Mecanisms underlying sensory interactions and complex perceptions

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MECANISMES SOUS-JACENTS AUX INTERACTIONS PERCEPTUELLES ET PERCEPTIONS COMPLEXES

Mechanisms underlying sensory interactions and complex perceptions

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Résumé

Les interactions perceptuelles entre différentes modalités sensorielles affectent la perception des aliments. Si ce phénomène a été largement étudié entre des stimuli olfactifs et gustatifs en solution aqueuse, peu d'études ont été menées avec des produits existants dans le commerce. Similairement, peu de travaux sont dédiés à l'étude des interactions multi-sensorielles impliquant l'olfaction, le goût et la perception tactile en bouche et à l'étude des perceptions dites complexes, c'est-à-dire qui impliquent plusieurs modalités sensorielles. Le premier objectif de ma thèse était l'exploration des mécanismes sous-jacents aux interactions perceptuelles existant entre l'olfaction et la gustation durant la consommation de produits réels, et en solution avec des stimuli olfactifs à une concentration infraliminaire. Cette approche a ensuite été étendue aux perceptions olfactive, gustative et tactile (en bouche) et à la perception complexe "rafraîchissante". Il a été mis en évidence que la familiarité d'un produit et la stratégie d'attention durant l'exposition affectent de façon critique les interactions perceptuelles. Pour la première fois il a été démontré qu'une concentration infraliminaire d'odorant associé au goût sucré (fraise) augmente la perception sucrée d'une solution de sucrose. La multiplicité des interactions sensorielles présente dans un milieu alimentaire complexe a également été mis en évidence à un niveau bimodal (ex: entre la perception amer et le froid) et tri-modal (ex: entre la perception olfactive, sucrée et froide). Finalement il été démontré que la perception rafraîchissante est construite sur la base d'une combinaison de déterminants sensoriels, d'habitudes alimentaires ainsi que de facteurs hédoniques et psychophysiologiques tels que l'énergie mental. Pour conclure, les connaissances acquises par ce travail soulèvent d'autres interrogations notamment à propos des mécanismes neuronaux sous-tendant la mémorisation des associations perceptuelles et des conditions requises en termes de durée et de fréquence d'exposition pour la mise en place de ces interactions.

Mots-clés: Olfaction, Gustation, Tactile, Interactions perceptuelles, Processus cognitifs, Perception rafraîchissante

Summary

Perceptual interactions between different sensory modalities affect overall perception of food. This phenomenon has been extensively investigated between olfactory and tastant stimuli in aqueous solutions. But few studies assessed olfactory and taste interactions in existing products (commercially available). Similarly multi-sensory perceptual interactions involving olfaction, taste and in mouth tactile perceptions and complex perceptions (i.e. a perception which is driven by more than one sensory dimension) have been poorly investigated. The first objective of my thesis was to investigate mechanisms underlying perceptual interactions between bimodal olfactory and taste perceptions in existing products and then in solutions with olfactory stimuli at a subthreshold concentration. This approach was extended to olfactory, taste and in mouth tactile perceptions and finally to "refreshing" complex perception. I showed that product familiarity and attentional strategy applied during exposure are critical factors modulating perceptual interactions. I demonstrated for the first time that subthreshold odorant concentrations related to sweet taste (i.e. strawberry) increase perceived sweetness of a sucrose solution. The multiplicity of perceptual interactions in complex food systems was demonstrated since I identified bimodal (e.g. between bitterness and coldness) and tri-modal (e.g. between mint aroma, sweetness and coldness) perceptual interactions. Finally I showed that refreshing perception is driven by positive and negative sensory drivers, food habits, together with hedonic and psychophysiological factors such as mental energy. To conclude, knowledge acquired during this work raised new questions; in particular related to neural mechanisms underlying memorization of perceptual associations and required conditions in terms of exposure duration and frequency for construction of such interactions.

Keywords: Olfaction, Taste, Tactile, Perceptual interactions, Cognitive processes, Refreshing perception

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Finally I dedicate this thesis to my family.

Content

Synthèse en français

Problématique

Afin de formuler des aliments avec des propriétés sensorielles satisfaisant les consommateurs, il est essentiel de mieux comprendre les facteurs influençant la perception des aliments. Parmi ces facteurs, les interactions perceptuelles sont reportées dans la littérature comme ayant un fort impact sur la perception. Cependant si de nombreuses d'études sont consacrées aux interactions bimodales entre olfaction et gustation en solution aqueuse: 1) aucune étude n'a été menée dans des produits existants dans le commerce avec pour but l'étude de l'impact de la familiarité du produit sur les interactions perceptuelles; et 2) peu de travaux sont dédiés à l'étude des interactions perceptuelles impliquant l'olfaction, la gustation ainsi que la perception tactile en bouche alors que paradoxalement l'ensemble de ces trois modalités sensorielles est systématiquement impliqué pendant la consommation des aliments. Il s'avère également que les perceptions complexes, c'est-à-dire qui ne sont pas expliquées par une seule dimension (par exemple onctueux), et généralement appréciés par les consommateurs, sont de façon surprenante peu étudiées scientifiquement.
Le but de cette thèse est d'apporter de nouvelles connaissances concernant les mécanismes sous-jacents aux interactions perceptuelles entre olfaction et gustation puis entre olfaction, gustation et perception tactile en bouche pour finalement explorer l'origine de la perception rafraîchissante. Les démarches et résultats sont expliqués dans un premier temps et ensuite discuté dans une seconde partie.

Démarche et résultats

Suite aux différentes questions soulevées dans le cadre de la revue de littérature (Partie 1), le sujet d'étude est organisé en deux parties comme présenté ci-dessous.

Partie 2: Interactions perceptuelles multi-sensorielles

Dans un premier temps nous nous sommes consacrés à l'étude des interactions perceptuelles entre l'olfaction et la gustation dans des produits existant dans le commerce et plus spécifiquement à l'étude de l'impact de la familiarité vis-à-vis du produit sur les interactions perceptuelles. Trois boissons amères et non sucrées différentes en termes de familiarité ont été utilisées: une boisson café familière, une boisson cacao reconstituée avec de l'eau (moins familière qu'une boisson café car reconstituée avec de l'eau), et du lait contenant de la caféine. Dans chaque produit a été ajouté un arôme vanille, sans composante gustative, mais perceptuellement associé au goût sucré. Pour chaque produit, les versions sans arôme et avec arôme ont été décrites par profil sensoriel. Nous avons démontré que la nature des interactions sensorielles entre olfaction et goût est modulée par la familiarité du produit. Comme supposé, l'ajout d'un odorant vanille dans la boisson café diminue la perception de l'amertume intrinsèque du café par l'effet suppressif de la sucrosité induite par l'arôme. Cependant quand l'arôme est ajouté dans le lait amer non familier, l'amertume de ce dernier est augmentée.

Ensuite, toujours dans le cadre des interactions entre olfaction et gustation agissant durant la consommation de produits réels, l'impact de la stratégie attentionnelle mise en place lors de la description de l'odeur de huit cafés a été évalué en comparant les résultats: 1) d'un groupe de panélistes expérimentés qui a utilisé la méthode de profil sensoriel, cette méthode induisant une stratégie attentionnelle analytique puisque chaque dimension sensorielle est appréhendée et décrite individuellement; et 2) d'un groupe de consommateurs de café qui a utilisé la méthode du tri avec libre génération de vocabulaire. Cette méthode de tri a été choisie car elle implique une stratégie d'attention synthétique, la perception de l'odeur café étant appréhendée de façon globale afin de pouvoir mener à bien cette épreuve. Cette approche est proche de la stratégie d'attention généralement adoptée spontanément pendant la consommation dans la vie courante. Les résultats ont mis en évidence que la nature de la stratégie attentionnelle mise en place lors de la description aromatique de huit cafés affecte fortement la caractérisation et la disposition relative des produits sur la carte sensorielle. Les consommateurs ont généré des termes appartenant au lexique de la gustation tels qu'amer ou sucré alors que les cafés étaient uniquement flairés.

Nous nous sommes intéressés par la suite à l'impact de stimuli olfactifs présentés à un niveau supraliminaire et infraliminaire sur l'intensité de la perception sucrée avec 1) un odorant communément associé au goût sucré (fraise) et; 2) un odorant non familier mais précédemment co-exposé avec du sucrose pendant cinq séances réparties sur une semaine. Nous avons démontré que l'ajout de l'arôme fraise à un niveau supra et infraliminaire dans une solution de saccharose à 15 g/L augmente la sucrosité. Cependant l'odeur nouvellement associée au goût sucré par co-exposition augmente la perception sucrée de la solution de saccharose uniquement lorsque l'odorant est présenté à un niveau supraliminaire mais n'a pas d'effet lorsque présenté à un niveau infraliminaire.

Finalement nous avons étendu notre champ d'investigation aux perceptions olfactive, gustative et tactile perçues dans un fluide visqueux sucré. La perception tactile a été abordées sous deux aspects: 1) la perception trigéminée (froid en bouche); et 2) la perception proprioceptive (épaisseur en bouche). En suivant un plan factoriel à deux niveaux, une gamme de huit produits aromatisés à la pêche et une gamme de huit produits aromatisés à la menthe ont été formulées. Les deux gammes contenaient en plus de l'odorant, de l'acide citrique et un agent cooling. Les deux espaces produits ont été validés en termes de variété sensorielle par une épreuve de tri avec libre choix de vocabulaire. Nous avons mis en évidence une large variété sensorielle entre les produits estimée suffisante pour l'étude par profil sensoriel des interactions sensorielles présentes dans ce modèle complexe. Les résultats de l'épreuve de tri ont également mis en évidence que le terme rafraîchissant est fréquemment cité pour décrire les groupes mis en place. Il s'avère donc que la richesse sensorielle des produits semble induire une perception complexe rafraîchissante. Les résultats du profil sensoriel ont mis en évidence qu'une grande diversité d'interactions perceptuelles affectent la perception des produits avec des interactions de type: 1) bimodale, par exemple entre la perception trigéminée froide et l'amertume et; 2) tri-modale, par exemple entre la perception olfactive menthe, le goût sucré et la perception trigéminée froide.

Partie 3 Perception complexe: rafraîchissant

Suite aux résultats précédemment décrits, la perception rafraîchissante a été étudiée plus en profondeur dans une dernière partie. Dans un premier temps une revue de littérature a montré que plusieurs déterminants sensoriels sont associés à la perception rafraîchissante et a mis en évidence l'importance de certains déterminants psychophysiologiques dans la construction de cette perception telle que la sensation de désaltération, de bouche hydratée (par opposition à une sensation généralement décrite comme "bouche sèche" ou "pâteuse") et d'augmentation de l'énergie mentale (terme regroupant les notions d'activité corticale, de performance cognitive et d'éveil perçu). De plus l'expérience alimentaire joue un rôle clé dans la mise en place de la perception rafraîchissante.
Cependant afin de connaître la contribution respective des différentes modalités sensorielles dans la perception rafraîchissante, une gamme de produits visqueux proche de celle utilisée précédemment, mais contenant en plus deux niveaux d'épaississant (xanthan) afin d'élargir l'espace sensoriel, a été décrite sensoriellement par un panel expérimenté en utilisant une liste de descripteurs et en termes d'intensité rafraîchissante par un groupe de 160 consommateurs. La technique statistique de cartographie des préférences interne a montré que: 1) plus les produits sont perçus sucrés, moins ils sont perçus rafraichissants par la majorité des 160 consommateurs et; 2) les 160 consommateurs ont été segmentés en trois groupes de taille similaire pour lesquels les déterminants sensoriels de la perception rafraîchissante varient. En effet, une forte intensité froide, une forte intensité acide et une faible intensité épaisse ont été mis en évidence comme étant pour chacun des trois groupes le principal déterminant sensoriel de la perception rafraîchissante.
La perception temporelle, c'est-à-dire au cours du temps, induite par ces mêmes produits après consommation a été évaluée par la technique de Dominance Temporelle des Sensations (DTS). La méthode DTS permet de suivre l'évolution au cours du temps d'un plus grand nombre d'attributs comparé aux méthodes classiques de Temps Intensité limitées à un ou deux termes. Les résultats de 48 panélistes entraînés à cette méthode ont montré que la perception laissée en bouche diffère entre produits jusqu' à trois minutes après la consommation des produits. De plus, en combinant ces résultats avec ceux obtenus lors du précédent test avec les 160 consommateurs, il s'avère que ces perceptions rémanentes pourraient être des facteurs importants sous-jacents à la perception rafraîchissante.
Finalement nous avons comparé l'impact de la consommation de deux produits congelées (contenant principalement de l'eau et du saccharose) et de même valeur énergétique, mais variant en intensité rafraîchissante, sur deux facteurs psychophysiologiques associés à la perception rafraîchissante: la sensation d'hydratation en bouche et l'énergie mentale. Le produit le plus rafraîchissant contenait un agent cooling et de l'acide citrique. La sensation d'hydratation en bouche a été évaluée en mesurant le flux salivaire et les propriétés lubrifiantes de la salive au moyen d'un tribomètre, un appareil reproduisant artificiellement les mouvements de friction entre la langue et le palais. L'énergie mentale a été mesurée lors d'une seconde étude en termes d'activité corticale par électroencéphalogramme, de performance cognitive durant une tache attentionnelle et de la sensation d'éveil par la notation d'une liste d'attributs relatifs à l'état d'humeur ressenti. Ces différents paramètres ont été mesurés avant et après consommation des produits. Suite à la consommation du produit le plus rafraîchissant, le flux salivaire est plus important et la

salive produite est plus lubrifiante, c'est à dire qu'elle induit une plus faible force de friction entre la langue et le palais. De même les mesures relatives à l'énergie mentale ont montré que le produit le plus rafraîchissant augmente l'activité corticale, principalement dans les fréquences alpha et beta, ainsi que les performances durant la tâche de vigilance. Cependant les deux produits n'ont pas significativement modifié l'éveil perçu par les sujets.

Discussion générale et perspectives

Les résultats de la partie consacrée à l'étude des interactions multi sensorielles ont montré que les interactions perceptuelles diffèrent entre des solutions modèles et des produits réels, puisque la familiarité du produit module ces interactions; et entre des approches attentionnelles analytique et synthétique.

L'augmentation de la perception sucrée et la diminution de l'amertume des boissons café et cacaotée causées par l'ajout d'odorant vanille étaient attendues. En effet l'association perceptuelle existant entre l'odeur vanille et le goût sucré est à l'origine de l'augmentation de la sucrosité et par conséquent de la diminution de l'amertume par un phénomène d'interaction suppressive entre les goûts sucré (induit par l'odeur) et amer. Cependant l'augmentation de l'amertume du produit non familier (lait cafeiné) par l'ajout de ce même odorant vanille reste inexpliquée. Ce produit a probablement été perçu de façon déplaisante par le biais de phénomènes de néophobie alimentaire et de rejet du goût amer largement décrits dans la littérature. L'ajout de vanille a certainement renforcé l'aspect non familier et donc déplaisant du produit. Le caractère déplaisant du produit a pu être reporté sur l'échelle dédiée à l'attribut amer, cette dimension sensorielle ayant une forte connotation hédonique négative (contrairement à la dimension sucrée généralement associée au plaisir). Pour résumer l'augmentation de l'amertume pourrait être la conséquence d'interactions entre dimensions sensorielle et hédonique.

Ces résultats ouvrent des perspectives d'études futures de neuro-imagerie en utilisant la technique d'imagerie par résonance magnétique fonctionnelle (IRMf) pendant la consommation de ces mêmes produits. Le but est de pouvoir expliquer d'un point de vue neuronal les différences de perception gustative observées après la consommation de produits familiers et non familiers aromatisés à la vanille, c'est-à-dire l'augmentation de la sucrosité et la diminution de l'amertume induites par le produit familier et l'augmentation de l'amertume induite par le produit non-familier. Deux hypothèses sont proposées:

- 1) une augmentation de l'activité de l'aire gustative primaire associée au goût sucré et au goût amer lors de la consommation de produit familier et non familier, respectivement qui signifierait que les modulations d'intensité gustatives perçues sont uniquement la conséquence d'une différence de traitement des stimuli sensoriels à un niveau périphérique (récepteurs sensoriels) et/ou à un niveau cortical primaire;
- 2) des différences induites par les deux produits au niveau de l'activation des aires intégratives (par exemple le cortex orbitofrontal) où convergent et sont intégrées les informations gustatives et olfactives ce qui validerait que des mécanismes cognitifs tels que le plaisir localisés dans ces même aires cérébrales modulent les processus d'intégration et consécutivement la perception gustative.

La seconde hypothèse semble la plus plausible et validerait l'explication proposée pour expliquer l'augmentation d'amertume causée par le produit non familier mis en évidence par les résultats psychophysiques, c'est à dire une interaction entre les dimensions sensorielle (amertume) et hédonique (déplaisant)

Les différentes stratégies d'attention mise en place par un panel sensoriel et par des consommateurs (c'est à dire dans la vie courante) modifie les interactions perceptuelles aussi bien lors de la description de produits familiers (l'arôme de café) que lors de la co-exposition à un nouvel odorant et à du saccharose. En effet considérer un produit de façon analytique: 1) pendant l'entraînement, c'est à dire en apprenant à disséquer ses caractéristiques individuellement; et 2) pendant le profil sensoriel où chaque attribut est évalué indépendamment, réduit l'effet des interactions sensorielles qui au contraire est augmenté lors d'une approche synthétique (ou holistique). Cependant la première étude consacrée au rôle de la familiarité a tout de même mis en évidence des interactions perceptuelles alors que l'approche utilisée était analytique (profil sensoriel). La persistance d'interaction peut être expliquée par l'entraînement qui n'a pas été mené spécifiquement sur les produits évalués par la suite mais sur des références externes. Par conséquence, les panélistes n'ont pas appris à décomposer les dimensions sensorielles des produits évalués ce pourrait expliquer que des interactions sensorielles ont pu quand même être observées avec une méthode analytique.

Afin de pouvoir évaluer l'efficacité de cette méthode analytique à mettre en évidence des interactions perceptuelles, il serait intéressant lors de futures études de combiner la notation standard d'attributs à l'aide d'échelle (profil sensoriel) avec d'autres méthodes qui ne nécessitent pas l'utilisation d'échelle comme par exemple l'épreuve de catégorisation. En effet le principe de cette méthode est de catégoriser un groupe de produits sur une dimension perceptuelle par exemple l'intensité du goût sucré, les catégories proposés par l'expérimentateur pouvant être à deux niveaux "faible intensité sucrée" et "forte intensité sucré", ou à trois niveaux incluant en plus des deux catégories précédentes la catégorie "moyennement sucré", ou plus. Ainsi en catégorisant un groupe de produits formulés avec plusieurs concentrations de sucre et d'odorant (par exemple vanille), il est possible de déterminer si l'odorant influence ou non la catégorisation des produits sur la perception sucrée et par conséquent si des interactions perceptuelles existent.

Nos travaux dédiés à l'impact d'un stimulus olfactif présenté à un niveau infraliminaire sur la perception sucrée, nous ont permis d'observer: 1) qu'un odorant communément présent dans des aliments sucrés augmente la sucrosité d'une solution de saccharose quand l'odorant est présenté au dessus du seuil de perception (supraliminaire) mais aussi quand l'odorant est présenté en dessous du seuil de détection (infraliminaire); et 2) qu'un odorant non-familier mais co-exposé de façon répétée pendant une semaine avec du saccharose augmente également par la suite l'intensité de la perception sucrée d'une solution de saccharose quand il est présenté à un niveau supraliminaire mais n'a pas d'effet quand il est présenté à un niveau infraliminaire. La littérature rapporte que les associations construites au cours de la vie entre différentes dimensions sensorielles nécessitent un apprentissage implicite et ensuite une consolidation impliquant la mémoire à long terme (de plusieurs jours à plusieurs mois) et à très long-terme (de plusieurs mois à la vie). A un niveau cérébral, des neurones à l'origine uni modaux et ne pouvant donc répondre qu'à une seule dimension sensorielle (par exemple olfactive) pourrait avoir évolués chez l'homme en neurones bimodaux suite aux stimulations répétées durant la vie à des même paires de stimuli olfactif et gustatif (par exemple arome fraise et goût sucré). Ces neurones bimodaux pourraient donc répondre à des stimuli olfactifs et gustatifs. Cette hypothèse semble être assez largement partagée comme étant le corrélat neuronal sous-tendant les interactions perceptuelles d'autant que des neurones bimodaux et multimodaux ont été identifiés chez le singe dans le cortex orbitofrontal. Dans notre étude, l'augmentation de la sucrosité est induite par un stimulus olfactif congruent avec le goût sucré présenté à un niveau supraliminaire mais aussi à un niveau infraliminaire. L'activation des récepteurs olfactifs, même à une concentration qui n'induit pas de perception consciente, pourrait tout de même conduire à une activation de neurones bimodaux olfactif-gustatif, en admettant que de tels neurones existent chez l'homme. Cependant dans le cas d'un nouvel odorant brièvement co-exposé avec du saccharose (durant une semaine), le processus d'apprentissage et de consolidation au niveau de la mémoire à court terme semble être suffisant pour induire des interactions perceptuelles quand présenté à un niveau supraliminaire mais insuffisant quand présenté à un niveau infraliminaire.

D'autres études psychophysiques sont nécessaires pour répondre à de nombreuses questions au sujet des processus de mémorisation et d'intégration impliqués dans la construction de nouvelles associations perceptuelles telles que: 1) leur robustesse au cours du temps (mois ou années), c'est-à-dire les nouvelles associations sont-elle ancrées dans la mémoire à long terme?; 2) la période optimale de co-exposition (durée de la phase de co-exposition, fréquence et durée des séances pendant cette phase) permettant de mémoriser de manière robuste la nouvelle association de façon à induire à cours terme (immédiatement après la co-exposition) et long terme (plus d'une semaine après la co-exposition) une augmentation de la sucrosité par ajout de l'odorant à un niveau infraliminaire? La mesure de l'activité cérébrale par Imagerie par Résonance Magnétique fonctionnelle (IRMf) en réponse à une exposition à un nouvel arôme en combinaison avec du saccharose avant, durant et de façon répété au cours du temps après l'apprentissage par co-exposition pourrait apporter des informations pertinentes sur la plasticité des aires cérébrales impliquées dans les processus d'intégration sensorielles.

L'étude des interactions perceptuelles entre olfaction, gustation et perception tactile a mis en évidence de nombreuses interactions soit entre deux modalités sensorielles, par exemple entre olfaction et perceptions sucré, amère et acide, soit entre trois modalités sensorielles notament entre olfaction, amertume et perception froide. Ces résultats démontrent que l'ajout d'un odorant dans un milieu sensoriellement complexe a un impact large et difficilement maîtrisable sur la perception globale.

Notre investigation des déterminants sensoriels de la perception rafraîchissante a permis d'identifier que la perception sucrée est pour la majorité des consommateurs de notre étude, négativement corrélée à la perception rafraîchissante. Ensuite trois caractéristiques sensorielles, une forte intensité froide, une forte acidité et une faible épaisseur, ont été identifiées comme positivement associées à la perception rafraichissante mais avec une importance variant entre consommateurs. Nous

pensons que ces différences entre consommateurs sont explicables dues à des différences d'habitudes alimentaires. En effet les consommateurs pour lesquels l'intensité froide est le principal déterminant sensoriel de la perception rafraîchissante sont consommateurs de chewing-gums à la menthe qu'ils consomment pour se rafraîchir l'haleine.
Il est connu que les composé acides stimulent le flux salivaire et induisent par conséquent une sensation de bouche hydratée (à opposer à une sensation de bouche sèche ou pâteuse) et que les agents cooling peuvent améliorer la capacité d'attention et stimuler l'éveil ressenti. Les résultats montrant que le produit surgelé perçu rafraîchissant grâce à l'ajout d'acide citrique et d'agent cooling sont donc en adéquations avec ces connaissances et démontrent pour la première fois qu'une augmentation de l'activité cérébrale impliquée dans les processus attentionnels (fréquence alpha) et d'intégration sensorimotrice (fréquence beta) est probablement à l'origine des meilleures performances obtenues lors de la tâche attentionnelle. D'autres investigations restent cependant nécessaires afin de valider si, pour être perçu rafraîchissant, un produit doit obligatoirement avoir un impact psychophysiologique.

Conclusion

Nos études nous ont permis d'étendre le champ des connaissances relatives aux mécanismes psychophysiques sous-jacents aux interactions perceptuelles en nous plaçant dans des conditions expérimentales proches de la réalité. Nos résultats nous ont permis d'émettre des hypothèses concernant les mécanismes de mémorisation et neuronaux sous-tendant nos interprétations. De plus pour la première fois nous avons appréhendé d'un point de vue sensoriel et psychophysiologique les déterminants d'une perception complexe. Ces nouvelles informations s'avèrent importantes dans le cadre du développement de nouveaux produits répondant mieux aux attentes du consommateur.

Introduction

Food acceptance is greatly affected by sensory properties perceived by olfactory, taste and tactile senses during consumption.

Olfactory perception results from the stimulation of the olfactory system by volatile molecules. Air-borne molecules enter the nasal cavity either from outside by sniffing through the nose which leads to orthonasal olfactory perception or during eating or drinking via the oropharyngeal cavity at the back of the mouth and throat inducing retronasal olfactory perception. Olfactory perceptions following orthonasal and retronasal stimulations are called "odour" and "aroma", respectively, according to the international standards (ISO 5492, 1995). But "flavour" instead of "aroma" is also currently used for describing the retronasal olfactory perception.

Regarding gustatory perception, the scientific community now agrees that at least five "basic" tastes exist: sweet, salty, sour, bitter and umami. These are perceived through taste receptor cells found in tongue taste buds immersed in the epithelium of taste papillae.

Tactile perception is classified as a part of the somatosensory system, which is concerned with four major modalities: 1) discriminative touch related to detection of pressure or vibration of objects in contact with the surface of the body, which allows sensing size, shape and microstructure; 2) thermosensation related to temperature detection; 3) proprioception related to detection of static position and movement of the jaws, tongue, hands, fingers, etc; and 4) nociception (pain) related to detection of tissue damage or events that could directly damage tissues and are perceived as painful.

Trigeminal perception corresponds to both thermosensation and nociception modalities and is caused 1) by chemical compounds leading for instance to hotness and irritation (induced by chilli pepper, capsaicin, black pepper piperine, etc), fizziness (soft drink carbonation) or coldness (menthol); and 2) by temperature.

To study the impact of ingredients and food processes on food perception, specific methodologies were developed in the field of sensory evaluation to qualify and quantify sensory dimensions perceived during consumption. Surprisingly, during sensory evaluation of diverse foods, correlations were highlighted between subjects' replies to stimulation of different sensory systems. For example, it was found that the sweetness rating of a fruity flavoured solution is positively correlated to the rating of the aroma intensity (Frank and Byram, 1988). This phenomenon was named sensory interaction and was historically found in bimodal food systems involving olfactory and taste stimuli. Later it was demonstrated that other perceptions such as touch can be similarly affected by sensory interactions. Multi sensory interactions were then highlighted in complex food system involving odorant, tastant and tactile stimuli. Because of their complexity, food systems may also induce characteristics resulting from simultaneous multi-sensory stimulations and modulate by cognitive factors such as familiarity and liking. Creaminess and freshness are two examples of complex perception widely used for a long time by food marketing since perceived as a product benefit by consumers but only recently scientifically investigated.

Sensory interactions can result in a modulation of the stimuli amount reaching sensory receptor mainly because of: 1) chemical interactions between sensory stimuli; and/or 2) physical or chemical interactions between a sensory stimulus and the food matrix. This type of interaction is named physicochemical interaction. Sensory interactions can also be the consequence of associations between different sensory modalities which are constructed during every day food experience. This type of interaction is named perceptual interaction.

To formulate food with sensory properties satisfying consumers, it is essential to acquire a better understanding of factors influencing food perception and more specifically of mechanisms underlying perceptual interactions in simple but also complex food as well as the mechanisms involved in complex perception, i.e. perception not easily described by simple attributes.

The scientific approach of this PhD is as follows:

PART 1: Literature review focussing on interactions between olfaction, taste, tactile perceptions and complex perceptions involved during food consumption with the following objectives:
- Detail the studies that provide substantial advanced learning in the field rather than proposing an exhaustive list of published works.
- Define work objectives accordingly.

PART 2: Exploration of bimodal perceptual interactions between olfaction and taste and then widening of the approach to multi-modal interactions involving tactile perception with a focus on:
- The role of familiarity and attentional strategy during exposure on the sensory characterization of commercial beverages with olfactory and taste stimulus at suprathreshold level;
- The impact of subthreshold olfactory stimuli on taste perception;
- The impact of olfaction on taste, trigeminal and texture perception.

PART 3: Extend the exploration of mechanisms underlying perceptual interactions to the investigation of refreshing complex perception in terms of:
- Sensory drivers
- Associated psychophysiological factors

PART 4: General discussion and perspectives

PART 1: Literature review

In the following review, results of the key studies in the field of sensory interactions are first presented (§1.1) by separating interactions observed between olfactory and taste stimuli (§1.1.1) and then involving tactile stimuli (§ 1.1.2). Second I focus on mechanisms underlying sensory interactions (§ 1.2). Finally the concept of complex perception is introduced (§ 1.3).

1.1 Examples of observed sensory interactions

1.1.1 Sensory interactions between olfaction and taste

1.1.1.1 Olfactory stimuli at a suprathreshold level

Pioneering studies reported that people describe a retronasal olfactory perception as a taste. More recently, in the 1970's, Murphy et al. (1977) showed that subjects attribute a sweet taste to ethyl butyrate, a volatile compound smelling of strawberry. This effect disappears when the retronasal olfaction is blocked by closing the nostrils.
The influence of strawberry odorant on sweetness was subsequently confirmed in a sucrose solution (Frank *et al.*, 1989) and in whipped cream (Frank and Byram, 1988). But in the latter study, no impact of peanut butter odorant on sweetness ratings of the whipped cream is showed. Peach odorant is also highlighted as a sweetness enhancer (Cliff and Noble, 1990). An increase in concentration of peach odorant leads to an increase in perceived maximum intensity and total duration of sweetness. Other odorants related to fruit were also found to enhance sweetness of sucrose solutions, for example pineapple and raspberry (Prescott, 1999a), maracuja and caramel (Stevenson *et al.*, 1999). In the same latter study, Stevenson et al (1999) showed that non-food-related odorants such as damascone or eucalyptol do not impact sweetness. They asked subjects to score the "smelled sweetness" (which corresponded to the sweetness evoked by the odour) and the in-mouth sweetness of several solutions flavoured with food and non-food related odorants. Critically, they observed that "smelled sweetness" panel rating is a good predictor of the odorant ability to change perceived in-mouth sweetness.
Regarding other tastes, a study by Djordjevic and colleagues (2004b) highlighted that soy sauce odour can increase perceived saltiness, but strawberry odour does not. Conversely, the same authors showed that strawberry odour appears to enhance sweetness but soy odour does not. A study demonstrated that bitterness in olive oil can be enhance by cis-3-hexenol, a volatile generally described as having a cut grass note (Caporale *et al.*, 2004). Finally, odours can also reduce perceived taste intensity. Specifically, a caramel odour produces a decrease in sour taste intensity (Stevenson *et al.*, 1999).
Many studies have highlighted that an odorant can modulate taste perception, but relatively few reported that a tastant can modulate aroma perception. A time-intensity study conducted in orange juices showed that an increase in sweetener concentration or citric acid concentration induces an enhancement of in-mouth fruitiness intensity (Bonnans and Noble, 1993). Similarly, an increase in sucrose concentration of a banana odorant solution enhances fruity aroma intensity (Hort and Hollowood, 2004).

1.1.1.2 Olfactory stimuli at a subthreshold level

Two studies focused on olfactory-taste interactions, when both odorant and tastant are presented at a subthreshold level (Dalton *et al.*, 2000; Pfeiffer *et al.*, 2005). They explored the impact of in mouth saccharine solution (sweet tastant) at a subthreshold concentration on the benzaldehyde threshold determination (a volatile compound generally described as having an almond note). Benzaldehyde was delivered orthonasally using an olfactometer in two conditions: with and without having saccharine in mouth. The olfactory threshold of benzaldehyde significantly decreases with the presence of the saccharin solution in mouth. In a second experiment, saccharin was replaced by monosodium glutamate (umami taste) at a subthreshold concentration. In that case, the olfactory threshold of benzaldehyde remains unchanged. More recently, a study showed that subthreshold concentrations of acetic acid can increase the perceived retronasal olfactory intensity of three volatile coffee aroma compounds presented at suprathreshold level. Such olfactory intensity enhancement was not observed with subthreshold concentrations of butyric acid (Miyazawa *et al.*, 2008).
In addition, Delwich and Heffelfinger (2005) showed that a sweetener, monosodium glutamate (MSG) and a pineapple odorant, which are not perceived individually at subthreshold level in water, are perceived in mixtures combining either: 1) the sweetener and the odorant; or 2) MSG and the odorant.

To summarize, many studies show that an odorant can enhance taste perception and that a tastant can modulate the retronasal olfactory intensity of an odorant. Such phenomena are observed with stimulus at suprathreshold level. In subthreshold conditions, sensory interactions were also highlighted

1.1.2 Sensory interactions involving tactile perception

Since in my PhD, I focus on cold trigeminal perception (thermosensation/nociception) and in mouth thickness (proprioception), other dimensions related to tactile perceptions are not detailed.

Regarding interactions involving thickness, several studies have been conducted in dairy products. It was reported that the increase of a low-fat stirred yoghurt thickness using a thickening agent decreases the intensity of perceived green-apple olfactory perception and reduces perceived sweetness (Paçi-Kora *et al.*, 2003). In the same matrix, results of two other studies showed that: 1) the addition of odorant related to fatty attributes (coconut, butter) enhances the yoghurt's perceived thickness (Saint-Eve *et al.*, 2004); and 2) an increase of yoghurt physical viscosity (using thickening agents) leads to a sweetness decrease (Paçi-Kora *et al.*, 2003). In a dairy dessert, it was found that, depending on the nature of the texture agent, the intensity of the fruity aroma changes (Lethuaut *et al.*, 2005). The intensity of the aroma is greater in soft dairy desserts composed of lambda-carrageenan than in dessert composed of iota or kappa carrageenan types. In another study, Lethuaut et al. (2003) showed that desserts with lambda-carrageenan are perceived as sweeter than desserts with iota-carrageenan a for similar sucrose content.

Studies related to sensory interactions involving the trigeminal perceptions were mainly carried out on pungency/hot perception induced by chemical compounds such as capsaicin. In a key observation, it was shown that pungency masks olfactory and taste perceptions (Prescott, 1999b; Reinbach *et al.*, 2007). Very few studies have explored interactions involving cold trigeminal perception. Recently it was demonstrated that an increase in cooling intensity using cooling agent enhances the melon olfactory intensity of a mixture containing a green colouring whereas cooling intensity increase does not change the pineapple odour intensity of a mixture containing a purple colouring (Petit *et al.*, 2007).

To conclude about observed sensory interactions, a large number of studies reports bimodal sensory interactions (mainly between olfactory and taste perception) in model solutions. Although olfactory, taste and tactile dimensions are involved during food consumption, few researchers investigated multi-sensory interactions

☞ ***In this context, the first part of my PhD was organized as detailed below:***
- First, a study of interactions between olfactory and taste modalities in existing products and further exploration of the odorant impact at subthreshold level on sweetness of sucrose solution;
- Second, widening of this approach to multi-sensory interactions in more complex products involving olfactory, taste and tactile perceptions. Tactile perception was apprehended in terms of trigeminal perception (coldness) and proprioception (thickness).

1.2 Mechanisms underlying sensory interactions

Observed sensory interactions can be explained by physicochemical or perceptual mechanisms. Physicochemical mechanisms are briefly reviewed (PART 1.2.1) and then perceptual mechanisms reported in the literature are reviewed in more detail (1.2.2).

1.2.1 Sensory interactions induced by physicochemical interactions

Chemical or physical interactions between 1) sensory stimuli (e.g. odorants, tastants), and/or 2) sensory stimuli and the food matrix ingredients (e.g. lipids, carbohydrates) lead to a modification of stimuli released from food and reaching receptors during consumption. Consequently, the perception related to the stimulus can be modified. Firstly the different physicochemical mechanisms occurring in food are presented (PART 1.2.1.1 and secondly the potential consequences on perception are discussed (PART 1.2.1.2).

1.2.1.1 Physicochemical mechanisms and their impact on release of sensory stimuli

The scientific community generally agrees that chemical bonds are mainly involved in physicochemical interactions between volatiles and components of the food matrix. Chemical bonds are classified into two categories: 1) high-energy bonds (covalent and ionic); and 2) weak-energy bonds (hydrogen bonds and Van Der Waals forces). Chemical bonds between aroma compounds and food ingredients reduce the volatile concentration in headspace because of a decrease in the air/food partition coefficient (ratio of the concentration of the aroma compound in the air phase to its concentration in the food). Volatile release is commonly measured in vitro by static-headspace analysis coupled with a gas chromatograph and mass spectrometer (GC-MS) for quantifying and identifying the volatiles. For example pectin addition in apple juice decreases isopropylalcohol release (Walker and Prescott, 2000). Isoamyl acetate release from dairy dessert is reduced by starch addition (Cayot *et al.*, 1998), one of the factors influencing volatile release being interactions with amylose. In addition a complementary study showed that release of isoamyl acetate, ethyl hexanoate, and linalool depend not only on amylose content but on amylose-amylopectin content ratio (Arvisenet *et al.*, 2002). The same type of chemical reactions can also account for a reduction in volatile compound release when a texturing agent such as xanthan is added to a model system (Bylaite *et al.*, 2005).

Physicochemical interactions between odorants and tastants have also been reported. For example, a study conducted in orange juice showed that increasing sucrose from 0 to 60 W/V% modulates release of 15 orange juice volatiles according to their hydrophobicity constant. Indeed release of volatiles with lower hydrophobicity constant (e.g. ethyl acetate) is increased whereas release of volatiles with higher hydrophobicity constant (e.g. octanal) is decreased (Nahon *et al.*, 1998). Similarly, in sweetened strawberry-flavoured yogurt, release of volatiles (ethyl butanoate, ethyl 3-methylbutanoate and (Z)-hex-3-enol) is significantly and differently impacted by the nature and the concentration of the sweet tastants (Mei *et al.*, 2004).

Modification of food rheological properties is another key factor affecting odorant and tastant release. A significant number of studies conducted in hydrocolloids gels or in aqueous solutions investigated the impact of rheological properties, mainly viscosity, on stimuli release by adding thickeners. According to Baines and Morris (1987) the modification of odorant and tastant release occurs at concentrations above the coil overlap concentration (C*) for a range of hydrocolloids. The main hypothesis is that an increase in viscosity reduces the odorant and tastant transfer from the matrix to the receptor, which is caused by a decrease in compound mobility. Consequently, the increase of viscosity in hydrocolloid gel and solutions is generally reported as decreasing odorant and tastant release (Baines and Morris, 1987; Hollowood *et al.*, 2002). However, more recent studies have shown that aroma release is not systematically related to C* concentration (Hollowood *et al.*, 2002; Cook *et al.*, 2003; Bylaite *et al.*, 2005).

The reduction of the water mobility due to the viscosity enhancement may also explain the decrease in perceptual intensity. This has been shown for a sweet tastant (Mathlouthi, 1984; Mathlouthi and Seuvre, 2008). The authors assumed that water mobility facilitates tastant transportation and detection.

Finally in-mouth food manipulation can also impact the release of volatile compounds. Blisset et al. (2006) showed that during mastication of a lemon-flavoured confectionery chew, differences between subjects in terms of chewing behaviour parameters such as chewing rate and force influenced volatile release and consequently aroma perception.

1.2.1.2 Impact of physicochemical interactions in food on perception

Some in vitro instrumental analyses conducted in parallel with sensory measurement showed that physicochemical interactions could be linked to differences in perception

(Walker and Prescott, 2000; Guinard and Marty, 1995; Relkin *et al.*, 2004). But other studies also showed differences in perception not related to differences in physicochemical measurements. For example, in model dairy desserts, changes in physical textural characteristics and in sucrose concentration modulated olfactory retronasal perception but not volatile release measured in vitro by static headspace gas chromatography (Lethuaut *et al.*, 2005). Therefore, the authors concluded that such perceptual change is not caused by physicochemical interactions.

More recently *in vivo* instrumental measurement was used to better understand the kinetics of stimuli release (mainly odorants) in *in vivo* conditions. Odorant concentration was monitored in exhaled breath by on-line instrumental measurement such as atmospheric pressure chemical ionisation mass spectrometry (APCI-MS), (Hewson *et al.*, 2008) or proton transfer reaction-mass spectrometry (PTR-MS), (Boland *et al.*, 2006; Lindinger *et al.*, 2008). But the limitation of these studies is that physicochemical measurements were conducted independently from sensory evaluation.

Sensory evaluation coupled with on-line in vivo instrumental measurement such as APCI-MS (Davidson *et al.*, 1999; Weel *et al.*, 2002; Lethuaut *et al.*, 2004; Pfeiffer *et al.*, 2006; Saint-Eve *et al.*, 2006); or PTR-MS (Buettner *et al.*, 2008) were carried out to more efficiently identify the respective contribution of physicochemical and sensory interactions to perception. Studies highlighted that: 1) physicochemical interactions have no impact on perception (Weel *et al.*, 2002); 2) physicochemical interactions explain some of the observed changes in perception (Saint-Eve *et al.*, 2004; Saint-Eve *et al.*, 2006); and 3) some of the reported perceptual modifications are not explained by physicochemical interactions and are considered as perceptual (Davidson *et al.*, 1999; Lethuaut *et al.*, 2004; Saint-Eve *et al.*, 2004; Saint-Eve *et al.*, 2006).

To summarize, some of the sensory interactions reported in the literature can be explained by physicochemical interactions but this is not systematic. It may be because physicochemical interactions cannot be identified for technical reasons (e.g. instrumental measurement is not sufficiently sensitive) or because the origin of sensory interaction is perceptual.

1.2.2 Role of perceptual mechanisms on sensory interactions

Cognitive factors are reported in the literature as being involved during the construction of perceptual interactions: food experience, attentional strategy during exposure, spatial and temporal co-occurrence during olfactory and taste perception and liking. The role of such factors in perceptual interaction is detailed below

1.2.2.1 Role of food experience

- Stimuli at suprathreshold level

The scientific community agrees that food experience plays an essential role in the construction of perceptual interactions (Prescott and Stevenson, 1995). The notion of congruency between sensory qualities is essential to our understanding of the perceptual interaction between senses. Congruency is the extent to which two stimuli are appropriate for combination in a food product (Schifferstein and Verlegh, 1996). Indeed many studies reported that olfaction can lead to an enhancement of taste intensity only when olfactory and taste stimuli are congruent, (Frank and Byram, 1988; Prescott, 1999a; Hort and Hollowood, 2004; Djordjevic *et al.*, 2004b).

Conversely, an odour can also decrease taste intensity when related to another taste, i.e. the sourness suppression by caramel odorant (Stevenson *et al.*, 1999) can probably be explained by sweetness evoked by caramel odorant since caramel aroma and sweetness are commonly associated in food. In addition, the symmetrically suppressive interactions observed between tastants, for instance sweet tastant and acid tastants (Keast and Breslin, 2003), could occur between a tastant and a taste induced by an odorant. Even though congruency between two stimuli is a mandatory condition to observe perceptual interactions, it has been shown that the level of congruency between sweetness and an odorant (ham, lemon and strawberry) can not predict the degree of sweetness enhancement by the odorant (Schifferstein and Verlegh, 1996).

Perceptual interactions between olfaction and taste can result from an association that is formed through food exposure, without any explicit attention or learning (Koster *et al.*, 2004; Koster, 2005a). This was demonstrated through repeated exposure to an unfamiliar odorant in solution (Petit *et al.*, 2007) with a tastant. This induced olfactory-taste association has been described as ‘learned synesthesia’ (Stevenson and Boakes, 1998). This conclusion is supported by another study (Prescott *et al.*, 2004). The authors showed that a single co-exposure of a sweet tastant and an unfamiliar odorant (prune) is sufficient to enhance the "smelled sweetness" of the odorant (prune). This result has been confirmed by a study showing that pairing a new odorant with sucrose leads to an enhancement of sweetness evoked by odorant sniffing (Yeomans *et al.*, 2006). In addition, saltiness rating decreases after odorant-sucrose pairing procedure.

Regarding a more complex system involving olfactory, trigeminal, and visual stimuli, a repeated exposure to an incongruent mixture combining pineapple odorant, cooling agent, and purple colouring, promotes perceptual interactions between olfaction and trigeminal perceptions whereas before the co-exposure such interactions do not exist (Petit *et al.*, 2007).

To summarize, food experience modulates perceptual interactions according to studies performed with sensory stimuli in model solutions. But the role of experience built through repeated exposure to every day commercial food on perceptual interactions remains unclear. Indeed, to our knowledge, the impact of the familiarity induced by a commercial product, which is built over time by repeated purchase and consumption, on perceptual interactions has never been investigated. This is critical since findings from experiments carried out with model solutions could differ to those obtained in familiar products.

☞ ***In this context the role of familiarity of drinks on olfactory and taste interactions was explored during my PhD in common and less common bitter drinks (coffee drink, cocoa drink and caffeinated milk).***

- Stimuli at subthreshold level

Consistently with findings obtained when stimuli are at suprathreshold level, perceptual interactions and the role of congruency have been evidenced with subthreshold stimuli. Indeed a subthreshold concentration of olfactory stimuli can reduce the detection threshold of a congruent taste stimuli (Dalton *et al.*, 2000; Pfeiffer *et al.*, 2005). This means that a mixture of subthreshold olfactory and taste congruent stimuli that are not perceived individually can lead to a perceptible stimulus. One study produced contradictory findings (Delwiche and Heffelfinger, 2005). The authors explored the impact on perception of subthreshold concentrations of pineapple odorant in mixture either with subthreshold concentrations of sweet tastant (congruent with pineapple aroma) or with subthreshold concentrations of umami tastant (incongruent with pineapple aroma). They showed that during discrimination tasks both mixtures (pineapple odorant-sweet tastant and pineapple odorant-umami tastant) are significantly perceived as different from water whereas individually, each of the three compounds are not significantly perceived as different from water. The authors argued that an additivity effect of subthreshold odorant and tastant concentrations reaching the threshold when mixed together and consequently congruency does not seem to play a role here.

To conclude, congruency between odorant and tastant stimuli seems to play a role, even if not systematic, in perceptual interactions produced by subthreshold concentrations of either one or both stimuli. Sensory measurements used in studies dealing with subthreshold odorant and sweet tastant stimuli are discriminative tests: threshold detection (Dalton *et al.*, 2000; Pfeiffer *et al.*, 2005) and triangular tests (Delwiche and Heffelfinger, 2005). These latter tests do not allow to describe the nature of the perception induced by the combination of olfactory and taste subthreshold stimuli. Only one study showed by descriptive tests that subthreshold carboxylic acids can increase olfactory intensity of suprathreshold concentrations of coffee volatiles (Miyazawa *et al.*, 2008).

Today it is not possible to confirm that perceptual interaction with stimuli at subthreshold concentrations can lead to an enhancement of sweet taste perception as observed in the literature for perceptual interactions with stimuli at suprathreshold concentrations. Such a confirmation would allow use of odorants related to sweetness at subthreshold level to reduce sucrose content, while keeping the same sweetness without modifying the product's olfactory characteristics.

☞ ***In this context, the impact of subthreshold concentrations of an odorant congruent with sweet taste on sweetness rating was explored in a sucrose solution. Firstly five odorants were evaluated at suprathreshold level in a sucrose solution. The odorant having the highest and the lowest sweetness enhancement properties were selected. Secondly we explored at subthreshold level the impact of both odorants on sweetness.***

1.2.2.2 Role of attentional strategy during exposure

The observed modulation of the intensity of a sensory quality by another independent one can be caused by experimental conditions which influence assessor attentional strategy during attribute rating. This has been suggested and named the "dumping effect" by Clark

& Lawless (1994) when applied to olfactory and taste perceptions. The authors showed that the dumping effect can lead to a transfer of the perceived olfactory intensity onto the taste scale when no olfactory intensity scale is available. This theory is supported by findings showing that taste ratings can be modulated by both odour perception and by attributes proposed by the experimenter (Frank *et al.*, 1993; Frank, 2002). For example, the sweetness of a sucrose solution is increased by a strawberry odorant. However, this effect disappears when a fruity aroma scale is made available in addition to a sweetness scale (van der Klaauw and Frank, 1996). But other paradigms different from attribute ratings highlighted perceptual interactions between sweetness and congruent odorants (Nguyen *et al.*, 2000; Djordjevic *et al.*, 2004a; White and Prescott, 2007). Consequently, increase of sweetness rating by odorant is not solely due to a response bias but involves perceptual interactions (Valentin *et al.*, 2006).

The attentional strategy applied during exposure to a new food can impact the construction of perceptual interactions. That was demonstrated by Prescott et al. (2004) who compared the impact of two different attentional strategies on construction of olfactory-taste interaction during co-exposure to a sucrose solution flavoured with an unfamiliar prune odorant. Two groups of assessors were co-exposed by triangle tests but the instructions varied between groups. The first group was asked to pick the sample with the strongest overall flavour. The second group was asked to pick the sweetest sample or the most intense in aroma (retronasal olfactory perception). The instructions received by the first group encouraged a synthetical attentional strategy, i.e. the subjects evaluated olfactory and taste stimuli as a whole, thereby promoting olfactory-taste interactions. In contrast, the instructions received by the second group led subjects to adopt an analytical attentional strategy, i.e. subjects considered olfactory and taste stimuli independently, which limited olfactory-taste interactions. As hypothesized, results of a post-exposure sweetness evaluation showed that the first group rated the flavoured sucrose solution sweeter than the unflavoured sucrose solution but not the second group.

To summarize, attentional strategy applied during exposure seems to play a key role in perception. However, the importance of attentional strategy during exposure, i.e. analytical vs. synthetical, on the construction of perceptual interactions has been only addressed in one study and it is worth validating these findings and further exploring this factor.

☞ ***Sensory information provided by a trained panel and by consumers may differ. Indeed trained panels follow an analytical attentional process and consumers a synthetical attentional process that may favour perceptual interactions.***

This hypothesis was checked by exploring the impact of attentional strategy comparing coffee aroma perception obtained: 1) by trained assessors using sensory profiling; and 2) by consumers using a holistic approach: a sorting task with verbalisation.

In addition during the set up of all different thesis protocols, the dumping effect was limited by using as many scales as sensory characteristics describing the products.

1.2.2.3 Role of olfactory stimulation pathway on perceptual interactions

Recently, an orthonasal and retronasal delivery odorant device has been developed for specifically investigating the role of the olfactory stimulation pathway on perceptual interactions (Heilmann and Hummel, 2004). The device consists in a olfactometer coupled to a two-tube system inserted into the nasal cavity, one being positioned into the epipharynx for stimulating the olfactory receptors orthonasally and the second one being placed in the antrum of the nasal cavity for simulating the olfactory receptors retronasally. This system allowed researchers to compare the direct impact of the spatial localisation of odorant on perception preventing any potential interactions for instance between odorant and saliva. Using this system, a recent study showed that an odorant can increase the intensities of thickness and creaminess, but only when the odour is presented retronasally (Bult *et al.*, 2007). According to the authors, spatial co-occurrence can facilitate perceptual interactions since these conditions naturally occur during everyday food consumption. However, another study using the same device demonstrated that differences in viscosities within a range of semisolid food products can modulate both perceived olfactory intensity of retronasal and orthonasal stimulations (Negoias *et al.*, 2008).

1.2.2.4 Role of temporal co-occurrence of stimulus delivery on perceptual interactions

A simultaneous presentation of odorant and tastant, i.e. temporal synchrony was shown to impact perceptual interactions between olfaction and taste (Pfeiffer *et al.*, 2005). Indeed sweetness of saccharine is increased when presented simultaneously in combination with benzaldehyde odorant. The effect is no longer observed when presented with temporal asynchrony, i.e., subjects taste and then spit out the solution of saccharine before sniffing the benzaldehyde sample. The same conclusions are valid for olfactory and texture interactions. A creamy odorant enhances the perceived creaminess of milk based food, but only when odorant and texture stimuli are delivered simultaneously (Bult *et al.*, 2007).

To summarize, respecting ecological conditions such as temporal and spatial co-occurrence of stimulus delivery during experiments can more efficiently promote perceptual interactions.

☞ ***Such conditions (temporal and spatial co-occurrence of stimulus delivery) were therefore applied in the different experiments conducted during my PhD.***

1.2.2.5 Impact of liking on perceptual interactions

In addition to taste acquisition, an odorant can acquire the hedonic valence, positive or negative of the paired tastant during a co-exposure (or learning phase). For instance pairing a tea drink with quinine during the exposure phase increases bitterness but also decreases the liking for the tea drink when tasted alone (Yeomans *et al.*, 2007). It is well known that bitterness is generally perceived as unpleasant, being genetically coded as a signaling system against potentially poisonous materials (Scott and Verhagen, 2000). Similarly, it has been previously shown that odorant pairing with sweetness during a conditioning period enhances the odorant liking when tasted alone (Zellner *et al.*, 1983; Brunstrom and Fletcher, 2008; Barkat *et al.*, 2008). In addition Barkat et al. (2008)

showed that the number of odorant and tastant co-exposures repetitions was more critical than the total co-exposure duration for enhancing odour pleasantness. Conversely to bitter taste, humans may be genetically pre-determined to like sweetness since sweet food are generally safe sources of energy and nutrients (Beidler, 1982). Learning, perhaps starting from *in utero* development, might also explain this phenomenon since sweet foods are generally more often experienced than bitter food during every day life (Reed *et al.*, 2006). Similarly, congruency between sensory stimuli is the consequence of life-time learning during food consumption. This may explain why congruency rating of sweetness in combination with several odorants was shown as positively correlated to pleasantness rating (Schifferstein and Verlegh, 1996) whereas experience of new/unfamiliar foods generally causes a neophobic reaction (Birch, 1999).

1.2.3 Neural correlate of perceptual interactions

The mammalian nervous system has the ability to integrate signals generated by physiological structures that are anatomically separated (Gibson, 1966; Marks, 1991) such as olfactory receptors and taste buds. According to the latter authors, the aim of such multi-sensory integration may be to enhance our efficiency for detecting and/or identifying stimuli. Resulting from the evolution process, neurons respond firstly independently to each of the five senses, then evolved to multi-sensory neurons (found in the superior colliculi of cats and primates) that integrate cues from three sensory modalities, vision, audition and somatosensation (Stein *et al.*, 1993; Stein *et al.*, 1993; Wallace *et al.*, 1996). By recording the electrophysiological activity of neurons, it has been shown that taste and smell information converge onto a single neuron in the primate caudal orbitofrontal cortex (Rolls and Baylis, 1994). On this basis such bimodal neurons might exist in humans and might be at the origin of flavour processing and of the representation of flavour (Rolls and Baylis, 1994; Critchley and Rolls, 1996).

1.2.3.1 Neural correlate of perceptual olfactory and taste interaction and impact of congruency

Neural correlate of olfactory and taste interactions in humans can be obtained by functional Magnetic Resonance Imagery (fMRI), a technique for mapping the functional activities of the brain. The principle consists in measuring the oxygenation (oxyhemoglobine/desoxyhemoglobine) which increases locally in areas activated due to increased fresh oxygenated blood supply. Activity measurement is compared to the baseline state.

fMRI imaging showed the convergence of taste and olfactory stimuli in the lateral anterior part of the orbitofrontal human cortex (de Araujo *et al.*, 2003). The authors showed that activation induced by tasting a sucrose solution flavoured with strawberry is higher than the sum of activation induced independently by each olfactory and taste stimuli in several area of the orbitofrontal cortex. Super-additivity was similarly highlighted with vanilla and sucrose (Small *et al.*, 2004). But, the latter findings showed that super-additivity does not occur with an incongruent salty solution flavoured with the same vanilla odorant. Small et al. (2004) also demonstrated that the key brain areas underlying perceptual olfactory taste interaction and the role of food experience are: insula, orbitofrontal cortex and anterior cingulate cortex.

The transfer of hedonic valence to odorant resulting from food experience can be explored at a neural level since neurons from the orbitofrontal cortex, which have the property to integrate different sensory stimuli, can also respond to pleasantness (de Araujo *et al.*, 2003).

1.2.3.2 Neural correlate of the impact of orthonasal vs. retronasal odorant presentation on perception

I previously reported that stimulation of the olfactory pathway can modify the perception of both odorants and the perceptual interactions involving taste and texture perception. By coupling the odorant delivery system developed by (Heilmann and Hummel, 2004) with the fMRI technique, Small et al. (2005) compared brain activations induced by food and non-food related odorants (chocolate, lavender, butanol and farnesol) when delivered orthonasally or retronasally. Chocolate aroma induces the highest difference in brain activation between both stimulation pathways. But scoring of olfactory intensity and pleasantness induced by chocolate does not differ between both olfactory stimulation pathways. Comparing activated brain areas in both olfactory conditions: the neural area involved in reward encoding responds more intensively when chocolate aroma is perceived retronasally than orthonasally. The authors suggested that this difference may be explained by the fact that chocolate, being a food-related odorant, is perceptually more associated with the mouth than the nose.

1.2.3.3 Neural correlate of perceptual interactions involving tactile perception

Electrophysiological neuron recordings in macaque orbitofrontal cortex using water stimuli at different temperatures and containing different concentrations of carboxymethyl-cellulose highlighted that the same neuron can either represent taste or temperature or viscosity changes but that other neurons can respond to both temperature and viscosity changes (Kadohisa *et al.*, 2004). Neurons from the orbitofrontal cortex can also react to trigeminal stimulation induced by capsaicin. More recently, neural electrophysiological recordings in macaques showed that some single neurons from insula, orbitofrontal cortex and amygdala respond to taste, temperature, viscosity and fat perception (Kadohisa *et al.*, 2005a; Kadohisa *et al.*, 2005b).

Using fMRI, it has been demonstrated that the same human neuron assembly can respond to both gustatory and tactile stimuli, e.g. astringency induced by aluminium potassium sulfate (Cerf-Ducastel *et al.*, 2001). A brain activation map was built by the authors showing a wide overlap of taste and tactile representations especially in subinsular and opercular regions according to fMRI results. In another study, combination of neurophysiological recordings in rhesus macaques and fMRI study in human subjects showed that human primary taste cortex and frontal operculum provide combined representations of the taste, temperature, viscosity and texture (Rolls, 2007).

To summarize, perceptual sensory interactions and the role of congruency evidenced at a psychophysical level have also been demonstrated at the brain level: 1) by fMRI in human highlighting an increase of activation in specific brain areas higher during simultaneous perception of congruent olfactory and taste stimulus compared with independent presentations of identical stimuli; and 2) by electrophysiological neuron recording in animal showing that different sensory stimuli can be integrated by single neurons. But similarly to studies conducted at psychophysical level, the role of congruency in perceptual interactions was widely investigated in model solutions whereas the impact of familiarity induced by realistic everyday food on neural representation of perception has not yet been explored.

☞ ***In this context a study was designed in order to explore at a neural level through fMRI the potential impact of product familiarity on olfactory and taste interactions. This work represented the neural correlate of the study conducted at a psychophysical level in* PART 2.1.1 *with coffee, cocoa and caffeinated milk drinks.***

1.3 Complex perception

We define a complex perception as a perception that is not easily described with simple attributes such as sweet or thick but that result from an integration of unitary percepts. Consequently product development delivering such perceptions remains challenging. Some studies have focused on complex perceptions such as creaminess (Richardson Harman *et al.*, 2000; Tournier *et al.*, 2007) and freshness ((Peneau *et al.*, 2006; Peneau *et al.*, 2007). Findings of these studies showed that olfaction, taste and tactile sensory dimensions are involved in creaminess and freshness. Moreover, food habits, demographic characteristics and liking contribute to the construction of these complex perceptions as detailed below:

1.3.1 Creaminess

Creamy (or unctuous in French) is a term commonly used by consumers for describing semi-solid products but is only very briefly defined in AFNOR as *moderate level of viscosity, example double cream* (ISO 5492, 1995). Merriam–Webster's Dictionary (1999) proposes the following definition: *which has the consistency of cream*. Richardson Harmann et al. (2000) and Tournier et al. (2007) enriched the understanding of creaminess by combining sensory profiling data from a trained panel and creaminess scoring from a group of consumers following preference mapping methodology on a range of dairy products. The first study highlighted that differences in creaminess scores are mainly explained by differences in consumer demographics and preferences but not by differences in product sensory attributes. The second study showed that creaminess perception results from a combination of several sensory characteristics such as homogenous intensity of the texture, fattiness or sweetness. However, sensory drivers positively associated with creaminess vary among consumers probably due to individual consumer's different food experience (Koster, 2005b; Koster *et al.*, 2004). Finally, the authors also noted a positive correlation between creaminess and liking.

1.3.2 Freshness

Freshness is a term not fully understood and briefly defined by AFNOR for fruits and vegetables as *a turgescent product with no signs of withering or ageing, the cells of which have not deteriorated* (ISO 7563, 1998).

An investigation conducted by Péneau et al. (2006) aimed at better understanding the term freshness from a consumer point of view. Six apples stored under different conditions were tested. Experimenters asked consumers during a local exhibition to score the importance of different sensory items and of other characteristics such as nutritional value, organic, cultivar. Freshness and liking were also scored. A key finding is that the perception of freshness is strongly related to taste, crispness, juiciness and liking. The concept of freshness seems to be interpreted differently according to demographic characteristics (e.g. gender) and consumption habits of consumers. A second study having the objective to characterize the apples from a sensory point of view highlighted that texture properties are key drivers of freshness (Peneau *et al.*, 2007).

☞ ***Within the framework of my PhD, i.e. starting from simple bimodal perception to complex multi-sensory perceptions, the last objective is to explore a complex perception. During our work dedicated to the investigation of perceptual interactions between olfactory, taste and tactile perceptions, a range of viscous liquid products were formulated. Tasting conducted for validating sensory differences among products revealed that refreshing attribute was elicited by a significant number of assessors. According to the limited literature, refreshing perception seemed to be consistent with our concept of complex perception and was therefore explored in PART 3.***

1.3.3 Refreshing

Refreshing perception has been poorly investigated but nevertheless is widely used by consumers and food marketing. The Merriam-Webster Dictionary & Thesaurus (2006) defines refreshing as *Serving to restore strength and animation, to revive, to arouse, to stimulate, to run water over or restore water to, with thirst quenching properties*, suggesting that refreshing is linked to physiological factors such as thirst-quenching and arousal. Some sensory and consumer studies have explored refreshing with regards to: 1) perceived food characteristics (Zellner and Durlach, 2003); and 2) expected food characteristics (Clydesdale *et al.*, 1992; Zellner and Durlach, 2002; Zellner and Durlach, 2003). From these data and in line with the dictionary definitions, refreshing seems to be associated with specific sensory characteristics (e.g. cold, liquid) related to water drinking and in relation with physiological states such as thirst

☞ ***The sensory determinant of refreshing and the role of physiological parameters in refreshing perception were explored in my work. First, a systematic study was conducted combining a conventional sensory profiling with trained assessors and refreshing intensity scoring by consumers to quantify the respective contribution of sensory attributes to refreshing perception. Sensory dimensions involved in refreshing perception over time were also investigated using an innovative temporal methodology, Temporal Dominance of Sensations (TDS). Finally, we related refreshing perception and physiological parameters: mental energy and saliva properties.***

Scientific approach

This literature review enabled us to highlight different gaps and questions related to perceptual interactions which were then investigated. We organized our work into two main research parts (Parts 2 and 3) as illustrated in Fig.3

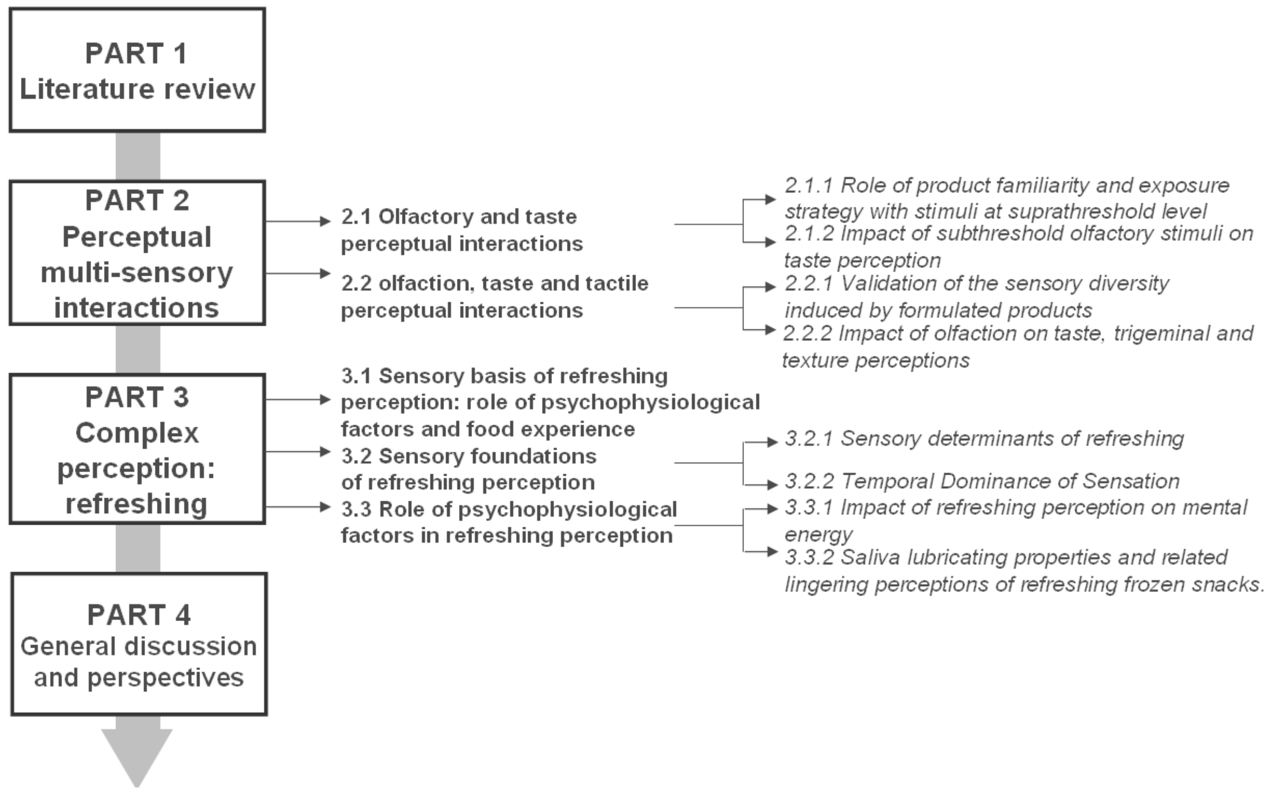

Fig.3: Overall representation of the PhD scientific approach

Based on the literature review two research axes were defined with the overall objective to explore mechanisms underlying perceptual interactions in realistic products. Firstly perceptual interactions were investigated starting from bimodal olfactory and taste interactions and the approach was widened to tactile perception. Secondly, perceptual and psychophysiological factors impacting refreshing perception were explored.

The two research parts are introduced below with the different experiments in terms of objectives and main findings. The details of material & methods, resultats and discussion of each experiment are then presented in each corresponding article.

PART 2: Perceptual multi-sensory interactions

The role of congruency on perceptual interaction has been widely investigated in solution between sensory stimuli and mainly odorant and tastant. Congruency between two stimuli results from repeated consumption of food containing these stimuli in combination. Congruency is therefore strongly related to the level of familiarity for these paired stimuli. To our knowledge, the impact of familiarity at an upper level, i.e. induced by the overall product, has never been investigated. Similarly, the exploration of perceptual interactions on product description was widely studied with experienced assessors using an analytical approach, generally a conventional sensory profiling where subjects are trained to evaluate independently each sensory attribute. Such a strategy can reduce the impact of perceptual interactions on perception. Results from a trained panel were never compared with a more synthetical procedure closer to how consumers apprehend foods during everyday consumption, i.e. considering perception as a whole without dissociating each sensory dimension. For improving basic knowledge about perceptual interaction mechanisms but also to explore perceptual interactions in a more realistic environment in terms of product and evaluation procedure, we investigated in *PART 2.1.1*: 1) the impact of existing products differing in familiarity on olfactory and taste interactions at psychophysics level and neural level by functional Magnetic Resonance Imagery (fMRI); and 2) the effect of two evaluation methodologies encouraging attentional strategy during tasting (sensory profiling using trained assessors) or synthetical strategy (sorting task with consumers) on description of coffee aroma.

The role of subthreshold stimuli was poorly investigated and no study has been designed to answer the following questions: 1) can subthreshold level of odorants increase taste; and 2) after co-exposure between a new odorant and a tastant, can this odorant at subthreshold level enhance perceived taste (as shown in the literature when presented at suprathreshold level)? These remaining questions are crucial regarding the overall understanding of perceptual interaction origin and mechanisms. In addition, the outcome of such a study could be applied to reduce sucrose content in food by adding subthreshold odorant concentration to keep similar sweetness and without perceived olfactory changes. Indeed health benefits are today key during consumer food choice, but without compromising pleasure. In the *PART 2.1.2* the impact of subthreshold odorant stimuli on sweetness was explored together with the impact of co-exposure on the construction of perceptual interactions between sweet taste and an unfamiliar odorant.

Investigations about perceptual interactions have mainly been carried out between olfactory and taste interactions. However few are known where olfactory, taste and tactile perceptions (including trigeminal and texture perception) are involved although this occurs during consumption of many foods. Cold trigeminal stimulation generated by cooling agents is also more and more used for product formulation, mainly in confectionary but also in hot beverages in order to create new sensory experiences for consumers. But few studies have explored perceptual interactions involving coldness. We therefore extended our approach to a more complex system combining olfactory, taste, cold trigeminal and proprioceptive (thickness) perceptions (PART 2.2). To reach this objective, ingredients related to olfactory (mint and peach odorant), taste (citric acid) and trigeminal (cooling agent) perceptions were added to a sweet viscous liquid (also termed "gel" in this work) basis according to an experimental design. Two formulation designs were built by adding for the first set of products a peach odorant at two concentrations, for the second set a mint odorant at two concentrations and for both sets a cooling agent and citric acid at two different concentrations. A total of eight mint-flavoured samples and eight peach-flavoured samples were therefore developed. After validating the range of samples in terms of sensory diversity (*PART 2.2.1*), we explored perceptual interactions according to a sensory profiling method (*PART 2.2.2*).

PART 2.1 Olfactory and taste perceptual interactions

PART 2.1.1 Role of product familiarity and exposure strategy with stimuli at suprathreshold level

The objective of this part was to better understand from both psychophysical and neural point of view how familiarity for the product modulates perceptual interactions between taste and olfaction. To achieve this objective I explored the impact of different odorants on a familiar black coffee drink, a cocoa-based beverage reconstituted with water (somewhat less familiar than the coffee drink) and an unfamiliar caffeinated milk beverage. The three products were profiled with and without odorant addition. We also planned to investigate the role of familiarity on perception at a neural level by functional Magnetic Resonance Imagery (fMRI) with the objective to highlight brain areas involved in taste modulation when product familiarity changed.

☞ Key results are presented below.
Product familiarity modulates perceptual interactions between olfaction and taste. Vanilla odorant increases sweetness of the familiar cocoa drink. But, in the unfamiliar caffeinated milk, vanilla odorant did not impact sweetness but enhanced bitterness.
The fMRI study is planned for October 08, consequently the findings are not here.

🗎 Details can be found in the article pp. 32-42:
Labbe,D., Damevin,L., Vaccher,C., Morgenegg,C., & Martin,N. (2006) Modulation of perceived taste by olfaction in familiar and unfamiliar beverages. Food Quality and Preference, 17, 582-589.

The second objective was to explore the impact of two different attentional strategies on perception: analytical and synthetical attentional strategies. To reach this objective, coffee aroma description done by sensory profiling with a trained panel (analytical strategy) was compared to coffee aroma description obtained by an holistic approach according to a sorting task with verbal description (synthetical strategy) carried out by a group of coffee consumers.

☞ Key results are presented below:
Attentional strategy during exposure strongly affects perception of coffee aroma. Indeed consumers group the coffees consensually but differently from the trained panel. The consumer holistic approach considering the product as a whole may promote the impact of previous food experience on perception, such as odour and taste association constructed during every day coffee exposure. On the contrary, the analytical approach followed by trained panelists probably reduces the impact of food experience on perception and consequently the role of interaction between sensory modalities.

Details can be found in the article pp. 43-51:
🗎 Labbe,D., Rytz,A. & Martin,N. (2006). Coffee aroma is perceived differently by consumers and by trained panelists. Proceedings of the 21st International Conference on Coffee Science, Montpellier, France

PART 2.1.2 Impact of subthreshold olfactory stimuli on taste perception

The impact of common odorants congruent with sweet taste on sweetness of a sucrose solution was investigated with odorant at subthreshold level. In addition, I explored through concomitant olfactory and taste exposure, whether an unfamiliar odorant can be perceptually associated with sweetness when presented at a subthreshold concentration. Moreover, the impact of the attentional strategy during exposure (analytical vs. synthetical) on building of olfactory and taste association was investigated to complement the part 2.1. Naive assessors were conditioned to sucrose and unfamiliar odorant paired stimuli either following: 1) an analytical strategy by sensory profiling training where subjects learnt to consider independently each sensory dimension; or 2) following a synthetical strategy by triangle tests where subjects were encouraged to consider the different sensory dimensions as a whole perception. Indeed since different sensory methodologies can involved different attentional strategies, understanding the impact of this factor on perception is key to be able to better select sensory tools according to the problematic.

☞ Key results are presented below:
The odorant having the highest boosting effect on sweetness when tasted at suprathreshold level showed the highest boosting effect on sweetness when presented at subthreshold level. Regarding the odorant having the lowest boosting effect on sweetness at suprathreshold level, no odorant impact on sweetness is evidenced at subthreshold concentration. Similarly to perceptual interactions with stimuli at suprathreshold level, congruency may play a role on perceptual interaction when a stimulus is at a subthreshold level.

Details can be found in the article pp. 52-62:
🖹 Labbe,D., Rytz,A., Morgenegg,C., Ali,S., & Martin,N. (2006) Subthreshold Olfactory Stimulation Can Enhance Sweetness. Chemical Senses, 32, 205-214.

Unfamiliar odorants co-exposed with sweet tastant acquire the property to enhance sweetness when presented at suprathreshold level, but only when co-exposure is done according to a synthetical approach. As hypothesized, considering the different sensory stimuli as a whole during exposure facilitates the construction of perceptual interactions compared to an analytical approach where sensory stimuli are considered individually. But when presented at subthreshold level, the odorant does not impact sweetness whatever the applied attentional strategy during co-exposure. Co-exposure through laboratory conditions could not reproduce perceptual associations as powerful as those constructed during every day life experience.

Details can be found in the article pp.63 -77:
🖹 Labbe, D., & Martin, N. Impact of novel odorants at supra and subthreshold level on sucrose perception further to an experimental implicit associative learning (Submitted to Chemical Senses)

PART 2.2: Perceptual interactions between olfactory, taste and tactile perceptions

PART 2.2.1 Validation of the sensory diversity induced by formulated products

The objective of this work was to ensure that the ingredients added to the viscous fluid products according to a factorial design deliver a wide sensory diversity mainly in terms of olfaction, taste, and trigeminal perception. Each of the two set of products, i.e. the eight mint flavoured products and the eight peach products were characterized by forty naive internal assessors following a sorting task with free description. This methodology was selected since our objective was to validate that products were different enough to be discriminated not only by a trained panel but also by naive people.

☞ Key results are presented below. Details can be found pp. 78-82.

Within each set, assessors consensually discriminate and describe samples validating that the sensory difference induced by the product range is wide enough to conduct *PART 2.2.2*. In addition, as refreshing attribute was elicited by a significant number of assessors, we chose to explore further this complex perception in **PART 3**.

PART 2.2.2 Impact of olfaction on taste, trigeminal and texture perceptions

The aim of this work was to systematically explore by sensory profiling perceptual interactions involving olfactory, taste and texture perception in the same two ranges of viscous fluid products developed in *PART 2.2.1*.

☞ Key results are presented below.

Olfaction strongly influences taste and cold trigeminal perception, but differently according to the odorant quality. For instance, sweetness is increased by both mint and peach odorant, however mint odorant enhances coldness but not peach odorant. Olfaction also modulates perceptual taste-taste and taste-trigeminal interactions. These findings highlight the large range of expected but also unexpected olfactory-trigeminal-taste perceptual interactions in complex food systems (for example bitterness enhancement by coldness).

Details can be found in the article pp. 83-93:

🗎 Labbe,D., Gilbert,F. and Martin,N. (2008) Impact of olfaction on taste, trigeminal, and texture perceptions. Chemosensory Perception, 1, 217-226.

PART 3: Complex perception: refreshing

First a review considering the sensory, consumer and psychophysiological aspects of refreshing perception was written (PART 3.1) to better identify gaps in this field. Then, we systematically explored the sensory foundations of refreshing perception (PART 3.2) using samples based on the liquid viscous solution used in PART 2.2. The sensory drivers were investigated through two approaches: 1) a punctual approach where perception was studied immediately after consumption (*PART 3.2.1*); and 2) a temporal approach focused on the role of temporal olfactory, taste and trigeminal perception perceived over a 5 min-period on refreshing perception (*PART 3.2.2*). The contribution of specific physiological factors in refreshing perception, selected according to literature review findings, was studied during two independent clinical studies (PART 3.3). The objective was to assess the impact of two frozen snack differing in refreshing intensity on mental energy (*PART 3.3.1*) and saliva properties (*PART 3.3.2*).

PART 3.1 Sensory basis of refreshing perception: role of psychophysiological factors and food experience

☞ Key results are presented below.
Based on a few articles dedicated to refreshing perception from sensory and consumer aspects, the main unsolved question was how a refreshing value (perceived or expected) can be attributed to foods or drinks. By covering in our review the field of psychophysiologics related to hydration/dehydration, we proposed that a product is refreshing when it shares some characteristics of water in terms of sensory properties (clear, cold, liquid) and can alleviate psychophysiological symptoms (e.g. thirst, mental fatigue) in a similar manner to water (e.g., acidic foods and drinks, cooling compounds).

Details can be found in the article pp. 96-115
🗎 Labbe,D., Almiron-Roig,E., Hudry,J., Leathwood,P., & Martin,N. Sensory basis of refreshing perception: role of psychophysiological factors and food experience (submitted to Physiology & Behavior).

PART 3.2 Sensory foundations of refreshing perception

First, a set of viscous fuid products (termed "gel" in this study) was described by sensory profiling using an expert panel and then scored for refreshing intensity by consumers (*PART 3.2.1*). Both data sets were related by the internal preference mapping methodology using refreshing scores rather than liking score. The sensory properties perceived over time were also investigated and linked to refreshing perception. Results from sensory profiling and from Temporal Dominance of Sensation were compared (*PART 3.2.2*).

☞ Key results are presented below.
Consumers agree quite well on the least refreshing products which are the sweetest, but they differ regarding the sensory drivers of the most refreshing products. Three clusters of consumers are identified for which refreshing was driven mainly by 1) cold-mint perception; 2) acidity; and 3) thickness. Food habits may partly explain the different key sensory drivers among consumer clusters.

Details can be found in the article pp. 116-126:

🗎 Labbe,D., Gilbert,F., Antille,N. and Martin,N. (2009) Sensory determinants of refreshing. Food Quality and Preference, 20, 100-109.

☞ TDS provides information on the dynamics of perception after product consumption that is not available using a conventional profiling method and that can bring added value for the understanding of refreshing complex perceptions.

Details can be found in the article pp. 127-133

🗎 Labbe,D., Schlich,P., Pineau,N., Gilbert,F. and Martin,N. (2009) Temporal Dominance of Sensations and Sensory Profiling: A Comparative Study. Food Quality and Preference, 20, 216-221.

PART 3.3 Role of psychophysiological factors in refreshing perception: mental energy and saliva

Mental energy was explored as a psychophysiological marker of refreshing perception in terms of mood, cognitive performances and brain electrical activity (*PART 3.3.1*). The role of saliva in refreshing perception was also investigated in terms of flow and friction coefficient (*PART 3.3.2*). The two study proposals have been approved by the ethical committee from the Centre Hospitalier Universitaire of Vaud at Lausanne. The impact of the two frozen snacks differing in refreshing intensity and of a glass of water (positive control for refreshing perception) was explored on: 1) mental energy in terms of mood, attentional performance and brain oscillations and 2) saliva in terms of salivary flow and saliva lubricating properties.

☞ Key results are presented below:
Compared to the standard frozen snack and the glass of water, consumption of the refreshing frozen snack increases: 1) brain electrical activity and mainly alpha waves that are generally associated with alertness mental state; and 2) subject attentional performances obtained during a visual detection task. Subjective alertness rating does not differ between the two frozen snacks.

Details can be found in the article pp. 134-152:

🗎 Labbe,D., Martin,N., le Coutre,J. & Hudry,J. The impact of refreshing perception on mental energy: changes in mood, cognitive performance and brain oscillations (submitted to International Journal of Neuroscience)

☞ After consumption of the refreshing product, saliva rate is higher and produced saliva is more lubricating compared to results obtained after consumption of the standard frozen snack and glass of water. This physical parameter change is associated with the intense salivating intensity remaining after the refreshing frozen snack consumption according to TDS measurement.

Details can be found in the article pp. 153-166:

🗎 Labbe,D., & Martin,N. Modulation of saliva flow, saliva lubricating properties and related lingering perceptions by refreshing frozen snacks (in preparation for Physiology & Behavior)

List of original communications

This thesis is based on the following original communications:

Publications in peer-reviewed journals & Proceedings

PART 2: Perceptual multi-sensory interactions

PART 2.1 Olfactory and taste perceptual interactions

PART 2.1.1 Role of product familiarity and exposure strategy with stimuli at suprathreshold level

- Labbe, D., Damevin,L., Vaccher,C., Morgenegg,C., & Martin,N. (2006). Modulation of perceived taste by olfaction in familiar and unfamiliar beverages. Food Quality and Preference, 17, 582-589.
- Labbe, D., Rytz, A. & Martin, N. (2006). Coffee aroma is perceived differently by consumers and by trained panelists. Proceedings of the 21st International Conference on Coffee Science, Montpellier, France

PART 2.1.2 Impact of subthreshold olfactory stimuli on taste perception

- Labbe, D., Rytz,A., Morgenegg,C., Ali,S., & Martin,N. (2006). Subthreshold Olfactory Stimulation Can Enhance Sweetness. Chemical Senses, 32, 205-214.
- Labbe, D., & Martin, N. Impact of novel odorants at supra and subthreshold level on sucrose perception further to an experimental implicit associative learning (Submitted to Chemical Senses)

Part 2.2 olfaction, taste and tactile perceptual interactions

PART 3.2.2 Impact of olfaction on taste, trigeminal and texture perceptions

- Labbe,D., Gilbert,F. and Martin,N. (2008) Impact of olfaction on taste, trigeminal, and texture perceptions. Chemosensory Perception, 1, 217-226.

Part 3: Complex perception: refreshing

Part 3.1 Sensory basis of refreshing perception: role of psychophysiological factors and food experience

- Labbe,D.,Almiron-Roig,E.,Hudry,J., Martin,N., & Leathwood,P. Sensory basis of refreshing perception: role of psychophysiological factors and food experience (Submitted to Physiology & Behavior)

Part 3.2 Sensory foundations of refreshing perception

- Labbe,D., Gilbert,F., Antille,N. and Martin,N. (2009) Sensory determinants of refreshing. Food Quality and Preference, 20, 100-109.

- Labbe,D., Schlich,P., Pineau,N., Gilbert,F. and Martin,N. (2009) Temporal Dominance of Sensations and Sensory Profiling: A Comparative Study. Food Quality and Preference, 20, 216-221.

PART 3.3 Impact on physiological factors on refreshing perception: mental energy and saliva

- Labbe,D, Martin,N, le Coutre,J, &. Hudry,J. The impact of refreshing perception on mental energy: changes in mood, cognitive performance and brain oscillations (submitted to International Journal of Neuroscience)

- Labbe,D., & Martin,N. Modulation of saliva flow, saliva lubricating properties and related lingering perceptions by refreshing frozen snacks (in preparation for Physiology & Behavior)

International symposium oral communications

- Labbe,D., Rytz,A. & Martin,N. Coffee aroma is perceived differently by consumers and by trained panelists. 21st International Conference on Coffee Science (ASIC), September 2006, Montpellier, France.

- Labbe,D., Rytz,A., Morgenegg,C., Ali,S. & Martin,N. Subthreshold Olfactory Stimulation Can Enhance Sweetness. 17th conference of the European Chemoreception Research Organisation (ECRO), September 2006, Grenada, Spain.

- Labbe,D., Gilbert,F., Antille,N., & Martin,N. Sensory determinants of refreshing. 2nd European Conference on Sensory Consumer Science of Food and Beverage, September 2006, The Hague, the Netherlands.

- Labbe.D., & Martin,N. Multisensory Processing in Flavour Perception, invitation at a satellite symposium in the frame of the International Multisensory Research Forum, July 2008, Hamburg, Germany

- Labbe,D., Martin,N., Rami,S., Le-Coutres,J. and Hudry,J. Mental energy enhancement by refreshing frozen snack. 18th conference of the European Chemoreception Research Organisation (ECRO), September 2008, Portoroz, Slovenia

First patent filling

- Labbe,D. & Martin,N. (2007). A sweet food composition with low sugar content. WO 110115
- Labbe,D., Puaud,M., & Lim,T. (2007). Production of food products with enhanced in mouth and mental refreshment. EP n°07120824.3

PART 2 Perceptual multi-sensory interactions

2.1 Olfactory and taste perceptual interactions

2.1.1 Role of product familiarity and exposure strategy with stimuli at suprathreshold level

2.1.1.1 Modulation of perceived taste by olfaction in familiar and unfamiliar beverages

Labbe,D., Damevin,L., Vaccher,C., Morgenegg,C. and Martin,N. (2006). Food Quality and Preference, 17, 582-589.

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Modulation of perceived taste by olfaction in familiar and unfamiliar beverages

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Abstract

The integration of olfactory and taste perception and the role of congruency and familiarity on perception have already been demonstrated in model solutions. The aim of the present study was to investigate the role of these factors in real food products. Therefore, we have investigated the impact of olfactory perception on perceived bitterness in a familiar (bitter cocoa beverage) and an unfamiliar (bitter milk) beverage. Sensory profilings with and without noseclip were conducted according to simultaneous product presentation. In a first experiment, an instant cocoa powder mixed with water was used to prepare a common base. Two types of flavourings were added: cocoa and vanilla, at three different levels (none, medium and high). Samples were compared within a flavouring type. In a second experiment a vanilla flavour was added at three levels to a milk base containing caffeine. The panellists scored bitterness, sourness, sweetness and body with noseclip. Without noseclip, overall aroma above the cup and in mouth were assessed in addition to the previous set of attributes. With noseclip, results showed that neither the cocoa nor the vanilla flavourings provided any additional taste to the beverages. Without noseclip, olfactory/taste interaction in the cocoa beverage led to an enhancement of bitterness induced by the cocoa flavouring and an increase in sweetness from the vanilla flavouring. On the contrary, in the caffeinated milk, the addition of vanilla flavouring did not significantly increase sweetness, but unexpectedly enhanced bitterness. This study is further evidence of the influence of olfaction on taste perception in complex matrices. In addition, our results suggest that taste–olfaction integration is product dependent and related to food experience, even when working with trained subjects. Furthermore, the unpleasantness due to the neophobia related to the consumption of a new product and to bitterness may enhance bitterness when the unfamiliarity of the product is increased by addition of vanilla flavouring to a bitter milk beverage.

Keywords: Psychophysics; Olfaction; Taste; Cognitive interaction; Familiarity; Food expectancy

1. Introduction

Interactions between olfaction and taste modalities are well known and have been extensively investigated in model solutions. Pioneer studies on interaction between odour and taste perception showed that subjects attributed a sweet taste to pure ethyl butyrate solutions and that the sweet perception of a citral–sucrose mixture was enhanced by increasing the citral concentration (Murphy & Cain, 1980; Murphy, Cain, & Bartoshuk, 1977). Time intensity studies also demonstrated that sweetness was enhanced by fruity odour whereas fruity odour was not increased by sweetness (Bonnans & Noble, 1993; Cliff & Noble, 1990). Frank, Shaffer, and Smith (1991) showed that perceptual similarity or dissimilarity between an odorant and a tastant in mixture was a good predictor of taste intensity change. An odour can acquire a taste quality when the odour–taste pair is perceived in food commonly experienced by consumers. Congruency was defined by Schifferstein and Verlegh (1996) as "the extent to which two stimuli are appropriate for combination in a food product". Frank and Byram (1988) reported that strawberry odour enhanced whipped cream sweetness whereas peanut butter did not affect sweetness rating. Lavin and Lawless (1998) also highlighted the enhancing effect of vanilla flavouring

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on perceived sweetness when added to milk among children and adults. Prescott (1999) showed that pineapple flavouring enhances perceived sweetness. Besides Stevenson, Prescott, and Boakes (1999) reported that an odour can also decrease taste intensity when the odour–taste pair is not congruent in food. In their experiment, caramel odour, related to sweet taste, decreases sour taste intensity.

The intensity rating of a sensory dimension also depends on the response alternative provided to the subjects. According to Lawless and Clark (1992) and Clark and Lawless (1994), taste enhancement by an odorant can be due to (1) a halo effect "where one positive characteristic of a product may induce positive opinions on other seemingly unrelated characteristics"; (2) a dumping effect, for instance a transfer of the perceived olfactory enhancement on the taste scale when no olfactory intensity scale is available. Consequently, it is important to provide the subjects with an exhaustive list of attributes to limit such bias. Frank, van der Klaauw, and Schifferstein (1993) and Frank (2002) confirmed that taste rating was modulated by both odour perception, called the "*perceptual factor*" (congruency) and also by the response alternative proposed by the experimenter called the ""*conceptual factor*".

Stevenson, Prescott, and Boakes (1995) tested the effect of learning on sweet perception associations to demonstrate the role of food experience. They showed that, through learning, a tasteless odor can acquire a sweet or a sour taste quality after being tested in mouth either with sucrose or with citric acid. Consequently odour–taste interactions may be the result of associations experienced and memorized through food exposure without any explicit attention or learning (Köster, 2005; Köster, Prescott, & Köster, 2004). Thus, memory seems to be strongly involved in food expectation (Mojet & Köster, 2005). Small et al. (2004) confirmed this sensory evidence at a neuronal level. Using neuroimaging, they showed that brain activation representing olfactory/taste interaction was dependent on previous subject experience with smell/taste combinations. Stein, Nagai, Nakagawa, and Beauchamp (2003) demonstrated that repeated exposure to different bitter beverages during a 7-day period significantly enhanced familiarity rating and modulated rating of taste modalities when comparing measurements conducted before and after the period of exposure. Indeed sweetness rating significantly increased whereas bitterness declined, but not significantly.

The attentional strategy applied also affects odorant-induced taste modulation. Prescott, Johnstone, and Francis (2004) reported that a synthetic exposure during joint exposure to either prune- or waterchesnut odour and a sucrose solution increased the ability of an odour to subsequently enhance the sweetness of the sucrose/prune (waterchesnut) mixture. A synthetic versus an analytical task instruction during exposure differentially affected the increase in sweetness of an odour/taste mixture judged afterwards. These findings suggest that procedures such as sensory profiling inducing an analytical attitude during exposure are less vulnerable to synthetic enhancement and therefore less prone to show odour-induced taste enhancement. Bingham, Birch, de Graaf, Behan, and Perring (1990) also highlighted an enhancement of sweetness by maltol addition in a sucrose solution when the evaluation was assessed by an untrained panel but not with a trained panel (used to evaluated products according to an analytical procedure).

The aim of this work was to investigate the impact of beverage familiarity on interaction between olfactory and taste perception. Indeed there is a need for the food industry to know whether such cognitive processes on food perception have similar effects in classical/familiar and new/unfamiliar products. In the present study, the impact of two different olfactory stimuli on bitter perception was investigated in two bitter beverages. Modulation of sweet and sour perception was also investigated to explore possible effects on these tastes. Beverages differed regarding their familiarity for consumers: a cocoa beverage and a caffeinated fat milk beverage frequently consumed and rarely/never consumed, respectively. Pure cocoa is a common bitter food frequently consumed in a beverage form as well as in chocolate tablet form. Even if the main consumption habit of cocoa powder is a liquid form with milk and sugar, in-house market research allows to strongly assume that for consumers cocoa is related to bitterness. Unlike cocoa, milk's main sensory characteristics in mouth are "creamy" and "sweet" (Lee, Lee, & Shin, 2003). Moreover milk is often used as a coffee additive to decrease harsh, bitter coffee taste and to increase acceptance (Cristovam, Russell, Paterson, & Reid, 2000). Adding caffeine to milk allowed us to obtain an unfamiliar bitter beverage. Our objectives were (1) to validate the enhancing impact on bitterness of odorant congruent with bitter taste by boosting the natural odour of cocoa beverage with a commercial cocoa flavouring; (2) to explore the effect of an odorant congruent with sweetness on bitter taste perception by adding vanilla aroma in the cocoa beverage; (3) to assess the role of beverage familiarity by measuring whether the bitter modulation induced by vanilla flavouring addition is the same in a familiar (cocoa) and an unfamiliar (caffeinated milk) beverage.

To avoid any confusion we will use the term "odour" to refer to the orthonasal olfactory perception evaluated above the cup, and "aroma" to refer to the retronasal olfactory perception i.e. the organoleptic attribute perceptible by the olfactory organ via the back of the nose (NF ISO 5492, 1995). The term "olfactory" regroups both odour and aroma perception.

2. Materials and methods

2.1. Beverage preparation

Two complex food matrices were investigated. A pure cocoa powder (Caillier-Nestlé, Switzerland) reconstituted at 14% in mineral water (Vittel Bonne Source, France) for the familiar bitter beverage and a caffeinated (0.08% of the reconstituted beverage) commercial 2.7% fat UHT milk for the non-familiar beverage. A previous sensory study clearly showed that the two beverages were only bitter without any sweet taste.

Two flavourings were added: (1) a dark chocolate flavouring to the cocoa beverage and (2) a vanilla flavouring to the cocoa and caffeinated milk beverages. The cocoa flavouring was described by the supplier (Firmenich, Switzerland) as "dark chocolate". It was chosen to match as closely as possible the flavour profile of the pure cocoa beverage and, therefore, to modulate only the cocoa intensity and not the quality of the product. Two concentration levels for cocoa flavouring were defined, on the basis of preliminary studies, to obtain a two-step enhancement of the cocoa beverage olfactory note. These two concentration levels were 0.15% and 0.24% of the reconstituted beverage (called "medium concentration" and "high concentration", respectively). The cocoa beverage without added flavour was defined as the reference. The same procedure was repeated with the vanilla flavouring (Givaudan, Switzerland) to select two concentration levels for modulating the olfactory characteristics of the cocoa and caffeinated milk beverages. The two vanilla flavouring concentrations were 0.05% and 0.10% of the reconstituted beverage.

For each beverage, and within a same flavouring type, four samples were prepared: one sample without added flavouring (reference), one sample flavoured at medium concentration (replicated for control), one sample flavoured at high concentration. Sample preparation was carried out 1 h prior to evaluation.

2.2. Subjects and tasting conditions

Ten external assessors, all women, with an average age of 45, were recruited for this study. They were used to participating in sensory panels for coffee beverage evaluation. For a previous study, they had been trained on basic tastes and on overall odour/aroma intensity evaluation. No specific training sessions were conducted for this study. Samples were coded with three-digit random numbers and served at 65 °C in a 50-ml cup. Rinsing was done between samples with water and unsalted crackers. Tests were conducted in an air-conditioned room (18 °C), under white light in individual booths. Data was acquired on a computer screen with FIZZ software (Biosystèmes, 1990).

2.3. Sensory procedure

Each of the three sets of four samples (i.e. cocoa beverages with cocoa flavouring, cocoa beverages with vanilla flavouring and caffeinated milks with vanilla flavouring) was assessed, separately, during a 1-h session, by sensory profiling with simultaneous product presentation. The four samples were presented all at once, but the sample order in the tray was randomized across subjects. Subjects had to score comparatively the four products for each successive attribute on a nine-point structured scale anchored at the extremities with "not intense" and "very intense". Sensory profilings were conducted with and without noseclip. Noseclips prevent olfactory perceptions and therefore only taste and texture in mouth were evaluated. Noseclip evaluation of the beverage with flavour addition was essential to ensure that flavourings did not provide any taste by themselves to the beverage when olfaction was prevented. The subjects scored bitterness, sourness, sweetness and body intensities for the evaluation with noseclip. Without noseclip, the assessors were asked firstly to smell the beverages and to score overall odour, and secondly to taste and to score the overall aroma and the taste and body attributes. "Body" attribute describes the perceived viscosity, fullness and weight in the mouth ranging from "thin, watery" to "thick, heavy".

Due to preparation constraints, sensory profilings were not balanced between the three 1-h sessions. Within each session and for each condition, sensory profiling was duplicated, samples being randomized over subjects according to a balanced experimental design.

2.4. Data analyses

Data analyses was analysed using FIZZ software. For each sensory profiling, the product effect at panel level was tested for each attribute, using a two-way analysis of variance with product as fixed and assessor as random factors. When the product effect was significant ($P < 0.05$) highlighting a significant difference between the four samples for a given attribute, two multiple range tests were performed: a Duncan test to compare the mean intensities between the four samples and a Dunett test to highlight sensory differences between each flavoured beverage and the non-flavoured reference. For the graphical representation, percentage of variation x' (%) between the average intensity (calculated across subjects) of the flavoured samples (Int.x) and the average intensity of the reference (Int.ref) was calculated according to the following formula:

$$x'\ (\%) = \left(\frac{\text{Int}.x - \text{Int.ref}}{\text{Int.ref}}\right) \times 100$$

3. Results

3.1. Cocoa and vanilla flavouring did not induce any taste

When evaluated with a noseclip, no significant taste and texture difference was observed (1) among the four cocoa beverages whatever the flavour added and (2) among the 4 caffeinated milk beverages with vanilla flavouring (Fig. 1). This means that cocoa and vanilla flavourings had no significant impact on taste nor on texture in mouth when olfactory perception was prevented.

3.2. Cocoa flavouring enhanced bitter perception of cocoa beverages

For cocoa beverages with cocoa flavouring evaluated without noseclip, two-way analyses of variance showed a

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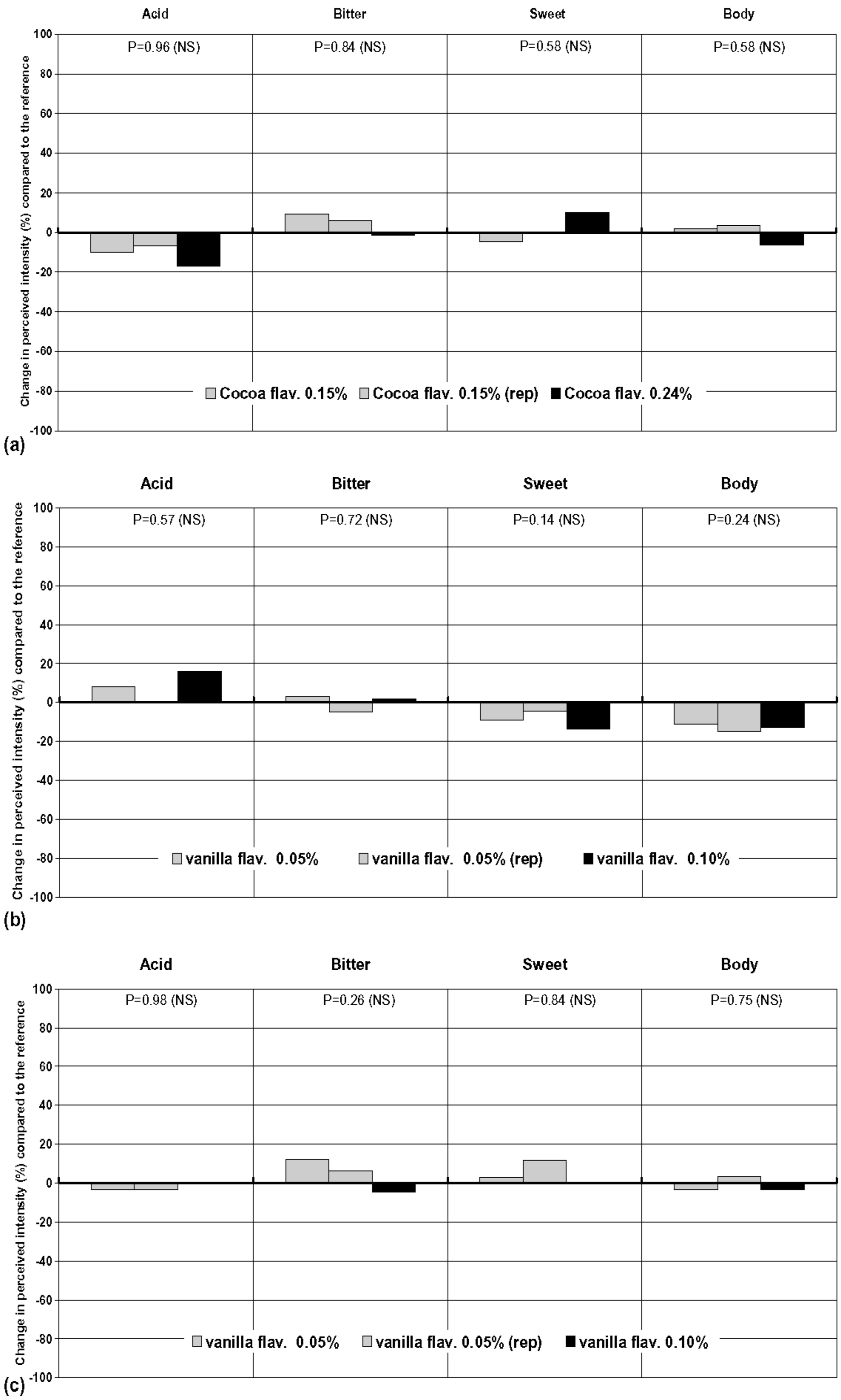

Fig. 1. (a c) Flavouring impact on cocoa beverage taste and texture. Evaluation conducted with noseclip for (a) cocoa beverage with cocoa flavouring; (b) cocoa beverage with vanilla flavouring; (c) caffeinated milk beverage with vanilla flavouring. According to the *F*-ratio of the product factor ANOVA: NS means "non-significant".

significant product effect for overall odour ($P < 0.0001$) and bitterness ($P < 0.05$). Cocoa flavouring enhanced perceived odour intensity. The three flavoured samples were significantly more intense than the reference sample, but no significant difference was perceived between the three flavoured samples according to the Duncan test at 5% (Fig. 2a). Panel mean scores for overall aroma intensity were higher for the three flavoured samples than for the reference (Fig. 2a), but product effect was not significant ($P = 0.15$).

Bitterness intensity was also boosted by the addition of cocoa flavouring for the three flavoured samples but only the high level of flavouring enhanced significantly the perceived bitterness compared to the reference according to the Dunett test (Fig. 2a). Even if differences among the four products for sourness and sweetness intensity were not significant ($P = 0.17$ and $P = 0.10$, respectively), there is a trend for a decreasing impact of cocoa flavouring on perceived sweetness (Fig. 2a).

No difference in body intensity due to the the cocoa flavouring was observed ($P = 0.88$). Mean panel scores for the flavoured samples were close to the reference (Fig. 2a).

3.3. Vanilla flavouring enhanced sweet perception of cocoa beverages

Without noseclip, the overall odour of cocoa beverages with vanilla flavouring was not perceived as different from the reference sample ($P = 0.33$). However overall aroma and sweetness intensity significantly differed among the four products ($P < 0.001$ and $P = 0.03$, respectively).The Dunett test pointed out that only the beverage with the highest vanilla flavouring concentration was significantly more intense than the reference for both attributes.

No significant differences in sourness, bitterness and body intensity were observed between the four samples (Fig. 2b).

3.4. Vanilla flavouring did not affect significantly taste perception of caffeinated milk beverage

Although the odour and aroma intensities of the flavoured beverages were significantly higher than the reference (Fig. 2c), no significant impact on taste and texture was observed. However, bitterness P-value was close to significance ($P = 0.06$) and a trend of increased bitterness with increased vanilla flavouring concentration was observed (Fig. 2c).

4. Discussion

4.1. Flavouring modulated cocoa beverage olfactory and taste perceptions

The addition of flavouring agents increased the olfactory intensity of the cocoa beverage. The cocoa flavouring added to the cocoa beverage led to a significant enhancement of the perceived overall odour whereas the addition of vanilla enhanced significantly the perceived aroma. This finding suggests that both olfactory cues (orthonasal and retronasal) modulate taste. These results are confirmed by Sakai, Kobayakawa, Gotow, Saito, and Imada (2001) who reported that taste enhancement by olfaction was effective when the odorant was presented by the orthonasal or by the retronasal route, provided that olfactory and taste stimuli were presented simultaneously. The differences observed between olfactory perception of cocoa odorant (more intense for orthonasal stimulation) and vanilla odorant (more intense for retronasal stimulation) may be explained by their physico-chemical characteristics. Diaz (2004) showed that differences between orthonasal and retronasal perception depend strongly on the physical characteristics of the aroma chemicals. Significant differences in olfactory intensity between samples with the medium and the high flavouring concentrations were also difficult to reach. But we had to limit concentration levels because increasing the two flavouring contents led to strongly perceived off-taste formation when evaluated by the panel with a noseclip. Nevertheless, even if differences in odour and aroma intensity between samples were not as marked as expected, the olfactory impact on taste perception remained coherent in the familiar cocoa beverage. Cocoa flavouring, congruent with bitterness, boosted the bitter perception. Vanilla flavouring, congruent with sweet taste, induced a sweet perception.

Moreover, simultaneously with bitterness enhancement, the cocoa flavouring reduced the sweet perception of the three flavoured cocoa beverages. These differences were not significant, but followed a clear trend ($P = 0.10$). Different hypotheses can be proposed to explain this decrease in sweetness: First a direct reducing effect of the aroma may explain this finding. Second a halo effect may occur, i.e. an increase in the unpleasant attribute, bitter, that leads to a decrease in the pleasant one, sweet (Clark & Lawless, 1994). Third but also the bitterness enhancement induced by the cocoa flavouring may have resulted in a suppression of the perceived sweet taste by odorant-induced taste/taste interaction. Beside the sweetness enhancement induced by the vanilla flavouring led to a slight bitterness decrease. These results suggested that symmetrically suppressive interactions between tastants (Keast & Breslin, 2003) seemed to be valid for odour-induced taste modulation. However the impact of the induced taste on the beverage taste perception was less powerful than the taste modulation induced by a congruent olfactory note. Indeed P-values of sweetness suppression induced by cocoa flavouring and bitterness suppression induced by vanilla flavouring were 0.10 and 0.34, respectively.

4.2. Olfactory/taste interaction are observed despite the analytical procedure

According to Prescott (1999) and Prescott et al. (2004), olfactory/taste interactions in water systems depend on the

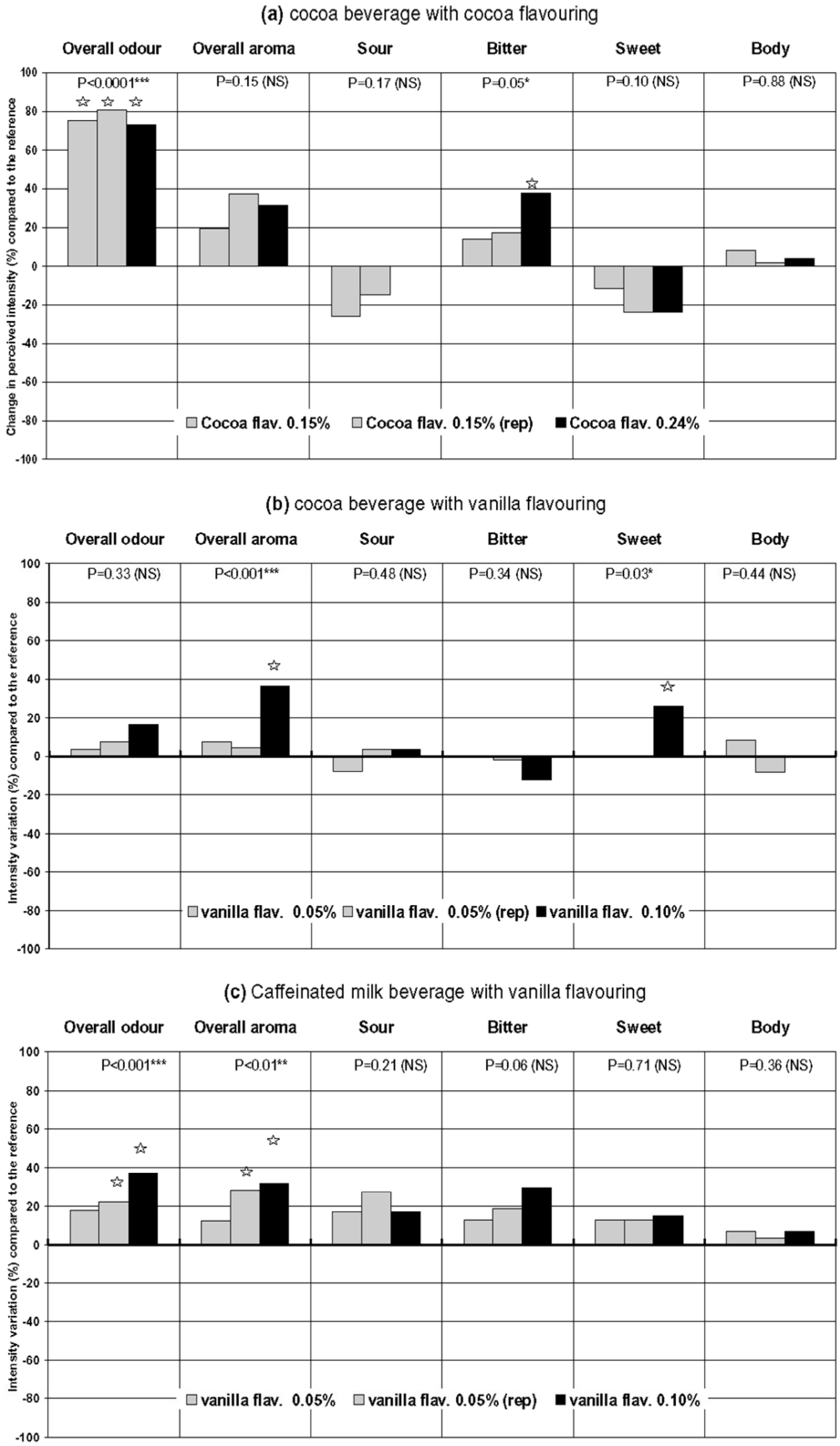

Fig. 2. (a c) Flavouring impact on beverage odour, aroma, taste and texture/evaluation conducted without noseclip. According to the *F*-ratio of the product factor ANOVA: NS means "non-significance"; *, **, *** indicate product effect at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. According to the Dunett test, a white star indicates a significant difference between each sample and the reference ($P < 0.05$).

exposure strategy applied to subjects. A synthetical exposure, encouraging subjects to evaluate olfactory and taste stimuli as a whole, promotes olfactory/taste interactions, whereas an analytical exposure, encouraging subjects to consider olfactory and taste stimuli independently, limits olfactory/taste interactions. Results of the present experiment show that adding cocoa and vanilla flavourings to the cocoa beverage induced a significant bitter and sweet enhancement, respectively. Although the attitude induced by the sensory profiling task was analytical, the outcome suggested a synthetical cognitive process. This may due to previous subject food experience regarding the tested beverages. It is conceivable that the subjects have been often exposed to cocoa beverages. Interaction between senses may be therefore more easily suppressed when occurring in model solution (Bingham et al., 1990) than in food commonly consumed. This finding should be kept in mind when interpreting the results of a descriptive analysis conducted on commercial products.

4.3. Familiarity of beverages affected olfactory/taste interactions

The importance of familiarity in the determination of sensory interactions can be further considered looking at the results obtained for the unfamiliar caffeinated milk beverage. Addition of vanilla flavouring to caffeinated milk did enhance sweetness, as in the familiar cocoa beverage, but unexpectedly enhanced bitterness. These observations therefore suggest that the degree of familiarity for the beverage may be an important factor in determining olfactory/taste interactions. But what is the cognitive mechanism leading to the enhancement of the perceived milk bitterness by vanilla? It is well known that bitterness is a signaling system against potentially poisonous materials (Scott & Verhagen, 2000). Consequently some individuals tend to avoid bitter tastes, which can partly explain aversion for certain foods such as brussels sprouts, cabbage or spinach (Drewnowski, Henderson, Levine, & Hann, 1999; Drewnowski, Henderson, Shore, & Barratt-Fornell, 1998). Additionally, a neophobic reaction to this new beverage may have occurred (Birch, 1999). Odour familiarity rating is usually strongly correlated to pleasantness rating (Stein et al., 2003; Sulmont, Issanchou, & Köster, 2002). The pleasant familiar vanilla note may therefore have induced a positive hedonic expectation, which was not fulfilled by the unfamiliar milk beverage's bitter taste. Based on these statements, the unfamiliar bitter milk beverage was probably judged unpleasant by tasters and the combination between this product and vanilla flavouring may have increased the product's unfamiliarity and consequently its unpleasantness. The subjects may have associated unpleasantness with bitterness, thereby boosting the perceived bitterness when the unpleasant sensation increased with addition of vanilla flavouring. Therefore, as for sensory interactions, a hedonic/sensory cognitive interaction may be at the origin of the taste modulation.

Moreover, a dumping effect may be involved in the bitterness enhancement (Clark & Lawless, 1994). Subjects had no liking scale available to report how they liked the product. They may have reported their hedonic judgment on the bitter scale, as we assumed that subjects associated bitterness with unpleasantness. The hedonic dimension could have been dumped onto the sensory dimension; this might explain the increase of the bitter taste attribute mean score.

4.4. Effect of single unfamiliar odorant/tastant co-exposure on sensory interaction

Prescott et al. (2004) showed that a single unfamiliar odorant/sweet tastant co-exposure is sufficient to produce a cognitive association between both stimuli. Authors also reported that the adoption of a synthetic perceptual strategy during co-exposure is necessary to produce an odour that will enhance sweetness. Results obtained for the bitterness and sweetness evaluation of the four caffeinated milk beverages during the first and the second evaluation were analysed separately by a two-way ANOVA (products × subjects). Indeed, the first evaluation may be considered as a first co-exposure between two stimuli in an unfamiliar context and the bitterness enhancement, highlighted when both evaluations were analysed conjointly, may be effective only during the second evaluation. But for both evaluations, flavoured caffeinated milk beverages were perceived as more bitter than the reference without any significant change in sweetness. Differences between the two replications were also assessed by a three-way analysis of variance (products × subjects × repetitions) on the three flavoured products. The repetition factor was significant for bitterness and sweetness ($P < 0.001$ and $P < 0.01$, respectively) Indeed the three flavoured milk beverages were perceived significantly more bitter and less sweet during the second evaluation than during the first evaluation (considered as the first exposition). Even if a single odorant/tastant co-exposure was not at the origin of the unfamiliar milk beverage bitterness enhancement by vanilla flavouring, this odorant/tastant co-exposure occurring during the first evaluation enhanced the olfactory impact on taste during the second evaluation.

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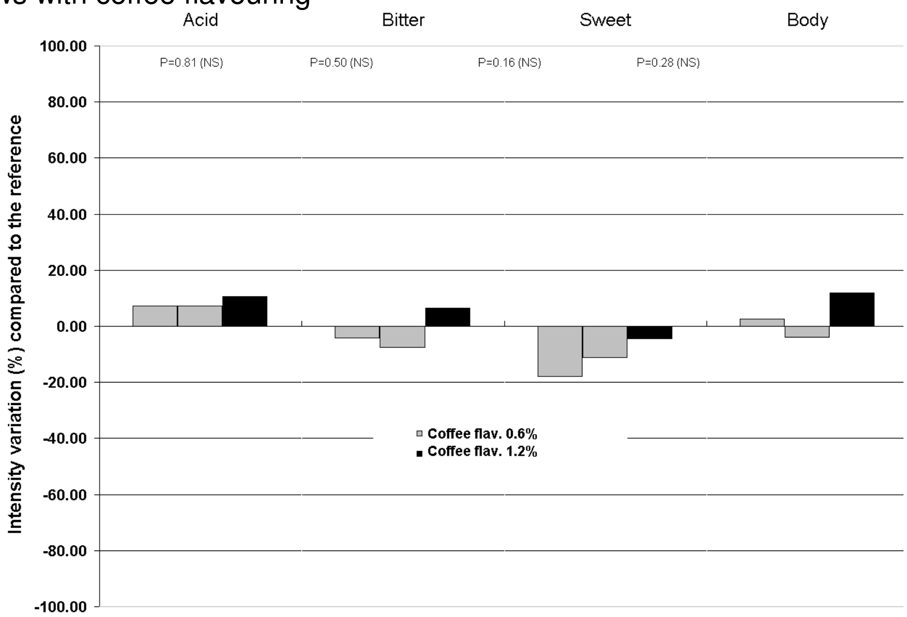
Additional unpublished results

In this published work, the selected drinks were a coca based beverage reconstituted with water assumed as familiar and a caffeinated milk assumed as unfamiliar. Latter, another bitter drink was tested in this study, a black coffee brew beverage assumed as the most familiar among the three drinks according to internal consumer insight.
Three products were therefore evaluated, a very familiar bitter coffee brew, a familiar bitter cocoa drink and an unfamiliar bitter milk. As for the cocoa drink, two sets of three coffee brews were prepared: 1) one set with a coffee brew reference, and two coffee brews flavoured with a natural coffee flavouring (produced internally) at two concentrations for boosting the intrinsic coffee odour and aroma; and 2) one set with a coffee brew reference, and two coffee brews flavoured with the same vanilla flavouring as the one used for flavoured cocoa and milk drinks. Product preparation, sensory evaluation procedure and statistical analyses were the same as described in
Results of the evaluation conducted with noseclip validates that the coffee flavouring does not taste by itself (Fig.1 a-b).

a) Coffee brews with coffee flavouring



b) Coffee brews with vanilla flavouring

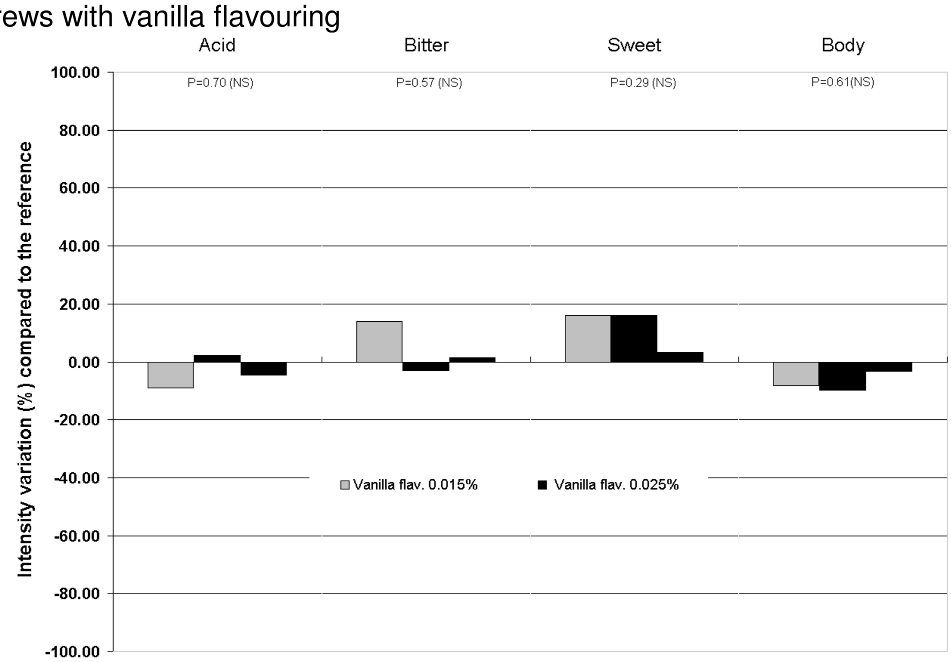
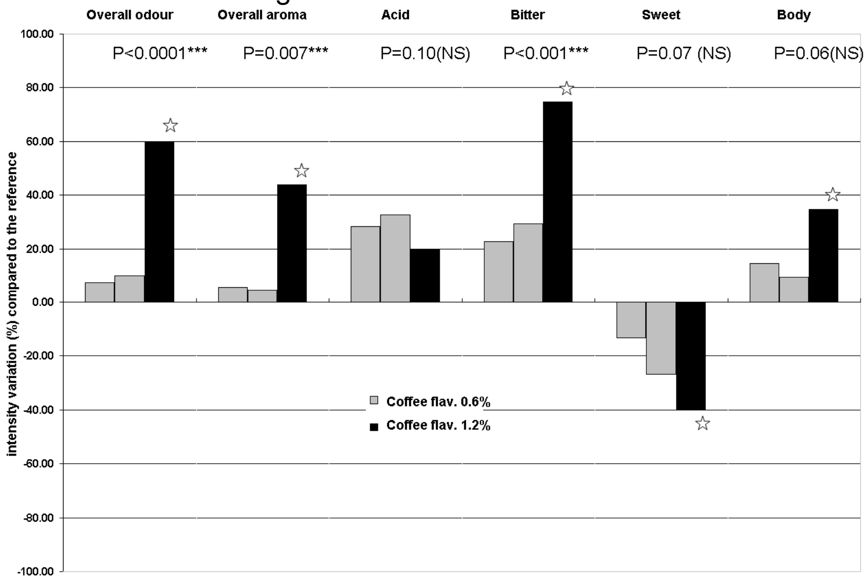


Fig.1 a-b: Flavouring impact on coffee brew taste and texture. Evaluation conducted with noseclip for a) coffee brew with coffee flavouring; b) coffee brew with vanilla flavouring; flavouring. According to the F-ratio of the product factor ANOVA: NS means "Non-Significant".

Without noseclip, the results obtained with coffee brews are consistent to those obtained with cocoa drink since: 1) coffee flavouring addition enhances bitterness and decreases sweetness (Fig.2) and; 2) vanilla flavouring addition induces sweetness. The mechanistic hypotheses underlying such taste modulation were detailed in the discussion part of the published article. Actually, since vanilla odour/aroma is congruent with sweet taste; sweetness probably reduces bitterness by symmetrical odour induced taste-taste interactions.

a) Coffee brews with coffee flavouring



b) Coffee brews with vanilla flavouring

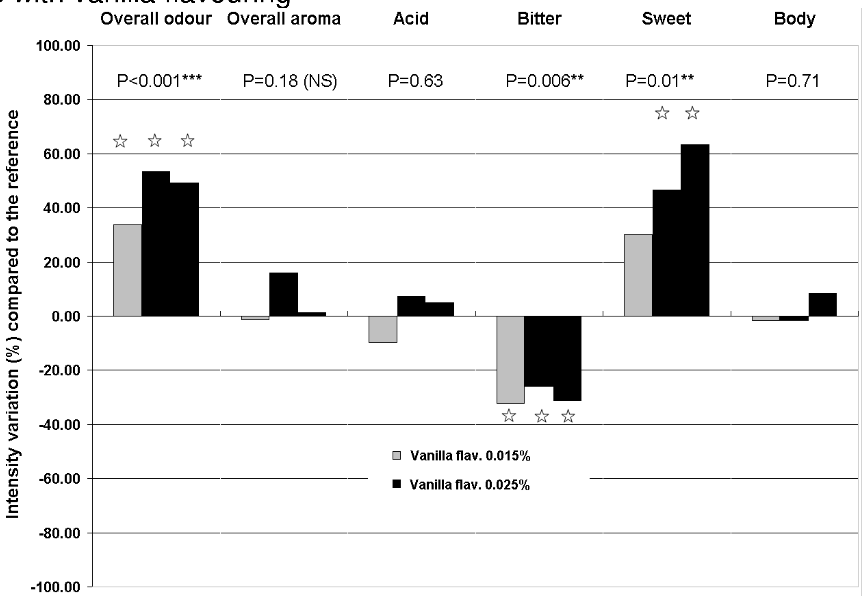


*Fig.2 a-b: Flavouring impact on beverage odour, aroma, taste and texture/Evaluation conducted without noseclip. According to the F-ratio of the product factor ANOVA: NS means "Non Significance"; *, **, *** indicate product effect at P<0.05, P<0.01, P<0.001, respectively. According to the Dunett test, a white star indicates a significant difference between each sample and the reference (P<0.05).*

The main finding of this additional experiment is that the taste modulation induced by vanilla flavouring follows the same direction as that obtained with the cocoa beverage in terms of sweetness enhancement and bitterness decrease but with a more powerful effect. Indeed the p-values of the sweetness increase induced by vanilla flavouring were 0.03 and 0.01 for the cocoa drink and coffee drink, respectively and the p-values of the bitterness reduction were 0.34 and <0.0001 the coca drink and coffee brew, respectively. These results reinforce our hypothesis that olfactory and taste integration is product dependent and related to food experience.

2.1.1.2 Coffee aroma is perceived differently by consumers and by trained panelists

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Coffee aroma is perceived differently by consumers and by trained panelists

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Summary

It is commonly accepted that coffee aroma can only be reliably described by experts or trained panels as coffee aroma results from a complex mixture of 20-40 key volatile compounds and their interaction. Without sensory training, it is unlikely that consumers can describe coffee aroma as precisely and consensually as a trained panel. But this does not mean that consumers are not able to detect and describe sensory difference between coffees. The aim of this study is to investigate the descriptive ability of consumers based on odour only (aroma above the cup) and to compare their description with those obtained by a trained panel. Odour description of eight different instant coffees was carried out by 1) 10 trained subjects according to the classical Quantitative Descriptive Analysis (scoring of the intensity of the different attributes of each product); 2) 40 coffee consumers using a sorting task (product grouping according to their similarities with a free description of each group). The analysis of sorting data showed that consumers grouped the eight coffees consensually but also differently from the trained panel. The present study highlighted a gap between sensory information collected from a trained panel and from consumers. This gap may be explained by differences between consumers and sensory panel in terms of evaluation approach, which may influence coffee perception. Indeed consumers have a holistic approach considering product sensory properties as a whole, which promotes the impact of cognitive processes (liking, interaction between senses, familiarity or expectation) on perception. On the contrary, trained panelists evaluate products with an analytical approach. They describe individually and independently each attribute of the sensory glossary following a procedure learnt during training. This training reduces therefore the impact of cognitive processes on perception.

Keywords: organoleptic properties, cognitive processes, sensory training, consumer

Résumé

Il est communément reconnu que l'arôme de café ne peut être décrit de manière fiable que par des experts ou un panel entraîné, l'arôme de café résultant d'un mélange complexe de plus de 20-40 composés d'arôme clé et de leurs interactions. Sans entraînement, il est peu probable que des consommateurs puissent décrire les arômes de café de façon aussi précise et consensuelle qu'un panel entraîné. Mais cela ne signifie pas que les consommateurs ne sont pas capables de détecter et décrire des différences sensorielles entre différents cafés. Le but de cette étude est d'explorer la capacité des consommateurs à décrire des cafés en se basant sur l'odeur uniquement et de comparer leur description à celle obtenue avec un panel entraîné. L'odeur de huit cafés instantanés a été caractérisée par 1) 10 sujets entraînés suivant la méthode classique de l'analyse descriptive quantitative (notation sur une échelle de l'intensité des différents attributs de chaque produit) ; 2) 40 consommateurs de café par la méthode de tri (groupement des produits en fonction de leurs similarités avec une description libre de chaque groupe). L'analyse des données de tri a montré que les consommateurs groupent les huit cafés de façon consensuelle mais qui diffère du panel entraîné. Cette étude a mis en évidence des différences entre les

Introduction

As for wine, it is commonly accepted that coffee aroma can only be reliably described by experts or trained panels. Coffee aroma is a complex mixture of 20-40 key volatile compounds and their interaction different volatile compounds and about 36 olfactory notes describing it are reported in "Le Nez du Café" (J. Lenoir, 2006). Without sensory training, it is unlikely that consumers can describe coffee aroma as precisely and consensually as a trained panel. But this does not mean that consumers are not able to detect sensory difference between coffees. On the contrary they can be very sensitive to aspect, olfactory, taste or texture properties. However the hedonic and cognitive aspects (interaction between senses, familiarity) may modulate consumer description whereas descriptive methodology used by a trained panel reduces the impact of cognitive mechanisms on perception.

Pioneer psychophysic studies demonstrated interactions between independent senses (Murphy et al. 1977, Murphy and Cain 1980). Especially, subjects attribute a taste to aqueous solution flavoured with an odorant. Indeed an odour can acquire a taste quality when the odour-taste pair is perceived in food commonly experienced by consumers. Sensory interactions are reported to result from associations experienced and memorized through food exposure without any explicit attention or learning (Köster *et al.*, 2004; Köster, 2005). Product familiarity also strongly modulates the strength of interactions and therefore perception (Labbe et al., 2006).

According to Prescott (1999) and Prescott, Johnstone and Francis (2004), the impact of cognitive mechanisms on perception depends on the exposure strategy applied to subjects. A synthetical exposure, encouraging subjects to evaluate, for instance, olfactory and taste stimuli as a unique flavour modality promotes olfactory/taste integration. Conversely, an analytical exposure, encouraging subjects to dissect their perception into independent sensory dimensions, limits interactions between senses.

The objective of the present study was to compare aroma grouping and description of a set of instant coffees between a group of consumers and a trained panel. To achieve this goal, aroma description of a same set of coffees was done by consumers and by a trained panel using methodologies mimicking best how they usually taste products: 1) a synthetical (or holistic) procedure for consumers, the sorting task with verbal description which consists in grouping samples according to their similarities and differences and in describing each group 2) an analytical method, the quantitative descriptive analysis, which is generally used by trained panel to specify the nature and the intensity of the sensory characteristics perceived when a product is evaluated (Stone, Sidel, Oliver, Woosley, & Singleton, 1974).

Material

The set of products consisted in eight soluble coffees varying in origin (X and Y) and aroma strength and balance (four conditions, 1, 2, 3 and 4 for each origin). In the present study, coffee samples will be therefore named according to these two factors i.e. from coffee X1 to X4 and from coffee Y1 to Y4.

Methodology

Sorting procedure with verbalisation task (holistic approach)

-Subjects

40 consumers used to drink black coffee participated to the evaluation

-Sensory procedure and tasting condition

During a first session, the panelists were informed about the principle of the sorting procedure. Within a second session, the panelists received the entire set of coffees at once. They were asked to sort the products served in cup at 65°C into groups having similar odour properties without tasting samples but only by smelling. No other recommendation was given, except that they had to make at least two groups. Then they were invited to describe their groups with their own vocabulary. Evaluation was conducted in an air-conditioned room (20 °C) and under white light in separate booths.

– Data analysis

The analysis of sorting data consisted in three steps (Cartier *et al.*, 2006):

a/ Product map using multidimensional scaling (MDS):

An individual binary dissimilarity matrix indicating whether two samples were grouped together was constructed for each panelist. The 40 individual matrices were summed and the resulting dissimilarity matrix was submitted to the classical metric multidimensional scaling MDS (Togenson, 1952) using NCSS software (Hintze, 2001).

b/ Product description using the vocabulary elicited to describe groups:

The vocabulary generated to describe the groups was used to build a contingency table for the products. Each term used to describe a group of samples was reallocated to each product of the group. We therefore assumed that all the products belonging to the same group could be described by the same terms. The resulting contingency table was then reduced and simplified: (1) Terms having similar meanings were grouped by the Sensory Analyst; (2) only terms having a quotation frequency higher or equal to 10% for the sample with highest elicitation rate were considered.

c/ Projection of product descriptions on MDS-map

The items describing products were projected on the MDS map using the correlation structure of product coordinates on the map and product descriptions in the contigency table.

Quantitative descriptive analysis (analytical approach)

-Subjects and training

A 10-subject panel used to evaluate coffee samples was used. The panelists received an updated training during 4 sessions on four odour attributes (overall intensity, roasty, earthy and fruity). Overall intensity and roasty attributes were proposed to the panel because they are prevalent criteria for coffee evaluation. Fruity and earthy were generated by the panel to completely cover the product space. During training each panelist had 1) to memorize the attributes and to detect them in coffee. References were presented for each attribute and simple discrimination tests were performed and 2) to rank products for each attribute and then to rate intensity levels on the scale. For each attribute, two products with two intensity levels (weak and strong) were presented.

-Sensory procedure and tasting condition

The eight samples were evaluated according to the quantitative descriptive analysis based on a randomized experimental design. Each sample was evaluated monadically using a 10 cm unstructured linear scale, anchored at the extremities with “not at all intense” and “very intense”. Data acquisition was realized on paper form generated by FIZZ software (Biosystemes, Couternon, France). Four samples were evaluated per session. Two sessions were therefore carried out. The presentation design, based on Williams Latin squares (Williams, 1949), balanced position and order effects. Samples were coded with 3-digit random numbers and served at 65°C in a 100-ml cup. Rinsing was done between samples with water and unsalted crackers. Tests were conducted in an air-conditioned room (20°C), under white light in individual booths.

– Data analysis

Firstly, data were submitted to two-way ANOVAs (origin and process aroma technology) on the product means (scores averaged across pnalists) to identify significant impact on coffee aroma description of the two factors, oigin and technology (differences were considered as significant at p-value= 0.05). Secondly, trained panel sensory attributes were projected on the MDS map resulting from consumer sorting using the correlation structure of product coordinates on the MDS map and product mean sensory profiles. Finally, a principal component analysis (PCA) was conducted on product means in order to obtain a sensory map, independent of consumers.

Results

Sorting task with sensory procedure

Figure 1 shows that consumers grouped consistently coffees according to their origin (axis 1) and also according to the aroma strengh and balance opposing conditions 1 and 2 to conditions 3 and 4 (axis 2). This map represents well the initial data, as shown by low stress (= 0.10), and high Pearson's correlation between actual and MDS dissimilarities (r= 0.93).

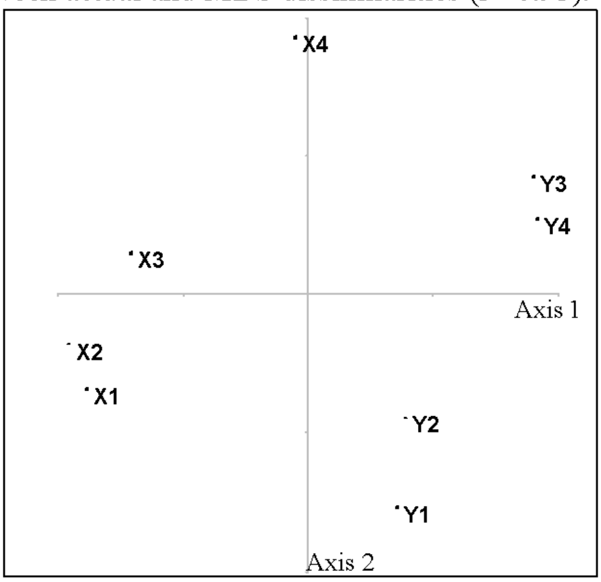

Figure 1: First factorial maps of MDS issued from sorting with consumers.

Projection of items that were elicited by at least 10% of consumers for a same sample showed a consistent textual characterisation among the panel (Figure 2). Coffees X were perceived “light” and coffee Y were perceived “burnt” and “roasted”. In addition coffees X1 and X2 were described as coffee, flat, mild and sweet and opposed to coffees Y3 and Y4 described as chemical, intense, bitter and acid. These results showed that consumers also used taste attributes (sweet, acrid, bitter) to describe olfactory perception.

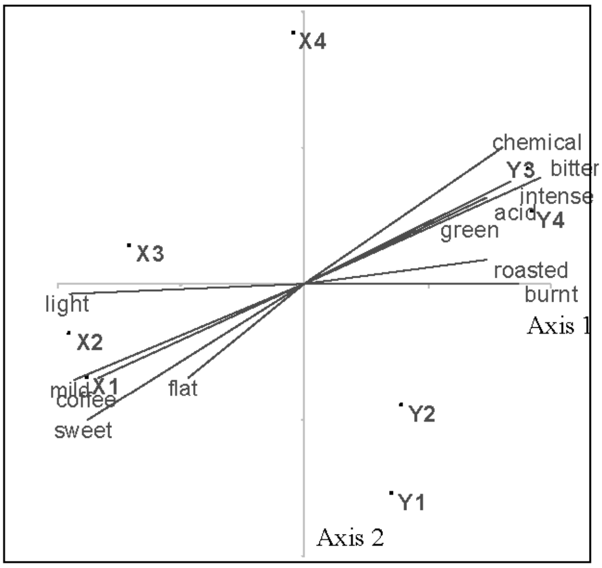

Figure 2: First factorial maps of MDS issued from sorting with consumers with projection of consumer items describing the products

Quantitative descriptive analysis

Projecting on the consumer map the four sensory attributes (underlined) based on QDA results (Figure 3) shows that trained panel description tended to account for only one dimension of the consumer space. Indeed three highly correlated attributes (overall intensity, roasted and earthy) differentiated aroma strength and balance conditions 3 and 4 and conditions 1 and 2 (two way ANOVAs p-values for aroma strength and balance factor of 0.03, 0.05 and 0.01 for overall, roasty and earthy attributes respectively). But trained panel attributes did not enable to discriminate samples according to the coffee origin (two way ANOVAs p-values for origin factor 0.20, 0.17, 0.59 and >0.99 for overall aroma, roasty, earthy and fruity attributes respectively). Fruity attribute is not well represented on the consumer map.

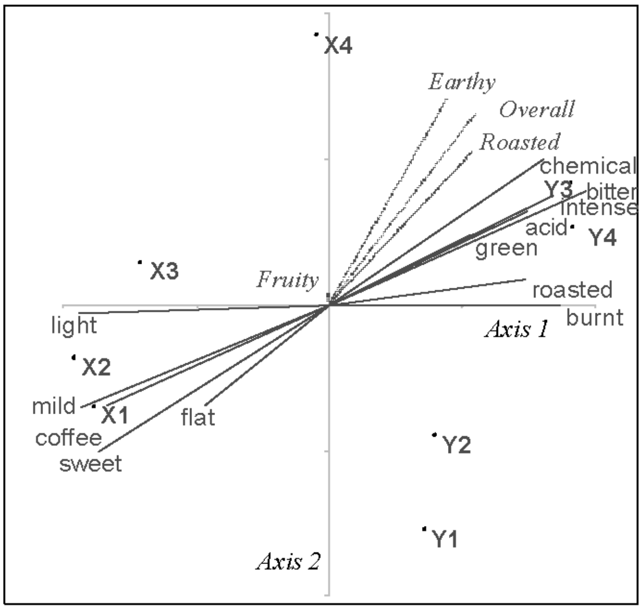

Figure 3: First factorial maps of MDS issued from sorting with consumers with projection of consumer items describing significantly the products and of trained panel attributes (italic font)

PCA of the trained panel scores (Figure 4) shows that, contrary to the consumers, origin was not the main factor of discrimination. X samples are central with very close sensory peoperties whereas Y samples are more scattered on the PCA map with larger different sensory differences. Even if fruity differences were not significant among Y samples (two way ANOVAs p-value for process aroma technology factor of 0.25), the fruity attribute had a tendancy to separate two couples of samples that were not discriminated by consumers: coffees Y1 vs. Y2 and coffees Y4 vs. Y3.

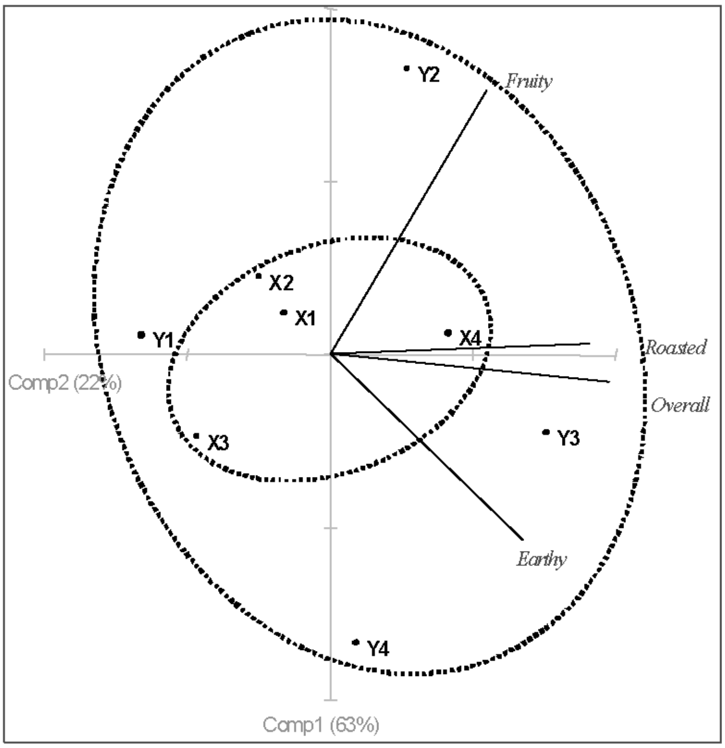

Figure 4: First factorial maps of PCA issued from quantitative descriptive analysis with trained panelists.

Discussion

Consumer description of aroma characteristics of the eight instant coffees differed largely from the sensory panel description. Indeed consumers distinguished coffees according to their origin (coffees X opposed to coffees Y) with a detailed description for each group. A different level of familiarity between the two coffee origins could explain this opposition.

For the trained panel, coffees X had no specific features (samples were close from each other and centred on the PCA map) and more diversity was perceived within coffees Y compared to consumers. Indeed the coffees Y were mainly differentiated in terms of fruitiness by the trained panel with a tendency to oppose samples Y1 and Y2 as well as the samples Y4 and Y3. Consumers did not consider this fruity characteristic, which seemed to be less important than other criteria to differentiate these coffees.

Within the X origin, consumers also differentiated the coffees according to the different technologies whereas the sensory panel did not. Preference may explain these differences especially in the present study where aroma differences, known to drive liking, were strong. Indeed pleasantness or rejection induced by coffee aroma may modulate perception.

Results of consumer verbalization showed that consumers used taste attributes to describe coffee aroma highlighting confusion between olfaction and taste and suggesting the role of perceptual associations acquired through food experience.

Our findings suggested that coffee aroma perception of consumer seems to be strongly modulated by hedonic and cognitive mechanisms. On the contrary for the trained panel, the impact of these factors may be reduced due to training where subjects learn to evaluate independently each sensory dimension (analytical strategy).

As a consequence of these differences, the common task of investigating the sensory drivers of consumer liking should always start with the holistic consumer liking to be explained using analytical sensory attributes (e.g. internal preference mapping) instead of investigating how analytical differences might influence consumer liking (e.g. external preference mapping).

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2.1.2 Impact of subthreshold olfactory stimuli on taste perception

2.1.2.1 Subthreshold olfactory stimulation can enhance sweetness.

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Subthreshold Olfactory Stimulation Can Enhance Sweetness

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Abstract

The impact of olfactory perception on sweetness was explored in a model solution using odorants at subthreshold concentrations. First, the impact of 6 odorants, previously described in the literature as congruent with sweetness, was investigated at suprathreshold level in a sucrose solution. Ethyl butyrate and maltol were selected as they had the highest and the lowest sweetness-enhancing properties, respectively. Second, the impact on sweetness of the 2 odorants was investigated at subthreshold concentrations. A system delivering a continuous liquid flow at the same sucrose level, but with varying odorant concentrations, was used. At a subthreshold level, ethyl butyrate but not maltol significantly enhanced the sweetness of the sucrose solution. This study highlights that olfactory perception induced by odorants at a subthreshold level can significantly modulate taste perception. Finally, contrary to results observed with ethyl butyrate at suprathreshold levels, at subthreshold levels, the intensity of sweetness enhancement was not proportional to ethyl butyrate concentration.

Key words: cognitive integration, familiarity, olfaction, psychophysics, taste

Introduction

Pioneer studies on interaction at a suprathreshold level between odor and taste perception using static sensory measurement (Murphy et al. 1977; Murphy and Cain 1980) or time–intensity evaluation (Cliff and Noble 1990; Bonnans and Noble 1993) showed that subjects attribute a taste to aqueous solutions flavored with an odorant. Retronasal olfactory perception can also be modulated by taste perception (Hort and Hollowood 2004).

Perceptual similarity between odorant and tastant in a mixture seemed to be a good predictor of taste intensity change (Frank et al. 1991). Indeed, an odor can acquire a taste quality when the odor–taste pair is perceived in food commonly experienced by consumers. Congruency is defined by Schifferstein and Verlegh (1996) as "the extent to which 2 stimuli are appropriate for combination in a food product." For instance, pineapple flavoring enhances perceived sweetness of a model solution (Prescott 1999). In a real food context, strawberry odor enhances whipped cream sweetness, whereas peanut butter does not affect sweetness rating (Frank and Byram 1988). Another study showed that vanilla flavoring enhances sweetness perceived by children and adults when added to milk (Lavin and Lawless 1998). Furthermore, Stevenson et al. (1999) reported that an odor can decrease taste intensity when the odor–taste pair is not congruent in the food. In their experiment, caramel odor, related to sweet taste, decreased sour taste intensity. Functional magnetic resonance imaging also provides evidence for the convergence of taste and olfactory stimuli in the lateral anterior part of the orbitofrontal cortex to produce flavor in humans (de Araujo et al. 2003).

Stimuli at a subthreshold concentration also have an impact on perception. Integration at a subthreshold level of congruent taste and olfactory stimuli presented orthonasally was demonstrated using a variant of the 2-alternative forced-choice method (Dalton et al. 2000). Indeed, the orthonasal olfactory threshold of benzaldehyde significantly decreased with the presence of a saccharin solution in mouth at a subthreshold concentration. The same experiment repeated with monosodium glutamate did not lead to any change in benzaldehyde sensitivity. As for olfactory/taste interaction at a suprathreshold level, interaction at a subthreshold level seems to occur only with familiar odorant/tastant pairs. These results about the impact of familiarity were confirmed by repeating the same experiment with another panel (Pfeiffer et al. 2005). In addition, the authors also highlighted that olfactory/taste interaction occurred when the olfactory stimulus was delivered retronasally. But in both cases (orthonasal and retronasal), integration with taste only occurred when stimuli were presented at the same time. The same authors also showed a lack of integration for the benzaldehyde/saccharin pair for 4 subjects. This result may be explained by a lack of familiarity with this taste/aroma pair. Delwiche and Heffelfinger (2005) also demonstrated an integration of odor and taste at a subthreshold level. Contrary to the results of Dalton and Pfeiffer, the authors showed that odor/taste integration is not dependent on familiarity. This study concludes that the impact of tastant and odorant is additive, regardless of the harmony of the taste/odor pair.

The goal of the present study was to investigate whether an odorant at a subthreshold level could enhance sweetness of a sucrose solution. The odorant was presented orally in a sucrose mixture clearly perceived as sweet. The aim of this protocol was to mimic everyday consumption of sweet food where synchrony of the odorant/tastant delivery is more likely to induce olfactory/taste integration (Pfeiffer et al. 2005). Indeed, there is a need for the food industry to extend understanding about the impact of olfactory/taste interaction on consumer perception.

Benzaldehyde, ethyl butyrate, furaneol, vanillin, maltol, and isoamyl acetate odorants were selected because of their reported enhancing properties on sweetness at a suprathreshold level (Lavin and Lawless 1998; Hollowood et al. 2002; Kato 2003; Baldwin et al. 2004; Cerf-Ducastel and Murphy 2004; Hort and Hollowood 2004).

Two experiments were designed to fulfill our objectives. The first experiment aimed at selecting the odorants having the highest and the lowest enhancing properties on sweetness at a suprathreshold concentration. The second experiment was carried out to quantify and compare the impact of the 2 selected odorants at a subthreshold level on the sweetness of a sucrose solution.

A liquid delivery system was used during the second experiment. Compared with standard in-cup tasting, this system continuously delivered a liquid flow with a constant sucrose concentration but with different odorant concentrations and without tasting interruption and sample change. Subjects were therefore less disrupted and influenced than with "cup tasting." This system prevented aroma evaporation during tasting and saved preparation time, even though the evaluation was conducted individually. The dilution error risk was also minimized. Hort and Hollowood (2004) explained that using the Dynataste system allowed to mimic beverage consumption over a realistic time period.

To avoid confusion we will use the term "odor" to refer to the orthonasal olfactory perception evaluated above the cup and "aroma" to refer to the retronasal olfactory perception (i.e., the organoleptic attribute perceptible by the olfactory organ via the back of the nose (NF ISO 5492 1995). The term "olfactory" regroups both odor and aroma perception.

Experiment 1: selection of 2 odorants having the highest and the lowest enhancing properties on sweetness among the 6 odorants tested at suprathreshold concentration

Subjects

Nine untrained students, 5 females and 4 males between 18 and 25 years old, were recruited for experiment 1. They had never participated in any sensory tasting, and no training session was conducted for this study.

Sample preparation

Mineral water (Vittel, France) with sucrose was used for odorant evaluation at a suprathreshold level for all solutions. As the aim was to obtain a solution perceived as sweet, a sucrose concentration of 5 g/l, and therefore, above the detection threshold was chosen according to Hong et al. (2005). We validated that this concentration was above threshold through a triangle test with 24 subjects (10 females and 14 males between 30 and 40 years old). The results showed that a 5 g/l sucrose solution prepared with Vittel water was significantly perceived as different from a pure Vittel water solution (P value < 0.0001). The triangle test was conducted using a nose clip to ensure that subjects discriminated samples based on the sweet taste of sucrose and not on a possible olfactory stimulation by the tastant as already reported by Mojet et al. (2004, 2005).

Each odorant (Sigma-Aldrich Chemie GmbH, Munich, Germany) was evaluated at low, medium, and high concentrations in the sweetened water solution. For each odorant, the 3 suprathreshold concentrations were defined on the basis of a preliminary tasting with 8 project members to obtain a perceptible 3-step enhancement of the olfactory note compared with the unflavored sweetened water (reference). This preliminary tasting conducted in pure Vittel water consisted for each compound in a ranking–scaling task of 8 odorant solutions on 2 attributes (overall odor intensity, overall aroma intensity). A linear scale whose extremities were defined as "not intense" and "extremely intense" was used. For each odorant, 3 concentrations (low, medium, and high) were selected according to the following sensory criteria: 1) the 3 concentrations for each odorant had clearly discriminable odor and aroma intensities and 2) for each level the selected concentrations of the 8 odorants were isointense. In addition, each solution was compared with pure water using a nose clip to ensure that the odorant did not induce any other perception than olfaction (taste, trigeminal, or texture). Odorant concentrations were as follows (ppm): benzaldehyde 10, 50, 100; ethyl butyrate 5, 10, 20; maltol 100, 500, 1300; furaneol 10, 75, 150; vanillin 100, 200, 400; isoamyl acetate 10, 50, 70. For all odorants, the highest concentration was still soluble in water (Chemfinder database, Cambridge Soft Corporation, Cambridge, UK, 2004). Sample preparation was carried out 1 h prior to evaluation using volumetric flasks with a magnetic stirring bar and covered with Parafilm M Barrier Film (Structure Probe, Inc., West Chester, PA).

Sensory procedure

For each odorant, four 30-ml samples (the unflavored sample and 3 flavored samples) were presented simultaneously. Samples were coded with 3-digit random numbers and served at room temperature in a 50-ml cup. The subjects assessed the samples in a predefined order, according to a replicated balanced experimental design over subject, and could retaste if needed. Each of the 6 sets of 4 samples was assessed during a 30-min session over 6 days. Due to technical constraints, the odorant order was not balanced over the 6 sessions and was the same for all subjects. The subjects ranked samples from the least to the most intense according to their odor, aroma, and sweetness intensity. Equality between samples was permitted. Vittel mineral water and unsalted crackers were used for rinsing between samples. Tests were conducted in an air-conditioned room (18 °C), under white light in individual booths. Data acquisition was carried out on a computer screen with FIZZ software (Biosystèmes 1990).

Data analyses

All data were analyzed using FIZZ software. For each attribute, the global difference between the 4 sums of ranks was tested using a nonparametric 2-way analysis on ranks (Friedman test, significance level $\alpha = 0.05$). For attributes showing significant differences, a multiple comparison test was applied (paired comparison with a Bonferonni adjustment with α=0.00833$(0.05/C_4^2)$, (Wolfe 1998).

Results

For each set of 4 samples, all odorants significantly enhanced odor (P value < 0.0001) and aroma (P value < 0.0001) compared with the reference. However, the olfactory impact on sweetness differed according to the odorant. Ethyl butyrate had the highest enhancing impact on sweetness, and maltol had the lowest enhancing impact on sweetness (See Figure 1a,b). They were therefore selected for the next step of the study. The magnitude of the sweetness-enhancing effect induced by the 4 other odorants was intermediate (benzaldehyde P value: 0.001, furaneol P value: 0.002, isoamyl acetate P value: 0.012, and vanillin P value: 0.520).

Experiment 2: odorant impact at subthreshold concentration on sweetness

Subjects

A new panel of 9 untrained subjects (6 females and 3 males between 18 and 25 years old) participated in parts A and B of the second experiment.

A. Determination of the lowest threshold value within the panel ([LTP]) for the 2 odorants selected from the first experiment

Sample preparation

Fifteen 1-l solutions of each odorant were prepared at room temperature with pure mineral water (Vittel, France) with odorant concentrations ranging from 0.1 to 5.94E-06 ppm for ethyl butyrate and from 2.5 to 1.5E-04 ppm for maltol. Each solution was prepared 1 h prior to the tasting.

Sensory procedure

The threshold of each subject for each odorant was determined in Vittel water using forced-choice ascending concentration series method of limit (ASTM Sensory Testing Methods 1991). For each odorant, the subjects performed a series of 15 three-alternative forced-choice discrimination tasks (3-AFCs). Each 3-AFC comprised 2 unflavored water samples and 1 flavored water sample. An ascending odorant concentration range was defined with a dilution factor of 2. The appropriate concentration range was determined for

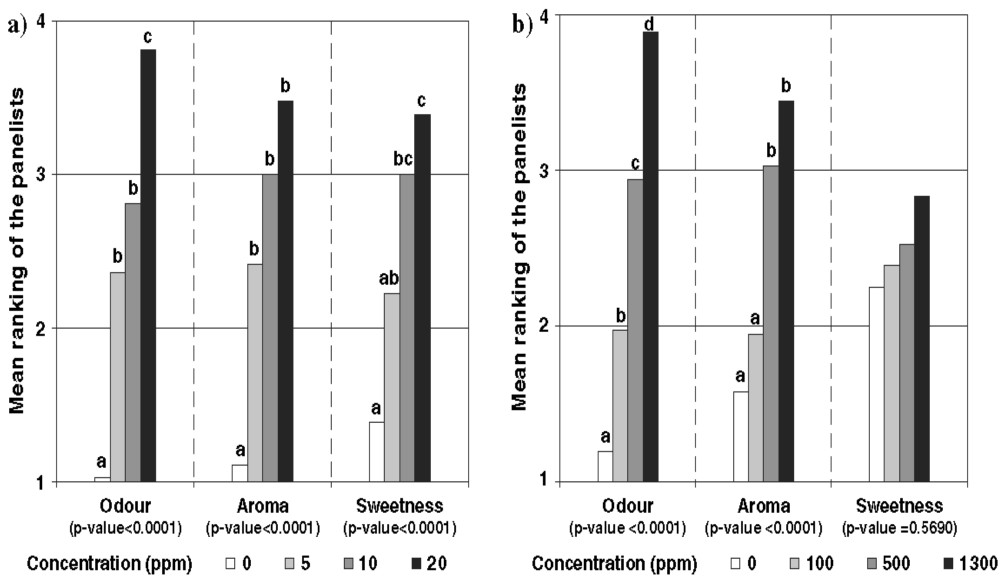

Figure 1 (a, b) Olfactory and sweetness modulation of aqueous sucrose solutions by a) ethyl butyrate and b) maltol. Samples with the same letter are not significantly different.

each odorant following benchscale preliminary trials including the range of threshold values reported in the literature. The 15 three-AFCs were evaluated in an ascending concentration order. The 30-ml solutions were tasted in plastic cups coded with 3-digit random numbers at room temperature in 50-ml cups. Samples were evaluated under the same conditions as previously described for experiment 1 with a rinsing between each of the 15 three-AFCs.

Data analyses

For each subject, the concentration above which all 3-AFC tests were correct was considered as the individual detection threshold concentration. However, when an incorrect response was given following at least 2 correct responses, the 15 three-AFC was repeated to obtain an unbiased threshold detection value. For each odorant, the lowest and the highest threshold concentration within the panel was defined and the geometric mean of individual threshold was calculated. The lowest individual threshold concentration within the panel [LTP] was chosen as a basis to determine the subthreshold concentrations evaluated in the next step of experiment 2. To prepare dilutions largely below any individual threshold, the 5 following concentrations were chosen: [LTP]/16, [LTP]/32, [LTP]/64, [LTP]/128, and [LTP]/256.

Results

The lowest and highest threshold concentrations within the 9 subjects were 1) 3.90E-04 ([LTP]) and 2E-01 ppm with a panel geometric mean of 1E-02 ppm for ethyl butyrate and 2) 1.21E-03 ([LTP]) and 1.25 ppm with a panel geometric mean of 1.24 ppm for maltol.

B. Investigation of the impact of odorant at a subthreshold level on sweetness intensity

Sample preparation (liquid delivery system)

To explore the impact of odorants at a subthreshold level on sweet perception, a liquid delivery system was developed, inspired by the Dynataste system of Hort and Hollowood (2004). The device was based on a programmable 4-channel preparative high-performance liquid chromatography pump (Merck-Hitachi, L 7150) and four 1-l reservoirs. The 4 reservoirs were linked to the high performance liquid chromatography (HPLC) mixing chamber, and the mixing chamber was linked to the subject's mouth with Teflon tubing. One reservoir (A) contained an aqueous sucrose solution, and the 2 other reservoirs (B and C) contained the same aqueous sucrose solution as reservoir A but with odorant at 2 concentrations. As explained by Hort and Hollowood (2004), by programming the flow rate of each pump, the composition of the delivered liquid can vary over time but the overall flow rate remains constant. In the present study, the device delivered online a solution with a constant in-mouth flow rate, thereby avoiding any variation of in-mouth tactile stimulation and a constant sucrose level but odorant concentration varied. The programming over time of the contribution of each channel to the final liquid flow delivered a solution with a 25-ml/s flow rate alternatively flavored and nonflavored. The 5 odorant concentrations were distributed into 2 delivery sequences as described in Table 1. The first sequence was divided into 6 steps (3 flavored and 3 unflavored), and the second sequence was divided into 4 steps (2 flavored and 2 unflavored stimuli) with a total of 10 steps for the 2 sequences. This flavored and unflavored liquid alternation ensured that tubing was rinsed between each odorant concentration delivery and therefore limited sensory adaptation. Each of the 10 steps was delivered for 18 s. Total duration of the 2 sequences was, therefore, 180 s.

Compared with the cup tasting in experiment 1 carried out for the odorant selection, the sucrose concentration of the liquid was increased from 5 to 15 g/l. This was because the subjects did not consider the stimulus as sweet enough during the familiarization sessions as, with this dynamic system, they had to swallow regularly and could not keep the liquid in mouth or retaste the samples.

Sensory procedure

For each of the odorants (ethyl butyrate and maltol), the 9 subjects evaluated during 180 s sequences 1 and 2 with a 180 s break between each sequence. The total duration of one session (sequence 1 and sequence 2) including the break was 360 s. The amount of liquid swallowed during a session

Table 1 Contribution over time of each channel of the delivery system to the in-mouth liquid flow

Stimuli n°	Delivery timing (s)	Odorant concentration	Contribution of each sucrose solution reservoir to the in-mouth liquid flow (%)		
			Reservoir A	Reservoir B	Reservoir C
			Sequence 1		
1	0–18	0	100		
2	18–36	[LTP]/256	50		50
3	36–54	0	100		
4	54–72	[LTP]/64	75	25	
5	72–90	0	100		
6	90–108	[LTP]/16		100	
			Break (180 s)		
			Sequence 2		
7	72–90	0	100		
8	90–108	[LTP]/32	50	50	
9	108–126	0	100		
10	126–144	[LTP]/128			100

Reservoir A: pure 1.5% sucrose solution; Reservoir B: 1.5% sucrose solution + odorant [LTP]/16; Reservoir C: 1.5% sucrose solution + odorant [LTP]/128.

based on a 25-ml/min flow rate was 75 ml. Each session was duplicated. A total of 4 sessions per judge was therefore conducted for the evaluation of the 2 odorants. To avoid contamination between odorants inside the pump tubing, the session order was not randomized between subjects. All subjects started with the evaluation of ethyl butyrate. To standardize among the 9 subjects the liquid delivery in mouth and swallowing, subjects were trained to pinch the Teflon tube extremity between the top and bottom incisors with 1-cm tube into the mouth. The subjects were invited to swallow regularly and normally. They scored over time, on a 11-box scale anchored at the extremities "not sweet at all" to "very sweet," the sweet taste intensity at 10 time points corresponding to ten 18-s steps. A computerized FIZZ session was coupled to the HPLC pump and synchronized with the appearance on PC screen of the sweetness scale 10 s after the beginning of each step. This time period took into account the 3 s needed for the pump to make the mixing and deliver the required concentration and the 7 s allowing subjects to experience and evaluate the stimulus before scoring it. The subjects could then score the sweetness intensity during the 8 remaining seconds. During evaluation, subjects were also asked to report on a sheet of paper any perception other than sweetness (olfactory or gustatory). Moreover, a debriefing session was carried out at the end of the second experiment to collect general comments of subjects. After exploring the impact of the 2 odorants, an additional session was conducted to validate that the sweet enhancement was not due to the device or the procedure. The sweetness intensity of an unflavored sucrose solution (15 g/l) was scored over time at 10 different points.

Before evaluation sessions, subjects were familiarized with the device and protocol during 2 sessions and especially with the constant flow of liquid into the mouth and the scoring procedure. This familiarization was carried out with unflavored sucrose solution only.

Data analyses

For each of the 5 odorant concentrations ([LTP]/16 to [LTP]/256), sensory data were transformed according to the formula $SC_n = SO_n - S_n$, where SC is the sweetness change, SO the sweetness of sucrose solution with odorant, and S the sweetness of sucrose solution without odorant evaluated before SO (see Figure 2). A confidence interval at 95% was calculated for the 5 SC panel mean scores. For the familiarization test with sucrose only, perceived changes in sweetness were also calculated and plotted as described above.

Results

Figure 3 shows that all subthreshold concentrations of ethyl butyrate significantly increased the perceived sweetness of the sucrose solution. In addition, sweetness enhancement by ethyl butyrate was constant, whatever the odorant concentration. Maltol did not consistently modify perceived sweetness (See Figure 4). The lowest concentration significantly enhanced sweetness, whereas the highest concentration significantly reduced sweetness. Intermediate concentrations 2, 3, and 4 did not significantly modify sweetness.

Changes in sweetness induced by subthreshold levels of ethyl butyrate and maltol are detailed for the different subjects in Figure 5. Seven subjects out of 9 (See bottom right part of the Figure 5) perceived the sucrose solution more frequently sweeter with ethyl butyrate than with maltol. Subject 6 found an equivalent high enhancement of sweetness with both ethyl butyrate and maltol. Sweetness perception of subject 1 was weakly enhanced by both odorants.

The evaluation of the sucrose solution without odorant addition conducted at the end of the study validated that sweetness enhancement was not due to the device and procedure used. As expected, results showed that perceived sweetness did not significantly differ over 10 consecutive evaluations (See Figure 6).

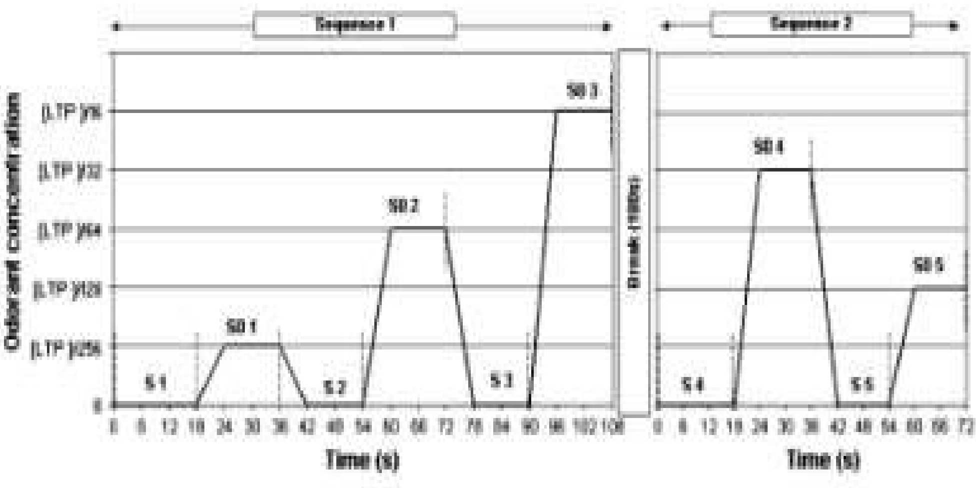

Figure 2 Schematic representation of the liquid flow evaluated over time. [LTP]: lowest threshold of the panel (3.90E-04 ppm for ethyl butyrate and 1.21E-03 for maltol); S: sweetness of sucrose solution without odorant; SO: sweetness of sucrose solution with odorant.

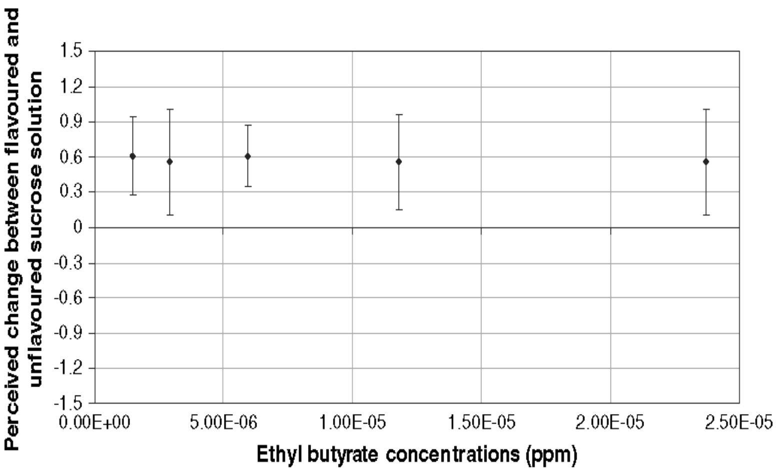

Figure 3 Effect of subthreshold ethyl butyrate concentrations on perceived sweetness of a 15 g/l sucrose solution. 95% confidence interval of means.

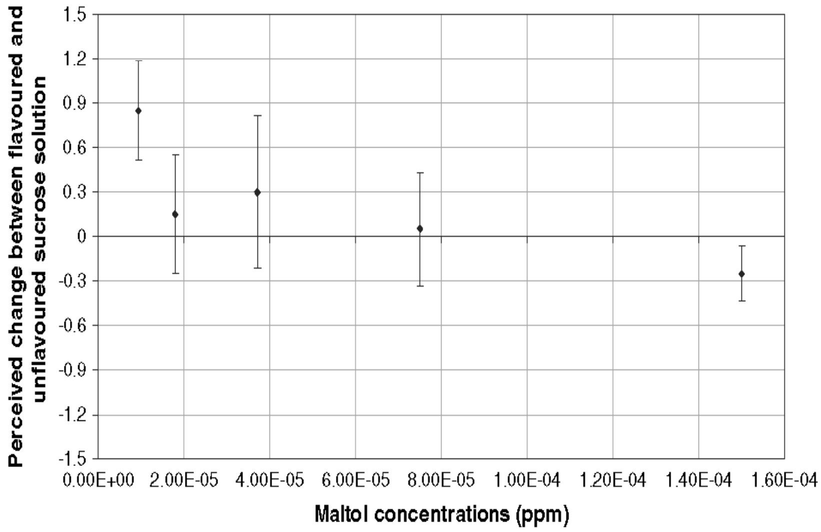

Figure 4 Effect of maltol concentrations on perceived sweetness of a 15 g/l sucrose solution. 95% confidence interval of means.

Discussion

Impact of odorant at a suprathreshold level on sweetness

Results obtained for all odorants at suprathreshold levels highlighted a clear consensus and discrimination of the 4 odorant concentrations regarding their odor and aroma intensity. Our results were partially in agreement with previous studies that reported the sweetness-enhancing properties of the 6 odorants tested. Ethyl butyrate, often described in the literature as having a strawberry note (Miettinen et al. 2004), seemed, in the present study, to be the odorant the most consistently associated with sweetness by the panel. Furaneol, isoamyl acetate, and benzaldehyde also significantly enhanced sweetness of the sucrose solution. Vanillin and maltol did not significantly boost sweetness even though these 2 compounds have already been reported to enhance sweetness. Indeed, Lavin and Lawless (1998) showed that vanilla flavoring, which has olfactory characteristics close to those of vanillin, enhances milk sweetness compared with plain milk. Kato (2003) showed that the maltol generated in roux (wheat flour and butter mixture) during heating enhances sweetness. These 2 studies were conducted in food, whereas in the present study, the odorant/tastant pairs were evaluated in water. The association between sweet taste and olfactory notes induced by vanillin and maltol may not be strong enough in a liquid system compared with more texturized food where these tastant/odorant pairs are usually experienced. Our hypothesis is in agreement with recent studies showing that odor/taste interaction results from associations experienced and memorized implicitly through food exposure (Köster et al. 2004; Köster 2005) and that food familiarity strongly modulates olfactory/taste interactions (Labbe et al. 2006). The role of food experience on sensory interaction at suprathreshold level has also been demonstrated at a neural level using neuroimaging (Small et al. 2004). Brain activation representing olfactory/taste interaction depends on the subject's previous experience with smell/taste combinations.

Impact of odorant at a subthreshold level on sweetness

The second part of the study was carried out with another panel of untrained subjects to avoid any bias induced by the first experiment. Indeed, previous odorant/sweet taste coexposure with odorant at a suprathreshold level may reinforce odor/taste cognitive association and therefore enhance the odorant's impact on taste perception (Labbe et al. 2006).

All subthreshold concentrations of ethyl butyrate enhanced sweetness, whereas maltol induced a significant enhancement for 1 concentration out of 5 only. Our results with maltol partially agree with Bingham et al. (1990), who, using a triangle test, showed that a subthreshold concentration of maltol (15 ppm) did not significantly change lemonade flavor perception. Sweetness enhancement by ethyl butyrate was neither due to the device nor due to the protocol. Consequently, 2 hypotheses can be proposed to explain this observation. First, sucrose enhances odorant release into the headspace due to physical–chemical interaction and led to a suprathreshold level of odorant. This may result in a sweetness scoring increase because of a perceptual olfactory–taste interaction or a dumping of olfactory perception on the sweetness scale (Frank et al. 1993; Clark and Lawless 1994). This hypothesis is unlikely for 3 main reasons: 1) Nahon et al. (1998) demonstrated that release of ethyl butyrate present in orange aroma was not enhanced by sucrose (at a concentration similar to that used in the present study) compared with the aqueous control, 2) in the present study, subjects did not report any perceived olfactory notes during evaluation with the liquid delivery system, and 3) 2 replicated triangle tests were performed by a 24-subject panel (10 females and 14 males between 30 and 40 years old) to check by sniffing the impact of the highest subthreshold ethyl butyrate concentration (3.2 ppm) on olfactory perception. Tests were conducted in water (comparing an unsweetened Vittel water solution with an unsweetened flavored Vittel water solution) and also in sweet water (comparing an unflavored 15 g/l sucrose solution with a flavored 15 g/l sucrose solution) to highlight any odorant/taste interactions. Each triangle test result showed that samples were similar at a 10% significance level (β), where similarity was defined as a maximum of 20% of assessors recognizing the difference. Therefore, at the concentrations used, ethyl butyrate did not induce any orthonasal olfactory stimulation.

Our second hypothesis is that ethyl butyrate enhances sweetness at subthreshold level through perceptual integration. This hypothesis probably explains our phenomenon

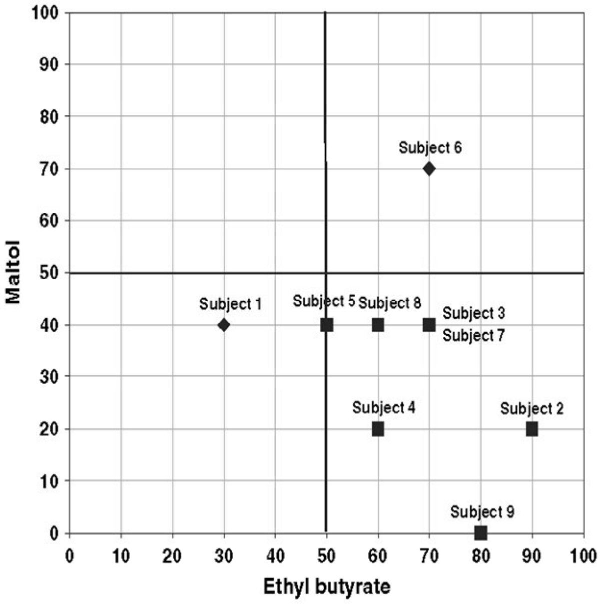

Figure 5 Percentage of times each subject judged the solution with odorant sweeter than the pure sucrose solution for maltol (vertical axis) and for ethyl butyrate (horizontal axis). $N = 10$ (5 concentrations × 2 replications).

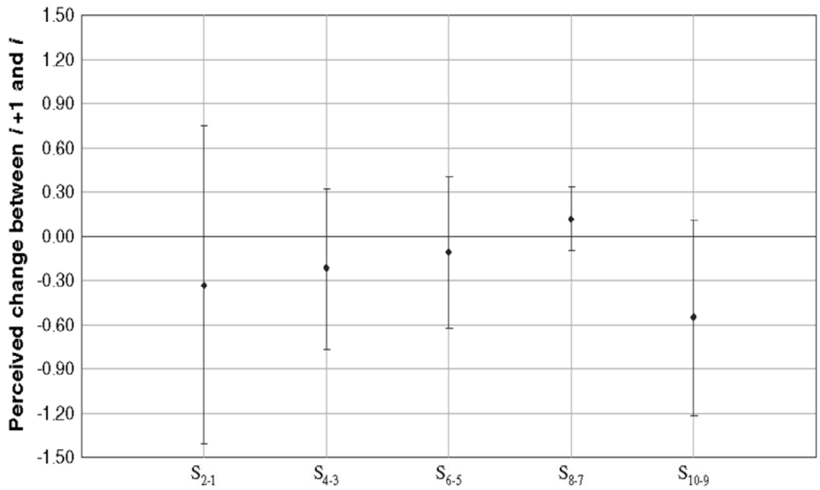

Figure 6 Sweetness evaluation of the unflavored sucrose solution over time (same calculations and representations as for Figures 4 and 6). 95% confidence interval of means, S_{i+1-i} account for sweetness score difference between evaluation i+1 minus evaluation i.

and supports previous findings by Dalton et al. (2000) and Pfeiffer et al. (2005).

Results obtained with ethyl butyrate and maltol at subthreshold levels were consistent with those at suprathreshold levels: significant effect of ethyl butyrate and nonsignificant effect of maltol on sweetness in both cases. Even if ethyl butyrate and maltol were both congruent with sweetness, the level of familiarity for ethyl butyrate/sucrose compared with maltol/sucrose pairs might explain the higher impact of ethyl butyrate on sweetness. Indeed, congruency between taste and olfaction has been reported to influence olfactory/taste central integration even with subthreshold concentrations of odorants (Dalton et al. 2000; Pfeiffer et al. 2005).

Our results showed that 2 of the 9 subjects were not consistent with the panel. Subject 6 showed considerable taste enhancement both with maltol and with ethyl butyrate. This subject might be as familiar with ethyl butyrate/sucrose association as with maltol/sucrose association. In contrast, subject 1 showed a weakly enhanced sweetness perception for both odorants. He/she might be less familiar with these odorant/tastant associations. Another explanation for differences between these 2 subjects may be sensitivity. Because thresholds varied widely among subjects, the extent to which the stimulus was below subthreshold also varied very much. To better assess the impact of subject odorant sensitivity on sweetness enhancement, the ratio between subthreshold stimulus concentration and individual threshold was calculated and plotted against individual sweetness enhancement. Figure 7 shows the results for the above-mentioned subjects (1, 6). The level of the subject's odorant threshold cannot explain differences in the sweetness enhancement between subjects. Indeed, for subject 1, the concentrations of ethyl butyrate used were closer to his/her threshold than the concentrations of maltol (10 000 times below threshold), but sweetness enhancement was weak for both odorants. For subject 6, both odorants strongly enhanced sweetness even though the concentrations of maltol and ethyl butyrate were largely below the subject's threshold values (1000 and 10 000 times lower for maltol and ethyl butyrate, respectively). Based on these observations, the ratio subthreshold

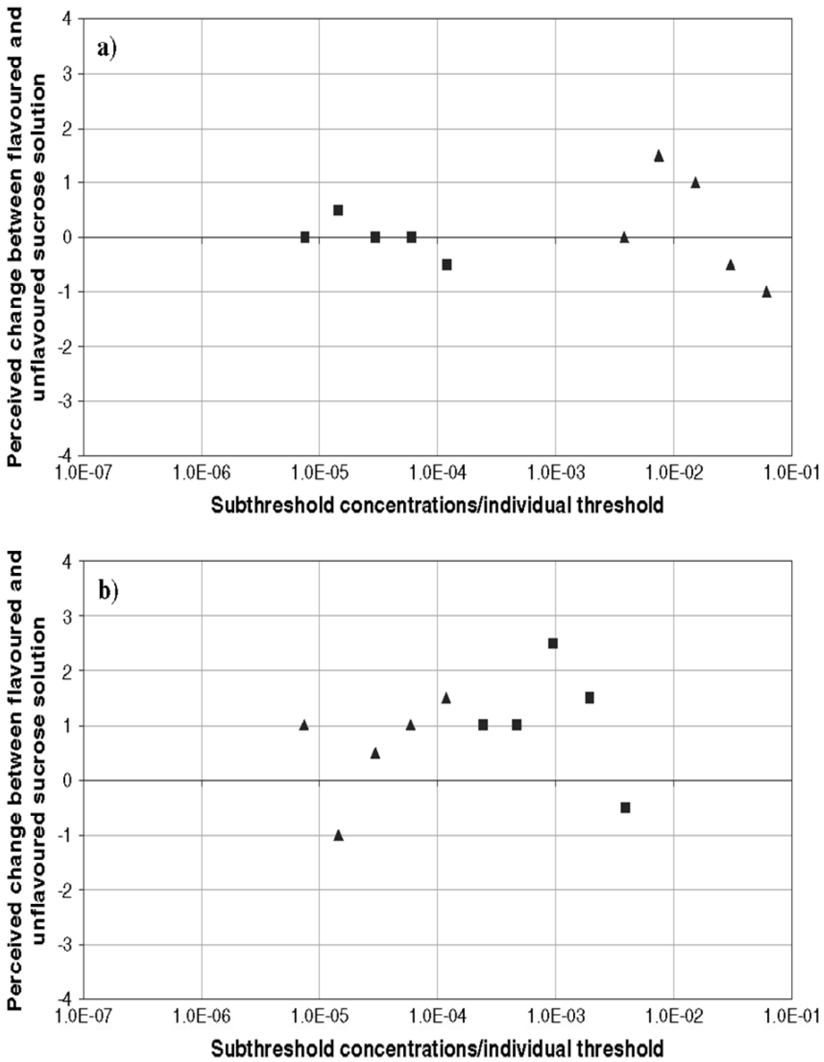

Figure 7 (a, b) Effect of the ratio subthreshold odorant concentration (5 concentrations × 2 replications)/individual threshold on the individual sweetness enhancement for ethyl butyrate (▲) and maltol (■). (a) subject 1, (b) subject 6.

concentration/individual threshold seems to have little impact on sweetness modulation. On the other hand, familiarity might explain the intersubject differences. Pfeiffer et al. (2005) also highlighted an absence of integration between saccharin taste and benzaldehyde odor for 4 out of 16 subjects. The authors assumed that these subjects were not familiar with the benzaldehyde/saccharin pair.

At subthreshold levels, the boosting impact of ethyl butyrate on sweetness does not seem to depend on its concentration: the sweetness enhancement was not proportional to ethyl butyrate concentration. This outcome suggests that odorant stimulation at subthreshold level led to an "on–off" taste modulation, which is different from taste modulation by an odorant at suprathreshold level. Conversely, results obtained in experiment 1 and reported in the literature on taste modulation by an odorant at suprathreshold level (Labbe et al. 2006) showed that sweetness enhancement was proportional to odorant concentration: the sweetness increased with increase in odorant concentration.

The main finding of the present study was that sweetness of a sucrose solution can be enhanced by subthreshold levels of an odorant. Given these results, it would be interesting to investigate the level of sucrose reduction that can be compensated by odorant addition while maintaining sweetness. In addition, the liquid delivery system should be improved in order to deliver 1) greater randomization within odorants, subjects, and sessions and 2) the same ratio between subthreshold concentration and individual threshold for each subject.

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2.1.2.2 Impact of novel odorants at supra and subthreshold level on sucrose perception further to an experimental implicit associative learning

Labbe,D., & Martin,N. to be submitted to Chemical Senses

Abstract

The impact of co-exposure to a novel odorant and sucrose on the construction of perceptual interactions was explored. The first objective was to validate the absence of associative learning when a group of subjects were co-exposed following a sensory profiling training approach compared to a group of subjects exposed according to a synthetical approach. The second objective was to explore the impact of the odorant at subthreshold level on sweetness further to the implicit associative learning. Sweetness of the sucrose solution was increased by the odorant only when scored by the group co-exposed according to the synthetical approach. We confirm that co-exposure following a sensory profiling training did not promote implicit learning likely because this approach encouraged subjects to consider sensory dimensions analytically. At subthreshold level, the odorant does not impact perception of the sucrose solution whatever the co-exposure approach. The potential role of neural integration processes and plasticity in these results is discussed.

Keywords: Olfaction, taste, Perceptual interaction, Associative learning, Suprathreshold, Subthreshold, neural

Introduction

Flavor is defined a s a complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting (ISO 5492, 1995). Regarding perceptual interactions involved in flavor perception, the most commonly reported interactions are between olfactory and sweet taste perceptions. First, the impact of strawberry odorant on sweetness was highlighted in a sucrose solution (Frank et al., 1989) , then, other odorants were also found to enhance sweetness of a sucrose solution, for example pineapple and raspberry (Prescott, 1999), maracuja and caramel (Stevenson et al., 1999). Such odorants are generally present in sweet food, these odorant stimuli are therefore congruent with sweet taste stimulus. Congruency between sensory qualities is a crucial factor for perceptual interaction between senses. Congruency is the extent to which two stimuli are appropriate for combination in a food product (Schifferstein, 2006). Studies showed that odorants incongruent with sweet taste do not increase sweetness contrary to congruent odorants (Stevenson et al., 1999; Djordjevic et al., 2004). The scientific community agrees that different sensory stimuli become congruent when conjointly and repeatedly experienced through everyday food consumption. Perceptual interactions result therefore from an implicit associative learning between sensory modalities (Koster, 2005; Koster et al., 2004).

The construction of perceptual interactions through implicit associative learning was also experimentally demonstrated by repeated exposures to a novel odorant in solution with sucrose or citric acid tastants (Stevenson et al., 1995). Further to the odorant and tastant co-exposure, the odorant acquires the taste property of the co-exposed tastant, sweet or sour. This implicit associative learning leading to olfactory-taste interactions was described as 'learned synthestesia' (Stevenson and Boakes, 1998). Results from a further work showed that: 1) pairing a new odorant with. sucrose leads to an enhancement of sweetness evoked by odorant sniffing; and 2) saltiness of a MSG and NaCl mixture decreases after odorant-sucrose paring procedure (Yeomans et al., 2006). The attentional strategy during exposure is an additional important factor influencing construction of perceptual associations. Prescott et al. (2004) compared the impact of two different co-exposure procedures on the construction of perceptual associations between a novel olfactory stimulus and sweet taste both at suprathreshold level. Each task encouraged subjects to consider olfactory and taste sensory dimensions either analytically or synthetically. Comparing post and pre-exposure results, the synthetical exposure strategy group rated the flavored sucrose solution sweeter than the unflavored sucrose solution but not the analytical exposure strategy group. However, another study showed that the construction of perceptual association may occur further to a sensory profiling training, a procedure still encouraging an analytical attentional strategy (Stevenson and Case, 2003). In the latter study, training consisted in differentiating the sensory dimensions during tasting of an odorant mixed with sucrose or citric acid in solution. All subjects also rated their liking of the solutions and according to Prescott et al. (2004), who discussed previous results from Stevenson and Case (2003), liking rating may have encouraged subjects to consider synthetically the olfactory and taste sensory dimensions what may explain why perceptual associations were built. The first objective of this study was to validate that the construction of perceptual associations between a novel odorant and sucrose is limited when co-exposure to the odorant and tastant mixture follows a sensory profiling training procedure, without hedonic evaluation.

A few studies focused on olfactory and taste interactions, when both odorant and tastant are presented at a subthreshold level (Dalton et al., 2000; Pfeiffer et al., 2005). Firstly, the authors explored the impact of an in-mouth saccharine solution (sweet tastant) at a subthreshold concentration on benzaldehyde threshold determination (a volatile compound generally described as having an almond-like odor). The olfactory threshold of benzaldehyde significantly decreases with the presence of the saccharine solution in mouth. In addition, Labbe et al. (2006) showed that sweetness rating of a sucrose solution is increased by a simultaneous retronasal olfactory stimulation by subthreshold ethyl butyrate odorant, commonly described as having a strawberry-like odor. More recently, Miyazawa et al. (2008) demonstrated that subthreshold concentrations of acetic acid increase the perceived retronasal olfactory intensity of three coffee aroma volatile compounds presented at suprathreshold level. The second objective of the present study was to explore whether after implicit associative learning between an odorant and a sweet tastant, odorant had an impact on sweetness when presented at subthreshold level.

Experiment 1: impact of two implicit associative learning procedures on construction of perceptual interaction with stimuli at suprathreshold level

Material and methods

Odorant selection

Two commercial odorants, elderflower (product code CD95904) from Givaudan S.A. (Dübendorf, Switzerland) at 1200 ppm and cactus (product code 505898 A) from Firmenich (Geneva, Switzerland) at 350 ppm were selected among twelve odorants during a preliminary study conducted with eleven subjects. The subjects were asked to taste and swallow the odorant solutions and to score familiarity induced by the odorants on a 10cm scale anchored at the extremities from the left to the right with "Not at all familiar" and "Extremely familiar". Odorants were evaluated in Vittel water solution with and without 7% sucrose. Among the twelve odorants, elderflower and cactus odorants were scored lower in familiarity (+/-standard error) in unsweetened water (3.8 +/-1.5 and 4.9 +/-1.5, respectively) and in a 7% sucrose solution (3.8 +/-1.6 and 3.7 +/-1.9, respectively).
An unflavored sucrose solution at 7% was also prepared. All one-liter solutions were prepared each morning prior to the test and stored at room temperature (22°C) until use.

Subjects and procedure

Twenty four naïve women between 40 and 45 years old took part in the study. Subjects were previously selected for normal olfactory and taste acuity based on the procedure ISO 8586-1 (1995).
The pre-exposure session (PRE) and the post-exposure session (POST) were conducted by all assessors and consisted in scoring: 1) sweetness of the two flavored unsweetened solutions, the two flavored sucrose solutions and the unflavored sucrose solution (which was replicated); and 2) familiarity (by smelling) and retronasal olfactory intensity of the two flavored unsweetened solutions.
The group of twenty four assessors was then split randomly into two groups of twelve, each group being co-exposed to sucrose with one of the two odorants. Within each group of twelve assessors: 1) six assessors were co-exposed following an analytical attentional strategy (ANA), i.e. sensory profiling training; and 2) six assessors were co-exposed following a synthetical attention strategy (SYN), i.e. triangle test.

Three exposure sessions lasting one hour were carried out for ANA and SYN groups using four solutions obtained by four successive dilutions of the flavored sucrose solution evaluated in PRE and POST with a dilution step of 1.2. The aim was to limit boredom by presenting solutions with different olfactory and taste intensities. However solutions had the same ratio of odorant and sucrose concentration to limit changes in the sensory olfactory and taste balance which could facilitate the dissection of the two sensory dimensions. Each of the four solutions was presented three times during each exposure session. Subjects were not informed about the replications of the three solutions. At the end of the exposure phase, assessors from ANA or SYN groups were exposed to the same number of times and volume of olfactory and taste stimuli, i.e. thirty-six flavored sucrose solutions of 50 ml with a constant odorant-sucrose ratio.
The odorant evaluated in PRE and POST was named either: 1) TEST when co-exposed with sucrose during the exposure phase; and 2) CONTROL when not co-exposed with sucrose during the exposure phase. Each of the five sessions, i.e. the PRE, POST and the three exposure sessions, were conducted on five separate and consecutive days (See Table 1).

Table 1: Steps and duration of experiments 1 and 2

Day 1	Days 2-3-4	Day 5	Days 6-7	Days 8-9	Day 10
Pre-exposure	Exposure	Post-exposure	Break	Odorant threshold determination	3 AFC tests
EXPERIMENT 1				**EXPERIMENT 2**	

- Sensory profiling training exposure (ANA)

The training aimed at promoting analytical attentional strategy during the co-exposure since assessors were encouraged by this procedure to consider independently olfactory and taste related perceptions. The six assessors exposed to cactus odorant and the six assessors exposed to elderflower odorant conducted the exposure sessions separately. The first session of the exposure consisted in sniffing and tasting the twelve flavored sucrose solutions and in describing with their own vocabulary the olfactory and taste characteristics of the solutions. During the second session the attribute list was reduced by removing redundant and confusing attributes (ISO 11035, 1995). The assessors tasted again the twelve flavored sucrose solutions and selected for each solution the attributes they considered as relevant. At the end of this session, sweetness and two attributes related to olfactory perception were kept for the third session. Finally the last training session consisted in a series of ranking tests. The four sucrose flavored solutions were ranked from the least to the most intense for each of the three attributes.

- Triangle test exposure (SYN)

The aim of conducting triangle tests was that the subjects were encouraged to acquire a synthetical attentional strategy so that they integrated olfactory and taste stimuli as a whole perception, namely flavor perception. Indeed subjects were asked to pick the odd sample based on overall perception and not to focus independently on each sensory dimension. The twelve assessors conducted a series of four triangle tests during each of the three sessions. For each of the three sessions, the six assessors exposed either to the cactus odorant or to the elderflower odorant carried out simultaneously the triangle tests in booths. Within each triangular test, three identical flavored sucrose solutions were presented. Each of the four triangle tests was conducted with one of the four flavored solutions previously described. The same four triangle tests were repeated during each of the three sessions.

Tasting conditions

Solutions were coded with three-digit random numbers and 50-ml portions were served in 100-ml plastic cups. Assessors were asked to sip and swallow the solutions. Rinsing was done between products with water and unsalted crackers for the PRE and POST evaluations and between each triangle test for the SYN group exposure sessions. For PRE and POST evaluations, the six samples (the two flavored unsweetened solutions, the two flavored sucrose solutions and the two unflavored sucrose solutions) were presented according to a presentation design, based on Williams Latin squares, balancing position and order effects. The design was identical for both evaluations. For each SYN group exposure session, the four triangle tests were presented in the same order within each group of six assessors being exposed to the same odorant. Data was acquired on a computer screen with FIZZ software Version 2.20E (Biosystemes, Couternon, France) for the PRE and POST evaluations and during the SYN group exposure. The same 10 cm scales as those described for the odorant selection were used for the PRE and POST exposure evaluations. Tests were conducted in an air-conditioned room (22°C), under white light in individual booths.

Statistical analyses

- *Unsweetened flavored solutions*

The objective was to determine if odorant and sucrose co-exposure impacted 1) odorant familiarity when the solution was sniffed; and 2) retronasal olfactory intensity and the sweetness evoked by the odorant when the solution was tasted. For each attribute, individual scores obtained in PRE were subtracted to individual scores obtained in POST (POST-PRE) within each odorant category (TEST and CONTROL) and within each exposure type (ANA and SYN). A positive value means that exposure induced an increase of the attribute intensity, a negative value indicates that exposure induced a decrease of the attribute intensity and a value close to zero means that exposure did not change perception.
An Odorant (TEST, CONTROL) x Attentional strategy (ANA, SYN) analysis of variance (ANOVA) with interactions was performed on POST-PRE scores to explore the impact of both factors on familiarity, aroma intensity and sweetness.

- *Sweetened flavored solutions*

The objective was to investigate if further to the exposure stage, the odorant impacted sweetness of the flavored sucrose solution. This was conducted in three steps. Step 1: the sweetness mean of the two unflavored sucrose solutions was calculated per assessor and for each stage (PRE, POST). Each value was used as a sweetness baseline for each stage. Step 2: the ability of the odorant to modulate sweetness was measured for each

period, each subject and each odorant by subtracting the sweetness of the unflavored sucrose solution calculated in step 1 from the sweetness of the flavored sucrose solution (called relative sweetness score). Step 3: the individual relative sweetness score calculated in PRE was subtracted from the individual relative sweetness obtained in POST (POST-PRE).
An odorant category (TEST, CONTROL) x attentional strategy (ANA, SYN) analysis of variance (ANOVA) with interactions was calculated to explore the impact of both factors on sweetness as defined in step 3.

Analyses of Variance (ANOVA) were calculated, using NCSS software version 2007 (Number Cruncher Statistical Systems, Karysville, Utah, USA.). Post-hoc pair comparisons were conducted by a Student t-test. Confidence level was set to 95% for all analyses.

Results

Results of PRE confirmed that cactus and elderflower odorant did not differ according to Student t-test (two-tailed paired): 1) in familiarity (p-value=0.23) with mean scores of 6.1 and 6.9; 2) in retronasal olfactory intensity (p-value=0.59) with mean scores of 6.8 and 6.6; and 3) in sweetness (p-value=0.51) with mean scores of 0.6 and 0.4, respectively.

Unsweetened flavored solutions

Evolutions of familiarity, retronasal olfactory intensity and sweetness between POST and PRE were not affected by the attentional strategy according to ANOVA with [F(1,44)=0.4], [F(1,44)=0.69] and [F(1,44)=0.10] for each of the three attributes, respectively.
Evolution of sweetness [F(1,44)=1.49] and retronasal olfactory intensity [F(1,44)=2.69] between POST and PRE were not affected by the odorant category (TEST, CONTROL). A trend existed regarding familiarity evolution [F(1,44)=3.27, p-value=0.07] according to the odorant category. As expected, increase in familiarity for the unsweetened flavored solution was higher when flavored with TEST than with CONTROL (See Figure 1).

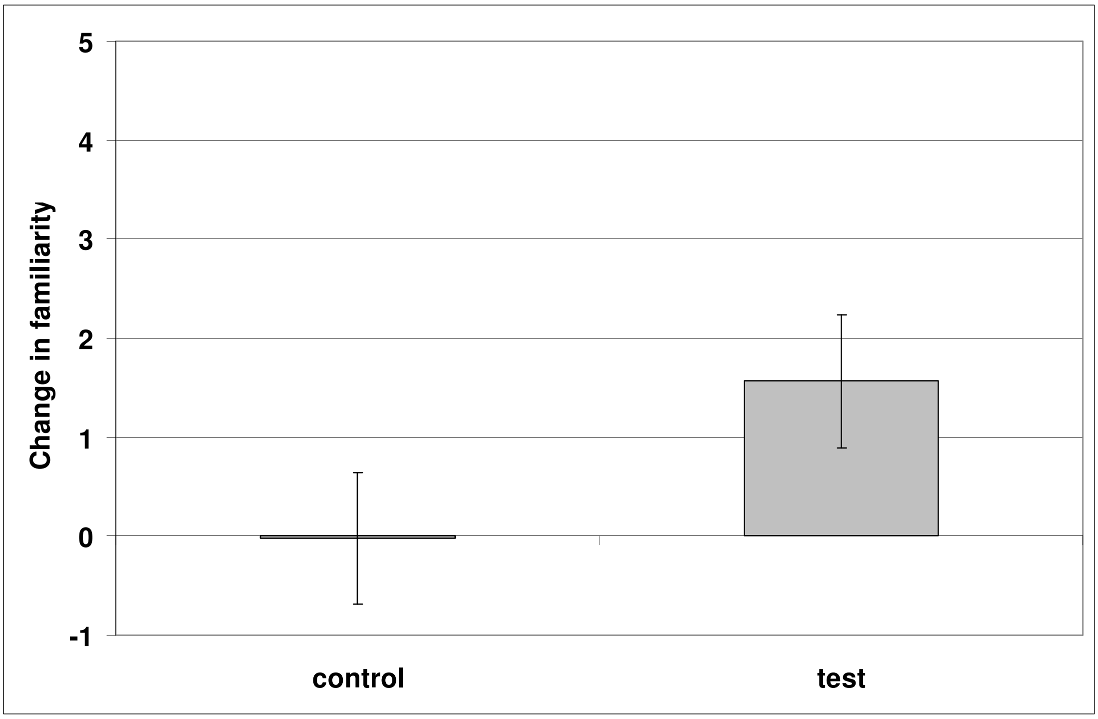


Fig.1: Panel mean score for change in familiarity (+/- SEM) of CONTROL and TEST unsweetened solutions

Sweetened flavored solutions

Change in sweetness between PRE and POST was not significantly impacted by attentional strategy (ANA vs. SYN) [F(1,44)=0.74] and odorant category (TEST, CONTROL) [F(1,44)=0.4]. But interaction between both factors (See Figure 2) was significant [F(1,44)=5.92, p-value<0.05]. Pair comparisons by Student-t test revealed a significant difference in sweetness for the TEST odorant between the two strategies (p-value<0.05) (See Figure 2). In addition, sweetness change of the sucrose solution flavored with TEST was significantly higher than zero for SYN (mean of 1.20 with a confident interval of 0.84) and significantly lower than zero for ANA (mean of -1.80 with a confident interval of 1.43).

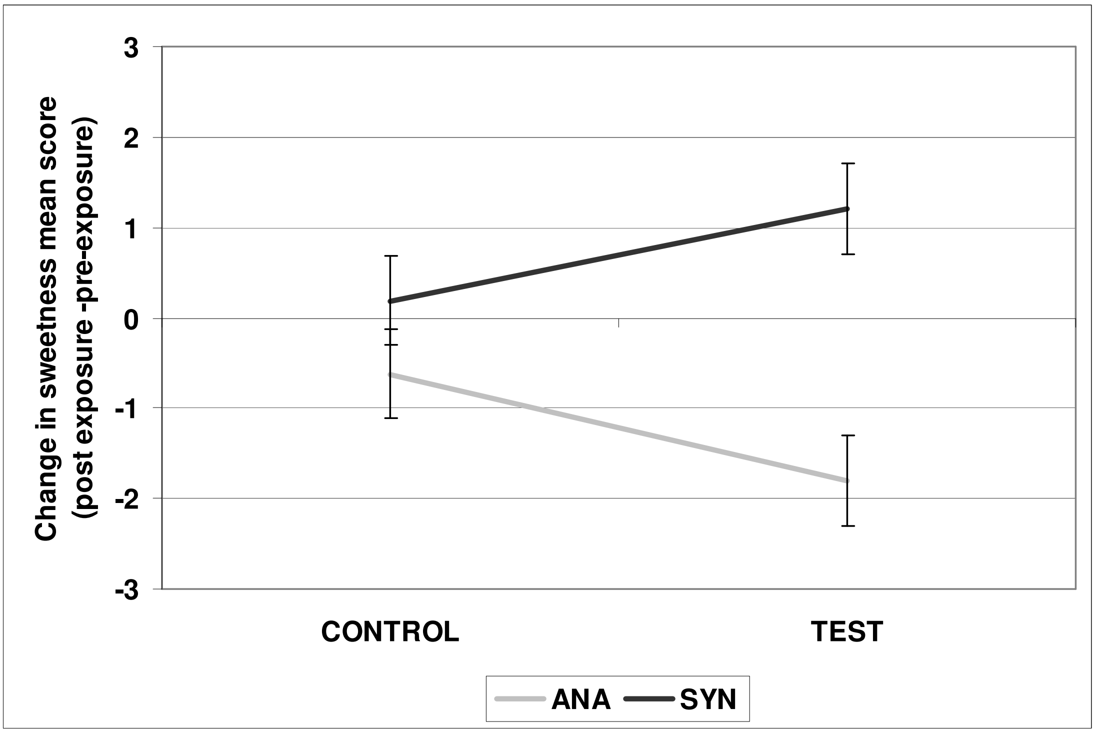


Fig.2: Panel mean score for change in sweetness (+/- SEM) of sucrose solution solutions flavoured with the control and test odorant according to the attentional strategy applied during exposure. A significant change is observed for solution flavoured with odorant test when evaluated by SYN group (sweetness increase) and by ANA group (sweetness decrease).

Experiment 2: Impact of olfactory stimuli at subthreshold level on sweetness after implicit associative learning

The second experiment was conducted to investigate the impact of the co-exposed odorant on sweetness of a sucrose solution when presented at subthreshold level. Two sucrose solutions, with and without odorant, were compared by a 3-AFC discrimination test. We supposed that a sucrose solution flavored at subthreshold level with the co-exposed odorant should be perceived differently from the unflavored sucrose solution due to sweetness change induced by perceptual interactions.
The experiment started after a two-days break (week-end) following the first experiment and consisted in two sessions for the determination of individual detection threshold and one session for the evaluation of the odorant impact at subthreshold concentration. These three sessions were conducted on three separated and consecutive days (See Table 1). The same twenty-four assessors as for the first experiment participated to the second experiment.

Material and methods

Procedure and statistical analyses

- Determination of individual odorant subthreshold concentrations

Firstly, the detection threshold of each odorant in mouth was determined for each of the twenty four subjects in Vittel mineral water using the Forced-Choice Ascending Concentration Series Method of Limit (ASTM, 1991) during two sessions (one for each odorant). Each subject performed for each odorant a series of fifteen 3-AFC tests with an ascending concentration of odorant using a dilution factor of 2 as described in Labbe et al. (2006). The ranges of concentrations used according to the supplier recommendations and preliminary trials were from 6.7E-04 to 11 ppm for cactus and from 4.8 E-03 to 78 ppm for elderflower. For each subject and odorant, the concentration above which all 3-AFC tests were correctly performed was considered as the individual detection threshold concentration. Finally the subthreshold value was obtained by dividing the threshold value by 64 to stand largely below the individual threshold.

- Criteria to conclude to an impact of subthreshold odorant concentration on sucrose solution perception

For each odorant, assessors carried out a 3-AFC test in a 7% sucrose solution, one of the three sucrose solutions being flavored with the subthreshold odorant concentration. A minimum of five 3-AFC tests out of six had to be solved to consider the flavored and unflavored sucrose samples as significantly different according to the binomial law with a confidence level set at 95%.

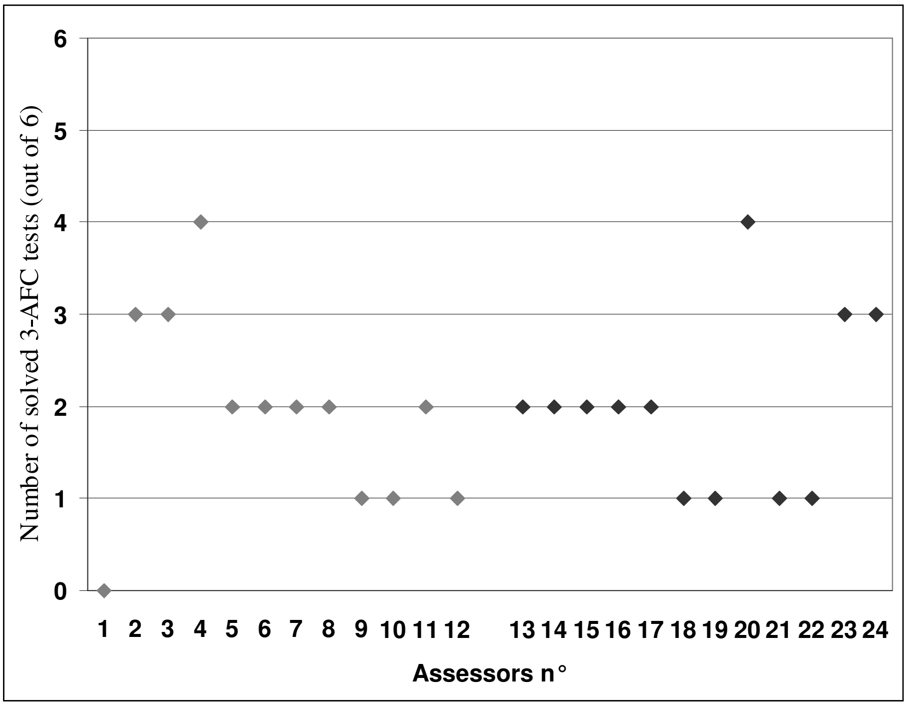
Tasting conditions

Solutions were coded with three-digit random numbers and 50-ml was served in 100-ml plastic cups. Assessors were asked to sip and swallow the solutions. Rinsing was done between each 3-AFC test with water and unsalted cracker. Data was acquired on a computer screen with FIZZ software Version 2.20E (Biosystemes, Couternon, France).

Results

The lowest and highest threshold concentrations within the twenty-four subjects were 1) 1.34E-03 and 1.10 ppm with a panel geometric mean of 4.2E-02 ppm for cactus and 2) 7.6E-02 and 2.45 ppm with a panel geometric mean of 3.8E-01 ppm for elderflower.
Only one subject out of twenty-four significantly distinguished the flavored from the unflavored sucrose solution for the TEST odorant (See Figure 3). Consequently we could not conclude to significant differences between odorant category and applied attentional strategy during exposure.

a)



b)

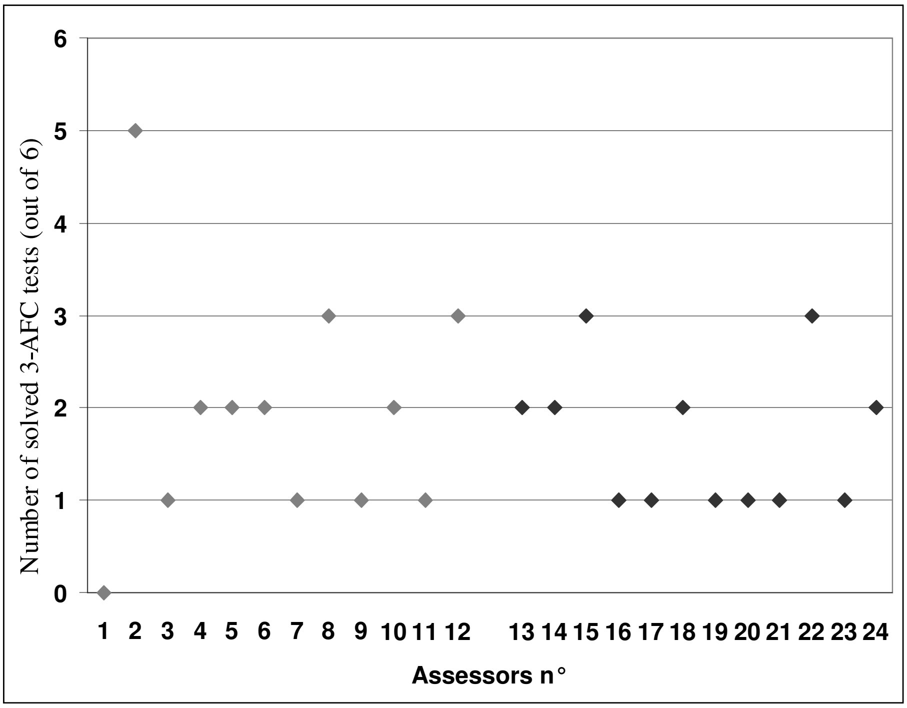


Fig.3: Performance of assessors in solving sucrose solution based triangle tests flavoured at subthreshold with a) the control odorant; and b) the test odorant. The 12 assessors from ANA group and the 12 assessors from SYN group are represented in grey and black, respectively.

Discussion

Impact of exposure approaches on implicit associative learning

After the odorant and sweet tastant co-exposure following a sensory profiling training approach, the co-exposed odorant did not enhance in-mouth sweetness. This finding validated that this approach encouraged subjects to consider perceptual dimensions analytically as assumed by Prescott et al. (2004). This finding showed that a descriptive profiling training where subjects learn to disconnect with reference solutions the different sensations that will be further conjointly experienced in the product tested could reduce the impact of perceptual interactions on product description (e.g. by tasting an odorant and a tastant first in mixture and then independently). Furthermore, sweetness of the flavored sucrose solution was perceived as being weaker after exposure than before exposure. As assessors were trained not to over evaluate sweetness of the flavored sucrose solution by dissociating olfactory and taste perceptions, this may have led them to under evaluate sweetness when the co-exposed odorant was present. Prescott et al. (2004) showed the same pattern but the authors do not suggest any interpretation.
Odorant and tastant co-exposure according to an approach encouraging a synthetical attentional strategy led to the construction of perceptual olfactory-taste association as previously shown by Stevenson and Case (2003), Prescott et al. (2004) and Yeomans (2006). Indeed when olfactory and taste stimuli were considered as a whole during in-mouth experience, the integration of both sensory dimensions was facilitated.
Whatever the exposure approach, when the unsweetened solution was in mouth, the odorant did not enhance sweetness. However Prescott et al. (2004) highlighted that after exposure, the smelled odor was perceptually associated with sweetness. In the present study, where we focused on in-mouth perception, a minimum amount of sweet tastant seemed to be required to induce the in-mouth perceptual association between odorant and sweetness.

Impact of implicit associative learning on sweetness with olfactory stimulus at subthreshold level

The impact of subthreshold olfactory stimulus on sweetness has already been demonstrated with common odors congruent with sweetness such as almond (Dalton et al., 2000; Pfeiffer et al., 2005) and strawberry (Labbe et al., 2006). In the present study, the expected enhancing effect of the odorant co-exposed with sucrose on sweetness was not obtained at subthreshold level whatever the attentional strategy applied during exposure. Indeed flavored and unflavored sucrose solutions were not discriminated through a 3-AFC procedure suggesting that sweetness of the flavored sucrose solution was not increased by perceptual interaction. The perceptual association built experimentally was probably not as strong as those constructed over life with familiar odors. This may explain why sweetness could not be modulated by a subthreshold concentration of a novel odor experimentally co-exposed with sucrose contrary to what shown with common odorants congruent with sweetness.

Role of neural integration processes in the construction of perceptual associations

Perceptual interactions between different sensory dimensions are underlain by neural processes as shown with suprathreshold olfactory and taste stimuli by Small et al. (2004). Using functional Magnetic Resonance Imagery (fMRI), the authors highlighted a supra-additive effect of a congruent vanilla odorant and sucrose mixture on neuron activity from insula/orbitofrontal cortex compared to the activation induced by each ingredient independently. The authors suggested that these observations may be explained by the presence of specific neurons from insula/orbitofrontal cortex which may integrate both olfactory and taste stimuli when congruent. Such bimodal neurons have been highlighted in macaque orbitofrontal cortex (Rolls and Baylis, 1994), and amygdala (Kadohisa et al., 2005) and may result from repeated and simultaneous exposure to a given olfactory and taste combination during life time. Other key structures, referred as to key nodes of the flavor network, have been recently proposed as candidates having a role in olfactory and taste integration processes such as the frontal operculum and the anterior cingular cortex (Small and Prescott, 2005).

Different studies also report olfactory and taste interactions with common odors congruent with sweet taste at a subthreshold concentration (Pfeiffer et al., 2005; Dalton et al., 2000; Labbe et al., 2006). We may argue that repeated exposure over life to odorants might lower the activation threshold of olfactory neuron receptors below the perceptual threshold level and may explain the perceptual association between odor at subthreshold level and sweetness. A few studies support the existence of odorant-specific plasticity in the peripheral olfactory system further exposure (Wysocki et al., 1989; Wang et al., 2004). Indeed authors showed that subjects anosmic to androstenon can acquire sensitivity to odorant through exposure. However, in our study, we did not observe sweetness modulation by the co-exposed odorant at subthreshold level. The short number of experimental exposure sessions to the novel odorant may not have been sufficient enough to lower the activation threshold of olfactory neuron receptors as we previously argued for familiar odorants.

Perspectives

As perspective, additional psychophysics and neuroimaging works could extend our understanding about the plasticity of the flavour network during associative learning and on how unitary perceptions are generated over time. The combined measurement, in response to stimulation by a sucrose solution containing a new odorant at subthreshold level: 1) of neural activity by fMRI of orbitofrontal cortex, amygdala, operculum and cortex cingular anterior structures; and 2) of perceptual impact of such odorant on sweetness, before and repeatedly after several exposure periods may bring relevant insights.

Acknowledgements

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2.2 Olfaction, taste and tactile perceptual interactions

2.2.1 Validation of the sensory diversity induced by formulated products

Introduction

The second main objective of my PhD work was to explore perceptual interactions in a model involving olfactory, taste and tactile perceptions. To our knowledge, the perceptual impact of cold trigeminal perception on other perceptions was so far little investigated. Since cooling agents are widely used by food companies in confectionary products and beverages, there is a clear need to better understand how this perception impacts the overall product characteristics. Based on this status, our exploration of perceptual interactions was extended to cold perception using a cooling agent. Two different flavourings and citric acid were selected as odorants and tastant. Finally a sweet viscous fluid was used as a model to increase perceptual complexity bringing sweet taste and thickness proprioception. Regarding tactile perception, two aspects were therefore studied: trigeminal perception (coldness) and proprioception (thickness).
Before exploring sensory differences between products and the role of perceptual interactions in product perception according to a sensory profiling approach, the first objective of this PART 2.2 was to ensure that formulated samples prepared according to a factorial design were different enough to be discriminated not only by a trained panel but also by naive people. To reach this objective, a sorting task with verbalisation was carried out by forty naive internal assessors for each of the two sets of products, i.e. the eight mint flavoured products and the eight peach-flavoured products.

Methods

Subjects

Forty naive assessors with an average age of 30 (twenty two women and eighteen men) from the Nestlé Research Center, were recruited for the study. They had never previously participated in sensory panels.

Products

A viscous solution containing fructose (16%), sucrose (32%), dextrose (32%), xanthan (0.5%), and water (19.5%) was used as a model for this study. With each type of odorant, a formulation design was built by adding for the first set a peach odorant (supplier reference 78130-33) at 0.10% and 0.20%, for the second set a mint odorant (supplier reference 11606) at 0.01% and 0.03%, and for both sets a cooling agent (WS-3) at 0.10% and 0.20%, and citric acid at 0.20% and 0.60%. The two odorants and the cooling agent were provided by Givaudan SA (Geneva, Dübendorf). The two sets of eight products were therefore based on a 2^3 formulation design and only differed by odorant type.

Tasting conditions

During a first session, the panelists were informed about the principle of the sorting procedure. The second and third sessions were dedicated to the evaluation. For both evaluation sessions, the panelists received the eight samples at once within a same odorant. They were asked to smell and taste the products and then to sort them into groups having similar sensory properties. No other recommendation was given, except that they had to make at least two groups. Then they were invited to describe their groups with their own vocabulary. The two flavoured sample sets, i.e. mint and peach, were randomized between subjects and the two sessions.
Evaluation was conducted in an air-conditioned room (20 °C) and under white light in separate booths.

Statistical analyses

Sorting data was analysed in three steps (Cartier *et al.*, 2006)

Product map using multidimensional scaling (MDS)

An individual binary dissimilarity matrix indicating whether two samples were grouped together was constructed for each panelist. The 40 individual matrices were summed and the resulting dissimilarity matrix was submitted to classical metric multidimensional scaling MDS (Togenson, 1952) using NCSS software version 2007 (Number Cruncher Statistical Systems, Karysville, Utah, USA).

Product description using the vocabulary elicited to describe groups

The vocabulary generated to describe the groups was used to build a contingency table for the products. Each term used to describe a group of samples was reallocated to each product of the group. We therefore assumed that all the products belonging to the same group could be described by the same terms. The resulting contingency table was then reduced and simplified: 1) terms having similar meanings were grouped by the Sensory Analyst; and 2) only terms having a quotation frequency higher or equal to 10% for the sample with the highest elicitation rate were considered.

Projection of product descriptions on MDS-map

The items describing products were projected onto the MDS map using the correlation structure of product coordinates on the map and product descriptions in the contingency table.

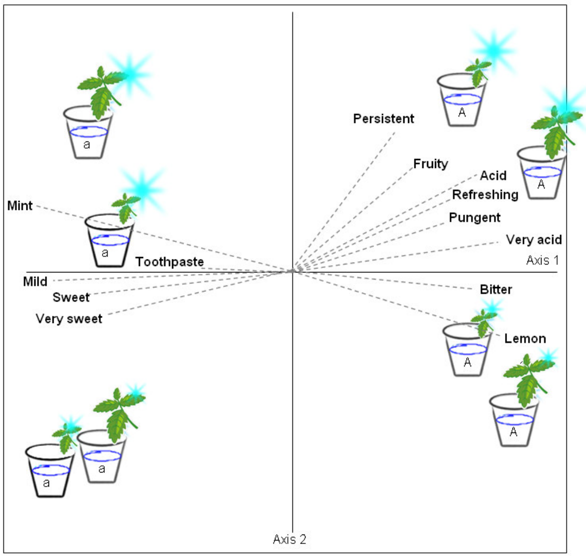
Results

Regarding the mint and peach flavoured products (Fig.1a-b), both maps represent well the initial data, as shown by low stress (= 0.23 and 0.18, for mint- and peach-flavoured products, respectively), and high Pearson's correlation between Euclidian distance between products represented on the two dimension map and MDS dissimilarities (r= 0.93 and 0.96, for mint and peach flavoured products, respectively).
For both set of products, assessors grouped consistently samples according to the level of citric acid (axis 1). Samples with the highest citric acid level are represented on the right hand side of the map and samples with the lowest citric acid level are represented on the left hand side of the map. Elicited terms were coherent with product formulation

since samples with the highest citric acid level were described as acid whereas samples with the lowest citric acid level as sweet.

The cooling agent level also impacted grouping of the mint flavoured samples. The four samples with the highest cooling agent level are opposed to the four samples with the lowest cooling agent level (Fig.1). For the peach flavoured samples (Fig.2a), the cooling agent level also impacted the grouping of samples (axis 2) but with a citric acid level interaction. Indeed the four products within each citric acid level are grouped into two pairs (based on the level of cooling agent) which are positioned differently according to the citric acid level. For both sample sets, products with the highest cooling agent and citric acid levels were described as refreshing with a frequency of 15% and 19% for the mint and peach flavoured range of samples, respectively.

a)



b)

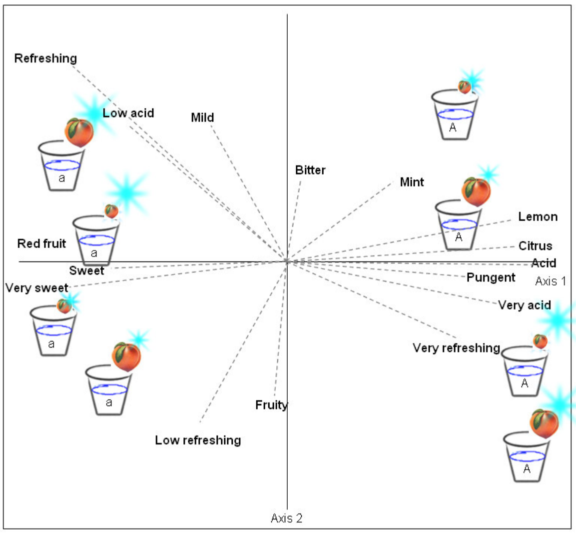


Fig.1: First factorial maps of MDS issued from sorting with assessors with projection of consumer items describing the products for:

a) mint flavoured samples containing the ingredients symbolized below with the respective concentrations

	Mint odorant		Cooling agent		Citric acid	
Level	Low (0.01%)	High (0.03%)	Low (0.10%)	High (0.20%)	Low (0.20%)	High (0.60%)
Symbol					a	A

b) peach flavoured samples containing the ingredients symbolized below with the respective concentrations

	Mint odorant		Cooling agent		Citric acid	
Level	Low (0.10%)	High (0.20%)	Low (0.10%)	High (0.20%)	Low (0.20%)	High (0.60%)
Symbol					a	A

Discussion

The ingredients added into the viscous liquid model according to a factorial design allowed to formulate eight products with a wide sensory diversity within a same odorant. Indeed assessors were able to 1) discriminate samples according to the level of cooling agent and citric acid; and 2) described products consensually and relevantly, i.e. with vocabulary corresponding to product composition.
The last step of the PhD work was to explore a complex conception, i.e. a perception not described with simple attributes such as sweet or thick but that result from an integration of unitary percepts. In the present study, within each odorant type, samples with both a high cooling and acid levels were described as refreshing. In addition samples refreshing and sweet descriptor were negatively correlated. Consequently, we considered refreshing perception as potentially a complex perception since driven by different sensory dimensions. The sensory foundations of refreshing perception were systematically and deeply studied in the PART 3.2 of the PhD work using a similar liquid viscous model system.

2.2.2 Impact of olfaction on taste, trigeminal and texture perceptions

Labbe,D., Gilbert,F. and Martin,N. (2008) Impact of olfaction on taste, trigeminal, and texture perceptions. Chemosensory Perception, 1, 217-226.

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Impact of Olfaction on Taste, Trigeminal, and Texture Perceptions

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Abstract Research on perceptual interactions has mainly been conducted on olfaction and taste. Few studies have extended the investigation to trigeminal and texture perceptions. The objective of the study was to explore systematical interactions between olfaction, taste, texture, and trigeminal perceptions with a specific focus on the role of olfaction. Ingredients inducing olfactory (odorant), taste (citric acid), and trigeminal (cooling agent) perceptions were systematically varied in a viscous model system. A panel assessed sensory properties of the products within the same odorant type under two different conditions: with and without noseclip. Olfaction strongly influenced taste and trigeminal perceptions but also modulated perceptual taste/taste and taste/trigeminal interactions. But results involving texture perception were inconclusive since the evaluation condition (with and without wearing a noseclip) might have impacted mastication and swallowing behaviors. These findings highlight the multiplicity and overlapping of olfactory/trigeminal/taste perceptual interactions in complex food systems.

Keywords Olfaction · Taste · Trigeminal · Psychophysics · Sensory Interaction

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Introduction

Perceptual interactions between olfaction and taste have been extensively explored, generally in aqueous systems. One of the first works reporting perceptual interactions between olfaction and taste showed that subjects attributed a taste to ethyl butyrate and citral odorants; this effect disappeared when the retronasal olfactory was prohibited by closing the nostrils (Murphy et al. 1977; Murphy and Cain 1980).

Further studies then demonstrated that taste qualities (sweetness and sourness) could enhance the fruitiness olfactory intensity perceived orthonasally (Bonnans and Noble 1993) and that odorant (caramel) could enhance sweetness but also decrease sourness (Stevenson et al. 1999) since caramel odor is congruent with sweetness but incongruent with sourness. The notion of congruency between sensory qualities was defined as *the extent to which two stimuli are appropriate for combination in a food product* (Schifferstein and Verlegh 1996). Recently, it was also demonstrated that taste identification is facilitated when an odorant congruent to the taste is sniffed beforehand (White and Prescott 2007).

The cognitive mechanisms involved in perceptual interactions between olfaction and taste have been widely studied. Today, the scientific community agrees that odor–taste interactions are the result of associations experienced and memorized through food exposure without any explicit attention or learning (Köster et al. 2004; Köster 2005, Le Berre et al. 2008). Perceptual interactions and the role of congruency were also highlighted at a neural level (Small et al. 2004). Indeed, the comparison between the sum of neural activation induced by a sucrose solution and by vanilla retronasal olfactory stimulation separately and by the overall neural activity induced by the perception of a sucrose solution flavored with vanilla showed a super-additive response. However, such results were not observed with a noncongruent odorant/tastant pair (vanilla odorant and salt tastant).

Perceptual interactions involving olfaction, taste, and in-mouth texture perception were also demonstrated in dairy products (Kora et al. 2003). The authors showed that the increase in thickness lead to a decrease in green apple olfactory perception and sweetness. Besides, the quality and the complexity of the flavoring influenced texture perception (Saint-Eve et al. 2004). Another study demonstrated that the change of viscosity and sucrose content could impact olfactory perception due to perceptual interactions (Lethuaut et al. 2005). The temporal and spatial co-occurrence of texture perception with other sensory cues may significantly strengthen perceptual interactions since these conditions naturally occur during everyday food consumption (Bult et al. 2007). The authors highlighted that perceptual interactions between olfaction and texture perception were effective when the olfactory stimulus was presented retronasally and more powerful when perceived simultaneously with swallowing. The importance of temporal synchrony in perceptual interaction mechanisms was also demonstrated between odorant and tastant stimuli (Pfeiffer et al. 2005).

Research dedicated to sensory interactions involving the trigeminal perceptions were mainly focused on pungency/burn sensation induced by chemical compounds such as capsaicin. As key finding, it was demonstrated that pungency could mask olfactory and taste perceptions, but this masking effect may probably be more linked to a desensitization induced by trigeminal compounds than to perceptual interactions (Prescott 1999; Reinbach et al. 2007). Regarding cold trigeminal perception, the effect of aroma and color, and of congruency and exposure on flavor and cooling perception was explored in water (Petit et al. 2007). It was shown that olfactory intensity was enhanced by coldness due to perceptual interactions in a congruent mixture (melon odorant, cooling agent, and green coloring) but not in an incongruent mixture (pineapple odorant, cooling agent, and purple coloring). However, when subjects were exposed during 5 weeks to the incongruent mixture, perceptual interactions between olfaction and trigeminal perceptions were promoted. These results are additional evidence of the role of food exposure on perceptual interactions.

Perceptual interactions between senses were therefore often studied in model solutions or commercial products involving two or three sensory modalities. To our knowledge, no systematic study on cross-modal sensory interactions combining olfaction, taste, trigeminal, and texture perceptions in a controlled system was reported in literature.

The goal of the present study was to better understand the impact of olfaction, in terms of odor type (peach vs. mint) and odor intensity, on taste, trigeminal, and texture perceptions, and on their interactions. To fulfill our objective, we formulated a xanthan-thickened solution containing fructose, glucose, and dextrose. A range of products was then prepared by adding in the model system an odorant (peach or mint), a cooling agent, and citric acid at two levels of concentration according to an experimental design. The products were characterized by sensory profiling with assessors carrying out the evaluation under two conditions: with and then without wearing a noseclip.

To avoid confusion, we will use the term “odor” to refer to the orthonasal olfactory perception and “aroma” to refer to the retronasal olfactory perception (i.e., the organoleptic attribute perceptible by the olfactory organ via the back of the nose (NF ISO 5492 1995). The term “olfactory” regroups both odor and aroma perception.

Material and Methods

Product Formulation

A viscous solution containing fructose (16%), sucrose (32%), dextrose (32%), xanthan (0.5%), and water (19.5%) was used as model for this study. With each type of odorant, a formulation design was built by adding for the first set a peach odorant (supplier reference 78130-33) at 0.10% and 0.20%, for the second set a mint odorant (supplier reference 11606) at 0.01% and 0.03%, and for both sets a cooling agent (WS-3) at 0.10% and 0.20%, and citric acid at 0.20% and 0.60%. The two odorants and the cooling agent were provided by Givaudan SA (Geneva, Switzerland). The two sets of eight products were therefore based on a 2^3 formulation design and differed by the odorant type only.

The two odorants were chosen because they are associated to different sensory modalities. Indeed peach odorant is associated with sweetness (Noble 1996) because of the frequent conjoint exposure to both stimuli in everyday food. Concerning mint odorant, this olfactory perception is related to coldness since menthol and menthol derivatives can bind thermo-receptor TRPM8 of the trigeminal nerve and induce cold perception and conjointly activate the olfactory system to produce a mint olfactory perception (Patapoutian et al. 2003). This temporal and spatial concomitance between olfactory and trigeminal perceptions may lead to a cognitive association between mint and cold as already observed in a study investigating sensory drivers of refreshing perception (Labbe et al. 2007). The mint odorant was specifically selected among a wide range of commercial products as, according to the supplier, it did not induce any cold perception. This was validated by four staff members who did not perceive any trigeminal stimulation when they tasted mint odorant in water at high level using a noseclip.

In order to avoid an effect of viscosity of the formulation on the study, the viscosity of 16 solutions was checked at 37 °C using a rheometer (Haake RS 100, Haake-Mess-Technik GmbH, Karlsruhe, Germany). A shear stress sweep was applied from 0.1 to 100 Pa. All solutions showed a similar shear-thinning behavior. All the solutions showed a yield stress value of 0.55±0.01 Pa and viscosity values of 3.973, 0.156, and 0.018 Pa s (±0.001) at shear rates of 1, 10, and 100 s^{-1}, respectively.

Sensory Characterization

Sensory Profiling and Tasting Conditions

Each of the two sets of products was characterized through a sensory profiling under two evaluation conditions, with and without noseclip. The product evaluation with noseclip was essential to validate that odorants did not confer any taste, trigeminal, and texture perception by themselves to the product. Once validated, any taste, trigeminal, or texture perception modulation depending on the odorant concentration and highlighted by the product evaluation when carried out without noseclip can therefore be attributed to a perceptual interaction between olfaction and other sensory dimensions.

Four samples were evaluated per session. Four sessions were dedicated to the set of samples flavored with a same odorant with the first two sessions with noseclip and the following two sessions without noseclip. The 16 products were therefore evaluated in eight sessions. Sessions were not balanced between odorant types due to preparation constraints. The presentation order of the products within a same odorant type and a same condition of evaluation (with noseclip or without noseclip) was randomized based on Williams Latin squares (Williams 1949). Products were coded with three-digit random numbers and served at room temperature (22 °C) in a 50-ml cup with a plastic coffee spoon. Rinsing was done between products with water and unsalted crackers. Data was acquired on a computer screen with FIZZ software Version 2.20E (Biosystemes, Couternon, France). Tests were conducted in an air-conditioned room (18 °C), under white light in individual booths.

Subjects, Attributes, and Training

Ten assessors, all women, with an average age of 40, were recruited for this study. They were used to participate in sensory panels on different product categories but had never evaluated viscous solutions. The subjects scored five attributes for the evaluation with noseclip (coldness, sourness, sweetness, bitterness, and thickness), and two attributes were added to the previous list for the evaluation without noseclip (overall odor and overall aroma). Subjects were familiar with evaluation of odor, aroma, taste, and texture attributes. For the purpose of this study, it was important that subjects were not trained and had not previously been exposed to the products. Indeed, an analytical exposure procedure (training) conducted before a sensory profiling as described in ISO 8586-1 standard (1995) may encourage subjects to consider sensory modalities independently and may limit perceptual interactions (Prescott et al. 2004).

A specific familiarization session was conducted for cold attribute using a cooling agent in water solutions at the low and high concentrations further used in the final products. Due to the temporality of the cold perception (Gwartney and Heymann 1995; Gwartney and Heymann 1996) induced by the cooling agent, a specific procedure was implemented to standardize the evaluation and the quantity of product to take in the mouth. For the evaluation of coldness, the subjects had to put one spoonful of product in their mouth and move it with their tongue during 8 s. Then, they had to swallow and to wait for 8 s after swallowing before scoring. For the evaluation of the attributes, overall aroma, sourness, sweetness, and bitterness, subjects swallowed a new spoonful of product. Finally, they took a last spoonful of product for the evaluation of thickness.

Statistical Treatment

For each set of eight products, the impact of the odorant (peach and mint) on coldness, sourness, sweetness, bitterness, and thickness was investigated by calculating a condition (with noseclip; without noseclip) × subject analysis of variance (ANOVA). Then, the impact of the odorant type was explored for the 16 products by calculating an odorant type (peach; mint) × subject ANOVA for all the attributes evaluated without wearing a noseclip. These two ANOVA were conducted separately instead of calculating a condition (with noseclip; without noseclip) × odorant type (peach; mint) × subject ANOVA since investigation of odorant type effect on perception when assessors wore a noseclip was not appropriate.

The effect of the three ingredient concentrations was studied by calculating an odorant concentration (low; high) × cooling agent (low; high) × citric acid concentration (low; high) × subject ANOVA with interactions on all the attributes and for each condition (with and without noseclip) within each odorant type (peach and mint).

ANOVAs were calculated, using NCSS software version 2007 (Number Cruncher Statistical Systems, Karysville, Utah, USA). Post hoc differences between means were calculated by a paired t test (two-tailed). Confidence level was set to 95% for all analyses.

Results

Impact of Evaluation Condition (With and Without Noseclip) on Taste, Trigeminal and Texture Perceptions

When evaluated without noseclip, peach flavored products were scored sweeter, less bitter, and less thick than when evaluated with noseclip (Fig. 1a). The mint flavored products were perceived colder, more intense in taste (sourness sweetness and bitterness), and less thick when evaluated without noseclip than when evaluated with noseclip (Fig. 1b).

Comparative Impact of Peach and Mint Odorant on Taste, Trigeminal, and Texture Perceptions

When evaluated with noseclip, attribute mean scores did not significantly differ between peach and mint-flavored samples except for coldness attribute (Fig. 1a and b). When evaluated without noseclip, aroma and odor intensities were significantly higher for peach-flavored samples than for mint-flavored samples. Mint-flavored products were perceived significantly more intense in coldness, sourness, sweetness, and bitterness than peach-flavored products. The difference in thickness according to odorant type was not significant (Fig. 2).

Impact of the Odorant Concentration on Perception in Both Evaluation Conditions

As expected, increase in both odorant concentrations significantly enhanced odor intensity of the products. The increase in mint odorant concentration enhanced coldness under both evaluation conditions (with and without noseclip), sweetness and thickness, when the evaluation was carried out without noseclip (Table 1, impact of odorant concentration). But increasing the peach odorant concentration had no impact on the intensity of the other perceptions. A significant interaction between mint odorant level and cooling agent level on sweetness was highlighted (p value<0.01). Increasing the mint odorant significantly enhanced sweetness of products having a high cooling agent level and did not modulate sweetness of products with a low cooling agent level (Fig. 3).

Impact of Cooling Agent Concentration on Perception Under Both Evaluation Conditions

The increase of cooling agent concentration significantly reduced aroma intensity of the peach-flavored products and did not change the mint-flavored product olfactory characteristics. Coldness was enhanced whatever the evaluation condition for peach-flavored products and only in the “with noseclip” condition for mint-flavored products. Sourness of all products significantly increased with increase in cooling agent concentration when the evaluation was conducted without noseclip. Increase of cooling agent concentration significantly reduced the sweetness of peach-flavored products and increased sweetness of the mint-flavored products during the evaluation conducted without noseclip. A modulation of texture perception of mint-flavored products when evaluated without noseclip was highlighted. Indeed thickness of the mint-flavored products was significantly enhanced (Table 1, impact of cooling agent concentration).

Impact of Citric Acid Concentration on Perception Under Both Evaluation Conditions

The increase of citric acid concentration did not change olfactory, trigeminal, and texture perceptions but enhanced sourness of the products whatever the odorant type. But when evaluated without noseclip, the sourness enhancement was not significant for mint-flavored products. Citric acid increase significantly reduced sweetness of peach-flavored products (Table 1, impact of citric acid concentration).

Discussion

Perceptual Interactions Between Olfaction, Taste and Trigeminal Perception

Both odorants did not confer any taste or texture perception since when evaluated with noseclip, mint and gel products showed similar results for taste- and texture-related attributes. This finding validated that perceived taste and texture modulations caused by odorants when products were evaluated without noseclip had an olfactory perceptual origin.

The odor and aroma of the peach products were higher than those of the mint products. However, perceptual interactions involving olfaction were more frequently observed within the mint products. This suggested that perceptual interactions induced by olfaction may depend more on the quality than on the intensity of the odorant.

Olfactory/Trigeminal Interactions

Olfactory perception modulated differently other perceptions according to the odorant quality. As expected, peach odorant did not enhance coldness but mint odorant did. This result suggested that peach olfactory perception may be perceptually less associated with coldness than mint. Peach and cold sensory dimensions are not often associated in food and therefore not commonly experienced together

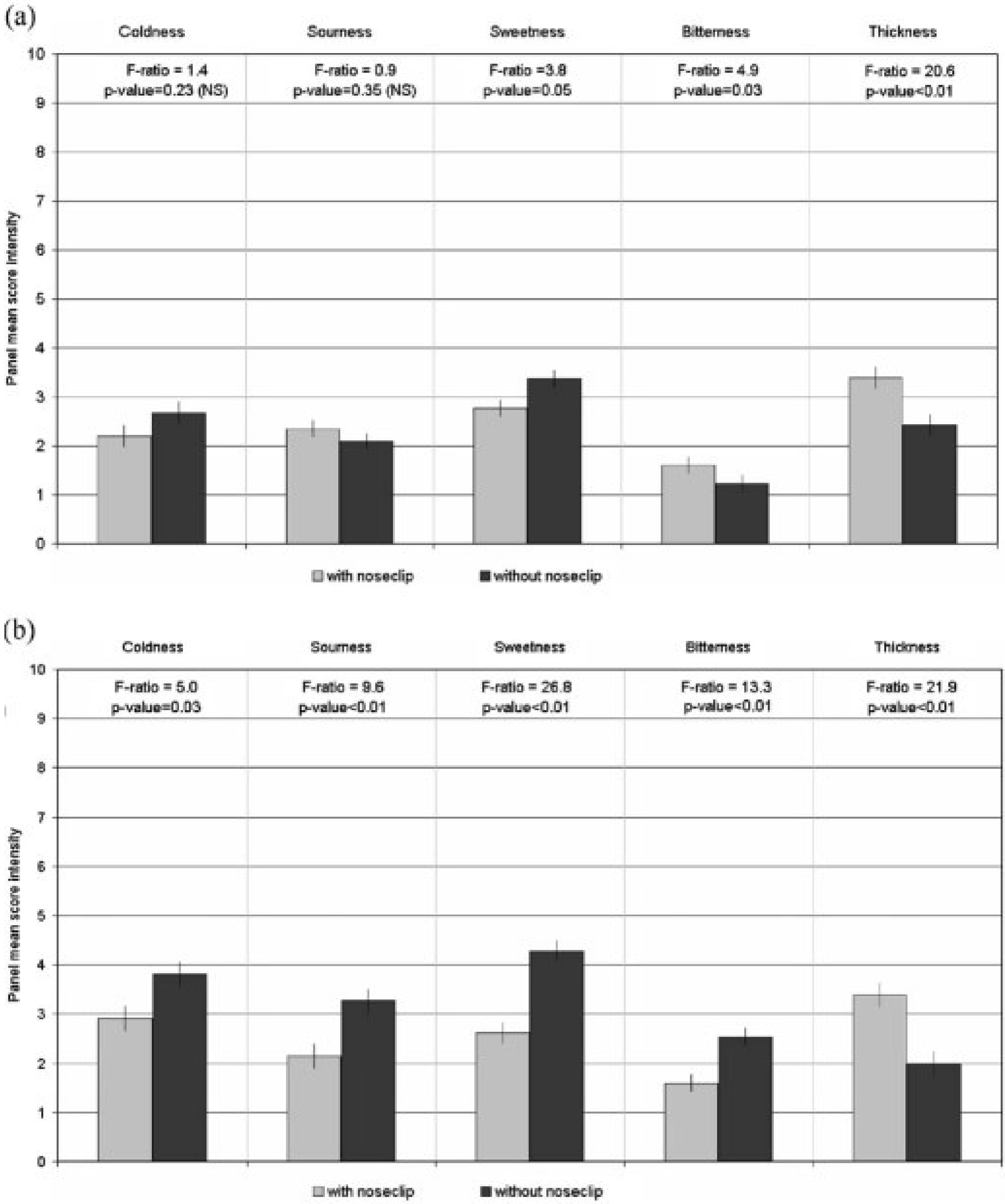


Fig. 1 **a**, **b** Impact of olfactory perception on attribute mean rating (±SEM) Evaluation conducted under two conditions: with and without noseclip for **a** peach-flavored products and **b** mint-flavored products. According to the *F* ratio of the evaluated ANOVA factor; NS means *p* value was "non-significant" with a confidence level of 95%

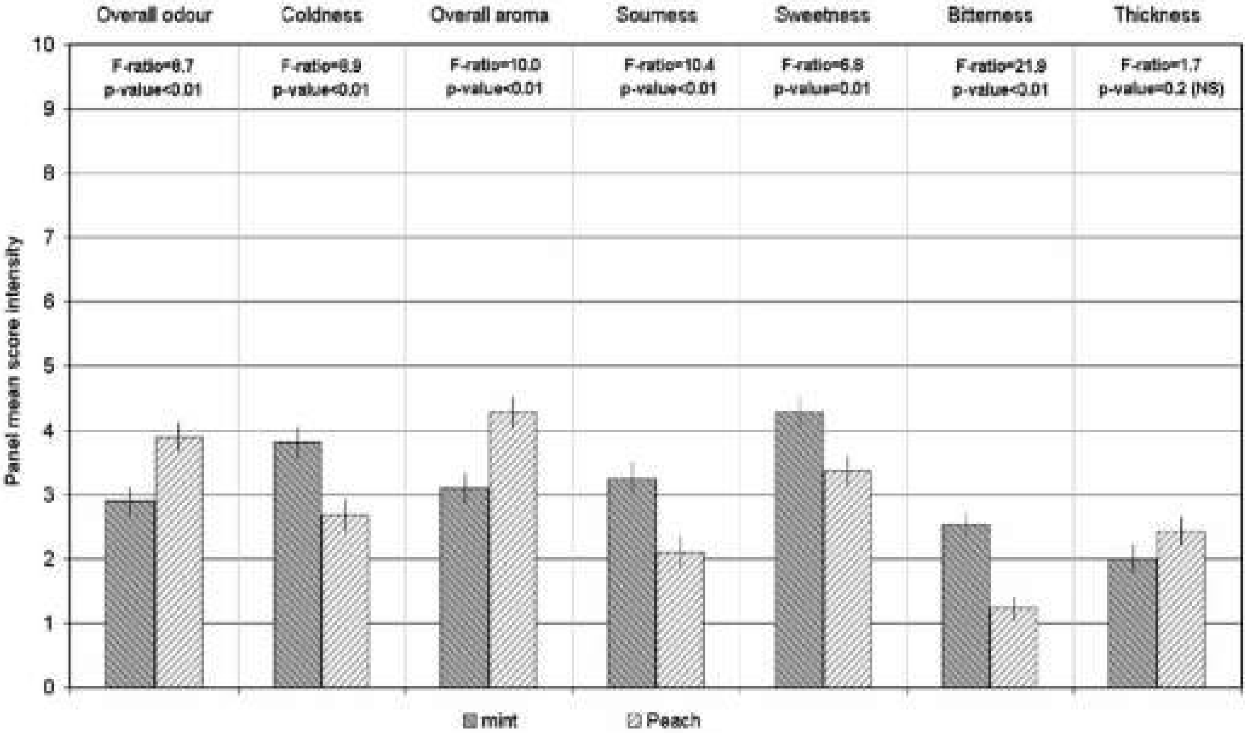


Fig. 2 Impact of olfactory quality induced by peach and mint odorant on attribute mean rating (±SEM). According to the *F* ratio of the evaluated ANOVA factor; NS means *p* value was "non-significant" with a confidence level of 95%

by consumers. On the contrary, the perceptual association existing between mint olfactory properties and coldness may be constructed and reinforced by exposure to toothpaste or chewing gum for example (Labbe et al. 2007). Indeed, these products usually combine both mint olfactory and cold trigeminal stimuli. Petit et al. (2007) demonstrated that exposure promoted perceptual interaction between trigeminal and olfactory perceptions.

In the present study, the increase in mint odorant concentration significantly enhanced coldness when products were evaluated not only without noseclip but also with noseclip (Fig. 4). Additionally, for both mint odorant concentrations, coldness was more intense when evaluated without noseclip than when evaluated with a noseclip, highlighting a perceptual olfactory/trigeminal interaction. It is likely that mint odorant induced cold trigeminal perception, which was not perceived during the control evaluation conducted with the noseclip. The difficulty to identify a compound with mint-like olfactory characteristics without trigeminal properties may suggest that compounds inducing mint-like odor/aroma may always have a specific structural configuration targeting binding sites of cold receptors. Findings from McKemy et al. (2002) support this assumption. Indeed, the authors highlighted that menthol, menthone, and eucalyptol, compounds with a mint-like olfactory characteristic, activated cold receptors, whereas cyclohexanol, a synthetic menthol analogue without mint olfactory characteristics, did not activate the same receptor.

Olfactory/Trigeminal/Taste Interactions

Sweetness was increased by mint and peach odorants. The perceptual interaction between fruity odor/aroma and sweet taste leading to a sweetness enhancement is often highlighted in the literature at suprathreshold level (Pfeiffer et al. 2006) and even at subthreshold level (Labbe et al. 2007). But there was no report of sweetness enhancement by mint odor, and such result was not expected. In addition, (1) sweetness enhancement increased with increasing odorant concentration, and (2) the mint-flavored products were evaluated sweeter when cooling agent concentration increased, but for the high mint odorant concentration only. This result illustrates complex tri-modal perceptual interactions between taste, olfaction, and trigeminal perception. The association of mint odor/aroma with conjoint sweet and cold stimuli is commonly present in common food and beverage products (mint sugar or chewing gums, syrup, ice cream, etc.), and this may explain why mint odorant specifically triggered a sweetness enhancement induced by a coldness increase.

Bitterness was reduced by peach odorant. This may be the result of the suppressive impact of odor-induced sweetness on bitterness (Fig. 1a). Sweetness of peach-flavored products was reduced by the increase of citric acid

Table 1 Impact of odorant concentration, cooling agent concentration, and citric acid concentration on attribute intensities (ANOVA *F* ratio; *p* value) for each odorant type and both evaluation conditions (with and without noseclip)

Attributes	Evaluation condition	Peach-flavored products	Mint-flavored products
Impact of odorant concentration			
Odor intensity	With noseclip		
	Without noseclip	5.3, 0.02+	11.4, <0.01+
Aroma intensity	With noseclip		
	Without noseclip	3.8, 0.05+	3.9, 0.05+
Coldness	With noseclip	NS	11.3, <0.01+
	Without noseclip	NS	10.8, <0.01+
Sourness	With noseclip	NS	NS
	Without noseclip	NS	NS
Sweetness	With noseclip	NS	NS
	Without noseclip	NS	11.7, <0.01+
Bitterness	With noseclip	NS	NS
	Without noseclip	NS	NS
Thickness	With noseclip	NS	NS
	Without noseclip	NS	5.9, 0.02+
Impact of cooling agent concentration			
Odor intensity	With noseclip		
	Without noseclip	NS	NS
Aroma intensity	With noseclip		
	Without noseclip	16.8, <0.01−	NS
Coldness	With noseclip	8.3, <0.01+	11.9, <0.01+
	Without noseclip	21.3, <0.01+	NS
Sourness	With noseclip	NS	NS
	Without noseclip	4.0, 0.05+	22.3, <0.01+
Sweetness	With noseclip	NS	NS
	Without noseclip	15.8, <0.00−	5.4, 0.02+
Bitterness	With noseclip	4.3, 0.04+	5.4, 0.02+
	Without noseclip	18.2, <0.01+	NS
Thickness	With noseclip	NS	NS
	Without noseclip	NS	5.1, 0.03+
Impact of citric acid concentration			
Odor intensity	With noseclip		
	Without noseclip	NS	NS
Aroma intensity	With noseclip		
	Without noseclip	NS	NS
Coldness	With noseclip	NS	NS
	Without noseclip	NS	NS
Sourness	With noseclip	5.2, <0.01+	3.9, 0.05+
	Without noseclip	16.4, <0.01+	NS
Sweetness	With noseclip	4.5, 0.04−	NS
	Without noseclip	6.8, 0.01−	NS
Bitterness	With noseclip	NS	NS
	Without noseclip	NS	NS
Thickness	With noseclip	NS	NS
	Without noseclip	NS	NS

According to the ANOVA *F* ratio of each ingredient concentration factor: NS means *p* value was "non-significant" (confidence level of 95%); "+" means the increase of ingredient concentration induced a significant enhancement of perceived intensity; and "−" means the increase of ingredient concentration induced a significant reduction of perceived intensity.

concentration. Regarding these two latter findings, symmetrical taste/taste suppressive interactions have already been highlighted between sweet and bitter tastants and between sweet and sour tastants (see Keast and Breslin 2003 for a review).

Comparing mint product evaluated with vs. without noseclip, we found that mint odorant increased sourness. It has been reported that cold temperature and cooling compounds such as menthol could stimulate salivary glands and therefore enhance salivary flow, inducing a mouth-

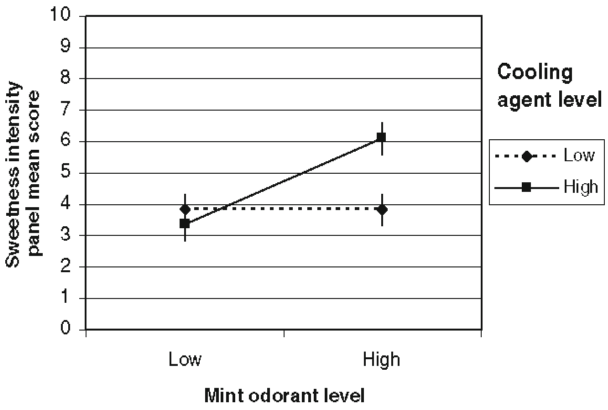


Fig. 3 Impact on sweetness (±SEM) of the significant interaction between mint odorant level and cooling agent level according to the *F* ratio of the ingredient increase interaction (ANOVA). A significant sweetness enhancement was induced by an increase in cooling agent concentration only for the high concentration of mint odorant with a confidence level of 95%

wetting sensation (Brunstrom et al. 2000; Eccles 2000). This physiological effect is very similar to that of acid tastants; consequently, the mouth-wetting sensation due to trigeminal and/or olfactory stimulation by mint odorant may be associated by the subject with higher sourness. The increase of cooling agent concentration enhanced the sour intensity of peach- and mint-flavored products only when evaluated without noseclip. Olfactory perception, and mainly mint odor, may play an essential role by promoting such cold trigeminal/acid taste interaction.

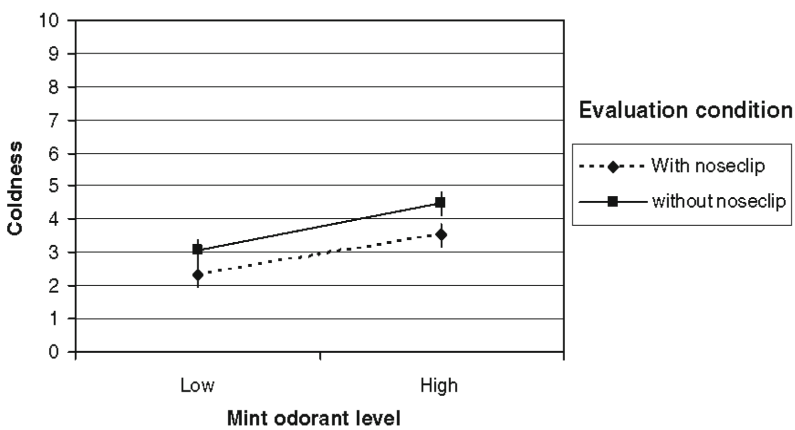
The mint-flavored products were perceived as more bitter when evaluated without noseclip. Bitterness may be reinforced by interactions with the mint odorant olfactory or trigeminal properties since increasing the cooling agent concentration also enhanced bitterness of all products when evaluated with noseclip.

Previous studies already reported that mint-like compounds induced bitter taste (Gwartney and Heymann 1995; Gwartney and Heymann 1996; Green and Schullery 2003; Labbe et al. 2007), but in the latter studies, olfaction was not suppressed, and to our knowledge, the relative contribution of trigeminal and olfactory properties of menthol to bitterness enhancement remains unknown. Interactions between burning/irritating trigeminal perception and bitterness have been extensively investigated in the literature (Prescott and Stevenson 1995). But few studies have been dedicated to cold trigeminal/bitter taste interaction, and it is uncertain whether the same mechanisms took place. Trigeminal/bitter taste interactions may have a physiological origin via peripheral transduction mechanisms consecutive to receptor binding by trigeminal compounds (for review, see Verhagen and Engelen 2006) or a perceptual origin, as shown recently by a study dedicated to bitter taste/trigeminal (burning) interactions (Lim and Green 2007). According to the authors, bitter taste enhancement by trigeminal perception may be the consequence of qualitative similarities between burning and bitter perception since both have a common role of preventing potentially harmful stimuli.

Impact of Olfaction on Texture Perception

Results related to texture perception appear contradictory. Products with a high mint odorant concentration were perceived thicker than products with a low mint odorant concentration when products were evaluated without noseclip, which suggests a perceptual interaction between odor and texture (Table 1, impact of odorant concentration). However, all products (whatever the odorant) were perceived less thick when evaluated without noseclip (Fig. 1a and b). This systematic decrease of thickness might be explained by the nasal airwaves obstruction that could likely change the mastication and swallowing behavior of the assessors between both evaluation conditions (with and without noseclip). As a consequence, the mixing behavior of product and saliva in the mouth might change depending on the evaluation condition. The use of aroma pulses

Fig. 4 Impact on perceived coldness (±SEM) of mint-flavored product of odorant level and evaluation condition. A significant coldness enhancement was induced by an increase in mint odorant concentration for both evaluation conditions with a confidence level of 95%



delivered by an olfactometer seemed therefore more relevant to dissociate olfactory and texture perceptions (Visschers et al. 2006).

Conclusion

As expected and already shown in the literature, our results highlight that olfaction modulates taste perception (sourness, bitterness, and sweetness) through perceptual interactions. The much less studied impact of olfaction on cold trigeminal perception was also highlighted. Olfaction also played a key role on taste/taste interaction and on cold trigeminal/taste interactions. Further investigations would be required to better understand (1) the interactions between olfactory (mint), trigeminal (cold), and bitter perception, and their origin (perceptual or physiological); and (2) the contribution to the overall trigeminal perception of perceived oral and nasal trigeminal stimulation. Finally, contradictory results regarding perceptual interactions involving texture perception may result from different in-mouth behavior when wearing or not a noseclip.

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2.2.3 Setting up of the range of liquid viscous products for the investigation of refreshing complex perception

As shown in the PART 2.2.1, refreshing term was spontaneously elicited by naive assessors during sorting task with free vocabulary generation conducted with liquid viscous solutions. In addition refreshing seems a complex perception, i.e. related to more than one sensory dimension since products spontaneously described as refreshing during the sorting task: 1) were opposed to products described as sweet; and 2) contained a high citric acid and cooling agent levels. The range of viscous liquid products was therefore used in the next part with the objective to investigate systematically the sensory drivers of refreshing after a re-formulation work to widener the sensory diversity as described below (Fig.1):

- Step 1: selection of the most different peach and mint flavoured products in terms of sensory perception among the 16 products used in the PART 2.2.

This was done on each set of products within the odorant type by performing a Principal Component Analysis on sensory profiling data obtained PART 2.2.1 with the sensory attributes as variables and the products as observations; and 2) selecting the most extreme products of the first biplot.

- Step 2: widening the sensory diversity in terms of in mouth texture perception. Indeed according to literature review on sensory foundations of refreshing perception presented in PART 3.1, thickness could be a driver of refreshing perception. The nine most extreme samples were therefore formulated with two levels of thickener (xanthan), two sensory profiling were carried out on samples within a same odorant type and a Principal Component Analysis was run. Again, the most extreme samples represented on the first biplot were selected. Five mint and four peach flavoured products were chosen for the PART 3.2.

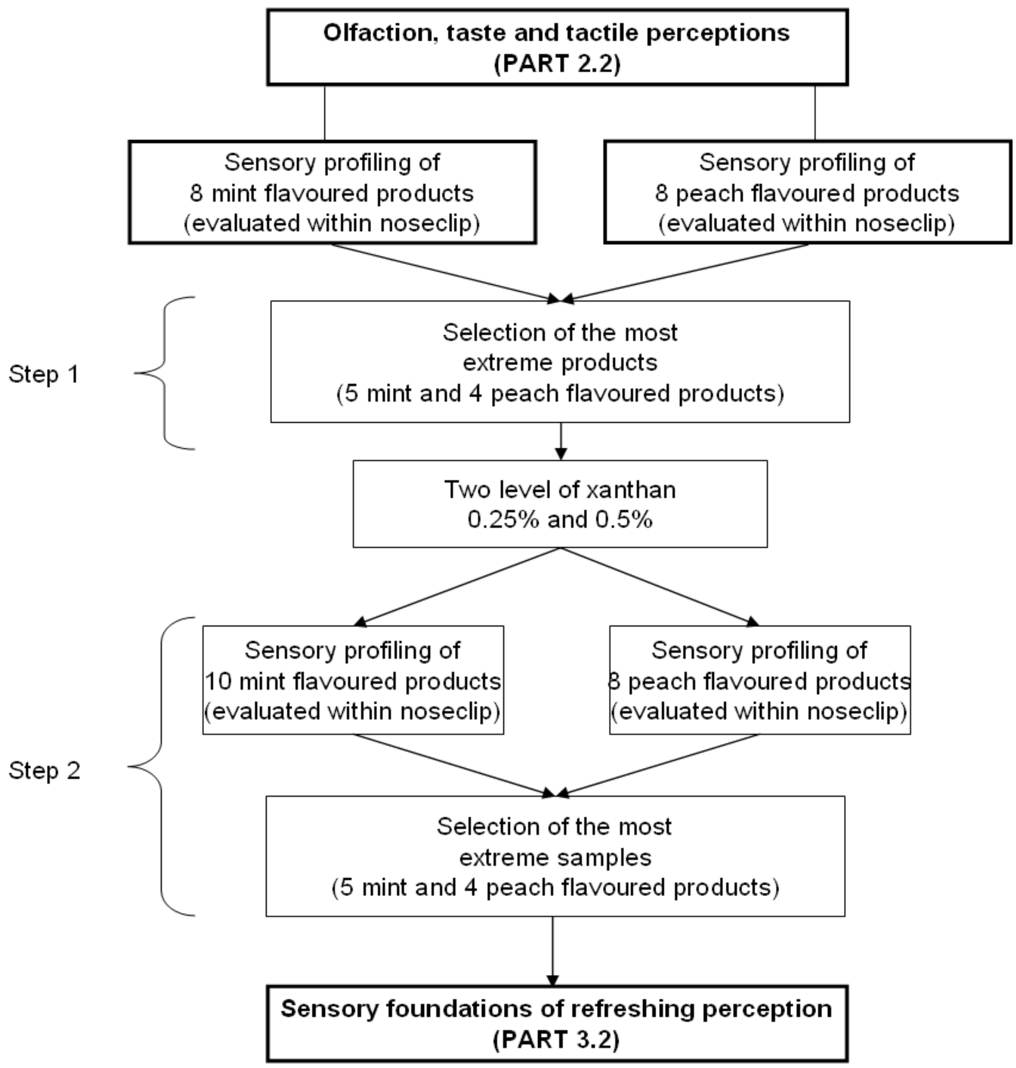

Fig.1: Schematic representation of performed formulation and evaluation steps to obtained samples used in PART 3.2.

PART 3: Complex perception: refreshing

3.1 Sensory basis of refreshing perception: role of psychophysiological factors and food experience

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Introduction

The Merriam-Webster Dictionary & Thesaurus (2006) defines "refreshing" as "serving to restore strength and animation, to revive, to arouse, to stimulate, to run water over or restore water to, with thirst quenching properties", suggesting that "refreshing" is linked to physiological factors such as thirst-quenching and arousal. To our knowledge, no peer-reviewed study has explored the relationship between the refreshing value of foods and drinks and these physiological factors. In contrast, a number of sensory and consumer studies have explored the refreshing attribute with regard to both expected and perceived characteristics of foods and drinks (Zellner and Durlach, 2003; Labbe et al., 2007). From these studies and in line with the dictionary definitions, we find that the perception of a food or drink as being refreshing is often associated with specific sensory characteristics related to psychophysiological states linked with water drinking and modulated by experience. This review explores the concept of refreshing following three axes. First, at the perceptual level, we examine the sensory drivers of refreshing perception. Second, studies of psychophysiological states in relation to water drinking are examined and potential links between psychophysiological factors and specific sensory characteristics in refreshing perception are reviewed. Third, studies on the influence of food experiences on perception of refreshing foods and drinks are explored. Finally, based on the reviewed studies, a model of the construction of refreshing perception is proposed.

Keywords: Refreshing, Sensory, Perception, Psychophysiology, Food experience.

Sensory drivers of refreshing perception

Sensory attributes related to food low temperature and cooling perception are among the most noteworthy drivers of refreshing perception (Zellner and Durlach, 2002). Indeed, cool drinks are typically described by consumers as being “refreshing”, especially during hot weather (Scriven et al., 1989). In addition, cold solid foods, such as ice cream, are also considered to be “refreshing” by many people (Zellner and Durlach, 2002).

Labbe and co-workers explored the sensory determinants of refreshing perception produced by a range of viscous fluid products varying in cooling agent, citric acid and thickener levels, and flavoured either with peach or with mint aroma (Labbe et al., 2007). A Swiss panel of 12 trained assessors described the nine products using a list of sensory attributes. Another group of 160 French consumers scored perceived refreshing intensity of the same products. The intensity of the attributes “cold” and “acid” (as defined by the trained assessors) is positively correlated with perceived refreshing intensity (rated by consumers). In contrast, increases in “sweetness” and “thickness” are negatively correlated with refreshing intensity. This latter finding is supported by two earlier studies. In the first, carried out in the UK with a range of non-alcoholic beverages (carbonated lemon, orange beverages, sparkling water, isotonic drinks, cola drinks and strawberry milk), thickness and sweetness are identified as negative drivers of thirst-quenching (McEwan and Colwill, 1996). In the second, a trained panel of American beer drinkers rated 18 different beers for various sensory attributes. Refreshing intensity correlates negatively with the intensity of the following attributes: “sweetness”, “thickness”, “flavour”, “astringency” and “after-taste”. In addition, the attribute “thirst-quenching” is also strongly correlated ($r = 0.95$) with perceived refreshing intensity (Guinard et al., 1998). These two studies demonstrate that perceived thirst-quenching and refreshing are closely linked and seem to be influenced by common sensory properties of drinks.

Non-oral sensory properties, such as colour, can also influence the perception of refreshing sensation induced by foods and beverages as shown by Zellner and Durlach (2003). In this study, three differently flavoured beverages (mint, lemon, and vanilla) were prepared in eight different colouring versions (clear, red, blue, green, yellow, purple, orange and brown). Three groups of American students tasted one set of eight beverages among the three flavours and rated perceived refreshing intensity. For all three flavours, colour significantly influences perceived refreshing intensity: for lemon, students rate the brown beverage as less refreshing than the clear, purple and yellow versions; for the mint, the brown and red versions receive the lowest refreshing intensity scores, and for vanilla, all colours are rated as about equally refreshing. Zellner and Durlach suggested that the clear beverages are often rated as most refreshing because of the association between clarity and water, while the low refreshing ratings for brown coloured lemon or mint flavoured drinks may have occurred because the colour is inappropriate (Zellner and Durlach, 2003).

Another study combined structured interviews on constructs of what constitutes oral freshness, with time-intensity measures of perceived oral freshness. The products investigated were different candies menthol and fruit flavoured, and different beverages (mineral water, cold water, apple juice and menthol water) [8]. This study was conducted in 12 European native English speakers. From the interviews, 42 individual factors are compiled and grouped according to six major clusters: in mouth cleanness, energy (including “bubble” factor), waterness (including factors related to the experience of “having water in mouth”) , coldness, taste (including mint and menthol factors) and smell. In the time-intensity study, cold water, menthol water and menthol candy produce the highest peak and mean scores for perceived freshness intensities. From this combination of approaches, the authors concluded that oral freshness is a multidimensional and dynamic concept and that the importance of each factor in the construction of this concept varies among subjects.

In summary, there is scientific evidence that olfaction, taste, texture and trigeminal stimulations can influence refreshing perception. The concepts of refreshing, thirst-quenching and freshness seem to be closely linked since they have common sensory drivers. This statement is reinforced by the strong positive correlations reported in the subjective ratings of refreshing and thirst-quenching intensities.

Psychophysiological origins of the sensory drivers of refreshing perception

The first part of this review highlighted the links between refreshing and sensory perceptions linked to water consumption (coldness, clarity, i.e. transparency, liquid texture, low sweetness, etc.). We now examine psychophysiological factors leading to water consumption in an attempt to better understand how and why refreshing value is attributed to these specific sensory characteristics.

Thirst

Thirst is the sensation of needing and/or wanting to drink. It has been variously described as “the perception of one’s need for drink” (French et al., 1995), as “the consequence of the need to moisten the mouth” (Brunstrom et al., 2000) and as “a physiological state linked to fluid deficit” (Saltmarsh, 2001). Physiologically, thirst is part of the mechanism that controls osmolality or volume of the extracellular liquid, and is regulated by the brain in the hypothalamus (Lahera and Tresguerres, 1992). Two types of physiological thirst are identified: “osmotic thirst”, the desire to drink related to an increase in solute concentrations in the extracellular fluid, and “volumetric thirst” linked to a drop in vascular volume such as during hemorrhage. These aspects of thirst do not however account for all “regular” drinking which seems to be driven by a combination of: 1) unpleasant dry-mouth sensations (Brunstrom et al., 2000); and/or 2) habits or social cues (French et al., 1995); and/or 3) the act of eating, possibly as an aid to the formation of a bolus that will be easier to swallow (Brunstrom, 2002).
Brunstrom et al. have proposed three stages for a drinking episode: initiation, maintenance and termination (Brunstrom et al., 2000). Initiation usually starts before physiological fluid need, due to in-mouth sensations and/or social cues (e.g., a meal). Initiation is followed by maintenance, corresponding to the period while thirst still persists at a high enough level to induce continued drinking. Drinking termination, or satiation, is triggered by oropharyngeal perceptions, gastric distension and/or post-absorptive mechanisms or cognitive factors. During dehydration, a 1-1.5 % increase in extracellular fluid osmolality is enough to induce a sensation of thirst (Lahera and Tresguerres, 1992). Even during rapid dehydration, as may occur during marathon running, thirst appears to provide an adequate indication of physiological water needs and stimulation to drink (Noakes, 2007). Termination of drinking might be expected to occur when extracellular fluid osmolality is normalized. However this does not seem to be the case. Even in dehydrated subjects, alleviation of thirst occurs before plasma dilution is significant. Osmolality usually starts to decrease about 10 minutes after cessation of drinking (Saltmarsh, 2001). This delay probably represents the time needed for the water to cross the intestinal wall and, via the blood, influence osmoreceptors in the brain. So if alleviation of thirst occurs before osmolality is restored, other pre-absorptive mechanisms

need to be involved. Stretch receptors present in the mouth, throat and stomach have been postulated as mediators of the transmission of information to the brain on how much liquid has been ingested (Saltmarsh, 2001), but additional factors may be implicated. Sensations leading to termination of drinking may be based on a combination of physiological responses to liquid passage through the oral, pharyngeal and gastric spaces. Psychological factors such as learning are also likely to play a role, and these are discussed in the last section of this review.

Under free-living conditions and with unrestricted access to water, young men become thirsty and drank before fluid deficit is developed (Phillips et al., 1984). This phenomenon is termed "anticipatory thirst" (Phillips et al., 1984). The mechanisms generating this thirst in advance of physiological needs for fluid are not completely understood but probably involve a learned anticipation of future fluid deficit, for example during exercise (Noakes, 2007) or to assist in mastication during eating (Brunstrom, 2002). In addition, levels of thirst and drinking decrease with age, making older people more vulnerable to dehydration (Kenney and Chiu, 2001) possibly due to impairment of such anticipatory thirst signals.

Using recent advances in neuroimaging techniques it is now possible to identify the brain areas involved in the emergence of sensations of thirst and thirst satiation in humans. The sensation of thirst was recently related to increased activity in phylogenetically ancient areas of the brain including the insula, the cerebellum and some parts of the hippocampal gyrus and anterior cingulate cortex which displays extensive connections to the hypothalamus (Egan et al., 2003). In addition, activity of this latter area was found to return to baseline level immediately after thirst satiation, whereas this occurres about 15 min later in the other areas. This time corresponds approximately to the time needed to complete normalization of osmolality. From these findings, the authors concluded that the anterior cingulate cortex is likely to be responsible for the emergence of thirst (Egan et al., 2003). Another neuroimaging study compared brain activity induced by the presence of water in the mouth as a function of thirst (de Araujo et al., 2003). In particular, thirst results in a higher level of activity in the caudal orbitofrontal cortex, compared with brain activity measured after thirst satiation. According to the authors, activity in the caudal orbitofrontal cortex in response to water may reflect the thirst level or motivational state of the subjects. With the emergence of these new techniques, brain responses to consummatory behavior can now be analyzed in a non-invasive manner, allowing better understanding of the mechanisms governing perceptions including refreshing and motivated behaviors.

In summary, it appears that thirst is often anticipatory (in that a sensation of thirst is perceived before significant dehydration has occurred) and that, even when dehydrated, thirst satiation seems to be triggered by relief of oropharyngeal symptoms such as mouth/throat dryness and unpleasant in-mouth perceptions and that this occurs well before extracellular fluid osmolality is normalized.

Mouth/throat dryness

Brunstrom (Brunstrom, 2002) suggested that drinking produces a progressive increase in parotid saliva flow and that this contributes to mouth wetting and hence to satiation of thirst. During dehydration in humans, infusion of water intragastrically restored hydration but thirst persisted until water was provided via the oral cavity (Figaro and Mack, 1997). In agreement with this, the perception of refreshing is often linked to alleviation of oropharyngeal symptoms (e.g. from mouth/throat dryness to mouth/throat wetting) rather than from completed rehydration (Brunstrom, 2002). The influence of oropharyngeal cues on drinking is complex and depends on hydration state, initial mouth dryness and volume consumed. In addition, for a given volume consumed, water at 5°C is perceived as more thirst-quenching than warmer (22°C) water (Brunstrom and MacRae, 1997). Eccles proposed that cold water is more effective than warm water in reducing thirst in humans because of the higher impact of cold water on salivation (Eccles, 2000). This idea was partly based on a previous study where parotid salivary flow was monitored and that demonstrated that water at 0°C produces more salivation than does water at room temperature (22°C) (Pangborn et al., 1970). It is also supported by other findings showing that the presence of water in the mouth at low temperatures (3°C or 10°C) is more efficient to increase salivation than warmer temperatures ranging from 22°C to 44°C (Brunstrom et al., 1997; Lee et al., 2006).

In the same way, menthone and menthol from peppermint oil are recognized as salivary flow enhancers, most likely in relation to the perception of coldness they induce (Haahr et al., 2004). Both are volatile compounds which give plants of the Menthe species their typical mint smell and flavour together with a cold or cooling perception. Coldness perception is mediated by specific receptors found in trigeminal cold-sensing neurons. These receptors are widely distributed (on the tongue, in the nasal cavity, and in the peripheral nervous system) and are activated by cold temperatures and by cooling agents such as menthone and menthol (for a review of mechanisms of temperature perception, see (Patapoutian et al., 2003)).

Other unpleasant in-mouth feeling experienced when thirsty may influence drinking behavior (Phillips et al., 1984; Brunstrom, 2002). Indeed, dry mouth has been identified as one of the factors linked to release of the volatile sulfur compounds responsible for bad breath (Feller and Blignaut, 2005). Water consumption may therefore contribute to the removal of this dry unpleasant taste and bad breath by its rinsing and cleaning actions.

In developed countries, extreme conditions of dehydration are rare. During everyday drinking, liquids are perceived as refreshing mainly because of their ability to alleviate oropharyngeal symptoms. Indeed, cold liquids are refreshing in part because they lead to mouth and throat wetness and in part because they enhance salivary flow due to cold stimulation. This may partly explain why cold or thin liquids are perceived as refreshing while astringent and thick liquids seem to dry the mouth even though the wetting effectiveness of astringent solutions is as efficient as that of water under dry mouth conditions (Guest et al., 2008).

Mental fatigue/energy

As stated in the two dictionary definitions cited in the introduction, refreshing perception is closely related to increased energy, arousal and stimulation. “Mental energy” has recently been described as a mood state linked to the ability to engage in cognitive work (O'Connor, 2004; Lieberman, 2006). It also refers to a functional state of the brain, such as cortical activation as measured with electroencephalography (EEG) (Lieberman, 2007). In this section, we examine how hydration status may affect mental energy in terms of mood, cognitive performance and cortical activation.

Mood

The effect of mild dehydration (1-3% loss of body weight as water) on subjective reports of physiological and mood states has been investigated during drinking restriction

(Shirreffs et al., 2004; Szinnai et al., 2005). As expected, dehydration induces increasing reported sensations of thirst and mouth dryness. Dehydrated subjects also reporte decreased ability to concentrate, reduced alertness and higher levels of fatigue, as compared to hydrated controls. These results confirm previous observations (Cian et al., 2001). The physiological changes and-or physical discomfort induced by moderate dehydration seem therefore to negatively influence subjective perceptions of mental energy.

Cognitive performance

Deterioration of cognitive performance during mild water restriction has been observed for a variety of cognitive tasks. Dehydrated subjects generally display lower performance in tasks involving sustained concentration or short-term memory when compared to hydrated controls (Gopinathan et al., 1988; Cian et al., 2001; Suhr et al., 2004; Bar-David et al., 2005). The physiological factors responsible for cognitive impairment associated with dehydration remain largely speculative and may be linked to general brain metabolism perturbation as has been reported in mice (Thurston et al., 1983) or to changes in plasma hormonal levels as observed humans (Maresh et al., 2006). For instance, the increased levels of the stress hormone cortisol observed by Maresh et al. (Maresh et al., 2006) after moderate dehydration (5% loss of body weight) should be enough to impair learning and memory processes (Het et al., 2005; Oei et al., 2006).

On one hand, water restriction, or dehydration, seems to increase mental fatigue and impair mental energy. But on the other, water consumption may also influence mental energy, either in a positive or negative manner, depending on prior thirst level. In an unpublished investigation on the psychostimulant effects of caffeine reported by Rogers et al. (Rogers et al., 2001), it was found that water consumption alone (used as a placebo treatment in the study) impaires visual attention processes compared to the “no drink” condition. Rogers and colleagues then examined the effect of water consumption on subjective alertness and performance of visual attention as a function of prior thirst status (Rogers et al., 2001). Water consumption induces immediate subjective sensations of being “alert” and “revitalized” in both the low and high thirst participants, while effects of water consumption on visual attention task performance dependes on prior thirst level (improved performance when thirst is high; decreased performance when low, as compared to a “no drink” condition). These findings suggest that the positive influence of refreshing on cognitive performance, but not subjective alertness, may be sensitive to hydration status. These results should, however, be treated with caution until they have been confirmed.

Besides water consumption, some other sensory stimulations driving refreshing perception have been shown to reduce mental fatigue and to improve mental energy. These effects have been consistently reported for coldness-cooling perception induced by odorous cooling compounds such as peppermint oil that contains both menthol and menthone. Peppermint is indeed widely reported to possess stimulating and invigorating properties. As compared to air, ambient exposure to peppermint oil was found to reduce sleepiness (Norrish and Dwyer, 2005), improve subjective alertness (Moss et al., 2008) and enhance performance in a range of cognitive tasks assessing attention and memory functions (Warm et al., 1991; Barker et al., 2003; McBride et al., 2004; Ho and Spence, 2005; Moss et al., 2008). Eccles suggested that the activation of nasal cold-receptors by menthol is similar to “taking a breath of fresh air,” leading to an increased level of alertness, or “cortical activation” (Eccles, 2000), as defined earlier (Oken and Salinsky, 1992). To illustrate his point, Eccles reported the example of the use of ‘smelling salts’, containing trigeminal stimulants such as menthol and ammonia, to arouse someone who has temporarily lost consciousness. From these observations, one could argue that the positive effects of smelling cooling compounds on mood and cognition might be mediated by neurophysiological mechanisms and, in particular, to the stimulation of the trigeminal system responsible for the coldness perception.

Cortical activation

Quantitative measurement of the functional state of the brain using EEG can provide evidence of changes in alertness, in terms of amplitude fluctuations of cortical oscillations (Oken and Salinsky, 1992). Consistent with subjective and performance data, recordings of EEG during water consumption reveale a greater enhancement of cortical activation in water-deprived subjects than in thirst-quenched subjects (Schmitt et al., 2000; Hallschmid et al., 2001). Following water consumption (1-3 min after drinking), cortical activation remaines enhanced in the low-alpha frequency range (8-10 Hz) only in water-deprived subjects (Hallschmid et al., 2002). These findings are consistent with other neuroimaging data on brain areas activated by water consumption (de Araujo et al., 2003). While activation of the insula does not differ in response to water as a function of hydration status, activation of the orbitofrontal cortex occurres only during the period before subjects had drunk to satiation; i.e., while drinking is still refreshing and rewarding. Taken together, these results suggest that increased cortical activation and activation of the insula observed during drinking reflect non-specific sensory and motor activity. In contrast, activation of the orbitofrontal cortex correlating with the process of thirst-quenching is consistent with the role of the orbitofrontal cortex in human motivation and related mood states. The persisting enhancement of cortical activation after thirst-quenching is hydration-dependent and may reflect direct measurement of refreshing perception.
Other EEG studies have reported increases in cortical activation induced by nasal stimulation with peppermint (Badia et al., 1990; Klemm et al., 1992) thus supporting Eccles' hypothesis (Eccles, 2000) of refreshing-induced alertness by cooling stimulation. Similar effects are induced by the chewing of gums flavoured with aromatic compounds including peppermint oil (Masumoto et al., 1998; Morinushi et al., 2000).
Consumption of mint or mint-flavoured candies, chewing gum or carbonated mineral water after a meal is common in many countries. Although, to our knowledge, the potential link has never been investigated scientifically, it may be in part driven by their perceived influence on alertness and-or digestion. If a person feels a lack of mental energy after eating (a phenomenon known as the post-prandial dip in alertness (Smith et al., 1990; Monk, 2005)), he or she may seek to improve feelings of energy by consuming a refreshing food or drink. A possible link with digestion is the observation that peppermint oil seems to facilitate digestion, possibly via a spasmolytic effect on gastrointestinal tract muscle (Grigoleit and Grigoleit, 2005).

Appetite

Appetite status may be another modulator of how we perceive the refreshing attributes of drinks (Rolls, 1993). To our knowledge, no systematic study has investigated the effects of appetite on refreshing perception of foods and drinks. The nearest sensation to appetite studied in relation to refreshing perception is the question on "filling" asked to volunteers during an investigation of the perceived "drinkability" properties of beer (defined as "the amount you think you can drink") (Guinard et al., 1998). Drinkability is increased by beers perceived as highly refreshing. In addition, refreshing and drinkability are both negatively correlated with strong flavour, viscosity, and aftertaste. Based on these results, the authors concluded that weaker-flavoured beers are perceived as less satiating and more refreshing than strongly-flavoured beers. This fits with previous findings (McEwan and Colwill, 1996; Guinard et al., 1998; Labbe et al., 2007) showing that high olfactory intensity, after-taste, sweetness and thickness are negative drivers of refreshing since they represent sensory properties of high caloric content food and beverage, generally not associated with water or other refreshing products.

Role of alliesthesia on refreshing perception

Alliesthesia, from the Greek word referring to altered sensation, is used to describe the observation that a given stimulus can induce a pleasant or unpleasant sensation depending on the subject's internal state (Cabanac, 1979). This phenomenon was investigated for the pleasantness of water drunk at different temperatures as a function of hydration status and body temperature (Boulze et al., 1983). Results showed that dehydration increases the perceived pleasantness of cold water (0°C) and decreases the pleasantness of warm water (50°C) compared to normal hydration status. In addition, cold water is even more pleasant during hyperthermia than during normothermia. These findings suggest that specific internal states, such as dehydration and hyperthermia, may reinforce the effects of both positive and negative sensory drivers on refreshing intensity. Alliesthesia is a phenomenon that should be taken into account when exploring refreshing perception.

In summary, refreshing perception induced by drinking water seems to alleviate some psychophysiological symptoms (including thirst, mouth dryness, and mental fatigue). Coldness induced by water and other cooling compounds may actively contribute to this process. As refreshing perception is associated with relief of psychophysiological symptoms, a transfer of refreshing valence to coldness may therefore occur through everyday associative learning. Further, internal incentives depending on physiological states such as dehydration or high body temperature may further influence refreshing perception.

Influence of experience on sensory drivers of refreshing

Role of learning during consumption

The degree to which a particular beverage or food is perceived as refreshing seems also to depend on learning from everyday eating and drinking experiences. As presented in the previous section, the positive experience of alleviation of unpleasant symptoms (thirst, mouth-dryness, mental fatigue, feeling too hot) following consumption of water or of another beverage leads to a learned association of the two and perception of the drink as refreshing. This can explain why Zellner and Durlach found that, among a group of American students asked to list foods, beverages and sensory characteristics they considered to be refreshing, water is the most frequently mentioned drink (90% of respondents) and cold temperature the most frequent sensory attribute (Zellner and Durlach, 2002). Learning may explain other associations with refreshing, such as the positive association with clear beverage colouring (Zellner and Durlach, 2003) and the negative associations with sweetness, thickness (McEwan and Colwill, 1996; Guinard et al., 1998; Labbe et al., 2007), intense flavour and after-taste (Guinard et al., 1998).

Acid tastants elicit a salivary reflex. As with water drinking, this salivation leads to mouth-wetting that is associated with thirst-quenching (French et al., 1995) and potentially with refreshing. This may, in part, explain why acid beverages (Brunstrom et al., 2000), and acid viscous fluids (Labbe et al., 2007) are often perceived as refreshing. Retronasal olfactory characteristics of acidic fruits such as orange are also associated with "refreshing" by consumers (Zellner and Durlach, 2002). A learned association between sourness and an accompanying orange aroma may explain why this odour is often added to products sold as "refreshing".

Labbe et al. noted that, in peach and mint flavoured viscous products, coldness, acidity and thickness are significantly correlated with perceived refreshing intensity (Labbe et al., 2007). On cluster analysis, they found that differences in the importance of these sensory characteristics for refreshing perception depends on the cluster considered. Coldness and acidity are the main positive drivers for refreshing in Clusters 1 (36% of respondents) and 2 (26%), respectively. For Cluster 3 (38%), coldness and acidity are less important but thickness is the main negative driver of refreshing. The speculation that these differences may be linked to past food experiences is supported by the further observation showing that Cluster 1 (coldness driven) included a greater proportion of heavy users of mint chewing gums compared to the other two clusters.

In summary, associative learning about sensory attributes often linked to positive psychological or physiological consequences of water drinking may sometimes generalize to the point where these attributes are perceived as refreshing in other contexts. This type of learning about food experiences has been elegantly explored in previous studies (Prescott, 1999; Koster et al., 2004; Mojet and Köster, 2005). However further work is needed to demonstrate that "flavour-refreshing" learning is as robust as other types of associative learning such as "sweet taste-olfaction" (Valentin et al., 2006; Auvray and Spence, 2007).

Marketing communication

Advertisers use the term "refreshing" as a claim in the belief that it may influence expectations about the product and later perception. These effects interact with personal attitudes and/or cultural conventions about particular foods and drinks, acting via memories and expectations. The influence of packaging features was investigated on expected characteristics (including refreshing) of an unfamiliar fruit juice among English consumers (Deliza et al., 2003). The authors manipulated background colour (orange/white), information (none/medium: "1 liter, pure"/high: "1 Liter, 100% Pure, Natural"), brand (none/minor UK brand/major UK brand), and image (drawing/photograph). Results confirmed that packaging features can indeed influence consumers' expectations concerning refreshing intensity that is improved by the following packaging conditions: white background, high information and major UK brand. The authors speculated that the effects of brand on expected refreshment might come from advertisements or from consumers' own experiences.

Another example of how consumer learning may be used in marketing communication is illustrated by a study exploring the expected refreshing properties of toothpastes as a function of colour (Lee and O' Mahony, 2005). The "refreshing look" of 20 commercial toothpastes of different colours was rated by 72 American consumers. Results showed that transparent blue toothpaste is expected to be the most refreshing and off-white the least refreshing. One can speculate that transparent blue may be perceptually the most closely associated to water and therefore to refreshing. This idea is supported by the results of a study showing that the same beverage evaluated in four differently coloured

glasses (blue, red, green and yellow) is perceived as most thirst-quenching when evaluated in the blue glass (Guéguen, 2003).
Zellner and Durlach reported that, for 50% of their sample of American students, sweetness is an expected characteristic of a thirst-quenching beverage (Zellner and Durlach, 2002). Using an open questionnaire study with 75 American students, Clydesdale and co-workers demonstrated that the four colours most frequently associated with thirst-quenching are clear, (36% of respondents), brown (24%), red (17%) and orange (12%) (Clydesdale et al., 1992). These findings contrast with sensory studies showing that brown (Zellner and Durlach, 2003) and sweetness (McEwan and Colwill, 1996; Guinard et al., 1998; Labbe et al., 2007) seem to be negative drivers of refreshing perception. Part of this controversy may be explained by the fact that in some circumstances, associating brown colour and sweetness with refreshing may result from experiences of drinking a cola beverage to quench thirst (Clydesdale et al., 1992; Zellner and Durlach, 2003). In addition, it is possible that frequently seeing advertising claims that a major brand of cola is refreshing may further influence these associations.

Pleasantness as a consequence of symptom alleviation

Several studies have demonstrated that pleasantness and refreshing perception (or related concepts) are positively correlated. In beverages: 1) for a wide range of different drinks, rated by English assessors, thirst-quenching intensity and acceptability scores are strongly correlated (McEwan and Colwill, 1996); 2) for drinks with different combinations of flavour and colour, rated by American students, refreshing and hedonic ratings follow similar patterns (Zellner and Durlach, 2003); 3) for bottled “nutritive drinks”, rated by Japanese assessors, scores for refreshing intensity and overall palatability are positively correlated (Kataoka et al., 2004); and 4) for the packaging rated as most liked is also expected by UK consumers to contain the most refreshing beverage (Deliza et al., 2003). Similar associations between pleasantness and expected refreshing intensity were obtained among American students for toothpastes (Lee and O' Mahony, 2005). Lastly, a study conducted with French consumers reported that refreshing intensity delivered by viscous fluids is strongly correlated with perceived pleasantness (Labbe et al., 2007).
Since, as this review has noted, refreshing perception is often linked to physiological needs, pleasure induced by refreshment may be a sign of satisfaction and fulfillment. This interpretation is supported by the work of Brunstrom et al. (1997) who asked participants to score preference for water at different temperatures (3, 13, 23 or 33 °C), under dry-mouth and non-dry mouth conditions. Pleasantness increases with lower temperatures and drier mouth feel, and this is correlated with higher levels of saliva production. Similarly, another study showed that respondents prefer and drink more cold water than warm water during and after exercise (Sandick et al., 1984). In addition, they scores 16 °C water as more pleasant after exercise than on control days, possibly due to the more noticeable relief of dry oral sensations induced by exercise. Guest et al. (2006) confirmed that cold water is preferred under dry mouth conditions but, in contrast to the findings of Sandick et al. (1984) and Brunstrom et al. (Brunstrom and MacRae, 1997), does not observe enhanced saliva flow. This can be due to the small amount (0.75 and 1.5 ml) of water ingested in the study of Guest and collaborators (Guest et al., 2006). Based on these observations, it seems that refreshing substances are often perceived as pleasant because they alleviate unpleasant feelings of dryness by either wetting the mouth of by stimulating saliva production. This is in line with the concept of alliesthesia where pleasantness is enhanced by the relief of physiological needs.

De Araujo and collaborators (de Araujo et al., 2003), in a study involving volunteers deprived of liquid for 6-8 hours, reporte a positive correlation between subjective pleasantness of water (as it quenches thirst) and activation of several brain regions including the orbitofrontal and anterior cingulate cortices. Other studies suggested that the reward value, or pleasantness, of oral temperature, and of taste and flavour of food are also represented in these brain regions (Rolls, 2006; Guest et al., 2007). Bringing together these different oral representations in the brain regions linked to reward provide evidence of neural integration of refreshing and pleasantness already shown at a perceptual level.

In summary, pleasure experienced during the consumption of a refreshing food or drink is likely to result from satisfaction of a psychophysiological need. In addition, the phenomenon of alliesthesia, as shown for water temperature and preference, probably plays a role in this association between refreshing perception and pleasure. Indeed, the internal state of humans in a context of water deprivation or in a "need to be refreshed" provides the motivation for consuming products with sensory properties that can alleviate the associated psychophysiological symptoms. In such a context, this may explain why sensory properties related to refreshing perception are also perceived as the most pleasant ones.

Conclusion

A schematic representation of how refreshing perception might be constructed is proposed in Fig. 1, below. First, a range of physiological symptoms can be alleviated by water drinking. In addition to body hydration mechanisms, water, through its specific sensory properties, has the ability to alleviate psychophysiological symptoms, leading to a refreshing perception and to pleasure. Everyday food and beverage experiences reinforce these associations between sensory experiences induced by water consumption and its related psychophysiological effects. Consequently, a refreshing value (perceived or expected) may be attributed to foods or drinks: 1) sharing some characteristics of water in terms of sensory properties (clear, cold, liquid); and 2) alleviating psychological or physiological symptoms in a similar manner to water (e.g., acidic foods and drinks, cooling compounds). In addition, food experiences can be expected to induce associative learning about perceptions conjointly present in refreshing products (e.g. mint olfactory perception and coldness) and new beliefs in terms of refreshing characteristics through marketing communication.

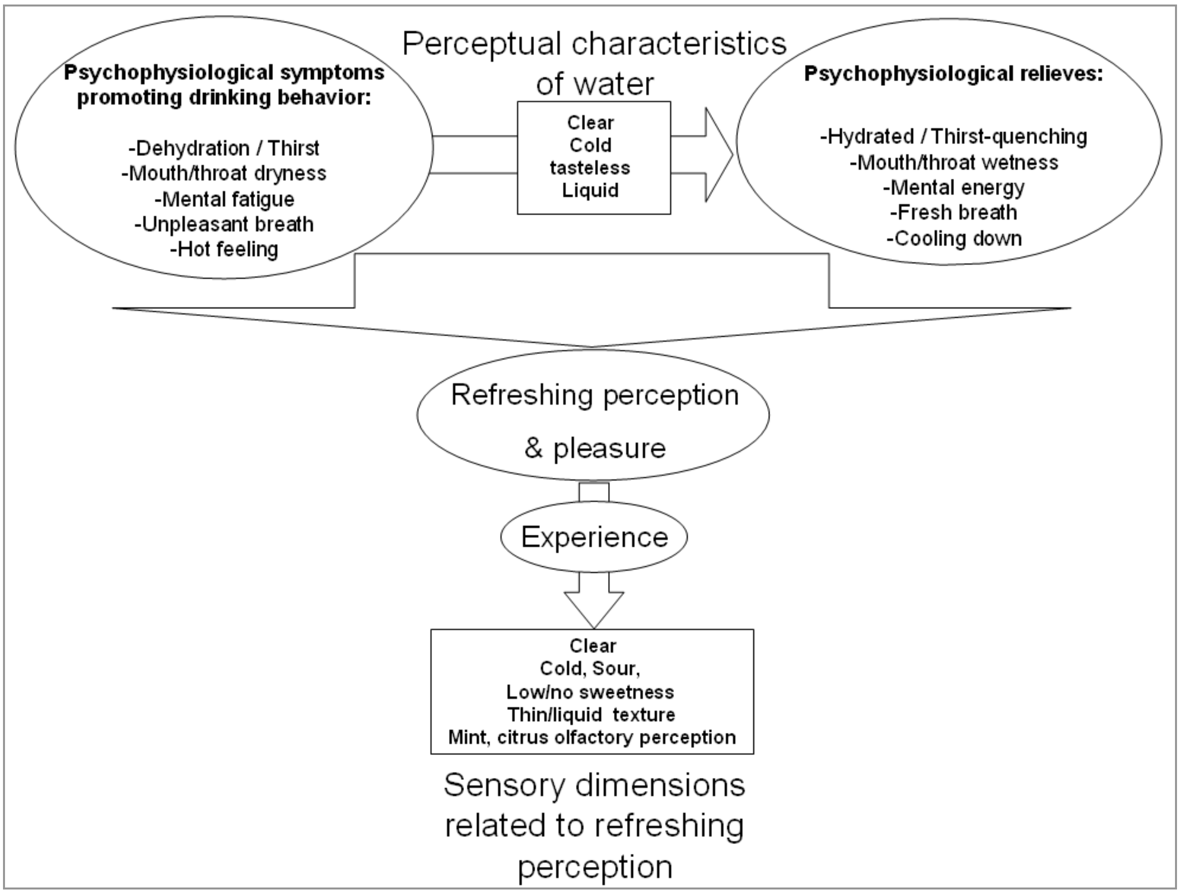

Figure 1: Schematic representation of the construction of refreshing perception
The alleviation of physiological symptoms by water drinking through its specific sensory properties leads to refreshing perception and pleasure. The daily consummatory experiences of water or any other food and beverage having sensory properties allowing to alleviate some of described psychophysiological symptoms induce the transfer of a refreshing value to a non exhaustive list of products attributes.

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3.2 Sensory foundations of refreshing perception

3.2.1 Sensory determinants of refreshing

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Sensory determinants of refreshing

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Abstract

To better understand how different sensory attributes combine to produce a refreshing sensation, we used a gel model system to vary the composition of ingredients selected to modulate the refreshing sensation: cooling agent, citric acid, peach and mint flavourings and xanthan. A group of 160 target consumers rated the refreshing intensity of the gels and a trained sensory panel evaluated the sensory properties of the same gels using Quantitative Descriptive Analysis®. An internal preference mapping methodology was applied to identify the contribution of the products' sensory characteristics to the refreshing scores given by consumers. Consumers agreed quite well on the least refreshing gels which were the sweetest, but they differed regarding the sensory drivers of the most refreshing gels. Three segments of consumers were identified for which refreshing was driven mainly by perception of cold/mint, acid and thickness, respectively. Food habits may be partly responsible for the different key sensory drivers among consumer clusters.

Keywords: Refreshing; Consumer test; Trained panel; Psychophysics

1. Introduction

Sensory determinants of complex perceptions such as refreshing, fresh, fatty or natural are multiple, involving olfaction, taste and texture modalities. In addition, physiological, psychological or social factors strongly modulate these perceptions. When looking at refreshing in the dictionary, the following definition can be found: *To restore strength and animation, to revive, to arouse, to stimulate, to run water over or restore water to, with thirst-quenching properties* (Merriam-Webster Dictionary & Thesaurus, 2006). According to this definition, refreshing seems to be mainly induced by water. Liquids may therefore be expected by consumers to be more refreshing than solids. This assumption is confirmed by a study (Zellner & Durlach, 2002) showing that, when asked to list ten foods or beverages they found refreshing, 86 American students most frequently listed water (90% of respondents). The students were also asked to *list ten characteristics of refreshing foods or beverages*. Results showed that temperature related attributes (cool, cold) were the sensory characteristics the most commonly reported (92% of respondents). A study conducted on beers with a wide range of sensory characteristics highlighted that the cooling sensation induced by beers was positively correlated to its refreshing sensation (Guinard, Souchard, Picot, Rogeaux, & Sieffermann, 1998). In addition, a study on freshness in oral care recently showed the association made by consumers between coldness and refreshing sensation (Westerink & Kozlov, 2004). In the latter study, freshness has the same meaning as refreshing in our work. Through subject interviews, the authors highlighted the different sensory, physiological and cognitive factors that contribute to oral freshness including "cool/coldness" and "taste" more often described by "menthol or mint" but also "citrus" by few participants. Citrus and peach have been reported elsewhere as fruit aromas associated with refreshing (Martin et al., 2005). The perceived or expected acidity of the fruits may explain why they are perceived as being refreshing. Acid perception has indeed been

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reported as a positive driver of refreshing characteristics in beverages (McEwan & Colwill, 1996).

Besides positive drivers of refreshing sensation (coldness, mint odour, peach odour or acidity), some attributes seem to be negative drivers of refreshing sensation such as thickness and sweetness. Both attributes were identified as negative drivers of refreshing sensation in soft beverages. A strawberry milk beverage was perceived as being less refreshing than a carbonated lemon drink (McEwan & Colwill, 1996). Beers with a high thickness and an intense flavour were perceived as poorly refreshing, thirst-quenching and drinkable (Guinard et al., 1998). Authors explained that the negative link between flavour and texture strength and "refreshing/cooling", "thirst-quenching" and "drinkable" is due to a cognitive association between intensity, caloric content and filling sensation. The negative correlation between thickness and refreshing sensation was also reported by Scriven, Gains, Green, and Thomson (1989) who asked 22 consumers about the contexts in which they typically consumed a range of 22 alcoholic beverages. Five perceptual dimensions common to most people, that explained beverage consumption context, were pointed out. One of them opposed "thirst-quenching" *vs.* "not thirst-quenching". Beverages having the highest thirst-quenching potential were beers and lagers as opposed to brandy, Irish cream and port wine that were thicker.

The above mentioned studies explored refreshing sensation in various commercial foods and highlighted the role of trigeminal perception (cold), odour (mint, peach), taste (acid, sweet) and texture (thickness) in refreshing sensation. But the respective roles of each perception have never been investigated simultaneously in a controlled system. The objective of this study is to better understand the respective roles of olfaction, taste, trigeminal and texture perception on refreshing sensation in a gel model system. Therefore we formulated a range of products with a wide sensory diversity by varying the composition in ingredients inducing different perceptions: a mint odorant, a peach odorant, a cooling agent, an organic acid and a thickener.

Several methodologies have been used in the literature to measure the contribution of sensory determinants to complex perceptions: (1) a trained panel for scoring both refreshing intensity of beers and their sensory characteristics (Guinard et al., 1998); (2) a group of consumers for scoring both freshness of apples and their sensory characteristics (Peneau, Hoehn, Roth, Escher, & Nuessli, 2006) and (3) a trained panel for scoring standard sensory attributes and a group of consumers for scoring either refreshing sensation of beers (McEwan & Colwill, 1996) or creaminess in dairy products (Richardson-Harman et al., 2000; Tournier, Martin, Guichard, Issanchou, & Sulmont-Rossé, 2007). The latter methodology was used in the present study. We assumed that combining analytical data (from Quantitative Descriptive Analyses® using a trained panel) and holistic data (refreshing intensity scoring with a group of consumers) would give a characterisation of refreshing sensation based on consumer perception. A refreshing mapping was built similarly to a standard internal preference mapping (Mc Fie & Thomson, 1988) but using refreshing scores instead of liking scores.

2. Materials and methods

2.1. Product formulation and selection of a reduced set of products with a wide range of sensory characteristics

A gel with visco-elastic properties containing fructose (16%) sucrose (32%), dextrose (32%) and water (20%) was used as model for this study. A mint odorant (supplier reference 112606), a peach odorant (supplier reference L-129046), a cooling agent (WS-3), these three additives being provided by Givandan SA (Geneva, Switzerland), citric acid and a Keltrol RD xanthan gum thickener provided by CP Kelco Ltd. (Knowsley, UK) were used to induce olfactory (mint and peach), trigeminal (coldness), taste (acidity) and texture (thickness) perception of different intensities. Two formulation designs were built, one for each odorant, with the four ingredients (odorant, cooling agent, citric acid and thickener) at a low and a high level. For each ingredient, the two concentrations were selected according to the following sensory criteria: the two concentrations for each ingredient clearly induced two discriminable perceptual intensities. As it was key to dissociate cold and olfactory perception, the mint odorant was selected so that it did not induce any cold perception. This was checked through preliminary tasting using a noseclip. Sixteen gel recipes were therefore prepared for the peach and the mint odorant, respectively (Table 1). The most different gels within each odorant type were selected based on two preliminary QDA® conducted independently on each set of products with ten trained subjects and a list of attributes generated by the panel. A total of nine products was kept for the main study (Table 1).

2.2. Sensory characterisation by a trained panel

Twelve external assessors, all women, with an average age of 45, were recruited for the study. None of the assessors participated in the two preliminary QDA®. They were used to participate in sensory panels on different product categories but not on gel products. A 90-min session was dedicated to term generation; three 60-min sessions were conducted for the training on the selected terms and the tasting procedure. Ten attributes were selected (overall odour, coldness, overall flavour, acidity, sweetness, bitterness, salivating, thickness, astringency, coldness persistency) following the term generation and selection phases, performed according to NF ISO 11035 standard (1995). Olfactory attributes were not specific to the flavouring type but were related to the overall perceived intensity (overall odour and overall flavour) to avoid a product clustering opposing peach and mint gels. The role of these two

Table 1
Two formulation designs with the ingredient concentrations corresponding to each level: (a) mint flavoured gels and (b) peach flavoured gels

a

	Mint [illegible]		Cooling agent		Citric acid		Gellan gum	
Level	Low ([illegible])	High ([illegible])	Low ([illegible])	High ([illegible])	Low ([illegible])	High ([illegible])	Low ([illegible])	High ([illegible])
Symbol								
1	✓		✓		✓		✓	
2	✓		✓		✓			✓
3	✓		✓			✓	✓	
4	✓		✓			✓		✓
5	✓			✓	✓		✓	
6	✓			✓	✓			✓
7	✓			✓		✓	✓	
8	✓			✓		✓		✓
9		✓	✓		✓		✓	
10		✓	✓		✓			✓
11		✓	✓			✓	✓	
12		✓	✓			✓		✓
13		✓		✓	✓		✓	
14		✓		✓	✓			✓
15		✓		✓		✓	✓	
16		✓		✓		✓		✓

b

	[illegible]		Cooling agent		Citric acid		Gellan gum	
Level	Low ([illegible])	High ([illegible])	Low ([illegible])	High ([illegible])	Low ([illegible])	High ([illegible])	Low ([illegible])	High ([illegible])
Symbol								
1	✓		✓		✓		✓	
2	✓		✓		✓			✓
3	✓		✓			✓	✓	
4	✓		✓			✓		✓
5	✓			✓	✓		✓	
6	✓			✓	✓			✓
7	✓			✓		✓	✓	
8	✓			✓		✓		✓
9		✓	✓		✓		✓	
10		✓	✓		✓			✓
11		✓	✓			✓	✓	
12		✓	✓			✓		✓
13		✓		✓	✓		✓	
14		✓		✓	✓			✓
15		✓		✓		✓	✓	
16		✓		✓		✓		✓

The nine products with a code number in bold are the products selected for the main sensory study and the consumer test.

attributes was to prevent product evaluation from a dumping effect, meaning a transfer of the olfactory perception on the other scales when no olfactory intensity scale is available. Overall odour and overall flavour attributes were not taken into account for the statistical analysis.

During training, subjects were familiarized with the specific vocabulary. They were also trained to rate intensity on a 10-cm unstructured linear scale, anchored at the extremities with "not at all intense" and "very intense". Due to the temporal progress of the coldness perception induced by the cooling agent (Gwartney & Heymann, 1995), a specific procedure was implemented to standardize the evaluation and the quantity of product to swallow (Table 2). Data acquisition was carried out on a computer screen with FIZZ software (Biosystèmes, 1990). Products were evaluated during two sessions with four and five products per session. The presentation design balanced position and order effects, based on Williams Latin Squares. Products were coded with three-digit random numbers and served at room temperature (22 °C) in a 50-ml cup. Rinsing was done between products with water and unsalted crackers. Tests were conducted in an air-conditioned room (18 °C), under white light in individual booths.

2.3. Evaluation of refreshing intensity by consumers

Consumers were recruited in France, 80 males and 80 females. As chewing gum and candy consumers were targeted, only participants between 18 and 35 years-old were selected for the recruitment step. We assumed that the difference in age between consumers and trained subjects (average age of 45) did not affect results as previous studies exploring the effect of ageing on perception showed that taste sensitivity remained unimpaired in normal healthy subjects until 60 (Cooper, Bilash, & Zubek, 1959; Schiffman, 1993 cited by Bitnes, Martens, Ueland, & Martens, 2007).

During this step, the consumer filled in a food habit questionnaire including exclusion criteria relating to the frequency of consumption of a confectionery (chewing gum or candy) assumed as refreshing because associated with claims as a breath refresher. To the question *How often do you consume sugars to refresh your breath?*, they had to tick off one of the seven following proposals: *Several times a day! At least once a day! Several times a week! At least once a week! Several time a month! Five to ten times a year! More rarely*. Consumers who chose one of the last two proposals were not recruited for the study.

The consumer test was carried out in hall. Consumers came once for a 60-min session. Each of the nine products was presented individually (sequential monadic way) according to a design based on Williams Latin Squares balancing position and order effects. The refreshing intensity of the nine products was scored on a 10 cm unstructured linear scale, anchored at the extremities with "not at all refreshing" and "very refreshing". Rinsing was done between products with water and unsalted crackers during the 5-min break. A 10-sheet questionnaire was used, one sheet per product evaluation which was collected after each scoring by one of the two supervisors present at the tasting place. An additional preference question was asked at the end of the test on the last sheet: *Which product do you prefer?* The codes of the nine products were presented with, for each consumer, the same presentation order as during the tasting. The consumer was asked to circle the preferred product among the nine.

2.4. Statistical treatment

A two-way analysis of variance (ANOVA) with interaction was performed for each sensory attribute to determine if the products could be discriminated by the trained

Table 2
Evaluation protocol and attribute definition used in the sensory evaluation with the trained panel

Evaluuvation protocol	Attribute	Definition (from not at all intense to very intense)
Smell above the cup and score the intensity of:	Overall odour	Globle intensity perceived orthonasally
Put one spoonful of gel in mouth and mix it with the tongue during eight seconds, then swallow Wait for eight seconds and score the intensity of:	Coldness	Perception related to temperature
	Overall flavour	Global intensity perceived retronasally
Swallow a second spoonful of gel and score the intensity of:	Acidity	Perception related to acid taste
	Sweetness	Perception related to sweet taste
	Bitterness	Perception related to bitter taste
Swallow a third spoonful of gel and score the intensity of:	Salivating	Perception related to the mouth lubrification
	Thickness	Perception related to the thickness of the gel being moved with the tongue
	Astringency	Perception related the tongue and mouth dryness
	Coldness	Linger temperature perception incluced by the three spoonfuls of gel
	Persistence	

panel. The factor *product* was set to fixed and the factor *assessor* was set to random. The Fisher's least significant difference (LSD) was then computed to determine if the differences between each pair of products were significant. Principal component analysis (PCA) on the matrix of correlations was performed as well as a mapping of the products based on the first two principal components (PCA biplot).

A two-way ANOVA with interaction was performed on the refreshing scores to determine if products were discriminated by the consumer panel. The factor *product* was set to fixed and the factor *consumer* was set to random. Fisher's LSD was then computed to determine if the differences between each pair of products were significant.

The relationships between refreshing scores and sensory profiles were investigated using the widely used internal preference mapping methodology (Mc Fie & Thomson, 1988; McEwan, 1996; Yackinous, Wee, & Guinard, 1999) where liking scores were replaced by refreshing scores. PCA was performed on the matrix of correlations. Consumers were considered as being the variables (refreshing scores) and products as being the observations. Sensory attributes were then superimposed and represented on the PCA biplot as supplementary variables (coordinates were obtained by orthogonal projection on the first two principal components). This allowed the refreshing dimensions to be interpreted in terms of sensory drivers.

The presence of clusters among consumers was investigated with the K-means algorithm (Hartigan & Wong, 1979) using normalized refreshing scores to give an equal weight to each consumer. The K-means clustering method belongs to the partitioning methods family. These methods attempt to directly decompose the data set into a set of disjoint clusters while minimizing some measure of dissimilarity within each cluster and maximizing the dissimilarity between different clusters. The K-means is the most commonly used partitioning method and, like all partitioning methods, requires that the number of clusters is fixed a priori. The algorithm tries then to minimize the average squared distance of the consumers from their nearest cluster centre. The algorithm stops when convergence is achieved or after a certain number of iterations.

For each identified cluster: (1) a two-way ANOVA with interaction was performed on the refreshing score average to determine if products were discriminated by consumers belonging to the cluster, (2) Fisher's LSD was then computed to determine if the differences in refreshing intensity between each pair of products were significant and (3) a Pearson's correlation coefficient was calculated between the refreshing intensity scores of consumers and each sensory attribute across products.

The confidence level was set to 5% for ANOVA tests, LSD tests and correlation coefficients and all statistical treatment were carried out using NCSS software (Hintze, 2001).

3. Results

3.1. Sensory characterisation of the gels

All attributes significantly discriminated the products based on ANOVA results (p-value < 0.05). Results of the PCA showed that 88% of the total information was represented by the first two principal components (73% and 15%, respectively). The third principal component only represented 6% of the total information. The first two principal components were therefore selected for the PCA biplot (Fig. 1). Variance in the first principal component was explained by differences between samples characterised by the sweet attribute (to the left) and a group of correlated attributes (to the right): cold, cold persistence, astringency, bitter and salivating. Variance in the second principal component was explained by differences between samples characterized by the acid attribute (to the top) and the thick attribute (to the bottom). The nine products are well distributed across these four main sensory dimensions.

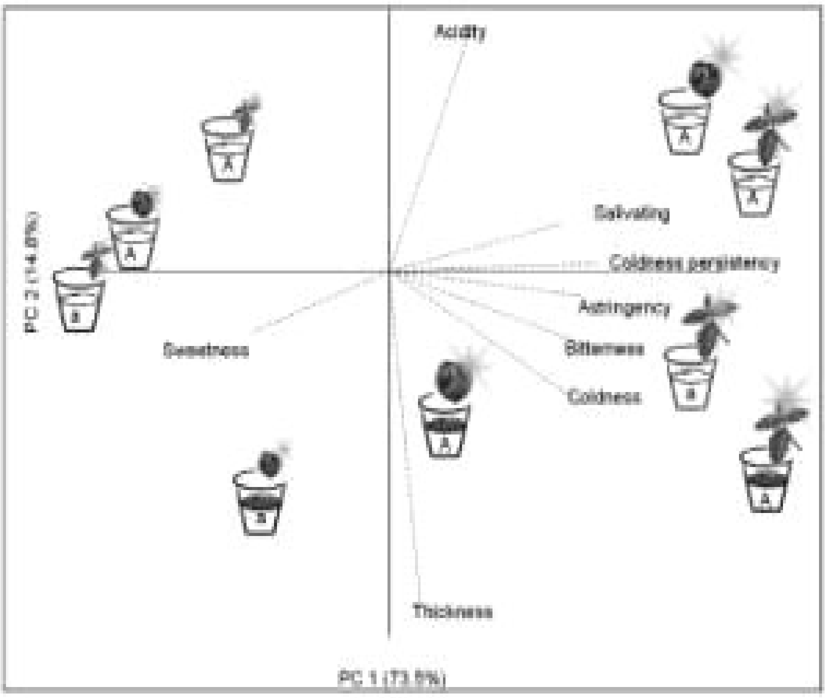

Fig. 1. Principal component (PC) analysis biplot (PC1 and PC2) representing the nine discriminating sensory attributes as variables and the nine products selected for the consumer test as observations. Product recipes and symbols representing the ingredients are explained in Table 1.

3.2. Consumer characterisation of the refreshing intensity and refreshing internal mapping

The refreshing score average across the 160 consumers for each gel showed that the gels induced a wide range of refreshing intensity, from 2.8 for the least refreshing gel to 6.6 for the most refreshing gel (Fig. 2). Based on the ANOVA performed on refreshing score average across products and LSD value (0.45), four groups of gels named a, b, c and d (Fig. 2) were significantly different in terms of refreshing intensity. Regarding the composition of extreme products, the two most refreshing gels (group a) contained the highest level of cooling agent and mint flavouring and the lowest level of thickener whereas the four least refreshing gels (group d) contained the lowest level of cooling agent and flavouring.

The PCA biplot represents the 160 consumers and the nine products (Fig. 3). Products that are close on the map were scored similarly by consumers. The position of each consumer illustrates how refreshing they perceived each product: the closer a consumer to a product, the more refreshing this product for that consumer. Most consumers are on the right hand side of the map, indicating that there was a high consumer agreement regarding the four least refreshing products. These products are characterised by a low level of odorant and cooling agent but are made with different odorants, thickener and citric acid levels. These four least refreshing gels were perceived as being the sweetest by the trained panel. Regarding the most refreshing gels, consumers were distributed across five gels with different sensory properties according to the sensory characterisation done by the trained panel (on the right hand side of the map).

To further investigate the sensory drivers of refreshing sensation, a new PCA biplot was performed using only the five most refreshing products (Fig. 4). K-means clustering highlighted three clusters of consumers (denoted by 1, 2 and 3, respectively). Consumers in cluster 1 (36% of consumers) scored mint flavoured gels as the most refreshing (Fig. 5a). The two peach flavoured gels were significantly scored less refreshing that the mint flavoured gels. Correlations, for the five retained gels, between cluster 1 refreshing scores and the sensory attribute intensities showed that cold and bitter attributes were significantly and positively correlated to refreshing intensity ($r = 0.95$ and 0.92, respectively) whereas sweetness was significantly negatively correlated to refreshing for these five gels ($r = -0.95$) (Fig. 6a). Cluster 2 represents 26% of consumers who principally scored the sample with a low level of citric acid as being significantly the least refreshing of the five samples (Fig. 5b). Highest positive and negative correlations between cluster 2 refreshing scores and sensory attributes were found for acid ($r = 0.49$) and astringent ($r = -0.68$), respectively (Fig. 6b). Cluster 3 represents 38% of consumers who scored the three products having the low thickener level as being more refreshing than the two products with the high thickener level (Fig. 5c). Regarding correlations between cluster 3 refreshing scores and sensory attributes, thick was significantly negatively correlated to refreshing for this cluster of consumers ($r = -0.90$) (Fig. 6c).

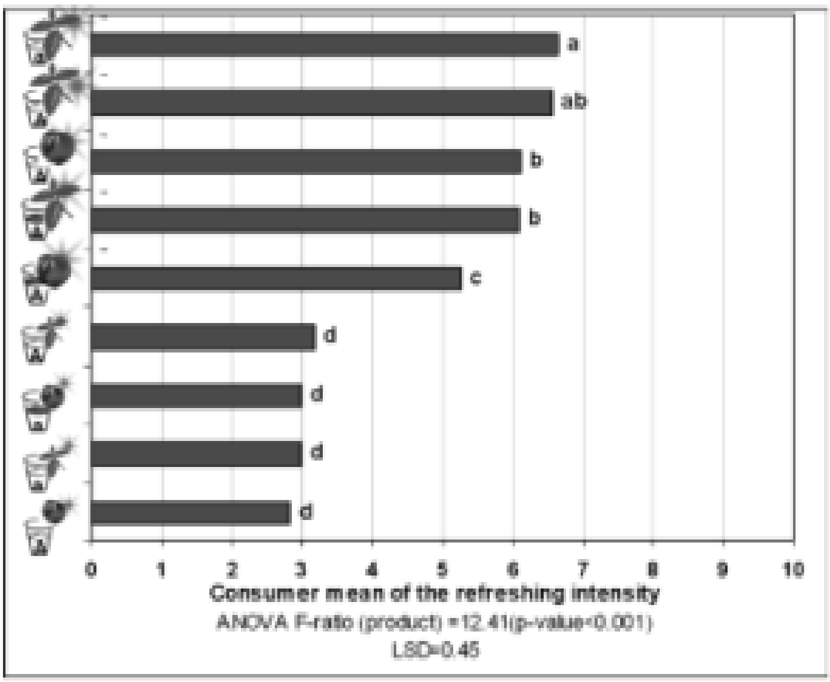

Fig. 2. Refreshing scores averaged across the 160 consumers for each gel. Products with a same letter are not significantly different according to the LSD value.

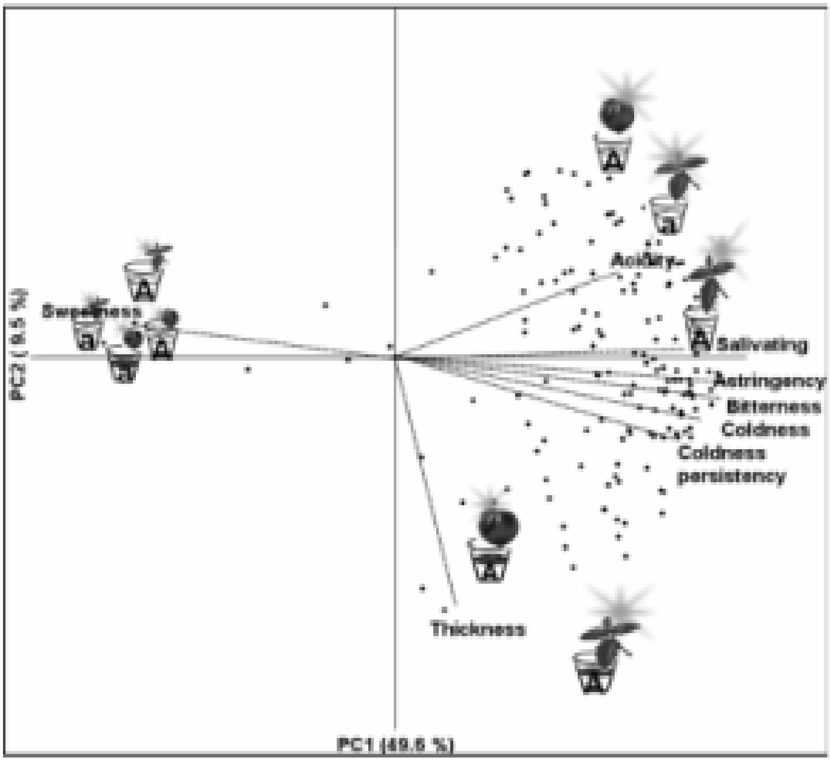

Fig. 3. Principal component (PC) analysis biplot (PC1 and PC2) representing the 160 consumers as variables and the nine products as observations. Sensory attributes were superimposed as supplementary variables.

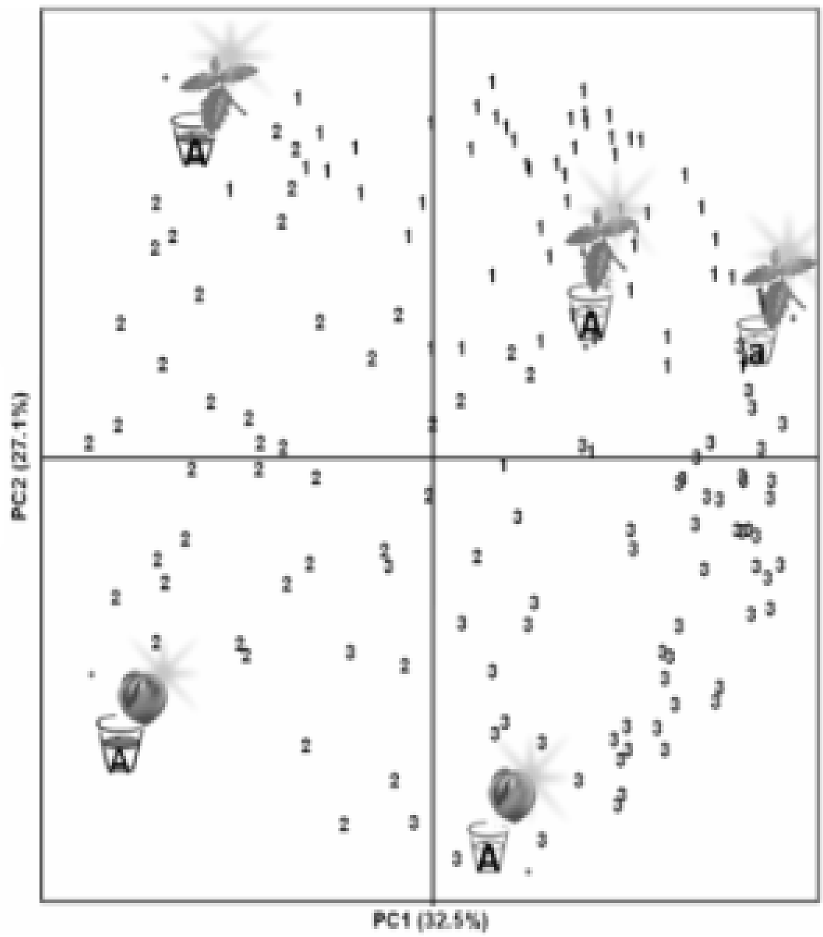

Fig. 4. Principal component (PC) analysis biplot (PC1 and PC2) representing the consumers as variables and the five most refreshing products as observations. The numbers "1", "2" and "3" represent consumers belonging to cluster 1 (36% of the 160 consumers), to cluster 2 (26% of the 160 consumers) and to cluster 3 (38% of the 160 consumers).

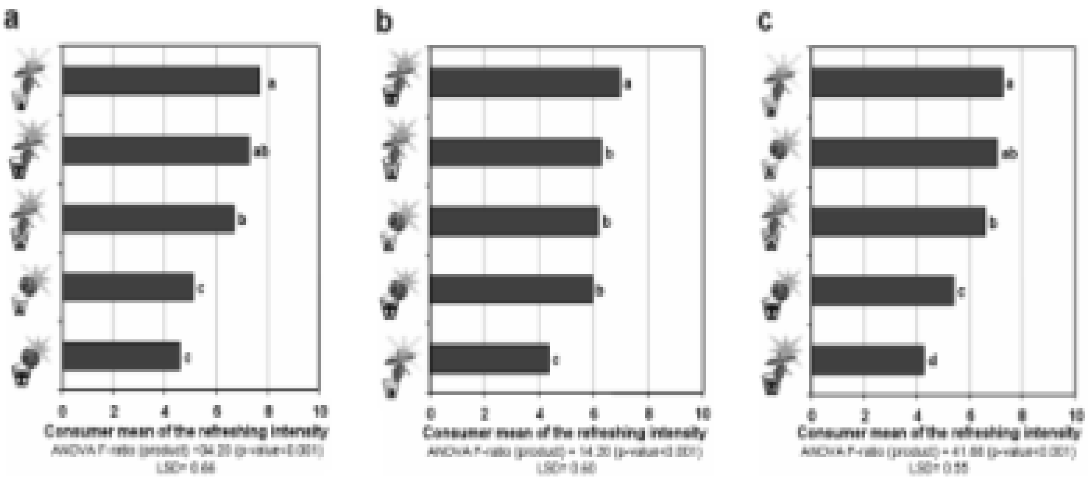

Fig. 5. Refreshing intensity mean score of consumers per cluster for the five most refreshing gels: (a) cluster 1; (b) cluster 2; (c) cluster 3. Products with a same letter are not significantly different according to the LSD value.

3.3. Preference for the gels

The refreshing intensity of the gels was strongly related to consumer preference. More than 2/3 of consumers (69%) preferred the product they scored the highest in term of refreshing. This percentage was slightly lower in cluster 2 (53%) than in clusters 1 and 3 (74% and 73%, respectively).

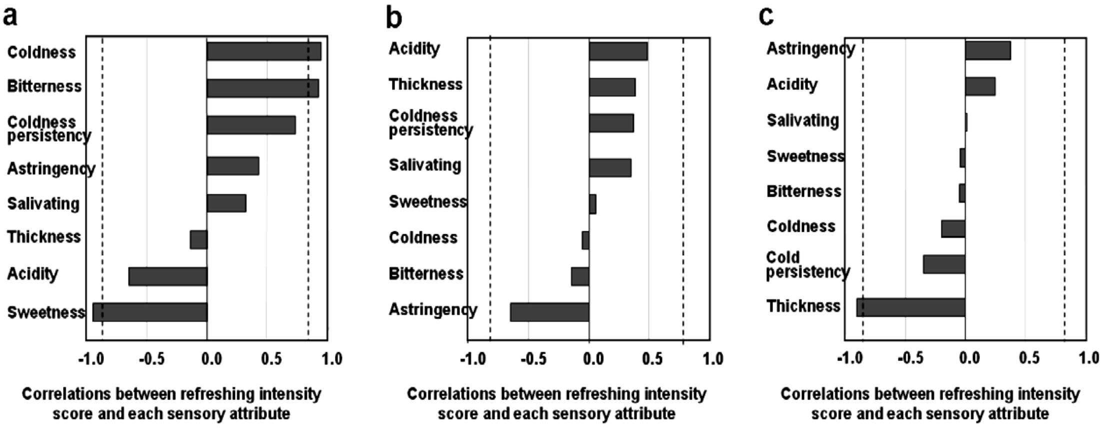

Fig. 6. Correlations between refreshing intensity mean score of consumer per cluster and sensory attribute mean scores of the trained panel for the five gels: (a) cluster 1; (b) cluster 2; (c) cluster 3. Correlation is significant when $|r| > 0.88$ (represented by the dotted black line).

4. Discussion

4.1. High sweetness reduced refreshing sensation for consumers

The sensory diversity of gels allowed consumers to well discriminate the products in terms of refreshing sensation and to obtain a wide range of refreshing intensity scores. The internal "refreshing" mapping with the nine gels clearly showed that sweetness reduced refreshing sensation for consumers. Indeed the four gels evaluated as the sweetest by the trained panel were scored by consumers as the least refreshing. Even if the formulation plan did not include a sweet tastant, sweetness was mainly modulated by the addition of citric acid which enhanced acidity and therefore reduced sweetness by taste/taste suppressive interaction (Keast & Breslin, 2003). This finding confirmed the negative impact of sweetness on refreshing already shown in beverages (McEwan & Colwill, 1996) and more specifically in beers (Guinard et al., 1998) and in low-sugar orange gels (Damasio, Costell, & Duran, 1997). On the contrary, the sensory drivers of the gels perceived as being the most refreshing differed among consumers.

4.2. Consumers were segmented according to their sensory drivers of refreshing

Three consumer clusters were identified for whom sensory drivers of refreshing differed. Following the same approach to investigate the complex perception of creaminess, Tournier et al. (2007) came to similar conclusions for the topic of creaminess for dairy products. They showed that consumers agree about the least creamy dairy products whereas the sensory drivers of creaminess varied among consumers. Three consumer clusters were also identified with specific sensory drivers of creaminess for each of them. On the other hand, for another set of dairy products, the four consumer clusters highlighted by Richardson-Harman et al. (2000) regarding drivers of creaminess were explained by differences in terms of demographics and preference but not in terms of sensory characteristics. In the latter study products mainly differed in their fat content and the low sensory diversity may explain these results.

4.3. Sensory drivers of refreshing

Sensory drivers positively associated with refreshing differed among consumers. Three main clusters were identified. For the cluster 1, the gels made with the mint odorant were scored as the most refreshing and cold was a key driver of refreshing sensation. This result agrees with findings of Guinard et al. (1998), Zellner and Durlach (2002) and Westerink and Kozlov (2004). In the latter study coldness and mint stimuli were reported as being strongly associated with oral refreshing concept for consumers. Unexpectedly, bitterness was correlated to refreshing whereas Guinard et al. (1998) showed that bitterness was a negative determinant of refreshing in beer. However this relation between refreshing and bitterness was likely not causal. Indeed bitter taste was probably induced by the cooling agent itself, as already reported for other trigeminal compounds such as menthol (Green & Schullery, 2003; Gwartney & Heymann, 1995, 1996). In addition, the bitterness did not seem to significantly impact consumer perception. Indeed bitter taste is generally reported as aversive to the consumer (Drewnowski & Gomez-Carneros, 2000) whereas in the present study, the two gels with the highest level of cooling agents and bitterness intensity were the preferred for this consumer cluster.

For consumers belonging to cluster 2, the sensory driver of refreshing sensation was a high acidity. This agrees with McEwan and Colwill (1996) who showed that a carbonated lemon drink was most refreshing than orange juice, orange squash, cola, isotonic, sparkling water, diet cola and strawberry milk for consumers mainly due to its high acidity intensity. This relationship between acid taste and refreshing sensation could be due to the salivation reflex elicited by acid tastants leading to a mouth-wetting effect which is expected to quench thirst (French, Read, Booth, & Arkley, 1995). The authors suggest that the drink might become a conditioned stimulus for salivation, further boosting the physiological signal which provides a basis for expected thirst-quenching. In the present study, the attributes acid and salivating were both correlated to refreshing sensation. This result supports the above mentioned explanation. In addition, astringency was negatively correlated to refreshing (and therefore to acidity and salivating). Since, according to literature, astringency is commonly described sensorially as mouth dryness and roughness (Lesschaeve & Noble, 2005), the negative correlation between astringency and refreshing is therefore consistent.

Consumers from the cluster 3 scored gels with a low thickness intensity as the most refreshing. This result confirmed findings of previous studies conducted: (1) in alcoholic beverages (Scriven et al., 1989), (2) in beers (Guinard et al., 1998) and (3) in soft drinks (McEwan & Colwill, 1996). Refreshing is a sensation strongly related to drinking and in-mouth water experience. Thick, which is more associated with solid food, therefore reduces refreshing sensation.

Regarding the impact of cold, acid and thick on refreshing, Ramsay and Booth (1991) and McEwan and Colwill (1996) pointed out that the refreshing property of a drink is modulated by the body state of the consumer (water deficient, feeling hot, dry mouth) and the desire to drink water for hydration purposes but also to wet the mouth and to cool. Food inducing sensory perceptions similar to those following a drink of water is likely to be perceived as refreshing.

Even if two out of the five most refreshing gels were flavoured with peach odorant, peach flavour was not pointed out as a major key driver of refreshing sensation contrary to mint odorant which had a high boosting impact on refreshing sensation for consumers. This was especially true for one consumer cluster. In addition, on average the mint flavoured gels were scored as more refreshing than the peach flavoured gels, for the same flavouring, citric acid, cooling agent and thickener levels (Fig. 2).

4.4. Food habits may have an impact on key sensory drivers of refreshing sensation

The reason why sensory drivers of refreshing differed between consumers (mint/cold for cluster 1, acidity for cluster 2 and thickness for cluster 3) may be difference among consumers regarding their food experience (Köster, 2005; Köster, Prescott, & Köster, 2004). Cluster 1 presented a frequency of heavy users of breath refreshers (with a several times a day consumption) of 25%, which was higher than in clusters 2 and 3 with a frequency of 12% and 15%, respectively. Many brands of sugar confectionary claimed as having breath refreshing properties are flavoured with menthol or compounds inducing similar sensations. The repeated exposure to mint aroma and cold stimulus paired during consumption of such confectionary for breath refreshing purpose may have led to a cognitive association between the combined mint/cold in mouth experience and refreshing sensation. This assumption may explain why this consumer cluster scored mint gels as the most refreshing.

4.5. Preference and refreshing sensation were related

The most refreshing gels seemed to be the preferred ones. For about 2/3 of consumers, the preferred product was the one they scored as the most refreshing. This agrees with studies showing that refreshing sensation and pleasantness/preference of food are strongly related (Clydesdale, Gover, Philipsen, & Fugardi, 1992; McEwan & Colwill, 1996; Deliza, Macfie, & Hedderley, 2003; Lee & O' Mahony, 2005). However this finding must be taken with precaution as the consumer focus on refreshing intensity scoring may have generated a carry over effect on preference.

Tournier et al. (2007) on another complex perception, creaminess, showed that correlation between creaminess and liking depended on the consumer cluster. That is also what we observed in the present study.

5. Conclusion

This study showed that refreshing sensation resulted from a combination of several independent sensory dimensions differing according to the consumer group. These differences of key sensory drivers according to consumers might be explained by food experience. In addition, refreshing seemed positively associated with preference.

Acknowledgments

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3.2.2 Temporal Dominance of Sensation and Sensory Profiling: A Comparative Study

Labbe,D., Schlich,P., Pineau,N., Gilbert,F. and Martin,N. (2009) Temporal Dominance of Sensations and Sensory Profiling: A Comparative Study. Food Qual Prefer, 20, 216-221.

Abstract

Temporal Dominance of Sensations (TDS) is a recent descriptive sensory method consisting in assessing repeatedly, until the sensations end, which sensation is dominant and in scoring its intensity. Compared to Time-Intensity, this method considers the multidimensionality of the perceptual space over time. The objectives of this study were first to compare description of viscous fluids containing different levels of odorants (peach and mint), citric acid, cooling agent and xanthan gum obtained with TDS and with a conventional descriptive method and then to explore the impact of mint and peach odorant on long lasting perception. TDS provided reliable information close to standard sensory profiling. In addition, TDS provided information on the dynamic of perception after product consumption that was not available using a conventional profiling method and that may be critical for the understanding of complex perceptions such as refreshing.

Keywords: Temporal Dominance of Sensations; Sensory profiling; Dynamic of perception

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Temporal dominance of sensations and sensory profiling: A comparative study

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ABSTRACT

Temporal dominance of sensations (TDS) is a recent descriptive sensory method consisting in assessing repeatedly, until the sensations end, which sensation is dominant and in scoring its intensity. Compared to time–intensity, this method considers the multidimensionality of the perceptual space over time. The objectives of this study were first to compare description of gels containing different levels of odorants (peach and mint), citric acid, cooling agent and xanthan gum obtained with TDS and with a conventional descriptive method and then to explore the impact of mint and peach odorant on long lasting perception. TDS provided reliable information close to standard sensory profiling. In addition, TDS provided information on the dynamic of perception after product consumption that was not available using a conventional profiling method and that may be critical for the understanding of complex perceptions such as refreshing.

1. Introduction

Sensory profiling is a descriptive approach widely used to qualify the nature and quantify the intensity of the sensory properties of food (Stone, Sidel, Oliver, Woolsey, & Singleton, 1974). By this method, product sensory properties are assessed immediately after sniffing or eating. A few seconds separate the sensory stimulation and its characterisation and quantification using a scale. However, perception is not a single event but a dynamic process with a series of events (Piggott, 1994). To take into account the dynamics of perception, the time intensity (T–I) method was developed by Larson-Powers and Pangborn (1978) to measure the intensity and the duration of sweetness, bitterness, sourness and flavour in different solutions. The lingering perceptions induced by sweeteners (Ujikawa & Bolini, 2004), bitter compounds (Pangborn, Lewis, & Yamashita, 1983), and trigeminal compounds such as menthol (Gwartney & Heymann, 1996) have been broadly investigated using this technique (See Cliff & Heymann, 1993 and Piggott, 2000 for a review). Perceptual interactions have also been highlighted between (1) olfactory perception and taste by dual-attribute T–I in chewing gum (Duizer, Bloom, & Findlay, 1997) and by conventional T–I in flavoured sucrose solution (Cliff & Noble, 1990), (2) olfactory perception and texture by conventional T–I in whey protein gels (Weel et al., 2002); and (3) more recently between hot trigeminal perception from capsaicin, flavour and texture in pork patties (Reinbach et al., 2007). But the main constraint of T–I is that the evaluation is limited to one or two sensory attributes at a time. The multidimensionality of the perceptual space over time is therefore not considered. For this reason, temporal dominance of sensation (TDS) has been recently developed (Pineau, Cordelle, Imbert, Rogeaux, & Schlich, 2003) and a study showed that TDS was more relevant for representing product perception pattern and highlighting interaction between attributes compared to T–I (Le Reverend, Hidrio, Fernandes, & Aubry, 2008). This descriptive sensory method consists in assessing iteratively at each specific time until the sensations ends, which sensation is dominant and in scoring its intensity. In this study the meaning of dominant was the most intense sensation.

The objectives of this study were: (1) to compare the sensory characterisation of nine gels done according to the sensory profiling method which was acquired in our previous study (Labbe, Gilbert, Antille, & Martin, 2007) and the TDS method; and (2) to explore over time the impact of the odorant type (mint and peach) on taste and trigeminal perceptions. In this context, we used a range of products a priori formulated to be different from a sensory point of view to test the methodology rather than the products.

2. Material and method

2.1. Product formulation

The composition and formulation of the nine gels are presented in Table 1, for further details see Labbe et al. (2007).

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	Peach odorant		Mint odorant		Cooling agent		Citric acid		Xanthan gum	
Level	Low (0.10%)	High (0.20%)	Low (0.01%)	High (0.03%)	Low (0.10%)	High (0.20%)	Low (0.20%)	High (0.60%)	Low (0.25%)	High (0.50%)
Symbol							a	A		
1			✓		✓			✓	✓	
2			✓		✓		✓		✓	
3				✓		✓		✓	✓	
4				✓		✓		✓		✓
5				✓		✓	✓		✓	
6	✓				✓			✓	✓	
7	✓				✓		✓			✓
8		✓				✓		✓		✓
9		✓				✓		✓	✓	

2.2. Methodologies

2.2.1. Sensory profiling

External assessors have been previously selected based on their performance according to results of screening tests selected in NF ISO 8586-1 (1993). Among them, twelve experienced assessors used to participate in sensory profiling study were recruited. A 90 min session was dedicated to term generation. Thirty terms were generated and ten attributes were finally kept (overall odour, coldness, overall flavour, acidity, sweetness, bitterness, salivating, thickness, astringency, coldness persistency) after the selection phases. This phase consisted in deleting: (1) attributes which did not characterise all products; and (2) synonymous and antonymous attributes (the most meaningful attribute to keep was defined by the panel). This step was performed based on the NF ISO 11035 standard (1995).

Three 60 min sessions were carried out for the training based on the NF ISO 8586-2 standard (1994). They were trained to rate attribute intensity using external references on a 10 cm unstructured linear scale, anchored at the extremities with "not at all intense" and "very intense". External references consisted of an aqueous solution containing ingredient related to each attribute at the same two concentrations as those detailed in Table 1. Olfactory attributes were not specific to the odorant type but were related to the overall olfactory intensity perceived either orthonasally (overall odour) or retronasally (overall flavour) to avoid a product clustering opposing peach and mint flavoured gels.

2.2.2. Temporal dominance of sensations

Forty three external assessors experienced in sensory profiling were selected as previously explained. A subset of five out of the ten attributes used in the sensory profiling was selected. Since one of the objectives was to explore the impact of olfactory quality on taste and trigeminal perceptions, the selected attributes were: overall flavour, coldness, sourness, sweetness and bitterness. They followed a similar training as assessors from the sensory profiling. In addition, a specific training session was conducted using solutions containing cooling and citric agents mixed at different concentrations for explaining the concept of dominance of sensation.

In the TDS methodology, all the attributes were presented simultaneously on the computer screen with their corresponding 10 cm unstructured linear scale, anchored at the extremities with "not at all intense" and "very intense" as for sensory profiling.

Once the subject had swallowed the product, he clicked on a start button on the screen to begin the evaluation. During an evaluation, the subject had to select the attribute considered as dominant and score this attribute on a linear scale. When the dominant perception changed, the subject had to score the new dominant sensation. The subject was free to choose several times the same attribute or conversely to never select an attribute as dominant. The test started after swallowing and lasted for 5 min since a preliminary study had shown that after this period the product persistence fully disappeared. The data collected during the tasting of each product for each subject were:

- the time when an attribute was selected as dominant;
- the name of the given attribute;
- the intensity scored for this attribute.

A duration parameter was also computed as the time elapsed between the elicitation of the given attribute and the following elicitation. This means an attribute is considered as dominant until another attribute is scored.

2.3. Tasting conditions

For both methods, data acquisition was carried out on a computer with FIZZ software Version 2.20E (Biosystemes, Couternon, France). All products were evaluated by all subjects during two sessions with four and five products per session. The presentation design balanced position and order effects, based on Williams Latin Squares.

Products were coded with three-digit random numbers and the 50 ml portion was served at room temperature (22 °C) in a 100 ml cup. Rinsing was done between products with water and unsalted crackers during a five min break. Tests were conducted in an air-conditioned room (18 °C), under white light in individual booths. For the sensory profiling method, each attribute was evaluated according to a specific procedure described in Labbe et al. (2007). For both methods, the 50 ml amount of product was fully swallowed in three sips.

2.4. Statistical treatment

2.4.1. Sensory profiling method

A two-way analysis of variance (ANOVA) with product and assessor as factors was performed for each sensory attribute to determine if the products could be discriminated by the trained panel. The product factor was set to fixed and the factor assessor was set to random. The Fisher's least significant difference (LSD) was computed to determine if the differences between selected pairs of products were significant. The confidence level was set to 95% for ANOVA tests and LSD tests.

Principal component analysis (PCA) on the matrix of correlations was performed, taking into account significant attributes, as well as a mapping of the products based on the first two principal components (PCA biplot).

All statistical treatments were carried out using NCSS software version 2007 (Number Cruncher Statistical Systems, Karysville, Utah, USA).

2.4.2. TDS method

In order to compare sensory profiling results with TDS results, the TDS SCORES were calculated (Pineau, Cordelle, & Schlich, 2004). For each evaluation, this parameter takes into account, during the 5 min of evaluation, the intensity and the duration of every elicitation of a given attribute. This SCORE value is therefore the average of the scores given to an attribute during an evaluation weighted by their duration, as defined according to the Eq. (1):

$$\text{SCORE} = \left(\sum_{\text{Scoring}} \text{Intensity} \times \text{Duration}\right) \Big/ \left(\sum_{\text{Scoring}} \text{Duration}\right) \qquad (1)$$

A two-way analysis of variance (ANOVA) was performed on the TDS SCORE as well as a PCA. Both statistical analyses were carried out with the same criteria as described for sensory profiling statistical treatment. The Fisher's least significant difference (LSD) was also computed with a confidence level of 95% to determine if the differences between selected pairs of products were significant.

In addition, TDS data were represented by curves showing for each product the percentage of subjects who selected the attribute as dominant at a specific time, i.e. the dominance rate. The frequency of selection of each attribute as dominant by all subjects is represented in percentage (Axis *Y*) for each 1.5 s step, which corresponds to the data collection interval, during the 5 min period (Axis *X*). The 200 points of the evolution curve were smoothed using a non-weighted moving average with a 10 points window. The curve showed over time the dominant attributes at a panel level. For each 1.5 s step, the proportion of scoring for an attribute was considered as significantly dominant when above $P_{s,}$ as defined according to the Eq. (2), and as dominant when between P_0 and P_s, in the case we considered results as a trend.

$$P_s = P_0 + 1.64\sqrt{\frac{P_0(1 - P_0)}{n}} \qquad (2)$$

With chance level $P_0 = 1 / (k + 1)$, k being the number of attributes, n the number of subjects and 1.64 the one-tailed normal law z value for $\alpha = 5\%$.

Differences among TDS curves for two selected products were investigated by subtracting the scoring proportion of homologous attributes at each time.

2.4.3. Correlations between sensory profiling and TDS attributes (univariate analysis)

A correlation coefficient (r) was calculated for each attribute between sensory profiling and TDS product mean scores in order to measure whether TDS attribute SCORES evidence the same product patterns as sensory profiling attribute intensities among the products. Considering the nine products and a confidence level of 95%, significance was reached for an r value above 0.67.

2.4.4. Biplot representations and comparison of the multidimensional structures using RV and RVD (multivariate analysis)

Sensory profiling and TDS biplots are computed from the sample*attributes matrices in which observations are the intensities or the SCORES. The RV coefficient (Robert & Escoufier, 1976) between these two matrices was calculated to evaluate the similarity of the product configurations in the sensory profiling space and in the TDS space. This coefficient ranges from 0 to 1. The closer to 1, the higher the similarity of the product configurations. The Dual RV or RVD coefficient was also calculated between the two matrices to evaluate the similarity of the correlations among attributes in the sensory profiling space and in the TDS space. This coefficient also ranges from 0 to 1 and high values indicate high similarities according to attribute correlations. The significance of these coefficients can be tested using permutation algorithms or analytical methods (Kazi-Aoual, 1993; Schlich, 1996, respectively).

3. Results

3.1. Comparison of sensory profiling and TDS results

3.1.1. Significant attributes and product ranking

The five attributes significantly discriminated the products based on ANOVA in terms of intensity and SCORE for sensory profiling method and TDS method, respectively (Table 2). Product ranking obtained with sensory profiling and TDS results was very close (Table 2) since the correlation coefficient was significant for coldness ($r = 0.95$), sourness ($r = 0.77$), sweetness ($r = 0.75$) and bitterness ($r = 0.67$). For overall flavour intensity, the correlation was not significant ($r = 0.54$).

3.1.2. Biplot representations

Results of PCA's showed that 80% and 94% of the total information were represented by the first two principal components for sensory profiling and TDS, respectively (Fig. 1a–b).

3.1.3. Attribute correlations

Overall, correlations between attributes for both sensory profiling and TDS biplots (Fig. 1a–b) were quite similar with a significant RVD coefficient of 0.84. Variance in the first principal component was explained by differences between samples in terms of sweetness (on the left) and of coldness and bitterness (on the right). Even though the overall structure is the same, some differences existed: (1) sourness was correlated to coldness and bitterness for TDS biplot (Fig. 1b) but not for sensory profiling biplot (Fig. 1a); and (2)

Table 2
Panel discrimination among the nine products for each attribute evaluated by sensory profiling and by DTS and product ranking comparison between both methods.

	Anova product factor F-ratio		
	Sensory profiling	TDS	Correlation coefficient
Coldness	15.7***	9.5***	0.95***
Overall flavour intensity	8.6***	6.3***	0.54(NS)
Sourness	3.6**	3.8***	0.77*
Sweetness	5.6***	3.1*	0.75*
Bitterness	9.2***	2.4*	0.67*

According to the F-ratio of the product factor ANOVA: *, ** and *** indicate a significant product effect at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. For the correlation coefficient, NS indicates a not significant correlation; * and *** indicate a significant correlation at $P < 0.05$ and $P < 0.001$, respectively.

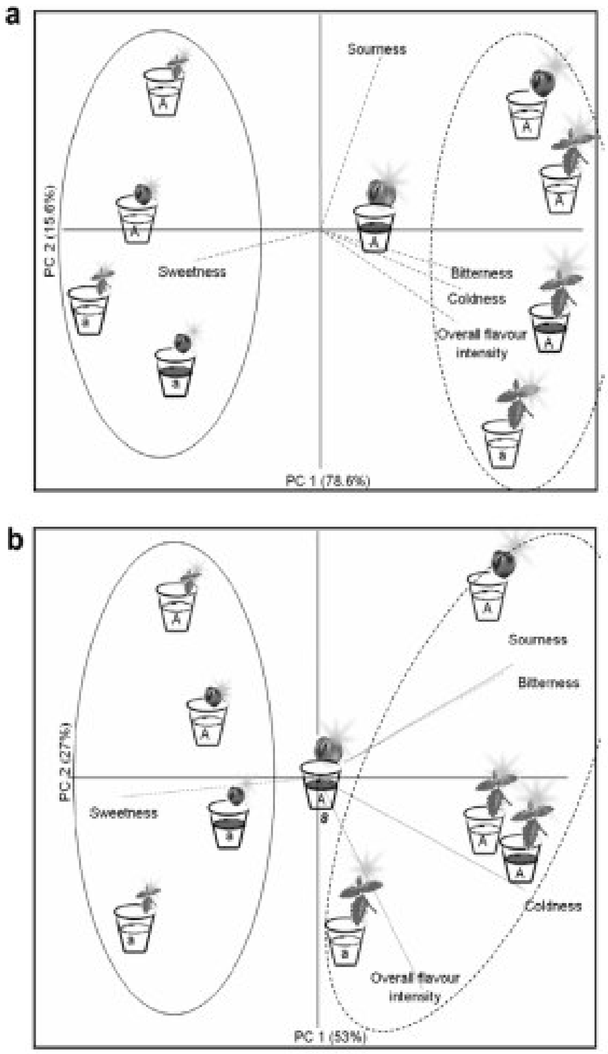

Fig. 1. Principal component analysis (PCA) biplots (PC1 and PC2) representing the nine products as observations and as variables (a) the attribute intensity panel mean from sensory profiling and (b) the attribute TDS SCORE parameters. Product recipes and symbols representing the ingredients are explained in Table 1. Group 1 products are circled with a continuous line and group 2 products are circled with a dotted line.

overall flavour intensity was correlated to coldness and bitterness for sensory profiling biplot (Fig. 1a) whereas it is only correlated with coldness in the TDS biplot (Fig. 1b).

3.1.4. Product positioning

Again, for both biplots, positioning was quite similar since the RV coefficient is close to 1 (RV = 0.78). The products were split into two groups of four products separated on the first axis. Group 1 on the left (Fig. 1a–b) contained products with low levels of cooling agent and flavouring and was perceived by the trained panel as intense in sweetness and weak in coldness, bitterness sourness and overall flavour intensity. Group 2 on the right (Fig. 1a–b) contained products with high levels of cooling agent and flavouring and was predominantly perceived as low in sweetness and intense in coldness and bitterness, sourness and overall flavour intensity.

3.1.5. Differences within groups

Considering the groups determined according to the PCAs, few differences between sensory profiling and TDS were highlighted in terms of overall flavour intensity and sweetness for group 2 products in terms of overall flavour intensity and sweetness (Fig. 2a–b). Indeed products described by the sensory profiling were not discriminated in terms of overall flavour intensity (Fig. 2a) whereas this attribute was discriminating according to TDS result (Fig. 2b). Conversely, sweetness discriminated products evaluated according to the sensory profiling method (Fig. 2a) but did not when products were evaluated by the TDS method (Fig. 2b).

3.2. Over time modulation of perception by flavourings

We selected a mint and a peach flavoured product having the same composition of other ingredients (high cooling agent, citric acid, and xanthan level). Regarding the temporal dominance after consumption, the two gels differed mainly in terms of coldness. For both gels the coldness curve was above the chance level 24 s after consumption (Fig. 3a–b) but mint flavoured gel induced a longer lasting perception since the curve decreased below the chance level 216 s after consumption whereas for the peach gel, the coldness curve was below the chance line 156 s after consumption.

Dominance rate differences (Fig. 3c) clearly highlighted that gels mainly differed in terms of long lasting coldness, coldness duration induced by the mint flavoured gel being longer compared to the peach flavoured gel.

4. Discussion

4.1. Product characterisation: TDS vs. sensory profiling

Product characterisations based on TDS SCORE, which considered the intensity and the duration of the sensory dimensions perceived over a 5 min period after consumption, and based on

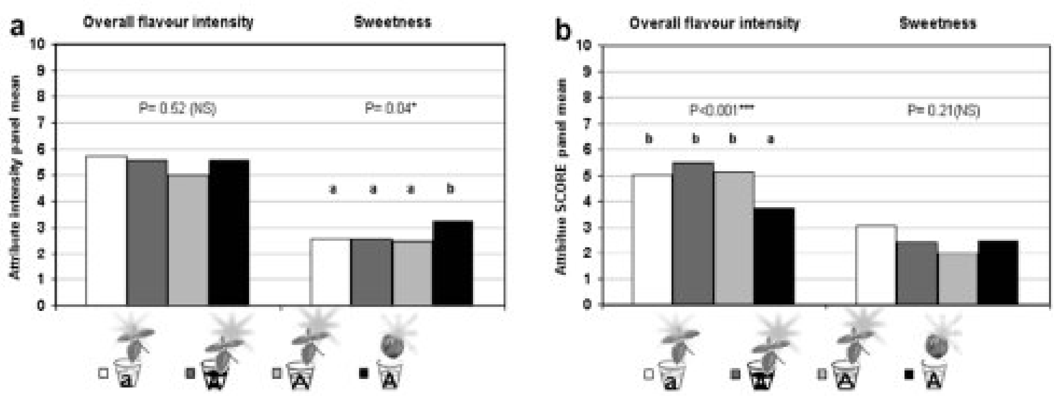

Fig. 2. Overall flavour intensity and sweetness panel mean scores for (a) sensory profiling biplot group 2 products and (b) TDS biplot group 2 products. According to the F-ratio of the product factor ANOVA (calculated within each four product group): NS means "Not Significant"; *, **, *** indicate product effect at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. According to the LSD (calculated within each four product group), products under the same horizontal line are not significantly different ($P < 0.05$).

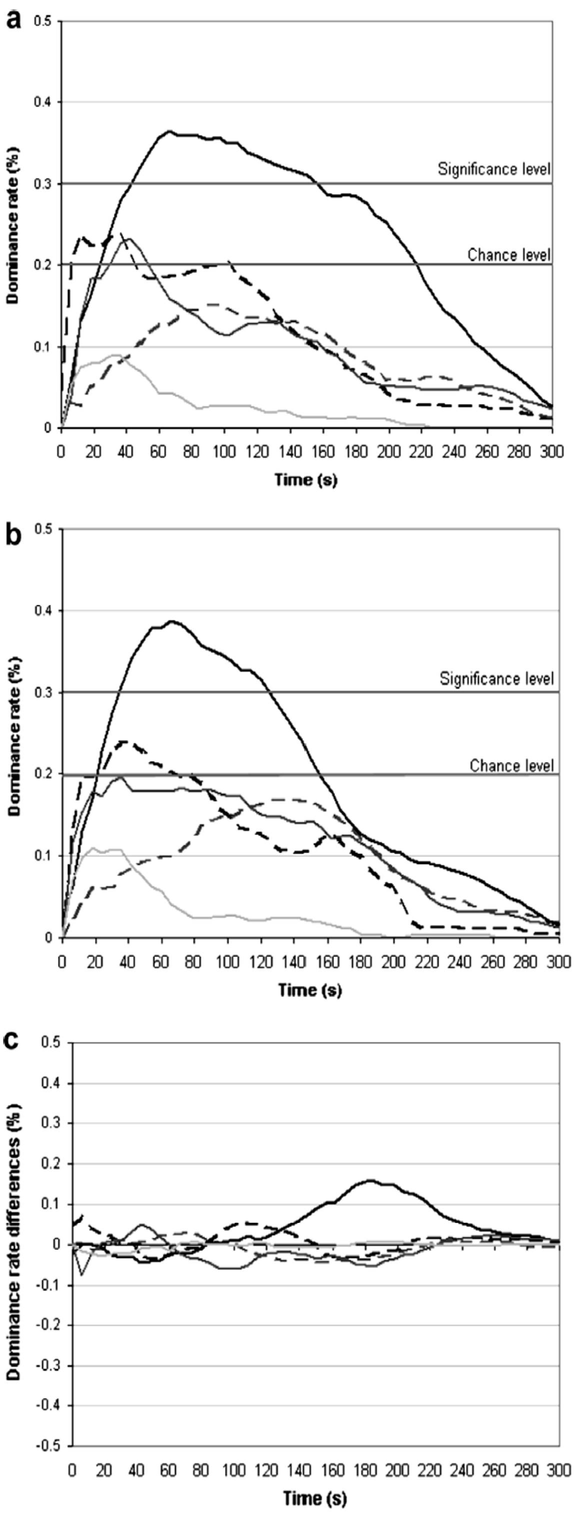

Fig. 3. Dominance rate in terms of subject percentage over a 5 min time period for (a) the peach flavoured gel (b) the mint flavoured gel and (c) dominance rate differences between the mint and peach products (The peach gel dominance rate is arbitrarily represented at 0 for all the attributes). Attributes are representing as following: overall flavour intensity, bitterness, coldness, sourness and sweetness. Product recipes and symbols representing the ingredients are explained in Table 1.

sensory profiling, which considered the sensory dimensions measured immediately after consumption did not significantly differ according to attribute correlation and product distance values. Indeed both methods showed that one group of products was characterised by their sweetness and the second group mainly by their coldness and bitterness.

Regarding differences, when gels were evaluated immediately after consumption by sensory profiling, bitterness and coldness provided the same information since both sensory dimensions were correlated. When gels were evaluated over time by TDS, coldness information differed from bitterness, the latter perception being strongly related to sourness according to TDS. In addition, product characterisation differed according to the evaluation method in terms of overall flavour intensity and sweetness discrimination. Even if results between both methods were globally not significantly different according to the multivariate analyses, these results showed that with products inducing a long lasting multi sensory perception, the dynamic of perception differs from the immediate perception.

4.2. Drivers of refreshing sensation in terms of sensory intensity over time and duration

The gels used in the present work were formulated in a previous study aiming at understanding the sensory determinants of refreshing sensation combining sensory profiling results and scoring by 160 consumers of refreshing intensity (Labbe et al. (2007). We highlighted that, considering the five most refreshing products among the nine, coldness was the main driver of refreshing for 36% of consumers. Correlations for these five gels between refreshing intensity score averages from the 36% consumers and (1) attribute score averages from sensory profiling; and (2) attribute SCORE from TDS highlighted the same trend in terms of sensory determinants of refreshing resulting from the dynamic (TDS) or punctual (sensory profiling) evaluation. But bitterness was identified as a positive driver of refreshing when evaluated by sensory profiling with a correlation coefficient of 0.91 but was negatively correlated to refreshing sensation when evaluated by TDS with a correlation coefficient of −0.51. This result showed that the same sensory characteristic perceived either immediately after consumption or over a five minute period may differently impact the refreshing sensation. Considering the study of Westerink and Kozlov (2004) showing that oral care freshness is a dynamic concept, the sensory characterisation by TDS may be more relevant for understanding drivers of refreshing sensations.

Further studies replicated this work are still required to draw definitive conclusions about the relevance of this method since: (1) this is the first attempt to relate TDS and sensory profiling data; and (2) sensory profiling data was not replicated (since performances of our panel are regularly checked in terms of individual discrimination ability and consensus at a panel level). In addition these findings are limited to our range of prototypes a priori formulated to differ in terms of sensory characteristics. A validation of TDS for a narrower product range, i.e. closer in terms of sensory differences (for example a range of commercial coffee drinks) would add value to this methodology.

5. Conclusions

Considering our range of prototypes, PCA and ANOVA results showed that TDS is a reliable tool providing a product positioning close to standard sensory profiling. In addition, TDS provided information on the dynamic of perception after product consumption that was not available using a conventional profiling method. These additional time-related drivers may be critical in the understanding of the complex refreshing perception. As additional research, it would be interesting to combine in the same evaluation session: (1) scoring by consumers of common olfactory, taste and trigeminal attributes using TDS; and (2) several punctual scorings of refreshing intensity using a standard 10 cm linear scale. The identification and monitoring of sensory drivers of refreshing sensation could be therefore investigated over time.

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3.3 Role of physiological factors in refreshing perception: mental energy and saliva

3.3.1 The impact of refreshing perception on mental energy: changes in mood, cognitive performance and brain oscillations

Labbe, D., Martin, N., le Coutre, J, &. J. Hudry to be submitted to Biological Psychology

Abstract

Previous sensory research has demonstrated that cold/cooling and sourness sensations improve thirst-quenching and subsequent refreshing perception in food products. Other research has suggested that thirst-quenching may improve mental energy in terms of mood, cognitive performance and brain activation during rest. The aim of the present research was to evaluate the effect of refreshing perception induced by an optimized citrus-flavoured frozen snack on mental energy during mental workload. Comparison treatments were a standard frozen snack differing in refreshing intensity but matched in flavour and energy content, and a glass of water. Two experiments were conducted in six and 18 healthy participants. Performances of sustained attention were assessed using a rapid visual information processing task and related cortical activation with electroencephalography (in Experiment I only). Results of Experiment I revealed that the optimized frozen snack improved cortical activation in the alpha and beta powers known to be involved in neural circuits of attention, working memory and sensory-motor integration. The assumption that such enhancement of cortical activation is in favor of optimal resources for task performance was demonstrated in Experiment II with a larger sample of participants.

Introduction

“Mental energy” is a term recently used by scientists to describe the psycho-physiological resources necessary to engage, concentrate and perform well on cognitive tasks (Lieberman, 2006; Lieberman, 2007; O'Connor, 2006). This term relates to a mood state, mainly in terms of fatigue and alertness, to cognitive performance, as well as to a functional state of the brain such as cortical activation measured with electroencephalography (EEG). Mental energy may be influenced by a number of variables including in particular the physiological status. Although the adverse effects on mental energy of related factors, such as heat and dehydration, have been largely examined, the scientific literature on refreshing and mental energy is quite limited.

"Refreshing" refers to a complex sensation similar to the one experienced during cool water drinking, and corresponding to the relief of unpleasant physical symptoms such as elevated body temperatures or mouth dryness (Brunstrom, 2002; Phillips, Rolls, Ledingham, & Morton, 1984). Specific sensory properties of food and beverages in favor of such a refreshing perception have been previously identified. Using a range of viscous products varying in their compositions (levels of sugar, citric acid, cooling and flavour), it has been shown that cooling was a key sensory driver of refreshing (Labbe, Gilbert, Antille, & Martin, 2008). The importance of cold sensations in refreshing is corroborated by other data showing that water at 5℃ is perceived as more thirst-quenching than warmer water at 22℃ (Brunstrom & Macrae, 1997). As suggested, this superiority of in-mouth cold stimulations may be due to both internal temperature and saliva flow changes in favor of thirst-quenching perception. Sourness is another sensory characteristic identified as a driver of refreshing in viscous products (Labbe et al., 2008) and of thirst-quenching in beverages (McEwan & Colwill, 1996). Sour tastants add a refreshing value to food products likely because they enhance the thirst-quenching experience by stimulating saliva flow. Olfactory stimulations associated with sour fruits, such as lemon and peach, seem as well to enhance refreshing intensity of yoghurts through a possible perceptual associative learning mechanism (Martin et al., 2005). Taken together, these sensory studies suggest that ingredients with cooling/cold and sour properties in food or beverages may improve mouth-feel, thirst-quenching intensity and therefore refreshing perception.

The idea that foods or beverages can reliably influence cognitive performance has received great attention over the past decade and the concept of “functional food” has been widely examined (for reviews see (Dye, Lluch, & Blundell, 2000; Messier, 2004). Beside energy provided by macronutrients, other factors may influence cognition such as refreshing induced by thirst-quenching. The impact of water consumption on some aspects of cognition and mood has been previously investigated with regard to thirst (Neave et al., 2001; Rogers, Kainth, & Smit, 2001). In the study of Rogers et al. (2001), 60 participants were randomly assigned to one of the following treatments: no-drink, 120 ml of water (10℃), 330 ml of water (10℃). They were a posteriori split into low- and high-thirst groups according to pre-treatment thirst ratings. Compared to the no-drink condition, the consumption of water induced immediate (2 min post-treatment), but not sustained, subjective sensations of being “alert” and “revitalized” in both the low- and high-thirst participants. In contrast, the effect of water consumption on performances of sustained attention and working memory depended on prior thirst level, as measured with a 6-min rapid visual information processing (RVIP) task. Hit rates were increased when thirst was high and decreased when thirst was low. These changes were observed both 25 and 50 min post-treatment and occurred in a dose-dependent manner. Neave et al. (2001) conducted a similar experiment in order to verify the benefits of drinking water upon mental energy in high-thirst participants. Thirst was induced by a ~11h (overnight) eating and drinking restriction procedure. In addition to a 3-min RVIP task and a mood questionnaire, the authors used a range of cognitive tasks measuring other aspects of memory and attention. Twenty-four participants were involved in a randomized, balanced crossover study employing a water (150 ml at 10℃) and a no-drink condition. Consistent with previous findings by Rogers et al. (2001), the consumption of water improved immediate subjective alertness as compared to the no-drink condition. However, cognitive performances were neither positively nor negatively affected by the consumption of water in participants in a high state of thirst. Substantial differences in methodology (e.g., thirst and hunger levels of participants, experimental paradigm, restriction procedure, treatments) between the two studies may explain the controversy in findings. Yet it remains difficult to conclude on the potential effect of refreshing induced by water consumption on mental energy, as measured with mood ratings and cognitive tasks of attention and memory.

Non-invasive measurement of the functional state of the brain with EEG can provide evidence of changes in mental energy, in terms of amplitude fluctuations of neuronal oscillations reflecting various states of cortical arousal and activation (Barry, Clarke, Johnstone, Magee, & Rushby, 2007). The effect of thirst-quenching on the brain state during low cognitive load (mental count) was investigated with EEG in water-deprived and thirst-quenched subjects after consumption of 400 ml of water (Hallschmid, Molle, Fischer, & Born, 2002). Results revealed an increase of brain oscillatory activity in the lower alpha band (8-10 Hz) in water-deprived subjects only, as compared to baseline measurements obtained before drinking. Based on this, the authors proposed the enhancement of 8-10 Hz neuronal oscillations as an electrophysiological marker of thirst-quenching, viewed as a reward response in relation to motivation. Similar changes in functional state of the resting brain have been reported in the alpha (8-12 Hz) and beta (13-30 Hz) bands after consumption of confectionery products with refreshing properties (Morinushi, Masumoto, Kawasaki, & Takigawa, 2000). These changes were obtained after chewing a flavoured gum the major constituents of which were aromatic oils with cooling (spearmint) and sour (lemon balm) properties. The convergence of recent evidence suggests that oscillations generated by the brain in different frequency ranges play a wide range of functions in human cognition (for explicit reviews see (Klimesch, 1999; Tallon-Baudry, 2003; Ward, 2003). Theta (4-8 Hz) and alpha oscillations are two particular frequency bands with strong cognitive correlates, largely assumed to reflect activity of multifunctional brain networks involved in alertness, attention and memory processes. Faster oscillations, such as those in the beta band, are thought to provide a means for the integration of anatomically distributed processing and for the formation of transient neuronal assemblies. Therefore, the enhancement of cortical oscillations obtained after water and chewing-gum consumption may reflect an improvement of mental energy for optimal task performance. However, in the absence of cognitive assessment, changes in brain oscillatory activity should be interpreted with caution.

The literature on the relationship between refreshing perception and mental energy is limited and inconsistent. Although it has been suggested that water drinking can enhance mood and sustained attention performance in thirsty subjects (Rogers et al., 2001), the improvement of cognitive performances was not replicated in subjects after an overnight eating-drinking restriction procedure (Neave et al., 2001). The impact of refreshing perception induced by water drinking or by food consumption on brain functional state has not been thoroughly investigated and related changes in cognitive functioning have not been assessed. There are a variety of reasons for the discrepancy in findings and for the lack of studies on the effect of refreshing on human brain functions. Tangible

refreshing is difficult to assess accurately using water as treatment since the effects will mainly depend upon prior physiological status (Hallschmid et al., 2002; Rogers et al., 2001). Inducing dehydration, or thirst, in a controlled and consistent manner is difficult and may provoke non desired psychophysiological consequences, such as hunger, stress or fatigue (Lieberman, 2007).
The aim of the present study was to explore further the relationship between refreshing perception and mental energy. We partly replicated the experiment of Rogers et al. (2001) and incorporated additional refreshing treatments and controls, as well as EEG measurements. The benefits of a sweet frozen snack with sensory properties optimized for refreshing perception was investigated on mood, cognitive performance (RVIP) and related brain oscillations. Comparison treatments were a glass of fresh water and a standard frozen snack matched in temperature and flavour with the optimized treatment. We hypothesized that refreshing perception is not only a result of physiological benefits consequent to eating or drinking, such as thirst-quenching, but also of benefits induced on mental energy. In particular, we anticipated that sensory properties previously identified as drivers of refreshing perception (Brunstrom & Macrae, 1997; Labbe et al., 2008; McEwan & Colwill, 1996) would enhance brain oscillations implicated in cognitive processes necessary to perform the task, (i.e. sustained attention and working memory) leading to optimal task performance.

Materials and methods

Participants

Healthy right-handed volunteers between the ages of 18 and 35 were selected among staff at Nestlé Research Center (Lausanne, Switzerland). Six participants (4 males) were included in Experiment I and 18 other participants (7 males) in Experiment II. They were healthy as determined by a medical questionnaire and had a BMI in the range of 18-30. Exclusion criteria included eating disorders, diabetes, neurological disease, history of head trauma, nose pathology or upper olfactory tract infection. All participants gave their written consent and were paid for participation. The two experiments described here were conducted in accordance with the Helsinki Declaration and were approved by the Ethical Committee for Clinical Research of Medicine and Biology Faculty (Lausanne University, Switzerland).

Treatment and design

A randomized, balanced, three-treatment crossover design was employed in order to investigate the effect of treatment on mental energy. Participants received either 70g of an Optimized frozen snack served at -17°C, 70g of a Standard frozen snack served at -17°C or a glass of 70 ml of Water served at 7°C. The Standard and Optimized frozen snacks were based on a citrus flavoured sucrose water recipe. The cooling agent 'L-127039' (Givaudan S.A., Dübendorf, Switzerland) were ingredients added to the frozen snack optimized for refreshing at a ratio of 1:6. The two frozen snacks were matched for energy (266 Kcal). The refreshing value of the Optimized frozen snack was previously validated in another study held in 160 French consumers. The Optimized product was judged significantly more refreshing than the standard frozen snack using 10-point rating scales from 'not at all refreshing' to 'extremely refreshing'.

Procedure

Participants completed three separate test sessions starting at 11:00 am as well as a prior practice session in order to familiarize themselves with the cognitive task and the subjective ratings to be completed during the study. They were informed to refrain from drinking alcohol for 12 h (overnight) prior to test sessions and to arrive at the EEG laboratory at 7:30 am to get a standardized breakfast. Participants were then informed that they should not drink or eat until the test session starts at 11:00 am. During one test session, participants completed the mood questionnaire and cognitive task in this order prior to treatment (Pre), immediately after treatment (Post1) and 15 min after treatment (Post2). Following completion of pre-treatment assessments, 5 min were allocated to consume the treatment. During the last period of the test session, 30 min after treatment (Post3), participants had ad libitum access to water while completing a mood and an additional distractive questionnaire of 10-15 min both. Without telling participants, the amount of water drunk was taken as an indirect measure of their thirst levels.
For experiment I, six participants completed the practice and test sessions individually while seated comfortably in front of a 17" computer screen, in a dimly lit, sound- and electrically-shielded room. For experiment II, three groups of six participants completed the sessions seating comfortably in front of a 17" computer screen, in dimly lit sensory booths wearing an ear muff headset for noise reduction.

EEG data recording and reduction

In Experiment I only, continuous EEG activity was acquired through a Biosemi Active Two system (Biosemi, Amsterdam, Netherlands) while participants performed the RVIP task. EEG was recorded from 16 scalp active electrodes attached to a headcap and referenced to the CMS-DRL ground (which functions as a feedback loop driving the average potential across the montage as close as possible to the amplifier zero). Recordings were sampled at 256 Hz with high- and low-pass filter settings of 0.33 and 128 Hz, respectively. EEG-epochs of 1000 ms were computed using CarTool software (http://brainmapping.unige.ch/Cartool.htm). The first EEG-epoch started randomly 500-1500 ms following the presentation of the first stimulus onset; and the last EEG-epoch started prior to the presentation of the last stimulus onset. In addition to the application of an automated artifact criterion of ±100 µV, visual inspection of EEG data was performed to exclude epochs containing artifacts due to facial movements or other sources of transient noise. Pre-treatment epochs were used as baseline measurements in order to isolate the task-specific activation changes induced by the treatments (Barry et al., 2007). Artifact-free epochs were subjected to a fast Fourier transformation using a 100% Hanning window. The mean power amplitude was computed for four separate frequency bands (delta: 1–3 Hz, theta: 4–7 Hz, alpha: 8–12 Hz and beta: 13–30 Hz) for each participant, scalp region (frontal: electrodes F3, Fz, F4, central: electrodes C3, Cz, C4, parietal: electrodes P3, Pz, P4), test period (Pre, Post1, Post2) and treatment (Optimized, Standard, Water).

Cognitive task

Performances of sustained attention were measured using a difficult version of RVIP task requiring working memory for completion. Single yellow digits (1-9) were presented in quick succession (100 digits/min) in a pseudo-random order on a black background during 6-min (via E-Prime software package, E-prime 1.1, Psychology Software Tools, Summit Software Company). Target sequences of three odd or even numbers had to be detected with a button press as fast and as accurately as possible. During one test, 48 possible hits (out of 600 trials) could be scored. Outcome measures for each test were the number of hits and the mean reaction time for correct target detection.

Mood ratings

Mood was assessed using the standardized “Bond and Lader questionnaire” consisting in 16 bipolar 100 mm scales anchored at either end by antonyms (e.g., alert-drowsy, calm-excited) according to three dimensions of mood: 'alertness' (9 items), ‘contentedness’ (5 items) and 'calmness' (2 items). Scores for each dimension were the average number of millimeters (max 100) from the individual scales contributing to the dimension.

Statistical analyses

EEG data were submitted to analyses of variance using a mixed model with repeated measurements for each frequency band (delta, theta, alpha, beta). The factors ‘treatment’ (Optimized, Standard, Water), ‘topography’ (Frontal, Central, Parietal, Occipital) and ‘period’ (Post1, Post2) were set as fix, and the factor subject set as random. Cognitive performances were submitted to analyses of variance using a mixed model with repeated measurements for each outcome measure of the RVIP task (number of hits, reaction times). The factors ‘treatment’ (Optimized, Standard, Water) and ‘period’ (Post1, Post2, Post3) were set as fix, and the factor subject set as random. Mood ratings were submitted to analyses of variance using a mixed model with repeated measurements for each mood dimension (alertness, calmness, contentedness). The factors ‘treatment’ (Optimized, Standard, Water) and ‘period’ (Post1, Post2, Post3) were set as fix, and the factor subject set as random. For EEG, performance and mood data, pre-treatment data were considered as baseline measurements and used as covariate in the model. The main advantage of this approach is elimination of between-subject variance in EEG power, performance and ratings. These analyses focused on correspondence between within-subject changes of subjective state and the changes in EEG spectral parameters, not on the raw mood or performance and EEG data. The effect of the treatments on ad libitum water intake was assessed by calculating analysis of variance with the factor treatment set as fixed and subject as random. All calculations were performed with NCSS software version 2007 (Number Cruncher Statistical Systems, Karysville, Utah, USA.). Post-hoc pair comparisons were assessed by Student t-tests. Confidence level was set to 95% for all analyses

Results

EEG data, Experiment I

Results of statistical analyses performed on EEG data from Experiment I as a function of treatment, topography and period for each frequency band are presented in Table 1.

Table 1: F and p-values obtained for the three-way ANOVA performed on EEG data for each frequency band

			Factors		Interactions		
Frequency band		Treatment (Tr)	Period (Pe)	Topography (To)	Tr*Pe	Tr*To	Pe*To
Delta	F	0.41	0.88	38.42	0.76	0.90	0.62
	p	0.66	0.34	<0.01	0.46	0.49	0.60
Theta	F	0.89	0.27	39.65	1.60	0.70	0.37
	p	0.41	0.61	<0.01	0.20	0.65	0.77
Alpha	F	2.83	30.96	4.19	15.64	1.53	5.49
	p	0.06	<0.01	<0.01	<0.01	0.16	<0.01
Beta	F	3.76	2.34	1.39	2.52	0.52	0.71
	p	0.02	0.12	0.24	0.08	0.79	0.54

Degrees of freedom were (2,372) for the factor Treatment; (1,372) for the factor Period; (3,372) for the factor Topography; (2,372) for the Treatment x Period interaction; (6,372) for the Treatment x Topography interaction and (2,372) for the Period x Topography interaction.

Fig. 1 illustrates the oscillatory activity in each frequency band as a function of treatment and period. The factor treatment had a significant impact only on beta power. Pairwise comparisons revealed that this effect was due to a higher beta power in the Optimized condition during Post2, as compared to the Standard and Water conditions during Post2 and to all three conditions during Post1 (Fig. 1D). A significant period effect and treatment x period interaction were obtained in the alpha power. These effects were caused by a significant higher power in this frequency range in the Optimized condition during Post2, as compared to the two other conditions during Post2 and to all conditions during Post1 according to pairwise comparisons (Fig. 1C). In addition, alpha power in the Water condition was significantly higher than in the Optimized and Standard conditions during Post1.

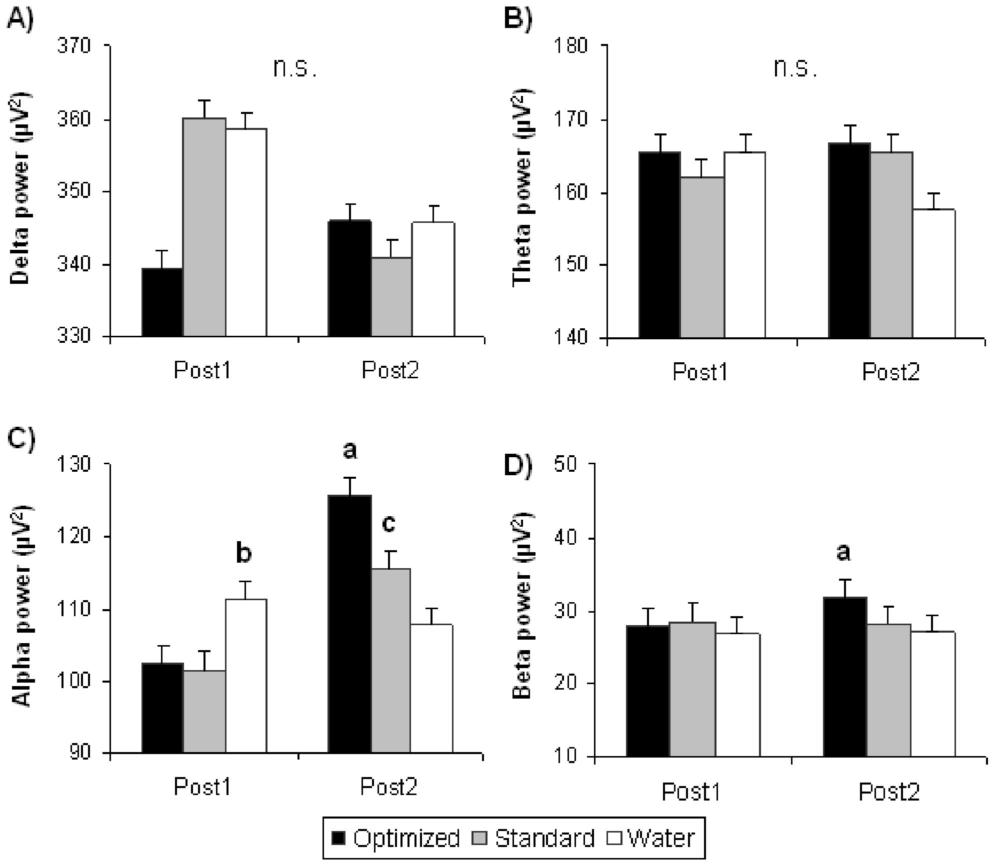

Fig.1: Mean changes in EEG power as a function of treatment and period of time for A) delta, B) theta, C) alpha and D) beta powers. Values are averaged across participants and scalp regions. a, significantly higher than all treatment conditions during Post1 and than Standard and Water conditions during Post2; b, significantly higher than Optimized and Standard conditions during Post1; c, significantly higher than Optimized and Standard conditions during Post1 and than Water condition during Post2; error bars, standard error of the mean; n.s., non significant effects or interactions as revealed by analyses of variance).

Topography had a significant effect on oscillatory activity in the delta, theta and alpha powers. The distribution of EEG power for each frequency band is given in Fig. 2 as a function of topography and, for illustrative value, as a function of the three periods of time. With time on test, EEG power tended to increase in the alpha and beta frequency bands, to decrease in the delta frequency band and to remain unchanged in the theta frequency band. Taking Pre values as covariate, these changes due to the factor period were significant only in the EEG alpha power (Fig. 2C). A significant period x treatment interaction was observed in this latter frequency band. Pairwise comparisons revealed that this effect was due to higher alpha power during Post2 than Post1 in the frontal, parietal and occipital regions. Other pairwise comparisons due to the factor topography revealed that delta power was significantly higher in the occipital than in the other scalp regions for all periods (Fig. 2A). Theta power was significantly higher in the frontal and occipital than in the central and parietal scalp regions. Alpha power was significantly higher in the occipital than in the other scalp regions (Fig. 2B).

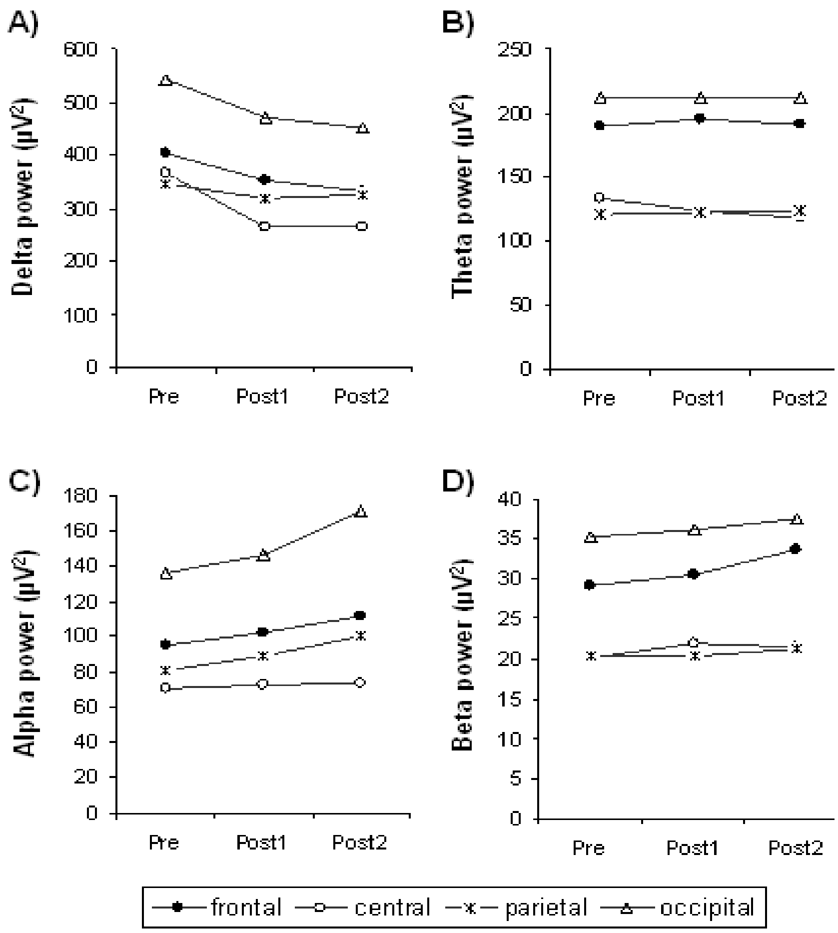

Fig.2: Mean changes in EEG power as a function of topography and period of time for A) delta, B) theta, C) alpha and D) beta powers. Values are averaged across participants and scalp treatments. Note that the Y axis scales were adapted for each frequency band.

Cognitive performances

Experiment I

For Experiment I, statistical analyses did not reveal any significant effect of the factors treatment and period for the number of hits ($F(2,24) = 0.35$; $p = 0.71$; n.s.; and $F(1,24) = 1.16$; $p = 0.29$; n.s.; respectively) and reaction times ($F(2,24) = 0.10$; $p = 0.9$; n.s.; and $F(1,24) = 0.21$; $p = 0.65$; n.s.; respectively). Interactions between the two factors were not significant for both measures of hits and reaction times ($F(2,24) = 1.56$; $p = 0.23$; n.s.; and $F(2,24) = 1.04$; $p = 0.36$; n.s.; respectively).

Experiment II

Performances of the RVIP task obtained in Experiment II are illustrated in Fig. 3. The factors treatment and period displayed a significant effect on the number of hits ($F(2,84) = 3.37$; $p = 0.04$; and $F(1,84) = 11.14$; $p < 0.01$; respectively). Pairwise comparisons revealed that the number of hits increased during Post2 as compared to Post1. In addition, the number of hits scored during Post2 was significantly higher in the Optimized condition as compared to the glass of water condition during Post2 and to all conditions during Post1. Such effects of the treatment and period factors were not observed for the reaction times ($F(2,84) = 2.09$; $p = 0.13$; n.s.; and $F(1,84) = 0.96$; $p = 0.33$; n.s.; respectively). No significant interaction was observed between the two factors for both measures of hits and reaction times ($F(2,84) = 0.63$; $p = 0.53$; n.s.; and $F(1,84) = 0.28$; $p = 0.75$; n.s.; respectively).

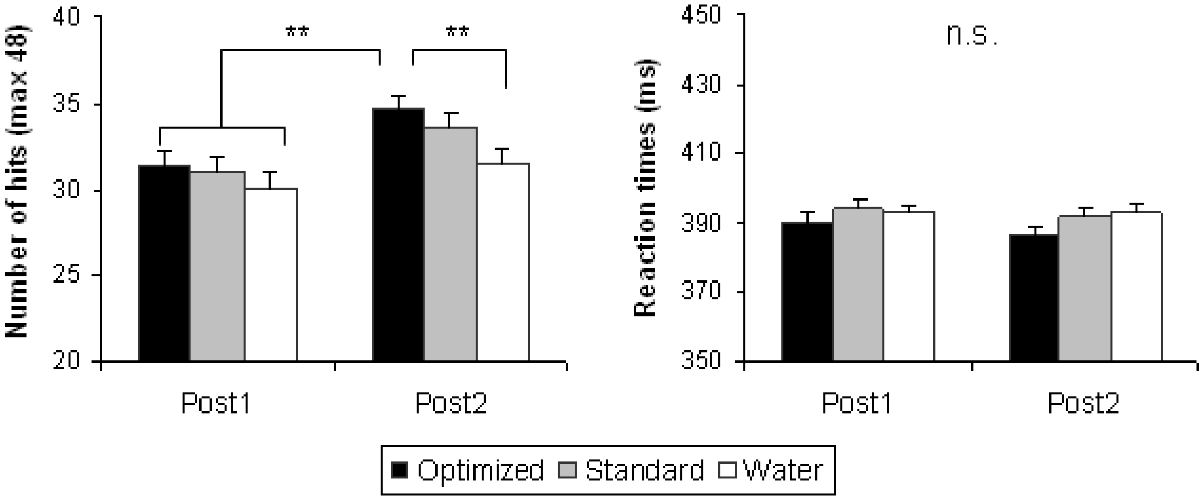

Fig.3. Mean changes in performances for the RVIP task obtained in Experiment II as a function of treatment and period of time for the number of hits (left panel) and reaction times (right panel). Error bars, standard error of the mean; n.s., non significant effects or interactions as revealed by analyses of variance.

Mood ratings

Experiment I

In Experiment I, alertness was impacted by the factor period ($F(2,53) = 7.96$; $p < 0.01$) due to a progressive decrease of ratings from Post1 (mean = 74.2 ± 3.4) to Post3 (mean = 63.1 ± 3.4). No significant effect of treatment or interactions between the two factors were observed on alertness ratings ($F(2,53) = 0.56$; $p = 0.58$; n.s.; $F(4,53) = 0.60$; $p = 0.66$; n.s.; respectively). Ratings of calmness and contentedness were not affected by the factors treatment ($F(2,53) = 0.92$; $p = 0.4$; n.s.; and $F(2,53) = 0.11$; $p = 0.74$; n.s.; respectively) and period ($F(2,53) = 0.62$; $p = 0.54$; n.s.; and $F(2,53) = 0.98$; $p = 0.38$; n.s.; respectively) nor by any interaction between the two factors ($F(4,53) = 0.30$; $p = 0.87$; n.s.; and $F(4,53) = 0.22$; $p = 0.80$; n.s.; respectively).

Experiment II

In Experiment II, alertness ratings were both impacted by the factors treatment ($F(2,135) = 17.3$; $p < 0.01$) period ($F(2,135) = 16.97$; $p < 0.01$). As in experiment I, mean alertness scores progressively decreased from Post1 (mean = 70.2 ± 2.1) to Post3 (mean = 60.4 ± 2.1). The effect of the factor treatment was due to significantly lower scores in the Water condition during Post1, Post2 and Post3, as compared to the Optimized and Standard conditions. Calmness was also both impacted by the factors treatment ($F(2,135) = 6.55$; $p < 0.01$) and period ($F(2,135) = 5.03$; $p < 0.01$). Mean calmness scores progressively increased from Post1 (mean = 63.4 ± 2.4) to Post3 (mean = 69.6 ± 2.4). These changes were due to significantly higher calmness ratings in the Water condition during Post1, Post2 and Post3, as compared to the Optimized and Standard conditions. Contentedness was impacted by the factor treatment ($F(2,135) = 7.52$; $p < 0.01$) but not by the factor period ($F(2,135) = 1.84$; $p = 0.16$) nor by an interaction between these two factors ($F(4,53) = 0.53$; $p = 0.72$). Contentedness ratings were significantly higher in the Optimized and Standard conditions than in the water condition.

Ad libitum water intake

For both Experiment I and II, the factor treatment did not affected the amount of water drunk ($F(2,10) = 1.38$; $p = 0.29$; n.s.; and $F(2,25) = 0.31$; $p = 0.73$; n.s.; respectively). However, in both experiments, the amount of water drunk tended to be smaller in the Optimized (Experiment I: 136.1 ± 26.9 ml; Experiment II: 109 ± 22.7 ml) than in the Standard conditions (Experiment I: 171.1 ± 26.9 ml; Experiment II: 113 ± 22.7 ml) and Water (Experiment I: 199.3 ± 26.9 ml; Experiment II: 135 ± 22.7 ml).

Discussion

The two experiments described here investigated the impact of refreshing perception induced by a solid food on mental energy, as measured by subjective mood reports, cognitive performances and related cortical activation. Refreshing perception was optimized in a frozen snack by adding specific ingredients associated with sensory properties previously identified as drivers of refreshing, namely cooling and sourness (Labbe et al., 2008; McEwan & Colwill, 1996). In a first experiment (I) including 6 participants, superior benefits of refreshing were found upon alpha and beta frequency ranges during Post2, suggesting an increase of brain activation for optimal task performance 15 min after treatment. This suggestion was corroborated at the mood and performance levels in a second experiment (II) including a larger sample of 18 participants.

Enhancement of brain oscillatory activity

In Experiment I, the beneficial effects of the frozen snack optimized for refreshing perception was examined on brain oscillations during completion of a 6-min RVIP task immediately (Post1) and 15 min after intake (Post2). Comparison treatments were a Standard frozen snack matched in flavour and energy content, and a glass of fresh Water. Power in two frequency bands (alpha and beta) was significantly affected by the treatment condition. Oscillatory changes in the alpha power were the most prominent and were affected by an interaction between the treatment and period factors (see Fig. 1C).
Three main changes in alpha oscillations are noteworthy. First, during Post1, EEG power in the alpha power was increased in the Water condition, as compared to both frozen snacks. This finding parallels those of a previous study of EEG and thirst-quenching reporting a general increase of EEG power in the alpha range immediately after drinking through a period of 7 min (Hallschmid et al., 2002). Second, whereas alpha power remained unchanged in the Water condition with time on test, oscillatory activity in this frequency range displayed a large increase in both frozen snack conditions from Post1 to Post2. Changes in EEG power subsequent to food consumption have been previously reported, mainly in terms of increased alpha and decreased delta activity during rest (Hoffman & Polich, 1998; Wang, Szabo, & Dykman, 2004). Such changes were seen as a general effect of arousal due to nutrient intake. Our finding that oscillatory activity in alpha power was enhanced in Post2 in the two frozen snack conditions compared to the glass

of water may also reflect an effect of nutrient intake, and in particular of carbohydrate. However, to our knowledge, the effect of carbohydrate on brain electrical activity has not been investigated over time. Third, the enhancement of alpha power during Post2 was significantly higher in the Optimized than Standard condition, suggesting a superior impact of the sensory properties optimized for refreshing perception. Similar oscillatory changes obtained in the beta frequency range (see Fig. 1D) reinforce this assumption of a superior impact of refreshing perception during Post2.

Possible mechanisms underlying changes in cortical activation induced by refreshing perception

Cooling sensations are mediated by specific receptors found in trigeminal cold-sensing neurons widely distributed over the tongue, throat and nasal cavity (Patapoutian, Peier, Story, & Viswanath, 2003). These receptors are activated both by cold temperatures and cooling agents. Their contributions to thirst quenching and consequent impact on arousal have been highlighted in a review article (Eccles, 2000). The author suggested that activation of the trigeminal system through a combination of cold and cooling stimulations trigger specific brain mechanisms leading to an increased level of arousal and cortical activation. A recent functional neuroimaging study in humans has identified a large network of brain areas activated by oral temperature processing during water drinking (Guest et al., 2007). This network includes the primary somatosensory cortices, parts of the primary taste cortex, the premotor cortex, the fronto-parietal opercular cortex and the orbitofrontal cortex. The assumption that trigeminal stimulations inducing cold/cooling sensations can improve mental energy is corroborated by other studies using volatile cooling compounds of menthol from peppermint oil that activates nasal cold-sensing receptors. The smell of peppermint was found to reduce the feeling of sleepiness (Norrish & Dwyer, 2005), to increase subjective alertness (Moss, Hewitt, Moss, & Wesnes, 2008) and to increase performances on a variety of cognitive tasks (Ho & Spence, 2005; McBride, Johnson, Merullo, & Bartow, Jr., 2004; Moss et al., 2008; Barker et al., 2003). It is interesting to note that olfactory properties of other odorants may not induce any benefit at all on mental energy, such as for example ylang-ylang (Moss et al., 2008), or may trigger opposite relaxing effects, such as lavender (Moss, Cook, Wesnes, & Duckett, 2003).

Sour tasting compounds activate specific taste cells located on the tongue and expressing proton sensing receptors responsible for sour perception. Of all taste qualities, sour taste induces secretion of the largest volume of saliva by salivary glands (Hodson & Linden, 2006). Increases of saliva flow rate and salivary cortisol concentration have been observed in response to mental stress induced by cognitive activity (Bakke et al., 2004). Acute exposure to stress, such as the one triggered by a cognitive demand or challenge, may act as a mood enhancer and cognitive facilitator (Duncko, Cornwell, Cui, Merikangas, & Grillon, 2007; Sandi & Pinelo-Nava, 2007). However, the link between changes in saliva flow rate and the release of stress hormones such as cortisol or adrenaline remains unexplored. By contrast, saliva has multiple well-known essential functions in relation to mouthfeel (lubrication) and to the digestive process, including the so-called ‘cephalic phase responses’ (Mattes, 2000). These physiological responses are believed to prime the body to better absorb and use ingested nutrients. They coincide with a rapid increase of insulin, promoting further glucose uptake into metabolic active cells. This facilitation of glucose availability induced by digestive processes might be improved by sour stimulation, possibly contributing to the superior impact of the optimized recipe on cortical activation upon the standard recipe. The possible link between sour stimulation and brain activation remains purely theoretical as the impact of sourness on mental energy has never been investigated. Further investigations are needed to answer this question.

Taken together, our EEG findings suggest that, beyond carbohydrate or other nutrient, the consumption of food with refreshing properties can enhance brain oscillations during cognitive workload in frequency bands known to be involved in cognitive processes. The activation of specific neural networks in response to trigeminal stimulation, together with a hypothetic hormonal release or facilitation of glucose availability by sour stimulation, is likely to improve mental energy.

Behavioral significance of enhanced brain oscillations during task performance

The success in detecting targets in the RVIP task is critically dependent on attentional resources and on successful maintenance of the neuronal representation of previously presented stimuli, up to three digits, in short-term memory. Numerous studies have investigated the functional significance of alpha oscillations with regard to cognitive processes. Whereas alpha oscillations typically increase during relaxation or when subjects are in a passive state, recent research describes these oscillations as key components of alertness, selective attentional processes and goal-directed behavior (Jensen, Gelfand, Kounios, & Lisman, 2002; Klimesch, Doppelmayr, Russegger, Pachinger, & Schwaiger, 1998; Palva, & Palva, 2007; Dockree, Kelly, Foxe, Reilly, & Robertson, 2007). Using a visual continuous performance task of attention and memory, Klimesch et al. (1998) found that alpha oscillations, and in particular in the posterior and bilateral central regions, play a crucial role in neural circuits responsible for working memory. In the present study, alpha oscillations were most prominent over the occipital region while participant performed the RVIP task, but the treatment condition was not affected by the topography. In addition to a clear involvement in cognitive processes, the power of brain oscillations in alpha and beta bands have been identified as valuable predictive measures of performance during tasks of sustained visual attention (Besserve et al., 2008; Dockree et al., 2007). Oscillatory activity in the beta or higher frequency ranges seems to play an important role in the functional organization of neuronal activity that underlies visual perception, attention and working memory (Gross et al., 2004; Tallon-Baudry, 2003). The possibility to predict performance based on EEG oscillatory activity is further supported by other data showing that oscillations below 20 Hz, especially those peaking between 7-12 and 16-20 Hz, are positively correlated with driving performances (Liang et al., 2005). From these EEG data, alpha and beta oscillations may account for an increase of antero-posterior synchrony with better performance, possibly reflecting the activation of a neural network involved visual-attention control.

In Experiment I, although clear effects of treatment condition on cortical activation were found in the 6 participants involved, these effects were not found at the task performance and mood levels. Nevertheless, using a larger sample of participants, we found effects of treatment at the behavioral levels in Experiment II (see Fig. 3) that corroborate EEG changes obtained during Post2 in Experiment I. The monitoring of changes in brain oscillations have been validated as a highly sensitive method to assess the effects of psychostimulants on the human brain (Siepmann & Kirch, 2002), even in the absence of related changes in cognitive performance (Deslandes et al., 2005). In Experiment II, a pattern of results comparable to the EEG changes observed in alpha and beta powers as a function of treatment and period was obtained for the number of hits and for the subjective ratings of alertness and calmness. Accuracy in detecting targets increased during Post2, and this improvement was significantly higher in the Optimized than in the

Water condition. Similarly, participants reported higher alertness and lower calmness levels in the Optimized condition during Post2. Interestingly, participants felt happier after consumption of any of the frozen snacks than the glass of water, contentedness ratings being lowest in the latter condition.

In conclusion, data presented here indicate that key sensory properties of refreshing perception can produce objective effects on cognitive performance, subjective effects on mood, as well as physiological effects on brain activation. In agreement with previous studies, our findings highlight the involvement of alpha oscillations in relation to refreshing perception, subjective alertness and processes of sustained attention. Whereas the benefits of trigeminal stimulations inducing cooling sensations are corroborated by other data, the involvement of sour stimulations remains largely unexplored. Further experiments will be conducted to elucidate the impact of individual sensory drivers of refreshing on mental energy and their potential additive or synergistic effects. In addition to providing insights into the impact of specific sensory stimulations into mental energy, the present data open the discussion on the origin of the association between refreshing and mental energy. In other words, is mental energy improved by certain sensory properties because their combinations induce a refreshing perception? Or, inversely, are combinations of certain sensory properties described as refreshing such as coldness and sourness because they improve mental energy?

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3.3.2 Modulation of saliva flow, saliva lubricating properties and related lingering perceptions by refreshing frozen snacks

Labbe,D., & Martin,N. (to be submitted to Physiology & Behavior)

Refreshing in food and drinks is a perception strongly related to mouth state after product consumption. Oral dryness and roughness are perceptions negatively related to refreshing whereas mouth wetting perception is a positive driver of refreshing perception. Since saliva seems to be related to mouth wetting, we explored if salivary flow and saliva lubricating properties could be potential markers of refreshing perception. To reach our objective, we explored saliva flow and saliva lubricating properties after the consumption of a frozen snack optimized to be perceived more refreshing than the standard snack. As key results, the optimized frozen snack induced the highest salivary rate and saliva production with the lowest friction coefficient. These results were validated perceptually. Indeed according to sensory results obtained with a group of 41 assessors, the optimized product delivered after consumption the most intense salivating perception. Our finding seems to validate the positive association between refreshing and mouth wetting perceptions.

Introduction

Refreshing perception is a complex perception induced by: 1) the perceptual combination of several key sensory stimuli such as high coldness, high sourness, low thickness and low sweetness (Labbe *et al.*, 2009a); and 2) the alleviation by these sensory stimuli of psychophysiological symptoms such as mental fatigue, thirst, mouth dryness and hot feeling (Labbe et al., 2008 submitted). The authors demonstrated that sensory drivers of refreshing and psychophysiological impact of related sensory stimuli were close to those experienced by water drinking.

Regarding psychophysiological parameters associated with refreshing perception, it was assumed that consumption of a refreshing product enhances mental energy in terms of cortical activation and cognitive performances thanks to these sour and cold sensory properties (Labbe et al., 2008 submitted). Consumption of a glass of water, a product generally associated with refreshing by consumers (Zellner and Durlach, 2002), has already been shown as enhancing subjective alertness and performance during a cognitive task (Rogers et al., 2001c) and increasing cortical activity (Hallschmid et al., 2001). These results were obtained only for subjects who were submitted to a drink restriction prior to the study ("thirst" condition). These findings suggest that the alleviation of thirst and related mental fatigue contribute to refreshing perception induced by water.

Refreshing perception may also be associated with the alleviation of oral dryness and consequently to mouth wetting perception (Brunstrom, 2002). This assumption was corroborated by perceptual findings showing that refreshing was negatively correlated to astringency (Guinard et al., 1998; Labbe et al., 2007), astringency being related to oral dryness and roughness (Lesschaeve and Noble, 2005). The two objectives of the present study were to compare the impact of two frozen snacks varying in refreshing intensity on oral wetting in terms of: 1) saliva flow rate and saliva lubricating effect; and 2) salivating perception. We hypothesized that the most refreshing product would induce the highest saliva flow rate and production of saliva with the highest lubricating properties and that physiological results could be validated by perceptual evidences. A glass of cold water (+8℃) was used as reference for the physiological experiment since cold water was reported as enhancing saliva flow rate (Pangborn *et al.*, 1970; Brunstrom *et al.*, 1997; Lee *et al.*, 2006)

At the end of the session subjects were free to drink ad libitum water. Amount of drank water was considered as an additional indicator of the product abilities to perceptually increase mouth wetting perception. Indeed thirst-quenching perception, which is strongly associated with refreshing perception (Guinard et al., 1998), was suggested as positively related to mouth wetting perception (Figaro and Mack, 1997; Brunstrom, 2002). Moreover thirst sensation has also been described as "the consequence of the need to moisten the mouth" (Brunstrom et al., 2000).

Experiment 1: physiological measures

Material and methods

Products

Two flavoured citrus frozen snacks were used (standard frozen snack and optimized frozen snack). They were similar to those described in the study from Labbe et al. (2008). They mainly contained water (70g) and sucrose (25g). Difference in refreshing intensity between both products was obtained by adding to the optimized frozen snack 0.04% of cooling agent L-127039 provided by Givaudan (Dübendorf, Switzerland) and 0.20% of citric acid as coldness and sourness are drivers of refreshing perception (Labbe et al., 2007). Preliminary studies validated that compared to the standard frozen snack, the optimized frozen snack was significantly perceived as being: 1) more intense in sourness and coldness by sensory profiling carried out with trained subjects; and 2) more refreshing in a consumer test. Frozen snacks were served at -17°C. Water was a glass of 70 ml of Vittel served at 8°C.

Subjects

Saliva was collected from six male volunteers between the ages of 18 and 35 selected among staff at Nestlé Research Center (Lausanne, Switzerland). They were healthy as determined by a medical questionnaire and had a BMI in the range of 18-30. Exclusion criteria were eating disorders, diabetes, neurological disease, history of head trauma, nose pathology or upper olfactory tract infection. All participants gave their written consent and were paid for participation. The study was conducted in accordance with the Helsinki Declaration and was approved by the Ethical Committee for Clinical Research of Medicine and Biology Faculty (Lausanne University, Switzerland). Subjects were required not to consume any food or drink after a standardized breakfast from 07:50 to 12:00 in order to ensure comparable conditions among subjects.

Saliva collection and product administration

Subjects arrived at 12.00 at the Metabolic Unit. First saliva was immediately collected before product consumption during 5 min (PRE). Subjects were seated and at rest, i.e. they did not stimulate salivation, but waited till they had too much saliva in their mouth to spit out into a beaker. Then, one of the three products (glass of water, standard frozen snack or optimized frozen snack) was administrated. The complete consumption of a frozen food lasted 5 min. One product was evaluated per day; the study was therefore completed in three days. Product order was randomized between sessions and subjects according to a Latin Square experimental design. During the sixty seconds following product consumption, they were asked to swallow normally in order to eliminate in-mouth product residues and finally during 5 min saliva was collected. All collected saliva samples were weighed and kept in their individual beakers at 37ºC in a water bath till instrumental analyses were conducted at 13.00 pm

Assessment of saliva lubricating effect by measurement of friction coefficient between tongue and palate using tribometry

Saliva lubricating properties directly affect in-mouth friction between tongue and palate. Indeed the higher the saliva lubricating effect, the lower the friction coefficient. In vivo measurement of friction coefficient between tongue and palate is technically challenging, this is why friction coefficient measurements were conducted using a tribometer. The device was developed to reproduce the friction movement between the tongue and the palate (Fig.1) (Ranc et al., 2005; 2006). The tribometer enforces a reciprocating sliding motion between a PCTFE (polychlorotrifluoroethylene) hemisphere which stands for the palate (it was chosen due to its Young's modulus that was close to the hard palate bone elastic modulus) and a rough silicone rubber which represents the tongue. It was selected due to its viscoelastic properties that were close to those of the tongue. Moreover, the Young's modulus of the silicone samples (1.5mPa) was in the same order of magnitude as the tongue elastic modulus.
Contact is ensured through application of a normal force. The tangential force is measured, and from this the friction coefficient is determined automatically. During the test, the temperature was maintained at 37°C with a temperature-controlled vessel to which the silicone surface can be fixed. The pin in PCTFE is first screwed at the end of the loading arm and comes into contact with the silicone surface with a contact load not exceeding 0.04 N. Then, a load of 0.5 N was applied thanks to dead weights that were suspended on the tribometer arm, to obtain a Hertzian pressure of 310 kPa that approximately corresponds to the pressure recorded in vivo with pressure transducers. To reproduce the velocity of tongue movements found in the literature, a triangular waveform with a frequency of 1 Hz (that corresponds to a velocity of 10 mms^{-1}) was applied. The displacement of the pin in PCTFE is measured thanks to the relative motion of a laser placed on the arm and recorded by a photodetector (Hamamatsu, France).
The systems allows measuring the tangential force magnitude (Ft) corresponding to the friction force, the normal force and the temperature. Thus, the friction coefficient (μ) is obtained by dividing the friction force by the applied normal force (Fn) magnitude according to the following equation:

$$\mu = \frac{Ft}{Fn}$$

μ: Coefficient of friction
Ft: Friction force (N)
Fn: Normal force (N)

The friction coefficient is first measured under dry conditions until the plateau region is reached. Then, the motion is stopped and the loading arm is lifted up with a wedge, to add 0.5 mL of saliva with a micropipette. The pin in PCTFE comes again into contact with the silicone surface and the motion is started again after stabilizing the temperature.
To calculate the average friction coefficient during dry and lubricated contact, at least 100 data points of the plateau region of the friction coefficient-versus-time curve are taken into account. All tests were performed in duplicate and new silicone surfaces and PCTFE pins are used for each test.

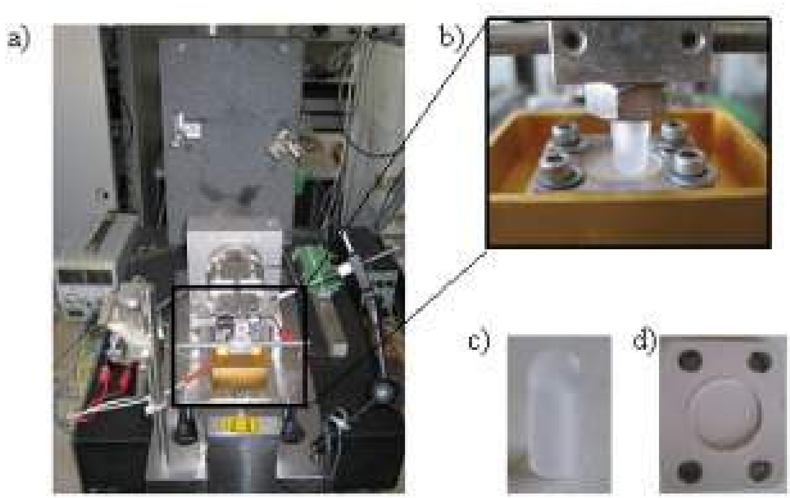


Fig.1 (a-d): Representation of a tribometer

a: The tribometer

b: The tribo-pair material does a reciprocating sliding motion between a hard hemisphere of PCTFE and a flat soft silicone surface to simulate the contact tongue/palate

c: A hard hemisphere in PCTFE

d: The silicone surface.

Amount of ad libitum drunk water

At the end of the session subjects were asked to fill in a questionnaire to occupy them for 10 min during during which they were free to drink ad libitum Vittel mineral water. Replies were not used as an outcome. An individual 1500 ml bottle of water was available and the spontaneous amount of water drunk was measured.

Statistical analysis

The amount of produced saliva was expressed in g collected during five min. Product impact on saliva production collected after product consumption (POST) was measured according to a product (Optimized frozen snack, standard frozen snack, glass of water) x Subject covariance analysis with interactions. Saliva production measurement before product consumption (PRE) was considered as baseline and used as a covariate variable. Product impact on friction coefficient in POST was measured according to a product (Optimized frozen snack, standard frozen snack, glass of water) x Repetition x Subjects covariance analysis with interactions. Friction coefficient measurement in PRE was considered as baseline and used as a covariate variable.

The effect of product consumption on the amount of drunk water at the end of the session was analysed by a product (Optimized frozen snack, standard frozen snack, glass of water) x Subject analysis of variance.

Analyses of Variance (ANOVA) were calculated, using NCSS software version 2007 (Number Cruncher Statistical Systems, Karysville, Utah, USA.). Post-hoc pair comparisons were conducted by a Student t-test. Confidence level was set to 95% for all analyses

Results

There was no significant impact of products on the salivary production after consumption [F(2,26)=0.73, P=0.49] (Fig.2). But comparing saliva product before and after consumption of the products, Student paired T-test showed a significant increase of the saliva production after consumption of the optimized frozen snack (p-value<0.05).

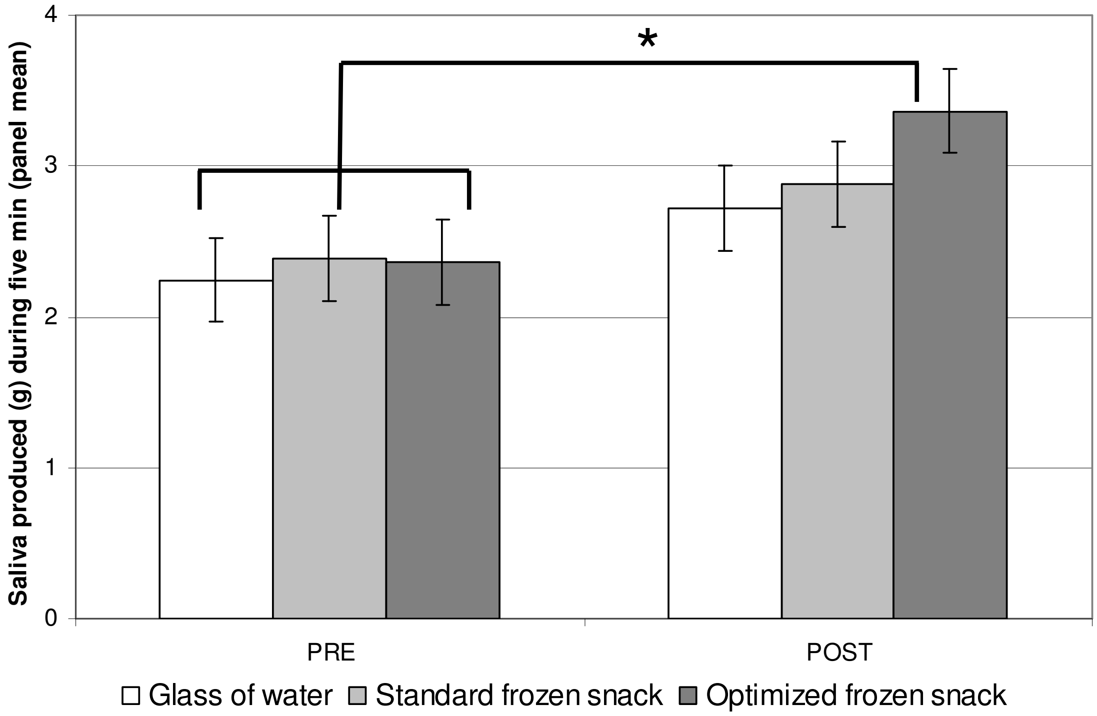


Fig.2: Impact of the three products on mean saliva production (+/- SEM). A star means a significant effect (p<0.05) according to Student t-test pair comparisons

There was a significant impact of products on friction coefficient [(F(2,61)=15.6, P<0.01). Saliva produced after consumption of the glass of water led to the highest friction coefficient whereas saliva produced after consumption of the optimized frozen food consumption had the lowest friction coefficient (Fig.3). According Student t-test paired, friction coefficient remained constant before and after consumption of the glass of water whereas it decreased after consumption of both frozen snacks (p-value<0.05%).

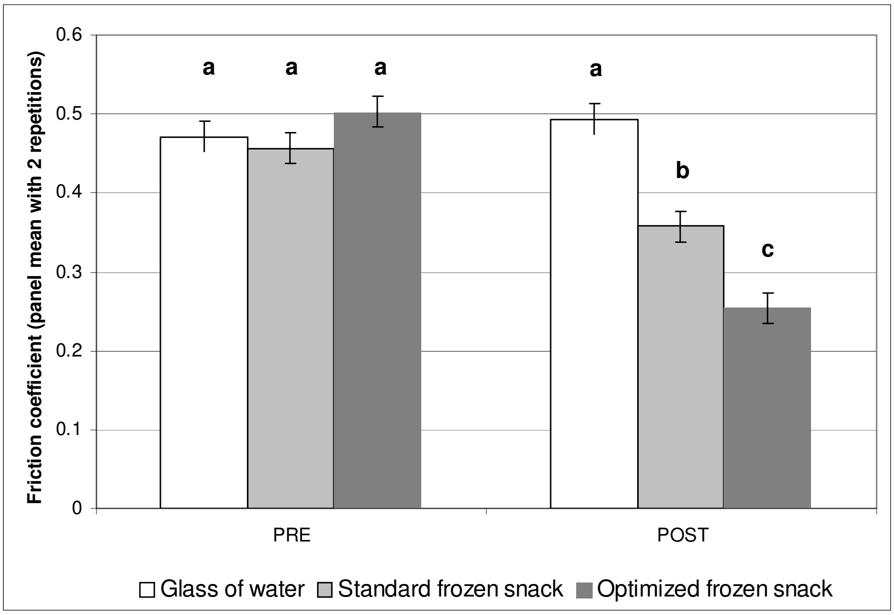


Fig.3: Impact of the three products on mean saliva friction coefficient (+/- SEM). Products with the same letter are not significantly different according to Student t-test pair comparisons (p<0.05)

The amount of drunk water at the end of the session did not significantly change according to the product consumed during the session [F(2,10)=0.87, P=0.44]. But the optimized refreshing snack consumption showed a trend in inducing the lowest volume of water consumption (Fig.4)

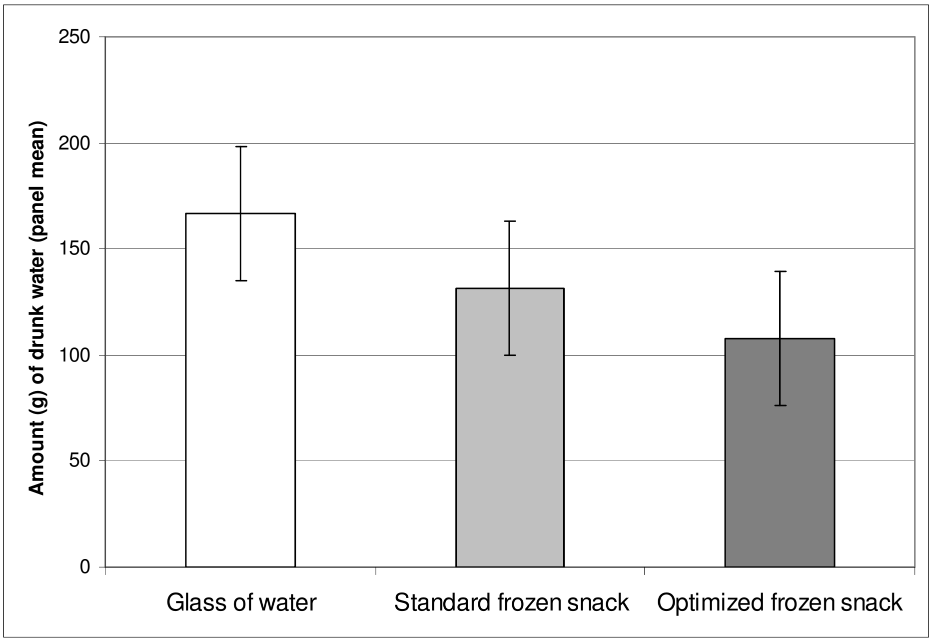


Fig.4: Product impact on the mean amount of drunk water (+/- SEM).
** means significant effects or interactions (p<0.05) as revealed by analyses of covariance*

Experiment 2: Sensory evaluation

Material and methods

Products

The two flavoured citrus frozen snacks of experiment 1 was used, but not the glass of water since sensory properties of water and frozen snacks are not comparable.

Subjects and attributes

An external panel of 41 subjects experienced in TDS measurement was recruited. They had previously been screened by tests selected in ISO 8586-1 (1995). They did not follow specific trainings for this study since they knew the TDS method and the five attributes used in this study: flavour intensity, sweetness, bitterness, coldness and salivating. The list of attributes was set up following a 90-min session where a list of terms was generated and then reduced according to ISO 11035 (1995). The reduction phase consisted in deleting: 1) attributes what did not characterise all products; and 2) synonymous and antonymous attributes (the most meaningful attribute to keep was defined by the panel). For instance sourness and astringency were not selected since they were synonymous and antonymous with salivating. All the attributes were presented simultaneously on the computer screen with their corresponding 10 cm unstructured linear scale, anchored at the extremities with “not at all intense” and “very intense”.

Method principle

TDS is an adapted time-intensity method which allows to consider the multidimensionality of the perceptual space over time (Pineau *et al.*, 2003; Labbe *et al.*, 2009b). This descriptive sensory method consists in assessing iteratively at each specific time until the perceptions end, which perception is dominant and in scoring its intensity. In this study *dominant* was the most intense sensation. The evaluation was conducted over 4 min during eating and then continued for 6 min after eating.

When the subject started to suck the product, he clicked on a start button on the screen to begin the evaluation. During an evaluation, the subject had to select the attribute considered as dominant and score this attribute on a linear scale. When the dominant perception changed, the subject had to score the new dominant sensation. The subject was free to choose several times the same attribute or conversely to never select an attribute as dominant.

Tasting conditions

Data acquisition was carried out on a computer with FIZZ software Version 2.20E (Biosystemes, Couternon, France). Products were evaluated by all subjects during one session and presented according to a design balancing position and order effects, based on Williams Latin Squares.

Products were coded with three-digit random numbers and the 70 ml portion was served at -17°C on a plastic plate. Rinsing was done between products with water and unsalted crackers during a 5-min break. Tests were conducted in an air-conditioned room (22°C), under white light in individual booths.

The products were consumed according to a standardized procedure, i.e. subjects were asked to suck every 5 s the product and to swallow normally during the evaluation. According to this procedure, duration required for complete product consumption was around 4 min.

Statistical analyses

TDS data were represented by curves showing for each product the percentage of subjects who selected the attribute as dominant at a specific time, i.e. the dominance rate. The frequency of selection of each attribute as dominant by all subjects is represented in percentage (Axis Y) each 1.5 s, which corresponds to the data collection interval, during the 5-min period (Axis X). The 200 points of the evolution curve were smoothed using a non-weighted moving average with a 10 points window. The curve showed over time the dominant attributes at a panel level. For each 1.5-s step, the proportion of scoring for an attribute was considered as significantly dominant when above P_s (the smallest dominance rate to reach to be significantly above the chance limit) as defined according to the equation (2), and as dominant when between P_0 (dominance rate obtained if all subjects scored by chance) and P_s, in this case we considered the results as a trend.

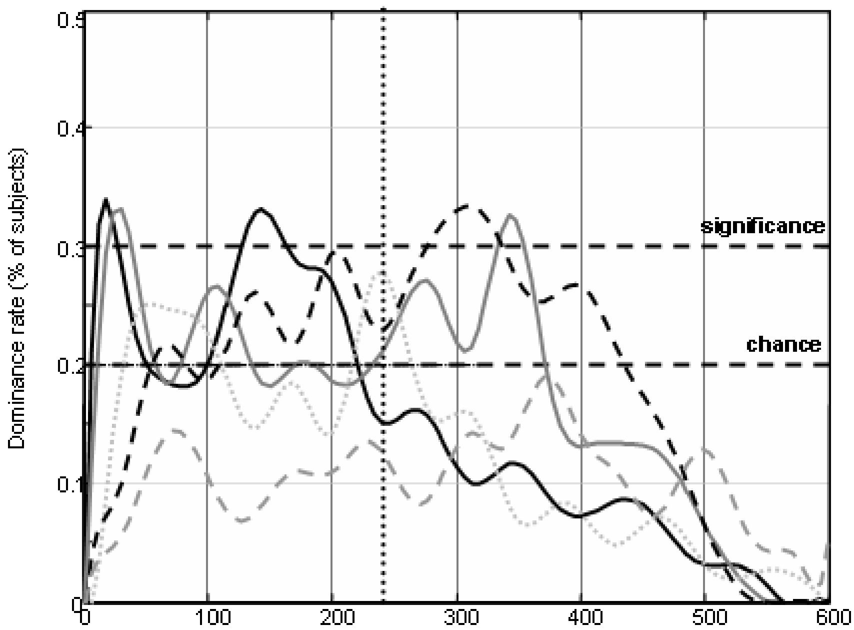
$$P_s = P_0 + 1.64\sqrt{\frac{P_0(1-P_0)}{n}}$$

With chance level P0 = 1/(k+1), k being the number of attributes, n the number of subjects and 1.64 the one-tailed normal law z value for α=5%.

Results

TDS curves showed that after consumption of the standard frozen snack, no clear dominant sensation could be highlighted (Fig.5a) since salivating and coldness curves were above the significant level only during a short time period. Regarding results obtained after optimized frozen food consumption (Fig.5b), lingering perception was clearly dominating in terms of salivating perception since the salivating attribute curve was above the significant level during 100 s and then slowly decreased to reach the chance level 500s after the end of consumption. In addition, for salivating attribute, panel agreement was higher for the optimized frozen snack than for standard frozen food with a maximal dominance rate of 40% and 33%, respectively.
Interestingly, during consumption, the flavour intensity was significantly different between products. Flavour intensity of the optimized frozen snack was perceived dominant during all 4 min consumption with a maximum dominant rate of 45% whereas flavour intensity delivered by the standard frozen snack was dominating during very short periods and with a lower panel agreement since the maximal dominance was 33 %.

a)



b)

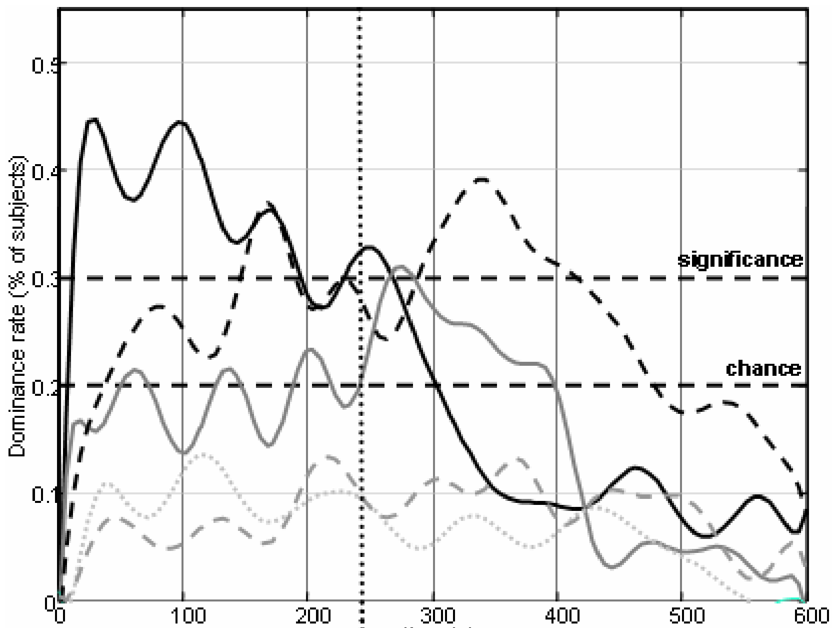


Fig.5a-b: Dominance rate in terms of subject percentage over a 10 min time period for a) standard frozen food and b) optimized frozen food. Attributes are representing as following:

——— *flavour intensity* — — *salivating* ——— *coldness* · · · · · *bitterness and*

— — *sweetness*

Discussion

The saliva flow rate after consumption did not significantly differ between the three products. But only the optimized product perceived as the most refreshing significantly enhanced the saliva flow rate after consumption. The mouth wetting ability of the product was shown as a physiological property of products perceived as refreshing and thirst quenching (Figaro and Mack, 1997; Brunstrom, 2002). This effect is probably due to the addition of citric acid that elicited a salivatory reflex (French et al., 1995). Cooling agent might also enhance saliva production since a previous study showed that cold water (at 0℃ and 3℃ caused more salivation than water at 22℃ or 33℃ (Pangborn et al., 1970; Brunstrom et al., 1997). In addition a study showed that a mouth pre-treatment with menthol, a cooling agent, increases in-mouth coldness of water (Green, 1984). The cooling agent might have potentialised the enhancing impact of the frozen snack on saliva flow.

Comparing both frozen snacks, optimized snack consumption led to saliva production with the highest lubricating properties in terms of friction coefficient. A saliva production with a high lubricating effect likely reduces perception of oral roughness, dryness or astringency that are negative drivers of refreshment (Guinard et al., 1998; Brunstrom, 2002; Labbe et al., 2007). This latter finding can physiologically explain the higher refreshing perception caused by the optimized frozen snack compared to the standard frozen snack. The physico-chemical mechanisms underlying the enhancement of saliva lubricating properties by citric acid and or cooling agent remains unclear. In addition our results are not consistent with a recent finding showing that citric acid aggregates and precipitates salivary protein leading to disruption of the salivary film lubricating the oral mucosa

At a perceptual level the optimized frozen snack was perceived significantly more salivating than the standard frozen snack in terms of dominance and duration. In addition, the higher dominance of fruity flavour intensity induced by the optimal frozen snack might be explained by: 1) an increase in volatile release by citric acid; or 2) perceptual interactions between olfactory and sour taste caused by citric acid. We support the perceptual interaction origin since such an effect was previously shown (Bonnans and Noble, 1993) and another study did not show effect of citric acid on volatile compounds release from orange and strawberry flavoured sucrose beverages (King et al., 2006).

Finally the amount of drunk water was the lowest after consumption of the optimized frozen snack consumption. Even if this result is only a trend, it might be related to the increase in saliva flow rate and salivating perception since Brunstrom et al. (2000) showed that an increase of saliva production during drinking reduces the need to continue drinking for mouth wetting purposes.

Conclusion

The physiological and perceptual outcomes validated our two working hypotheses, i.e. saliva flow rate was the highest and saliva production was the most lubricating after consumption of the optimized frozen snack. Consequently this product delivered a more intense oral wetting perception. This finding may partly explain that the optimized frozen snack was perceived more refreshing than the standard frozen snack by consumers since oral wetting is a positive driver of refreshing perception. Additional studies are required to understand: 1) the impact of citric acid and cooling agent on saliva flow rate and lubricating properties; and 2) mechanisms underlying the increase in saliva lubricating effect by citric acid and cooling agent comparing saliva chemical composition when collected after consumption of each frozen snack.

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PART 4: General discussion and perspectives

4.1 Familiarity and exposure strategy modulate perceptual interactions

The role of product familiarity and exposure strategy, i.e. analytical task (sensory profiling) vs. synthetical task (sorting task with free verbalisation), in the modulation of perceptual interactions during food experience has been clearly established in the set of experiments performed for the PhD work. Differential effects of product familiarity on perceptual interactions were demonstrated using sensory profiling. As expected, vanilla odorant increased sweetness in coffee and coca drinks whereas it surprisingly increased bitterness in caffeinated milk. We proposed that an interaction between unpleasantness and bitterness can explain this finding. The planned fMRI study will aim at investigating the neural correlates of these psychophysical findings. Two main assumptions are considered to explain the mechanisms underlying changes in taste perception induced by vanilla odour in both the familiar and unfamiliar drinks. First, modulation of taste may be driven by bottom-up processes that are involved in sweet and bitter perceptions through a differential activation of taste receptors and/or primary taste cortex. Second, other top-down processes may result in differential activation of the integrative orbitofrontal cortex responsible for flavour integration and reward/pleasure evaluation. We favor the second mechanism since we assumed that unpleasantness could be at the origin of bitterness enhancement during sensory/reward integration.

Concerning the impact of exposure strategy on coffee odour perception, we showed differences between product sensory maps built up from sensory profiling and sorting data. We assumed that perceptual interactions modulate perception and therefore product grouping done by consumers. This is highlighted by the use of taste-related words, such as 'bitterness', by consumers to describe the coffee smell. This term was relevantly used to describe Robusta coffees which are known to be bitter due to their chemical composition. In addition specific coffee olfactory notes (e.g. roasty, earthy) are typical of Robusta coffees and can therefore predict coffee taste (Lindinger *et al.*, 2008). Olfactory taste association constructed during prior repeated exposure to coffee may impact coffee aroma perception. The role of attentional strategy on perceptual interactions was also confirmed during co-exposure to unfamiliar odorant and sweet taste. Indeed synthetical attentional strategy exposure (triangle test) to the combined stimuli promoted the construction of perceptual interactions whereas the analytical strategy (sensory profiling) did not. This is probably because assessors from the analytical attentional strategy group learnt during training to dissociate perceptual dimensions on the studied products before conducting the sensory profiling. This approach limited the construction of perceptual interactions and their impact on sensory profiling results.

4.2 Multiplicity of perceptual interactions between olfactory, trigeminal and tactile perceptions

As expected, and already shown in the literature, we highlighted that olfaction modulates taste perception (sourness, bitterness, sweetness) through perceptual interactions. As new findings, we also demonstrated that olfaction impacts cold trigeminal perception and modulates taste-taste and trigeminal-taste interactions. The multiplicity and overlapping of olfactory-trigeminal-taste perceptual interactions in complex food systems should be taken into account in product formulation since the use of odorants may have unexpected effects on overall perception. More investigations are required to better understand if interactions between cold trigeminal and bitter perceptions have only a perceptual origin or if physiological factors are involved, for instance via common peripheral transduction mechanisms consecutive to receptor binding by trigeminal compounds.

4.3 Food experience contributes to construction of refreshing perception

We demonstrated that refreshing perception induced by our range of viscous liquid products is driven by four sensory dimensions: sweetness, coldness, acidity and thickness. A large majority of consumers agreed that the sweeter the product the less refreshing. Considering the three other sensory characteristics, a high coldness, a high acidity and a low thickness enhance refreshing intensity, but the importance of these drivers differ among consumers. Among the group of consumers who mainly associated refreshing perception with coldness, we identified a significantly higher proportion of chewing gum heavy users for breath refreshing purposes. Since these chewing gums generally contain cooling agents, this might explain why consumers strongly associated coldness and refreshing perception. The limited number of questions asked in the consumer questionnaire did not allow to explain the association between refreshing perception and either high acidity or low thickness for each of the other consumer groups. But we might assume that consumers who frequently drink sour fruit juice such as orange for refreshing purpose are more prone to associate acidity and refreshing. As for perceptual interactions, food experience may strongly impact the construction of complex perceptions.

The other novelty of this work about refreshing complex perception compared to studies previously done on creaminess (Richardson Harman *et al.*, 2000; Tournier *et al.*, 2007) and freshness (Peneau *et al.*, 2006; Peneau *et al.*, 2007) was the identification of psychophysiological determinant strongly related to refreshing perception, i.e. saliva properties and mental energy. However, the following questions have still no answer: 1) what is the respective contribution of the citric acid and cooling agent in modification of saliva properties and in mental energy enhancement; and 2) is the impact on psychophysiological determinant required to perceive a food as refreshing?

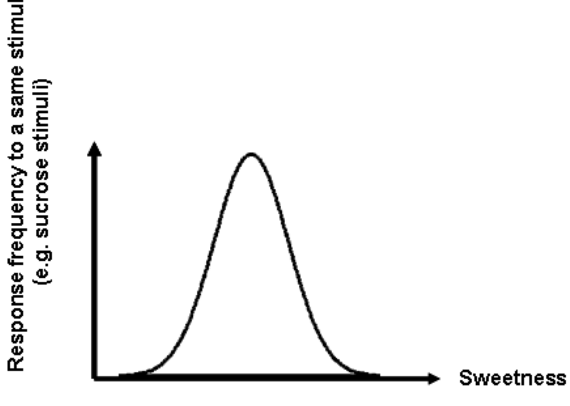
As next step, it would be interesting to recruit consumers having filled a food habit questionnaire related to refreshing food and to test the impact of three liquid viscous product containing either only citric acid or only cooling agent or neither citric acid nor cooling agent (and therefore very sweet) on refreshing intensity rating and mental energy. It would then be possible to explore the relationship between consumer food habits and potential consumer groups differing in terms of refreshing sensory drivers (acidity or coldness) and to highlight if mental energy is more impacted by cold or acid product according to the consumer group.

4.4 Alternatives to attribute rating (sensory profiling) for further exploring perceptual interactions and understanding consumer preference

We successfully used the attribute rating approach in my PhD work to explore: 1) the role of familiarity on perceptual interaction; 2) the impact of attentional strategy during exposure on the construction of perceptual interaction via sweetness rating procedure; 3) multi-sensory interactions in complex food systems; and 4) the sensory drivers of refreshing perception. Indeed by limiting the exposure to the studied products during training, assessors do not learn to dissociate specifically all product sensory dimensions. Attribute rating therefore remains suitable for exploring perceptual interactions and understanding consumer perception. For further exploring mechanisms underlying perceptual interactions and understanding consumer preference, it would be interesting to combine the attribute rating method with different paradigms with a more synthetical approach as we did with the sorting task. However the sorting task procedure did not allow the exploration of perceptual interactions between specific senses.

Nguyen et al. (2000) used a method based on Garner (1974) filtering paradigm developed for investigating the notion of *separability* and *integration* between two sensory dimensions in the field of visual and auditory perceptions. The notion of separable dimension was defined by Garner (1974) as *the possibility to give a perceptual judgment on one dimension independently from perceptual information provided by another dimension*. Nguyen and co-workers (2000) applied this psychophysics method to chemical senses. They asked assessors to make categorization judgments according to the level of the sweetness intensity of a range of four samples combining factorially two concentrations of sucrose and two concentrations of vanillin. First assessors are trained to taste and memorize the four different samples and then they had to categorize samples as a function of sweetness (high vs. low). Categorization performance on sweet intensities was impacted by vanilla. This finding demonstrated that assessors were unable to ignore the olfactory signal when categorizing the solutions according to taste probably because of perceptual interactions. Same findings were obtained on sourness categorization using four samples combining factorially two levels of lemon odorant and acid tastant.

Signal detection theory is based on the Thurstone's model stating that a same repeated stimulus induces a slightly different perception distributed according to a normal law (see Fig.1). According to the signal detection theory (Green and Swets, 1966), when one has to distinguish a signal, she/he has to distinguish in reality a signal plus a noise from a noise (Fig.2).



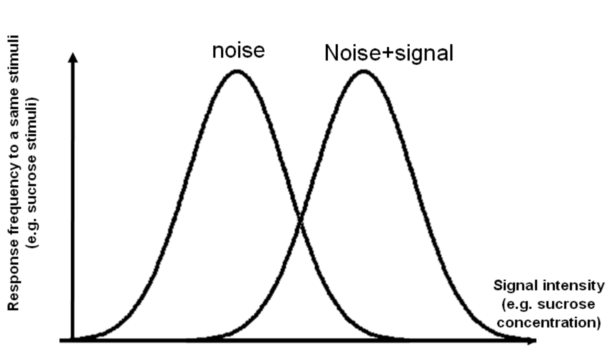


Fig.1: Distribution of perceived intensity for a same stimuli (according to Thurstone's model)

Fig.2: Representation of the Signal Detection Theory

A noise is a group of spontaneous and disorganized stimulations having an external or internal origin (Reuchlin, 1978). Subject response is related to: 1) his sensitivity to the signal, i.e. his capacity to distinguish "noise" from "noise+signal", this can be translated for example by the discrimination between "control" and "test" samples during a different from control task; and 2) his strategy, i.e. some subjects have a tendency to reply more often "control" and other subjects "test". Subjects are therefore called "conservator" and "liberal", respectively. This means that the perceptual difference separating the test from the control product is smaller for the liberal subject than for the conservator subject. During discriminative tasks the sensitivity and strategy indices can be calculated (O' Mahony, 1988). Signal Detection Theory approach can also be applied to categorization task on a specific product sensory dimension. In this situation, subjects reply is generally "low intensity" or "strong intensity" instead of "control" or "test".

To summarize, the discriminative and categorization task based methods have the following advantages compared to attribute rating: 1) they engage a synthetical approach since the subjects do not dissociate their overall percept into sub-unit perceptions using a list of attribute; and 2) they allow to measure the respective contribution to the subject response of his sensitivity to the signal and of his behavior (liberal vs. conservator). To our knowledge, no other study has been published about the exploration of perceptual interactions using this approach. This could mainly be explained by technical issues related to the complexity of the training which required that the panelists memorise all the samples as explained by Dacremont (2007) who reviewed the potential applications of such approach in exploring perceptual interactions between chemical senses. The applied statistical treatments when the number of sensory dimensions and/or levels within each dimension is higher than two are very complex. Another potential application of this approach is to measure strategy index of assessors and to use this information for panel formation according to the objective of the test. For instance if the objective of a discriminative task is to validate that a new coffee recipe (Test) is similar to the current coffee product (Control) commercialized from a long time and targeting hard core consumers, the use of conservator assessors is more adequate since product consumers are probably highly sensitive to slight differences between test and control products.

As another alternative to attribute rating for exploring perceptual interaction, White and Prescott (2007) used a rapid detection task like paradigm for exploring perceptual interaction between olfactory (strawberry and grapefruit) and taste (sweet and sour) interactions. Assessors had to identify as fast as possible a tastant by pressing one of two buttons related to sweet and sour response. By presenting randomly an olfactory stimuli (strawberry, pineapple or a blank) simultaneously to a tastant (sweet or sour) according to a 3x2 factorial design, the authors highlighted: 1) grapefruit olfactory perception led to faster identification of citric acid (lower reaction time for pressing the button "sour") than sucrose (sweet), whereas the strawberry odorant led to a reverse pattern. A previous study demonstrated perceptual interactions between olfactory and taste perceptions using a same type of detection task paradigm (Djordjevic *et al.*, 2004a).

To conclude about alternatives to sensory profiling for investigating perceptual interactions, several different paradigms successfully demonstrated perceptual interactions in model solutions comprising olfactory and taste stimuli. But using such methods for highlighting perceptual interaction between more than two sensory dimensions remains challenging. In addition more methodological development is required for considering such methods as potential synthetical alternatives to conventional sensory profiling allowing to better understand consumer preference.

4.5 Role of memory and neural integration processes in the construction of perceptual association

Regarding the integration of olfactory and taste perceptions, we observed in our study *Subthreshold olfactory stimulation can enhance sweetness* (pp. 52-62) that: 1) common odorants related to taste (e.g. strawberry-sweet) can enhance taste intensity with olfactory stimulus at both suprathreshold and subthreshold level); and 2) unfamiliar odorants (e.g. cactus) co-exposed with sucrose can enhance sweetness during post exposure tests when presented at suprathreshold level but not when then presented at subthreshold level. It is worthwhile to indicate that the post exposure tests with cactus odorant were conducted with a suprathreshold concentration the day after co-exposure and with a subthreshold concentration one week after exposure which may partly explain the results.

Olfactory and taste associations occurring during life experience involve first implicit/incidental learning and then consolidation into long term (from days to months) and or long-lasting (from months to life) memories during repeated co-exposure over life time. At the brain level, simultaneous olfactory and taste stimulations are integrated in the orbitofrontal cortex to generate flavour perception as shown in monkey (Rolls and Baylis, 1994) and humans (Rolls, 2006). Other key structures, referred as to *key nodes of the flavour network,* have been recently implicated in olfactory and taste integration processes (Small and Prescott, 2005). These include the anterior insula, frontal operculum and anterior cingular cortex. The amygala has also been identified as an essential structure of this flavour network in humans (de Araujo *et al.*, 2003). Electrophysiological research in monkies has identified: 1) unimodal neurons in the orbitofrontal cortex that respond specifically to input from one sensory modality amongst taste, smell or touch; and 2) multimodal neurons responding to input from several of these sensory modalities (e.g. Kadohisa et al. (2005a). Synaptic connections between unimodal olfactory and taste neurons may develop in the orbitofrontal cortex as a result of repeated and simultaneous exposure to a given olfactory and taste combination during life time. This assumption is reinforced by the presence of olfactory-taste bimodal neurons in this brain area identified in monkeys (Rolls and Baylis, 1994; Critchley and Rolls, 1996; Kadohisa *et al.*, 2005a; Kadohisa *et al.*, 2005b; Kadohisa *et al.*, 2005a; Kadohisa *et al.*, 2005b); which may result from evolution processes. Such a complex neural integration system allows sensory inputs from separate sensory modalities to converge into a unitary perception. Moreover, in the flavour network, the amygdala has been proposed to potentiate cortical information processing of stimuli that become associated through life experience (Dalton *et al.*, 2000). The impact of food experience in terms of congruency between olfactory and taste stimuli has also been evidenced at the brain level in humans (de Araujo *et al.*, 2003; Small *et al.*, 2004).

In the light of psychophysical findings obtained in our study and previous research (see Valentin and co-workers (2006) for a review) perceptual interactions between sucrose and a congruent suprathreshold odorant appears as the consequence of long lasting memorization processes within the flavour network. In these types of sensory interactions generated during life time, a congruent odorant presented at subthreshold level can also impact sweetness. A possible mechanism explaining this phenomenon is the activation of olfactory receptors occurring below the detection threshold (unconscious perception) but still leading to projections on bimodal neurons responding to these specific olfactory-taste combinations in the flavour network. In our study, co-exposure to unfamiliar odorant and sweet taste combinations during a week implied learning processes and consolidation into short term memory (from seconds to days). Short term learning under experimental conditions seems sufficient to induce sweetness enhancement with suprathreshold odorant previously co-exposed with sucrose. By contrast, when tested one week after co-exposure, this effect was not obtained for odorant presented below threshold level. The perceptual association learnt at short-term level may not have been consolidated into longer term memory. Other studies dealing with the impact of co-exposure between unfamiliar odorant and taste carried out the post-exposure test for validating the construction of perceptual interactions only immediately after co-exposure (Dalton *et al.*, 2000; Prescott *et al.*, 2004; Pfeiffer *et al.*, 2005; Yeomans *et al.*, 2006; Miyazawa *et al.*, 2008). To our knowledge, the robustness of perceptual associations experimentally constructed has never been investigated over time.

Additional psychophysics work is therefore needed. Indeed many aspects of memory and neural integration processes involved in the construction of perceptual associations during unfamiliar odorant and taste co-exposure remain unknown: 1) the robustness over months or years of newly memorized associations (long-lasting memory); and 2) the optimal period of co-exposure (i.e. learning phase duration, frequency and duration of sessions during the learning phase) needed to robustly memorized new associations leading at short term (immediately after co-exposure) and longer term (more than a week after co-exposure) to taste modulation with a subthreshold odorant concentration.

The measurement of brain activity with fMRI in response to taste- new aroma co-exposure before, during, and repeatedly after learning (from 1 week to several months) may bring relevant insight into the plasticity of the flavour network and how unitary perceptions are generated.

4.6 Anthropological approach of perceptual interactions

To enlarge our knowledge about mechanisms driving perceptual association, our psychophysical investigations were completed by an anthropological approach. This was conducted in the frame of the study *Impact of concomitant exposure to new aroma and sweet taste stimuli on perceptual interaction between tastant and odorant at supra and subthreshold concentrations (pp.* ***Error! Bookmark not defined.*** *p.77).* Indeed, additionally to the two groups exposed according to either an analytical or a synthetical attentional strategy, a third group was exposed according to the analytical strategy with individual interviews after each training and profiling sessions. A total of five interviews were therefore conducted by M-N. Ottavi (PhD student in Anthropology at the University of Nice Sophia Antipolis) for each of the twelve assessors. The interview method is based on a validated technique based on the self-confrontation method. The analyses of the interviews is currently on-going and should allow to highlight: 1) naive theories elaborated by each subject for describing the flavour perception (aroma and taste); and 2) the respective contributions during theory elaborations of the sensory training and of the dynamic construction (i.e. through the five daily interviews done consecutively over one week) of flavour representations (individual and collective).

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