University of Reading

The effects of taste sensitivity and repeated taste exposure on children's intake and liking of turnip (*Brassica rapa* subsp. *rapa*); a bitter *Brassica* vegetable

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Thesis submitted for the

Degree of Doctor of Philosophy (PhD)

Abstract

Taste sensitivity plays an important role in influencing food preferences and thus nutritional status. It has been reported that children have low vegetable consumption. Differences in bitter taste sensitivity between individuals may influence vegetable consumption, especially *Brassica* vegetables. Glucosinolates (GSLs) are present in high amount in *Brassica* vegetables, and these compounds contain a thiourea group, which is partly responsible for the bitter taste of *Brassica* vegetables. The thiourea group also exists in 6-propylthiouracil (PROP), and the ability to taste it is genetically determined. Variations in the bitter taste receptor of *TAS2R38* predominantly explain the differences in response of PROP perception. Additionally, phenotypic measure of fungiform papillae density (FPD) has been shown to contribute to taste sensitivity, and gustin (*CA6*) gene has been proposed to be involved in the development of papillae. Existing literature has shown that repeated taste exposure can modify the acceptance of initially disliked/novel foods. However, no previous study has considered taste sensitivity within a repeated taste exposure study design.

The main objective of this thesis was to investigate the effects of taste genotypes (*TAS2R38* and *CA6*) and phenotypes (PROP taster status and FPD) on the effectiveness of repeated taste exposure of an unfamiliar *Brassica* vegetable (turnip) on intake and liking in children aged 3 to 5 years. To support this main objective, we also determined the effects of cooking method on the sensory profile and consumer liking of turnip, and identified and quantified GSLs in turnip. Using parental reported questionnaires about children's preferences, this thesis also explored whether taste sensitivity would have effects on overall vegetable intake and liking in children.

Our findings revealed that turnip liking is dependent on cooking method, where we found that roasted-turnip was the most preferred, and boiled-pureed turnip was the least preferred. Sweetness in turnip increased liking, while bitterness decreased liking. Although

TAS2R38 genotype had a significant impact on bitter perception in turnip, where the PAV/PAV consumers tended to score higher bitter intensity than the PAV/AVI and AVI/AVI consumers, it did not influence taste liking. Our chemical analysis showed that there were 12 individual GSLs found across our turnip samples. Gluconasturtiin was the most abundant GSL, and we found significant differences in individual GSL content (except glucoalyssin) between samples. As expected, GSLs were positively correlated with bitter taste, and negatively correlated (except glucobrassicanapin) with sweet taste.

In our main study, intake and liking of steamed-pureed turnip significantly increased after exposure, but there were no significant effects of taste genotypes and phenotypes. Furthermore, we found significant increases in intake and liking of the vegetable at follow-up, compared to pre-intervention. From the parent-reported questionnaires, we found no significant effects of taste genotypes and phenotypes on intake of vegetables collectively (*Brassica*, non-*Brassica* and total vegetables). However, there were some significant effects of these genotypes and phenotypes on intake of certain vegetables. For liking, FPD was found to have had a significant impact on *Brassica* and total vegetables where the low and high FPD groups had higher liking than the medium FPD group. From the questionnaire results, we concluded that vegetable intake and liking were positively correlated, suggesting that as intake increases, liking increases and vice versa.

In conclusion, cooking method predicts turnip liking, and 12 GSLs in turnip were positively correlated with bitterness. Repeated taste exposure is effective in increasing the acceptance of an unfamiliar bitter vegetable in children, and has long-term positive effects. Taste sensitivity did not have a significant impact on the effectiveness of repeated taste exposure. However, there were significant effects of taste genotype (*TAS2R38*) and phenotypes (PROP taster status and FPD) on intake of specific vegetables, and only FPD influenced parent-reported liking of vegetables from the 3 to 5 year-old children.

Table of Contents

List of figures

List of tables

Glossary of terms

AHC: Agglomerative hierarchical cluster

ANOVA: Analysis of variance

API-ES: Atmospheric pressure ionization-electrospray

AVI: Alanine-Valine-Isoleucine

***CA6*:** Gustin

CVD: Cardiovascular disease

DNA: Deoxyribonucleic acid

DW: Dry weight

FAO: Food and Agriculture Organisation

FFQ: Food frequency questionnaire

FPD: Fungiform papillae density

gLMS: General labelled magnitude scale

GSL: Glucosinolate

HPLC: High-performance liquid chromatography

ITC: Isothiocyanate

KCL: Potassium chloride

LC-MS: Liquid chromatography mass spectrometry

NaCl: sodium chloride

NDNS: National Diet and Nutrition Survey

NHS: National Health Service

PAV: Proline-Alanine-Valine

PCA: Principal component analysis

PROP: 6-n-propylthiouracil

PTC: Phenylthiocarbamide

QDA: Quantitative Descriptive Analysis

RRF: Relative response factor

SD: Standard deviation

SEM: Standard error of the mean

SNP: Single nucleotide polymorphism

T2R: Taste type 2 receptors

WHO: World Health Organisation

CHAPTER 1: Literature review

1.1 Health benefits of fruit and vegetable consumption

A diet high in fruit and vegetables is promoted globally (Slavin & Lloyd, 2012) and studies show that their consumption is associated with decreased risk of chronic diseases. Slavin and Lloyd's (2012) review demonstrates that dietary fibre in fruit and vegetables has a role in cardiovascular disease (CVD) prevention and may help prevent obesity. A meta-analysis of case-controlled studies shows vegetables have protective effects against cancers of the oesophagus, lung, stomach, colorectum and breast (Riboli & Norat, 2003). The World Health Organisation (WHO) has listed 'low fruit and vegetable consumption' as one of the risk factors for total burden of disease (World Health Organisation, 2002) and Lock, Pomerleau, Causer, Altmann and McKee (2005) suggest that an intake of 600 g of fruit and vegetables per day in adults has the potential to reduce the total burden of disease by 1.8% and ischaemic heart

disease and ischaemic stroke by 31% and 19% respectively. The authors conclude that increased fruit and vegetable intake in the daily diet may reduce the risk of lung, stomach, oesophageal and colorectal cancer by 12%, 19%, 20% and 2% respectively.

Studies have reported that risk of CVD starts to develop from childhood. A study that involved 2204 subjects showed that CVD risk factors (BMI, serum lipid levels and blood pressure) in childhood are correlated with values measured in adulthood; concluded from a 27-year follow up (Juhola et al., 2011). Another study showed similar results, concluding that cardiovascular risk in childhood persists through adulthood (Joshi et al., 2014). Maynard, Gunnell, Emmett, Frankel and Davey Smith (2003) suggested that early diet intervention has an impact on adult health, as their study showed that fruit consumption in childhood has a protective effect on cancer risk in later life.

In addition to reducing risk of disease, evidence indicates a diet high in fruit and vegetables can reduce obesity. In a prospective dietary study of 206 adults, a 10-year follow up revealed an average weight gain of 3.41 kg/person. However, with an intake of 249 to 386 g fruit/day, the risk of gaining ≥3.41 kg over 10 years reduces by 69% and with an intake of >333 g vegetables/day, this risk reduces by 82% (Vioque, Weinbrenner, Castelló, Asensio, & Garcia de la Hera, 2008).

The World Health Organisation (WHO)/Food and Agriculture Organisation (FAO) recommend a minimum intake of 400 g of fruit and vegetables per day (excluding potatoes and other starchy tubers) to prevent chronic diseases such as diabetes, obesity and heart disease (WHO, 2004). The recommendation is the same as UK guidelines that recommend 5 portions of fruit and vegetables per day (at 80 g per portion) (Bates et al., 2014). The guideline is recommended for those aged 11 years and over (Bates et al., 2016). According to National Health Service (NHS), younger children should also consume at least 5 portions of fruit and vegetables a day, where one portion is equal to the amount they can fit in their hand (National Health Service, 2015).

Despite the health benefits of vegetables being heavily promoted, vegetable intake is often reported to be low among children. The National Diet and Nutrition Survey (NDNS) in the UK from 2008 to 2012 showed that the mean intake of vegetables was 72 g per day for children aged 1.5 to 3 years, 97 g per day for children aged 4 to 10 years and 112 g per day for children aged 11 to 18 years. Only 9% of 11 to 18 years old children consumed 5 portions of fruit and vegetables as recommended by the UK guidelines (Bates et al., 2014). Low vegetable intake occurs not only in the UK; Reinaerts, Nooijer, Candel and Vries (2007) reported that children aged 4 to 12 years old in the Netherlands only consume an average of 60 g of vegetables per day. In addition, Magarey, Daniels and Smith (2001) showed that the mean intake of vegetables in Australian children aged 2 to 7 years is between 60 to 98 g per day.

In summary, children must be encouraged to eat vegetables as it has been established that a diet rich in vegetables provides health benefits as it may help prevent or reduce many chronic diseases.

1.2 Food neophobia

Many researchers have suggested that low consumption or avoidance of certain foods is due to food neophobia. Pelchat and Pliner (1995) defined food neophobia as "the reluctance to try unfamiliar foods or dislike for the flavour of unfamiliar foods" (p.153). Cooke, Wardle and Gibson (2003) found that greater food neophobia in 2 to 6 year-old children was related to lower consumption of vegetables, fruits and meat. These data were based on a questionnaire which included a measure of child food neophobia and a food frequency questionnaire completed by 564 mothers. They suggested that these foods are being avoided because they may contain toxins especially in vegetables and food neophobia serves to protect humans from ingesting these potentially dangerous foods. Similar results were found in a study by Russell and Worsley (2008) that revealed food neophobia in 2 to 5 year-old children has the strongest effects on intake of vegetables followed by meat and fruits. These studies suggest that food neophobia is crucial in determining children's dietary intake and food preferences. In addition, Knaapila et al. (2015) reported that food neophobia is associated with low consumption of vegetables, poor quality of diet and high body mass index (BMI) in Finnish adults. Moreover, the same research group argued that food neophobia limited familiarity with spices (Knaapila et al., 2017).

Food neophobia is associated with age and tends to decrease as age increases. Cashdan (1994) found that food neophobia is low in children under 2 years old, substantially increases between 2 to 3 years, and slowly decreases thereafter. Pelchat and Pliner (1995) also argued that food neophobia is more pronounced in younger children than older children given their findings that children aged 6 to 8 years were more willing to try novel foods than children aged

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3 to 5 years. McFarlane and Pliner (1997) in a study on 10 to 79 year-old participants reported that food neophobia continues to decrease from childhood, through adolescence to adulthood. Cooke and Wardle (2005) suggested that as age increases, children are more exposed to a variety of foods, and thus neophobia decreases. Ton Nu, Macleod and Barthelemy (1996) argued that older children (10 to 15 years) tend to have greater autonomy about the foods they eat at home and eating away from home becomes common. Eating away from home provides children with more opportunities to exert their autonomy as well as increased exposure to previously novel foods and different norms, for example peers' food preferences.

1.3 Development of food preferences in children

As discussed above, the rejection of unfamiliar foods due to food neophobia is common in younger children but it becomes a less prominent feature as children get older. Other factors also influence the development of food preferences, including innate preferences and exposure to foods. Humans are born with an innate preference for sweet tastes and a tendency to reject bitter tastes (Galindo, Schneider, Stähler, Töle, & Meyerhof, 2012). Desor, Maller and Turner (1973) demonstrated infants' (1 to 3 days of age) innate preference for sweet tastes by recording their greater ingestion of a sugar solution versus water. Moreover, the findings demonstrated that infants showed greater preferences for sugar solutions at higher concentrations. Newborns exhibited negative hedonic responses when given bitter solutions (urea and quinine) but exhibited positive hedonic responses when given a sweet solution (sucrose) (Ganchrow, Steiner, & Daher, 1983). Bitter tastes are innately disliked and avoided because bitter tasting foods potentially contain toxic compounds (Glendinning, 1994). According to Drewnowski and Gomez-Carneros (2000), humans have a low bitter taste threshold but a high sweet taste threshold; the bitter taste of quinine can be detected at 25 μmol/L while the sweet taste of sucrose is detected at 10000 μmol/L.

Such innate preferences may influence food choice in later life as a study in the UK found that among the top favourite foods of 4 to 5 year-old children were sweet foods which included cream, cakes, pastries, fruit pie, sponge pudding, custard and dairy desserts, and the least liked foods were vegetables (Wardle, Sanderson, Gibson, & Rapoport, 2001). Among the lowest rated vegetables by children aged from 4 to 16 years in the UK were bitter tasting vegetables (swede, sprouts and turnip) (Cooke & Wardle, 2005). Similar results were shown in a study among children aged 2 to 8 years in the USA (Skinner, Carruth, Bounds, & Ziegler, 2002). Consistent across many studies around the world is the result that vegetables are reported to be the least favoured foods, which are associated with bitter tastes. Ton Nu, Macleod and Barthelemy (1996) determined food preferences among 222 French participants aged between 10 to 20 years old and found green vegetables, for example endives, spinach, sprouts and cabbage were among the 10 most disliked foods. Pérez-Rodrigo, Ribas, Serra-Majem and Aranceta (2003) found that 47% of a Spanish population of 3534 individuals aged 2 to 24 years-old reported dislike for vegetables (artichokes, cauliflower, spinach, asparagus, carrot, lettuce and tomato). The study also reported that individuals with low consumption of vegetables were among those who reported dislike for vegetables. Yngve et al. (2005) argued that there are similar patterns in vegetable intake in children aged 11 years across 9 European countries (the Netherlands, Belgium, Portugal, Denmark, Sweden, Austria, Norway, Iceland and Spain) and they are all below the national and international guidelines. In addition, the study argued that vegetable preparation is determined by culture where they found that northern countries consumed more raw vegetables, while Portugal and Spain consumers had vegetables predominantly as soup. Besides, parents tend to offer foods that are readily accepted by their children (Wardle et al., 2001), providing more exposures to the foods, which then may contribute to higher food liking, and parents typically stop offering foods that their children reject or dislike (Carruth, Ziegler, Gordon, & Barr, 2004).

Familiarisation of foods starts as early as in the uterus and continues throughout life. Before the introduction to solid foods, foetus and breast-fed babies have already experienced flavours from their mother's diet. Flavours are transmitted from foods to amniotic fluid and later to breast-milk (Birch, 1999). Schaal, Marlier and Soussignan (2000) reported that infants develop odour preferences related to mothers' diet during pregnancy. The study found that infants who had been exposed to anise flavour prenatally (ingested by mothers during pregnancy) showed positive responses when anise odour was presented, whereas infants in a control group showed negative or neutral responses. Similarly, in another study, Mennella, Jagnow and Beauchamp (2001) revealed that exposure to flavours that occur during the pregnancy and breastfeeding periods can modify infants' acceptance of similar flavours during weaning. Their study found that infants showed less negative facial expressions while eating carrot-flavoured cereal relative to plain cereal if they had been exposed to the carrot flavour either prenatally (mothers drank carrot juice during the last trimester of pregnancy) or postnatally (mothers drank carrot juice during the first 2 months of lactation).

Breastfeeding not only facilitates infants' acceptance of specific flavours during weaning, but it also facilitates acceptance of novel flavours compared to formula-fed infants. Maier, Chabanet, Schaal, Leathwood and Issanchou's (2008) findings supported this statement with breast-fed infants (5 to 6 months) in their study consuming and liking (as rated by mothers and observers) novel vegetables (zucchini, tomato and peas) more than formula-fed infants. In a recent paper describing follow-up at 6 years old, results revealed that the breast-fed infants continued to have higher consumption of vegetables compared to the formula-fed infants (Maier-Nöth, Schaal, Leathwood, & Issanchou, 2016).

Children's food preferences can be influenced by their family members' preferences as they have been exposed to similar foods. A meta-analysis of 5 studies concluded that there is a similarity in food preferences between children and their mothers and fathers (Borah-Giddens

& Falciglia, 1993). In a study to determine food preferences among children, Skinner et al. (1998) found a strong concordance between children and their fathers, mothers and siblings. The study assessed food preferences of 118 children aged 28 to 36 months by using questionnaires comprising a list of 196 foods commonly eaten in the USA. In a child/mother pair longitudinal study where children were recruited at 2 months of age and followed until they were 8 years old, results demonstrated a strong correlation between mothers and children for liked, disliked and never tasted foods and the concordance only decreased by 2% at the end of the study when the children reached 8 years old (Skinner et al., 2002). The study concluded that the mothers' influences on food preferences remain strong even though children are exposed to other influences outside the family.

In addition to incidental exposure through experiences, familiarity with foods has also been explored through intentional repeated exposure regimes. Many intervention studies have been done to determine the effectiveness of repeated taste exposure on unfamiliar and disliked foods. A study conducted by Wardle, Herrera, Cooke and Gibson (2003) that involved 5 to 7 year-old children tasting a novel and disliked vegetable (sweet red pepper) for 8 days, showed that intake of this vegetable increased significantly from just over 1 piece of sweet red pepper before exposure to more than 9 pieces after exposure, furthermore the liking score also increased. In addition, the study reported that intake and liking of the vegetable in the exposure group were higher compared to both a reward group (in which children received stickers if they ate vegetable) and the control group.

In another repeated exposure study with 49 seven-month old infants, they were fed disliked and liked vegetable purees on alternate days over a period of 16 days (Maier, Chabanet, Schaal, Issanchou, & Leathwood, 2007). Initially, the mean intake of the disliked vegetable was substantially lower than the liked vegetable (39 ± 29 g versus 164 ± 73 g (mean ± SD)), however at day 8 of exposure, the mean intake of the disliked vegetable increased substantially to 174 ±

54 g, which was comparable to the mean intake of the liked vegetable, 186 ± 68 g. Furthermore, infants' liking (rated by mothers using a 9-point scale) also showed a similar pattern.

Another study involving 3 to 6 year-old children compared 2 strategies to encourage vegetable consumption in children; the two strategies were mere exposure and flavour-flavour learning using a liked dip. Each child was asked to taste 2 disliked vegetables, one without a dip (mere exposure) and the other one with a liked dip (flavour-flavour learning), twice weekly over a period of 4 weeks. The results showed that liking increased after 6 exposures for both strategies and remained higher until the end of 8 tasting trials; with liking from mere exposure being higher than flavour-flavour learning (Anzman-Frasca, Savage, Marini, Fisher, & Birch, 2012). In a similar study, Bouhlal, Issanchou, Chabanet and Nicklaus (2014) compared repeated taste exposure with 2 flavour-flavour learning tests (in which salt and spice (nutmeg) were used separately) of an unfamiliar vegetable (salsify) puree in toddlers aged 2 to 3 years. The results demonstrated that children in the repeated taste exposure group had the highest increase in

intake (64 ± 11 g (mean ± SE)) compared to flavour-flavour learning with nutmeg (36 ± 11 g) and flavour-flavour learning with salt (23 ± 11 g). The increase in intake remained high in all groups after 6 months. These results revealed that repeated taste exposure is a simpler and better strategy to increase vegetable acceptance than flavour-flavour learning.

Repeated exposure increases familiarity of a stimulus which then increases liking of it. There are a few theories explaining how exposure works in increasing liking of a stimulus. Zajonc (1968) suggested that repeated exposure to a particular stimulus would enhance positive attitude to that stimulus. On the other hand, Kalat and Rozin (1973) proposed a 'learned safety theory' as a mechanism of food acceptance. The theory explains that a food is safe to eat if it does not cause any negative effect after repeated taste exposure to the food.

1.4 PROP taster status

Although innate preferences and familiarity to foods are partially responsible for the development of food preferences, individuals may perceive foods differently due to variability in taste sensitivity. For example, some individuals have higher sensitivity to bitter tastes than others, therefore they may not accept bitter foods as readily as the less sensitive individuals. There are a number of methods to test taste sensitivity, and one of them is to test sensitivity to 6-n-propylthiouracil (PROP), which is a bitter compound. Tepper, Christensen and Cao (2001) classified super-, medium- and non-tasters using a suprathreshold (above threshold) method. Participants were asked to rate bitterness and saltiness from 3 levels of PROP solutions (0.032, 0.32 and 3.2 mmol/l) and sodium chloride solutions (NaCl) (0.01, 0.1 and 1.0 mmol/l) on a labelled magnitude scale (LMS). Non-tasters were classified as those who rated PROP intensity lower than NaCl, medium-tasters rated the intensity of both PROP and NaCl as similar, and super-tasters rated PROP intensity higher than NaCl. Meanwhile, Zhao, Kirkmeyer and Tepper (2003) determined PROP taster status by placing PROP and NaCl paper disks on the tip of the tongue. The PROP paper disks were prepared by impregnating filter paper disks in a 50 mmol/l PROP solution while NaCl disks were impregnated in a 1.0 mol/l NaCl solution, then dried in an oven at 121°C for 1 hour. Participants who rated the PROP disk below ≤15 mm (over 100-mm on a LMS; labelled from 'barely detectable' to 'strongest imaginable') were classified as non-tasters, those who rated ≥67 mm were classified as super-tasters, and medium-tasters were in between these limits. The NaCl rating was to help determine those participants who give a borderline rating to PROP. For example, participants who gave a rating of PROP at 15 mm and gave a higher rating of NaCl, were categorised as non-tasters. When these 2 methods were tested together, Zhao et al. (2003) found that the classification of PROP taster status was similar for both tests, thus concluding both suprathreshold and PROP paper disk tests are reliable in classifying PROP taster status.

However, measuring PROP taster status in children is not as straightforward as in adults as the methods used in adults (as discussed above) requires participants to rate the bitter intensity of PROP on a complex scale, which may be difficult for children to use. Instead of using a complex scale, a simple forced-choice method is normally used to determine children's PROP taster status, however this method only categorises children into either tasters or non-tasters (Keller, Steinmann, Nurse, & Tepper, 2002; Mennella et al., 2005). This difference in PROP classification method between adults and children may lead to discrepant findings in studies of taste sensitivity and food preferences. Therefore, bitter taste sensitivity measurements other than PROP taster status should be considered in order to increase confidence in study results.

1.5 Fungiform papillae density (FPD)

FPD is also used as a phenotypical measure of taste sensitivity. According to Prescott (2012), when a food enters the mouth, chemical compounds from the food are released which stimulate taste receptors to perceive sourness, sweetness, saltiness or bitterness. People with a high density of taste buds on their tongue will perceive all tastes as more intense compared to those with a low density of taste buds. It is said that the human tongue has between 3000 and 8000 taste buds (Prescott, 2012). A high number of fungiform papillae (FP) can be found at the dorsal anterior tongue in humans (Segovia, Hutchinson, Laing, & Jinks, 2002) and the measurement of FPD can act as a tool to retrieve information about taste functions (Shahbake, Hutchinson, Laing, & Jinks, 2005). FP are mushroom-liked shapes that are embedded with taste buds which contain taste receptor cells and trigeminal (touch) fibres (Feeney, O'Brien, Scannell, Markey, & Gibney, 2014).

A large study that involved 2371 adults aged 21 to 84 years concluded that FPD tends to decrease with age (Fischer et al., 2013). Segovia et al. (2002) found that children aged 8 to 9 years have higher FPD (91/cm^2) than adults aged 18 to 30 years (68/cm^2), similarly, children had higher taste bud density (571/cm^2) than adults (359/cm^2). This study also reported that the papillae diameters in children are smaller and more symmetrical in shape than adults. With children having more FPD and taste buds, they might have higher sensitivity to tastes than adults.

Individuals with higher FPD often rate the intensity of PROP bitterness to be stronger than those with lower FPD (Duffy et al., 2010; Yackinous & Guinard, 2002). Moreover, Essick, Chopra, Guest and McGlone (2003) reported that in 83 adult females (52 Asians and 31 Caucasians) between the ages of 18 to 35 years, super-tasters of PROP have the highest number of papillae (143.7/cm^2), compared to medium- (106.5/cm^2) and non-tasters (54.4/cm^2). Other than bitter tastes, Hayes and Duffy (2008) found that creaminess and sweetness ratings for milk/sugar mixtures to be higher in those with high FPD. Higher FPD is also associated with low liking for both high fat and high sodium foods as well as greater saltiness in salt solutions (Hayes, Sullivan, & Duffy, 2010). In Spence, Hobkinson, Gallace and Fiszman's (2013) review, the predominant attributes recognised in fatty foods result from mouthfeel, tactile sensations in the mouth, rather than true taste sensations. As mentioned previously, FP contain trigeminal fibres which explains those with higher FPD perceive fatty foods as more intense than individuals with lower FPD.

1.6 *TAS2R38*

Variations in individual PROP sensitivity are genetically predisposed. Bitter tastes are detected by taste type 2 receptors (T2R) located mainly in taste buds (cells) within the papillae on the surface of the tongue. These receptors also can be found in the palate and epiglottis (Garcia-Bailo, Toguri, Eny, & El-Sohemy, 2009). Up until now, 25 T2R bitter receptors have been discovered in humans and each one of these receptors reacts differently to various bitter