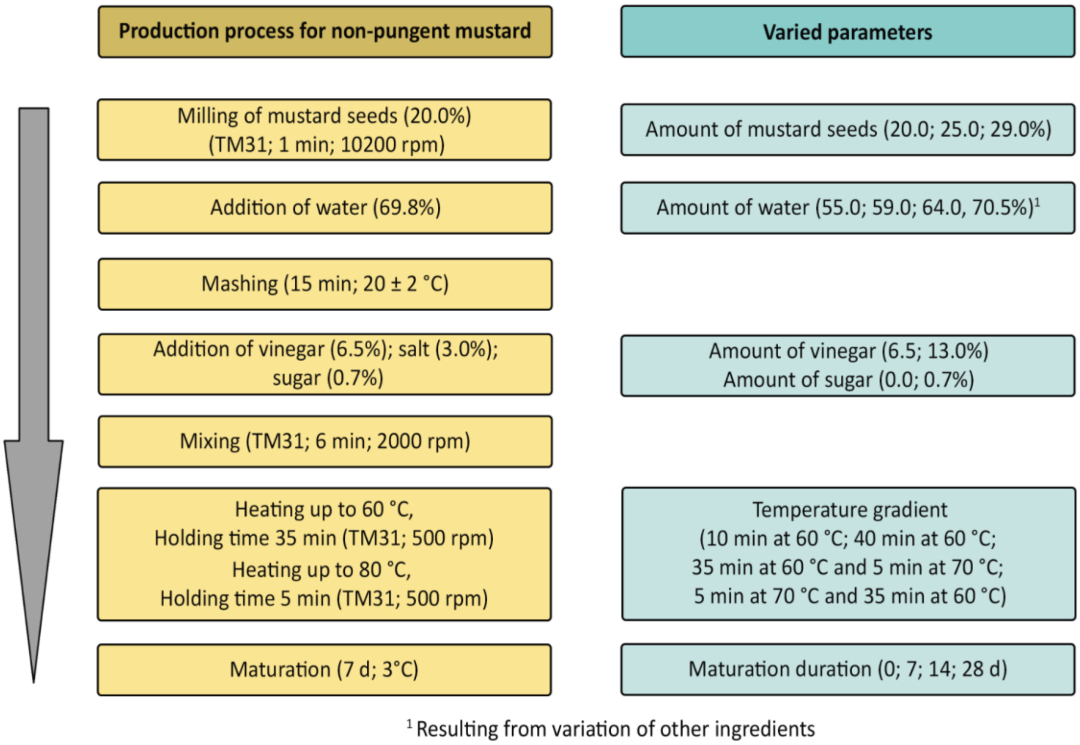

Figure 7.2 Development of non-pungent mustard carrier matrix including the final production process (left) and varied parameters (right)

During product development several process parameters and mustard composition were varied, as shown in Figure 7.2. The reduction of mustard pungency was achieved by partial hydrolysis of GSL. The addition of water to the ground mustard seeds activates and accelerates the formation of isothiocyanates, as water is a substrate in this reaction (Dai & Lim, 2014). Subsequently, the enzyme myrosinase was inactivated by heat to stop the formation of isothiocyanates. According to Sindhu et al. (2012), enzyme inactivation reduced pungency. Nevertheless, partial hydrolysis of GSL was desired to decrease the bitterness of the mustard matrix (Sindhu et al., 2012). Furthermore, the temperature profile was adapted to remove the volatile isothiocyanates from the mustard matrix (Mustakas et al., 1962), and few degradation products affecting the sensory properties were formed. Mustakas et al. (1962) developed a process to produce non-pungent edible mustard oils. Therefore, the ground mustard was mashed with water to induce the conversion of sinigrin to AITC and subsequently underwent a combined moisture and heat treatment completed with distillation to remove volatile oil. Moreover, Sindhu et al. (2012) used an approach based on the same principle to reduce bitterness and retain pungency in mustard powder.

In summary, the 5th hypothesis was partially verified as the use of the mustard model carrier was suitable and the variation in between commercially mustard products was too wide to be covered by one model carrier. The use of simple water- and oil-based carriers was indispensable to understand the influence of the matrix on the complex process of AITC induced-pungency perception.

6th Hypothesis

Localization of the AITC pungency perception in the mouth, throat, and nose depends on the AITC concentration.

The location of the AITC pungency perception shifted with increasing AITC concentrations from a predominant sensation on the tongue to the throat, which was independent of the carrier matrix. Additionally, the pungency perception in the nose increased with increasing AITC concentrations. This observation was confirmed by all sensory studies conducted. In addition, the location of the sensation depended on the time of the rating as previously reported for capsaicin and piperine by Rentmeister-Bryant and Green (1997). Panelists described an increase in the pungency perception in the throat and nose after each swallowing act. This may be explained by the specific release of volatile stimuli during sample incorporation and swallowing (Cayot et al., 2008). The release process consists of three phases (Buettner et al., 2002). In the first phase, the sample is incorporated into the oral cavity, diluted with saliva, and volatile compounds merge into the gas phase (Doyennette et al., 2011). In this phase, AITC can only stimulate receptors located in the oral cavity (tongue and palate) to induce a pungency sensation. In the second phase, the product saliva mixture is swallowed and passes through the pro-pharynx (Buettner et al., 2002). Depending on the viscosity, a layer of product saliva mixture is left on the pharynx (Doyennette et al., 2011). AITC is distributed into the throat and may stimulate the TRP ion channels located there, depending on the viscosity of the AITC carrier, and the duration of the pungency perception after swallowing may vary. In the third phase, the so-called "swallow-breath" (Buettner et al., 2002), volatilized aroma compounds are distributed in the air and pass into the pharynx and upper airways (Doyennette et al., 2011). Volatilized AITC may circulate in the throat and nose to induce a pungency sensation. With increasing dilution of the AITC-containing product with saliva, the AITC concentration in the mixture decreases, resulting in a reduced pungency perception over time. Thus, the temporal aspect of the localization of the pungency perception may be explained by the dynamics of sample incorporation and swallowing. Furthermore, the hydrophobicity of AITC may influence its localization as AITC partitions into the oil phase (Zhang et al., 2010). AITC may only induce pungency sensations diluted in aqueous phases (McNulty & Karel, 1973). With the increasing dilution of the product with saliva, more AITC partitions into the water and air phases to induce a pungency sensation (McNulty & Karel, 1973). However, the overall AITC concentration reduces owing to dilution. Additionally, fat is able to form a coating in the mouth and pharynx, inhibiting AITC from reaching the receptors located in the oral cavity and throat (Lynch et al., 1993).

Other studies reported the locations of the capsaicin-induced burn (Allison et al., 1999; Rentmeister-Bryant & Green, 1997; Schneider et al., 2014b). Contrary to these findings, Allison et al., 1999 described a more intense capsaicin-induced pungency sensation on the tongue than in the throat. Nevertheless, Rentmeister-Bryant and Green (1997) and Schneider et al. (2014b) reported the higher responsiveness of the throat than of the tongue to capsaicin. These contradictory findings may be explained by the different concentration levels of the stimuli used in these studies.

In general, changes in the study design, especially the predefined sample intake process and timing, may influence the pungency sensation of the panelists. Furthermore, precise localization of the pungency sensation is difficult. A large trained panel, particularly on the localization of pungency, might have led to more reliable and consistent results.

7th Hypothesis

The sensory pungency evaluation (TI parameters) of mustard products can be correlated with the quantified AITC content of these products.

To review the 7th hypothesis, an HPLC quantification method for AITC in mustard products needs to be adapted. Therefore, the HPLC method published by Franco et al. (2016) was adapted, and the extraction procedure suggested by Pelosi et al. (2014) was also adjusted. The mustard sample preparation process and HPLC method are illustrated in Figure 7.3.

The sample preparation method published by Pelosi et al. (2014) was adapted by varying the composition and addition sequence of the extraction agent, the duration of sample homogenization on an orbital shaker and applied speed, utilization of the ultrasonic bath, application of an Ultra-Turrax instead of an ultrasonic bath, and the temperature of centrifugation. Initially, the extraction method proposed by Tsao et al. (2002) was tested; however, its use was rejected. The insufficient separation of suspended solids caused rapid clogging of the filter. Additionally, quantification of AITC in the supernatant suggested that AITC was not completely extracted and dissolved in the extraction medium. Tsao et al. (2002) used water as the extraction medium. Because of the lipophilic nature of AITCs, unblended water may not be an adequate extraction medium.

The HPLC method was validated as a multiple-point internal standard quantification method with a calibration curve $y = 0.9929 * x * 0.0041$. Improvement of the quantification precision was achieved by integrating the dilution factor of the sample (6.5) and the correction of the quantification curve by relocating the point of interception into the origin, resulting in the following quantification equation:

$$y = 0.99 * x * 6.5$$

where x = (AITC concentration / BITC concentration) and y = (AITC peak area / BITC peak area)

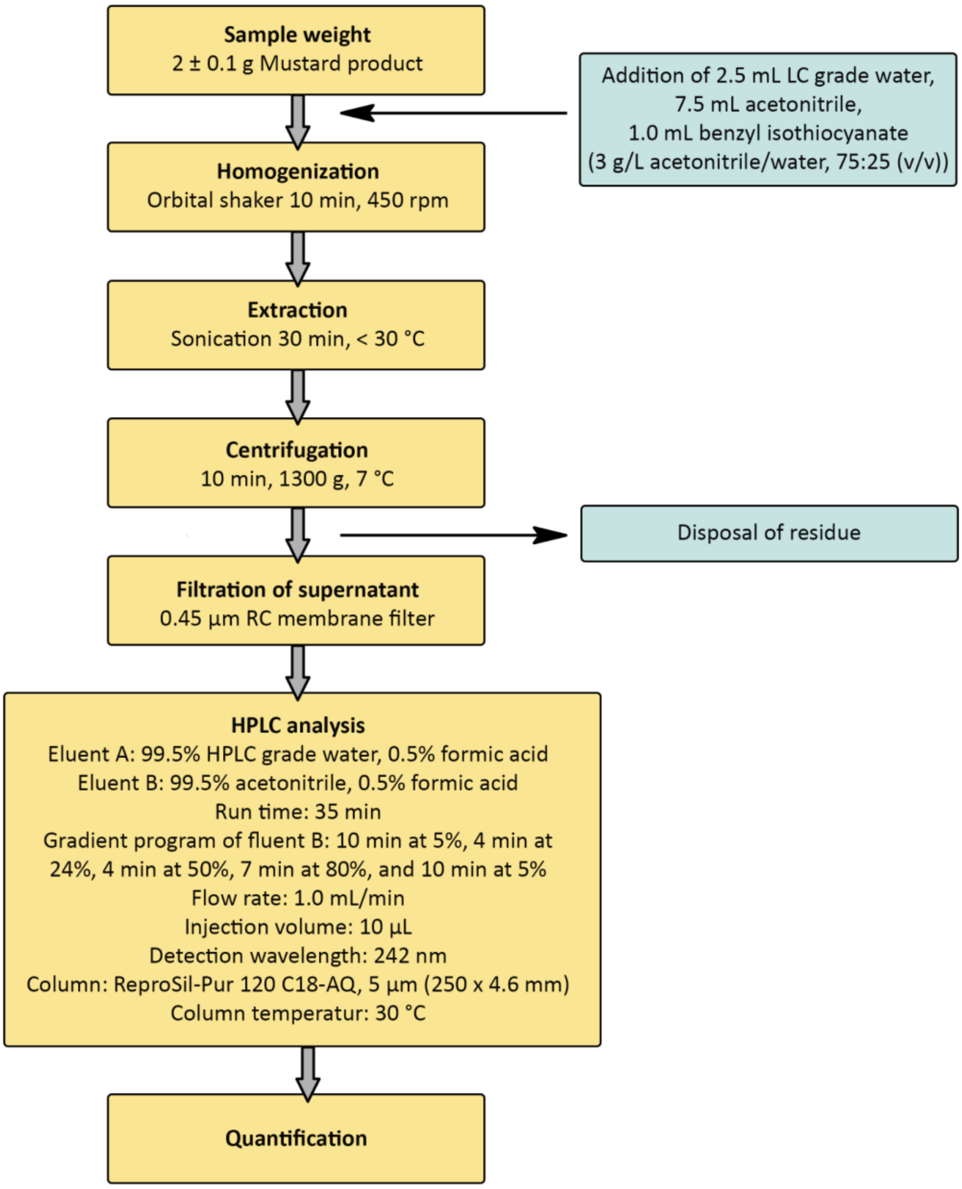

Figure 7.3 HPLC method for the quantification of AITC in mustard products

The sensory-perceived maximum pungency intensity (I_{max}), TI parameter *AUC,* and duration of pungency perception decline (DUR_{Dec}) could be positively correlated with the AITC content of commercially available mustard products.

The prediction of I_{max} of mustard products may be carried out using the following equation:

$$I_{max} = -5.9129 + 0.1207 * \text{moisture content} + 0.0349 * \text{AITC concentration}$$

This model explains approximately 85% of the variance in I_{max}. The models for the sensory parameters *AUC* and DUR_{Dec} are displayed in Table 7.3.

Table 7.3 Results of the regression analysis for the correlation of the maximum perceived pungency intensity (*I_{max}*), area under the curve (*AUC*) and duration of decreasing phase (*DUR_{Dec}*) each with the AITC concentration of commercial mustard products

	I_{max}	*AUC*	DUR_{Dec}
Model equation	$I_{max} = -5.9129 + 0.1207*MC + 0.0349*c_{AITC}$	$AUC = -149.1065 + 3.1831*MC + 1.4458*c_{AITC}$	$DUR_{Dec} = -22.4310 + 0.5918*MC + 0.2201*c_{AITC}$
Correlation coefficient	0.9435	0.9597	0.9543
R^2 adjusted	0.8464	0.8894	0.8752

MC is the moisture content in %M.

c_{AITC} is the AITC concentration in mg/100 g.

Further improvement of the established models could be achieved by integrating additional independent variables such as the content of the ingredients 4-HBITC, carbohydrate, especially sugar, vinegar, and fat.

Mustard products may contain the pungency-inducing compound 4-HBITC (Shankaranarayaba et al., 1972). Therefore, tests to quantify 4-HBITC using the developed HPLC method were carried out. Analysis of the 4-HBITC standard showed that during the process, the substance was decomposed into several unidentified compounds. Nevertheless, some improvements (change in solvent composition, sample handling and cooling during sample storage in the HPLC autosampler) enabled the analysis of 4-HBITC with a low measurement precision. The resulting quantification equation is as follows:

$$y = 2.6869 * x * 6.5$$

where x = (4-HBITC concentration / BITC concentration) and y = (4-HBITC peak area / BITC peak area)

The amounts of AITC and 4-HBITC determined in various commercially available mustard products are displayed in Table 7.4.

Table 7.4 Allyl isothiocyanate (AITC) and 4-hydroxybenzyl isothiocyanate (4-HBITC) concentrations of commercial mustard products

Mustard product[1]	AITC concentration fresh weight[2] (mg/100 g)	4-HBITC concentration fresh weight[2] (mg/100 g)
A	43.9 (± 3.5)	32.9 (± 1.6)
B	28.8 (± 0.7)	16.2 (± 2.6)
C	21.3 (± 0.4)	30.4 (± 3.0)
D	38.1 (± 4.9)	12.5 (± 3.1)
E	99.7 (± 1.7)	0 (± 0)
F	135.6 (± 1.8)	0 (± 0)
G	183.1 (± 1.9)	0 (± 0)
H	34.0 (± 0.7)	0 (± 0)

[1] Mustard product designation according to Eib, Schneider, et al. (2020b)

[2] Values in mg/100 g ± *SD* of fresh weight; $n = 3$

The influence of the 4-HBITC content of mustard products on the correlation of the chemical-analytically and sensory-determined parameters was evaluated by adding the 4-HBITC concentration to the previously described regression analysis. Integration of the 4-HBITC content in the regression analysis increased the correlation coefficients and the adjusted R^2 for I_{max} (correlation coefficient = 0.9778; R^2 = 0.9232), *AUC* (correlation coefficient = 0.9748; R^2 = 0.9130), and DUR_{Dec} (correlation coefficient = 0.9889; R^2 = 0.9612). However, the effect was not significant for I_{max} and *AUC*.

A precise quantification of 4-HBITC was not achieved. As previously reported by Ekanayake et al. (2012) and Kuebler (2010), the instability of 4-HBITC hinders its quantification capability. This might be a reason for the insignificant correlation of the 4-HBITC content of commercial mustard products with the sensory parameters I_{max} and *AUC*.

However, inclusion of the chemical-analytically determined 4-HBITC content, carbohydrate content, and fat content, as labeled on the products, into the regression analysis did not influence the determination coefficient R^2 significantly. Further research is needed to improve the established models. First, the starch, sugar, and fat content should be analytically determined as the content may deviate from the specifications provided by the producer. Second, the HPLC quantification method for 4-HBITC needs to be improved to obtain reliable results.

Summarily, the 7th hypothesis was verified, and positive correlations between the AITC concentration and sensory parameters I_{max}, *AUC* and DUR_{Dec} were confirmed.

7.2 Outlook

Taken together, pungency-imparting compounds of mustard products were analyzed using human sensory and chemical–analytical evaluations. Models that describe the influence of the AITC content of mustard products on the sensory TI parameters (I_{max}, *AUC* and DUR_{Dec}) have been established based on the evaluations.

Finally, the classification of mustard products into pungency categories needs to be considered. To define these categories, classification into four categories (mild, medium pungent, pungent, and extra pungent) seems adequate as this covers the majority of mustard products. A recommendation to classify mustard products based on the AITC content is illustrated in Figure 7.4.

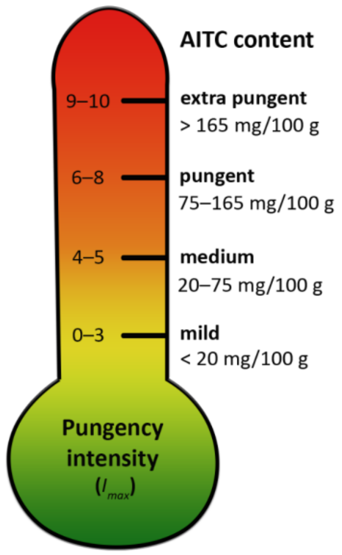

Figure 7.4 Classification of mustard products into pungency categories based on the AITC content and maximum pungency intensity (I_{max})

However, this proposed classification is affected by numerous factors. Despite the fact that the ingredients for mustard products are explicitly defined (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015), products differ substantially in their composition (Eib, Schneider, et al., 2020b). Significant differences in the sensory evaluation of commercial mustard products among each other and with the mustard-based carrier samples with comparable AITC concentrations may be explained by these variations in the product composition. In addition to the AITC concentration, the moisture and fat content are the major components affecting pungency perception. A significant influence on the sensory TI parameters I_{max}, *AUC* and DUR_{Dec} has been confirmed for the moisture content of commercial mustard products (Eib, Schneider, et al., 2020b). Despite the fact that the statistical evaluation did not confirm the influence of fat content on the sensory TI parameters of commercial mustard products, other aspects support this assumption. The detection threshold of AITC in oil-based carriers was approximately five times higher than that in water-based carriers, demonstrating the lower responsiveness to AITC in fat (Eib, Ramos Gajek, et al., 2020). The results of the TI study support this hypothesis, showing significantly shorter pungency sensations and significantly lower pungency intensities for similar AITC concentrations in oil- than in water-based matrices (Eib, Schneider, et al., 2020a). TI parameters (I_{max}, *AUC* and DUR_{Dec}) increased with decreasing fat content of the carrier in the order oil-based matrix < mustard-based matrix < water-based matrix. These observations may be explained by the partitioning of AITC into the oil phase (Carey et al., 2002; McNulty & Karel, 1973; Zhang et al., 2010) and the ability of fat to form a mouth-coating, preventing AITC from accessing the receptors (Lynch et al., 1993).

Alongside the influence of the matrix, additional factors may influence the sensory perception of AITC-induced pungency. Among these are a preference for pungency (Ludy & Mattes, 2012; Nilius & Appendino, 2011), cultural background (Gutierreza & Simona, 2016; Rozin & Schiller, 1980), gender (Byrnes & Hayes, 2015; Spinelli et al., 2018), sensation-seeking behavior (Logue & Smith, 1986), and genetic differences such as saliva flow and composition (Horne et al., 2002; Mosca & Chen, 2017). Furthermore, the consumption frequency influences the responsiveness to pain-inducing stimuli (Lawless et al., 1985; Prescott & Stevenson, 1995). Thus, human sensory studies are multifactorial influenced measurement tools.

To support and reduce necessary sensory measurements in the food industry, chemical–analytical methods can be implemented. In this context, the pungency-inducing chemical irritant AITC was quantified in mustard products using HPLC. Thus, reproducible, reliable, and fast chemical–analytical measurements of the AITC content can be used to evaluate the expected pungency intensity of mustard products.

The use of the above-described pungency classification in the food industry would enable comparable categorization of commercial mustard products. Consequently, standardization of the pungency categories would facilitate consumers' product choice based on preferred pungency intensity.

Furthermore, the AITC concentration may decrease during product life cycle because of the volatility of the compound and depending on storage conditions (Stahl et al., 2009). As this may alter the sensory product properties and lead especially to the reduction of the pungency intensity, the date of expiry might be affected. With defined pungency categories based on the AITC concentration, compliance with the product pungency classification can be tested alongside the products shelf life.

AITC is the pungency-imparting compound in further food products (Chin et al., 1996; G. S. Clark, 1992; Gilbert & Nursten, 1972; Sultana, Porter, et al., 2003). A transferability of the results to wasabi or horseradish pastes is conceivable and may be realizable with low effort.

7.3 Discussion of own approach

To enhance the outcome of the presented studies, some modifications would have been advantageous. All sensory tests were carried out in water- and oil-based matrices. Thus, significant differences in the perception of AITC depending on the matrix were identified. Further, the influences of fat and water content on the pungency perception of AITC could have been evaluated more precisely in oil-water emulsions with varying ratios. Additional mustard ingredients such as sugar and vinegar might have a significant influence on pungency perception and sensory tests, and evaluation of the impact would have been useful. However, the experimental effort would have been immense.

The determination of the sinigrin detection threshold confirmed the previously reported findings of Fenwick et al. (1983). Therefore, sensory tests with sinigrin would not have been necessary. Consequently, the sinigrin detection threshold was only determined in the prestudy and no further tests were carried out in the main study. Nevertheless, the sinigrin threshold determination in the prestudy enabled the comparison of sinigrin and AITC detection thresholds obtained under similar test conditions.

In addition to the sensory tests with AITC, the pungency-inducing 4-HBITC should have been evaluated as it is the major pungent compound resulting from the enzymatic hydrolysis of sinalbin in yellow mustard seeds. To estimate the impact of 4-HBITC on the overall pungency impression of mustard products, determination of the detection threshold and temporal intensity measurements in various matrices with the pure compound would be necessary. Because of the instability and hydrophobicity of 4-HBITC (Bones & Rossiter, 2006; Choubdar et al., 2010), sensory tests are hardly realizable. Additionally, 4-HBITC, which is allowed for human use, is not available from chemical vendors. Thus, sensory tests evaluating the pungency induced by 4-HBITC cannot be conducted.

In general, for all sensory studies, a high number of panelists, a balanced ratio of male and female panelists, as well as users and nonusers would have been advantageous. Additionally, an increased training intensity for the TI panelists might have improved the variability between the panelists.

Sensory and chemical–analytical analyses, of the correlation between sensory parameters and AITC concentration were carried out with eight commercial mustard products. Although a good correlation was achieved, a broader mustard product variety would have enhanced the validity of the correlation.

The chemical–analytical HPLC method was predominantly developed to quantify AITC. However, the quantification of 4-HBITC was also carried out. Despite the fact that the integration of the 4-HBITC content in the regression analysis did not improve the correlation, its influence on mustard pungency may be relevant. The 4-HBITC quantification results varied greatly because of its instability. Thus, improvement of the quantification method is necessary to obtain reliable, more precise results and to repeat the regression analysis.

Another optimization of the quantification method would be the simultaneous determination of the AITC and sinigrin as well as the 4-HBITC and sinalbin content. This would allow the degree of GSL degradation to be estimated, or check whether sinigrin and sinalbin were fully degraded during mustard production. Nevertheless, the method development is time consuming and costly.

In general, the use of an HPLC method for AITC quantification is discussable. In addition to the quantification, liquid chromatography mass spectrometry (LC-MS) allows the identification of additional compounds and lowers detection limit. AITC is volatile; therefore, quantification via GC is a selective and sensitive method with low sample preparation effort, as previously described (Elfoul et al., 2001; Stahl et al., 2009). However, both methods would be applicable for AITC quantification but high purchase and operating expenses, as well as suboptimal practicability in industrial processes, discourage the use of GC and LC-MS methods in this context.

The storage conditions and duration influence the AITC content of mustard products. Therefore, shelf-life tests evaluating the AITC concentration change would enable statements on sensory alterations, especially decrease in pungency intensity. As storage conditions in the market and in a consumer's home vary, several experimental set-ups would be necessary. Stahl et al. (2009) demonstrated that the storage of mustard products at 4 °C did not significantly influence the AITC content. However, storage at 4 °C is not realistic in the market and in a consumer's home until the opening of the products and a decline in the AITC concentration at higher storage temperatures is likely.

7.4 Conclusion

In summary, this work demonstrates that the AITC concentration in mustard products can be positively correlated with the sensory TI parameters I_{max}, *AUC,* and DUR_{Dec}. Additionally, the sensory pungency perception of AITC is influenced by the composition of the carrier matrix, especially the water and fat content. Knowledge of the sensory studies with AITC in model matrices can be transferred to commercial mustard products. Further, classification of mustard products into pungency categories (mild, medium, pungent, and extra pungent) was achieved considering the AITC concentration, moisture content, and TI parameter I_{max}. Finally, a classification model for mustard products according to the AITC content has been proposed.

8 Summary

Food products that induce a pungent sensory sensation are popular globally in almost all traditional cuisines. This includes products such as chili, black pepper, wasabi, cinnamon, horseradish, and mustard. The character of pungent sensation depends on the irritating chemical stimulus (McDonald et al., 2016). Thus, cooling and warming, burning and tingling sensations, and slow or rapid increase of pungency perception can be elicited by these compounds in varying areas of stimulation. This study focused on the chemical stimulus AITC, a quality-determining ingredient in mustard, horseradish, and wasabi. Pungency is an essential quality characteristic of several mustard products; however, limited and inconsistent information on the relationship between pungency perception and the amount of pungent compounds in mustard products is available. It is known that AITC is involved in the pungency sensations induced by mustard products. Therefore, fundamental tests to assess the sensory perception of AITC are necessary, as only a few studies addressing this issue have been published. On these grounds, the sensory perception of AITC in mustard products was in the scope of this work. Thus, the aim was to study the relationship between sensory-perceived pungency and quality-determining pungency-inducing ingredients of mustard.

The perception of pungency is a chemesthetic sensation elicited when chemical irritants stimulate free nerve endings of the trigeminal system. Sensations of pungency are different from taste and olfaction and do not belong to basic tastes. The trigeminal nerve belongs to the somatosensory system; therefore, pungency is a sensation of pain. (McDonald et al., 2016)

Commonly, the term "mustard" describes paste-like products composed of ground or partially ground mustard seeds or mustard flour, water, and acids such as vinegar, alcoholic beverages, or grape juice. Only the seeds of the *Brassicaceae* species *S. alba* (yellow/white mustard), *B. nigra* (black mustard), and *B. juncea* (brown mustard) may be used for mustard paste production. (Federation of Associations and Enterprises of Industrial Culinary Product Producers in Europe, 2015) In general, the consumption of mustard is characterized by sharp, sulfurous aroma notes, pungency sensations, and a lachrymatory effect (G. S. Clark, 1992; Gilbert & Nursten, 1972). During mustard production, bitter glucosinolates, sinigrin, and sinalbin are enzymatically degraded into isothiocyanates, AITC, and 4-HBITC, which are primarily accountable for mustard flavor and pungency (Fenwick & Heaney, 1983; Ghawi et al., 2014).

The pungency level of mustard products ranges from mild to extra hot and is usually declared on the product label. Despite the fact that the pungency level is a quality-determining attribute, no explicit guideline for mustard classification is available, with the exception of a report of a German regional analytical authority recommending a minimum AITC content of 70 mg/100 g for pungent mustard products (Staatliches Lebensmitteluntersuchungsamt Oldenburg, 1998).

Consequently, mustard classification based on sensory and chemical–analytical data would improve the comparability of products. To achieve the aim of this thesis, sensory and chemical–analytical approaches were chosen, and their correlation was reviewed.

To begin with, the threshold, the lowest concentration at which a stimulus can be detected, was determined for sinigrin in a water-based matrix and AITC in water and oil-based matrices. Ten panelists in a prestudy and 64 in a main study assessed repeatedly ascending stimulus concentrations using a 3-AFC test. The group threshold (BET) for sinigrin in the water-based matrix was 10.9 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.4). For AITC in a water-based matrix, the BETs in the pre and main studies were 0.1 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.6) and 0.1 mg/100 mL (*SD* $\log_{10}$ BET ± 0.6), respectively. Additionally, for AITC in an oil-based matrix, the BETs in pre and main studies were 0.5 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.6) and 0.6 mg/100 mL (*SD* of $\log_{10}$ BET ± 0.5), respectively. Significant differences ($p < 0.001$) in the thresholds of sinigrin and AITC in the water-based matrix were confirmed using two-way ANOVA (product/matrix and panelist). Further significant differences were identified for the thresholds of AITC in water- and oil-based matrices in the pre ($p < 0.01$) and main ($p < 0.001$) studies. Individual panelists did not significantly influence the results. The evaluation showed that AITC thresholds in water- and oil-based matrices determined in the pre and main studies were at comparable levels. The group threshold for sinigrin in a water-based matrix was in accordance with the previously reported threshold of 10.6 mg/100 mL by Fenwick et al. (1983).

Furthermore, the influence of mustard consumption frequency on the detection threshold level was assessed. The panel of the prestudy consisted of 3 mustard “users” and 7 mustard “nonusers,” whereas that of the main study consisted of 10 mustard “users” and 54 mustard “nonusers.” No significant influence of the mustard consumption frequency on the thresholds was identified. Nevertheless, the thresholds of the mustard “users” tended to be slightly lower than those of the mustard “nonusers” in all tests, indicating that mustard “users” tend to be more sensitive to mustard.

In both study parts, the panelists were asked to localize the area of sensation (tongue, throat, or tongue and throat) by completing a questionnaire. Sinigrin was predominantly perceived as bitter (in the water-based matrix at concentrations of 1 to 16 mg sinigrin/100 mL). Further, the AITC-induced sensation was described as being pungent in water- and oil-based matrices. In both the pre and main studies, the pungent sensation was prevalently perceived on the tongue (in the water-based matrix at concentrations of 0.01 to 0.32 mg AITC/mL; in the oil-based matrix at concentrations of 0.04 to 1.08 mg AITC/100 mL).

In conclusion, the determination of the detection threshold of AITC in water- and oil-based matrices indicated a significant influence of the carrier matrix on the AITC thresholds.

To analyze the influence of the carrier matrix on the sensory perception of AITC, a TI study was carried out. The time course of the perception of AITC in water-, oil-, and mustard recipe-based matrices was assessed in two study parts. In the first part, 10 assessors tested various concentrations of AITC in water- and oil-based matrices, and in the second part, 19 panelists assessed mustard-based carrier samples with various AITC concentrations. To adjust the pungency induced by AITC in the mustard recipe-based carrier, a method to produce mustard paste lacking perceivable pungency was established. Previously defined AITC concentrations (50, 100, and 200 mg AITC/100 g mustard carrier) were added to the mustard paste lacking perceivable pungency. In both study parts, several TI parameters such as I_{max} (maximum intensity), T_{Imax} (time to reach maximum intensity), T_{End} (end time of pungency perception), DUR_{Dec} (duration of decreasing phase), AUC (total area under the curve), and AUC_{Dec} (area under the decreasing phase) were recorded and analyzed.

The main findings of the TI study were that AITC-induced pungency perception depended significantly on the carrier matrix and the added AITC concentration.

The evaluation of the TI parameters indicated that perceived pungency intensity decreased in the order water-based matrix > mustard recipe-based matrix > oil-based matrix. Additionally, time-related parameters showed that AITC perception in a water-based matrix lasts longer than in oil- and mustard-based matrices. Nevertheless, the differences between mustard- and oil-based matrices were not clear for the tested parameters and concentrations. The I_{max}, T_{End}, and DUR_{Dec} values increased with increasing AITC concentration in all matrices. This indicates that increasing the AITC concentration led to an increase in the intensity and duration of pungency perception.

Furthermore, assessors located the area of pungency sensation (lip, nose area, palate, tongue, and throat) at the I_{max} of each sample. Panelists located the sensation at multiple locations in the oral cavity (tongue, palate, and throat) and in the nose area. Nevertheless, the location was also dependent on the AITC concentration. At low AITC concentrations (in the water-based matrix, 5 and 10 mg AITC/mL; in the oil-based matrix, 10 and 50 mg AITC/mL), the pungency perception was predominantly described to be on the tongue. Further, at high AITC concentrations (in the water-based matrix, 50 and 100 mg AITC/mL; in the oil-based matrix, 100 and 200 mg AITC/mL; in the mustard-based matrix, 100 and 200 mg/100 g) the pungency sensations were additionally and prevalently perceived in the throat and palate. At the highest concentration (in the water-based matrix, 100 mg AITC/mL; in the oil-based matrix, 200 mg AITC/mL; in the mustard-based matrix, 200 mg/100 g), additional intense sensations in the nose were frequently reported. During the time course of perception, the first AITC-induced pungency sensation was mostly reported to be on the tongue in all matrices and at all concentration levels. Several panelists reported an increase in pungency intensity after swallowing the sample. Additionally, the location shifted from a burn in the mouth to pungency sensations in the nose and throat after swallowing. The perception of pungency in the throat was described to be long-lasting, whereas sensations in the nose and mouth diverted rapidly.

As the sensory perception of AITC was significantly affected by the carrier matrix, several commercial mustard products were tested in a further TI study. To discuss the influence of the AITC concentration of the products, an HPLC method to quantify the AITC content of mustard products was implemented. The relationship between the sensory pungency assessment and chemical-analytically determined AITC concentration was reviewed subsequently. Commercial mustard products from different brands and with different pungency labels, from sweet to medium hot, hot, and extra hot, were examined. Several TI parameters were evaluated, and significant differences between the products were observed. In particular, the maximum perceived pungency intensity varied significantly ($p < 0.05$) between products, even if the pungency labeling was similar. The values of the parameters I_{max}, DUR_{Dec}, and *AUC* increased proportionally with an increase in the AITC concentrations of the products. Besides, in the products classified as hot I_{max}, DUR_{Dec}, and *AUC* values were higher than in sweet mustard products.

To evaluate the influence of relevant ingredients of the product matrix on the sensory evaluation, the moisture content and nutritional facts labeled on the analyzed products were included in the evaluation. The analysis revealed clear differences in the moisture, fat, and carbohydrate content, which may explain the differences in the sensory evaluation in addition to the effect of the AITC concentration of the products.

Furthermore, the assessors located (lip, nose, palate, tongue, and throat) the pungency sensation at I_{max} using a questionnaire. Pungency sensations in the throat were reported to increase with an increase in product pungency levels. Additionally, products with high AITC content (136 and 183 mg AITC/100 g) provoked sensations in the nose region more frequently than others.

The AITC concentration of the sensory tested commercially available mustard products was quantified using HPLC. The results showed that the concentration varied greatly between the analyzed mustard products. The values ranged from 21.3 to 183.1 mg/100 g fresh weight.

Moreover, the results of AITC quantification and sensory TI measurements were correlated using regression analysis. Statistically significant positive correlations between the AITC concentrations and the TI parameters I_{max} (correlation coefficient 0.803), DUR_{Dec} (correlation coefficient 0.856), and *AUC* (correlation coefficient 0.891) were observed. Integration of the moisture content of the products as independent variables into the multiple regression analysis showed that both the AITC concentration and moisture content significantly influenced the pungency perception induced during mustard product consumption. The regression analysis with both the products AITC concentration and moisture content as independent variables showed an improvement in the correlations between the AITC concentration and TI parameters I_{max} (correlation coefficient 0.944), *AUC* (correlation coefficient 0.960), and DUR_{Dec} (correlation coefficient 0.954). In summary, the regression analysis revealed a positive correlation between chemical and sensory evaluations and showed that a higher AITC concentration in mustard products induces a more intense pungency sensation. Additionally, the pungency sensations intensified with increasing moisture content of the mustard products. Similar observations were made in other studies evaluating AITC perception in water-based carriers.

Consequently, with the established regression model, mustard products can be classified into defined pungency categories. Therefore, the pungency categories mild, medium pungent, pungent, and extra pungent were defined based on the maximum pungency intensity I_{max} and the AITC concentration. The categorization is classified as follows: Mild, the AITC content should be < 20 mg/100 g and I_{max} value between 0 and 3 on a pungency intensity scale from 0 to 10; Medium pungent, pungent and extra pungent, an AITC content of 20–75 mg/100 g and I_{max} values between 4 and 5, an AITC content of 75–165 mg/100 g and I_{max} values between 6 and 8, and an AITC content of > 165 mg/100 g and I_{max} values between 9 and 10, respectively.

Nonetheless, numerous factors may influence this classification. Among these are differences in the product composition such as fat and moisture content, storage conditions, and consumer-related factors such as cultural background, gender, preference for pungency, and the consumption frequency of pungent products (Byrnes & Hayes, 2015; Carey et al., 2002; Gutierreza & Simona, 2016; Ludy & Mattes, 2012; Lynch et al., 1993; McNulty & Karel, 1973; Prescott & Stevenson, 1995; Rozin & Schiller, 1980).

In summary, human sensory evaluations are time consuming, cost intensive, and multifactorially influenced. The presented pungency classification may facilitate the reduction of necessary sensory tests in quality control and product development of mustard products in the food industry. The use of a fast, reliable, and reproducible quantification method for AITC concentration and the presented regression model may help in estimating the sensory maximum pungency intensity of mustard products. Additionally, the use of an AITC-based pungency classification in the mustard-producing industry may help to standardize pungency labels on commercial mustard products.

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10 Annex

Digital annex

The digital annex is available on the data storage medium enclosed to this thesis, including FIZZ sensory software data, chromatography data, questionnaires from the sensory studies, and statistical evaluations.

Supporting information to Chapter 6

Sabrina Eib, Désirée Janet Schneider, Oliver Hensel and Ingrid Seuß-Baum

Journal of Food Science **2020**, 85(9), 2728-2736. Supporting Information

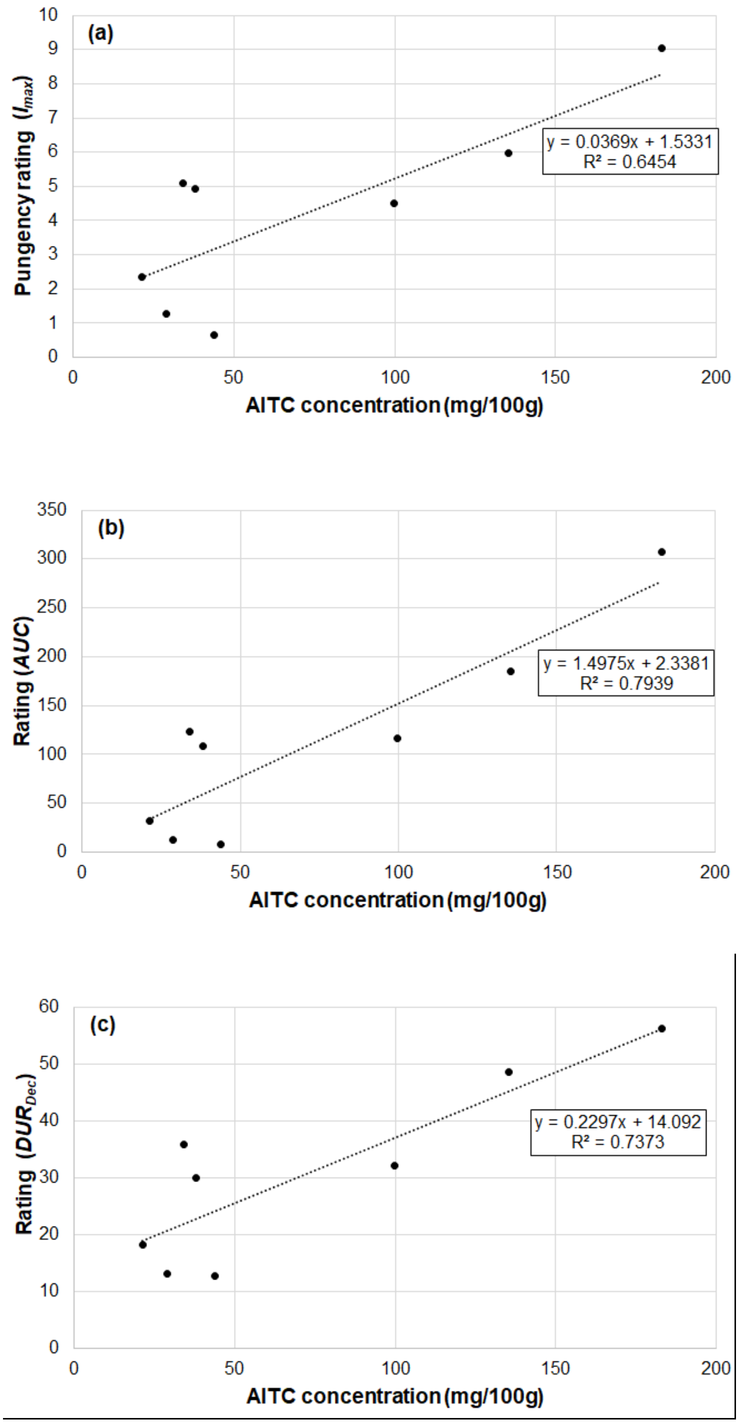

Figure 10.1 AITC concentration plotted against pungency rating I_{max} (a), area under the curve rating *AUC* (b), and duration of decreasing phase rating DUR_{Dec} (c) (mean values).

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