1 Communication

2 What temperature of coffee exceeds the pain

3 threshold? Organoleptical methodology as basis for

4 cancer risk assessment including feasibility study

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12 Abstract: The International Agency for Research on Cancer (IARC) evaluates "very hot (>65°C) 13 beverages" as probably carcinogenic to humans. However, there is a lack of research regarding 14 what temperatures consumers actually perceive as "very hot" or as "too hot". A methodology for 15 organoleptical assessment of such threshold temperatures was developed. The participants were 16 asked to mix a very hot coffee step by step into a cooler coffee. Because of that, the coffee to be 17 tasted was incrementally getting hotter during the test. The participants took a sip at every 18 addition, until they perceive the beverage as too hot for consumption. The protocol was evaluated 19 using 87 participants. Interestingly, the average pain threshold of the test group (67°C) and the 20 preferred drinking temperature (63°C) iterated around the IARC threshold for carcinogenicity. The 21 developed methodology was found as fit for the purpose and may be applied in larger studies.

Keywords: coffee; temperature; esophageal cancer; thermosensing; sensory thresholds; methodological
 study

24

25 1. Introduction

Since 2016, the cancer risk in connection to hot beverage consumption has received increased scrutiny from science and consumers alike. The reason for this has been the classification of very hot beverage consumption by the International Agency for Research on Cancer (IARC) into group 2A as "probably carcinogenic to humans" [1,2]. Specifically, the risk of developing oesophageal carcinoma increases with the consumption of very hot beverages as shown by a number of epidemiological studies [3-8]. Beverages above 65°C are considered "very hot" [1,2].

32 There are only a few studies available that researched the perception of temperature when 33 consuming hot drinks. In general, the so-called thermoreceptors are responsible for the sensation of 34 heat and cold. These receptors are free nerve endings located in the skin and mucous membranes. 35 When an action potential occurs, these receptors relay the stimulus to the nervous system, triggering 36 a sensation [9,10]. The thermoreceptors are located 0.1 to 0.6 mm below the skin surface, in the 37 dermis. These receptors are located not only on the surface of the skin, but also inside the body, e.g. 38 on the internal organs and their mucous membranes [11]. The thermoreceptors can be divided into 39 cold and warm ones. These react during cooling or warming with an impulse increase and thus 40 leading to an action potential. Important factors in the sensation of temperature are the absolute 41 temperature, the steepness of the change in the temperature which affects the skin during a certain 42 time and the size of the irritated body surface. In addition, the thermal conductivity of the object or 43 the fluid plays a role [11]. Above temperatures of about 44-45°C, the human begins to develop a 44 painful heat sensation. Pain stimuli are absorbed by so-called pain receptors. These receptors are 45 also free nerve endings located in the epithelia of the skin and mucous membranes. These receptors 46 do not approach a specific organ but run in the intercellular clefts of the epithelium. The pain 47 receptors react differently to different stimuli, e.g. on heat, pressure and strain [11]. Only after a 48 series of action potentials and the exceedance of the threshold value, a pain stimulus is triggered 49 [11].

50 Only a few experimental approaches are available for the determination of drinking 51 temperatures by means of organoleptic tests. Graham et al. [12] adapted the method of Pearson and 52 McCloy [13] to estimate the preferred drinking temperature of hot drinks. Hot water, with an initial 53 temperature of 80 to 85°C, was filled in a porcelain cup. Each time the water cooled down 2°C, 54 participants were asked to sample the water and give their assessment of the current temperature 55 [12]. Another study also determined which temperatures of the hot drinks are perceived as 56 preferable by consumers. For this purpose, a mixing method of coffee with different temperatures 57 has been developed. The participants were asked to mix their coffee to that temperature they usually 58 would consume the beverage. At first, very hot coffee has been in the cup to be tasted, which was 59 gradually mixed with colder coffee. Having reached the optimum temperature of the coffee, the 60 temperature was measured and documented [14].

61 The aim of this study was to develop a method to elucidate which temperatures of hot 62 beverages are perceived as too hot. That is the temperature at which consumers can no longer drink 63 the coffee without feeling pain. Since none of the literature approaches was completely convincing, 64 we developed a new methodology based on the study of Lee and O'Mahony [14] but with an inverse 65 experimental design because we judged it as being inappropriate to start from the pain stimulus 66 directly in the very first tasting. Therefore, in our design the temperatures of coffee are gradually 67 increased until the pain stimulus level is reached.

68 2. Materials and Methods

The mixing method according to Lee and O'Mahony [14] is used with some modifications. In contrast to the reduction of temperature in the original protocol, the coffee temperature is gradually increased by adding very hot coffee until the participants perceive the drink as being too hot. In each step, the participants re-test the hot drink after a temperature increase of 2-3°C.

The experimental set-up is shown in Figure 1. Each participant receives a cup of cold water, a cup as spittoon, a thermometer (Testo 108, Testo, Lenzkirch, Germany), a beaker, a thermos flask with very hot coffee (Instant coffee, Brand "Gut und Günstig", Edeka, Hamburg, Germany) and an isolation cup (Styrofoam cup, 400 ml, Thermo Cup EPS Neutral, white, Gastro-Sun.de, Blankenhain, Germany) with the "colder" coffee.

Before the start of the study, the participants are trained through a short briefing so that all of them have understood the test procedure and any questions that may arise can be clarified before the start of the test. The test sheet (Figures A1-A2, Appendix A), which is given to the participant to be filled in during the test run, also contains the test instructions. Then it contains a table, in which the temperatures of the tasted coffees are entered. For each tasted temperature of the hot drink, the participant gives a judgement about the taste sensation and may make a brief remark in his own words. Finally, the participants indicate how often they usually ingest hot drinks.

The coffee is prepared each time with the same amount of water (200 g) and soluble coffee powder (4.5 g). To ensure a similar starting temperature for all participants, a pot of coffee is prepared 15 minutes before they arrive. This coffee is cooled down to the desired temperature using a thermostatted water bath with temperature control. The desired starting temperature for all participants is 60°C.

The very hot coffee, however, is poured only shortly before the start of the experiment to ensure its high temperature. In order to be able to hinder the rapid cooling of the coffee, tests are carried out with a cup holder made of foam (Florence foam holder for 12 glasses/cups, 330 x 245 x 60 mm, Haba BV, Maasland, The Netherlands). With the help of the foam, the cups are isolated and thus delay the cooling. In order to minimize the cooling of the coffee in the thermos flasks as well, they are preheated before the experiments by adding boiling hot water. The water is removed from the 96 thermos flasks shortly before the test persons arrive. By using a water dispenser (Bunn H3EA Hot 97 Water Dispenser, Bunn, Springfield, Illinois, USA) for the preparation of the "very hot" coffee, 98 constant temperatures of about 96°C can be achieved. This coffee is prepared only after the brief 99 introduction of the participants to keep these temperatures as long as possible.

100 The participants gradually mix in the test phase in an ascending fashion very hot coffee (about 101 96°C) to the colder coffee (starting temperature 60°C) and measure the temperature of the mixture. 102 The added amount is standardized by a small beaker (0.04 L), which leads to a temperature increase 103 of 2-3°C. Then the coffee is tasted. The process of mixing is repeated by the participants until their 104 personal optimum drinking temperature is reached, followed by the temperature, which is 105 perceived as unpleasant or too hot at which the trial is stopped.

106 For a pilot feasibility test of the study protocol, all students, staff and professors of the 107 Albstadt-Sigmaringen University were invited by e-mail to participate. A total of 87 people (65 108 female and 22 male) and thus about 5% of the university location Sigmaringen members participated 109 in the study. The informed consent of the participants was obtained with signatures in writing 110 following an oral information about the trial. The study protocol was approved by the ethics 111 commission at Landesärztekammer Baden-Württemberg (Stuttgart, Germany) at 19.12.2017, Az.

112 F-2017-094.



- 113 Figure 1. Experimental set up provided to each participant. The isolation cup contains coffee at 60°C 114 and the thermos flask coffee at 93°C. The small beaker cup is used to gradually add the hot coffee.
- 115 Two further cups with cold water for spilling if required and an empty cup for spitting are provided. 116
- The thermometer is used to measure the temperature in each tasting step.

117 3. Results

118 The raw results of each participant are presented in Appendix B, Table A1. Descriptive 119 statistical analysis shows that on average between participants, coffee is perceived as "too hot" from 120 temperatures beyond 66°C (Table 1). The standard deviation is 3°C, which appears to be comparably 121 low in the context of organoleptical testing. The highest mentioned temperature for the pain 122 threshold of one of the participants is 71°C and is thus well above the specified IARC threshold of 123 65°C. The range shows that the participants have a very different perception of the threshold 124 temperature. Temperatures from 58°C to 71°C are determined as maximum tolerable temperatures.

125 This results in a span of 13°C for the pain threshold. Peer-reviewed version available at Foods 2018, 7, 83; doi:10.3390/foods70600

126	Table 1. Summarized results of the pilot study (n=87)						
	Temperature Mean Standard Minimum Maximum Range Media						
			Deviation				
	Pain threshold ^a	66°C	3°C	58°C	71°C	13°C	66°C
	Preferred drinking temperature	63°C	3°C	55°C	70°C	15°C	63°C
127	^a Temperature perceived as "too hot"						

127

Figure 1 shows the distribution of the temperatures perceived as "too hot". Again, it can be seen that most of the participants' responses for the pain threshold are at temperatures around 66°C to

68°C (n = 31; 36%). The category of pain threshold temperatures above 68°C includes 19 participants
(22%). Of the 87 participants, 24 persons (28%) already consider temperatures below 65°C to be "too

133 hot"; one participant already considered a temperature below 60°C as being "too hot" (1%).



134Figure 1. Histogram of the distributions for temperatures perceived as "too hot" and as135"desirable/preferred" (n=87). The curves show the normal distribution for both data sets.

136 In addition to the maximum temperature perceived as "too hot", the study also determined, 137 which temperatures the participants feel most desirable or preferable for coffee consumption. The 138 descriptive statistical analysis gives a mean value of 63°C with a standard deviation of 3°C. The 139 values range from a minimum of 55°C to a maximum of 70°C. Among the respondents are 19 140 participants (22%), who find their coffee at temperatures above 65°C to be optimally temperated. 141 Among them are two participants (2%) who even find the drinking desirable at temperatures above 142 68°C. However, of all participants, a large majority of 63 persons (72%) has their personal drinking 143 temperature below 65°C.

144 The distributions for both temperatures (Figure 1) are normal at the 0.05 level (Shapiro-Wilk 145 test). At the 0.05 level, the population means are significantly different (One-Way Analysis of 146 Variance). The average difference between the desirable and pain threshold temperatures was 3°C 147 (standard deviation 2°C).

148 4. Discussion

149 During an initial trial, the rapid cooling of the coffee and thus a difficulty of gradually 150 increasing the temperature of the beverage was observed, especially when normal porcelain coffee 151 cups were used. Based on previous research on cooling of coffee in different materials [15,16], the 152 experimental setup was modified to include insulation in a foam cup holder and the use of a 153 styrofoam cup. The cups remain in the foam holder during the experiment, only for drinking the cup 154 is removed. Furthermore, it is important that the hot coffee for increasing the temperature is made as 155 hot as possible, in our case using preheated thermos flasks and a hot water dispenser. Through these 156 measures, it has been possible to almost linearly increase the temperature in the cup.

157 Our study protocol was well usable for its purpose and no inconsistencies were observed 158 besides one participant who already judged the initial coffee as "too hot", but this problem can be 159 circumvented by allowing the coffee to cool for some minutes or to decrease the initial temperature.

160 According to the literature, there may be a considerable intra-individual variation in pain 161 threshold tests (12°C on average [14]). While such differences were not researched in our study (only 162 one trial per participant), the inter-individual variation in our trial is lower than this intra-individual 163 value from the literature. This can potentially be explained by differences in the experimental design 164 (starting from hot to cold in [14]). We believe that large receptor responses such as pain may hinder 165 or restrict taste testing for some time, so that the starting point should be the low stimulus and not 166 the high stimulus (similar to, e.g. standard procedures for taste tests for salt, acidity etc., see ISO 8586 167 [17], which always start at the lowest concentration). The strength of coffee may also play a role in 168 the sensation of temperature [14]. However, only one single coffee type was used in this study and 169 the coffee in the study by Lee and O'Mahony [14] was much stronger. Coffee type may therefore 170 potentially explain the larger variances in Lee and O'Mahony [14] compared to this study. A larger 171 bitterness response, for example, may interact with the temperature response. In addition, this study 172 does not assess whether there are differences between consumers drinking their coffee with milk or 173 without milk. Before further use, the protocol should be validated as to whether reproducible results 174 are achieved both intra-individual and inter-individual within taste-testing panels and for different 175 beverage types and preparations.

The results of the pilot test study show that, on average, the participants judge coffee as being "too hot" just above the threshold of 65°C suggested by IARC for "very hot" beverages that are probably carcinogenic to humans [1,2]. This consumption preference of German consumers may explain the fact that an increased incidence of oesophageal cancer has not been described in connection to hot beverage consumption in Germany, while in other countries, such as for tea in Iran or mate in South America, where such epidemiological associations were mainly described, the preferred consumption temperature was typically much higher than 70°C [3-8].

183 Nevertheless, some participants in Germany tolerate temperatures well above this threshold
184 and thus may be exposed to an increased risk of oesophageal cancer. Therefore, the question arises
185 how this group of consumers can tolerate such high temperatures without experiencing pain.

According to Lee et al. [18], the threshold for sensation of pain on the tongue starts at temperatures of about 46-48°C. However, our study temperatures clearly exceed this level. Obviously, the liquid has much higher temperatures than the surface of the tongue when heated by the coffee. But measurements of Lee et al. [18] showed that coffee at a temperature of 60 ° may raise the surface of the tongue to 53°C, which is above the postulated pain threshold of the tongue.

191 The increase in the temperature at the tongue surface results from the contact temperature 192 between the liquid and the tongue. The contact temperature is the point at which two bodies touch 193 each other at different temperatures. This temperature can be estimated by the simple formula 1 [19]:

$$T_{\kappa} = T_2 + \frac{b_1}{b_1 + b_2} * (T_1 - T_2)$$

T_{\keta} = Contact temperature (1)

194 The skin temperature is about 37.4°C with a thermal effusivity of about 1.3 kWs^{0.5}m⁻²K⁻¹. The 195 thermal effusivity of water, on the other hand, is 1.6 kWs^{0.5}m⁻²K⁻¹ [19]. If the coffee has a temperature 196 of 70°C, thus the contact temperature estimation will be about 55°C. On the other hand, the formula 197 results in a temperature of 57°C, which would cause the tongue to be heated at around the pain 198 threshold temperature of 48°C. These theoretical estimations well confirm the measurements of the 199 proband's tongue surfaces of Lee et al. [18]. Recommended maximum temperatures for water to 200 avoid burning were 65°C for contact periods up to 1 s duration and 60°C for contact periods of 3-4 s 201 [20]. These would be potentially be exceeded by the average coffee drinking behaviour in our study 202 collective.

Still, many of the participants in our study perceive coffee temperatures of around 60°C and also much higher as desirable and certainly not too hot. This discrepancy is explained by Lee et al. [18] by the fact that the temperatures may not be kept in the mouth long enough. A swallowing process lasts only a few seconds, so the temperature exposure acting on the mucous membranes in the mouth and on the tongue is not sufficiently long and thus not able to reach an action potential. Thus, no pain is transmitted despite high temperatures, so that consumers are able to drink the hot drinks without pain.

Finally, another hypothesis is that the pain potential can possibly be reduced by a habituation effect through continuous hot beverage consumption over the lifetime [21]. Hence, this habituation effect may also explain the large inter-individual differences of up to 15°C.

213 5. Conclusions

214 Some previous studies considered the question whether temperatures of very hot beverages 215 actually influence cancer risk or other disease outcomes [1,3,12,13]. In fact, previous experimental 216 studies have shown that some consumers may ingest their coffee at temperatures that can burn the 217 epithelium of the oesophagus [14]. It has been shown that the participants on average perceive coffee 218 most preferable at temperatures of about 60°C. People who drink their coffee black prefer slightly 219 higher temperatures than participants who drink their coffee with milk. Similarly, "weaker" (less 220 concentrated) coffee is preferred at higher temperatures [14]. Our findings basically corroborate 221 these observations, suggesting that the temperature at which coffee is considered preferable in 222 Germany is typically <65°C, and hence below the threshold for carcinogenic risk [1,2]. The 223 developed methodology is well-suitable for the purpose to obtain temperature preferences of 224 beverages and next steps could include intra- and inter-individual validation work and field testing 225 with larger collectives of probands.

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experiments; J.D. analysed the data; J.D. wrote the initial draft in the form of a Bachelor thesis; D.W.L.
summarized the draft as scientific paper and translated into English; G.W. revised the final draft; J.D., G.W.
and D.W.L. revised and approved the final version.

- 231 **Conflicts of Interest:** The authors declare no conflict of interest.
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290 Appendix A

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Figure A1. Experimental sheet for each participant of the trial (original in German)

Ab welcher Trinktemperatur werden Heißgetränke als zu heiß empfunden? Untersuchung zum Schmerzschwellenwert als Grundlange für eine Krebsrisikoanalyse.

- Versuchsbogen -

Mein Geschlecht: weiblich: O männlich: O

Mein Geburtsjahr:

Anleitung zur Durchführung des aufsteigenden Mischverfahrens: Sie selbst erhöhen bitte stufenweise die Trinktemperatur des Kaffees in Ihrem Trinkbecher um jeweils ca. 2 - 3 °C.

- Dazu geben Sie jeweils ein kleines Becherglas voll heißem Kaffee zum kälteren Kaffee in Ihrem Trinkbecher.
- Lesen Sie die neue Temperatur am Thermometer ab und notieren Sie sie in der Tabelle unten.
- Probieren Sie nun vorsichtig einen kleinen Schluck des Kaffees im Trinkbecher. Sie müssen den Kaffee nicht schlucken, sondern können ihn nach dem Probieren ausspucken. Notieren Sie nun durch Ankreuzen in der Tabelle Ihre Empfindung.

Temperatur in °C						
Wahrnehmung	o zu kalt					
	o optimal					
	o zu heiß					
Kommentar, Notiz						
					1	
Temperatur in °C						
Wahrnehmung	o zu kalt					
	o optimal					
	o zu heiß					
Kommentar, Notiz						

Meine optimale Trinktemperatur liegt bei °C

So häufig trinke ich üblicherweise Heißgetränke:

- > 4 Tassen pro Tag
- 2-3 Tassen pro Tag ο
- 1 Tasse pro Tag ο
- seltener 0

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Figure A2. Experimental sheet for each participant of the trial (English translation)

What temperature of hot beverages is perceived as too hot? Pain threshold analysis as basis for cancer risk assessment.

My sex: female: O male: O

Year of birth:

Instructions for carrying out the ascending mixing procedure: You yourself will gradually increase the drinking temperature of the coffee in your cup by approximately 2 - 3 °C.

- Add a small beaker full of hot coffee to the colder coffee in your cup.
- · Read the new temperature on the thermometer and record it in the table below.
- Carefully try a small sip of the coffee in the cup. You do not have to swallow the coffee but can spit it out after testing. Finally note your sensation by ticking the appropriate field in the table.

Temperature in °C						
Sensation	o too cold					
	o optimal					
	o too hot					
Comment, note						
Temperature in						
°C						
Sensation	o too cold					
	o optimal					
	• too hot	o too hot				
Comment, note						
My optimal drinking temperature is °C						
$M_{\rm V}$ frequency of hot beverage consumption: $\sim \sim 24$ cups per day						
wy nequency of not beverage consumption.						

2-3 cups per day

- 2-3 cups per day
 1 cup per day
- o i cup per uay

• seldom (<1 cup per day)

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298 Appendix B

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Table A1. Raw results of pilot study (n=87)

			Preferred	Coffee
Sex	Year of	Temperature	drinking	consumption
	birth	perceived as	temperature	behaviour
		"too hot" (°C)	(°C)	(cups per day)
female	1998	63.3	61.7	seldom
female	1998	67.6	65.0	seldom
female	1998	67.1	65.0	2-3
female	1998	69.5	66.1	2-3
female	1997	67.1	63.4	2-3
female	1997	65.2	63.6	seldom
female	1996	64.1	62.2	2-3
female	1996	67.3	65.0	>4
female	1996	65.4	62.5	2-3
female	1996	68.3	64.5	2-3
female	1996	69.0	66.0	2-3
female	1996	63.0	59.6	2-3
female	1996	64.5	61.7	seldom
female	1995	65.6	61.0	2-3
female	1995	62.4	59.0	1
female	1995	66.4	61.0	>4
female	1995	69.8	67.6	2-3
female	1995	68.8	68.2	>4
female	1995	65.4	61.9	>4
female	1995	63.2	61.2	>4
female	1995	68.2	64.6	2-3
female	1995	66.3	63.5	2-3
female	1994	67.3	66.0	2-3
female	1994	67.0	62.0	1
female	1994	63.8	61.0	2-3
female	1994	69.5	66.0	2-3
female	1994	65.7	62.0	2-3
female	1994	64.2	62.4	1
female	1994	66.4	64.5	1
female	1994	71.2	70.0	> 4
female	1993	69.0	63.2	2-3
female	1993	67.6	66.0	2-3
female	1993	67.5	67.3	> 4
female	1993	66.5	63.0	2-3
female	1993	65.0	63.0	2-3

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1992

1989

1987

1982

1982

male

male

male male

male

64.2

63.0

70.5

61.9

67.3

62.0

60.5

65.6

58.4

65.0

2-3

2-3

>4

>4

>4

s.org) NOT PEER-	REVIEWED P	osted: 26 April	2018	doi:10.20944/preprints
	Peer-reviewed	version available	at Foods 2018 ,	7, 83; <u>doi:10.33</u>	<u>90/foods7060083</u>
	female	1993	69.0	64.0	seldom
ł	female	1992	67.4	66.0	1
ł	female	1992	66.9	63.0	>4
ł	female	1992	67.3	64.5	> 4
ł	female	1991	65.8	63.0	>4
i	female	1991	66.1	60.0	seldom
ł	female	1990	63.5	60.8	2-3
i	female	1989	61.5	59.7	2-3
t	female	1988	69.6	67.7	>4
ł	female	1983	64.8	62.6	2-3
t	female	1983	64.0	61.0	2-3
t	female	1981	66.2	64.0	2-3
t	female	1979	64.9	58.0	>4
t	female	1978	64.1	60.4	2-3
t	female	1976	66.7	60.5	2-3
t	female	1972	60.2	< 60.0	2-3
t	female	1969	69.3	67.0	>4
i	female	1969	67.7	63.3	2-3
t	female	1966	67.3	66.0	>4
i	female	1966	67.1	64.0	2-3
t	female	1966	68.6	67.5	2-3
i	female	1965	65.7	60.2	>4
ł	female	1964	65.9	59.3	seldom
i	female	1963	67.9	67.4	>4
ł	female	1962	67.8	63.7	>4
t	female	1962	70.2	69.3	2-3
ł	female	1961	68.9	67.0	2-3
t	female	1959	68.0	63.3	2-3
t	female	1958	66.0	60.0	> 4
t	female	1955	67.9	63.8	2-3
	male	1997	68.5	66.0	2-3
	male	1996	65.0	62.5	2-3
	male	1995	67.8	63.4	2-3
	male	1995	66.0	61.5	2-3
	male	1995	65.3	63.3	2-3
	male	1993	62.0	60.0	2-3

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male	1975	67.3	65.0	2-3
male	1975	67.4	60.0	>4
male	1968	62.0	60.0	2-3
male	1965	67.1	61.0	>4
male	1964	66.3	60.0	>4
male	1962	58.0	55.0	2-3
male	1962	64.5	60.0	>4
male	1961	60.2	57.4	> 4
male	1954	64.3	62.3	2-3
male	1953	68.4	62.8	> 4
male	1945	65.2	61.7	>4

300