



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

# Food Hedonic Properties: Color and Flavor

---

Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



Department  
Drug Science  
and Technology

## **Food Hedonic Properties: Color and Flavor**

### **Hedonic profile of food**

Relevance and relation to food choice

Aroma, taste, trigeminal perception (coolants and spicy perception)

Cross-modal interactions

### **Pigments**

Psychological effects of food colors

Natural pigments examples: anthocyanins, betalaines, carotenoids

Melanoidins - Maillard reaction products

### **Case studies**

Plant-based meat color and flavor

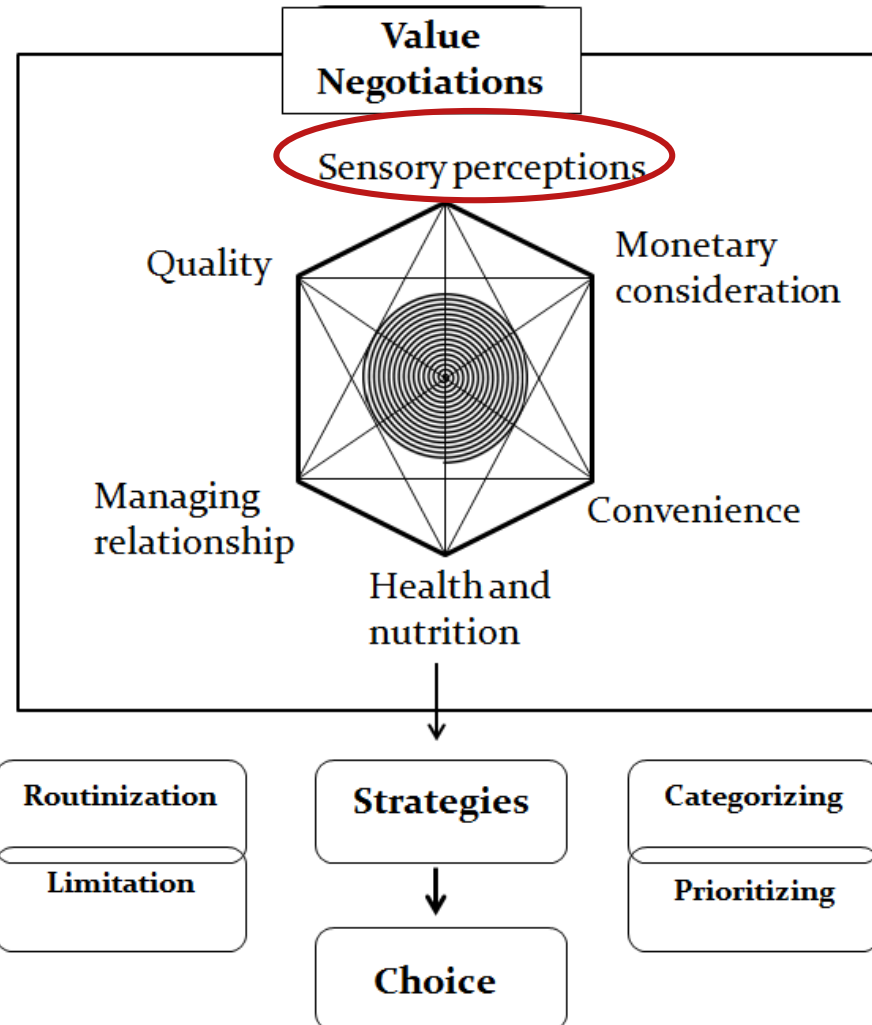
# The Food Choice

Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

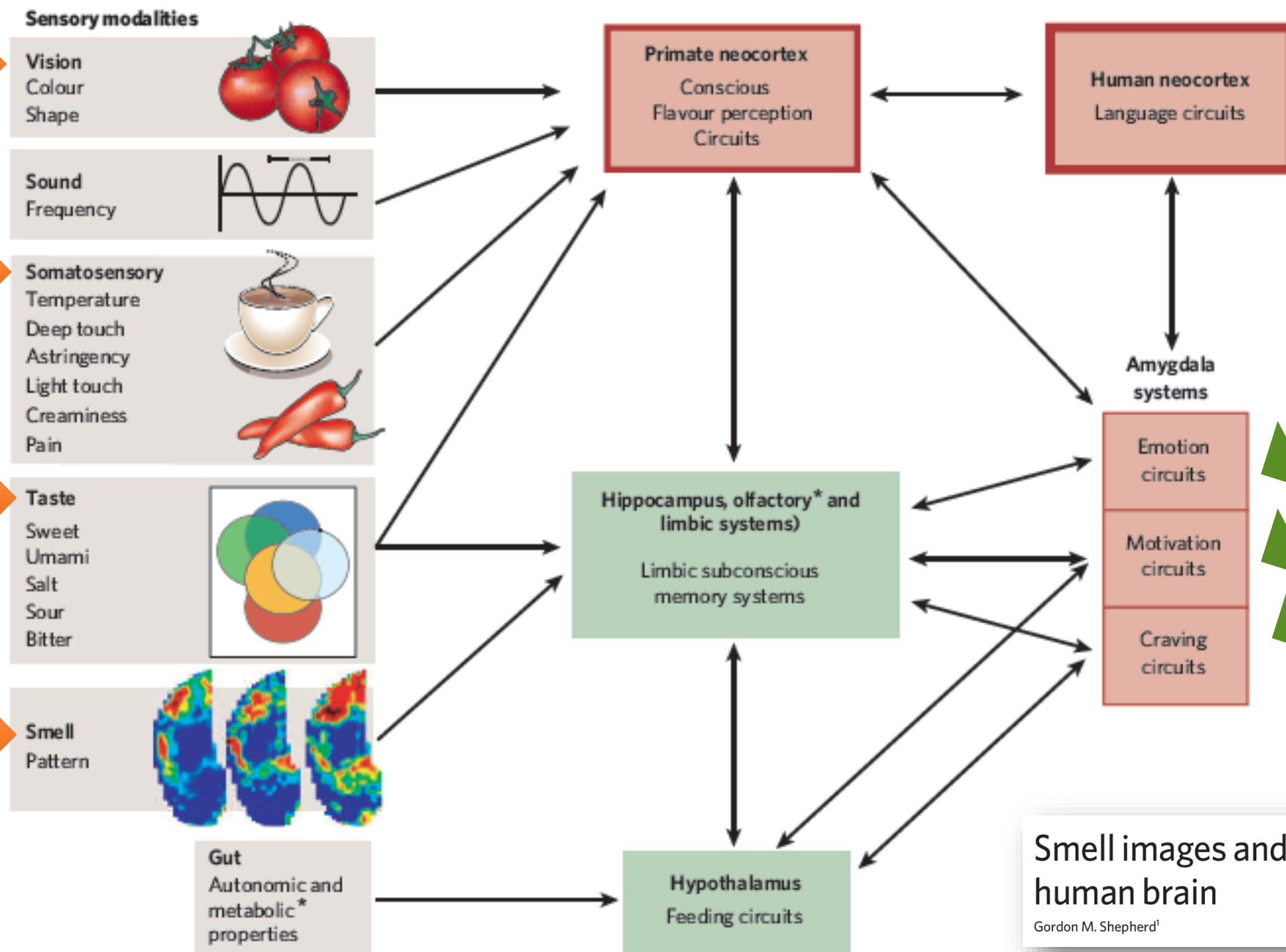


Nowadays people are faced with many food products and they're involved in diverse eating situations that can make food-choice decisions complex. As a consequence, many disciplines examine the **process** of **decision making** in food behaviour but, despite the efforts, it remains a topic not well understood.

As long as food is potentially available and accessible everywhere and anytime, **individuals experience food choice events daily**. A single **individual** and his/her **social influences** are **both** at the **base** of a **food choice**. In particular food **choice decisions are multifaceted and multistructured events that incorporate conscious decisions but also automatic, habitual and subconscious ones**.

An essential but comprehensive portrait of food choice process has been proposed in 1996 by Furst *et al.* (Furst T. 1996).

Authors observed that people simplify the task of making decisions by using an individualized set of categories.



Smell images and the flavour system in the  
human brain

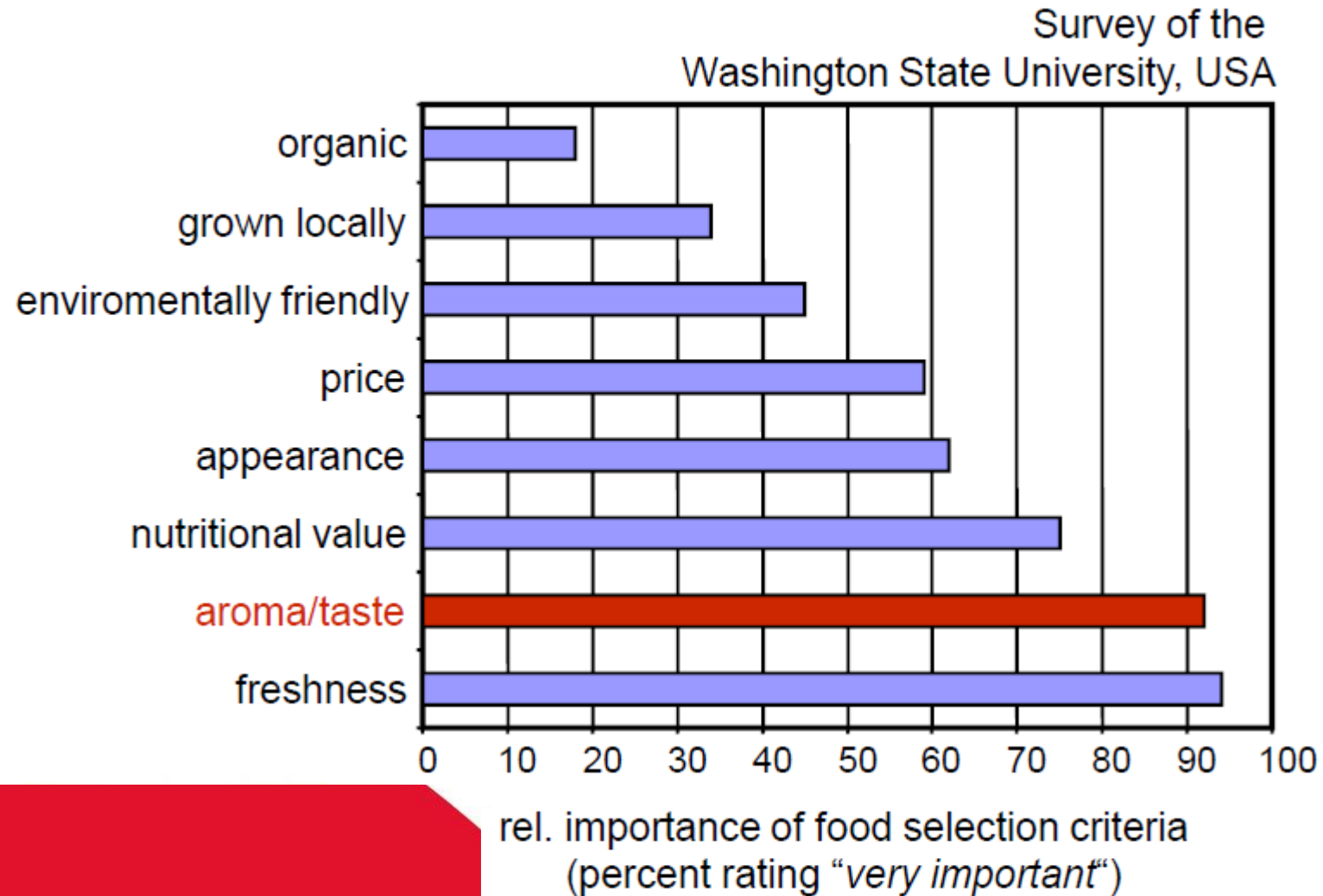
Gordon M. Shepherd<sup>1</sup>

Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO



# The Sensory System

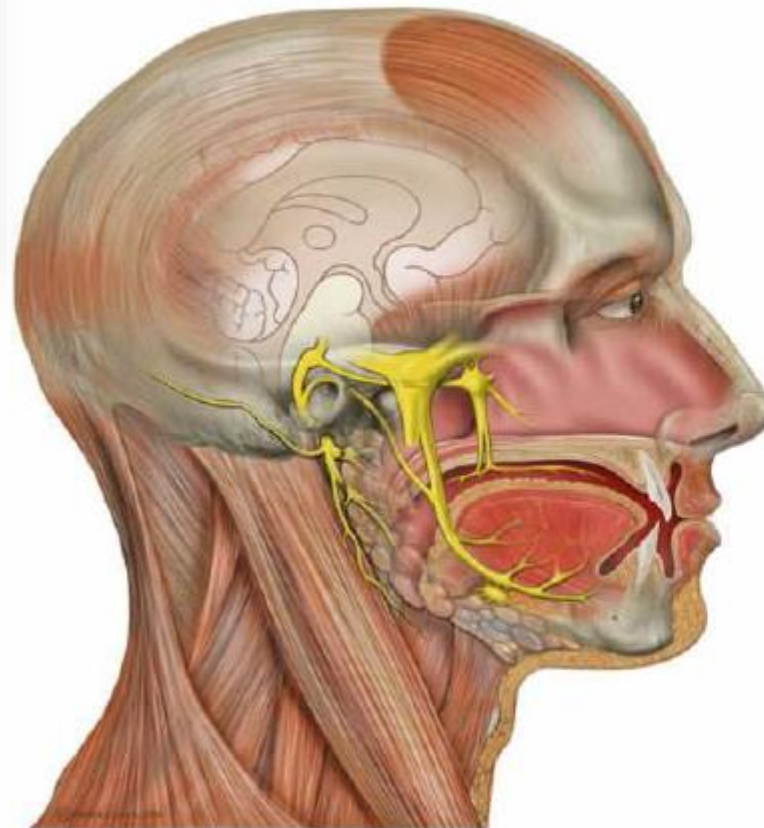
Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



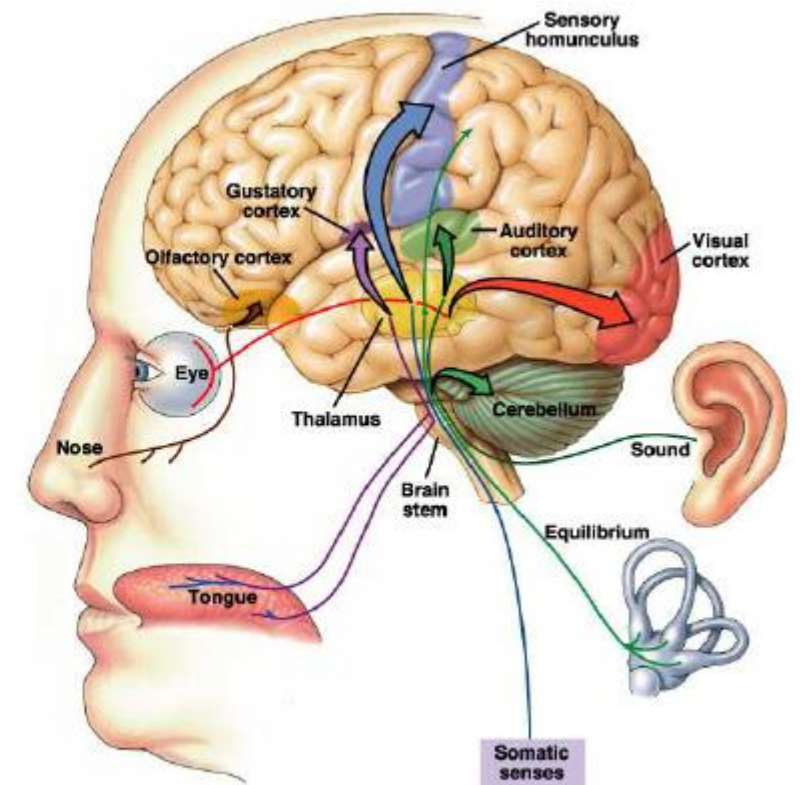
Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

odor	(nose)
taste	(mouth)
sight	(eyes)
sound	(ears)
touch	(mouth, skin)
temperature	(mouth, skin)



trigeminal perception



odor

taste



Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

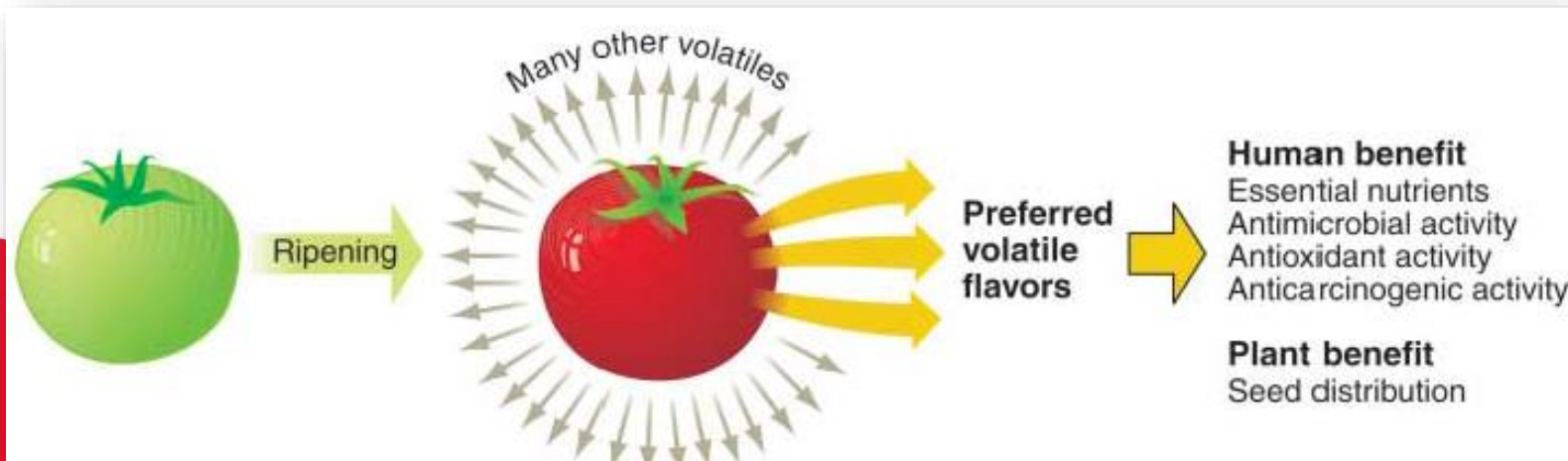
REVIEW

# Plant Volatile Compounds: Sensory Cues for Health and Nutritional Value?

Stephen A. Goff<sup>1\*</sup> and Harry J. Klee<sup>2</sup>



Olfaction and molecular coding



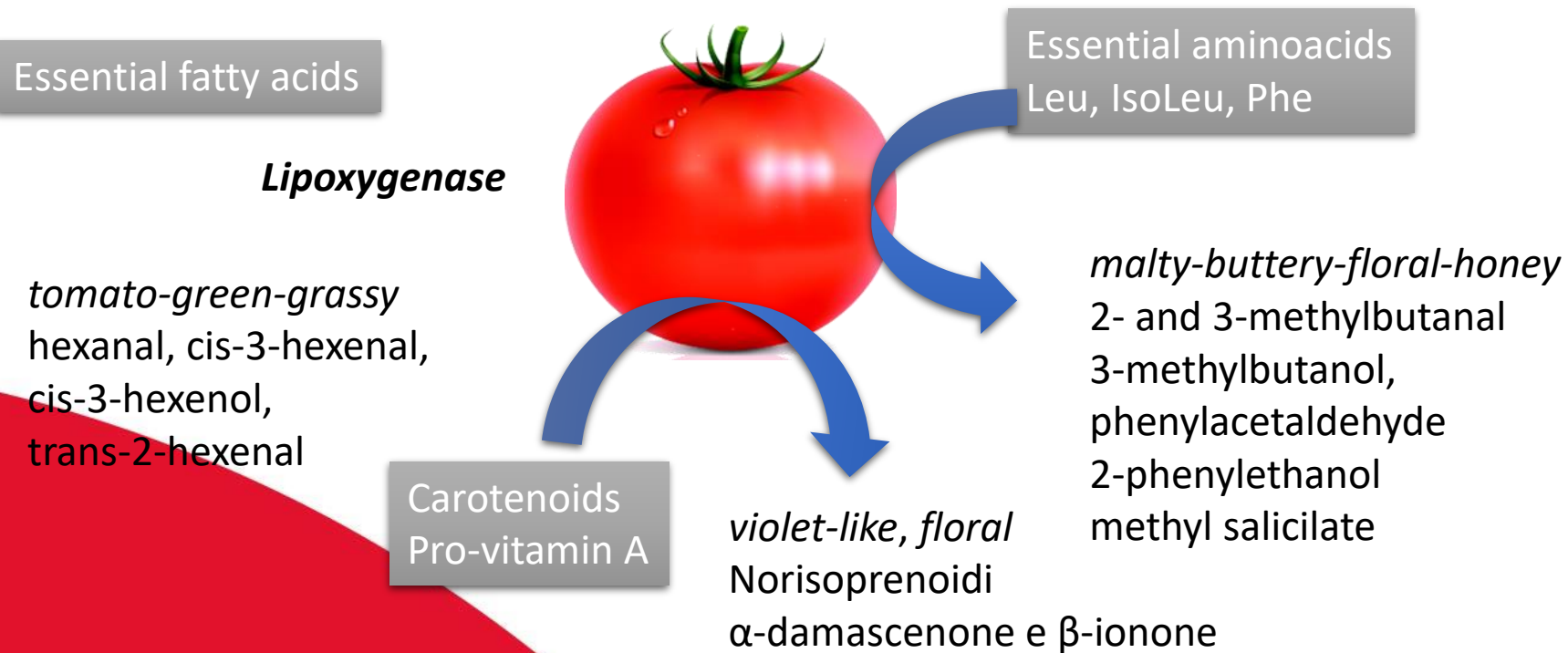
# Olfaction and molecular coding

Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO





# Olfaction and molecular coding

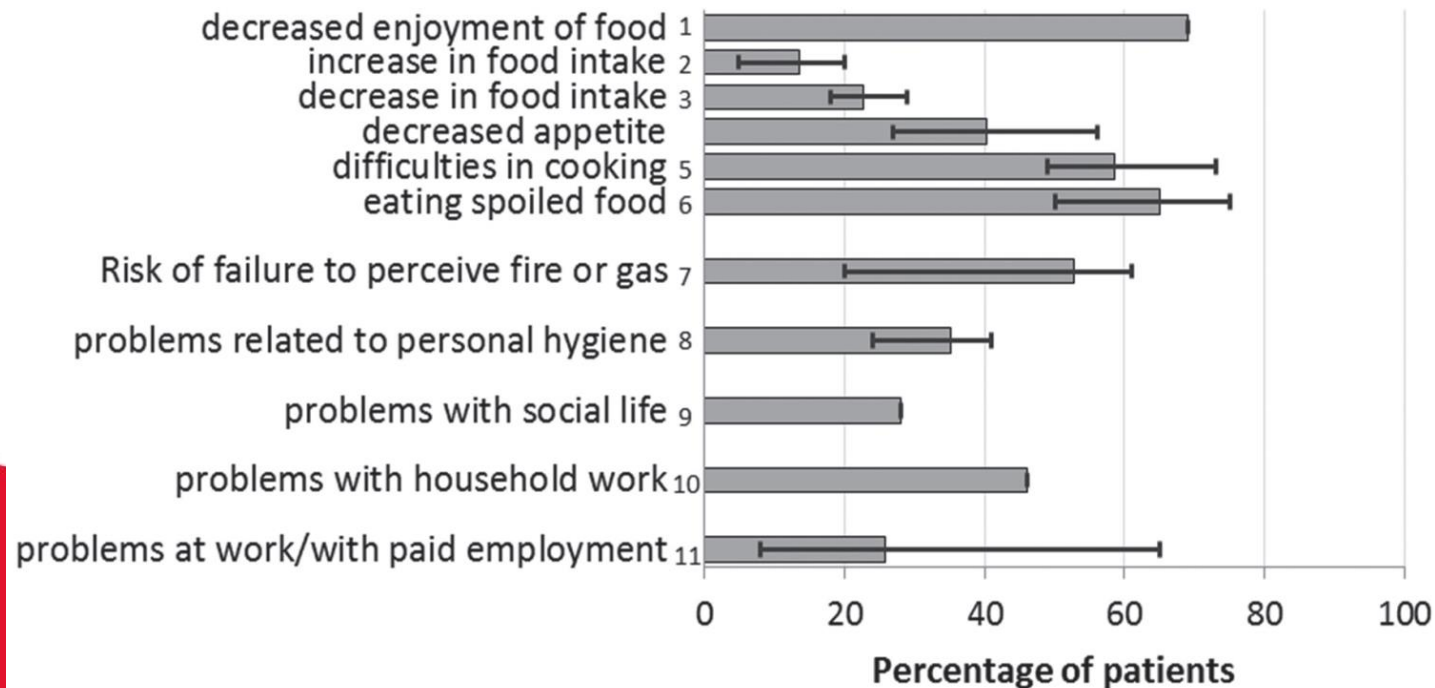
Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

## Daily life problems in patients with smell disorders



Chem. Senses 39: 185–194, 2014

doi:10.1093/chemse/bjt072  
Advance Access publication January 15, 2014

Olfactory Disorders and Quality of Life—An Updated Review

Ilona Croy<sup>1,3</sup>, Steven Nordin<sup>2</sup> and Thomas Hummel<sup>3</sup>

Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

Unsolved Mystery

## The Human Sense of Smell: Are We Better Than We Think?

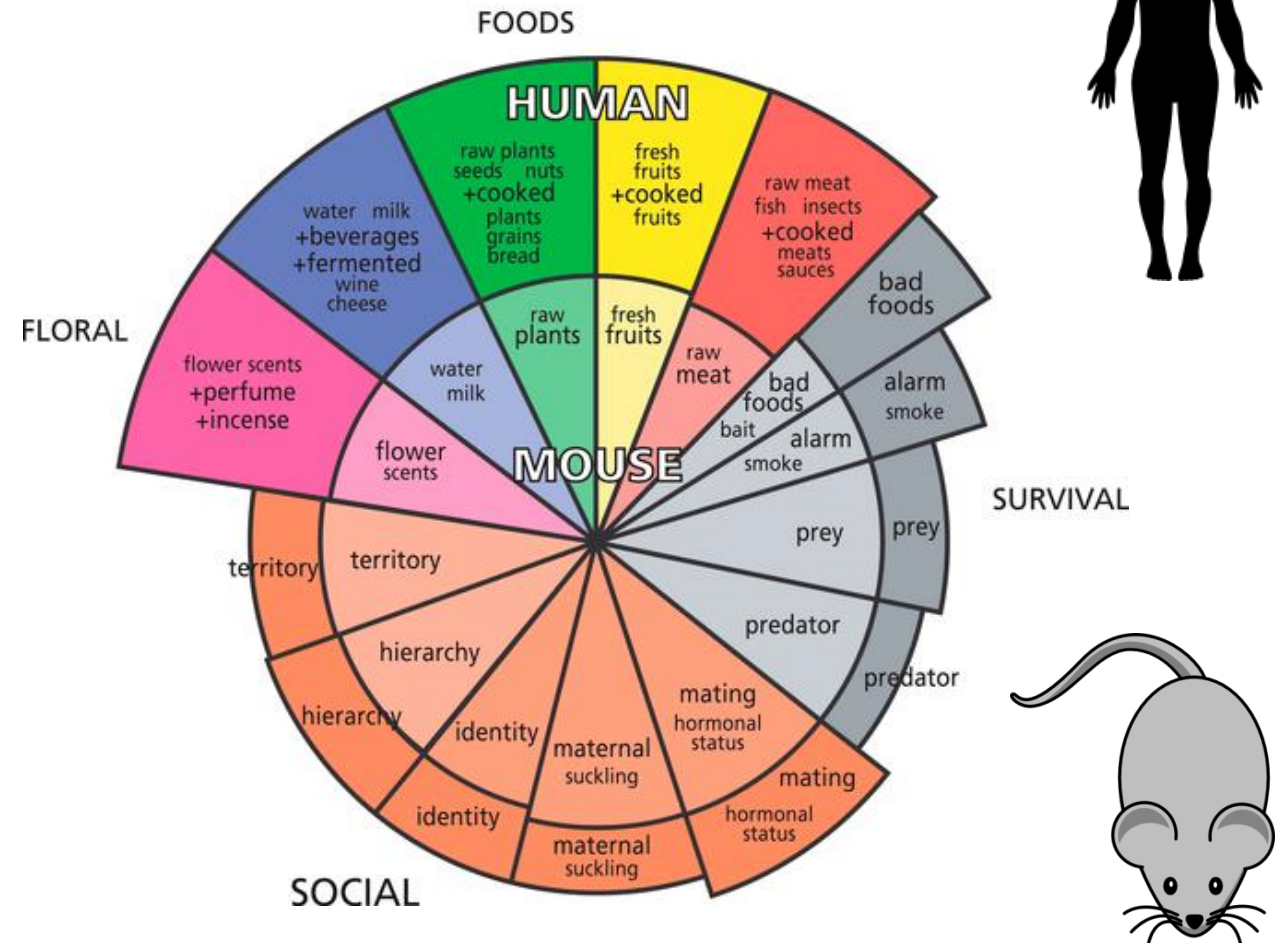
Gordon M. Shepherd

The inner sphere represents the different categories of odors / odorants for the mouse; the external sphere concerns man. The area of each sector is relativized to the importance of the specific function on the entire repertoire while the relative weight of mouse / human is highlighted by the size of the area.

It is evident that the sphere of odorants linked to food is dominant in humans.

Shepherd GM (2004) The Human Sense of Smell: Are We Better Than We Think?.  
PLoS Biol 2(5): e146. doi:10.1371/journal.pbio.0020146  
<http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.0020146>

RELATIVE ODOR WORLDS OF MOUSE AND HUMAN



# Olfaction and molecular coding

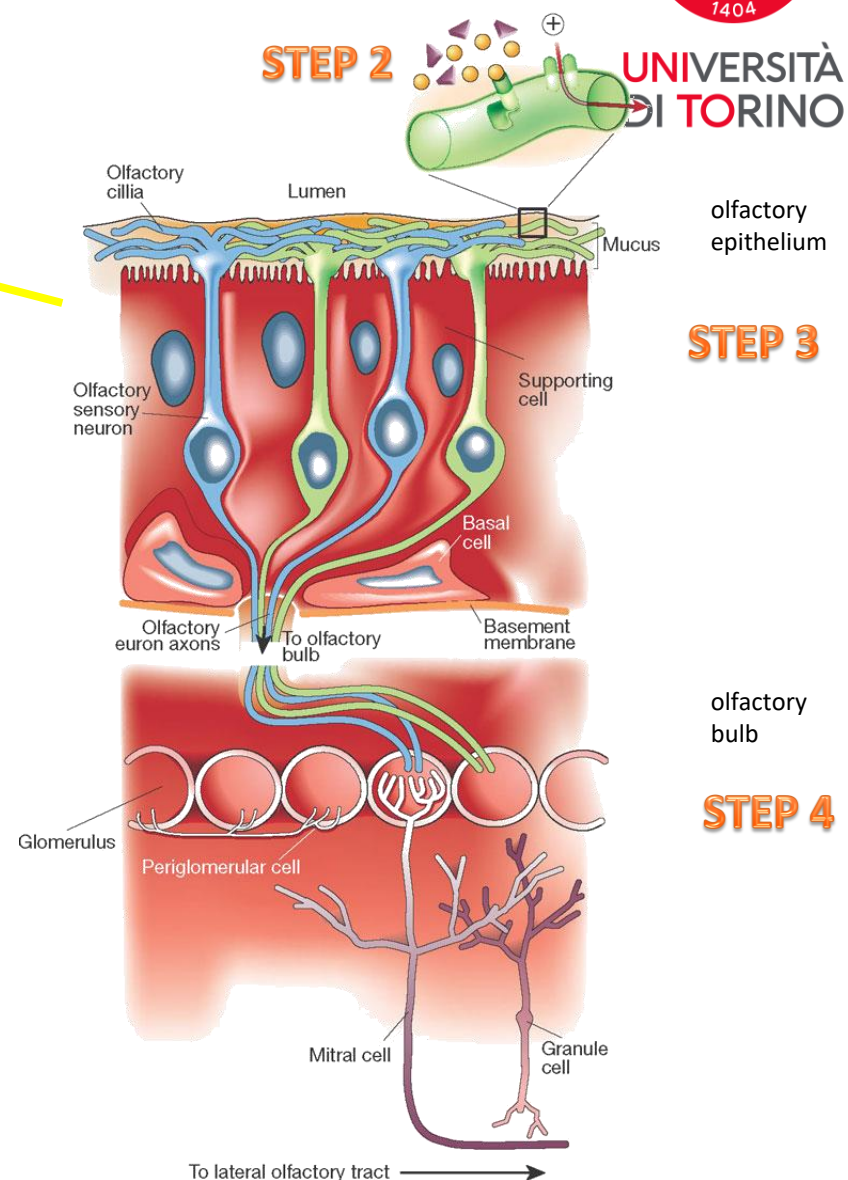
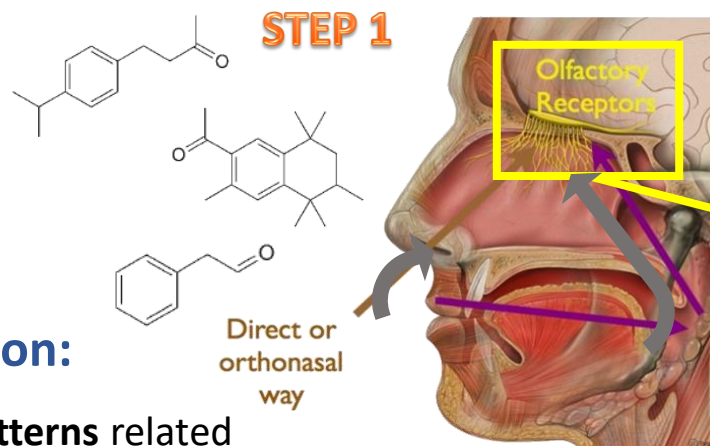
Chiara Emilia Irma Cordero <chiara.cordero@unito.it>



Department  
Drug Science  
and Technology

## Chemistry behind olfaction:

- ✓ Identification of chemical patterns related to the **combinatorial code of olfaction**  
analytical chemistry - separation science space
- ✓ Identification of ligands for olfactory receptors (pharmacological space)
- ✓ Identification of chemical features related odor quality and odor threshold  
(SAR studies – medicinal chemistry space)
- ✓ Characterization of perireceptor events that modulate odorant-receptor interaction  
(chemical biology space)



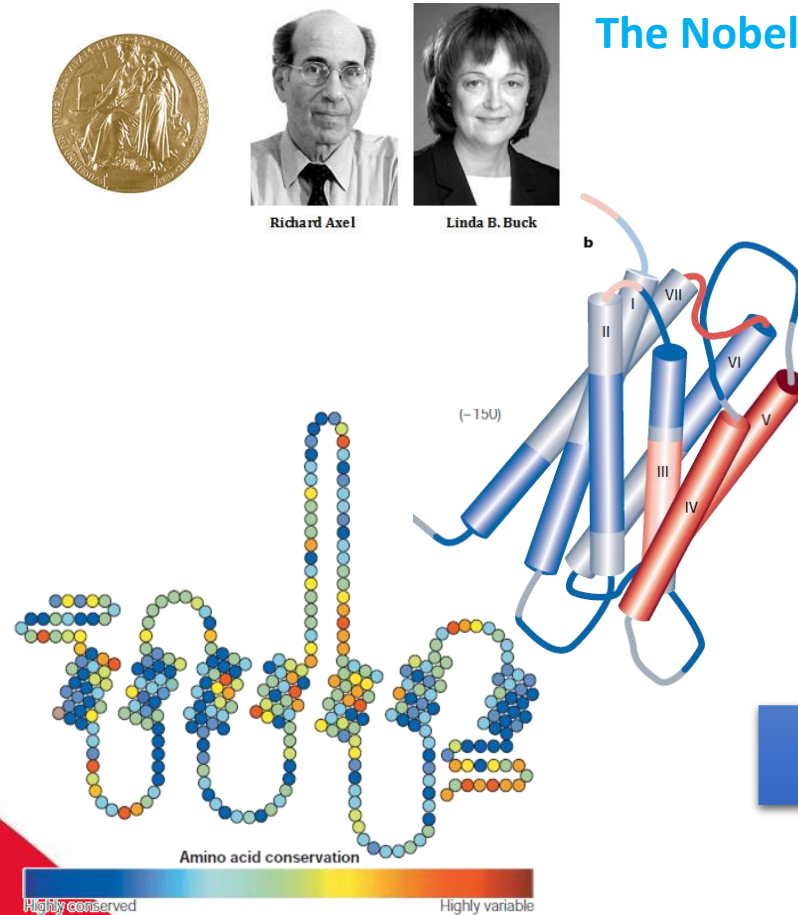


## Olfactory Receptors (ORs) and the Receptor Code

ORs are members of a superfamily of up to 1000 different **G-protein coupled receptors** ( $G_{olf}$ ) with seven trans-membrane (7TM) domains.

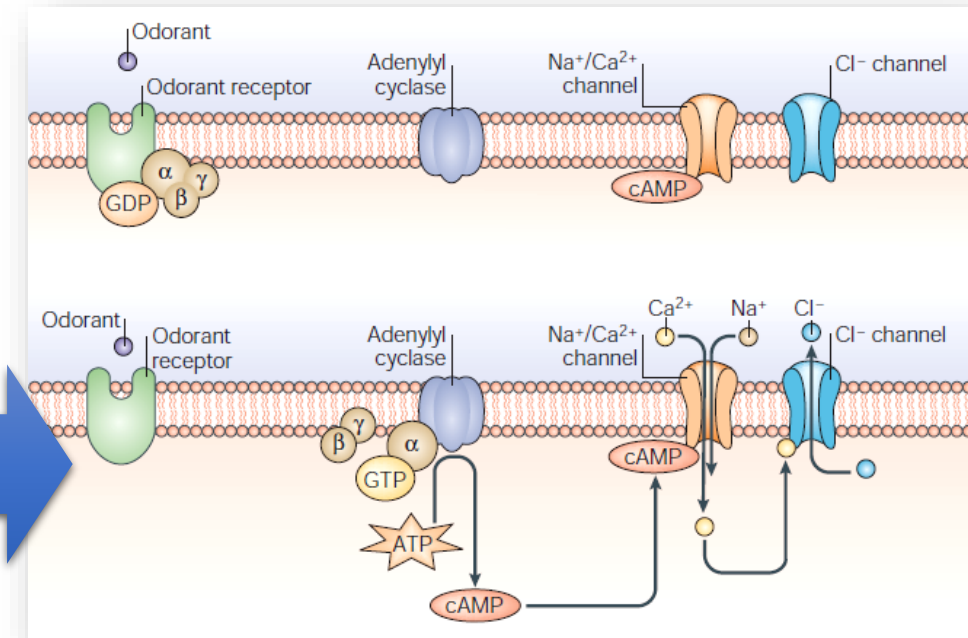
The location of odorant binding is thought to be a hydrophobic pocket in trans-membrane regions 3, 4, and 5 of the seven-membrane-spanning receptor.

Canonical pathway of signal transduction in olfactory sensory neurons (OSN)



The Nobel Prize Medal for Physiology or Medicine Year 2004

*"for their discoveries of odorant receptors and the organization of the olfactory system"*



Chiara Emilia Irma Cordero <chiara.cordero@unito.it>

## Odor and olfaction - Aroma perception

Unsolved Mystery

### The Human Sense of Smell: Are We Better Than We Think?

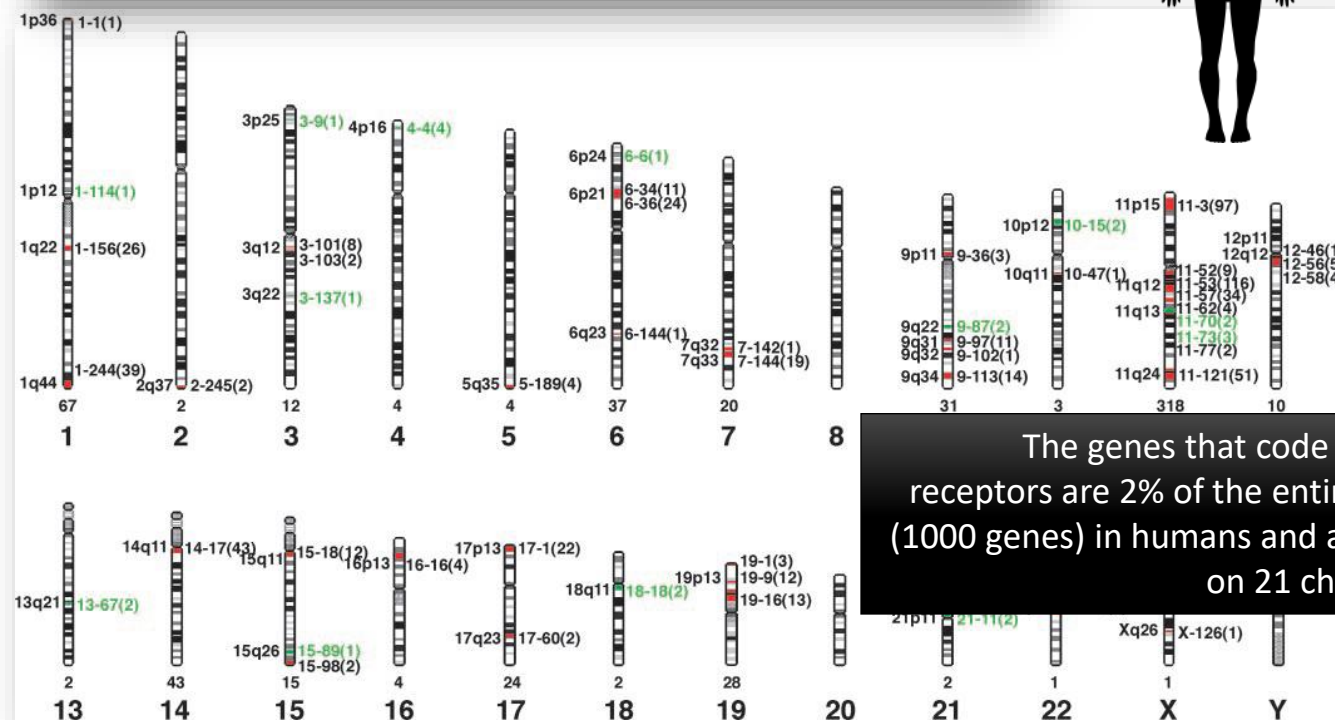
Gordon M. Shepherd

The reduced repertoire of olfactory receptor genes in the human is thus offset by the expanded repertoire of higher brain mechanisms. Rather than being restricted to a tiny part of the brain, olfactory processing of complex smells, such as those produced by human cuisines, draws on the enlarged processing capacity of the human brain.

PNAS | February 24, 2004 | vol. 101 | no. 8 | 2585

## The human olfactory receptor gene family

Bettina Malnic<sup>\*†</sup>, Paul A. Godfrey<sup>†‡</sup>, and Linda B. Buck<sup>\*§</sup>



The genes that code for olfactory receptors are 2% of the entire repertoire (1000 genes) in humans and are scattered on 21 chromosomes.

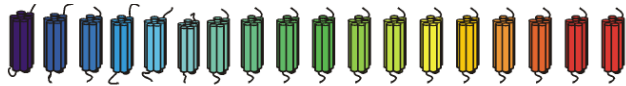
Chromosome locations of human OR genes. Six hundred thirty OR genes were localized to 51 different chromosomal loci distributed over 21 human chromosomes. OR loci containing one or more intact OR genes are indicated in red; loci containing only pseudogenes are indicated in green.

# Olfaction and molecular coding

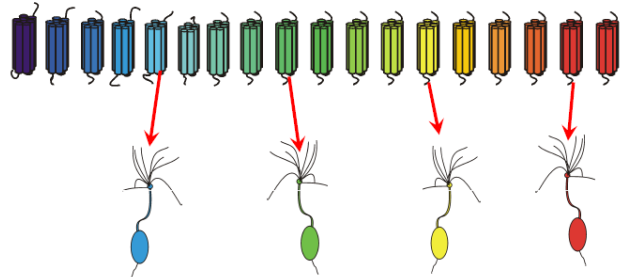


Department  
Drug Science  
and Technology

## 1. Humans ≈ 400 different ORs



## 2. Each OSN expresses only one kind ORs



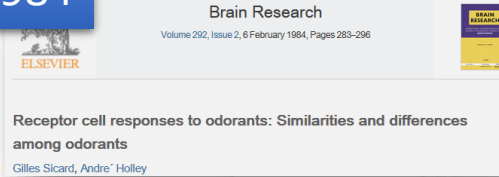
Selectivity study on 60 olfactory neurons tested with 20 different odorants.

Each neuron is equivalent to one column, each odorant to one row. The size of the dots symbolizes the number of action potentials.

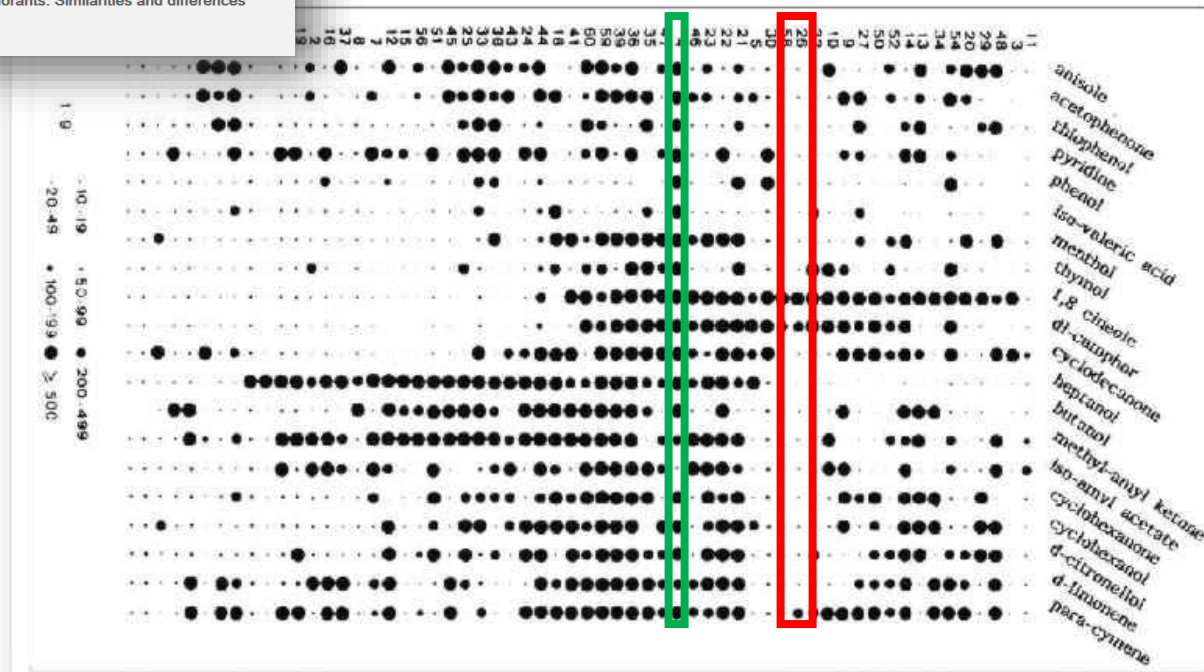
Neurons 26 and 58 react specifically on cineole and camphor.

Neuron 4 reacts on all odorants except isoamyl acetate.

1984



## 3. ORs are not highly specific multiple activation/modulation



### Receptor Code

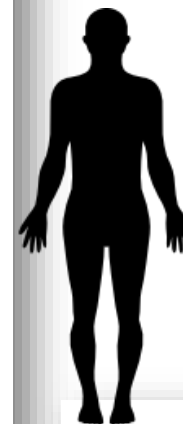
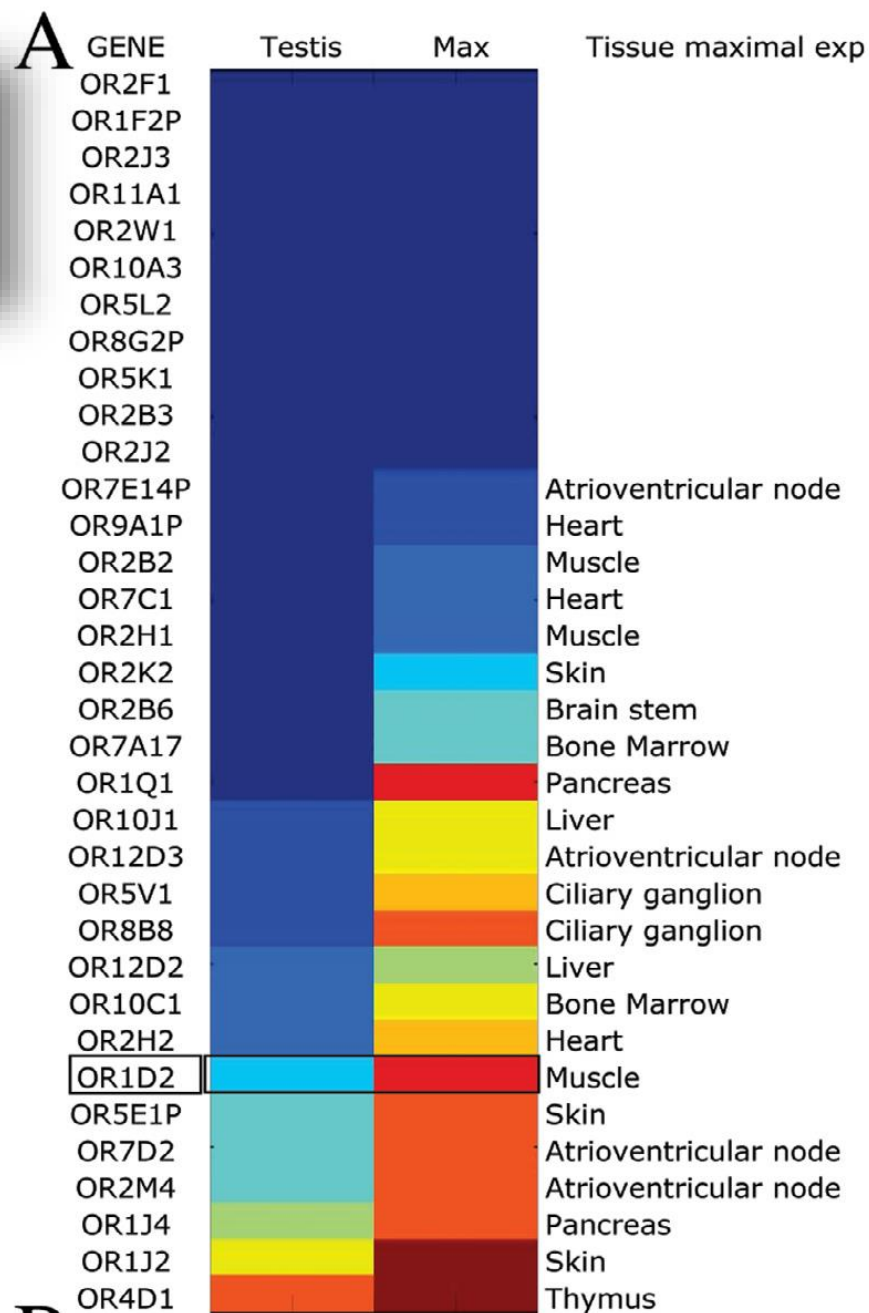
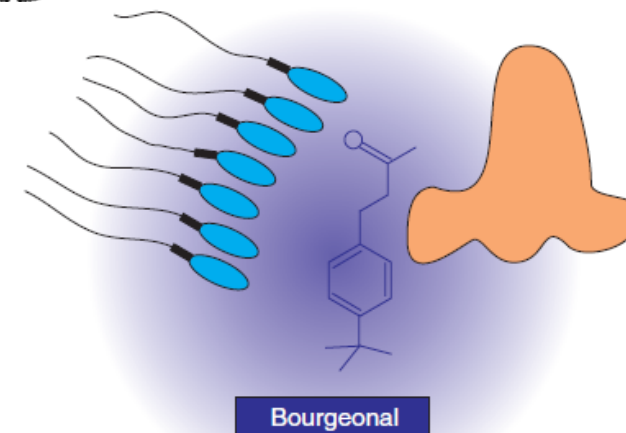
Complex activity pattern of many differently tuned olfactory neurons that project in specific regions of the olfactory bulb



## Olfactory receptors in non-chemosensory tissues

NaNa Kang &amp; JaeHyung Koo\*

Department of Brain Science, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 711-873, Korea

UNIVERSITÀ  
DI TORINODepartment  
Drug Science  
and Technology

Current Biology

Figure 1. Testes and the nose both express members of the odorant receptor super-family of G protein-coupled receptors. The testes odorant receptor hOR 17-4, previously shown to interact with the floral odor bourgeonal, is now shown to be expressed in the nose as well. This suggests that hOR 17-4 has evolved a dual role in chemoreception: perhaps guiding sperm to the egg and providing a conscious perception of odors through the nose. A gradient of bourgeonal is shown to attract sperm (left) and to be smelled by the nose (right).

Research article

## Widespread ectopic expression of olfactory receptor genes

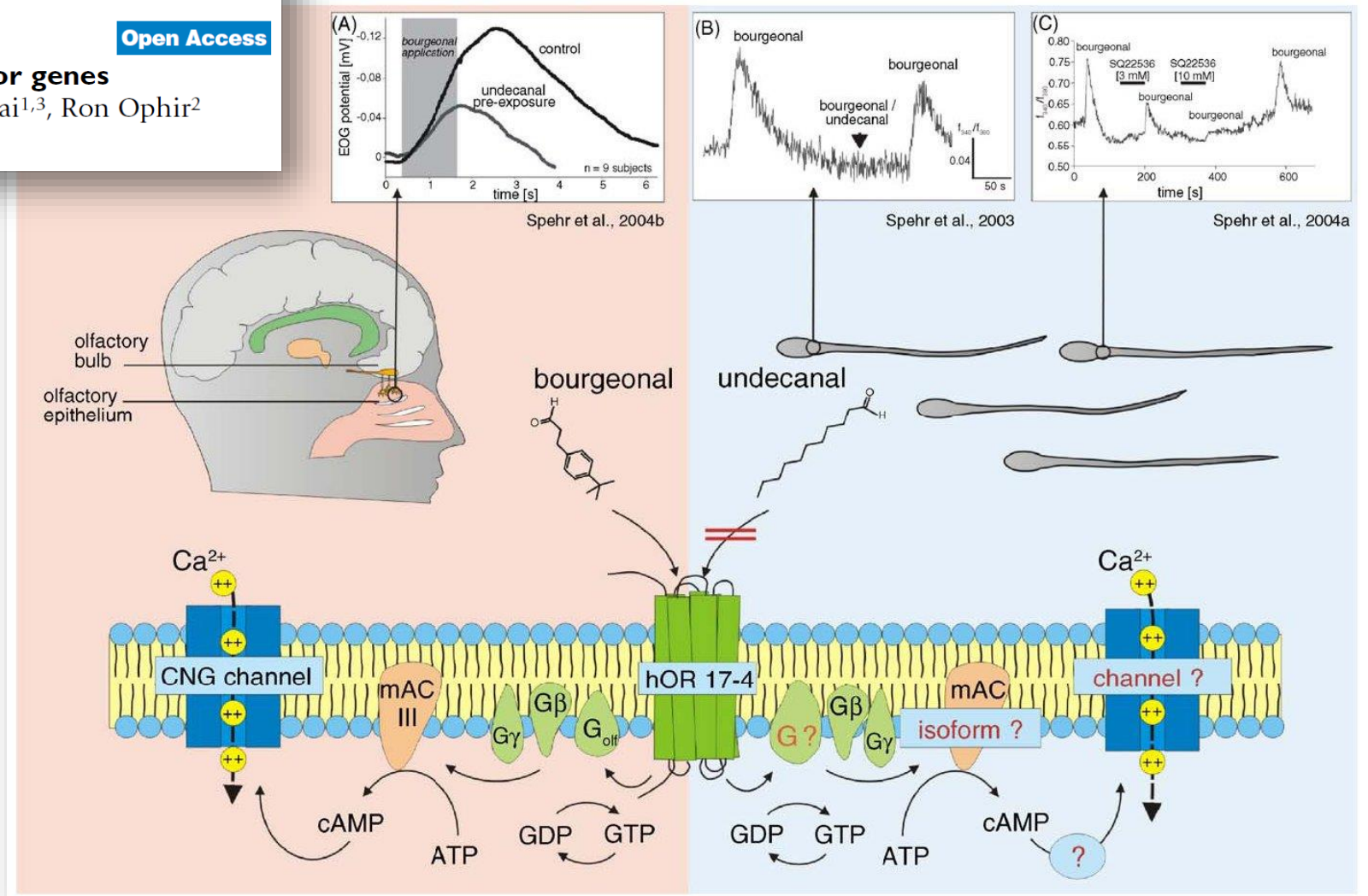
Ester Feldmesser<sup>1</sup>, Tsviya Olender<sup>1</sup>, Miriam Khen<sup>1</sup>, Itai Yanai<sup>1,3</sup>, Ron Ophir<sup>2</sup> and Doron Lancet<sup>\*1</sup>

Open Access

**Bourgeonal**: powerful chemotactic agent for spermatozoa

Reduced olfactory sensitivity to bourgeonal has been linked to the phenomenon of idiopathic infertility

Reduced expression of the OR 17-4 receptor and / or its mutation spermatozoa lose "orientation" (chemotaxis)



## Olfaction and molecular coding

Network that correlates the set of odorants (chemical ligands to olfactory receptors) and characterize the aroma of a food and the technological process of transformation.

Food groups:

- ✓ cheeses and milk derivatives
- ✓ alcoholic beverages
- ✓ cooked fish
- ✓ juices of the genus Citrus
- ✓ fruit juices (Pomaceae)
- ✓ thermized yeast

Process type:

green: raw food

blue: fermentation

red: boiling and cooking in water

orange: dry thermal processes





# Taste perception and molecular coding

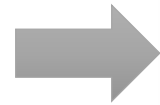
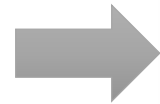







Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

Each "base" taste corresponds to a class of nutrients:

- ✓ **sweet** presence of carbohydrates;
- ✓ **umami** presence of proteins and amino acids;
- ✓ **salty** presence of osmolytes (NaCl);
- ✓ **bitter** presence of potentially toxic substances;
- ✓ **acid** informs about "freshness" (fermentations).



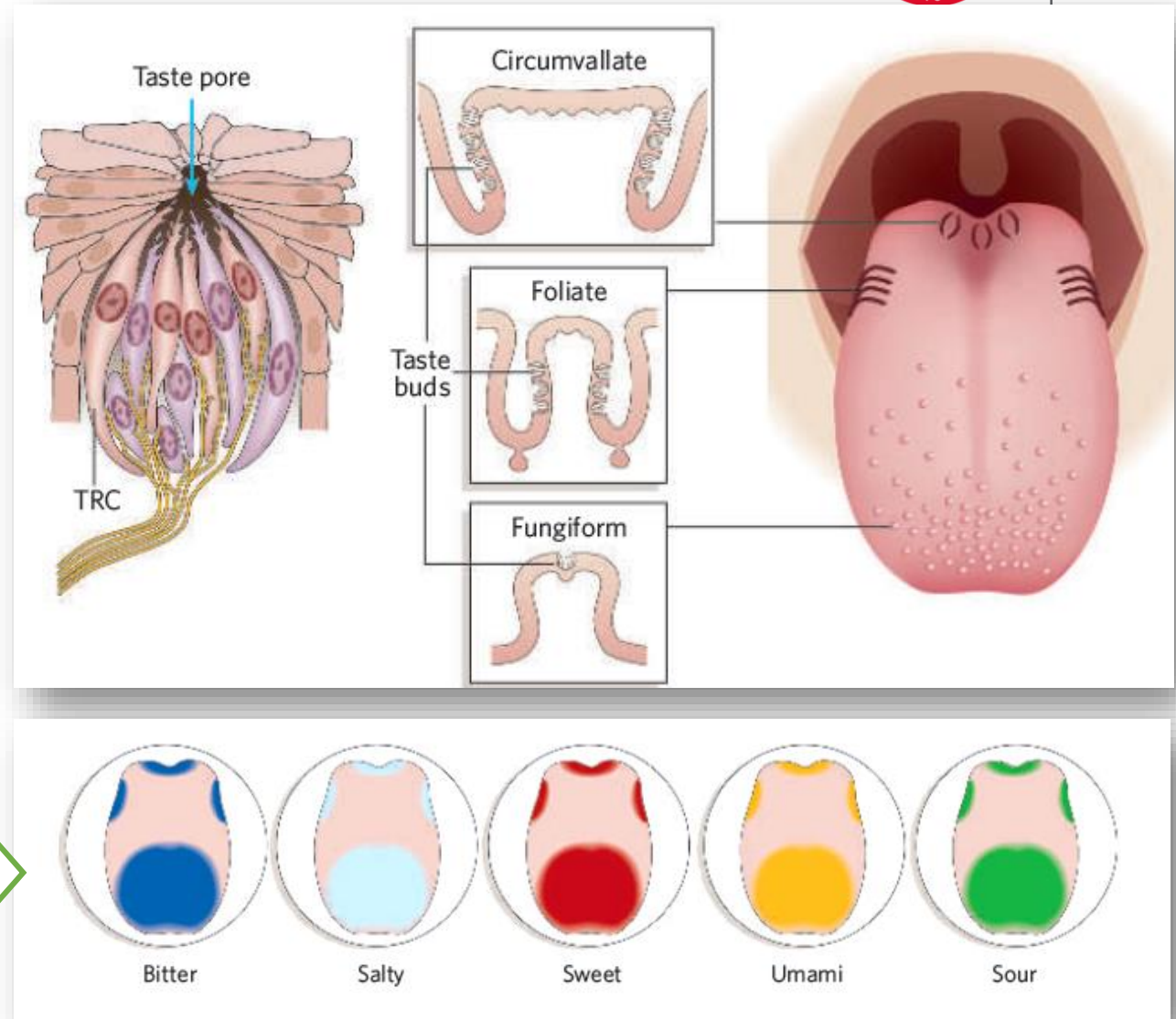
quality		function	hedonic	substance	threshold (mol/L)
bitter		warning	aversive	quinine sulfate nicotine	0.000008 0.000016
sour		warning	aversive attractive	HCl citrate	0.0009 0.0023
sweet		calory detector	attractive	sucrose glucose	0.01 0.08
salty		osmo- regulation	attractive	NaCl	0.01
umami		calory detector	attractive	Na-glutamate	0.01

# Taste perception

The taste receptors, organized in "taste buds" (gustatory buttons) are located within structures called "papillae" distributed in the oral cavity from the oropharynx to the tongue.

Each papilla (circumvallate, foliate and fungiform) includes from 30 to 70 taste cells that express different types of chemoreceptors, each cell is responsible for modulating a single modality: acid, sweet, bitter, etc ...

The perception of the five basic tastes (bitter, salty, sweet, umami and sour) is "widespread" and not localized as was previously thought.

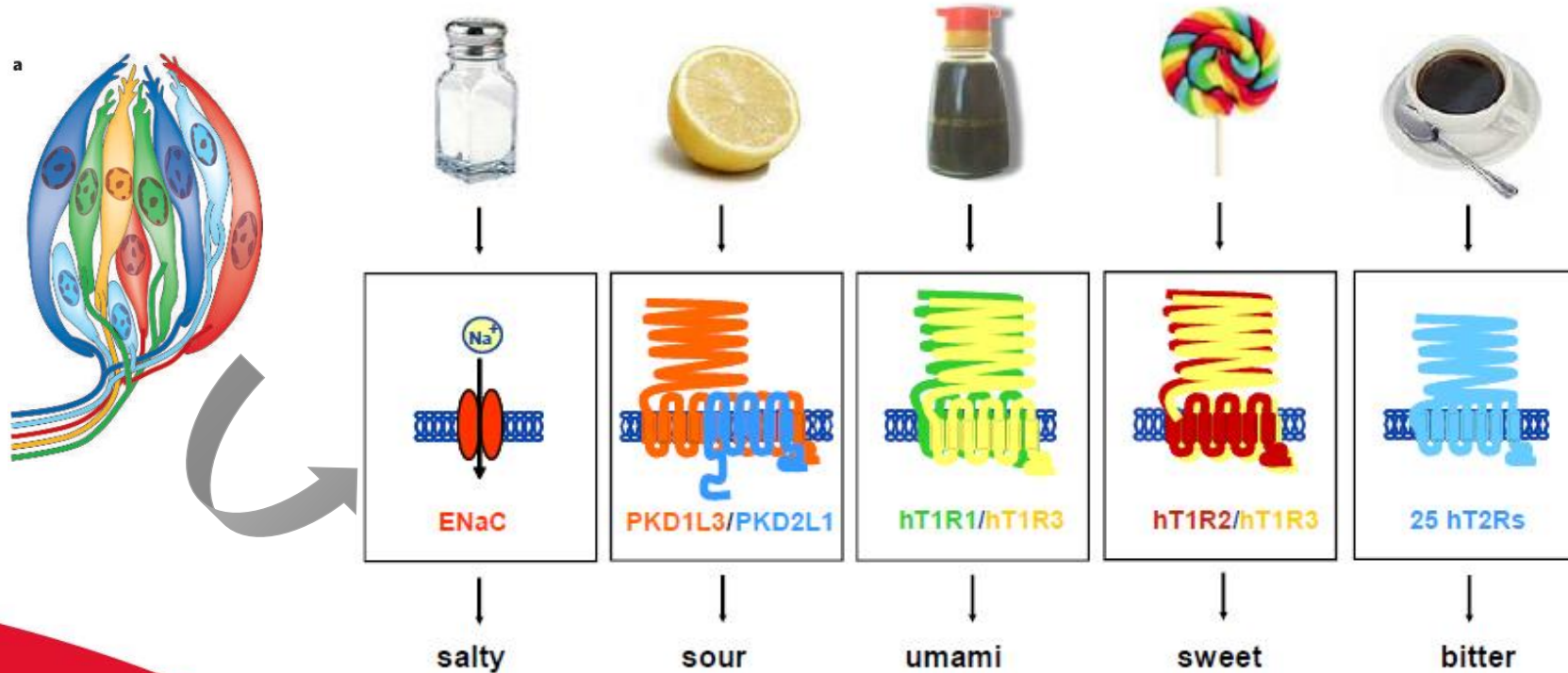


# Taste perception



Department  
Drug Science  
and Technology

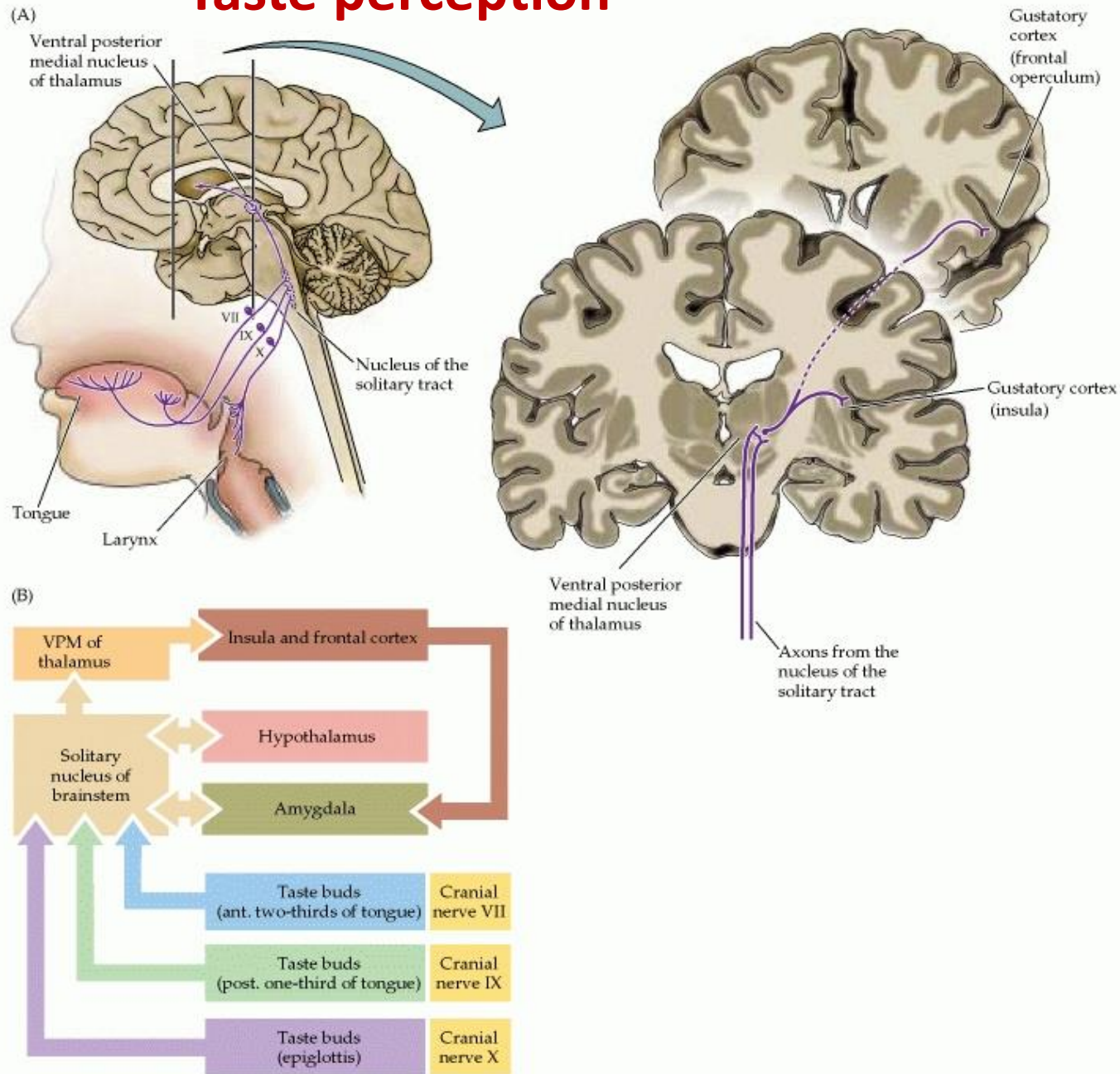
UNIVERSITÀ  
DI TORINO



Each taste cell expresses a different receptor (or a combination of several receptors), which once activated by binding with an agonist (taste molecule) gives rise to a cascade of events that will ultimately lead to the transfer of the signal to the nerve fibers. afferent (and therefore to the CNS).



# Taste perception



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

# Taste perception

The receptors responsible for perception: bitter, sweet and umami are defined

## METABOTROPICS

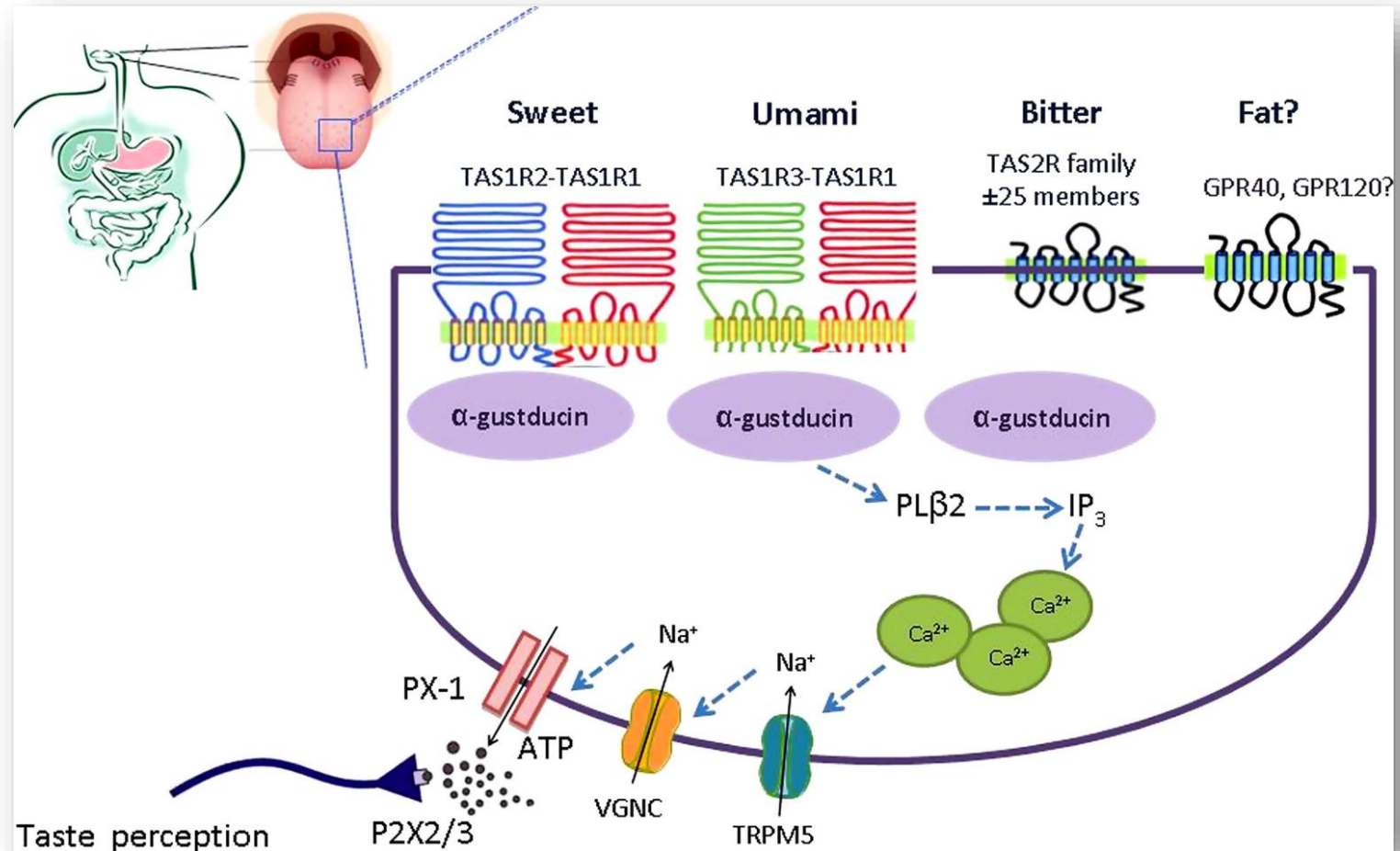
### Taste Receptor type 1 and Type 2 (T1R and T2R)

They are membrane receptors connected to G-proteins that activate signaling pathways that result in the entry of  $\text{Ca}^{2+}$  ions into the cell with the consequent release of a neuromediator into the inter-synaptic space.



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO



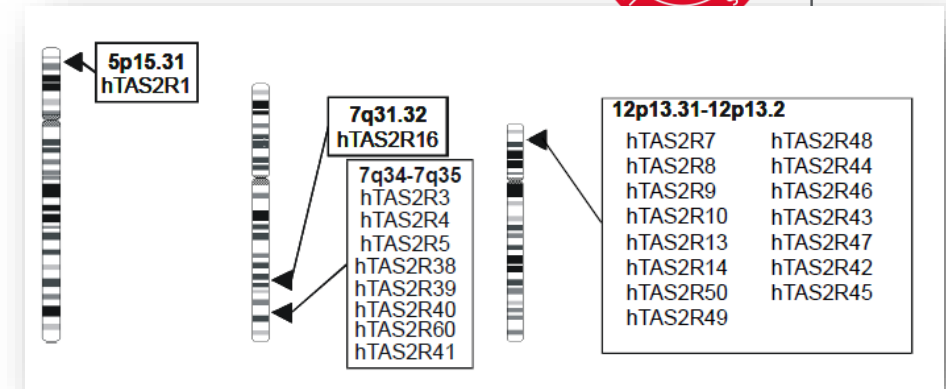
# Taste perception



## Example:

HT2R bitter receptors (at least 25 isotypes)  
located on three chromosomes: 5, 7, 12.

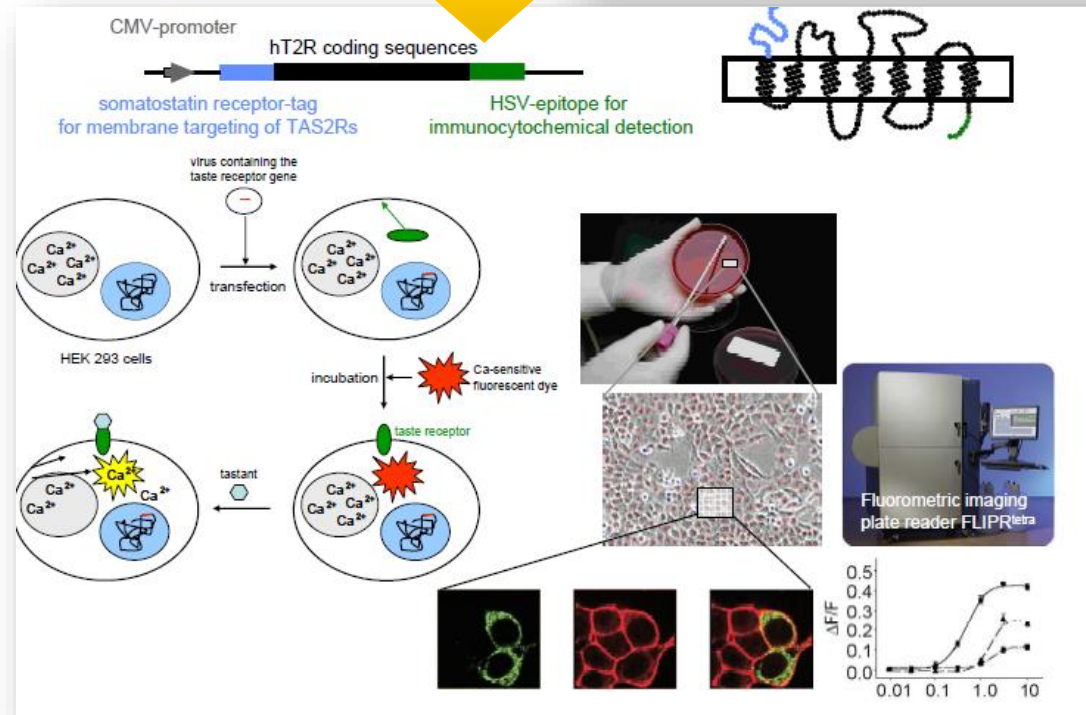
Their functional expression in heterologous systems allowed to know  
their "pharmacological" characteristics of selectivity and activation  
threshold.



Receptor expression in cell lines from  
human embryos >> kidney cells.

DNA is transferred by viral vector, cells  
in culture will express the receptor.

By means of **calcium imaging** with  
fluorescent markers the responsive  
cells (activated by the ligand) will be  
highlighted.








# Taste perception

Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO



As with aroma compounds, a single ligand might activate multiple taste receptors simultaneously, generating a receptor activation code that will result in a final perception.

		T2R																													
compound		1	3	4	5	7	8	9	10	13	14	16	38	39	40	41	42	43	44	45	46	47	48	49	50	60					
	(-)-α-thujone										x																				
	sodium benzoate										x																				
	picrotin										x																				
	picrotoxinin										x											x									
	absinthin																					x									
	limonin																				x										
	herbolide A																				x										
	salicin											x																			
	amygdalin											x																			
	arbutin											x																			
	helicin										x																				
	(-)-epicatechin													x																	
	phenylethylisothiocyanate												x																		
	PTC												x																		
	PROP			x									x																		
	isohumulone	x									x																				
	humulone	x													x																
	Gly-Leu	x		x							x	x																			
	acesulfam K																		x	x											
	saccharin							x											x	x											
	O-caffeoyl quinide						x				x			x																	
	sucrose octaacetate						x		x												x										
	caffeine								x										x		x										
	quinine HCl	x		x		x			x		x				x				x		x										
	strychnine			x		x	x		x		x				x	x			x	x		x									
denatonium benzoate			x			x		x	x					x				x	x		x	x									



# Taste perception



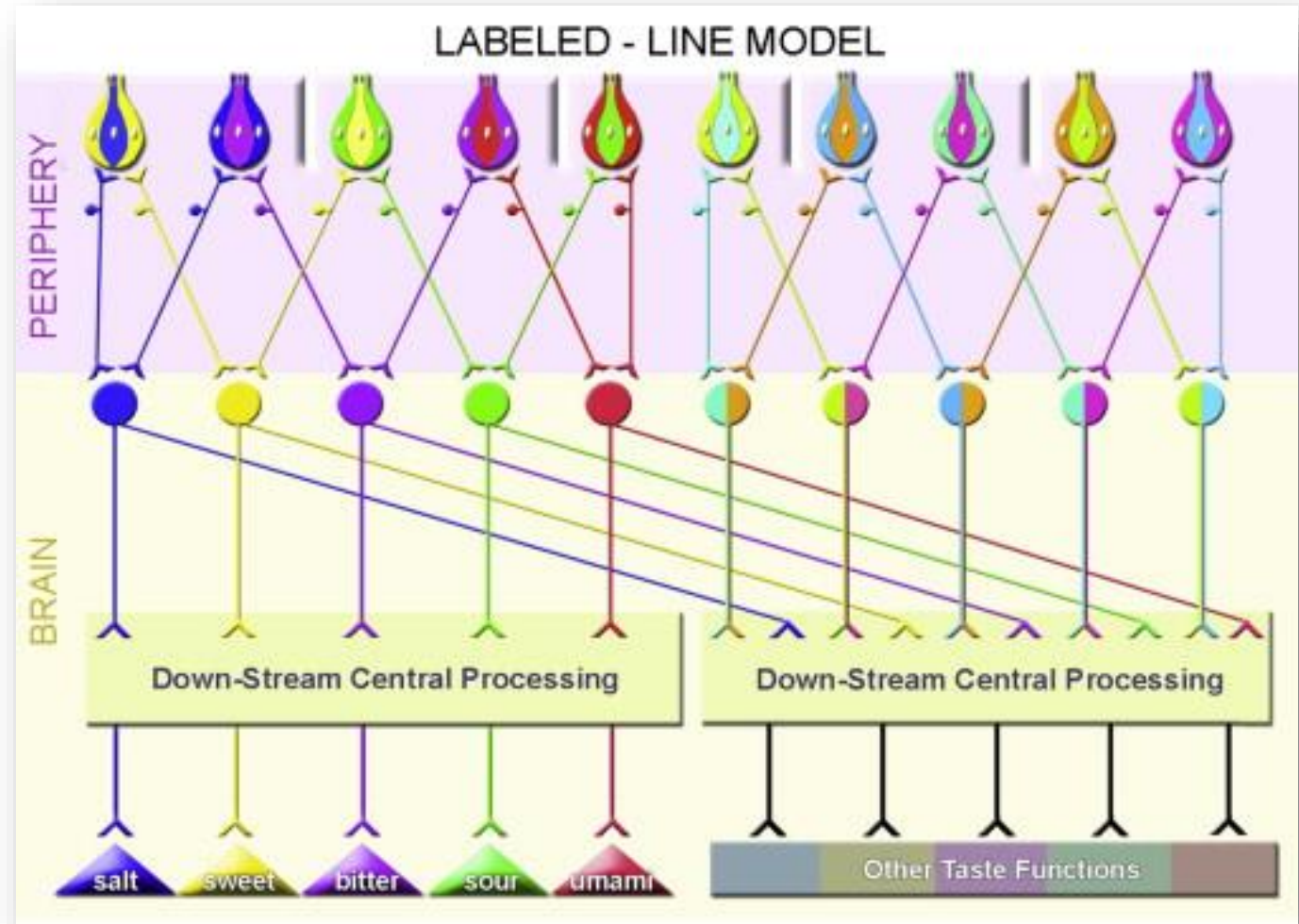
Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

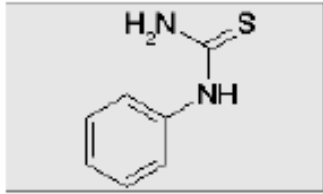
The interaction with the "agonists" (taste molecules) causes the simultaneous activation of different receptors. The final perception will be the outcome of the integration of the individual signals by the CNS. Also in the case of taste we refer to as "Taste Code".



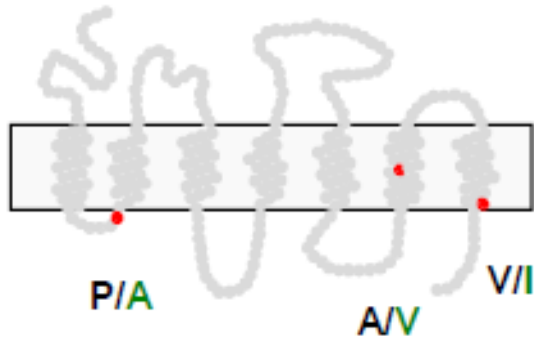
Cells responsible for the "transduction" of bitterness will transfer the signal to the CNS in presence of receptor agonists. Where there are genetic mutations, an anomaly in perception can occur...



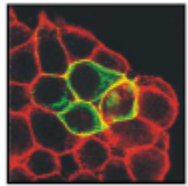
# Taste perception



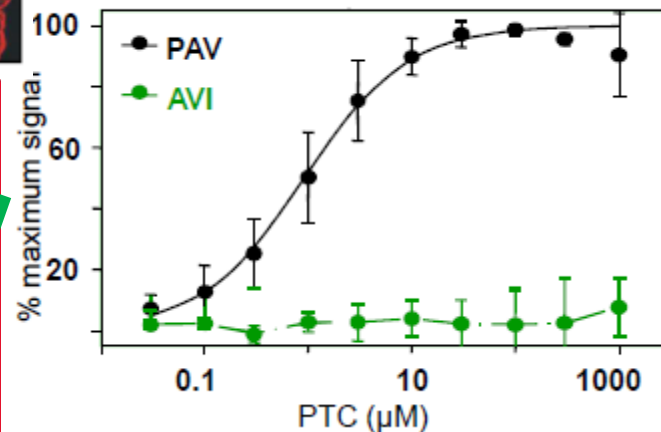
**Phenylthiocarbamide (PTC)** is sensed intense bitter of 50% of the population, and as tasteless of the other 50%.



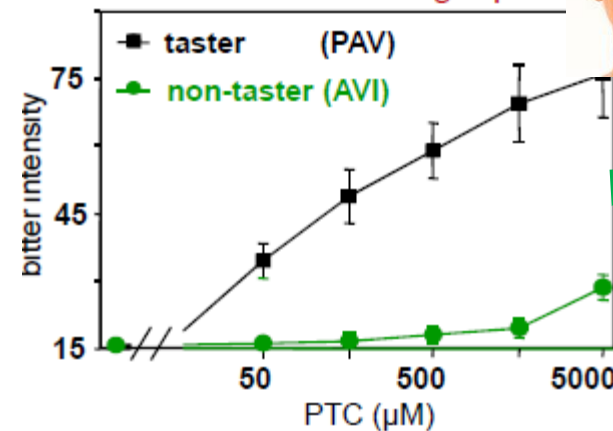
The receptor responsible for perception is hT2R38; the mutation involves the aa of the primary sequence (red dots) which are substituted:  
Proline / Alanine-Alanine / Valine-Valine / Isoleucine



hT2R38 function in transfected cell culture



panelist genotyping and human PTC tasting expt.





# Taste perception



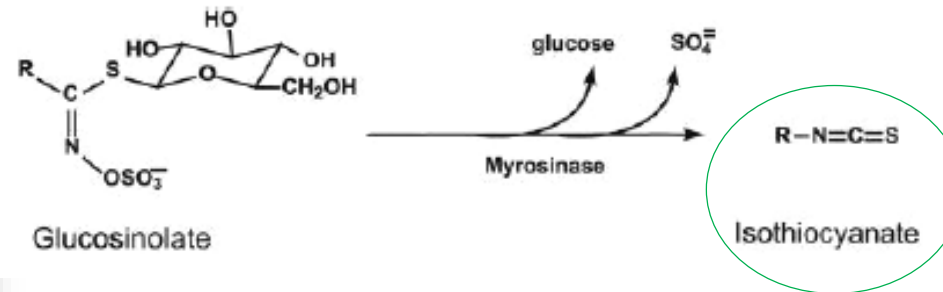
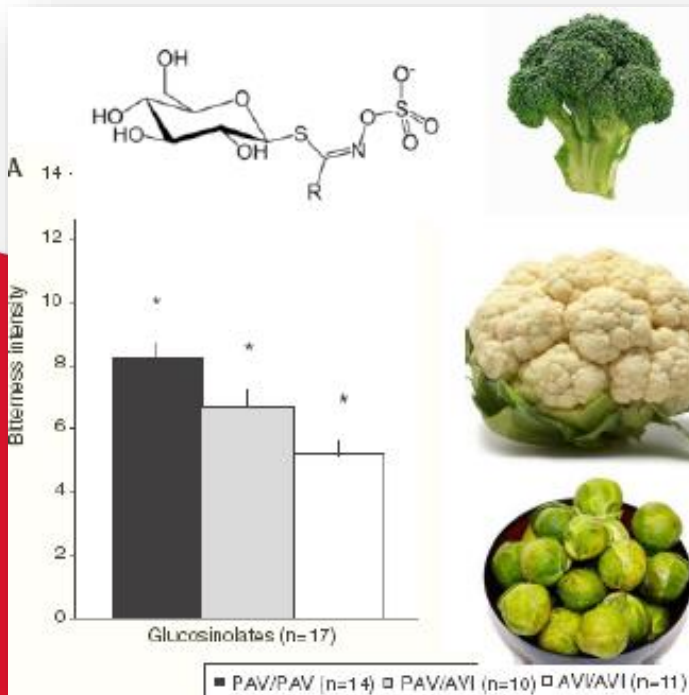
Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO



The receptor responsible for perception is hT2R38;  
the mutation involves the aa of the primary sequence  
(red dots) which are substituted:  
Proline / Alanine-Alanine / Valine-Valine / Isoleucine

## Glucosinolates- *Brassicaceae*



It is accepted that the population expresses different sensitivity to bitterness, due to gene mutations, therefore it is plausible to think of food preferences as a "phenotypic" expression of the individual genetic set-up.

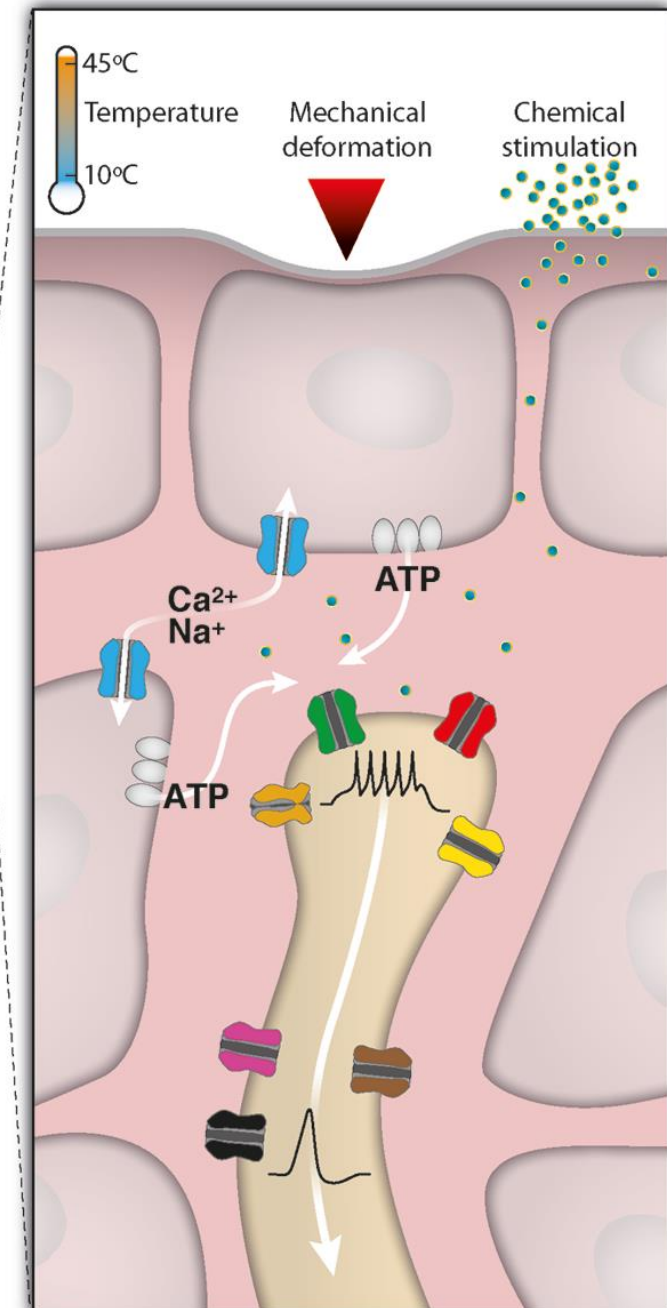
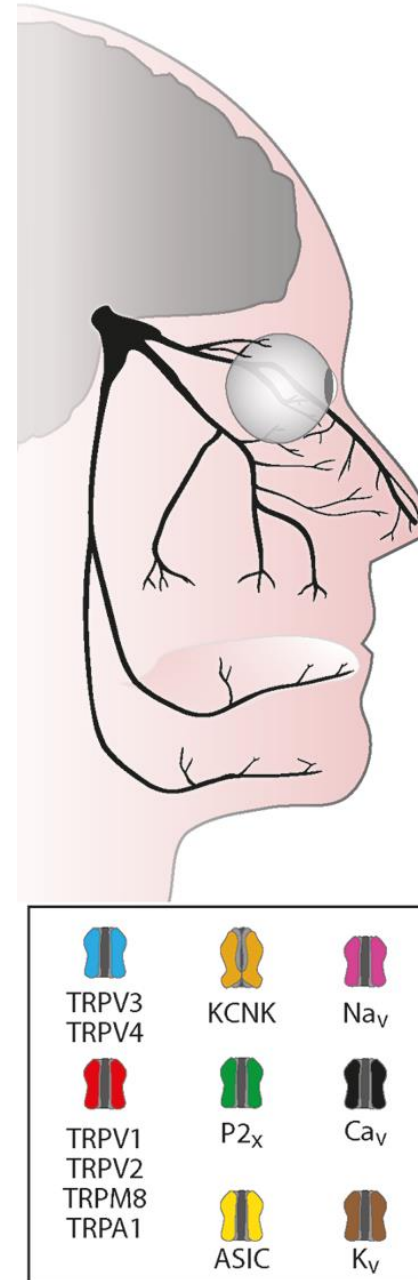
# Somatosensation - Trigeminal perception

To complete the picture of sensory perception during food consumption, an important role is played by **somatosensory sensations**.

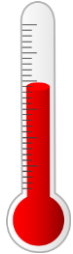
The trigeminal nerve (V cranial nerve) in addition to sensations of **temperature** and **texture** (mechanical deformation and consistency of the food) of a food is able to **mediate** a **chemical stimulation** that **results** in very "particular" perceptions:

- ✓ **cooling;**
- ✓ **pungent;**
- ✓ **astringent;**
- ✓ **spicy, burning;**
- ✓ **hot;**
- ✓ **tingling / pricking**

thanks to the presence of **metabotropic receptors** similar to taste receptors.



# Somatosensation



The metabotropic receptors of chemesthetic mediation show a strong correlation with thermal perception

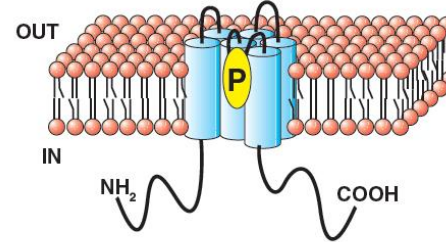
Some compounds such as **capsaicin** (*Capsicum*) **sensitize** the **perception** of **heat** and **trigger** a sensation of "**hot**" "**burning**"



Compounds such as **menthol** (*Mentha* spp) **enhance** the **perception** of **cold** and, it has been shown, **amplify** the **intrinsic sensitivity** of **nerve endings** at low **temperatures**.

The explanation lies in the temperature-mediated allosteric modulation (changes in the conformation of the membrane receptor) of TRPs receptors.

## TRPs : transient receptor potential

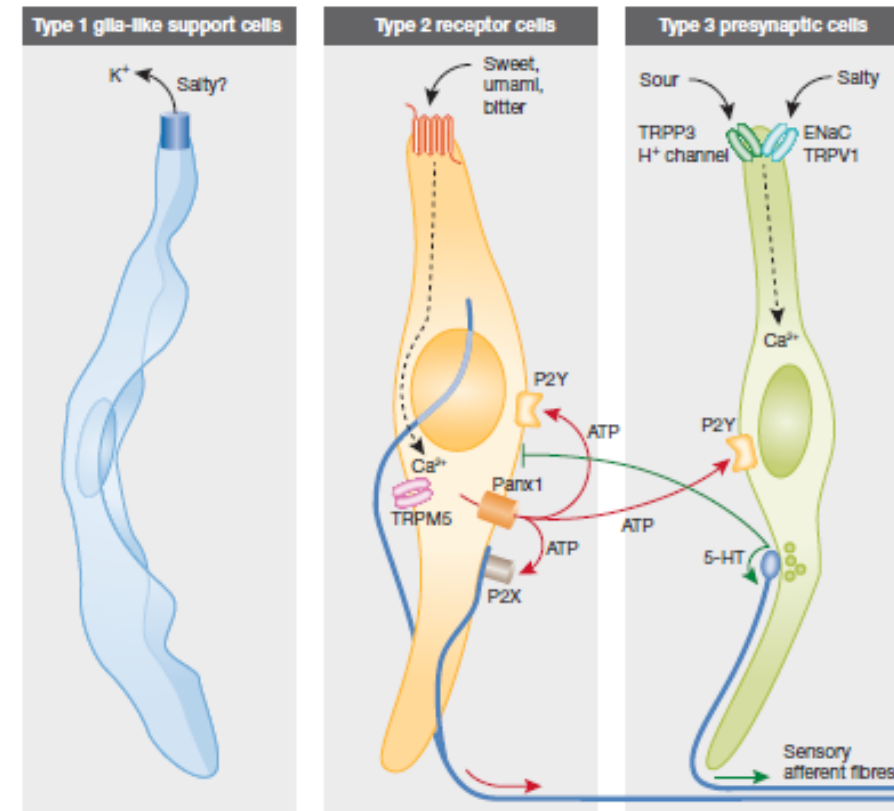


**TRP** receptors are ubiquitous, those expressed on **taste cells** (taste cells Type 2 and Type 3) are in fact **ion channels** that **regulate neuronal activation**.



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology



# Somatosensation



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO



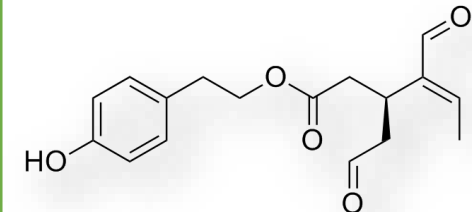
**Szechuan pepper** contains  $\alpha$ -sanshool binds as an agonist to the terminals of the trigeminal nerve in the oral cavity by activating TRP receptors and certain potassium and sodium channels. The effect is a sort of "sensory confusion" that is perceived with the sensation of "tingling" equivalent to the effect obtained by placing a rechargeable battery on the tongue.



The **olive tree** (*Olea europea*) and in particular **extra-virgin olive oil** contains a phenolic derivative, oleocanthal, which stimulates a sensation of "tingling" and "irritation" in the throat. It has shown its action as an **agonist of TRPA1** receptors, exactly like the well-known anti-inflammatory ibuprofen, which coincidentally has the same effect when introduced into the oral cavity.



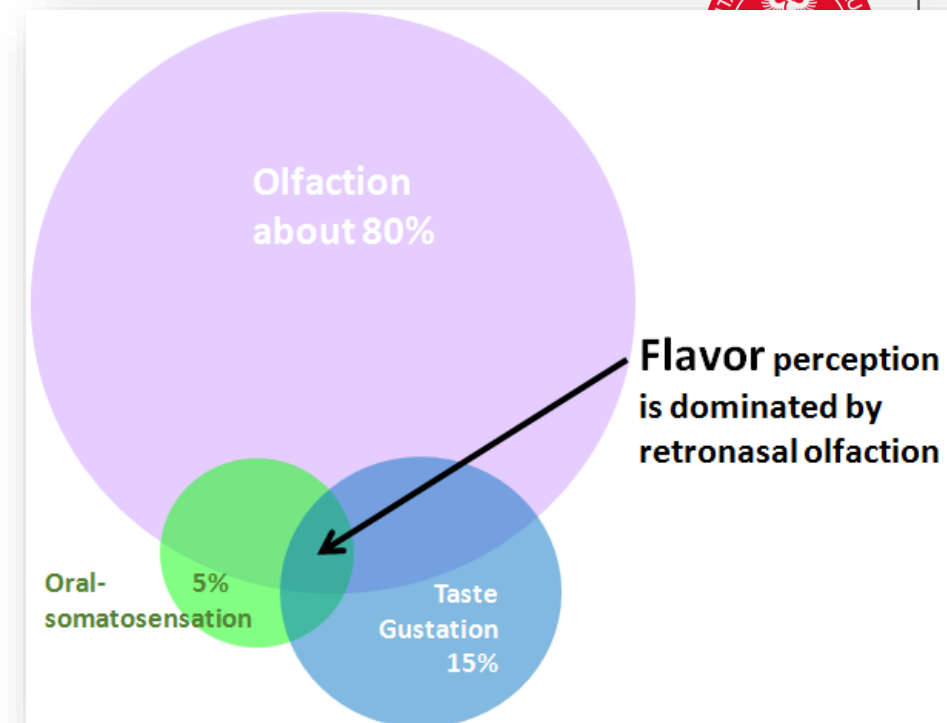
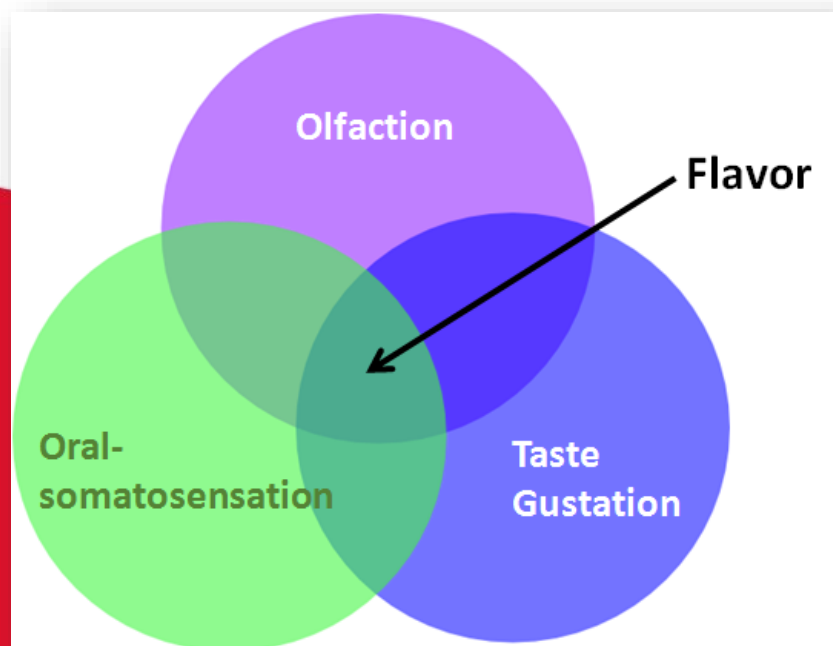
Mediterraneans, having developed tolerance to the itchiness of oleocanthal, would have benefited from its positive effects unlike peoples defined as "fat and umami oriented" (North Americans) who, on the contrary, do not tolerate it. The latter develop diseases such as Alzheimer's with a higher incidence (Feart et al. 2009).





**Flavor** involves the combination of gustatory and olfactory stimuli. **Just retronasal aromas combined with gustatory cues** give rise to flavors; trigeminal inputs also contribute but as second-line player.

As for vision, audition and oral somatosensation, the jury is currently still out as to which if any of these senses should be considered as constitutive of flavor perception or, rather, as factors that merely modulate the experience of flavor.



It has been suggested that as much as 80%–90% of the taste of food comes from the nose (e.g., Chartier, 2012; Stuckey, 2012), however the contribution of olfaction is strictly dependent on the foodstuff under consideration.

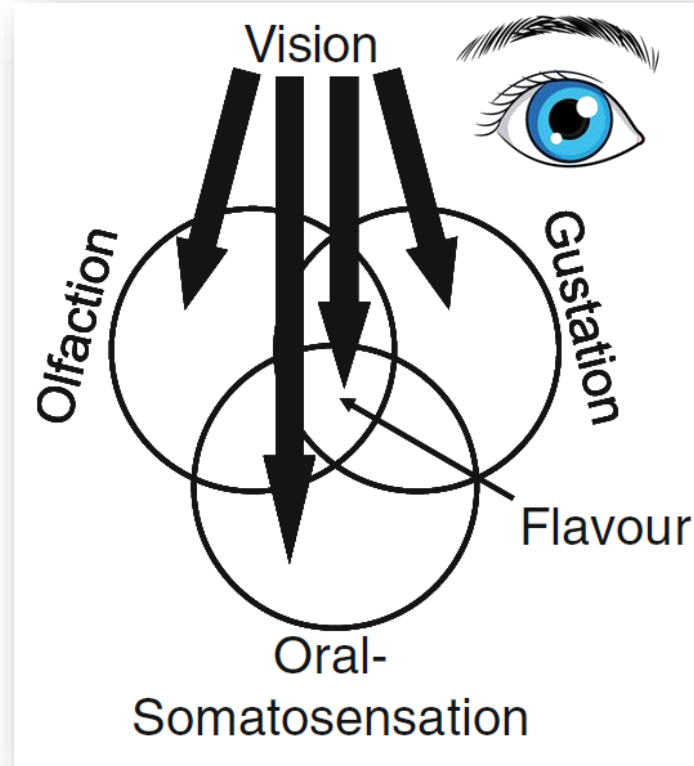
This evidence is opening the door to the concept of **crossmodal interactions and their impact of actual perception**.

**CROSSMODAL EFFECTS** ... a compatibility effect between attributes or dimensions of a stimulus (i.e. an object or event) in different sensory modalities (be they redundant or not)

International Standards Organization (ISO-5492 1992) defined **flavor** as a “complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting. The flavor may be influenced by tactile, thermal, painful and/or kinaesthetic effects”.

Visual and auditory cues may modify a food's flavor, but they are not intrinsic to it, at least according to the ISO definition

There is robust psychophysical evidence that **visual** (color) cues can modulate people's perception of the **identity** and **intensity** of both orthonasally and retronasally presented odors.



Visual cues, such as a food's color, may modify the perception of a food's flavor by influencing the gustatory qualities of the food, by influencing the olfactory attributes of the food (as perceived orthonasally and/or retronasally), by influencing the oral–somatosensory qualities of the food, and/or by influencing the overall multisensory flavor percept.

**Gestalt: an organized whole that is perceived as more than the sum of its parts.**



# Visual contribution to multisensory Flavor perception



## Food color influence TASTE intensity

Maga 1974 - fundamental study on perceptual thresholds (sensitivity)

**Table 2** Summary of the results from Maga's (1974) study highlighting the effect of the addition of color on participants' sensitivity to each of the four traditional basic tastants when dissolved in solution (and compared to performance when the tastants were presented in uncolored solutions)

Color of solution	Taste			
	Sour (citric acid)	Sweet (sucrose)	Salty (sodium chloride)	Bitter (caffeine)
Red	No effect	No effect	No effect	Decrease
Yellow	Decrease	Decrease	No effect	No effect
Green	Decrease	Increase	No effect	No effect

Johnson and Clydesdale demonstrated that changing the level of food coloring had a significant effect on participants' perception of the sweetness of both odorless and cherry-flavored solution, with the darker-colored solutions being rated as 2–10% sweeter than the lighter-colored.



Department  
Drug Science  
and Technology

UNIVERSITATEA

Red facilitate detection of sweet tastant in a solution. Color intensity - dark red enhance the sweet taste rating (2-10%) in odorless or flavored solutions

Maga (1974) argued that there is a natural correlation between redness and sweetness levels as many fruits ripen.



That is, many fruits show a transition from colors at the green end of the spectrum, through yellow, to colors at the red end of the spectrum.

The consensus view would currently seem to be that color cues do not influence the perception of saltiness. By contrast, the perception of sweetness can be modified by the addition of red (or green) food coloring although this effect is not always observed.

# Visual contribution to multisensory Flavor perception



## Food color influence FLAVOR intensity

DuBose et al. (1980) reported that overall flavor intensity was affected by color intensity, with higher color intensity solutions giving rise to stronger flavor evaluation responses by participants for orange- (but not for cherry-) flavored beverages.

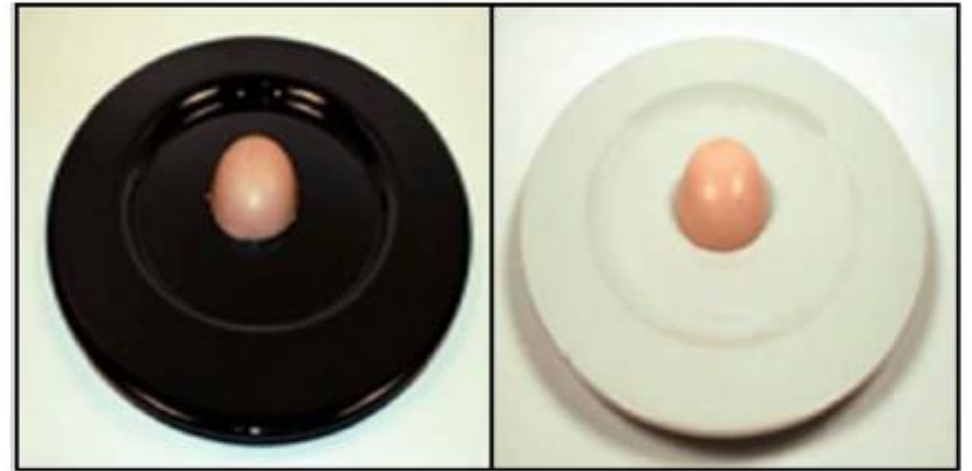


Kostyla (1978) reported that the addition of yellow color to sweetened cherry-, raspberry-, and strawberry-flavored beverage decreased flavor ratings by around 4%. Blue color reduced fruit flavor by 20% (and the addition of red coloring increased sweetness by 5–10%).



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology



**Figure 3. Can You Taste the Plate?**

Black and white plates with red frozen strawberry dessert. Participants rated the dessert tasted from the white plate as tasting significantly sweeter and more flavorful than exactly the same food when served from the black plate instead. Figure reprinted with permission from Figure 2 of [Piqueras-Fiszman et al. \(2012\)](#).



## Food color influence FLAVOR intensity

DuBose et al. (1980) reported that overall flavor intensity was affected by color intensity, with higher color intensity solutions giving rise to stronger flavor evaluation responses by participants for orange- (but not for cherry-) flavored beverages.



Kostyla (1978) reported that the addition of yellow color to sweetened cherry-, raspberry-, and strawberry-flavored beverages decreased flavor ratings by around 4%. Blue color reduced fruit flavor by 20% (and the addition of red coloring increased sweetness by 5–10%).



People are very poor at identifying orthonasally presented odors in the absence of any other sensory cues regarding the odor's identity. By contrast, people appear to be much better at identifying the presence of the basic tastes (e.g., sweet, sour, salty, and bitter). A priori, then, it would seem likely that participants' judgments of odor (and hence of flavor) identity would be much more likely to be influenced by the presentation of an incongruent visual color cue than would their judgments of the identity of a specific tastant.



## Food color and FLAVOR identity

Odors are more rapidly identified when colored appropriately than when uncolored or else inappropriately colored.

**Table 3** Partial summary of the results from DuBose et al. (1980) (experiment 2)

Reported flavor	Color of drink		
	Red (%)	Orange (%)	Green (%)
Cherry	70	41	37
Orange	0	19	0
Lime	0	0	26

The results highlight the profound effect that food coloring can have on participants' flavor identification responses. The participants in this study had to try and identify 16 different sequentially presented beverages created by fully crossing the factors of flavor (cherry-, orange-, or lime-flavored, or flavorless) and color (red, orange, green, colorless). The participants were given a checklist of 14 possible responses (including 12 fruit flavors) to choose from when trying to identify each of the drinks (strawberry, raspberry, lemon, lime, grape, apple, cherry, orange, blueberry, lemon lime, grapefruit, apricot, other, or no flavor). The table highlights the distribution of responses from the three most common flavor responses for the cherry-flavored drink. The numerical values indicate the percentages of each flavor response for each color

# Visual contribution to multisensory Flavor perception



## Food color influence FLAVOR identity



To note: it is entirely possible that humans simply use salient visual cues as a cognitive shortcut.

This confound has been identified in the literature documenting the visual capture, or dominance, over perceived auditory localization.

Response bias: influence of color cue on flavor identification;

Perceptual effect: color cue modulate flavor perception.



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

There is empirical support for the claim that the color of a food/drink can exert a powerful influence on people's flavor identification responses.

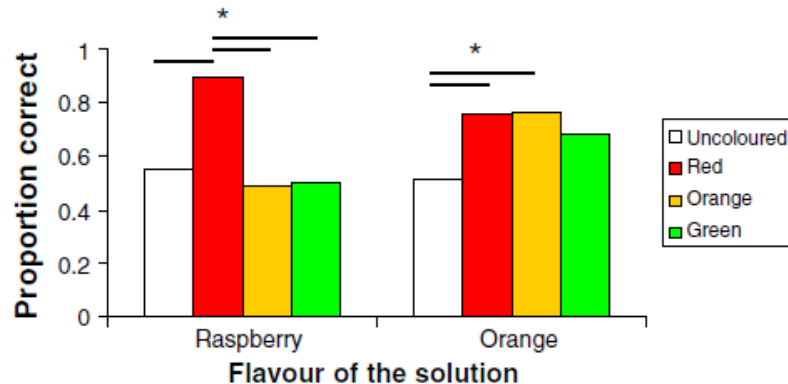
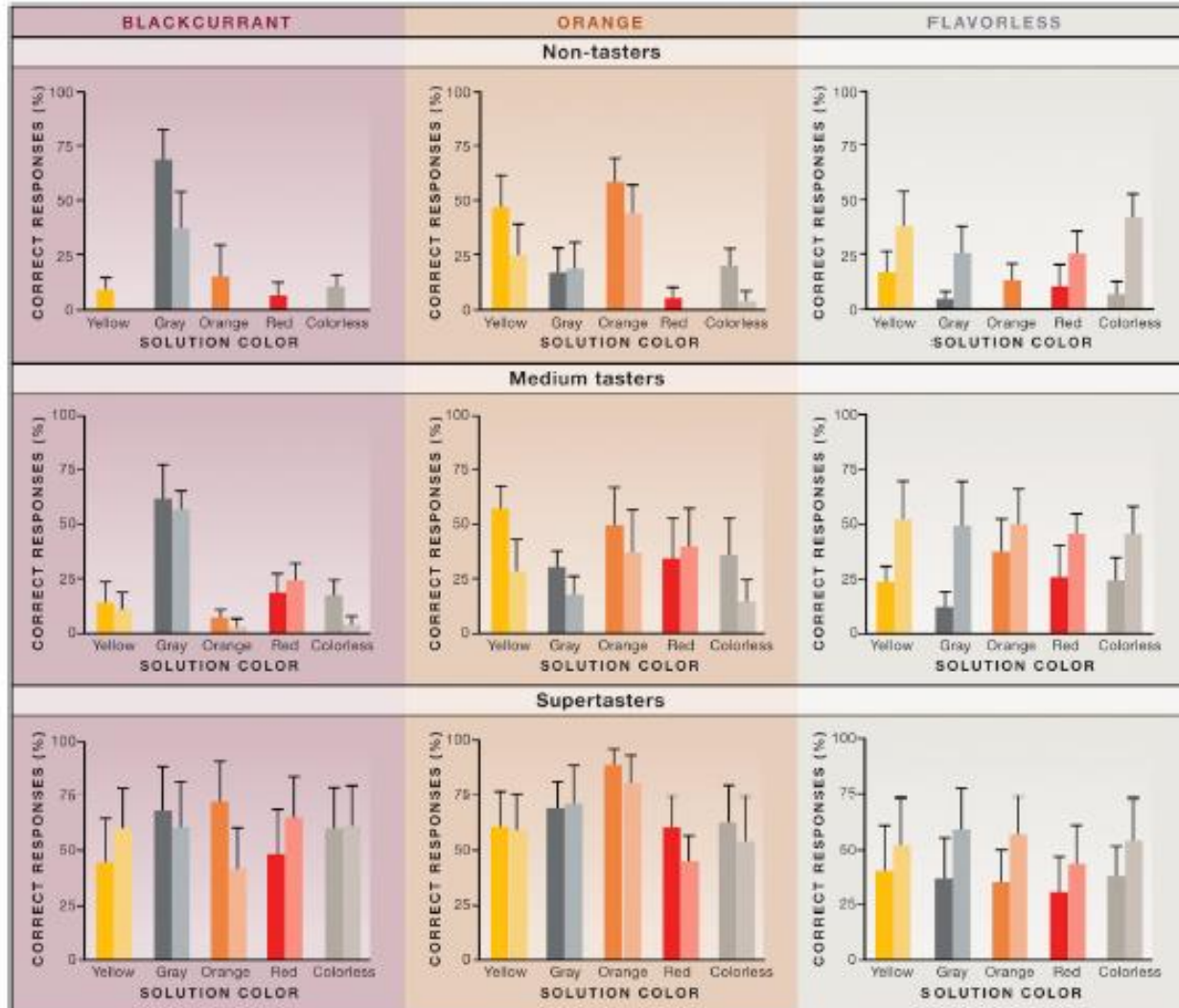


Fig. 2 Summary of the results of Stillman's (1993) study in which 310 participants were given a single drink to taste and identify. The horizontal lines indicate those comparisons between conditions that were significant (asterisk) using independent samples chi-square tests

310 untrained observers (Stillman - 1993) were each given a beverage to taste and identify and were informed that its color was independent of the flavor. The drinks were either raspberry- or orange-flavored and were either colored red, yellowish orange, green, or else left uncolored. The results showed that participants were significantly better at correctly identifying the raspberry flavor when the drink was colored red than when it was colored green, orange yellow, or else was presented as a colorless solution. The orange-flavored solution was identified significantly more accurately when the drink was colored orange, yellow, or red than when it was presented as a colorless solution.



## Food color influence FLAVOR identity



Influence of Color on Flavor Identification as a Function of Taster Status

Mean percentages of correct flavor identification responses for the three groups of participants (non-tasters, medium tasters, and supertasters) for the blackcurrant, orange, and flavorless solutions presented in Zampini et al.'s study (2008) of the effects of color cues on multisensory flavor perception in humans.

The darker columns represent solutions where fruit acids had been added, and the lighter columns represent solutions without fruit acids. The error bars represent the between-participants standard errors of the means. Figure reprinted with permission from Figure 1 of Zampini et al. (2007).

# Visual contribution to multisensory Flavor perception

## Expectancy based effects of food coloring

It seems likely that whenever we see a food of a certain color, that color, together with any other contextual cues (e.g., is it a food or drink item, hot or cold, transparent or opaque - “visual appearance” cues), will lead us to generate specific expectations regarding the likely flavor of that food.

Such color-induced flavor expectations (what Hutchings 1977 called “anticipatory” effects) may lead to the “misidentification” of flavors when a drink is subsequently tasted.

Misidentification at this (cognitive) level will presumably also provide access to a wealth of semantic information about the “misidentified” flavor which in turn might reasonably be expected to influence participants’ judgments/responses.



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

**Table 4** Results of the study of Zampini et al. (2007) (experiment 1) assessing the flavor expectations generated by a group of UK participants on being presented with clear plastic beakers containing transparent drinks that had been colored green, orange, yellow, blue, gray, red, or left colorless

Color of drink	Expected flavor (% of participants with that expectation)
Green	Lime (69%), apple (20%), melon (11%)
Orange	Orange (91%), aniseed (5%), toffee (4%)
Yellow	Lemon (89%), pear (5%), apple (4%), melon (2%)
Blue	Spearmint (86%), raspberry (9%), cream soda (5%)
Gray	Blackcurrant (53%), licorice (40%), cherry (4%), aniseed (4%)
Red	Strawberry (46%), raspberry (27%), cherry (27%)
Colorless	Flavorless (51%), cream soda (16%), vanilla (15%), aniseed (15%), spearmint (2%), melon (2%), pear (2%)

The table shows the percentage of trials in which participants reported that they expected a particular colored drink to taste of a certain flavor



## Visual contribution to multisensory Flavor perception

### Expectancy based effects of food coloring

Shankar et al. (2009) recently investigated the nature of any cross-cultural differences in color-flavor expectancy effects.

**Table 5** Results of the cross-cultural study of Shankar et al. (2010) assessing the flavor expectations elicited by differently colored drinks in two groups of participants, one from the UK, the other from Taiwan

COLOR	British Participants (N=20)	Taiwanese Participants (N=15)
Brown	Cola (14), Cherry (3), Blackcurrant (2)	Grape (6), Mulberry (3), Cranberry (3)
Blue	Raspberry (8), Mint (4), Blueberry (3)	Mint (7), Cocktail (3)
Yellow	Lemon (11), Pineapple (2), Grape (2)	Yellow Soda (4), White Wine (2)
Orange	Orange (13)	Cranberry (2), Strawberry (2), Apple(2)
Green	Mint (11), Lime (4), Apple (4)	Mint (5), Apple (3), Lime (2), Kiwi (2)
Clear	Water (16), Lemon (2)	Water (14)
Red	Cherry (8), Strawberry (4), Cranberry (3), Raspberry (3),	Cranberry (5), Strawberry (2), Cherry (2), Wine (2)

The table highlights the three most commonly expected flavor responses (chosen by more than one participant). The shaded rows indicate the colored drinks (brown, blue, yellow, and orange) that elicited significantly different flavor expectations from the two groups of participants

# Visual contribution to multisensory Flavor perception



Department  
Drug Science  
and Technology

UNIVERSITÀ



## Expectancy based effects of food coloring

The four amuse bouche served at Synaesthesia by *Kitchen Theory* (see <https://kitchen-theory.com/>). The spoons are brought to the table in a random arrangement, and it is the diner's job to sort the tastes by color. The spoons in the figure are shown in the intended order. This dish was inspired by the latest cross-cultural research demonstrating the robust crossmodal correspondences that exist between color and taste (see [Wan et al., 2014b](#)). Picture adapted and reprinted with permission from Eva-Luise Schwarz/FOUR magazine.

<https://kitchen-theory.com/portfolio-item/synaesthesia/>





In the West, we describe the aromas of strawberry, caramel, and vanilla as smelling “sweet” (Stevenson and Boakes, 2004).

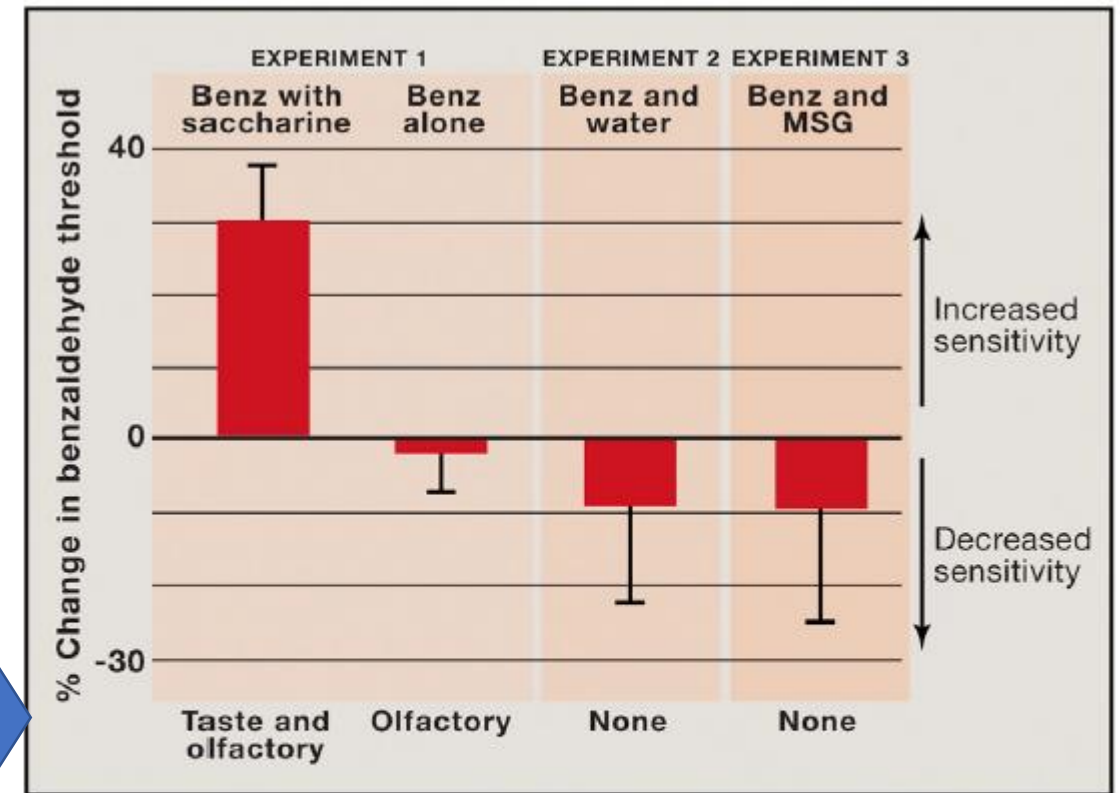
This is more than merely a [synaesthetic](#) or [metaphorical use of language](#) (Stevenson and Tomiczek, 2007).

**Olfactory stimuli that have regularly been paired with sweet, bitter, salty, or even sour-tasting foods can, in fact, come to enhance the associated taste quality, even when they are presented at a sub-threshold level.**

There can be no doubt that such crossmodal interactions make it all the more difficult to try and draw a clear line between experiences of taste and of flavor.

Following ingestion of 10 mL of the respective solutions (saccharin, monosodium glutamate (MSG), or deionized water), subjects sniffed the odor of benzaldehyde (cherry/almond odor) at sub-threshold level.

Subjects’ sensitivity to the odor was increased in the presence of congruent taste (i. e., saccharin) in the mouth, while their odor sensitivity was decreased in the presence of incongruent taste (i.e., monosodium glutamate) or of deionized water.



**Figure 1. Multisensory Interactions between Olfaction and Gustation in Multisensory Flavor Perception**

Results of a series of experiments by Dalton et al. (2000), showing the integration of orthonasal olfactory and gustatory cues. Figure reprinted with permission from Figure 6.2 of Spence and Piqueras-Fiszman (2014).



**Japanese** participants tend to show **perceptual enhancement in the Mono Sodium Glutamate condition, but not in the saccharin condition** (i.e., the opposite pattern to that shown by western participants in Dalton et al., 2000; see Breslin et al. 2001). It turns out that pickled condiments containing the savory almond combination are common in Japanese cuisine, whereas sweet almond desserts (just think of Bakewell Tart) are more commonly experienced in the west. These results therefore suggest that our **brains learn to combine tastes and smells that regularly co-occur in our home cuisine.**

The underlying idea here then is that, **while everyone's brain may use the same rules to combine the inputs from their senses, the particular combinations of tastants and olfactory stimuli (and possibly also visual stimuli) that lead to multisensory enhancement (or suppression, when the taste and smell don't match; see, e.g., de Araujo et al., 2003) depends on the combination of ingredients and, hence, of sensory cues that tend to co-occur in the cuisine of the region where people have grown up.**

Table 1 Examples of aroma → taste interactions.

Aroma/ Odour	taste	Effect	Products
Almond	Sweet	+	MS
Caramel	Sweet	+	MS
Cocoa	Bitter	+	Chocolate beverage
Garlic	Salty	+	Thickened MS
Green apple	Sour	+	Yoghurts
Lemon	Sour	+	MS
	Sweet	+	MS
Maracujá	Sweet	+	MS
Peach	Sweet	+	MS
Strawberry	Sweet	+	MS, whip cream, yoghurts
Vanilla	Bitter	+	Caffeine milk
	Sour	+	MS
	Sweet	+	MS, chocolate beverage
Chocolate	Sour	0	MS
Eucalyptol	Sweet	0	MS
Ham	Sweet	0	MS
Lemon	Sour	0	MS
Peanuts	Sweet	0	Whipped cream
Strawberry	Salty	0	MS
Vanilla	Sour	0	MS
Vanilla	Sweet	0	Caffeine milk
Wintergreen	Sucrée	0	MS
Angélica	Sweet	-	MS
Chocolate	Sweet	-	MS
Damascone	Sweet	-	MS
Maltol	Sweet	-	MS

MS: model solution, +: odour-induced taste enhancement, -: odour-induced taste suppression

Famous British chef Heston Blumenthal to say of sound that it was “the forgotten flavor sense.”

Auditory cues play an important role in the multisensory perception of food attributes such as crispy, crackly, crunchy, carbonated, and creamy (see Spence, 2015).

**And while a number of these might initially seem to be oral-somatosensory in nature, it turns out that our in-mouth experience can be radically changed by modifying the sounds of mastication.**



UNIVERSITÀ

LETTER FROM OXFORD NOVEMBER 2, 2015 ISSUE

## ACCOUNTING FOR TASTE

*How packaging can make food more flavorful.*



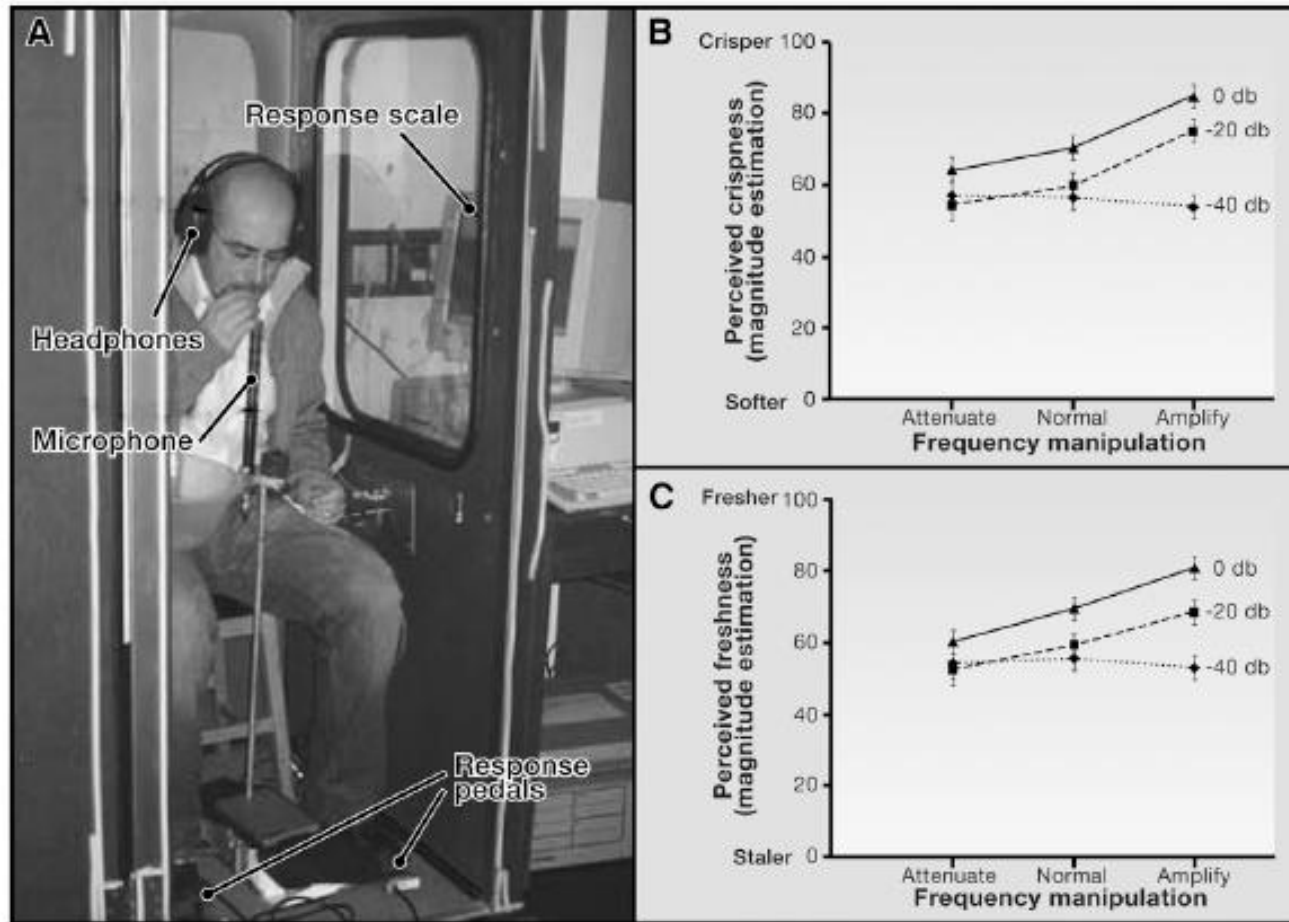
By Nicola Twilley



**Table 47.3** A list of studies showing cross-modal correspondences between olfactory and auditory cues

Olfactory cues	Auditory cues	References
<i>Orthonasal odors</i>		
Essential oils	Pitch	[47.66]
Fragrances and essential oils	Pitch (200 and 1000 Hz)	[47.60]
Wine aroma kit	Pitch	[47.63]
Aroma kit	Pitch	[47.65]
Wine aroma kit	Timbre (instrumental note)	[47.63]
Aroma kit	Timbre (instrumental note)	[47.65]
<i>Retronasal odors (flavors)</i>		
Flavors (foods and flowers)	Pitch	[47.61]
Flavors (milk)	Pitch	[47.62]
Flavors (foods and flowers)	Timbre (instrumental note)	[47.61]
Flavors (milk)	Timbre (instrumental note)	[47.62]
Flavors (chocolate)	Timbre (instrumental note)	[47.64]

People’s perception of the crispness and freshness of potato chips (crisps to the readers in the UK) could be systematically modified (by around 15%) by changing the self-generated crisp biting sounds that participants heard when biting into a selection of this not altogether healthy dry snack food.



**Figure 5. The “Sonic Chip” Experiment**  
(A) Schematic view of the apparatus and participant in [Zampini and Spence \(2004\)](#) study demonstrating the influence of biting sounds on crispness and freshness perception. The door of the experimental booth was closed during the experiment, and the response scale was viewed through the window in the left-side wall of the booth. Mean responses for the soft-crisp (B), and fresh-stale (C) response scales for the three overall attenuation levels (0 dB, -20 dB, or -40 dB) against the three frequency manipulations (high frequencies attenuated, veridical auditory feedback, or high frequencies amplified) are reported. Error bars represent the between-participants standard errors of the means. Figure reprinted with permission from Figure 1 of [Zampini and Spence \(2004\)](#).



# Food colorants



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

A progressive empowerment of food industry has been observed in the last decades, mainly incited by the increasing demands by consumers.

More delightful, nutritive, attractive, healthy and high sensorial quality products are already available, and therefore more specific and applied methods/techniques need to be developed and then implemented to achieve the industrial goals and consumer desires.

It is not new that several external conditions, such as light, air, temperature, moisture and storage conditions play a crucial role on the food color loss.

Thus, food colorants are mainly applied to offset and overcome those unpleasant characteristics, as also to homogenize the color of foodstuffs, through correction of color variations and/or enhancement of the naturally occurring food color, and even making available colorless products.

The final result arising from this intervention is the appearance of specifically targeted and requested products by consumers, and the commonly named “fun foods”, that significantly improves their attractiveness and consequent worldwide demand.

# Food colorants



## Synthetic food colorants

With the growing and continuous search, numerous synthetic food colorants were developed to be added to improve food products quality and organoleptic characteristics, however, over time, **most of them were banned due to the clearly evident side effects, signals of toxicity** at short and long terms, as also **health impairment abilities**, including their possible **carcinogenic effects**.



**Natural food colorants** revealed to be as much effective as those derived from chemical synthesis, with the subsequent benefits of: being more safe, providing health benefits besides conferring organoleptic features, exerting two or more benefits as food ingredients (in fact several food additives exerting colorant effects also act as antioxidants and even preservatives), and lastly **contributing functional properties to food products**.

**Brown** to **black** food colorants still continue to be highly explored:

- ✓ **Caramel** – E150 (ADI 160–200 mg/kg b.w.), authorized to be used in sauces, biscuits, crisps, pickles and several alcoholic and non-alcoholic beverages;
- ✓ **Brilliant black** – E151 (1 mg/kg b.w.), used in cheeses, wine, sauces, and drinks;
- ✓ **Vegetable carbon** – E153 (not established), used in jam and jelly crystals;
- ✓ **Brown FK** – E154 (0.15 mg/kg b.w.), authorized in smoked and cured fish, meat and crisps;
- ✓ **Brown HT** – E155 (1.5 mg/kg b.w.), used in biscuits, chocolate and cakes.



L'UNIVERSITÀ

Department  
Drug Science  
and Technology

# Food colorants



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

However, other naturally-occurring food colorants have been also studied, namely anthocyanins, beet colorants, carotenoids and phenolic compounds.

Annato, carminic acid and some curcuminoids, particularly curcumin, have been also limited investigated, while many others still to be examined and their use is not yet authorized with an E code.



## Anthocyanins

Anthocyanins are the most widely studied natural food colorants, being obtained from flowers, fruits, leaves and even whole plants.

Commercial anthocyanins, namely cyanidin 3-glucoside, pelargonidin 3-glucoside and peonidin 3-glucoside have been also used, and their effectiveness has been increasingly assessed.

It is really important to highlight that external interferences highly affect the anthocyanin pigment colors, namely pH, temperature, humidity, salinity, stress conditions and even storage conditions.

# Food colorants



Department  
Drug Science  
and Technology

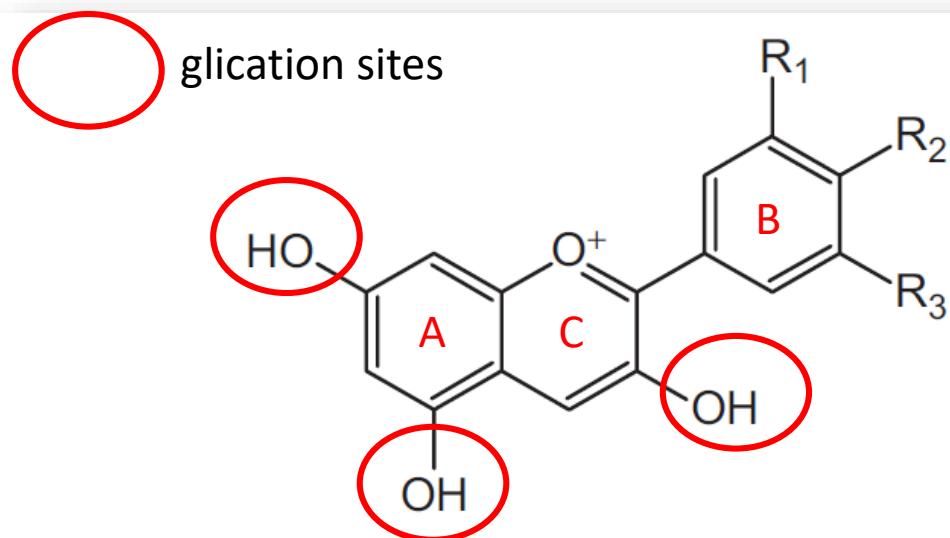
UNIVERSITÀ  
DI TORINO

## Anthocyanins

Anthocyanins form a subgroup of flavonoids, which are characterized by their typical C6-C3-C6 structural backbone.

They are almost ubiquitously found in higher plants, with the exception of 10 plant families of the *Caryophyllales*, where betalains may be found.

Anthocyanidins may be substituted at different positions with one or more saccharide moieties, thus giving rise mainly to 3-glycosides and 3,5-glycosides. Among sugar substituents, glucose, rhamnose, xylose, galactose and arabinose are predominant.



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
Pelargonidin	H	OH	H
Cyanidin	OH	OH	H
Delphinidin	OH	OH	OH
Peonidin	OCH <sub>3</sub>	OH	H
Petunidin	OCH <sub>3</sub>	OH	OH
Malvidin	OCH <sub>3</sub>	OH	OCH <sub>3</sub>

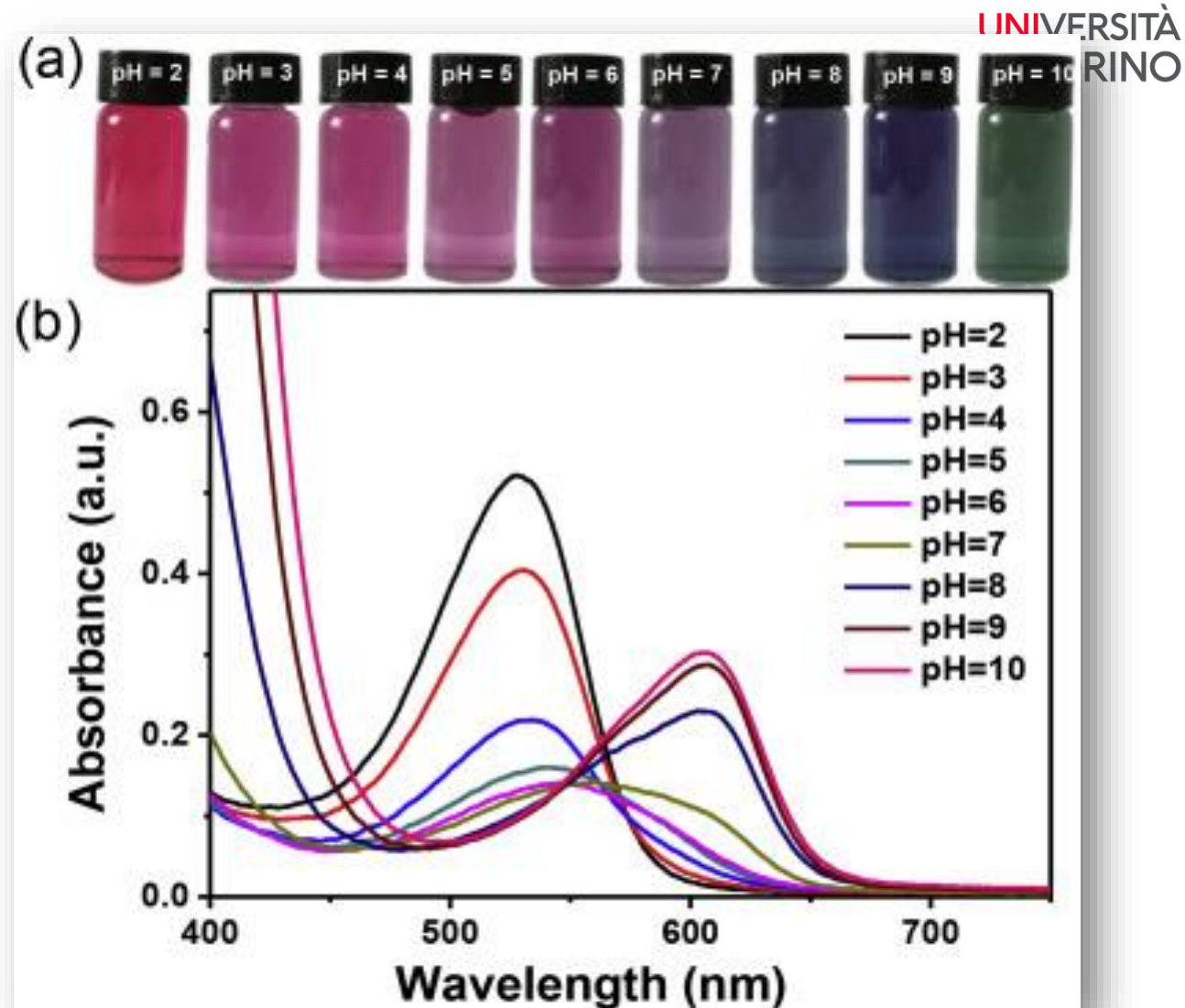


# Anthocyanins



Generally, the color of nonacylated and monoacylated anthocyanins is largely determined by the substitution pattern of the aglycone B-ring.

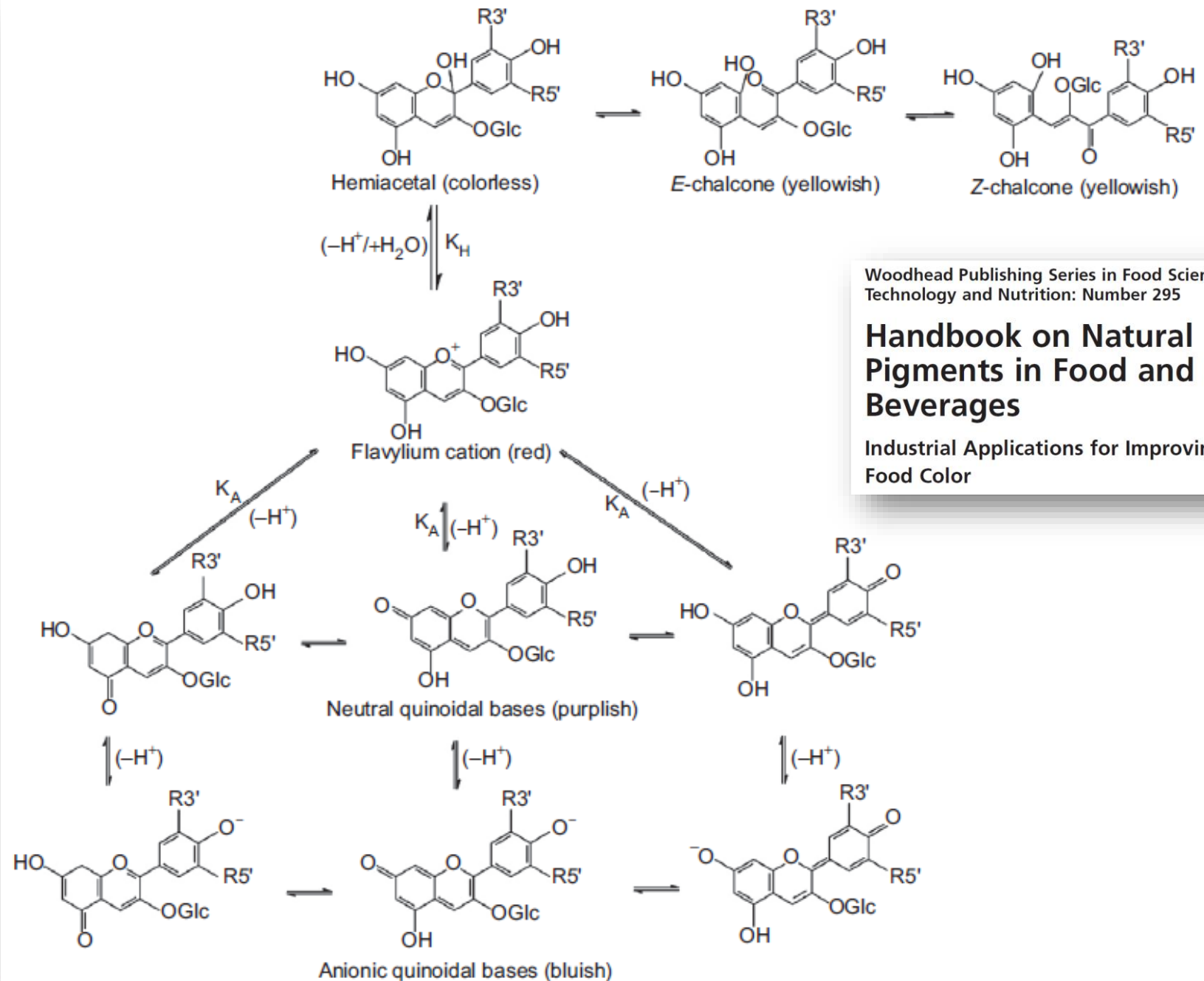
- ✓ An increasing number of hydroxyl groups (pelargonidin → cyanidin → delphinidin) causes a bathochromic shift (blue nuances).
- ✓ Increasing methylation (cyanidin → peonidin → malvidin) brings about a hypsochromic shift (purple/red nuances).
- ✓ Acylation with cinnamic acids causes a bathochromic shift of the pigment, which can be observed as bluish colors.



# Anthocyanins

Generally, the color of nonacylated and monoacylated anthocyanins is largely determined by the substitution pattern of the aglycone B-ring.

- ✓ An increasing number of hydroxyl groups (pelargonidin → cyanidin → delphinidin) causes a bathochromic shift (blue nuances).
- ✓ Increasing methylation (cyanidin → peonidin → malvidin) brings about a hypsochromic shift (purple/red nuances).
- ✓ Acylation with cinnamic acids causes a bathochromic shift of the pigment, which can be observed as bluish colors.



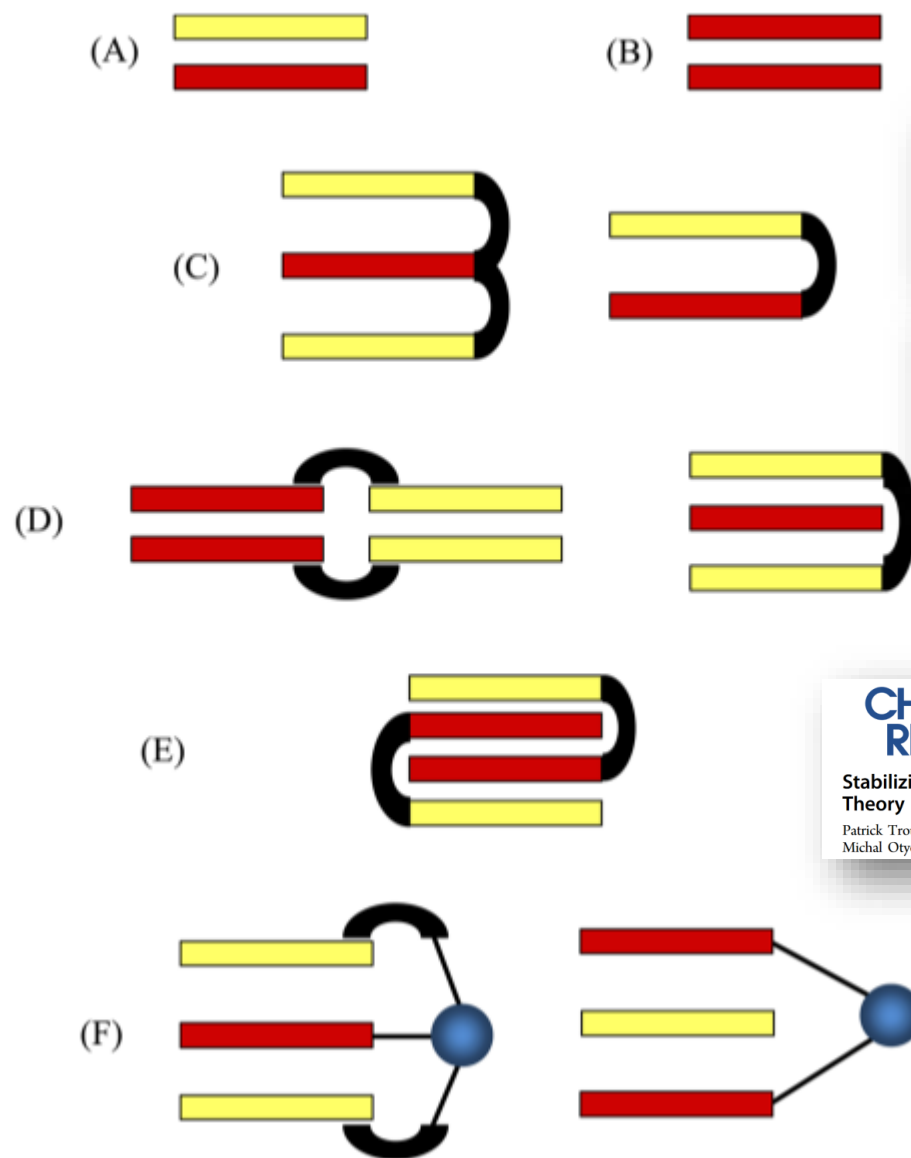
# Anthocyanins

Anthocyanins color is a function of:

- ✓ pH (see previous slide);
- ✓ intramolecular and intermolecular copigmentation, self-association;
- ✓ chelation with metal ions.

(A) Intermolecular copigmentation,  
(B) self-association,  
(C) intra-molecular copigmentation in acylated anthocyanins,  
(D) self-association of acylated anthocyanins,  
(E) intercalation in intermolecular copigmentation,  
(F) copigmentation in metal-anthocyanin complexes.



**Scheme 2.  $\pi$ - $\pi$  Stacking Interactions in Anthocyanins and Their Complexes<sup>a</sup>**



UNIVERSITÀ

Department  
Drug Science  
and Technology

 Pigment (anthocyanin)  
 Copigment

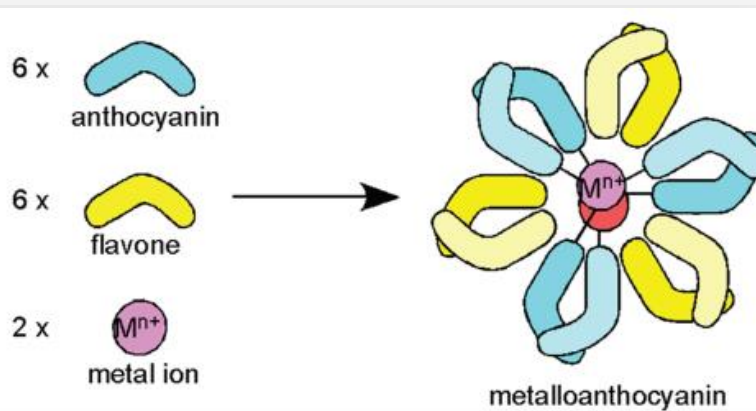
 Acyl link  
 Metal ion

**CHEMICAL  
REVIEWS**

Stabilizing and Modulating Color by Copigmentation: Insights from Theory and Experiment

Patrick Trouillas,<sup>a,†,‡</sup> Juan C. Sancho-García,<sup>§</sup> Victor De Freitas,<sup>||</sup> Johannes Gierschner,<sup>⊥</sup> Michal Otyepka,<sup>\*,‡</sup> and Olivier Dangles<sup>@</sup>

# Food colorants



**Figure 10.** Structure of commelinin. Blue: malonylawobanin (MA), yellow and orange: flavocommelin (FC), red:  $Mg^{2+}$  (A). A side view of a left-handed stacking of two MA units that coordinate to different  $Mg$  ions. (B) A side view of a copigmentation of MA and FC in a right-handed stacking arrangement. (C) A side view of a left-handed stacking of two FC units. (D) A side view of commelinin. (E) A skew view of a copigmentation between MA and FC. (F) A skew view of the self-association of two FC units. Adapted with permission from ref 1. Copyright 2009 The Royal Society of Chemistry.

## Anthocyanins



Department  
Drug Science  
and Technology

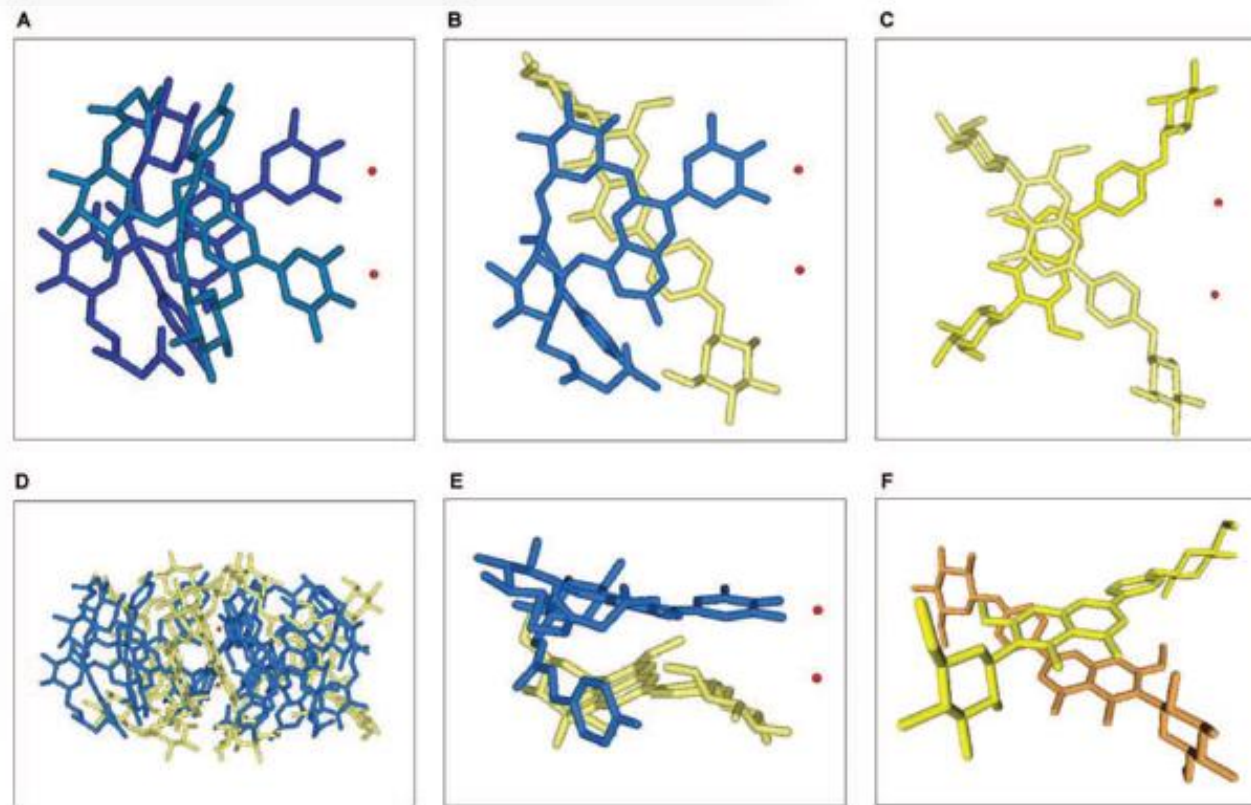
UNIVERSITÀ  
DI TORINO

CHEMICAL  
REVIEWS

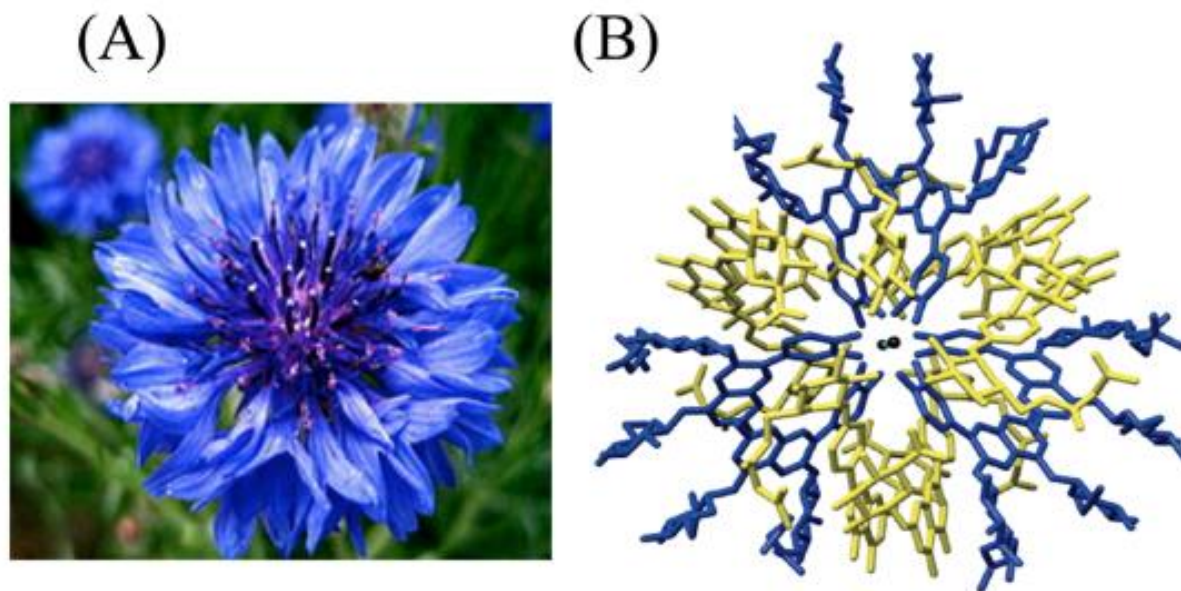
Review  
pubs.acs.org/CR

### Stabilizing and Modulating Color by Copigmentation: Insights from Theory and Experiment

Patrick Trouillas,<sup>\*,†,‡</sup> Juan C. Sancho-García,<sup>§</sup> Victor De Freitas,<sup>||</sup> Johannes Gierschner,<sup>⊥</sup> Michal Otyepka,<sup>\*,‡</sup> and Olivier Dangles<sup>@</sup>







**Figure 12.** (A) Structure of protocyanins from *Centaurea cyanus* (cornflower). (B) X-ray diffraction crystal structure, showing the two metal ions  $\text{Fe}^{3+}$  and  $\text{Mg}^{2+}$  in the center of the supramolecular assembly. Adapted with permission from ref 1. Copyright 2009 The Royal Society of Chemistry.

## CHEMICAL REVIEWS

Review  
pubs.acs.org/CR

### Stabilizing and Modulating Color by Copigmentation: Insights from Theory and Experiment

Patrick Trouillas,<sup>\*,†,‡</sup> Juan C. Sancho-García,<sup>§</sup> Victor De Freitas,<sup>||</sup> Johannes Gierschner,<sup>⊥</sup>  
Michal Otyepka,<sup>\*,‡</sup> and Olivier Dangles<sup>@</sup>

# Food colorants



Pigments known as betalains occur in *centrospermae*, e. g., in red beet and also in some mushrooms (the red cap of fly amanita). They consist of **red-violet betacyanins** ( $\lambda_{\text{max}} \sim 540 \text{ nm}$ ) and **yellow betaxanthins** ( $\lambda_{\text{max}} \sim 480 \text{ nm}$ ).



Their color is ascribed by the betalain structure's resonating double bonds (A).

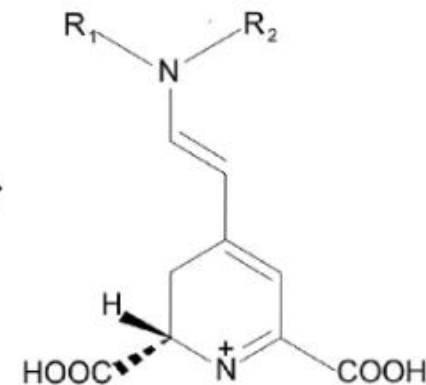
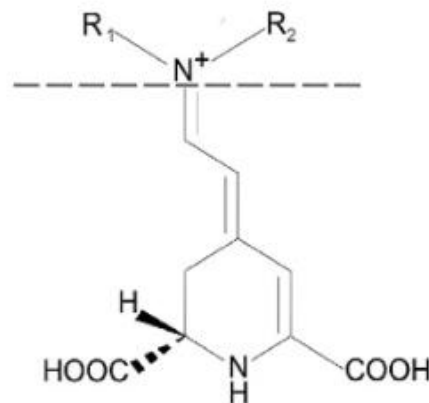
**Betacyanins** (B) are derivatives of betanidin, an iminium adduct of betalamic acid and cyclo-DOPA, whereas **betaxanthins** (C) result from the condensation of  $\alpha$ -amino acids or amines with betalamic acid.

## Betalains

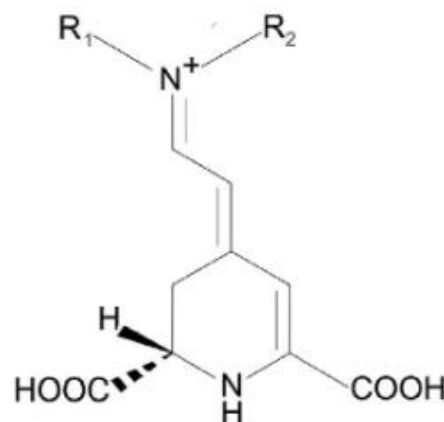


Department  
Drug Science  
and Technology

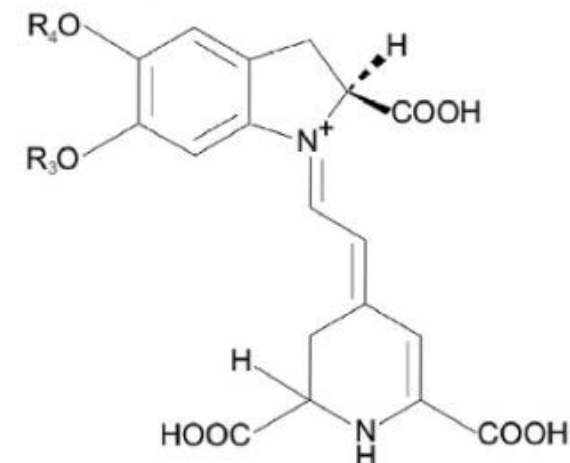
A



B

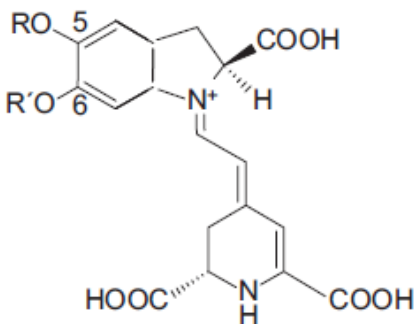


C



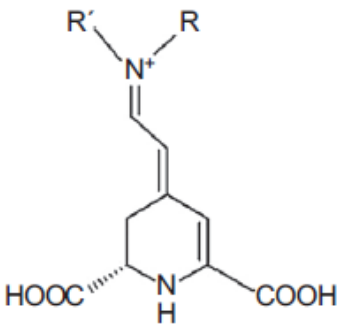


(a) Major betacyanins in betalain-rich sources



Compound	R	R'	Sources
Amaranthin	2-O-(β-glucuronic acid)-β-glucose	H	<i>Amaranthus tricolor</i> (Cai et al., 2005)
Betanin	β-glucose	H	<i>Beta vulgaris</i> , <i>Hylocereus polyrhizus</i> , <i>Chenopodium</i> (Stintzing et al., 2002b; Kugler et al., 2004)
Hylocerenin	5-O-methyl-glutaryl-β-glucoside	H	<i>Hylocereus polyrhizus</i> (Stintzing et al., 2002a)
Phyllocactin	5-O-malonyl-β-glucoside	H	<i>Hylocereus polyrhizus</i> (Stintzing et al., 2002a)

(b) Major betaxanthins in betalain-rich sources



Compound	R	R'	Sources
Dopaxanthin	H	L-Dopa	<i>Bougainvillea</i> sp. (Kugler et al., 2007)
Vulgaxanthin I	H	Glutamine	<i>Beta vulgaris</i> (Stintzing et al., 2002b)
Indicaxanthin	H	Proline	<i>Opuntia ficus-indica</i> (Stintzing et al., 2002b)



# Betalains



Department  
Drug Science  
and Technology

There are a number of known edible sources of betalains. These include red and yellow beetroot (*Beta vulgaris* L. ssp. *vulgaris*), colored Swiss chard (*B. vulgaris* L. ssp. *cicla*), leafy or grainy amaranth (*Amaranthus* sp.) and cactus fruit (*Opuntia* sp. and *Hylocereus* sp.).

Betalains have different concurrent properties that might improve food functions:

- ✓ antioxidants
- ✓ cancer prevention
- ✓ anti-lipidemic

(A) Red beet (B) Amaranth (C) Prickly pear (D) Red pitahaya





- high pigment content
- high degree of glucosylation
- high degree of acylation
- low  $a_w$
- matrix constituents
- pH 3-7
- antioxidants
- chelating agents
- low temperature
- darkness
- nitrogen atmosphere

+

**Betalain  
stability**

-

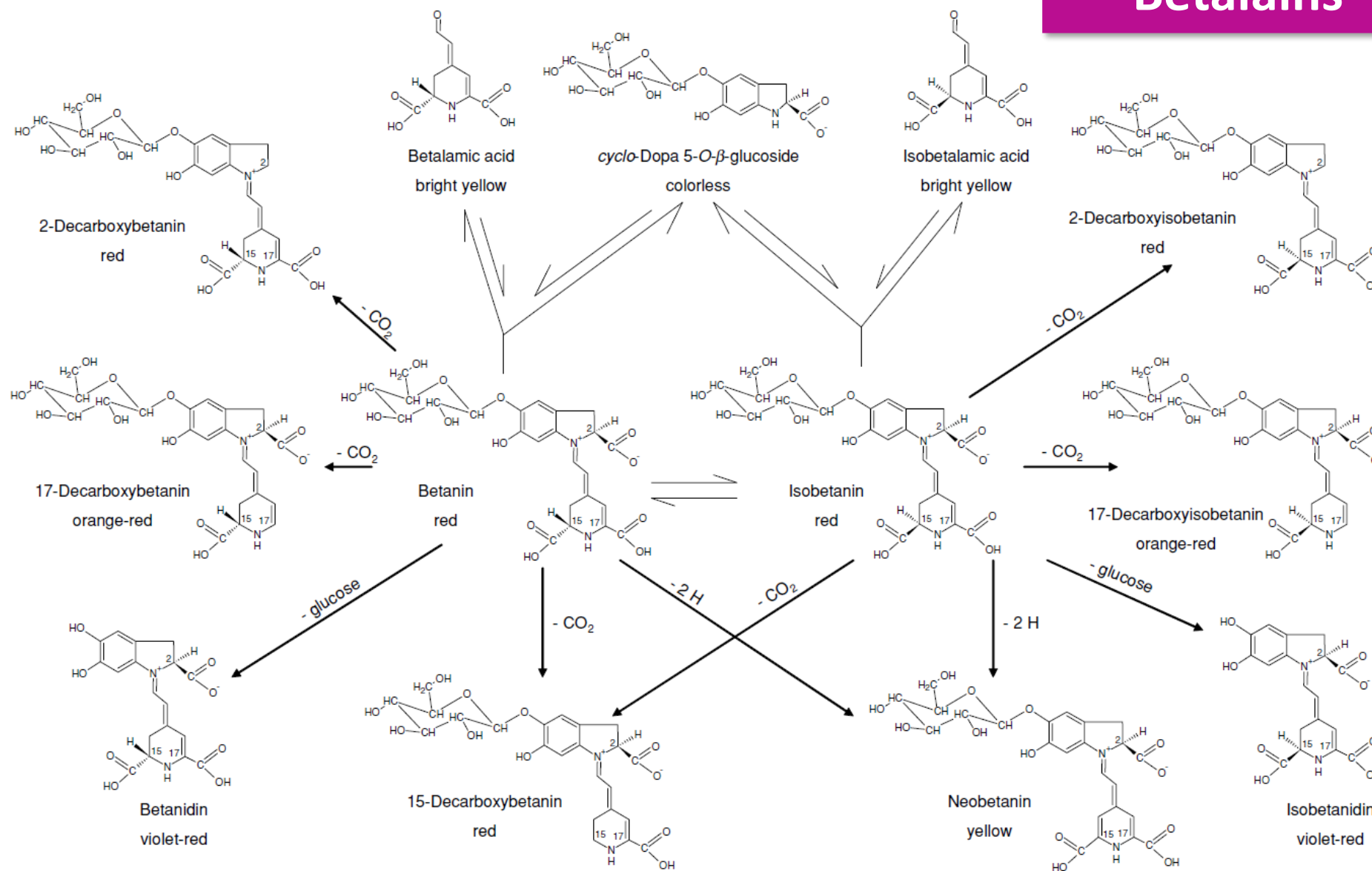
- low pigment content
- low degree of glucosylation
- low degree of acylation
- high  $a_w$
- degrading enzymes  
(POD, PPO, glucosidases)
- pH < 3 or > 7
- metal cations
- high temperature
- light
- $O_2$
- $H_2O_2$

JFS R: Concise Reviews/Hypotheses in Food Science

## Betalain Stability and Degradation— Structural and Chromatic Aspects

KIRSTEN M. HERBACH, FLORIAN C. STINTZING, AND REINHOLD CARLE

# Betalains



## Degradation Pathways and Effects on Chromatic Stability:

- ✓ Isomerization
- ✓ Deglycosylation
- ✓ Hydrolysis
- ✓ Decarboxylation
- ✓ Dehydrogenation
- ✓ Combined and/ or multiple decarboxylation and dehydrogenation

## Controlling betalain degradation pathways

**Betaxanthins** are generally less stable than betacyanins, resulting in a greater color loss.

**Betacyanin** degradation is generally accompanied by a marked color change as a result of the formation of yellow degradation products, that is, betalamic acid, neobetacyanins, and betaxanthins, respectively.

To minimize color shifts of betacyanin-containing solutions:

- ✓ minimum thermal exposure
- ✓ exclusion of light and oxygen
- ✓ addition of antioxidative or chelating additives
- ✓ cool storage below 10°C over at least 24 h allows optimum pigment regeneration.

### Additional alternatives:

- ✓ bluish color can be achieved by systematic deglycosylation of betanin by endogenous or added  $\beta$ -glucosidase.
- ✓ red or orange hues can be adjusted by directed thermal treatment of red beet juice.
- ✓ generation of dehydrogenated betacyanins by heating at low pH.
- ✓ addition of amino acids prior to thermal treatment promotes betaxanthin formation - need of additional labeling (no natural pigments)

# Carotenoids



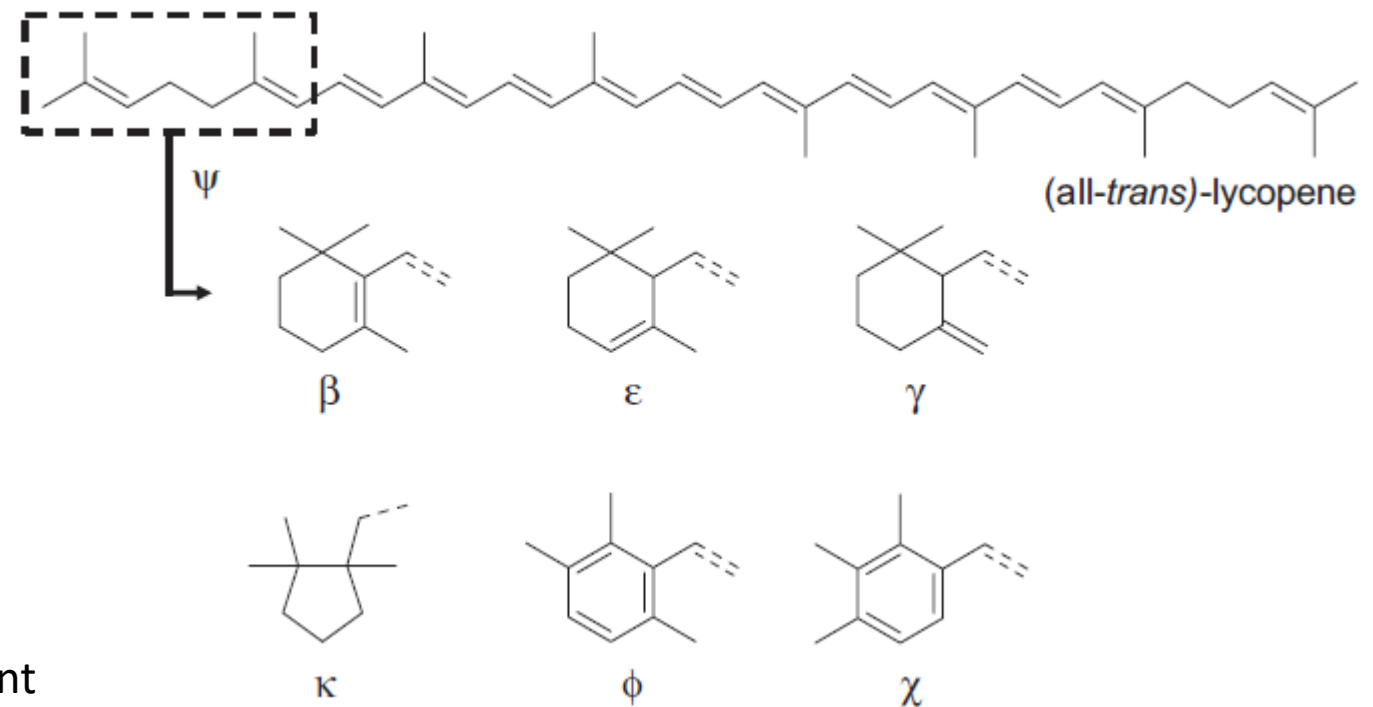
Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

Carotenoids are lipid-soluble pigments widely distributed in nature. While they are biosynthesized primarily by plants and algae, as well as by yeasts, fungi, archaea, and eubacteria, carotenoids are also found throughout the animal kingdom due to selective absorption along the food chain.

They play an outstandingly important role in the photosynthetic apparatus and are well recognized for their beautiful and diverse **yellow, orange,** and **red** colors. These **colors** are **caused** by their chemical structure with a **long polyenic carbon chain**. The **characteristic C<sub>40</sub> isoprenoid skeleton** and its **manifold derivatizations** result in numerous different structures and a broad range of physical, chemical, and biological properties, including the aforementioned colors.

(All-*trans*)-lycopene and the six naturally formed cyclic end-groups of carotenoids





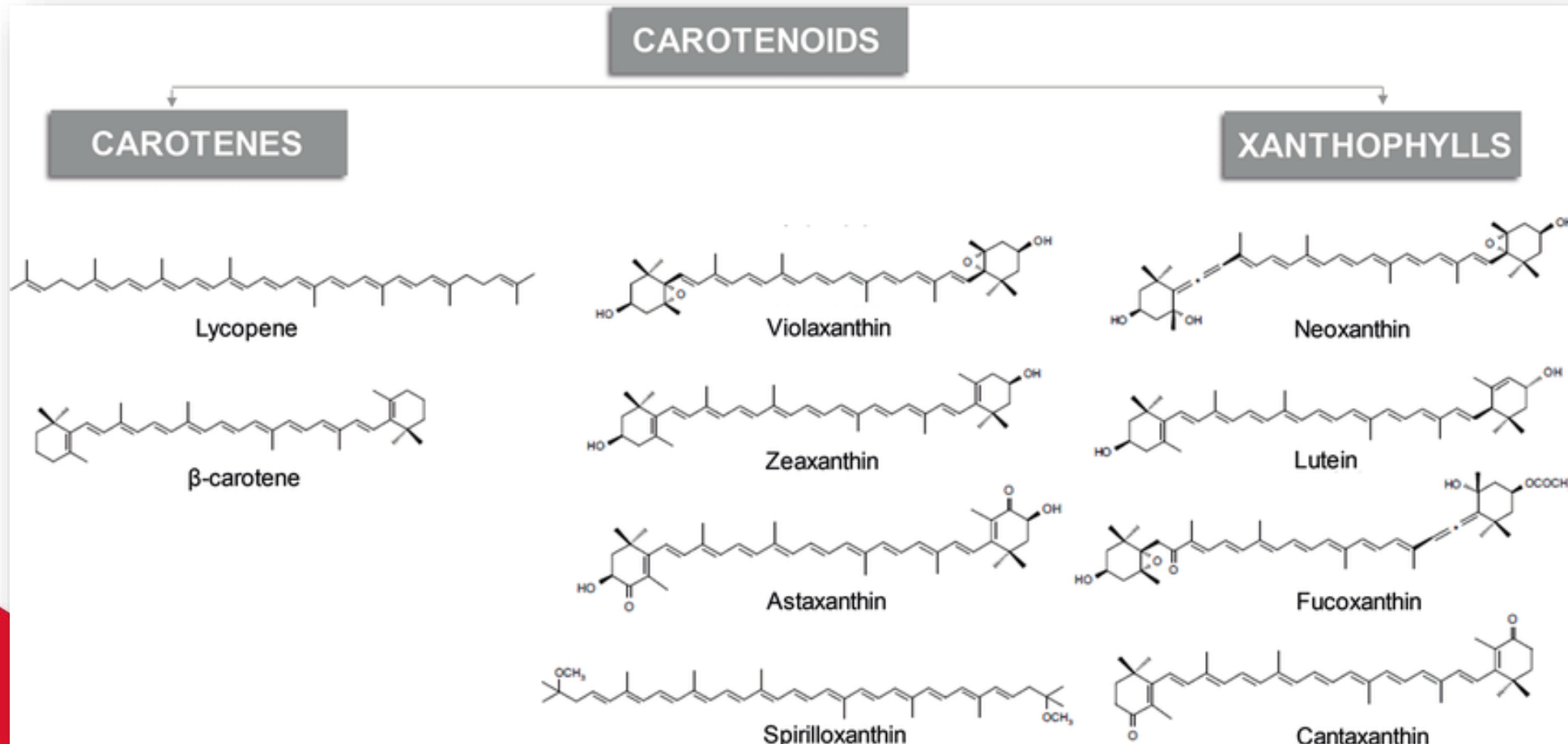
# Carotenoids



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

Subsequent to the **hydroxylation**, **esterification** with various acyl groups or **glycosylation** may alter xanthophyll structure. The **xanthophylls** of fruits and flowers are mainly esterified with long-chain fatty acids, rendering them more hydrophobic. The introduction of hydrophilic sugar moieties occurs in some bacteria and fungi and in some plants such as saffron, which contains a series of glycosyl ester such as crocin (crocetin digentiobioside)



# Food colorants



Carotenoids are fairly stable in their original biological environment as long as cell and chromoplast integrity is ensured, because they are embedded in a complex matrix that imparts numerous protective effects.

When plant material is turning overripe and leaves are wilting, carotenoids are confronted with different detrimental factors such as oxidizing enzymes, excessive light exposure, and oxygen. As a result, their concentrations decrease.

Food processing almost inevitably includes disruption of the plant material and often involves treatment at elevated temperatures, which also affect carotenoid stability.

## Carotenoids



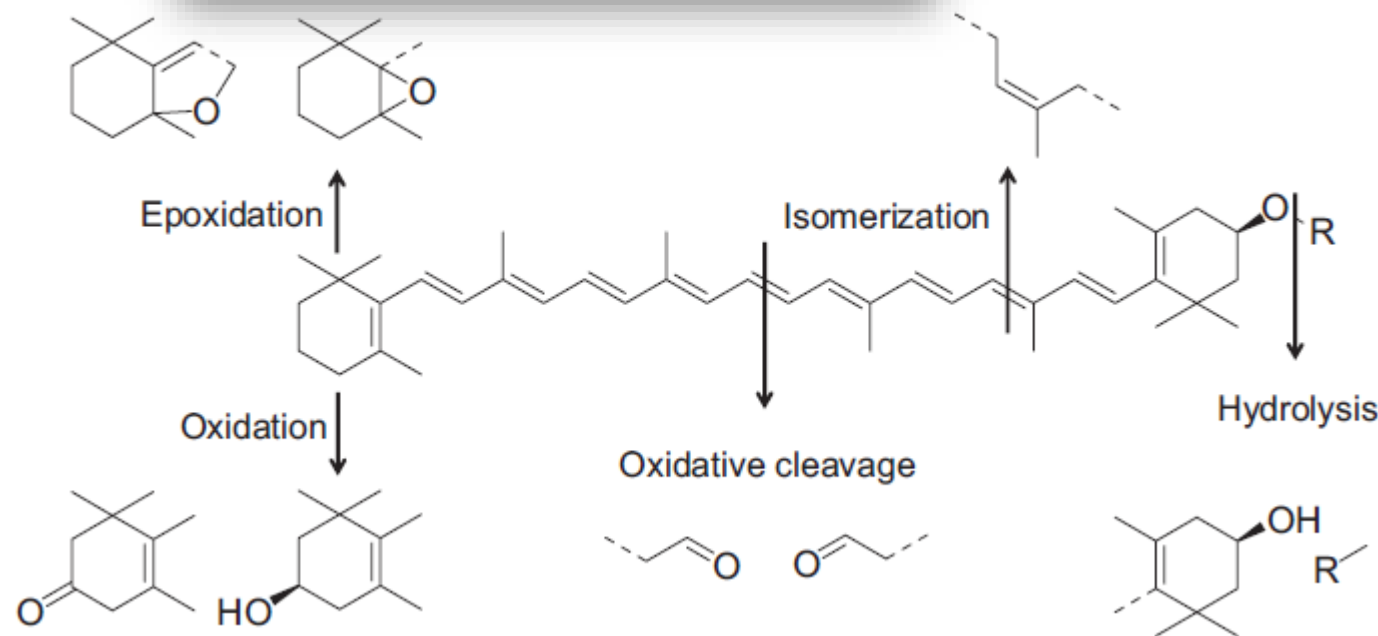
Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

Critical Reviews in Food Science and Nutrition, 50:515-532 (2010)  
Copyright © Taylor and Francis Group, LLC  
ISSN: 1040-8398  
DOI: 10.1080/10408398.2010.55889



### Factors Influencing the Chemical Stability of Carotenoids in Foods



Major possible reactions of a  $\beta$ -cryptoxanthin ester. The oxidative alteration and degradation of carotenoids follow different pathways depending on the surrounding environment. Several factors such as heat, light exposure, enzymatic actions, and the presence of metal ions may facilitate oxidation and lead to different products.

# Food colorants



## Carotenoids



Department  
Drug Science  
and Technology

UNIVERSITÀ  
DI TORINO

*Critical Reviews in Food Science and Nutrition*, 50:515-532 (2010)  
Copyright © Taylor and Francis Group, LLC  
ISSN: 1040-8398  
DOI: 10.1080/10408398.2010.505889

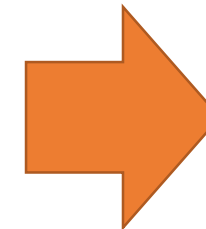


### Factors Influencing the Chemical Stability of Carotenoids in Foods

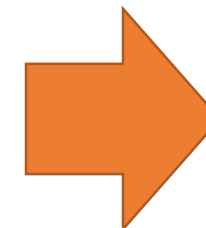
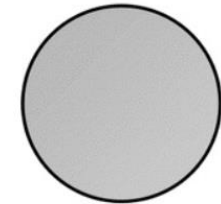
Understanding the mechanisms of carotenoid degradation is essential for developing technologies for the incorporation of these compounds into functional foods. Carotenoids could be added to foods as pure compounds, oleoresins of foods (e.g. paprika), or as dried food products (e.g. tomatoes).

While these options may be viable in some foods, they may be limited in others by problems with solubility, flavor, and stability. An alternate possibility for incorporating carotenoids into foods would be to incorporate them into emulsion or nanostructure delivery systems that if necessary could be further encapsulated by drying operations.

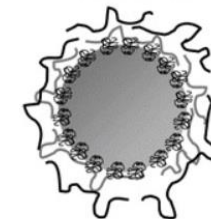
These dispersed forms of carotenoids would have the advantage of being more easily dispersed into food products. In addition, delivery systems could be designed to help decrease the degradation of the carotenoids. By identifying the predominant carotenoid degradation pathway likely to occur in a particular food product, delivery systems could be engineered for optimal stability.



Conventional  
Emulsion



Multilayer  
Emulsion



## Conventional emulsions

These emulsions are typically produced by homogenizing oil and aqueous phases along with emulsifiers at high pressure. This process results in oil droplets coated in the surfactant that form an interfacial layer between the oil and aqueous phases.

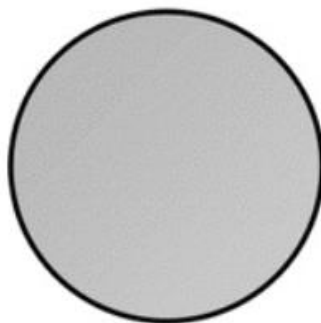
Emulsions are easy to incorporate into aqueous based products at a relatively low cost and they could retain their antioxidant properties once diluted into the food if their chemical and physical properties are retained.

## Carotenoids

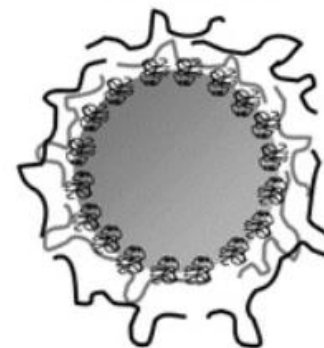


Department  
Drug Science  
and Technology

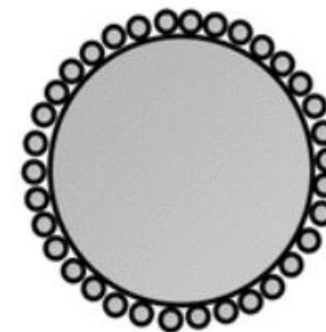
Conventional  
Emulsion



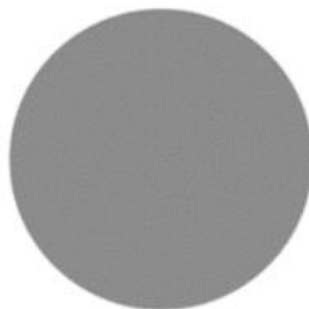
Multilayer  
Emulsion



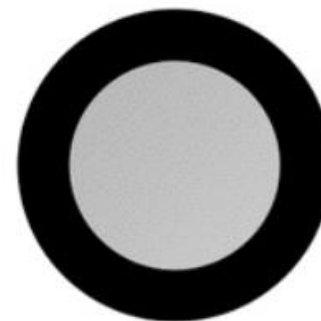
Colloidosome



Solid Lipid Particles



Homogeneous



Core-Shell



Dispersed



## Multilayer Emulsions

Multilayer emulsions are produced by forming layers around an oil droplet.

Typically, a conventional emulsion is made followed by addition of a polyelectrolyte of opposite charge (e.g. polysaccharide or protein). The opposite charges attract to one another, causing the polyelectrolyte to adsorb to the droplet surface. This process can be repeated, again using the principle of electrostatic attraction to form additional layers.

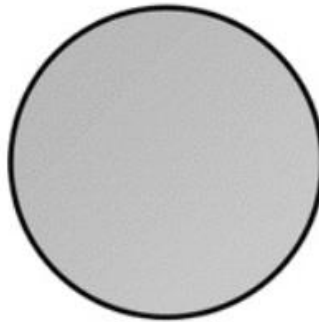
The thicker interfacial membrane present in these emulsions may provide added protection to carotenoids from prooxidants in the aqueous phase of the product.

## Carotenoids

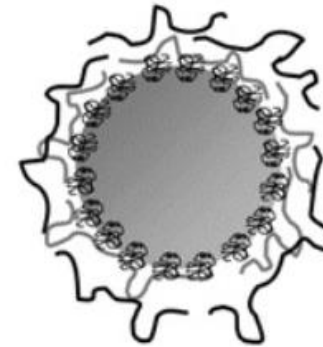


Department  
Drug Science  
and Technology

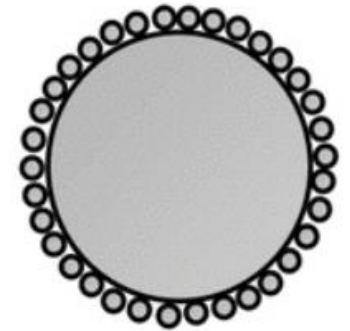
Conventional  
Emulsion



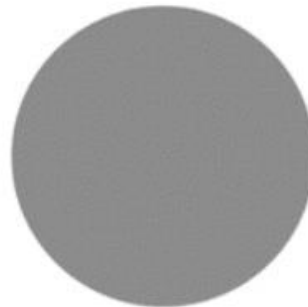
Multilayer  
Emulsion



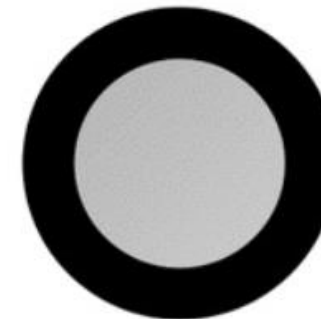
Colloidosome



Solid Lipid Particles



Homogeneous



Core-Shell



Dispersed

## Solid-Lipid Particles

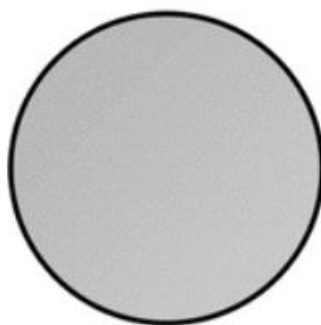
Solid-lipid particles can be produced using the similar methods to those used for creating conventional emulsions. However, the lipid components are carefully chosen so that all or part of the lipid in the emulsion droplets solidifies after processing and cooling. A key component to solid-lipid particle production is to homogenize the lipid and aqueous phases at a temperature that is higher than the melting point of the lipids

## Carotenoids

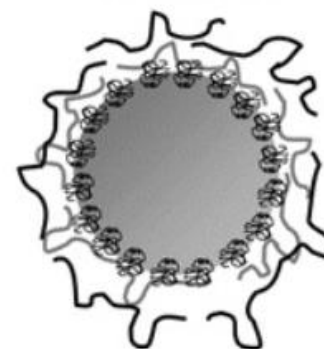


Department  
Drug Science  
and Technology

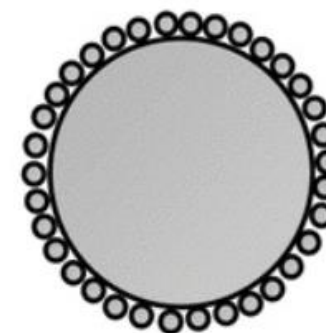
Conventional  
Emulsion



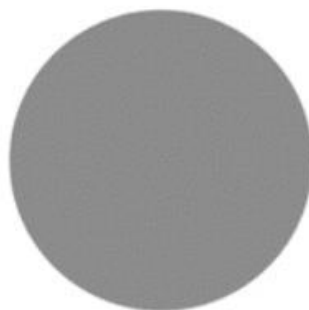
Multilayer  
Emulsion



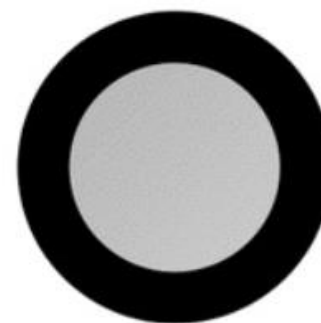
Colloidosome



Solid Lipid Particles



Homogeneous



Core-Shell



Dispersed

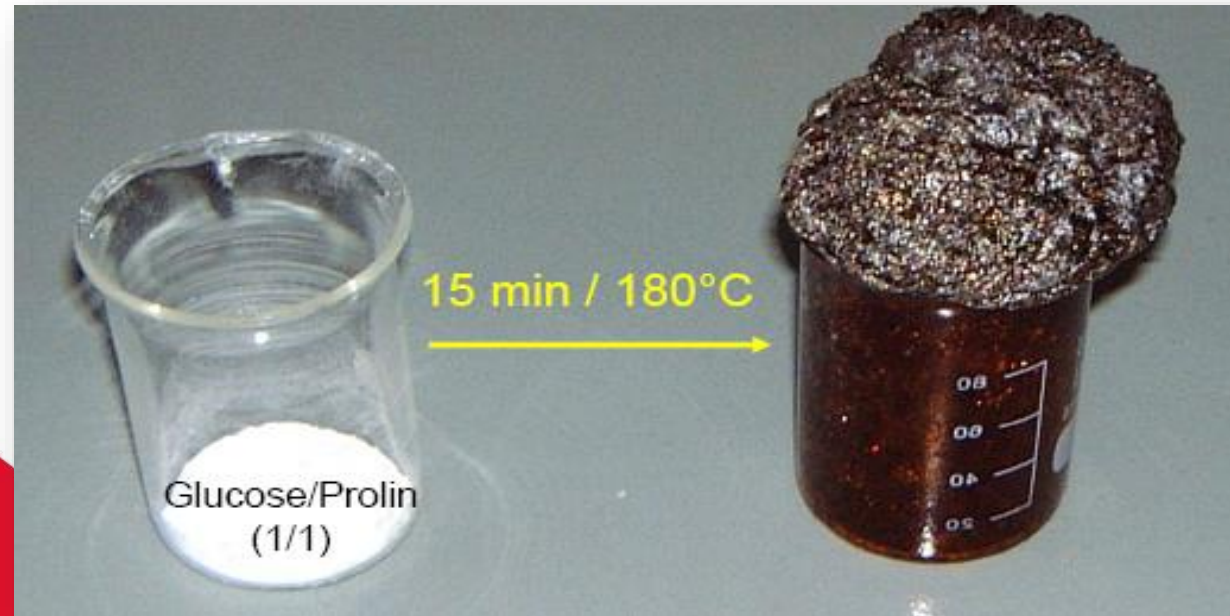
# Food colorants

## Maillard reaction



Reaction between reducing sugars and amine compounds, studied by the French biochemist Louis Camille Maillard and also called the **non-enzymatic browning reaction**.

It contrasts with the well-known phenomenon of enzymatic browning that occurs in the polyphenolic fraction of plant foods by specific enzymes: polyphenol oxidase.



## Melanoidins



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

# Food colorants

## Maillard reaction



**Maillard reaction**, or nonenzymatic browning, includes the **formation of N-glycosides** and their successive reactions (e.g., oxidation, dehydration, hydrolysis etc.). N-Glycosides are widely distributed in nature (nucleic acids, NAD, coenzyme A). They are formed in food whenever **reducing sugars** occur together with **proteins, peptides, amino acids** or **amines**. They are obtained more readily at a higher temperature, low water activity and on longer storage.

On the **sugar** side, the reactants are mainly **glucose, fructose, maltose, lactose** and, to a smaller extent, reducing **pentoses**, e. g., ribose.

On the side of the **amino component**, **amino acids** with a **primary amino group** are more important than those with a secondary because their concentration in foods is usually higher. Exceptions are, e. g., malt and corn products which have a high proline content. In the case of proteins, the  **$\epsilon$ -amino groups of lysine** react predominantly.

# Melanoidins

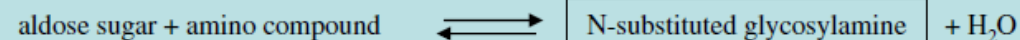


UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

*Initial stage (colourless; no absorption in near-UV)*

Sugar-amine condensation:

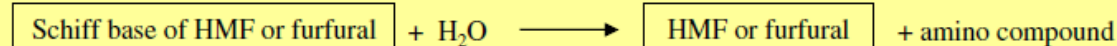
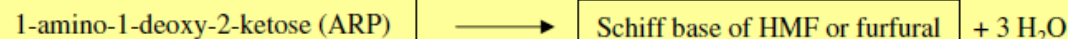


Amadori rearrangement:



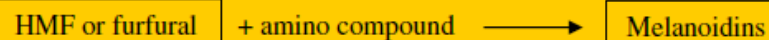
*Intermediate stage (colourless or yellow; strong absorption in near-UV)*

Sugar dehydration:



*Final stage (highly coloured)*

Aldehyde-amino polymerization; formation  
of heterocyclic nitrogen compounds:



(brown nitrogenous polymers and copolymers)



# MAILLARD REACTION

reshuffling atoms,  
over heat, to make  
flavor molecules

PROCESS

70°F/21°C

212°F/100°C

250°F/110°C

**flavor-full**  
(Maillard Reaction/browned)

300°F/149°C

330°F/166°C

400°F/204°C

**raw**  
(uncooked)

**bland**  
(steamed)

**sweet**  
(caramelized)

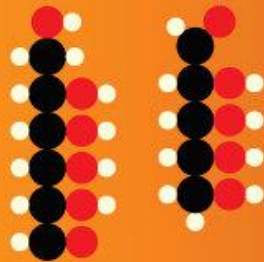
**no taste**  
(burned)



More + more-varied proteins (meat  
vs. veggies) = more (stronger) flavors.

Only the surface reaches the temperature at which the Maillard Reaction  
(discovered by chemist Louis Camille Maillard in the 1910s) can occur.

**browned onions**  
(Maillard Reaction happens  
before caramelization)

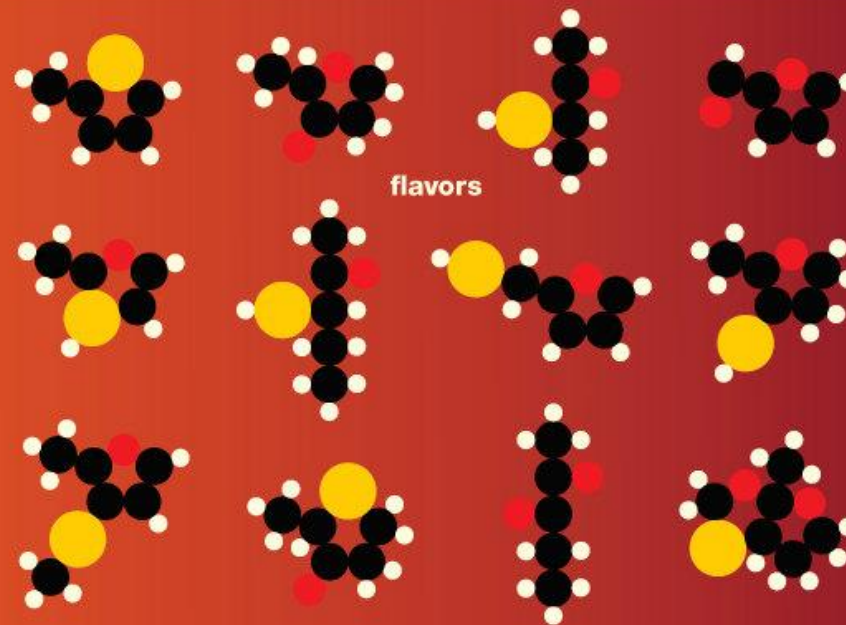


(some of the)  
amino acids  
from protein



+heat  
→

flavors



● H ● O ● N ● C ● Sulfur

Heat and water causes hydrolysis or the breaking  
of the peptide bonds [in protein]. Enzymes in your body  
perform this at lower temperatures and more efficiently.  
-Michael Klopfer

IOANA



UNIVERSITÀ  
TORINO

Department  
Drug Science  
and Technology



Department  
Drug Science  
and Technology

UNIVERSITÀ  
TORINO





# Food colorants

## Maillard reaction



## Melanoidins

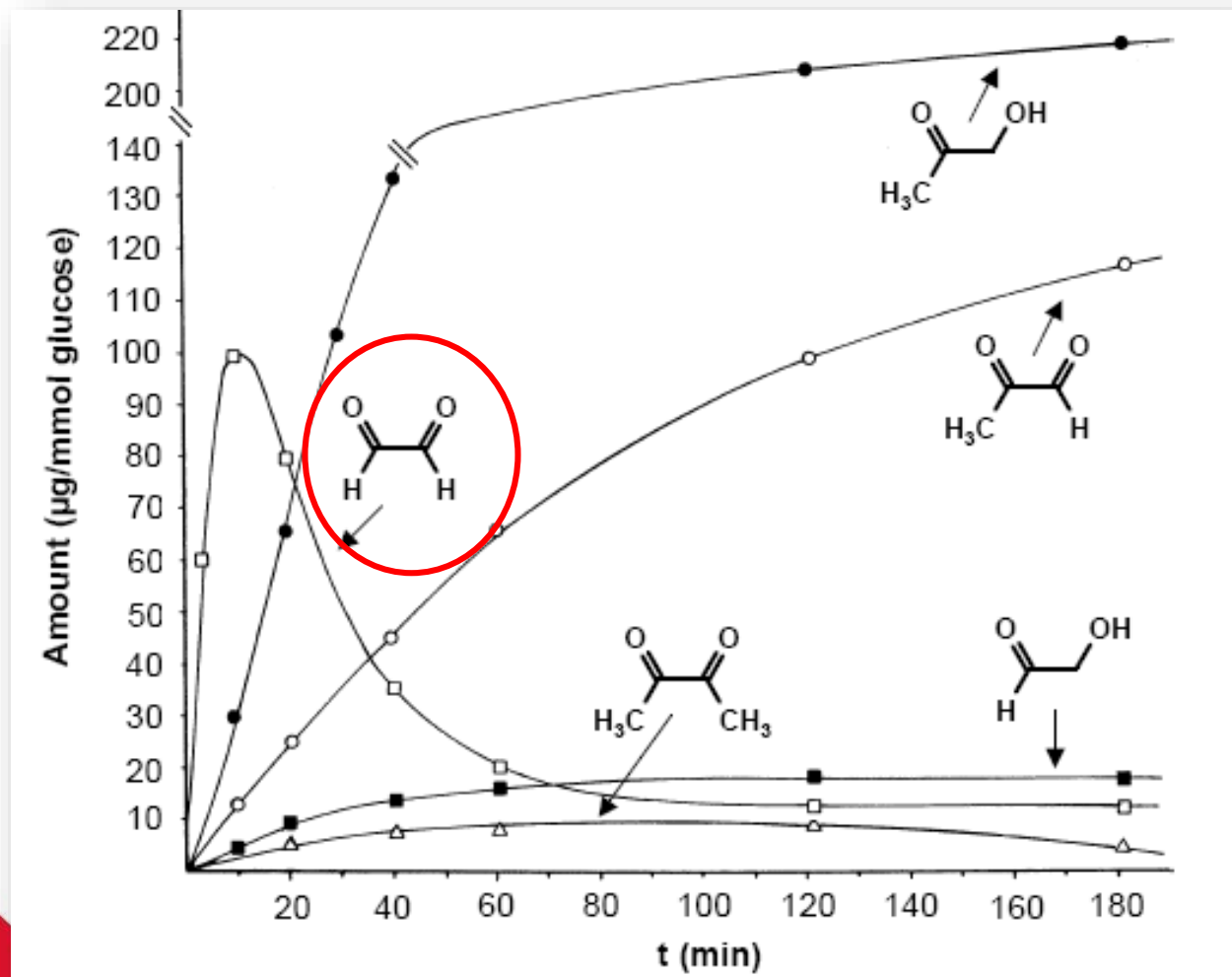


UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

Formation of **glyoxal**, and other  $\alpha$ -dicarbonyl derivatives, in a glucose / alanine system at pH 7.0.

Glyoxal is the primary degradation product of Amadori compounds under oxidizing conditions, it is formed at relatively low glucose concentrations.



# Food colorants

## Maillard reaction



The same reaction mechanism, which involves the cleavage of  $\alpha$ -dicarbonyl compounds can also lead to the formation of radical compounds: the so-called **crosspy-radicals**.

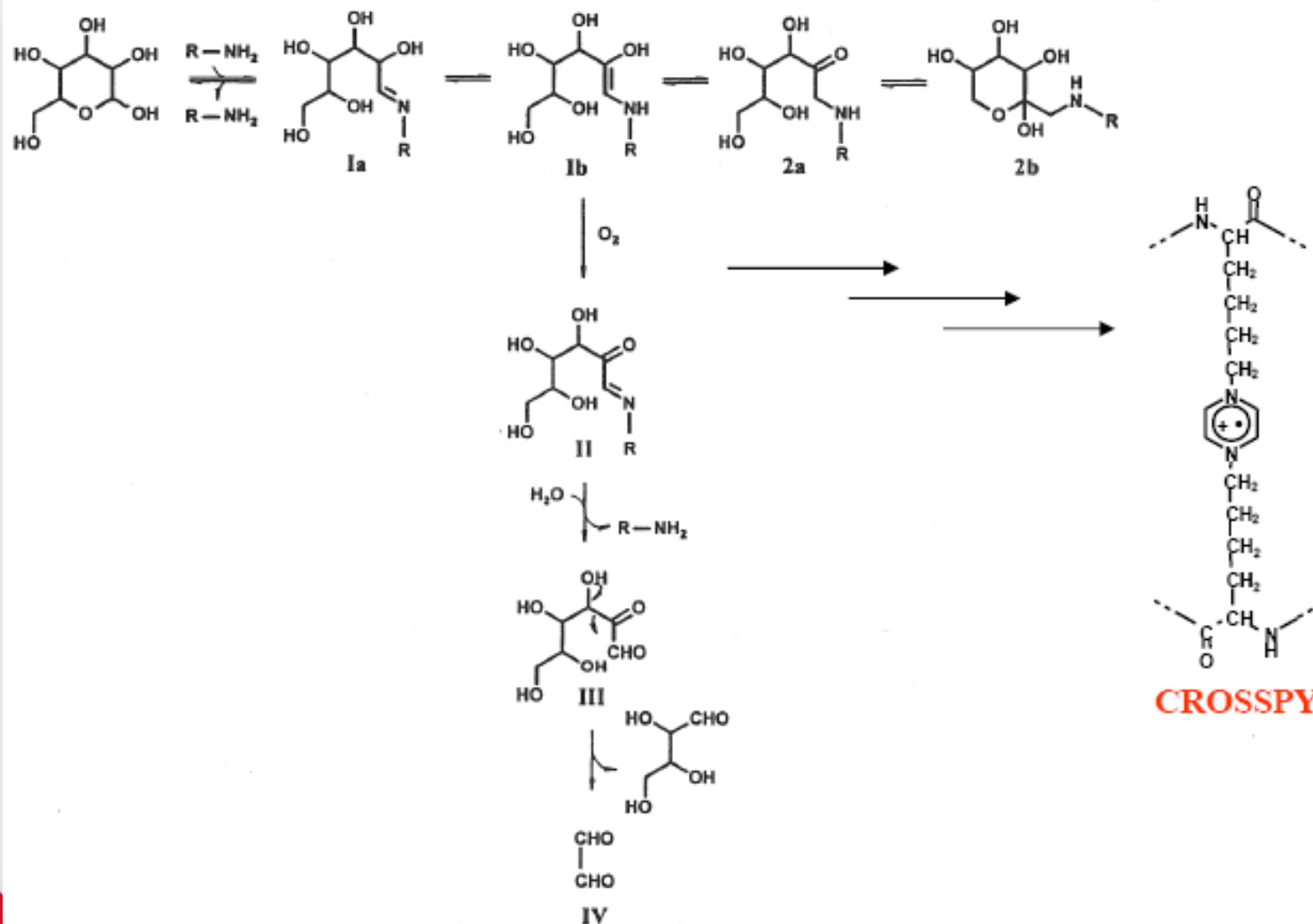
These compounds represent the key intermediates in the formation of brown pigments (responsible for the browning of heat-treated foods) defined as **melanoidins**.

## Melanoidins



Department  
Drug Science  
and Technology

UNIVERSITÀ





# Food colorants

## Maillard reaction



## Melanoidins



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

The graph on the side describes the development of brown pigments (melanoidins - solid line) and the kinetics of formation of crosspy radicals (dashed line) as a function of time in a model system composed of **glucose and N-acetyl-L-lysine**

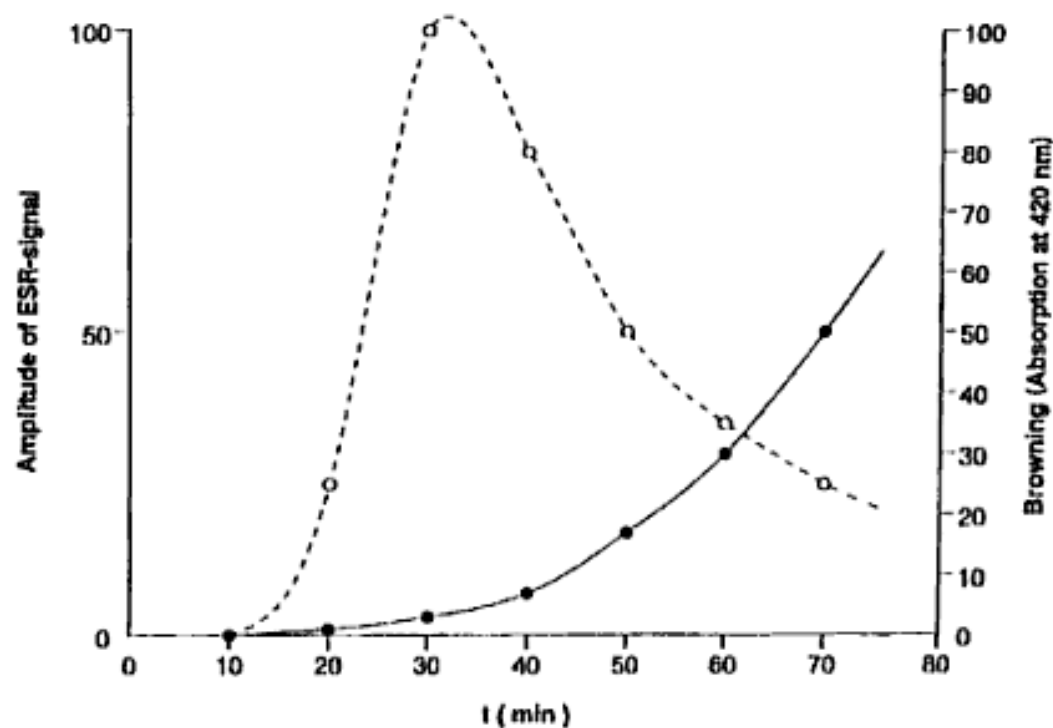


Figure 2.16: Time course of browning development and Crosspy formation in a thermally treated mixture of glucose and *N*<sub>α</sub>-acetyl-L-lysine.

# Food colorants

## Maillard reaction



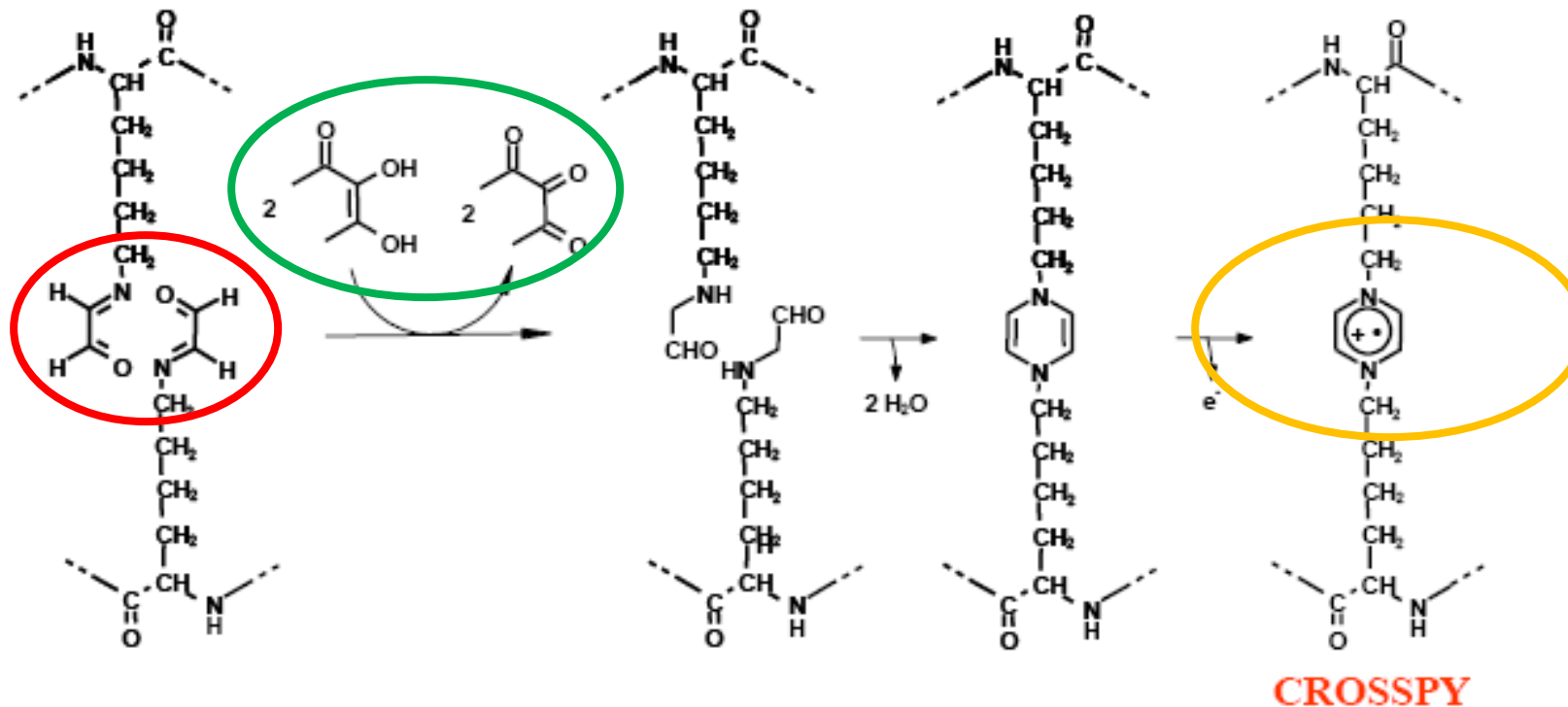
# Melanoidins



Department  
Drug Science  
and Technology

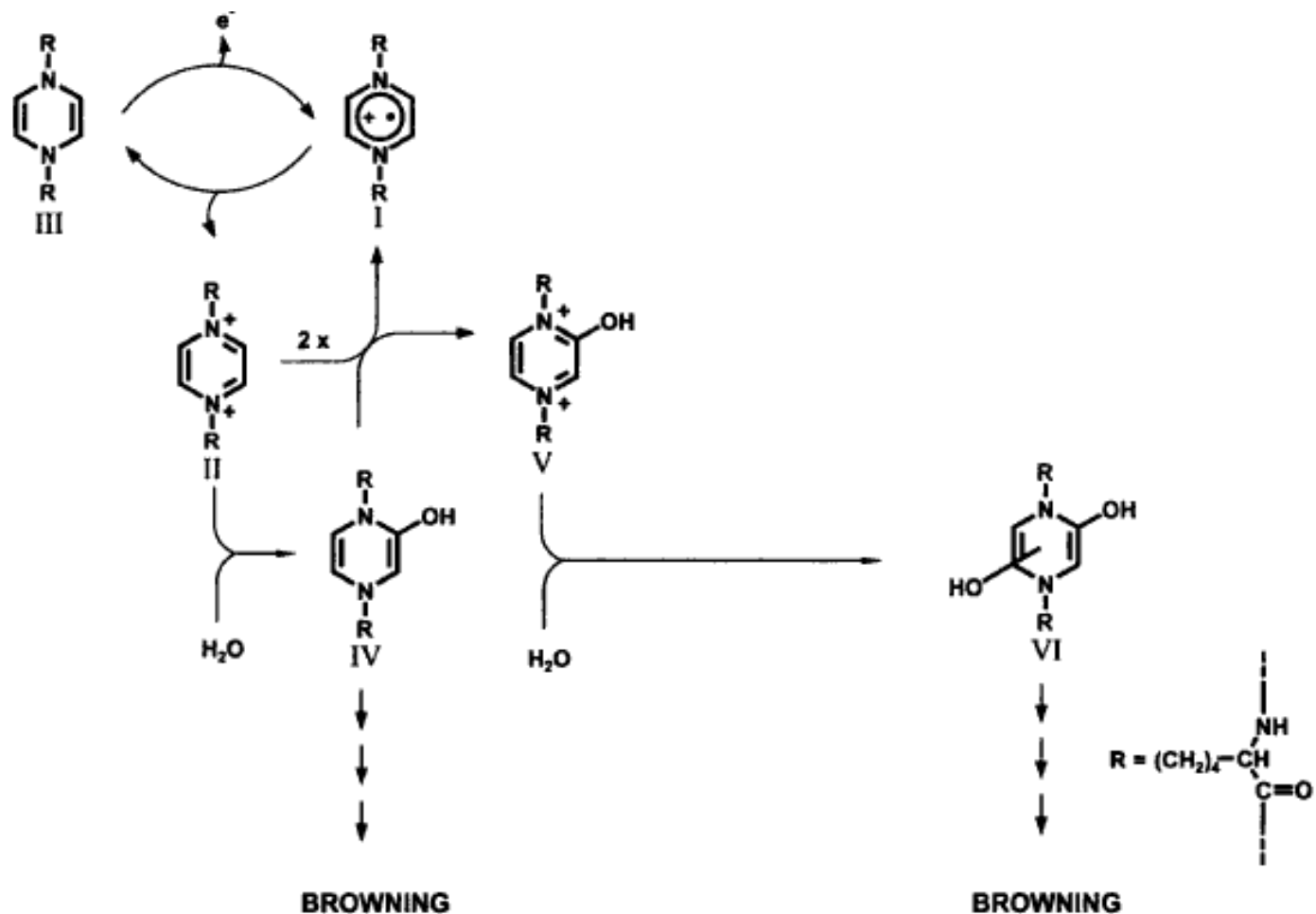
UNIVERSITÀ  
DI TORINO

The formation of **crosspy radicals** involves the reaction of **two glyoxal molecules** with an  $\epsilon$ -amino group of a lysine residue of a protein to form a glyoxal-imine which by reduction (with molecules called **reductons**), forms two terminal aldehyde functions that dimerize to forming a dihydro pyrazine which, by losing an electron, forms the pyrazine cation **CROSSPY** (**pyrazinium radical cation**).



# Food colorants

## Maillard reaction



# Melanoidins



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

The reaction scheme reported here summarizes the hypothesis with which the crosspy radicals (I) react to form brown pigments. The radical would form the diquaternary salt (II) and the relative 1,4-dihydropyrazine (III) by disproportionation. The diquaternation salt subsequently forms 2-hydroxy-1,4-dihydropyrazine (IV) by hydration. The latter reacting with the diquaternary salt regenerates the crosspy radical and forms the bishydroxy-dihydropyrazine (VI) and the hydroxylated quaternary salt on the heterocycle (V). Compounds IV and VI are very strong nucleophiles and are the precursors of brown pigments.

# Food colorants

## Maillard reaction

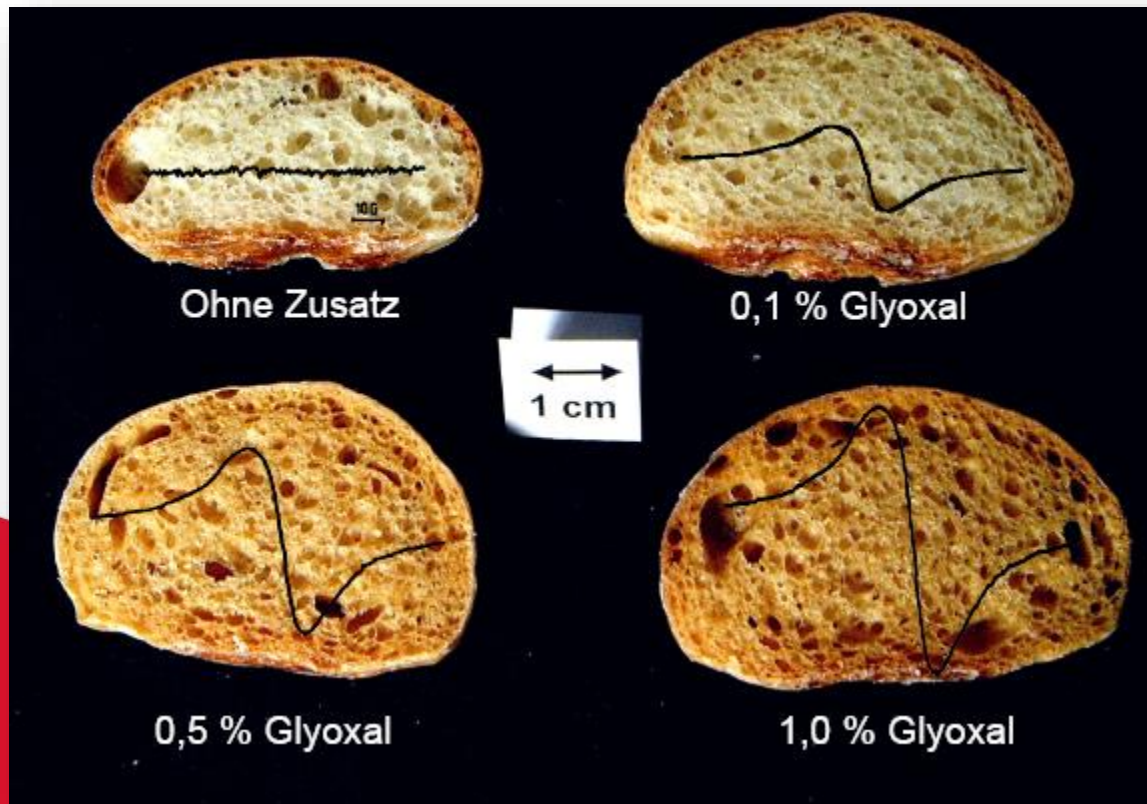


# Melanoidins



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology





# Food colorants

## Maillard reaction



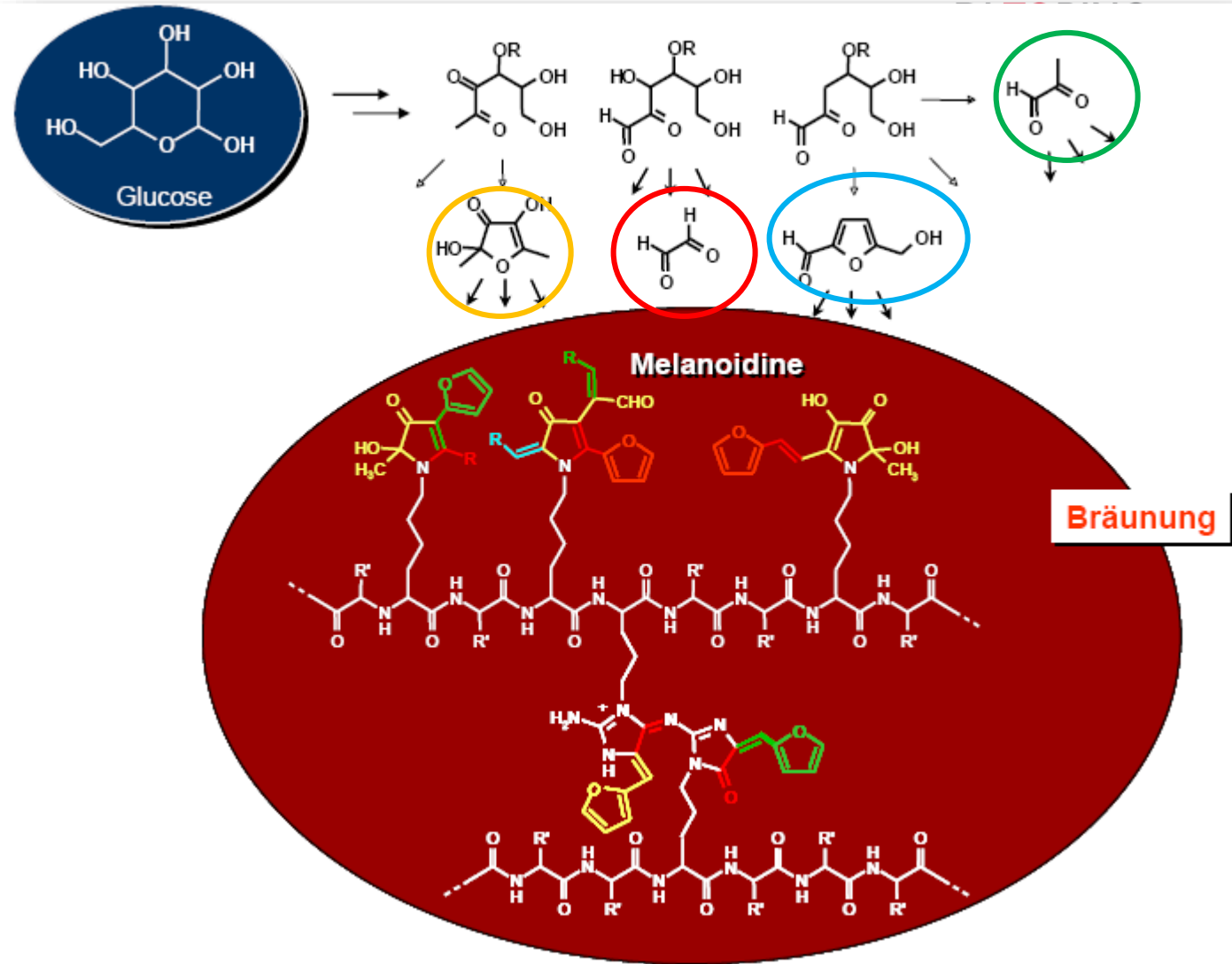
Brown pigments (precisely defined melanoidins) are formed by reaction between Lys and Arg residues, present in proteins, and the secondary products of the Maillard reaction: **glyoxal**, **methyl-glyoxal**, **5-hydroxymethyl-furfural**, **acetylformoin**. They generate chromophores which impart the characteristic brown color to cooked foods.

## Melanoidins



Department  
Drug Science  
and Technology

UNIVERSITÀ



# Food colorants

## Maillard reaction

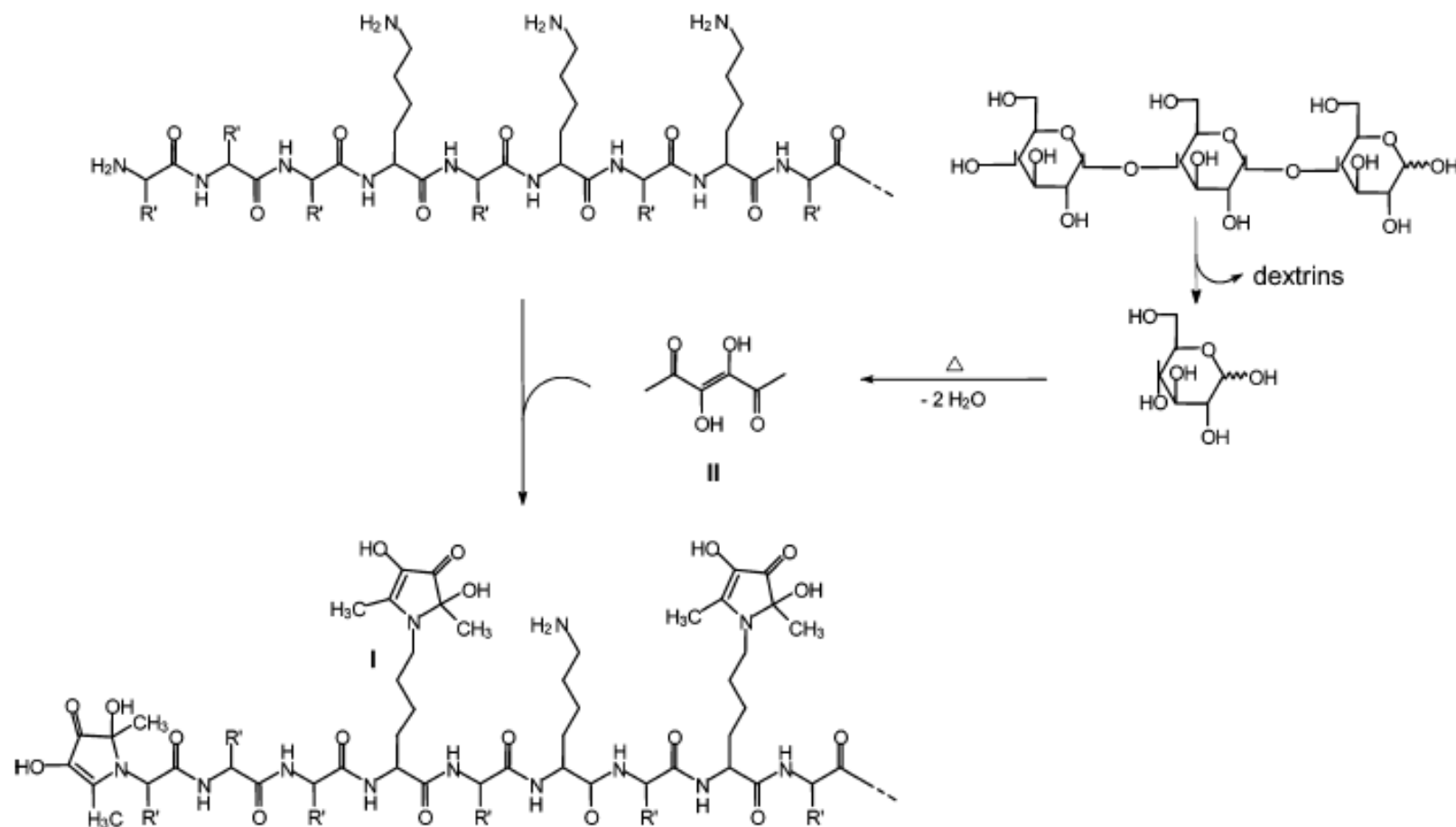


# Melanoidins



UNIVERSITÀ  
DI TORINO

Department  
Drug Science  
and Technology

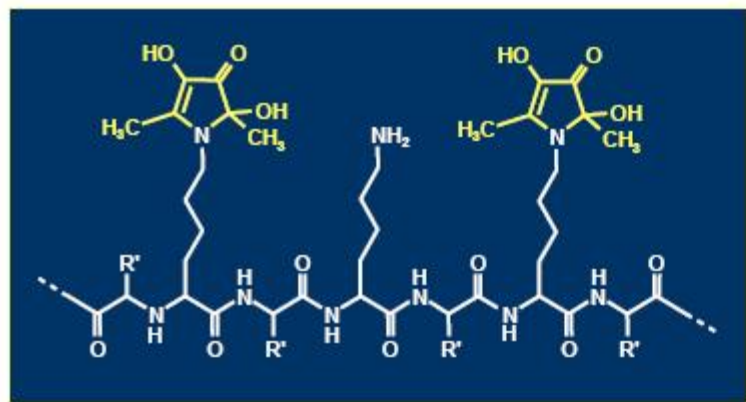


re 1. Reaction scheme on the formation of protein-bound pronyl-L-lysine (I) from proteins and starch via the key intermediate acetylformoin (II).

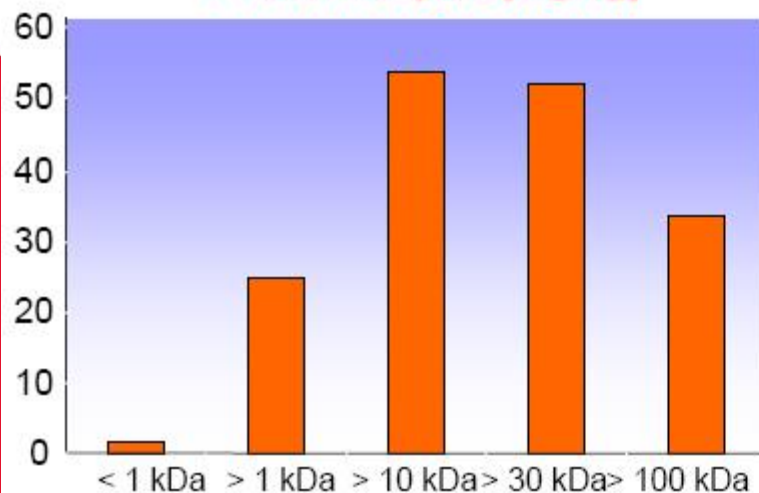
M. Lindenmeier, T. Hofmann *J. Agric. Food Chem.*, Vol. 52, No. 2, 2004

# Food colorants

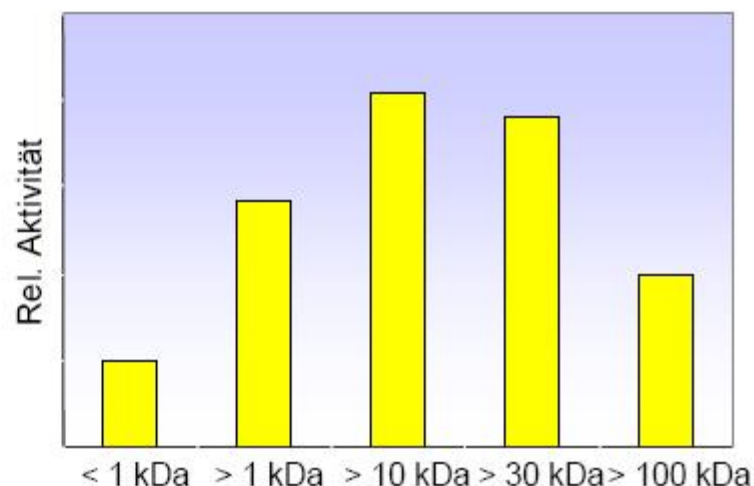
## Maillard reaction



**PRONYL-Lysin [mg/kg]**



**Antioxidative Aktivität**



# Melanoidins



UNIVERSITATEA

Department  
Drug Science  
and Technology

Pronyl-Lys, a product of the reaction between the Lys residues of proteins present in cereals and acetylformoin, has been attributed a remarkable antioxidant activity in-vitro and in-vivo. The histograms shown indicate the distribution in pM of the pigments and the relative antioxidant activity of the fractions.