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Consumption of garlic positively affects hedonic perception of axillary body odour

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ABSTRACT

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

1. Introduction

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

Nowadays, its well-known medical properties involve several major domains including antioxidant, immunostimulant, cardiovascular, bactericidal, and oncological effects. Several studies report that garlic consumption significantly increases antioxidant activity in various tissues (Banerjee, Dinda, Manchanda, & Maulik, 2002; Wei & Lau, 1998), presumably by reducing reactive oxygen species or by interacting with them to protect vascular endothelial cells from oxidant injury (Amagase, 2006). Garlic ingestion might also influence the immune response, as it stimulates proliferation of lymphocytes, influences macrophage phagocytosis, and enhances activities of natural killer cells and lymphokine-activated killer cells (Amagase, Petesch, Matsuura, Kasuga, & Itakura, 2001; Lamm & Riggs, 2001). Furthermore, significant effects of garlic on the cardiovascular system, such as platelet aggregation inhibition (Srivastava & Tyagi, 1993), decreases in fibrinolytic activity (Butt, Sultan, Butt, & Iqbal, 2009), and an antihypertensive effect (Ried, Frank, Stocks, Fakler, & Sullivan, 2008) have been reported, perhaps due to its influence on plasma lipid metabolism. Garlic is also known to have inhibitory activity on various pathogenic bacteria, viruses and fungi (Ankri & Mirelman, 1999). Moreover, several epidemiological studies reported associations between garlic

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consumption and lower risk of acquiring (or death caused by) several types of cancer (Hsing et al., 2002; Mei et al., 1982; Steinmetz, Kushi, Bostick, Folsom, & Potter, 1994; Zheng et al., 1992). Suggested mechanisms of garlic's anticancer efficacy, based on experimental studies, include its antioxidant action, inhibition of DNA adduct formation, antiproliferating activities (Shukla & Kalra, 2007), induction of apoptosis, and cell cycle arrest (Iciek, Kwiecień, & Włodek, 2009).

Apart from the wide range of health benefits attributed to garlic consumption, adverse effects have also been reported. The most common of these is unpleasant garlic breath and body odour (Amagase, 2006; Borrelli, Capasso, & Izzo, 2007). Suarez, Springfield, Furne, and Levitt (1999) attempted to reveal the mechanism behind this effect and investigated the origin of odoriferous gases (i.e., gut versus mouth) following garlic ingestion. Concentrations of all sulphur-containing gases decreased after 3 h except for allyl methyl sulfide, which became the predominant sulphur gas present in breath (Suarez et al., 1999). Other studies showed that garlic consumption may also affect the odour of human breast milk and amniotic fluid. After ingestion of garlic capsules by breast-feeding women, the odour of breast milk was perceived by adult panelists as more intense; moreover, infants spent more time attached to their mother's breast and fed more vigorously (Mennella & Beauchamp, 1991). Similarly, babies without previous experience of garlic spent more time breast-feeding and more time attached to the nipple after their mothers ingested garlic capsules compared to infants whose mothers repeatedly consumed garlic during an experimental period. These findings suggest that infants repeatedly exposed to garlic flavour in mother's milk become habituated to the flavour (Mennella & Beauchamp, 1993). Another study showed that garlic consumption influenced the odour of amniotic fluid, as samples obtained from women who had ingested garlic capsules were judged to be stronger or more garlic-like than samples collected from women consuming placebo capsules (Mennella, Johnson, Staley, & Beauchamp, 1995).

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

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

2. Material and methods

2.1. Participants

2.1.1. Odour donors

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

2.1.2. Raters

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

2.2. Odour sampling procedure

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

Each participant received a written list of instructions. The day before sampling and during the sampling day, they were asked to refrain from (i) using perfumes, deodorants, antiperspirants, aftershave and shower gels, (ii) eating meals containing garlic, onion, chilli, pepper, vinegar, blue cheese, cabbage, radish, fermented milk products, marinated fish, (iii) drinking alcoholic beverages or using other drugs and (iv) smoking. They were further asked to avoid strenuous physical (e.g., jogging, aerobic) and sexual activities or sharing bed with their partner or pet during the sampling. The night before sampling and in the following morning the donors were asked to wash without using soap or shower gel. The next evening, between 5-7 pm, they visited the laboratory where they received their garlic dose (see above) and subsequently washed their armpits using non-perfumed soap (Neutral, DM-drogerie markt, www.dm-drogeriemarkt.cz, Prague). They then fixed a cotton pad (elliptical in shape, approximately 9 × 7 cm at their longest axis, Ebelin cosmetic pads, DM-drogerie markt, www.dm-drogeriemarkt.cz, Prague) to either armpit using surgical tape (Omnipur, DM-drogeriemarkt, www.dm-drogeriemarkt.cz, Prague). They then left the laboratory and continued to wear the pads for the following 12 h. Collection of axillary odours by use of cotton pads has been employed in several previous studies (e.g., Ferdenzi et al., 2011; Havlicek & Lenochova, 2006; Havlíček, Lenochová, Oberzaucher, Grammer, & Roberts, 2011; Roberts et al., 2011). To avoid odour contamination from odour donors' own clothing, or by other extrinsic ambient odours, the donors were asked to wear new white 100% cotton T-shirts (previously washed without washing powder) as their first layer of clothing. We did not control their food intake before the onset of the study, however, they were asked not to consume further meals while wearing pads. The next morning, they put the pads into zip-lock plastic bags and handed these back to the experimenters. The samples were immediately placed in a freezer at −32 °C; so long as samples are thawed before rating, freezing has no significant effect on hedonic ratings (Lenochova, Roberts, & Havlicek, 2008; Roberts, Gosling, Carter, & Petrie, 2008).

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

2.3. Odour rating procedure

Ratings took place in a quiet, ventilated room. The temperature across all three studies was between 20.9 and 22.4 °C (30 %–37 % humidity). One randomly selected stimulus (a pad worn in the left or right armpit) from each participant was enclosed in a 250 ml opaque jar labelled by a code. The armpit (left, right) from which the odour stimulus was used was kept constant across both testing sessions. The pads were presented as pairs, with each pair consisting of samples acquired from the garlic and non-garlic condition of a particular donor. Raters were instructed not to use the same value within each pair (samples of one person) for any of the assessed variables (e.g., pleasantness). This procedure (the equivalent of a forced-choice test) is designed to detect subtle effects as it clarifies differences between the tested groups. Each rater assessed all collected paired samples from the donors (i.e., 10, 16 and 16 pairs of pads across the first, second and third study, respectively). However, to avoid odour adaptation, the samples were randomly split into sub-sets (2 groups in study 1, 3 groups in studies 2 and 3), and raters were given a break between assessing each set. During breaks, raters were offered mineral water and completed an additional questionnaire. They were asked to rate male body odour samples and these were assessed on a 7-point scale for their (i) pleasantness, (ii) attractiveness, (iii) masculinity and (iv) intensity. Both ends of each scale were anchored by verbal descriptions (e.g., very unpleasant and very pleasant). In the event that raters found any of the samples too weak to assess, they were asked to select “I cannot smell the sample” instead of using the rating scales; these samples were not included in further analysis. The ratings were written down immediately after sniffing each stimulus, but the time spent sniffing was not restricted.

2.4. Statistical analysis

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

3. Results

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

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

Most of the significant effects of garlic consumption found in study 2 were confirmed in study 3. Specifically, we found significant differences in the ratings of attractiveness ($F_{(1, 26)} = 7.638$, $p = 0.010$, $\eta^2 = 0.227$), masculinity ($F_{(1, 26)} = 4.269$, $p = 0.049$, $\eta^2 = 0.141$) and intensity ($F_{(1, 26)} = 14.930$, $p = 0.001$, $\eta^2 = 0.365$), but not in the ratings of pleasantness ($F_{(1, 26)} = 0.355$, $p = 0.557$, $\eta^2 = 0.013$) (Fig. 2). Again, no significant effect of time of day was found for the ratings of pleasantness ($F_{(1, 26)} = 0.253$, $p = 0.619$, $\eta^2 = 0.010$), attractiveness ($F_{(1, 26)} = 0.295$, $p = 0.592$, $\eta^2 = 0.011$), and intensity ($F_{(1, 26)} = 3.297$, $p = 0.058$, $\eta^2 = 0.131$), although the

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

		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
	η2		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
	η2		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
	η2		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
	η2		0.015			0.218			0.365	
Total ratings			140			640			448	
Analysed ratings			128			570			414	

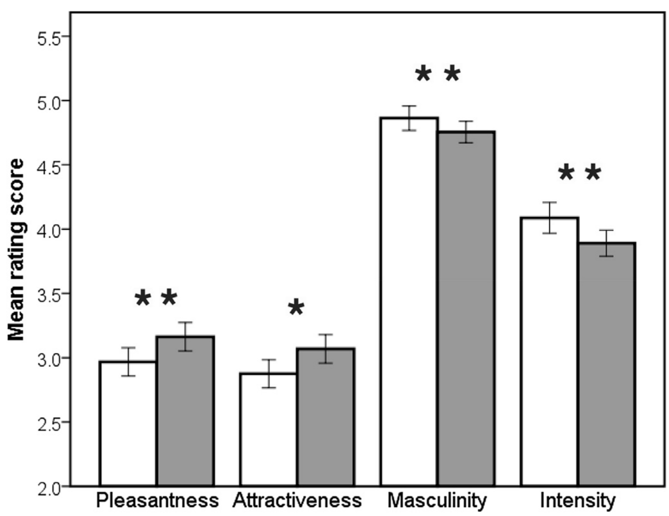

Fig. 1. Mean ratings (±SE) of 16 pairs of male axillary odours on pleasantness, attractiveness, masculinity and intensity in the experimental (garlic; grey bars) and control condition (non-garlic; white bars) judged by 40 female raters (study 2). Ratings were on a 7-point scale (e.g., 1 – very unpleasant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

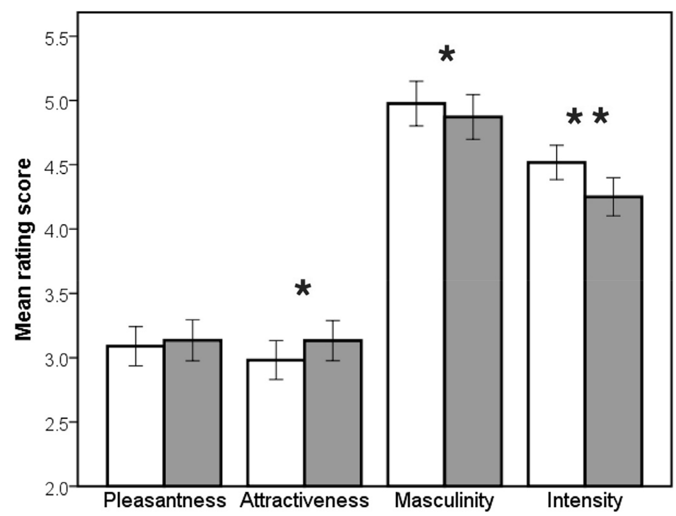

Fig. 2. Mean ratings (±SE) of 16 pairs of male axillary odours on pleasantness, attractiveness, masculinity and intensity in the experimental (garlic; grey bars) and control condition (non-garlic; white bars) judged by 28 female raters (study 3). Ratings were on a 7-point scale (e.g., 1 – very unpleasant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

differences were significant for masculinity ratings: masculinity was rated lower during the first half of the session ($F_{(1, 26)} = 7.676$, $p = 0.010$, $\eta^2 = 0.228$).

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

4. Discussion

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

4.1. Effects of garlic on body odour

Our results showing that consumption of garlic affects quality of axillary odour are consistent with previous studies testing the influence of garlic consumption on quality of human breast milk and amniotic fluid. It was observed, for instance, that infants were attached to the breast for longer periods of time, and ingested more milk, when the mother consumed garlic capsules (Mennella & Beauchamp, 1991). Interestingly, the authors interpreted the increase in suckling behaviour either in terms of prior experience with this flavour during pregnancy and early months of lactation, or in terms of response to a novel odour (Mennella & Beauchamp, 1993). However, our findings suggest an alternative explanation: garlic may have improved the odour and flavour of breast milk for infants in a similar way as occurred in our study with axillary odour. Indeed, when adult panellists rated the odour of breast milk (Mennella & Beauchamp, 1991) and amniotic fluid (Mennella & Beauchamp, 1993), the samples collected after ingestion of garlic capsules were rated as more intense and smelling like garlic. Due to the experimental protocol, which did not differentiate the two distinct qualities (intensity and garlic odour), it is not entirely clear whether the odours were actually more intense or the panellists really smelled the garlic odour. Moreover, blinded conditions were not met as the panellists were asked to indicate which samples smelled “more like garlic” and therefore could be influenced by expectations of garlic odour. In contrast, our experimental design followed a double-blinded protocol and neither the raters nor the experimenters were aware, at time of testing, of the condition under which the individual samples were collected. Our raters were aware that they were rating male body odour samples. One might argue that such specific knowledge could skew the ratings (e.g., positively for intensity, negatively for attractiveness), thus creating floor or ceiling effects which would in turn decrease the likelihood of finding significant differences. However, visual inspection of the data distribution and significant results suggest that this was not the case here, and such knowledge of the nature of the odour stimuli is not unusual in odour rating studies.

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

One might question whether or not our experimental design allowed the active compounds in garlic sufficient time to manifest their effects. It was recently shown that the initial levels of volatiles released from the breath decrease about 4 h after garlic consumption. Initial levels are assumed to be volatiles released from the stomach. However, a second peak of an increase of the volatiles was observed about 6 h post-ingestion and it is thought that these compounds are being released from the blood (Rosen et al., 2000). Such a time period approximately corresponds to half of our sampling time, and implies that our odour sampling procedure allowed sufficient time for the odour to emerge at the axillary region. In study 2, we found an increase in pleasantness and attractiveness ratings after garlic consumption, while in study 3 this effect was observed only for the attractiveness ratings. The slightly different outcomes between study 2 and study 3 could be ascribed to the somewhat different nature of the stimuli (fresh garlic versus garlic capsules with soybean oil). Soybean oil could deliver only the fat soluble fractions of the garlic extract, while fresh garlic could also contain the water soluble fractions. Thus, some of the substances responsible for the increase in pleasantness might not have been released after the use of soybean oil in study 3.

4.2. Health benefits associated with garlic consumption

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

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

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

Garlic has been also shown to have significant effect on the cardiovascular system. Such areas include platelet aggregation inhibition through suppression of thromboxane production (Srivastava & Tyagi, 1993), antioxidant effects and decrease in fibrinolytic activity (Butt et al., 2009), and antihypertensive effects (Ried et al., 2008), perhaps due to its influence on plasma lipid metabolism. Garlic decreases total serum cholesterol, low density lipoprotein cholesterol and triglycerides (Qureshi et al., 1983), and enhances the ratio of high density lipoproteins to low density lipoproteins (Kamanna & Chandrasekhara, 1982), and could, therefore, be a valuable component of atherosclerosis-preventing diet (Gonen et al., 2005).

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

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

4.3. Health benefits could lead to increased odour attractiveness

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

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

From an evolutionary perspective, formation of preferences for diet-associated body odours was possibly shaped by means of sexual selection (Fialová, Roberts, & Havlíček, 2013). Previous research indicates that many animal species use diet-associated cues to select mates in good physical condition. For example, it was shown that secondary sexual displays reveal the individual quality of potential mates via links between foraging success or diet and phenotypic characteristics such as diet-dependent coloration and odour quality (Ferkin, Sorokin, Johnston, & Lee, 1997; Walls, Mathis, Jaeger, & Gergits, 1989). With regard to food quantity, food deprivation in meadow voles results in decreasing odour attractiveness for opposite sex conspecifics, but odour attractiveness is restored after re-feeding (Pierce & Ferkin, 2005). As the health benefits of garlic consumption include antioxidant, immunostimulant, cardiovascular, bactericidal, and anti-carcinogenic effects (Butt et al., 2009), it is plausible that human odour preferences have been similarly shaped by sexual selection. This idea is consistent with a study by Olsson et al. (2014), which showed that innate immune activation induced by injection of an endotoxin is detectable by other individuals through more aversive body odour (Olsson et al., 2014).

5. Conclusion

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

Ethics statement

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

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.appet.2015.11.001.

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