



Research report

Individual astringency responsiveness affects the acceptance of phenol-rich foods

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ABSTRACT

Sensory responses greatly vary between individuals, and individual sensory experiences influence eating behaviour. Three groups responding differently to phenolic astringent stimuli (Low Responding, LR, $n = 20$, Medium Responding, MR, $n = 37$ and High Responding, HR, $n = 20$) were identified from a population of 77 subjects, based on the maintenance vs fluctuation of salivary characteristics after repeated stimulation of the masticatory and taste/somatosensory systems. The effect of LR, MR and HR status on perceived astringency and liking for phenol-containing apple, grape and carrot juices spiked with increasing tannic acid (TA) concentrations was examined. TA induced a greater increase of perceived astringency in HR, compared to MR and LR subjects. A decrease in liking for spiked juices was found in HR and to a lesser extent in MR and LR subjects. No significant differences were found comparing MR and LR groups for both astringency intensity and liking data. Liking for and familiarity with 37 food items, as well as preference for 14 phenol-rich foods and beverages, each paired with a less astringent counter-product, were also examined. An internal preference map was computed on liking scores and product subgroups were identified. An effect of LR/HR status was found for two food subgroups consisting of coffee without sugar, tea without sugar, raw chicory and milk chocolate, tea with sugar, coffee with sugar. LR subjects rated the products with the most astringency higher and those with the least astringency lower than did HR subjects. LR subjects also rated their familiarity with highly astringent products higher than did HR subjects. Thus, individual differences related to the physiological salivary response to oral stimulations affect responses to astringent stimuli and can influence the overall acceptability of phenol-rich food items.

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Introduction

Vegetable and fruit consumption decreases the risk of chronic diseases and several dietary guidelines (Dietary Guidelines for Americans, 2005; INRAN, 2003) encourage the consumption of plant-derived foods and beverages. Phenolic compounds are found in a wide variety of fruit and vegetables. Their presence in human food is of nutritional interest since they may be responsible for beneficial as well as detrimental effects on health (Halliwell, Rafter, & Jenner, 2005). Phenolic compounds can be divided into fifteen classes depending on their chemical structure. Flavonoids constitute the largest and most diverse family of phenolic compounds. Their strong radical scavenging activity probably accounts for their role in preventing diseases related to oxidative stress, such as coronary heart disease and various forms of cancer (Arts & Hollman, 2005). Tannins constitute a complex group of flavonoid-based polymers capable of binding and eventually precipitating proteins. The antioxidant activity has been demonstrated for tannins, but harmful effects have also been

reported for these compounds, such as inhibition of digestive enzymes, lowering of dietary protein digestibility, depressed growth in rats, altered food consumption, and acute hepatotoxicity (Mueller-Harvey, 2006).

Oral sensations experienced when eating involve many different sensory pathways: taste (salty, sweet, sour, bitter and umami), retronasal olfaction and somatosensory pathways responsible for temperature, mouthfeel/texture and chemesthetic (burning, cooling and astringent) sensations. Food product selection and consumption strongly depends on the product's sensory properties and on the intensity of experienced sensations (Duffy, 2007; Mattes, 2006).

Sensory properties of flavonoids and tannins can be summarized by two main descriptors: bitter and astringent, both eliciting negative consumer reactions when perceived at high intensities (Jaeger, Axten, & Wohlers, 2009; Lesschaeve & Noble, 2005). Human beings long sensitized to the bitter taste of plant toxins consider excessive bitterness the principal reason for food rejection (Drewnowski & Gomez-Carneros, 2000). The tactile sensation of astringency on the human palate has been defined as a complex group of sensations involving dryness of the oral surface and tightening and puckering sensations of the mucosa and muscles around the mouth (Lee & Lawless, 1991). Astringency

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arises from the interaction of dietary tannins with lubricating salivary proteins (Dinnella, Recchia, Vincenzi, Tuorila, & Monteleone, 2010; Nayak & Carpenter, 2008). It has been proposed that the sensation of astringency represents a warning cue to discourage animals from ingesting foods too high in tannins and thus protecting them from their potential harmful anti-nutritional effects (Shimada, 2006).

Responsiveness to tastes varies across individuals depending on several factors such as gender, age, hormonal status and medications (Duffy, 2007; Pangborn, 1981). Genetic factors can explain individual differences in psychophysical responses to oral sensations (Reed, Tanaka, & McDaniel, 2006). Genetic studies of the sense of taste have revealed the existence of multiple receptor genes for bitterness induced by thiourea compounds (phenylthiocarbamide – PTC, 6-n-propylthiouracil – PROP) (Reed, Nanthakumar, North, Bell, & Bartoshuk, 1999). PTC/PROP taster status may influence ingestive behaviours by affecting the acquisition of food preference (Keller, Steinman, Nurse, & Tepper, 2002) and liking for bitter vegetable foods and beverages (Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006; Drewnowski & Gomez-Carneros, 2000; Duffy & Bartoshuk, 2000; Kaminski, Henderson, & Drewnowski, 2000; Yeomans, Prescott, & Gould, 2009). Inherited sweet taste preference has been demonstrated (Keskitalo et al., 2007). Based on the relation between perceived sweetness intensity and hedonic response, subgroups of sweet likers and dislikers have been identified (Looy & Weingarten, 1991; Pangborn, 1970). The ability to perceive the thermal taste (a phantom taste evoked by tongue thermal stimulation) (Cruz & Green, 2000) was associated with relatively higher responsiveness to 5 prototypical taste stimuli (Green & George, 2004) and it is considered as a further marker of individual sensitivity variation to oral sensations.

Individual variation of saliva characteristics modulates the sensitivity to astringency induced by phenolic stimuli (Condelli, Dinnella, Cerone, Monteleone, & Bertuccioli, 2006; Fischer, Boulton, & Noble, 1994; Horne, Hayes, & Lawless, 2002). Two groups of subjects, Low Responding (LR) and High Responding (HR) to phenolic astringent stimuli have been identified based on their ability to maintain constant salivary characteristics after repeated oral stimulation (Dinnella, Recchia, Fia, Bertuccioli, & Monteleone, 2009). A nearly constant salivary protein concentration and profile after both masticatory and taste/somatosensory system stimulation characterized the group with lower sensitivity to astringency (LR), while a strong depletion of glycosylated salivary proteins was found in the more sensitive group (HR) (Dinnella et al., 2010). Physiological differences in protein secretory pathways as well as morphological characteristics of the parotid glands can explain the different behaviour of LR and HR groups after protracted oral stimulation.

The pronounced variation in sensory perceptions, due to the physiologically different salivary responses, may lead to different hedonic responses during exposures to foods and beverages containing phenolic compounds. The present work therefore investigates the effect of LR/HR status on the acceptance of phenol-containing foods.

Materials and methods

Overview

The experimental plan consisted of the following stages:

1. Development of a questionnaire tracking the choice of, preference for, and familiarity with phenol-rich food items.

2. Development of fruit and vegetable stimuli with increasing phenolic content providing different levels of perceived astringency within a similar intensity range.
3. Selection of LR ($n = 20$), MR ($n = 37$) and HR ($n = 20$) groups from a population of 77 subjects based on salivary protein characteristics after repeated oral stimulation.
4. Evaluation of LR, MR and HR status effect on liking for juices at different astringency levels.
5. Training to recognize sourness, bitterness and astringency intensities, and their subsequent ratings in juice samples.

Subjects

Seventy-seven subjects, 33 males and 44 females, aged from 21 to 33 years were recruited from the University of Florence students. The subjects had no history of disorders in oral perception. They were paid for their participation in the study. The Ethic Committee of the Department of Agricultural Biotechnology, University of Florence approved the protocol. Written informed consent was obtained from each subject after the description of the experiment.

Collection of liking and perceived intensity data

Stimuli

Three branded products purchased at a local retailer were used: apple (A), grape (G) and carrot (C) juices. Tannic acid (TA, Sigma–Aldrich) was used to modify the phenolic content of the juices. Three levels of TA concentration were considered for each juice (TA0, TA1, TA2). TA0 refers to pure juices; TA1 to 1.0 g/l TA in apple and carrot juices, and 1.5 g/l TA in grape juices, TA2 refers to 1.5 g/l in apple and carrot juices and to 2.0 g/l TA in grape juice. TA concentrations were chosen in pilot tests by experienced laboratory personnel to provide three approximately similar astringency levels for all juices.

Collection of liking data

Two sample sets were prepared. The first set was composed of the three different pure juices (TA0). The order of the juice presentation was randomized across subjects. The second set was composed of three subsets each consisting of the same juice at two TA levels (TA1 and TA2; 6 samples in total). The order of the subset presentation was randomized across subjects within and between pairs. Stimuli (15 ml each) were presented at room temperature in 35 ml-plastic cups labelled with three-digit code numbers. Subjects were asked to hold the whole sample in their mouth for 10 s, spit it out, wait for a further 20 s and rate the overall liking on a 9-point hedonic scale (Peryam & Pilgrim, 1957). This fully anchored 9-point category scale ranges from 1 (“dislike extremely”) to 9 (“like extremely”), with a neutral point at 5 (“neither like nor dislike”). After each sample, subjects rinsed their mouths with distilled water for 40 s, had some plain crackers for 40 s and finally rinsed their mouths with water for a further 40 s. Between subsets, subjects rinsed for 60 s. Evaluations were performed in individual booths under white lights.

Collection of perceived intensity data

Prior to intensity evaluations, subjects were trained to recognize and rate the perceived intensity of sourness, bitterness and astringency using the following standard (Sigma–Aldrich) aqueous solutions: citric acid: 0.25, 0.38, 0.50 g/l (sour); quinine monohydrochloride dihydrate: 0.025, 0.037, 0.050 g/l (bitter); aluminium potassium sulphate: 0.3, 0.6, 0.9 g/l (astringent). During training sessions the subjects rated the perceived intensities on a Labelled Magnitude Scale (LMS, 100 mm line) (Green,

Shaffer, & Gilmore, 1993), a quasi-logarithmic scale with the bottom labelled as “barely detectable” (1.4 mm) and the top as “strongest imaginable oral sensations, including pain” (100 mm). Intermediate labels include “weak” (6.1 mm), “moderate” (17.2 mm), “strong” (35.4 mm) and “very strong” (53.3 mm) oral sensations. Instructions for using the scale were given according to Green et al. (1993). Subjects participated in a total of four training sessions.

Nine samples (3 juices \times 3 TA levels) were evaluated for sourness, bitterness and astringency. Evaluations were performed on two sample sets of 5 and 4 juices each, with a 15 min break between them. Sample presentation was evenly balanced to control for both order and carry over effects on sensory responses. The order of attribute evaluation was balanced to minimize a possible “proximity” effect. The evaluation and rinsing procedure described above for hedonic evaluation was used. The perceived intensity of each sensation was rated on the LMS. To eliminate visual clues, samples were evaluated in individual booths under red lights.

Collection of saliva samples and total protein content assessment

The saliva was collected as described by Dinnella et al. (2009). Participants were instructed to avoid foods and beverages with high phenolic content for at least 8 h before the session started. A list of such products was provided.

Subjects were also instructed to refrain from smoking, eating, and drinking for 2 h before the session. The session (60 min) started at 9.00 am. Subjects received tap water to rinse their mouths, then they were instructed to mechanically evoke saliva by chewing Parafilm (3 cm \times 3 cm) for a total collection time of 15 min (first saliva collection, S1). After a 30 min break, subjects received an aqueous solution of 3.0 g/l TA (15 ml) to induce the reflex parotid gland salivation. They rinsed their mouths with water and evoked saliva by chewing Parafilm for a further 15 min (second saliva collection, S2). Immediately after saliva collection, subjects received an aqueous solution of 1.4 g/l TA (15 ml) and rated the perceived astringency, bitterness and sourness on the LMS. The order of attribute evaluation was balanced in order to minimize a possible “proximity” effect. The same sample evaluation and rinsing procedure described above for hedonic evaluation was used. Evaluations were performed in individual booths under red lights.

Saliva samples were put in an ultrasonic water bath at the maximum output for 5 min at 37 °C. The pellet eventually still present in the salivary sample was discarded while the clear upper phase was recovered and analysed for the total protein content (SPs) by Biuret method (Kallithraka, Bakker, Clifford, & Vallis, 2001). The effect of stimulation on saliva protein concentration was expressed in terms of SPs *D*-value, computed as the arithmetic difference between the protein concentrations in the S2 and S1 samples (Dinnella et al., 2009).

Table 1

Checklist of 37 food items. Average phenol content refers to fresh (FW) or dry weight (DW). Potential astringency is referred to by + (moderate) or ++ (strong); – (not typically astringent).

Product		Phenol content	Potential astringency	Reference
<i>Astringent</i>				
Red wine	Young	0.7 mg/ml tannins	+	Waterhouse, 2002
	Aged	1.0 mg/ml tannins	++	
Wine	White	0.25 mg/ml	+	Waterhouse, 2002
	Red	1.7 mg/ml	++	
Chocolate	70% cocoa	–	+	
	95% cocoa	–	++	
Chocolate	Milk	52.2 μ mol/g DW	+	Vinson, Proch, & Zubik, 1999
	Dark	126 μ mol/g DW	++	
Fruit	Extremely ripe	0.02–1.8 mg/g FW	+	Ayaz, Demir, Torun, Kolcuoglu, & Colak, 2008
	Not very ripe	0.03–6.8 mg/g FW	++	
Banana	Extremely ripe	0.2 mg/g FW	+	Bugaud, Alter, Daribo, & Brillouet, 2009
	Not very ripe	220 mg/g FW	++	
Pear	Extremely ripe	2.6 mg/g DW	+	Bai, Wu, Manthey, Goodner, & Baldwin, 2009
	Not very ripe	4.7 mg/g DW	++	
Tea	Sweetened	0.4 mg/ml	+	Yan, Hu, & Yao, 2009
	Unsweetened		++	Drewnowski & Gomez-Carneros, 2000
Coffee	Sweetened	50 mg/ml	+	
	Unsweetened		++	
Coffee	With milk	50 mg/ml	+	Narain et al., 2004
	Black		++	
Vegetables	Cooked	1.7 mg/g FW	+	Faller & Fialho, 2009
	Raw	3.0 mg/g FW	++	Kuti & Konuru, 2004
Artichoke	Cooked	–	+	
	Raw		++	
Chicory	Cooked	–	+	
	Raw		++	
Radicchio	Cooked	–	+	
	Raw		++	
<i>Bitter and astringent</i>				
Apple juice		0.83 mg/ml	–	Oszmianski, Woidilo, & Kolniak, 2009
Grape juice		0.5 mg/ml	–	Belitz, Grosch, & Schieberle, 2003
Carrot juice		0.3 mg/ml	–	Ninfali & Bacchiocca, 2003
<i>Bitter</i>				
Endive		1.3 mg/kg FW	–	Drewnowski & Gomez-Carneros, 2000
Grapefruit		0.5 mg/ml	–	Drewnowski & Gomez-Carneros, 2000
Bilberry juice		0.12 mg/ml	–	Koponen et al., 2008
Soy milk		2.5 mg/g DW	–	Xu & Chang, 2009
Tonic water		–	–	
Sanbitter		–	–	

All sensory data were collected using the FIZZ computer system (version 2.40G, Biosystemes, Couternon, France).

Questionnaire

A checklist of 37 foods was prepared: 14 pairs each consisting of the same food at two levels of potentially perceived astringency, 3 products both astringent and bitter and 6 foods characterized by bitterness as the predominant oral sensation (Drewnowski & Gomez-Carneros, 2000) (Table 1). The food pairs were formed based on different phenolic contents (Faller & Fioalho, 2009) and compositions (Waterhouse, 2002), or on added ingredients that counteract the perceived astringency (Narain, Paterson, Piggott, Dhawan, & Reid, 2004).

A 7-page questionnaire included four types of questions: (1) demographic, (2) choice from 14 pairs of phenol-containing foods, (3) familiarity with and (4) liking for the 37-item checklist. Participants expressed their choice for one of the products within a pair using a 7-point scale where “1” indicate that the choice was definitively for the product on the left side and “7” that the choice was definitively for the product on the right. The number 4 was considered as a neutral point. The points 2, 3, 5 and 6 were used to indicate any other intermediate judgements. The position of products at left vs right was counterbalanced across the 14 pairs. Subjects rated familiarity with and preference for the complete 37-item checklist. The familiarity scale consisted of five options, labelled 1 “I do not recognize the product”, 2 “I recognize the product, but I have not tasted it”, 3 “I have tasted, but I do not use the product”, 4 “I occasionally eat the product” and 5 “I regularly eat the product” (Bäckström, Pirttilä-Backman, & Tuorila, 2004). A nine-point hedonic scale (Peryam & Pilgrim, 1957) was used for the collection of liking data.

Results

Effect of tannic acid concentration on intensity and liking ratings of juices

Perceived intensity and liking data from each juice were independently submitted to a one-way ANOVA model to estimate the TA concentration effect (three levels: TA0, TA1 and TA2).

Astringency intensities increased with TA concentration in all juices (Table 2). Bitterness was significantly affected by TA levels in all the three juices. The results of the LSD post hoc test showed that bitterness ratings from TA2 were significantly higher than those from both TA0 and TA1 samples, while no significant differences were found when comparing TA0 and TA1 juices. No significant effect of TA concentration was determined on sourness.

Table 2

Effect of tannic acid (TA) concentration on astringency, bitterness and sourness ratings from juices (one-way ANOVA).

		$F_{2,228}$	p	Mean		
				TA0	TA1	TA2
Apple	Astringency	56.4	<0.001	9.7 ^c	31.4 ^b	39.5 ^a
	Bitterness	11.2	<0.001	3.3 ^b	4.5 ^b	10.7 ^a
	Sourness	0.8	0.440	24.3	24.7	21.4
Grape	Astringency	46.8	<0.001	14.0 ^c	33.3 ^b	41.7 ^a
	Bitterness	7.9	<0.001	5.5 ^b	6.5 ^b	11.8 ^a
	Sourness	1.2	0.310	25.5	25.1	21.2
Carrot	Astringency	35.8	<0.001	7.3 ^c	21.0 ^b	28.6 ^a
	Bitterness	3.3	0.040	17.2 ^b	18.9 ^b	24.3 ^a
	Sourness	0.6	0.550	16.2	17.8	14.9

Mean values followed by different letters are significantly different ($p < 0.05$).

Table 3

Effect of tannic acid (TA) concentration on liking scores from juices (one-way ANOVA).

	$F_{2,228}$	p	Mean		
			TA0	TA1	TA2
Apple	30.9	<0.001	6.7 ^a	5.6 ^b	4.6 ^c
Grape	14.3	<0.001	6.6 ^a	5.6 ^b	5.3 ^b
Carrot	1.1	0.320	2.6	2.9	2.6

Mean values followed by different letters are significantly different ($p < 0.001$).

A significant effect of TA concentration on liking for apple and grape juices was found (Table 3). The results of LSD post hoc tests showed that the mean ratings regularly decreased with TA concentration in apple juice. In grape juice, liking ratings of TA0 were significantly higher than those of TA1 and TA2 samples, whereas no significant differences were found when comparing TA1 and TA2 juices. TA concentration did not significantly affect liking ratings of carrot juices.

Subjects grouping

Individual responsiveness to phenolic astringent stimuli was recently demonstrated to relate to SPs D -values (Dinnella et al., 2009). Subjects were grouped according to three levels of variation (low, L; medium, M; high, H) of SPs D -values. Characteristic values of a percentile distribution (first and third quartiles) were used to define three groups: Low Responding (LR, $n = 20$), Medium Responding (MR, $n = 37$) and High Responding (HR, $n = 20$) subjects. Mean salivary protein concentration determined in the three subject groups after first and after second stimulation is reported in Table 4.

Responsiveness to phenolic astringent stimuli of the three groups was evaluated based on the ratings of 1.4 g/l TA sample tasted immediately after the second saliva collection. Astringency, bitterness and sourness ratings were independently submitted to a one-way ANOVA model to estimate the group effect (three levels: LR, MR, HR subjects). Subject groups differed significantly for the intensity of perceived astringency ($F_{2,74} = 8.48$; $p < 0.001$). Mean astringency ratings of HR subjects (35.74 ± 2.04) were significantly higher than those of the other two groups, while no differences were found comparing LR (20.98 ± 2.04) and MR (18.48 ± 1.50) ratings. The sensory results confirm that subjects capable of maintaining constant salivary protein concentration after both mechanical and chemical stimulation were less responsive to astringent stimuli than subjects in which the same stimulations induced a significant decrease of salivary protein concentration.

Physiological characteristics and perceptive responses to phenolic stimuli of the three selected subject groups have been extensively discussed elsewhere (Dinnella et al., 2009, 2010).

Effect of individual astringency responsiveness on liking for juices

Responsiveness of the LR, MR and HR groups to astringency, bitterness and sourness of pure and TA spiked juices was investigated. The modification of perceived intensities induced by adding TA to juices was computed as arithmetic difference between astringency, bitterness and sourness rated in TA2 and in pure juices ($d_{\text{Intensity}} = \text{Intensity T2} - \text{Intensity T0}$). Astringency, bitterness and sourness $d_{\text{Intensity}}$ values were independently submitted to a two-way ANOVA to estimate the group (three levels: LR, MR and HR subjects) and the juice (three levels: apple, grape and carrot) effects, and their interaction. Only significant F ratios are listed below.

The results confirmed that groups differed significantly in their perception of astringency ($F_{2,222} = 6.54$; $p \leq 0.001$). A significant

Table 4

Mean salivary protein concentration (mg/ml) and relevant *D* values determined in Low Responding (LR, *n* = 20), Medium Responding (MR, *n* = 37) and High Responding (HR, *n* = 20) subjects after first (S1) and after second stimulation (S2).

	LR			MR			HR		
	S1	S2	<i>D</i>	S1	S2	<i>D</i>	S1	S2	<i>D</i>
Mean	3.67	3.40	-0.26	3.59	2.79	-0.79	6.76	4.22	-2.54
SE	0.23	0.28	0.17	0.24	0.18	0.14	0.69	0.38	0.42
Min	2.39	1.67	-1.76	1.55	1.40	-2.90	3.01	2.18	-7.24
Max	6.57	6.62	1.01	7.77	5.46	0.54	15.74	8.50	-0.06
<i>p</i> -Value			0.14			<0.001			<0.001

SE: standard error; Min: minimum; Max: maximum values.

effect of juice on astringency dIntensity values was found ($F_{2,222} = 5.59$; $p \leq 0.001$). No significant effect was found for group \times juice interactions. No significant group effect for bitterness and sourness dIntensity values was found.

One-way ANOVA models were computed independently for each juice on dIntensity astringency values to further investigate group effects. dIntensity values for astringency were higher in HR than that in MR and LR subjects (apple: $F_{2,74} = 3.44$; $p = 0.04$, grape: $F_{2,74} = 3.06$; $p = 0.05$, carrot: $F_{2,74} = 2.81$; $p = 0.06$), meaning that TA

induces a greater increase of perceived astringency in HR than in the rest of subjects (Fig. 1A).

The liking for pure and TA2 juices was also computed as the arithmetic difference between liking for TA2 and T0 samples (dLiking = Liking T2 – Liking T0). dLiking values were submitted to a two-way ANOVA to estimate the group (three levels: LR, MR and HR subjects) and the juice (three levels: apple, grape and carrot) effects. Significant group and juice effects on dLiking values were found ($F_{2,222} = 3.76$; $p = 0.02$; $F_{2,222} = 24.72$; $p \leq 0.001$). No significant effect of group \times juice interactions was found.

The group effect on liking for vegetable juices was further investigated by performing one-way ANOVA model on dLiking values computed for each juice (Fig. 1B). Adding TA induced a greater decrease in liking in HR than in MR and LR subjects for apple juice ($F_{2,74} = 3.62$; $p = 0.03$). TA induced a greater decrease in liking in HR than in LR subjects while no significant differences were found comparing dLiking data from MR to both HR and LR groups for grape juice ($F_{2,74} = 2.13$, $p = 0.10$). No significant variation of liking for carrot juice was found when comparing data from different subject groups.

Effect of individual astringency responsiveness on preference for phenol-rich foods

The liking data for phenol-rich food expressed by all 77 subjects were analysed by means of an Internal Preference Map (IMP). Results are shown in the score plot (Fig. 2A) and in the correlation plot (Fig. 2B). The variance explained by the model after the first two significant dimensions was 41%. Individual respondents are represented on the correlation plot by points which can be considered as end-points of vectors from the origin. These vectors are not exact representations of each individual's scores, but are projections onto the preference dimension demonstrating the best fit of the original data. The direction of the vector represents the direction of increasing personal 'preference' for a consumer (Monteleone, Frewer, Wakeling, & Mela, 1998). The first dimension of the correlation plot indicates that, on average, subject preference is oriented towards the products on the right side of the first dimension of the score plot. The less preferred products are positioned on the left side of the first dimension of the score plot and consist of carrot juice, soy milk, raw chicory, coffee without sugar, tea without sugar. Wines (aged red wine, red wine and white wine), vegetables, coffee wo milk and chocolate, positioned on the right of the map, represent the most preferred products.

Subjects are spread more widely along the second dimension of the correlation plot. Looking at the second dimensions of both score and correlation plots and considering the potential astringency of the products, three product subgroups were identified. Subgroup 1, consisting of extremely ripe fruits (extremely ripe fruit, extremely ripe banana and extremely ripe pear), is positioned on the bottom of the second dimension of the score plot. These products are potentially less astringent than unripe fruit (unripe fruit; unripe banana and unripe pear) that are positioned on the upper side of the second dimension of the score plot. Also subgroups 2 and 3, positioned on the opposite sides of the score plot, were identified.

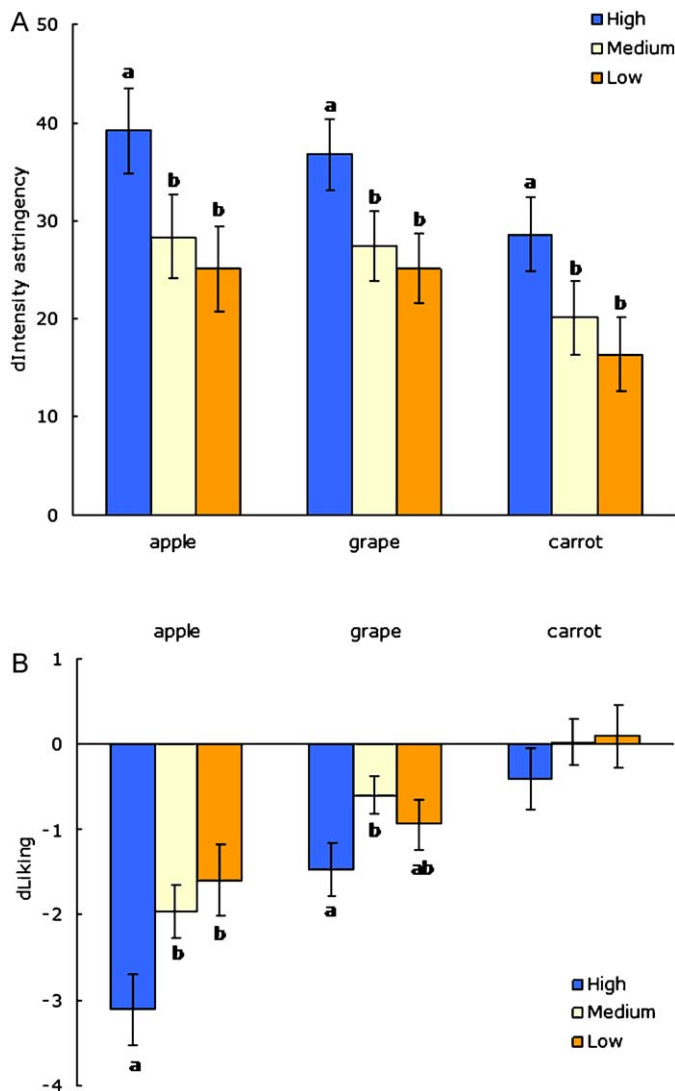


Fig. 1. dIntensity (Intensity T2 – Intensity T0) (A) and dLiking (Liking T2 – Liking T0) (B) values of juices, induced by adding TA to juices computed for Low (LR, *n* = 20), Medium (MR, *n* = 37) and High Responding (HR, *n* = 20) subject groups. Bars represent standard errors. Mean values followed by different letters are significantly different ($p < 0.05$).

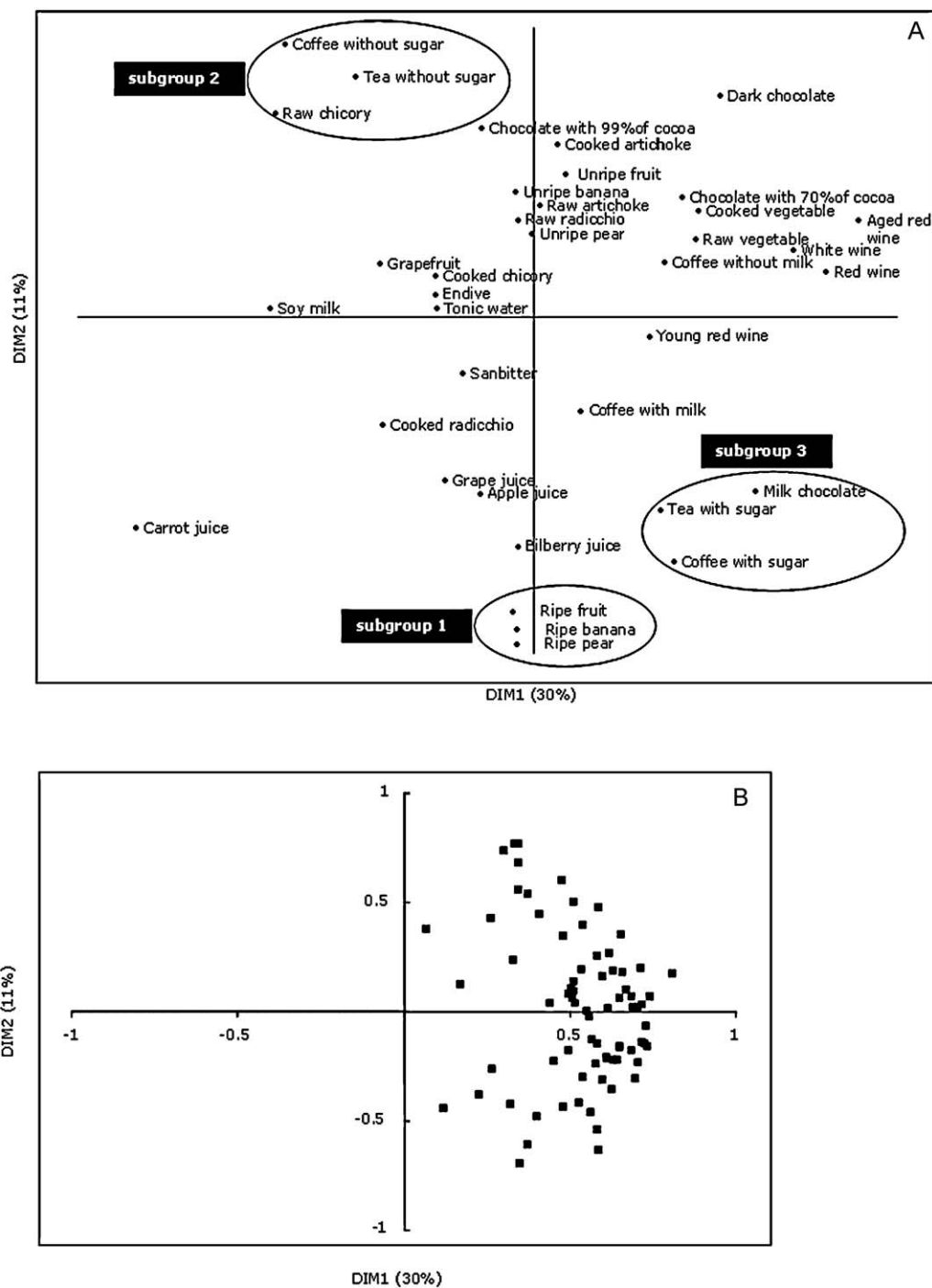


Fig. 2. Internal preference map computed on preference scores for 37 food products from 77 subjects (A) and subject score plot (B).

Subgroup 2 (coffee w/o sugar, tea w/o sugar and raw chicory) is positioned on the upper left of the score plot. These products are characterized by a high potential astringency. Subgroup 3 (milk chocolate, tea w/ sugar and coffee w/ sugar) is positioned on the lower right of the bi-dimensional product space. These products are characterized by a low potential astringency.

Sensory data from both TA model solution and juice evaluation clearly showed that the perceptions of the MR group are not significantly different from those of LR group. Thus, only the two extreme subject groups (HR and LR) were considered in questionnaire data analysis.

The effect of HR and LR status on food subgroup preference was investigated. A 2-way ANOVA model was performed on

preference scores independently for each food subgroups using subject groups (2 levels: HR and LR) and products (3 levels) as factors. No subject group ($F_{1,114} = 0.08$; $p = 0.78$), product ($F_{2,114} = 0.10$; $p = 0.90$) or subject group \times product interaction ($F_{2,114} = 0.10$; $p = 0.90$) effects were found for food subgroup 1. A significant subject group effect was found in both food subgroup 2 and 3 ($F_{1,114} = 4.72$; $p = 0.03$; $F_{1,114} = 3.21$; $p = 0.08$, respectively), no significant product ($F_{2,114} = 0.42$; $p = 0.66$; $F_{2,114} = 1.39$; $p = 0.25$, respectively) or subject group \times product interaction ($F_{2,114} = 0.07$; $p = 0.93$; $F_{2,114} = 0.34$; $p = 0.71$, respectively) effects were found. Mean preference scores of HR subjects were lower for products with the most astringency potential (subgroup 2) and higher for products with the least astringency

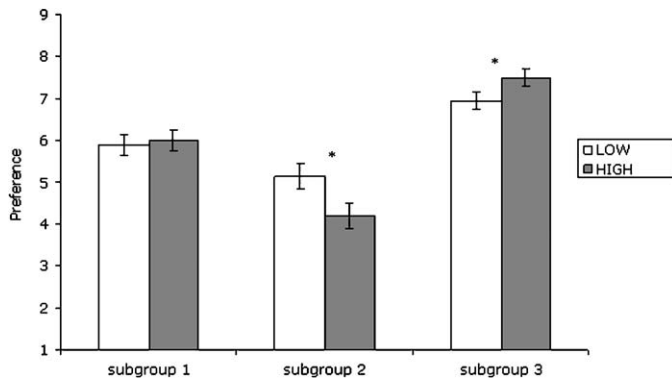


Fig. 3. Preference scores for food subgroups rated by Low Responding (LR, $n = 20$) and High Responding (HR, $n = 20$) subject groups. Subgroup 1 (low astringency potential): extremely ripe fruit, extremely ripe banana, extremely ripe pear; subgroup 2 (high astringency potential): coffee wo sugar, tea wo sugar, raw chicory; subgroup 3 (low astringency potential): milk chocolate, tea w sugar, coffee w sugar. Bars represent standard errors. * $p \leq 0.10$.

potential (subgroup 3) compared with those of the LR subjects (Fig. 3).

Relationship between preference and familiarity

Scores on the first dimension of the preference map and rank sum of familiarity scores were ordered by ranking for the 77 subjects. A significant linear correlation was found between these data sets ($r = 0.86$; $p \leq 0.001$). The scores ranking order was congruent with the familiarity ranking order particularly for the most preferred food items. However, this relationship was not found in a few cases. Coffee without sugar, tea without sugar, cooked radicchio and extremely ripe fruit resulted more familiar than preferred, whereas grape, apple and bilberry juices were less familiar than preferred. Preference and familiarity ranks show that several food items, belonging to the same pair, were ranked regardless of their astringency potential. Coffee w/wo sugar and tea w/wo sugar were ranked according to their potential astringency; the most astringent being much less preferred and familiar than the least astringent product.

Astringency responsiveness and familiarity with phenol-rich foods

The effect of individual responsiveness to astringent stimuli on familiarity was investigated by comparing the scores expressed for the most astringent products of the 14 food pair items by HR and LR subjects. Kruskal–Wallis one-way ANOVA indicates that LR subjects tended to rate familiarity with the most astringent products of the 14 food pair items higher than did HR subjects ($\chi^2 = 2.85$; $p = 0.09$, rank sum 81,604 and 75,476, respectively). The effect of HR/LR status on familiarity was further investigated by considering the number of regular consumers of highly astringent products. The self-reported familiarity score of 5 separated regular consumers (F) from the rest of subjects (UF, self reported familiarity score ≤ 4). Five homogeneous astringent food subgroups were considered (coffee/tea: coffee wo sugar, tea wo sugar, coffee wo milk; chocolate: dark, 70% and 90% cocoa; vegetables: raw artichoke, raw radicchio, raw chicory, raw vegetables; fruit: not perfectly ripe pear, not perfectly ripe banana, not perfectly ripe fruit; wine: aged red and red). Chi-square was computed on the number of F and UF subjects found in HR and LR groups independently for each food subgroup (Table 5). The number of regular consumers of highly astringent coffee/tea, chocolate, vegetables and fruit tend to be greater in the LR than in the HR group. Individual responsiveness to astringent stimuli was not associated with the number of regular consumers of red wine.

Table 5

Pearson chi-square (χ^2) computed on the number of subjects familiar (F) and unfamiliar (UF) with five astringent food groups identified in High Responding and Low Responding subject groups.

Astringent food group	HR		LR		χ^2	p
	F	UF	F	UF		
<i>Coffee/tea</i>						
Tea wo sugar	2	18	6	14	2.30	0.13
Coffee wo sugar	3	17	6	14		
Black coffee	13	7	14	6		
Tot	18	42	26	34		
<i>Chocolate</i>						
Dark chocolate	4	16	8	12	8.08	0.00
Chocolate 99% cocoa	1	19	3	17		
Chocolate 70% cocoa	2	18	9	11		
Tot	7	53	20	40		
<i>Vegetables</i>						
Raw artichoke	2	18	4	16	2.60	0.10
Raw radicchio	4	16	7	13		
Raw chicory	0	20	1	19		
Raw vegetables	11	9	14	6		
Tot	17	63	26	54		
<i>Fruit</i>						
Unripe banana	3	17	5	15	2.30	0.12
Unripe pear	3	17	3	17		
Unripe fruit	4	16	9	11		
Tot	10	50	17	43		
<i>Wine</i>						
Aged red wine	8	12	6	14	1.23	0.26
Red wine	13	7	10	10		
Tot	21	19	16	24		

F: self-reported familiarity score = 5; UF: self-reported familiarity score ≤ 4 .

Astringency responsiveness and the choice of phenol-rich foods

Before data analysis, pairs were ordered so that ratings less than 4 always indicate a choice for the product with the most astringency potential while ratings higher than 4 indicate a choice for the product with the least potential astringency. Mean choice scores for

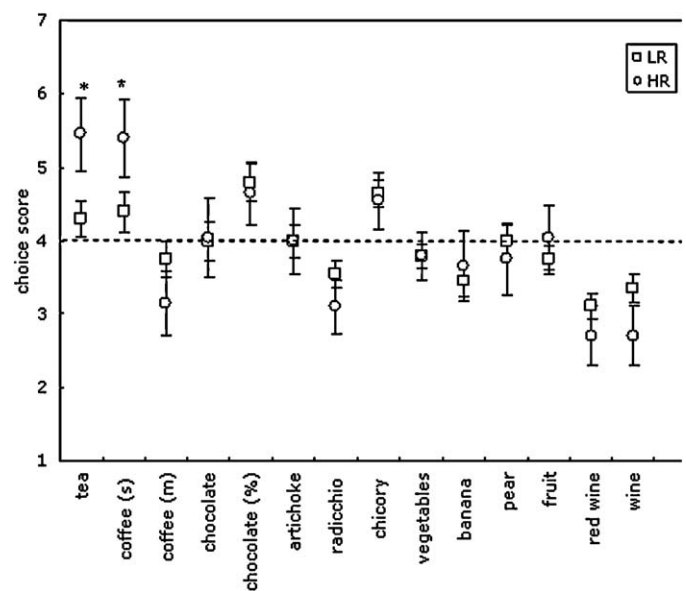


Fig. 4. Choice scores for food pairs rated by Low Responding (LR, $n = 20$) and High Responding (HR, $n = 20$) subject groups. Scores < 4 indicate choice for the most astringent product in the pair. Scores > 4 indicate choice for the least astringent product in the pair. Bars represent standard errors. * $p \leq 0.15$.

the 14 food pairs rated by the all the 77 subjects were submitted to a one-way ANOVA to estimate the pair effect on choice. A significant pair effect on choice was found ($F_{13,988} = 8.77$; $p \leq 0.001$). The choice was oriented to the most astringent product within the following pairs: red wine aged/young, wine red/white, coffee wo/w milk and radicchio raw/cooked. On the other hand, within tea wo/w sugar, coffee wo/w sugar, chicory raw/cooked, chocolate 90/70% cocoa and artichoke raw/cooked pairs the choice was oriented to the least astringent product. A similar choice pattern was found in HR and LR subjects (Fig. 4). Scores expressed by HR and LR subject groups were submitted to *t*-test for each pair independently. Choice scores from HR were significantly higher than those from LR subjects within tea wo/w sugar ($t_{38, 2.02} = 1.74$; $p = 0.09$) and coffee wo/w sugar ($t_{38, 2.02} = 1.43$; $p = 0.15$) pairs meaning that the HR group choice was oriented towards the least astringent product. Overall, however, the effect of HR/LR status was negligible.

Discussion

Effect of tannic acid on sensory properties and liking for juices

Pure apple, grape and carrot juices are complex stimuli which induce different oral sensations. Moderate/strong sourness, weak/moderate astringency and bitterness were perceived in pure apple and grape juices. Pure carrot juice was perceived as weakly astringent and moderately sour and bitter. Water solutions of tannic acid are described as highly astringent, bitter and weakly sour (Robichaud & Noble, 1990). A marked modification of the sensory profile was found when TA was added to a juice. As expected, a strong enhancement of astringency was observed in all the TA spiked juices and a small but significant increase in bitterness was found in all the TA2 samples. Thus, astringency became the dominant attribute in TA spiked apple and grape juices whereas both astringency and bitterness characterized spiked carrot juices. Mean hedonic values indicated that pure apple and grape juices were definitely liked by subjects. The decrease in hedonic ratings of spiked apple and grape juices, compared with corresponding pure samples, confirm the general negative effect of astringency and bitterness on acceptability (Lesschaeve & Noble, 2005). However, the effect of perceived astringency intensity on the hedonic response strongly depends also on the product. In apple juices liking decreased with increased intensities of astringency. Based on liking scores, both pure and TA1 apple juice can be considered well-liked products. The further increase in astringency intensity in the TA2 apple juice sample resulted in the product being disliked (mean liking score <5). On the other hand, increasing astringency in grape juice samples only induced a slight decrease in liking, and all the three samples can be considered as well-liked products irrespective to their astringency intensity. No significant effect of TA on liking for carrot juices was observed, despite the increased intensities of both astringency and bitterness. The very low hedonic ratings expressed for pure carrot juice probably account for this result. Overall, the intensity and hedonic ratings indicate that the importance of the perceived intensities of generally disliked sensations, such as bitterness and astringency, in determining the overall liking can vary widely depending on the food product. Increasing intensity of disliked sensations can be critical for well-liked foods, depending on their overall sensory profile, whereas it can only slightly influence the liking for products with low acceptability.

Effect of Low, Medium and High Responding status on astringency and hedonic ratings of juices

Astringency arises from rupture of the lubricating saliva film that lines the oral cavity (de Wijk & Prinz, 2006). A two step molecular model of salivary protein/dietary phenol interaction has

been hypothesized for astringency elicitation (Nayak & Carpenter, 2008). The first step might involve a dynamic salivary protein film (prolin rich proteins, amylases, cystatins and hystatins) exerting a phenol-sequestering role, thus protecting against the negative effects of phenolic compounds on nutritional uptake. The second step might be based on phenol interactions with an adsorbed glycoprotein layer with the consequent oral cavity delubrication and astringency elicitation.

Individual differences in perceived astringency are correlated with the change in salivary protein content after repeated stimulations (SPs *D*-value) (Dinnella et al., 2009). Based on a subject's ability to react to oral stimulation, groups with different responsiveness to astringent phenolic stimuli can be identified. LR and MR groups show SPs *D*-values around zero indicating a high ability to maintain a constant salivary protein concentration. Negative *D*-values characterize the HR group since a strong salivary glycoprotein depletion takes place in this subject group after repeated oral stimulations. A decrease of parotid protein secretion is involved in the depletion of whole saliva protein concentration induced by oral stimulation in the HR group, thus resulting in a temporary lowering of the overall saliva defence level against phenolic compounds. Thereby an increased responsiveness to astringent stimuli provides a warning cue (Dinnella et al., 2010).

Based on SPs *D*-values distribution, three subject groups were selected with different responsiveness to astringency induced by TA (a widely used phenolic astringent standard). The different responsiveness of subject groups to TA induced astringency was confirmed by intensity data from juices. The intensity modification induced by juices with different TA concentrations was lower in LR and MR than in HR group. Thus, salivary status affects perceived astringency intensity when it is induced by complex stimuli whereby several oral and olfactory sensations are simultaneously perceived. The salivary variables used to group subjects do not affect the sensitivity to either bitterness or sourness (Dinnella et al., 2009). Juice intensity data indicate that salivary characteristics responsible for individual differences in astringency response are not related to differences in perceived response to bitterness or sourness.

Hedonic responses to apple and grape juices show that the individual responsiveness to astringency determines the overall liking for the complex stimuli. The greater the increase of perceived intensity, the greater is the decrease in liking induced by adding TA to juices. The hedonic responses to products with a low acceptability were not affected by the intensity of perceived astringency. This explains the lack of a significant effect of salivary status on carrot juice liking.

Effect of Low Responding and High Responding status on the acceptance of phenol-rich foods

Preference patterns are determined by a broad set of factors such as existing habitual behaviours, learning mechanisms, individual beliefs and expectations of sensory and other properties (Mela, 1999). Astringency potential represents only one of the preference drivers for the 37 food items considered in the questionnaire, as clearly shown by their position on the bi-dimensional space of IMP. The internal preference map identified a group of items (wines, vegetables, coffee and chocolate) which are highly preferred irrespective of their astringency potential. In fact, similar scores on the first bi-plot dimension characterize both products of the relevant food pairs (wine red/white; red wine aged/young; coffee wo/w milk; vegetables raw/cooked and chocolate dark/milk). Moreover, raw and cooked vegetables, red and white wines show similar scores also on the second dimension thus indicating an analogous preference pattern by all the subjects. These products are largely represented in the daily Italian diet

(Monteleone & Dinnella, 2009) and the close positive correlation between preference and consumption frequency is very well known (Aldridge, Dovey, & Halford, 2009). On the contrary, for some pairs, sensory differences among items are mainly driven by astringency, bitterness and sourness (e.g., ripe/unripe fruit; coffee wo/w sugar; tea wo/w sugar; dark/milk chocolate). Items from these pairs were differently positioned on the second dimension of the preference map. Based on ripeness level, fruit pair items show the same scores on the first preference map dimension but are positioned on the opposite side of the second dimension thus indicating subjective differences in the preference patterns. An effect of astringency responsiveness might have been expected when comparing HR and LR preference scores for fruit items. Ripening induces several modifications of fruit sensory properties including colour, olfaction, texture and taste other than astringency intensity (Billy, Mehinagic, & Royer, 2008; Cascales, Costell, & Romojaro, 2005; Manganaris, Vasilakakis, & Mignani, 2008). The overall modification of sensory profile induced by ripening can be responsible for the different preference patterns of fruit pair items thus explaining the lack of individual astringency responsiveness effect on preference.

Coffee and tea wo/w sugar pair items were positioned on the opposite side of the preference map according to their astringency potential. The food pairs with the lowest astringency potential were more preferred than those with the highest astringency potential. LR/HR status significantly affects the preference expressed for these items with preference patterns driven by astringency intensity.

Analysis of familiarity data shows that individual variation in astringency responsiveness influences knowledge and consumption frequency for both food products important from the nutritional point of view, such as fruit and vegetables, and for voluptuary products such as stimulant beverages and chocolate. HR/LR status does not seem to affect familiarity with wine. This is likely to be related to the wide variation of the sensory profiles of wine products as well as to the complexity of their preference and consumption patterns (Lockshin, Jarvis, d'Hauteville, & Perrouy, 2006).

The comparison between preference and familiarity ranking orders confirms that, within a given population, the astringency intensity does not necessarily drive the acceptability of products. This is even more evident for foods in the upper part of the ranking, in fact they are ordered irrespective to their astringency potential. However, for a limited number of foods, preference and familiarity ranking order are not congruent. The discrepancy between self-reported knowledge/consumption and preference further underlines the multimodality of overall food acceptance. Health related beliefs for apple and blackberry juices could have driven their high preference scores but these products are not well known among Italian consumers.

All the data from the questionnaire make evident the effect of HR/LR status on the acceptance of coffee and tea at different astringency potentials. This may be related to their extensive presence in the daily diet of Italian consumers, and to the easy modulation of their astringency level by appropriate sweetening.

Conclusion

Physiological differences in individual perceptual abilities represent one of the variables influencing food acceptance. The present data show, for the first time, that individual differences in physiological salivation responses to oral stimulation, significantly affect responsiveness to complex astringent stimuli and influence the overall acceptance of selected phenol-rich food items. An increase in tannic acid concentration in juices induces a more marked decrease of liking in subjects highly responsive to

astringent phenolic compounds than in those less responsive to these stimuli. Although astringency is a disliked sensation, the questionnaire data show that no clear-cut relationship exists between acceptance and astringency intensity, probably due to the complex, multiphase formation of preference patterns. Overall, however, the individual sensitivity to astringent stimuli potentially influences the consumption of phenol-rich foods recommended for a healthy diet and thus may be considered as one of the factors influencing food habits.

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