New molecular and cell-based approaches to assess food astringency and bitterness

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Make healthy food be tasty

The BeTASTy project seeks to understand the physical-chemical mechanisms behind unpleasant taste and mouthfeel perceptions and why they vary among individuals, potentially revolutionising the food industry and promoting healthier, more sustainable eating habits.

In a world increasingly conscious of health, sustainability and climate impact, the shift towards plant-based diets is more than a trend—it's a necessity. Unhealthy diets are a major health concern, and it is estimated that one in five deaths globally is associated with poor diet, contributing to almost 40 per cent of the global burden of disease. In fact, unhealthy dietary practices are known as risk factors for non-communicable chronic diseases, including cardiovascular diseases, cancer and diabetes.

Encouraging the population to adopt healthier diets throughout their lifespan is a societal challenge and a priority, which is why nutrition is included in one of the goals of the 2030 Agenda for Sustainable Development. The food industry is now in the spotlight as they are constantly challenged to create novel and healthier solutions by reformulating existing food products or by creating new ones with minimal processing and maximal health profit. Also, the food industry has been focused on developing products combined with functional compounds, such as alternative proteins and phytonutraceuticals to meet consumer demand for functional food that improves human health, performance and well-being. The search for sustainable alternative protein sources with neutral climate impact is imperative to align with global environmental sustainability. Plant-based food and derived products are healthy, accessible and sustainable alternatives for animal-derived protein. However, plant-based foods are often described as having unpleasant mouthfeel and taste properties, particularly astringency and bitterness. This poses a significant barrier in the development of novel plant-based food products, as several studies show that consumers' food choices are driven mainly by flavour, no matter the health benefits, challenging the food industry to 'make healthy plant-based food be tasty'.

Understanding the connection between astringency and bitterness

Astringency is a tactile sensation of dryness and roughness perceived in the oral cavity triggered by chemicals like acids and metals. In food, astringent compounds are mainly phenolic compounds derived from plants, such as tannins (found in red wine) or theaflavins (found in tea). In addition to phenolic compounds, plant-based proteins have also been reported to induce astringency. Bitter-tasting compounds are diverse and typically found in plant-based food, having been produced by plants as a defence mechanism. Examples include caffeine (found in coffee) or hop bitter acids (found in beer). Moreover, bitterness is also induced by bitter peptides found in protein-rich foods, which can be formed during food processing. They can be found in animal-derived products like cheese and plant-based food like soy protein products.

Astringency and bitterness are perceived through different physical-chemical mechanisms in the oral cavity. The exact mechanism by which food nutrients like phenolic compounds cause the sensation of astringency is still unknown. Astringency can be triggered by compounds that precipitate salivary proteins or interact directly with epithelial cells. A hypothesis argues that astringency can also result from the activation of mechanoreceptors in the mouth, but so far, no evidence has been found to support this hypothesis. While the exact mechanism of astringency is still unknown, it is likely that all three described mechanisms contribute to this sensation or that different astringent compounds activate different mechanisms. Although most of the studies focused on the salivary protein's precipitation and, more recently, on the interaction with epithelial cells, less is known about the involvement of mechanosensation in astringency sensing. Mechanoreceptors are innervated by trigeminal ganglion neurons, which are responsible for the sense of food texture in the oral cavity. Once they are activated, the resulting cell depolarisation triggers a complex signalling cascade, providing information to the central nervous system about the mouthfeel characteristics. While the primary sensation of astringency involves the tactile and mechanical response in the oral cavity, trigeminal ganglion neurons are believed to contribute by conveying these mechanical sensations to the brain. On the other hand, the bitterness perception mechanism is well understood. Bitterness results from the

activation of specific bitter taste receptors (TAS2Rs) found in the oral cavity. Bitter taste receptors are mainly found on the taste bud and can be activated by bitter substances, leading to increased intracellular calcium levels and cell membrane depolarisation. This complex taste transduction response concludes on the release of neurotransmitters, allowing us to perceive the bitter taste. Some astringent compounds, particularly phenolic compounds, can also activate bitter taste receptors.

An important challenge arises from the fact that astringency and bitterness are not perceived equally by different people. The interindividual variances include differences in oral mucosa, saliva and age-related changes, which may also influence oral food processing and sensory properties. Optimisation of taste properties in the food industry shall not obey the 'one size fits all' rule, as interindividual perceptions seem to have a determinant role in defining sensory tasting. Also, the simultaneous presence of bitter and/or astringency components on the food matrix might influence taste perception.

Understanding the mechanisms of astringency and bitter taste is crucial for comprehending the intricate molecular interactions involved in taste perception and sensations. This knowledge paves the way for developing advanced methods to modulate unpleasant tastes in food and beverages, enhancing consumer satisfaction and broadening the possibilities for food innovation.

Creating novel approaches to tackle unpleasant taste research

Current methods to quantify astringency and bitterness have significant limitations due to the intricate nature of food matrices and the wide range of individual differences in sensory perception. An integrated and comprehensive approach combining advanced analytical techniques with deeper knowledge of sensory science and human variability is needed to address these complexities. BeTASTy will develop innovative methodologies to advance our knowledge of intricate molecular interactions involved in astringency and bitterness.

The activation of bitter taste receptors is being studied *in vitro* using fluorescence-based assays and cellular model cells expressing TAS2Rs. The activation of TAS2Rs by bitter compounds triggers a complex signalling cascade that increases intracellular calcium levels, which can be monitored using calcium-specific fluorescent probes. Studying the activation of TAS2Rs by polyphenols, plant-based peptides, or proteins, and the influence of salivary proteins as key components of astringency perception, is crucial for understanding taste. Moreover, we will employ this technology to explore how certain compounds (e.g. carbohydrates) interact with bitter molecules and identify those that enhance or inhibit bitterness. This insight will support the development of strategies to mask or reduce unpleasant tastes in food and beverages, enhancing their palatability. Finding natural bitter inhibitors is a natural solution to make plant-based foods more appealing, supporting a shift towards sustainable and nutritious diets.

To approach astringency, the molecular interactions of astringent compounds are being explored with a novel in-house developed cellular model combining tongue and buccal mucosa cell lines, which are capable of mimicking a similar environment to that observed in a human mouth. The importance of a cell-based model relies on deepening the intricate interactions that occur within the oral cavity and that lead to astringency perception. The model combines epithelial cells with saliva and mucin (the last two being largely produced by mouth glands and epithelial tissues in most animals). This cellular model will be used to further evaluate the interaction of several types of astringent compounds with the oral constituents.

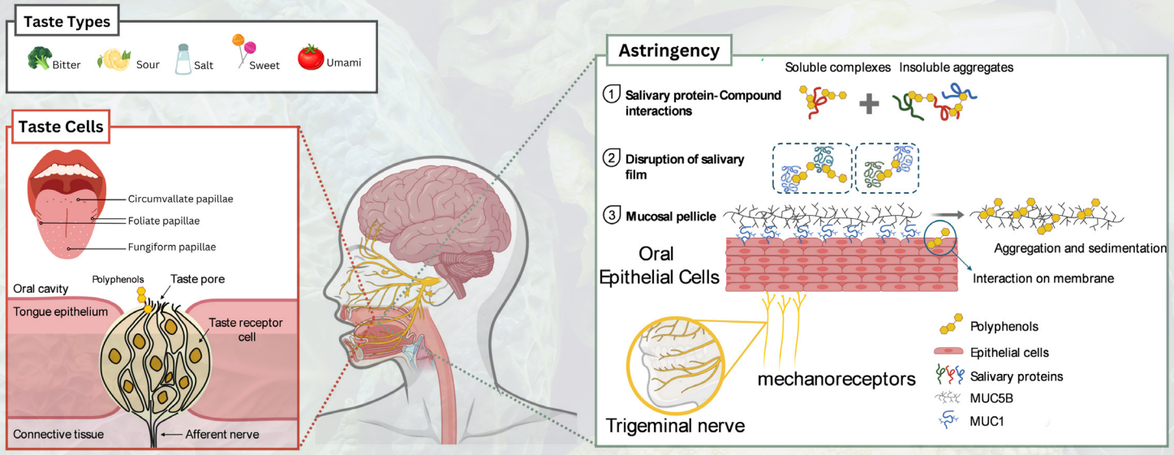

Figure 1: The complexity of taste and flavour perception in the oral cavity. Taste buds are found inside small bumps (papillae) scattered throughout our tongue and are responsible for the perception of the five basic tastes: bitter, sour, umami, sweet and salty. Taste buds are composed of a group of cells, including taste receptor cells containing specific receptors for each taste. The taste receptor cells connect the taste sensation in the mouth to the nerves that transmit taste signals to the brain. Astringency is a sensory phenomenon that is characterised by a dry, puckery and rough sensation when consuming polyphenol-rich food or beverages. This sensation is the result of the interaction of astringent compounds with salivary proteins, disruption of the salivary film, interactions with the mucosal pellicle or oral epithelial cells and the activation of trigeminal mechanoreceptors. (Credits to Carlos Eloy Guerreiro and Rita Vilaça. Created with Biorender.com and Canva Pro.)

Is mechanosensation involved in astringency perception?

Despite major advances in understanding astringency, some compounds/polyphenols fail to precipitate salivary proteins and yet induce an astringency sensation. Recent studies suggest a new mechanism to impact the sensation of astringency, namely the disruption of the salivary film, interaction with the mucosal pellicle and oral epithelial cells, or activation of oral trigeminal mechanoreceptors.

BeTASTY will explore this new hypothesis by including mechanoreceptors in the oral cavity model. These proteins sense external pressure or stretching and elicit an internal signal, which, in this case, will be transmitted to the nervous system. Since, in opposition to the bitter taste, receptors for the astringency sensation are unknown, we will focus on cell membranes to see if an effect on them can somehow impact the mechanoreceptors. We expect to understand further the interactions occurring in the mouth, which will provide more tools for effectively modulating taste.

Creation of cell-free biosensors for the food industry

In the food industry, astringency and bitterness are usually assessed by sensory panels, which can be biased and fatigued, leading to misleading results. Recruiting and training panellists is also time-consuming and costly. Therefore, the industry needs a rapid, reliable and practical method for assessing astringency and bitterness. Biosensor systems using biological components of taste, such as taste buds or receptor cells, are gaining popularity. These systems work by coupling these sensitive elements with transducers that convert signals into optical or electrical outputs. However, accurate and reliable biosensors for astringency are still lacking. Based on the knowledge gained from the above molecular and cell-based approaches, we aim to develop biosensors containing specific biological protein components involved in both astringent and bitter taste perception to assess astringency and bitterness in food matrices independently.

By delving into the molecular basis of unpleasant taste and mouthfeel experiences, BeTASTy will advance knowledge on astringency and bitterness research that will be used to create more palatable and enjoyable products, ultimately improving dietary choices and nutrition on a global scale.

BeTASTy
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PROJECT NAME

BeTASTy

PROJECT SUMMARY

Mouthfeel properties are crucial for human survival, nutrition, health and well-being. Unpleasant taste and mouthfeel properties in plant-based food, such as bitterness and astringency, are a challenge for food industries as flavour is key for consumer's food choices. BeTASTy will contribute to deciphering the physiological and neural mechanisms involved in the interindividual unpleasant taste and mouthfeel perceptions through innovative molecular and cell-based approaches.

PROJECT LEAD PROFILE

Susana Soares is an Assistant Professor at the University of Porto – Faculty of Sciences (FCUP, Portugal) and an Assistant Researcher at the Portuguese Research Centre for Sustainable Chemistry (LAQV/REQUIMTE, Portugal) and the principal investigator of BeTASTy. Prof. Susana and her team are focused on developing integrative molecular and cellular approaches to understand and modulate unpleasant food flavour properties.

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