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Remote testing for PROP taster status assessment using solutions and paper disks

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ABSTRACT

Solutions and paper disks are widely used methods for classifying people by their ability to perceive 6-n-propylthiouracil (PROP). Recently, remote sensory testing carried out under the supervision of the panel leader, has been found to be a valid alternative to the traditional lab-setting, offering several practical advantages. This study aimed to validate a protocol for PROP status assessment in remote condition and to compare the results obtained using solutions and paper disks. Individual differences in bitterness responsiveness and the relative classification between two different testing conditions (remote and laboratory) and two stimuli (solutions and disks) were compared. 77 subjects (18-30 years; 60 % women) participated in two sessions (one in laboratory and one in remote setting) and rated the perceived intensity of PROP solutions and disks. Mean PROP ratings did not vary across remote and lab conditions (p = 0.844). PROP phenotype classifications in remote-solution and lab-disk conditions were comparable to lab-solution reference condition (with a misclassification rate of 27 %and 31 % respectively, all occurring within contiguous groups). In contrast, the classification in remote-disk condition was not fully comparable (misclassification in respect to lab-solution of 48 % in contiguous groups, and 1.3 % in non-contiguous groups). One-solution test appears as a reliable procedure for PROP status assessment in live remote testing. Also, impregnated paper disks represent a suitable alternative to solution testing in conventional lab setting for practical reasons. However, paper disks could represent a valid option for data collection outside the lab, but they require rigorous in-person control by the sensory personnel when used.

1. Introduction

The ability to perceive the bitter taste of the 6-n-propylthiouracil (PROP status) varies greatly among individuals and is considered as an oral acuity index widely studied in sensory research (Tepper, 1998; Tepper, 2008; Tepper, Banni, Melis, Crnjar, & Barbarossa, 2014). Individual differences in responsiveness to PROP are mainly due to the genetic polymorphism of the TAS2R38 gene which occurs in two common haplotypes, PAV, the dominant taster haplotype and AVI, the recessive non taster haplotype (Bufe et al., 2005; Kim et al., 2003). Three phenotypes are usually identified to indicate the PROP status in the general population: Non Taster (NT), consisting in individuals perceiving low PROP bitterness intensity (<17-moderate on the general Labelled Magnitude Scale-gLMS), Medium Taster (MT), consisting in individuals perceiving moderate PROP bitterness intensity (≥17-moderate and <53very strong on gLMS) and Super Taster (ST) consisting in those with very high PROP intensity perception (253-very strong on gLMS) (Fischer et al., 2013; Hayes, Sullivan, & Duffy, 2010; Monteleone et al., 2017).

PROP phenotype positively associated with variations in perception for a wide range of oral sensations (Tepper, 2008). Tasters generally reported heightened responsiveness to basic tastes (bitter, salt, sour, sweet, and umami) compared to Non Tasters (Bajec & Pickering, 2008a; Dinnella et al., 2018; Fischer et al., 2014; Hayes, Bartoshuk, Kidd, & Duffy, 2008; Nolden, McGeary, & Hayes, 2020; Piochi, Dinnella, Spinelli, Monteleone, & Torri, 2021; Prescott, Ripandelli, & Wakeling, 2001; Robino et al., 2022). However, the high variability in bitter taste receptors (Bayer et al., 2021) could lead to complex relationships between PROP and responsiveness to other bitter compounds (Delwiche, Buletic, & Breslin, 2001a). Tasters also perceive oral irritation (Bartoshuk et al., 1993; Karrer & Bartoshuk, 1991; Nolden et al., 2020; Piochi et al., 2021; Prescott & Swain-Campbell, 2000; Spinelli et al., 2018; Yang, Ma, Cao, Wang, & Zheng, 2014), and tactile stimuli including astringency (Bajec & Pickering, 2008a; Essick, Chopra, Guest, & McGlone, 2003; Hayes & Duffy, 2007; Melis et al., 2017; Piochi et al., 2021; Robino et al., 2022; Yackinous & Guinard, 2001), at higher intensity than Non Tasters. Moreover, PROP status has been also

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associated with fat taste sensitivity (Kirkmeyer & Tepper, 2003; Melis, Sollai, Muroni, Crnjar, & Barbarossa, 2015; Tepper & Nurse, 1998). PROP status is then considered a general marker of oral responsiveness and it is often taken into account in studies aimed at investigating factors underlaying individual differences in food preferences and dietary habits (Bell & Tepper, 2006; Deshaware & Singhal, 2017; Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006; Duffy et al., 2010; Ervina et al., 2021; Kaminski, Henderson, & Drewnowski, 2000; Keller, Steinmann, Nurse & Tepper, 2002; Masi, Dinnella, Monteleone, & Prescott, 2015; Menghi et al., 2023; Mennella, Pepino, & Reed, 2005; Monteleone et al., 2017; Tepper, 2008, Tepper & Nurse, 1998; Wijtzes et al., 2017; Yeomans, Tepper, Rietzschel, & Prescott, 2007).

Several psychophysical methods are used to classify individuals by PROP taster status (Tepper, Christensen, & Cao, 2001). The most common screening procedures fall into two general categories that include thresholds and suprathreshold methods (Tepper, 2008; Tepper et al., 2017). Threshold measures determine the lowest PROP concentration that an individual can distinguish. These techniques have been used extensively (Blakeslee & Salmon, 1935; Drewnowski, Henderson, and Shore, 1997; Fischer, Griffin, England, & Garn, 1961; Galindo-Cuspinera et al., 2009; Harris & Kalmus, 1949), but they are time and laborintensive, poorly predict suprathreshold intensities, and often fail in distinguishing Medium from Super Tasters (Bartoshuk, 2000; Bartoshuk, Duffy, & Miller, 1994; Keast & Roper, 2007; Mojet, Christ-Hazelhof, & Heidema, 2005; Tepper, 2008; Tepper et al., 2017). Suprathreshold techniques instead include taste intensity assessment at higher concentrations using solution series (Bartoshuk, Duffy, & Miller, 1994; Drewnowski, Henderson, & Shore, 1997; Galindo-Cuspinera et al., 2009; Hayes, Sullivan, & Duffy, 2010; Tepper, Christensen, & Cao, 2001; Zhao, Kirkmeyer, & Tepper, 2003), a single solution (Bajec & Pickering, 2008b; Monteleone et al., 2017; Porubcan & Vickers, 2005; Prescott, Soo, Campbell, & Roberts, 2004; Tepper et al., 2001), or impregnated filter paper disks (Bartoshuk, Duffy, Reed, & Williams, 1996; Fischer et al., 2013; Zhao et al., 2003). These techniques are faster, less demanding and more discriminant than threshold measurements (Bartoshuk et al., 1994; Tepper, 2008; Tepper et al., 2017). Furthermore, suprathreshold measures reflect intensity perception in the "real world" (Bartoshuk, 2000) and represent the preferred option when the aim is investigating the association of oral responsiveness and food preferences (Ervina et al., 2021; Kaminski et al., 2000; Keller et al., 2002; Masi et al., 2015; Monteleone et al., 2017, Wijtzes et al., 2017).

The most common scales used to collect suprathreshold PROP ratings are the Labelled Magnitude Scale (LMS) (Green, Shaffer, & Gilmore, 1993), anchored at the top with "the strongest imaginable oral sensation", and its variant, the general Labelled Magnitude Scale (gLMS) (Bartoshuk et al., 2004), anchored at the top with "the strongest imaginable sensation of any kind". Typically, participants are first acclimated to the scale use through different training strategies including rating different cross-modal stimuli. Some authors ask participants to rate the intensities of different imagined/remembered sensations of varying magnitudes and modalities (Bajec & Pickering, 2008b; Bartoshuk et al., 2002; Green & Hayes, 2004; Kalva, Sims, Puentes, Snyder, & Bartoshuk, 2014; Porubcan & Vickers, 2005; Webb, Bolhuis, Cicerale, Hayes, & Keast, 2015). Other authors instead, ask participants to evaluate heaviness of visually identical weights (Delwiche et al., 2001a; Keast & Roper, 2007; Pickering & Robert, 2006; Webb et al., 2015; Yousaf, Zheng, Yi, & Tepper, 2022), or to rate the intensities of audible tones (Bartoshuk et al., 2004; Delwiche et al., 2001a; Duffy, Peterson, & Bartoshuk, 2004; Hayes et al., 2008; Hayes et al., 2010).

One-solution test at 3.2 mM PROP concentration, either its original version (Prescott et al., 2004) or with replicate (Masi et al., 2015), and impregnated paper disks (Zhao et al., 2003) represent the most popular techniques due to their ease of execution. Paper disks are easy to handle and portable thus are increasingly used in field studies and with vulnerable population who have difficulty to move to the laboratory for the test (Ervina et al., 2021; Ervina, Berget, & Almli, 2020; Melis et al.,

2020; Melis et al., 2021).

In the past years, restrictions put in place to control the spread of the Covid-19 pandemic have limited the possibility to conduct sensory tests in the classic laboratory setting, so that researchers were prompted to find a valid alternative to move sensory testing out of it, but still trying to maintain an adequately controlled environment. This effort had been made easier thanks to the spread of novel digital technologies, that allow collecting data in a less expensive way, anywhere, anytime and in real time (Dinnella et al., 2022; Meiselman, 2013). The best solution provided is the remote sensory testing in which tests are carried out online in videoconference under the constant supervision of the panel leader, who has the possibility to monitor the whole procedure and to interact with the participants (Albiol Tapia & Lee, 2022; Dinnella et al., 2022; Ervina et al., 2021; Fuentes, Gonzalez Viejo, Hall, Tang, & Tongson, 2021; Gonzalez Viejo, Fuentes, De Anda-Lobo, & Hernandez-Brenes, 2022; Gonzalez Viejo, Zhang, Khamly, Xing, & Fuentes, 2021; Menghi et al., 2023; Venkatesh & DeJesus, 2021). The methodology brings with it advantages both from the laboratory setting, such as the adequate control of the sources of variability, and from the home tests, such as being time- and costs- saving for subjects who do not have to travel to the laboratory to carry out the test, thus facilitating participant recruitment and overcoming logistic limitations (Dinnella et al., 2022).

The present study aims at developing and validating a protocol for PROP status assessment in remote condition and at comparing the results obtained with two common methodologies, paper disks and onesolution test. To this aim, PROP ratings from one-solution test and paper disks with replicates were collected in conventional lab setting and in remote conditions. Subjects' classifications (NT, MT, and ST) obtained from PROP ratings in different conditions (lab and remote settings) and from different stimuli (solution and paper disk) were compared.

2. Materials and methods

2.1. Study overview

A within-subject design was adopted. Subjects took part in two sessions on separate days one week apart, one in remote and one in laboratory settings. Subjects were assigned to four different groups to balance the order of evaluation conditions (L, lab and R, remote settings) and type of stimulus (S, solution and D, paper disk) (Table 1). Therefore, PROP ratings were collected in four different evaluation conditions: solution in lab (LS), solution in remote (RS), disk in lab (LD), and disk in remote (RD).

2.2. Subjects

Participants were recruited in the Florence area (Italy), by means of announcements published on university websites, social networks, emails, pamphlet distribution, and by word of mouth. Seventy-seven healthy subjects aged 18–30 years (mean age 26 years; 60 % women) participated in the study. At the time of recruitment, respondents were asked to complete an online questionnaire on their sociodemographic characteristics and food allergies/intolerances. Participants were also asked to provide their address to send them the kit for the remote testing

Table 1			
Sequence of evaluat	ion conditions	by subject	group.

Subject Group	Day1	Day2	
	Lab Setting (L)	Remote Condition (R)	
Group $1 \ n = 20$	Solution (S) – Disk (D)	Solution (S) – Disk (D)	
Group 2 $n = 20$	Disk (D) – Solution (S)	Disk (D) – Solution (S)	
	Remote Condition (R)	Lab Setting (L)	
Group $3 n = 19$	Solution (S) – Disk (D)	Solution (S) – Disk (D)	
Group 4 $n = 18$	Disk (D) – Solution (S)	Disk (D) – Solution (S)	

evaluation. Written informed consent from all participants was obtained according to the GDPR (General Data Protection Regulation) 2016/679. The study was conducted according to the principles established in the Declaration of Helsinki for medical research involving humans. In line with national regulations given that the research was not medical, the protocol was not submitted for approval to an ethical committee. Subjects received a gift card payment to motivate their participation in the study.

2.3. PROP taster status assessment

2.3.1. Stimuli

A 3.2 mM PROP solution was prepared by dissolving 0.5477 g/L of PROP (Sigma Aldrich, Milano, IT) into deionized water at room temperature (Prescott et al., 2004). Solutions were prepared in advance and kept under refrigeration conditions (6 $^{\circ}$ C) for a maximum of 48 h. Solutions were taken off from the refrigerator 30 min before each session to bring them back to room temperature for tasting.

1.5 cm diameter filter paper disks (Whatman, Sigma Aldrich, Milano, IT) were threaded onto a piece of polyester thread using a sewing needle, soaked for 30 s into a 50 mmol/L PROP solution at boiling temperature, dried overnight at room temperature (Zhao et al., 2003) and used for tasting sessions within three weeks from preparation. The stability in time of the intensity of paper disks impregnated with PROP was preliminarily assessed in a study with 33 subjects (mean age 28 years; 58 % women) who tasted disks prepared within a period of 35 days (described in detail in Supplementary material 1). The amount of PROP per disk was quantified using a spectrophotometer. The absorbance of PROP eluted in methanol solution was measured at a peak wavelength of 275 nm. The mean amount of PROP per disk was determined using the extinction coefficient for PROP and resulted to be 0.347 \pm 0.012 mg in line with Zhao et al. (2003).

2.3.2. General procedure

Subjects participated in two sessions, with a duration of approximately 45 min each. On day one (in lab or in remote conditions, depending on the subject group), participants were introduced to the general organization of the test. Before PROP testing, participants were trained to the use of the general Labelled Magnitude Scale (gLMS: 0 = not detectable, 100 = strongest imaginable sensation of any kind) (Bartoshuk et al., 2004), and were asked to rate the intensity of imagined/remembered sensations from different modalities. Subjects were then asked to evaluate the bitterness intensity of PROP solutions and disks. On day two (participants switched from lab to remote conditions and vice versa) instructions for the use of the gLMS were quickly recalled and the PROP evaluation was repeated as in day one. Subjects were not aware of the aim of the study or of the nature of the stimuli and were told that it was a study on bitter perception. Data were collected using the software Compusense20 (Compusense Inc, Ontario, Canada).

Evaluations in remote condition were performed by real-time video calls from the assessor's home under the guidance of the panel leader according to the guidelines indicated in Dinnella et al. (2022). An evaluation box with all the equipment needed for the evaluation (samples, plastic cups, napkins, instruction sheet to prepare the evaluation station and samples, and crackers for rinsing procedures) was delivered to participants' homes the day before the test. Subjects were asked to put solutions in the refrigerator as soon as they received the box and were reminded to take them off 30 min before the test through a short message (SMS). Subjects were also instructed to have available a small mirror to help the correct disk positioning on the tongue and plain water for rinsing procedure. Video calls were carried out using Google Meet online platform (Google LLC, Mountain View, California, USA). Subjects were provided by email with the links to access to the videocall and to the software for data collection. The panel leader opened the video call, invited participants to set up the working station according to the instructions and recalled evaluation aim and procedure. Then participants connected to the software for data collection and started the evaluation. Participants were asked to keep the camera on throughout the evaluation session to allow the panel leader to monitor for their compliance to the evaluation procedure. After the instruction section that was done with a maximum of 4 participants at a time, tests were performed individually at the subject's own pace with the presence of the panel leader that could be called or intervene in case of need.

2.3.3. Training and acclimatation to the gLMS scale use

Subjects were trained to the use of the gLMS scale (general Labelled Magnitude Scale: 0- not detectable, 1- barely detectable, 6- weak, 17-moderate, 35- strong, 53- very strong, 100- strongest imaginable sensation of any kind) (Bartoshuk et al., 2004) on day 1, either in the lab or in remote conditions, depending on the subject group. Verbal instructions were given explaining to subjects to treat the top of the scale as the most intense sensation they could ever imagine experiencing. For practice on the use of the scale participants were asked to rate the intensity of a list of imagined/remembered sensations from different modalities including loudness, brightness, pain, and tastes (Bajec & Pickering, 2008b; Bartoshuk et al., 2002; Kalva et al., 2014; Porubcan & Vickers, 2005; Webb et al., 2015) adapted from Hayes, Allen, and Bennett (2013) (Table 2).

Instructions to the scale use were quickly recalled to participants on day 2, and they were asked to rate only the sensation "brightness of the sun directly looking at it on a clear day" (top-of-the-scale sensation).

Two criteria were adopted to conclude that subjects correctly used the scale: 1) intensity of "brightness of the sun directly looking at it on a clear day" higher than very strong (gLMS > 53) and lower than the strongest imaginable sensation of any kind in both sessions; 2) intensity of "loudness of a whisper" lower than "loudness of a conversation" lower than "loudness of a plane passing over your head" (Bartoshuk et al., 2002). No subjects systematically failed the criteria, and all were retained for data analysis.

2.3.4. Evaluation procedure

The stimuli were presented in duplicate identified with three-digit codes in a randomized and balanced order. Solutions (10 ml) were presented in plastic cups and disks on Petri dishes or in sealed plastic bags (in lab setting and remote conditions, respectively). Furthermore, in the first session, subjects were provided with a blank paper disk, to acclimate to the methodology, learning how to correctly put the disk along the midline of the tongue around 0.5 cm from the tip and to carry out the evaluation (Zhao et al., 2003). Subjects were instructed to hold the whole PROP solution in the mouth for 10 s or to place the disk on the tip of the tongue until moistened with saliva (25 s), then to expectorate/ remove it, and after a further 20 s to rate bitterness intensity on the gLMS. Subjects had a 3 min break between solution replicates and a 90 s break between disk replicates to control for carry-over effects. During

Table 2

List of the imagined/remembered sensations evaluated in the training to the scale use.

Imagined/remembered sensations				
Loudness of a whisper				
Loudness of a conversation				
Loudness of a plane passing over your head				
Brightness of a dimly lit room				
Brightness of a well-lit room				
Brightness of the sun directly looking at it on a clear day				
Warmth of a summer breeze				
Pain of biting your tongue				
The heat when you put your hand in boiling water				
Freshness of the mint toothpaste				
Bitterness of a black coffee				
Sweetness of cotton candy				
Sourness of a lemon				
Oral burn of a bite from fresh pepper				

the break a mouth rinsing procedure was applied (water, plain crackers with 1,25 % NaCl, and water again) (Monteleone, Condelli, Dinnella, & Bertuccioli, 2004). A 10 min break was observed between the evaluation of solutions and disks. The whole testing process was guided with appropriate timers and instructions on the screen.

2.4. Data analysis

To verify that the training in the use of the gLMS was effective, a Two-Way ANOVA model with interactions was applied on intensity ratings from imagined/remembered sensations (factors: Sensations, 14 levels; Condition, 2 levels: remote, lab).

Replicate and condition effects on PROP ratings were analyzed by means of Two-Way ANOVA models with interactions for each stimulus (solution and disk) separately (factors: Replicates, 2 levels: 1, 2; Condition, 2 levels: remote, lab). Given the lack of effect of replicates (as single factor or in interaction), in the following analyses an average of the two replicates was considered. Effects of evaluation condition and stimulus on mean PROP ratings were analyzed by means of a Two-Way ANOVA model with interactions (factors: Condition, 2 levels: remote, lab; Stimulus, 2 levels: solution, disk). Distributions of data in the four different conditions (LS; RS; LD; RD) were analyzed by means of descriptive statistical tools. The first and third quartile limits of the percentile distributions of PROP ratings in each of the four evaluation conditions were computed and used to categorize subjects in Non Tasters (NTs < 1st quartile), Super Tasters (STs > 3rd quartile), and Medium Tasters (1st quartile < MTs < 3rd quartile). This approach is preferred when the populations studied are small and not homogeneous in terms of characteristics that could influence PROP perception (i.e. gender and age), rather than the use of the arbitrary cut-offs used in the literature for the general population (Monteleone et al., 2017). The degree of agreement between classifications among groups (NT, MT, ST) obtained in the four evaluation conditions (LS; RS; LD; RD) were compared by means of contingency tables (Zhao et al., 2003). Relationships between ratings in the different conditions were explored using linear regressions. Lab-solution (LS) was chosen as a reference condition. A One-Way ANOVA model was performed on bitterness ratings from RS, LD, and RD considering the subject segmentation obtained from the percentile limits of the rating distribution from the reference condition (LS) as a factor (3 levels: NT, MT, and ST).

Significance level was set at 95 %. LSD post hoc test was applied to test the differences between means. The XLSTAT statistical software package version 2022.1 (Addinsoft, Paris, France), was used for data analysis.

All the analyses were performed on PROP raw data and on normalized PROP ratings relative to a non-taste sensation ("the heat when you put your hand in boiling water") to improve the validity of comparisons across individuals as suggested by Bartoshuk et al. (2002). Results were very similar with both data, with a slight lower number of misclassifications comparing the different conditions with normalized data. To avoid duplication, only results on normalized data are reported here.

PROP intensity ratings were normalized adapting the procedure described by Porubcan and Vickers (2005). "The heat when you put your hand in boiling water" sensation was selected instead of "the brightness of the sun when looking directly at it" considering that it was rated in the upper part of the gLMS (mean = 74 on the gLMS; between "very strong" and "the strongest imaginable sensation of any kind" labels), it showed the lowest standard deviation value (SD = 18.8) in comparison to the other imagined/remembered sensations evaluated higher than very strong (gLMS > 53) and was not correlated to PROP ratings (r \leq 0.13, p \geq 0.256).

PROP ratings were normalized against the non-taste sensation "the heat when you put your hand in boiling water" adopting the following formula: PROP/(NTSs/NTSm), where PROP is the individual PROP intensity rating; NTSs is the individual intensity of "the heat when you put your hand in boiling water"; NTSm is the mean intensity of "the heat when you put your hand in boiling water" for the whole sample.

3. Results

Intensities of imagined/remembered sensations rated in lab and remote conditions were compared. The average scores were significantly different and sorted in ascending order from "loudness of a whisper", which was rated the lowest, to "the brightness of the sun directly looking at it on a clear day" and "the heat when you put your hand in boiling water", which were both rated the highest ($F_{(13,1050)} = 93.25$, p < 0.0001) (Fig. 1). The intensities of imagined/remembered sensations rated in lab setting and remote conditions did not significantly differ ($F_{(1,1050)} = 0.08$, p = 0.773) and the interaction sensations*conditions was not significant ($F_{(13,1050)} = 0.79$, p = 0.675).

Effects of replicate and conditions on PROP intensity of solutions and disks were independently assessed. No significant replicate effect were found either in solutions ($F_{(1,304)} = 0.13$, p = 0.721) or in disks ($F_{(1,304)} = 0.01$, p = 0.923). Condition did not affect mean PROP ratings of solutions ($F_{(1,304)} = 0.02$, p = 0.882) and disks ($F_{(1,304)} = 0.58$, p = 0.448). Replicate*condition interactions were not significant for solutions ($F_{(1,304)} = 0.21$, p = 0.646) and disks ($F_{(1,304)} = 0.01$, p = 0.944). Thus, replicate mean ratings in each condition for solutions and disks were calculated and used for further analyses.

Effects of condition (lab and remote) and type of stimulus (solution and disk) on mean PROP ratings were assessed. Condition did not significantly affect mean ratings ($F_{(1,304)} = 0.04$, p = 0.844), while the type of stimulus significantly affected intensity ratings ($F_{(1,304)} = 65.92$, p < 0.0001), with bitterness of solutions that was rated higher than bitterness of disks (31.54 vs 13.38 on the gLMS). No effect of interaction was found ($F_{(1,304)} = 0.16$, p = 0.692) (Fig. 2).

The characteristic values of the percentile distributions of PROP ratings from the four evaluation conditions (LS, RS, LD, and RD) were computed and used to categorize subjects for PROP status. First and third quartile limits of solution rating distributions were 13 and 50 in lab setting and 13 and 45 in remote conditions. For disks, the first and third quartile limits were 3 and 21 in lab condition and 3 and 18 in remote condition. Lab-solution (LS) was chosen as a reference to compare subject segmentation from the other conditions (RS; LD; RD) (Table 3). Relatively few misclassifications were observed. RS, LD, and RD conditions showed progressively larger misclassifications. Furthermore, in RS and LD conditions misclassifications occurred only between contiguous classes (i.e., NT/MT; MT/ST) while in RD condition also one NT/ST misclassification (non-contiguous classes) was observed.

Relationships between PROP intensity ratings between reference (LS) and other conditions (RS; LD; RD) are shown in Table 4. All regressions were significant (p < 0.0001), and r² correlation coefficients indicated that the goodness of fit progressively decreased from RS to LD, until reaching the lowest value for RD condition. The linear regression between LD and RD was significant (p < 0.0001) with r = 0.47 and r² = 0.22.

The effect of PROP group segmentation according to the percentile limits from the reference condition (LS) on the mean ratings from RS, LD, and RD was assessed to verify if the segmentation according to the solution in lab could also be applied to discriminate among PROP taster groups when evaluations were performed in remote (solution and disk) or in lab condition with disk (Table 5). Bitterness mean ratings from RS, LD, and RD significantly differed among taster groups (RS: $F_{(2,74)} = 59.07$, p < 0.0001; LD: $F_{(2,74)} = 41.87$, p < 0.0001; RD: $F_{(2,74)} = 10.47$, p < 0.0001). Subject classification in PROP taster groups based on the reference condition allowed the significant discrimination among ST, MT, and NT when ratings were collected in RS and LD conditions, while only ST group was discriminated from MT and NT when ratings were collected in RD.



Fig. 1. Mean intensities of imagined/remembered sensations. Different letters indicate significant differences according to LSD post-hoc test ($p \le 0.05$).



Fig. 2. Mean PROP bitterness intensity of solutions and disks evaluated in lab setting and remote conditions. Different letters indicate significantly different ratings according to LSD post-hoc test (p < 0.0001).

Table 3

Contingency table comparing subject reference classification (lab solution - LS) with those obtained in the other conditions: remote solution (RS), lab disk (LD), remote disk (RD). The total number of subjects for each taster group in each condition is reported in brackets, the number of misclassified subjects in each condition is reported in bold.

		Lab Solution		
		NT (19)	MT (39)	ST (19)
Remote Solution	NT (19)	13	6	0
	MT (38)	6	28	4
	ST (20)	0	5	15
Lab Disk	NT (17)	11	6	0
	MT (41)	8	28	5
	ST (19)	0	5	14
Remote Disk	NT (17)	7	10	0
	MT (40)	11	21	8
	ST (20)	1	8	11

Table 4

Results of linear regressions between PROP ratings in the reference lab solution condition and in the remote solution, lab disk and remote disk conditions, respectively.

	r^2	r	F	p-value
Lab solution - Remote solution	0.76	0.87	231.88	< 0.0001
Lab solution - Lab disk	0.63	0.79	128.58	< 0.0001
Lab solution - Remote disk	0.24	0.49	23.58	< 0.0001

Table 5

Effect of PROP group segmentation according to the percentile limits from the reference condition (lab-solution - LS) on the mean ratings from solution evaluation in remote condition (RS), disk evaluation in lab setting (LD) and in remote condition (RD): mean and p values. Different letters in a column indicate significant different values according to the LSD post-hoc test ($p \le 0.05$).

	Remote solution	Lab disk	Remote disk
ST	60.80a	31.18a	23.30a
MT	27.91b	10.55b	10.50b
NT	10.62c	4.07c	6.68b
p-value	< 0.0001	< 0.0001	< 0.0001

4. Discussion

Results of the present study showed that remote sensory testing could be a valid alternative to the conventional lab setting also when performing a quite complex test requiring careful training and strict adherence to the evaluation condition as in the case of PROP status assessment.

Subjects were successful trained to the scale use either in lab setting or in remote conditions. In fact, the discrimination among mean intensities of imagined/remembered sensations was not affected by the training condition and was in agreement with previous data from lab setting (Bartoshuk et al., 2002; Hayes et al., 2013). Furthermore, all subjects participating in the study met the criteria for concluding their correct understanding and use of the gLMS. The training to the scale use was performed only on day 1 which was in lab setting for half of the participants and in remote condition for the remaining half, thus the training was performed in the two conditions by two different group of subjects and this further strength the value of the finding.

The study design included a sample replicate in the evaluation. However, in consideration of the lack of significance of the replicate effect on mean ratings in both lab and remote conditions it can be stated that including a replicate in the evaluation does not bring any advantage to the test; for this reason, this can be avoided to reduce the test duration and the participants' sensory fatigue.

Mean PROP ratings of the same type of stimulus (solution or disk) are not significantly affected by the condition adopted for data collection (lab or remote) thus confirming the general match between the results of sensory tests performed in conventional lab setting and in live remote conditions (Dinnella et al., 2022; Gonzalez Viejo et al., 2022; Gonzalez Viejo et al., 2021; Venkatesh & DeJesus, 2021).

Quartiles limits of distribution of normalized PROP ratings of solutions evaluated both in lab and remote conditions were found to be slightly lower than the cut-offs used in previous studies to categorize participants according to the scale labels in Non Tasters (NT \leq 17, moderate, gLMS) and Super Tasters (ST \geq 53, very strong, gLMS) (Dinnella et al., 2018; Fischer et al., 2013; Hayes et al., 2010; Monteleone et al., 2017). These results are somehow unexpected considering the relatively low mean age (26 y.o.) and the gender distribution (60 % women) of the subject group participating in the present study. In fact, PROP responsiveness significantly decreases with aging and women are more responsive to PROP than men (Bartoshuk et al., 1994; Dinnella et al., 2018; Monteleone et al., 2017; Tepper et al., 2017). The relatively small size of the sample considered in the present study might have resulted in the observed slight shift in rating distribution.

Subject classifications based on solution evaluation in lab and remote conditions were comparable with a relatively low number of misclassifications between contiguous PROP taster groups, such as NT and MT, or MT and ST respectively (average misclassification = 27 %). Solution ratings in lab and remote conditions were strongly correlated and the linear model explained 76 % of variance. Furthermore, subject classification in PROP taster groups obtained from solution evaluations in lab setting can be effectively applied on PROP ratings from solution evaluation in remote condition resulting in significant differences in PROP mean ratings between ST, MT, and NT groups. Thus, these results indicate that one-solution test for PROP status assessment can be effectively carried out in live remotely condition.

Mean values of PROP ratings of paper disk were significantly lower than those obtained from solution evaluations. These findings are consistent with the idea that lower intensity ratings are observed in regional (disk) compared to whole mouth (solution) stimulation. Humans have taste receptors in several regions of the oral cavity such as on the surface area of the tongue, soft palate, pharyngeal and laryngeal regions of throat (Breslin & Huang, 2006). It has been hypothesized that taste perception is influenced by the areas of the oral cavity that are stimulated (Colvin, Pullicin, & Lim, 2018; Delwiche, Buletic, & Breslin, 2001b; Delwiche, Halpern, & Lee, 1996; Feeney & Hayes, 2014; Running & Hayes, 2017) and that the intensity of a tastant aligns with the number of receptors that are stimulated (Delwiche et al., 2001b; Spence, 2022) with an increased perception when the tastant can reach receptors on the different taste-responsive areas of the oral cavity (Breslin & Huang, 2006; Delwiche et al., 1996; Feng, Huang, & Wang, 2014; Miller & Bartoshuk, 1991; Ponnusamy et al., 2023; Spence, 2022) as it happens with tastant solution stimulation that contacts taste buds from multiple regions of tongue (anterior and posterior), soft palate, larynx and pharynx (Miller & Bartoshuk, 1991). On the other hand, paper disk predominantly stimulates only receptors of the contact area thus resulting in lower intensity perception. However, the cut-off values to classify the subjects obtained with paper disks in this study were considerably lower even than those reported by Zhao et al. (2003) for subject classification (NT: LMS \leq 15; ST: LMS \geq 67) and adopted in other studies (Melis et al., 2020; Melis et al., 2021; Oftedal & Tepper, 2013; Tepper, Williams, Burgess, Antalis, & Mattes, 2009). This may be explained by the fact that a different scale was used, gLMS in this study and Labeled Magnitude Scale (LMS) in the case of Zhao et al. (2003). The evaluation of PROP bitterness in the context of intensity of any kind may clearly have impacted the evaluation lowering the ratings. It has been

suggested that the most intense sensation "of any kind" (gLMS) would presumably be more intense in memory than the most intense "oral experience" (LMS), thus tastant intensity is rated significantly lower on gLMS than on LMS (Horne, Lawless, Speirs, & Sposato, 2002).

In our study subject classifications based on solution and disk evaluations in lab condition were comparable with a relatively low number of misclassifications between contiguous PROP taster group (average misclassification = 31 %). Ratings of solutions and disks in lab setting showed an acceptable correlation and the linear model explained 63 % of variance. Furthermore, subject classification in PROP taster groups obtained using solutions evaluated in lab setting can be effectively applied on PROP ratings obtained using disks evaluated in the same condition and significant differences in PROP mean ratings between ST, MT, and NT groups were observed.

On the other hand, correlations between ratings of solutions in lab and disks in remote, as well as between disk ratings in the two conditions, were relatively lower (with 24 % and 22 % of explained variance, respectively). The number of misclassifications between disk in remote and the reference condition was relatively high (average misclassification = 49 %) with classification changes also between non-contiguous taster groups (i.e. NT/ST). Furthermore, a lower discrimination of the reference classification was observed when applied to disk ratings in remote condition with significant differences only between ST and the rest of the sample.

Taken together these results indicate that solutions and disks can be both used for PROP status assessment in conventional lab setting. However, caution should be used when comparing PROP responsiveness from whole and regional stimulation given the significant differences in intensity ratings for the concentrations commonly used. The poor performance of PROP status assessment using paper disk in remote condition needs further investigations. Possibly the relatively higher complexity of the procedure, the need for the accurate control for a proper regional stimulation, and the variation of PROP responsiveness across tongue regions (Ponnusamy et al., 2023) can at least partially account for the observed results. It is possible to speculate that in remote condition the experimenter is not able to provide adequate assistance to subjects helping them to correctly position the disk on the tongue and properly perform the test. This can result in lower data stability and higher misclassification.

In this study all PROP ratings data were normalized because it is known that rescaling the PROP ratings relative to a non-taste sensation ("the heat when you put your hand in boiling water") allows to make more valid comparisons across individuals (Bartoshuk et al., 2002). Raw data were also analyzed with very similar results. The most relevant difference was found in the comparisons between reference and other conditions subject classifications, specifically considering remote-disk condition where normalization reduced the numbers of misclassifications between non-contiguous classes NT/ST evidenced in the raw data analysis.

5. Conclusions

This was the first methodological study aimed at exploring the possibility of assessing the responsiveness to PROP in remote condition using two popular methodologies: one-solution test and impregnated paper disk.

One-solution test appears a reliable procedure for PROP status assessment in live remote testing. This would help the further application of this measure in large population studies aimed at exploring the association between individual variation in oral responsiveness and food preferences and eating habits. The availability of reliable remote testing procedure will also promote sensory investigations in vulnerable population groups (i.e. people with diseases or conditions) for which the participation in lab setting evaluations can be difficult. Moreover, the results show that live remote testing, if meticulously planned and carried out, is well suited also for complex sensory tests for which accurate

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training procedure are requested.

Impregnated paper disks resulted a suitable alternative to solutions in conventional lab setting with the advantages of easier handling and convenience (disks can be prepared in advance at least up to 3 weeks). Paper disk evaluation could represent a valid option also for data collection out of the lab setting (i.e. school, hospital), provided that the strict control in person by sensory personnel can be guaranteed.

6. Author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

7. Ethical statement for food quality and preference

Hereby, I Claudia Rorandelli consciously assure that for this manuscript the following is fulfilled:

1) This material is the authors' own original work, which has not been previously published elsewhere.

2) The paper is not currently being considered for publication elsewhere.

3) The paper reflects the authors' own research and analysis in a truthful and complete manner.

4) The paper properly credits the meaningful contributions of coauthors and co-researchers.

5) The results are appropriately placed in the context of prior and existing research.

6) All sources used are properly disclosed.

7) All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

8) This study was conducted according to the guidelines established in the Declaration of Helsinki.

9) Written informed consent was obtained from all participants according to the GDPR (General Data Protection Regulation) 2016/679.

CRediT authorship contribution statement

C. Rorandelli: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **A. Lippi:** Conceptualization, Investigation, Writing – review & editing. **S. Spinelli:** Conceptualization, Data curation, Formal analysis, Supervision, Writing – review & editing. **L. Pierguidi:** Formal analysis, Software, Writing – review & editing. **E. Monteleone:** Conceptualization, Formal analysis, Writing – review & editing. **C. Dinnella:** Conceptualization, Data curation, Formal analysis, Supervision, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodqual.2023.105045.

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