Highlights

- Food neophobia encompasses the rejections of familiar food and beverages
- Food neophobia influences liking of strong but not mild tasting food and beverages
- High, medium and low neophobics do not differ in individual chemosensory responsiveness
- Perceptual and hedonic differences are probably mediated by high levels of arousal in food neophobics
- Food neophobia related differences in liking of strong tasting items are independent from age and gender

Associations between food neophobia and responsiveness to "warning" chemosensory sensations in food products in a large population sample

Laureati, M.[1], Spinelli, S.[2], Monteleone, E.[2], Dinnella, C.[2], Prescott, J.[2,3], Cattaneo, C.[1], Proserpio, C.[1], De Toffoli, A.[2], Gasperi, F.[4], Endrizzi, I.[4], Torri, L.[5], Peparaio, M.[6], Arena, E.[7], Bonello, F.[8], Condelli, N.[9], Di Monaco, R.[10], Gatti, E.[11], Piasentier, E.[12], Tesini, F.[13], Pagliarini, E.[1]*

[1]University of Milan, Italy
[2]University of Florence, Italy
[3]TasteMatters Research & Consulting, Sydney, Australia
[4]Edmund Mach Foundation, San Michele all'Adige, Italy
[5]University of Gastronomic Science, Bra, Italy
[6]CREA – Research Centre on Food and Nutrition, Rome, Italy
[7]University of Catania, Italy
[8]CREA – Research Center for Enology, Asti, Italy
[9]University of Basilicata, Potenza, Italy
[10]University of Naples - Federico II, Italy
[11]IBIMET-CNR, Bologna, Italy
[12]University of Udine, Italy
[13]University of Bologna, Italy

[*] Corresponding author: Monica Laureati, PhD. Address: Department of Food, Environmental and Nutritional Sciences (DeFENS), Via Celoria 2, 20133, Milan, Italy. Tel.:+39 2 50 31 91 88; fax.: +39 2 50 31 91 90. E-mail address: monica.laureati@unimi.it

Abstract

The aim of the present study is to explore the association between food neophobia and chemosensory responsiveness and to determine whether this association translates into different food liking and preference patterns. Data were collected on 1225 respondents (61% females, age 20-60 years) as part of the Italian Taste project. Respondents completed the *Food Neophobia Scale* (FNS) as well as a food preference and familiarity questionnaire for a number of foods and beverages categorized as mild or strong tasting. Moreover, they evaluated attribute intensity and liking of an actual food (dark chocolate pudding) varying in the level of sweetness, bitterness and astringency. Taste function was evaluated by measuring fungiform papillae density (FPD), responsiveness to PROP (6-n- propylthiouracil) and to water solutions representing various oro-sensory qualities.

High, medium and low neophobic subjects did not differ for FPD and chemosensory responsiveness. Reported liking was significantly lower for high neophobics than low neophobics only for those vegetables and beverages characterized by high levels of warning stimuli (i.e. bitterness, sourness, astringency and alcohol), whereas almost no differences were found for the bland versions of food items. High and medium neophobics rated astringency and, to a lesser extent, bitterness of the dark chocolate pudding, as more intense than low neophobics and liked the most bitter and astringent variants significantly less than low neophobics.

Differences in liking, however, do not seem to be mediated by food neophobics' superior taste functioning but rather by higher levels of arousal when eating food and/or drinking beverages that are perceived as potentially unpleasant and dangerous. Finally, the effect of food neophobia was evident not only for potentially unusual items in the Italian food context, but even for items that might be considered highly familiar.

Keywords: Food neophobia, arousal, liking, fungiform papillae, prop, bitterness, astringency

1. Introduction

Food neophobia, defined as the reluctance to eat unfamiliar foods, is a characteristic that all omnivores, including humans, share (Pliner & Hobden, 1992). This food behavior is a heritable trait (Knaapila et al., 2007) which has been preserved from one generation to another making some individuals extremely selective about food, presumably as a means to avoid the potential toxicity of an unknown food source. Even in modern society, where food safety is generally guaranteed and the protective purpose of food neophobia has lost importance, up to 35% of individuals show a selective attitude toward food (Kauer et al., 2015; Zickgraf & Schepps, 2016). Similar percentages have been reported in two large-scale studies on USA (Meiselman, King, & Gillette, 2010) and New Zealand (Jaeger et al., 2017) population samples, with high neophobic individuals accounting, respectively, for 40-45% and 30% of the total population.

Food neophobia (FN) and food selectivity are considered maladaptive behaviors as they decrease diet variety, thus having potentially important nutritional consequences. Recent evidence suggests that, in adults, FN is negatively related to daily fruit and vegetables intake and to diet variety in general (Jaeger et al., 2017; Zickgraf & Schepps, 2016). Moreover, an association between FN and increased body mass index has been observed (Proserpio et al., 2018) as neophobic individuals may choose to eat familiar food which is more energy dense than fruit and vegetables (Knaapila et al., 2011) or may be less willing to try healthy alternative versions of familiar products (Monteleone et al., 2017; Schickenberg, van Assema, Brug, & de Vries, 2008).

Although FN has been studied extensively, especially in children, relatively little information is available on its causal origins and relationship to eating behavior in adults. Knaapila et al. (2011) reported high neophobic reactions for fruit and vegetables, fish and meat but no effect of FN was observed on frequency of use of energy dense foods in a large sample of young adults. Similar findings have been reported in children (Cooke et al., 2003), but it remains unclear why FN is particularly high for certain food categories. Some authors suggested that this behavior may be due to other personality traits (Dovey et al., 2008), whereas others reported perceptual (Coulthard & Blissett, 2009) or genetic reasons (Knaapila et al., 2007; 2011). More likely, the specificity of FN is due to the concurrence of all these factors.

An important aspect for novel food refusal is the expectation that the sensory properties of food may be unpleasant (Pliner et al., 1993). In this context, individual difference in taste responsiveness may play an essential role in moderating this effect. Polymorphisms in the TAS2R38 gene may lead to variation in the perception of the bitterness of 6-n-propylthiouracil (PROP), with individuals classed as ‘supertasters’ (STs), ‘medium tasters’ (MTs) or ‘nontasters’ (NTs) (Bartoshuk, Duffy, & Miller, 1994). Despite some contradictory data in the literature, higher taste responsiveness to PROP has been associated with greater perception of a variety of oro-sensory stimuli including sensations from bitter/astringent fruits and vegetables, fruit juices, and alcoholic beverages (Dinehart et al., 2006; Lanier et al., 2005; Melis et al., 2017; Tepper et al., 2009). Moreover, when compared with PROP non-tasters, PROP tasters perceive sourness (Prescott et al., 2004) and the burning sensations from ethanol and spices (Prescott et al., 2000) more intensely. In general, STs also express greater dislike and more frequent rejection of astringent, bitter and sour fruits and vegetables compared to NTs (Hayes et al., 2013; Monteleone et al., 2017; Sandell et al., 2015). Moreover, a greater PROP responsiveness seems to be associated with diets rich in saturated fatty acid and added sugars, in contrast to plant-based diets (Stevenson et al., 2016). Since FN is considered an adaptive, evolutionary response, which prevents from the ingestion of poisonous substances more commonly found in fruits and vegetables (i.e., bitter, sour, and astringent compounds) (Pliner & Salvy, 2006), it is reasonable to hypothesize that food neophobics might be more sensitive to such "warning" chemosensory signals, detecting even subtle changes of these stimuli in food.

Quite surprisingly, there has been very little research carried out to ascertain whether taste responsiveness varies according to degree of FN, and whether individual differences in perception may contribute to influence food preference and choice among neophobics and neophilics. Törnwall et al. (2014), in a large-scale study on twins, showed large differences in liking of foods with specific flavor qualities (e.g. sour fruits, berries, spicy foods and spices), but showed no differences in the liking of bland foods (salty-and-fatty foods, sweet-and-fatty foods, and fish), as a function of FN. The food neophilic group (food adventurous), expressed higher liking for sour and spicy foods compared to the less neophilic group (basic) and had more tolerance for capsaicin burn when tasted in model food. Interestingly, the two groups did not differ in their PROP responsiveness, or in their ratings of the intensity of sour and pungent stimuli.

Ullrich et al. (2004) reported a more complex association between taste responsiveness, rejection of novel food and food preference. They classified subjects according to their frequency of trying new foods as food adventurous or non-adventurous and found that food adventurousness was strongly associated with greater liking of bitter, hot, and pungent foods in PROP tasters, but not in PROP NTs. Only PROP tasters that were less adventurous showed a dislike of bitter, hot, and pungent foods. However, a comparison in PROP responsiveness between the two groups was not explicitly reported.

Although these findings suggest an association between FN, taste responsiveness and food preference, it is unclear whether the food rejection shown by food neophobics is mediated by a physiological predisposition to hypersensitivity or instead by higher levels of arousal when approaching new foods. With the possible exception of Törnwall et al. (2014), in which a model food (strawberry jelly) was used, to our knowledge, there have been no studies of FN in large population samples that have evaluated real foods varying in their sensory properties. Indeed, one of the limits of the existing literature on FN is that conclusions are drawn on small datasets thus limiting the explanatory power of FN in relation to other factors associated to food choice and health (Jaeger et al., 2017). Therefore, there is a need for further exploration of FN in larger population samples in order to examine its causal origins and its impact on food preferences and choices and its potential consequences on human health.

The present paper is part of the *Italian Taste* project, a large-scale study aimed at exploring the associations among biological, genetic, physiological, sociocultural, psychological and personality-related factors, describing the dimensions of food liking, preference, behavior and choice, and their relevance in determining individual differences within a given food culture framework (Monteleone et al., 2017).

Assuming that those high in FN tend to reject foods, in particular vegetables that are often characterized by "alarm" sensations such as sourness, bitterness and astringency, we wanted to explore whether the reluctance to consume such foods might reflect greater chemosensory responsiveness. The hypothesis is that food neophobics show higher taste responsiveness, which lead them to perceive "warning" chemosensory sensations as more intense than do neophilics. The increased responsiveness in food neophobics might justify the reduced liking for a variety of foods with high levels of "warning" sensations often experienced in many vegetables and healthy products. To test this hypothesis, we studied a sample of 1225 individuals who were assessed for taste functioning by measuring fungiform papillae density (FPD) and PROP responsiveness as well as the intensity of solutions representing the basic tastes and astringency. Respondents also completed the Food Neophobia Scale (FNS) and a food preference and familiarity questionnaire for a number of foods and beverages that could be easily categorized as mild or strong tasting. Food preference for warning stimuli was also tested using a real product (i.e., chocolate pudding) which was evaluated for liking and intensity of sweetness, bitterness and astringency.

2. Material and methods

2.1. Participants

Data were collected on 1225 Italian consumers (61% female; age range 20-60 years). Male and female mean ages were 37.0 years (SD=13.1) and 36.8 years (SD=12.7), respectively. The age distributions of males and females were not significantly different. In order to explore possible age-related differences, respondents were divided in three age groups: 18-30 years (41%), 31-45 years (27%), 46-60 years (32%). Participant recruitment details for the project are detailed in Monteleone et al. (2017).

Data on PROP responsiveness, attribute intensities and liking for the product (chocolate puddings) were collected on 1149 respondents (61% females; age range 20-60 years, males mean age 36.6 years ± SD 13.1, females mean age 36.4 years ± SD 12.7). This reduced data set was due to the fact that two of the 19 research units involved in the project differed from the others for these measurements, showing a higher frequency of ratings close to the maximum of the scale, probably due to the lack of compliance with the procedure for training subjects to the gLMS and LAM scale use (Monteleone et al., 2017).

The study was conducted in agreement with the Italian ethical requirements on research activities and personal data protection (D.L. 30.6.03 n. 196). The study protocol was approved by the Ethics Committee of Trieste

University where the genetics unit of the project is based. The respondents gave their written informed consent at the beginning of the test according to the principles of the Declaration of Helsinki.

2.2. Measurements

A detailed description of the *Italian Taste* project data collection is provided in Monteleone et al. (2017). In the present study, we limited the description to the measurements of interest. Briefly, respondents were invited to the laboratory to participate to several activities throughout two separate days. Prior to the laboratory sessions, participants completed at home an online questionnaire about their familiarity with a series of food items. During the first day, respondents were introduced to the general aim of the study and received instructions on the use of the hedonic and intensity rating scales as well as on the administration of the questionnaires. Then, they were asked to perform the hedonic test on four chocolate pudding samples. The hedonic test was followed by the administration of the food preference questionnaire, the FNS questionnaire and the evaluation of PROP solutions. During the second day, respondents were reminded of the general aim of the study and asked to rate the intensity of the water solutions (i.e., sweet, bitter, salty, sour, umami, astringent) and, after a short rest, the intensity of sweetness, bitterness and astringency of the chocolate pudding samples. The second session ended with the assessment of fungiform papillae density.

2.2.1. Questionnaires

2.2.1.1. Food familiarity and preference

The food familiarity and food preference questionnaires were developed to measure, respectively, familiarity with, and liking for, a series of food items including vegetables, beverages and sweets/desserts. The item selection reflected variations in familiarity (more/less familiar foods) and taste (mild/strong). Taste classification was based on previous literature data and published sensory databases (Dinnella et al., 2011; Lease et al., 2016; Rouseff, 1990; Wiener et al., 2012). The rationale for choosing these three specific food categories was that vegetables and beverages include items that can be easily categorized as mild or strong tasting, whereas sweets/desserts are clearly recognizable as mild items. This categorization would have been difficult with foods such as meat, fish or bakery products that, on their own, vary little in flavor intensity.

Food familiarity was assessed using a 5-point labeled scale (Tuorila et al., 2001): 1= “I do not recognize it”; 2= “I recognize it, but I have never tasted it”; 3= “I have tasted it, but I don’t eat it”; 4=”I occasionally eat it; 5= “I regularly eat it”. In order to minimize possible influences of familiarity on the association between food neophobia and reported liking of mild/strong tasting food products, within each food category, only items with mean familiarity score > 3.5 were retained, for a total of 16 vegetables, 13 beverages and 15 sweets/desserts. Reported liking was assessed using the 9-point hedonic scale (Peryam & Pilgrim, 1957) anchored at the extremes: 1=“extremely disliked” and 9= “extremely liked” using as middle point of the scale 5= “neither liked nor disliked”. If the participant had never tasted the food in question, they could choose the answer “I have never tasted it". The presentation order of the items within each product category as well as the product category order were randomized across participants.

2.2.1.2. Food neophobia assessment

Food neophobia was quantified using the Food Neophobia Scale (FNS) developed by Pliner & Hobden (1992). The FNS consists of ten statements evaluated with a 7-point agreement scale ranging from 1="I strongly disagree" to 7="I strongly agree". The individual FNS scores were computed as the sum of ratings given to the ten statements, after the neophilic items had been reversed; thus, the scores theoretically ranged from 10 to 70, with higher scores reflecting higher FN levels. The FNS frequency distribution was calculated and respondents were divided into 3 groups according to their FN level: low, medium and high (see results section 3.2 for details).

The original FNS was translated to Italian by two independent bilingual Italian native-speakers and, then, back translated into English (Supplementary material). The two versions were compared to identify discrepancies and reach consensus for an updated version, which was reviewed by an expert in semantics and adjustments were made when necessary to select the most appropriate translation. The final version of the Italian FNS was pilot tested with a small sample of subjects to confirm the clarity of the items and instructions for completion of the instrument. In order to assess temporal stability of the Italian version of FNS, the scale was administered twice on a sub-sample of 117 respondents (48.5% females, age range 21-60 years, mean age=39.4 years, SD=11.6) with a minimum and maximum time interval of 8 and 14 months, respectively, between the two administrations.

2.2.2. Liking and intensity ratings of a real food product

A dark chocolate pudding (prepared by dissolving in water a pudding mix: Budino da zuccherare, Cameo S.p.A., Italy with added cocoa powder: Cacao Amaro Perugina, Nestlè, Italy) was selected for the study according to the following criteria: i) being widely consumed and distributed in Italy; ii) being simple and reproducible to prepare (e.g. ready-made product), to handle (e.g. to be consumed at room temperature) and homogeneous in composition and to be easily portioned (e.g. semi-solid). Four samples varying in sucrose concentration were produced by adding different amounts of sucrose (C1=38 g/kg; C2=83 g/kg; C3=119 g/kg; C4=233 g/kg) to the base dark chocolate pudding. The addition of sucrose was expected to increase sweetness, while decreasing bitterness and astringency. The choice of sugar concentrations was based on published psychophysical data, preliminary tests (unpublished data) and a pilot study performed in 10 sensory laboratories with an average number of 5 subjects per lab to ascertain that all four prototypes were clearly discriminated according to the target sensations (i.e., sweetness, bitterness, astringency).

Liking and intensity of the target sensations were evaluated in separate days. During the first session, respondents were asked to rate their liking for each of the chocolate pudding samples using the Labeled

Affective Magnitude Scale, LAM (0–100) (Schutz & Cardello, 2001). During the second session, respondents evaluated the intensity of three sensations, namely sweetness, bitterness and astringency for each of the samples using the Generalized Labeled Magnitude Scale, gLMS (0–100) (Bartoshuk et al., 2004). The experimenters provided instructions for the use of both scales prior to tasting.

In each session, the samples were served at room temperature and presented simultaneously in plastic cups coded with 3-digit numbers. Each sample consisted of 15 g of chocolate pudding. The respondents were instructed to eat the entire amount provided prior to rating liking/intensity. An interval of 90 s was imposed between tastings, during which water (tap or mineral water) was provided for palate cleansing. The sample presentation order was systematically varied according to a William's Latin square.

2.2.3. Responsiveness to PROP and water solutions

A supra-threshold 3.2 mM PROP solution was prepared by dissolving 0.5447 g/L of 6-n-propyl-2-thiouracil (European Pharmacopoeia Reference Standard, Sigma Aldrich, Milano, IT) into deionized water (Prescott, Soo, Campbell, & Roberts, 2004). Subjects were presented with two identical samples (10 ml) in plastic cups, coded with three-digit numbers. Subjects were instructed to hold each sample (10 ml) in their mouth for 10 s, then expectorate, wait 20 s and evaluate the intensity of bitterness using the gLMS (Bartoshuk et al., 2004). Subjects had a 90 s break in order to control for carry-over effect after the first sample evaluation. During the break, subjects rinsed their mouth with water for 30 s, had some plain crackers for 30 s, and finally rinsed with water for a further 30 s. The average bitterness score was used for each subject.

Respondents were grouped according to their PROP status based on arbitrary cut-offs (Fischer et al., 2013; Hayes et al., 2010). Non-tasters (NTs) were 25.6% of total sample (arbitrary cut-off gLMS $\leq$ 17, moderate), whereas Super-tasters (STs) were 29.3% (arbitrary cut-off gLMS $\geq$ 53, very strong). The rest of the respondents were considered as Medium-tasters (MTs).

Six water solutions, corresponding to the five basic tastes and astringency were rated for intensity using the gLMS. The concentration of the solutions were decided based on published psychophysical data (Feeney & Hayes, 2014; Hayes, Sullivan, & Duffy, 2010; Masi, Dinnella, Monteleone, & Prescott, 2015) and previous preliminary trials conducted with one hundred untrained subjects (unpublished data) in order to select solutions equivalent to moderate/strong on a gLMS (sourness: citric acid 4 g/kg; bitterness: caffeine 3 g/kg; sweetness: sucrose 200 g/kg; saltiness: sodium chloride 15 g/kg; umami: monosodium glutamic acid salt 10 g/kg; astringency: potassium aluminum sulfate 0.8 g/kg). Respondents were informed about the sensory quality that they were tasting.

2.2.4. Fungiform papillae density

The anterior portion of the dorsal surface of the tongue was swabbed with household blue food coloring, using a cotton-tipped applicator. This made the fungiform papillae (FP) easily visible as red structures against the blue background of the stained tongue. Digital pictures of the tongue were recorded (Shahbake, Hutchinson, Laing, & Jinks, 2005) using a digital microscope (MicroCapture, version 2.0 for 20x-400x) (Masi et al., 2015). For each participant, the clearest image was selected, and the number of FP was counted in two 0.6 cm diameter circles, one on right side and one on left side of tongue, 0.5 cm from the tip and 0.5 cm from the tongue midline. The number of FP was manually counted by two researchers independently according to the Denver Papillae Protocol (Nuessle, Garneau, Sloan, & Santorico, 2015). The average of these two scores was used for each subject. The individual FPD was then calculated by reporting the number of FP to a common unit area of 1 cm^2. A FPD frequency distribution was calculated and respondents were divided into 3 groups: Low FPD (LFP; respondents in the lowest quartile: FPD $\leq$ 12.37, 25.7%), Medium FPD (MFP; respondents in the second and third quartiles, 12.37 < FPD < 29.16, 49.5%) and High FPD (HFP; respondents in the highest quartile: FPD $\geq$ 29.16, 24.8%).

2.3. Data analysis

2.3.1. Validation of the Italian version of the FNS

Reliability of the scale was assessed by calculating internal consistency (Cronbach's α) and temporal stability by test–retest evaluation. Correlations between items, item total correlation with FNS score and the relationship between mean values for each item and for total FNS score in the test–retest evaluation were measured using Pearson's correlation coefficients. Analysis of Cronbach's α with deleted variables was performed in order to investigate whether all the items contributed in the same way to the construct.

Consistent with previous studies (Fernandez-Ruiz et al., 2013; Laureati, Bergamaschi et al., 2015), the relationship between each item was further evaluated with Principal Component Analysis (PCA). Data were standardized (i.e., scaled to unit variance) prior to modeling and cross validation was chosen as validation method. A correlation loadings plot was used to find significant variables (>50% explained variance) (Westad et al., 2000). The external validity of FNS was evaluated analyzing the relationship between FNS scores and mean vegetables reported liking and familiarity through Pearson's correlation coefficients.

2.3.2. Association among food neophobia, taste responsiveness, liking and attribute intensities

The association between FN, taste responsiveness and reported liking (vegetables, beverages and sweets) was explored through 3-way ANOVAs considering Neophobia level (Low, Medium, High), Gender and Age (18-30 years, 31-45 years, 46-60 years) and their 2-way interactions as factors. When a significant effect of Age and Gender was found, data were further analyzed separately for males and females and for the three age groups through 2-way ANOVA considering Neophobia level, either Gender or Age and the respective interactions as independent variables in order to have better insights on the relative contribution of these factors on dependent variables. Post-hoc comparisons using the Bonferroni test adjusted for multiple comparison were used. Familiarity data were analyzed through Friedman's test. The association between FN, liking and attribute intensities of a food was investigated through 2-way ANOVA considering Neophobia level (Low, Medium, High), Samples (C1-C4) and their interaction as factors. A p-value of 0.05 was considered as threshold for statistical significance. The SAS/STAT statistical software package version 9.3.1 (SAS Institute Inc., Cary, USA) and The Unscrambler X software (CAMO Software AS, Oslo, Norway) were used for the data analysis.

3. Results

3.1. Validation of the Italian version of the FNS

The reader is referred to Appendix 1 for the presentation of the results about internal reliability and external validity of FNS Italian version. Briefly, the scale displayed high internal consistency (Cronbach's $\alpha = 0.87$) and test–retest reliability. The correlation between the first and second administration of the whole scale was 0.77 ($p<0.01$). PCA results showed that the second principal component separated reversed from unreversed items, indicating the ability of the instrument to measure two distinctive dimensions that describe opposite reactions to food, namely food neophobia and food neophilia. The FNS score was significantly and negatively related to reported vegetables liking ($r=-0.19$, $p<0.0001$) and familiarity ($r=-0.15$, $p<0.0001$) indicating satisfactory predictive validity.

3.2. Food neophobia scores segmentation

The FNS frequency distribution was calculated and respondents were divided into three groups according to their neophobia level. The group with Low FN (the neophilic group), corresponded to 26.9% of the total sample and had a FNS score within the lowest quartile (FNS score ≤ 18, mean FNS score=14.2). The medium FN group accounted for 46.9% of the total sample and included respondents within the second and third quartiles (18<FNS score<36, mean FNS score =26.1). The group with high FN (the neophobic group) corresponded to 26.2% of the total sample and had a FNS score within the highest quartile (FNS score ≥ 36, mean FNS score =43.3).

3.3. Taste responsiveness is not affected by food neophobia level

Mean values of FPD and responsiveness to PROP, basic tastes and astringency as a function of FN are reported in Table 1. Three-way ANOVA showed no effect of FN level on any of the oro-sensory variables considered. An effect of the main factors Age and Gender was found for FPD (Gender: $F_{(1,1105)}= 5.44$, $p<0.05$; Age: $F_{(2,1105)}= 60.71$, $p<0.0001$), responsiveness to PROP (Gender: $F_{(1,1135)}= 14.70$, $p<0.0001$; Age: $F_{(2,1135)}= 3.19$ $p<0.05$), umami (Gender: $F_{(1,1134)}= 4.64$, $p<0.05$; Age: $F_{(2,1134)}= 5.74$, $p<0.01$) and astringency (Gender: $F_{(1,1134)}= 5.47$, $p<0.05$; Age: $F_{(2,1134)}= 3.78$, $p<0.05$). Post-hoc tests with Bonferroni adjustment revealed that females had higher FPD and were more responsive to PROP but scored lower for umami and astringency than did males. FPD decreased considerably with increasing age. Accordingly, younger subjects perceived PROP, umami and astringency as more intense than the older ones. None of the 2-way interactions were significant.

INSERT TABLE 1 ABOUT HERE

3.4. Food neophobia level influences liking of strong but not mild tasting food and beverages

3.4.1. Vegetables

Results from 3-way ANOVA with interactions showed that the main factors Age and Gender were significant for most vegetables independently of taste categorization (mild/strong). In all cases, females and older subjects liked vegetables more than did males and younger people (only sweet corn showed a significant, negative relationship with age), probably due to the increased awareness of healthy eating with age and in females (Margetts et al., 1997). The FN x Gender interaction was significant only in one case (Cucumber: $F_{(4, 1197)}$=3.24, p<0.05), and the FN x Age interaction was significant in two cases (Broccoli: $F_{(4, 1201)}$=3.21, p<0.05; Eggplant: $F_{(4, 1201)}$=2.45, p<0.05). In general, ANOVA conducted on females and males separately produced comparable results, as did the analysis performed on the three age groups, suggesting that Gender and Age are not confounding effects of FN on reported liking of mild/strong tasting vegetables. The results on the effect of FN on vegetable liking and familiarity are reported in Table 2 averaged across gender and age. Food neophobia had a significant effect on liking of all vegetables with a strong taste, while the effect on mild vegetables was observed only for one (i.e. green beans) out of eight items. Post-hoc comparisons showed that, in general, low food neophobics (neophilics) liked vegetables significantly more than did medium and high food neophobics, who showed no differences. The analysis of familiarity data showed that, with the exception of three strong tasting items (i.e., asparagus, broccoli and radish), all vegetable items were well known and commonly used by subjects with different levels of FN. Overall, results indicate a strong association, independent of age and gender, between FN and liking for those vegetables characterized by “warning” chemosensory sensations such as bitterness and/or astringency.

INSERT TABLE 2 ABOUT HERE

3.4.2. Beverages

Results from 3-way ANOVA with interactions showed that the main factor Age was often significant. When the association between age and liking was negative and a concomitant FN effect was observed, the relative contribution of age and FN on beverages reported liking cannot be established unequivocally. This was only the case for one item, namely alcoholic aperitifs. To analyze further the relative contribution of FN and age on reported beverage liking, the analysis was performed separately for the three age categories (18-30 y, 31-45 y, 46-60 y), confirming that Age was not a confounding effect of FN. In other words, if a beverage was significantly more or less liked according to age, the trend was the same in all the three FN groups (low, medium, high). The FN x Age interaction was significant only for red wine ($F_{(4,1189)}$=2.39, p=0.05); red wine was equally liked by the three age categories in low and medium neophobic people, whereas liking for red wine increased significantly according to age in the high neophobic group.

Gender was often a significant effect for liking, with males providing higher liking ratings for beverages than females, except for non-alcoholic aperitif. In order to better understand the relative contribution of gender and FN on beverages liking, a separate analysis was performed for males and females, which provided a very similar outcome for both genders. No FN x Gender and FN x Age interactions were significant.

Mean beverage liking and familiarity ratings by taste categorization (mild/strong) and FN, averaged across gender and age, are reported in Table 3. FN had a significant effect on liking for all beverages with a strong taste. Post-hoc comparisons indicated that, overall, low neophobics liked these beverages significantly more than did high neophobics, whereas medium neophobics lay in between. The effect of FN on beverages with a mild taste was significant for sweetened tea and soft drinks. In this case, the reported liking was in the opposite direction, in that high food neophobics liked these beverages significantly more than low neophobics. The analysis of the familiarity data provided similar results with mild beverages being either equally familiar or more familiar to food neophobics than to neophilics and strong beverages being in general less familiar to neophobics than neophilics. Overall, these results indicate that, for beverages, a strong taste, which comprised warning sensations such as bitterness, astringency and alcohol bite plays an important role in modulating liking in food neophobic individuals. Moreover, this behavior is independent of age and gender.

INSERT TABLE 3 ABOUT HERE

3.4.3. Sweets and desserts

Results from 3-way ANOVA with interactions showed that Age and Gender were significant for most items. As expected, the association between age and liking of sweets and desserts was always negative, probably due to increased health concerns with increasing age and/or decreased liking for sweetness with age. Moreover, post-hoc comparisons showed that females gave higher liking scores than males for all items, with the exception of honey. Although women are reported to have high food health awareness, there is also evidence of higher cravings for sweets in females than males (Roininen et al., 2001; Tuorila et al., 2017). To analyze further the relative contribution of FN, age and gender on liking for sweets and desserts, separate analyses were performed for females and males and for the three age classes. These analyses returned very similar outcomes for females and males as well as for the three age groups, confirming that age and gender were not confounding effects of FN in reported liking of sweets and desserts.

Mean liking and familiarity ratings for sweets and desserts mean by taste categorization (mild/strong) and FN averaged across gender and age are reported in Table 4. Obviously, for this food category, all sweets and desserts are considered to have a mild taste, with few exceptions (i.e. dark chocolate, dark chocolate pudding, lemon sorbet, strawberries with sugar and lemon). Food neophobia did not have any effect on reported liking of sweets and desserts, with the exception of honey ($F_{(2, 1097)}=4.12$, $p<0.05$), dark chocolate ($F_{(2, 1209)}=7.95$, $p<0.0001$) and dark chocolate pudding ($F_{(2, 1196)}=3.20$, $p<0.05$), which were liked less by high and medium neophobics than low neophobics. Moreover, FN affected liking for milk chocolate ($F_{(2, 1204)}$=3.79, $p<0.05$), however, in this case food neophobics provided significantly higher liking ratings than subjects with low FN. Familiarity data analysis provided similar results with sweets and desserts being either equally familiar or more familiar to food neophobics than neophilics with the exception of honey, which was less familiar among neophobics than neophilics. Overall, the present results are a confirmation that when a food is not perceived as a "warning" stimulus, FN plays a marginal role on liking, independently of age and gender.

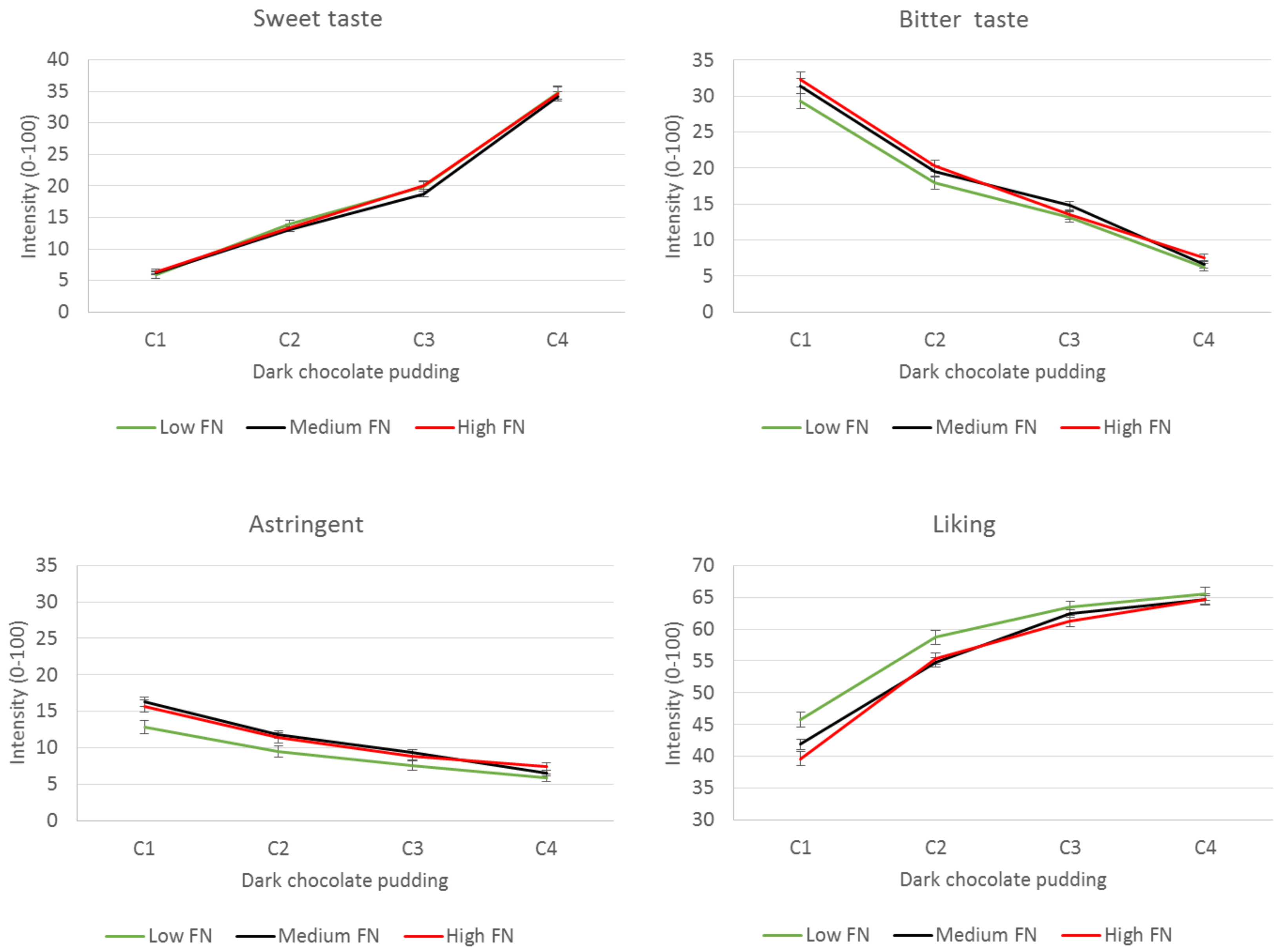
INSERT TABLE 4 ABOUT HERE

3.5. Food neophobia level influences the perception and liking of warning sensations in real food

Mean intensity ratings for sensory attributes and mean liking by product for each FN level are depicted in Figure 1 a-d. Two-way ANOVA with interaction showed that sweetness (Figure 1 a) increased with sugar concentration (main Sample effect: $F_{(3, 4564)}$=1067.47; $p<0.0001$), with no significant differences among the three FN groups (main Neophobia level effect: $F_{(2; 4564)}$=0.92; p=0.39; 2-way interaction: $F_{(6; 4564)}$=0.75; p=0.61). Accordingly, bitterness (Figure 1 b) decreases with increased sugar concentration (main Sample effect: $F_{(3, 4564)}$=666.68; $p<0.0001$), with the low food neophobic group providing lower intensity ratings than the medium and the high food neophobic groups, although the main factor FN just failed to reach significance ($F_{(2; 4564)}$=2.30; p=0.09). The interaction Sample x Neophobia level was not significant ($F_{(6; 4564)}$=0.56; p=0.76).

Astringency (Figure 1 c) decreased with increasing sugar concentration (main Sample effect: $F_{(3, 4564)}$=109.46; $p<0.0001$). The neophilic group provided intensity ratings which were systematically lower than the other two groups (main Neophobia level effect: $F_{(2; 4564)}$=6.61; $p<0.01$). The interaction was not significant. This reduced perception of bitterness and astringency by low food neophobics was reflected in an increased liking (Figure 1 d) for the most bitter and astringent samples compared to high and medium food neophobics (main Sample effect: $F_{(3, 4564)}$=384.86$p<0.0001$; main Neophobia level effect: $F_{(2; 4564)}$=8.06; $p<0.001$), although the 2-way interaction was not significant. Separate analyses performed on females and males and on the three age classes produced a similar outcome, confirming that gender and age are not confounding effects of FN in the perception of warning sensations and liking of chocolate pudding.

Figure 1. Mean intensity ratings for sweet taste (a), bitter taste (b), astringency (c) and mean liking ratings (d) by product (C1 less sweet sample, C4 sweetest sample) and by neophobia. Error bars are standard errors.



4. Discussion

4.1. Validation of the Italian version of the Food Neophobia Scale

The original version of the FNS, developed and validated on a representative sample of Canadian students, has been widely used to assess willingness to try new foods in studies conducted around the world after translation in different languages. Although the FNS has been already used in the Italian translation (Demattè, Endrizzi, & Gasperi, 2013) with good internal consistency, this is the first study to validate the instrument on a large sample of the Italian population (n=1225). Internal consistency of the FNS scores in the present study was comparable to that reported in previous research involving large population samples of Finns (Knaapila et al., 2015; Tuorila, Lähteenmäki, Pohjalainen, & Lotti, 2001), Swiss (Siegrist, Hartmann, & Keller, 2013), Spanish (Fernández-Ruiz et al., 2013), Swedish (Hursti & Sjödén, 1997) and New Zealand (Jaeger et al., 2017). Altogether, these results confirm that FNS is a robust and efficient tool even when translated in other languages (Ritchey, Frank, Hursti, & Tuorila, 2003).

4.2. Characteristics of food neophobia

We found a somewhat high proportion of neophobic people, in that a quarter of this sample had a food neophobia score higher than 36. Considering that we studied a population sample of adults, in which FN is expected to be low compared with childhood, a proportion of this magnitude has significant implications for food choices. As already observed in previous studies, we found an effect of both age (Meiselman et al., 2010; Siegrist et al., 2013; Tuorila et al., 2001) and gender (Hursti & Sjödén, 1997; Siegrist et al., 2013; Tuorila et al., 2001) on FN. Although these two factors did not seem to be confounding effects of FN on perception and liking of mild/strong tasting foods and beverages, we recommend considering both age- and gender-related differences when exploring the association between personality traits, food perception and preference. Other studies have indeed found that sociodemographic factors, especially gender, mediate the effect of personality traits on food liking and choice of spicy food (Spinelli et al., 2017 submitted).

4.3. Association between food neophobia, perception and liking of warning sensations in real food

The present large-scale study aimed to better understand the association between FN and chemosensory responsiveness and to determine whether this association translated in different food liking and preference patterns. We hypothesized that the rejection of specific food categories such as fruits and vegetables could be in part due to food neophobics' increased perception of strong and disliked oro-sensory stimuli, which often characterize plant food. Most fruits and vegetables are indeed rich in phenolic compounds and other substances that impart bitterness, astringency and sourness to the food (Drewnoski & Gomez-Carneros, 2000). Such oro-sensory qualities are considered biologically important "warning' signals. Bitterness and sourness are notoriously two sensory properties for which humans have an innate dislike and aversion, as they represent potential sources of toxic compounds and rotten and/or unripe food, respectively (Laureati, Pagliarini et al., 2015). Astringency also elicits negative consumer reactions when perceived at high intensities (Dinnella et al., 2011), probably because tannins may have anti-nutritional effects in animals and humans by reducing the digestibility of dietary proteins (Melis et al., 2017). Since FN is a conservative behavior, which keeps the organism's feeding behavior 'locked in on a safe track' (Schulze & Watson, 1995, p. 230), it can be reasonably hypothesized that food neophobics may have developed a hypersensitivity to warning sensations that makes them extremely cautious when approaching unfamiliar food, especially if it tastes bitter, astringent or sour.

We found that reported liking was significantly lower for high and medium food neophobics than low food neophobics only for those vegetables and beverages which were characterized by higher levels of alarm stimuli (i.e. bitterness, sourness, astringency and alcohol), whereas almost no differences were found for the bland versions of vegetables and beverages and for sweets and desserts. This pattern was confirmed when tasting an actual food, as high and medium food neophobics liked the most bitter and astringent versions of a dark chocolate pudding significantly less than did low food neophobics. The clear hedonic-related differences between individuals with low and high neophobia levels for warning signals were substantiated by differences in perception, as high and medium food neophobics systematically rated astringency and, to a lesser extent, bitterness, as more intense than low food neophobics. The fact that astringency was clearly better discriminated by high and medium food neophobics than low food neophobics, whereas a tendency was found for bitterness is interesting and merits further explanation. Our data indicated that samples C1 and C2 of chocolate puddings were rated as "strong-moderate" for bitterness on the gLMS (mean intensity ratings: C1=31.3; C2=19.3), while as "moderate-weak" for astringency (mean intensity rating: C1=15.0; C2=11.0). Thus, we would have expected to find a more robust effect of food neophobia level on bitterness rather than on astringency. One explanation may be that when a critical sensation is clearly perceptible (i.e. bitterness), the higher arousal of neophobic subjects is difficult to detect. In other words, both neophilics and neophobics could be in an aroused state, thus neophobia-related differences could not be seen. By contrast, when the concentration of the sensation is subtle, the difference between neophobics and neophilics becomes evident. In line with this assumption, previous research has shown that food neophobics are characterized by a higher arousal level and a generalized enhanced vigilance than food neophilics when confronted with food stimuli (Pliner & Melo, 1997), which could lead them to detect minimal changes in sensory qualities of food. This pattern seems to be in agreement with liking data as neophobia-related differences were only detected for the most astringent and bitter samples. Interestingly, we did not find any difference between subjects with different FN levels for markers of chemosensory responsiveness (PROP sensitivity and FPD) and response to oro-sensory stimuli (i.e., astringency, sweetness, sourness, umami, saltiness and bitterness by caffeine). The fact that water solutions of chemosensory stimuli were all clearly perceptible (they were chosen to represent a "moderate/strong" intensity on the gLMS) is a further confirmation that differences in oro-sensory perception between food neophobics and food neophilics may be evident only at low concentrations. In other words, our data seem to suggest that higher arousal in food neophobics could increase perceptual sensitivity via increased alertness when approaching food and that arousal could be unpleasant, therefore producing dislike of stimulus.

Recently, a few studies have investigated the relationship between sensory responsiveness and FN, reporting a significant correlation between childhood FN and taste/smell sensitivity using parental report data (Coulthard & Blissett, 2009) and a significant and positive association between smell (but not taste) reactivity and FN in toddlers using behavioural measurements (Monnery-Patris et al. 2015). Interestingly, Farrow & Coulthard (2012) found that children's sensory sensitivity mediated the relationship between anxiety and selective/neophobic eating, suggesting that greater sensitivity to sensory information may explain why more anxious children are more likely to be selective eaters. A role for anxiety mediation in food neophobia has also been pointed out in adults (Pliner and Hobden, 1992; Pliner et al., 1993, 1995), and neophilics were found to exhibit lower physiological arousal (pulse, GSR, respirations) than neophobics when presented with food stimuli (Raudenbush & Capiola, 2012). Platte, Herbert, Pauli & Breslin (2013) demonstrated also that healthy individuals with moderate levels of anxiety were more sensitive to bitter and sweet. We may thus hypothesize that food neophilics liking of stronger sensory qualities (i.e., in our study the most astringent and bitter chocolate pudding samples) does not depend on individual taste functioning but rather on a psychological mechanism of anxiety triggered by the perception of warning sensations. A similar hypothesis was proposed by Spinelli et al (submitted) to explain the effect of anxiety related traits such as neophobia, sensitivity to disgust and to punishment on pungency liking and sensory response. From this perspective, differences between neophilics and the other groups are associated with a different arousal intensity, influenced by the trait of neophobia, which can modulate sensory and hedonic responses. In other words, food neophobics would not be hypersensitive to alarm signals but the perception of such signals would put them in an arousal state that could be thought to heighten the sensory responses to the stimuli. This is consistent with the assumption that the perception of danger and fear of negative consequences of eating novel food, as well as the expectation that sensory characteristics may be unpleasant, is a fundamental principle of food rejection (Pliner & Salvy, 2006).

Similar to our findings, Törnwall et al. (2014) reported an increased liking for spicy food in people defined as "adventurous" - a term that can be assimilated into the concept of food neophilia - but no differences in taste ability, as measured by PROP responsiveness, were found between adventurous and non-adventurous individuals. Moreover, as we also found in the present study, large differences were shown between adventurous and non-adventurous individuals in liking of foods with specific flavor qualities (e.g. sour fruits and berries and spicy foods and spices), but reported no differences in the liking of bland foods (e.g. salty-and-fatty foods, sweet-and-fatty foods). Kauer et al. (2015) found that "selective" eaters were more likely to reject foods that were bitter or sour but not sweet. Knaapila et al. (2011) reported similar results in a large sample of young adults, whereas Cooke et al. (2003) observed this behavior in children, showing high neophobic reaction for fruit and vegetables as well as fish and meat but not starchy, sweet or fatty snack foods.

These findings are in line with Rozin's (1988) argument that foods that are generally accepted are those that (are expected to) taste good (e.g. sweets) and those that are seen to be beneficial for survival (e.g. energy dense food). Such foods share sensory characteristics (i.e. saltiness, sweetness, fattiness) that are signals of nutrients and are thus inconsistent with the need to be wary. Thus, individuals with high levels of FN may indeed perceive energy dense food as "safe", resulting in increased preference ratings and familiarity for those foods. Further confirmation of this assumption is provided by the fact that in the present study we found not only that food neophobics disliked foods and beverages with strong taste but, in some cases, they even reported greater liking than neophilics for energy dense food and beverages (i.e. milk chocolate, sweetened tea and soft-drinks). The implication of this finding is that FN may contribute quite substantially to the quality of the diet, leading neophobics to opt for more caloric versions of food, as shown in previous studies (Jaeger et al., 2017; Knaapila et al., 2011, 2015; Zickgraf & Schepps, 2016). Moreover, the comparison between our data and data on children (Cooke et al., 2003; Russell & Worsley, 2008) seems to indicate that the rejection of healthy food such as fruit and vegetables and the preference for high-energy dense food are not behaviors observable only in childhood but in all ages. Thus, finding solutions to reduce neophobic reactions in early age groups – or dealing with it in adulthood and third age – should be an important aim of future studies.

Somewhat at odds with the FN in terms of food novelty, we also found that FN seems to be generalized to food that can be considered highly familiar, at least in our representative sample of Italian consumers. In fact, both in the food preference questionnaire and in the actual tasting test we selected food items and beverages that scored high on familiarity in order to avoid unwanted effects of low familiarity on hedonic responses. The analysis of familiarity data showed that, as expected, food neophobics differed from food neophilics in the familiarity of several food items considered especially for items with strong taste. Thus, it cannot be excluded that familiarity instead of the perception of alarm stimuli played a role in the large hedonic differences observed according to neophobia level. The direction of this association is difficult to predict. Indeed, strong tasting foods and beverages may be less familiar to food neophobics due to their (disliked) taste, which in turn reduces the frequency of consumption and the familiarity toward such foods, thus leading to a vicious circle and possibly to changes in FN level over the lifespan.

Consistent with our findings, Jaeger et al. (2017) also found that the effect of food neophobia extends beyond rejection of unfamiliar/unusual foods to encompass many commonplace food items. It is not easy to explain how such a broad effect of food neophobia might arise. Tuorila et al. (2001) speculated that people scoring high in FN are possibly not only those who have fear of new foods; they may also be individuals who have little interest in foods. Similarly, Jaeger et al. (2017) hypothesized that those high in FN have, in general, less positive associations with food throughout their lives, as a results of more frequent encounters with foods that they wish to avoid. Finally, although in our study we did not include a measure for pickiness, we cannot exclude that the behavior we observed is also representative of pickiness, which is defined as the refusal of familiar and unfamiliar food severe enough to interfere with daily routines to an extent that is problematic (Taylor et al., 2015). Despite the fact pickiness and food neophobia are sometimes considered as distinct constructs, these two behaviors have been reported to be highly correlated (Taylor et al., 2015).

5. Conclusion

The present large-scale study has expanded the existing knowledge on the association between food neophobia, taste responsiveness, and food preference, thus contributing to the understanding of psychological and sensory-driven barriers to healthy food consumption. Our main outcome is that neophobia-related differences in reported liking were found only for foods and beverages characterized by high intensities of warning sensations (i.e. bitter, astringency, sourness and alcohol). These hedonic differences were confirmed also using a real food, especially when the concentration of the warning sensation was subtle. This pattern of findings is independent of age and gender and does not seem to be mediated by food neophobics superior taste functioning but rather by higher levels of general trait anxiety, which lead them to be on alert when eating food and/or drinking beverages that are perceived as potentially unpleasant and dangerous. However, it should be underlined that in the present study no measures of anxiety were performed, thus further perspectives of study should aim to better understand the role of anxiety trait in relation to food neophobia and food consumption. Finally, the effect of food neophobia was evident not only for potentially unusual items in the Italian context, but even for items that might be considered highly familiar to the Italian population.

As a final remark, it should be highlighted that the actual product chosen in this study to test the relation between FN, food preference and chemosensory responsiveness (i.e. chocolate pudding) is a rather familiar product in Italy, thus it would be interesting to replicate the study in order to verify whether the effect of FN would be stronger when using novel and unfamiliar foods.

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Author Contributions

ML undertook the data analyses and wrote the manuscript; ML, SS, EM, CD contributed to plan the data analyses; ML, SS, EM, CD, ADT, EP, JP discussed the interpretation of the results; ML, SS, EM, CD, LT, FG, IE, EP collaborated in the design of the Italian Taste project; all authors helped with data collection, reviewed and offered critical comments on the manuscript.

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Appendix 1.

Validation of the Italian version of the FNS

Results - Reliability of the scale

FNS internal consistency was 0.87, much greater than the suggested value of 0.70 given by Nunnally and Bernstein (1994). The correlation among items was always positive and highly significant (p<.0001) with Pearson's correlation coefficients ranging from 0.20 to 0.72. Item total correlation with FNS score ranged from 0.48 for item 8 to 0.71 for item 10. The analysis of Cronbach's alpha with deleted variables did not show significant increase or decrease in the standardized alpha coefficients, thus suggesting that all items were measuring the same construct.

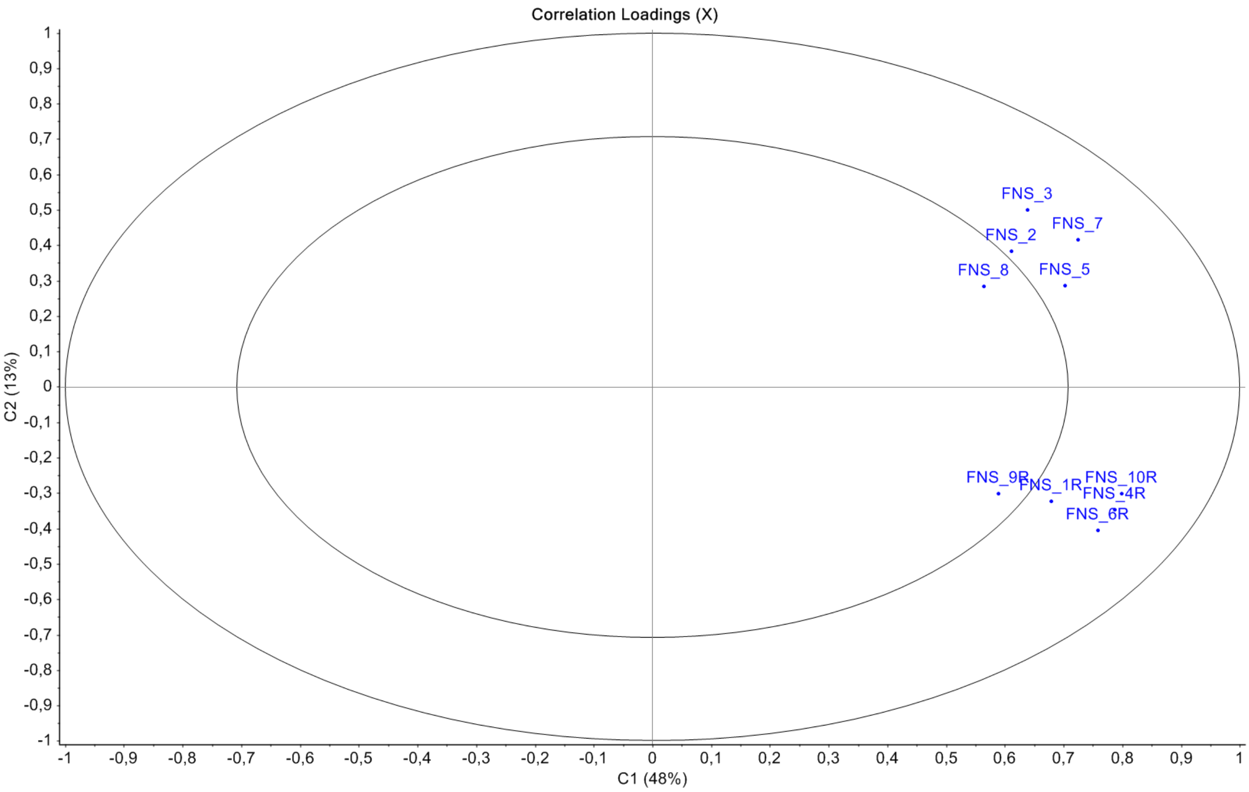
Overall mean FNS scores and individual item scores in the test–retest evaluation are reported in Table A1. The correlation between responses in the first and second administration of the FNS was high in all cases, indicating good stability of the measurement over time. The correlation between the first and second administration of the whole scale was 0.77 (p<0.01).

Table A1. Mean value, standard deviation (SD) and Pearson's correlation coefficient of each FNS item and total *FNS score (n = 117) in the test-retest evaluation. R indicates the neophilic items for which the score was reversed.*

Item	**Test**		**Retest**		**Pearson's r**	**p-value**
	Mean	**SD**	**Mean**	**SD**		
1R	3.6	1.6	3.3	1.6	0.64	<0.0001
2	2.8	1.5	2.6	1.4	0.33	<0.001
3	2.2	1.5	2.3	1.4	0.23	<0.05
4R	2.7	1.8	2.9	1.9	0.64	<0.0001
5	2.1	1.3	2.1	1.4	0.45	<0.0001
6R	2.7	1.8	2.8	1.9	0.78	<0.0001
7	2.2	1.4	2.3	1.5	0.54	<0.0001
8	2.9	1.9	2.8	1.8	0.45	<0.0001
9R	2.8	2.1	3.0	2.0	0.53	<0.0001
10R	3.1	1.9	3.1	1.8	0.81	<0.0001
FNS	**27.1**	**10.8**	**27.2**	**10.9**	**0.77**	**<0.01**

The relationship between the items was further investigated through PCA (Fig. 2). The total variance explained by the first two PCs was 61%. PC1 accounted for 48% of total variance whereas PC2 explained a further 13%. All items were positively related on PC1. Moreover, Figure 2 clearly shows that PC2 separates reversed (negative correlation) from unreversed items (positive correlation), indicating the ability of the instrument to measure two distinctive dimensions that describe opposite reactions to food, namely food neophobia and food neophilia. Since correlation loadings plot showed that items 8 and 9 explained less than 50% of the explained variance, a further analysis was conducted omitting these two items. The Cronbach's alpha resulting from the 8-item scale was 0.87. Moreover, the correlation between the 8-item scale and the original 10-item scale was r=0.975 (p<0.0001), indicating that no improvement would have been obtained by the omission of items 8 and 9.

Figure A1. Correlation Loadings Plot obtained by PCA performed on scores of each item (n = 1225). Concentric circles show the locus of 100 and 50% explained variance.



Results - Predictive validity

Despite the correlation coefficients were somewhat low, FNS score was significantly and negatively related to vegetables reported liking (r=-0.19, p<0.0001) and familiarity (r=-0.15, p<0.0001).

Results - Comparison with other FNS versions

The comparison of internal consistency of the FNS scores between the present study, the original FNS on a sample of Canadian subjects (Pliner & Hobden, 1992) and previous research involving Finns (Knaapila et al., 2015; Tuorila, Lähteenmäki, Pohjalainen, & Lotti, 2001), Swiss (Siegrist, Hartmann, & Keller, 2013), Spanish (Fernández-Ruiz et al., 2013), Swedish subjects (Koivisto-Hursti & Sjödén, 1997) and New Zealand (Jaeger et al., 2017) provided similar results. This indicates that the internal consistency of the FNS does not change substantially in relation to cultural aspects, as also reported by Ritchey et al. (2003).

Table A2. Descriptive statistics and Cronbach's alpha of the FNS as measured in the present study and comparison with other studies with similar subjects' age range (SD=standard deviation).

Paper	N	Age range	FNS Range	FNS Mean	SD	Cronbach's α
Present paper (Laureati et al.)	1225	18-60	10-69	27.4	11.7	0.87
Fernández-Ruiz et al. (2013)	309	25-60+	10-66	31.7	11.0	0.82
Jaeger et al. (2017)	1167	18-72	10-68	27.4	-	0.83
Knaapila et al. (2015)	2191	18-57	10-70	28.5	11.0	0.88
Koivisto-Hursti & Sjödén (1997)	722	10-66	10-66	25.6	-	0.81-0.90
Pliner & Hobden (1992)	75-135	18-74	10-68	34.5	11.9	0.88
Siegrist et al. (2013)	4436	21-99	-	-	-	0.80
Tuorila et al. (2001)	1083	16-80	10-70	33.9	11.4	0.85