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PERCEPTIVE AND BEHAVOURIAL DETERMINANTS OF FRUITS AND VEGETABLES CONSUMPTION IN CHILDHOOD: STRATEGIES TO PREVENT OBESITY

Scientific field AGR/15

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PREFACE

This thesis represents work to fulfil requirements for a PhD, in PHD school of Food Biotechnology, at the Department of Food, Environmental and Nutritional Sciences (DeFENSE), University of Milan.

The study applying the Food Dudes educational program carried out in Italy, was funded by Regione Lombardia and was part of the project *'Food and Fun: Consumi alimentari e stili di vita nei bambini della scuola primaria. Analisi e ricerca per prevenire sovrappeso e obesità'*. The Danish research study was part of the OPUS project *'Optimal well-being, development and health for Danish children through a healthy New Nordic Diet'*. Besides, some Italian and Danish industries participated in the project group providing many of the foods used in all the studies of this thesis. The studies are mainly been conducted in the Sensory & Consumer Science Laboratory of the DeFENS of Milan, in the years 2011-14. One study was carried out during a research stay abroad in 2013 at Department of Food Science / Sensory and Consumer Science of the University of Copenhagen, in collaboration with prof. Wender Bredie. Core parts of most studies have been conducted on site at participating schools in the Milan and Copenhagen areas, respectively. All the study details are given in the following chapters.

The overall aims of this PhD thesis project were to deepen knowledge on how measure perceptive, and behavioural determinants of fruits and vegetables consumption, and on their relationship with nutritional status in children. Sensory approaches were also applied trying to change children's consumption in a more healthy direction, with the ultimate goal of understand how child obesity could be prevent.

The PhD thesis is structured in three chapters, each one devote to the above mentioned technological sphere. In particular, *chapter 1* explore, develop and validate a questionnaire to assess food neophobia in Italian primary school children. *Chapter 2* investigate how perceptive and behavioural variables are related to nutritional status, in adult and children. Finally, *chapter 3* is focused on how increase fruits and vegetables in children and it presents in details the effect of two different strategies in increase the consumption of these products in Italian and danish school-aged children.

ABSTRACT

In the recent years, prevention and treatment of child obesity have been of primary importance in public health all over the world. Although it's well known that regular consumption of fruits and vegetables is associated with reduction of weight and health benefits, the intake of these products is far below the recommended 5 serving per day, especially among children (WHO, 2012). Among the many factors influencing the prediction of fruits and vegetables consumption, the hedonic dimension plays a major role. It is result of complex interactions between perceptive and behavioural determinants, and it is streactly related to familiarity and neophobia (Nicklaus et al., 2005). Evidence for a negative relationship between neophobia traits and fruits and vegetables consumption in children has been reported (Falciglia et al., 2000), despite that the role of food neophobia, familiarity and preferences on nutritional status are unclear.

Over the past 30 years, research on children's food habits has identified several variables that can increase the consumption of different foods. According to the social learning theory, modeling by significant others can be highly influential in establishing food behaviour changes. Children are also more likely to imitate a model whose behaviour they see being rewarded, who is of the same age or slightly older than themselves or who they like or admire. Another influential strategy to modifying food habits is to induce prolonged exposure to a stimulus (Olsen et al., 2012). Repeated exposures to a specific food increase familiarity of foods and extend preferences for those initially rejected (Cooke et al., 2011). A different approach to extend specific products consumption is to increase variety of food offered. Variety within a meal is known to increase intake. Thus, it may be used to increase fruits and vegetables liking and to promote their intake in children (Forestell et al., 2007).

The present PhD thesis addresses this theme in three chapters. The aim of the *chapter 1* was to explore, develop and validate a questionnaire to assess food neophobia in primary school Italian children. In the *chapter 2* two studies regarding nutritional status related to sensory dimension are presented. The first study explored the relationship between taste thresholds and body composition in adults. This is a preliminary survey carried out to deepen the knowledge on this field with a view to develop new researches in children. The second study of this chapter investigated the relationship between food preferences, neophobic traits and body composition in children. The aim of *chapter 3* was to investigate the effect of different strategies in increasing children's fruits and vegetables consumption. In partucular, the first experiment was carried out in Italy, involving Italian children of the Municipality of Milan; the second experiment was conducted on a group of Danish elementary school children of Copenhagen area.

Cooke, L., J., Chambers, L., C., Añes, E., V., & Wardle, J. (2011). Facilitating or undermining? The effect of reward on food acceptance. A narrative review. Appetite, 57, 493-497.

Falciglia, G.A., Couch, S.C., Gribble, L.S., Pabst, S.M., & Frank, R. (2000). Food neophobia in childhood affects dietary variety. Journal of American Dietetic Association, 100, 1474-1478.

Forestell, C.A., &, J.A. (2007). Early Determinants of Fruits and Vegetables Acceptance. Pediatrics, 120 (6), 1257-1254.

Nicklaus, S., Boggio, V., Chabanet, C., & Issanchou, S. (2005). A prospective study of food variety seeking in childhood, adolescence and early adult life. Appetite, 44, 289-297.

Olsen, A., Ritz, C., Kraaij, L.W., & Møller, P. (2012a). Children's liking and intake and vegetables: A school-based intervention study. Food Quality and Preference, 23, 90-98.

WHO, World Health Organization (2012). Population-based approaches to childhood obesity prevention. WHO Document Production Services, Geneva, Switzerland.

RIASSUNTO

Negli ultimi anni la prevenzione e il trattamento dell'obesità hanno rivestito un ruolo di primaria importanza per salute pubblica in tutto il mondo. Anche se è ormai noto che un regolare consumo di frutta e verdure è associato a una riduzione di peso e a benefici per la salute, il consumo di tali prodotti è ben lontano dalle cinque porzioni al giorno raccomandate, specialmente nei bambini (WHO, 2012). Tra i differenti fattori che influenzano il consumo di frutta e verdura, la dimensione edonistica gioca un ruolo fondamentale. Essa è il risultato di complesse interazioni tra determinanti percettive e comportamentali ed è strettamente associata al concetto di familiarità e neofobia (Nicklaus et al., 2005). Falciglia et al, (2000) hanno riportato evidenze di una correlazione negativa tra neofobia alimentare e consumo di frutta e vedura, nonostante ciò il ruolo della neofobia, della familiarità e delle preferenze sullo stato nutrizionale non è chiaro.

Negli ultimi 30 anni, ricerche sulle abitudini alimentari dei bambini hanno identificato diverse variabili che possono aumentare il consumo di alcuni alimenti. In accordo con la teoria sociale dell'apprendimento (Bandura 1977), l'imitazione di comportamenti può avere una forte influenza nel cambiare le scelte alimentari. In aggiunta, i bambini hanno molte più probabilità di imitare un modello, quando il loro comportamento è premiato, sia tale modello coetaneo o poco più grande, e in particolare se esso viene da loro ammirato. Un'altra strategia influente nella modificazione delle abitudini alimentari sono le esposizioni ripetute a uno specifico alimento. Assaggi ripetuti, infatti aumentano la familiarità, con la possibilità di estendere la preferenza anche per alimenti che inizialmente erano sgraditi (Cooke et al., 2011). Un approccio differente per implementare il consumo di prodotti specifici è quello di aumentare la varietà degli alimenti offerti. E' noto che la varietà in un pasto aumenta il consumo. Quindi, questo fattore potrebbe essere usato per promuovere il consumo di frutta e verdura nei bambini (Forestell et al., 2007).

La presente tesi di dottorato affronta questo tema in tre capitoli. Lo scopo del *Capitolo 1* è stato quello di esplorare, sviluppare e validare un questionario per valutare la neofobia alimentare in bambini italiani della scuola elementare. Nel *Capitolo 2* sono presentati due studi che riguardano lo stato nutrizionale in relazione alla dimensione sensoriale. Nel primo studio è stata indagata la relazione tra soglie gustative e composizione corporea negli adulti. Tale studio è stato condotto in via priminare sugli adulti per approfondire le conoscenze in questo campo d'indagine in vista di mettere a punto nuove ricerche sui bambini. Il secondo studio di questo capitolo si è occupato di indagare la relazione tra preferenze alimentari, tratti neofobici e composizione corporea nei bambini. Lo scopo del *Capitolo 3* è stato di esaminare l'effetto di diverse strategie nell'aumentare il consumo di frutta e verdura da parte dei bambini. In particolare, il primo esperimento presentato, è stato condotto in Italia, coivolgendo bambini della scuole elementari del comune di Milano; mentre il secondo ha visto la partecipazione di bambini danesi della città di Copenhagen.

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1. EVALUATION OF CHILDREN'S FOOD NEOPHOBIA

How to measure this variable in Italian children?

1.1 INTRODUCTION

1.1.1 Food neophobia in children

Food neophobia, defined as a reluctance to eat unfamiliar foods (Birch & Fischer, 1998), is a characteristic that all humans share. The term 'neophobia' derives from Rozin's 'omnivore's dilemma' (Rozin & Vollmecke, 1986) that it must both approach and avoid novel foods. The reluctance of new foods is a process described as an evolutionary survival mechanism, serving a protective function in a potentially hostile food environment. At the same time, the omnivores must also be willing to try novel foods.

In children, food neophobia has the aim to avoid consuming toxic compounds once they are able enough to consider and consume 'objects' found when outside parental vision but in the modern society it leads children to naturally reject foods that they have no experience with. Foods that do not 'look right' to the child will initially be rejected based on vision alone. This neophobic predisposition to avoid new foods may seem maladaptive for species that need to consume a varied diet to obtain adequate nutrition and might also explain why neophobia changes during childhood (Birch & Fischer, 1998).

1.1.2 Food neophobia characteristics

Age and gender differences as well as individual differences are present in the strength of neophobic response. The hypothetical evolutionary variation in food neophobia raised the question whether the variation in food neophobia, considered as a personality trait, had a genetic component. Some authors showed that food neophobia might be genetically inherited as familial similarities in this behavioural trait were observed (Pliner et al., 2006; Cooke et al., 2007). Koivisto-Hursti & Sjoden (1997) demonstrated a moderate relationship between parents and children's food neophobia. Knaapila et al. (2007) measured food neophobia within Finnish families and British twin pairs finding, for both populations, that about two thirds of variation in neophobia in genetically determined.

Among the studies conducted on neophobia in children, some found boys more neophbic than girls (Koivisto & & Sjoden, 1996; Cooke et al., 2006; Reverdy et al., 2008) and other have not (Pliner & Hobden, 1997; Rigal et al., 2006; Fernàndez-Ruiz et al., 2013). The contradicting results of literature suggest that more research into gender effects is still needed.

Food neophobia in children is characterized by an interesting developmental trajectory that shows a decreasing of the neophobic response with age. Birch et al. (1999) showed that among infants who were just beginning the transition to solid foods, the food neophobia appears to be minimal. In this study, 4-6 months infants were introduced to new fruits or vegetables by feeding one new food to the infant over series of 10 meals. The results indicated that only one feeding with a new food was enough to increase an infant's intake of that food significantly from a mean of 30 g at the first feeding to a mean of 60 g at the second feeding. Furthermore, it was shown that neophobia response decreasing appeared to generalize to similar foods, so if an infant has experience with one vegetable, it can influence other vegetables's acceptance and intake. Pliner and colleagues investigated age differences in food neophobia among children and adolescents, comparing 3-8 years old with 10-20 year old children (Pelchat and Pliner, 1995; Pliner, 1994; Pliner and Loewen, 1997). They evidenced that during childhood, the strength of the neophobic response changes. Similar results were also found from many other experimenters (Koivisto & Sjöden, 1996, Tuorila et al., 2010). These findings suggest that during development the relationship between age and the neophobic response may be curvilinear. Food neophobia is low at the weaning, it increase when children became more mobile, reaching a peak between 2 and 6 years of age. Then, neophobia response decreases with individual's age, until it reaches a relatively stable zenith in adulthood (Cooke et al., 2006). This curvilinear relationship is illustrated in **Figure 1.1**

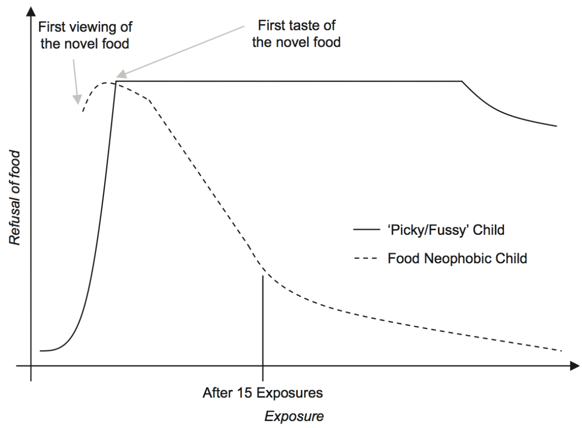

Figure 1.1 A lifespan model for levels of food neophobia in humans (Dovey et al. 2008).

1.1.3 Food neophobia and willingness to try novel foods

Food neophobia, as a behaviour involving the rejection of novel foods, is related with the willingness to consume them (Pliner & Hobden, 1992). Many studies on neophobia traits revealed that it moderately or strongly predicts willingness to try food products (Pliner et al., 2006; Dovey et al., 2008). In particular, Tuorila et al. (2001), affirmed that food neophobia had no effect on willingness to try familiar foods, but it significantly decreased the willingness to try the unfamiliar others.

Reverdy et al., (2008), identified two kind of food neophobia: the first as a personality characteristic, evaluated with a questionnaire; and the second as a behaviour implying the rejection of novel food and measured in term of willingness to try real stimuli.

In a research conducted by Tuorila et al. in 2010, the relationship between reluctance to try unfamiliar food and liking for the food was investigated. Data showed that willingness to taste a food is a powerful predictor of the subsequent hedonic experience of an unfamiliar food. Children who were willing to try a stimulus, but in spite of that tasted it, rated the liking negatively and largely similarly to those who rated their expected liking without tasting. This finding has large importance considering that pleasantness of foods is known to predict choice and repeated consumption. Moreover, the results confirm many earlier experiments on children evidencing the negative effect of the pressure to consume on subsequent responses to food.

1.1.4 Measuring food neophobia

Pliner and Hobden (1992) have developed a scale to measure the trait of food neophobia in human. This scale consists of a questionnaire of five positive (neophilic) and five negative (neophobic) statements about food or situations related to food consumption. People complete the questionnaire indicating the level at which they agree or disagree with the 10 statements in a 7-point Likert scale from ''strongly disagree'' to ''strongly agree''. A total FNS score is then calculated by the sum of ratings to each item after having reversed the scores of the neophilic ones; the higher the FNS score the higher the food neophobia level. This scale has been validated with adults in 1992 and has been used later to develop a tool to measure food neophobia in children (Pliner, 1994). Pliner's work consisted in an observational study of children's (5, 8 and 11 years) neophobic behaviour through the measurement of children's willingness to try familiar and unfamiliar foods when presented with them and the

corresponding parent's measurement of the predicted child's willingness and neophobic behaviour.
The Child Food Neophobia Scale (CFNS) proposed by Pliner (Pliner, 1994) is filled by parents and consists of the FNS with items couched in terms of child's behaviour. In her study, Pliner found a moderate relationship between neophobia degree shown by children in choosing foods to be tasted in a laboratory context (calculated as the ratio of novel foods accepted to familiar foods accepted) and both parents' belief about how willing their child was to try unfamiliar food and parent's prediction about their child's neophobia degree (Pliner, 1994). Since 1994, CFNS has been widely used to measure food neophobia in children of different age (Cooke et al., 2006; Cooke et al., 2003; Dovey et al., 2011; Coulthard & Blissett, 2009; Galloway et al., 2003; Howard et al., 2012; Mustonen et al, 2012; Mustonen & Tuorila, 2010; Russell & Worsley, 2008; Wardle et al., 2005). This means that most of the studies published on children willingness to try novel food came from parents' report of their child degree of neophobia and not from an actual children statement. Information about children's food behaviour obtained from questionnaires provided to the parents may be misleading because, though parents play a crucial role in their children diet, it underestimates the role of the child in the process (Aldrige et al., 2009). In addition, parents may sometimes project their own behaviours onto those of their children (Mata et al., 2007) and child and maternal food neophobia are not always significantly correlated (Tan & Holub, 2012). There are also examples in the literature of the use of FNS (Falciglia et al., 2000; Koivisto & Sjödén, 1996, 1997) or slightly modified version of it (Skinner et al., 2002) with children. However, in these studies, children, especially the youngest ones, completed the questionnaire helped by parents, thus, it is not clear whether the resulting scores reflected the children's or their parents' views of their degree of food neophobia. The obvious problem of asking children to respond to the adults FNS items is that children may not understand situations described in some of the items (e.g. "ethnic restaurants", "dinner party") and the younger ones may have difficulties with the 7-point Likert scale. Furthermore, some of the FNS vocabulary is inappropriate for children (e.g. "constantly", "particular").
Only recently researchers have tried to set up questionnaires tailored for children to study their food neophobia degree. We are aware of two examples in the literature of such questionnaires. The first is a modified version of the adults FNS with a reduced number of items used with French children aged 8-10 years (Reverdy et al., 2008); this version, however, has not been validated. The second is a validated French version (Rubio et al., 2008) of the Food Situations Questionnaire (FSQ) (Loewen and Pliner, 2000) used with 5-8 years old children. Also, very recently Hollar et al. (2013) developed the Fruits and Vegetables Neophobia Instrument (FVNI) validated with primary school children (8-10 years) in order to specifically study children's attitude towards new fruits and vegetables. This questionnaire was developed starting from both the FNS and the CFNS and consists of two subscales (one for fruits and one for vegetables) each composed by 9 items in which the word "food" is replaced by "fruits" and "vegetables", respectively.
Developing scales to measure food neophobia directly with children has important implications for the study of childhood eating behaviour and may be an effective tool to be used to measure children willingness to try new food when applying school-based food educational programs. However, when setting up questionnaires tailored for children several precautions should be taken, such as modifying the items in order that they describe situations likely to be familiar to them, employing terms that are age-appropriate, and using a response format that can be easily understandable. Also, there has been concern about the meaning and interpretation of individual FNS statements in different populations and cultures. Thus, care should be taken also in adapting the questionnaire according to children's food culture.
In view of the importance of studying FN in children and of the lack of information about this personality trait in Mediterranean countries, the specific aim of first part of my PhD research

project was to develop and validate a self-report measure of food neophobia designed for Italian primary school children. This was an ecological study performed at school in order to be as much as possible representative of an everyday life situation.

1.2 MATERIALS AND METHODS

1.2.1 Preliminary experiment: design of the questionnaire

In this first stage of the study, the original version of the adults FNS (Pliner & Hobden, 1992) was carefully examined to establish whether the items, vocabulary and response format would be appropriate for Italian primary school children. Then, to ascertain that children understood all the items and the scale, the questionnaire, after careful translation into Italian, was tested in a pilot study with a group of 30 children (16 girls and 14 boys who ranged in age from 6 to 10 years) recruited from a childhood community center in Milan. Children received the questionnaire in a collective setting (small groups of 5 to 6 children) and were asked to respond to each item using the 5-point scale. During the completion of the questionnaire, an experimenter recorded questions and comments raised by the children.

1.2.2 Main experiment: validation of the questionnaire

The ICFNS (Laureati et al., 2015) was first presented to 561 children (none of whom were involved in the preliminary experiment) in their classrooms immediately prior to their mid-morning break in the presence of their teacher and an experimenter. Children received the ICFNS, and the experimenter explained to them how to complete the questionnaire. For each item, the children were asked to indicate the degree to which they considered the statement to be true for them, using the 5-point facial scale. To evaluate the reliability of the scale, the same children were invited to repeat the test one week later. Only 491 of the initial 561 children were present at this second test administration. The second test was administered using the same conditions as the first test, that is, the questionnaire was self-administered in a collective setting. The administration method was identical for all children.

To assess the predictive validity of the ICFNS, the same 491 children were invited to participate in a third task one week later consisting of an evaluation of the children's willingness to taste and liking of two unfamiliar foods, namely daikon and radish. Only 475 (radish) and 468 (daikon) of the 491 children participated in the food task. The vegetables were served to the children in plastic cups at 10:30 am, immediately prior to the mid-morning break in the classroom in the presence of their teacher and an experimenter. The presentation order of the two items was balanced across the children. The children were presented with each of the items and asked to indicate whether each food item was familiar or not (answer: yes/no), whether they were willing to taste the food item (answer: yes/no). Then, they were requested to take a small bite and indicate how much they liked it on a 7-point hedonic facial scale (Pagliarini et al., 2003; Pagliarini et al., 2005).

1.2.3 Data Analysis

For each child, a neophobia score ranging from 8 to 40 was calculated (neophilic items scores were reversed). High scores indicated higher food neophobia. Reliability of the ICFNS was assessed calculating internal consistency (Cronbach's alpha) and temporal stability by test-retest evaluation. Mean values for each item in the test-retest evaluation were compared through paired t-tests ($p < 0.05$). Correlations between items were measured using Pearson correlation coefficients. The relationship between each item in the test-retest evaluation was further evaluated with Principal Component Analysis (PCA).

The relationship between the ICFNS scores and children's willingness to try and how much they liked unfamiliar foods was analyzed by calculating Pearson correlation coefficients and

PCA. In addition, to further interpret the relationship between children's liking and willingness to try unfamiliar foods and neophobia, the frequency distribution of FNS scores was calculated and children were divided into 3 groups: ''low neophobia'' (children in the lowest quartile, ICFNS scores 6 17, n = 124), ''medium neophobia'' (children in the second and third quartile, ICFNS scores P18 and 624, n = 208) and ''high neophobia'' (children in the highest quartile, ICFNS scores P 25, n = 143). Liking data were examined using a mixed model ANOVA that included subjects (random effect), neophobia level (fixed effect) and their interaction as factors. Willingness data were compared using Chi-Square test. 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.

1.3 RESULTS AND DISCUSSION

1.3.1 Preliminary experiment

After this first examination, we had concerns that children would not properly understand the term "ethnic." Thus, the original scale was reduced to 8 items, 4 related to neophilic and 4 related to neophobic attitudes. More specifically, the items "Ethnic food looks too weird to eat," "I like trying new ethnic restaurants" and "I like foods from different countries," which were present in the original FNS, were removed and replaced by the item "I like trying new food and tastes from other countries".

We also decided to reduce the number of response options of the agreement scale from 7 to 5 because we felt that the youngest children may have difficulty discriminating between 7 response options. Additionally, each response on the 5-point scale was represented by a facial expression, which we believed would help the child better understand the level of agreement or disagreement for each of the 8 items (from left to right, the 5-point scale was "Very false for me," "False for me," "So-so," "True for me," "Very true for me").

The pilot test revealed that the children had difficulty understanding one item with a double negative (i.e., "If I don't know what a food is, I won't try it") and were not familiar with the situation described by the item "At dinner parties, I will try new food." Thus, these two items were slightly modified to eliminate the double negative and to include situations that are more familiar to children (i.e., "When I am at a friend's party, I will try new food"). With these adjustments, children seemed to properly understand the meaning of all of the items. Questionnaire internal consistency evaluated in the pilot study was 0.77. The Italian Children Food Neophobia Scale (ICFNS), that was developed to be used in the main study, is included in **Table 1.1**

Table 1.1 Original version of the FNS and Italian version adapted for children (ICFNS). Items 4, 5 and 10 of the original FNS were replaced by item 4 of the ICFNS. R indicates the neophilic items for which the score was reversed.

English items (FNS)	**Italian items (ICFNS)**
1. I am constantly sampling new and different foods (R)	1. Mangio quasi tutti i giorni cibi nuovi e diversi dal solito (R)
2. I don’t trust new foods	2. Non mi fido dei cibi nuovi
3. If I don’t know what a food is, I won’t try it	3. Se un cibo è nuovo, non lo assaggio
4. I like foods from different countries (R)	4. Mi piace provare sapori e cibi strani, diversi dal solito e provenienti da altri Paesi (R)
5. Ethnic food looks weird to me	
6. At dinner parties, I will try new food (R)	5. Quando sono alla festa di un amico mi piace assaggiare cibi nuovi (R)
7. I am afraid to eat things I have never had before	6. Ho paura di assaggiare un cibo che non ho mai mangiato prima
8. I am very particular about the foods I eat	7. Sono molto schizzinoso quando si tratta di mangiare
9. I will eat almost anything (R)	8. Mangio tutto, ma proprio tutto! (R)
10. I like to try ethnic restaurants (R)	

1.3.2 Main experiment

In the main experiment, the validity of the questionnaire was tested evaluating internal consistency, test-retest reliability and predictive validity.

Internal consistency and reliability of the questionnaire

The internal consistency of the ICFNS was satisfactory (Cronbach’s alpha = 0.73; n =8). Total mean ICFNS scores and individual item scores for the test-retest evaluation are reported in **Table 1.2**.

Table 1.2 Mean scores and standard deviation of each item of the ICFNS (n=491). Comparison between mean values by paired t-test (p<0.05). (R) indicates the neophilic items for which the score was reversed.

Item	Test		Re-		p-value
	Mean	SD	Mean	SD	
1(R)	2.7	1.2	2.8	1.3	n.s.
2	2.8	1.4	2.7	1.4	n.s.
3	2.9	1.4	2.8	1.4	n.s.
4(R)	2.3	1.4	2.3	1.3	n.s.
5(R)	2.3	1.3	2.3	1.3	n.s.
6	2.5	1.3	2.5	1.3	n.s.
7	2.6	1.4	2.6	1.4	n.s.
8(R)	2.9	1.3	2.9	1.3	n.s.
Overal	21.0	5.8	20.7	6.1	n.s.

The correlation between first and second administration of the whole scale was 0.61. According to paired t-test comparisons, responses to the items and the total scores did not differ significantly ($p<0.05$) between the first and second test administration, which indicates good repeatability over the two sessions. Both the internal consistency and reliability over the test and retest sessions were satisfactory, with coefficients comparable to those found in previous research on children food neophobia assessment (Loewen & Pliner, 2000; Reverdy et al., 2008).

The relationship between the items in the test-retest evaluation was further investigated through PCA (**Figure 1.2**).

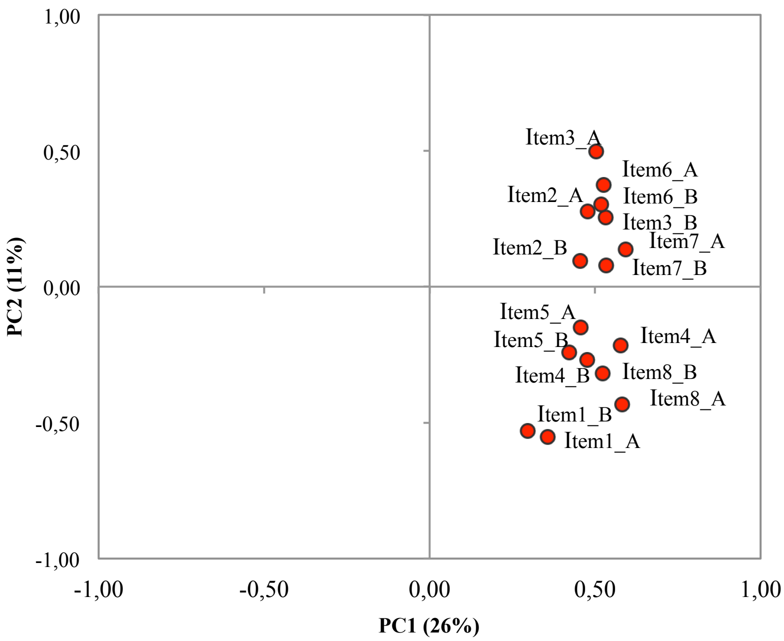

Figure 1.2 Principal Component Analysis performed on scores of each item (Items 1-8) at test (A) and re-test (B) evaluation (n=491).

The total variance explained by the first two PCs was 37%. All of the items showed a positive correlation with PC1 (26% explained variance), whereas PC2 (11% explained variance) separated the neophobic items (1, 4, 5 and 8; negative loadings on PC2) from the neophilic items (2, 3, 6 and 7; positive loadings on PC2). Items at first and second evaluations were positioned near in the bi-dimensional space, confirming a satisfactory temporal stability of children's food neophobia measured by the ICFNS. Thus, PCA, in addition to confirm the validity of test-retest demonstrated that items accomplish the purpose of measuring two opposite "directions" of the same variable (neophobia). This additional information corroborates the validity of the ICFNS.

Predictive validity of the questionnaire

To test the predictive validity of the ICFNS, the children were asked to perform a food task consisting of an evaluation of their willingness to try and how much they liked two unfamiliar foods (i.e., daikon and radish). The results confirmed that daikon and radish were considered unfamiliar to 95% and 70% of the children, respectively. The children's willingness to try and liking of the two food items were calculated according to children's food neophobia level (i.e., low, medium and high neophobia). The results are reported in **Figure 1.3 a-b**.

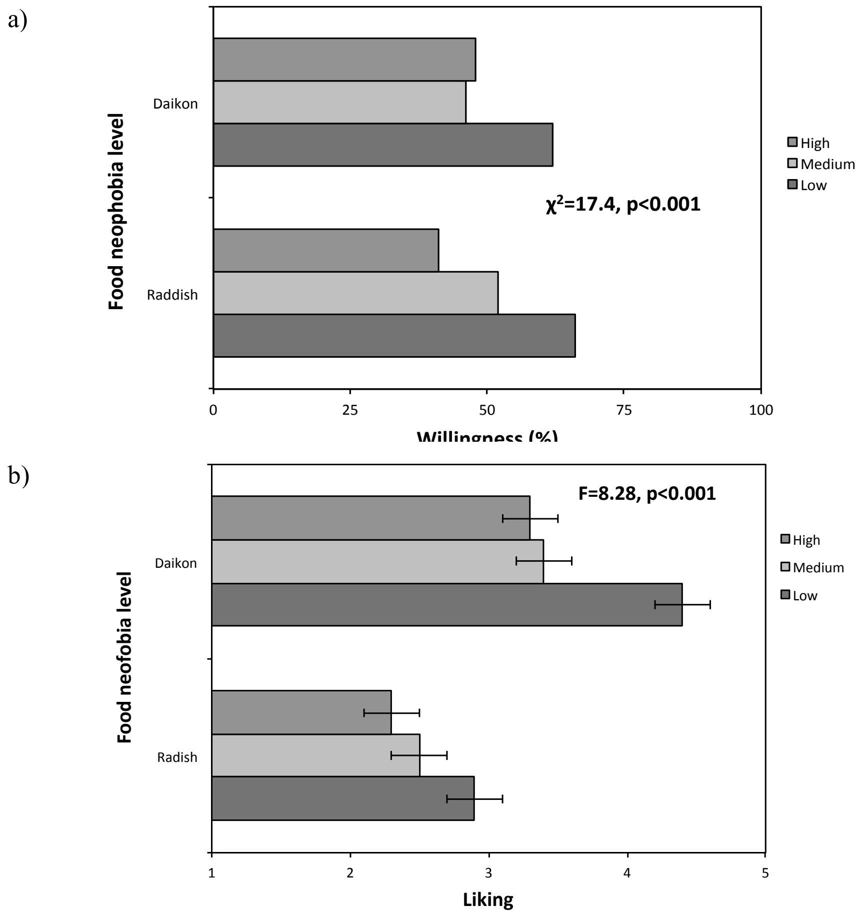

Figure 1.3 (a-b) Willingness to try (a) and liking (b) of unfamiliar foods, daikon and radish, by children’s food neophobia level.

Willingness to try the two food items differed according to neophobia level; the children with low levels of food neophobia were significantly more willing than the children with high levels of food neophobia to taste both daikon (χ^2=9.1, df=2, p<0.05) and radish (χ^2=17.4, df=2, p<0.001). Additionally, the degree to which the children liked unfamiliar foods decreased with increasing levels of neophobia because the children with a high level of food neophobia liked both daikon (F=8.28, p<0.001) and radish (F=2.51, p=0.05) significantly less than did the children with low food neophobia.
Results from the ICFNS and children’s reports of their liking and willingness data were then analyzed using PCA to investigate the relationship among the variables from a multidimensional point of view (**Figure 1.4**).

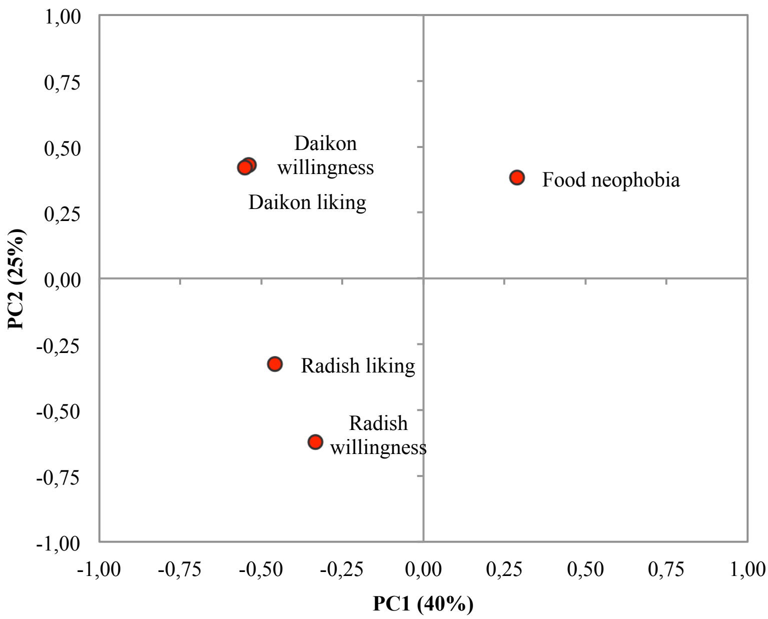

Figure 1.4 Principal Component Analysis performed on food neophobia scores and willingness to try and liking of unfamiliar foods (daikon and radish).

The total variance explained by the first two PCs was 65%. The ICFNS had a positive loading on PC1 (40% of the explained variance) and was negatively correlated with the children's willingness to try and liking of both daikon and radish, which loaded negatively on PC1. As expected, willingness and liking both food items were positioned very near one another in the multidimensional space, meaning that children's willingness to taste a novel food reflected the degree to which they reported liking that food. These results indicate that the more neophobic children are (as evaluated by ICFNS), the less willing they are to try a new food and the less they like it.

The results related to familiarity revealed that our food sample was appropriate because most of the children considered these items to be unfamiliar.

Predictive validity data showed that the ICFNS scores reflected the children's liking of and willingness to taste unfamiliar food. Although the ICFNS coefficients were low overall, they were significantly and negatively correlated with willingness to taste and liking of unfamiliar food. Our correlation values were comparable to those obtained in previous studies that used a behavioural food task to examine the external validity of questionnaires related to children's willingness to try unfamiliar food (Loewen & Pliner, 2000; Pliner, 1994; Reverdy et al., 2008). The relatively weak relationship between the ICFNS and children's willingness to try new food may be explained by the fact that the two tests measure different things. Actually, the ICFNS is a measure of a personality trait, whereas the behavioural assessment of children's willingness to try new food evaluates the expression of this trait (Rigal et al., 2006) and strongly depends on the specific situation surrounding the measurement (Reverdy et al., 2008). The predictive validity of the ICFNS was further confirmed when the children were divided according to the degree of neophobia. It was shown that neophobic children were significantly less willing to taste the new food and reported liking the new food significantly less than their neophilic peers. Thus, we can reasonably state that self-reports by children are good predictors of their willingness to taste and liking of novel food.

There is evidence in the literature of the predictive validity of questionnaires evaluating neophobia through the use of food pictures and listing (Rubio et al., 2008). Although some authors report that the use of pictures of food is a reliable method to evaluate children's food preferences (Guthrie, Rapoport, & Wardle, 2000), we believe that providing children with real food in an actual food context (e.g., midmorning snack at school) is the best way to achieve more representative and ecologically valid results. The naturalistic environment is an important point to consider when studying
factors linked to children's food behaviour. In everyday life, the perceived danger of food may be greater than that felt in the safety of a laboratory so that the effects of food neophobia may be underestimated when measured in a laboratory setting (Russell & Worsley, 2008).

Questionnaire results according to children's age
Because the children involved in the study were 6 to 9 years old and large differences from a cognitive point of view are reported in this age range (ASTM, 2003; Guinard, 2001), the ICFNS scores were calculated according to the children's age. Comparisons of the ICFNS scores obtained in the test-retest evaluation by age group are reported in **Table 1.3**.

Table 1.3 Mean scores of each item of the ICFNS by children's age (6 years, n=110; 7 years, n=156; 8 years, n=136; 9 years, n=89). P-values are obtained according to paired t-tests (p<0.05).

Age		Item								
		1R	**2**	**3**	**4R**	**5R**	**6**	**7**	**8R**	**Overall**
6	Test	2,9	2,9	3,3	2,7	2,5	2,9	3,0	2,8	23,1
	Re-test	2,6	2,8	3,4	2,1	2,2	3,1	3,5	3,1	22,8
	p	*	**n.s.**	**n.s.**	***	*	**n.s.**	**	*	**n.s.**
7	Test	2,9	3,0	2,9	2,3	2,3	2,4	2,5	2,9	21,2
	Re-test	3,0	2,8	2,8	2,4	2,1	2,4	2,3	2,8	20,5
	p	**n.s.**	**n.s.**	**n.s.**	**n.s.**	*	**n.s.**	**n.s.**	**n.s.**	**n.s.**
8	Test	2,7	2,8	2,7	2,3	2,2	2,5	2,3	3,0	20,5
	Re-test	2,8	2,6	2,5	2,4	2,0	2,3	2,2	2,8	19,5
	p	**n.s.**	**n.s.**	*	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
9	Test	2,2	2,4	2,7	1,9	2,3	2,1	2,5	2,8	18,9
	Re-test	2,8	2,6	2,4	2,3	2,1	2,3	2,4	3,0	20,0
	p	***	**n.s.**	**n.s.**	**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	*

Although the overall ICFNS scores between the test and retest were not significantly different (p<0.05) for all age groups, younger children (6 years old) had clear difficulties reliably answering five out of the 8 items: all 4 of the neophilic items and 1 neophobic item. All the other age groups performed the task well and were reliable in answering all of the items, with the exception of item 1 for the oldest children.
We also analyzed the correlations between the ICFNS scores and the food task (i.e., willingness to try and liking of unfamiliar food) by age group (**Table 1.4**).

Table 1.4. Correlations between ICFNS scores, willingness to try and liking of daikon and radish by age groups. (*) p<0.10; * p<0.05; ** p<0.01; *** p<0.001.

Age (years)	Liking		Willingness	
	Daikon	Radish	Daikon	Radish
6	-0.10	-0.12(*)	0.01	-0.19*
7	-0.05	-0.23**	-0.11	-0.22**
8	-0.23**	-0.18*	-0.26**	-0.23**
9	-0.39***	-0.17*	-0.21*	-0.32**

The results revealed that the overall ICFNS scores were negatively and significantly correlated with both willingness to try and liking unfamiliar food. However, for younger children (6 and 7 years old) coefficients were correlated to willingness to try and liking radish but not daikon.
The questionnaire validity was further investigated by considering variations according to the children’s age. It was shown that the results for younger children (6 years old) were not reliable between the first and second administrations of the questionnaire. Additionally, in contrast to the older children (8 and 9 years old), the correlation coefficients between the ICFNS and the food task were not significant for one of the two food items used among the 6-year-old children. The results for the 7-year-old children appeared to be reliable in the test-retest evaluation, but their ICFNS scores, like those of the 6-year-old children, were significantly correlated with willingness to try and liking data for only one of the two food items. This finding suggests that younger children and especially the 6 year olds may have difficulty understanding the task. Because validation of a new tool implies using the same methodology and standardized administration for all subjects, the administration method was the same across all age groups of children in the present study. This may explain the lower performance of the youngest children. It is likely that 6-year-old children need specific instructions and help when asked to complete the questionnaire. Examples of administration methods adapted for younger children (e.g., questionnaires administered in an individual instead of collective setting and questions read aloud by an experimenter) are present in the literature and have shown a positive result when validating questionnaires among children as young as 5 years old (Rubio et al., 2008).

1.4 CONCLUSIONS

In conclusion, it was shown that simple, age-appropriate vocabulary and items modified to describe situations likely to be familiar to children facilitated the self-administration and understanding of the questionnaire.

We believe that we have successfully accomplished the goal of developing a questionnaire to measure food neophobia, which relies on children’s reports rather than their parent’s reports. More specifically, the questionnaire is tailored for Italian primary school children and takes into consideration aspects related to their specific culture. The questionnaire can be reliably used with Italian primary school children starting from the age of 8 years and most likely as early as 7 years. For 6-year-old children, adapted administration methods are recommended to achieve reliable results.

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2. PERCEPTIVE AND BEHAVIOURAL VARIABLES AND OVERWEIGHT/OBESITY

Are they related?

2.1 INTRODUCTION

2.1.1 Overweight and obesity

Overweight and obesity is on the rise worldwide and it increasing concerns because of its negative effects on people's health and on the sustainability of current and future healthcare systems.

WHO define overweight and obesity as abnormal or excessive fat accumulation that may impair health? An individual's degree of adiposity cannot be immediately measured; however, there are some proxies based on anthropometric features that can be easily measured and are recognized internationally (WHO, 2012). In particular, the Body Mass Index (BMI), measured as the ratio between weight (in kilograms) and height (stated in m^2), is the indicator most used. In adults, a BMI of 25 kg/m2 is used as a cut-off point for overweight, and 30 kg/m^2 as a cut-off point for obesity. In children, BMI change substantially with age and also varies between genders, for this reason BMI values are not commonly used in children, rather, BMI *percentiles* or BMI *z scores* are used.

Overweight and obesity are very common and affect not only the Western countries but also many emerging, despite the many public strategies that have been implemented over the past several decades. In United States about 68% of Americans are overweight, and 34% of the adult population appears to fall within the identification criteria for the definition of conditions of obesity. Furthermore, it is possible to identify a percentage equal to 4.7% of the American adult population that could be included in the so-called "extreme obesity" category (BMI>40).

These phenomena are also growing strongly in Europe and the obese adult population is 13.4% share but large differences are found between the different countries. Obesity prevalence over the past decade has increased from 10% to 40%, with higher rates in Eastern Europe compared to Western Europe (WHO, 2002). In Italy, it is noted that 29.6% is overweight and 10.5% is obese. However, this distribution of individuals throughout Italy shows a remarkable difference between the North and the South: in fact, all the regions of southern Italy and its islands have a percentage of overweight and obesity that is higher than the national average (ISTAT, 2010). These diseases are increasing also in Asian and South American countries and the speed with which the phenomenon is growing in is worrisome, especially in the female population (Gortmaker et al., 2011).

2.1.2 Effects of overweight and obesity in children

In the past few decades also in childhood obesity there has been a steep rise, with one third of children becoming overweight or obese by the time they are 2 years old. The number of obese children and/or their levels of adiposity continue to increase, and the distribution of fat is shifting towards central adiposity. This critical phenomenon is linked to obesity later in life. Indeed, international scientific literature has supplied proof of a strong or moderate relationship between being overweight/obese in childhood and being overweight/obese in adulthood (Dietz, 1998; Sandhu et al., 2006). Moreover, this disease can persist throughout the entire life of the individual, with significant consequences on increasing the likelihood of contracting obesity related chronic diseases in adulthood.

Overweight and obesity major effects on health can be summarized as being problems of a metabolic nature (insulin resistance, glucose intolerance, hypertension) and non-metabolic in nature, such as bone and joint diseases (valgus of the lower limbs, joint pain, reduced mobility, flat feet), skin diseases (strie rubrae, acanthosis nigricans), liver diseases (fatty liver), and respiratory diseases (desaturations and nocturnal apnea).

In addition, people who were overweight or obese in their youth have greater exposure to cardio-circulatory diseases (hypertension, coronary diseases), musculoskeletal diseases (early onset of arthritis due to the increase in static-dynamic demands on the spine and lower limbs,

subjected more to the weight load), metabolic diseases (diabetes mellitus, high cholesterol, high triglycerides, etc.), up to the development of tumours in the gastro- intestinal tract (increases in body weight and other obesity indices in youth (but not only) can translate into later increases in blood pressure (Dietz, 1998).
Emotion effects are not to be excluded. Many overweight and obese subjects experience discrimination, beginning even in early childhood. Moreover, this discrimination often leads to bullying and teasing with a consequently decreasing of self-esteem.
Thus, the widely held notion that children can grow out of obesity should be solidly put a rest. Instead, to help obese children to become normal weight active efforts should be made (Dietz, 1998).

2.1.3 Overweight and obesity causes

To deal effectively with this widespread overweight and obesity epidemic, it is important to identify its determinants. The origins and causes of this phenomenon are manifold and complex, but at the individual level, the main culprit in the majority of cases of obesity is a combination of sedentary lifestyles and wrong eating behaviours. It's well know, in fact, that physical exercise to prevent an excessive increase in body weight is crucial during child's growth, since it promotes and helps to modify the ratio between lean mass (muscle tissue) and fat mass (adipose tissue). Moreover, various aspects of food habits play a key role on children choice and can thus, affect risk of overweight and obesity. Only a limited number of cases are instead due to genetics, health reasons, or psychiatric illnesses.
The spread of *sedentary lifestyles* in childhood often depend on family and social environment, which leave few, if any, possibilities to perform basic physical activities. For example, it's commonly know the growing average time that children spend in front of the television or at the computer because their parents tend to be busy at work until the evening hours. Furthermore, numerous international data show that children and adolescents leave home less and less, and they participate less frequently in physical education activities (in particular, adolescent girls)(Swinburn, 2000), since parents are very apprehensive about their safety (Gordon-Larsen et al., 2004).
The worst *eating habits* that can lead to overweight, and obesity are related to an excessive caloric intake and a reduced consumption of fruits and vegetables (FV). The factor involved in the high total number of calories intake is the caloric density. Fats increase the caloric density of food with its energy supply of 9 kcal/g. Since the feeling of satiety is influenced by the food's volume and since low caloric density foods are more voluminous than high caloric density ones, food high in fat tend to induce less satiety than the others. To make matter worse, fats give food greater appeal (consistency, freshness, etc.) and, therefore encourage their consumption, increasing the total energy contribution. Moreover, heat-generating characteristics (energy cost for the digestion, absorption, metabolization, and storage) of lipids ranges between 2% and 4% of the energy content of the lipids taken in, much less than the energy costs for carbohydrates (5%-24%) and proteins (25%-3, 2008).
Finally, in the last few decades, an increasing in consumption of product added with sugar and soft drink has made the situation worst.
FV have high water contents, they are recommended as a source of vitamins, in particular vitamin C, and minerals, especially potassium. Plant foods are also high in dietary fibre and phytoestrogens that are related to a reduction of risk of intestinal chronic diseases (Slavin et al., 2008). Moreover, fibre food content decreases energy density and can decrease satiety (Slavin et al., 2007). Despite these benefic effects are well know, the consumption of these products is still very low in worldwide populations.

2.1.4 Taste sensitivity and nutritional status

Among individual factors, taste sensitivity plays an important role in food preferences, choices, and thus consumption. Taste sensitivity can be defined as the minimum concentration at which the subject is able to perceive a specific taste quality, such as sweet, sour, salty and bitter (Sørensen et al., 2003). A growing literature suggested that the ability to taste phenylthiocarbamide/6-n-propylthiouracil (PROP), synthetic compounds identified as major ligands for bitter-taste-receptor genes (TAS2R38), influences dietary behaviour (Bufe et al., 2005; Kim et al., 2003). In particular variation in taste sensitivity to bitter has been associated with differences in preferences for and selection of bitter FV, as well as sweet foods, added fats, spicy foods, and alcoholic beverages (Dinehart et al., 2006; Keller et al., 2002; Duffy et al., 2004). Past studies failed to show any association between sweet thresholds and nutritional status (Donaldson et al., 2009), while more recent studies described a difference between overweight and normal weight subjects (Simchen et al., 2006; Monnause et al., 2008). In particular, it has been shown that PROP phenotype is related to body mass index (BMI) in females and that sweet (sucrose) as well as salty (sodium chloride) taste sensitivity are higher in young overweight/obese individuals compared with normal weight controls (Sartor et al., 2011). This suggests that overweight and obese subjects may have a reduced or distorted sensory sensitivity that might increase the desire and ingestion of food, thus leading to excessive energy intake and weight gain (Tepper et al., 2008). Moreover, a possible interaction between tasting profile such as sweet liking or supertasting status with metabolic syndrome has been suggested in adolescence (Pasquet et al., 2007) and more recently in the adults (Turner-McGrievy, 2013). Finally, other investigators have reported that taste sensitivity may be affected by short-term caloric deprivation in both overweight and lean subjects, with lower thresholds of perception in fasted state than in satiated state (Zverev, 2004). Thus, it could be suggested an implication for taste sensitivity also in diet-induced weight loss program. However, evidence in regard to this issue is still in lack. The main purposes of the first experiment were: to study the relationship between nutritional status and taste sensitivity; and to investigate the relationship between metabolic syndrome parameters and taste sensitivity in normal weigh, overweigh and obese adults.

2.1.5 Liking, food neophobia and nutritional status

It is widely agreed that food acceptance and food choice are largely driven by taste preferences and liking, particularly among the children in contemporary western environments. For this reason, it is often assumed that overweight individuals differ from their normal-weight counterparts in that they prefer foods that are thought implicated in the development of obesity. Despite this opinion, earlier findings on the association between adiposity and food liking for common foods are inconclusive in adults and relevant research in children is limited (Hill, et al., 2009).

Food neophobia is one of the main factors influencing the quality of children's diets and the development of food preferences (Russell & Worsley, 2008). Although food neophobia has been evolutionarily useful, in a modern society where food safety is guaranteed, it can have a negative effect on food choices, particularly for children, because food neophobic individuals avoid new food experiences and thus lack dietary variety (Cooke et al., 2003a). Evidence for a negative relationship between food neophobia and dietary variety in children has been reported (Koivisto & Sjöden, 1996; Falciglia et al., 2000; Skinner et al., 2002), as neophobic children are less inclined to eat certain types of foods (e.g., fruits, vegetables and foods of animal origin) than their more neophilic peers (Galloway et al., 2003; Nicklaus et al, 2005; Cooke et al., 2006). Although the negative effects of food neophobia on children's everyday food intake are increasingly well documented, the role of food neophobia in children nutritional status remains unclear. Falciglia et al. (2000) showed a tendency toward a higher caloric intake in the neophilic children, while Zalilah et al. (2005) suggested that neophobic children had a greater prevalence of both overweight and underweight.

To the best of our knowledge, very few studies have investigated the relationships among food neophobia, food preferences and nutritional status in an ecological environment, particularly in children. We are aware of the study of Knaapila et al. (2011), who conducted a multidisciplinary investigation of the origins of food neophobia and its relationship with a series of variables, among which were personality traits, the pleasantness of the food and the body mass index.

The aim of the second experiment was to assess food neophobia and the liking of FV in a representative sample of primary school children in an ecological environment (i.e., at school) and to evaluate how food neophobia and liking are related to the children‘s nutritional status. We hypothesized that food neophobia is associated with a reduced liking of FV, that boys are more food neophobic than girls and that food neophobia and food preferences are affected by the children‘s age. We also hypothesized that both food neophobia and the liking of FV are related to the children‘s body mass index (BMI).

EXPERIMENT A

2.2a MATERIALS AND METHODS

2.2.1 Participants

41 overweight (OW; F:M, 34:7) and 52 obese (OB; F:M, 32:20) patients of the International Center for the Assessment of Nutritional Status (University of Milan, Italy) only for weight and dietetic concern, and 56 healthy normal-weight (NW F:M, 36:20) volunteers were recruited. Major study inclusion criteria were age <65 years (range: 18-64), euthyroidism, no diabetes, no alcohol drinking, no diet to lose weight in the last 6 months, no restrained eating behaviour and absence of well-established dysgeusia. Binge eating disorder was also excluded according to current diagnostic criteria. On the same day, all the patients underwent a full nutritional assessment and taste sensitivity analysis in fasting state.

2.2.2 Nutritional assessment and presence of metabolic syndrome

Nutritional assessment was performed after 8-12 hours of fasting and included: - Medical history and physical examination, including blood pressure measurement. - Anthropometric evaluation by collecting body weight (to the nearest 0.1 kg) and standing height (SH; to the nearest 0.1 cm) through the same calibrated scale provided of a telescopic vertical steel stadiometer (SECA 90 220; Germany) and kept the patient dressing only underwear. Body mass index (BMI) was derived accordingly (weight [kg]/height [m^2]). Waist circumference was also measured (to the nearest 0.5 cm) at the midpoint between the iliac crest and the last rib. - Body composition by a four-polar impedence meter (BIA; Human IM Scan, DS-Medigroup, Milan, Italy). Whole-body resistance was measured on the left side of the body at frequency of 50 kHz (R50) following international guidelines and fat free mass was calculated using the formula for healthy adults proposed by Deurenberg et al. (Deurenberg et al., 1991). Percentage of body fat mass (BF%) was derived accordingly. - Resting energy expenditure (REE) assessment by indirect calorimetry (Sensor Medics Vmax-29N; Anheim, CA). Concentrations of carbon dioxide and oxygen were measured with the ventilated-hood technique. Therefore, gas concentrations were used to determine REE with the Weir equation (Weir, 1949). - Venous blood sampling in fasted state for the evaluation of glucose, high density lipoproteins (HDL) and triglycerides. - Dietary recall by the same well trained dietician to evaluate eating behaviour, eating habits and food preferences which were almost taken into account during diet preparation. The updated criteria from the International Diabetes Federation (Tong et al., 2007) were used to define metabolic syndrome (MetS+). That is to say, subjects had to have ≥ 3 of the following: (1) waist circumference > 94 cm in men and > 88 cm in women; (2) serum triglyceride ≥ 150 mg/dL; (3) HDL-cholesterol < 40 mg/dL in men and < 50 mg/dL in women; (4) blood pressure ≥ 130/85 mmHg; and (5) fasting plasma glucose level ≥ 100 mg/dL. Participants treated with antihypertensive or triglyceride-lowering medications were considered as hypertensive or hypertriglyceridemic, respectively. Subjects in the control group were not evaluated for waist circumference and body composition.

2.2.3 Taste sensitivity analysis

Taste sensitivity determination was performed at the sensory laboratory of the Department of Food, Environmental and Nutritional Sciences (DeFENS- University of Milan, Italy). Participants were asked not to smoke, eat or drink anything except water before the test. Recognition taste thresholds were evaluated by means of the three-alternative-forced-choice method (3-AFC) (ASTM, 2004). Sucrose, caffeine, sodium chloride and citric acid were used to elicit sweet, bitter, salty and sour tastes, respectively. For each compound, five concentrations were prepared in mineral water. Concentration range of each taste stimulus was chosen on the basis of threshold values reported in the literature (Mojet et al., 2001; Mojet et al., 2003). Concentration ranges were established in order that the lowest concentration was clearly below and the highest concentration clearly above the level at which subjects are able to detect or recognize the stimulus. A preliminary test was carried out to adjust concentration ranges since in some cases subjects occasionally recognized the lowest concentration or did not recognize the highest concentration of the stimuli. The final ranges of concentration (expressed in g/L) and dilution factors used to elicit the four basic tastes are reported in **Table 2.1**

Table 2.1 Compounds used to elicit the 4 basic tastes with relevant dilution step and concentration range.

Taste	Compound	Dilution step	Concentration range (g/L)
Sweet	Sucrose	3	1.23-100.00
Bitter	Caffeine	0.2 log	0.16-1.00
Salty	Sodium cloride	3.5	0.50-75.00
Sour	Citric acid	3.5	0.33-50.00

The solutions were prepared the same day of the session and tested at room temperature. For each basic taste participants were presented with 5 triads of samples marked with three-digit numbers. Each triad consisted of one cup containing the stimulus and two cups containing an equal volume of blank (mineral water). The 5 triads proceeded from weaker to progressively stronger concentration, with the position of the cup containing the stimulus randomized over trials and assessors. For each triad, participants were instructed to indicate which sample was different from the other two. If assessors were uncertain, they were instructed to guess (forced choice procedure). At the beginning of each session, and before each triad, the assessors were instructed to rinse their mouth with mineral water. Data were self-recorded by the subjects on paper sheets. The individual threshold for each sensory stimulus was calculated as the geometric mean of the concentration at which the last miss occurred and the next higher concentration that was correctly recognized (ASTM, 2004). In addition, from the above mentioned threshold values, an individual global taste acuity score (GTAS) was determined, as recently reported by Monneuse et al. (2008). For every basic taste we divided patients into tertiles according to taste sensitivity threshold data. We attributed the score 3, 2 and 1 to increasing threshold values and the sum of these scores defined the GTAS. Therefore, the higher the GTAS the higher the acuity.

2.2.4 Weight loss program outcomes

Adherence and compliance to the program were defined as drop-out since the next visit and weight loss ≥5% in 3 months, respectively.

2.2.5 Statistical analysis

Variables were presented as frequencies or percentages if categorical (sex, smoking and menopause status, metabolic syndrome) and as mean ± standard deviation if continuous (age, BMI, body fat mass, waist, taste thresholds). As preliminary results indicated that data on tastes sensitivity were not normally distributed, values were log-transformed to achieve a near-Gaussian distribution. Categorical variables were compared by chi-square ($\chi 2$) test and comparison between groups for continuous variables was performed by Student t-test (two-group comparisons) or ANOVA analysis (multiple-group comparisons) followed by post-hoc comparison of means by Tukey's test. Then, to avoid the effect of possible confounders such as gender and age linear regression models were built to test the independent relationship between: i) taste sensitivity (dependent variable) and both BMI and MetS (independent variables); ii) outcomes, namely dropout and successful weight loss (as dependent variables), and taste sensitivity (each taste as independent variable).

2.3a RESULTS AND DISCUSSION

2.3.1 Taste sensitivity according to nutritional status and metabolic syndrome

The features of the population investigated are presented in **Table 2.2**.

Table 2.2 Features of the population according to weight status.

	Controls (n=56)	Overweight (n=41)	Obese (n=52)	p*
Sex (M:F)	20:36	7:34	20:32	0.061
Age (years)	41.6 ± 12.3	46.9 ± 11.5	45.8 ± 11.6	0.060
Range	24-66	20-64	19-64	
Smoking (n)	30 (53.6)	22 (53.7)	33 (63.4)	0.511
Menopause (n)	15 (41.7)	16 (47.0)	15 (46.9)	0.404
Body mass index (Kg/m^2)	22.1 ± 1.7	27.9 ± 1.6	34.8 ± 4.6	**<0.001**
Body fat mass (%)	-	45.6 ± 5.2	47.6 ± 5.1	0.054
Waist (cm)	-	91.5 ± 7.5	106.2 ± 18.2	**<0.001**
Metabolic syndrome (n)	0 (0)	8 (19.5)	25 (48.1)	**0.004**
Taste thresholds**				
Sweet (log g/L)	0.74 ± 0.44	0.78 ± 0.40	0.85 ± 0.48	0.418
Salty (log g/L)	0.23 ± 0.54	0.13 ± 0.48	0.36 ± 0.58	0.099
Sour (log g/L)	-0.21 ± 0.54	0.34 ± 0.40	-0.05 ± 0.67	0105
Bitter (log g/L)	-0.34 ± 0.35	0.21 ± 0.29	0.24 ± 0.30	0.151
GTAS	8.0 ± 1.9	8.0 ± 1.6	7.3 ± 2.1	0.132

Data are reported as mean±standard deviation or counts (%). **GTAS**, Global Taste Acuity Score. * P-values according to Chi-square, parametric (ANOVA analysis) and non parametric (Kruskal-Wallis or Wilcoxon-Mann-Whitney) tests, where appropriate. ** for two-group comparisons: no significant differences were detected by Wilcoxon-Mann-Whitney test.

Normal-weight controls, OW and OB patients were matched for age, gender and smoking and hormonal status. A higher prevalence of MetS characterized obese patients when compared to those overweight despite similar BF%. At baseline, nosignificant difference was detected neither in any of the taste sensitivity nor in GTAS, although we observed a general trend to higher threshold values with increasing BMI. However, sex and age-adjusted multiple regression models revealed (**Table 2.3**) a significant association between BMI and both sour taste and GTAS, with lower sensitivity with increasing BMI.

Table 2.3 Multiple regression model between taste sensitivity and nutri-metabolic parameters

	Sour	Bitter	Salty	BMI	BF%*	Waist*	MetS*	MetS criteria*
Sour	-	-	-	0.20^{a}	0.05	0.27^{c}	0.21^{a}	0.21^{a}
Bitter	0.34^{e}	-	-	0.14	-0.09	-0.13	0.02	0.03
Salty	0.26^{c}	0.23^{b}	-	0.10	-0.08	-0.11	0.23^{a}	0.19
Sweet	0.24^{d}	0.33^{e}	0.26^{c}	0.15	-0.15	010	0.08	-0.01
GTAS	-	-	-	-0.13^{a}	-0.15	-0.05	-0.08	-0.11

*For BF%, waist circumference, presence of metabolic MetS and the num- ber of MetS criteria correlations refer to overweight/obese patients (n = 93). Values are standardized coefficients adjusted for age and sex, ^{a}p < 0.05; ^{b}p < 0.01; ^{c}p < 0.002; ^{d}p < 0.005; ^{e}p < 0.001, between BMI and both sour taste and GTAS, with lower sensitivity with increasing BMI. BMI: Body mass index; BF%: Percentage of body fat mass; MetS: Metabolic syndrome; GTAS: Global Taste Acuity Score.

These results evidenced an independent effect of BMI on taste sensitivity for sour and global taste acuity. Moreover, obese individuals showed in general a tendency to higher taste thresholds than lean subjects.
Although the association between BMI and taste has been largely investigated, very few data are available on the relationship between taste thresholds and body mass index and our findings appear partially in contrast with those already provided. Pasquet et al. (2007) observed that massively obese adolescents have lower thresholds for taste recognition than normal-weight controls. Obrebowski et al. (2000) found that children and adolescents with simple obesity have lowered electrogustometric thresholds. The authors attributed this behaviour to obesity-related metabolic disturbances rather than to body mass. Similarly to our study, Simchen et al., (2006) have recently investigated the association between taste qualities (sweet, sour, bitter and salty) and BMI in a group of adults. They observed an age dependent relationship with respectively lower and higher sensory capabilities in overweight subjects aged < 65 years and ≥ 65 years for sour and bitter tastes. However, despite the investigation by Simchen et al. (2006) has been performed in a larger cohort, the authors have recognized not to have controlled for an important potential confounder such as restrained eating behaviour, a factor that has been considered by us during recruitment. Besides, body composition and fat distribution assessments were helpful to better characterize our subjects nutritional status, as the pathophysiology of metabolic complications is substantially related to overall and compartmental body fatness (Tong et al., 2007).

Table 2.4 Taste in overweight and obese patients according to metabolic syndrome

	MetS+ (n=33)	MetS- (n=60)	p*
BMI (Kg/m^2)	33.7 ± 5.2	30.7 ± 4.2	0.002
BF%	47.7 ± 6.0	46.4 ± 4.7	0.281
Waist (cm)	106.2 ± 14.2	96.1 ± 16.3	0.004
Sweet (Log g/L)	0.87 ± 0.41	0.80 ± 0.47	0.396
Salty (Log g/L)	0.43 ± 0.56	0.16 ± 0.52	0.029
Sour (Log g/L)	-0.01 ± 0.69	-0.27 ± 0.49	0.069
Bitter (Log g/L)	-0.22 ± 14.2	-0.23 ± 14.2	0.956
GTAS	7.4 ± 2.0	7.7 ± 1.9	0.440

p values according to unpaired Student *t*-test or Wilcoxon-Mann-Whitney test. *MetS+ *vs* MetS- within the same group (overall or women or men). BMI: Body mass index; BF%: Percentage of body fat mass; MetS: Metabolic syndrome (+, presence; -, absence); GTAS: Global Taste Acuity Score.

MetS+ subjects presented higher thresholds for salty when compared to MetS- patients while no significant difference was detected for the other tastes and GTAS (Wilcoxon-Mann-Whitney test; **Table 2.4**). As assessed by multiple regression model, the association between salty taste and MetS appeared to be independent of sex, age and BMI.
These results seem in conflict with the recent findings by Pasquet et al. (2006) who found a female-specific but positive association between taste sensitivity for sweet and salty tastes and the number of obesity-related metabolic disorders in a group of adolescents. This inconsistency may be ascribed to the different approach used to measure taste thresholds and to the fact that, contrary to Pasquet et al. (2006) study, adolescents were not considered in the present experiment. The positive association found between higher threshold for salty taste and Mets probably is dependent, at least partially, on association between higher threshold for salty taste and hypertension as suggested by Rabin et al (2009). Indeed, hypertension is a major component of the metabolic syndrome (Tong et al., 2007). It should be pointed out that the association between metabolic syndrome and taste acuity still needs to be clarified as several changes in perception could occur throughout life for example in reason of hormonal and psychological factors.

EXPERIMENT B

2.2b MATERIALS AND METHODS

2.2.1 Participants

Five hundred and twenty-eight (267 boys and 261 girls) aged between 6 and 9 years (mean age: 7.8 ± 1.1 years) who attended three urban public primary schools participated in this cross-sectional study. The schools were selected in the center and the larger metropolitan area of Milan (Italy). Food neophobia, the liking of FV and the BMI of all of the participants were assessed at school.

The children were selected based on a consent form that was completed by the parents. The parents were asked to read a short explanation of the study and to complete a questionnaire in which they were asked to indicate whether their child had any food allergy or followed a specific diet. All of the children involved in the study met the following criteria: healthy, not on a specific diet, and not subject to food allergies. The study was performed in adherence with the principles established by the Declaration of Helsinki. The protocol was approved by the Institutional Ethics Committee at the study site.

2.2.2 Nutritional status evaluation

Anthropometric measurements were taken at the schools by trained technicians according to standardized procedures (Lohman et al., 1988). Height was recorded to the nearest 0.1 cm using a stadiometer and weight was measured to the nearest 0.1 kg using a high-precision mechanical scale. The BMI was calculated as the weight (kg) per height^2 (m^2). The gender-specific BMI-for-age percentiles and Z scores were calculated using the 2000 CDC Growth Charts (Center for Disease Control and Prevention, 2000). In accordance with CDC guidelines, a Z-score below the 5th percentile represented underweight, a score between the 5th and 85th percentile represented a normal weight, a score at or above the 85th percentile and below the 95th percentile represented overweight, and a score at or above the 95th percentile represented obesity (Center for Disease Control and Prevention, 2000).

2.2.3 Evaluation of food neophobia

The ICFNS (Laureati et al., 2015) was first presented to all the children. The administration method was the same across all age groups of children, except for 6-years-old children for whom the administration was simplified (e.g., questionnaires administered in small groups of 5-6 children and questions read aloud by the experimenter). A neophobia score ranging from 8 to 40 was calculated for each child (neophilic items scores were reversed).

2.2.4 Evaluation of liking

The liking test was performed one week after the food neophobia evaluation. The children received small portions of FV (fruits: apple, pear, grapes and miyagawa; vegetables: fennel, radish, broccoli and carrot). A portion of approximately 40 g, of each FV, was served raw to the children immediately prior to their mid-morning snack. To increase ecological validity, children were tested in a familiar environment, their classroom (because Italian children usually eat in their classrooms). FV were selected based on availability in season, ease of handle and storage.

The FV were fresh and were cut into standardized, uniform-sized pieces; they were presented to the children at room temperature in plastic cups encoded with the word “fruits” or “vegetables”. For each stimulus, the children were asked to rate their degree of liking using a 7-point hedonic-facial scale (Pagliarini et al., 2003; Pagliarini et al., 2005).

2.2.5 Data analysis

The food neophobia and liking data were first analyzed using an analysis of variance (ANOVA) test that considered the *School* (school 1-3), the *Age* (6-9 years), the *Gender*, and the *Stimulus* (the FV provided) as factors.
The relationship among the *BMI*, *Food neophobia* and *Liking* data was evaluated using principal component analysis (PCA). Autoscaling was performed on the data prior to any modeling. Cross validation was chosen as the validation method. To further interpret the relationship between liking of FV and food neophobia, the children were divided according to their level of neophobia into 3 groups: 'low' (children with scores in the lower 25th percentile of food neophobia scores, score ≤ 17, n=141), 'medium' (children with scores between the 25th and 75th percentiles, score ≥ 18 and ≤ 24, n=234) and 'high' (children with scores in the upper 25th percentile, score ≥ 25, n=154). The data were subjected to an GLM ANOVA that considered the *Neophobia level* ('low','medium' and 'high'), *Stimulus category* (FV) and their interaction as factors and liking as a dependent variable.

2.3b RESULTS

2.3.1 Nutritional status evaluation

The distribution of the socio-anagraphic (gender and ethnicity) and nutritional status (BMI classification) variables according to age is reported in **Table 2.5**. Age was not related to gender (p=0.81) or to the BMI classes (p=0.76). Of the total sample, 8 children (1.5%) were underweight, 369 (69.9%) were of normal weight, 132 (25.0%) were overweight and 19 (3.6%) were obese. Only 5.3% of the entire sample was non-Caucasian.

Table 2.5 Selected characteristic of the sample and association with age classes (Statistical differences between groups were determined by using the χ^2 test).

Age	*Gender*		*BMI*				*Ethnicity*	
	Boys	Girls	Under-weight	Normal weight	Over-weight	Obese	Caucasian	Other
6	50.70% (70)	49.30% (68)	1.40% (2)	68.80% (95)	26.80% (37)	2.90% (4)	94.20% (130)	5.80% (8)
7	52.00% (91)	48.00% (84)	2.30% (4)	72.60% (127)	20.60% (36)	4.60% (8)	95.40% (167)	4.60% (8)
8	51.50% (70)	48.50% (66)	0.70% (1)	71.30% (97)	25.00% (34)	2.90% (4)	97.80% (133)	2.20% (3)
9	45.60% (36)	54.40% (43)	1.30% (1)	63.30% (50)	31.60% (25)	3.80% (3)	88.60% (70)	11.40% (9)
Total	50.60% (267)	49.60% (261)	1.50% (8)	69.90% (369)	25% (132)	3.60% (19)	94.70% (500)	5.30% (28)
***p* value**	**0.806**			**0.763**			**0.22**	

Although the children in the sample involved in this study showed a prevalence of excess weight, consistent with the most recent national survey of primary-school children (Ministero della Salute, 2012) that was conducted, which reported about 30% overweight/obese individuals, the percentage of obese children was low (4%) compared with the mean Italian percentage (22.2% overweight and 10.6% obese children). However, the lower percentage of obese children found in the present study is coherent with the results of the previously mentioned survey that was conducted in the Lombardia region (6% obese children) and is due to the lower prevalence of overweight children generally observed in the north of Italy.

2.3.2 Evaluation of food neophobia

The mean food neophobia scores by age and gender are reported in **Figure 2.1**. According to the LSD post-hoc test, boys were more neophobic than girls (F=4.82, p=0.03) and the four age classes differed significantly (F=8.67, p<0.001), with a reduction of the neophobic attitude with increasing age. These results confirmed literature data (Koivisto-Hursti & Sjoden, 1996; Nicklaus et al., 2005; Rigal et al., 2006; Dovey et al., 2008). Boys being more neophobic than girls regardless of the age class (F=0.77, p=0.51). However, the differences between the boys and girls were more pronounced at 6 and 7 years and vanished with increasing age. No differences (F=0.19, p=0.83) were detected in the food neophobia scores among the three schools. The food neophobia results did not change when the analysis was performed only on the Caucasian children.

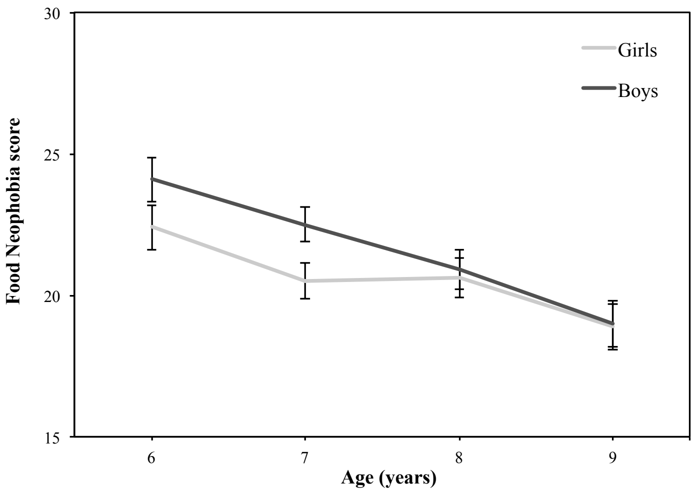


Figure 2.1 Mean food neophobia scores (range 8-40) ± SEM according to age and gender.

2.3.3 Evaluation of liking

The mean hedonic scores by school. age, gender and stimulus are reported in **Table 2.6**.

Table 2.6 Mean liking scores and standard error of the mean (SEM) by school, age, gender and type of stimulus with relevant significance for each factor. Mean values with different superscripts are significantly different (p<0.05) according to LSD post-hoc test.

Variable	**Liking scores**		
	Mean	SEM	F-value (p-value)
School			F=1.60 (p=0.20)
School 1	4.7	0.1	
School 2	4.7	0.1	
School 3	4.6	0.1	
Age (years)			F=6.66 (p<0.001)
6	4.8 [a]	0.1	
7	4.8 [a]	0.1	
8	4.6 [a]	0.1	
9	4.3 [b]	0.1	
Gender			F=0.01 (p=0.94)
Boys	4.6	0.1	
Girls	4.6	0.1	
Stimulus			F=154.22 (p<0.0001)
Apple	6.0 [a]	0.1	
Grapes	5.9 [a]	0.1	
Carrot	5.5 [b]	0.1	
Pear	5.4 [b]	0.1	
Miyagawa	5.0 [c]	0.1	
Fennel	4.3 [d]	0.1	
Radish	2.6 [e]	0.1	
Broccoli	2.4 [e]	0.1	
Stimulus category			
Fruits (overall, n=4)	5.6 [a]	0.1	F=814.28 (p<0.0001)
Vegetables (overall, n=4)	3.7 [b]	0.1	

Liking of products was independet to age (F=6.66, p<0.001). According to the LSD post-hoc test, nine-year-old children showed significantly lower liking scores than the younger children, which were similar. Overall, fruits were more liked than vegetables (F=814.28, p<0.0001) and differences were found among stimuli (F=154.22, p<0.0001). According to the LSD post-hoc test, apple and grapes were the most favored stimuli, followed by carrot and pear. Carrot was the only type of vegetables that received a comparable or even higher liking score than the fruits. Miyagawa was the least liked type of fruits probably due to its sourness. Fennel received significantly lower liking scores than the fruits, whereas radish and broccoli received comparable and very low ratings. No significant differences in the liking scores were found for the school (F=1.60, p<0.20) or the gender (F=0.01, p<0.94). The same results were obtained when the analysis was performed only on Caucasian children.

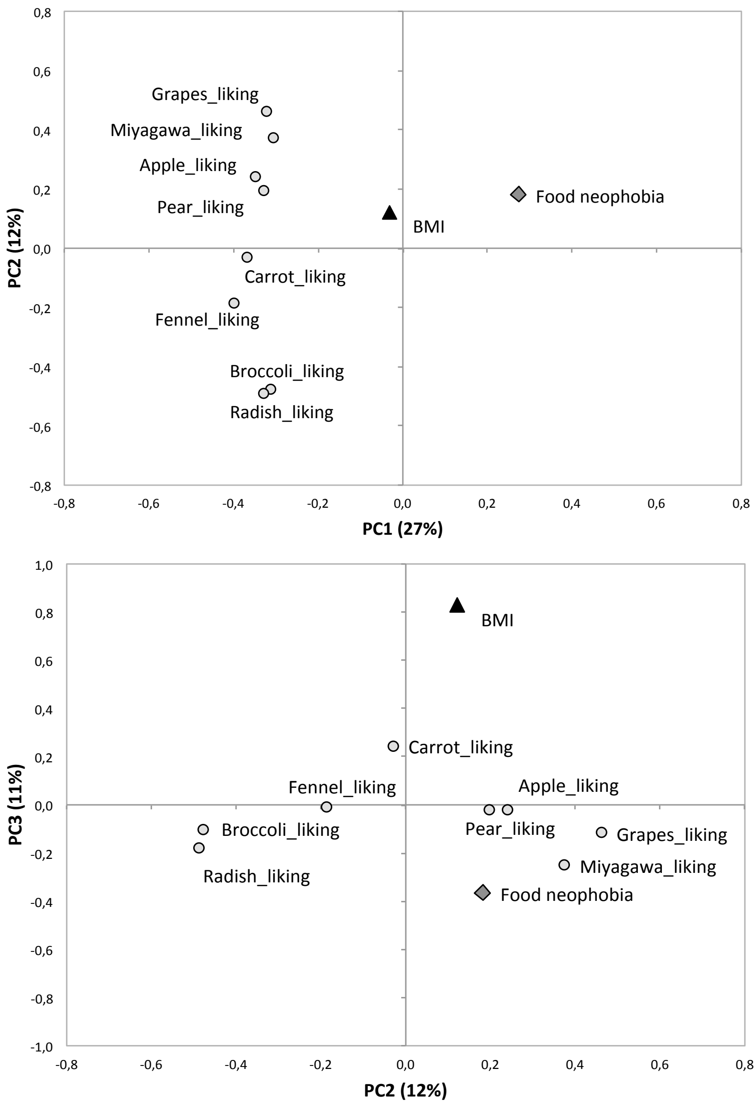
2.3.4 Relation among nutritional status, food neophobia and food liking

The BMI, food neophobia and liking data were subjected to PCA to examine the results from a multidimensional point of view (**Figures 2.2 a-b**).

Figure 2.2 (a-b). PCA loadings plots (PC1 vs PC2, fig. 1a; PC2 vs PC3, fig. 1b) showing the relationship between food liking, food neophobia and BMI measurements.

a)

b)



The first two PCs explained 39% of the variance (**Figure 2.2a**). The BMI was unrelated to either the food neophobia or the liking values. Food neophobia was positioned in the positive part of PC1 (23% of the explained variance) and was negatively correlated with all of the liking values, which were in turn positioned in the negative part of PC1. The negative correlation between food neophobia and the liking for FV was confirmed by the data shown in **Table 2.7**.

Variables	BMI	FN	Pear liking	Apple liking	Miyagawa liking	Grapes liking	Broccoli liking	Carrot liking	Fennel liking	Radish liking
BMI	1	-0.05	0.05	0.03	-0.01	0.00	0.00	0.05	-0.02	-0.01
FN	-0.05	1	-0.15 *	-0.16 *	-0.11	-0.11	-0.21 **	-0.28 **	-0.20 **	-0.12
Pear liking	0.05	-0.15 *	1	0.22 **	0.23 **	0.25 **	0.18 **	0.17 *	0.25 **	0.17
Apple liking	0.03	-0.16 *	0.22 **	1	0.28 **	0.25 **	0.24 **	0.23 **	0.21 **	0.11
Miyagawa liking	-0.01	-0.11	0.23 **	0.28 **	1	0.30 **	0.13	0.16 *	0.19 **	0.13
Grapes liking	0.00	-0.11	0.25 **	0.25 **	0.30 **	1	0.11	0.27 **	0.19 **	0.14
Broccoli liking	0.00	-0.21 **	0.18 **	0.24 **	0.13	0.11	1	0.18 **	0.22 **	0.40
Carrot liking	0.05	-0.28 **	0.17 *	0.23 **	0.16 *	0.27 **	0.18 **	1	0.41 **	0.15
Fennel liking	-0.02	-0.20 **	0.25 **	0.21 **	0.19 **	0.19 **	0.22 **	0.41 **	1	0.34
Radish liking	-0.01	-0.12	0.17 *	0.11	0.13	0.14 *	0.40 **	0.15 *	0.34 **	1

Table 2.7 Pearson's correlation coefficients for BMI, food neophobia (FN) and liking of FV (* significant for $p<0.05$, ** significant for $p<0.01$).

The correlation coefficients were somewhat low (range: -0.11 to -0.28) but were significant ($p<0.05$), considering the large number of individuals ($n > 200$). Food neophobia also showed positive coordinates in PC2. The relationship between food neophobia and the liking values could be further interpreted from examining **Figure 2.2b**. For this figure, PC2 was plotted against PC3, which explained a further 11% of the variance. As seen, the liking values were distributed along PC2, with the vegetables liking values in the negative part of PC2 and the fruits liking values in the positive part. The distribution of the liking values along PC2 perfectly reflected the direction of the children's preference evidenced by the ANOVA results, with broccoli and radish being the least liked items, followed by fennel and carrots and then by all of the fruits. Food neophobia was positioned near to the fruits' liking values, but it was still opposite to vegetables' liking scores. Thus, even if a negative correlation between food neophobia and the liking scores was evident overall (**Table 2.7**), **Figure 2.2b** clearly indicated that food neophobia was related to liking; more specifically, food neophobic children tended to give higher liking scores to fruits compared with vegetables; therefore, the liking for vegetables seemed to be the main indicator of the children's food neophobia. The BMI variable showed high loading on PC3 and low loading on PC2 thus confirming that this variable is unrelated to the food neophobia and liking scores.

This result is consistent with the findings of Hill et al. (2009), who did not find any relationship between adiposity and the reported liking for a series of foods, among which were FV. Conversely, Knaapila et al. (2011) found a moderate but significant positive correlation between the BMI and food neophobia in women aged 20-25 years. In their study, Knaapila and colleagues speculated that the link between food neophobia and nutritional status might be bidirectional. Therefore, neophobia might manifest in a diet with a limited variety of foodstuffs, thus reducing the frequency of using foods overall, and in turn, the energy intake; in contrast, food neophobics could prefer to consume traditional foods with a higher energy density compared with healthier food such as FV, resulting in a higher BMI.

To further interpret the relationship between liking of FV and food neophobia, the children were divided according to their level of neophobia into 3 groups: 'low', 'medium' and 'highly' neophobic children. Differences were for the neophobia level among stimulus category ($F=3.81$, $p<0.05$).

Table 2.8 Liking measurements of FV according to children's food neophobia level (*** significant for p<0.001, n.s. not significant)

Variables	Neophobia level		
	Low	Medium	High
Fruits	4.5	4.4	4.3
Vegetables	4.3	3.7	3.3

As shown in **Table 2.8**, vegetables' liking scores significantly ($p<0.0001$) decreased with increasing neophobia, whereas fruits' liking scores remained stable whatever the level of the children's neophobia. In other words, vegetables liking contributed more in discriminating children according to their neophobia level than fruits liking. This result confirms PCA results and suggests that vegetables' liking is a better indicator of children's food neophobia than is fruits' liking.

Our results confirm previous findings that high food neophobia is associated with low food liking (Russell & Worsley, 2008) and low consumption of FV (Galloway et al., 2003; Cooke et al., 2003, 2006; Wardle et al., 2005). This association appears to exist in adults, as reported by Knaapila et al. (2011), who found that in young adults, high levels of food neophobia are associated with low pleasantness of food in general and the reduced use of vegetables. It remains unclear why food neophobia is particularly high for certain categories of food. It appears that children food neophobia is highly related FV as well as to fish and meat but not to starchy, sweet or fatty snack foods (Cooke et al., 2003). Some authors suggested that this behaviour may be due to personality traits (Dovey et al., 2008), whereas others reported perceptive (Coulthard & Blissett, 2009) and even genetic reasons (Knaapila et al., 2011). The fact that vegetables are less liked than fruits is well known and has been confirmed by previous reports indicating that vegetables are among the least favored food of children (Skinner et al., 2002; Perez-Rodrigo et al., 2003; Cooke & Wardle, 2005). This pattern of preferences is consistent with the evidence for innate tendencies to prefer sweet tastes and to dislike bitter tastes (Birch, 1999). Indeed, most fruits are sweet, whereas vegetables are often perceived as bitter due to compounds that are specifically found in cruciferous vegetables (*e.g.,* broccoli, cauliflower and kale) Recent evidence has shown that this behaviour, which is particularly prevalent among children, may be explained in part by genetic factors. Polymorphism in the TAS2R38 gene may lead to a variation in the perception of the bitterness of 6-n-propylthiouracil (PROP), which can in turn influence dietary patterns. Indeed, it has been reported that PROP supertaster children are less likely than the nontasters to have tried/tasted cruciferous vegetables (Feeney et al., 2014).

2.4 CONCLUSION

In the first experiment we explored the relationship between taste sensitivity and nutritional status, in normal weigh, overweigh and obese adults. The role of taste thresholds in physiophatoly of overweight and obesity deseases need to be clarified.

In the second experiment conducted on children, we investigated two important determinants of children's nutritional status, food neophobia and their liking of FV. Most of the hypotheses we formulated were confirmed, as we found a negative association between food liking and food neophobia, with vegetables being the best predictor of children's food neophobia. Additionally, our data confirmed previously reported findings that food neophobia is more pronounced in boys than in girls and decreases with the children's age. Finally, we did not find any association between the BMI and either food neophobia or food liking. To our knowledge, very little information exists about the associations among food neophobia, food liking and nutritional status in Italian children.

The present study had a number of strengths. First, it was an ecological study conducted in an actual mealtime situation. Secondly, the relatively large sample of children makes us confident that the lack of association between the BMI and food neophobia or food liking is not a consequence of an inadequate power. However, this study was cross-sectional. Therefore, we cannot explore the onset and causality of these associations. Longitudinal studies are needed to examine the course of food neophobia and its possible effect on nutritional status.

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3. INCREASING FRUITS AND VEGETABLES CONSUMPTION IN CHILDREN

How is it possible?

3.1 INTRODUCTION

3.1.1 Fruits and vegetables consumption in children

It is well known that regular consumption of fruits and vegetables (FV), as already mentioned in the previous chapters, is associated with health benefits (Antova et al., 2003; Kraak, Story, & Swinburn, 2013). Also, emerging evidence suggests that increasing FV consumption is one of the factors, which may assist dietary weight management strategies to prevent obesity (Ledoux, Hingle, & Baranowski, 2010). Nevertheless, children and adolescents in many countries, including Italy and Denmark, eat below the recommended daily amount of FV. A European study found consistency in daily intake of fruits across the European population, but the picture was not as consistent for vegetables intake (Yngve et al. 2005). As mentioned, the recommended Danish daily intake of FV for children and adolescents over 10 years is 300g of vegetables and 300g of fruits excluding potatoes (Yngve et al. 2005). Fruits consumption in Denmark is higher than that of vegetables and girls consume more compared to boys (Pedersen et al. 2010; Yngve et al. 2005). There is a long list of publications showing that girls tend to consume and like FV more compared to boys and that the gender effects are larger as children move from mid to late adolescence (Brug et al. 2008; Cooke et al. 2005; Reynolds et al. 1999). Brug et al. (2008) argue that one reason why girls consume more FV may be due to the fact that they have stronger taste preferences towards this food category compared to boys. Furthermore, the energy requirements of boys of all ages are larger than those of girls, therefore boys' preference for more energy dense food groups, rather than FV, may serve an adaptive purpose (Cooke et al. 2005; Wardle et al. 2004). Older children and adolescents are aware of the health benefits of FV (Hill et al. 1998). An additional reason could be that girls pay more attention to dietetics compared to boys (Cooke et al. 2005; Nu et al. 2007).

While some food preferences are genetically predisposed, many are acquired through childhood and adolescence and are primarily determined by cultural factors (Rozin et al. 1986). Numerous studies, including longitudinal ones, have found that eating behaviour and food preferences formed in early childhood can persist into later childhood and even into the start of adult life (Nicklaus et al. 2004; Skinner et al. 2002; Skinner et al. 2011). This makes food preferences of children and adolescents even more important to study. Though it should be noted that preferences for different food groups, such as for cheese and vegetables, do not necessarily persist to the same extent. Nicklaus et al., (2004) found that preferences for vegetables were found to increase with age. The study also showed that different preferences remained depending on whether the subject segments was males or female.

Moreover, recent studies on children's food preferences showed that vegetables are still the least liked food category, particularly among school-aged children and adolescents. (Lanfer et al., 2011). Considering that the pattern of FV consumption persists into adulthood, it is important to establish healthy habits from childhood.

Increasing FV consumption has been reported as a global public health nutrition priority. However, minimal progress has been made in developing effective means to ensure an adequate intake of these foods because FV continue to be among the most disliked foods by children (Chapman & Armitage, 2012; Skinner, Carruth, Bounds, Ziegler, & Reidy, 2002).

3.1.2 Modelling, rewards and exposure in increasing fruits and vegetables consumption

Over the past 30 years, research on children's food habits has identified several variables that can influence their liking and consumption of different foods. According to the social learning account of Bandura (1977), modelling by significant others can be highly influential in establishing food behaviour changes. Models that have been shown to be effective with children include cartoon characters, peers, mothers, unfamiliar adults and teachers. In contexts other than food consumption, research has also shown that children are more likely to imitate a model whose behaviour they see is being rewarded, who is of the same age or slightly older than themselves or who they like or admire. Children are also more likely to imitate the behaviour of multiple rather than single models (Lowe, Horne, Tapper, Bowdery, & Egerton, 2004). Another influential variable for modifying food habits is to induce prolonged exposure to a stimulus. According to Zajonc's "mere exposure" theory (Zajonc, 1968), repeated exposure to a specific food decreases food neophobia and increases the liking and consumption of that food (Cooke, Chambers, Añes, & Wardle, 2011; Wardle, Herrera, Cooke, & Gibson, 2003b). The mechanism by which repeated exposure reduce neophobia is thought to be a "learned safety" behaviour (Kalat & Rozin, 1973). This hypothesis proposes that repeated ingestion of an unfamiliar food without negative consequences leads to increased acceptance of that food.

For several years, researchers have been focusing on establishing psycho-educational programs aimed at improving eating habits and lifestyles in children. For example, recent studies reported a positive influence of sensory education on French and Finnish children's food-related behaviour (Mustonen et al., 2009; Mustonen & Tuorila, 2010; Reverdy, Chesnel, Schlich, Köster, & Lange, 2008; Reverdy, Schlich, Köster, Ginon, & Lange, 2010).

The program used in the first experiment, the 'Food Dudes' program, is based on the previously mentioned core principles derived from the literature on the determinants of children's food preference, namely modelling, reward and repeated exposure, which encourage children to taste FV. The 'Food Dudes' program has been applied in countries such as Ireland, the United Kingdom and the United States (Horne et al., 2009; Lowe et al., 2004; Wengreen et al., 2013) with encouraging findings. The results showed a large and lasting increase in children's FV consumption, which can be generalized to the home setting. This intervention has never been tested in Italy. Therefore, in view of the differences in food habits between the Italian population and British and American people, it might be interesting to apply this program to children with a different food cultural heritage.

This research project consisted of the application of the 'Food Dudes' intervention in a large cohort of Italian children and the measurement of the impact of such an intervention in reducing food neophobia and increasing liking for FV.

3.1.3 Effect of variety in increasing fruits and vegetables consumption

Children population surveys indicate the need to increase the intake of FV (Fox et al., 2010). Indeed, in most countries daily consumption of FV is well below the recommendations of five portions a day (Lorson, et al., 2009).

A way to stimulate the consumption of FV could be to enhance variety through provision of many different types of foods (Temple et al., 2008). Variety within a meal is know to increase the intake, with a larger amounts of food consumed in meals characterize by high variety. Roll et al., (Rolls et al., 1982), have shown that during a meal the pleasantness of food which have been eaten declines more than the pleasantness of food which as not been eaten. They explained this phenomenon as probably caused in part by a sensory or cognitive factors, since these changes in pleasantness are apparent immediately after the end of a meal, before there as been time for much absorption. Because these changes are depend on the sensory properties of each food are termed the phenomenon "sensory-specific satiety" (Rolls et al., 1981). Given such relatively specific decrease in the pleasantness of a food eaten, if after a first food is eaten a second is offered, further eating occurs, so that total food intake with a variety of foods is greater than with a single type of food. Is also showed that increasing variety of foods tends to increase the consumption when the foods are presented both simultaneously and, sequentially, in different courses (Norton et al., 2006).

One of the first experiments conducted on food variety in children aged 8-12 years old, suggested that it influenced intake of low energy density foods as well as high energy density alternatives (Epstein et al., 2009). Temple et al., (2008) investigated the effects of a varied diet in children, and showed that offering a high variety of foods could increase energy intake, but the response was not related to energy density. This result means that, also for low energy density foods, increasing the variety of foods offered may increase their consumption. Thus, variety may be used as an alternative approach for promoting intake of particular healthy products, such as FV.

Variety has rarely been used to encourage children's vegetables consumption. Roe et al., (2013) tested several familiar FV as snack with pre-school children, and successfully found that providing a variety of foods increased the likelihood that children would select some of them as well as the amount they actually chose and ate.

Most of the previously mentioned studies considered food variety as the variation of number of products but relatively little is known about what food variety actually means for children. It is likely that varying sensory properties such as taste, texture, size, shape, and colour will influence perceived variety; as such attributes have previously been demonstrated to affect SSS (Rolls et al., 1982). Regarding vegetables, for instance, shape is known to be influential for liking of vegetables in children aged 9-12 years, they prefer to have their vegetables cut and presented in more complex serving styles. Moreover in their study Olsen et al. (2012) demonstrated that size did not matter when vegetables were cut, but when served whole or as a chunks the ordinary size was preferred than baby-size.

Even if it's well know that serving a wide variety of food increase the intake, this strategy has not been largely explored in children with the aim to improve consumption of healthy products. Moreover, the second experiment of this chapter has the advantage to compare two different types of food variety. The main purpose of the study was to investigate whether and how three levels of two different variety sets influence acceptance and intake of snack servings in school children. Eating habits and frequency of consumption of FV were also investigated, through a questionnaire completed by the children's parents.

EXPERIMENT A

3.2.a MATERIALS AND METHODS

3.2.1 Participants
An informative questionnaire and a consent form were distributed to 620 children's parents. A total of 591 children (about 90%), who returned the consent form completed by one of the parents, were considered for the study. Thirty-one children were excluded because suffered from food allergies, followed a specific diet or temporarily assumed drugs that may influence taste and smell perception. A total of 560 children (278 girls and 282 boys) aged 6 to 9 years were finally enrolled in the study.
Of the three schools, of the metropolitan area of Milan, that agreed to participate, one school was selected to be the experimental group and the other two schools served as the control group. The choice of using separate schools for the experimental and control groups derived from the need of avoiding children from the two groups meeting and exchanging information about the intervention; however the schools shared the same refectory and had the same class schedule.
Thirty classes were enrolled: six 1st graders (four for the experimental group), nine 2nd graders (four for the experimental group), eight 3rd graders (four for the experimental group), and seven 4th graders (three for the experimental group). Children from the experimental (N = 374) and control (N = 186) groups were matched for gender ($X^2 = 0.67$; $p = 0.41$), age ($X^2 = 3.66$; $p = 0.30$) and BMI ($X^2 = 0.54$; $p = 0.55$).

3.2.2 Stimuli
Both the experimental and the control groups received four different pairs or FV: 1) apple and fennel; 2) pear and radish; 3) grapes and broccoli; 4) miyagawa and carrot. Products were selected based on availability in season, ease of handling and storage. In addition, stimuli were chosen in order to have FV that were familiar to Italian children. A portion of approximately 40 g each FV was provided daily in the 16-day intervention phase, during the mid-morning break at school. Stimuli, served fresh and raw were cut into standardized pieces of uniform size. At first presentation of each food stimulus, children were also asked to indicate whether they had already tasted it. All items were familiar for more than 93% of children, except for radish, which was known only by 60% of them.

3.2.3 Food neophobia and liking evaluation
Food neophobia was calculated through the ICFNS (Laureati et al., 2015). Liking was measured using a 7-point hedonic facial scale (Pagliarini et al., 2003). Food liking and neophobia evaluations were performed in the classrooms in the presence of a teacher and an experimenter.
Before each test, the children received a brief explanation about the use of the scales and how to complete the booklet. The administration method was simplified for 6-year-old children for whom questionnaires were administered in small groups of five to six children and questions were read aloud by the experimenter.

3.2.4 Experimental design
The experiment consisted in four phases (**Figure 3.1**).

Phases	PRE-INTERVENTION (baseline)			INTERVENTION			after 6-months	FOLLOW UP	
Days	1	2 3 4 5	6 7 8 9	1 2 3 4 5 6 7 8 9 10 11 12	13 14 15 16	17		1	2 3 4 5
Tests	Food Neophobia evaluation t0	Liking evaluation (4 pairs of F&V) t0'	Liking evaluation (4 pairs of F&V) t0''		Liking evaluation (4 pairs of F&V) t1	Food Neophobia evaluation t1		Food Neophobia evaluation t2	Liking evaluation (4 pairs of F&V) t2
		F&V distribution		F&V distribution + videos, letters and rewards					F&V distribution

Pre-intervention phase (baseline) (9 days)

Food neophobia was measured on the first day, FV were served on the subsequent 8 days. Liking of stimuli was evaluated twice to investigate possible boredom effects due to mere exposure.

Intervention phase (16 days)

Children received each FV pair four times. Experimental group was subjected to the 'Food Dudes' program, whereas the control group was only exposed to the stimuli. The 'Food Dudes' intervention included three principles: taste exposure (FV distribution), modelling (videos and letters) and rewards (gadgets).

• Videos: The peer modelling videos included six 6-min episodes featuring the 'Food Dudes' heroes, a group of teenagers (two boys and two girls), that eat (and are observed to enjoy) a variety of FV to battle the enemies. By doing this, they encourage all other children to do the same.

• Letters: the teacher read everyday aloud a letter addressed to the children from the 'Food Dudes'. The purpose of these letters was to remind the children of the target foods of the day, give general feedback on their consumption on the previous day and promise rewards for all children who ate their FV at the next snack time.

• Rewards: The rewards consisted of stickers, pens, pencil cases, rulers, erasers and certificates. These items have been shown to have a wide appeal for primary school children (Lowe et al., 2004). A reward was given, for the 16-day of intervention phase, only to children who were willing to taste a piece of both the FV of the day. Food Dudes containers were provided to encourage parents to supply children with FV in their lunchboxes now that these foods were no longer provided in school. As maintenance progressed, the rewards were gradually withdrawn and replaced with certificates for children who brought FV from home.

To verify the effectiveness of the program, during the last 4 days of the intervention phase, liking for each FV pairs was evaluated in both the experimental and control group. In addition, on the day after the end of the FV serving period, food neophobia was measured.

Follow-up phase (5 days)

Six months after the end of the intervention phase, children of both the experimental and the control groups, were exposed to the same four FV pairs. At this stage, liking and food neophobia were measured again to verify the effectiveness of the program over the long term.

3.2.5 Data analysis

The data were first analyzed at baseline to evaluate children's food neophobia and liking before the application of the program. Analysis of variance (ANOVA) was performed considering *Age*, *Gender* and their interaction as factors and food neophobia and liking scores as dependent variables. Because no differences were detected in food neophobia or liking scores between the three schools, this variable *School* was not further considered for data analysis.To evaluate the effectiveness of the program in reducing food neophobia and increasing liking, the data were analyzed through repeated measures GLM ANOVA considering *Time* (pre-intervention, intervention and follow-up) as a within-subject factor and *Group* (experimental, control), *Gender*, *Age* (6–9 years) and *Product* (FV) as between-subject factors.

3.3.a RESULTS AND DISCUSSION

3.3.1 Food neophobia evaluation

The neophobia scores obtained at baseline (pre-intervention, t0), intervention phase (t1) and follow-up (t2) for the experimental and control groups are shown in **Figure 3.2**.

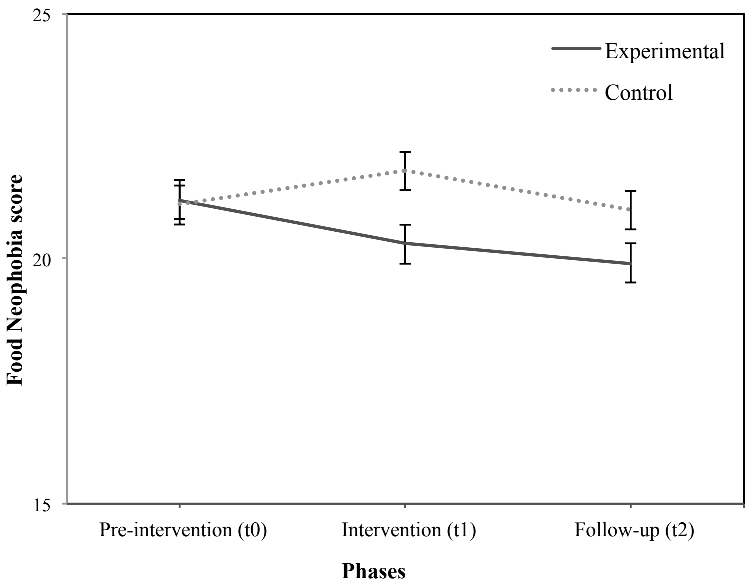

Figure 3.2 Food neophobia score (range8–40) ± SEM for experimental and control groups, at pre-intervention, intervention phase and follow-up (Laureati et al., 2014).

Differences were found in food neophobia scores among the three phases ($F = 4.54$, $p < 0.01$). Before the application of the program (pre-intervention, t0), the mean food neophobia scores for the experimental and control groups were comparable, indicating that children were initially homogeneous in terms of neophobic behaviour. After 16 days, the end of the intervention for the experimental group and the end of the repeated administration of FV for the control group, the scores differed significantly: the experimental group showed significantly lower ratings than the control group ($p < 0.01$). At follow-up, the difference between the two groups was still significant ($p < 0.01$). If we consider the scores over time within each group of children, food neophobia remained stable over time for the control group, whereas a systematic, significant decrease was observed for the experimental group. In particular, for the experimental group, the scores at intervention and follow-up were significantly lower ($p < 0.05$) than those at baseline. This results indicate that the intervention is effective in reducing food neophobia and, most importantly, that this effect is also observed over the long term (6 months).

A significant effect on age on the neophobia scores over time was found ($p < 0.05$). In particular, in the experimental group, scores gradually decreased over time for children aged 6–8 years, whereas there was a significant increase in food neophobia scores at 9 years. This result suggests that young children appear to benefit slightly more from the intervention than older children.
This results represent a very interesting finding. Similar results were reported by Mustonen and Tuorila (2010) and Reverdy et al. (2008), who found that children older than 9.5 years were less susceptible to neophobia reduction than younger children after exposure to a sensory education program. Accordingly, Loewen and Pliner (1999) observed that the evolution of neophobia after exposure to food stimuli was different depending on whether children were older or younger than 9 years old, most likely because children around this age develop a different neophobic reaction due to different optimal levels of arousal. Therefore, the age of 9 years appears to be a critical period in a child's life with respect to food behaviour development regardless of his/her country of origin, as similar patterns can be found in Italian, French, Finnish and Canadian children.

3.3.2 Liking evaluation

Liking scores averaged by type of FV at the two pre-intervention phases (t0', t0''), the intervention phase (t1) and follow-up (t2) for the experimental and control groups are shown in **Figure 3.3**.

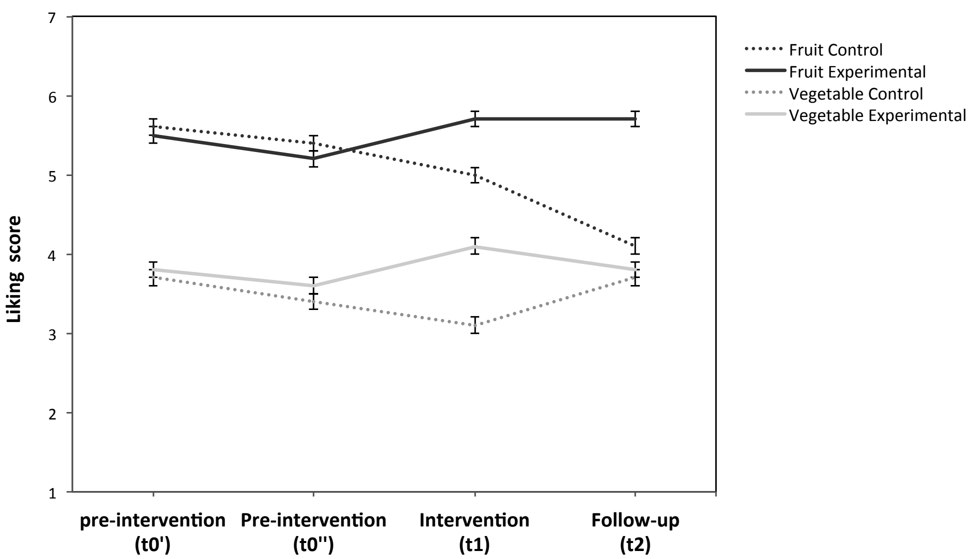

Figure 3.3 Liking score (range1–7) ± SEM for FV, for experimental and control group, at pre-intervention (t0', t0''), intervention phase (t1) and follow-up (t2) (Laureati et al., 2014).

Differences were show in liking scores of the two product categories among the four phases ($F = 52.95$, $p < 0.0001$). At baseline (t0' and t0''), the experimental (red and green solid lines) and control (red and green dotted lines) groups were comparable in terms of liking for both FV. After the intervention (t1), hedonic scores were significantly higher for the experimental group versus the control group for both fruits ($p < 0.0001$) and vegetables ($p < 0.0001$).

These results demonstrate the effectiveness of the program in increasing children's liking in the short term. At follow-up (t2), the liking scores of the experimental group were still higher than those of the control group but only for fruits ($p < 0.0001$).
Observing control group trend over time, hedonic scores for the control group decreased systematically, suggesting that taste exposure alone had little impact in increasing liking. This finding appeared to be confirmed by the fact that hedonic scores for both FV and for both groups of children (control vs experimental) decreased significantly over the two liking evaluations at pre-intervention (t0' and t0''). However, for the control group an increase of vegetables liking was seen at follow-up. This was mainly due to an increase of liking for the two most disliked items, namely broccoli and radish (**Table 3.1**).

Table 3.1. Liking scores (range 1–7, SEM = 0.1 for all values) for each food item provided to both the experimental and control groups at pre-intervention (t0', t0''), intervention phase (t1) and follow-up (t2).

Product	Groups	Apple	Miyagawa	Broccoli	Carrot	Pear	Grapes	Fennel	Radish
t0'	Experim.	6,0	5,0	2,5	5,7	5,4	5,9	4,4	2,6
	Control	6,0	5,0	2,4	5,2	5,7	5,8	4,1	2,9
t0''	Experim.	5,8	4,2	2,2	5,5	5,3	5,5	3,8	2,5
	Control	5,6	4,4	2,0	5,4	5,6	5,6	3,9	2,5
t1	Experim.	6,1	5,4	2,8	5,8	5,5	5,6	4,7	2,9
	Control	5,4	3,9	1,9	4,3	5,4	5,5	3,7	2,3
t2	Experim.	5,9	5,8	3,3	5,4	5,3	5,8	3,9	2,5
	Control	4,2	3,8	3,7	4,5	4,0	3,9	3,7	3,7

These reduction of liking scores during the two measurements at baseline (t0' and t0'') and by the systematic decrease of liking over time in the control group supported the hypothesis that the combination of several approaches appears to be more effective in motivating children to try new foods and appreciate FV. These results are likely to be ascribed to boredom effects that arise due to exposure alone. Indeed, it has been reported that repeated tasting may induce an increased feeling of boredom when participants are exposed to the same stimuli over a short period and that the monotony may lead to a temporary decrease in the consumer's acceptance for the food (Olsen et al., 2012). Also, the fact that liking of vegetables for the control group increased at follow-up and reached initial (baseline) values suggests that exposure has less effect in increasing liking when a food is initially well accepted (all fruits and carrot and fennel), whereas it might be more successful with very disliked items (all vegetables, especially broccoli and radish). Initial liking and familiarity of the stimulus are, indeed, strong determinants of repeated exposure effectiveness (Sulmont-Rossé et al., 2008).
The outcome of a higher liking degree for fruits than vegetables observed in the present study is well known and confirmed by previous reports indicating that vegetables are among the least favored food among children (Cooke & Wardle, 2005; Perez-Rodrigo et al., 2003; Skinner et al., 2002). This pattern of preferences is consistent with the evidence for innate tendencies to prefer sweet tastes and to dislike bitter tastes (Birch, 1999). Indeed, most fruits are sweet, whereas vegetables are often perceived as bitter due to specific compounds (e.g., glucosinolates) that are found in cruciferous vegetables (e.g., broccoli, cauliflower and kale) (Forestell & Mennella, 2007).
For the experimental group over the time, liking scores increased significantly ($p < 0.0001$) after the intervention for both stimuli. Liking remained stable after 6 months for fruits but decreased significantly for vegetables ($p < 0.0001$).

In particular, we observed that liking scores of the experimental group after the intervention and at follow up were higher than those of the control group only for younger children (6–8 years) ($F = 4.70$, $p < 0.001$). Thus, as already verified for food neophobia, younger children appeared to benefit more from the intervention than older children.
Evidence from a meta-analysis study conducted on 21 school-based interventions showed that multi-component programs are more effective than single-component programs in increasing food acceptance among children (Evans et al., 2012). Most of the single-component interventions are based on repeated exposure, which has been shown to be effective in increasing liking and intake with infants, preschoolers and school children (Wardle et al., 2003a, 2003b). However, there is evidence that when exposure is associated to another reinforcement (e.g., reward), the intervention has a more durable effect (Cooke et al., 2011). Reverdy et al. (2008) used an approach consisting of sensory lessons provided at school to French children aged 8–10 years. They found that neophobia scores decreased as a function of education; however, the effect was only temporary. The same intervention was used by Mustonen and Tuorila (2010) in Finland with children aged 8–11 years. In this case, the program was extended to include further sensory lessons to deepen children's knowledge of food. With this improved version of the program, a stronger decrease was observed in food neophobia but only for younger children. Furthermore, this outcome is in agreement with the strong age effects we observed in our previous work, Laureati et al., 2015, for both food neophobia and liking. More specifically, we found that 9-year-old children are less neophobic than younger children, most likely because experience with food increases with age, and this makes older children more willing than younger children to taste new food. At the same time, the age of 9 years seems to be critical in relation to food appreciation, as 9-year-old children gave lower liking scores for FV than did younger children. This result is also in line with the findings of Pagliarini et al., (2005), who reported age-related differences in children's food preferences for several foods served at the school canteen, including FV. Accordingly, Cooke and Wardle (2005) reported that the number of liked foods decreases with increasing age. We hypothesize that this behaviour is due to the acquisition of a more critical attitude toward food with increasing age as a consequence of exposure to a more varied diet, although this apparently contradicts the finding of increased neophilia among older children in the present study. However, it is important to note that the increase in the willingness to try new foods that comes with increasing age does not necessarily mean that these foods are also more liked.

EXPERIMENT B

3.2.b MATERIALS AND METHODS

3.2.1 Participants

One-hundred-fifty-three children from a public school in Copenhagen (Denmark) were enrolled in the study. Participant characteristics are described in **Table 3.2**.

Table 3.2 Description of participants.

Children	Number or mean ± SEM
Gender distribution	69 boys, 63 girls
Age (years)	9.60 ± 0.05
BMI (kg/m^2)	17.2 ± 0.2
BMI z-score[1]	0.39 ± 0.08

[1]The WHO2007 data are used as reference population (De Onis et al., 2007).

A total of seven classes were involved: four 3rd graders (9-10 years) and three 4th graders (10-11 years). One 3rd class was involved in a pilot study (n=18), whereas the other children (n=135) took part in the main experiment. Teachers and parents were thoroughly informed about the study and parents gave written consent and completed a short questionnaire on food allergy or whether their child followed a specific diet prior to study start. None of the children involved in the present experiment followed specific diets. Children who suffered from allergies for pilot study products were not served these products in their servings. Children's participation in the study was voluntary and classes received mixed toys as a small reward for their participation.

3.2.2 Pilot experiment

A pilot experiment was carried out to select the stimuli for the main experimental study. Fifteen snack foods were tested for liking, familiarity, frequency of consumption, and preferred serving styles by one of the school classes. The presented FV were: pear, red apple, dried cranberry, almond, parsley root, beetroot, white cabbage, plum, green apple, red currant, hazelnut, parsnip, carrot, kohlrabi, and Brussels sprout. Products were selected on the basis of suitability as a snack food, being organic and Danish, as well as seasonality, availability from supplier and being reasonably easy to handle and portion.

Testing took place in the classroom. Children were instructed how the questions were phrased and how to use the scales. This was practiced a couple of times in plenum. For each food, children were asked about familiarity (before tasting the food): "Do you know this food?' on a 3-point scale: "No, I've never seen it before", "I've seen it, but not tasted", and "Yes, and I've tasted it". Then children tasted the food and rated liking: "How much do you like this food?", on a 7-point hedonic facial scale adopted from Chen et al., (1996) with the descriptors: "really bad", "very bad", "bad", "okay", "good", "very good", and "really good". Finally, children assessed their frequency of consumption: "How often do you eat this food?" and answered through a 5-point scale with the descriptors: "Never", " A few times a year", " A few times a month", "A few times a week", "Every day".

During tasting, children were offered a small piece/bunch (around 30 g) of each food one at a time in a mid-morning break.

In order to choose stimuli shapes for the main study, after the tasting session, children rated their liking of pictures of green apple and carrot presented in four different serving styles: small chunks, slices, sticks, and triangles. Children evaluated liking of each serving shape individually: “How much do you like this serving style?” and answered on the 7-point hedonic facial scale described above. Children were instructed not to influence their classmates with facial expression or comment about the food they tasted, and it was carefully emphasized that there were no right or wrong answers. Results from the pilot study are shown in **Tables 3.3** and **3.4**. Familiarity is reported as the percentage of children answering “I know this vegetables and I've tasted it” (score 3), while frequency of consumption was measured as the sum of the percentage of children answering “A few times a week” and “Every day” (scores 4 and 5).

Table 3.3 Familiarity degree (score 3), average liking scores (range 1-7, means ± SEM), and frequency of consumption degree (scores 4 and 5), of the pilot study products. Average liking scores with different letters are significantly different ($p<0.05$).

Products	Familiarity %	Liking score	Frequency %
Pear	83.3	$6.6^{a} \pm 0.3$	55.6
Red apple	94.4	$6.3^{a} \pm 0.4$	83.3
Cranberry	44.4	$5.1^{b} \pm 0.4$	22.2
Almond	83.3	$5.6^{b} \pm 0.4$	50.0
Parsley root	16.7	$3.6^{d} \pm 0.4$	0.0
Beetroot	22.2	$3.8^{d} \pm 0.4$	5.6
White cabbage	61.1	$5.2^{b} \pm 0.4$	27.8
Plum	94.4	$6.4^{a} \pm 0.3$	55.6
Green apple	88.9	$6.4^{a} \pm 0.2$	72.2
Red currant	50.0	$4.9^{bc} \pm 0.4$	0.0
Hazelnut	66.7	$4.6^{c} \pm 0.5$	11.1
Parsnip	22.2	$4.2^{c} \pm 0.4$	11.1
Carrot	94.4	$6.3^{a} \pm 0.2$	100.0
Kohlrabi	16.7	$4.1^{c} \pm 0.5$	5.6
Brussel sprout	44.4	$4.3^{c} \pm 0.5$	5.6

Table 3.4 Average liking scores (means ± SEM) of pictures of green apple and carrot presented in different shapes (small chunks, sticks, slices and triangles). Average liking scores with different letters are significantly different ($p<0.05$).

Products	Shape	Liking score
Green apple	chunks	$5.4^{b} \pm 0.4$
	slices	$5.8^{b} \pm 0.3$
	sticks	$5.9^{b} \pm 0.3$
	triangles	$6.6^{a} \pm 0.3$
Carrot	chunks	$4.2^{c} \pm 0.5$
	slices	$5.7^{b} \pm 0.3$
	sticks	$5.2^{b} \pm 0.4$
	triangles	$6.7^{a} \pm 0.2$

3.2.3 Main experiment

Stimuli

Selection of stimuli for the eating sessions was based on liking of the products rated in the pilot study, but also based on practical considerations. Practical issues included for instance ease of handle and storage. Several products were excluded due to browning during storage. Familiar and liked products were chosen to minimize the effect of rejection of novel or disliked foods.

The final products chosen and their serving shapes were: carrot chunks (a), sticks (b), slices (c); green apple chunks (d), slices (e), triangles (f); plum chunks (g); white cabbage quadrangular pieces (h); dried cranberry (i); almond (l). Two stimuli sets were served: 'Classical Variety' (CV), in which the number of different foods varied, and 'Perceived Variety' (PV), in which only serving styles shape of green apple and carrot were varied. For each set, three levels of variety were tested: low (2 stimuli), medium (4 stimuli), and high variety (6 stimuli). Stimuli were arranged in transparent plastic boxes giving 6 different serving styles. The total amount of each serving box was 200 g, thus the amount of each product changed on the basis of set and level of variety. Exact content of the 6 servings is described in **Table 3.5**. All snack foods were served raw, except for cranberries and almonds.

Table 3.5 Serving sets stimuli presentation, ('Classical Variety', CV and 'Perceived Variety', PV) and their variety levels (*l*ow, medium and high).

	'Classical Variety' (CV) (number of different food varies)	**'Perceived Variety' (PV) (serving style varies)**
Low (2 items)	green apple (slices) 100g carrot (sticks) 100g	green apple (chunks) 100g carrot (chunks) 100g
Medium (4 items)	green apple (slices) 50g carrot (sticks) 50g dried cranberry (whole) 50g white cabbage (pieces) 50g	green apple (chunks) 50g carrot (chunks) 50g green apple (slices) 50g carrot (sticks) 50g
High (6 items)	green apple (slices) 33g carrot (sticks) 33g dried cranberry (whole) 33g white cabbage (pieces) 33g plum (slices) 33g almond (whole) 33g	green apple (chunks) 33g carrot (chunks) 33g green apple (slices) 33g carrot (sticks) 33g green apple (triangles) 33g carrot (slices) 33g

Experimental study design

The study consisted of three separate phases (**Table 3.6**): Baseline measurements, eating sessions, overall rated and ranked liking measurements, anthropometrics measurements and parent's questionnaire.

Table 3.6 Overview of the main study experimental design.

Phase of the experiment	Duration	Measurement
Baseline measurements	2 days	Measurement of familiarity, liking and frequency of consumption of each selected snack vegetables included in the serving sets
Eating session: Intake measurements	3 weeks (2 sessions per week)	Presentation of the six serving sets to each class. Measurements of each serving set intake.
Overall rated and ranked liking measurements	1 days	Measurement of visual overall rated liking and overall ranked liking for the six serving sets served
Anthropometrics measurements	1 day	Measurement of children's height and weight
Parents' questionnaire	2 weeks	Delivery and collection of the parent's questionnaire to each child involved

- Baseline measurements: Baseline measurements were performed during the first two days, at midmorning at school. To increase ecological validity, children were tested in a familiar environment, their classroom (because Danish children usually eat in their classrooms). The number of children in the 6 classes ranged from 18 to 25. The experimenters were thoroughly trained in order to give all instructions in a standardized manner. Testing of familiarity, liking and frequency of consumption of each selected snack vegetables, followed the same procedure as described for the pilot study, with the main difference being that in the main study, the following foods were assessed: apple, carrot, almond, plum, cranberry, cabbage, cucumber and celery. These last two products were added as warm up samples and were chosen because they were known, from earlier experience (Olsen, et al., 2012), to be respectively among the most preferred and the most disliked vegetables for children.
- Intake measurements: In the eating session phase, 2 sessions were scheduled per week, with at least a 2 day break between visits, for a total duration of 3 weeks. Each of the six different servings was randomly presented to each class in a balanced manner. All children in a class always received the same serving at a given day. At the beginning of children's habitual midmorning break, a serving box was delivered to each child, and they were invited to eat as much or as little as they wished during the duration of the break (20 minutes). Parents had been instructed not to give their children any snack foods for this break during study days. To determine individual intake, each box was marked with the child's name and the exact amount of each food item in a serving was determined with a 1 g precision. Participants were instructed not to share food with each other, and if anything was spilled, it was putted back to the box when the child had finished eating. After serving, the boxes were taken back to the lab to establish exact intake of each food item. No second servings were allowed. To ensure compliance, experimenters and teacher were asked not to comment on the stimuli and not eat anything in classroom. A jug of water was also offered to the children during the eating session. Products were prepared few hours before serving, apples were dipped in water added citric acid to avoid browning, all the products served at room temperature
- Overall rated and ranked liking: After the end of the serving phase, in the following week, the overall assessment of the 6 serving sets took place. To allow direct comparisons between them, all six serving sets were presented simultaneously to the children in their plastic boxes. Children evaluated the servings individually outside the classroom and observing them they performed a visual overall liking test (7-point facial scale) and then a visual ranking test by elimination by asking them the following question: "Which of these servings do you prefer?". The box chosen

as the favorite one was removed and the question was repeated for the remaining servings until a full ranking was given.
- Anthropometric measurements: Children were weighted and measured for height in stocking feet and light clothing. Height and weight were converted to body mass index (BMI= kg/m2), and BMI z-scores were calculated using the WHO 2007 data as reference.
- Parent's questionnaire: Questionnaire included basic demographic questions about the parents (age, sex, education level, job, height, weight) and the child (height, weight, breast feeding, weaning). Children's FV consumption was assessed using the following questions: 'How much does your child like fruits/vegetables?' (answer ranged from 1, 'very bad', to 5, 'very good'); 'How often does your child eat fruits/vegetables?' (answer ranged from 1,'everyday', to 5, 'never'); 'How many portions of fruits/vegetables does your child eat per day?'; 'Are there usually vegetables available at your home?'; 'Are there usually vegetables, that your child likes, available at your home?' (answer ranged from 1, 'always', to 5, 'never'); 'Who prepare snack vegetables for your child?' (Answers: 'my child prepares', 'sometimes my child prepares', 'I prepare', 'my child doesn't eat vegetables for snack'). Child Eating Behaviour Questionnaire (CEBQ) sub-scales (Wardle et al., 2001) were also used to have insights on children's food habits. The following variables of the CEBQ were measured (score ranged from 1 to 5): Satiety Responsiveness, Fussiness, Food Responsiveness (FR), Enjoyment of food (EF), Desire to drink (DD), Emotional undereating (EU), Emotional overeating (EO). Parents also answered questions about child food neophobia traits through a reduced version of 6 items of Food Neophobia Scale (FNS) (score ranged from 6 to 30) (Pliner & Hobden, 1992).

3.2.4 Data analysis

GLM ANOVA was used to analyze intake and liking data. *Children* and *Class* were considered as random factors in the model, whereas *Set of variety*, (CV and PV), *Level of variety* (low, medium and high) and their interaction, as well as, *Gender, Age* and *BMI z-score* were considered as fixed factors. When the ANOVA showed a significant effect ($p<0.05$), post-hoc LSD test was applied. Pearson correlation coefficients were calculated to investigate the relationship between intake and liking data.
ANOVA was also used in order to investigate the effect of questionnaire variables (*FV consumption, Availability at home, Availability of liked vegetables at home, Availability of preparation scores, CEBQ variables, Food neophobia*) on intake and liking data.

3.3.b RESULTS AND DISCUSSION

3.3.1 Baseline measurements

Familiarity was calculated as the percentage of children answering the question "I know this vegetables and I've tasted it" (score 3), while frequency of consumption was measured as the sum of the percentage of children answering the question "A few times a week" and "Every day" (scores 4 and 5, respectively). Average familiarity, liking scores and frequency of consumption of each snack vegetables are presented in **Table 3.7**.

Table 3.7 Average familiarity (score 3), liking scores (range 1-7) ± SEM, and frequency of consumption (scores 4 and 5) of the study products (n=135). Average liking scores with different letters are significantly different (p<0.05).

Stimuli	Familiarity %	Liking score	Frequency %
Apple	98.3	$6.5^{a} \pm 0.1$	86.6
Carrot	97.5	$6.2^{ab} \pm 0.1$	89.1
Almond	94.8	$5.9^{b} \pm 0.1$	63.0
Plum	90.9	$5.9^{b} \pm 0.1$	52.1
Cranberry	64.0	$5.0^{c} \pm 0.1$	18.5
White cabbage	54.2	$4.3^{d} \pm 0.1$	11.8

Mean liking scores ranged from 6.5 to 4.3. All the stimuli were evaluated as familiar for more than 50% of the children, whereas the number of participants with a weekly/daily frequency of consumption largely varied (from 86.6 % to 11.8%). Familiarity data perfectly reflected liking scores. The most preferred products, apple and carrot, were familiar for more than 95% of participants; liking of almond and plum decreased gradually with the decreasing of their familiarity and frequency of consumption. Finally, cranberry and white cabbage were the least liked stimuli, although they received a somewhat high liking score; they were known by participants but the weekly/daily consumption was very low. Cranberry and white cabbage, which were included in the less consumed serving sets, were less liked compared to the other products, probably due to the fact that they were less familiar and for their sour and bitter taste, respectively. Apple, carrot and plum liking scores were high probably because they were perceived as sweet and familiar. Almond is difficult to place, but is definitely energy dense, which is known to be well liked (Birch, 1992).

3.2 Intake measurements

Intake data by set and level of variety obtained in the eating sessions are shown in **Figure 3.4**.

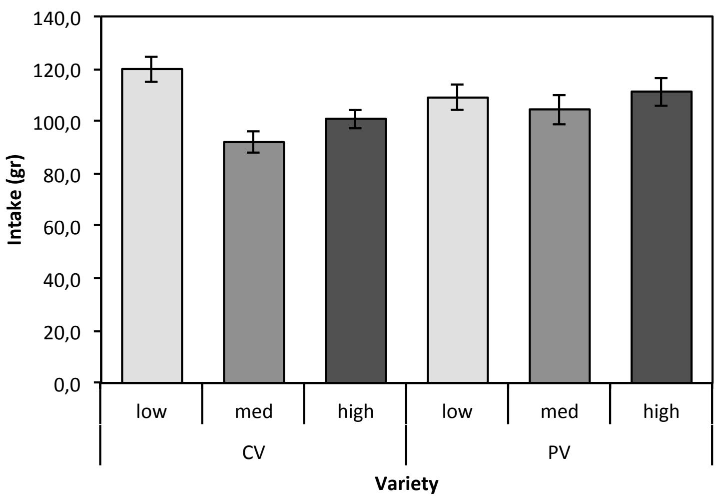

Figure 3.4 Mean intake values (± SEM) of Classical Variety (CV) and Perceived Variety (PV), and their variety levels (low, medium, high).

A significant effect (F=3.88; p<0.01) of the interaction between sets of variety and variety levels on intake was found. In the CV set, intake values were significantly different among the three levels, with a significantly higher consumption of the low level (containing apple and carrot), followed by high and medium levels. No differences in intake were found in the PV set among levels of variety. Furthermore, comparing the low level of the two sets of variety, CV and PV, which contained the same stimuli (i.e., apple and carrot) cut in different ways (apple slices and carrot sticks; apple chunks and carrot chunks), intake values did not differ.
Based on analysis of parents questionnaires (n=46), servings intake was influenced by the availability preparation (F=7.82, p<0.05). Half of the parents declared that sometimes their child prepares his/her own snacks vegetables, while the other half consisting in parent pairs that usually prepare the snacks vegetables for their child. In all the CV set levels (low, medium and high), the intake was higher for the children that are sometimes involved in their snack preparation than those that are not. On the contrary, for the PV levels an opposite trend was observed, showing a higher intake of servings for children that don't participate in snack preparation, except for high level.
The intake data according to the type of stimuli included within each serving set are illustrated in **Figure 3.5**.

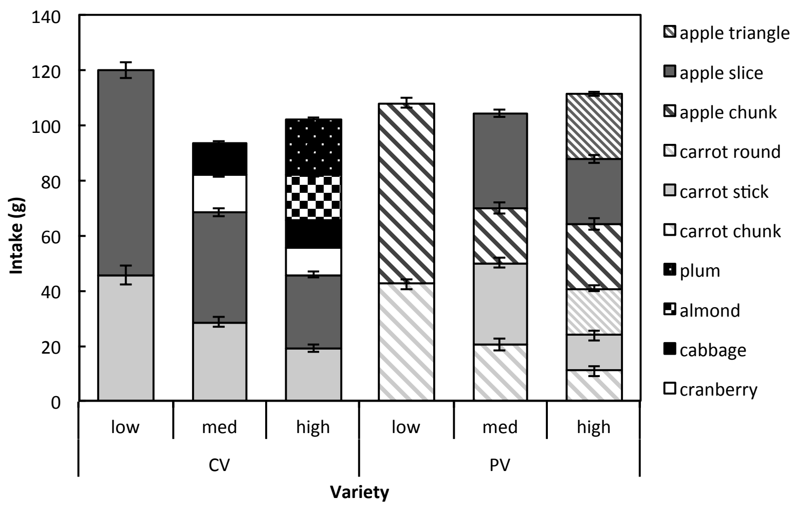

Figure 3.5 Intake of single snack item composed Classical Variety (CV) and Perceived Variety (PV).

Results showed that apple was the most consumed snack in all the servings (p<0.001). In the CV medium set (p<0.001) apple intake was followed by carrot stick, whereas cranberry and white cabbage were consumed in low amounts and did not differ from each other. In the high level of CV set, significant differences among the snacks intake were found (p<0.001). Carrot stick, plum, almond intake amounts did not differ and they were followed by cranberry and white cabbage. Comparing the PV medium and high levels, no differences among the intake of the snacks presented in different shapes (carrot chunk, stick and slices; apple chunk, slice and triangle) were found in both set levels.
Therefore, we can affirm that, adding less liked stimuli (i.e., white cabbage and cranberries) to high and medium serving sets probably produced a gradual decrease in intake values. This suggests that intake is more affected by acceptability and familiarity for the single stimulus included in the serving snack than by variety. Another hypothesis that might be forwarded to

explain low intake with higher variety levels is that the medium and high levels of the CV serving set were more satiating compared to the others due to the presence of dried cranberry and almond. Thus, the reduced consumption of these servings could be due to difficulties of eating the entire portion in the limited time period of the mid-morning break.

Moreover, observing the results of intake in grams, we can note the children consumed an average intake of approximately 100 grams. Information about daily consumption of FV during the intake measurement days were not obtained in the current study, and it is therefore not clear whether the offered mid-morning snack substituted the daily FV consumption or not. However, an average intake of about 100 grams of the stimuli sets is equal to half the amount that Danish children normally eat in one day (Yngve et al., 2005), and corresponds to about 20% of the recommended daily intake. In Danish children, about 4% of vegetables and 9-10% of fruits eaten in one day are consumed as a mid-morning snack, whereas the majority of vegetables are consumed at lunch and dinner, where fruits are mainly eaten as an afternoon snack (Pedersen et al., 2010). If 100 grams of FV are already eaten in the mid-morning, it should be manageable to consume the recommended amount of FV. Energy intake of each snack item composing serving sets was also calculated (**Figure 3.6**).

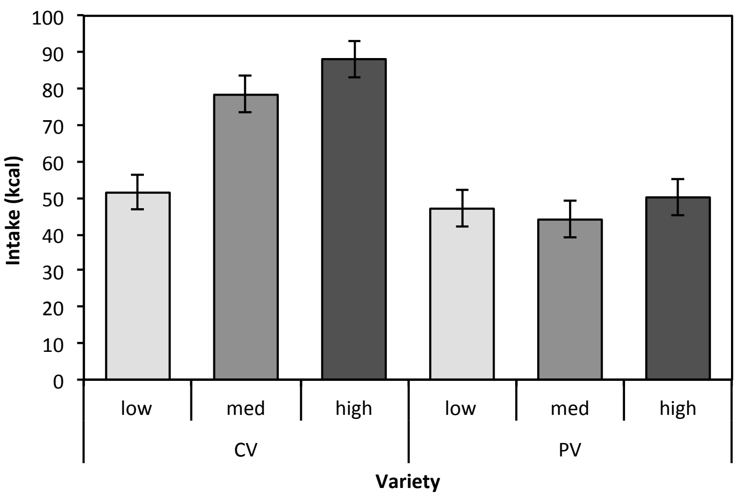

Figure 3.6 Energy intake (kcal) ± SEM of snack vegetables composing serving sets, Classical Variety (CV) and Perceived Variety (PV), and their variety levels, low, med, high.

A significant effect (F=18.2; p<0.01) of the interaction between sets of variety and variety levels on energy intake values was found. Considering the CV set, a higher amount of kilocalories was obtained in the medium and high levels compared to low level, while, no significant differences were found in the energy intake among the three levels of PV set. Moreover, no difference in the energy amount was obtained for CV low if compared to PV low (both containing equal amounts of apple and carrot), since they were composed of the same stimuli in equal amounts, confirming intake data. In addition, the absence of this difference, indicates that adding more foods to a serving to increase variety in a meal has greater effect on energy intake than adding different cuttings of the same foods. Previous research indicated that the more different the sensory properties (taste, smell, color, shape, texture) of the food are, the greater the effect of variety (Rolls, et al., 1981). For PV set the properties of the stimuli might not be different enough. Previous research has shown that increasing the number of colors of jelly beans in an organized serving led to greater consumption (Kahn & Wansink, 2004). Based

on this, changing the appearance of apple and carrot by adding more cuttings of them was thought to have similar effects. This was, however, not the case. The fact that adding more foods has greater effect on intake than adding more cuttings can be considered a positive finding from a practical point of view since parents do not have to cut foods into several different shapes to increase the child's consumption.
The effect of level of variety of the CV set on energy intake but not intake in grams might be related to the fact that the stimuli used to increase variety, added extra energy to these sets. Especially, the almonds and cranberries contributed with a great energy amount. If the stimuli had been more similar with regard to energy content, it would have been easier to state that the effect was clearly due to variety.

3.3 Overall rated and ranked liking measurements

Mean rated liking scores by serving set are reported in **Figure 3.7**. A significant effect (F= 5.9; p<0.01) of the interaction between serving sets and variety levels on overall rated liking score was found.

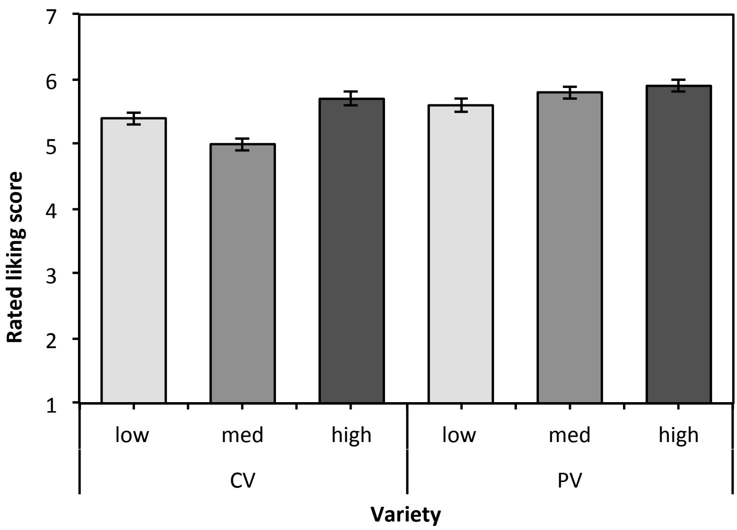

Figure 3.7 Mean rated liking scores (range 1-7) for Classical Variety (CV) and Perceived Variety (PV), and their variety levels (low, medium, high).

Even though not significant, an increase of liking according to levels of variety was seen for the PV set. Considering the CV set, the high level received significantly higher scores than the other levels, followed by the low and the medium level that was the least appreciated. The comparison between CV low and PV low levels showed no significant differences in the overall liking scores.
The signifinat lower rated liking of CV medium set may be explained by the low liking obtained at the baseline for white cabbage and cranberry, which were added to CV medium set to enhance variety. Results of PVs liking scores reflected the intake data. Thus, we can assume that stimuli shapes were not influent enough to make a change in children preference. A previous study has demonstrated that more advanced cuttings were preferred over simple cuttings (Olsen et al.,), which was also confirmed in the pilot study of the present work. The

results of Olsen et al. showed that liking of the stimuli sets affected the intake of the particular stimuli set. However, this doesn't seem to have large effects, since the energy intake of the CV set was higher than that of the PV set, although PV was more liked. It is possible that, in the present study, more advanced cuttings, e.g. stars or dinosaurs, would have led to higher intakes and liking.

Analysis of parents questionnaires showed differences in overall liking for child fussiness mean scores ($F= 3.57$, $p<0.05$). To further interpret the relationship between overall liking of serving sets and their levels and fussiness, the children were divided according to their level of fussiness into 3 groups: group 1 (children with scores in the lower 25th percentile of fussiness scores, score ≤ 1.8, n=12), group 2 (children with scores between the 25th and 75th percentiles, $1.9 \leq$ score ≤ 3.3, n=19) and group 3 (children with scores in the upper 25th percentile, score ≥ 3.4, n=15). Within CV set three levels (low, medium and high), liking scores significantly decreased with the increasing of child fussiness; whereas in PV levels the overall liking scores increased with the increasing of level of fussiness.

This results show that fussiness was also found to influence negatively children's liking. Fussiness is strictly related to food neophobia (Dovey, et al., 2008) and it describes the unwillingness to eat many familiar foods (Galloway et al., 2003). The negative relationship between children willingness to eat, liking and consumption of FV found in the present study is consistent with the results from several previous works (Koivisto et al., 1997, Laureati et al., 2014).

Overall liking was also measured asking children to rank the 6 serving styles according to their preference. Results are presented in **Figure 3.8**.

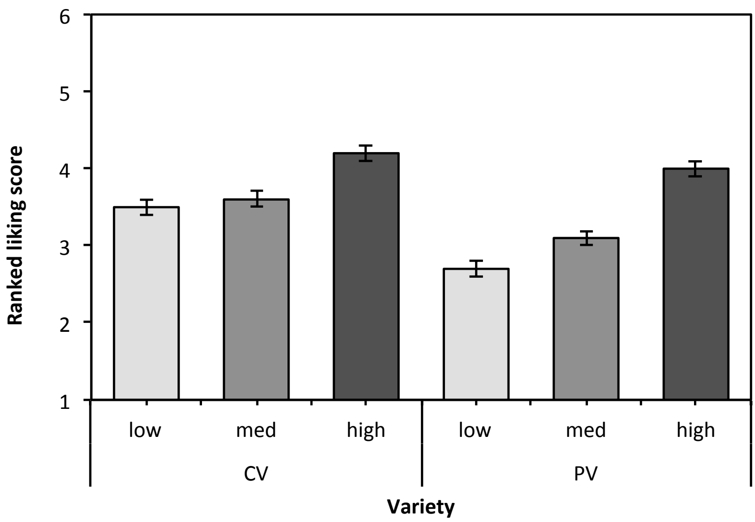

Figure 3.8 Ranked liking score for Classical variety (CV) and Perceived variety (PV), and their variety levels, low, med, high.

The high levels of variety was ranked significantly higher than low and medium levels ($p<0.001$) in both CV and PV sets. Comparing rated and ranked liking results, a similar trend was seen according to serving set. However, ranked liking provided a clearer hedonic discrimination than rated liking between variety levels in both serving sets.

This finding indicates the difficulties in measuring preference and how different methodologies can provide different results. Indeed, ranking is a forced procedure that requires individuals to judge foods against each other. Ranking has been used successfully in other study (Kildegaard et al., 2011) but it is possible that children found difficulties understanding instructions.
The correlation between intake in grams of each serving set and their rated and ranked liking scores was calculated (**Table 3.8**). With exception of low levels, significant relationships between snack vegetables intake and the corresponding rated liking were found. On the contrary, the intake was not correlated with ranked liking.

Table 3.8 Correlation coefficients and their p values between intake of serving sets (CV and PV) and levels (low, med, high) and corresponding rated and ranked liking scores.

Serving sets		**Rated liking**		**Ranked liking**	
		r	p value	r	p value
CV set	low	0.14	0.12	0.07	0.44
	med	0.32	<0.001	0.16	0.08
	high	0.29	<0.001	0.20	0.06
PV set	low	0.12	0.16	-0.02	0.84
	med	0.23	0.01	-0.17	0.06
	high	0.22	0.01	0.08	0.36

We can affirm that rated liking is a better measurement method than ranked liking to predict children consumption of the snack vegetables servings.

3.4 CONCLUSION

A number of studies have been published in the last decade concerning the effectiveness of school-based interventions in modifying food consumption in children; this is due to the increasing risk of obesity worldwide. It has been suggested that proper education at school and at home may decrease the consumption of junk food and increase the consumption of more healthy foods, such as FV (Reverdy et al., 2008).

In conclusion, our data suggest that the 'Food Dudes' school-based intervention can have positive effects on Italian children's food attitude, reducing food neophobia and increasing liking for both FV. With the exception of vegetables liking, these effects were maintained at 6 months after the intervention. It may be advisable to perform several iterations of the intervention to maintain a high level of liking for vegetables.

Finally, the 'Food Dudes' program has been applied with encouraging results in countries such as Ireland, UK and US, which have important culture-related differences as compared with Italy. The positive outcome of the present study seems to indicate that this multi-component intervention based on food exposure, peer-modeling and reward can be successfully applied to primary school children regardless of the cultural heritage and the specific dietary habit of a population.

The results from our study confirm previous findings indicating that a suitable age for the commencement of school-based programs could be 8 years or even earlier, as younger children appear to be more likely to change their food behaviour than older children. Early intervention is also likely to maximize health benefits because eating habits in childhood are strongly predictive of those in adulthood.

One of the strengths of the present paper is that it is an ecological study conducted in an actual mealtime situation. The naturalistic environment is an important point to consider when studying factors linked to food behaviour, especially with children. Moreover, the relatively large sample of children makes us confi- dent about the adequate power of the study design. One weakness of this study is that we involved 6-year-old children in our mea- surements and, despite that children of that age can perform hedonic test reliably (Guinard, 2001), some problems may arise in under- standing the food neophobia task. In this context, the administration procedure was slightly modified for 6-year-old children in order to make the task easier for them. Examples of administration methods adapted for younger children (e.g., questionnaires administered in an individual instead of collective setting and questions read aloud by an experimenter) are present in the literature and have shown a positive result when validating questionnaires among children as young as 5 years old (Rubio et al., 2008). Finally, one obvious weak- ness is that we did not measure children's actual consumption of FV, thus we cannot conclude that the decreased neophobia and in- creased liking would have translated in an actual higher FV intake by children.

In the second experiment we investigate whether and how different levels of variety influence children intake and liking of FV snacks in school-children. The hypothesis of a relationship between level of variety and children's acceptance was only partially confirmed. Energy intake increased with the levels of variety in CV set, while an increase of ranked liking was observed in PV set levels. No other systematic effects of either intake in grams or rated liking were observed. The results indicate that adding more foods or different cuttings of the same foods to a serving in a meal doesn't influence children's intake. Results also suggest that consumption is more affected by acceptability and familiarity for the stimuli than by variety. Adding more different foods to a serving leads to higher energy intakes than adding more cuttings of the same foods, although servings with several cuttings seemed to be more liked.

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4. CONCLUSION

4. CONCLUSION

Firstly, we developed and validated a questionnaire to measure food neophobia in Italian school children, the ICFNS. It represents a valuable tool for practitioners to evaluate interventions or policies focused on decreasing children's food neophobia and increasing their preference for, and promoting a positive attitude toward, the consumption of healthy foods such as fruits and vegetables (FV).
Regarding the survey conducted on nutritional status and thresholds, we can conclude that perspetive studies are arguably the best way to assess the relationship between taste acuity and the development of overweight and obesity desease in adults, with the goal of carrying out a future study in children.
Our results demonstrated that children body composition is not related liking of FV and neophobia, by means of ICFNS, whereas our study confirmed the hypothesis of the relationship between food liking and neophobia. In particular, children with low liking of vegetables are characterized by high neophobia traits. Such relationship might be explained as follows: children like what they know and eat what they like.
Our findings could expand the knowledge in an open research field, allowing for a better understanding of crucial behaviours in the pathogenesis of overweight and obesity in childhood. Moreover, it is well know that individuals vary in their sensitivity to stimuli across various sensory domains, whereby individuals who are sensitive to stimuli only need small amounts of sensory variation to detect changes in stimulation. In the case of food, individuals who are sensitive to stimuli would be able to detect small changes in the sensory characteristics of food. These findings suggest that individuals with food neophobia may detect smaller changes in the appearance, smell, texture and taste of food and consequently they are more prompt to reject it. Therefore, future experiments could be to adressed towards understanding whether children with food neophobia are more sensitive to sensory attributes. In addition it can be interesting to examine whether sensory sensitivity is associated with reduced consumption of FV as well as investigating the mechanisms that really hinder the acceptance of these products.
The educational program applyed in Italy was shown to be effective in changing eating behaviour in Italian primary school children, more in neophobic behaviour than in vegetables liking. These results indicate that the overthrow of food neophobia is the first step to obtain a long lasting change in children's food habits. However, further efforts such as iterations of the educational intervention, are needed to increase liking for FV, with the goal to obtain an effective change in children food choices.
Furthermore, we can affirm that exposing children to daily servings of different liked products did not increase liking. On the contrary, boredom effects were observed for most than vegetables. Thus, exposure should be associated with other multidisciplinary approaches, such as peer modeling and rewards, when applying interventions with children.
To deeply understand whether this increasing of liking results in a rising of consumption, collection of intake data could be carried out.
Regarding the OPUS Danish project, although we didn't obtained any clear effect of variety of snacks on consumption of vegetables, we observed that exposing children in natural condition, at school, to a snack consisting of fruits, nuts and vegetables may be a good method to increase children's familiarity and thus intake of these foods.
In conclusion, all results of our studies confirm previous findings indicating that a suitable age for the commencement of school-based programs could be 8 years or even earlier, as younger children appear to be more likely to change their food behaviour than older children. Early intervention is also likely to maximize health benefits because eating habits in childhood are strongly predictive of those in adulthood.

LIST OF PAPER, ORAL COMMUNICATIONS AND POSTERS

Peer-reviewed pubblications:

Paper I Bergamaschi V., Olsen, A., Laureati, M., Zangenberg, S., Pagliarini, E., Bredie, W.L.P. Variety in snack servings as determinant for acceptance in school children.
Appetite, Under revision

Paper II Laureati, M., Bergamaschi, V., Pagliarini, E. (2015) Assessing childhood food neophobia: validation of a scale in Italian primary school children.
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Paper III Laureati, M., Bertoli S., Bergamaschi V., Leone A., Lewandowski L., Giussani B., Battezzati A., Pagliarini E. (2015). Overweight, food neophobia and food liking in children: is there a relationship?
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Paper IV Laureati, M., Bergamaschi, V., & Pagliarini, E. (2014). School-based intervention with children: peer-modeling, reward and repeated exposure reduce food neophobia and increase liking of fruits and vegetables.
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I Effectiveness in reducing food neophobia and increasing acceptance in fruit and vegetable. Pagliarini E., Laureati M., Bergamaschi V. Oral presentation in *International Conference on Multidisciplinary Perspectives on Child and Teen Consumption*, IULM University, Milan, December 12-13-14, 2012.

II Liking, familiarity and neophobia in the prevention of child obesity by applying a new educational program. Bergamaschi V., Pagliarini E. Poster in *XVII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology*, University of Bologna, Cesena, 19-21 September, 2012.

III Effect of education on food behavior: A survey on 6-9 years old Italian children. Laureati M., Bergamaschi V., Pagliarini E. Poster (P1.34) in *Eurosense 2012: 5th European Conference on Sensory and Consumer Research,* Bern 2012.

IV Taste sensitivity in weight excess and management. Laureati M., Bergamaschi V., Bertoli S., Battezzati A., Pagliarini E. Poster in: *The International Symposium on Olfaction and Taste (ISOT),* Sweden 2012.

V Programma Food & Fun: primi risultati di un progetto di educazione alimentare nelle scuole del comune di Milano.[Food & Fun: preliminary results of a educational program in the schools of Milan] Bergamaschi V., Di Iorio M., Laureati M. Poster in *Nutrimi, Forum Internazionale di Nutrizione Pratica,* Milan 2012.

VI Taste memory in children: incidental vs intentional learning. Laureati M., Bergamaschi V., Pagliarini E. Poster in 9th *Pangborn Sensory Science Symposium*, Toronto, 2011.

VII Sostenibilità e valutazione sensoriale. L'effetto bio sulle scelte di consumo. Castelli L., Jabes D., Laureati M., Bergamaschi V. Oral presentation in *Convegno Nazionale AIP sezione "Psicologia per le organizzazioni": Senso e prospettive dell'applicare.* Milan 2011.