UNIVERSITÀ DEGLI STUDI DI MILANO

Ph.D. SCHOOL IN FOOD SYSTEMS

Department of Food, Environmental and Nutritional Sciences

XXIX Cycle

BEHAVIORAL AND PHYSIOLOGICAL DRIVERS OF OBESITY: AN INVESTIGATION USING A SENSORY APPROACH

[Scientific field – AGR/15]

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2016/2017

Many are stubborn in pursuit of the path they have chosen,
few in pursuit of the goal.

Friedrich Nietzsche

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FOREWORD

The experiments presented in this Ph.D. thesis were mainly conducted in the Sensory & Consumer Science Laboratory at the Department of Food, Environmental and Nutritional Sciences (University of Milan) during the years 2014-16. A part of the Ph.D. project was done in collaboration with the International Center for the Assessment of Nutritional Status (ICANS) and with the Department of Medical Sciences and Rehabilitation, IRCCS Istituto Auxologico Italiano. An experiment (founded by NOW, The Netherlands Organization for Scientific Research, Veni grant no. 451-11-021, awarded to Sanne Boesveldt) was carried out during a period abroad in 2015 at Division of Human Nutrition, at the Wageningen University, in collaboration with prof. Cees de Graaf and dr. Sanne Boesveldt.

The overall aim of this Ph.D. thesis was to study behavioral and physiological drivers of obesity using a sensory approach.

The thesis is organized in five chapters. After an introductory chapter (**chapter 1**), which provides background information from the literature about the topics of the present thesis, the rational and aims are described in **chapter 2**.

Results and discussion are presented in **chapter 3.** This chapter is structured in three paragraphs referred to the three main specific topics of the Ph.D. project. In particular, the *first paragraph* is focused on the study of the taste sensitivity, food neophobia and food liking in relationship with Body Mass Index (BMI). *The second paragraph* pays attention on the study of multisensory interactions and food liking in relationship with BMI and gender. Finally, *the third paragraph* is focused on the investigation of ambient odor exposure on salivation, appetite and food intake.

Chapter 4 provides the general conclusions drawn from this thesis and the future perspectives of study. The material and methods of all the experiments conducted during the Ph.D. are reported in **chapter 5.**

ABSTRACT

It is well known that the pathology of obesity is considered a disease with a multifactorial etiology. However, fairly poor data have been reported on the influence of variables which are deeply-rooted in human mind and determine food habits. Recent evidences have suggested that factors related to the sensory perception may explain weigh excess. Indeed, food perception and food liking are the result of multiple sensory modalities, including visual, olfactory, gustatory, and somatosensory inputs. In particular, the odor and taste cues of foods play a pivotal role in food choice, acceptability and, thus, energy intake. Despite the relationship between sensory perception and food intake is evident, the studies available on this topic are very few in number and results are rather contradictory and not easy to compare.

The overall aim of this Ph.D. thesis was to study behavioral and physiological variables involved in the phenomenon of obesity using a sensory approach. Specific goals were: 1) the evaluation of taste sensitivity, food neophobia and food liking in normal-weight and obese subjects; 2) the study of multisensory interactions (odor-taste-texture) in a model food (custard dessert) and food liking in relationship with gender and nutritional status; 3) the evaluation of the influence of ambient odor exposure on salivation, appetite and food intake.

The results showed that obese subjects differed in terms of taste sensitivity from normal-weight subjects. More specifically, the obese subjects involved were less sensitive to taste stimuli compared to the lean subjects. These differences may lead subjects with higher BMI to prefer foods rich, for example, in sugar and fat in order to compensate their reduced sensitivity. This hypothesis is supported by the liking scores, provided by obese subjects, to the high energy dense food products which were significantly higher compared to the scores given by the normal-weight subjects. No differences in food neophobia scores have been found in the two groups of subjects.

The multisensory interactions (odor-taste-texture) occurred differently in relation to BMI and, to a lesser extent, to gender. Indeed, a model food (custard dessert) modified with aromas and thickener agents produced strongest sensory interactions (odor-taste, odor-flavor and odor-texture) in subjects with higher BMI, especially in women, compared to the control group. The addition of a stimulus signaling high-calorie

products, such as butter aroma, modified the perception of different sensory characteristics in a more effective way in obese subjects compared to the normal-weight. For example, obese subjects perceived the modified samples, added with butter aroma, as sweater without the addition of sugar.

Finally, the ambient odors exposure affected behavioral and physiological responses involved in eating behavior. In particular, the exposure to odor signalling high-energy dense food products increased the total eaten amount of a model food (chocolate rice), the salivation and the appetite. The ability of odors to influence the amount of food ingested, and therefore the amount of energy assimilated by individuals, could be a useful instrument to prevent overeating in obese individuals steering food intake away from high energy unhealthy foods, towards healthier choices.

In conclusion, it is evident that investigate the phenomenon of obesity applying an innovative sensory approach is interesting in order to better understand and stem the complex issue of overeating. Indeed, it could be possible to drive food preferences, food choices and food intake focusing on sensory cues. How the mechanism of brain integration occurs in subjects with different nutritional status might be taken in to account in order to develop new food products with a reduced caloric intake but satisfying for the consumer.

RIASSUNTO

La patologia dell'obesità attualmente può essere considerata una malattia a eziologia multifattoriale. I dati relativi allo studio delle variabili che sono profondamente radicate nella mente umana e che determinano le abitudini, tuttavia, sono relativamente scarsi. Studi recenti hanno ipotizzato che i fattori legati alla percezione sensoriale forniscono informazioni utili per indagare il fenomeno dell'incremento di peso. La percezione e il gradimento del cibo, infatti, sono il risultato di molteplici stimoli sensoriali, visivi, olfattivi, gustativi, e somatosensoriali. In particolare, gli stimuli olfattivi e gustativi giocano un ruolo centrale nella scelta degli alimenti, nell'accettabilità e, di conseguenza, nell'assunzione di energia. Nonostante la relazione tra percezione sensoriale e assunzione di cibo sia evidente, gli studi disponibili anche su questo argomento sono limitati, i risultati sono contraddittori e non facilmente confrontabili.

L'obiettivo generale di questa tesi di dottorato è stato quello di studiare le variabili comportamentali e fisiologiche coinvolte nel fenomeno dell'obesità utilizzando un approccio sensoriale, attraverso: a) la valutazione della sensibilità gustativa, della neofobia alimentare e del gradimento alimentare in soggetti normopeso e obesi; b) lo studio delle interazioni multisensoriali (odore-gusto-consistenza) in un alimento modello (crema dessert) e del gradimento in relazione al sesso e allo stato nutrizionale; c) la valutazione dell'influenza dell'esposizione agli stimoli olfattivi nell'ambiente circostante sulla salivazione, sull'appetito e sull'assunzione di cibo.

I risultati hanno dimostrato che i soggetti obesi hanno una distorta sensibilità gustativa rispetto ai soggetti normopeso. In particolare, i soggetti obesi coinvolti sono risultati meno sensibili agli stimoli gustativi rispetto ai soggetti normopeso. Queste differenze nella percezione gustativa potrebbero portare i soggetti con un elevato indice di massa corporea (IMC) ad avere diverse preferenze alimentari rispetto ai soggetti normopeso, prediligendo prodotti alimentari ricchi per esempio di zuccheri e grassi, in grado di sopperire alla ridotta sensibilità. Questa ipotesi è avvalorata dai punteggi di gradimento significativamente più elevati dati dai soggetti obesi ai prodotti ad alta densità energetica rispetto ai soggetti normopeso. Differenze significative non sono state invece riscontrate tra gli indici di neofobia alimentare nei due gruppi di soggetti coinvolti.

Le interazioni multisensoriali (odore-gusto-consistenza) si sono dimostrate diverse in relazione all'IMC e, in misura minore, al sesso. Infatti, l'aggiunta di aromi e agenti addensanti a un prodotto modello (crema dessert) ha generato maggiori interazioni sensoriali (odore-gusto, odore-flavor e odore-consistenza) soprattutto nelle donne con elevato IMC rispetto al gruppo di controllo. L'aggiunta di uno stimolo riconducibile a prodotti ad alta densità energetica, come per esempio l'aroma di burro, ha infatti modificato la percezione di caratteristiche sensoriali in modo più efficace nei soggetti obesi rispetto al gruppo di controllo. Gli individui obesi hanno, per esempio, percepito l'aumento della dolcezza in seguito all'aggiunta di aroma burro senza una effettiva aggiunta di zucchero al prodotto.
Infine, l'esposizione agli odori nell'ambiente circostante ha influenzato sia le risposte comportamentali sia quelle fisiologiche coinvolte nel consumo alimentare. L'esposizione agli odori riconducibili a prodotti ad alta densità energetica, in particolare, ha aumentato la quantità consumata di un prodotto modello (riso al cioccolato), la salivazione e l'appetito. La capacità degli odori di influenzare la quantità di cibo ingerito e, quindi, la quantità di energia assimilata, potrebbe essere uno strumento utile per prevenire l'eccessivo consumo di cibo da parte degli individui sovrappeso e obesi e guidarli verso scelte più sane.

In conclusione, lo studio dell'obesità utilizzando un approccio sensoriale risulta interessante e innovativo per una miglior comprensione del fenomeno e per la messa a punto di strategie che ne contrastino lo sviluppo. Gli stimoli sensoriali, infatti, potrebbero essere utilizzati nell'indirizzare i consumatori a una minore assunzione di cibo e verso scelte di prodotti alimentari più salutari. Inoltre, considerando i meccanismi di integrazione degli stimoli sensoriali a livello cerebrale si potrebbero sviluppare nuovi prodotti alimentari a ridotto apporto calorico, soddisfacenti per il consumatore.

Chapter 1

General introduction

The obesity epidemic and sensory perception

Globally, the sharp rise in the rates of obesity is a significant public health concern. According to the World Health Organization (WHO, 2016), the obesity epidemic has more than doubled between 1980 and 2014. In 2014, over 1.9 billion adults were overweight, with over 600 million being obese. Worryingly, a similar picture is revealed also in children (Cuschieri & Mamo, 2016).

The growth of this pathology is not restricted to the industrialized countries as this increase is often intense in developing countries too. Indeed, the increasing global prevalence of obesity has led to what has been defined the Obesity Epidemic (Hill & Peteres, 1998). A number of negative consequences, such as cardiovascular disease and hypertension, are linked with this pathology (Flegal et al., 2007).

Although the growing prevalence of obesity is well documented, explanations for the emerging epidemic appear more elusive. Indeed, obesity is considered as a disease with a multifactorial etiology. Further the genetic, which is a risk factor in elucidating an individual susceptibility to weight gain, obesity requires lifestyle influences to arise (Qi & Cho, 2008; Lifshitz & Lifshitz, 2014). Essentially, obesity occurs from an imbalance between the quantity of the energy consumed and the amount expended. Moreover, the growth of this pathology reflects changings in society that have been accompanied to extensive modifications in the environment. These modifications have created an environment, rich of energy dense processed foods, promoting the development of obesity.

Diet certainly constitutes an important part of such environmental influences, indeed poor diet is a key adaptable determinant of lifestyle related diseases. Modifying food choices and improving diet quality is a main concern in improving population health (Cox et al., 2016). In this context, developing new formulations of good-tasting but low-calorie food is one of the leading priorities for researchers in today’s food industry (Tomaschunas et al., 2013). Unfortunately, creating innovative formulations is not an easy task, since the food perception and food liking are the result of multiple sensory modalities, including visual, gustatory, olfactory, and somatosensory inputs (Small & Prescott, 2005).

The sensory properties of foods and beverages are effective before, during and after an eating event. They direct subjects towards a food source, guide preferences, portion selection and the fullness after consumption (McCrickerd & Forde, 2016). The sensory food cues, such as the sight, smell and taste of a food, could promote overconsumption when they enhance the palatability of foods (Sorensen et al., 2003). Indeed, food liking, which is defined as the positive hedonic evaluation of food's sensory characteristics, is definitely an important driver of food selection and intake, but it is just one aspect of the sensory experience (Yeomans, 1998).

The primary experience of eating is important since people learn to eat in response to sensory cues, by creating associations between the early experience of a food's sensory characteristic and the post-ingestive effects of nutrient delivery. Therefore, this learned integration of pre- and post- ingestive signals can be translated as increased liking for nutrient-rich foods and information about a food's potential satiating power (Brunstrom, 2007), which modify food selection and intake.

Moreover, the sensory properties of food can elicit cephalic phase responses, such as salivation (Wooley & Wooley, 1973), gastric acid secretion (Feldman & Richardson, 1986) and the release of some gastrointestinal hormones (Smeets et al., 2010) in order to improve nutrient processing throughout the gastrointestinal tract. These anticipatory physiological responses are triggers of a cascade of events (digestive and endocrine) which increase the efficiency of the digestion and metabolism, but also directly and indirectly regulate meal size and duration (Power & Schulkin, 2008). This demonstrates that sensory signals present before and during consumption play a key role in optimizing energy intake regulation.

Genetic variation in taste perception

The sensory perception of food varies significantly between individuals influencing eating behavior and therefore the nutritional status and health of the subjects (Tepper, 2008). It has been suggested that these differences could be due to numerous factors, such as gender (Bartoshuk et al., 1994), age (Mojet et al., 2001), salivary composition (Spielman, 1990) and genetic variation (Bajec & Pickering, 2008).

The most studied genetic source of individual variation is the ability to taste the bitter compounds 6-n-propylthiouracil (PROP) (e.g. Yackinous & Guinard, 2002; Tepper, 2008; Tepper et al., 2009). PROP responsiveness is typically expressed categorically as PROP taster status, which consists of three groups: PROP non-tasters (least responsive), PROP medium-tasters (intermediate responsiveness) and PROP super-tasters (most responsive) (Bartoshuk, 1993). The PROP responsiveness is also associated with sensitivity to other oro-sensory stimuli. Indeed, the increased sensitivity of super-tasters to tastant solutions appears to translate into a better sensitivity also to the same taste qualities in food. In this context, it has been proposed that super-taster perceive saltiness, sweetness, and sour more intensely than medium and no taster (e.g. Duffy et al., 2003; Prescott et al., 2004; Hayes & Duffy, 2007). Moreover, PROP phenotype may be a marker for underlying differences in fat preference and dietary fat intake (Tepper & Nurse, 1998). In fact, humans seem to discriminate among fatty acids, probably based on the presence of double bond, and genetic variation in taste sensitivity to PROP seems to affect chemosensory responses to unsaturated fatty acids. In particular, subjects who could detect the bitter taste of PROP reported higher taste intensity values for linoleic acid compared with PROP non-tasters (Ebba et al., 2012).

Moreover, Goldstein and colleagues (2005) showed an inverse association between PROP taste sensitivity and BMI. Accordingly, Tepper and Ullrich (2002) suggested that BMI tended to be higher in male no tasters and lowest in male supertasters while no differences or trends were noted in women.

Other authors suggested that PROP sensitivity is also correlated with increased densities of fungiform papillae, with the supertasters having the highest densities followed by tasters and non-tasters (Miller & Reedy, 1990; Bartoshuk et al., 1994; Duffy et al., 1994; Tepper & Nurse, 1998). Indeed, the fungiform papillae, so-called for their mushroom-like appearance when viewed in transection, are supposed to be correlated to the overall taste bud number. For this reason, the density of these papillae on the tongue is often used to evaluate the taste function. However, this association is still unclear, since some authors found a correlation between the fungiform papillae density and taste sensitivity (Zuniga et al., 1993; Bartoshuk et al., 1994; Doty et al., 2001), while others did not found this correlation (Garneau & Derr 2013; Feeney & Hayes 2014).

How does taste perception affect eating behavior and differ between individuals?

The 'taste' of food, used in a colloquial sense to mean all aspects of the sensory perceptions of foods or overall palatability (how much it is liked or disliked), steer consumer food choice (Nasser, 2001). Indeed, the importance of taste cannot be overstated (Sobal et al., 2006) considering that it guides food preference and liking which are main factors driving food choice and eating behavior (Drewnowski et al.,1999; Ly & Drewnowski, 2001; Tepper et al., 2009). The basic tastes (sweet, salty, bitter, sour and umami) are supposed to signal the nutrient composition of foods, with sweet taste representing carbohydrates and savory taste associated with electrolytes and protein (van Dongen et al., 2012), whereas sour and bitter taste signal foods that could be harmful when ingested.

There are predispositions to respond preferentially to the basic tastes: at birth, sweet taste is preferred and sour and bitter are rejected (Steiner, 1977; Ventura & Mennella, 2011); preference for salt emerges by approximately 4 months (Beauchamp et al., 1986). These predispositions, which are readily modified via early experience, affect the infants' behavioral responses to these tastes, including their intake. As adults, the majority of our energy intake still comes from food sources that can be described as sweet or salty, while little energy comes from foods described as bitter or sour (Mattes, 1985), highlighting the close association between our general taste preferences, food choice and intake. However, this taste hedonics can also be modified through experience and learning, and are thought to account for considerable variation in individual food choices (de Houwer et al., 2001; Eertmans et al., 2001).

Although it has been established that taste plays a pivotal role in food choice, acceptability and, thus, energy intake (Drewnowsky 1997, Mennella et al., 2005), the extent of specific taste perception in relation to weight status is not well understood. Earlier studies (Malcolm et al., 1979; Frijters & Rasmussen-Conrad, 1982) did not showed a relationship between taste thresholds and nutritional status, whereas more recent studies (Simchen et al., 2006; Monneuse et al., 2008; Bertoli et al., 2014) described a difference between overweight and normal-weight subjects. In particular, lower taste perception ability with an increase in weight has been found. However, some reviews (De Graaf, 2005; Mela & Rogers, 1988) have reported that obese subjects exhibit ‘‘normal’’ chemosensory function and liking for specific tastes or aromas. These results show that the relationship between taste perception and nutritional status remains unclear.

Recently, in addition to the study of the perception of basic tastes, attention has been concentrated on the sensitivity to fat stimulus, which could be associated to obesity. The perception of fat in relation to nutritional status has been explored in some recent studies (Stewart et al., 2010, 2011; Stewart & Keast, 2012) in which an inverse association between BMI and both fatty acid taste sensitivity and fat intake was found.

Accordingly, a strong liking for highly fatty foods in subjects with higher BMI has been recently revealed (Deaglaire et al., 2015). These findings suggest that reduced sensitivity to fats may be an aspect that contributes to the pathogenesis of obesity, although it is important to recognize that causality cannot be inferred from association studies. However, other studies reported non-significant associations between fat sensitivity and weight status (Alexy et al., 2011; Salbe et al., 2004; Simchen et al., 2006), thus underlining inconsistencies in literature data.

The role of odor perception in eating behavior

Besides the taste, the other sensory properties of food, such as the smell, are important factors for regulating what and how much we eat (De Graaf & Kok, 2010; Sørensen et al., 2003). It is well known that the olfactory modality plays a key role not only during consumption, but also before eating. Indeed, odors are omnipresent in our food environment and olfaction is critical in flavor perception and food preferences since it is organized to identify foods more holistically and to be readily influenced by learning and experience (Bartoshuk, 1991; Duffy & Bartoshuk, 1996). Unfortunately, less is known about the development of olfactory preferences than what is known about taste preferences (Engen, 1978; Lawless, 1985).

In the late 1960s, Schachter and colleagues proposed the ‘externality theory’ of human obesity (Schachter, 1968; Schachter & Rodin, 1974). In this context, Herman and Polivy (2008) suggested that certain people, such as obese and dieting individuals, are more affected by external food-related cues (e.g. smell and sight) than normal-weight subjects. In humans, ‘food-cue exposure’ can have a deep effect on our motivation and physiological readiness to eat. Despite this, it remains unclear whether differences in cue reactivity represent a risk factor for overweight and obesity.

Literature data suggest that odors can direct appetite and food choices to foods that are signaled by the odor specifically. Rolls and Rolls (1997) demonstrated that signs of satiety for a specific product occur after smelling their respective odor. They found that pleasantness ratings for banana and chicken odors decreased after 5 min of smelling a plastic cup containing banana or chicken, respectively. More recent studies have demonstrated that non-attentively perceived fruity odors affect food choices, guiding participants towards more fruit and/or vegetable choices (Gaillet et al., 2013; 2014). For example, Ramaekers et al. (2014) found that food odors, such as bread and chocolate, stimulated appetite and choice for congruent foods. Similarly, in recent research, Zoon et al. (2016) found that odors signaling high energy dense foods increased appetite for high energy dense products but not for low energy products, and *vice versa.*

Moreover, it has been suggested that cue exposure promotes the selection of larger portion sizes (Ferriday & Brunstrom, 2008) and it increases the amount of food that is consumed in a meal (Fedoroff et al., 1997¸ Fedoroff et al., 2003; Ferriday & Brunstrom, 2008). Tetley and colleagues (2010) in a research in which overweight and lean adults were exposed to a food cue (pizza) in a satiated state, showed that the overweight participants reported a greater increase in desired pizza portion-size.

Although it is plausible that food odors contribute to the regulation of food intake, and consequently energy intake, scientific evidence is scarce to support this hypothesis. Studies that have looked into behavioral responses to food cues mainly use subjective ratings (e.g. Rolls & Rolls, 1997; Ferriday & Brunstrom, 2008; Tetley et al., 2010). These ratings may provide some indication about food preferences, but they may not represent actual food choice and intake. Research into the effects of olfactory food cues on actual eating behavior is scarce. Larsen et al. (2012) examined this, but did not find an effect of ambient cookie odor exposure on cookies intake. Some studies showed a decrease in intake upon odor exposure (Coelho et al., 2009; Ramaekers et al., 2014), while other researchers found an increased intake (Fedoroff et al., 2003; Ferriday & Brunstrom, 2008) or reported no effect of odor exposure on *ad libitum* intake (Ruijschop et al., 2009; Zoon et al., 2014; Ramaekers et al., 2014).

In order to better understand factors that may lead to overweight, it is crucial to elucidate the different factors (including food odor exposure) involved in the processes leading up to actual intake. Odor can influence food consumption through taste enhancement or through suppression (Rozin, 1982; Stevenson et al., 1999). Unpleasant ambient odors are likely to shorten the duration of a meal and to suppress food consumption. Yet the reverse is not necessarily true. It is not known whether favorable odors necessarily increase consumption volume. It has been found, for instance, that regardless of whether a person tastes a food or simply smells it, sensory-specific satiety (SSS) can occur within a reasonably short time (Rolls & Rolls,1997). SSS was defined as a larger decrease in pleasantness of eaten foods relative to the decrease in pleasantness of uneaten foods (Rolls et al., 1981). SSS is the opposite of sensory-specific appetite (SSA), which means that exposure to food cues specifically increases the appetite for the cued food relative to the appetite for other foods (Fedoroff et al., 2003). This suggests that odors might not necessarily increase consumption other than by simply initiating it.

Multisensory interactions in food perception

As mention previously food perception is the result of multiple sensory modalities, including visual, gustatory, olfactory, and somatosensory inputs. Integration of information from physiologically distinct sensory modalities is a general property of the mammalian nervous system (Small & Prescott, 2005). Its purpose is to enhance the detection or identification of stimuli, particularly in those cases where a single sensory modality provides ambiguous, incomplete, or low perceptibility information (for instance, integration of odors and taste). From a neuroanatomical point of view taste and smell are very different senses. Taste is perceived primarily on the tongue whereas odors are perceived in the upper part of the nasal cavity either directly or via the back of the mouth. However, this neuroanatomical dissociation does not mean that taste and odor perception are independent. Indeed, information coming from the gustatory and the olfactory systems are likely to be combined at a higher level of processing in the brain to give rise to a unique perception referred to as "flavor" (Valentin et al., 2006).

Much of the human psychophysical evidence for integration of odors and tastes is derived from data showing interactions between these distinct modalities when they are experienced in mixtures that is, as part of a flavor.

For instance, a commonly reported effect of interaction is the ability of food odors such as strawberry or vanilla to enhance sweetness when added to solutions of a tastant, such as sucrose (Small & Prescott, 2005). The increase in sweetness intensity is not mediated by chemical interactions between the odorant and tastant, since preventing odor volatiles from reaching olfactory receptors (for example, by closing the external nares during tasting) remove the effect. Also, the odorants are typically tasteless when experienced alone in solution. In other words, inter odor-taste action refers to a modification in perceived taste intensity in the presence of an odor.

Of course, odor-taste integration happens when the odor-taste mixture is perceived in an “appropriate” context. For instance, it has been shown that strawberry odor enhanced the sweetness of a sucrose solution. Contrariwise, saltiness was not increased by strawberry odor (Small & Prescott, 2005). Other examples of odor-taste interaction were obtained in presence of several aromas including almond, caramel, coffee, lemon, peach and vanilla for several sweeteners such as sucrose, fructose, aspartame and saccharine (Valentin et al., 2006). The possibility of very early cortical integration of the sensory components of flavor is consistent with the fact that taste perception is almost always accompanied by oral somatosensation and retronasal olfaction.

Moreover, experiencing a new odor determin a comparison with memories of previously encountered odors. If in the initial experience of the odor it is combined with a taste, a configurational stimulus is fixed in memory. Subsequently, smell the odor alone will arouse the most similar odor memory, that is, a flavor, which will include both the odor and the taste component. Thus, for example, smelling caramel odor elicits memorial representations of caramel flavors, which includes a sweet component. In other words, perceptions are being constructed from a combination of both ‘‘real’ tastes and taste properties of the odor that are encoded in memory. Such explanations are consistent with data showing that memorial representations of chemosensory qualities can combine with physically present stimuli to produce mixtures that show very similar psychophysical interactions to those of identical combinations of physically present qualities (Stevenson & Boakes, 2004).

These ‘‘mental mixtures’’ have been demonstrated with combinations of different tastes, odors, as well as odor/taste mixtures (Algom & Cain, 1991; Algom et al., 1993; Stevenson & Prescott, 1997). If the acquisition of taste-like properties by odors reflects associative learning, then it is reasonable to predict that odors that elicit taste perceptions develop the ability to activate gustatory neurons, either as a direct function of the olfactory perception or as a result of a reactivation of a flavor memory (Small & Prescott, 2005).

In this context, it is clear that studies on odor-taste mixtures are particularly interesting if used to modulate the perception of taste within food. This would have enormous implications for the formulation of food with reduced addition of sugar, fat, salt that can help in reducing caloric intake and encourage good health.

Not only odor-taste but also odor-texture mixtures have been studied in relation to food intake. Warwick et al. (1993) demonstrated that combining olfactory (vanilla) and gustatory (aspartame) sensations enhanced the satiating effect of a high-fat meal, compared with a bland version of the same meal. Accordingly, recent research supports the idea that the sensory characteristics of a drink can modulate its satiety value. McCrickerd et al. (2012; 2014) demonstrated that regardless of their real energy content, thicker drinks were more filling than thin versions, presumably because the perceived creaminess elicited the texture and flavor attributes typically associated with nutrients. Indeed, the thicker drinks were expected to be more filling and to suppress hunger to a greater extent than thin versions, regardless of their actual energy content. The addition of creamy flavors did not affect expected satiety but did enhanced the expectation that the drinks would be filling, presumably because perceived creaminess has both textural (thickness and smoothness) and flavor (dairy, vanilla and sweetness) attributes typically associated with nutrients (McCrickerd, et al., 2014). Thus, it is clear that oro-sensory characteristics of food are important for the development of satiety. In fact, humans learn to associate the sensory characteristics of a food with its caloric value post-consumption (Laureati et al., 2008; Morin-Audebrand et al., 2009), and this strongly influence satiation and therefore the amount of eaten foods (McCrickerd et al., 2012). This result might be explained by the fact that¸ similarly to odor-taste interaction, the perception of foods or beverages flavors reflect information derived from multiple sensory afferents, including gustatory, olfactory, and somatosensory fibers. Although many studies have been conducted on sensory interactions among tastes, odors and perceived fattiness (Maga, 1974; Lavin & Lawless, 1998; Frank et al., 1993), none of them have considered these mechanisms in relation to subjects' nutritional status. This could have enormous implications for the formulation of food products with reduced sugar, fat, and salt, which would help to reduce caloric intake and tackle the obesity epidemic. In this context, it would be interesting to verify whether obese individuals perceive sensory characteristics, and therefore their integrations, in the same way as normal-weight subjects do.

Role of food preferences and food neophobia in eating behavior

Food preferences are innate reflections of the body's need for nutrients and are learned through experience with food and eating. Considering that food preferences and food selection are causally linked to the current prevalence of overweight and obesity, there is a large literature focus on this association (Birch, 1999).

The genetic predispositions that primarily influence food preferences include: a) the tendency to prefer sweet and salty foods and to reject sour and bitter foods; b) the reluctance to eat novel foods (food neophobia); c) the predisposition to learn preferences by associating foods with the contexts and consequences of eating them.

Although these predispositions are common additional researches have been conducted to identify genetic variability contributing to the development of food preferences. There are evidences suggesting that genetic differences account for relatively little of the variance in food preferences and that environmental factor are essential (Reed et al., 1997; Perusse & Bouchard, 1994).

As mentioned previously, the reluctance to eat novel foods (food neophobia) could affect food preferences and eating behavior. Food neophobia is literally the "fear of the new," and it is defined as the reluctance to taste a new food (Raudenbush at al., 2003). Humans are omnivores, and they can eat a wide range of foods. This advantage allows our species to easily adapt to a new food environment. However, humans reveal an ambivalent reaction in front of a new food: a combination of curiosity and fear (Rozin & Vollmecke, 1986).

The fear of new, potential dangerous food products, serves a protective function (Rozin, 1976), however, it may seem maladaptive for species that require consuming dietary variety to obtain adequate nutrition.

Literature evidence suggest that food-neophobic are hesitant to try or to buy novel foods (e.g. Arvola et al., 1999; Tuorila et al., 2001, Schickenberg et al., 2008; Henriques et al., 2009; Chung et al., 2012). Indeed, food neophobics have less variety in their diet than do food neophilics (Falciglia et al., 2000), which could clearly affect their energy intake and nutritional status. In this context, food neophobia is considered another factor that could be involved in the development of obesity.

In particular, the food neophobia attitude is typical in children and some authors have suggested that the lack of dietary variety in children's diets is directly associated with intake of certain foodstuffs (Dovey et al., 2008). Specifically, in these children, intake of fresh products such as fruits and vegetables is replaced by unhealthy processed foods characterized by their high hedonic value that results from their sugar, fat and salt content (Dennison et al., 1998). This limited but energy dense hyper-caloric diet is widely considered to be a key contributing factor to the rise in the rates of childhood obesity (Falciglia et al., 2000; Rigal et al., 2006) as well as the increase in the prevalence of non-communicable diseases (e.g. type II diabetes) in children (Kaufman, 2002). Associations between food neophobia and food intake were observed in an undergraduate student sample (Eertmans et al., 2005) and it has been found that consumption of healthy foods is associated with a lower level of food neophobia (MacNicol et al., 2003; Mustonen et al., 2012). Indeed, in children, high food neophobia is associated with low liking (Russell & Worsley, 2008) and low consumption of fruits and vegetables (e.g Wardle et al., 2005; Cooke et al., 2006; Coulthard & Blissett, 2009).

However, it is not clear whether these associations hold in adults and how the neophobic attitude could influence the nutritional status of the subjects. Indeed, food neophobia may restrict besides the variety of the diet, also the quantity, and thereby the energy content but, on the other hand, food neophobics may replace fruit and vegetables with more energy-dense foods, leading to increase energy intake (Knaapila et al., 2015). Inconsistent results are reported in literature regarding the relationship beetween food neophobia and BMI. Knaapila and colleagues (2011) reported a small correlation in young adult women although not in men. However, other evidence suggested that food neophobics have significantly higher BMI than food neophilics, both children and adults (Knaapila et al., 2015; Finistrella et al., 2012). Even if, it has also been suggested that people having a higher sensitivity to taste stimuli are more open to food experience (Ullrich et al., 2004) the relationship between food neophobia and nutritional status might be investigated.

Overall, it is clear that the factors which are involved in food perception, and in eating behavior are several, and that it is necessary to better understand mechanisms which could play a role in the phenomenon of weight gain.

Chapter 2

Rationale and aims

Fairly poor data have been reported on the influence of variables which are deeply-rooted in human mind and determine food habits. Recent evidences have suggested that factors related to sensory perception may explain weigh excess. Despite the relation between sensory perception and food intake is evident, the studies available on this topic are few in number and results are rather contradictory and not easy to compare.

The overall aim of this Ph.D. thesis was to study behavioral and physiological determinants of obesity using a sensory approach. Specific goals were:

a) to compare taste sensitivity in normal-weight and obese subjects using different methods: taste thresholds for the 4 basic tastes (sweet, salty, sour and bitter) and the fat stimulus and the number of the fungiform papillae. Food liking and food neophobia according to subjects' BMI were also investigated in order to study the relationship between these variables and taste sensitivity;

b) to study food cross-modal sensory interactions, in relation to BMI and gender, in model foods by designing formulations with varying odorant-thickener concentrations in order to generate strong expectations of satiety and increase the perceived intensity of sensory attributes. The perception of sensory properties and liking of the models foods were investigated;

c) to investigate the effects of ambient odor exposure on behavioral and physiological measurements. The influence of odors signaling different types of foods (high and low in energy-density, sweet and savory products) on appetite, saliva production and food intake was evaluated. This research has been conducted at Wageningen University.

Chapter 3

Results and discussion

a) Taste sensitivity, food neophobia and liking

How these variables are related to the nutritional status?

The sensory properties of food are important determining factors of food choice. Taste sensitivity varies among individuals, and even when several studies have described differences between obese (OB) and normal-weight (NW) subjects concerning taste perception, the data are contradictory and not easy to compare.

In this context, taste sensitivity in NW and OB subjects was evaluated with the taste thresholds for the 4 basic tastes (sweet, salty, sour, and bitter) and for the fat stimulus. The number of the fungiform papillae (FP) in relation to nutritional status was also evaluated as additional measurement of taste acuity. We hypothesized that OB subjects could be less sensitive than NW subjects and maybe one factor that lead to this different perception might be related with the morphology of the tongue (e.g., FP).

A second research goal was to evaluate food liking and food neophobia according to subjects' BMI in order to study the relationship between these variables and taste sensitivity. We expect that any difference in taste sensitivity in relation to the nutritional status could reveal a different attitude toward foods (e.g., prefer high energy foods and not have a varied diet).

One hundred three adults, fifty-one OB patients admitted to the International Center for the Assessment of Nutritional Status (ICANS, Univeristy of Milan) and fifty-two NW subjects among the students and employees of the Faculty of Agriculture and Food Sciences of the University of Milan were recruited in the **Experiment 1** (see participant's characteristics in **Table 1**). NW and OB subjects were balanced according to gender ($\chi 2 = 1.58$; $p = 0.21$) and age ($df = 101$; $t = 1.72$; $p = 0.09$).

Table 1. Characteristics of study participants: data are reported as mean values ± SD or counts (modified from Proserpio et al., 2016a)

	All (n=103)	**NW (n=52)**	**OB (n= 51)**
Sex (F:M)	55:48	27:25	28:23
Age (years)	40.17 ± 10.79	38.38 ± 11.65	42.00 ± 9.61
BMI (Kg m^{-2})	27.76± 7.10	21.57 ± 1.95	34.08 ± 4.29

Taste sensitivity evaluation

The mean taste threshold values and the mean of fungiform papillae number (FP) in NW and OB subjects are shown in **Figure 1.**

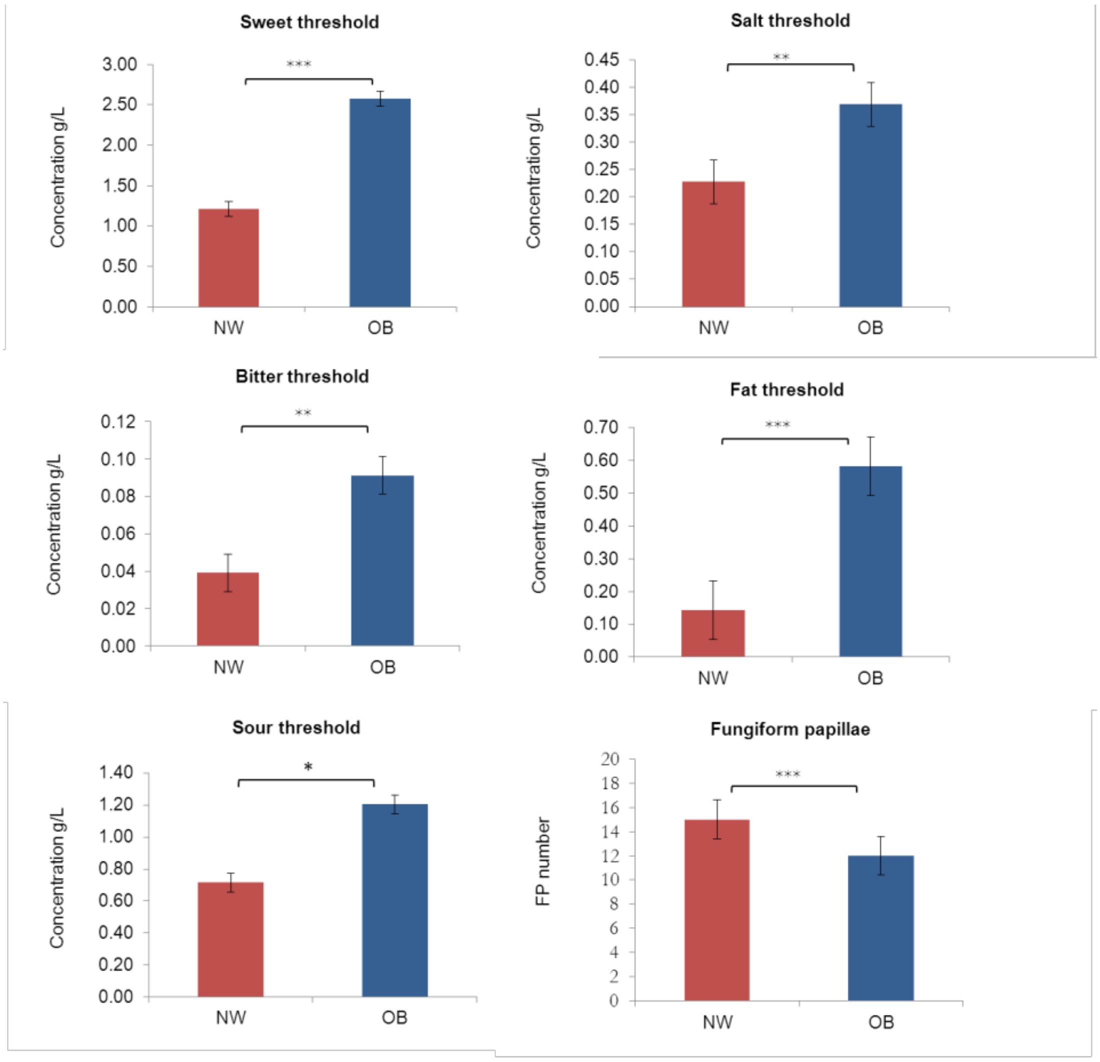

Figure 1. Mean taste thresholds (g/L) and mean FP (± SEM) in relation with nutritional status. * p< 0.05, ** p< 0.01, *** p< 0.001 (modified from Proserpio et al., 2016a)

As reported in **Figure 1** OB subjects showed a significant lower taste sensitivity than the control group for all the basic tastes and also for the fat stimulus: sweet taste: df=101, t=3.48, p<0.001; salty taste: df=101, t=2.98, p<0.01; bitter taste: df=101, t=3.00, p<0.01; fat sensation: df=101, t=4,42, p<0.001, sour taste: df=101, t=2.15, p <0.03. Moreover, OB subjects had a significant lower FP number compared to the NW subjects (df=101, t=4,04, p<0.001).

Accordingly to our results, Stewart and colleagues (2010; 2011) have recently suggested that overweight and obese individuals are less sensitive to fatty acids; therefore, this reduced taste acuity might lead to the consumption of excess dietary energy and weight gain. However, some studies have reported no significant association between fat sensitivity and the nutritional status (Alexy et al., 2011; Salbe et al., 2004).

The sense of taste and the somatosensory system in the oral cavity are the main pathways involved in fat perception and both sensory systems (the sense of taste and the somatosensory system) have a shared anatomical unit: the fungiform papillae (Mattes, 2009). Taste buds in the fungiform papillae enclose fatty acid receptors (Galindo et al., 2011) and mechanoreceptors (Whitehead et al., 1994). Consequently, a higher amount of fungiform papillae may intensify the perception of fat via strengthened tactile and chemosensory perception. Currently, there is little evidence about the impact of the number of fungiform papillae on fat perception. Only Hayes and Duffy (2007) investigated the relationship between the fungiform papillae number on the tongue and the perception of the fat-related attribute creaminess. In this context, they showed that subjects with a higher fungiform papillae number gave higher creaminess scores to milk–cream mixtures compared to subjects with a lower number of fungiform papillae. Moreover, the amount of fungiform papillae seems to be associated not only with the perception of fat but also in general with taste perception. Indeed, Essick and colleagues (2003) proposed that lingual tactile perception and taste sensitivities reflect individual differences in the density and diameter of fungiform papillae. This hypothesis seems to be confirmed by the present results, displaying that the fungiform papillae number is higher in lean subjects, who are also more sensitive than obese subjects to all the stimuli investigated.

The present results agree with most of literature data showing that subjects with higher BMI are less sensitive to both bitter (Tepper & Ullrich, 2002; Goldstein et al., 2005) and sweet (Bartoshuk et al., 2006; Simchen et al., 2006; Overberg et al., 2012) tastes than lean subjects. However, in some studies, no association between sweet (Salbe et al., 2004; Alexy et al., 2011) and bitter (Kaminski et al., 2000; Yackinous & Guinard, 2002; Drewnowski et al., 2007) tastes sensitivity and weight status was stated, and one study even reported a positive relationship (Paquet et al., 2010).

Little is known about the sensitivity to salt and sour tastes in relation to the nutritional status of the subjects. Literature evidences reported reduced taste sensitivity in subjects with higher BMI for salty (Simchen at al., 2006; Overberg et al., 2012) and sour (Simchen et al., 2006; Bertoli et al., 2014) tastes, but again, the results are often inconsistent, with studies showing either no relationship between BMI and sensitivity (Alexy et al., 2011; Overberg et al., 2012) or a positive association (Paquet et al., 2010).

The discrepancy between studies may be attributable to differences in the methods used to measure taste perception. In particular, the discrepancies could be due to the stimuli used to elicit the tastes.

Relationship between food neophobia and taste sensitivity

Internal consistency of the questionnaire (The Food Neophobia Scale, FNS; Pliner & Hobden, 1992) translated into Italian was satisfactory (Cronbach's alpha=0.83; n=10).

No significant differences were found in the food neophobia scores among subjects according to BMI (NW=28.21±9.80; OB=28.59±9.82).

Obviously, not only factors associated with taste perception could lead to weight gain. Evidence suggests that body weight could be associated to personality traits such as food neophobia (Raudenbush al., 2003).

Indeed, food neophobia might manifest as a limited variety of food in the diet, thus leading to a reduced overall food intake and, in turn, to a reduced energy intake; in contrast, food neophobics could prefer to consume traditional food with a higher energy density compared with healthier food, which results in a higher BMI (Laureati et al., 2015). However, the results of the present study did not find a relationship between BMI and food neophobia, accordingly to findings observed in children (Laureati et al., 2015) and young adults (Knaapila et al., 2011).

To further interpret the relationship between taste sensitivity and food neophobia, the subjects were divided according to their level of taste sensitivity for each sensation and FP into 2 groups:

a) “high sensitivity”: adults with a taste threshold less than the median taste threshold group and FP density above or equal than the median FP density group; sweet: 1.61 g/L, salt: 0.35 g/L, bitter: 0.03 g/L, fat: 0.14 g/L; 0.61 g/L and FP: 13;

b) “low sensitive”: adults with taste threshold above or equal to the median taste threshold group and FP density less the median FP density group; sweet: 1.61 g/L, salt: 0.35 g/L, bitter: 0.03 g/L, fat: 0.14 g/L; 0.61 g/L and FP: 13.

As reported in **Table 2,** only a significant difference in food neophobia scores between the two groups was found for the salty taste (df=101; t= 2.85; p<0.01). In particular, high sensitive subjects appear to be more neophobic than the low sensitive.

Table 2. Food neophobia score (FNS, mean value ± SD) in relation with taste sensitivity: “low sensitive” and “high sensitive”. Values in bold show the significant differences (modified from Proserpio et al., 2016a)

Taste sensitivity	**Food neophobia score (FNS)**			
	Low sensitive	**High sensitive**	**t**	**p**
Bitter	29.4 ± 9.06	27.49 ± 10.34	1.01	0.315
Sweet	28.60 ± 10.06	28.23 ± 9.64	0.19	0.848
Salty	25.6 ± 9.43	30.82 ± 9.47	2.85	**0.005**
Sour	28.64 ± 10.47	28.29 ± 9.57	0.16	0.870
Fat	29.27 ± 9.92	27.54 ± 9.63	0.91	0.364
FP	28.34± 10.18	25.93 ± 10.60	1.78	0.077

Regarding the relationship between food neophobia and taste sensitivity, it seems from the present results that subjects who are more sensitive to salty taste are significantly more neophobic than less sensitive individuals, which suggests that higher taste sensitivity might lead to neophobic reactions. In agreement with the present findings, Carter and colleagues (2000) reported that taste sensitivity for bitterness is positively related to the food neophobia attitude.

Liking assessment

Mean hedonic ratings for different product categories (carbohydrates, seasoning, sweets, fruits, dairy products, animal derivatives and vegetables) for NW and OB subjects are reported in **Table 3.**

Table 3. Mean hedonic ratings ± SD in NW and OB subjects. Values in bold show the significant differences (modified from Proserpio et al., 2016a)

Food products category	NW (n=52)	OB (n= 51)	t	p
Animal derivatives	6.29 ± 2.33	7.42 ± 2.00	2.63	**0.01**
Sweets	5.43 ± 1.96	6.14 ± 2.10	1.78	0.08
Seasoning	5.07 ± 2.28	6.03 ± 2.24	2.17	**0.03**
Carbohydrates	7.01 ± 2.67	7.91 ± 1.47	2.11	**0.04**
Dairy products	6.22 ± 2.67	6.59 ± 2.60	0.70	0.50
Fruits	6.58 ± 2.03	7.02 ± 2.17	1.04	0.30
Vegetables	5.83 ± 1.84	6.23 ± 1.94	1.05	0.30

As shown in **Table 3** subjects differed in liking scores for carbohydrates, seasoning, and animal derivatives. In particular, OB gave significantly higher scores for these food product categories than did NW.

The foods investigated in the liking questionnaire were also categorized according to energy density: “low energy dense” (<100 kcal/100 g) and “high energy dense” (>100 kcal/100 g). The mean liking data provided by OB and NW subjects are reported in **Figure 2.**

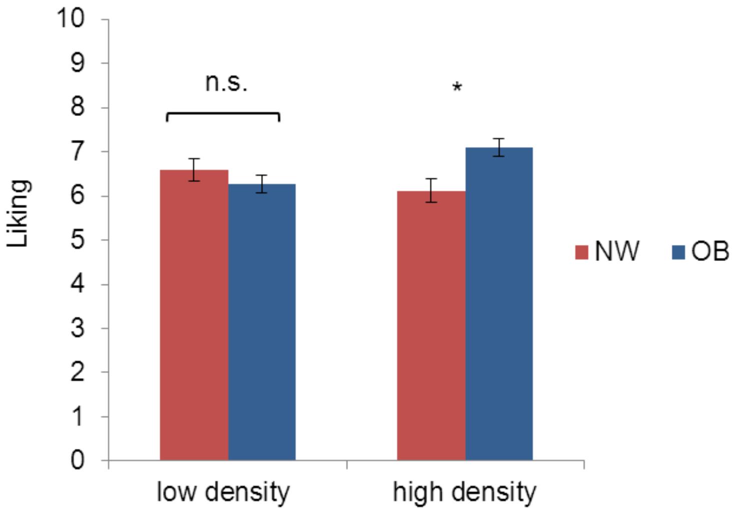

Figure 2. Mean hedonic ratings (± SEM) for "low energy dense" and "high energy dense" products in relation to subjects' nutritional status. * p< 0.05 (modified from Proserpio et al., 2016a)

As can be observed, NW and OB subjects provided comparable liking scores for "low energy dense" food products (df= 101; t=-1.05; p=0,29), whereas OB subjects gave significantly higher liking scores (df= 101; t=-2,51; p<0,05) for "high energy dense" food products compared to NW subjects.

The assumption that overweight and obese people have a higher liking for certain types of taste stimuli that contributes to an excess energy intake has been investigated previously (Mela & Rogers, 1988). In accordance with the present findings, it has been reported a positive relationship between liking and both fat-sweet content and high-calorie products (Cox et al., 2016).

In particular, fat preference may have a greater impact on body weight compared with sweet preference. In this context, literature data show that obese women may prefer foods that are less sweet but higher in fat compared with normal-weight women (Drewnowski et al., 1985). This difference could be due to genetic and behavioral factors, but this relationship is still under discussion.

Some limitations should be considered when evaluating the results of this study. First, the two groups of subjects haven't been matched on possible factors that may affect their attitudes towards foods, such as restraint eating, health attitudes and also cognitive factors affecting their attention. Second, olfactory thresholds, which could be useful to study in a more exhaustive way subjects' perception, were not evaluated.

Third, taste threshold assessed in water are not representative of real foods, thus future researches are needed to study the different perceptions in models foods and considering all the sensory modalities. Fourth, only a single stimulus for each sensation was used; it might be interesting to evaluate whether different stimuli affect the results. Fifth, the assessment of food liking was made without really administering the food products. These aspects should be considered in future investigations of the perceptive and behavioral factors which could be involved in the development of obesity.

b) Multisensory interactions in food perception

How do these mechanisms occur in NW and OB subjects and affect food liking?

Deepening the study of the associative learning basis of sensory perception and preference has important practical implications. It is well-known that there is a multifactorial control of food intake and thereby body weight (Salbe al., 2004). In particular, the cross-modal sensory interactions have been reported to be effective in increasing satiety since they can elicit a perceived enhancement of gustatory, olfactory and/or somatosensory sensations through a mechanism of brain integration (McCrickerd et al., 2012, 2014).

Nowadays, few studies have been conducted to investigate whether and how OB people differ from NW subjects in the way they integrate sensory inputs and how this can translate in a difference in hedonic perception.

In this context, the effects of variation in aromas and thickening agent on sensory attributes perception and liking of a model custard dessert were evaluated in NW and OB women. Subjects rated their liking and the intensity of sensory properties (sweetness, vanilla and butter flavors, and creaminess) of 3 block samples (the first varied in vanilla aroma, the second varied in butter aroma and the third varied in xanthan gum). We expected that sensory integration of the sensory signals would occur differently according to subjects' BMI, and that the overall liking of the model foods would be changed in women with a different nutritional status.

The attention has been focused to these modalities (sweetness, vanilla and butter flavors, and creaminess) since they have been reported to influence liking, satiety and, thus, they might play a role in body weight control. Indeed, it is recognized that the perception of sweet taste is directly associated to liking and the increase of weight (Rodin et al., 1976, Laureati et al., 2015). Vanilla aroma, as well, is known to be related to the increased perception of sweet taste (Small & Prescott, 2005), whereas we assumed that butter aroma, might elicit the perception of thickness and fattiness. We also modified the creaminess of the custard desserts since texture has been suggested to play a key role in the increase of satiety (McCrickerd et al. 2012, 2014).

Forty-one OB women with BMI over 30 kg/m^2 (mean age= 50.29 ± 11.49; BMI= 35.70 ± 4.14 kg/m^2), who were patients admitted to the Department of Medical Sciences and Rehabilitation (Istituto Auxologico Italiano), and forty-one NW women among the students and employees of the Faculty of Agriculture and Food Sciences of the University of Milan (mean age= 47.58 ± 9.75 years; BMI= 21.90 ± 2.90 kg/m^2) were recruited in the **Experiment 2**. The two groups of women were balanced according to age (df= 80; t = 1.15; p= 0.25).

Intensity ratings and liking of custards modified with vanilla aroma

The perceived intensity of sweetness, vanilla and butter flavors and creaminess of samples with increasing concentration of vanilla aroma (ST=unmodified sample, V_1 and V_2 experimental samples prepared by adding either 0.1% or 0.3% of vanilla aroma) for NW and OB women are reported in **Figure 3(a-d)**.

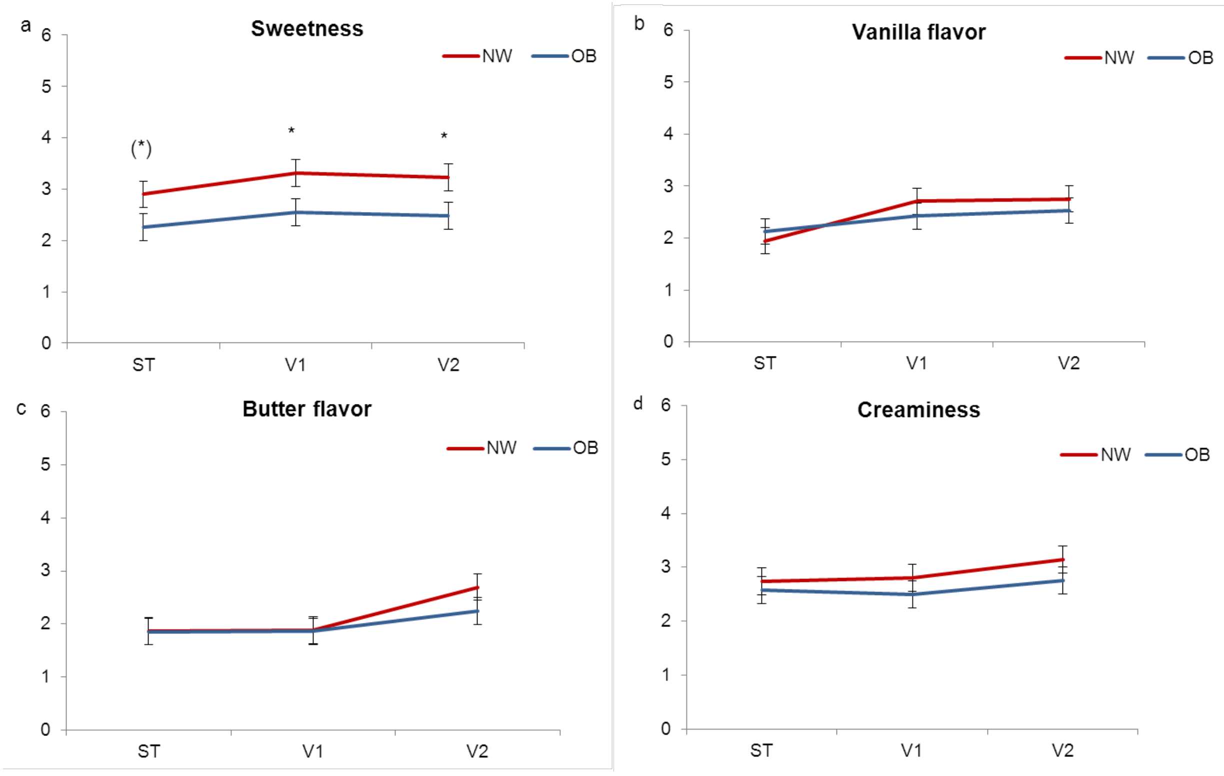

Figure 3(a-d). Intensity ratings (±SEM) of sweetness, vanilla flavor, butter flavor and creaminess for the samples differing in vanilla aroma (ST, V_1, V_2) for NW and OB women. For each attribute and each sample, significant differences between OB and NW women, detected according to t-test, are indicated by (*) for p<0.10 and * for p<0.05, respectively (modified from Proserpio et al., 2016b).

ANOVA results revealed that the interaction BMI*Samples had a significant effect ($F_{(5,240)}$=2.59, p< 0.05) only on the perceived intensity of sweetness (**Figure 3a**).

Contrary to our expectations the addition of vanilla aroma did not increased the sweetness perception in either groups. However, NW women (red line) gave significantly higher scores than OB subjects to all samples (ST: p<0.10; V_1: p<0.05; V_2: p<0.05). It is possible to hypothesize that the addition of vanilla aroma was not enough to lead to detectable changes in taste perception. However, this doesn't seem to be the case, as the addition of vanilla aroma was clearly perceived by NW women. Indeed, NW women rated V_1 (p<0.05) and V_2 (p<0.05) samples as more flavored than the standard (**Figure 3b**).

Accordingly to the present results, Green et al. (2012) found that the enhancement of sweet taste was inconsistent with added vanillin in custard dessert. However, other previous studies showed that adding food odors, such as strawberry or vanilla, to water solutions of tastants such as sucrose, enhanced their sweetness (Frank & Byram

1988; Frank et al., 1989). The difference between the results could be due to the different matrices used to investigate the multisensory interactions. Indeed, results obtained with simple-single ingredient system, such as water solutions, may not be applicable to real life situations compared to complex food matrices (Drewnosky et al., 1985).

Concerning butter flavor (**Figure 3c**), only the sample with the highest concentration of vanilla aroma (V_2) was perceived by NW women as more intense ($p<0.05$) than the other samples. Thus, an odor-flavor interaction was found only in the control group of NW women, who perceived an increase in butter flavor.

The addition of vanilla odor did not increase the perceived creaminess in either OB or NW women (**Figure 3d**). Contrary to the present findings, some authors showed that texture attributes, such as fattiness and creaminess, were affected by flavorings (De Wijk et al., 2003).

One hypothesis that can be forwarded to explain the lack of effect of adding vanilla on perception of other sensory modalities is that this flavoring was added to boost the aroma of custard that, in its original formulation, was already vanilla-flavored. In some cases, it has been showed that simple flavor amplification (e.g., adding vanilla to a vanilla dessert) might be less effective than flavor enhancement (e.g., adding butter flavor to a vanilla dessert) (Drewnoski et al., 2002; Laureati et al., 2008).

The liking scores obtained for the samples with increasing concentration of vanilla aroma (ST, V_1, V_2), for NW and OB women are shown in **Figure 4.**

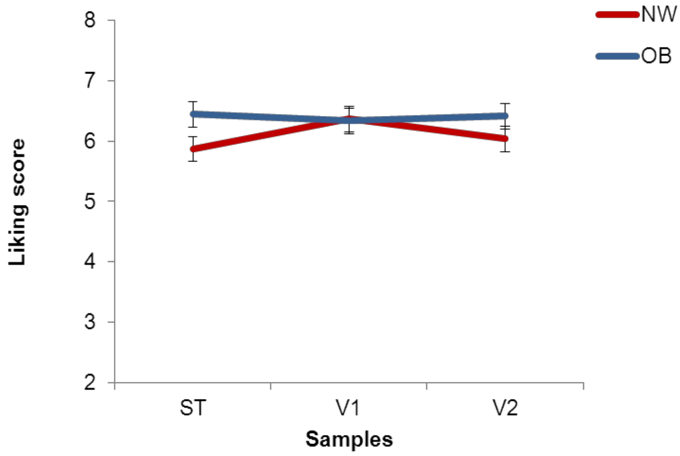

Figure 4. Liking score (± SEM) obtained for the blocks of samples differing in vanilla aroma (ST, V_1, V_2) for NW and OB women subjects (modified from Proserpio et al., 2016b).

ANOVA results showed that the interaction BMI*Samples hadn't a significant effect on the liking scores. Indeed, the addition of vanilla aroma to the standard recipe did not influence significantly liking in either OB (blue line) or NW women (red line).

The lack of the difference obtained in the hedonic scores could be related to the poor changes in the sensory attributes perception between the modified samples (V_1 and V_2) and the standard custard (ST).

Intensity ratings and liking of custards modified with butter aroma

The perceived intensity of sweetness, vanilla and butter flavors and creaminess of samples differing in butter aroma (ST=unmodified sample, B_1 and B_2 experimental samples prepared by adding either 0.05% or 0.1% of butter aroma) for NW and OB women are reported in **Figure 5(a-d)**.

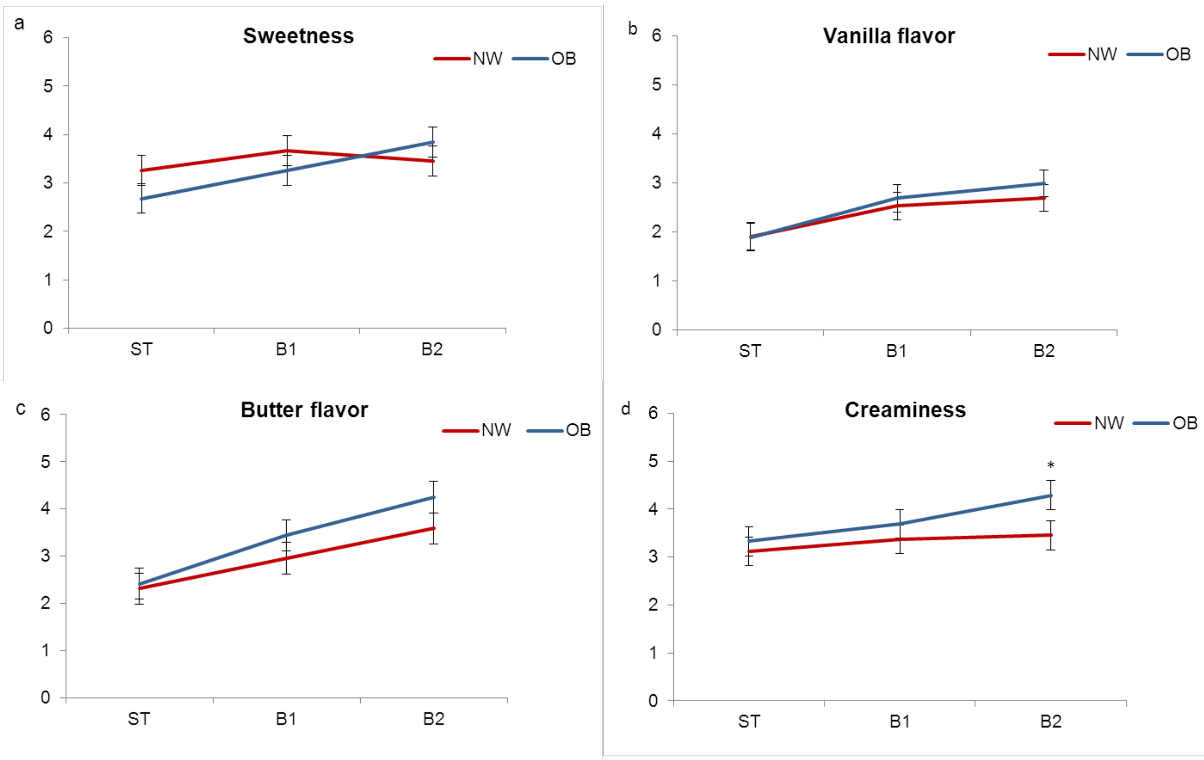

Figure 5(a-d). Intensity ratings (±SEM) of sweetness, vanilla flavor, butter flavor and creaminess for the samples differing in butter aroma (ST, B_1, B_2) for NW and OB. For each attribute and each sample, significant differences between OB and NW women, detected according to t-test, are indicated by * for p<0.05 (modified from Proserpio et al., 2016b)

ANOVA results revealed that the interaction BMI*Samples had a significant effect on the perceived intensity of butter flavor ($F_{(5,240)}=5.09$, $p<0.001$) and a marginal effect on creaminess ($F_{(5,240)}=1.78$, $p=0.10$).

The addition of butter aroma increased the butter flavor perception in both groups (**Figure 5c**), but OB women discriminated both B_1 ($p<0.05$) and B_2 ($p<0.0001$) from the standard custard, whereas NW subjects perceived an increase of the intensity only at the highest concentration (B_2, $p<0.01$). Only OB women, who rated the B_2 samples as creamier ($p<0.05$) than the standard, perceived an increase in creaminess (**Figure 5c**).

Sweetness intensity (**Figure 5a**) was increased by the addition of butter aroma only in the group of OB women, who rated the B_2 sample significantly sweeter ($p<0.01$) than the standard. Moreover, OB women gave to B_2 a higher score than NW subjects ($p<0.05$).

Vanilla flavor perception was enhanced by the addiction of butter aroma similarly in both groups, with the B_1 ($p<0.05$) and B_2 ($p<0.0001$) samples perceived as comparable between them and more intense than the ST (**Figure 5b**).

Considering samples modified with butter aroma odor-taste, odor-flavor and odor-texture interactions were highlighted in OB women, and an odor-flavor interaction in the control group.

Butter aroma addition, without adding calories, increased obese females' perception of sweet taste, vanilla flavor and creaminess, which are all desirable sensory attributes in a custard dessert. Therefore, adding an odor that is normally associated with a high-fat food led to different cross-modal integrations in a more effective way, while maintaining high the liking degree of obese women as shown in **Figure 6.**

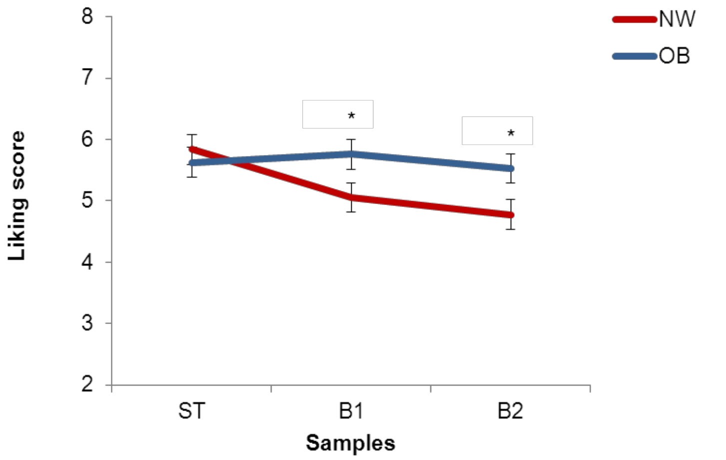

Figure 6. Liking score (± SEM) obtained for the blocks of samples differing in butter aroma (ST,B_1,B_2) for NW and OB women subjects**.** For each sample, significant differences between OB and NW women detected according to t-test are indicated by * for p< 0.05 (modified from Proserpio et al., 2016b).

ANOVA results revealed that the interaction BMI*Samples had a significant effect ($F_{(5,240)}$=2.94, p<0.05) on the liking scores.

OB women gave higher liking scores for B_1 (p<0.05) and B_2 (p<0.05) samples than NW women. Moreover, considering the group of OB women, the addition of the aroma did not influence the liking, because all samples had comparable scores. By contrast, significant differences were observed in NW women, with the standard custard (ST) being significantly more liked than the two modified samples B_1 and B_2 (p<0.01; p<0.05 respectively) which were comparable to each other.

OB women gave to samples added with butter aroma liking scores that were higher than NW women, supporting the hypothesis that a greater liking could result in more consumption of this type of products by overweight women. Everyone tend to eat more of a palatable food than one that is not. However, those who are OB respond to pleasant foods by eating even more than do NW individuals (Nisbett, 1968; Salbe et al., 2004). Accordingly, overweight subjects have been reported to prefer fatty and sweet foods (Drewnoski et al., 1985; Cox et al., 1998; Bartoshuk et al., 2006). In the context of preventing overconsumption and thus overweight, intensifying a flavor may help to elicit the link between the flavor of a food and its metabolic outcomes. Considering that the consumption of sweet and fat foods can directly activate reward mechanisms in the brain related to energy density (Lenoir et al., 2007; De Araujo et al., 2008), food odors have the important role of providing a unique sensory signature that identifies a source of nutrition (Hajnal, 2009). This assumption is supported by recent neuroimaging studies that identified specific brain regions for sensory information on the mouthfeel, viscosity, odor, and even the pleasantness of fat. These brain loci are also accessed by satiety and physiological signals (De Araujo & Rolls, 2004; Rolls, 2004).

Intensity ratings and liking of custards modified with thickener agent

The perceived intensity of sweetness, vanilla and butter flavors and creaminess of samples differing in creaminess (ST=unmodified sample, XG_1 and XG_2 experimental samples prepared by adding either 1% or 1.5% of xanthan gum) for NW and OB women are reported **in Figure 7(a-d)**.

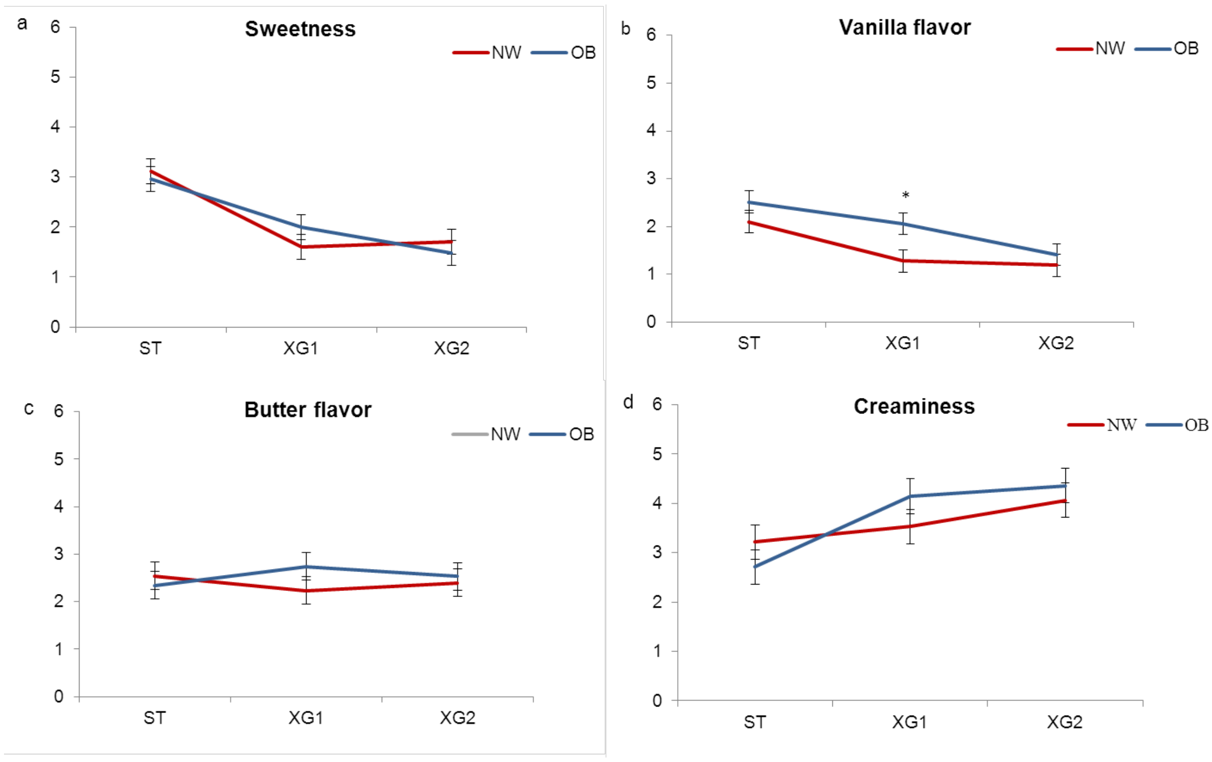

Figure 7(a-d). Intensity ratings (± SEM) of sweetness, vanilla flavor, butter flavor and creaminess for the samples differing in creaminess (ST, XG_1, XG_2) for NW and OB women. For each attribute and each sample, significant differences between OB and NW women, detected according to t-test, are indicated by * for $p<0.05$ (modified from Proserpio et al., 2016b).

ANOVA results revealed that the interaction BMI*Samples had a significant effect on the perceived intensity of sweetness ($F_{(5,240)}= 8.23$, $p<0.0001$), vanilla flavor ($F_{(5,240)}=5.59$, $p<0.0001$) and creaminess ($F_{(5,240)}= 3.25$, $p<0.01$).

The addition of the thickening agent decreased the perceived intensity of sweet taste (**Figure 7a**) and vanilla flavor (**Figure 7b**) in both groups of subjects. OB and NW women perceived the XG_1 ($p<0.0001$) and XG_2 ($p<0.0001$) samples of equal intensity and as less sweet than the standard (**Figure 7a**). NW women perceived a decrease of vanilla flavor intensity (**Figure 7b**) already in the XG_1 sample ($p<0.05$), whereas OB females only at the highest concentration (XG_2, $p<0.0001$). Moreover, the XG_1 sample was rated significantly higher ($p<0.05$) for vanilla flavor by OB women than the control group (**Figure 7b**). The addition of the thickening agent produced an increase in the perceived intensity of creaminess only in OB women, who rated the XG_1 ($p<0.01$) and XG_2 ($p<0.01$) samples as creamier than the standard (**Figure 7d**).

Considering the samples modified with the thickener agent texture-taste and texture-odor interactions were found in both groups of subjects, but in a negative and unexpected direction. The addition of the thickener, indeed, although devoid of taste and smell, significantly decreased the perception of sweet taste and vanilla flavor. This is probably the explanation of the decreased liking scores observed in both groups of women, as shown in **Figure 8.**

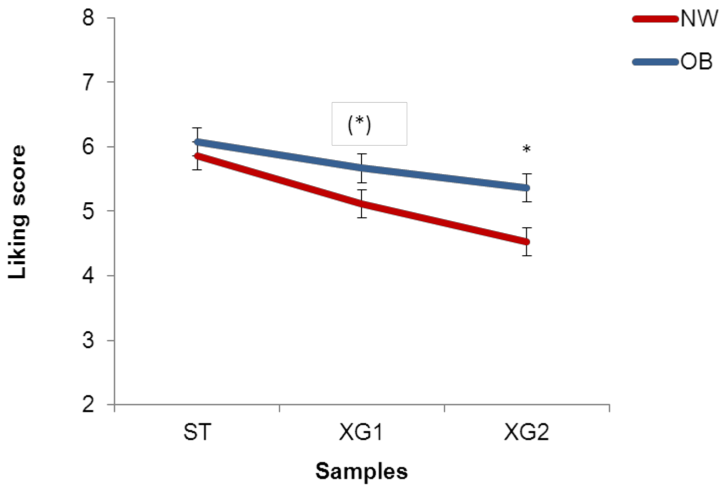

Figure 8. Liking score (± SEM) obtained for the blocks of samples differing in creaminess (ST, XG_1, XG_2) for NW and OB women**.** For each sample, significant differences between NW and OB women detected according to t-test are indicated by (*) and * for $p<0.10$; $p< 0.05$, respectively (modified from Proserpio et al., 2016b).

ANOVA results showed that the interaction BMI*Samples had a significant effect ($F_{(5,240)}=6.10$, $p<0.001$) on the liking scores.

OB women liked the samples with the addition of xanthan gum significantly more than NW women (XG_1: $p<0.10$, and XG_2: $p<0.05$). However, in both groups of women, liking decreased with increased creaminess, especially for NW women. For this group, the standard custard (ST) was significantly more liked than both added samples (XG_1, $p<0.05$; XG_2, $p<0.0001$), which were comparable to each other. For OB subjects, only the sample XG_2 was significantly less pleasant than the unmodified sample ST ($p<0.05$).

This outcome could be explained by physico-chemical effects such as limiting access of tastants and odorants to their receptors. In agreement with the present results, some authors proposed that somatosensory stimuli can interact with taste and smell, modifying their perception (Hollowood et al., 2002; Weel et al., 2002); indeed, increasing the viscosity of a solution has been reported to decrease, in some cases, both taste and flavor intensity (Pangborn & Szczesniak, 1974; Baloga et al.,1994).
It would be interesting in future studies to modify creaminess through the addition of another thickening agent that increase instead of decreasing the liking and the perceived intensity of desirable sensory characteristics in a custard dessert. Indeed, more dense products could create a higher expectation of satiety and therefore decrease consumption (McCrickerd et al., 2014).

Overall, obese subjects seemed to pay more attention to stimuli signaling high-calorie products, such as butter aroma. The addition of the butter aroma, which modified the perception of different sensory characteristics for OB women, could have interesting implications from the viewpoint of food products development. It is plausible that obese individuals might be more satisfied from consuming these products, even with a lower calorie density. In this perspective, the study of multisensory integration in relation to nutritional status could have implications in the development of lower-calorie foods that are still satisfying for the consumer.

BMI and gender-related differences in multisensory integration of food modalities deserve further investigation in view of the possible implications they may have in nutritional approach of overweight individuals.
The query of whether men and women differ in their ability to smell has been studied for over a hundred years. Literature data suggest that, for at least some odorants, women are more sensitive in odor detection, identification, discrimination, and memory tests than men (Doty & Cameron, 2009). However, findings on this topic remain controversial (Amoore & Venstrom, 1966; Venstrom & Amoore, 1968; Punter, 1983).

The investigation of food cross-modal interactions in relation to BMI and gender was deepened. To this purpose, the perception of sensory properties (sweetness, vanilla and butter flavors and creaminess) and liking in model custards added with increasing concentration of butter aroma, signaling high-energy dense product, were investigated. We expected that sensory integration, and thus the liking of the samples, occurs differently in relation to nutritional status and gender. This is one of the first studies that examined how multisensory perception, and thus liking, occurred in relation to both the nutritional status and gender.

A standard custard dessert was modified with different concentrations of butter aroma, since it has been proposed that the addition of an aroma, which is reminiscent of something high-energy dense and fat affects the perception of some sensory modalities, such as sweetness, more in OB women than in NW women (Proserpio et al., 2016b). It has been choose to add an aroma which was not present in the original custard recipe, since this could be more effective in changing the intensity perception of sensory stimuli (Laureati et al., 2008) than adding an aroma already present in the product, such as vanilla.

Forty-six obese subjects among patients referred to the Istituto Auxologico Italiano (Milan, Italy) and forty-five normal-weight subjects among the students and employees of the Faculty of Agriculture and Food Sciences of the University of Milan were involved in **Experiment 3** (see participant's characteristics in **Table 4**).

Table 4. Characteristics of study participants (data are reported as mean values ± SD or counts) (modified from Proserpio et al., 2017a)

	All (n= 91)	**NW (n=45)**	**OB (n=46)**
Sex (F:M)	47:44	21:24	26:20
Age (years)	44.79 ± 9.74	41.64 ± 8.22	47.86 ± 10.02
BMI (Kg m^{-2})	29.86 ± 8. 72	22.03 ± 2.14	37.52 ± 5.07

Sweetness perception

The mean intensity ratings (± SEM) of perceived sweetness by BMI and gender, provided for each sample (ST=unmodified sample, B_1 and B_2 experimental samples prepared by adding either 0.05% or 0.1% of butter aroma), are shown in **Figure 9.**

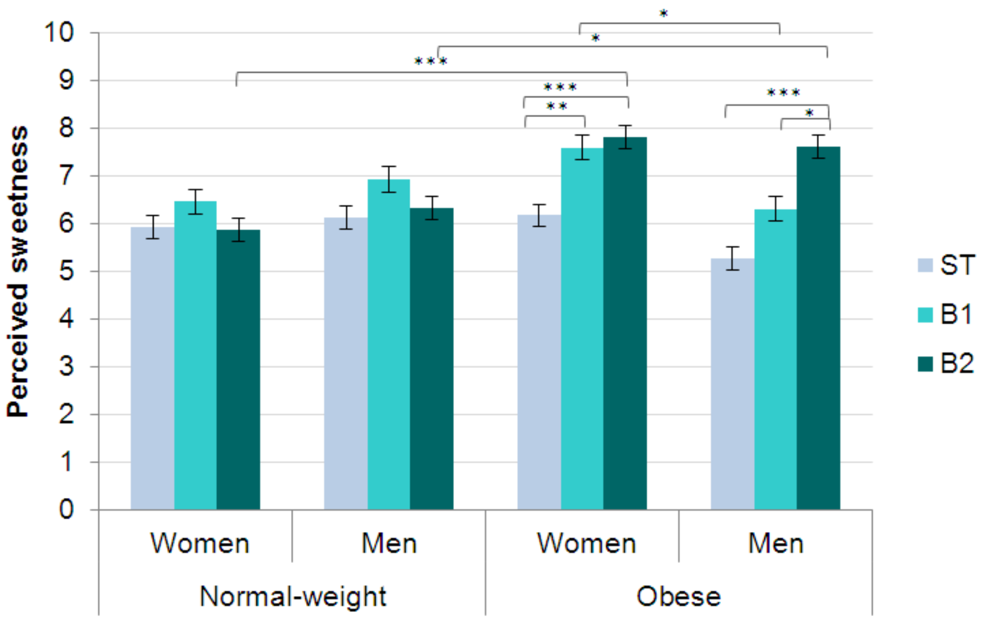

Figure 9. Mean sweetness intensity ratings (± SEM), provided for each sample, by BMI and gender. * p<0.05, ** p<0.01, *** p<0.001 (modified from Proserpio et al., 2017a)

A significant BMI effect on perceived sweetness was found ($F_{(1,261)}$= 13.36, p<0.001). This effect was mainly driven by B_2 sample which was perceived significantly sweeter by OB subjects compared to NW subjects (M=7.71 ± 0.25; M=6.10 ± 0.24, respectively).

Accordingly to our results, several researches have provided strong support to the ability of some odors to modify taste qualities. Indeed, during eating, odors that typically induce sweet taste appear to be related to previous co-exposure with a sweet taste (Stevenson et al., 1998; Prescott, 2004). For example, the odors of strawberry, mint and caramel produced sweetness enhancement in Western countries where people often experience those odors with sucrose (Nguyen et al., 2002). In this case, it could be possible to hypothesize that butter, used usually as an ingredient also in the preparation of sweet products, elicits an enhancement of the perceived sweetness.

A Gender effect was not found ($F_{(1,261)}$= 2.26, p=0.13). Moreover, the interaction Gender*BMI*Samples had a significant effect on perceived sweetness ($F_{(7,261)}$= 7.50;

p<0.0001). According to *post hoc* comparison, considering the NW subjects, the sweetness perception was not affected by the increasing concentration of butter aroma and no gender-related differences were found. Contrariwise, considering the OB subjects, the addition of butter aroma to the custard dessert influenced significantly the perceived sweetness in both sexes. Indeed, OB women perceived the modified samples, which were comparable to each other, as sweeter than the unmodified sample ST (B_1: p<0.01; B_2: p<0.0001). OB men perceived an increase in sweet taste only in sample B_2 (p<0.0001), which was perceived also sweeter than B_1 (p<0.05). Gender-related differences in sweetness perception only for B_1 sample were found, with OB women providing higher scores (p<0.05).

Significant differences were observed between NW and OB subjects. The sample with the highest concentration of butter aroma (B_2) was perceived as significantly sweeter by OB subjects than by NW subjects for both sexes (women: p<0.0001; men: p<0.05).

Vanilla flavor perception

The mean intensity ratings (± SEM) of perceived vanilla flavor by BMI and gender, provided for each sample, are shown in **Figure 10.**

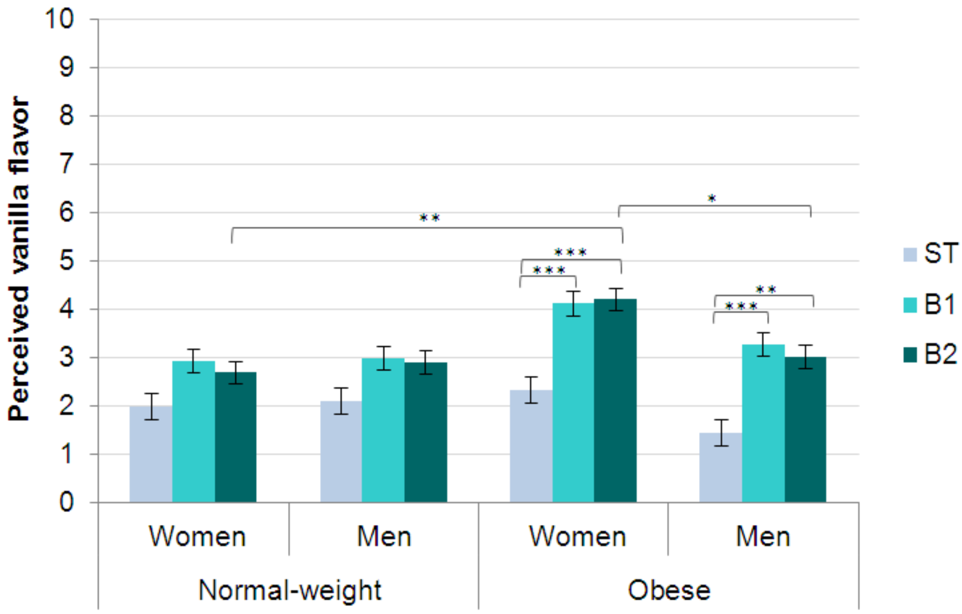

Figure 10. Mean vanilla flavor intensity ratings (± SEM), provided for each sample, by BMI and gender. * p<0.05, ** p<0.01, *** p<0.001 (modified from Proserpio et al., 2017a)

A significant BMI effect on perceived vanilla flavor was found ($F_{(1,261)}$= 9.40, p<0.01), with OB subjects who generally gave higher scores compared to NW subjects (M=3.06 ± 0.11; M=2.60 ± 0.11, respectively). Indeed, the addition of butter aroma increased significantly the perceived vanilla flavor only in OB subjects.

A Gender effect was also found ($F_{(1,261)}$= 7.78, p<0.01), with women providing higher scores compared to men (M=3.04 ± 0.10; M=2.62 ± 0.11, respectively). Moreover, the interaction Gender*BMI*Samples had a significant effect on perceived vanilla flavor ($F_{(7,261)}$= 3.27; p<0.01). According to *post hoc* test, considering the NW subjects, a similar trend in vanilla flavor perception between women and men was found. Vanilla flavor perception was not affected by the increasing concentration of butter aroma and no gender-related differences were found. Considering the OB subjects, the addition of butter aroma influenced significantly the perceived vanilla flavor. Indeed, the modified samples B_1 and B_2, which were comparable to each other, were perceived by both women and men with a more intense vanilla flavor than the unmodified sample ST (women B_1: $p<0.0001$; B_2: $p<0.0001$; men B_1: $p<0.001$; B_2: $p<0.01$). Gender-related differences were found, as OB women provided higher scores than men to all the samples, even if the difference was significant only for B_2 ($p=0.05$). NW and OB subjects differed in vanilla flavor perception only for B_2 sample, which was perceived as significantly more intense by OB women than by NW women ($p<0.01$).

There is less evidence of odor-odor interactions in the literature. In the present study the addition of the butter aroma increased the vanilla flavor perception in the OB subjects, especially women. Early research has shown that flavor interaction can result in various changes in perceived flavor when complex stimuli are used (Sydow et al., 1974). The present results could be explained by the assumption that OB subjects are over-responsive to external food cues and less responsive to internal cues, such as hunger or distress (Schachter,1968; Schachter & Rodin, 1974). In this context, Herman and Polivy (2008) suggested that certain people, such as obese and dieting individuals, are more affected by external food-related cues, such as palatability, smell, sight, texture and all cues that appeal directly to the five senses, than normal-weight subjects. Moreover, it has been shown that the exposure to the sight and smell of pizza, a high-energy dense product, in overweight individuals elicited a significantly greater salivary response and desire to eat than lean controls (Ferriday & Brunstrom, 2011). There is also evidence suggesting that olfactory stimulation with high-energy dense related food cues significantly influenced consumers' food choices (Chambaron et al., 2015). In this perspective the ‘cue reactiveness’ could be considered as a potential predisposing factor for overweight. Thus, the addition of an aroma could generate a greater and earlier satisfaction and probably enhance satiety. This assumption could be supported by earlier studies suggesting that the brain reaction to a retro-nasally sensed food odor may signal the perception of food enhancing feelings of satiation (Ruijschop et al., 2011).

Butter flavor perception

The mean intensity ratings (± SEM) of perceived butter flavor by BMI and gender, provided for each sample, are shown in **Figure 11.**

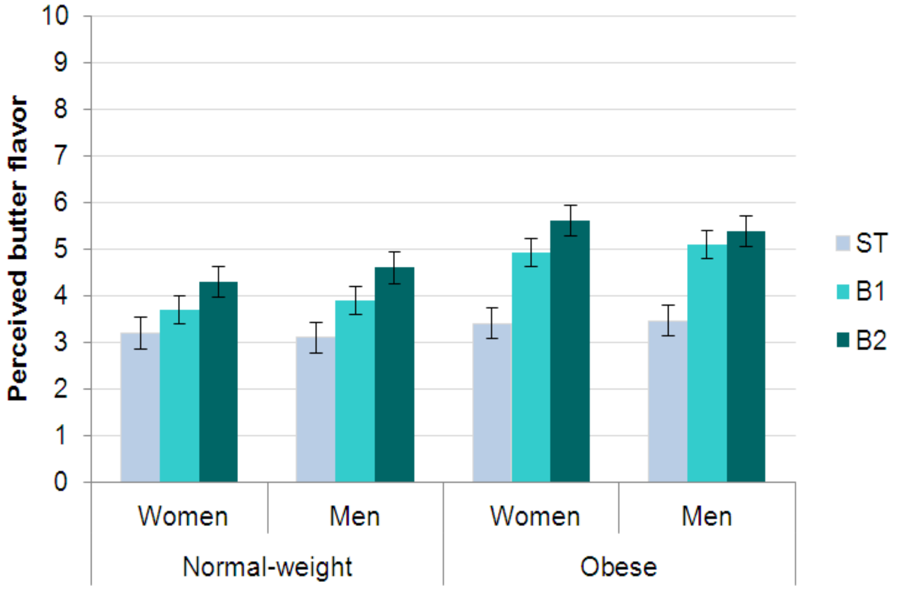

Figure 11. Mean butter flavor intensity ratings (± SEM), provided for each sample, by BMI and gender (modified from Proserpio et al., 2017a)

A significant BMI effect on perceived butter flavor was found ($F_{(1,261)}$=14.22, p<0.001). In particular, even if the addition of butter aroma was perceived by all subjects, OB generally gave higher scores compared to NW subjects (M=4.65 ± 0.13; M=3.96 ± 0.13, respectively).

The main factor Gender and the interaction Gender*BMI*Samples were not significant ($F_{(1,261)}$=0.04, p=0.85; $F_{(7,261)}$=0.96, p=0.46, respectively). The increasing concentration of butter aroma, without change the nutritional content of the custard, was perceived by all subjects, even if in a more intense way by OB than NW subjects.

Creaminess perception

The mean intensity ratings (± SEM) of perceived creaminess by BMI and gender, provided for each sample, are shown in **Figure 12.**

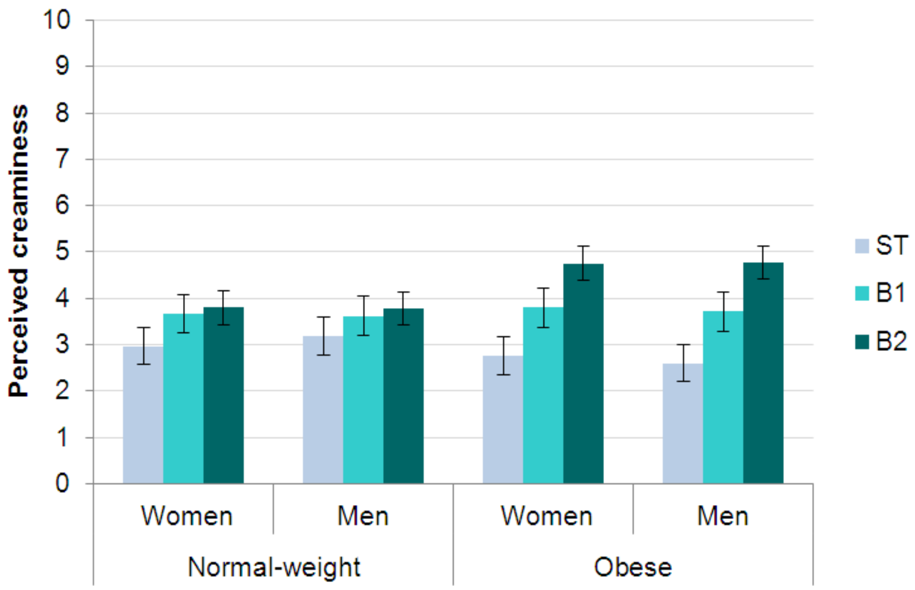

Figure 12. Mean creaminess intensity ratings (± SEM), provided for each sample, by BMI and gender (modified from Proserpio et al., 2017a)

The main factors BMI*,* Gender and the interaction Gender*BMI*Samples had not a significant effect on perceived creaminess ($F_{(1,261)}$= 0.72, p=0.40; $F_{(1,261)}$= 0.16, p=0.70; $F_{(7,261)}$= 1.61, p=0.14).

The addition of butter aroma did not produce a significant increase in creaminess perception in the modified samples (B_1 and B_2) independently from subjects' nutritional status and gender. However, a positive trend (p<0.07), driven mainly by B_2 sample, was found only in OB subjects who perceived this sample as the creamiest.

It has been demonstrated that the addition of a cream aroma to slightly complex matrices, like as semi-solid or gel form, can affect the texture perception with the increase in fat perception, creaminess and thickness (Tepper & Kuang, 1996; De Wijk et al., 2003; Bult et al., 2007). This could have interesting implication in the food development since a product perceived as creamier, without increasing the calories content, could create a higher expectation of satiety and therefore decrease consumption (McCrickerd et al., 2014). Contrary to expectation, gender-related differences in sensory perception were found only in the OB group although there are data supporting the different odor perception in relation to gender independently from the nutritional status (van Elst et al., 2000; Doty & Cameron, 2009). Even though the results are controversial (e.g. Cain, 1982; Lehrner, 1993; Koelega, 1994; Kobal et al., 2000), where gender differences exist in terms of odor detection, women are usually more sensitive to odors and perform better than men in olfactory functions. The present results showed that only OB women perceived more intense than OB men the sweetness and vanilla flavor, which were enhanced by the use of butter flavor. This could be supported by the results provided by Drewnowski and colleagues (1992), which showed that there is a tendency of obese women to prefer sweet food with a high fat content probably leading them to be more attentive to this type of stimuli.

Liking assessment

Mean liking scores (± SEM) by BMI and gender, provided for each sample, are shown in **Figure 13.**

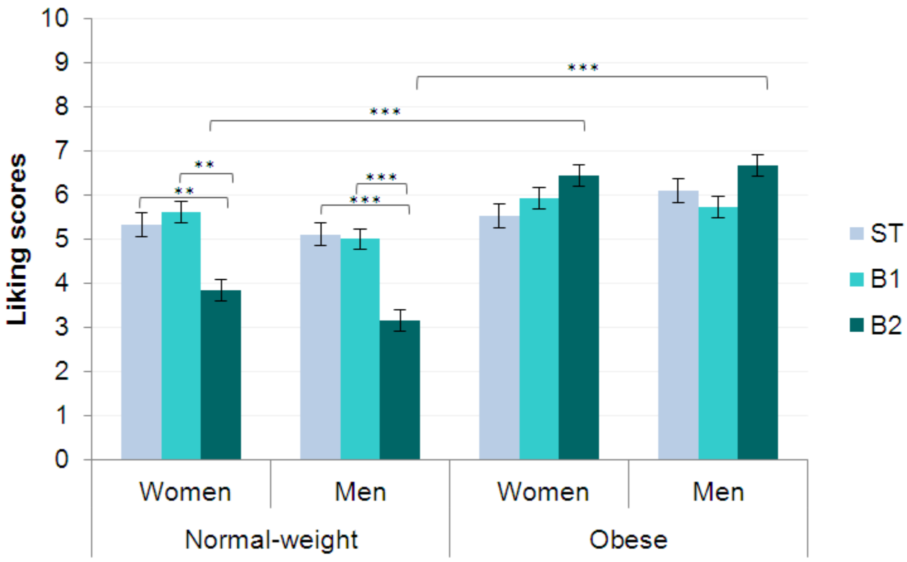

Figure 13. Mean liking scores (± SEM), provided for each sample, by BMI and gender. ** p<0.01, *** p<0.001 (modified from Proserpio et al., 2017a).

A significant BMI effect on liking scores was found ($F_{(1,261)}$= 83.29, p<0.0001), with OB subjects who generally gave higher scores compare to NW subjects (M= 6.06 ± 0.11; M= 4.67 ± 0.11, respectively).
A Gender effect was not found ($F_{(1,261)}$= 0.99, p=0.32). Moreover, mixed models analysis revealed that the interaction Gender*BMI*Samples had a significant effect on liking ($F_{(7,261)}$=9.80; p<0.0001). According to *post hoc* test, considering the NW subjects, a similar trend in liking scores was found in women and men, who both gave to the sample with the highest concentration of butter aroma (B_2) significantly lower liking scores compared to the samples ST (women: p<0.01; men: p<0.0001) and B_1 (women p<0.01; men: p<0.0001), which were comparable to each other. NW subjects were negatively influenced by the addition of butter aroma, while OB subjects provided significantly higher liking scores than the lean controls to the most flavored custard. No gender-related differences within the NW group were observed. Considering the OB subjects, for both women and men, liking increased with the addition of butter aroma though not significant (p<0.27). No gender-related differences within the OB group were observed. Significant differences in hedonic scores were seen between NW and OB subjects. The sample with the highest concentration of butter aroma (B_2) was liked significantly more by OB subjects than by NW subjects for both sexes (p<0.0001).

The significant differences in hedonic scores for the flavored custards observed between NW and OB subjects could be explained by the different results also obtained regarding the cross-modal interactions. Indeed, butter aroma, which is cognitively associated to fat foods, produced odor-taste and odor-odor interactions only in OB subjects. In other words, OB subjects perceived as sweeter and more flavored the modified custards without the addition of calories. Support to these findings is provided by Zellner and colleagues (1983) who hypothesized that sweetness perception reflects in changing the liking, increasing the hedonic response. Accordingly to our results, it has been proposed that overweight subjects prefer fatty and sweet foods (Bartoshuk et al., 2006). There is evidence suggesting that the hedonic response to foods is driven widely by our olfactory system. Moreover, overweight and obese subjects have better sensitivity and higher liking to food-related odors which have a clear association to high-energy dense food, such as chocolate (Stafford & Whittle, 2015).
Overall, the multisensory processes involved in food perception seem to occur differently in relation to BMI and, to a lesser extent, to gender. The observed

differences between obese women and men in sensory integration could have interesting implications in order to propose *ad hoc* dietary interventions in relation to gender. Moreover, deepening the study of the cross-modal interactions could help to better understand the processes driving the food acceptability considering that sensory properties perception influences the energy intake.

c) Odor cues in eating behavior

How does the ambient odor exposure influence salivation, appetite and food intake?

In view of the rapidly increased prevalence of overweight and obesity, it is important to clarify the different factors (including food odor exposure), involved in the processes leading up to actual intake. In this context, it is important to gain insight into how and under what conditions normal weight/lean people are affected by sensory food cues, such as the smell of food. It has been suggested that the modern Western food environment, which exposes individuals to copious cues of highly palatable and high energy dense foods, is driving the current obesity epidemic (Brownell et al., 2009).

The effects of ambient odor exposure, in a detectable but mild concentration, on behavioral and physiological measurements in normal-weight individuals were investigated. Our primary interest was to evaluate the influence of odors, signaling different types of food products, on appetite, saliva production and *ad libitum* food intake of a model food (chocolate rice). The participants were exposed to five different ambient odor conditions: beef (high energy savory), chocolate (high energy sweet), melon (low energy sweet), cucumber (low energy savory) and no odor. We hypothesized that food intake and appetite would increase upon exposure to congruent (e.g. exposure to chocolate odor, appetite/intake of chocolate product) versus incongruent odors (e.g. exposure to beef odor, appetite/intake of chocolate product). We further hypothesized that saliva production would increase upon exposure to food odors.

Thirty-two healthy, normal-weight women around Wageningen University were recruited in **Experiment 4** (see participants' characteristics in **Table 5**). Participants were asked to filling out a questionnaire on impulsivity behavior (BIS-11; Patton et al., 1995) and on reward sensitivity (BIS/BAS; Franken et al., 2005; Carver & White,1994).

Table 5. Characteristics of study participants (data are reported as mean values ± SD) (modified from Proserpio et al., 2017b).

Characteristic	Subjects (n=32)
Age (years)	21.4 ± 5.30
BMI (Kg m^{-2})	21.7 ± 1.90
BIS11 (Barratt Impulsiveness Scale)	67.2 ± 5.43
BIS/BAS:	
- Bis score (Behavioral Inhibition System)	15.1 ± 1.99
- Bas score (Behavioral Activation/Approach System)	25.2 ± 3.66

Influence of ambient odor exposure on intake

A significant effect of odor condition on participants' food intake was found ($F_{(4;123)}$=2.70; p<0.05). The amount of chocolate rice eaten in the various conditions is reported in **Figure 14**.

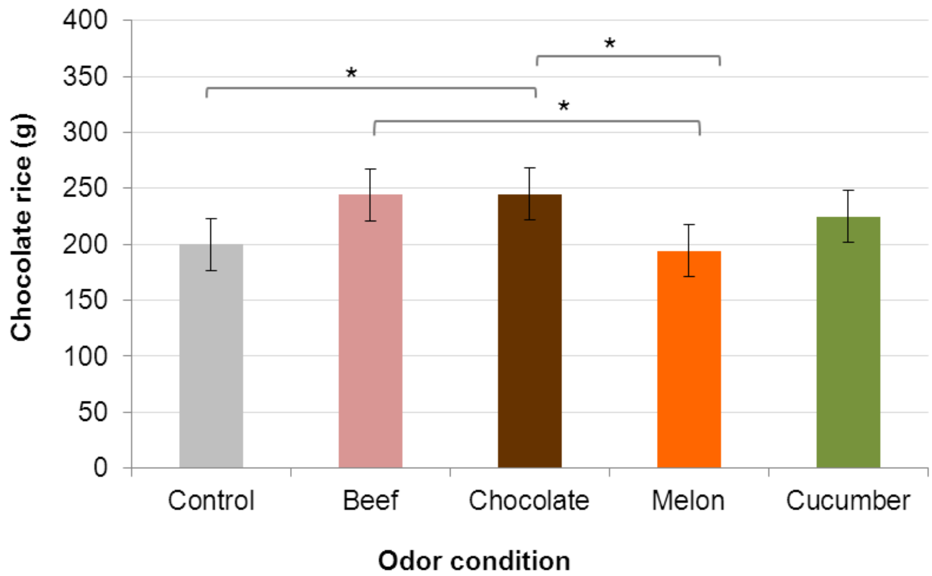

Figure 14. Mean total amount of chocolate rice (in g) eaten *ad libitum* after 30 minutes of odor exposure (error bars showing SEM). Significant differences (p<0.05) in intake between odor conditions are indicated by * (modified from Proserpio et al., 2017b)

Post hoc comparisons revealed that intake was significantly higher after chocolate odor exposure (mean ± SEM: 245.85 ± 24.79 g) compared to no odor exposure (206.93 ± 24.93 g; p<0.05) and to melon (193.55 ± 24.79 g; p<0.01). Similar results were found

regarding beef odor exposure (242.09 ± 24.79 g). Indeed, the *ad libitum* intake under this condition was significantly higher than during melon ($p<0.05$) and marginally significantly higher than during no odor exposure ($p=0.073$).
Considering the covariates, only 'session' (the order of odor conditions) influenced the effect of odor condition on *ad libitum* intake, though the odor effect remained significant ($p<0.05$). In particular, *ad libitum* intake during the first session was significantly lower ($F_{(1,122)}=11.56$; $p<0.01$) compared to the other four sessions, which were comparable to each other.

Categorizing the odors according to energy-density **(Figure 15)**, there was a significant effect of odor category on the amount of chocolate rice eaten ($F_{(2,125)}=4.40$; $p<0.05$). According to *post hoc* analysis, odors signaling high energy dense food products (chocolate and beef) increased significantly food intake (mean: 243.97 ± 22.84 g) compared to odor signaling low dense food products (melon and cucumber, mean: 207.08 ± 22.84 g; $p<0.01$) and control condition (206.94 ± 24.93 g; $p<0.05$).

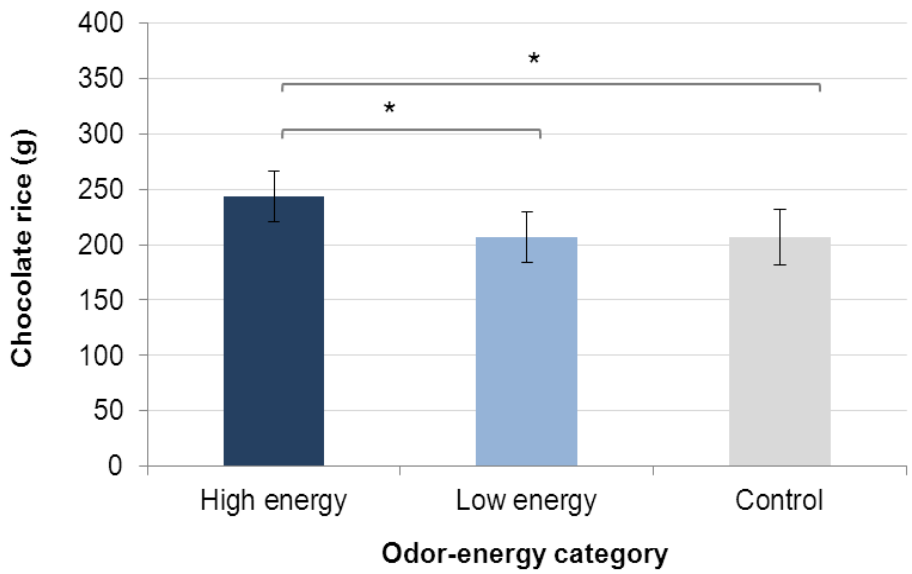

Figure 15. Mean total amount of chocolate rice (in g) eaten *ad libitum* after 30 minutes of odor exposure to odor signaling low and high energy dense products (error bars showing SEM) . Significant differences ($p<0.05$) in intake between odor conditions are indicated by *.

Categorizing the odors into sweet (melon and chocolate) and savory products (beef and cucumber), no significant differences the amount of chocolate rice eaten was found **(Figure 16).**

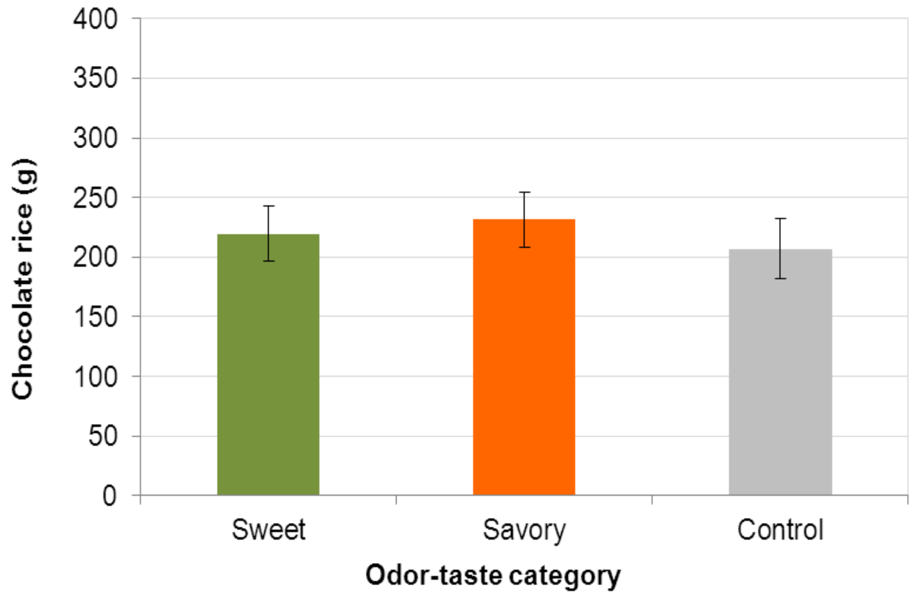

Figure 16. Mean total amount of chocolate rice (in g) eaten *ad libitum* after 30 minutes of odor exposure to odor signaling low and high energy dense products (error bars showing SEM) .

The present results are one of the first to systematically show an effect of ambient odor exposure, in a detectable but mild concentration, on actual food intake. In particular, a significant increase of the amount of chocolate rice eaten upon chocolate and beef odor exposure was found.

Even if previous studies, using both visual and olfactory cues, likewise revealed an increase in food intake (Cornell et al., 1989; Jansen et al., 2003), these results have been inconsistent in the literature (Fedoroff et al., 1997; Coelho et al., 2009; Larsen et al., 2012). Indeed, some results showed a negative odor effect on intake (Coelho et al., 2009; Ramaekers et al., 2014) while other researchers found a positive effect (Fedoroff et al., 2003; Ferriday & Brunstrom, 2008) and other findings reported no effect of odor exposure on *ad libitum* intake (Ruijschop et al., 2009; Zoon et al., 2014; Ramaekers et al., 2014).

Actually, there appears to be a gap between self-report ratings of eating behavior and actual consumption. Indeed, Ferriday and Brunstrom (2011), involving lean and overweight subjects, demonstrated that the exposure to the sight and smell of pizza increased participants' desire to eat but not the actual food intake. The inconsistent results of these researches could be due to the different concentrations of the odors and thus differences in consciousness of the subjects towards the food cues.

Accordingly to this hypothesis, in a recent study in which ambient odors were presented at clearly noticeable intensities, food consumption was not affected by odor exposure (Zoon et al., 2014).

It could be discussed that the effect found in our result was driven by liking of the odors rather than energy-density signaling (i.e. that odors representing high energy dense foods are more liked than low energy food/odors). However, odors and food were carefully selected and similar in liking to prevent this possible confound. Given that beef, melon and cucumber odors had similar liking ratings (and only chocolate odors was rated higher), it is likely that the increasing food intake upon chocolate and beef odor exposure can be attributed to the fact that both these odors signal high energy dense food products, similar to the chocolate rice. Indeed, the total eaten amount was more affected during high energy odor condition compared to the low energy one. Unexpectedly, when categorizing the odor conditions according to taste category (sweet/savory), no significant differences were found on intake. Indeed, the odors signaling sweet food products did not increase food intake of chocolate rice compared to savory odors. It is possible that the chocolate rice elicited mixed associations in our participants, as rice is often associated with a savory meal while chocolate is typically linked to sweet meals.

Unlike previous research, in which high impulsive individuals or participants who are more reward sensitive had more difficulties resisting appetizing foods, leading to a higher intake (Beaver, 2006; Tetley et al., 2010; Hou et al., 2011; Kakoschke et al., 2015), in the present study, no significant effects of personality traits, such as impulsivity or reward sensitivity were found on food intake. Perhaps our research sample did not include participants with a wide-enough range of impulsiveness and reward sensitivity to detect a relation with food intake.

Influence of ambient odor exposure on salivation

A significant effect of odor condition on participants' salivation was found ($F_{(4;439)}$=3.05; $p<0.05$). Mean saliva production during the different odor conditions are reported in **Figure 17.** *Post hoc* comparison revealed that saliva production was significantly higher during chocolate exposure (mean ± SEM: 0.496 ± 0.052 g) compared to control condition (0.417 ± 0.052 g; $p<0.05$) and to cucumber (0.417 ± 0.052 g; $p<0.05$).
Similar results were found regarding beef odor exposure (0.492 ± 0.052 g); saliva production under this condition was significantly higher compared to no odor exposure ($p<0.05$) and to cucumber ($p<0.05$).

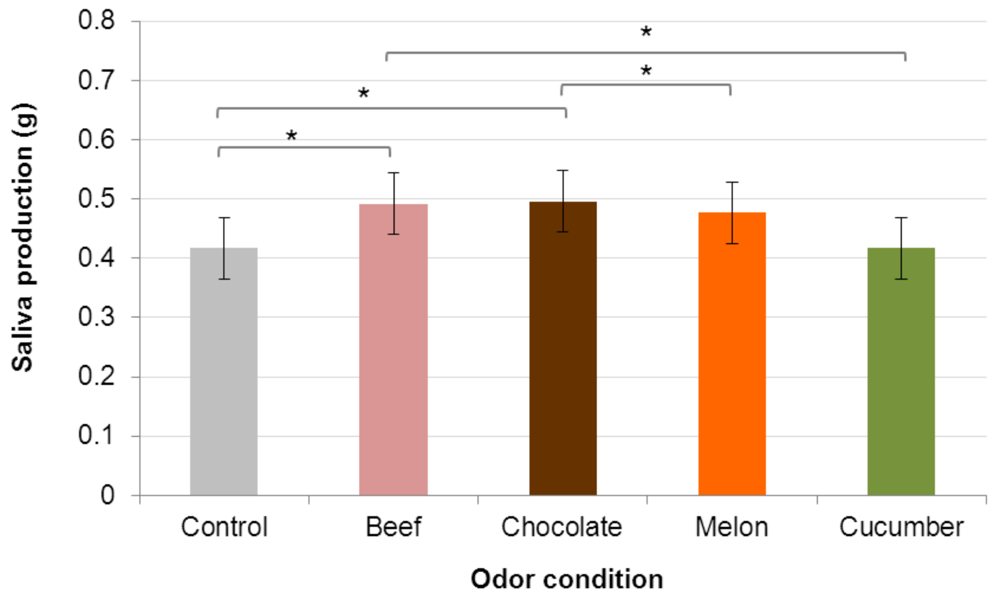

Figure 17. Mean saliva production (in g; averaged over the time points) and error bars showing SEM during the different odor conditions. Significant differences ($p<0.05$) in saliva production between odor conditions are indicated by * (modified from Proserpio et al., 2017b).

A significant effect of time point (saliva was measured at baseline, after 3 and 10 minutes of odor exposure) on salivation was found ($F_{(2,439)}$=7.16; $p<0.01$): saliva production decreased as measured over time. Considering the covariates, only 'session' (the order of odor conditions) influenced the effect of odor condition on saliva production, though the odor effect remained marginally significant (p=0.061). Specifically, salivation during the first session was significantly higher ($F_{(1,438)}$=24.42; $p<0.0001$) compared to the other four sessions, which were comparable to each other.

When categorizing the odors according to energy-density (**Figure 18**), there was a significant effect of odor category on salivation (F=4.28; p<0.05). According to *post hoc* analysis, odor signaling high energy dense food products significantly increased the saliva production (0.494 ± 0.050 g) compared to no odor exposure (0.417 ± 0.052 g; p<0.01) and to odor signaling low dense food products (0.447 ± 0.050 g; p<0.05).

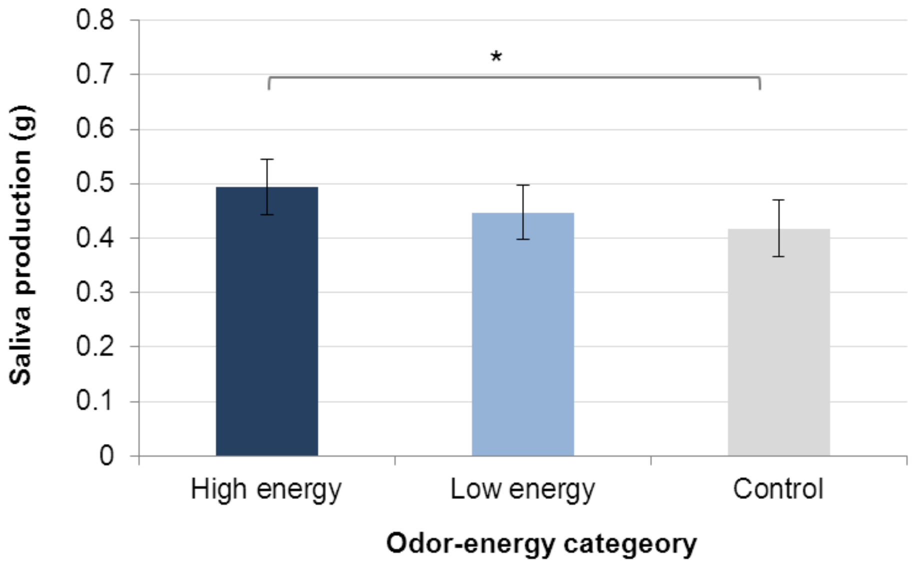

Figure 18. Mean saliva production (in g; averaged over the time points) and error bars showing SEM during the exposure to odor signaling low and high energy dense products. Significant differences (p<0.05) in saliva production between odor conditions are indicated by *.

Categorizing the odors into sweet and savory products (**Figure 19**), there were significant differences on salivation (F=3.19; p<0.05), showing that odor signaling sweet products significantly increased the saliva production (0.487 ± 0.050 g) compared to no odor exposure (0.417 ± 0.052 g; p<0.05). No significant differences were found in salivation between odor signaling sweet and savory products (p=0.156).

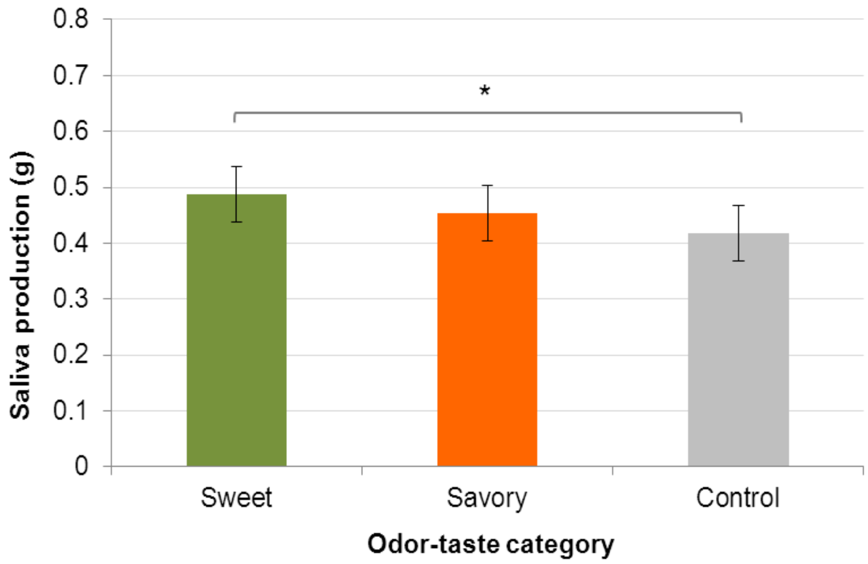

Figure 19. Mean saliva production (in g; averaged over the time points) and error bars showing SEM during the exposure to odor signaling low and high energy dense products. Significant differences ($p<0.05$) in saliva production between odor conditions are indicated by *.

This study revealed not only effects of ambient odor exposure on behavioral outcomes but also on physiological measurements. Indeed a significant odor effect on saliva production over time was found.

Beef and chocolate odors, which increased the *ad libitum* intake, also enhanced salivation. Though for many years it has been claimed that the mere sight of food is capable of *“making the mouth water”* (Masurovsky, 1939; Rosenweig, 1959), researchers suggested that not only sight, but also smell could affect salivary flow rates (Pangborn,1968; Shannon, 1974; Pangborn et al., 1979). However, conflicting results have been reported regarding the ability of odors to induce salivation. Some findings support the hypothesis that salivation can be stimulated by seeing or smelling appetizing foods, as a preparatory response for food intake (Masurovsky, 1939; Rosenweig, 1959; Ferriday & Brunstrom, 2011; Ilangakoon & Carpenter, 2011; Pangborn et al., 1979; Pangborn, 1968), while it should be noted that in other studies no increase in salivation from seeing or smelling an appetizing food product was reported (Kerr, 1961; Engen, 1982; Crowder & Schab, 1995; Larsen et al., 2012; Ramaekers et al., 2014; Lashley, 1916). The lack of salivary increase in these studies may be due to small sample sizes (Spence, 2011) or measurement of inappropriate salivary glands. For example, Lee and Linden (1992) showed that exposure to food odors, such as tomato, vanilla, peppermint, chocolate, lemon, and beef elicited greater salivation in some salivary glands (the submandibular) but not others (the parotid). Differences between studies could be due also to the use of different methods to measure the salivation (e.g. counting swallows, or spitting), to the measurement of the whole mouth saliva instead of salivation from specific glands, or the time points used to collect the saliva.

Moreover, our results showed that saliva production decreased over time. This is in line with previous research demonstrating that after prolonged exposure to food cues, people get used to these cues, leading to a decrease in salivary response (Epstein et al., 2009). In addition, ongoing salivary flow may have been affected by inserting the first cotton roll, and absorbing all saliva present in the mouth most of itself; or participants might have been influenced by the procedure and felt uncomfortable using the cotton rolls. It is possible for future research to examine salivation using other approaches such as counting swallows and spitting method (Nederkoorn et al., 2001).

Influence of ambient odor exposure on sensory specific appetite

The interaction between odor condition and product category on specific appetite ratings was not significant ($F_{(16;9481)}$=0.84; p=0.634), indicating no sensory specific appetite. However, there was a significant effect of odor condition on overall appetite scores ($F_{(4;9481)}$=5.08; p<0.0001), as well as of time point (specific appetite scores were assessed at baseline, after 1, 8 and 15 minutes of odor exposure: $F_{(3;9481)}$=3.77; p<0.05).

Appetite scores were higher during all odor conditions, regardless of the specific odor, compared to the no-odor control condition (**Figure 20**), and increased during odor exposure. Considering the covariates, only ‘session’ (the order of odor conditions) had a significant impact, though the odor effect remained significant (p<0.05). In particular, during the first session the appetite scores were higher ($F_{(1;9480)}$=15.45; p<0.0001) compared to the other sessions, which were comparable to each other.

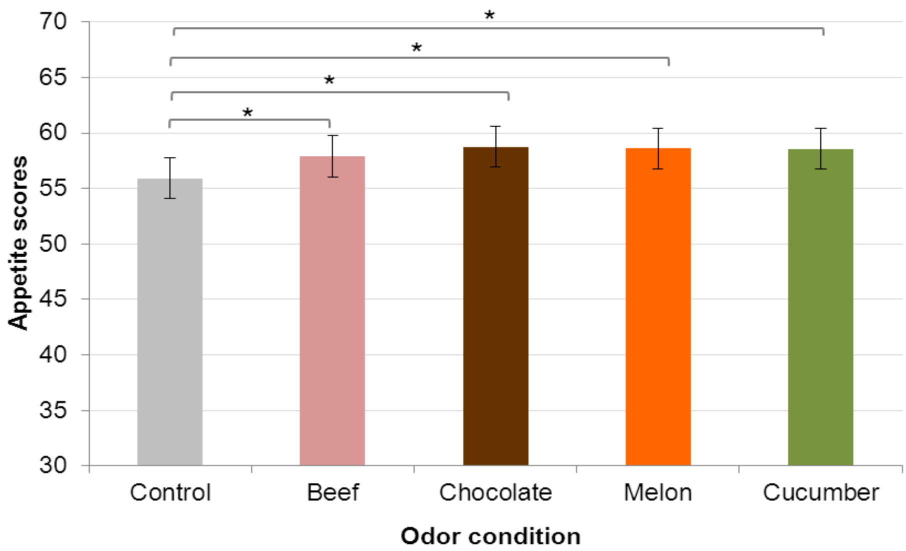

Figure 20. Mean appetite ratings (of all specific products, rated on 100mm VAS) and error bars showing SE, averaged over the time points, during the different odor conditions. Significant differences between odor condition are indicated by * (modified from Proserpio et al., 2017b).

In the current study, we could not demonstrate a sensory-specific appetite effect of odor exposure. This was an unexpected result considering that various studies have now reliably shown that odors (Ramaekers et al., 2014; Zoon et al., 2016) and both odor and visual cues (Ferriday & Brunstrom, 2008; 2011) can specifically induce appetite for the cued food. However, our results show that appetite scores were higher during odor exposure compared to the no-odor control condition, regardless of the specific odors, and increased over time, demonstrating a clear effect of odor of exposure.

It is important to consider that in our study, using odors in a detectable but mild concentration, the results for the 'implicit' measurement (food intake, unknowingly measured and salivation) were greater and more specific than for the explicit measure (specific appetite ratings). This is in line with evidence that food choices and eating behavior, are driven mainly by non-conscious processes (Laureati et al., 2008; Gaillet et al., 2013; Laureati et al., 2013). In particular, it has been proposed that that odors are better able to influence behavior outside of awareness than in conditions in which it is possible to reliably identify the odor (Smeets & Dijksterhuis, 2014).

For our outcome measures (intake, saliva, appetite) it was found an order effect of odor condition that might have been produced by familiarization with the test setup or food product. It could be possible to hypothesize that the participants maybe attempting to control their intake more on the first session compared to subsequent sessions. This can be solved by adding a practice session to future studies. Also, this study focused exclusively on female university students, restricting the generalizability of the current findings. It could be interesting to involve also overweight or restrained participants of both sexes in order to investigate the possibility to steer food intake away from high energy unhealthy foods, towards healthier choices. This could have important implications for reducing overweight.

Chapter 4

Conclusions and future perspectives

Even if the causes which are involved in weight gain had been studied for long time it is necessary to better understand the factors which determine food preferences, choices and thus food intake. The food cues and their perception clearly play a central role in eating behavior.

The researches presented in this Ph.D. thesis give an overview of the potentiality of a sensory approach in the investigation of the obesity phenomenon.
The taste sensitivity and the multisensory interactions of sensory stimuli occurred differently accordingly to the nutritional status of the subjects. In particular, the obese subjects involved were less sensitive to all the taste stimuli. As future perspectives it will be interesting to evaluate also the olfactory thresholds, which could be a useful instrument to explore in a more exhaustive way subjects' perception. Moreover, it might help to match subjects according to other possible factors that could affect their performance as restraint eating, attitudes towards foods, and also cognitive factors affecting their attention.
Due to the reduced sensitivity reported by obese subjects it is possible to hypothesize that they tend to prefer "strong" taste food products with a high-energy content, rich for example in fat and sugar, to be more satisfied. In this context, the addiction of aromas and thickener agents could be used in order to affect the sensory perceptions through a mechanism of brain integration. Indeed, the results showed in the present thesis revealed that the sensory attribute perception was more affected by the addiction of aromas (e.g. butter aroma reminiscent of something fat), in obese subjects, especially women, compared to the normal-weight subjects. Deepening the study of the cross-modal interactions could help to better understand how the sensory properties perception impacts food preferences and the energy intake. It would be interesting to investigate if it could be possible to improve the acceptability and the eaten amount of healthy low-energy dense food products using the multisensory interactions. Thus, this approach could encourage healthier habits using food products with low energy content which are still pleasant for the subjects.

Moreover, the results of the present thesis showed that the odor exposure to stimuli signaling energy-dense food products, in a detectable but mild concentration, affected food intake and saliva production in a congruent way, increasing the intake of a high-calorie product. The ability of odors to influence the amount of food ingested, and therefore the amount of energy assimilated by subjects, could have important consequences in the context of the reduction and prevention of obesity. It could be possible to increase the intake of low rather than high-energy dense, healthier foods by means of congruent odor exposure. Odor exposure might then be a useful instrument to prevent overeating in obese individuals, but may also help malnourished individuals at risk for underweight. Considering that odors are primary triggers of a cascade of events that may finally lead to food intake, as future prospective, it would be interesting to involve also overweight or restrained participants in order to explore the possibility to steer food intake away from high energy unhealthy foods, towards healthier choices. This could have important implications for reducing overweight.

In conclusion, the results of this Ph.D. thesis suggest that a sensory approach in the investigation of variables which are deeply-rooted in human mind and determine food habits is useful in order to better understand and to stem the complex issue of overeating. Actually, food preferences, food choices and food intake could be guided by sensory cues. Moreover, new food products, with a reduced caloric intake but satisfying for the consumer, could be developed taking in account how the mechanism of brain integration occurs in subjects with different nutritional status.

Chapter 5

Materials and methods

Experiment 1

Participants

One hundred three adults gave informed consent and completed the study. Fifty-one (N= 28 women; N= 23 men) obese (OB) patients admitted to the International Center for the Assessment of Nutritional Status (University of Milan, Italy) and fifty-two healthy volunteers of normal-weight (NW) (N= 27 women; N= 25 men) were recruited. The exclusion criteria were individuals aged > 65 years, individuals' ageusie or subjects undergoing medical treatment that could modify taste perception. All subjects were invited to the sensory laboratory that was designed according to ISO guidelines (ISO 8589, 2007), before lunch from 12.00 to 13.00, and were assessed for their taste sensitivity (taste thresholds and fungiform papillae density) in pre-prandial condition. Subsequently, they were asked to complete a questionnaire concerning food neophobia and food liking. The entire session took approximately 1 hour. Data were collected using the Fizz v2.31 software program (Biosystemes, Couternon, France).

Every subject was asked for informed consent before the assessments were made. The present study was performed according to the principles established by the Declaration of Helsinki, after the protocol was approved by the Institutional Ethics Committee of the University of Milan (protocol number 91/14).

Anthropometric assessment

Anthropometric evaluations were made by collecting body weight (to the nearest 0.1 kg) and standing height (to the nearest 0.1 cm) using the same calibrated scale on a telescopic vertical steel stadiometer (SECA 220; Germany), with the subjects dressed only in underwear. BMI was derived accordingly [weight (kg)/height (m2)]. Waist circumference was also measured (to the nearest 0.5 cm) at the midpoint between the iliac crest and the last rib (Lohman et al., 1988).

Stimuli for taste thresholds evaluation

Sucrose, caffeine, sodium chloride, citric acid and oleic acid were used to elicit sweet, bitter, salty, sour and fat sensation tastes, respectively. Seven concentrations of each compound were prepared in mineral water (Levissima, Spa, Italy). The concentration range for each taste stimulus was chosen based on the threshold values reported in the literature (Mojet et al., 2001; Bertoli et al., 2014). Concentration ranges were established such that the lowest concentration was clearly below and the highest concentration was clearly above the level at which subjects could detect or recognize the stimulus. Preliminary tests were carried out to adjust the concentration ranges because the subjects occasionally recognized the lowest concentration or did not recognize the highest concentration of the stimuli in some cases. The final concentration ranges (expressed in g/L) and dilution factors used to elicit the sensations are shown in **Table 6.**

Table 6. Compounds used to elicit the stimuli with relevant dilution step and concentration range.

Compounds	Concentration range (g/L)		Log Steps
	lowest	highest	
Sucrose	0.16	40	0.4
Sodium chloride	0.06	4	0.4
Caffeine	0.003	2	0.4
Citric acid	0.33	50	0.5
Oleic acid	0.02	30	0.5

Sucrose, sodium chloride, citric acid and caffeine were dissolved in water, prepared on the same day as the session and tested at room temperature. Initially, to study the sensitivity to fat, an emulsion of 5% w/v (1.8 × 10−1 M) oleic acid (OA, Sigma-Aldrich, Spa, Milano) in deionized water with 12% gum arabic (Sigma-Aldrich, Spa, Milano), 0.01% xanthan gum (Sigma-Aldrich, Spa, Milano), and 0.01% ethylenediaminetetraacetic acid (Sigma-Aldrich, Spa, Milano) was prepared (Tucker et al., 2014). Subsequently, the OA concentration was reduced to 3% because we realized that it was an identifiable concentration during the initial tests. Oleic acid emulsion was prepared in 200 mL batches by homogenization (IKA T18 Basic Ultra Turrax) for 20 min at 15500 rpm and then diluted by 0.4 log steps to create a range of

7 stimulus concentrations. Samples were made less than 24 h before testing, stored under nitrogen in glass containers, and served at room temperature.

Procedure for taste thresholds assessment

Taste thresholds were evaluated using the 3-AFC (Three Alternative Forced Choice) method reported in ASTM E-679-04. This standard describes a reliable procedure to determine a sensory threshold for any compound dissolved in any liquid. For each stimulus, participants were presented with 7 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 a blank solution (mineral water). The 7 triads proceeded from a weaker to a progressively stronger concentration, and the position of the cup containing the stimulus was randomized over trials and assessors. For each triad, participants were instructed to indicate which sample was different from the other two (ASTM E 679-04). If the subjects 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. To mask the visual and olfactory component (particularly regarding the samples containing emulsions of oleic acid in water), the entire evaluation was carried out under red light and with a nose clip. 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 E 679-04). Participants were asked not to smoke, eat or drink anything except water before the test.

Fungiform papillae assessment

The fungiform papillae density was measured according to Nachtsheim & Schlich (2013). The subjects' tongues were stained with a blue food dye (F.lli Rebecchi, Color Dolci, Spa, Milano, Italy). A circle of filter paper (6 mm diameter) was placed on the center of the tongue approximately 1–2 cm from the tip. Several photos of the tongue were taken using a 12-megapixel digital camera (FUJIFILM USA, Inc., Hollywood, CA, USA) in a brightly light room using the camera's macro mode with no flash. The best photograph was selected to measure the papillae density, and Adobe Photoshop was used to mark the area in which papillae were to be counted according to Bakke & Vickers (2011). To do this, three circles were drawn in the front of the anterior tongue using the filter paper as a template (**Figure 20**). The FP were counted inside the marked circles. Only FP that were at least 50% inside a circle were counted. The FP were counted independently by three researchers. There was no significant difference ($F=2.07$; $p=0.13$) between the researchers' counts, so the mean of the counts was calculated.

Figure 21. Example of image taken for fungiform papillae (FP) count showing the placement of the template (6 mm diameter) and the three counted areas.

Food neophobia assessment

The Food Neophobia Scale (FNS), which was developed by Pliner and Hobden (1992) was translated into Italian (see **Table 7**). In the first stage of the study, the original version was carefully examined to establish whether the items, vocabulary and response format would be appropriate for Italian adults. The wording for some items had to be changed slightly to retain the same meaning as the original items. Some of the items in other studies on food neophobia were also slightly changed such that they were meaningful to the study participants (Siegrist et al., 2013; Flight et al., 2003; Henriques et al., 2009; Laureati et al., 2015). The FNS consists of ten statements, such as ''I don't like new foods,'' each offering seven graded response alternatives, from ''strongly disagree'' (1) to ''strongly agree''(7). Half of the statements are worded in reverse relative to food neophobia, so responses to these statements were reversed when calculating the score. The FNS score was calculated as a sum of the responses, yielding a range of 10–70. The items indicated with R in **Table 7** were reversed.

Table 7. Original English items of the food neophobia scale, and Italian translation of the items

English items	***Italian items***
1. I am constantly sampling new and different foods (R)	1. Mangio costantemente cibi nuovi e diversi dal solito (R)
2. I do not trust new foods	2. Non mi fido di nuovi alimenti
3. If I do not know what is in a food, I won't try it	3. Se non conosco un alimento, non lo provo
4. I like foods from different countries (R)	4. Mi piacciono i cibi provenienti da diversi paesi (R)
5. Ethnic food looks too weird to eat	5. Il cibo etnico mi sembra strano
6. At dinner parties, I will try a new food (R)	6. Ai pranzi e cene con gli amici mi piace assaggiare cibi che non conosco (R)
7. I am afraid to eat things I have never had before	7. Ho paura a mangiare qualcosa che non ho mai assaggiato prima
8. I am very particular about the foods I will eat	8. Sono molto schizzinoso quando si tratta di mangiare
9. I will eat almost anything (R)	9. Mangerei quasi tutto (R)
10. I like to try new ethnic restaurants (R)	10. Mi piace provare ristoranti etnici (R)

(R) Reversed items

Food liking assessment

Each subject completed a 26-item food liking questionnaire. The subjects were asked to indicate their liking on a linear scale anchored at the extremes "I don't like it at all" (rated 0) to "I like it a lot" (rated 10) for the following food categories: vegetables (e.g., carrots, broccoli and tomatoes); fruits (e.g., banana, cherry and apple); carbohydrates (e.g., pasta, bread and rice); seasonings (e.g., butter and olive oil); meat and fish (e.g., white meat, red meat and fish); dairy products (e.g., milk, cheese); and sweets (e.g., chocolate, snacks). The products were chosen based on their energy content: "low energy dense" (<100 kcal/100 g) and "high energy dense" (> 100 kcal/100 g).

Statistical analysis

The matrix of the correct and incorrect answers produced separately by each judge was used to calculate the individual taste thresholds. The geometric mean of the value to the last wrong answer and the first correct answer was chosen to represent the best estimate of the threshold for each subject (ASTM E 679-04). After verifying that taste sensitivity, food liking and food neophobia data were normally distributed independent t-tests were performed to compare normal-weight and obese subjects. Statistical analysis was performed using STATGRAPHICS PLUS v.16 software (Manugest KS Inc., Rockville, USA). To further interpret the relationship between sensitivity and food neophobia, the subjects were divided according to their level of taste acuity for each sensation and FP into 2 groups: “high sensitive” (adults with a taste threshold less than the median taste threshold group and FP density above or equal than the median FP density group; sweet: 1.61 g/L, salt: 0.35 g/L, bitter: 0.03 g/L, fat: 0.14 g/L; 0.61 g/L and FP: 13); “low sensitive” (adults with taste threshold above or equal to the median taste threshold group and FP density less the median FP density group; sweet: 1.61 g/L, salt: 0.35 g/L, bitter: 0.03 g/L, fat: 0.14 g/L; 0.61 g/L and FP: 13).

Experiment 2

Participants

A total of eighty-two women gave informed consent and completed the study. Forty-one obese women with BMI over 30 kg/m^2, who were patients (mean age= 50.29 ± 11.49; BMI= 35.70 ± 4.14 kg/m^2) admitted to the Department of Medical Sciences and Rehabilitation (Istituto Auxologico Italiano), and forty-one normal-weight control women (mean age= 47.58 ± 9.75 years; BMI= 21.90 ± 2.90 kg/m^2) were recruited. Only subjects who liked custard desserts were recruited. The two groups of women were balanced according to age (t = 1.15; p= 0.25). The exclusion criteria were as follows: aged > 65 years, experienced ageusia, or women who were on a medical treatment that could modify taste and odor perception. Every subject was asked for informed consent before the assessments were made. The present study was performed according to the principles established by the Declaration of Helsinki.

Stimuli

Samples consisted of 7 different formulations of custard desserts made with custard powder that does not require cooking (Elah Dofour S.p.a., Novi Ligure, Italy). The custard ingredients were sugar, modified starch, dextrose, thickener (carrageenan), flavorings and coloring. The standard custards (ST) were prepared by adding 75 g of custard powder to 350 mL of skim milk. The experimental products were prepared by adding different concentrations of two flavoring compounds (either vanilla or butter; Flavourart, Oleggio, Italy) or a thickener agent (xanthan gum; Sigma-Aldrich, S.p.a., Milano, Italy) to this standard formulation (**Table 8**). Preliminary experiments, consisting in a series of triangle tests (Lawless & Heymann, 2010), were carried out with a separate group of 20 young adults in order to obtain suitable concentrations (i.e., differences among the standard and the modified versions should be subtle but detectable) of flavoring and thickener compounds to be added to the standard custard.

Table 8. Concentration of vanilla flavor, butter flavor and xanthan gum added to the standard custard to obtain the experimental samples (ST=standard custard, V=vanilla-flavored custard; B=butter-flavored custard, XG=xanthan gum modified custard).

Samples	Vanilla aroma	Butter aroma	Xanthan gum
ST	-	-	-
V_1	0.1%	-	-
V_2	0.3%	-	-
B_1	-	0.05%	-
B_2	-	0.1%	-
XG_1	-	-	1%
XG_2	-	-	1.5%

Procedure

The sessions with obese and normal-weight women were organized in the same way. Groups of ten subjects were invited to take part in two separate sessions that took place on two separate days.

In the first session, liking assessment of the custard dessert samples was evaluated in the pre-prandial condition. Subjects received 3 blocks of samples, each consisting of 3 stimuli: the vanilla aroma block (ST, V_1, V_2), the butter aroma block (ST, B_1, B_2) and the xanthan gum block (ST, XG_1, XG_2). Subjects were given a mandatory 3 min break between testing blocks. Subjects had to evaluate their liking for each sample using a labeled hedonic scale (LAM), anchored by the extremes of “greatest imaginable dislike” (rated 0) and “greatest imaginable like” (rated 10) (Schutz & Cardello, 2001).

In the second session, one week later, the same groups of subjects evaluated the sensory properties (sweet taste, vanilla and butter flavors, and creaminess) of the same 3 blocks of samples. All women were first instructed on how to use the generalized Labeled Magnitude Scale (gLMS). Following the instructions, the women rated some of their remembered and/or imagined oral sensations on the gLMS (Green et al., 2012). For example, women were asked to remember the bitterness of black coffee, the brightness of a dimly lit room, the brightness of a well-lit room, and the sweetness of cotton candy. After the explanations, the women were instructed to place a small plastic spoonful of the custard in their mouths; the women then rated sweetness, vanilla flavor, butter flavor and creaminess for each sample. Subjects were given a mandatory 3 min break between the 3 blocks.

Experimental conditions

Sessions were conducted in quiet rooms under similar light conditions. During the test sessions, subjects were seated separately. All stimuli were prepared on the same day of the session and were presented at room temperature (20-22 °C). For the evaluation of the custard desserts, the subjects received 20 g of each custard. The stimuli within each block were randomly presented, whereas the presentation order of the blocks was fixed for all subjects, with the vanilla aroma block always appearing first, followed by the butter aroma and the xanthan gum blocks. This choice derived by the very saturating nature of the creaminess-modified samples. Samples were coded with different three-digit numbers in each of the tests. Each session took approximately 45 minutes.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) with the interaction *BMI x Samples* as factor and liking scores and sensory attributes (sweetness, vanilla flavor, butter flavor and creaminess) as dependent variables. Differences among samples within each BMI group and differences between obese and normal-weight women's liking and intensity ratings for each sample were evaluated through t-test analysis (pdiff SAS option). All data were analyzed using SAS v.9.3 software (SAS Institute Inc., Cary, USA).

Experiment 3

Participants

Ninety-one subjects completed the study. Forty-six obese subjects were recruited among patients referred to the Istituto Auxologico Italiano (Milan, Italy). Forty-five normal-weight subjects were recruited among the employees of the Faculty of Agriculture and Food Sciences of the University of Milan. Only subjects who liked custard desserts were involved in the study. The exclusion criteria were as follows: aged > 65 years, experienced ageusia, or subjects who were on a medical treatment that could modify taste and odor perception. This study was approved by the Ethic Committee of the IRCCS Istituto Auxologico Italiano, and written informed consent was obtained from all subjects after individual explanation. The study performed also the principles established by the Declaration of Helsinki.

Stimuli

Samples consisted of 3 different formulations of custard desserts made with custard powder (ingredients: sugar, modified starch, dextrose, thickener (carrageenan), flavorings and coloring. Elah Dofour S.p.a., Novi Ligure, Italy). 75 g of custard powder were added to 350 mL of skim milk to make the standard custard (ST). The experimental samples were prepared by adding either 0.05% (B1) or 0.1% (B2) of butter aroma (Flavourart, Oleggio, Italy) to the standard custard. In order to obtain subtle but detectable differences between the ST and the added samples pilot triangle tests (Lawless & Heymann, 2010), with a separate group of 20 adults, were performed. All stimuli were prepared on the same day of the session and were presented at room temperature (20-22 °C). For the evaluation of the custard desserts, the subjects received 20 g of each custard. The stimuli were randomly presented. Samples were coded with different three-digit numbers in each of the tests.

Procedure

Subjects were invited to take part in two separate sessions in different days. Liking assessment of the custard samples was evaluated, during the first session, in a non-satiated state. Subjects rated their liking using a labeled hedonic scale (LAM), anchored by the extremes “greatest imaginable dislike” (rated 0) and “greatest imaginable like” (rated 10) (Schutz & Cardello, 2001).

The same subjects, one week later, evaluated the intensity of a series of sensory properties (sweet taste, vanilla and butter flavors, and creaminess) of the custard samples using the generalized Labeled Magnitude Scale (gLMS) (Bartoshuk et al., 2004). After a brief explanation on how to use the gLMS (Green et al., 2012) the subjects were instructed to place a small plastic spoonful of the custard in their mouths. Each session took approximately 20 minutes.

Statistical analysis

For each dependent variable (liking, sweetness, vanilla flavor, butter flavor and creaminess) a linear mixed models procedure was performed considering *Subjects*, *BMI* (NW and OB), *Samples* (ST, B1 and B2), *Gender* (women and men) and the 3-way interaction *Gender*BMI*Samples* as factors. The 3-way interaction was useful to assess differences in liking and sensory perception between women and men in both NW and OB subjects. Subjects were considered as random effect in all the analyses, whereas the other factors were considered as fixed effects. When a significant difference ($p<0.05$) was found, Tukey’s post-hoc test was used. All statistical analyses were performed using SAS v.9.3 software (SAS Institute Inc., Cary, USA).

Experiment 4

Participants

Eighty seven normal-weight (BMI: 18-25 kg m^{-2}) women candidates recruited around Wageningen University were invited for a screening session in which body weight (kg) and height (m) were determined. Restraint score (1–5) was determined by using the Dutch Eating Behavior Questionnaire (DEBQ, Van Strien, 2005). Higher scores indicate higher dietary restraint; in order to only include people with a normal eating behavior subjects that scored > 2.9 on the restraint subscale were excluded (Van Strien, 2005). Only normosmic subjects, i.e. score ≥ 12 on the Sniffing Sticks 16 items odor identification test (Hummel et al., 2007), that were in good general health, not using medication other than paracetamol and oral contraceptives were included. We also excluded subjects that were vegetarian or vegan, had any food allergies or intolerances, or were habitual smokers. Subjects that did not like the odor or the test meal used in the study (< 40 mm on a 100 mm VAS) were excluded in order to not negatively affect physiological and behavioral responses. After the screening session, thirty-two healthy, normal-weight women were selected.

To ensure that participants were unaware of the true purpose of the experiment, they were informed that the aim of this study was to investigate the effect of individual variation in saliva production and eating behavior. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethical Committee of Wageningen University. Written informed consent was obtained from all subjects and they received financial compensation for their contribution.

Olfactory stimuli

The participants were exposed to five different ambient odor conditions: beef (high energy savory; International Flavors and Fragrances, IFF, 10878095; 0.02% in demineralized water), chocolate (high energy sweet; IFF, 10810180; 5% in Propylene Glycol), melon (low energy sweet; IFF 15025874; 20% in Propylene Glycol), cucumber (low energy savory; IFF 73519595; 100%) and no odor. All odors were distributed in identical air-conditioned rooms (Restaurant of the Future, Wageningen, the Netherlands) using vaporizers (Zaluti, Oosterhout, The Netherlands) set to release them in a detectable but mild concentration, as determined by a pilot study.

The pilot study was carried out with four separate groups of subjects, each one consisting of 20 subjects (total n=80), who had to indicate how intense the ambient odor was (100mm VAS, not at all–very) and categorize the odors into low/high energy dense and sweet/savory or neutral food products. The pilot study showed that the odors were perceived as detectable but mild (chocolate: 45.20 ± 8.49; beef: 44.26 ± 7.78; melon: 43.13 ± 9.65; cucumber: 43.65 ± 14.12). Moreover, 70% of the participants categorized correctly the chocolate odor as high-energy dense sweet, 72% categorized the beef odor as high-energy dense savory, 67% categorized the melon odor as low-energy dense sweet and finally 65% of the participants categorized the cucumber odor as low-energy dense savory.

The pleasantness of the odors was evaluated during the screening sessions involving the participants of the experimental sessions. The chocolate odor obtained higher liking score (M=69.40 ± 22.97) than the other odors, which were comparable to each other (beef M=50.55 ± 28.05; cucumber M=56.06 ± 19.60; melon M=55.56 ± 23.49).

Procedure

Participants attended five separate test sessions on different days, between 8:30 and 16:30. Test sessions and participants were spread out evenly across the day. The participants attended each session at the same time of the day, and had at least one day wash-out period between their sessions. They were asked to refrain from eating and drinking anything but water and weak tea in the 3 hours before the test session. Two participants, separated from each other by a screen, were tested in each of the rooms. The order of odor conditions was randomized but not fully balanced, since there were four time slots per day and five odor conditions per test day.

Upon arrival, only in the first session, participants started by filling out a questionnaire on impulsivity behavior (BIS-11, Barratt Impulsiveness Scale ; Patton et al., 1995) and on reward sensitivity (BIS/BAS, Behavioral Inhibition System, Behavioral

Activation/Approach System, Franken et al., 2005; Carver & White, 1994) in a non-odorous room. Further, in each session, participants filled out a questionnaire on general appetite (hunger, fullness, satiety, prospective consumption, desire to eat, and thirst), as well as appetite for fifteen specific products, all measured on 100mm computerized visual analogue scales (VAS, not at all-very). Saliva was collected using cotton rolls placed under the tongue for 60s (Ferriday & Brunstrom, 2011; Peck, 1959).

After 10 minutes, participants entered one of the test rooms where they were exposed to one of the ambient odor or no-odor control conditions. The participants were given instructions on a computer (EyeQuestion, Version 3.11.1, Logic8 BV) to repeat the specific appetite questionnaire (1, 8, and 15 min after entering the odorous room) and to collect saliva (3 and 10 min after entering the odorous room). After approximately 30 minutes of exposure, *ad libitum* food intake was measured, providing a food product (chocolate rice) that was congruent with one of the odors the subjects were exposed to. The timeline of the study procedure for each of the five sessions is reported in **Figure 22**.

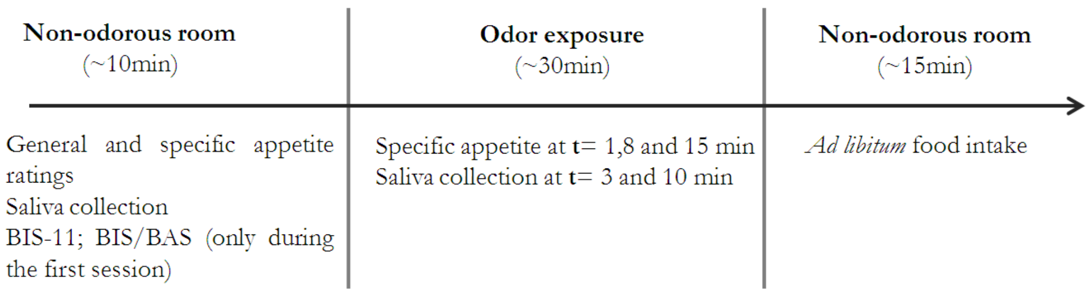

Figure 22. Schematic timeline of study procedure for each of the five sessions.

Specific appetite ratings

After 1,8 and 15 minutes of odor exposure, participants filled out the appetite questionnaire, rating how much they would want to eat 15 different food products, at that moment. The 15 products, and thus the specific appetite scores, were given in a randomized order at every time point. Three products were included for each category (see also Zoon et al., 2016): high energy sweet (HESw), high energy savory (HESa), low energy sweet (LESw), low energy savory (LESa) and three neutral food products (in terms of flavor) were added as control. All of them can be considered as snack foods in the Netherlands. HESw products included pieces of chocolate, cake and stroopwafel (a Dutch caramel syrup waffle); HESa were beef croquette, cheese cubes and crisps; LESw products were a slice of melon, an apple and strawberries; LESa products included pieces of cucumber, tomato salad and raw carrot; bread, croissants and pancake were included as neutral products.

Salivation

Saliva production was measured after 3 and 10 minutes of odor exposure, using the absorption of saliva by cotton rolls, a technique that provides a sensitive single measure of whole-mouth saliva volume (Ferriday & Brunstrom, 2011; Peck, 1959). Pre-weighed plastic bags were given to the subjects containing a single cotton roll and, at specific time points, they were instructed to place the cotton roll in their mouth under the tongue for 60 s in the most comfortable way, and to keep their tongue relaxed. Moreover, they were instructed to swallow as usual before insert the cotton roll. After this period, the participants removed the cotton roll and returned it to the plastic bag, which was then weighed a second time by the experimenter. The difference was calculated to assess amount of saliva production.

Food intake

Food intake (g) was measured after about 30 minutes of odor exposure. During the screening session, liking for two different food products (beef rice and chocolate rice), congruent with two of the odors used during the exposure, was measured. Rice was chosen as test meal since it is commonly eaten and it is easily manipulated into sweet and savory versions (Griffioen-Roose et al., 2010). The chocolate version, that was the preferred one, was chosen for *ad libitum* intake. Participants were instructed to eat the chocolate rice as much as they wanted until they felt comfortable satiated and to consume water only after eating. The subjects received a portion of chocolate rice weighing 600g (800 Kcal; for ingredients see **Table 9**), an amount that allowed for *ad* *libitum* intake, and were unaware that it was weighed before and after the test session to determine food intake.

Table 9. Ingredients to prepare 1 Kg of chocolate rice.

Ingredients	Amount (g)
Rice	130
Water	379
Semi skimmed milk	304
Margarine	30
Sugar	113
Vanilla aroma	20
Cacao powder	25

Statistical analysis

All main analyses were performed following a linear mixed models effects procedure in IBM SPSS Statistics for Windows, Version 22.0 (IBMCorp., Armonk NY). A p-value of <0.05 was considered significant.

Baseline hunger (composite score of hunger, fullness and satiety (reversed scores), prospective consumption, desire to eat scores) and thirst ratings were not different between the odor conditions, and therefore not included in subsequent analyses. Participants were added as random factor in all the analyses. To assess differences between odor categories, for all analyses, odors were also divided into high energy dense products (chocolate and beef), low energy dense products (melon and cucumber odor), sweet products (chocolate and melon), and savory products (beef and cucumber).

To determine the influence of odor exposure on food intake, a basic model was constructed with *ad libitum* intake of chocolate rice (g) as dependent factor, and ‘odor condition’ (four odors and no odor-control condition) as fixed factor. To check for possible confounding or modulating effects, separate analyses were performed by adding ‘hours’ (morning sessions= from 8:30 until 12:30; afternoon sessions= from 13:30 until 16:30), ‘session’ (the order of odor conditions), BIS11 scores, and BIS/BAS scores (impulsivity and reward sensitivity), as covariate to the model.

To determine the influence of odor exposure on saliva production, a basic model was constructed with amount of saliva (g) as dependent factor, and ‘odor condition’, ‘time point’ (saliva was measured at baseline, after 3 and 10 minutes of odor exposure), and their interaction, as fixed factors. The interaction was not significant and thus subsequently removed from the model. Additional analyses were performed to check for possible confounding or modulating effects, by adding ‘hours’ and ‘session’ as covariate to the model.

Appetite ratings (100mm VAS) were analysed by adding specific appetite scores (for all 15 products) as dependent factor, and ‘odor condition’, and ‘time point’ (specific appetite scores were assessed at baseline, after 1, 8 and 15 minutes of odor exposure), and ‘product category’ (the food products were categorized in: neutral products, HESa, HESw, LESa and LeSw), and their interactions as fixed factors. The interactions between odor condition and product category, and between odor condition and time point were not significant, and therefore removed from the model. Additional analyses were performed to check for possible confounding or modulating effects, by adding ‘hours’, ‘session’, BIS11 scores, and BIS/BAS scores as covariate to the model.

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Papers with Impact Factor

1) Proserpio, C., Laureati, M., Bertoli, S., Battezzati, A., & Pagliarini, E. (2016). Determinants of obesity in italian adults: The role of taste sensitivity, food liking and food neophobia. *Chemical Senses,* 41, 169–176.
http://dx.doi.org/10.1093/chemse/bjv072

Chemical Senses, 2016, Vol 41, 169–176
doi:10.1093/chemse/bjv072
Original Article
Advance Access publication December 15, 2015

OXFORD

Original Article

Determinants of Obesity in Italian Adults: The Role of Taste Sensitivity, Food Liking, and Food Neophobia

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Accepted 17 November 2015.

Abstract

Recent evidence has suggested that factors related to sensory perception may explain excess weight. The objective of this study was to consider multiple aspects while investigating the phenomenon of obesity. One goal was to compare taste acuity (taste threshold and density of fungiform papillae) in both normal weight and obese subjects. Thresholds for 4 basic tastes and the fat stimulus were investigated. A second research goal was to study the relationship between food neophobia and food liking according to the body mass index and taste sensitivity. The results showed that obese subjects seem to have higher threshold values and a reduced number of fungiform papillae than do normal weight subjects. Food neophobia did not vary with nutritional status, whereas differences were found for food liking, with obese subjects showing significantly higher liking ratings for high energy dense products compared with normal weight subjects.

Key words: BMI, food preferences, fungiform papillae, overweight, taste threshold

2) Pagliarini, E., Laureati, M., Dinnella, C., Monteleone, E., **Proserpio, C**., & Piasentier, E. (2016). Influence of pig genetic type on sensory properties and consumer acceptance of Parma, San Daniele and Toscano dry-cured hams. *Journal of the Science of Food and Agriculture*, 96(3), 798-806.
http://dx.doi.org/ 10.1002/jsfa.7151.

Research Article

Received: 20 November 2014 Revised: 20 February 2015 Accepted article published: 26 February 2015 Published online in Wiley Online Library: 11 March 2015

(wileyonlinelibrary.com) DOI 10.1002/jsfa.7151

Influence of pig genetic type on sensory properties and consumer acceptance of Parma, San Daniele and Toscano dry-cured hams

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Abstract

BACKGROUND: This study investigated the sensory properties and acceptability of different Protected Designation of Origin (PDO) dry-cured hams. For each PDO, two genotypes were selected: IL×LW (reference hybrid) and Goland (commercial hybrid).

RESULTS: According to descriptive analysis, genetic variance affected few attributes describing Toscano and San Daniele ham sensory quality. The commercial hybrid Parma ham was distinct from the traditional one, the Goland genotype being significantly higher in red color, saltiness, dryness and hardness and showing a lower intensity of pork-meat odor/flavor and sweetness than the IL×LW genotype. Consumer acceptance was mainly influenced by the PDO technology. A genotype effect on acceptance was only observed in Toscano ham. Principal component regression analysis revealed that Toscano ham was the preferred sample. Considering that the consumers involved were from Tuscany, it is likely that Toscano ham was preferred owing to their higher familiarity with this product.

CONCLUSION: Sensory properties of ham samples were better discriminated according to their PDO than their genotype. Likewise, consumer liking was more affected by the specific PDO technology than by genetic type. Toscano ham was the most preferred and most familiar product among Tuscan consumers, indicating that familiarity with the product was the best driver of dry-cured ham preference.

Keywords: dry-cured ham; crossbreeding; Protected Denomination of Origin; acceptability; familiarity

3) Laureati, M., **Proserpio, C.,** Jucker, C., & Savoldelli, S. (2016). New sustainable protein sources: consumers' willingness to adopt insects as feed and food children fish accepted. *Italian Journal of Food Science*, Vol. 28(4), 652-667.
http://dx.doi.org/10.14674/1120-1770%2Fijfs.v476

PAPER

NEW SUSTAINABLE PROTEIN SOURCES: CONSUMERS' WILLINGNESS TO ADOPT INSECTS AS FEED AND FOOD

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ABSTRACT

The aim of the study was to investigate the willingness of Italian consumers to adopt insects, suitable candidates for providing sustainable animal proteins, as part of animal and human diets. Furthermore, we evaluated the effect of information about the benefit of introducing insects into the diet on consumers' acceptance. The results showed that respondents were clearly not ready to accept insects as food, whereas a major positive trend was observed regarding their use as feed. The principal factors affecting the Italian consumers' readiness to adopt insects as food and feed were age, gender, cultural background and food neophobia. Contrary to our expectation, subjects' involvement in sustainability issues did not play a role in the acceptance of insects. Information about the environmental and nutritional benefits of introducing insects as food had a marginal but positive effect on their visual acceptability.

Keywords: consumer acceptance, entomophagy, familiarity, novel protein sources, sustainability

4) **Proserpio, C.**, Laureati, M., Invitti, C., Pasqualinotto, L., Bergamaschi, V., & Pagliarini, E. (2016). Cross modal interactions for custard desserts differ in obese and normal weight Italian women. *Appetite,* 100, 203-209. http://dx.doi.org/10.1016/j.appet.2016.02.033

Appetite 100 (2016) 203–209

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Cross-modal interactions for custard desserts differ in obese and normal weight Italian women

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ARTICLE INFO

Article history:
Received 15 October 2015
Received in revised form 18 January 2016
Accepted 16 February 2016
Available online 18 February 2016

Keywords:
Obesity
Food liking
Sensory perception
Brain integration

ABSTRACT

The effects of variation in odors and thickening agents on sensory properties and acceptability of a model custard dessert were investigated in normal weight and obese women. Subjects rated their liking and the intensity of sensory properties (sweetness, vanilla and butter flavors, and creaminess) of 3 block samples (the first varied in vanilla aroma, the second varied in butter aroma and the third varied in xanthan gum). Significant differences were found in acceptability and intensity ratings in relation to body mass index. The addition of butter aroma in the custard was the most effective way to elicit odor-taste, odor-flavor and odor-texture interactions in obese women. In this group, butter aroma, signaling energy dense products, increased the perception of sweetness, vanilla flavor and creaminess, which are all desirable properties in a custard, while maintaining a high liking degree. Understanding cross-modal interactions in relation to nutritional status is interesting in order to develop new food products with reduced sugar and fat, that are still satisfying for the consumer. This could have important implications to reduce caloric intake and tackle the obesity epidemic.

5) Laureati, M., Cattaneo, C., Bergamaschi, V., **Proserpio, C**., & Pagliarini, E. (2016). School children preferences for fish formulations: The impact of child and parental food neophobia. *Journal of Sensory Studies*, *31*(5), 408-415.
http://dx.doi.org/10.1111/joss.12224

Received: 21 October 2015 | Revised: 15 July 2016 | Accepted: 17 July 2016
DOI 10.1111/joss.12224

ORIGINAL ARTICLE

WILEY Journal of Sensory Studies

School children preferences for fish formulations: The impact of child and parental food neophobia

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Abstract

Child food preferences influence food choice and consumption. Thus, understanding the factors leading to the development of food likes and dislikes is important for enhancing nutritional healthy diets. This study was aimed to investigate children's acceptance of, and preferences for, three different trout formulations served at school lunch. Liking and preference were studied in relation to age, gender, and neophobic traits. Parental food neophobia, fish-eating habits, and frequency of seafood consumption in family life conditions were also investigated. The results indicated that children's liking was strongly dependent on cooking methods, and the proper choice of recipes is likely able to minimize children neophobic attitudes. Parental food neophobia was related to child neophobic behavior and to how fish is prepared at home, with neophilic parents more prone to cook fish in healthy ways.

Practical application

Children fish liking is strongly dependent on product preparation and cooking methods, and the proper choice of recipes could minimize neophobic attitudes. Nutritionists, dieticians, and product developers should consider the sensory aspects to promote more sustainable and appealing refectory meals to increase acceptability and consumption at school and at home.

6) **Proserpio, C.**, Laureati, M., Invitti, C., Cattaneo, C., & Pagliarini, E. (2017). BMI and gender related differences in cross-modal interaction and liking of sensory stimuli. *Food Quality and Preference*, *56*, 49-54.
http://dx.doi.org/10.1016/j.foodqual.2016.09.011

Food Quality and Preference 56 (2017) 49–54

Contents lists available at ScienceDirect

Food Quality and Preference

journal homepage: www.elsevier.com/locate/foodqual

BMI and gender related differences in cross-modal interaction and liking of sensory stimuli

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ARTICLE INFO

Article history:
Received 12 July 2016
Received in revised form 27 September 2016
Accepted 27 September 2016
Available online 28 September 2016

Keywords:
Obesity
Sensory perception
Custard dessert
Acceptability
Food odors
Energy density

ABSTRACT

Sensory experience and food liking are due to the simultaneous perception of multiple sensory modalities. Among all, the olfactory functions play a major role in detecting food sensory properties and in influencing liking. The differences in sensory perception and their interactions with other modalities are poorly studied in relation to BMI and gender. The aim of the present study was to investigate whether the cross-modal interactions and liking occur differently in normal-weight (NW) and obese (OB) subjects belonged to both sexes. 45 NW subjects ($BMI = 22.03 \pm 2.14\ kg/m^2$) and 46 OB subjects ($BMI = 37.52 \pm 5.07\ kg/m^2$) attended two sessions. In the first session, the liking of three custard desserts with increasing concentration of butter aroma, a high-energy dense related aroma, was evaluated. In the second session, the intensity of sensory properties (sweetness, vanilla flavor, butter flavor and creaminess) of the samples was assessed. Results revealed that OB subjects gave higher liking scores to the sample with the major amount of butter aroma compared to NW subjects. This difference is due to odor-taste and odor-odor interactions elicited by high-energy dense related aroma in the OB group. Moreover, OB women were found to perceive the sweetness and vanilla flavor more intense than OB men. Understanding the multisensory processes involved in food perception and liking in relation to BMI and gender could have important implication into the control of energy intake and this approach could be useful in order to prevent overconsumption.

7) Proserpio, C., de Graaf, C., Laureati, M., Pagliarini, E., & Boesveldt, S. (2017). Impact of ambient odors on food intake, saliva production and appetite ratings. *Physiology & Behavior, 174, 35–41.*

http://dx.doi.org/10.1016/j.physbeh.2017.02.042

Physiology & Behavior 174 (2017) 35–41

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Impact of ambient odors on food intake, saliva production and appetite ratings

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HIGHLIGHTS

- Effect of ambient odor on appetite, salivation and food intake was investigated.
- A significant odor effect on food intake and salivation was found.
- Odors signaling high-energy dense products increased food intake and salivation.
- Appetite increased significantly with odor exposure and increased over time.
- Odor exposure did not induce specific appetite for congruent products.

ARTICLE INFO

Article history:
Received 20 July 2016
Received in revised form 27 January 2017
Available online 01 March 2017

Keywords:
Olfactory cues
Appetite
Food intake
Energy density
Salivary response
Eating behavior

ABSTRACT

The aim of this study was to investigate the effect of ambient odor exposure on appetite, salivation and food intake. 32 normal-weight young women (age: 21.4 ± 5.3 year; BMI: 21.7 ± 1.9 kg/m^2) attended five test sessions in a non-satiated state. Each participant was exposed to ambient odors (chocolate, beef, melon and cucumber), in a detectable but mild concentration, and to a control condition (no-odor exposure). During each condition, at different time points, participants rated appetite for 15 food products, and saliva was collected. After approximately 30 min, *ad libitum* intake was measured providing a food (chocolate rice, high-energy dense product) that was congruent with one of the odors they were exposed to. A significant odor effect on food intake ($p = 0.034$) and salivation ($p = 0.017$) was found. Exposure to odors signaling high-energy dense products increased food intake (243.97 ± 22.84 g) compared to control condition (206.94 ± 24.93 g; $p = 0.03$). Consistently, salivation was increased significantly during chocolate and beef exposure (mean: 0.494 ± 0.050 g) compared to control condition (0.417 ± 0.05 g; $p = 0.006$). Even though odor exposure did not induce specific appetite for congruent products ($p = 0.634$), appetite scores were significantly higher during odor exposure ($p < 0.0001$) compared to the no-odor control condition and increased significantly over time ($p = 0.010$). Exposure to food odors seems to drive behavioral and physiological responses involved in eating behavior, specifically for odors and foods that are high in energy density. This could have implications for steering food intake and ultimately influencing the nutritional status of people.

8) Proserpio, C., Laureati, M., Invitti, C., & Pagliarini, E. Reduced taste sensitivity and increased food neophobia characterize obese adults. *S*ubmitted to *Food Quality and Preference.*

Paper without Impact factor

1) Proserpio, C., Laureati, M & Bergamaschi, V. (2012). Psiche e memoria condizionano il gusto del consumatore [Psyche and memory influences consumer taste]. *Intersezioni*, n° 9, January 11th, 2012.

Oral communications

1) **Proserpio, C.** Sovrappeso e obesità: fenomeni che è possibile indagare con un approccio sensoriale. *V Conference of the Italian Sensory Society (SISS)*, San Michele all'Adige (Trento), Italy, November 26th-28th, 2014.

2) **Proserpio, C.**, Laureati, M., Jucker, C., Savoldelli, S. Insetti nel piatto: cosa ne pensa il consumatore? NUTRIMI, 10° forum di nutrizione pratica, Milan, Italy, April 21st-22nd, 2016.

3) **Proserpio, C.** Study of behavioral and physiological determinants of obesity using a sensory approach. 21th Workshop on the Developments in the Italian Ph.D. Research on Food Science Technology and Biotechnology, University of Naples Federico II, Portici, September 14th-16th, 2016.

4) **Proserpio, C.,** de Graaf, C., Laureati, M., Pagliarini, E., Boesveldt, S. Food odors influence behavioral and physiological parameters of human eating behavior. 7th European Conference on Sensory and Consumer Research, Dijon, France, September 11th-14th, 2016.

5) **Proserpio, C.,** Laureati, M., Pagliarini, E. Sensibilita' gustativa e interazioni multisensoriali in relazione allo stato nutrizionale. *VI Conference of the Italian Sensory Society (SISS)*, Camplus Living Bononia Bologna, November 30th – December 2nd, 2016. Awarded with Adacta International in Sensory & Consumer Science

Poster presentations

1) Proserpio, C. Food formulation for reducing overweight: a new sensory approach. *19th Workshop on the Developments in the Italian Ph.D. Research on Food Science Technology and Biotechnology*, University of Bari, Italy, September 24^{th}-26^{th}, 2014.

2) Proserpio, C., Laureati, M., Pagliarini, E. Behavioral and perceptive determinants of obesity in italian adults. 11th *Pangborn Sensory Science Symposium*, Gothenburg, Sweden, August 23^{rd}-27^{th}, 2015.

3) Laureati, M., Cattaneo, C., **Proserpio, C.**, Pagliarini, E. Do children like fish? Perceptive and behavioral factors related to children's acceptance of fish school formulations. 7th European Conference on Sensory and Consumer Research Dijon, France, September 11^{th}-14^{th}, 2016

4) Laureati, M., Cattaneo, C., **Proserpio, C.**, Pagliarini, E. Sensory profiling of fibre-enriched apple purées by using the Check-All-That-Apply method with school aged children. 7th European Conference on Sensory and Consumer Research Dijon, France, September 11^{th}-14^{th}, 2016.

5) Proserpio, C., Laureati, M., Invitti, C., Pagliarini, E. Impact of BMI and gender on cross-modal interactions in custard desserts. 7th European Conference on Sensory and Consumer Research, Dijon, France, September 11^{th}-14^{th}, 2016. Awarded with the European Sensory Science Society (E3S)

Congress communications

1) Pagliarini, E., **Proserpio, C.**, Laureati, M., Invitti, C. Brain integration and liking of sensory stimuli in relation to gender and BMI. VIII Congresso Nazionale SIO, Roma, Italy, September 29th-October 1st, 2016

2) Proserpio, C., Invitti, C., Laureati, M., Cattaneo, C., Pagliarini, E. Esiste una relazione tra sensibilità gustativa, neofobia e obesitá? *VI Conference of the Italian Sensory Society (SISS)*, Camplus Living Bononia Bologna, November 30th – December 2nd, 2016

ACKNOWLEDGEMENTS

First of all, I would like to thank prof. Ella Pagliarini for giving me the opportunity to conduct this Ph.D. and for her teachings, both educational and professional. Warm thanks to dr. Monica Laureati for the scientific and personal support during these three years. I learned really more than what I expected, not only from a scientific point of view. Thanks again!

Thanks to prof. Cees de Graaf and dr. Sanne Boesveldt for giving me the opportunity to spend a period abroad at Wageningen University where I have lived a great experience, one of the best of my life. Thanks to all the friends that I met in the Netherlands, in particular to Irene, Camilla and Luca, who contributed to make this experience unforgettable!

Special thanks go to my family, especially to my parents, who have always been there for me. Thanks for the support, for always believed in me and for teaching me to be a positive person. Warm thanks to Alexander who started on my side this experience and supported me during these years.

Thanks to all the friends and the colleagues, especially to Camilla, Cristina, Silvia, Maria Eletta, Margherita and Valentina for their friendship during this period. This experience would not have been the same without you.

Last but not least, thanks to all my friends, especially to Eleonora, Anna, Carmen and Lorenzo, who played a key role in these years. You have made all the times best.

Thanks to everyone who played a role in my professional and personal training. This is only the beginning!