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1 Consumption of garlic positively affects hedonic perception of axillary body odour

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20

21 **Abstract**

22 Beneficial health properties of garlic, as well as its most common adverse effect - distinctive breath
23 odour - are well-known. In contrast, analogous research on the effect of garlic on axillary odour is
24 currently missing. Here, in three studies varying in the amount and nature of garlic provided (raw
25 garlic in study 1 and 2, garlic capsules in study 3), we tested the effect of garlic consumption on
26 quality of axillary odour. A balanced within-subject experimental design was used. In total, 42 male
27 odour donors were allocated to either a “garlic” or “non-garlic” condition, after which they wore
28 axillary pads for 12 hours to collect body odour. One week later, the conditions were reversed.
29 Odour samples were then judged for their pleasantness, attractiveness, masculinity and intensity by
30 82 women. We found no significant differences in ratings of any characteristics in study 1.
31 However, the odour of donors after an increased garlic dosage was assessed as significantly more
32 pleasant, attractive and less intense (study 2), and more attractive and less intense in study 3. Our
33 results indicate that garlic consumption may have positive effects on perceived body odour
34 hedonicity, perhaps due to its health effects (e. g., antioxidant properties, antimicrobial activity).

35

36 **Keywords**

37 *Allium sativum*, diet, health, antioxidant, sexual selection

38

39 **Introduction**

40 Garlic (*Allium sativum*) is an integral part of Euro-Asian local cuisines, both for its specific aroma
41 as well as its taste. It is also associated with a wide range of health benefits. For instance, records
42 from Ancient Egypt suggest that pyramid builders were fed with garlic to acquire extra vigor
43 (Rivlin, 2001), and several cloves of garlic were found in the tomb of the pharaoh Tutankhamun.
44 Pliny the elder prescribed garlic for treating gastrointestinal disorders, asthma, madness, tumors,
45 and worms. Furthermore, it was used for medical purposes by other ancient medical authorities such
46 as Hippocrates, Aristophanes and Galen (Block, 1985). The antibacterial properties of garlic were
47 recognized by Louis Pasteur and, during the Second World War, garlic was used as an antiseptic in
48 the prevention of gangrene (Afzal, Ali, Thomson, & Armstrong, 2000). Garlic has thus acquired a
49 longstanding reputation as a therapeutic medicinal agent.

50 Nowadays, well-known medical properties involve several major domains including antioxidant,
51 immunostimulant, cardiovascular, bactericidal, and oncological effects. Several studies reported

52 that garlic consumption significantly increases antioxidant activity in various tissues (Banerjee,
53 Dinda, Manchanda, & Maulik, 2002; Wei & Lau, 1998); presumably by reducing reactive oxygen
54 species or by interacting with them to protect vascular endothelial cells from oxidant injury
55 (Amagase, 2006). Garlic ingestion might also influence immune response, as it stimulates
56 proliferation of lymphocytes, influences macrophage phagocytosis, and enhances activities of
57 natural killer cells and lymphokine-activated killer cells (Amagase, Petesch, Matsuura, Kasuga, &
58 Itakura, 2001, Lamm & Riggs, 2001). Furthermore, significant effects of garlic on the
59 cardiovascular system, such as platelet aggregation inhibition (Srivastava & Tyagi, 1993), decreases
60 in fibrinolytic activity (Butt, Sultan, Butt, & Iqbal, 2009), and an antihypertensive effect (Ried,
61 Frank, Stocks, Fakler, & Sullivan, 2008) have been reported; perhaps due to its influence on plasma
62 lipid metabolism. Garlic is further known to have inhibitory activity on various pathogenic bacteria,
63 viruses and fungi (Ankri & Mirelman, 1999). Moreover, several epidemiological studies reported
64 associations between garlic consumption and lower risk of acquiring (or death caused by) several
65 types of cancer (Mei et al., 1982, Hsing et al., 2002, Zheng et al., 1992, Steinmetz, Kushi, Bostick,
66 Folsom, & Potter, 1994). Suggested mechanisms of garlic's anticancer efficacy, based on
67 experimental studies, include antioxidant action, inhibition of DNA adduct formation,
68 antiproliferating activities (Shukla & Kalra, 2007), induction of apoptosis and cell cycle arrest
69 (Iciek, Kwiecień, & Włodek, 2009).

70 Apart from the wide range of health benefits attributed to garlic consumption, adverse effects have
71 also been reported. The most common of these is unpleasant garlic breath and body odour
72 (Amagase, 2006; Borrelli, Capasso, & Izzo, 2007). Suarez et al. (1999) attempted to reveal the
73 mechanism behind this effect and investigated the origin of odoriferous gases (i.e., gut versus
74 mouth) following garlic ingestion. Concentrations of all sulfur-containing gases decreased after 3
75 hours except allyl methyl sulfide, which became the predominant sulfur gas present in breath
76 (Suarez et al., 1999). Other studies showed that garlic consumption may also affect the odour of
77 human breast milk and amniotic fluid. After ingestion of garlic capsules by breast-feeding women,
78 the odour of breast milk was perceived by adult panelists as more intense; moreover, infants spent
79 more time attached to their mother's breast and fed more vigorously (Mennella & Beauchamp,
80 1991). Similarly, babies without previous experience of garlic spent more time breast-feeding and
81 more time attached to the nipple after their mothers ingested garlic capsules compared to infants
82 whose mothers repeatedly consumed garlic during an experimental period. These findings suggest
83 that infants repeatedly exposed to garlic flavor in mother's milk become habituated to the flavor
84 (Mennella & Beauchamp, 1993). Another study showed that garlic consumption influenced the

85 odour of amniotic fluid, as samples obtained from women who had ingested garlic capsules were
86 judged to be stronger or more garlic-like than samples collected from women consuming placebo
87 capsules (Mennella, Johnson, Staley, & Beauchamp, 1995).

88 In contrast to the evidence reviewed above, there is currently no direct empirical evidence that
89 garlic consumption similarly influences axillary odour. One may expect such an effect as the studies
90 involving breast and amniotic fluid odour indicate that volatile molecules are transported from the
91 digestive system to the bloodstream. Subsequently, the volatile molecules could be transported from
92 arteries to eccrine or other skin glands which subsequently secrete these compounds onto the skin
93 surface. Furthermore, several anecdotal accounts point to the smell of garlic from the skin
94 (Amagase et al., 2001; Borrelli et al., 2007; Stevinson, Pittler, & Ernst, 2001). Indeed, previous
95 studies have shown that diet can also affect axillary odour (for review, see Havlicek & Lenochova,
96 2008). For instance, Havlicek and Lenochova (2006) found that body odour of individuals on a non-
97 meat diet was perceived as more pleasant, attractive and less intense compared to the same
98 individuals on a meat diet.

99 Our main aim in this study was therefore to test the effect of garlic consumption on axillary odour
100 hedonicity. We conducted three studies, varying the amount of garlic consumed and the nature and
101 origin of the odoriferous molecules involved.

102 **Material and methods**

103 *Participants*

104 **Odour donors**

105 Ten men, mean age 25.2 (range 18 - 31 years), body weight 73.4 kg (range 55 - 96 kg) and body
106 height 179.5 cm (range 174 - 186 cm), mostly students at Charles University in Prague, participated
107 in the first study. Another 16 men, mean age 25.1 (range 20 - 34 years), body weight 75.3 kg (range
108 54 - 103 kg) and body height 179.4 cm (range 169 - 193 cm) took part in the second study; in the
109 third, a further 16 men, mean age 25.8 (range 19 - 35 years), body weight 75.3 kg (range 62 - 105
110 kg) and body height 179.9 cm (range 170 - 188 cm) participated. All were recruited via posters or
111 contacted via e-mail by JF. All were non-smokers, reported no dermatological or other diseases at
112 the time of the study, and did not shave their armpits. The axillary shaving was kept constant as it
113 might affect perceived quality of the axillary odour (Kohoutová, Rubešová, & Havlíček, 2011). The
114 donors were given 400 CZK (approximately 20 USD) as compensation for their time and potential
115 inconvenience caused by the prescribed diet.

116 **Raters**

117 Fourteen women (mean age 24.6; range 20 - 35 years) took part in the first study. In the second and
118 third studies, a further 40 (mean age 22.5; range 19 - 32 years) and 28 (mean age 22.6; range 19 - 36
119 years) raters took part. . All were Charles University students and were contacted via e-mail or
120 posters. All were using hormonal contraception, to avoid changes in olfactory perception during the
121 menstrual cycle (Doty, 1981; Navarrete-Palacios, Hudson, Reyes-Guerrero, & Guevara-Guzman,
122 2003; Martinec Nováková, Havlíček, & Roberts, 2014). In the first study, the raters received a
123 chocolate bar, while in the second and third studies they were given 100 CZK (approximately 5
124 USD) as compensation for their time.

125 *Odour sampling procedure*

126 We used a balanced within-subject design in which odour donors were randomly assigned to one of
127 two groups (A, B). Odour donors in group A were in the “garlic” condition during the first session,
128 while those in group B were in the ”non-garlic” condition; conditions were reversed in the second
129 session which took place one week apart. In study 1, donors in group A ate a slice of bread with 6g
130 of crushed garlic (approximately 2 cloves of fresh garlic) mixed with fresh cheese, while those in
131 group B ate a slice of bread with fresh cheese only. The dosage of garlic (6g) we used corresponds
132 to the recommended daily amount (Amagase et al., 2001; Staba, Lash, & Staba, 2001) and was also
133 employed in previous studies assessing garlic gases in the oral cavity and intestines (Suarez et al.,
134 1999). To test the effect of dosage, in study 2 we doubled the original dose (to 12g). In the third
135 study, donors in group A were given 12 commercially available Walmark Alicin 1000 mg garlic
136 capsules (http://www.walmark.eu/eu/pages/products.aspx?nl_product_id=1017), each capsule
137 containing 1000 mg of garlic extract which corresponds to 12g of fresh garlic dissolved in soybean
138 oil, while those in group B received a placebo (capsules with soybean oil).

139 Each participant received a written list of instructions. The day before sampling and during the
140 sampling day, they were asked to refrain from (i) using perfumes, deodorants, antiperspirants,
141 aftershave and shower gels, (ii) eating meals containing garlic, onion, chilli, pepper, vinegar, blue
142 cheese, cabbage, radish, fermented milk products, marinated fish, (iii) drinking alcoholic beverages
143 or using other drugs and (iv) smoking. They were further asked to avoid strenuous physical (e. g.,
144 jogging, aerobic), and sexual activities or sharing bed with their partner or pet during sampling. The
145 night before sampling and in the following morning the donors were asked to wash without using
146 soap or shower gel. The next evening, between 17 and 19 hours, they visited the laboratory where
147 they received their garlic dose (see above) and subsequently washed their armpits using non-
148 perfumed soap (Neutral, DM-drogerie markt, www.dm-drogeriemarkt.cz, Prague). They then fixed

149 a cotton pad (elliptical in shape, approximately 9 x 7cm at their longest axis, Ebelin cosmetic pads,
150 DM-drogerie markt, www.dm-drogeriemarkt.cz, Prague) to either armpit using surgical tape
151 (Omnipur, DM-drogeriemarkt, www.dm-drogeriemarkt.cz, Prague). They then left the laboratory
152 and continued to wear the pads for the following 12h. Collection of axillary odours by use of cotton
153 pads has been employed in several previous studies (e. g., Ferdenzi et al., 2011; Havlicek &
154 Lenochova, 2006; Havlíček, Lenochová, Oberzaucher, Grammer, & Roberts, 2011; Roberts et al.,
155 2011). To avoid odour contamination from odour donors' own clothing, or by other extrinsic
156 ambient odours, the donors were asked to wear new white 100% cotton T-shirts (previously washed
157 without washing powder) as their first layer of clothing. We did not control their food intake before
158 the onset of the study, however, they were asked not to consume further meals while wearing pads.
159 The next morning, they put the pads into zip-lock plastic bags and handed these back to the
160 experimenters. The samples were immediately placed in a freezer at -32°C; so long as samples are
161 thawed before rating, freezing has no significant effect on hedonic ratings (Lenochova, Roberts, &
162 Havlicek, 2008; Roberts, Gosling, Carter, & Petrie, 2008).

163 Donors' conformity with the instructions was checked by a questionnaire. No serious violations,
164 particularly on the day of sampling, were found. In the first study, one donor in the experimental
165 condition reported eating garlic and another used a shower gel during the first session. During the
166 second session one donor reported eating onion; two others had a glass of beer (0.5 l) and one used
167 a perfume. In the second study, two donors reported using shower gel and one donor had wine
168 during the first session. During the second session one donor used shower gel, one ate a fermented
169 milk product and the other had a glass of beer. In the third study, two donors reported eating onion
170 and another two using shower gel; one had one glass of wine during the first session. During the
171 second session, two donors had consumed fermented milk products, one ate onion, and another had
172 a glass of wine (2 dcl). However, exclusion of these samples from the statistical analysis did not
173 significantly affect the findings.

174 ***Odour rating procedure***

175 Ratings took place in a quiet, ventilated room. The temperature across all three studies was between
176 20.9 and 22.4°C (30%-37% humidity). One randomly selected stimulus (a pad worn in the left or
177 right armpit) from each participant was enclosed in a 250 ml opaque jar labelled by a code. The
178 armpit (left, right) from which the odour stimulus was used was kept constant across both testing
179 sessions. Pads were presented as pairs, with each pair consisting of samples acquired from the garlic
180 and non-garlic condition of a particular donor. Raters were instructed not to use the same value
181 within each pair (samples of one person) for any of the assessed variables (e. g., pleasantness). This

182 procedure (the equivalent of a forced-choice test) is designed to detect subtle effects as it clarifies
183 differences between the tested groups. Each rater assessed all collected paired samples from the
184 donors (i. e., 10, 16 and 16 pairs of pads across the first, second and third study, respectively).
185 However, to avoid odour adaptation, the samples were randomly split into sub-sets (2 groups in
186 study 1, 3 groups in studies 2 and 3), and raters were given a break between assessing each set.
187 During breaks, raters were offered mineral water and completed an additional questionnaire. Raters
188 were asked to rate male body odour samples and stimuli were assessed on a 7-point scale for their
189 (i) pleasantness, (ii) attractiveness, (iii) masculinity and (iv) intensity. Both ends of each scale were
190 anchored by verbal descriptions (e.g., very unpleasant and very pleasant). In the event that raters
191 found any of the samples too weak to assess, they were asked to select “I cannot smell the sample”
192 instead of using the rating scales; these samples were not included in further analysis. The ratings
193 were written down immediately after sniffing each stimulus, but the time spent sniffing was not
194 restricted.

195 *Statistical analysis*

196 Kolmogorov-Smirnov tests showed normal data distribution in all of the three studies. We
197 computed mean odour ratings for both of the tested conditions (garlic and non-garlic). The odour
198 samples might undergo further changes during the rating session. To test for this potentially
199 confounding variable, we split the ratings assessed during the first and second half of the session
200 and entered this variable into the analysis (e.g., in study 1, ratings made by raters 1 - 7 were
201 compared to ratings by raters 8 – 14). As our design was within-subjects, the mean ratings were
202 subsequently compared using repeated-measures ANOVA with the time of day as a between-
203 subject factor (entered as a binominal variable). The data were analysed using mean subjects
204 (raters) ratings as the unit of analysis to test possible changes in the perception of axillary odour
205 sampled under different conditions. Each study was then analysed separately. Note that although
206 raters, rather than donors, were used as the unit of analysis, the ratings explicitly incorporated a
207 comparison between pairs of odour samples from the same donors. Our experimental design and
208 analytical approach therefore produces results that should be generalizable across donors, while
209 controlling for individual variability of rater’s olfactory perception. The statistical package SPSS
210 v.20 was used for all testing.

211

212 **Results**

213 Table 1 shows descriptive statistics for each rated variable, including mean scores (and SD), the
214 total number of ratings, and the number of analyzed ratings after exclusion of samples that were
215 judged to be too weak to be detected by some raters. In study 1, we found no significant differences
216 between experimental (garlic) and control conditions (non-garlic) for ratings of pleasantness ($F_{(1, 12)}$
217 $=0.466$, $p=0.508$, $\eta^2=0.037$), attractiveness ($F_{(1, 12)}=1.135$, $p=0.308$, $\eta^2=0.086$), masculinity ($F_{(1, 12)}$
218 $=0.414$, $p=0.532$, $\eta^2=0.033$) and intensity ($F_{(1, 12)}=0.182$, $p=0.677$, $\eta^2=0.015$), with no significant
219 effect of time of day on pleasantness ($F_{(1, 12)}=3.582$, $p=0.083$, $\eta^2=0.230$), attractiveness ($F_{(1, 12)}$
220 $=2.997$, $p=0.109$, $\eta^2=0.200$), masculinity ($F_{(1, 12)}=2.973$, $p=0.110$, $\eta^2=0.199$) and intensity ($F_{(1, 12)}$
221 $=2.186$, $p=0.165$, $\eta^2=0.154$) ratings with raters as the unit of analysis.

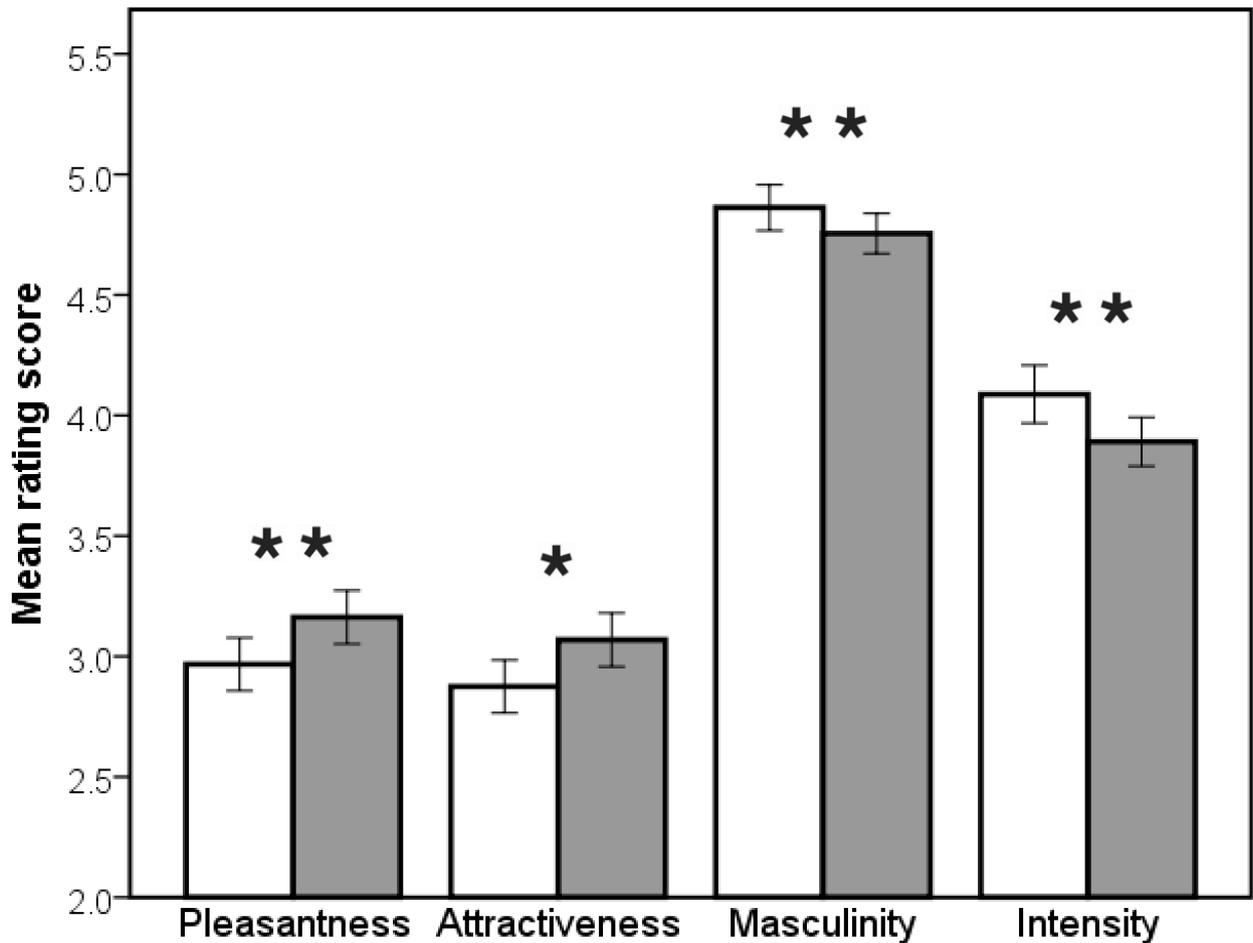
222

		Study I		Study II		Study III	
		Control	Experimental	Control	Experimental	Control	Experimental
Pleasantness	Mean	3.053	3.147	2.968	3.162	3.089	3.135
	SD	0.729	0.663	0.696	0.701	0.805	0.845
	η^2	0.037		0.257		0.013	
Attractiveness	Mean	3.058	3.198	2.915	3.070	2.981	3.132
	SD	0.698	0.574	0.694	0.708	0.797	0.826
	η^2	0.086		0.136		0.227	
Masculinity	Mean	4.642	4.581	4.904	4.723	4.975	4.871
	SD	0.901	0.734	0.600	0.526	0.922	0.924
	η^2	0.033		0.186		0.141	
Intensity	Mean	4.113	4.128	4.120	3.888	4.517	4.251
	SD	0.978	0.510	0.764	0.642	0.704	0.783
	η^2	0.015		0.218		0.365	
Total ratings		70 pairs		320 pairs		224 pairs	
Analyzed ratings		129 samples		570 samples		414 samples	

223

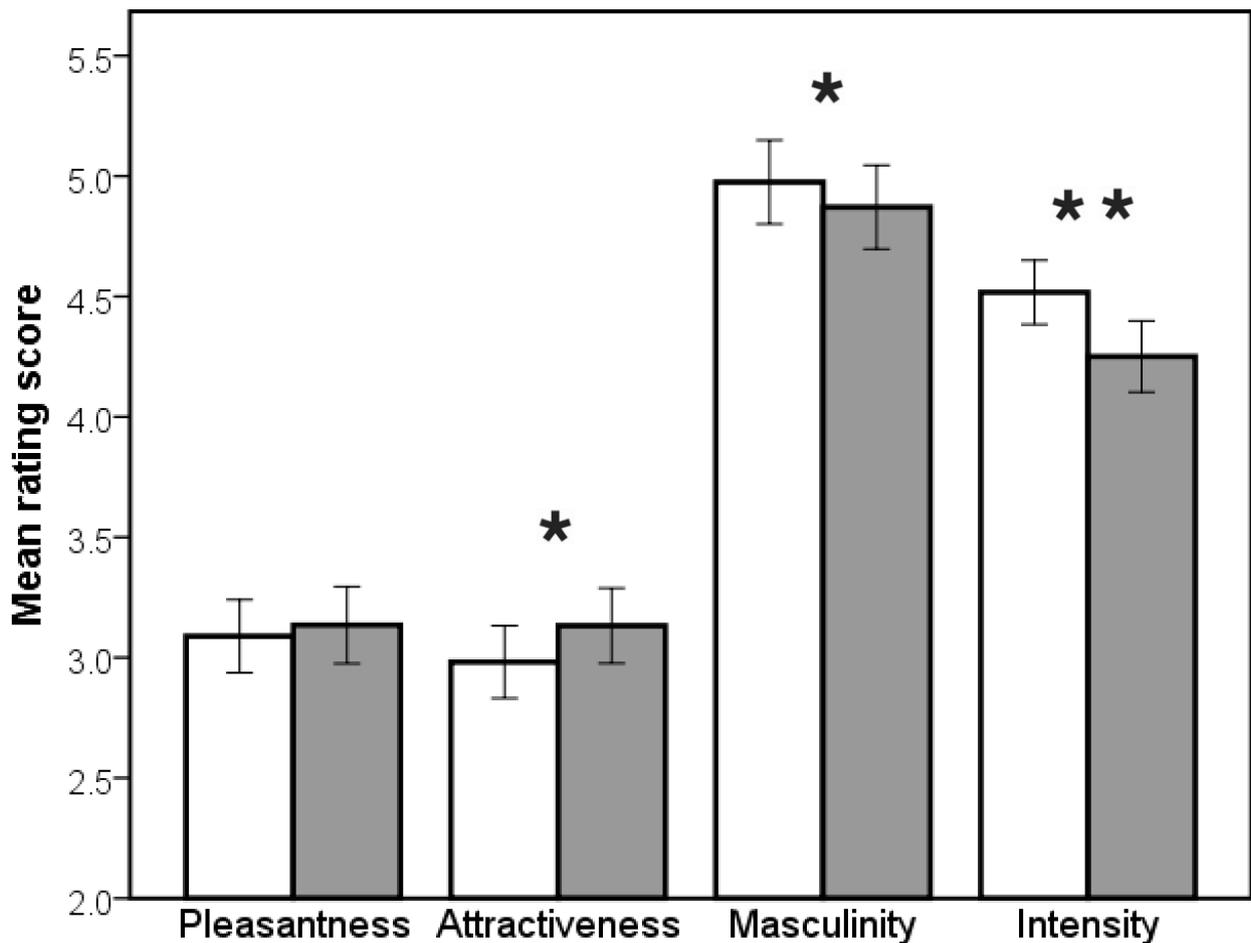
224 **Table 1** Overview on mean pleasantness, attractiveness, masculinity and intensity ratings when raters were used as a unit of analysis in control
225 (non-garlic) and experimental (garlic) conditions, number of total and analyzed ratings.

226 When garlic dosage was increased in study 2, odour of donors in the experimental (garlic) condition
 227 was judged as significantly more pleasant ($F_{(1, 38)}=13.115$, $p=0.001$, $\eta^2=0.257$), attractive ($F_{(1,$
 228 $38)}=6.006$, $p=0.019$, $\eta^2=0.136$), masculine ($F_{(1, 38)}=8.685$, $p=0.005$, $\eta^2=0.186$) and less intense ($F_{(1,$
 229 $38)}=10.615$, $p=0.002$, $\eta^2=0.218$) compared to control conditions (Fig. 1), with no significant effect of
 230 time of day on pleasantness ($F_{(1, 38)}=0.249$, $p=0.621$, $\eta^2=0.007$), attractiveness ($F_{(1, 38)}=0.093$,
 231 $p=0.762$, $\eta^2=0.002$), masculinity ($F_{(1, 38)}=0.664$, $p=0.420$, $\eta^2=0.017$) and intensity ($F_{(1, 38)}=0.054$,
 232 $p=0.818$, $\eta^2=0.001$) ratings.
 233



234
 235 **Fig. 1** Mean ratings (\pm SE) of 16 pairs of male axillary odours on pleasantness, attractiveness,
 236 masculinity and intensity in the experimental (garlic; grey bars) and control condition (non-garlic;
 237 white bars) judged by 40 female raters (study 2). Ratings were on a 7-point scale (e. g., 1 – very
 238 unpleasant and 7 – very pleasant). Asterisks indicate level of significance in paired t-tests; * $p < 0.05$
 239 level, ** $p < 0.01$ level.
 240

241 Most of the significant effects of garlic consumption found in study 2 were confirmed in study 3.
 242 Specifically, we found significant differences in the ratings of attractiveness ($F_{(1, 26)}=7.638$,
 243 $p=0.010$, $\eta^2=0.227$), masculinity ($F_{(1, 26)}=4.269$, $p=0.049$, $\eta^2=0.141$) and intensity ($F_{(1, 26)}=14.930$,
 244 $p=0.001$, $\eta^2=0.365$), but not in the ratings of pleasantness ($F_{(1, 26)}=0.355$, $p=0.557$, $\eta^2=0.013$) (Fig.
 245 2). Again, no significant effect of time of day was found for ratings of pleasantness ($F_{(1, 26)}=0.253$,
 246 $p=0.619$, $\eta^2=0.010$), attractiveness ($F_{(1, 26)}=0.295$, $p=0.592$, $\eta^2=0.011$) and intensity ($F_{(1, 26)}=3.297$,
 247 $p=0.058$, $\eta^2=0.131$), although the differences were significant for masculinity ratings: masculinity
 248 was rated lower during the first half of the session ($F_{(1, 26)}=7.676$, $p=0.010$, $\eta^2=0.228$).
 249



250
 251 **Fig. 2** Mean ratings (\pm SE) of 16 pairs of male axillary odours on pleasantness, attractiveness,
 252 masculinity and intensity in the experimental (garlic; grey bars) and control condition (non-garlic;
 253 white bars) judged by 28 female raters (study 3). Ratings were on a 7-point scale (e. g., 1 – very
 254 unpleasant and 7 – very pleasant). Asterisks indicate level of significance in paired t-tests; * $p < 0.05$
 255 level, ** $p < 0.01$ level.

256 The relationships between mean scores for odour donors on the different rated variables were
257 analyzed by Pearson's correlations (Supplementary tables 2, 3, and 4); we did this separately for
258 the garlic and non-garlic conditions. In all three studies, we found very strong positive correlations
259 between pleasantness and attractiveness ratings (r 's = 0.97 - 0.99), and very strong negative
260 correlations between pleasantness and intensity (r 's = -0.74 – -0.93) and between attractiveness and
261 intensity (r 's = -0.74 – -0.94). Masculinity ratings were positively related to intensity (r 's = 0.1 –
262 0.89) and negatively related to both pleasantness (r 's = -0.39 – -0.89) and attractiveness (r 's = -0.27
263 – -0.87).

264

265 **Discussion**

266 The main aim of this study was to test whether garlic consumption affects human axillary odour. In
267 study 1, we found no significant effect of consumption of 6g of crushed garlic (approximately 2
268 cloves of fresh garlic) on perceived quality of axillary odour. To test whether this might be due to
269 insufficient dosing, we doubled the dose of consumed garlic in study 2. As predicted, we then found
270 a significant influence of garlic consumption on axillary odour, although unexpectedly we found
271 that odour collected after consumption of the garlic was rated as more pleasant rather than less
272 pleasant, and less (rather than more) intense. The robustness of these findings was subsequently
273 confirmed in study 3, with the use of garlic capsules instead of raw garlic.

274 *Effects of garlic on axillary odour*

275 Our results showing that consumption of garlic affects quality of axillary odour are consistent with
276 previous studies testing the influence of garlic consumption on quality of human breast milk and
277 amniotic fluid. It was observed, for instance, that infants attached to the breast for longer periods of
278 time, and consumed more milk, when the mother consumed garlic capsules (Mennella &
279 Beauchamp, 1991). Interestingly, the authors interpreted the increase in suckling behaviour either in
280 terms of prior experience with this flavour during pregnancy and early months of lactation, or in
281 terms of response to a novel odour (Mennella & Beauchamp, 1993). However, our findings suggest
282 an alternative explanation: garlic may have improved the odour and flavour of breast milk for
283 infants in a similar way as occurred in our study with axillary odour. Indeed, when adult panellists
284 rated the odour of breast milk (Mennella & Beauchamp, 1991) and amniotic fluid (Mennella &
285 Beauchamp, 1993), samples collected after ingestion of garlic capsules were rated as more intense
286 and smelling like garlic. Due to the experimental protocol, which did not differentiate the two
287 different qualities (intensity and garlic odour), it is not entirely clear whether the odours were

288 actually more intense or the panellists really smelled the garlic odour. Moreover, blinded conditions
289 were not met as the panellists were asked to indicate which samples smelled “more like garlic” and
290 therefore could be influenced by expectations of garlic odour. In contrast, our experimental design
291 followed a double-blinded protocol and neither raters nor experimenters were aware, at time of
292 testing, the condition under which the individual samples were collected. Our raters were aware that
293 were rating male body odour samples. One might argue that such specific knowledge could skew
294 the ratings (e.g. positively for pleasantness, negatively for attractiveness), thus creating floor or
295 ceiling effects which would in turn decrease the likelihood of finding significant differences.
296 However, visual inspection of the data distribution and significant results suggest that this was not
297 the case here, and such knowledge of the nature of the odour stimuli is not unusual in odour rating
298 studies.

299 Several compounds responsible for garlic’s specific aroma have been identified. When garlic is
300 chopped or crushed, the clove’s membrane disrupts and odorless allin (S-allylcysteine sulfoxide) is
301 transformed into allicin (diallyl thiosulfinate) by action of the enzyme allinase (C-S liase) (Block,
302 1985). The main components of the volatile oil are sulfur compounds, especially allicin, which are
303 responsible for the typical odour of garlic. However, allicin is unstable and converts readily into
304 mono-, di- and trisulfides, and other compounds such as ajoene and vinylthiines (Shukla & Kalra,
305 2007). It therefore seems that allicin itself cannot be responsible for garlic’s biological activity but
306 is an intermediate product on the metabolic pathway towards other biologically important sulfur
307 compounds (Iciek et al., 2009). Obviously, garlic negatively influences the individuals’ breath on
308 account of sulphur containing gases which does not seem to apply to the body odour. The
309 compounds contributing to garlic odour might not reach the skin glands in perceptible quantities,
310 because the sulphurous constituents are highly volatile and many leave the body through the mouth
311 (Suarez et al., 1999). This is attributable to the lack of gut and liver metabolism of this gas or to
312 rapid metabolism of the other gases. It was therefore concluded that breath odour after garlic
313 ingestion initially originates from the mouth and subsequently from the gut (Suarez et al., 1999).

314 One might question whether or not our experimental design allowed chemicals from garlic
315 sufficient time to manifest its effects. It was recently shown that initial levels of volatiles released
316 from the breath decrease about 4 hours after garlic consumption. Initial levels are assumed to be
317 volatiles released from the stomach. However, a second peak of an increase of the volatiles was
318 observed about 6 hours and it is thought that these compounds are being released from the blood
319 (Rosen et al., 2000). Such a time period approximately corresponds to half of our sampling time,
320 and implies our odour sampling procedure allowed sufficient time for odour to emerge at the

321 axillary region. In study 2, we found an increase in pleasantness and attractiveness ratings after
322 garlic consumption, while in study 3 this effect was observed only for attractiveness ratings. The
323 slightly different outcomes between study 2 and study 3 could be ascribed to the somewhat different
324 nature of the stimuli (fresh garlic versus garlic capsules with soybean oil). Soybean oil could deliver
325 only the fat soluble fractions of the garlic extract, while fresh garlic could also contain the water
326 soluble fractions. Thus, some of the substances responsible for the increase in pleasantness might
327 not have been released after the use of soybean oil in study 3.

328

329

330 ***Health benefits associated with garlic consumption***

331 At face value, our results appear counterintuitive as several previous studies reported participants'
332 complaints about unpleasant breath and body odour after garlic ingestion (Borrelli et al., 2007;
333 Staba et al., 2001; Stevinson et al., 2001). However, the observed positive effect on hedonic quality
334 of axillary odour could be attributed to its well-established health benefits which involve several
335 major domains: i) antioxidant, ii) immunostimulant, iii) cardiovascular, iv) bactericidal, and v)
336 oncological effects (we review these factors below).

337 Several studies reported that garlic consumption significantly increases the antioxidant activity of
338 cells (Banerjee et al., 2002; Wei & Lau, 1998). It presumably reduces reactive oxygen species (e. g.,
339 superoxide anion, hydroxyl radical, hydrogen peroxide) or interacts with them to protect vascular
340 endothelial cells from oxidant injury (Amagase, 2006). The antioxidant activity is exerted by
341 scavenging free radicals, enhancing antioxidant enzymes superoxide dismutase, catalase and
342 glutathione peroxidase, and increasing levels of cellular glutathione. These mechanisms may play a
343 role in the cardiovascular, antineoplastic, and cognitive effects of garlic (Banerjee et al., 2002).

344 Immune responses are influenced by various intrinsic and extrinsic factors, but diet plays a crucial
345 role in regulation and proper functionality of immune system. Garlic consumption stimulates
346 proliferation of lymphocytes, macrophage phagocytosis, enhances activities of lymphokine-
347 activated killer cells (Amagase et al., 2001) and bone-marrow cellularity. Consumption of garlic
348 results in stimulated synthesis of nitric oxide (NO) and, in turn, interferon- α (IFN- α), which could
349 be beneficial in viral or proliferative diseases (Bhattacharyya, Girish, Karmohapatra, Samad, &
350 Sinha, 2007). Consumption of garlic also protects against suppression of immunity by
351 chemotherapy and UV radiation (Lamm & Riggs, 2001).

352 Garlic has been also shown to have significant effect on the cardiovascular system. Such areas
353 include platelet aggregation inhibition through suppression of thromboxane production (Srivastava

354 & Tyagi, 1993), antioxidant effects and decrease in fibrinolytic activity (Butt, Sultan, Butt, & Iqbal,
355 2009), and antihypertensive effects (Ried et al., 2008), perhaps due to its influence on plasma lipid
356 metabolism. Garlic decreases total serum cholesterol, low density lipoprotein cholesterol and
357 triglycerides (Qureshi et al., 1983) and enhances the ratio of high density lipoproteins to low density
358 lipoproteins (Kamanna & Chandrasekhara, 1982), and could therefore be a valuable component of
359 atherosclerosis-preventing diet (Gonen et al., 2005).

360 Garlic is further known to have inhibitory activity on various pathogenic bacteria, viruses and fungi.
361 It is active against proliferation of many Gram-negative and Gram-positive bacteria, including
362 *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *Clostridium*,
363 and *Mycobacterium tuberculosis*. Even some bacteria resistant to antibiotics, such as methicillin-
364 resistant *Staphylococcus aureus*, multidrug-resistant strains of *Escherichia coli*, *Enterococcus spp.*,
365 and *Shigella spp.* were found to be sensitive to garlic (Ankri & Mirelman, 1999). Allicin and ajoene
366 appears to be the main chemicals responsible for these wide-spectrum antimicrobial effects due to
367 the multiple inhibitory effects they have on various thiol-dependent enzymatic systems. Studies also
368 suggest that garlic has an antifungal effect and antiviral activity against several viruses including
369 cytomegalovirus, influenza B, *Herpes simplex* virus, and human rhinovirus (Ankri & Mirelman,
370 1999). There are several possible mechanisms for these properties, including decreasing oxygen
371 uptake by bacteria and viruses, reducing their growth, inhibiting their synthesis of lipids, proteins
372 and nucleic acids, and causing membrane damage (Harris, Cottrell, Plummer, & Lloyd, 2001).

373 Several epidemiological studies have revealed associations between garlic consumption and lower
374 risk of acquisition or death from stomach cancer (Mei et al., 1982). It has been shown that garlic
375 consumed either in food or as a food supplement is effective against prostate cancer (Hsing et al.,
376 2002; Key, Silcocks, Davey, Appleby, & Bishop, 1997) and decreases risk of cancer of the larynx
377 (Zheng et al., 1992) and esophagus (Yu et al., 2005), and of lung (Wu, Kassie, & Mersch-
378 Sundermann, 2005) and gastric and colon cancer (Steinmetz et al., 1994). Although the precise
379 mechanism of this anti-carcinogenic efficacy of garlic is still unknown, several hypotheses have
380 been proposed: antioxidant action, inhibition of mutagenesis by inhibiting the metabolism,
381 inhibition of DNA adduct formation, antibacterial properties, antiproliferating activities (Shukla &
382 Kalra, 2007), inhibition of carcinogen activation, induction of apoptosis and cell cycle arrest, and
383 modulation signal transduction (Iciek et al., 2009).

384

385 ***Health benefits could lead to increased odour pleasantness***

386 The health effects of garlic consumption may also be responsible for our findings. Garlic ingestion
387 could affect axillary odour indirectly through the antioxidant properties documented in previous
388 studies. Sulphur-containing compounds from garlic are known to protect endothelial vascular cells
389 and vessels against oxidative stress (Borek, 2001) which is caused by highly reactive oxygen
390 molecules and may therefore play a significant role in the defence against free radical-mediated
391 disorders (Wei & Lau, 1998). Although the precise mechanism of this effect is still debated, garlic
392 could either decrease or prevent production of the reactive molecules and/or their metabolites.
393 Furthermore, garlic is known to enhance levels of three antioxidant enzymes: superoxide dismutase,
394 catalase and glutathione peroxidase, which destroy toxic peroxides, and other reactive molecules
395 including glutathione (Borek, 2001). Changes in levels of these molecules, their metabolites or
396 other oxidative stress related compounds might affect quality of the axillary odour.

397 Another possible mechanism for how garlic indirectly affects axillary odour is via its antibacterial
398 action. The main sources of axillary odour are compounds produced by apocrine glands (Beier,
399 Ginez, & Schaller, 2005). However, fresh secretion of these glands is practically odourless (Shelley,
400 Hurley, & Nichols, 1953); characteristic axillary odour consequently originates from the metabolic
401 activity of skin bacteria (e. g., Gram-positive bacteria: *Propionibacterium*, *Staphylococcus*,
402 *Micrococcus*, *Corynebacterium*, *Acinetobacter*, *Malassezia*) (Bojar & Holland, 2002). It is
403 conceivable that consumption of garlic might reduce density of bacterial microflora either in
404 general or strain-specific fashion. As a consequence, this might lead to decreasing armpit odour
405 intensity. It is further known that perceived odour intensity is negatively linked to its pleasantness
406 and attractiveness (Doty, 1981; Havlicek, Dvorakova, Bartos, & Flegr, 2006). This would explain
407 not only the effect of lower odour intensity, but also the inverse effect of higher pleasantness and
408 attractiveness odour ratings following garlic consumption.

409 From an evolutionary perspective, formation of preferences for diet-associated body odours was
410 possibly shaped by means of sexual selection (Fialová, Roberts, & Havlíček, 2013). Previous
411 research indicates that many animal species use diet-associated cues to select mates in good
412 physical condition. For example, it was shown that secondary sexual displays reveal individual
413 quality of potential mates via links between foraging success or diet and phenotypic characteristics
414 such as diet-dependent coloration and odour quality (Ferkin, Sorokin, Johnston, & Lee, 1997;
415 Walls, Mathis, Jaeger, & Gergits, 1989). With regard to food quantity, food deprivation in meadow
416 voles results in decreasing odour attractiveness for opposite sex conspecifics, but odour
417 attractiveness is restored after re-feeding (Pierce & Ferkin, 2005). As the health benefits of garlic

418 consumption include antioxidant, immunostimulant, cardiovascular, bactericidal, and anti-
419 carcinogenic effects (Butt et al., 2009), it is plausible that human odour preferences have been
420 similarly shaped by sexual selection. This idea is consistent with a study by Olsson et al. (2014),
421 who showed that innate immune activation induced by injection of an endotoxin is detectable by
422 other individuals through more aversive body odour (Olsson et al., 2014).

423

424 **Conclusion**

425 Our study shows that axillary odour, in contrast to oral odour, is positively affected by garlic, and
426 these two sources of odour should be strictly differentiated. One may thus speculate on the relative
427 strength and salience of these effects in social interactions. Certainly, breath odour plays a crucial
428 role in most social interactions, but human axillary odour is also an important factor in intimate
429 relationships (Havlicek et al., 2008), although these odour sources are surely interrelated. Future
430 studies may thus try to disentangle relative contribution of these two effects. Furthermore, our
431 suggestions concerning the exact mechanism by which garlic consumption may shape axillary body
432 odour is speculative and future studies should determine possible differences in axillary secretion
433 related to garlic consumption and aim to identify specific compounds responsible for possible
434 differences in axillary microflora. Another area which deserves further attention includes
435 physiological processes following garlic consumption, metabolism of particular compounds and
436 subsequent action in body tissues. Our data thus add an additional line of evidence for wide-spread
437 health effects of garlic consumption.

438 **Ethics statement**

439 This study was conducted according to the guidelines laid down in the Declaration of Helsinki and
440 all procedures involving human subjects/patients were approved by the Institutional Review Board
441 of Charles University, Faculty of Science (approval number 2010/12). Written informed consent
442 was obtained from all subjects/patients.

443 **Conflict of interest**

444 None.

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453

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