Instituto de Agroquímica y Tecnología de Alimentos

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Diseño de alimentos lácteos con capacidad saciante.

Relación entre estructura, procesamiento oral y percepción

Tesis doctoral

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HACEN CONSTAR QUE:

El trabajo de investigación "Diseño de alimentos lácteos con capacidad saciante. Relación entre estructura, procesamiento oral y percepción" que presenta D. Pere Morell Esteve por la Universidad Politécnica de Valencia, ha sido realizado bajo nuestra dirección en el Departamento de Ciencia de Alimentos del IATA-CSIC y en el Grupo de Investigación de Microestructura y Química de Alimentos de la Universidad Politécnica de Valencia, reúne las condiciones para optar al grado de Doctor.

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Resumen

La presente tesis estudia cómo aumentar las expectativas de la capacidad saciante en productos lácteos analizando el efecto de la incorporación de determinados ingredientes.

El estudio de la reformulación de alimentos con alta expectativa sobre su capacidad saciante contribuye a moderar la elección de las raciones de alimentos y a regular la ingesta. En la presente tesis se han estudiado principalmente dos de los factores que más influyen en la percepción de capacidad saciante de alimentos semisólidos: a) el aumento en el tiempo de procesado oral que produce saciedad debido a una prolongación de las señales orosensoriales, y b) el alto contenido en proteínas.

El aumento del tiempo de procesado oral lo producen, por ejemplo, mayores consistencias, recubrimiento oral o sensaciones de cremosidad, que son características que se pueden modificar por adición de hidrocoloides. Se emplearon almidón nativo y modificado, goma guar y λ-carragenato para aumentar la viscosidad y consistencia de batidos lácteos. La caracterización reológica de los productos reveló patrones de comportamiento muy diferentes dependiendo del tipo de hidrocoloide. La adición de la saliva en los estudios demostró modificar las percepciones sensoriales, por lo que su inclusión resulta recomendable y más realista. Se estudió también la adición de hidroxipropilmetilcelulosa (HPMC) y el efecto de dos componentes normales en productos lácteos (sólidos lácteos y nata). Además del efecto dominante del HPMC en la temporalidad de las percepciones, la presencia de glóbulos de grasa o sólidos de la leche modularon dichas percepciones.

Para aumentar el contenido en proteína se seleccionó como alimento el yogur, al que se añadió el doble de la cantidad de proteína de distintas fracciones lácteas y almidón. El yogur con el doble de leche en polvo fue globalmente el más aceptado y más cercano a las características "ideales" según los consumidores, con una serie de atributos sensoriales que contribuyen a la sensación de saciedad esperada. Por otro lado, la digestión oral *in vitro* mostró que los gránulos de almidón procedentes de almidón

físicamente modificado permanecen inalterados después del ataque de la α-amilasa de la saliva, y serían los responsables de la mayor consistencia y cremosidad.

Adicionalmente, se encontró que la sensación de astringencia resultaba negativa; los estudios tribológicos permitieron interpretar las características sensoriales. Las propiedades lubricantes de algunas muestras no reflejaron la diferencia de astringencia sensorial indicando que ésta no era una percepción puramente táctil causada por un aumento en la fricción. La adición de almidón modificado físicamente redujo significativamente los valores de los coeficientes de fricción relacionados con los polímeros de almidón solubles y la conservación de gránulos en el almidón.

Todas las estrategias abordadas permitieron comprender y ahondar en los conocimientos que conducen a cómo reformular un alimento para aumentar la percepción de saciedad. Dichas estrategias son extrapolables a otras categorías de alimentos lo que amplía el alcance de los resultados obtenidos en la presente tesis.

Resum

La present tesi estudia com augmentar les expectatives de la capacitat saciant en productes lactis analitzant l'efecte de la incorporació de determinats ingredients.

L'estudi de la reformulació d'aliments amb alta expectativa sobre la seua capacitat saciant contribueix a moderar l'elecció de les racions d'aliments i a regular la ingesta. En la present tesi s'han estudiat principalment dos dels factors que més influeixen en la percepció de capacitat saciant d'aliments semisòlids: a) l'augment en el temps de processat oral que produeix sacietat a causa d'una prolongació dels senyals orosensorials, i b) l'alt contingut en proteïnes.

L'augment del temps de processat oral el produeixen, per exemple, majors consistències, recobriment oral o sensacions de cremositat, que són característiques que es poden modificar per addició d'hidrocol·loïdes. S'emprà midó natiu i modificat, goma guar i λ-carragenat per augmentar la viscositat i consistència de batuts lactis. La caracterització reològica dels productes revelà patrons de comportament molt diferents depenent del tipus d'hidrocol·loïde. L'addició de la saliva en els estudis demostrà modificar les percepcions sensorials, per la qual cosa la seua inclusió resulta recomanable i més realista. S'estudià també l'addició d'hidroxipropilmetilcel·lulosa (HPMC) i l'efecte de dos components normals en productes lactis (sòlids lactis i nata). A més de l'efecte dominant del HPMC en la temporalitat de les percepcions, la presència de glòbuls de greix o sòlids de la llet van modular les citades percepcions.

Per augmentar el contingut en proteïna es va seleccionar com a aliment el iogurt, a què s'afegí el doble de la quantitat de proteïna de diverses fraccions làcties i midó. El iogurt amb el doble de llet en pols fou globalment el més acceptat i més pròxim a les característiques "ideals" segons els consumidors, amb una sèrie d'atributs sensorials que contribueixen a la sensació de sacietat esperada. D'altra banda, la digestió oral *in vitro* mostrà que els grànuls de midó procedents de midó físicament modificat romanen inalterats després de l'atac de l'α-amilasa de la saliva, i serien els responsables de la major consistència i cremositat.

Addicionalment, es trobà que la sensació d'astringència resultava negativa; els estudis tribològics permeteren interpretar les característiques sensorials. Les propietats lubricants d'algunes mostres no reflectiren la diferència d'astringència sensorial indicant que esta no era una percepció purament tàctil causada per un augment en la fricció. L'addició de midó modificat físicament reduí significativament els valors dels coeficients de fricció relacionats amb els polímers de midó solubles i la conservació de grànuls en el midó.

Totes les estratègies abordades permeteren comprendre i aprofundir en els coneixements que condueixen a com reformular un aliment per augmentar la percepció de sacietat. Les citades estratègies són extrapolables a altres categories d'aliments, cosa que amplia l'abast dels resultats obtinguts en la present tesi.

Abstract

This thesis studies how to increase the expected satiating ability in dairy products analyzing the effect of the incorporation of certain ingredients.

The study of food reformulation with high expected satiating ability contributes to moderate size meal choice and to regulate food intake. In this thesis, two factors influencing the satiating ability perception of semi-solid foods have been mainly studied: a) the increase in oral processing time that produces prolonged orosensory signals, and b) the increase in protein content.

The increase in oral processing time is produced by, for example, higher consistencies, mouth-coating or creaminess sensations. These features can be modified by addition of hydrocolloids. Native starch, modified starch, λ-carrageenan and guar gum were added to increase the viscosity and consistency of milkshakes. Rheological characterization of the products revealed very different behavior patterns depending on the type of hydrocolloid. The addition of saliva in the studies showed modified sensory perceptions, so its inclusion is desirable and more realistic. The addition of hydroxypropyl methylcellulose (HPMC) and the effect of milk solids and cream in milk-based desserts was also studied. In addition to HPMC dominant effect on the temporality of perceptions, the presence of fat globules or milk solids modulated such perceptions.

Yogurt was selected to increase protein content, adding twice the original amount of protein from different milk fractions, and starch. Yogurt with milk powder addition was the most accepted sample and it was closer to the 'ideal' characteristics of a satiating yogurt according to consumers, with a series of sensory attributes that contribute to the feeling of expected satiety. On the other hand, *in vitro* oral digestion showed that physically modified starch granules remain unchanged after the attack of α-amylase, and would be responsible for its consistency and creaminess.

In addition, it was found that the sensation of astringency was undesirable; tribological studies allowed to interpret yogurt sensory characteristics. The lubricity of some

samples did not reflect the difference in astringency between samples indicating that this was not a purely tactile perception caused by an increase in friction. The addition of physically modified starch significantly reduced friction coefficients values due to soluble starch polymers and starch granules preservation.

All the strategies allowed to gain understanding on how to reformulate food increasing its satiating ability perception. Such strategies could be extrapolated to other food categories, what broadens the scope of the results obtained in this thesis.

Introducción

1. Concepto de saciedad

La saciedad es la sensación de plenitud que se produce después de comer y por la cual se suprime la necesidad de volver a comer por un período determinado de tiempo. Esa sensación desempeña un papel importante en el control de cuánta cantidad y cuántas veces se come. Si una persona se encuentra llena o saciada después de una comida, transcurrirá más tiempo antes de volver a sentir hambre, y comerá menos en la próxima comida. Por el contrario, si una persona no se siente muy llena, probablemente tendrá hambre más rápido y tendrá ganas de comer antes o de comer más en la próxima comida. Por lo tanto, aumentar la capacidad saciante de los alimentos podría suponer un método para controlar la ingesta diaria de alimentos y, en última instancia, el peso corporal (Hoad et al., 2004).

Existen dos conceptos englobados en el término saciedad (en inglés se distinguen con términos distintos: "satiation" y "satiety"). Ambos son conceptos importantes para entender el control del apetito. El primero, "satiation", tiene lugar durante una comida y es el que conduce a finalizarla. El segundo, "satiety", comienza al finalizar una comida y evita que el hambre retorne (Bellisle and Tremblay, 2011).

La ingestión de alimentos y bebidas desencadena una sucesión de señales de diversa naturaleza (sensoriales, cognitivas, hormonales y metabólicas) que inhibirán la necesidad de comer. Esta serie de complejas y sucesivas influencias superpuestas se han conceptualizado en un diagrama conocido como la "cascada de la saciedad". La versión original del diagrama fue diseñada por Blundell, Rogers y Hill (1987), pero desde entonces ha sido modificado por muchos autores (Backus, 2006; Blundell, 1991; Halford and Harrold, 2012; Harrold, Dovey, Blundell, and Halford, 2012; Kringelbach, Stein, and van Hartevelt, 2012). La investigación en las últimas décadas ha revelado la contribución y la interacción de muchos factores, por lo que, las modificaciones más recientes se han vuelto más complejas, incluyendo influencias preprandiales y postprandiales. La cascada de la saciedad proporciona un marco conceptual para

entender el impacto que tienen los alimentos sobre la saciedad (Blundell et al., 2010) y muestra los principales factores que influirán en el proceso de la saciedad (Figura 1).

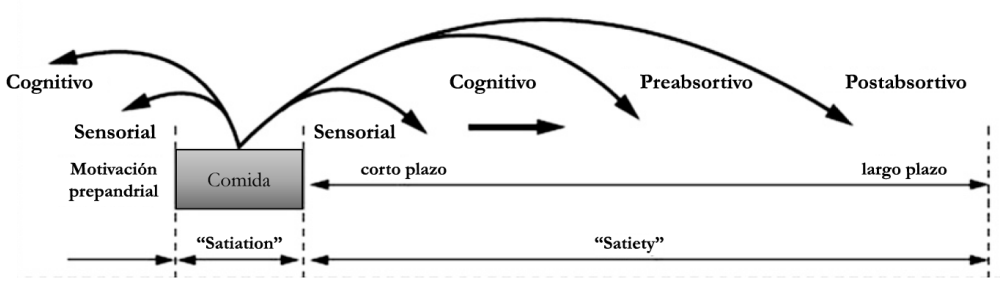

Figura 1. Representación del diagrama conocido como Cascada de la Saciedad (Harrold et al., 2012).

Los macronutrientes que componen los alimentos, su densidad energética, su estructura y los atributos sensoriales, contribuyen a la modulación de la saciedad (Blundell et al., 2010). De hecho, las influencias sensoriales son determinantes en la cantidad de alimentos que ingerimos en una comida. La variedad de señales sensoriales relacionadas con los alimentos (gusto, textura, visión olfativa y señales auditivas) afectan a la palatabilidad y también a la cantidad de comida que se consume. Los estímulos relacionados con la palatabilidad actúan sobre las vías hedónicas en el cerebro, estimulando una respuesta emocional positiva y aumentando el deseo de consumir más alimentos (Berthoud, 2007; Bobroff and Kissileff, 1986). La importancia de los procesos hedónicos (cuyos mecanismos cerebrales se solapan parcialmente con los sistemas responsables del control homeostático) está bien documentada. El placer al ingerir un alimento podría ser tan influyente, o tal vez más que la satisfacción de las necesidades energéticas (Blundell and Bellisle, 2013).

Las influencias cognitivas, las condiciones sociales y las ambientales también afectan de manera significativa al comportamiento a la hora de comer. La saciedad ("satiation") tiene una importante dimensión cognitiva; el riesgo es que la evaluación de estas sensaciones puede interferir con los mecanismos fisiológicos responsables de la

saciedad. Pedir a los sujetos que centren su atención en los alimentos y masticar (Weijzen, Liem, Zandstra, and De Graaf, 2008), o distraer a los sujetos a la hora de comer (Brunstrom and Mitchell, 2006) disminuye e incrementa la ingesta, respectivamente.

Otros parámetros como el tamaño de las porciones (Fisher, Rolls, and Birch, 2003; Young and Nestle, 2002), la falta de sueño (Knutson, 2007; Magee, Iverson, Huang, and Caputi, 2008), la actividad física (De Castro, 1994; Miles, 2007), la televisión y otras distracciones (principalmente en niños) (Manios et al., 2009) y en adolescentes (Van den Bulck and Van Mierlo, 2004) pueden hacer que las personas sean menos sensibles al control del apetito conduciendo a un aumento en la ingesta de energía. Varios estudios han determinado que también hay componentes genéticos involucrados en varios aspectos fisiológicos de la ingesta de alimentos, tales como el llenado y el vaciado gástrico (de Castro, 2004).

Después de acabar de comer, una cascada de influencias inhibe la necesidad de comer durante un período variable de tiempo. Además de las influencias cognitivas y sensoriales, los procesos hormonales y metabólicos previos a la ingesta prolongan el tiempo antes de la siguiente comida. Numerosas manifestaciones hormonales y señales cerebrales relacionadas con la saciedad han sido documentadas en los últimos años (Blundell and Bellisle, 2013).

2. Concepto de saciedad esperada

Las personas aprenden sobre el contenido energético de un alimento mediante la exposición repetida y vinculando las sensaciones postingestivas del apetito con los atributos sensoriales del alimento (Yeomans, Weinberg, and James, 2005). El tamaño de las raciones está controlado en gran medida por una disminución aprendida en el apetito hacia el final de una comida, que se ha definido como saciedad aprendida (Booth and Davis, 1973; Brunstrom, 2007). La saciedad aprendida dará lugar a expectativas

sobre la capacidad saciante que tiene cada alimento. Este efecto esperado que posee un determinado alimento puede jugar un papel importante en las decisiones sobre el tamaño de las porciones (Brunstrom and Rogers, 2009) y, por consiguiente, en el consumo de energía. Aprender sobre el contenido energético de un alimento puede cambiar estos efectos esperados sobre la saciedad (Wilkinson and Brunstrom, 2009) con cambios en el tamaño de las porciones.

Las expectativas sobre el efecto saciante de un alimento específico están basadas, entre otras cosas, en sus atributos sensoriales (Sørensen, Møller, Flint, Martens, and Raben, 2003). Uno de los atributos sensoriales más importante es la textura. Hogenkamp, Stafleu, Mars, Brunstrom y de Graaf (2011) observaron que el aumento en la consistencia de varios productos lácteos resultó en un aumento en la capacidad de saciedad esperada. Esto coincide con los resultados de varios estudios que mostraron que los alimentos sólidos producen mayor saciedad que los alimentos líquidos con el mismo contenido energético (Mattes and Rothacker, 2001). En concordancia con lo expuesto, algunos estudios demostraron de manera consistente que la ingesta *ad libitum* de alimentos sólidos o semisólidos era menor que la de los alimentos líquidos con una composición similar de macronutrientes (Hogenkamp, Mars, Stafleu, and de Graaf, 2010; Zijlstra, Mars, de Wijk, Westerterp-Plantenga, and de Graaf, 2008). La saciedad esperada de un alimento específico también depende del contenido energético del alimento seleccionado. Sin embargo, existen estudios que muestran que las expectativas no cambian cuando se consume repetidamente alimentos de baja o alta densidad energética que son similares en apariencia (Hogenkamp et al., 2011).

3. Procesado oral y percepción sensorial

Durante el procesado oral de los alimentos hay que tener en cuenta dos efectos importantes que afectan a la percepción sensorial: las variaciones individuales y las propiedades de los alimentos. El primero refleja la variación de la fisiología oral en

particular (debido a la edad, sexo, estado de salud, etc.), mientras que el segundo refleja el efecto de las características propias del alimento (como la reología, textura, estructura, sabor, etc.). Ambas variaciones desempeñan un papel importante en cómo un alimento se procesa oralmente y se percibe (Chen, 2009).

La boca es la primera unidad del tracto gastrointestinal, a través de ella la comida entra en el sistema digestivo. En la cavidad oral, la comida se manipula y procesa mecánica y químicamente para permitir su paso a través de la faringe y el esófago hacia el estómago. Este primer paso afectará principalmente a la estructura del alimento y a sus propiedades físico-químicas. A través de la acción mecánica del masticado la matriz alimentaria es desintegrada en partículas de menor tamaño; el mezclado de estas partículas con la saliva permite la formación del bolo y su enfriamiento o calentamiento para alcanzar la temperatura corporal (Turgeon and Rioux, 2011; van Aken, 2010). El tiempo de residencia de la comida en la boca depende de su naturaleza. Los sólidos se desintegran hasta que alcanzan un tamaño lo suficientemente pequeño para que su ingestión sea segura. Los alimentos fluidos tienden a ingerirse de manera rápida, por lo que tienen un proceso y tiempo de mezclado con la saliva mucho menor (van Aken, 2010). La saliva ejerce un efecto de lubricación que facilita la ingestión. Desempeña un papel vital en el procesamiento oral de los alimentos y en el mantenimiento de la salud oral. La saliva puede interactuar también con los componentes de los alimentos, y puede conducir a la formación de nuevas estructuras (Chen, 2009).

La percepción de saciedad se modifica por la exposición sensorial a los propios alimentos (Hogenkamp and Schiöth, 2013). La textura ha sido aislada como un componente sensorial del alimento que desempeña un papel clave en la saciedad (Chambers, McCrickerd, and Yeomans, 2015; McCrickerd and Forde, 2016), por lo tanto, puede servir como una señal predictiva fiable (Davidson and Swithers, 2005). Señales como la cremosidad, por ejemplo, se asocian con alimentos ricos en nutrientes (Bertenshaw, Lluch, and Yeomans, 2008, 2013) y por lo tanto, es normal que una persona relacione esta sensación con la idea de que un alimento muy cremoso lo saciará.

Esto apoyaría la idea de que las sensaciones orosensoriales como el sabor y la textura actúan dirigiendo el comportamiento alimentario para asegurar el consumo eficiente de alimentos ricos en nutrientes (McCrickerd, Lensing, and Yeomans, 2015; Woods, 2009). Esta es la razón por la que exposiciones orosensoriales más largas contribuyen al desarrollo de la saciedad a través del desencadenamiento de respuestas anticipadas relacionadas con las asociaciones aprendidas entre las características sensoriales de un alimento y sus consecuencias metabólicas (Yeomans et al., 2005) y muy probablemente estas asociaciones influyan en las expectativas explícitas sobre el efecto que un alimento tendrá sobre el apetito (Blundell et al., 2010; Brunstrom, Shakeshaft, and Scott-Samuel, 2008).

Debido a su naturaleza fluida, las bebidas requieren menos tiempo de procesado oral que equivalentes calóricos sólidos, minimizando la exposición orosensorial (de Wijk, Zijlstra, Mars, de Graaf, and Prinz, 2008; Tieken et al., 2007; Zijlstra et al., 2009). Según McCrickerd, Chambers, Brunstrom y Yeomans (2012) el procesado mecánico de los alimentos en la boca y la masticación están estrechamente relacionados con los procesos cognitivos relacionados con la saciedad (Forde, Van Kuijk, Thaler, De Graaf, and Martin, 2013), con las respuestas de la fase cefálica preparatoria y con la liberación de hormonas relacionadas con el apetito (Li et al., 2011). Un aumento en la exposición orosensorial puede resultar en un aumento en la detección de energía-nutriente, y un intervalo de tiempo más largo para que las señales de saciedad lleguen al cerebro.

4. Ingredientes asociados a la saciedad

De lo expuesto se deduce, que la sensación de saciedad, no está sólo basada en la cantidad de alimentos ingeridos, sino también en su composición, estructura y propiedades físicas y sensoriales.

Existe una amplia variedad de componentes o ingredientes capaces de incrementar la saciedad fisiológica, es decir, estimular la respuesta hormonal que tiene lugar al ingerir

el alimento, y por lo tanto aumentar la saciedad. No obstante, el desarrollo de productos incorporando estos ingredientes puede presentar dificultades para las industrias de alimentos: pueden surgir problemas tecnológicos, los costes de demostrar la eficacia en humanos son elevados y la legislación y etiquetado pueden resultar complejos (Paeschke and Aimutis, 2011).

Las fibras y las proteínas (véase artículo de revisión) son reconocidas como los nutrientes con mayor potencial para el desarrollo de alimentos saciantes (Halford and Harrold, 2012).

4.1. Hidratos de carbono

4.1.1 Fibra alimentaria

La fibra alimentaria tiene una larga historia de definiciones. Una de las más completas y recientes es la de la Comisión de la Unión Europea (2008): polímeros de carbohidrato con tres o más unidades monoméricas, que no son digeridos ni absorbidos en el intestino delgado humano y que pertenecen a las siguientes categorías: 1) polímeros de carbohidrato comestibles, presentes naturalmente en los alimentos tal y como se consumen; 2) polímeros de carbohidratos comestibles que han sido obtenidos de alimentos crudos por medios físicos, enzimáticos o químicos y que tienen un efecto fisiológico beneficioso demostrado por evidencia científica generalmente aceptada y 3) polímeros de carbohidratos sintéticos comestibles que tienen un efecto fisiológico beneficioso demostrado por una evidencia científica generalmente aceptada (Fiszman and Varela, 2013).

Se pueden distinguir dos tipos de fibra alimentaria:

a) insoluble: integrada principalmente por hemicelulosa, celulosa y lignina. Estas sustancias, apenas sufren procesos fermentativos o de hidrólisis por las bacterias en el colon, no son capaces de disolverse en agua, pero sí son capaces de retener el agua en

su matriz estructural formando mezclas de baja viscosidad; esto produce un aumento de la masa fecal que acelera el tránsito intestinal. Actúan de diferentes formas con respecto a la saciedad: a) incrementan el tiempo de masticación; b) actúan como material de relleno aportando volumen (con baja densidad calórica) aunque suelen tener una palatabilidad no muy alta; c) atrapan nutrientes en el intestino delgado, liberándolos más lentamente. Estas fibras se encuentran en todos los granos integrales, salvado de trigo y algunos vegetales (Fiszman and Varela, 2013).

b) soluble: integrada principalmente por pectinas, gomas y mucílagos, inulina, fructooligosacáridos y algunas hemicelulosas. Estas sustancias son solubles en agua y en contacto con la misma forman un retículo donde la atrapada, originándose así soluciones de gran viscosidad. Estas fibras son fermentables por los microorganismos del intestino grueso, produciéndose gas y ácidos grasos de cadena corta que el organismo puede absorber en pequeñas cantidades. Además de por la viscosidad que proporcionan estas sustancias, esta fermentación también puede contribuir a la saciedad ya que los ácidos grasos de cadena corta podrían influenciar la respuesta de determinadas hormonas relacionadas con la saciedad (Fiszman and Varela, 2013).

Las fibras han sido estudiadas como medio para proporcionar saciedad y retrasar la absorción de glucosa durante más de 30 años (Kay and Stitt, 1978; Wilmshurst and Crawley, 1980). Los efectos que tienen en la sensación de saciedad muchas de las fibras hidrosolubles de alto peso molecular que imparten viscosidad a sus disoluciones son causados por mecanismos relacionados con la ralentización de la actividad enzimática, distensión de la cavidad gástrica y retraso del vaciado gástrico, lo cual puede incrementar o prolongar las señales de saciedad del estómago (Fiszman and Varela, 2013).

Aunque formular alimentos con fibra alimentaria con el fin de aumentar la saciedad no es nuevo, no existen muchos productos en el mercado con estas características. Esto se debe fundamentalmente a que, para conseguir esta sensación de saciedad, se han de usar concentraciones altas de estos compuestos, lo que va en detrimento de las características sensoriales del producto, y de sus propiedades durante el procesado. Además, en el caso de

productos líquidos, estos altos niveles suponen una consistencia que dificulta la ingestión. Por otro lado, si se usan en productos sólidos, intentarán hidratarse mientras se mastican e ingieren produciendo un bolo espeso y pegajoso (Paeschke and Aimutis, 2011).

En cuanto a las fibras que se suelen emplear para obtener una mayor sensación de saciedad en la formulación de alimentos, se emplean básicamente dos tipos con propiedades distintas: las neutras, como la goma de guar, la goma de garrofín, la goma xantana, o la hidroxipropilmetilcelulosa que desarrollan viscosidad por el mecanismo de entramado molecular; y los cargados, como alginatos, pectinas o carragenatos, que pueden desarrollar una viscosidad adicional mediante asociación con iones mono y divalentes.

A continuación, se enumeran las fórmulas químicas de los polisacáridos que se han usado en la presente tesis doctoral (Figuras 2, 3 y 4):

Goma guar

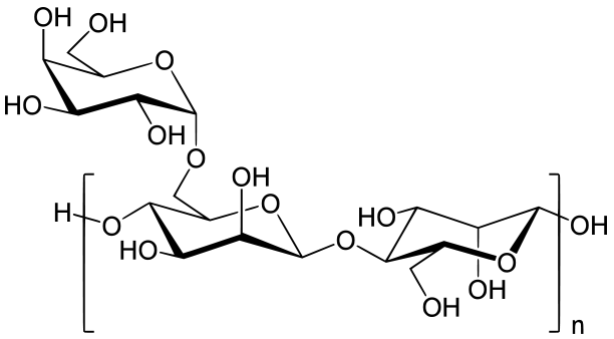

Figura 2. Fórmula estructural de una cadena de goma guar.

Carragenatos

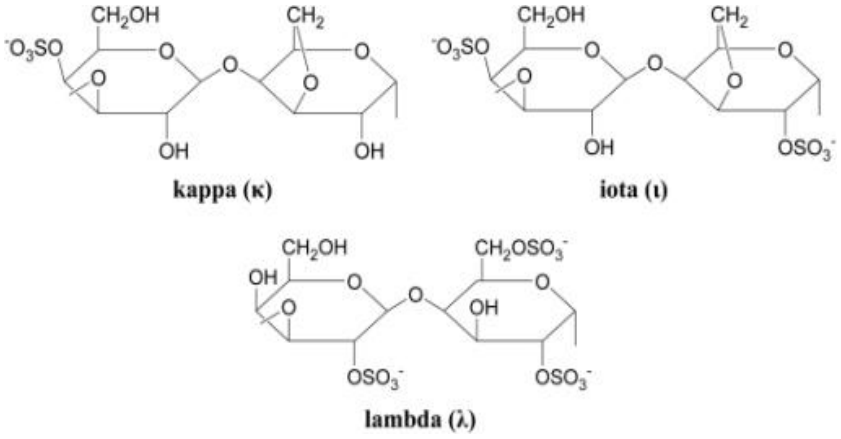

Figura 3. Estructura idealizada de las unidades de carragenatos de los tipos ϰ, ι y λ (Damodaran, Parkin, and Fennema, 2010).

Hidroxipropilmetilcelulosa (HPMC)

La HPMC es una modificación sintética del polímero natural (la celulosa) (Burdock, 2007).

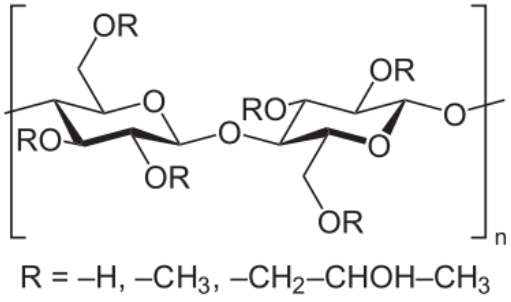

Figura 4. Estructura de la hidroxipropilmetilcelulosa.

4.1.2. Almidón

4.1.2.1. Almidón modificado químicamente

La modificación química se lleva a cabo introduciendo determinados grupos funcionales en la molécula de polímero del gránulo de almidón en su forma nativa que producen cambios en sus propiedades fisicoquímicas. Estas modificaciones producen cambios en la gelatinización y en la retrogradación. Además, ayuda en la estabilización de enlaces intra e intermoleculares en diferentes posiciones y localizaciones. Factores tales como el origen del almidón, las condiciones de la reacción, el grado de sustitución, el tipo y la distribución del agente sustitutivo a lo largo de la molécula afectan las propiedades funcionales y químicas de los almidones modificados. La modificación química de almidones se realiza generalmente mediante acetilación, hidrólisis ácida y oxidación. Sin embargo, ha habido una tendencia creciente a combinar diferentes tipos de técnicas químicas para crear nuevos tipos de modificación (Din, Xiong, and Fei, 2015).

En uno de los trabajos de esta tesis se emplea específicamente dialmidón hidroxipropilado de alto entrecruzamiento, es decir, un almidón con dos modificaciones químicas. Estas modificaciones hacen que el almidón tenga mayor resistencia al cizallamiento, estabilidad, resistencia a la congelación-descongelación, y transparencia y, aporta mayor viscosidad con baja tendencia a retrogradarse.

4.1.2.2. Almidón físicamente modificado

La tendencia actual hacia los alimentos naturales y el aumento de la preocupación de los consumidores han llevado a una clara preferencia por los etiquetados "limpios" (Arocas, Sanz, and Fiszman, 2009) mediante modificaciones físicas (Singh, Kaur, and McCarthy, 2007). La modificación física es simple, económica y segura porque no requiere la utilización de agentes químicos o biológicos (Ashogbon and Akintayo, 2014; Kaur,

Ariffin, Bhat, and Karim, 2012) y permite una etiqueta sin aditivos añadidos que actualmente es un valor añadido para el mercado.

La modificación física implica el tratamiento de gránulos de almidón nativo con diferentes combinaciones de temperatura/humedad, presión y cizallamiento (Che et al., 2009; Che, Li, Wang, Dong Chen, and Mao, 2007; Huang, Lu, Li, and Tong, 2007; Lewandowicz and Soral-Śmietana, 2004; Lim, Han, Lim, and BeMiller, 2002; Zoulikha Maache-Rezzoug et al., 2009; Nemtanu and Minea, 2006; Pinto et al., 2012; Szymońska, Krok, Komorowska-Czepirska, and Rebilas, 2003; Zarguili, Maache-Rezzoug, Loisel, and Doublier, 2006). La modificación física también incluye el desgaste mecánico para cambiar el tamaño de los gránulos de almidón mediante fricción, colisión, impacto, cizallamiento y otras acciones mecánicas para alterar las estructuras cristalinas y las propiedades del gránulo de almidón (He et al., 2014; Huang et al., 2007). Estos procesos disminuyen la temperatura, la entalpía de la gelatinización y la viscosidad aparente de las muestras tratadas, e incrementa su solubilidad en agua fría (Che et al., 2007; Huang et al., 2007).

Las modificaciones que destruyen la integridad molecular abarcan todos los procesos de pregelatinización (secado en tambor, secado por pulverización y cocción por extrusión) donde se pierde parcialmente el empaquetamiento de las cadenas en el gránulo de almidón junto a la despolimerización parcial de los componentes del almidón (Ashogbon and Akintayo, 2014). Además, existen numerosos tratamientos físicos que conducen a nuevas propiedades funcionales del almidón que se deben a la modificación de la estructura cristalina del gránulo conservándose su integridad. Entre ellos, los procesos más investigados son la hibridación (Lewandowicz, Jankowski, and Fornal, 2000; Maache-Rezzoug and Allaf, 1999) y los tratamientos de calor-humedad (Gonzalez and Perez, 2002; Szymońska et al., 2003), procesos en los que se pueden emplear una gran variedad de condiciones (Haghayegh and Schoenlechner, 2011). Recientemente se han investigado numerosos nuevos métodos de modificación física: almidón sobrecalentado (Steeneken and Woortman, 2009); calentamiento en seco (Lim et al.,

2002); tratamiento mediante presión osmótica (Pukkahuta, Shobsngob, and Varavinit, 2007); congelación y descongelación profunda múltiple (Szymońska et al., 2003); proceso de caída de presión instantánea controlada (Maache-Rezzoug et al., 2009; Zarguili et al., 2006); activación mecánica con molino de bolas de agitación (Huang et al., 2007); micronización en molino de bolas al vacío (Che et al., 2007); tratamiento con campos eléctricos pulsados (Han, Zeng, Zhang, and Yu, 2009); y descargas eléctricas (Nemtanu and Minea, 2006). La aplicación de almidón físicamente modificado al desarrollo de nuevos alimentos sugiere un futuro prometedor para la industria alimentaria (Haghayegh and Schoenlechner, 2011).

Con el fin de aunar y discutir la información previa necesaria al desarrollo de la presente tesis, se elaboraron dos trabajos de revisión que se exponen a continuación.

El primer artículo revisa los principales trabajos que estudian la capacidad saciante que proporcionan las proteínas, tanto a corto como a largo plazo. Además, revisa de manera exhaustiva los mecanismos fisiológicos, cognitivos y sensoriales mediante los cuáles las proteínas ejercen su efecto saciante. La revisión permite conocer cómo contribuyen las proteínas a las propiedades físicas y sensoriales de los alimentos y cómo se integran en la matriz alimentaria. Proporciona herramientas adecuadas para diseñar alimentos saciantes con alta cantidad de proteínas teniendo en cuenta la satisfacción del consumidor y su percepción de saciedad. El artículo de revisión fue publicado con el título "Revisiting the role of protein-induced satiation and satiety" en la revista Food Hydrocolloids.

El segundo artículo de revisión repasa las principales técnicas *in vitro* que se emplean actualmente para simular el procesado oral de los alimentos y la digestión incipiente en boca mediante la saliva. Se abarcaron fuentes multidisciplinares (odontología, fisiología, foniatría, medicina, nutrición, tecnología de alimentos, análisis sensorial, etc.) estudiando y clasificando las últimas investigaciones sobre la percepción de la textura como consecuencia de una "trayectoria oral": es decir, fases consecutivas de reducción de tamaño de partícula, insalivación y formación de un bolo con características reológicas y extensionales adecuadas para tragar de modo confortable y seguro. Se revisaron conocimientos de todas las áreas que ahondan en las percepciones en boca durante el consumo del alimento y que pueden estar relacionadas con la generación de sensaciones de saciedad anticipada. Fue publicado con el título "Understanding the relevance of in-mouth food processing. A review of *in vitro* techniques" en la revista Trends in Food Science & Technology.

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Revisiting the role of protein-induced satiation and satiety

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Abstract

'Satiation' and 'satiety' are key terms that have come to be widely used to help understand processes involved in appetite control. Satiation is considered to be the signals or processes that bring a meal to an end, whereas satiety is the signals or processes, following the end of a meal, that inhibit eating before hunger returns. Protein is the most effective food macronutrient providing a satiating effect. Thus, formulating foods with increased protein contents can help to modulate food intake, promoting body weight loss and body weight maintenance thereafter. Mechanisms explaining protein-induced satiety are primarily nutrient-specific, but they are of course not mathematically related to satiety. Different proteins cause different nutrient-related responses of anorexigenic hormones. Glucagon-like peptide-1 (GLP-1) release evoked by a high protein meal is stimulated by the carbohydrate content. Also, cholecystokinin (CCK) and peptide YY (PYY) release is stimulated by a high-protein meal. Sensory, cognitive, post-ingestive and post-absorptive signals will determine jointly the feeling of satiation and satiety. Oral perception cues also contribute increased expectations of satiating capacity when the oral residence time and in-mouth handling are longer and more laborious. In the present review, the authors want to obtain an overview of the satiating ability of dietary protein and its role in satiation and satiety. This could be really significant in showing the food industry the path for developing protein-rich satiating foods in response to consumer demand.

Keywords: high protein diet, gluconeogenesis, amino acids, energy expenditure, hormones, oral exposure.

1. Introduction

The global obesity epidemic is an issue that commands the resources of many public health organizations and demands the input of health professionals and scientists to develop new approaches to prevent and treat it. It also invites researchers to revisit some previously accepted effects of macronutrients, specifically that of protein on energy balance, to determine if greater than expected benefits might be obtained from diet manipulations (Arentson-Lantz, Clairmont, Paddon-Jones, Tremblay, and Elango, 2015).

Many studies have investigated the effects of protein on satiety (Figure 1) and satiation, and most but not all have found that, at sufficiently high levels, protein has a stronger effect than equivalent quantities of energy from either carbohydrate or fat (Blundell, Lawton, Cotton, and Macdiarmid, 1996; Holt, Brand Miller, Petocz, and Farmakalidis, 1995; Veldhorst, et al., 2008; Veldhorst, et al., 2009a, 2009b, 2009c; Weigle, et al., 2005). Introducing the concepts of satiation, satiety and the factors that influence them through the "satiety cascade" lie outside the scope of the present review. Protein has taken centre stage as the highest satiety food constituent because of considerable research indicating that increasing the protein composition of the diet without changing the net energy load can lead to enhanced feelings of satiety (Paddon-Jones, et al., 2008).

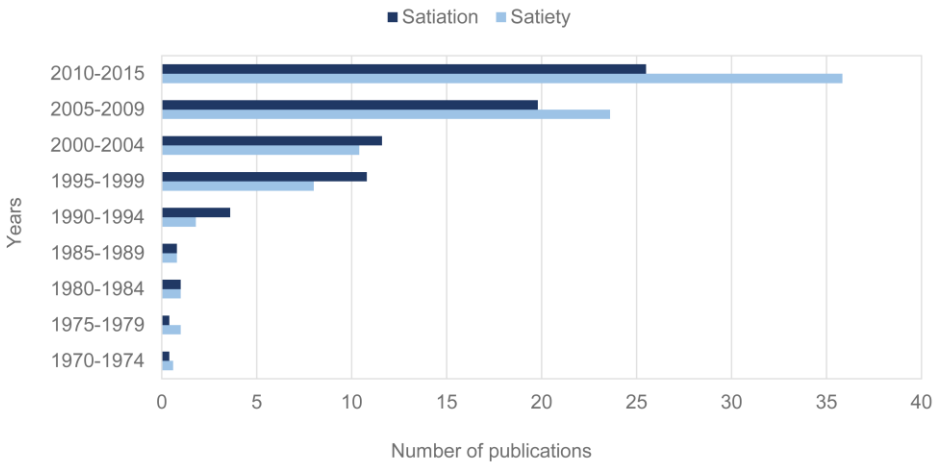

Figure 1. Evolution of the number of publications with satiation "in the abstract, title or key words" since 1970 (data from Scopus)

Protein is an indispensable nutrient; its ingestion as a source of amino acids is necessary for almost all biological processes. Accordingly, food intake is sensitive to protein and the response to the protein content of meals and diets is controlled at different levels, from peripheral organs to the brain. Protein intake induces complex signals including neuropeptides secreted in the gut, metabolic hormones and blood amino acids, as well as derived metabolites released in the blood (Journel, Chaumontet, Darcel, Fromentin, and Tomé, 2012). The mechanisms described for protein-induced satiety are 1) increases in concentrations of 'satiety' hormones, 2) increases in energy expenditure, 3) increases in concentrations of amino acids, and 4) the process of gluconeogenesis (Veldhorst, et al., 2008).

Knowing why to select the food constituents, what they contribute to the food's physical and sensory properties and how they are integrated into the food matrix will provide suitable tools for designing high-protein satiating foods (Morell, Hernando,

Llorca, and Fiszman, 2015a). An additional value is that this design would have direct implications for the consumer's satisfaction and the perception of satiety. It is difficult to reformulate food products to enhance their satiating capacities because the constituents can themselves influence energy density, palatability, texture and a number of other factors involved in feeding behaviour (Fiszman and Varela, 2013). Some of the effects overlap, to an uncertain degree, emphasizing the need for integrative multidisciplinary research. In recent years, the food market has seen a rise in the sale of enhanced satiety products (categorically different to reduced-energy diet foods), which claim to be effective in staving off hunger and seem to be well received by the public (Bilman, van Kleef, Mela, Hulshof, and van Trijp, 2012; Chambers, McCrickerd, and Yeomans, 2015; Hetherington and Havermans, 2013).

Human eating is a complex and varied behaviour (Harrold, Dovey, Blundell, and Halford, 2012). A radical change in diet cannot and should not be recommended, but a feasible lifestyle adaptation to a moderately higher-protein, higher-fibre, controlled energy diet should be possible (Hill, 2006).

2. Physiological aspects of protein satiating ability

2.1. Protein-related hormones

Both short- and long-term signals of appetite control act directly through receptors in the brain or indirectly via the nervous system on areas of the brain involved in appetite control pathways (Hillebrand, De Wied, and Adan, 2002; Morris and Hansen, 2009). The pathways can broadly be divided into anorexigenic (inhibit feeding) and orexigenic (stimulate feeding) pathways. Each pathway can be both stimulated and inhibited by signals from the gut, pancreas and adipose tissue.

Protein-induced satiety coincides with a relatively high increase in concentrations of anorexigenic hormones or a larger decrease in orexigenic hormones (Batterham, et al.,

2006; Hall, Millward, Long, and Morgan, 2003; Lejeune, Westerterp, Adam, Luscombe-Marsh, and Westerterp-Plantenga, 2006; Smeets, Soenen, Luscombe-Marsh, Ueland, and Westerterp-Plantenga, 2008; Veldhorst, et al., 2008).

Satiation involves gastric distension and gastrointestinal peptide release (Cummings and Overduin, 2007). Gastric distension could be used as a biomarker of satiation (De Graaf, Blom, Smeets, Stafleu, and Hendriks, 2004). It is sensed by mechanoreceptor neurons in the stomach and relayed to the hindbrain via vagal afferent and spinal sensory nerves (Ritter, 2004). The gut hormone CKK appears to be involved in satiation (Wren and Bloom, 2007). GLP-1 is a potential biomarker for satiety that can be measured from blood samples and can be seen to rise for two hours after a meal (De Graaf, et al., 2004). There is some evidence that a high protein meal in combination with carbohydrate stimulates GLP-1 release (Smeets, et al., 2008), yet this also depends on the carbohydrate content. Batterham, et al. (2003) observed significantly higher plasma PYY responses to a high protein meal, reaching a plateau after 1–2 hours and remaining high for approximately six hours. Similarly to CCK, GLP-1 also acts as a short-term regulator of feeding behaviour. The endogenous release of GLP-1 after a meal has been shown to reduce meal size and also to increase the time to the next meal, thus affecting both satiation and satiety (Williams, Baskin, and Schwartz, 2009). Leptin has also been suggested as a biomarker for satiety (De Graaf, et al., 2004). However, since high leptin levels do not appear to increase satiety reliably it cannot be assumed that changes in leptin will cause a related change in appetite.

The physiological systems underlying the control of satiation and satiety involve associations between peripheral physiology (stomach emptying and gastrointestinal peptides) and metabolism (glucose homeostasis and adiposity), which in turn are linked to various brain processes (Blundell and Bellisle, 2013).

2.2. Energy expenditure via protein-induced thermogenesis

One of the mechanisms that classically have been suggested to explain the satiating power of protein is energy expenditure. Daily energy expenditure consists of three components: basal metabolic rate, diet-induced thermogenesis and the energy cost of physical activity (Westerterp-Plantenga, 2004). A relationship between energy expenditure and protein-induced satiety has mainly appeared in relation to a high protein diet, and to a lesser extent after a single high protein meal. The theoretical basis of this relationship may be that increased energy expenditure at rest implies increased oxygen consumption and an increase in body temperature that may lead to feeling deprived of oxygen and thus promote satiety (Westerterp-Plantenga, Rolland, Wilson, and Westerterp, 1999; Westerterp-Plantenga, Lemmens, and Westerterp, 2012).

It has been known for many years that the ingestion of dietary proteins stimulates energy expenditure in the postprandial period immediately after meal ingestion.

The thermic effect of a food is the increase in energy expenditure above baseline following consumption. It can be defined further as the energy required for digestion, absorption, and disposal of ingested nutrients (Halton and Hu, 2004). Certainly, the typical thermic effect of protein is 20%–35% of energy consumed, while for carbohydrate it is usually between 5% and 15% less (Westerterp-Plantenga, et al., 1999). These values have found support and been confirmed for protein and carbohydrate/ glucose in several human clinical trials (Acheson, et al., 2011). Protein not only increases energy expenditure (Halton, et al., 2004; Johnstone, et al., 2002) but also decreases energy intake through mechanisms that influence appetite control (Anderson, Tecimer, Shah, and Zafar, 2004b; Lejeune, Kovacs, and Westerterp-Plantenga, 2005; Nickols-Richardson, Coleman, Volpe, and Hosig, 2005; Schoeller and Buchholz, 2005; Weigle, et al., 2005). Westerterp-Plantenga, et al., (2012) stated that from combining the studies on protein intake, it can be concluded that protein intake causes an acute increase in diet-induced energy expenditure and, when sustained over three days, results in an increase in the sleeping metabolic rate.

An interesting theory concerning thermogenesis as it relates to protein intake is the Stock Hypothesis (Stock, 1999). This author cited 12 human overfeeding studies to support the view that diets high in protein increase thermogenesis in an effort to homeostatically waste energy when fed an unbalanced diet. This would make sense from an evolutionary perspective, in that such an increase in thermogenesis would help ensure an adequate supply of nutrients while avoiding the risks to survival associated with excess weight gain. An area of research worth mentioning concerns the theory that the obese have a blunted thermic effect in general and in relation to fat in particular (Granata and Brandon, 2002).

In fifteen studies concerning thermogenesis reviewed by Halton, et al., (2004), the thermic effect of food was measured in a variety of ways. The results suggested that high-protein diets exert a greater effect on energy expenditure than low-protein ones. One limitation was that the numbers might be underestimated, as most studies were conducted for a period of only 6-7 h and many believe that the thermic effect of protein continues for longer.

One important reason for the difference in the thermic effects of food may be the fact that the body has no protein storage capacity, so it requires immediate metabolic processing. Protein synthesis, the high ATP cost of peptide bond synthesis and the high cost of urea production and gluconeogenesis are often cited as reasons for the higher thermic effect of protein (Mikkelsen, Toubro, and Astrup, 2000). Energy expenditure is not clearly dependent on the protein source, although there is some evidence that animal proteins produce higher energy expenditure than vegetable proteins, resulting in reduced appetite (Westerterp-Plantenga, 2003).

2.3. Amino acids

Dietary protein and amino acids, including glutamate, generate signals involved in the control of gastric and intestinal motility, pancreatic secretion, and food intake. The

signals include postprandial meal-induced visceral and metabolic signals and associated nutrients, gut neuropeptides, and hormonal signals. Protein reduces gastric motility and stimulates pancreatic secretions (Blundell, et al., 2013).

The amino acid composition of the protein is a determinant of the metabolic efficacy of protein oxidation (hence, heat production) because there are large differences in the efficacy with which amino acids are oxidized. This is due to the wide variety of carbon chains and co-factors that result from amino acid catabolism (Boirie, et al., 1997; Dangin, et al., 2001; Dangin, Boirie, Guillet, and Beaufrère, 2002; Van Milgen, 2002).

Both the quality and type of protein appear to be involved in hunger suppression. Protein quality is mainly determined by the amino acid composition. Some proteins are considered 'incomplete' or 'lower quality' proteins because they are lacking one or more essential amino acids or have an inadequate balance of them. High protein diets with proteins that predominantly consist of ketogenic amino acids may result in increased plasma ketone body concentrations, which in turn contribute to increased satiety (there are seven ketogenic amino acids; exclusively ketogenic: leucine and lysine; ketogenic and glucogenic: tyrosine, threonine, isoleucine, phenylalanine and tryptophan) (Westerterp-Plantenga, et al., 2012).

Metabolites, including certain amino acids, contribute to the perception of postprandial satiety. As early as in 1956, Mellinkoff suggested a relationship between serum amino acid concentration and fluctuations in appetite. This theory is termed the aminostatic hypothesis. Whether induced by feeding protein or amino acids, or by infusing amino acid mixtures, a rise in the serum amino acid concentration appeared to be accompanied by a waning of appetite. The subsequent increase in appetite was accompanied by a fall in the amino acid concentration. This author has also suggested that a high concentration of blood or plasma amino acids that cannot be channelled into protein synthesis may serve as a satiety signal for a food intake regulating mechanism and thereby result in depressed food intake. More recently, it has been proposed that protein- and amino acid-induced satiety could be associated with the branch-chain

amino acids found in complete proteins, which could be involved in the regulation of amino acid oxidation and gluconeogenesis (Aldrich, et al., 2011). In addition, the good balance of indispensable amino acids usually obtained from dietary protein is sensed by the protein synthesizing machinery in specialized cells in the brain (Fromentin, et al., 2012; Gietzen and Aja, 2012) which influences subsequent feeding-related responses.

Until now hardly any clear differences in satiating properties between different protein types have been shown, mainly due to the design of the studies, which have not used just one single protein. An important issue that should also be taken into account is timing, due to marked differences in protein metabolism kinetics. In their review on whey proteins in the regulation of food intake and satiety, Luhovyy, Akhavan, and Anderson (2007) showed that the satiating power of a high protein meal may be used optimally when the timing of the meal interval synchronizes with timing of the amino acid profiles. The speed of absorption of dietary amino acids by the gut varies according to the type of dietary protein ingested, and since amino acids are potent modulators of protein synthesis, breakdown and oxidation, different patterns of postprandial aminoacidemia might well be found to influence satiety or satiation. Dietary carbohydrates are commonly classified as slow or fast because it is now well recognized that their structure affects their speed of absorption, which in turn has a major impact on the metabolic and hormonal response to a meal. However, less is known about how postprandial protein kinetics could be affected by the speed of absorption of dietary amino acids. The latter is very variable, depending on gastric and intestinal motility, luminal digestion, and finally mucosal absorption. This concept of slow and fast proteins could be applied to distinguishing different kinetic types of proteins (Boirie, et al., 1997).

2.4. Gluconeogenesis

Finally, the mechanism of gluconeogenesis has been mentioned as contributing to satiety in relation to protein, or better, to food intake regulation, at least in the animal model.

Azzout-Marniche, et al., (2007) suggested that hepatic gluconeogenesis is stimulated by a high-protein diet in rats. As gluco-receptors are able to send a satiety signal to the brain via the vagal nerve, stimulation of gluconeogenesis could be involved in the satiating effect of protein through the modulation of glucose signalling to the brain (Melanson, Westerterp-Plantenga, Saris, Smith, and Campfield, 1999; Westerterp-Plantenga, Lejeune, Smeets, and Luscombe-Marsh, 2009). The study by Azzout-Marniche, et al. (2007) also showed that a diet with a high protein content and without any carbohydrates did not stimulate gluconeogenesis sufficiently to signal satiety. Therefore, mechanisms other than amino acid-induced gluconeogenesis are involved in protein-induced satiety.

Duraffourd, et al., (2012) showed that μ-opioid receptors present in nerves in the portal vein walls respond to peptides to regulate a gut-brain neural circuit that controls intestinal gluconeogenesis and satiety in rats. Moreover, transient blood glucose declines have been shown to be related to the signal of meal initiation (Melanson, et al., 1999). Thus, an amino acid-induced gluconeogenesis may prevent a decrease in glycaemia and thereby contribute to satiety. Gluconeogenesis was increased and appetite was lower when healthy human subjects consumed a high-protein diet in comparison with the consumption of a normal-protein diet. Although appetite was strongly suppressed by the high-protein diet, there was no correlation between gluconeogenesis and appetite ratings. Gluconeogenesis in humans is thought to remain relatively stable in varying metabolic conditions (Nuttall, Ngo, and Gannon, 2008).

Veldhorst, Westerterp, Van Vught, and Westerterp-Plantenga (2010) showed that increased concentrations of β-hydroxybutyrate and increased dietary fat oxidation

contribute to the appetite suppression effect of a high-protein diet. Several studies have shown increased concentrations of β-hydroxybutyrate coinciding with reduced appetite in human subjects (Johnstone, Horgan, Murison, Bremner, and Lobley, 2008; Veldhorst, Westerterp, and Westerterp-Plantenga, 2012), thus potentially contributing to appetite suppression.

Although the mechanistic basis of protein-induced satiety is still unknown it is likely to involve several complementary routes (Figure 2) that can be altered by diet composition; the challenge will be to identify which conditions promote satiety (Johnstone, 2013).

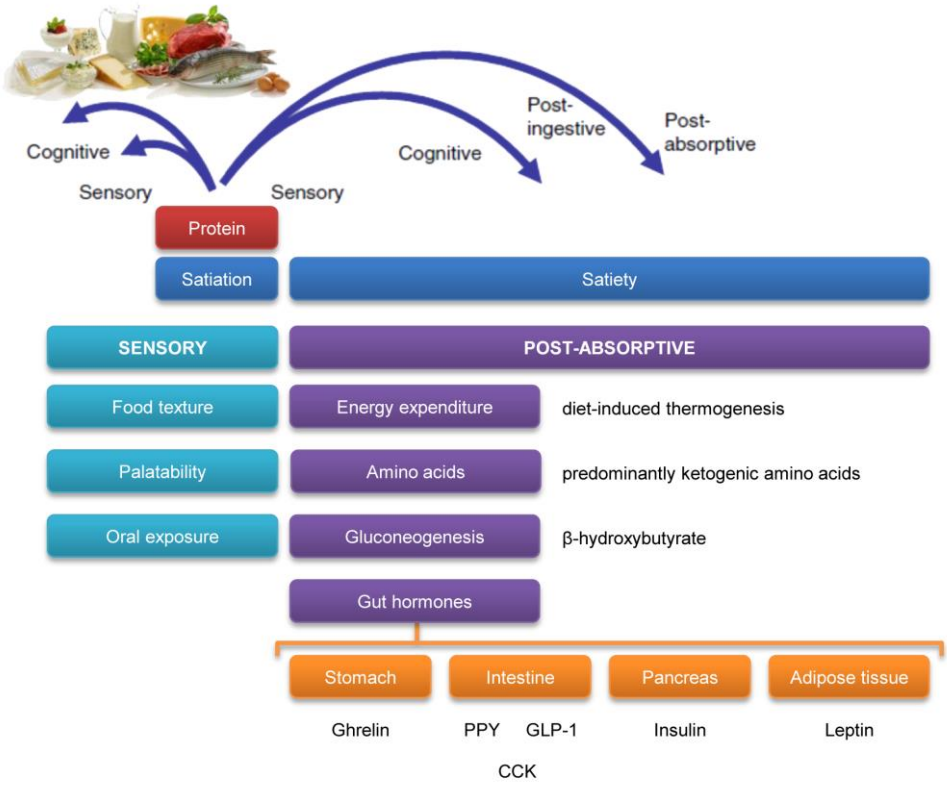

Figure 2. Schematic view of factors related to protein influence on satiation and satiety. PPY: peptide YY; GLP-1: glucagon-like peptide-1; CCK: cholecystokinin.

3. High protein diets

There is currently no formal definition of 'high protein' as a percentage of energy in a meal or diet. The composition of the diet can be determined as the absolute amount of protein (grams), the % of total energy as protein or the amount of protein ingested per kg body weight (Johnstone, Gonzalez, and Harrold, 2012).

In a review of the safety and efficacy of high protein diets, Eisenstein, Roberts, Dallal, and Saltzman (2002) suggested that protein intakes higher than 25% energy should be defined as 'high' and over 35% energy as 'extremely high' based on the US dietary recommended intakes which give 10–35% as the acceptable range of protein intake. In the context of research on prevention and treatment of overweight and obesity, relatively high-protein diets have come into focus as having the potential to act on the different metabolic targets regulating body weight (Westerterp-Plantenga, et al., 2006) and thereby providing the required conditions for successful weight maintenance after weight loss (Veldhorst, et al., 2008). High-protein diets reported in weight loss studies often include ~30% of energy intake as protein. In general, protein as a percentage of energy is doubled from 15% to 30% (Halton, et al., 2004). There are many variants, such as the Dukan diet (Dukan, 2010), the Zone diet (Gardner, et al., 2007) and the CSIRO diet (Wyld, Harrison, and Noakes, 2010). Both the safety (St. Jeor, et al., 2001) and efficacy of high protein diets have been questioned, particularly in combination with low-carbohydrate advice (Astrup, 2005). Low-carbohydrate diets are described as ketogenic diets since ketone bodies such as β-hydroxybutyrate and acetoacetate are produced by the liver, replacing glucose as an energy source for the brain. One example of such a low-carbohydrate diet is the Atkins' diet (Johnstone, et al., 2012).

The consumption of a high protein diet induces an immediate strong depression in food intake followed by a progressive but incomplete return to the level of energy intake of the control diet in animals (Fromentin, et al., 2012; Jean, et al., 2001).

The commonly poor palatability of high-protein diets has been documented (McArthur, Kelly, Gietzen, and Rogers, 1993), but with respect to protein intake its relative importance remains unclear. It is possible that the appetite-suppressing effect of dietary protein is partially induced by poor palatability. Taken together, experiments indicate that the overall behavioural response more probably originated in an initial lower palatability of the food combined with an enhanced satiety effect of the high-protein diet and a delay required for metabolic adaptation (Fromentin, et al., 2012).

In the laboratory, the satiating effects of high protein foods or meals have been compared to iso-energetic lower protein counterparts, typically using a "preload" methodology where the measure of satiety is subjective post-consumption ratings of appetite and/ or food intake. The majority of these types of studies indicate that high protein foods deliver better satiety than energy-matched foods with lower levels of protein (e.g. (Astbury, Stevenson, Morris, Taylor, and MacDonald, 2010; Bertenshaw, Lluch, and Yeomans, 2009; Booth, Chase, and Campbell, 1970; Fischer, Colombani, and Wenk, 2004; Hill and Blundell, 1986; Rolls, Hetherington, and Burley, 1988; Teff, Young, and Blundell, 1989), though this is not always reported (de Graaf, Hulshof, Weststrate, and Jas, 1992; Vozzo, et al., 2003).

In general, it is accepted that a reduced-carbohydrate, high-protein diet is associated with better fat loss and relatively less lean mass loss.

4. Designing protein-rich satiating food

4.1. Effect of type and amount of protein

A large number of behavioural studies indicate that both the quantity and quality of dietary protein can influence food intake and metabolism markedly, and that dietary protein intake may be prioritized over energy intake (Morrison and Laeger, 2015).

Different proteins appear to involve different satiety mechanisms and the different mechanisms appear to be related mainly to different food components (Anderson, et al., 2004b; Diepvens, Häberer, and Westerterp-Plantenga, 2008; Veldhorst, et al., 2008).

4.1.1. Short-term studies

A number of studies have investigated the short-term effects of different protein sources on satiety. Twelve years ago, Halton, et al. (2004) reviewed the literature on the effects of high protein foods and diets on satiety. At least in the short-term, they found convincing evidence that high-protein meals are more satiating than lower protein meals and stated that the mechanisms remain elusive. Later, in a meta-regression approach, Krieger, Sitren, Daniels, and Langkamp-Henken (2006) examined 87 short-term studies and found that protein intakes of >1.05 g/kg of actual (rather than desirable) body weight were associated with 0.6 kg better retention of lean mass; in studies of more than 12 weeks' duration, this increased to 1.2 kg.

Veldhorst, et al. (2009a, 2009b, 2009c) have published many preloading studies that have suggested that in iso-energetic amounts, high-protein diets are more satiating than normal protein meals. In short, the evidence supports the conclusion that higher-protein meals tend to increase satiety when compared to lower-protein ones, at least in the short term.

Fish and meat proteins. Uhe, Collier, and O'Dea (1992) measured the relative satiating effects of protein in beef, chicken and fish over a period of three hours. Visual analogue scale (VAS) measurements of satiety were found to be significantly higher after the subjects consumed fish than after beef or chicken; subsequent energy intake was not measured. Borzoei, Neovius, Barkeling, Teixeira-Pinto, and Rössner (2006) looked at the satiating effects of beef and fish. With fish, they found a non-significant increase in

satiety by appetite ratings and a significant decrease in energy intake at a subsequent meal. However, both studies were relatively small (6 and 23 subjects, respectively) and the participants only included lean men. The authors hypothesized that these results might be due to differences in amino acid content or the slower rate of digestion of fish proteins, and also suggested serotonergic factors as a possible explanation of extended observations of fish protein enhanced satiety.

A series of seminal papers have addressed the satiating effect of dietary compounds introduced by duodenal infusion in rats, where glucose, fatty acids, and meat protein hydrolysates clearly affected the release of CCK and resulted in lower dietary intake due to smaller and less frequent meals (Young, et al., 2013). Johnstone, et al. (2008) indicated that high-protein (30% protein) meat-based weight-loss diets are highly satiating and reduce ad libitum food intake over a 4-week period.

Cudennec, Fouchereau-Peron, Ferry, Duclos, and Ravallec (2012) and Nobile, et al. (2016) showed that hydrolysates produced from blue whiting muscle reduced short-term food intake, which was correlated to an increase in the CCK and GLP-1 plasma levels. Moreover, they demonstrated that their chronic administration led to a decrease in body weight gain.

Milk proteins. The fact that different proteins may affect satiety differently has been studied especially with respect to whey and casein protein. Hall, et al. (2003) found an increased satiety response to whey compared with casein, involving post-absorptive increases in plasma amino acids together with some hormones or peptides as potential mediators of that response. These authors compared the effects of drinks containing very large amounts of whey or casein and found that compared with the casein preload, the whey reduced the energy intake by 19% at a subsequent meal. However, the buffet meal was offered 90 min after the preloads, which is probably too soon to be a realistic sensitive time frame. Anderson and Moore (2004a) considered that this difference

between casein and whey proteins could be attributed to clotting of the casein (unlike the soluble whey) in the acidic media of the stomach, giving it a longer gastric emptying effect and longer exposure to gastric peptic hydrolysis. Since whey contains high concentrations of branched chain amino acids (leucine, isoleucine and valine), it has been suggested that these perform a unique metabolic role, enhancing satiety due to the extra-hepatic metabolism and interactions with insulin-signaling pathways (Diepvens, et al., 2008). In addition, other nutrients such as vitamin D and fatty acids might also have a role in the impact of dairy supplementation.

Other recent studies have shown that dairy protein supplements favourably influence appetite sensations and time of request of meal (Douglas, Ortinau, Hoertel, and Leidy, 2013), as well as subsequent energy compensation (Akhavan, Luhovyy, Brown, Cho, and Anderson, 2010).

Anderson, et al., (2004b) compared the effects on subsequent energy intake of liquid preloads containing 45–50 g of whey protein, soy protein, egg albumen or sucrose and a control (water). Whey and soy protein suppressed energy intake at a meal provided one hour later, but egg albumen and sucrose did not, resulting in a greater energy intake overall (preload plus meal). Whey was more effective than soy. In Akhavan, et al., (2010), the ingestion of a preload supplemented with whey protein resulted in a subsequent decrease in energy intake that was larger than the energy content of the supplement.

Bowen, Noakes, Trenerry, and Clifton (2006) compared energy intake, ghrelin, and CCK after different carbohydrate and protein preloads in overweight men. Although they did not find different effects from different proteins (casein and whey), they observed that the protein preloads induced a larger satiating effect than the carbohydrate ones. In addition, they referred to differences in appetite-regulatory hormone responses by body mass index status.

The satiating properties of caseinomacropeptide have been considered to involve an increased release of CCK and inhibition of gastric secretions. However, most human studies conducted to date with caseinomacropeptide appeared to have failed to demonstrate a clear satiating effect followed by a reduction in food intake (Nongonierma and FitzGerald, 2015).

Mycoprotein. Mycoprotein, a high-protein food produced from a fungal source, has also been tested for its effects on satiety. Burley, Paul, and Blundell (1993) and Turnbull, Walton, and Leeds (1993) compared the effects on satiety of a mycoprotein-based meal and a chicken-based one with the same protein content. In both studies, subsequent energy intake was lower after the mycoprotein than after the chicken meal. Although the meals were matched for energy and protein, it should be noted that the mycoprotein meal was higher in fibre, which may have affected the satiety response. It is therefore not possible to draw conclusions about the specific effects of protein on satiety in this case (Benelam, 2009). Williamson, et al., (2006) compared the effects on satiety of mycoprotein, chicken and tofu preloads with matched protein contents. The energy intake at the test lunch was lower after both the tofu and mycoprotein preloads than after the chicken preload, while there was no significant difference in appetite ratings.

Egg, soy, and other protein sources. Different types of isolated protein added to meals have also been investigated for their potential effects on satiety. Lang, et al. (1998) looked at the effects of egg albumen, casein, gelatine, soy protein, pea protein, and wheat gluten on satiety, using a preload design, and found no significant differences in their effects on appetite ratings.

In a separate experiment, the effect of soy protein was compared with a combination of soy protein and carbohydrates (either glucose or amylose) on energy intake at a meal one hour later. Soy protein alone caused a significant reduction in energy intake, but

when the protein content was reduced and carbohydrate added, there was no significant reduction in the energy intake (Anderson, et al., 2004b). Overall, the evidence suggests that the source of protein itself, at levels feasible in foods, does not have a large and consistent effect on subsequent appetite and food intake. Neacsu, Fyfe, Horgan, and Johnstone (2014) concluded that appetite control and weight loss were similar for vegetarian and meat-based diets. The gut hormone profile was similar for both, which suggest that vegetarian diets can be as effective as meat-based diets for appetite control during weight loss.

Some studies have shown that eggs eaten at breakfast are more satiating than cereals in normal weight subjects (Fallaize, Wilson, Gray, Morgan, and Griffin, 2013) and are also more satiating than bagels in overweight (Ratliff, et al., 2010) and obese subjects (Vander Wal, Marth, Khosla, Jen, and Dhurandhar, 2005). Despite a similar energy density and macronutrient composition, consuming an egg-based breakfast compared to a cereal-based breakfast significantly reduced short-term, but not long-term, energy intake, influencing the fullness rating (Bayham, Greenway, Johnson, and Dhurandhar, 2014). This was also so in children (Kral, Bannon, Chittams, and Moore, 2016). In these studies, the satiating effect of eggs was demonstrated not only by decreased feelings of hunger, but also by a lower energy intake.

Marsset-Baglieri, et al. (2015) concluded that despite important differences in protein kinetics and their subsequent effects on hormone secretion, eggs and cottage cheese had a similar satiating power. This strongly suggested that with a dose of proteins that is compatible with supplement strategies, i.e. 20–30 g, a modulation of protein kinetics is ineffective in increasing satiety.

4.1.2. Long-term studies

Boirie, et al. (1997) studied the effect of two milk proteins – casein and whey protein – on postprandial whole-body protein metabolism. They combined oral and intravenous

administration of proteins. According to the speed at which amino acids appeared in the bloodstream, the authors classified whey as a fast protein and casein as a slow protein. The postprandial amino acid levels differ a lot depending on the mode of administration of the dietary protein: a single protein meal results in an acute but transient peak of amino acids whereas the same amount of the same protein given in a continuous manner, which mimics slow absorption, induces a smaller but prolonged increase (Boirie, et al., 1997; Wolever, 1994). The slowly absorbed casein promotes postprandial protein deposition by an inhibition of protein breakdown without an excessive increase in amino acid concentration, whereas a fast-dietary protein stimulates not only protein synthesis but also oxidation.

Halton, et al. (2004) stated that in long-term studies conducted over a few days, higher post-absorptive satiety and thermogenesis are sustained irrespective of the protein source. There is no clear consensus that one type of protein is more satiating than another. Weigle, et al., (2005) looked at the effects of both iso-caloric and ad libitum high-protein diets. Their subjects were given a diet that provided either 15% or 30% of the energy from protein (carbohydrate was kept constant at 50% of energy and fat varied from 35% to 20% of energy). For the first four weeks, when the high- and low-protein diets were isocaloric, the subjects reported significantly higher satiety ratings on the high-protein diet. For the following 12 weeks, the macronutrient proportions of the diet remained constant but the subjects were allowed to eat ad libitum from the foods provided. This resulted in a spontaneous reduction in energy intake among those on the high-protein diet, and weight loss at the end of the study. The reduction in energy intake did not appear to cause a reduction in satiety, according to self-reported appetite ratings.

Lejeune, et al., (2006) conducted a study on protein and satiety in respiration chambers, allowing energy expenditure to be assessed. For four days, subjects were fed either an adequate-protein diet (10% of energy from protein) or a high-protein diet (30% of energy from protein), which were iso-caloric. VAS measurements showed that satiety was significantly higher and hunger was significantly lower on the high-protein diet,

despite the energy intake being the same. Sleeping metabolic rate and diet-induced thermogenesis were significantly higher on the high-protein than on the adequate-protein diet. These results confirmed those of similar studies performed by the same research group in 1999 (Westerterp-Plantenga, et al., 1999).

Skov, Toubro, Rønn, Holm, and Astrup (1999) showed large weight loss benefits at 6 months on a high-protein weight loss regime. According to Clifton (2006a, 2006b), groups consuming a high-protein, moderate-carbohydrate diet have an increased likelihood of maintaining weight loss at 12 months and beyond, with minimal risk of side effects. In addition, high-protein diets provide a potential benefit of improved compliance during weight loss attempts: in a 12-month study, Due, Toubro, Skov, and Astrup (2004) reported substantially greater compliance in subjects consuming a higher protein diet (25% of energy from protein).

In conclusion, protein intake enhances satiety, but there is not clear evidence to indicate whether there is a difference in the effect size dependent on the source of the protein, i.e. from animal or plant-based food.

4.2. Satiety implications of increasing oral exposure by adding protein

Satiety is a complex object which needs to be studied from both a metabolic and a behavioural point of view (Allirot, et al., 2014). Oral exposure is affected by a number of food characteristics (de Wijk, Zijlstra, Mars, de Graaf, and Prinz, 2008; Hutchings, et al., 2009), such as food viscosity (de Wijk, et al., 2008; Viskaal-van Dongen, Kok, and de Graaf, 2011), bite size, oral processing time, or chewing frequency (Bolhuis, Lakemond, de Wijk, Luning, and de Graaf, 2011; Morell, Fiszman, Varela, and Hernando, 2014; Zijlstra, et al., 2009), among others. Morell, et al. (2015a) showed that adding whey protein to yogurts raised the expectations of their satiating ability significantly in a consumer test (n=121) and attributed this effect to the higher viscosity of these yogurts in comparison to the control samples (no extra protein added). This

suggested that an increased time or intensity of the orosensory exposure (i.e. food present in the oral cavity) would contribute to controlling further energy intake.

A review by Hogenkamp and Schiöth (2013) found that satiety and satiation are modified not only by cognitive processes but also by sensory exposure to food. Years before, French and Cecil (2001) showed that satiety was greater when a food was consumed orally than when the same food was infused into the gastro-intestinal tract. It has also been observed that because of their fluid nature, beverages require less oral processing time than semi-solid and solid caloric equivalents, minimizing oro-sensory exposure (de Wijk, et al., 2008; Morell, et al., 2014; Tieken, et al., 2007; Zijlstra, et al., 2009). A number of studies have indicated that "liquid" energy fails to suppress subjective appetite (Leidy, Apolzan, Mattes, and Campbell, 2010; McCrickerd, Chambers, Brunstrom, and Yeomans, 2012), eliciting weaker suppressive appetite responses than "more solid" iso-caloric versions of the same food product (Bertenshaw, et al., 2009; Mattes and Rothacker, 2001; Zijlstra, Mars, Stafleu, and de Graaf, 2010). According to McCrickerd, Chambers, Brunstrom and Yeomans (2012), the possible reason is that longer oro-sensory exposures contribute to the development of satiety through triggering anticipatory responses related to learned associations between the sensory characteristics of a food and its caloric value post-consumption (Yeomans, Weinberg, and James, 2005). These associations are likely to influence explicit expectations about the effect a food will have on appetite (Blundell, et al., 2010; Brunstrom, Shakeshaft, and Scott-Samuel, 2008). In line with this, Morell, Ramírez-López, Vélez-Ruiz, and Fiszman (2015c), using food photograph visual scales, reported that a milk-based dessert with added HPMC and protein and a thick, dense texture elicited the perception of having a high satiating capacity. The taste, smell and texture of a food all contribute to the representation of its flavour, but food texture (or form) has been isolated as a sensory component of food that plays a key role in satiety (Chambers, et al., 2015; McCrickerd and Forde, 2016). Food texture, therefore, may serve as a reliable predictive cue for further sensations of satiety (Davidson and

Swithers, 2005). Creamy texture cues have been associated with nutrient-rich foods (Bertenshaw, Lluch, and Yeomans, 2008; Bertenshaw, et al., 2009; Bertenshaw, Lluch, and Yeomans, 2013) and satiety-relevant sensory cues can be used to estimate the satiating power of foods. This would support the view that oro-sensory sensations such as taste and texture cues act as nutrient sensors (Woods, 2009), directing eating behaviour to ensure the efficient consumption of nutrient-rich or nutrient-lacking foods (McCrickerd, Lensing, and Yeomans, 2015).

Textured foods require mastication, which will slow down consumption rates and enhance orosensory exposure times (Zijlstra, Mars, de Wijk, Westerterp-Plantenga, and de Graaf, 2008). The mechanical processing of food in the mouth might be one way in which the nutrient content of a food is estimated. Indeed, chewing has been associated with satiety-related cognition (Forde, van Kuijk, Thaler, de Graaf, and Martin, 2013), preparatory cephalic phase responses and appetite peptide release (Li, et al., 2011), but relationships with satiety signals have not always been reported (Mattes and Considine, 2013; Teff, 2010).

Differences in *ad libitum* intake after treatment with either liquid yogurt consumed with a straw, or liquid yogurt or semi-solid yogurt both consumed with a spoon, have been explained by eating rate, which was faster with a straw and led to reduced oral processing time (Hogenkamp, Mars, Stafleu, and de Graaf, 2010). The daily energy intake increased with decreasing viscosity (Juvonen, et al., 2009), while the ad libitum intake increased by 30% in the beverage group compared to the semi-solid group in another study (Zijlstra, et al., 2008). Controlling for the effort involved in consuming the test load did not influence intake, but the eating rate, measured as the volume consumed per minute, was positively associated with intake, whereas there were no differences between any of the treatments in the ratings for hunger, fullness, and a desire to eat (Zijlstra, et al., 2008). The subjects consumed greater quantities of a beverage test load compared to a semi-solid test load, although these differences were eliminated after standardizing the duration of oral processing (de Wijk, et al., 2008).

More solid products require more labour and time in the mouth, causing longer oro-sensory exposure, which in turn may result in a greater timespan to allow satiety signals to induce meal termination or evoke satiety (Hogenkamp, et al., 2013). An increase in proxies of orosensory exposure may result in an increase in nutrient-energy-sensing, and a longer timespan for satiety signals to reach the brain.

Increasing the food texture complexity could also be an interesting strategy for prolonging oral exposure (Marcano, Morales, Vélez-Ruiz, and Fiszman, 2015). A few examples of added-protein milk-based desserts with enhanced satiating capacity have recently been published (Morell, et al., 2015a; Morell, Piqueras-Fiszman, Hernando, and Fiszman, 2015b). Literature on the development of novel food items with enhanced satiating capacity is still scarce. Marcano, Varela, and Fiszman (2015) selected a system that was basically made of fresh cheese, eggs, sugar, milk, and starch as a model for designing a satiating dairy pie with increased protein content.

Long-term intervention studies have reported that slowing down the normal eating rate may alter the physiological responses to food beneficially (Galhardo, et al., 2011) and may be a useful therapy to include in programs that aim to reduce obesity (Ford, et al., 2010). This suggests that aspects of oral processing and eating rate can make a beneficial contribution to controlling our energy balance and meal size.

The physical properties of solids may require increased energy expenditure to break them down and their incomplete degradation may promote inefficient energy and nutrient absorption (Conley, et al., 2011). Stomach distention and retention of stomach contents produce feelings of fullness (Cuomo, et al., 2011). Rapid gastric emptying could result in faster gastrointestinal transit, which, in turn, could result in decreased absorption and blunted nutrient-based feedback signalling (e.g., CCK or GLP-1 release) (Moukarzel and Sabri, 1996). Nutrient sensing in the proximal and distal gastrointestinal tract can trigger physiological responses that slow transit time, e.g., CCK (Schwartz and Moran, 1998) and GLP-1 secretion (Flint, Raben, Ersbøll, Holst, and Astrup, 2001).

There is considerable evidence on the relationship between endocrine status, appetite, and ingestive behaviour, but it is very mixed (Apolzan, Leidy, Mattes, and Campbell, 2011; Cassady, Considine, and Mattes, 2012; Juvonen, et al., 2009; Leidy, et al., 2010; Tieken, et al., 2007; Zijlstra, et al., 2009), leading to questions about whether the relationships are associative or causal under physiological conditions. It may be that measurable circulating concentrations of endocrine factors are poor predictors of the more important influence of central effects (Havel, 2001; Ionut, Hucking, Liberty, and Bergman, 2005). Taken together, the evidence suggests that different food states lead to different patterns and magnitudes of endocrine responses and, in consequence, to different effects on feeding.

Food scientists could take advantage of this knowledge about food texture and food form as sensory aspects that play basic roles in satiety. The length of orosensory exposure influences satiety and satiation, so researchers have to take into account that adding protein will influence the characteristics of a food, such as its viscosity, oral processing time, and chewing frequency, in order to attain the proper design of really worthwhile enhanced-satiety products.

5. New protein-rich food development

A new product development process should involve the following critical stages: idea generation, concept development, product development, launch and post-market monitoring. New ideas have been derived from human biology and physiology, nutritional epidemiology, food technology, consumer learning and consumer psychology in choice and consumption contexts. A concept constitutes the outline of a product idea; a crucial challenge in new product development is the extent to which the physical product can actually live up to its expectations in enhancing feelings of satiety and satiation. Once the product is ready for its market launch it will be successful both in terms of its commercial and public health ambitions, but satiety-enhancing product

features need to be convincingly and responsibly communicated to consumers (Van Kleef, Van Trijp, Van Den Borne, and Zondervan, 2012).

A number of satiety/satiation-related patents have taken out over the past ten years. One of the principal approaches has consisted in adding a number of proteins such as whey proteins, caseinmacropeptide, glycomacropeptides, whey protein hydrolysate, lactalbumin, sodium caseinate, intact pea and wheat protein, hydrolysed yeast proteins, codfish, egg, or egg hydrolysate to foods. There are patents that mention the protein formulation's potential for stimulating hunger-control related neurotransmitters or enzymes. Others use the fact that certain proteins have an unfolding transition in the stomach's pH range, as is the case for some cross-linked globular proteins that can form hydrogels.

From the food development point of view, it is important to note that the pleasantness of eating fish, for example, is not the same as that of eating meat, and that the type and quality of the fats or oils of the fish or meat consumed influence the feeding response. Also, the versatility of milk proteins is greater than that of other types of protein from several points of view, such as predisposition and times when they are consumed. Similarly, many other factors influence a particular choice (Paddon-Jones, et al., 2008).

High protein food products invariably contain other energy-yielding nutrients, usually both carbohydrates and fats. Therefore, in order to optimize high satiety products, the carbohydrate-to-fat ratio should also be considered (Chambers, et al., 2015).

Processing is another factor to be taken into account, since proteins normally require optimal pH control, protein concentration, heat treatment and evaluation of the risks from other ingredients (gums, minerals) in order to prevent flocculation, turbidity or sedimentation. In spite of this, proteins are a wide-ranging group, some of which are highly functional and have been developed and optimized by the industry for different food systems.

Reformulating foods to increase their protein content can also affect the sensory properties of the final system. An understanding the mechanism of texture perception is essential when developing food products to both meet nutritional needs and maintain an acceptable level of sensory quality (Çakır, et al., 2012). The appearance of unpleasant flavours and texture features has been reported in high-protein satiating yogurts formulated with whey proteins (Morell, et al., 2015a) and in high-protein satiating bars (Little, Gregory, and Robinson, 2009).

Finally, in the development of satiety-enhancing food products it is crucial to be aware that they require an integrated multidisciplinary perspective of the problem (Allirot, et al., 2014), in relation to both public health governance and corporate ambitions and in relation to nutritional, food technology, communication and consumer sciences. The efforts of various segments of the food industry to provide foods with a lower energy density in response to recommendations from nutrition experts and insistent demands from consumers, in spite of the technical difficulty, deserves a little more attention (Bellisle and Tremblay, 2011). The consumer and health benefits of satiety-enhancing products need to be better defined. It is not clear what the consumer understands by satiety (Fiszman, Varela, Díaz, Linares, and Garrido, 2014) or whether consumers know how satiety products should be incorporated into their daily diet (Halford and Harrold, 2012).

Products designed to incorporated and claim high amounts of protein or meet a specific preferred ratio of protein/ other macronutrients is one of the most interesting potential ways to market satiety-related food items. Although bars and sakes are currently the most common novel formulations of food and meals seem to be promising.

6. Conclusion and future trends

As general conclusions, according to Johnstone (2013), there is some suggestive evidence for enhanced protein-induced satiety effects are likely to be amplified,

particularly in studies where negative energy balance is induced. The optimal amount of protein, type, timing, and interactions with other intervention (e.g. exercise) is still unclear.

Energy from protein, in a sufficient dose, has a greater effect on satiety than an equivalent amount of energy from carbohydrate or fat in the short-term. The literature reviewed indicates that increasing the protein content of a food is an effective way to deliver enhanced satiety.

On the other hand, it should be taken into account that manipulating the macronutrient content of a food while keeping its energy level constant makes it difficult to be certain whether the effects are caused by the superior satiating effect of protein, reduction of the less satiating nutrients (carbohydrate and fat), or a combination of the two.

When ad libitum high-protein diets are compared with lower-protein diets, larger weight losses appear with the former in longer-term studies. This may be a result of the higher satiating effect of protein, although appetite measurements are often not taken in these studies. Differences in study designs make it difficult to pinpoint the optimum dose or percentage of energy needed to observe significant effects of protein on satiety. It is generally accepted that at least 50 g of protein in a food or meal is necessary to see a significant effect on satiety, but that currently there is not sufficient information to describe a dose–response relationship. The protein content of food, and its source, is a solid determinant of short-term satiety and of how much food is eaten. However, the role of protein in the regulation of long-term food intake and body weight is less clear, but several lines of evidence suggest that further research to define its role is merited.

Finally, protein-rich food development seems to be a good strategy for designing satiating food.

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Understanding the relevance of in-mouth food processing. A review of *in vitro* techniques

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Abstract

Oral processing of food is the first step in the eating process. Although the food undergoes a number of changes during mastication that influence the subsequent steps, this stage has very often been neglected in studies of digestion, bioavailability, flavor release, satiety potential, glycaemic index determination, etc. The present review draws on different sources such as nutrition, medicine, phoniatry and dentistry to explain some *in vitro* oral processing methods and techniques that could be transferred to food technology studies to mimic *in vivo* comminution, insalivation, and bolus formation, describing, as a necessary reference, the respective *in vivo* physiological processes they attempt to imitate.

Developing a deeper understanding of all the aspects of in-mouth process will help food technologists to give this crucial step the necessary attention its due importance and to consider better ways to incorporate it into their studies.

Introduction

Food is a mixture of proteins, carbohydrates and lipids that interact physically and chemically in an aqueous environment to create a food-specific native or processed structure. Differences in the chemical composition of foods are therefore associated with differences in their macrostructure and texture which affect various food characteristics, including resistance to hydrolysis or to breakdown during oral food processing and simultaneous (oral) or subsequent (gastric, intestinal digestion. In-mouth actions results from a dynamic process in which the textural characteristics of food are continuously analyzed by the oral sensory systems (Pineau, et al., 2009). Chen (2009) reviewed the physiology as well as the rheological principles of food texture and sensory perception, since food texture is the main factor that determines the different processes for transforming food into a material that is ready to be swallowed.

In a pioneering work, Hutchings and Lillford (1988) stated that texture perception in the mouth is a dynamic sensory monitor of changes made to a food. They proposed a groundbreaking general model, defining the breakdown path of the food during oral processing through three aspects or dimensions: the mechanical and rheological behavior of the food (degree of structure), the oral experience via saliva participation (degree of lubrication), and the sequences of oral processing (time). Involving the oral experience and time in texture studies was a significant development which turned texture appreciation from a static process into a dynamic one. Several years later Prinz and Lucas (1997) proposed the optimum swallow model, in which swallowing was defined as the moment when the food bolus reaches a peak cohesive force, driven by the interaction between the food particles (degree of structure) and saliva (degree of lubrication). In this way, the duality of separating thresholds for food particle size and for particle lubrication is eliminated: swallowing is initiated when it is sensed that a batch of food particles is binding together under viscous forces so as to form a bolus.

In plain words, digestion is the process of breaking food down into simpler substances that can be absorbed by the body. Food digestion in humans depends on both the

chemical and physical characteristics of the food and on how it changes as it passes through the different areas of the digestive tract. Within this framework, the relevance of oral processing up to the instant of swallowing is evident.

Inside the mouth, food undergoes a number of changes. Some of them, such as comminution, are not strictly speaking digestive processes but are undoubtedly necessary before these can take place, and could be considered a "pre-digestive step".

During in-mouth food processing, food is subjected to several major mechanical and chemical modifications. The solid food is fractured by the teeth and diluted and broken down by saliva. These joint actions induce its progressive comminution and adherence of the resulting smaller particles through saliva impregnation, formed into a cohesive bolus and finally swallowed (Van der Bilt, Mojet, Tekamp, and Abbink, 2010). It would appear that saliva is involved at every step, not only as a digestive medium but as a lubricant, providing surface smoothness and weak inter-particle adhesive forces (Lillford, 2011). Although mastication seems a simple process, it involves many factors: the physiological characteristics of the individual performing the chewing action, such as facial anatomy, gender, age, personality type, time of day, or dentition status, as well as the properties of the food being chewed, such as hardness, moisture content, fat content, food portion size, or food structure, all have an effect on the formation of the food bolus (Bornhorst and Singh, 2012). The bolus is eventually swallowed when its structural characteristics have become suitable for safe swallowing.

Over the years, researchers from different disciplines such as nutrition, pharmacy, medicine or dentistry have been working on this subject. However, it is in the last decade that food technology research has fully approached oral processing, with enormous interest, as the bridge between food texture, microstructure and sensory perception (Stieger and Van de Velde, 2013). As it constitutes a short step (about 20–30 seconds) in the overall ingestion process compared with the length of the gastric and intestinal stages (1–10 hours), it has often been neglected in studies such as those dealing with food digestion.

In vitro studies covering bioavailability, determination of the carbohydrate glycaemic index, transportation and absorption of nutrients, flavor release, evaluation of the satiety potential of ingredients or whole food systems, etc. are only a few examples of current interests in the area of food science and technology research where the release of some food components from their physicochemical dietary matrix is necessary. This release begins in the mouth. Depending on the scope of each specific study, the selection of methods for mimicking oral actions in *in vitro* studies has to consider a number of factors.

The principal aim of the present work is to give an overview of the main strategies that could be used in Food Technology research for *in vitro* studies in which oral processing plays a role. For this purpose, it offers a review of the main equipment and techniques that have been designed to reproduce human mouth processing, emphasizing the newest of these. The physiological actions they attempt to imitate are necessary references and are also described. This paper will help Food Technology researchers to choose the proper tool for their *in vitro* studies.

Oral comminution

In vivo scenario

The oral breakdown or disruption of food during mastication is highly variable, depending on the food itself (texture, dryness, hardness, size) and on the characteristics of each person (dental health, degree of hunger, particular habits). Many authors have pointed out that the pre-swallow bolus is characterized by a specific particle size distribution that is similar across individuals for the same food (Jalabert-Malbos, Mishellany-Dutour, Woda, and Peyron, 2007; Mishellany, Woda, Labas, and Peyron, 2006). Nevertheless, some studies have also revealed important inter-individual differences in food bolus formation and in chewing behavior (Loret, et al., 2011;

Tárrega, Yven, Sémon, and Salles, 2011; Tournier, Grass, Zope, Salles, and Bertrand, 2012).

A recent study by Hwang, et al., (2012), with banana, tofu, cooked rice, and biscuits eaten by healthy subjects, showed that the particle size distribution of the ready-to-swallow bolus depended essentially on food type and on mechanical properties of the food such as hardness, cohesiveness, and adhesiveness, and not on individual differences. Mishellany, et al., (2006), working with three nuts and three vegetables, showed that the sizes of the bolus particles just before swallowing were comparable in all subjects, whereas the number of cycles and duration of sequences varied widely between individuals. They stated that fracture and fragmentation of food (ingestion involving fracture of particles by the incisors) were closely correlated with the ratio of toughness to Young's modulus in foods with approximate linear stress-strain relationships (the stress-strain gradient provides the Young's modulus value of the food and toughness is the work required to fracture it). Since the stress-strain relations of a number of food products are distinctly nonlinear, more complex fracture models have to be introduced in these cases (Lucas, Prinz, Agrawal, and Bruce, 2004). Of course, other factors such as water content, the ability to absorb saliva (Hutchings and Lillford, 1988) and the fibrous structure of the food also influence the way in which they are broken down (Mishellany, Woda, Labas, and Peyron, 2006).

In a study by Jalabert-Malbos, et al., (2007), foods that were swallowed rapidly (14–20 masticatory cycles) were soft and had a high-water content, like egg white, pickled cucumbers, mushrooms or olives. The boluses obtained from these foods contained many large particles. Harder foods such as coconuts and carrots needed more cycles and longer mastication before swallowing, probably because more time was needed to process the food and to disrupt the fibers. They also needed more complete insalivation to produce a lubricated bolus that was safe to swallow. To be swallowed easily, particles must be smaller than 2 mm, with the exception of soft particles that are not liable to injure the upper digestive mucosae. Jalabert-Malbos, Mishellany-Dutour, Woda, and

Peyron (2007) showed that for a range of foods, sizes from 0.4 to 4 mm with a median of around 2 mm were found in boluses when ready for swallowing. Mastication reduced bread to an increasing number of small particles. Le Bleis, Chaunier, Della Valle, Panouillé, and Réguerre (2013) found that mastication reduced two types of bread of different textures into an increasing number of small particles. However, the number of small particles did not always increase with the number of masticatory cycles, probably because many small particles are lost during intermediary swallows that are not generally analyzed (Jalabert-Malbos, Mishellany-Dutour, Woda, and Peyron, 2007).

One important parameter that describes the bolus just before swallowing is its median particle size (d50), defined as the theoretical sieve size through which 50% of its mass can pass (Jalabert-Malbos, Mishellany-Dutour, Woda, and Peyron, 2007; Ngom, Diagne, Aïdara-Tamba, and Sene, 2007). The d50 value is a useful way to classify foods used in masticatory evaluation (Veyrune, Opé, Nicolas, Woda, and Hennequin, 2013) according to how easily they are processed in the mouth to form a suitable bolus.

The *in vivo* results highlight two characteristics of mastication in humans. Firstly, the intra-individual variability of food bolus particle size distribution is very narrow. Secondly, there is a contrast between the narrow inter-individual variability of the food bolus d50 and the much broader variability of the physiological variables among individuals, such as duration of the sequence, number of strokes, and electromyographic activity (Jalabert-Malbos, Mishellany-Dutour, Woda, and Peyron, 2007; Mishellany, Woda, Labas, and Peyron, 2006; Peyron, Mishellany, and Woda, 2004).

Quantitative electromyography (EMG) has been used to explain the physiological process of mastication, to assess muscle function, and also to diagnose temporomandibular disorders (González, Montoya, and Cárcel, 2001). EMG emerged timidly in the late '80s (Boyar and Kilcast, 1986) as a new tool in texture evaluation. It is a non-invasive technique that does not interfere with the mastication process and gives a detailed account of the activity of the masticatory muscles. EMG offers the possibility of monitoring muscle activity during mastication (González, Montoya,

Benedito, and Rey, 2004; González, Montoya, and Cárcel, 2001). The results obtained provide time-dependent information to characterize food texture. By monitoring the activities of the facial muscles, this technique makes it possible to correlate food physics with the physiology of oral processing and the sensory perception of food (González, Montoya, Benedito, and Rey, 2004).

Electrognathography, also known as jaw tracking (JT), is a three-dimensional method for tracking mandibular movements that provides information on mandibular velocity and direction as well as the extent of the jaw movements.

EMG and JT are the methods most commonly used to study the relationships between oral processing and food texture. Together with mechanical and sensory analyses, these two techniques constitute a powerful combination for characterizing the complex nature of food texture (Chen, 2009). A number of EMG and JT parameters are used to understand changes in chewing behavior in relation to different textural properties. The typical measurements are number of chews, chewing time, chewing frequency, total or mean muscle activity, peak muscle activity, jaw movement amplitudes, and jaw-opening and -closing velocities, as well as opening, closing, and occlusal phase durations. These parameters can be examined over the complete chewing sequence or over different parts of it (Koç, Vinyard, Essick, and Foegeding, 2013). A new intraoral bite force recorder which would allow the study of natural mastication without an increase in the occlusal vertical dimension was recently proposed by Shimada, Yamabe, Torisu, Baad-Hansen, Murata, and Svensson (2012) for subsequent analysis of the relation between electromyographic (EMG) activity of jaw-closing muscles, jaw movements and bite force during mastication of five different types of food.

Oral physiology also exerts an important influence on chewing (Van der Bilt, Engelen, Pereira, Van der Glas, and Abbink, 2006), as do characteristics such as bite force, chewing performance and salivary flow rate. Chewing performance can be determined by quantifying the degree of fragmentation through sieving artificial (for example, silicon rubber cubes) or real food. Other methods involve evaluating the ability to mix

and knead a food bolus using two-colored chewing gum or paraffin wax (Van der Bilt, Mojet, Tekamp, and Abbink, 2010).

Besides teeth, masticatory muscles, and the temporomandibular joint, the tongue plays an important role in orofacial motor behavior such as mastication and swallowing. As Kakizaki, Uchida, Yamamura, and Yamada (2002) stated, the neuronal network plays a major role in triggering and sequencing the neuromuscular events associated with movements, and the tongue and masticatory muscles have been shown to be active in a well-coordinated manner during semiautomatic movements. It is believed that the tongue senses the size and lubrication status of food particles. Chewed food particles of the right size are pushed by the elevated tongue to the back of the oral cavity (Mioche, Bourdiol, Monier, and Martin, 2002; Okada, Honma, Nomura, and Yamada, 2007), while large particles are selected for further size reduction. From a physiological point of view, it is the combined action of pushing, pulling, and twisting by the tongue that transports the food particle, either to push it back to the molar teeth for further size reduction or to pull it to the back of oral cavity for bolus formation. The structural characteristics of the tongue, which is made up of 17 muscles, allow it to perform a wide range of movements to seal the bolus content anteriorly and laterally and generate pressure for its posterior propulsion. The videofluorography technique has made it possible to track and analyze the tongue movement during mastication by gluing small lead markers to the teeth and tongue surface (Taniguchi, et al., 2013).

Nevertheless, there are other food bolus characteristics that could influence the exact conditions for starting to swallow. Data involving not only granularity but also the rheological properties of the food bolus need to be collected in order to gain a better understanding of the link between physiological properties and the final d_{50} values observed just before swallowing. It could be hypothesized that the moderate correlation seen between the number of cycles and pre-swallow d_{50} reflects a need to attain certain rheological states that are partially independent of particle size. Mishellany-Dutour, et al., (2007) reported that subjects who display long masticatory sequences, with many

cycles, probably masticate less efficiently but still need to achieve certain rheological conditions in terms of the viscosity, cohesiveness or stickiness of the final bolus.

Recently, some devices have been developed to measure tongue function objectively during swallowing. Some of these methods have limitations for measuring tongue-palate contact function quantitatively. For example, dynamic palatography can be effective in showing temporary changes in tongue contact position but cannot measure the amplitude of tongue pressure (Taniguchi, Tsukada, Ootaki, Yamada, and Inoue, 2008). Developed for dysphagia rehabilitation and often used by phoniatricians, this method consists of instrumentation which records linguopalatal contacts during continuous speech and is used to evaluate areas of the palate contacted by the tongue.

A technique reported by Kieser, et al., (2008) allowed accurate measurement of tongue pressure during swallowing, using an intraoral appliance with multichannel pressure sensors. These sensors are capable of measuring absolute pressures to a chrome-cobalt palatal appliance with a labial bow. However, the details of the movement of the tongue surface during different functions remain unclear. Sugita, Inoue, Taniguchi, Ootaki, Igarashi, and Yamada (2006) recorded tongue pressures at two sites on the palate during swallowing of model gels with different consistencies, and demonstrated that bolus consistency affected the tongue pressure of the anterior and posterior portions against the hard palate in different ways. The results suggested that a basic pattern of tongue pressure is maintained during swallowing but is modulated differently, by sensory feedback between the anterior and posterior portions of the tongue, to complete the propulsion of the bolus in the oral cavity.

In vitro **scenario**

A few artificial mouths that simulate mastication have been developed in recent years. One of these, called the chewing simulator (Salles, et al., 2007), makes it possible to set and control some of the masticatory variables, such as the number of masticatory cycles,

the amplitude of the mechanical movements or the bite force. Another, the BITE Master II, can measure variables to be replicated such as fractal force and energy to fracture, but in this case only for the first bite (Meullenet and Gandhapuneni, 2006).

Most of the existing prototypes have been developed for dental or orthodontic research and use compressive forces with teeth that have anatomical shapes. However, the complex shapes of natural teeth are operative because of the action of the central nervous system and it is very difficult to mimic this. In most machines, only one functional variable (e.g. speed, deformation or piston movement) can be controlled at a time (Woda, et al., 2010).

Other machines are oriented towards the mechanical properties of the mouth and make no attempt to reproduce the conditions in which foods are processed within a closed mouth (Hoebler, et al., 2002). Conserva, et al., (2008) developed a machine for *in vitro* study of the stress transmitted to a bone-implant in dentistry. Daumas, Xu and Bronlund (2005) developed another, called the mechatronic chewing device, to evaluate the dynamic changes in the texture of foods quantitatively, reproducing human chewing behavior. In this device, the jaw mechanism design first needs to be modelled and analyzed through simulations with the corresponding mathematical model.

Arvisenet, Billy, Poinot, Vigneau, Bertrand, and Prost (2008) also developed an artificial mouth. Their aim was not to reproduce the human mouth exactly but to determine whether mastication conditions have an effect on the release of volatile compounds.

Comprehension of the physiology of taste perception is a key to preparing some food products. Using a newly patented mastication simulator called AMADEUS (Automated Mastication for Artificial Destructuration and Extensive Understanding of Sensoriality), Guilloux, et al. (2013) obtained salt release kinetics and compared the results with sensory data.

Researchers from the University of Auvergne developed a mastication simulator called the Artificial Masticatory Advanced Machine (AM2) (Figure 1 and Figure 2) (Monique, et al., 2007).

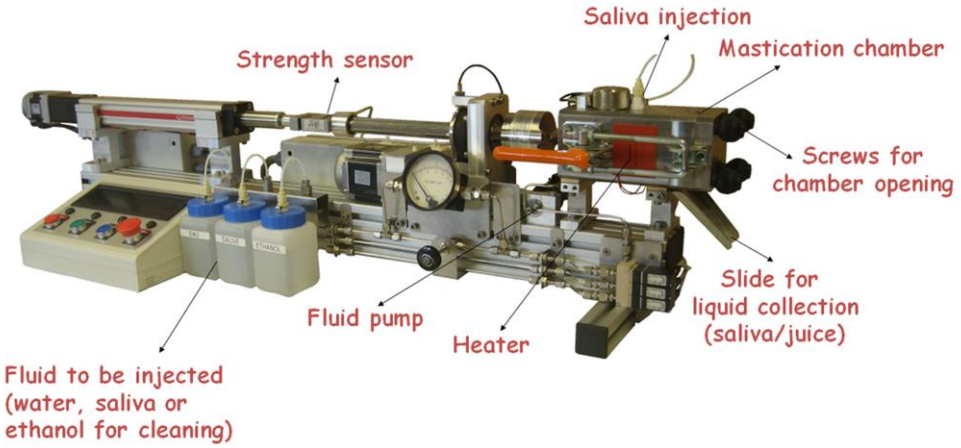

Figure 1. General view of the Artificial Masticatory Advanced Machine (AM2) masticator simulator.

It simulates the mastication function, producing a bolus, while allowing permanent control of the process and collection of the whole bolus at any time. This simulator produces a food bolus with physical properties similar to those of the food bolus produced after natural mastication just before deglutition of the same food. In the AM2, a number of mastication variables are replicated and controlled. The experimenter can select the type of constraints exerted on the food, the number of masticatory cycles, the cycle duration and the duration of the mastication sequence, the force range applied to the food, the mastication chamber temperature and the quantity of artificial saliva. As pre-swallow food particle size distribution is a good indicator of food bolus characteristics, Mishellany-Dutour, et al., (2011) used d_{50} to check the efficiency of AM2. They compared the d50 particle size values obtained in healthy human subjects with those obtained using the AM2. The results showed that the AM2 was able to simulate the d_{50} food bolus particle size of peanuts and carrots produced by humans.

Food bolus d_{50} values obtained *in vitro* and *in vivo* at different times during the mastication process were also similar.

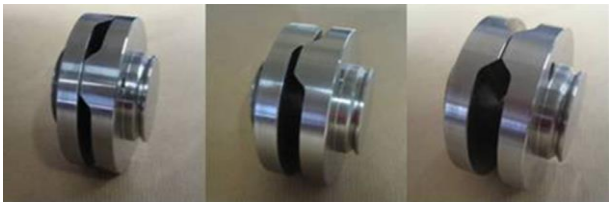

Figure 2. The AM2 masticatory chamber. It is a cylindrical cavity whose two ends are formed by the stationary "maxillary disk" and the moving "mandibular disk"; this can move back and forth along and rotate around the central axis of the cylinder. Both AM2 disks are shown in the different positions during operation.

In simulating mastication with mechanical devices, the intention has been to break down solid foods into particles of a similar average size to those achieved by chewing.

If equipment to simulate the masticatory process is not available, the sample can simply be minced. Experiments with rice, spaghetti and sweetcorn have shown that mincing is an appropriate means of mimicking mastication, giving similar starch content values to the mean values obtained by chewing. Hoebler, Devaux, Karinthi, Belleville, and Barry (2000) compared the particle sizes of food after human mastication and *in vitro* mincing. The particles obtained after human mastication were described as heterogeneous in size and shape, moist, limp, and not easily wet-sieved. The results showed that mincing gave an acceptable reproduction of the particle size distribution of bread, pasta and tortiglioni after *in vivo* mastication. The variability in size and distribution of the minced bread particles was high, but satisfactory for the purpose of *in vitro* simulation of mastication. Applied to foods of differing sizes (spaghetti and tortiglioni) and physical textures (bread and pasta), mincing allowed large amounts of food to be broken down, and thus

seems to be a suitable means of mimicking chewing in a wide range of foods. This method of breaking down food is simple, suitable for routine analysis and easy to use in an *in vitro* procedure.

As discussed above, some devices have been developed to measure *in vivo* tongue function. A technique reported by Ishihara, et al., (2013) has established an *in vitro* evaluation system for determining the deformation of both the tongue and the food, particularly tongue-palate compression, using an artificial tongue made of silicone rubber and an aluminum plate that mimics the hard palate in a conventional uniaxial compression apparatus. They used this method to determine the fracture profiles of gels prepared from different agar sources.

Consequently, existing *in vitro* models can be improved by including an *in vitro* oral phase that mimics chewing behavior. When exact imitation is not feasible, at least a particle size characterization of the sample (prior to subsequent steps) should be carried out (Van Buggenhout, et al., 2010).

Quantifying the bolus particle size distribution

To quantify the particle size distribution of chewed foods, the method most commonly used has been sieving. Image analysis (IA) is another frequently used method to characterize the size and shape of the bolus particles (Hoebler, Devaux, Karinthi, Belleville, and Barry, 2000). This method has been used to determine whether the size and shape properties of a ready-to-swallow food bolus were independent of the subjects (Peyron, Mishellany, and Woda, 2004). Chen, Khandelwal, Liu and Funami (2013) used image analysis to study the correlation between the particle size distribution of food bolus and the hardness of the food. Le Bleis, Chaunier, Della Valle, Panouillé, and Réguerre (2013) also used IA to characterize the degree of fragmentation and heterogeneity of boluses from two types of bread. Mishellany, Woda, Labas and Peyron (2006) listed a number of additional methods that have been used to quantify particle

size during *in vitro* digestion studies, such as laser diffraction, microscopy, sedimentation analysis and diffusion of light.

Six natural foods using sieving and laser diffraction methods were compared by Peyron, Mishellany and Woda (2004); after *in vivo* mastication, they noted that each of these two methods analyzed only one interval of the full range of particle sizes. Particles smaller than the aperture of the finest sieve were lost by sieving and laser diffraction lost large particles because of its technical limits. Therefore, food boluses of raw vegetables consisting of larger particles are better characterized by sieving but laser diffraction is the best method for measuring the granularity of dry and brittle foods such as nuts, because these contain a high percentage of small particles.

The use of IA to ascertain the particle size of food has been described as rapid, accurate and reliable, providing precise particle enumeration over a wide range of sizes with detailed two-dimensional data and obviating the unpleasant and time-consuming sieving and laser diffraction processes. However, the IA technique has the same limitation as the sieving method with respect to the range of values: the smallest particles in the food boluses are missed because they are eliminated during preparation, which involves diluting, washing and arranging the samples, so distribution curves obtained with IA are similar to those obtained by sieving. Importantly, however, the IA technique offers an additional insight, as the particle shape can be observed and quantified by the particle shape index.

Arvisenet, Billy, Poinot, Vigneau, Bertrand, and Prost (2008) studied food boluses with low levels of distinguishable particles by using an image texture analysis technique, the grey level co-occurrence matrix method (GLCM). They showed that this method can provide reliable differentiation using images of apple crunched in an artificial mouth under different compression movement frequency conditions and with different rotation speeds. Hoebler, Devaux, Karinthi, Belleville, and Barry (2000) showed that GLCM can be used to investigate food bolus formation during mastication of different breads and different types of pasta. The use of GLCM textural features for image

classification enabled an average of 67% of images to be classified correctly into their respective chewing cycles. Tournier, Grass, Zope, Salles, and Bertrand (2012) used GLCM in four different breads and identified contrast as the best marker of food degradation.

Hence, the choice of one method rather than another will depend on both the goal of the proposed study and the nature of the food. It should also be considered that whatever technique is used, not all the particles will be spat out even when the material obtained by rinsing out the oral cavity is added to the sample (Mishellany, Woda, Labas, and Peyron, 2006).

Insalivation

***In vivo* scenario**

The oral food stage is short but it also plays another important role: hydrating and lubricating the food by mixing it with saliva. The saliva interacts with the food components, leading to structure formation or structure breakdown (Chen, 2009).

Human saliva is a complex biological fluid, consisting mainly of water (99.5% w/w), various proteins (0.3% w/w), small organic compounds and inorganic salts. It has a pH of around 6.8, rising to around 7-8 after food ingestion. Saliva is typically secreted at a rate of about 0.2 to 4 ml per minute, with a total saliva output of 500 to 1500 mL per day (McClements and Li, 2010). Resting or unstimulated salivary flow is the result of low-level autonomic stimulation by the higher brain centers. Salivary secretion is upregulated above the resting rate by taste and chewing and to a lesser degree by smell stimulation (Carpenter, 2013).

The major protein component of human saliva is mucin. Other proteins in saliva include various enzymes such as α-amylase, immunoglobulins, antibacterial proteins, proline-rich proteins (up to 45 % of the total weight of protein) and peptides such as histatins

and cystatins (Sarkar, Goh, and Singh, 2009). The parotid gland contributes the greatest flow (as much as 60% of the total) to stimulated saliva but less to resting salivary flow. It secretes a serous substance that contains no mucins but is rich in amylase and in proline-rich proteins. The submandibular and sublingual glands contribute more to the resting salivary flow rate and their saliva is rich in mucins. Mucins are high-molecular-weight glycoproteins with an elongated structure that contribute significantly to the viscoelastic behavior of saliva.

Amylase is the single most abundant protein in saliva and is involved in the initial digestion of starch-containing foods. Because of this, when the food under study is rich in starch the oral digestion step has been taken into consideration, as in studies with potatoes (Parada and Aguilera, 2009), pasta (Petitot, et al., 2009) or a starch-based custard dessert (Engelen, et al., 2003). During insalivation, which is particularly important for starchy semi-fluid foods, the rapid action of salivary amylase reduces the viscosity (Hoebler, et al., 2002).

Since the activity of salivary amylase is greatly reduced as soon as it reaches the acidic environment of the stomach, pancreatic amylase is much more likely to be involved in the digestion of starch in foods, in the opinion of Carpenter (2013). Also, in studies on pancreatic digestion pancreatic activity has been found to overwhelm salivary amylase activity, so Woolnough, Bird, Monro, and Brennan (2010) considered that oral digestion can be neglected.

Structural variability among foods can give rise to different rates of starch hydrolysis as a consequence of their different degree of accessibility to enzymes. Hoebler, Devaux, Karinthi, Belleville, and Barry (2000) found that in cereal-based products, about 50% of bread starch and 25% of pasta starch were hydrolyzed during the short period of oral processing. Butterworth, Warre, and Ellis (2011) stated that some uncertainty still remains with regard to the physiological significance of salivary amylase. According to Nantanga, Chan, Suleman, Bertoft, and Seetharaman (2013), who worked with cooked starch treated with saliva from six participants at equal activity under conditions

mimicking oral digestion, further research is needed to understand whether the hydrolyzate structure obtained, rather than the level of amylase activity, is the determinant of oral digestion of starch.

Lingual lipase is another salivary digestive enzyme. This enzyme breaks down a small fraction of dietary triglycerides in the oral cavity and stomach. However, lingual lipase is considered to be of limited significance in lipolysis for healthy individuals (Pedersen, Bardow, Jensen, and Nauntofte, 2002).

Many factors such as the flow rate, time of day, type and size of the salivary glands, duration and type of the stimulus, diet, drugs, age, sex and blood type affect the amount and composition of saliva secreted in humans (Vingerhoeds, Blijdenstein, Zoet, and Van Aken, 2005). When subjects display marked differences in their saliva composition their potential for oral interaction with food may differ, as in the subsequent release and perception of taste compounds (Neyraud, Palicki, Schwartz, Nicklaus, and Feron, 2012). The role of saliva in the perception of the taste, flavor and texture of foods has been also taken into account. During consumption, food mixes with saliva, so it is not the food itself but the products of its interactions with saliva which we perceive. Consequently, the role of saliva in perception appears to be essential (Neyraud, Palicki, Schwartz, Nicklaus, and Feron, 2012). For example, the action of the enzyme α-amylase, initiating the digestion of starch, can result in a drop in the perceived thickness of certain food products, as commented above. In addition, the large salivary proteins influence lubrication and hence, possibly, the perception of attributes such as smoothness and astringency (Engelen, et al., 2003). Saliva also plays a major role in the detection and perception of fat, as it is directly involved in the orosensory detection of triglycerides and their hydrolysis products (Feron and Poette, 2013).

The perception of texture attributes is strongly related to the way the food is processed during food intake, mastication, and swallowing and during the cleaning of the mouth after swallowing. It is also modulated by the interaction with other basic properties, such as taste and aroma attributes. The most important dynamic feature of an eating

process in association with texture perception is the change of length scale. Understanding the in-mouth processes at the colloidal scale turned out to be essential to grasping the interplay between perception, oral physiology and food properties. In this regard, two aspects have to be taken into account: first, food particles are chewed and reduced in size from centimeter scale initially to sub-millimeter scale at the point of swallowing, and second, a thick film of food-saliva mixture between oral surfaces (i.e. tongue and hard palate) is gradually reduced to a final thin film of a few micrometers (Van Vliet, Van Aken, de Jongh, and Hamer, 2009). These changes have important implications for the perceived texture and, more importantly, for the underpinning mechanisms applied for texture perception (Chen and Stokes, 2012).

Saliva acts as a buffering system (De Almeida, Grégio, Machado, de Lima, and Azevedo, 2008), affecting the degree to which sourness is perceived. Significant decreases in perception with increasing salivary flow rates were observed for citric acid and sodium chloride. Although this can partially be explained by a dilution effect, bitterness and sweetness remained unaffected by the salivary flow conditions (Heinzerling, Stieger, Bult, and Smit, 2011).

In vitro scenario

The important role of saliva in the oral processing of foods makes it clear that saliva needs to be used in *in vitro* studies. Exact reproduction of human saliva is especially difficult because of its complexity, unstable character and inter-individual variability, as well as its dependence on the type of saliva stimulation (Roger-Leroi, Mishellany-Dutour, Woda, Marchand, and Peyron, 2012). In addition, its complex composition varies over the day. It is thus only possible to imitate an average saliva composition (Gal, Fovet, and Adib-Yadzi, 2001).

The compositional complexity of simulated saliva fluids (SSF) used in the literature varies widely depending on the objectives of the research. Some researchers use a simple

buffer solution without any additional component to simulate oral conditions. Others use simulated saliva fluids that contain many of the components found in human saliva, such as acids, buffers, minerals, mucins and enzymes (McClements and Li, 2010). In the food technology field, in studies where digestion processes are to be emulated, the SSF should be as similar as possible to naturally occurring saliva. For example, Van Ruth, Grossmann, Geary, and Delahunty (2001) found that significant differences in the volatility of compounds when artificial saliva or water was added indicated that saliva replacement was inadequate in aroma release studies.

Some recipes for preparing simulated saliva solutions can be found in the literature (Björklund, Ouwehand, and Forssten, 2011; Gal, Fovet, and Adib-Yadzi, 2001; Leung and Darvell, 1997; Mishellany-Dutour, et al., 2011; Sarkar, Goh, and Singh, 2009).

As mentioned above, during oral processing the effect of saliva on the food can lead to impressive changes in rheological and other related properties. Saliva acts as a glue, holding the fragmented solid particles together. The lubrication or tribological qualities of saliva are central to many of its food processing roles, such as facilitating the swallowing of the food bolus and its transport through the body. Surprisingly, according to Bongaerts, Rossetti and Stokes (2007) there are few studies on the lubricating properties of whole human saliva in terms of how it is influenced by surface roughness or surface compliance.

The results from the *in vitro* study carried out by Engelen, et al., (2003) suggested that for a semi-solid food like custard, breakdown by α-amylase in the mouth is limited because the time it spends in the mouth (about 4-5 seconds) is too short for the saliva and custard to become properly mixed, so the effects of breakdown are undoubtedly present but not extensive. In contrast, during mastication of solids the mixing is more vigorous, and probably more efficient, enabling the enzyme to come into contact with more starch particles rather than being confined to the initial surface. Therefore, enzyme activity is more valuable for breaking down solid foods that remain in the mouth for a longer time, such as bread and other cereal products. Using a mixing simulator, Prinz,

Janssen and de Wijk (2007) demonstrated with video images of the recovered samples that saliva-induced structure breakdown exerts a dramatic effect on the viscosity of starch-based custards despite the incomplete mixing of custard and saliva that occurs *in vivo*. Several authors (Ferry, Hort, Mitchell, Lagarrigue, and Pamies, 2004; Sorba and Sopade, 2013) used the Rapid Visco Analyser (Newport Scientific, Warriewood, Australia) to measure the decrease in viscosity over time on adding amylase to starch pastes.

To quantify the susceptibility of starch-based semisolid foods to salivary α-amylase and the rate of enzyme-induced structure breakdown, Janssen, Terpstra, de Wijk, and Prinz (2007) developed a measuring system, the Structure Breakdown Cell (SBC), consisting of a helical rotating vane. This system aims to achieve near-perfect mixing with saliva while monitoring the resulting change in the torque required to rotate the vane through the food sample. The use of complex geometries in rotational rheometry offers numerous benefits for the mechanical characterization of saliva-induced breakdown, compared with the conventional geometries used in rotational rheometry, as it is more effective in simulating the mixing process in the mouth and tracking the evolution of the structure.

"Melting", defined by Engelen, et al., (2003) as the rate of decrease in thickness and spreading of the product in the mouth, is a sensory attribute that could be affected considerably by the presence of salivary enzymes. Since starch is broken down by the salivary enzyme α-amylase, sensory melting could be affected more by saliva than by water. However, why does saliva affect melting more than an α-amylase solution? A possible reason is that the α-amylase in the water solution is less active than in saliva. Early work by Erickson (1992) has provided support for this explanation by showing that the presence of chloride ions is essential for α-amylase to reach full activity. The molecular basis for this effect was further studied by Qian, Ajandouz, Payan, and Nahoum (2005). Studies performed with mice have indicated that α-amylase is more active in saliva than in the gland. It can therefore be speculated that other components

of saliva (e.g. hydrolyzing enzymes) or products originating in microorganisms can also influence the activity of salivary α-amylase. The choice of kinetic models for studying starch amylolysis *in vitro* is also a subject of some controversy (Butterworth, Warren, and Ellis, 2011).

As described above, several masticatory apparatuses have been employed to date to produce a food bolus with the closest possible resemblance to that resulting from *in vivo* chewing. To achieve the goal of producing the expected food bolus, Roger-Leroi, Mishellany-Dutour, Woda, Marchand, and Peyron (2012) stated that it is mandatory to develop artificial saliva with chemical and rheological characteristics that are close to those of human saliva and proposed a formulation that satisfies the major requirement of viscosity.

Bolus formation

Bolus characterization

Understanding the dynamic changes in food structure that take place during oral processing is a key factor for texture design. A knowledge of bolus rheology is one of the more important approaches to such understanding. From a rheological point of view, the bolus should behave as a weak gel for ease of mastication and swallowing. A homogeneous and cohesive state allows the mass flow of bolus through the pharyngeal phase, increasing swallowing comfort (Ishihara, Nakauma, Funami, Odake, and Nishinari, 2011).

Prinz and Lucas (1997) stated that the decisive factor for swallowing should be the combined effect of particle size and oral lubrication with the participation of saliva. According to these authors the optimum moment for swallowing is defined in terms of a peak cohesive force between food particles: a swallow should be triggered when it is sensed that a batch of food particles is binding together under viscous forces so as to

form a bolus. As Chen and Lolivret (2011) commented, experimental evidence suggests that rather than maximum consistency, appropriate flow-ability is a likely trigger point for swallowing. They proved this with different food boluses expectorated by volunteers and simulated boluses made with SSF, using a tensile method in which the boluses were stretched vertically and the force at separation was recorded as a function of stretching distance. Some other experimental evidence in the literature supports this premise. With the help of magnetic resonance imaging (MRI) and videofluorescence techniques, for example, Buettner, Beer, Hannig, and Settles (2001) observed that a food bolus became highly stretched or extensionally deformed during swallowing. This was further confirmed by Kumagai, Tashiro, Hasegawa, Kohyama, and Kumagai (2009), who observed the velocity profile of various bolus flows in the pharynx by the Ultrasonic Pulse Doppler method. Pereira, Gavião, Engelen, and van der Bilt (2007) demonstrated that the addition of fluid could significantly reduce the number of chewing cycles for some dry foods because of enhanced bolus flowability in the presence of extra fluid. The importance of bolus stretchability was also confirmed by Seo, Hwang, Han, and Kim (2007) on investigating sensory and instrumental slipperiness and compliance of foods during swallowing by human subjects using non-invasive techniques. All this experimental evidence suggests that maximum consistency is not a criterion for the point of swallowing and that the key criterion in swallowing is stretchability (Chen and Lolivret (2011).

Peyron, et al., (2011) were also of the opinion that particle size and bolus hardness are not the only decisive factors in the swallowing threshold, since d50 and hardness values barely change after the middle of the masticatory sequence. Particle size (Peyron, Mishellany, and Woda, 2004), lubrication by saliva and bolus wetting (Gavião, Engelen, and Van der Bilt, 2004) are initial contributing factors to the final rheological values obtained for the swallowing threshold.

On the other hand, the several critical thresholds for swallowing may not be reached simultaneously in a bolus: the swallowing threshold is probably an integrative process

that combines the perceptions of the various bolus properties enabling swallowing (Peyron, et al., 2011). Evidently, the swallowing threshold comprises many components. As formation of a swallowable bolus is assumed to be a key driving constraint, to avoid dangerous aspiration of small particles, each individual uses his or her physiological resources to chew a given food until a safe bolus is made and the swallowing threshold is reached.

Current techniques for studying bolus rheology

Ishihara, Nakauma, Funami, Odake, and Nishinari (2011) listed a number of techniques for inspecting the physiology of swallowing, such as videoendoscopy, the ultrasonic (ultrasound) method and acoustic analysis, not only for clinical studies but also for texture studies (Kumagai, Tashiro, Hasegawa, Kohyama, and Kumagai, 2009; Saitoh, et al., 2007). Other techniques such as Doppler velocimetry might allow direct information concerning bolus velocity to be obtained without the need to track the boundaries of a bolus (e.g. in videofluoroscopy) (Engmann and Burbidge, 2013).

Videofluorography (VI) (Okada, Honma, Nomura, and Yamada, 2007; Ono, Hori, Masuda, and Hayashi, 2009) and the real-time MRI technique (Buettner, Beer, Hannig, and Settles, 2001; Kulinna-Cosentini, Schima, and Cosentini, 2007), both developed for medical applications, have been used successfully to provide insight into the visual evidence of food transformation and transportation at different stages of oral processing (Figure 3). It is foreseeable that the use of such imaging techniques, together with the classic mechanical and sensory methods, will be a powerful combination in characterizing food texture (Chen, 2009).

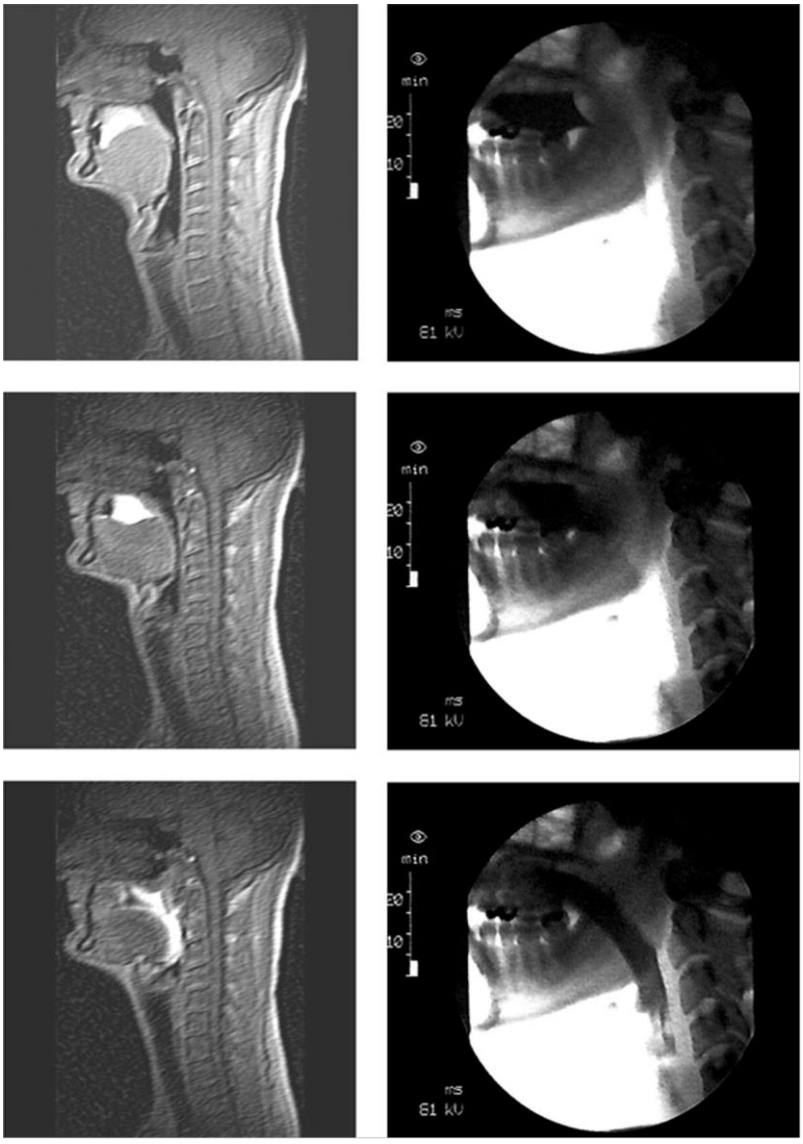

Figure 3. Oral and pharyngeal segments of a subject. Dynamic sequence in the sagittal view shows a normal peristaltic wave with propagation of the bolus. Upper: during rest; middle: at the beginning of swallowing; bellow: complete swallowing (velopharyngeal closure prevents nasal penetration). Left: videofluorography images; right: magnetic resonance images.

VI is currently one of the best ways of evaluating the swallowing function because it enables visualization of the movement of all the anatomical components related to chewing and swallowing (Ono, Hori, Masuda, and Hayashi, 2009). These components include the lips, cheeks, jaw, tongue, hyoid bone, pharynx, larynx, and esophagus. This technique also makes it possible to visualize the passage of a food or drink containing a contrast medium (typically barium sulfate powder or soluble iodine complexes) in two dimensions (sagittal and frontal). However, its application involves radiation exposure and is therefore limited to patients with severe dysfunction in chewing and swallowing.

Kulinna-Cosentini, et al., (2007) have proved that MRI is a feasible, non-invasive method for swallowing evaluations because it has excellent potential for providing fully three-dimensional static images of the gastroesophageal junction and its anatomical structures involved in swallowing, and their degree of variation. In comparison to VI, MRI offers several advantages: it provides a better evaluation of soft tissues, the ability to acquire various series of images with excellent time resolution, and – if adequately processed, which is no trivial challenge (Engmann and Burbidge, 2013) – the possibility of resolving three-dimensional details from different angles without changing the patient's position, but its main advantage is the lack of ionizing radiation to the patient.

Currently, these physiological measurements suffer from limitations. For instance, videoendoscopy presents low quantitative performance because of the 2D projection character of the technique. The ultrasonic method is applicable preferably to females, as they lack the thyroid cartilage which could interfere with the transit of the ultrasonic pulse. Acoustic analysis is an alternative approach for recording swallowing profiles that has been utilized for diagnostic purpose as a non-invasive method in both healthy and dysphagic individuals (Lazareck and Moussavi, 2004), but has been used less in the field of food technology.

Despite the aid of the above techniques, difficulties in measuring the rheological properties of boluses still remain owing to personal physiological differences, including mastication ability and saliva secretion, which sometimes lead to poor reproducibility of

experiments. This could be one of the reasons why more research on bolus rheology has been conducted from a physiological perspective, in medical research, than by food scientists from the food technology point of view. Different stages of the swallowing mechanism, which involve different fluid mechanics regimes (from creeping flow to turbulent flow conditions) depending on the boundary conditions and bolus rheology, need to be studied (Engmann and Burbidge, 2013). It is important for food scientists to establish experimental procedures to prepare a bolus *in vitro* with high reproducibility (Ishihara, Nakauma, Funami, Odake, and Nishinari, 2011).

In silico **scenario**

The last few decades have been witnessing the rise of alternative research models, the so-called *in silico* approaches, using computational environments. The expression *in silico*, imitating the common biological Latin expressions *in vivo* and *in vitro*, refers to performing experiments using computers (Noori and Spanagel, 2013).

In silico models are gaining importance in the food science and technology field. The development and validation of such models require more and more in-depth knowledge of the physiological mechanisms of mastication. Mathematical models of oral processing are proposed, generally based on geometrical considerations, to emulate certain physiological features during mastication. *In vitro*, *in vivo* and *in silico* approaches have been compared when studying the dynamics of the perception of saltiness and solute release from model dairy products of varying composition and rheological behavior (Panouillé, et al., 2010). In another study, the mechanical human mastication of commercial breakfast cereals was modelled by using X-ray tomography data to quantify crack propagation in brittle airy products (Hedjazi, Guessasma, Martin, Della Valle, and Dendievel, 2012). Le Révérend, Loret and Hartmann (2012) studied how force is distributed along the mandibular arch and how force distribution is related to the space available to fit foods between the teeth.

In silico models have found a number of applications in characterizing mastication. Of special interest are the studies on aroma release and its particularities, some of which are more closely related to oral processing. Tréléa, et al., (2008) described a mechanistic mathematical model for aroma release in the oropharynx reaching the nasal cavity during consumption of flavored yogurt. The model was based on the physiology of the swallowing process and was validated via mass spectrometry measurements of aroma concentration. According to the authors, this work constitutes a first step towards computer-aided product formulation. An elastohydrodynamic model of swallowing was developed by De Loubens, Magnin, Doyennette, Tréléa, and Souchon (2011) to quantify physical mechanisms that explain pharyngeal mucosa coating. Considering complex physiological conditions, the results were applied to predicting aroma release kinetics. Using a coupled biomechanical-SPH (Smoothed Particle Hydrodynamics) model, Harrison et al. (2012) studied food breakdown and flavor release during mastication. SPH is a numerical method that allows complexities such as fluid free surfaces or solid fracture and interactions with complicated deforming boundaries and chemical dynamics to be modelled. De Loubens, Magnin, Doyennette, Tréléa, and Souchon (2010) developed an experimental device in order to gain insight into the biomechanics of the pharyngeal peristalsis; the results demonstrated the influence of food bolus viscosity on flavor release. Déléris, et al., (2012) developed a mathematical model of mass transfer in the mouth during eating that made it possible to identify the parameters and properties associated with the product, or with the subject eating the product, that explain stimuli release in the mouth. To examine the effect of various oral and gastric factors, the disintegration profiles obtained by measuring the mass retention of different artificially masticated boluses were fitted to a linear-exponential model, demonstrating that the bread structure and moisture content were key features controlling the process (Bornhorst and Singh, 2012).

Model predictions have generally been in good agreement with the experimental data, so, *in silico* approaches could be a promising tool in food oral processing studies.

Conclusions

While we are eating, a whole series of transformations take place in the mouth before swallowing. Thanks to research in a number of very different disciplines we are gradually but constantly learning more about these processes, and in greater detail.

Physically, the food is broken down in the mouth into smaller particles in preparation for the following stages: gastric and intestinal digestion. Physiologically, the processes that take place in the mouth must be viewed from three different angles. The first is the beginning of starch digestion, thanks to the α-amylase in the saliva, the second is the chewing process (number of chews, chewing time, chewing frequency, bite force, fracture energy, oral – or simulation chamber – temperature, quantity and type of saliva) in relation to the food involved (size, shape, viscosity, cohesiveness, hardness, stickiness) and the third is that the particles obtained have to be formed into a cohesive, hydrated bolus that can be swallowed safely and comfortably.

While it is practically impossible to reproduce such a complicated mechanism as in-mouth processing, there are tools that can achieve similar results. Researchers should ask themselves which steps, in relation to the food in question and the parameters to be analyzed, necessarily precede the procedures they wish to apply in their study.

The choice of one method or another will depend on the physical state of the food (liquid or solid), and its initial mechanical and structural properties. For example, a researcher who wishes to study how a food's texture affects its consumer acceptability needs to consider the in-mouth sensations aroused by all the chewing and insalivation mechanisms involved through to formation of the bolus to be swallowed, and not merely measure some single mechanical property as an indicator of texture, while the researcher who wants to know how the lipids contained in a given food could be digested by pancreatic lipases needs to consider which of the structural breakdowns the food undergoes is responsible for releasing the fat from the matrix. In addition, a cohesive, consistent bolus has many different properties to those of a food that is simply

minced and diluted in water or in artificial saliva. The question is: do all these differences affect the results of my study?

The path of research related to the oral processing of food is very broad and many crossroads and shortcuts may be encountered along the way. Only a profound knowledge of the processes and a clear vision of the aims of the study will make it possible to take the right course.

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Objetivos

OBJETIVO GENERAL

El objetivo general de esta tesis es estudiar la capacidad saciante de diferentes productos lácteos, formulados con adición de distintos ingredientes, y su relación con la estructura y percepción sensorial.

OBJETIVOS PARCIALES

- Estudiar cómo la consistencia modula la percepción de la capacidad saciante de alimentos con alto contenido en proteína.

- Estudiar cómo afecta la dinámica de la trayectoria oral a las expectativas de saciedad y a la aceptabilidad por parte del consumidor analizando los cambios físicos y estructurales durante la digestión oral *in vitro*.

- Analizar el efecto de la adición de diferentes proteínas lácteas sobre las características sensoriales y su relación con las expectativas de saciedad.

- Estudiar la relación entre la sensación de astringencia y las características tribológicas en yogur y su atenuación por adición de un almidón físicamente modificado.

Estructura de la tesis

La presente tesis doctoral se enmarca dentro del proyecto del Ministerio de Ciencia e Innovación titulado "Formulación de alimentos con proteínas e hidrocoloides de efecto saciante. Reología, estructura y percepción sensorial y del consumidor. Estudio de trayectoria oral y digestión *in vitro*" (AGL2012-36753-C02-01).

El desarrollo de alimentos que aporten saciedad es uno de los puntos de gran interés para el control del apetito y el peso. Existen determinados componentes que, de forma demostrada, proveen capacidad saciante. Un gran número de trabajos han estudiado estos componentes o ingredientes desde un punto de vista clínico. Sin embargo, existen pocos trabajos que estudien cómo actúan en un sistema alimentario real: cuáles son las interacciones con los otros componentes más frecuentes de los alimentos (proteínas, grasa, fibras o hidratos de carbono) y cómo modifican su reología, textura, estructura, procesamiento oral y en consecuencia su palatabilidad y aceptación. En la introducción de esta tesis, ya se han abordado estos temas, y se han adjuntado dos artículos de revisión correspondientes a los mecanismos de procesamiento oral "*in vitro*" y la relación de las proteínas con la saciedad.

En cuanto a los resultados, la tesis aborda dos grandes bloques: uno, en el que se estudian distintos ingredientes con capacidad de modular la consistencia, estructura, trayectoria oral y percepción sensorial de distintos alimentos de origen lácteo; en él se analizan qué cambios químicos, físicos y sensoriales ocurren en los sistemas seleccionados en relación con la capacidad saciante esperada del alimento final. El segundo bloque se centra en el diseño de yogures con proteína añadida. Se estudia cómo afecta la adición de distintas proteínas lácteas a la microestructura, reología y percepción sensorial de cada una de las muestras. Además, de acuerdo con los resultados obtenidos en el primer bloque se añaden también hidrocoloides con el fin de mejorar la consistencia y las propiedades sensoriales de las muestras y evitar sensaciones como la astringencia producida por la adición de proteínas.

Un primer estudio tuvo como hipótesis de partida de que batidos lácteos (espesados con distintos hidrocoloides) para producir viscosidades similares durante la

manipulación previa a la ingesta, pueden generar sensaciones de "saciedad esperada" diferentes. Se analizaron las propiedades reológicas, viscosidad, consistencia, módulo elástico y viscoso antes y después de la digestión oral *in vitro*, así como los cambios microestructurales producidos y su relación con los parámetros reológicos. Se analizaron los descriptores sensoriales que definen cada muestra y en qué medida éstos influyen en la aceptabilidad por parte de los consumidores; para ello se aplicó la técnica C.A.T.A. ("check-all-data-apply"). Todos los resultados se relacionaron con la intensidad de capacidad saciante esperada que percibieron los consumidores. Este trabajo se publicó con el título "Hydrocolloids for enhancing satiety: relating oral digestion to rheology, structure and sensory perception" en la revista "Food hydrocolloids".

Se realizó un segundo estudio experimental sobre un postre lácteo adicionado esta vez con un mismo hidrocoloide, hidroxipropilmetilcelulosa (HPMC), pero en concentraciones crecientes. El postre se formuló con dos variantes: con el doble de leche en polvo y con adición de nata. para conocer la influencia de otros factores de composición sobre la capacidad saciante esperada. Se realizó una caracterización reológica para estudiar las diferencias estructurales que existían entre las distintas muestras. Con el fin de demostrar el efecto de cada uno de los factores (sólidos lácteos, nata, y nivel de HPMC) sobre la saciedad esperada de las muestras, se utilizaron escalas visuales de alimentos de comparación para evaluar la saciedad esperada relativa (modificado de Brunstrom and Rogers 2009). A su vez se llevó a cabo un estudio sensorial mediante el uso de una técnica relativamente nueva llamada "Temporal Dominance of Sensations" (TDS). Los resultados conjuntos se relacionaron de modo muy interesante para profundizar en las características que los consumidores asocian a saciedad. El trabajo ha sido publicado con el título "Relating HPMC concentration to elicited expected satiation in milk-based desserts" en la revista "Food Hydrocolloids".

En el segundo bloque se trabajó con yogur. Se sabe que la proteína es el macronutriente con mayor capacidad saciante, por lo que se decidió añadir el doble de la cantidad de

proteína que habitualmente tiene un yogur, de distintas fuentes proteicas. Se evaluaron 5 yogures añadiendo: una cantidad extra de leche desnatada en polvo, concentrado de proteína de suero, caseinato cálcico o mezcla (50:50) de proteína de suero concentrado con caseinato cálcico; por último, se realizó un yogur control sin adición de proteínas. Se analizaron las diferencias en firmeza, así como las diferencias en la percepción sensorial (a través de las preguntas CATA) relacionándolo con la capacidad saciante esperada y valoraciones hedónicas de las distintas muestras. Además, a las preguntas C.A.T.A. se añadió un "yogur saciante ideal" para mostrar la dirección que hay que seguir para la reformulación del yogur con proteína añadida. El trabajo ha sido publicado con el título "How is an ideal satiating yogurt described? A case study with added-protein yogurts" en la revista "Food Research International".

En la misma línea se decidió ahondar más en el sistema yogur y se evaluó la adición de distintas proteínas lácteas y almidón físicamente modificado. Este último ingrediente puede mejorar la percepción de textura cremosa y permite obtener un etiquetado limpio del producto. Se estudió cómo afectaban estas adiciones a las propiedades microestructurales, reológicas, de viscosidad y consistencia del producto, así como al tránsito oral y, en consecuencia, a la aceptación final por parte del consumidor. Para determinar la capacidad saciante, se llevaron a cabo pruebas sensoriales, en las que participaron más de cien consumidores. Se pudo observar que la adición de almidón para las distintas muestras aumentó significativamente la percepción de su capacidad saciante, por lo que su utilización podría ser una herramienta útil en el diseño de este tipo de productos lácteos. El trabajo se publicó con el título "Yogurts with an increased protein content and physically modified starch: rheological, structural, oral digestion and sensory properties related to enhanced satiating capacity" en la revista "Food Research International".

Los estudios anteriores demostraron que uno de los mayores problemas a los que se enfrenta la adición de proteínas es el desarrollo de astringencia. La astringencia se describe normalmente como un grupo de sensaciones complejas que implica la

percepción de sequedad y rugosidad de las superficies orales. Se sabe que la astringencia en los productos lácteos ácidos está relacionada con las interacciones de la proteína del suero con la saliva, por lo que podría evaluarse mediante el estudio del comportamiento dinámico de la mezcla de proteína de suero-saliva en la boca durante la ingestión. Por ello se pensó en realizar estudios de tribología de estos sistemas. Los resultados ayudarían a entender el fenómeno que ocurre con el fin de desarrollar sistemas con una palatabilidad aceptable. En el mismo conjunto de muestras se llevó a cabo también un "flash profiling" para poder relacionar los parámetros tribológicos con la percepción sensorial. El trabajo se publicó bajo el título "The role of starch and saliva in tribology studies and the sensory perception of protein-added yogurts" en la revista "Food and Function".

En resumen, se estudiaron todos los aspectos que ayudan a desarrollar un producto lácteo saciante, incluyendo el análisis de los ingredientes responsables de la modulación de la consistencia y la percepción sensorial de los distintos sistemas. Además, se diseñó y estudió un alimento semisólido adecuado y versátil para la elaboración de un alimento saciante. Las publicaciones científicas derivadas de los resultados de esta tesis se presentan a lo largo de los dos capítulos en el siguiente orden:

Capítulo 1:

Hydrocolloids for enhancing satiety: Relating oral digestion to rheology, structure and sensory perception. Morell, P., Fiszman, S. M., Varela, P., and Hernando, I. (2014). Food Hydrocolloids, 41, 343-353. (DOI: 10.1016/j.foodhyd.2014.04.038).

Relating HPMC concentration to elicited expected satiation in milk-based desserts. Morell, P., Ramírez-López, C., Vélez-Ruiz, J. F., and Fiszman, S. (2015). Food Hydrocolloids, 45, 158-167. (DOI: 10.1016/j.foodhyd.2014.11.011).

Capítulo 2:

How is an ideal satiating yogurt described? A case study with added-protein yogurts. Morell, P., Piqueras-Fiszman, B., Hernando, I., and Fiszman, S. (2015). Food Research International, 78, 141-147. (DOI: 10.1016/j.foodres.2015.10.024).

Yogurts with an increased protein content and physically modified starch: rheological, structural, oral digestion and sensory properties related to enhanced satiating capacity. Morell, P., Hernando, I., Llorca, E., and Fiszman, S. (2015). Food Research International, 70, 64-73. (DOI: 10.1016/j.foodres.2015.01.024).

The role of starch and saliva in tribology studies and the sensory perception of protein-added yogurts. Morell, P., Chen, J., and Fiszman, S. (2017). Food & Function, 8, 545-553. (DOI: 10.1039/C6FO00259E).

Resultados y discusión

Capítulo 1:

Ingredientes con capacidad de modular la consistencia, estructura, trayectoria oral y percepción sensorial de distintos alimentos de origen lácteo

Hydrocolloids for enhancing satiety: relating oral digestion to rheology, structure and sensory perception

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Abstract

Satiety expectations can be closely related to the structural changes that take place in the mouth. An important role of hydrocolloids is to impart viscosity, which has a key effect on the feelings of richness, mouth coating and fullness. In this study, native and modified corn starch, λ-carrageenan and guar gum were used to formulate milkshakes. Expected satiety was rated (106 consumers) and the perception of various attributes was studied. The rheological properties of the milkshakes without and with added saliva were analysed and observed with a light microscope during *in vitro* oral digestion.

Disintegration of the swollen starch granules by saliva was observed mainly in the modified starch sample. The structure of the milkshakes prepared with λ-carrageenan and guar gum was preserved better. It could be hypothesized that the starch would provide lower expected satiety due to the extensive in-mouth disintegration. However, the sensory analysis showed that the modified starch milkshakes obtained the highest expected satiety scores, with consumers finding them homogeneous, thick in the mouth and very creamy. These results suggested that consumers related satiety more with the thick and creamy characteristics at the very start of the consumption than with the loss of structure in mouth. Sensory properties affect the assessment of the satiating capacity, especially texture, which is directly related to the orosensory exposure and, therefore, to the feeling of fullness that the milkshakes elicit. The present study casts light on the factors affecting in-mouth perception of different hydrocolloids used to design foods with enhanced satiety.

Keywords: milkshake, hydrocolloid, satiety, rheology, microstructure, sensory perception, *in vitro* oral digestion.

Introduction

Nowadays, opportunities to consume energy-dense, unhealthy snacks are encouraged by the obesogenic environment that has developed in the western world, particularly among adolescents (Fiszman and Varela, 2013). One of the main trends in weight management is to develop foods and beverages that provide satiety or hunger satisfaction (Tecklenburg, 2009). A wide range of ingredients is available for the creation of satiety-related products. Hydrocolloids have been studied for over 30 years as a means to provide satiety and blunt glucose absorption (Wong, 1974; Kay and Stitt, 1978; Wilmshurst and Crawley, 1980).

Hydrocolloids provide viscosity and play a role in developing foods with high satiating capacity. An enormous number of studies have assessed the satiating capacity of a long list of soluble gums that are viscous in solution. The effect on the feeling of satiety of most of this type of compounds which impart viscosity to their solutions is caused by mechanisms which are related with slowing down enzyme action efficacy and/or with gastric antrum distension (as they absorb large quantities of liquid) and/or delaying gastric emptying, which, in turn, may increase or prolong the satiety signals from the stomach (Fiszman and Varela, 2013). Based on availability to impart viscosity, almost any gum could act as a potential satiety-enhancing agent. From a compositional point of view there are basically two types of gums, with some variation within each type: neutral hydrocolloids, including guar, locust bean gum and konjac, which simply hydrate to a fully extended form, creating viscosity through polymer entanglement, and charged hydrocolloids such as alginate, pectin, carrageenan and gellan gum, which also develop maximum viscosity with full hydration in water but may also develop additional viscosity through association with mono- and divalent ions and, in some cases, hydrogen ions (Aimutis, Paeschke, Sun, Johnson, Sweeney, Patist, Vander Pol, and Finocchiaro, 2007; Wolf, Blidner, Garleb, Laie, and Schenz, 2007; Boers, Strom, and Wiseman, 2008). If these hydrocolloids are dispersed in a milk beverage, some calcium ions released from the milk micelles can help them to form gels during acidification in

the stomach. The meals formulated with this type of hydrocolloid form lumps in the stomach, producing large volumes. Although gastric emptying is similar, the sense of fullness for the same gastric volume is significantly greater than for meals without any hydrocolloid (Hoad, Rayment, Spiller, Marciani, Alonso, Traynor, Mela, Peters, and Gowland, 2004; Fiszman and Varela, 2013).

Perceived satiety is limited in the case of foods that can be consumed quickly and with little effort, such as liquid or semiliquid foods (Hogenkamp and Schiöth, 2013). It has been suggested that greater an increased length or intensity of orosensory exposure (i.e. food present in the oral cavity) would contribute to the development of satiety and further control of the energy intake (McCrickerd, Chambers, Brunstrom, Norton, Mills, and Yeomans, 2012). Oral exposure is affected by the characteristics of a food (de Wijk, Zijlstra, Mars, de Graaf, and Prinz, 2008; Hutchings, Bronlund, Lentle, Foster, Jones, and Morgenstern, 2009) and viscosity is one important attribute to take into account (Viskaal-van Dongen, Kok, and de Graaf, 2011). Related properties such as the perception of mouthcoating or creaminess that some hydrocolloids impart during consumption could be interesting and worth researching. Other factors that determine orosensory exposure include bite size, oral processing time and chewing frequency (Zijlstra, de Wijk, Mars, Stafleu, and de Graaf, 2009; Bolhuis, Lakemond, de Wijk, Luning, and de Graaf, 2011). Hogenkamp, Brunstrom, Stafleu, Mars and de Graaf (2012) observed that an increase in the perceived thickness of several dairy products resulted in a consistent increase in the expected satiating capacity of the foods.

Consequently, expectations about the satiety effects of a specific food are based, among others, on its texture attributes (Sørensen, Møller, Flint, Martens, and Raben, 2003; Hogenkamp, Mars, Stafleu, and de Graaf, 2012). In this sense hydrocolloids, including starches, offer a complete field of possibilities for arousing these sensations. Attributes such as sliminess or creaminess are important in selecting one hydrocolloid rather than another for thickening a liquid or semisolid food to provide good satiating capacity.

However, almost no attention has been paid to relating the distinctive oral sensations communicated by hydrocolloids with their potential to elicit expectations of satiety.

Based on the foregoing, the authors of the present study hypothesized that the oral digestion (as in the case of starches) and oral processing of different hydrocolloids could play a critical role in the sensations related to perceptions of expected satiety (Morell, Hernando, and Fiszman, 2014).

The aim of the present work was to analyse the effect of adding different hydrocolloids imparting similar initial viscosities during pouring or handling to milkshakes on the expected satiety they elicited in consumers. Rheological and microstructural studies before and after an *in vitro* oral digestion were performed and analysed in relation with oral perceptions. Additionally, sensory and non-sensory attributes of the milkshakes were evaluated with a "*check-all-that-apply*" consumer study.

2. Materials and methods

2.1. Milkshake ingredients

The ingredients used in the preparation of the milkshakes were powdered skimmed milk (kindly supplied by Central Lechera Asturiana, Asturias, Spain), native corn starch (C Gel 03401), hydroxypropyl distarch phosphate (C PolarTex 06748) and guar gum (Viscogum MP41230) (all three from Cargill, Inc., Minneapolis, Minn., U.S.A.), λ-carrageenan (Secolacta BR, from Hispanagar S.A. Burgos, Spain), aspartame and acesulfame K (both from EPSA Aditivos Alimentarios, Valencia, Spain), cochineal carmine (Roha Europe S.L.U., Valencia, Spain), strawberry flavour (Firmenich S.A., Barcelona, Spain) and distilled water.

2.2. Artificial saliva

Artificial saliva was prepared according to the method described by Mishellany-Dutour, Peyron, Croze, François, Hartmann, Alric, and Woda (2011), with some modifications. All the reagents were of analytical grade. The components were sodium bicarbonate (5.208 g/L), potassium phosphate dibasic trihydrate (1.369 g/L), sodium chloride (0.877 g/L), potassium chloride (0.477 g/L), calcium chloride dehydrate (0.441 g/L), mucin from porcine stomach type II (PGM) (Sigma, M2378) (2.16 g/L), α-amylase type VI-B from porcine pancreas (Sigma, A3176) (8.70 g/L (200.000 units)) and HPLC grade doubly-distilled water. To perform the *in vitro* oral digestion, the ratio of saliva to sample was1:4 (Sanz and Luyten, 2006).

2.3. Sample preparation

Four milkshakes consisting of 100 mL water, 10 g powdered skimmed milk, 0.0175 g aspartame, 0.0075 g acesulfame K, 0.001 g cochineal carmine and 0.1 mL strawberry flavour were each formulated with a different hydrocolloid.

The quantity of hydrocolloid was selected through a preliminary study to obtain similar viscosities in the milkshake samples during pouring or handling. For this purpose, the apparent viscosity of the milkshakes was measured using a viscometer (Haake Viscotester 6 R Plus, Thermo Scientific, Walthman, Mass., U.S.A.), equipped with spindle 2, at 6 rpm at a temperature of 10 ºC. Measurements were performed in triplicate. The final amounts selected for each hydrocolloid were 4.56 g of native corn starch (sample NS), 4.56 g of modified waxy corn starch (sample MS), 0.665 g of guar gum (sample GG) and 0.215 g of λ-carrageenan (sample λ-C). Since the only difference between the samples was the variation in the hydrocolloid concentration, the samples may be considered equicaloric.

Powdered skimmed milk, distilled water and the corresponding hydrocolloid were placed in a cooking device (Thermomix TM 31, Wuppertal, Germany). The milkshakes were heated to 70 ºC for 5 minutes at 1250 rpm (Hoad, Rayment, Spiller, Marciani, Alonso, Traynor, Mela, Peters, and Gowland, 2004). The sweeteners, colouring and strawberry flavour were added after cooling at ambient temperature. The samples were placed in glass beakers (250 mL), covered with plastic film, and stored at 4-5 ºC for 24 hours before performing the tests.

2.4. Rheological measurements

Measurements were made in a controlled stress rheometer RS1 (Thermo Haake, Karlsruhe, Germany), using a parallel plates geometry of a 6-cm diameter and 1-mm gap, and monitored by a RheoWin software package (version 2.93, Haake). During the measurements with a Phoenix P1 Circulator device (Thermo Haake), the temperature was kept at 10 ± 1 ºC, selected as representative of the usual consumption temperature of milkshakes. Measurements were made of each formulation with and without the saliva treatment (*in vitro* oral digestion). All the samples were allowed to rest for 5 min before each measurement in the rheometer cell. For the *in vitro* oral digestion, a saliva:sample ratio of 1:4 was used (Sanz and Luyten, 2006). All the measurements were made in triplicate.

2.4.1. Flow behaviour

Sample flow was measured by recording the shear stress values when shearing the samples with a linearly increasing shear rate from 1 to 200 s^{-1} for a period of 60 s and in reverse sequence for the same time. The areas under the upstream data point curve (A_{up}) and under the downstream data point curve (A_{down}), as well as the hysteresis area (A_{up}–A_{down}), were obtained using Rheowin Pro software (version 2.93, Thermo Haake).

If applicable, the percentage of relative hysteresis area (Dolz, González, Delegido, Hernández, and Pellicer, 2000; Tárrega, Durán, and Costell, 2004) was calculated according to equation 1:

$$A_r = (A_{up} - A_{down})/A_{up} \times 100 \qquad \text{(equation 1)}$$

The data from the ascending segment of the shear cycle were fitted to the Ostwald-de Waele model (equation 2) using Rheowin Pro software (version 2.93, Thermo Haake):

$$\sigma = K\,\dot{\gamma}^{n} \qquad \text{(equation 2)}$$

where σ (Pa) is the stress, K (Pa s^n) is the consistency index, $\dot{\gamma}$ is the shear rate and n is the flow index.

In time-dependent and non-Newtonian shear-thinning products, perceived thickness is difficult to predict with rheological parameter values since flow in the mouth is a combination of shear and elongational flow (van Vliet, 2002). However, some authors have found that oral thickness correlates well with different rheological indices. According to Wood (1968), apparent viscosity at a shear rate of 50 s^{-1} has practical utility as a possible instrumental index of perceived thickness in semisolid foods. It has been used by many authors (Cook, Hollowood, Linforth, and Taylor, 2003; Arancibia, Costell, and Bayarri, 2013). Consequently, the apparent viscosity values at a shear rate of 50 s^{-1} (η_{50}) were also calculated as follows:

$$\eta_{50} = K \cdot \dot{\gamma}_{50}^{n-1} \qquad \text{(equation 3)}$$

2.4.2. Viscoelastic properties

Prior to the mechanical spectrum measurements, the linear viscoelastic region (LVR) was determined. Stress sweeps were run between 0.02 and 300 Pa at a frequency of 1 Hz in all systems, at stress values that ensured an LVR for each system. The frequency

sweeps were performed over f = 0.01–10 Hz and the values of the storage modulus (G') and the loss modulus (G") as a function of the frequency were calculated using Rheowin Pro software version 2.93.

2.5. Microstructure

The equipment used for microscopic examination during simulated oral *in vitro* digestion was a Nikon ECLIPSE 80i (Nikon Co., Ltd., Tokyo, Japan) light microscope (LM). A 20 μL sample of each formulation was placed in the concavity of a glass slide and observed at 10x magnification (objective lens 10x/0.30□ /0.17 WD 16, Nikon, Tokyo, Japan). A camera (ExWaveHAD, model no. DXC-190, Sony Electronics Inc, Park Ridge, New Jersey) was attached to the microscope and connected to the video entry port of a computer, then 5 μL of saliva were added. During the simulated oral digestion, a video film was recorded. The images were captured each second and stored in 640x480 pixel format using the microscope software (Linksys 32, Linkam Scientific Instruments Ltd., Surrey, UK). The software interfaced directly with the microscope, enabling image recording control.

2.6. Sensory analysis

For each sample, consumers had to score the intensity of their expected satiety and answer a *Check-all-that-apply* (CATA) questionnaire.

Consumers: A total of 106 untrained consumers, of ages ranging between 18 and 61 years, were recruited from the Universidad Politécnica de Valencia. Of the participants, 61% were women and 39% men.

Samples: The four samples were coded with random three-digit numbers and were presented to the consumers in a balanced rotation order, following Williams' design (MacFie, and Thompson, 1988). The consumers were instructed to rinse their mouths with water between samples. The milkshakes were served in plastic cups, without a spoon, at drinking temperature (8 to 10 ºC).

Intensity of expected satiety: The consumers scored their perception of expected satiety on a 9-point scale (from 1= "not very satiating" to 9= "very satiating") for each sample. They were not asked to drink a large volume of the sample, only to perform a "sip test", and were asked to rate their "expected satiety" with an evoked context: "Please taste the sample. How satiating do you think this milkshake is? Imagine drinking a whole bottle of this milkshake (like the one we are showing you). How full do you think it would make you feel?" The consumers had a real bottle of milkshake (white plastic, no label, 50 mL) in their booths so that they could imagine the volume they would drink.

Generation and selection of terms for the CATA (check-all-that-apply) questionnaire: The questionnaire included 47 attributes, including sensory and non-sensory terms. A panel of ten assessors, skilled in quantitative descriptive analysis, evaluated the four samples to develop the sensory attributes that would be included in the CATA list. They were first given a number of samples, a brief outline of the procedure and a list of potential attributes taken from the literature. They were then asked to choose and write down the most appropriate attributes to describe all the sensory properties of the milkshakes and/or to suggest new ones. At the end of a one-and-a-half-hour session, including a round table discussion, a consensus on the list of sensory attributes was reached. This procedure was proposed by Stone and Sidel (2004) in order to obtain a complete description of a product's sensory properties.

CATA questionnaire: The final questionnaire included the following terms, sensory: adhesive [adhesivo], aerated/with bubbles [aireado/con burbujas], thick appearance [apariencia espesa], creamy appearance [apariencia cremosa], homogeneous appearance [apariencia homogénea], liquid appearance/fluid [apariencia líquida/fluida], thick in mouth [consistente en boca], dense [denso], creamy [cremoso], jelly-like [gelatinoso], gummy/elastic [gomoso/elástico], grainy [granuloso], heterogeneous [heterogéneo], homogeneous [homogéneo], light [ligero], liquid [líquido], very creamy [muy cremoso], very dense [muy denso], not very dense [poco denso], not very creamy [poco cremoso], heavy [pesado], slippery [resbaladizo], satiating [saciante], soft [suave], not very satiating [poco saciante], viscous [viscoso], very satiating [muy saciante], much strawberry flavour [mucho sabor a fresa], very sweet [muy dulce], not very sweet [poco dulce], gummy flavour [sabor a goma], floury flavour [sabor a harina], legume flavour [sabor a legumbre], bitter flavour [sabor amargo], artificial flavour [sabor artificial], strange flavour [sabor extraño], dairy flavour [sabor lácteo], not much strawberry flavour [poco sabor a fresa], starchy flavour [sabor a almidón], mouthcoating after swallowing [deja recubrimiento bucal después de tragar], bitter aftertaste after swallowing [retrogusto amargo luego de tragar], sweet aftertaste after swallowing [retrogusto dulce después de tragar]; non-sensory: it is a healthy snack [es un tentempié saludable], it could replace a meal [podría sustituir una comida], I would eat it as brunch [lo tomaría de almuerzo], I would eat it as an afternoon snack [lo tomaría de merienda], I would eat it for breakfast [lo tomaría de desayuno]. The instructions given to the participants were: "Please check all the attributes that apply to the milkshake you are tasting". The order in which the 47 attributes were presented was randomized between products and across consumers.

After assessing the four milkshake samples in balanced rotated order, at the end of the questionnaire the consumers were asked to rate their "ideal satiating milkshake". They did so by answering the same questions (expected satiety and CATA questionnaire) after being placed in a new evoked context: "Now, imagine your "ideal satiating milkshake" (strawberry flavour). It would be yummy and you would feel well after drinking it. It

would help you to feel full for some time or would replace a meal." They were asked to rate their "expected satiety" in the same way as for the real samples: "How satiating do you think this milkshake would be? Imagine drinking a whole bottle of this milkshake (like the one we are showing you). How full do you think it would make you feel?" (Piqueras-Fiszman and Spence, 2012).

2.7. Statistical data analysis

Analysis of variance was performed on the rheological data using the Statgraphics Plus 5.1 software package (Statistical Graphics Co., Rockville, Md., U.S.A.). Fisher's least significant difference (LSD) test was used to evaluate mean value differences ($p<0.05$).

The chi-square test was used to study differences in the consumers' perception of the milkshakes based on the CATA responses. For each milkshake, the frequency of selection of each attribute was determined by counting the number of consumers that checked that term to describe that sample. Cochran's Q test (Manoukian, 1986) was carried out on the CATA data in order to identify significant differences between samples for each of the attributes. A multiple factor analysis (MFA) was run on the CATA frequency counts table to understand the positioning of the four milkshakes as perceived by the consumers, together with their ideal. Hierarchical cluster analysis (HCA) was run on the first four factors of the MFA to cluster the samples in terms of their sensory profile (dissimilarity: Euclidean distance, agglomeration method: Ward's method). All statistical analyses were performed using XLSTAT statistical software (version 2010.5.02, Microsoft Excel®, Barcelona, Spain).

3. Results and discussion

3.1. Flow behaviour

Figure 1 shows the flow behaviour curves obtained with and without *in vitro* oral digestion of the four samples. Without oral digestion, all the samples showed pseudo-plastic behaviour (n<1) (Figure 1A). Sample GG showed the least time-dependence, followed by sample λ-C (both with negligible thixotropic area values). The thixotropic area value for the NS samples was 28.36. Bearing in mind that this area is related to the energy needed to destroy the structure of the materials (Arancibia, Costell, and Bayarri, 2013), the sample that would suffer a certain level of rheodestruction would be NS.

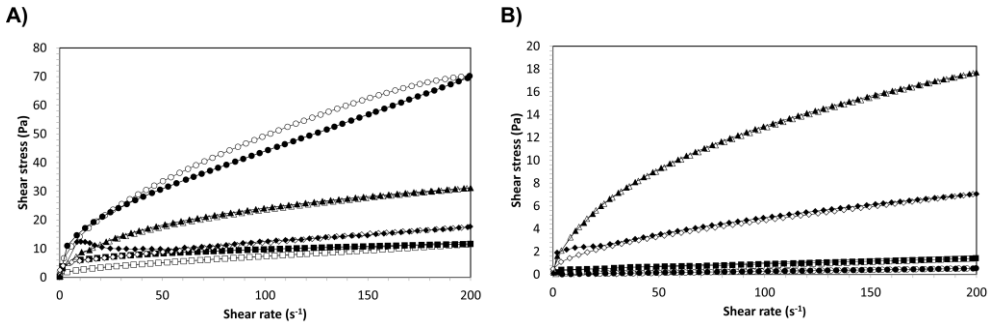

Figure 1. Flow behaviour of the four milkshakes: A) before *in vitro* oral digestion, B) after *in vitro* oral digestion. Upward shear rate (black symbols), downward shear rate (white symbols). Native starch (squares), modified starch (circles), guar gum (diamonds), λ-carrageenan (triangles)

The milkshake formulated with modified starch (MS) exhibited antithixotropic behaviour (thixotropic area value: -295.57) that has previously been described for this type of starch as being a result of shear-induced structure formation (Tattiyakul and Rao, 2000). The MS sample presented the highest viscosity values over the entire shear stress range studied (Figure 1 A). Cross-linked waxy maize starch (MS) is a high amylopectin starch (almost free of amylose), which has been chemically modified to

make the starch granules resistant to thermal and mechanical treatment. A gelatinized MS dispersion can be described as just a suspension of swollen particles. These systems normally exhibit a shear-thinning flow behaviour and a solid-like viscoelastic behaviour, properties which are mostly governed by the volume fraction and the deformability of swollen starch granules, depending on the concentration (Nayouf, Loisel, and Doublier, 2003). However, it has been reported that in the presence of milk proteins the solid-like behaviour of MS alone tends to disappear and a liquid-like behaviour appears, showing that this system is much less structured than starch-only suspensions.

Sample λ-C presented an irregular shape at the start of the curve. This overshoot probably reflects the resistance to deformation of a structured system which has broken down partially with increasing shear rates, as Lizarraga, Piante Vicin, González, Rubiolo, and Santiago (2006) observed when studying the flow curves of whey protein concentrate and λ-carrageenan mixtures.

Following oral digestion (Figure 1B), the systems with the highest viscosity were GG and λ-C. Both starches lost a certain degree of pseudoplasticity and a large part of their viscosity. This loss was noticeably greater in the MS sample, which had been the most viscous before digestion and became the least viscous sample after digestion (Table 1). It was also found that, as expected, the MS sample lost its thixotropy after digestion.

Fitting the flow curves to the Ostwald - de Waele model gave the consistency index (K), flow index (n) and apparent viscosity at 50 s^{-1} (η_{50}) values for the four systems before and after oral digestion (Table 1). Before digestion, sample λ-C presented a significantly ($p<0.05$) higher consistency index than the other samples and the least Newtonian flow behaviour (lowest n value).

Table 1. Mean values (n=3) of consistency index (K), Flow index (n), and viscosity at 50 s^{-1} (η_{50}) of the milkshakes without and with *in vitro* oral digestion.

Sample*	Predigestion			Postdigestion		
	K (Pa·s^n)	**n**	**η_{50} (Pa·s)**	**K (Pa·s^n)**	**n**	**η_{50} (Pa·s)**
NS	3.54 [a] (0.19)	0.21 [a] (0.02)	0.16 [a] (0.02)	0.09 [a] (0.05)	0.54 [a] (0.12)	0.01 [a] (0.00)
MS	3.39[a] (0.1)	0.56 [b] (0.02)	0.61 [b] (0.06)	0.01 [b] (0.01)	0.81[b] (0.20)	0.00 [b] (0.00)
GG	3.70[a] (0.19)	0.40[c] (0.01)	0.36 [c] (0.00)	1.41 [c] (0.06)	0.48 [a] (0.01)	0.18 [c] (0.00)
λ-C	6.98 [b] (1.68)	0.15 [d] (0.05)	0.25 [d] (0.00)	0.62 [d] (0.01)	0.46 [a] (0.00)	0.07 [d] (0.00)

Different letters in the same column indicate statistically significant differences ($p<0.05$).

* NS: native starch; MS: modified starch; GG: guar gum and λ-C: λ carrageenan.

After digestion, the MS sample went from having significantly ($p<0.05$) the highest apparent viscosity (η_{50}) before digestion to being significantly ($p<0.05$) the least viscous sample and having significantly ($p<0.05$) higher flow index values than the rest, denoting the closest behaviour to Newtonian flow of all the samples. These values could be attributed to the effect of starch digestion in contact with the α-amylase enzyme added to the saliva, in addition to the logical dilution effect of the added water – the major ingredient of the artificial saliva – which naturally also affected the rest of the samples. To assess the dilution effect of the water in the saliva, measurements were made in the same samples after adding the saliva without the enzyme (α-amylase). The water in the saliva was found to have a significant dilution effect, causing all the systems to present lower K and greater n values following digestion (data not shown).

3.2. Viscoelastic properties

Figure 2 shows the evolution of the storage modulus (G') and loss module (G") values as a function of the frequency (mechanical spectra) of the milkshakes formulated with the four different hydrocolloids, with and without *in vitro* oral digestion of the hydrocolloid.

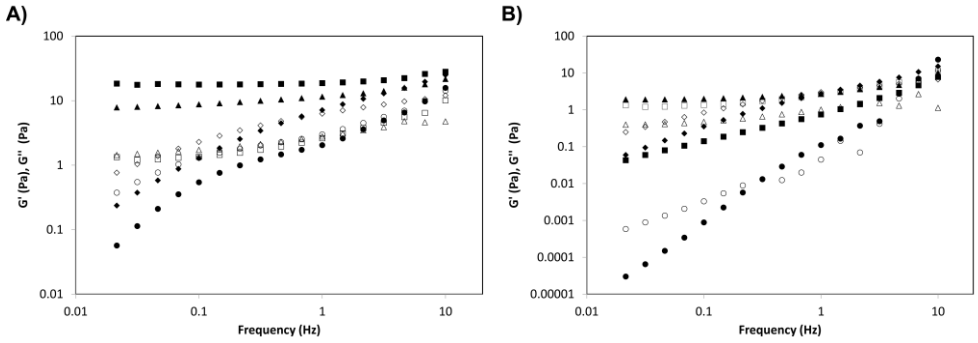

Figure 2. Viscoelastic properties of the four systems: A) before *in vitro* oral digestion, B) after *in vitro* oral digestion. G' (black symbols), G" (white symbols). Native starch (squares), modified starch (circles), guar gum (diamonds), λ-carrageenan (triangles)

Before contact with the saliva, the MS samples showed non-gelling behaviour with typical features of random coil polymers, strong frequency dependence and G' – G" crossover (Figure 2A), whereas the G' values were higher than those for G" in samples NS and λ-C, denoting a gel-like behaviour.

Comparison of the mechanical spectra and the G' and G" values at 1 Hz of the four systems before and after oral digestion *in vitro* (Table 2) indicates that in addition to the dilution effect already mentioned, the NS sample lost its gel-like behaviour following oral digestion, showing higher values for G" than for G'.

Table 2. Mechanical spectra of the samples measured at 1 Hz of the samples without and with *in vitro* oral digestion.

Sample	Predigestion*		Postdigestion*	
	G'	**G"**	**G'**	**G"**
	(Pa)	**(Pa)**	**(Pa)**	**(Pa)**
NS	18.72 [a]	2.61 [a]	0.75 [a]	0.78 [a]
	(0.57)	(0.29)	(0.06)	(0.05)
MS	2.04 [b]	2.99 [b]	0.11 [b]	0.04 [b]
	(0.02)	(0.01)	(0.03)	(0.03)
GG	7.13 [c]	6.29 [c]	2.73 [c]	3.00 [c]
	(0.19)	(0.16)	(0.24)	(0.24)
λ-C	11.50 [d]	2.78 [ab]	2.74 [c]	1.02 [a]
	(0.79)	(0.03)	(0.35)	(0.13)

*Different letters in the same column indicate statistically significant differences ($p<0.05$).

*G': storage modulus; G": loss modulus.

Following oral digestion, MS became the least structured sample owing to the major destruction of the granules caused by enzyme action combined with the dilution effect.

After digestion, samples GG and λ-C presented higher values for both viscoelastic moduli than the starch-based samples – although evidently lower than before the addition of saliva – indicating much weaker gel behaviour as a result of the dilution. The milkshake formulated with GG presented a significantly ($p<0.05$) higher viscous modulus (loss module value) than any of the other samples.

The present study did not aim to characterize the rheological behaviour of the samples (which is already known from the literature through the hydrocolloids they contain) but to relate the variations in this behaviour – which differed considerably between the samples in the presence of saliva – to the expectations of satiety aroused by the beverages formulated with the different hydrocolloids.

3.3. Microstructure

For comparison, Figure 3 shows the optical microscopy (LM) images obtained during the *in vitro* simulation of the digestion process, when the samples had been in contact with the saliva for 0 seconds, 10 seconds, 45 seconds and 3 minutes. Initially (0"), when the samples retained their original structure, it can be seen that the NS sample (Figure 3A) still showed swollen but entire starch granules. However, some areas were already stained blue (with lugol) owing to amylose leaching as a result of gelatinization. Swollen granules with brown staining can be seen in sample MS (Figure 3B). This is characteristic of cross-linked waxy starch granules. Some individual blue-stained granules can also be seen, corresponding to starch granules with a higher amylose content. Also, the MS granules were more tightly packed than in the NS sample. This is because the modified starch has a greater capacity to swell during gelatinization without losing its granular form, owing to its high degree of cross-linking.

Some blue-colored gum particles are visible in sample GG (Figure 3C), while the formation of a pink and brown network can be seen in sample λ-C (Figure 3D).

As regards the changes that took place after the artificial saliva was added, it can be seen that the structure of both the GG (Figures 3C, G, K and O) and λ-C (Figures 3D, H, L and P) systems remained practically unchanged even 3 minutes after adding the saliva. The only effect, slight but visible, was that both matrices had been diluted by the water content of the saliva.

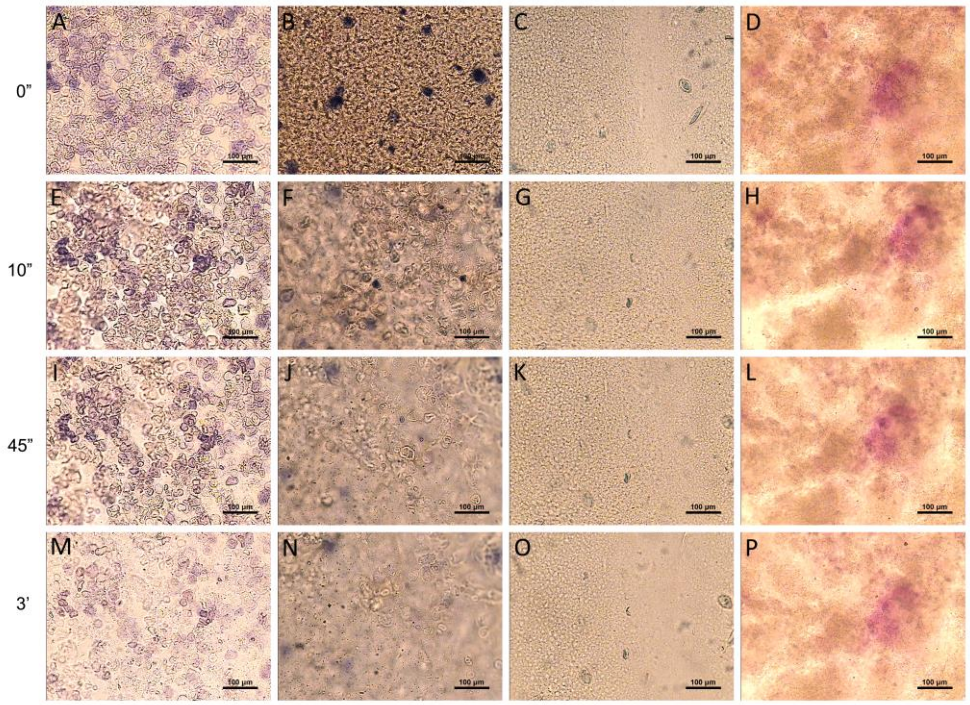

Figure 3. Optical microscopy. Lugol staining. Evolution of milkshake microstructure during *in vitro* simulation of the digestion process at 0 s, 15 s, 45 s and 3 min after saliva addition. A, E, I and M: milkshake formulated with native starch. B, F, J and N: milkshake formulated with modified starch. C, G, K and O: milkshake formulated with guar gum. D, H, L and P: milkshake formulated with λ-carrageenan. Magnification 10X

Following digestion, the expected dilution effect was joined by changes in the granule structure in both the native starch (Figures 3E, I and M) and the modified starch (Figures 3F, J and N). These changes, which were greater in the MS samples, consisted in a loss of granule structure as a result of granule digestion by the α-amylase. Specifically, in the images obtained 10 seconds after adding the saliva it can be seen that the loss of granule structure was practically imperceptible in the NS sample (Figure 3E)

but very marked granule disintegration had taken place in the MS sample (Figure 3F). Moreover, 3 minutes after adding the saliva almost all the granular structures had disappeared in the MS sample (Figure 3N), whereas the NS sample still retained a large part of its remaining granular structures (Figure 3M).

The microstructural changes in the MS sample observed by LM may be related to the changes in its flow behaviour and viscoelastic properties. As mentioned above, the MS sample presented significantly ($p<0.05$) the highest apparent viscosity value at 50 s-1 before digestion but significantly ($p<0.05$) the lowest after digestion, compared to all the others. The more swollen the granule the more easily the α-amylase reaches the starch chains. Substitution through hydroxypropylation of the MS gives rise to greater swelling of the granules, which affords the enzyme more access and therefore leads to faster digestion and loss of granule structure (Östergård, Björck, and Gunnarsson, 1988; Björck, Gunnarsson, and Østergård, 1989).

3.4. Sensory analysis

3.4.1. Expected satiety scores

One hypothesis considered in this study was that the expected satiety would be lower in the samples prepared with starch, bearing in mind that their structure is broken down in the mouth through the action of the saliva.

However, an examination of the expected satiety scores of the four samples, measured on a 9-point scale (from "not at all satiating" to" very satiating"), showed that while the highest score (6.3) was given to MS, this was the only sample not to differ significantly ($p>0.05$) from the "ideal satiating milkshake" score (6.7). The milkshakes formulated with NS, GG and λ-C, with scores of 5.4, 5.8 y 5.2 respectively, were rated significantly lower ($p>0.05$) than the "ideal satiating milkshake" value.

This result is related to the attributes the consumers perceived in the MS sample (Figure 4), such as homogeneous, thick in the mouth and very creamy, which seem to generate expectations of satiety.

Consequently, the consumers associated the perception of satiety from this type of sample with consistency and creaminess at the moment it was placed in the mouth (first sip), in other words, during the initial moment of consumption, and with loss of the milkshake matrix structure as a result of the short phase of in-mouth handling and salivary action, which produces a sensation of melting or dissolving that has been defined as the speed in which the samples disappear during mastication (Alting, Fred van de, Kanning, Burgering, Mulleners, Sein, and Buwalda, 2009) and could potentially be related to the perception of creaminess. This point will be discussed further in the next section. A detailed description of the sample profiling, as well as the ideal product, can be obtained through a closer look at the CATA results.

3.4.2. Sensory profiling through CATA

Check-all-that-apply (CATA) questionnaires consist of multiple choice lists of words or phrases from which respondents have to select those they consider apply to the question. This technique has been increasingly applied in food research (Ares, Jaeger, Bava, Chheang, Jin, Gimenez, Vidal, Fiszman, and Varela, 2013). The main advantage of this type of question is that it allows multiple options to be selected instead of limiting respondents to only one answer or forcing consumers to focus their attention on specific attributes to be evaluated (Smyth, Dillman, Christian, and Stern, 2006). The lists of words or phrases in the CATA questions can be related or unrelated to the sensory characteristics of the product, as they can include terms related to non-sensory characteristics such as occasions when used, product positioning and emotions (Varela and Ares, 2012).

Chi-square tests gave significant results when comparing the profiles of the four real samples, and also when comparing the four tasted samples with the ideal, meaning that different profiles were obtained through the CATA questionnaire. To understand these differences between individual attributes better, Cochran Q tests were run on all the attributes, both among the real (tasted) samples and between these and the ideal (Table 3). Most of the results discriminated significantly between individual samples and between the samples and the ideal. The exceptions were "slippery" and "very satiating" when comparing the five treatments, and "starchy flavour" in relation to the real (tasted) samples.

Table 3. Number of selections of each attribute for the real samples and the ideal sample, and Cochran's Q test results

Attributes	**NS**	**MS**	**GG**	**λ-C**	**Cochran's Q (between the 4 real samples)**	**IDEAL**	**Cochran's Q (between the 4 samples and the ideal)**
Sensory							
Adhesive	11	10	24	5	0	1	<0.0001
Aerated/with bubbles	20	10	15	51	<0.0001	23	<0.0001
Artificial flavour	42	34	61	28	<0.0001	2	<0.0001
Bitter aftertaste	9	5	26	7	<0.0001	2	<0.0001
Bitter flavour	2	1	10	4	0.001	0	<0.0001
Creamy	39	48	28	26	0.02	68	<0.0001
Creamy appearance	39	60	37	29	<0.0001	74	<0.0001
Dairy flavour	44	48	13	43	<0.0001	61	<0.0001
Dense	30	55	65	16	<0.0001	12	<0.0001

Floury flavour	18	10	7	6	0.017	0	<0.0001
Grainy	10	0	3	34	<0.0001	2	<0.0001
Gummy flavour	10	11	38	9	<0.0001	0	<0.0001
Gummy/elastic	8	20	23	3	<0.0001	0	<0.0001
Heavy	13	24	40	5	<0.0001	0	<0.0001
Heterogeneous	8	2	5	29	<0.0001	6	<0.0001
Homogeneous	54	64	57	39	0.003	43	0.001
Homogeneous appearance	64	75	63	38	<0.0001	67	<0.0001
Jelly-like	5	11	24	8	<0.0001	2	<0.0001
Legume flavour	7	1	30	0	<0.0001	0	<0.0001
Light	32	15	8	41	<0.0001	39	<0.0001
Liquid	33	15	6	40	<0.0001	28	<0.0001
Liquid appearance/fluid	42	23	18	42	<0.0001	28	<0.0001
Mouthcoating	39	33	48	24	<0.0001	17	<0.0001
Much strawberry flavour	12	16	3	17	<0.0001	72	<0.0001
Not much strawberry flavour	38	43	61	40	0.027	6	<0.0001
Not very creamy	12	2	4	24	0.007	3	<0.0001
Not very dense	24	5	2	32	0.003	19	0.001
Not very satiating	18	8	9	16	0	3	<0.0001
Not very sweet	24	20	40	26	0.004	31	0.0022
Satiating	31	41	28	24	0.027	52	0
Slippery	13	16	19	27	0.07	14	0.063
Soft	52	41	23	60	<0.0001	52	<0.0001
Starchy flavour	11	7	15	7	0.194	0	0.003
Strange flavour	22	18	60	12	<0.0001	1	<0.0001

Sweet aftertaste	31	32	12	32	0.001	48	<0.0001
Thick appearance	31	47	57	29	<0.0001	11	<0.0001
Thick in mouth	29	45	36	18	0	27	0
Very creamy	5	18	15	6	0.002	17	0.003
Very dense	14	22	49	3	<0.0001	1	<0.0001
Very satiating	10	15	12	6	0.099	16	0.078
Very sweet	15	27	9	21	0.001	38	<0.0001
Viscous	16	30	40	15	<0.0001	4	<0.0001
Use and Attitudes							
It is a healthy snack	13	13	5	20	0.001	43	<0.0001
It could replace a meal	8	13	4	2	0.003	20	<0.0001
I would eat it as a brunch	14	21	8	19	0.018	45	<0.0001
I would eat it as a afternoon snack	27	31	14	30	0.009	55	<0.0001
I would eat it for breakfast	19	20	5	22	0	45	< 0.0001

*NS: native starch; MS: modified starch; GG: guar gum and λ-C: λ carrageenan.

As regards the frequency of term selection for the ideal satiating milkshake, consumers would like a drink with particular flavour and texture characteristics, described as "dairy flavour", "much strawberry flavour", "very sweet" and "sweet aftertaste", and also as "creamy", "creamy appearance", "homogeneous", "homogeneous appearance", "light" "soft" and "satiating". It may be pointed out that the attributes "very creamy", "very satiating", "very dense" and "thick" were not frequently selected to describe the ideal sample, suggesting that these terms could be associated with a milkshake which is too heavy or calorie-dense, which are not necessarily desirable for this product category. Regarding use and attitude, consumers would not be interested in replacing a meal with

their ideal milkshake, but rather in having it as a snack at different times of the day. However, almost half the consumers agreed that it would have to be "a healthy snack".

In the profiles of the four samples, MS emerged as the most similar to the consumers´ ideal, particularly as regards "creaminess", "homogeneity", and "dairy flavour", although not in a number of other aspects. MS was not perceived to be as "light", as "much strawberry flavour", or as "sweet" as the consumers imagined their ideal. Furthermore, sample MS was perceived as quite "dense", "thick" and "viscous" – attributes which were not desired in the ideal sample. In terms of the perception of "satiating" in the CATA test, sample MS had the highest satiety rating of the samples tested, much as expected, but the use and attitude characteristics were not in line with consumers' expectations, as MS was not considered particularly suitable for consumption as a snack.

Regarding the strawberry flavour, none of the samples tasted were perceived as possessing that attribute particularly strongly. Indeed, the authors formulated the milkshakes with a subtle strawberry flavour in order to give it a nice, familiar taste and avoid the bias that a strong flavour probably would have introduced.

Sample GG was perceived as the farthest from the ideal, according to its detailed CATA profile. It lacked the perceptions of "creaminess", "dairy flavour", "softness", "light", "strawberry flavour" and "sweetness". Also, many consumers highlighted undesired textural attributes such as "viscous", "dense/very dense", "thick appearance", "mouthcoating", "jelly-like" and "heavy". In addition, GG was associated with the negative flavour attributes of "artificial", "gummy", "legume flavour" and "strange", probably related to the origin of the hydrocolloid (guar gum). Aravind, Sisson, and Fellows (2012) also found a floury mouthfeel and starchy aftertaste in pasta containing guar gum. In the present work, acceptability was not measured as the experimental samples were not "rounded" as they would be in a market launch, since the objective was to acquire an idea of expected satiety in relation to the thickeners rather than a

measurement of liking. However, the negative terms associated with sample GG could be an indication of dislike that could have influenced the satiety assessment.

Most of the use and attitudes terms described the ideal sample as "it is a healthy snack", "it could replace a meal" and "I would eat it for breakfast, "as an afternoon snack" or "as brunch". However, many consumers did not relate any of the real (tasted) samples to those terms, suggesting that the consumers did not find them applicable to the samples and that more development would be needed to bring them closer to the ideal in terms of fitness for use.

The Multiple Factor Analysis (MFA) of the frequency of selection of the sensory terms in the CATA test helped in visualizing the differences and similarities between the samples (Figure 4 A and B). MFA studies several groups of variables (numerical and/ or categorical) in the same set of individuals, considered in a unique framework (Escofier and Pagès, 1994). The first two factors accounted for most of the data variability, 86.76% (55.97 % and 30.79% respectively). The five samples (the 4 real ones and the ideal) were well separated in the perceptual space.

The ideal sample appeared in the top left corner of the sample map (with positive values for the first factor and negative ones for the second). It was described as "creamy", "creamy appearance", "very sweet", "sweet aftertaste", "much strawberry flavour" and "dairy flavour", and correlated negatively to "gummy", "bitter", "artificial" and "starchy", which are normally considered negative attributes. Again, it is noteworthy that consumers mainly associated their ideal satiating milkshake with creaminess but not with other textural parameters such as thickness, denseness or viscosity, as they would like a "light", "soft" beverage. It would be interesting to compare these outcomes with those of other kind of products, particularly other satiating beverages and solid foods, to gain a better understanding of whether this is the case for this particular product or whether these requirements could be generalized to other categories.

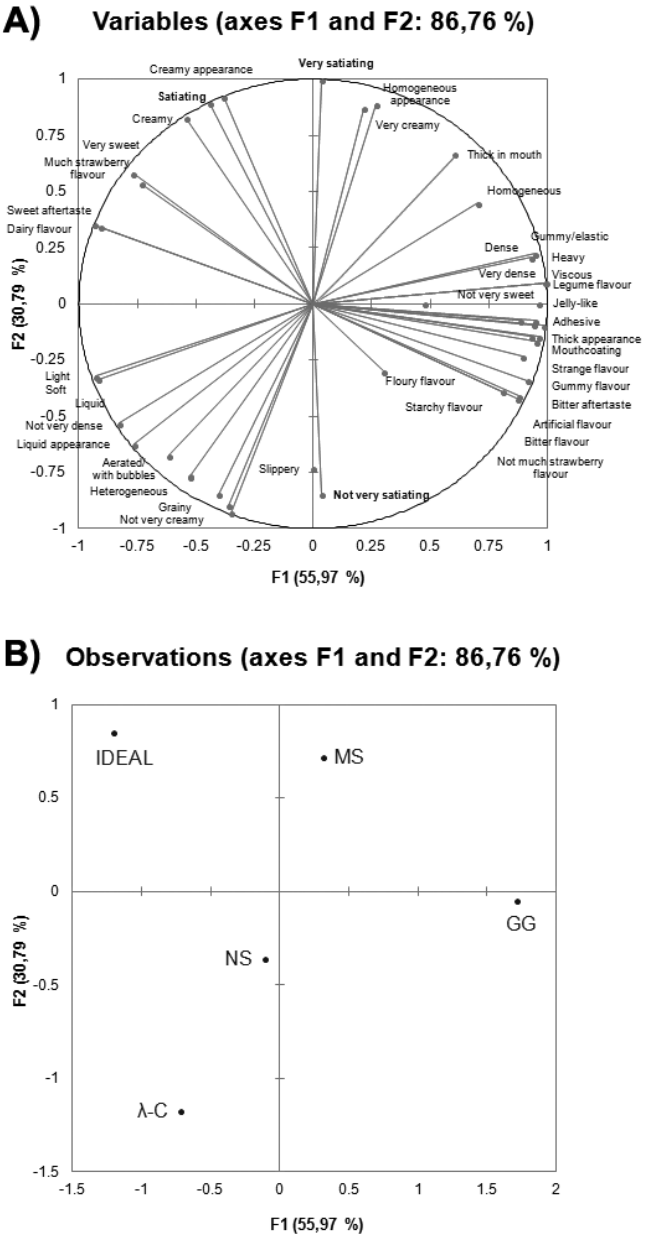

Figure 4. A) Representation of the terms from the CATA questionnaire, B) representation of the four milk beverage samples and the ideal satiating milkshake in the first two dimensions of MFA of the CATA counts.

Sample λ-C was located in the bottom left corner of the space defined by the two first factors of the MFA. It was perceived as "liquid", "soft", "light", "not very dense", "aerated", "heterogeneous", "grainy", and "not very creamy". It was identified as "not very satiating" in the CATA test, in agreement with its low expected satiety rating and most probably related to its low creaminess and its fluid texture.

Sample GG appeared with positive loadings for the first factor (on the right of the map). As discussed above, it was perceived as "jelly-like", "viscous", and "mouthcoating", as quite dense and as related to all the negative flavour parameters, so was very far from the ideal. This sample is an excellent example of how creaminess was one of the main drivers of satiation expectations in milkshakes: although it was a thick, heavy drink, it was not especially creamy, and the consumers therefore perceived it as not particularly satiating.

Food creaminess is not a primary sensory property but an integrated sensory perception (or sensory experience) derived from combined sensations of visual, olfactory, gustatory, and tactile cues, among which smoothness has been suggested as the most important sensory texture-related feature (Chen and Eaton, 2012). According to Alting, et al., (2009), perceived creaminess resulted from the perception of the in-mouth melting sensation, to which hydrolysis by amylase present in the saliva during mastication is a contributing factor. Moreover, de Wijk, van Gemerta, Terpstraa and Wilkinson (2003), found that "melting" was inversely correlated to "thick" in custard desserts, and that these two sensations were related to the perception of creaminess. They also found that adding carrageenan to custards reduced the perception of "melting". Added to this, for similar samples, de Wijk, Prinz and Janssen (2006) found that sensory ratings for melting reflected physical starch break-down, at least to some degree, and that they were primarily determined by the surface properties of the food. The perception of creaminess is very complex and when comparing the influence of different hydrocolloids, it is not only textural parameters that are important, but also lubrication and homogeneity. It has been demonstrated that starches can provide

enhanced creaminess in milk products, possibly because their enzymatic breakdown may also have beneficial effects on lubrication, due to the release of fat droplets from the starch matrix, and this could be more pronounced in low fat products (R. A. de Wijk, Terpstra, Janssen, and Prinz, 2006).

The findings for the λ-C and GG samples are also in line with the results of Tárrega, Martínez, Vélez- Ruiz and Fiszman (2014), where the viscous component was shown to be more connected with expected satiety than the elastic component in the rheological behaviour of several gums. This was the case for MS, which presented the highest values for η_{50} and G" in relation to G' than the rest of the samples.

For sample GG in particular, the number of negative flavour attributes selected probably influenced the perception of satiety. According to Yeomans (2010), this result can be related to the fact that the size of an eating event can be modified by the palatability or hedonics (immediate sensory appeal) of the item being ingested, among other factors.

The samples formulated with starch (MS and NS) were in the middle of the map. To study the relation between the real tasted samples and the consumers' ideal further, a hierarchical cluster analysis (HCA) was run on the first four factors of the MFA to cluster the samples in terms of their sensory profiles, as defined by the CATA results. The HCA highlighted four groups. One contained samples MS and NS. They were the closest to the ideal, which formed a cluster on its own. Samples λ-C and GG each also formed their own separate groups, with GG as the farthest from the ideal. The HCA showed that the samples formulated with starch were quite similar in various aspects that brought them together globally in the sensory description and made them the tasted samples closest to the ideal. As discussed above, many of their attributes were not in line with the consumers' expectations of an ideal satiating milkshake, but this study has certainly provided a better understanding of what would be needed to get closer to the ideal milkshake as pictured by consumers.

Ares, Varela, Rado, and Giménez (2011) proposed the use of CATA questionnaires linked to preferences for real samples and to an ideal product as a method to gain a better understanding of the drivers of liking. In the present study, this idea was adapted and applied to acquire a deeper knowledge of the intrinsic and extrinsic factors that fuel expected satiety. The results give a picture of how satiety expectations are related to the sensory and other characteristics of the products, and provide directions for product design improvement. With these results, it was also possible to relate the effect of incorporating different hydrocolloids into the milkshakes to the expected satiety they elicited in the consumers' sensory perception and to interpret the results in the light of their rheological properties.

4. Conclusions

The hydrocolloids used for thickening milk-based beverages can play an important part in the expectations of satiety these products arouse. The rheological characterisation of the beverages over a wider range of shear rates reveals very different behaviour patterns depending on the type of hydrocolloid. As some of these hydrocolloids undergo changes during oral digestion, which differ according to their structure and formulation, a comparison of their rheological behaviour before and after exposure to saliva is of great interest as a first step in studying the satiating capacity they can bring to a food such as a milkshake. The temporality of these perceptions was not considered in the present study but it could be of interest to sum add this dimension to the perception of the expected satiety.

As the results of the present study show, it seemed evident that GG and λ-C were not considered as creamy, thick in the mouth and homogeneous as the starches were. Characteristics such as heterogeneous, dense, viscous, gummy or jelly-like were related to these hydrocolloids, which were not considered as satiating as the starches were. The perception of satiating capacity would appear to be related more to the feeling of

creaminess when the milkshake enters the consumer's mouth and with the loss of structure that takes place during oral processing, which causes a sensation known as "fondant", "melting" or "disappearing". This sensation is perceived as being quite unlike the jelly-like, thick, slimy or slippery sensations associated with other hydrocolloids.

The methods used in the present study should be considered just another tool for investigating the perception of expected satiety more deeply. The type of sensations perceived is only one of a number of factors that need to be taken into account when formulating satiating foods. These factors cannot and should not be restricted to the product's apparent viscosity in the pack before it is consumed.

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Relating HPMC concentration to elicited expected satiation in milk-based desserts

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Abstract

Previous research has shown that multiple factors influence perceptions of the satiating capacity of a food. Sensory properties, especially texture, play a role in eliciting expected satiation/satiety. The present work analysed some rheological and texture-related cues to the expected satiation that nine milk-based desserts prepared with three different levels of hydroxypropylmethylcellulose, HPMC (1.5, 2.0 and 2.5 g) elicited in consumers (n=113). Two variations were introduced in order to obtain different textures: adding diary cream and extra skimmed milk powder. The temporality (appearance and duration) of sensory perceptions was assessed by the temporal dominance of sensations method and was related to the expected satiating capacity elicited by the desserts. Relative expected satiation (RES) was measured on four nine-point picture scales showing nine increasingly larger standardized portions of the comparison food. The participants were also asked to score their liking for the samples on hedonic scales.

In the low HPMC concentration samples, the first sensations to appear were thin and creamy, while fondant and a little mouth-coating sensation emerged close to swallowing. In the high HPMC concentration samples, with longer consumption times, thick, gummy and creamy appeared in the first stages, and adhesive and mouth-coating when close to swallowing.

The RES results depended greatly on the HPMC concentration, but also on the addition of cream or extra milk powder which elicited significantly higher RES values than for the samples without additions. The HPMC concentration in the system and the temporality of the sensations it elicited (orosensory exposure) seemed to modulate the desserts' satiating ability.

Keywords: HPMC, relative expected satiation, milk-based dessert, temporal dominance of sensations, sensory perception.

1. Introduction

Healthy satiating products used as between-meal snacks target consumer satisfaction at a particular time, because of their filling effects, and encourage improved dietary habits if used as a way to prevent weight gain (Tárrega, Martínez, Vélez- Ruiz, and Fiszman, 2014). There is a need to formulate healthy, low-energy products that are affordable, attractive, convenient and, importantly, as tasty and gratifying as those they are intended to replace (Halford and Harrold, 2012).

Previous research has shown that multiple factors (energy content, volume, structure, major components) influence a food product's satiating capacity. Among these variables, sensory properties – especially texture – play a role in expected satiation/satiety (how filling a food is likely to be and to what extent it is likely to stave off hunger until the next meal) (Hogenkamp, Stafleu, Mars, Brunstrom and de Graaf, 2011; Yeomans and Chambers, 2011), as part of the pre-absorptive signals from the cognitive and sensory systems (Scheibehenne, Todd and Wansink, 2010; Zijlstra, de Wijk, Mars, Stafleu and de Graaf, 2009). A number of studies indicate that "liquid" energy fails to suppress subjective appetite (Hulshof, de Graaf and Weststrat, 1993; Leidy, Apolzan, Mattes and Campbell, 2010), eliciting weaker suppressive appetite responses than equi-caloric "more solid" versions of the same food product (Mattes and Rothacker, 2001; Zijlstra, Mars, Stafleu and de Graaf, 2010). Among liquids, "thick" versions suppress hunger to a greater extent than equi-caloric flavour-matched "thin" ones (Mattes and Rothacker, 2001; Zijlstra, et al., 2009). According to McCrickerd, Chambers, Brunstrom and Yeomans (2012), this would be because longer orosensory exposures contribute to the development of satiety. The mechanism involved would be related to triggering anticipatory responses due to learning about the associations between the sensory characteristics of a food and its caloric value post-consumption (Birch and Deysher, 1985; Booth, Mather and Fuller, 1982; Shaffer and Tepper, 1994; Yeomans, Weinber and James, 2005), and these associations are likely to influence

explicit expectations about the effect a food will have on appetite (Blundell, et al., 2010; Brunstrom, Shakeshaft andScott-Samuel, 2008).

McCrickerd, et al., (2012), working with fruit yogurt drinks, stated that people were sensitive to subtle changes in their sensory quality (thick texture and creamy flavour) and that these characteristics could increase the expectation that a drink will be filling (anticipated satiation) and will suppress hunger over time (expected satiety). In their research, thick texture rather than creamy flavour was found to have the biggest influence on satiety expectations irrespective of the drink's actual energy content. Increased viscosity connoted higher energy content and this could reduce hunger independently of the actual energy content, confirming their hypothesis. A recent study by Tárrega, et al., (2014) of milk-based snacks designed to have very different rheological profiles showed that the higher the viscosity the higher the expected satiety reported by consumers, while systems with a higher elastic component in their viscoelastic structure elicited poorer satiety perceptions in consumers. Consequently, hydrocolloids like hydroxypropyl methylcellulose (HPMC) seem to be more appropriate for increasing the expected satiety/satiation delivered by the product. A new challenge that arose from that study was to discover how the concentration of this hydrocolloid affects the perception of expected satiation.

HPMC is a non-fermentable, semisynthetic cellulose derivative with the physiological properties of a soluble dietary fibre. It imparts creaminess and lubricity to pourable and spoonable sauces and dressings (Akoh, 1998) and has a number of interesting properties that are described elsewhere (Hung, et al., 2009; Hung, Anderson, Albers, Langhorst, andYoung, 2011). Little information is available concerning HPMC behaviour in ternary systems, i.e. systems formed with water and proteins (Jara, Pérez, and Pilosof, 2010). Pérez, Wargon, and Pilosof (2006) studied the influence of HPMC on whey protein gelation and structural properties and found that the overall texture and stability of the whole system depended not only on the polysaccharides and proteins, but also on the nature and strength of their interactions.

Food products designed for satiety and hunger control usually employ fat reduction, but this affects both flavour and texture perceptions (Kritchhevsky, Akoh, and Min, 2002), such as creaminess and other related in-mouth sensations which contribute to orosensory cues that could help to elicit satiety. Since it is now well established that food perception occurs through cross-modal sensory integration (Small and Prescott, 2005) (i.e., the senses of smell, taste, and touch interact to form the perception), it is obvious that changing one modality, such as viscosity or creaminess, can affect the overall sensation perceived (Bayarri, Taylor and Hort, 2006) and hence would probably influence the perception of expected satiation. All these results suggest that modifying the textural features of a food item could be a way to increase its expected satiating capacity.

Over many years, a number of authors have used different methods to measure expected satiety/satiation. Pilgrim and Kamen (1963) explored predictors of food consumption in a large-scale study across a range of foods and found that perceived "fillingness" was the best predictor of food choice, better than macronutrient composition or palatability. De Graaf, Stafleu, Staal, and Wijne, (1992) were able to estimate and rate how long consumers expected the foods would appease hunger by showing food photographs. This approach is quickly and easily implemented. However, it remains unclear that people can reliably estimate the time course of their satiety when particular foods are consumed, and ratings of this kind are subject to known sources of bias (Poulton, 1979). On this basis, it would seem likely that understanding expectations about satiety and satiation may contribute insight into decisions about meal size. Brunstrom and Rogers (2008) employed a relatively bias-free method to measure expected satiety, known as the "method of constant stimuli", which can quantify differences in expectations across foods. In the present study, based on Brunstrom, et al., (2008), expected satiation was also assessed by presenting picture food scales to consumers. Instead of pair-comparison, however, four nine-point picture scales, each

with increasing amounts of the corresponding product, were presented following Tárrega, et al., (2014).

In recent years, temporality features of sensory attributes (such as merging and duration) have received attention from researchers. It could be hypothesised that they could also have a role in eliciting expected satiation sensations. The relation between the temporality of sensations and eliciting satiation has not been investigated before. A recent study by Morell, Varela, Fiszman and Hernando (2014) of milkshakes thickened with several hydrocolloids showed that with this kind of sample, consumers associated the perception of satiety with consistency and creaminess at the time it is placed in the mouth (first sip), during the very first moment of consumption, showing the importance of the temporality of some sensory perceptions in satiation. TDS, which has not been used in many previous investigations, is presented as a tool to assess the perceived satiating power of the samples.

The aim of the present work was to analyse the relevance of some rheological and texture-related cues to the expected satiation elicited by milk-based desserts prepared with different levels of HPMC. The effects of adding diary cream and extra milk powder to the formulation were also studied. The temporality (appearance and duration) of perceptions was assessed by the TDS method and was related to the expected satiating capacity elicited by the desserts.

2. Materials and methods

2.1 Sample composition and preparation

Nine white chocolate-flavoured milk-based desserts were formulated (Table 1). Skimmed milk powder (10 g/100 g, Central Lechera Asturiana™, Siero, Spain) and HPMC (Methocel K4M, Dow Wolff Cellulosics, Bomlitz, Germany) at three levels (1.5, 2.0 and 2.5 g/ 100g) were used as the basic recipes. Two variations were introduced in

order to obtain different textures: extra skimmed milk powder (20 g/ 100g) and diary cream (36.5 g/100 g) (33 g/ 100g fat content, 2.0 g/100 g protein content, Hacendado™, Renedo de Piélagos, Cantabria, Spain), added to the samples with each of the three levels of HPMC.

Table 1. Composition and code of the experimental samples. All samples contain 0.2 mL of white chocolate flavour, and 0.025g of sweetener.

Sample	Ingredients (g/100 g)				Total protein content (g)	Total fat content (g)
	HPMC	SMP	Water	Cream		
L-HPMC	1.5	9	89.5	0	3.4	0.05
M-HPMC	2	9	89	0	3.4	0.05
H-HPMC	2.5	9	88.5	0	3.4	0.05
L-HPMC+M	1.5	16.5	82	0	6.8	0.1
M-HPMC+M	2	16.5	81.5	0	6.8	0.1
H-HPMC+M	2.5	16.5	81	0	6.8	0.1
L-HPMC+C	1.5	8	61.5	29	4.13	12.1
M-HPMC+C	2	8	61	29	4.13	12.1
H-HPMC+C	2.5	8	60.5	29	4.13	12.1

*HPMC: hydroxypropylmethylcellulose; SMP: skimmed milk powder.

The skimmed milk powder, the sweetener (EPSA, Valencia, Spain, 0.05 g/100g of a blend of 3.5:1.5 aspartame: acesulfame K) and the HPMC powders were dispersed in mineral water (Aquarel™, Barcelona, Spain) at 350 rpm at room temperature for 15 min in a cooking device (Thermomix TM 31, Wuppertal, Germany). The white chocolate

flavour (0.1 g/100 g, 555267T Firmenich SA, Geneva, Switzerland) was added and mixed in for an additional 5 min. The cream was added while stirring gently (120 rpm, Heidolph, Schwabach, Germany) for 3 min to prepare the added-cream samples. The samples were kept under refrigeration (4°C) until they were analysed (24 hours). All the samples were prepared three times for triplicate measurement on different days.

2.2 Rheological measurements

Rheological measurements were carried out in an ARG2 controlled stress rheometer (TA Instruments, New Castle, DE, USA), using serrated parallel-plate geometry (40-mm diameter; 1-mm gap), and monitored by ARES Software version V5.7.0 (T.A. Instruments, New Castle, DE, USA). A temperature of 10±1°C was selected as representative of the usual consumption temperature of milk-based desserts. It was maintained by an F30-C circulating water bath (Julabo GmbH, Seelbach, Germany) during the measurements. A fresh sample was loaded for each run. All the samples were allowed to rest for 5 min in the rheometer cell before each measurement.

2.2.1. Flow behaviour

Flow behaviour was measured by recording the shear stress values when shearing the samples at linearly increasing shear rates from 1 to 200 s^{-1} over 60 s and down in reverse sequence for the same time. To quantify the time dependence of the flow, the relative thixotropic area (A_t) was calculated as the difference between the area under the upstream data point curve (A_{up}) and the area under the downstream data point curve (A_{down}). If applicable, the percentage relative hysteresis area (Dolz, González, Delegido, Hernández, and Pellicer, 2000; Tárrega, Durán, and Costell, 2004) was calculated according to equation 1:

$$A_r = (A_{up} - A_{down})/A_{up} \times 100 \qquad \text{(equation 1)}$$

The data from the ascending segment of the shear cycle were fitted to the Ostwald-de Waele model (equation 2) using Rheowin Pro software (version 2.93, Thermo Haake):

$$\sigma = K\,\gamma n \qquad \text{(equation 2)}$$

where σ (Pa) is the shear stress, K (Pa s^n) is the consistency index, γ is the shear rate (s^{-1}) and n is the flow index.

In time-dependent and non-Newtonian shear-thinning products, perceived thickness is difficult to predict with rheological parameter values since flow in the mouth is a combination of shear and elongational flow (van Vliet, 2002). However, some authors have found that oral thickness correlates well with different rheological indices. According to Wood (1968), apparent viscosity at a shear rate of 50 s^{-1} has practical utility as a possible instrumental index of perceived thickness in semisolid foods. It has been used by many authors (Arancibia, Costell and Bayarri, 2013a; Cook, Hollowood, Linforth and Taylor, 2003). Consequently, the apparent viscosity values at a shear rate of 50 s^{-1} (η_{50}) were also calculated as follows:

$$\eta_{50} = K \cdot \gamma^{n-1} \qquad \text{(equation 3)}$$

2.2.2. Viscoelastic properties

Viscoelastic properties were measured by small amplitude oscillatory shear tests. Before obtaining the mechanical spectra, the linear viscoelastic region (LVR) of each sample was determined. Stress sweeps were then performed, applying a stress wave amplitude within the LVR over the frequency range from 10 to 0.01 Hz, and the values of the storage modulus (G'), and the loss modulus (G") as a function of the frequency were plotted using the Rheology AdvantageTM data analysis software, version V5.7.0 (T.A. Instruments, New Castle, DE, USA).

2.3. Sensory evaluation

2.3.1. Measurement of expected satiation

A total of 113 consumers (untrained, 64 females, mean age 23.9 years) participated in the test. All of them were recruited from the staff and student population of the Polytechnic University of Valencia, were consumer of dairy products and declared no food allergies or lactose intolerance. The samples were served at consumption temperature (10.0 ± 0.5ºC) in small white plastic cups (30 ml) coded with random three digit numbers. Expected satiation was assessed using four picture scales of "comparison foods", based on Tárrega, et al., (2014). The food products selected for comparison in the present study were: apple, sandwich (ham and cheese), Kit-Kat® chocolate bar and Oreo® biscuits (covered with white chocolate). Each of the four nine-point picture scales showed 9 photographs of increasing amounts of one of the comparison products, numbered from 1 to 9 (Figure 1). Each scale was printed individually (80x300 mm). The energy content of the scales ranged from 13 to 133 kcal for apple, from 75 to 675 kcal for sandwich, from 58.5 to 526.5 kcal for chocolate bar and from 88.3 to 794.7 kcal for biscuit.

The test consisted of two parts. In the first part the panellists were provided with the four picture scales and answered the following question for each scale: "Assuming it is 5:00 pm and you want to eat something to keep you going until dinner at 9:00 pm, please indicate the amount you would eat of each food on the picture scale". In the second part of the test, six milk-based dessert samples (low and high HPMC levels, each with or without added milk powder or added cream: L-HPMC, L-HPMC+M, L-HPMC+C, H-HPMC, H-HPMC+M and H-HPMC+C) were served monadically in a randomized order. Each panellist was asked to eat a single spoonful of the sample and select the amount of food on each picture scale that would be equally as satiating as a whole pot of the sample (they were given an empty 135-mL plastic pot for size estimation purposes). Mineral water was provided for rinsing the mouth between samples.

The RES values were calculated as the amount (in kcal) of the comparison food selected as being as satiating as a whole pot of the sample, divided by the amount that the consumer had previously indicated for the same food scale (in kcal).

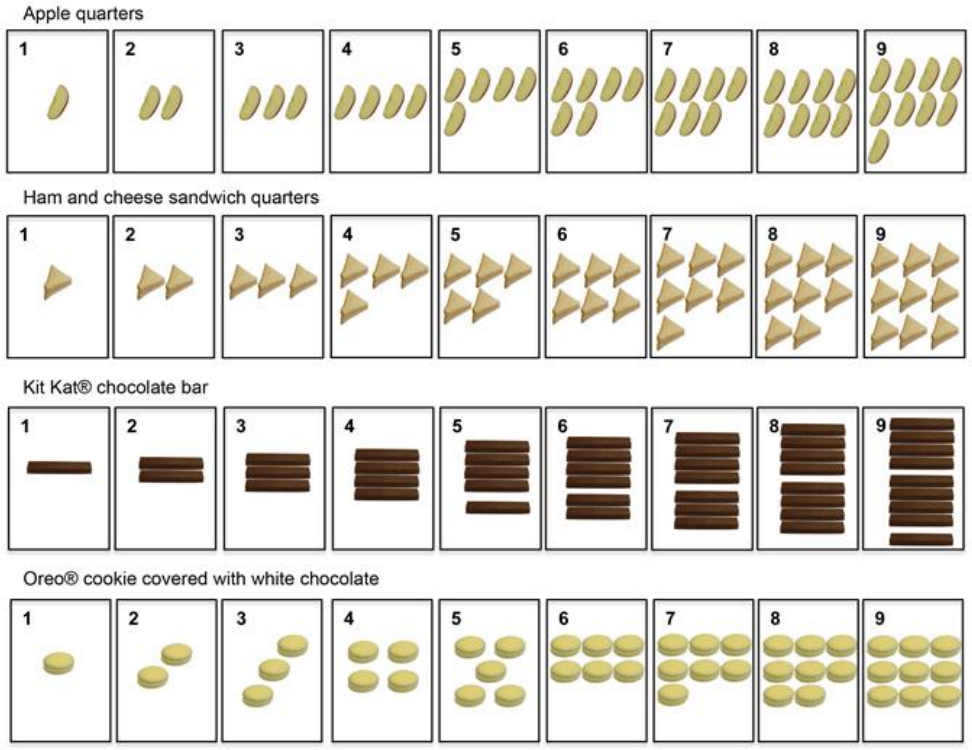

Figure 1. Four picture scales used as "comparison food" for evaluating the expected satiation elicited by the milk-based snacks.

2.3.2 Liking assessment

For each sample, the participants (n=113) were also asked to rate their global liking, liking for the flavour and liking for the consistency on 9-box structured hedonic scales (from 1= "I dislike it very much" to 9= "I like it very much").

2.3.3 Temporal Dominance of Sensations (TDS) measurement of texture attributes

Panel training. The sensory panel consisted of fourteen assessors recruited from the scientific staff and students of IATA, with ages ranging from 20 to 60 years, who had previous experience in TDS analysis of different food items. Four 1-hour preliminary sessions were conducted to select the TDS terms and familiarize the panel with the sample characteristics. In the first session, the panellists described four different milk-based desserts, with the lowest and the highest level of HPMC (1.5 and 2.5 g/100 g, respectively), in order to generate a list of textural attributes that appeared over the time of consumption. During the second and third sessions, the attributes cited most often in the first session were discussed and defined for the panellists to make the final selection. The selected terms were thin, thick, gummy, mouth-coating, creamy, fondant and adhesive (Table 2). In the fourth session, the panel participated in a simulated TDS session in order to solve any software operation questions (Pineau, et al., 2012).

Table 2. Attribute definitions generated by the trained panel.

Attribute	Description
Thin	Liquid, light consistency
Thick	Semi-solid, heavy consistency
Gummy	Cohesive, difficult to disintegrate to a state suitable for swallowing
Mouth-coating	Presence of a film covering the palate and other parts of the mouth
Creamy	Soft, smooth and homogeneous texture
Fondant	Quick disappearance in the mouth
Adhesive	Degree of adherence to certain parts of the mouth

Formal TDS assessment. The TDS tests were performed on three different days in order to assess three replications. Six samples were evaluated (those with the lowest and highest HPMC content, 1.5 g/100 g and 2.5 g/100 g, and with double the milk powder and added cream). The samples were labelled with three-digit numbers. A Williams Latin square design was followed. The samples were served monadically in a randomized order across the panel at consumption temperature (10 ± 1°C). The panellists were instructed to put the complete spoonful (10 ml) offered into their mouth and immediately click on the start button, then after swallowing the sample and when no new sensations were perceived, to click on the stop button. During the consumption, the subject was asked to consider which of the seven textural attributes shown on the screen was perceived as dominant at each time by clicking on the button for the corresponding attribute. At each time the subject can click on only one attribute; when the dominant perception changed, the subject had to click the new dominant sensation. The subject was free to choose several times the same attribute or conversely to never select an attribute as dominant. Mineral water (Aquarel™, Barcelona, Spain) was used for cleansing the mouth between samples. The actual consumption times of each

subject (three repetitions) for the six samples were computed and analysed in order to study the effect of sample composition on time of consumption across subjects.

2.4. Statistical data analysis

Analysis of variance (ANOVA) was performed on the rheological results, the relative expected satiation values and the liking scores using the Statgraphics Plus 5.1 software package (Statistical Graphics Co., Rockville, Md., U.S.A.). The effect of HPMC concentration, added protein or added cream and their interaction were studied applying a two-way ANOVA. Fisher's least significant difference (LSD) test was used to evaluate mean value differences ($p<0.05$).

For the TDS analysis, the data acquired were recorded using FIZZ software (version 2.45, Biosystemes, Couternon, France), following the method proposed by Pineau, et al., (2009). As proposed by Lenfant, Loret, Pineau, Hartmann, and Martin (2009), the attribute chosen as dominant and the times when the dominance started and stopped were collected for each panellist run. The effect of the sample composition on the consumption time across subjects was studied applying ANOVA. As the duration of ingestion differed from one subject to another for plotting the TDS curves the data were normalized by adjusting them according to each subject's individual duration of mastication (Albert, Salvador, Schlich, and Fiszman, 2012).

Finally, when the TDS curves were plotted, two additional lines were drawn for the chance and significance levels. The chance level refers to the dominance rate that an attribute could obtain by chance. Its value is inversely proportional to the number of attributes ($P_0=1/p$, where p is the number of attributes). The significance level is the minimum value this proportion should equal if it is to be considered significantly ($p<0.05$) higher than P0.

3. Results and discussion

3.1. Rheological properties of the milk-based desserts

3.1.1. Flow behaviour

The flow curves obtained for the milk dessert samples with the lowest (1.5 g/100g) and highest (2.5 g/100g) HPMC levels are shown in Figure 2. As the samples with 2 g/100g of HPMC showed intermediate behaviour between the others and did not provide further information the results are not shown.

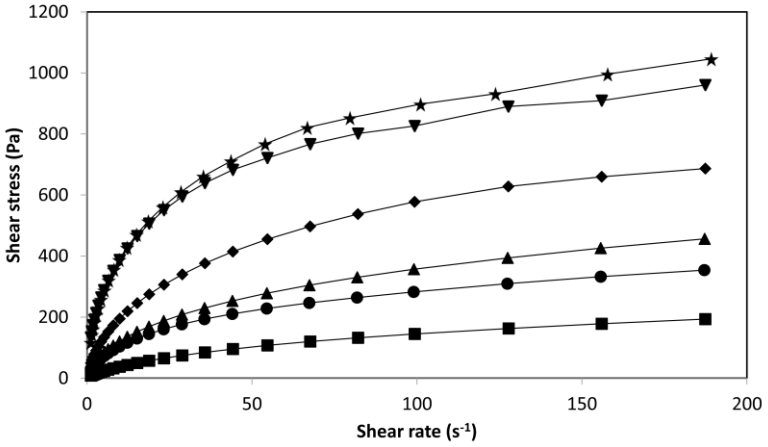

Figure 2. Flow behaviour of the six milk dessert samples. Rising shear rate. L-HPMC (squares), L-HPMC+M (circles), L-HPMC+C (diamonds), H-HPMC (triangles), H-HPMC+M (inverted triangles), and H-HPMC+C (stars). See Table 1 for sample codes.

The experimental results in the upward curve were adjusted to the Ostwald- de Waele model and the consistency index (K), flow index (n) and apparent viscosity at 50 s^{-1} (η_{50}) values for the nine systems were calculated (Table 3).

Table 3. Mean values (n=3) of flow behavior and viscoleastic parameters: consistency index (K), flow index (n), thixotropy area, viscosity at 50 s^{-1} (η_{50}), storage modulus (G') and loss modulus (G") of the milk desserts.

Sample*	Flow behaviour				Viscoelastic properties	
	K (Pa·s^n)	n	η_{50} (Pa·s)	thixotropic area (Pa·s)	G' (Pa)	G'' (Pa)
L-HPMC	10.5 ^{a} (0.6)	0.57 ^{a} (0.01)	96.1 ^{a} (6.1)	3807.3 ^{a} (661.2)	4.31 ^{a} (0.51)	7.48 ^{a} (0.17)
M-HPMC	35.1 ^{ab} (8.5)	0.44 ^{bc} (0.07)	191.4 ^{ab} (12.0)	6155 ^{ab} (1209.0)	14.96 ^{a} (5.45)	19.85 ^{a} (2.51)
H-HPMC	65.4 ^{bc} (7.6)	0.41 ^{bc} (0.05)	329.2 ^{cd} (93.9)	9079.7 ^{b} (4420.2)	39.74 ^{ab} (32.06)	45.91 ^{ab} (21.77)
L-HPMC+M	47.0 ^{bc} (7.3)	0.39 ^{bc} (0.07)	218.5 ^{b} (39.6)	9353.7 ^{b} (2115.1)	30.67 ^{ab} (5.47)	26.92 ^{ab} (2.5)
M-HPMC+M	91.8 ^{cd} (7.2)	0.38 ^{cd} (0.05)	406.4 ^{de} (101.0)	17570 ^{c} (5398.6)	56.21 ^{b} (7.57)	51.51 ^{b} (2.53)
H-HPMC+M	196.5 ^{e} (17.4)	0.31 ^{de} (0.04)	652.8 ^{f} (78.4)	24866.7 ^{d} (996.2)	108.85 ^{de} (20.61)	102.55 ^{c} (18.08)
L-HPMC+C	42.4 ^{ab} (4.0)	0.45 ^{bc} (0.01)	250.8 ^{bc} (20.7)	8764.3 ^{b} (2140.2)	67.08 ^{bc} (24.04)	48.85 ^{b} (10.86)
M-HPMC+C	100.2 ^{d} (13.5)	0.4 ^{bc} (0.02)	471.5 ^{e} (34.2)	16550 ^{c} (3074.9)	97.31 ^{cd} (27.88)	82.56 ^{c} (13.55)
H-HPMC+C	228.0 ^{e} (50.4)	0.29 ^{e} (0.06)	706.1 ^{f} (31.4)	46576.7 ^{e} (345.0)	139.95 ^{e} (37.44)	136.57 ^{d} (27.75)

*Different letters in the same column indicate statistically significant differences ($p<0.05$).

*See sample codes in Table 1.

Two-way ANOVA with interaction was applied to the flow rheology results. One analysis considered the effects of HPMC concentration (three levels) and cream addition (two levels) and their interactions, and another considered the effects of HPMC concentration (three levels) and extra milk powder addition (two levels) and their interactions. The results from the ANOVA analysis of rheological parameter values showed that the effect of the interaction between HPMC concentration and cream addition was only significant ($p<0.05$) for the thixotropy area values, but not for the other parameters. This significant interaction indicated that when cream was added to the samples, the thixotropy area values were affected differently depending on the HPMC concentration. No significant interactions between HPMC concentration and milk powder addition were found.

All the samples studied showed pseudoplastic behaviour ($n<1$), and exhibited shear-thinning flow behaviour and large areas of thixotropy (Table 3). Every sample showed hysteresis loops when sheared during a complete cycle (data not shown), indicating that the sample flow was time-dependent. This thixotropic behaviour increased with HPMC concentration. Considering that the hysteresis loop area represents the energy needed to destroy the structure of a material (Bayarri and Costell, 2011), the samples with 2.5 g/100g of HPMC needed higher energy to break their structure, especially H-HPMC+C as compared with the other samples formulated with the addition of milk powder or without any extra addition. This would indicate that the flow behaviour of these systems was not only influenced by HPMC concentration, but also by the presence of cream fat or milk solids. Not only HPMC molecular interactions but also fat globules contributed to the higher flow resistance of these samples. The thixotropic area value for H-HPMC+C was significantly the highest ($p < 0.05$).

The experimental results obtained for HPMC desserts showed that a higher HPMC concentration produced a more structured system, which broke down partially with increasing shearing time.

Besides the dominant effect of the amount of HPMC, the presence of fat globules (cream) or milk solids also played an important role. On comparing the shear stress and shear rate profiles of the samples at low shear rates, the highest slope values corresponded to samples with the highest concentration of HPMC (2.5%) and added ingredients (H-HPMC+M and H-HPMC+C), from which it may be concluded that samples with added cream or protein need higher shear stress to break down. The samples formulated with added cream also presented higher consistency index values than the other samples with the same concentration of HPMC. As a result, samples H-HPMC+C and H-HPMC+M presented the highest consistency values ($p < 0.05$).

As expected, K values increased significantly with HPMC concentration, probably caused by an increase in resistance to flow due to particle-particle interaction. At lower HPMC concentrations the HPMC molecular chains are in their most extended conformation, while at higher polymer concentrations, extended HPMC chains start to overlap and entangle, resulting in a transient network structure (Arancibia, Bayarri, and Costell, 2013b).

With regard to the flow behaviour index (n), increased HPMC concentration significantly decreased its values, which ranged from 0.29 for H-HPMC+C to 0.57 for L-HPMC, and the HPMC samples without protein or cream addition presented the significantly highest values ($p < 0.05$) compared with the rest of samples. The increase in flow pseudoplasticity due to increased HPMC concentration can be explained by the orientation of the HPMC macromolecules, as they align more easily in the direction of flow at lower concentrations (Rozema and Beverloo, 1974). Arancibia et al. (2013b) obtained similar results when comparing CMC as a thickener in o/w emulsions.

It is well known that flow in the mouth is a combination of shear and elongational flow (van Vliet, 2002), and apparent viscosity at a shear rate of 50 s-1 has practical utility as a possible instrumental index of perceived thickness in semisolid foods. The H-

HPMC+M and H-HPMC+C samples had the significantly highest ($p<0.05$) apparent viscosity (η_{50}) values.

3.1.2. Viscoelastic properties

Figure 3 shows the evolution of the storage modulus (G') and loss modulus (G") values as a function of the frequency (mechanical spectra) of the samples studied (samples with 2.0g/100g of HPMC are not shown). The samples without additions (L-HPMC, M-HPMC and H-HPMC) showed a non-gelling behaviour with typical viscoelastic features of a random coil polymer solution: strong frequency dependence and G' – G" crossover (Figure 3), whereas the G' values were higher than those for G" in the samples formulated with added milk powder and added cream, denoting a weak gel-like behaviour.

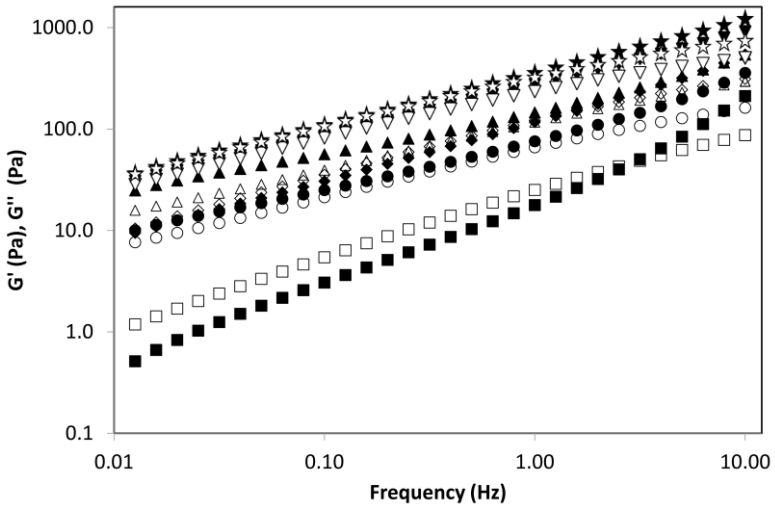

Figure 3. Mechanical spectra of the six systems. G' (black symbols), G" (white symbols). L-HPMC (squares), L-HPMC+M (circles), L-HPMC+C (diamonds), H-HPMC (triangles), H-HPMC+M (inverted triangles), and H-HPMC+C (stars). See Table 1 for sample codes.

Comparison of the mechanical spectra and the G' and G" values at 1 Hz of the nine systems (Table 3) showed that only the samples without cream or extra milk powder (L-HPMC, M-HPMC and H-HPMC) displayed a non-gel-like behaviour (G" values higher than G'). H-HPMC+C presented the significantly highest elastic and viscous moduli values ($p < 0.05$). As the HPMC concentration increased, the mechanical spectra pattern changed (indicating a transition from an entangled polymer solution to a more structured system), the dynamic moduli became less frequency-dependent and the elastic contribution gradually prevailed over the viscous contribution.

The present study did not aim to characterize the rheological behaviour of the samples (which can easily be deduced from the literature) but to relate the variations in their behaviour to the expectations of satiation aroused by the desserts formulated with the different amounts of HPMC and the interactions with protein and cream, as discussed below.

3.2. Measurements of relative expected satiation (RES) of milk-based desserts

Beliefs and expectations can have a marked effect on satiety and this effect can persist well into the inter-meal interval (Brunstrom, Brown, Hinton, Rogers, and Fay, 2011). According to Brunstrom et al., (2008), expectations play a causal role in the satiety that is experienced after a food has been consumed. These expectations arise from pre-absorptive signals from the cognitive and sensory systems (Scheibehenne et al., 2010; Zijlstra, et al., 2009). What is currently less clear is the extent to which these expectations integrate with post-ingestive signals to determine satiety (Chambers, Ells, and Yeomans, 2013).

"Expected satiation" is a utility value, i.e. a benefit the individual expects after the food has been consumed, adding an extra value to it. It may be noted that the precise correspondence between expected satiety and expected satiation remains unclear. In particular, researchers might consider whether decisions about the amount of food to

consume are governed to a greater or lesser extent by expected satiety or by expected satiation, and whether there are individual differences in their relative importance (Brunstrom et al., 2008).

In the present study, the differences among the characteristics of the formulated milk-based desserts were based on increasing HPMC concentration. At each HPMC level, cream or extra milk powder were also tested to contribute textural and flavour changes. Consumers were asked to evaluate the expected satiation capacity of six samples: the three with the lowest HPMC concentration (L-HPMC, L-HPMC+M, and L-HPMC+C) and the three with the highest (H-HPMC, H-HPMC+M, and H-HPMC+C). The intermediate HPMC concentration samples were not evaluated in order to avoid consumer fatigue. The expected satiety score that each participant awarded each dessert on each of the four 9-picture food scales was transformed into the corresponding energy load (kcal). Although the pattern of differences in expected satiation between samples was similar for the four different scales, the actual energy values selected showed considerable variations depending on the food product (scale) used for comparison. The average expected satiation elicited by the samples varied from 62.8 to 77.0 kcal on the apple scale and, at the opposite extreme, from 311.4 to 432.2 kcal on the Oreo® biscuit scale (Table 4). These scale-dependent (comparison food-dependent) differences confirmed that the differences in expected satiation elicited by the different milk-based desserts are not linked to energy density alone: other factors such as familiarity or liking could play a role (Brunstrom, Collingwood, and Rogers, 2010; Brunstrom, et al., 2008). The RES values were calculated as the amount of food selected as being equally as satiating as the sample (supposing they had eaten the whole pot they were given with the comparison food scales), divided by the amount that the consumer had previously indicated on the same food scale that he or she would eat to keep going from 5 p.m. to 9 p.m. (Table 4). The results obtained from this calculation were no longer scale-dependent. The resulting value (RES) gives the amount of sample that would be as satiating as the amount of the comparison food that the consumer would

eat in the described circumstances: the higher the RES value of the milk-based dessert, the higher the expected satiation it elicited.

Table 4. Expected satiation values in each scale (Kcal). RES values and liking scores of the six samples. See Table 1 for sample codes.

Sample	Scale-dependent Expected Satiation (kcal)				RES	Liking (9-box scale)		
	Apple scale	Sandwich scale	Kit Kat® scale	Oreo® scale		Global	Taste	Consistency
L-HPMC	62.8^{a}	267.1^{a}	225.3^{a}	311.4^{a}	1.02^{a}	3.8^{b}	3.2^{b}	4.6^{c}
L-HPMC+M	68.4^{ab}	319.1^{b}	266.1^{b}	369.5^{b}	1.13^{b}	4.2^{bc}	4.1^{c}	5.0^{c}
L-HPMC+C	72.0^{bc}	317.8^{b}	263.8^{b}	371.8^{b}	1.19^{b}	5.9^{d}	5.9^{d}	5.9^{d}
H-HPMC	73.1^{bc}	336.2^{b}	279.7^{b}	386.5^{bc}	1.22^{bc}	2.4^{a}	2.3^{a}	2.8^{a}
H-HPMC+M	77.0^{bc}	357.9^{b}	289.9^{b}	410.5^{bc}	1.33^{d}	2.5^{a}	2.5^{a}	2.6^{a}
H-HPMC+C	75.3^{c}	355.3^{b}	292.0^{b}	432.2^{c}	1.32^{d}	4.3^{c}	4.4^{b}	3.6^{b}

*Different letters within the same column indicate significant differences ($p < 0.05$) according to Fisher's test.

The RES results depended primarily on the HPMC concentration (Table 4). The samples prepared with the lowest level of HPMC (1.5%) elicited significantly lower RES values than those for the samples with the highest HPMC concentration (2.5%). In particular, sample L-HPMC (prepared with the lowest HPMC level and without any extra addition) was, as expected, significantly the least satiating ($p < 0.05$) (based on its fluid-like characteristics). The RES values elicited by the samples with the lowest level of HPMC and added cream and added milk powder (L-HPMC+M and L-HPMC+C)

presented significantly higher RES values than L-HPMC but did not differ significantly between each other.

The same pattern was observed for the three samples with the highest concentration of HPMC. The samples with extra cream or milk powder were scored as more satiating than the sample without any extra addition (Table 4).

These results suggested that the HPMC concentration is the critical factor for suggesting satiating power to the consumers. Cream and milk powder additions also played a role in eliciting satiating capacity, since within each HPMC-concentration set of samples (L and H), those containing cream (+C) or extra milk powder (+M) showed significantly higher values of RES than the corresponding samples with no addition (Table 4). The rheological behaviour results confirmed that the consistency values increased significantly with HPMC concentration, mainly caused by an increase in resistance to flow due to particle-particle interaction. Apart from the dominant effect of the amount of HPMC, the presence of fat globules (in added-cream samples) or milk solids (in the added-milk powder ones) contributed the highest consistency values ($p < 0.05$) over the entire shear stress range studied, although to a lesser extent than the HPMC concentration. In the same way, the G' values of the H-HPMC samples were higher than those of the L-HPMC samples, demonstrating the higher viscosity of the more concentrated desserts. ANOVA for each rheological parameter (data not shown) confirmed that RES values were significantly higher for higher values of K, η_{50}, thixotropic area, G' and G" and for lower values of n.

3.3. Liking measurement

The participants were asked to rate their global liking, liking for the flavour and liking for the consistency of each sample on 9-box structured scales. L-HPMC+C received the significantly highest scores for global, flavour, and consistency liking ($p < 0.05$) (Table 4). Liking scores for the rest of the samples depended on the liking modality.

Global and flavour liking scores evolved completely in parallel. The samples with added cream were the most liked for both HPMC concentrations (occupying the first and second place for both global and flavour liking). This means that the cream contributed a flavour that was well recognized and assessed as pleasant.

Regarding the liking for the samples' consistency, a clear preference appeared for the three samples with the lowest HPMC concentration. There were no significant differences between samples H-HPMC and H-HPMC+M but the added-cream sample had the best-liked consistency. It should be noted that cream contributes not only a pleasant texture, due principally to its fat content, but also a pleasant taste.

On comparing the liking and RES results, it can be seen that the less fluid samples (HPMC 2.5%) were the most satiating but not the best liked. When designing a satiating food (or any other), acceptability cannot be left out of the equation. In this case, if the desired effect is high expected satiety then a formulation with a high HPMC content (2.5%) should be considered, but with the addition of cream (H-HPMC+C), even though this adds fat, increasing the calorie level. McCrickerd et al. (2012) found that altering the sensory characteristics of a drink to give it a slightly thicker texture or a creamier flavour both generated expectations that the product would be more satiating. In this case, it would be possible to add a creamy flavour that would contribute this flavour note without adding calories. However, in the present case the cream may have contributed not just a creamy flavour, but also other sensory features such as texture. For their part, the more fluid samples (HPMC 1.5%) obtained lower RES values and the consumers' preferred sample was again the one with added cream (L-HPMC+C). Food likes and dislikes are thought to reflect underlying associations that form between the sensory characteristics of a food and its post-ingestive effects (Brunstrom, 2005). Previous work has suggested that expected satiety is based on the same flavour-nutrient associations (Brunstrom and Shakeshaft, 2009).

3.4. Temporal Dominance of Sensations (TDS) curves

TDS is a recent descriptive sensory method that consists in assessing continually which sensation is dominant and scoring its intensity, until the sensations end. The panellists are presented with the complete list of attributes on a computer screen and are asked to assess which of the attributes they perceive as dominant (i.e. the most striking perception at a given moment). In the course of the evaluation, when the panellist considers that the dominant attribute has changed, he or she has to score the new dominant attribute, and so on, until the perception ends (Pineau, et al., 2009).

As Varela, Pintor and Fiszman (2014) stated, dynamic perception techniques such as TDS have made it possible to show how different attributes are perceived at different times during ice cream consumption and how they are modulated by the presence of hydrocolloids.

In order to investigate the type of sensations elicited by the desserts and their sequential appearance over time in greater depth, a TDS test was conducted. Figure 4 (A to F) shows the TDS graphs for the same six milk-based desserts assessed for RES (L-HPMC, L-HPMC+M, L-HPMC+C, H-HPMC, H-HPMC+M, and H-HPMC+C). Each curve represents the attributes chosen as dominant at each moment during the evaluation. Two groups of samples were easily detected. One was made up of samples L-HPMC, L-HPMC+M and L-HPMC+C, prepared with the lowest concentration of HPMC, and the other was formed by those with the highest HPMC concentration: samples H-HPMC, H-HPMC+M, and H-HPMC+C.

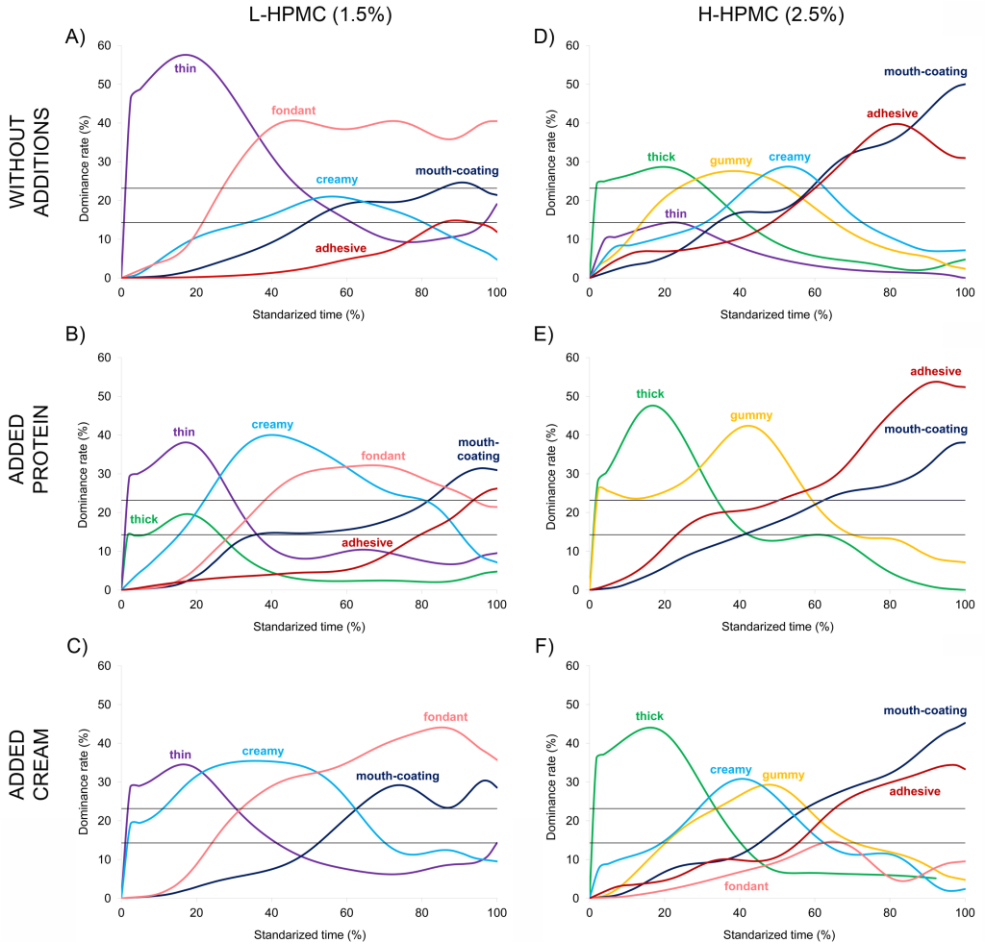

Figure 4. Normalized TDS curves by sample. A) L-HPMC, B) L-HPMC+M, C) L-HPMC+C, D) H-HPMC, E) H-HPMC+M and F) H-HPMC+C.
See Table 1 for sample codes.

Samples with a low HPMC concentration

Thin was the first sensation elicited by L-HPMC and had a high and long dominance rate (up to 60% of the consumption time). Fondant was the second dominant sensation (appearing as significant for 21% of the consumption time) and lasted up to the end of

consumption. This attribute was defined as a "disappearing from the mouth" sensation, corresponding to a quite thin sample with low cohesiveness. A creamy sensation appeared but did not exceed the significance level for this sample. Thin and fondant sensations are probably related to a light texture, in other words, a sample that only requires short oral processing and it is soon ready to be swallowed.

For samples L-HPMC+M and L-HPMC+C, thin also appeared as the first dominant sensation, but creamy appeared distinctly for these two samples, over the significance level, and earlier than in sample L-HPMC. The creamy sensation lasted up to 70% and 90% of the consumption time respectively. Finally, fondant appeared later than in the L-HPMC sample. Only sample L-HPMC+C presented some significant mouth coating sensation, denoting a greater sensation of residence in the mouth.

Samples with a high HPMC concentration

The set of samples with the highest HPMC level presented a very different profile of TDS curves. Thick was the first dominant sensation for the three samples and lasted up to approximately 40% of the consumption time. Its dominance rate did not exceed 28% for sample H-HPMC but was very much higher for H-HPMC+C and H-HPMC+M, indicating that the additions (both cream and milk powder) added thick sensations in the mouth. Indeed, H-HPMC (without extra additions) thin sensation appeared although in the limit of the chance level. This was in accordance with their rheological properties, as the samples with the highest HPMC level (2.5 g/100g of HPMC), especially H-HPMC+C, needed higher energy levels to break their structure compared with the other samples (higher thixotropic area). An increase in consistency (higher K values) due to the higher HPMC concentration was also perceived in the TDS analysis.

Gummy was the second sensation which appeared during the consumption time of these samples. This sensation appeared alongside creamy in samples H-HPMC and H-HPMC+C, but with a lower dominance rate, whereas in sample H-HPMC+M creamy

was not perceived to a significant degree but gummy presented a higher and longer dominance rate than for the other two samples.

Finally, the three samples with 2.5% HPMC were perceived as adhesive and mouth-coating from 50% of the consumption time up to the end of this time. Both attributes appeared almost together and evolved in parallel, so these attributes could be thought to describe similar sensations of oral structure coating and residence in the mouth (McCrickerd, et al., 2012).

The actual consumption times (taken before normalising the data to construct the TDS curves) of each sample for each subject (three repetitions) were additionally analysed through ANOVA. The results showed that not only higher HPMC concentration but addition of cream or milk powder significantly increased the consumption time across subjects, being the times for the samples H-HPMC+C and H-HPMC + M significantly the longest. This means that regardless the different time that different subjects used to consume the same sample, in average the composition had a significant effect on this time.

TDS and its relation with satiating capacity

From the analysis of the TDS curves for the two set of samples (L and H) it was clear that dominant sensations such as thin and fondant was the general pattern of the TDS curves for low-concentration HPMC samples which elicited lower values of RES than H samples (Table 4); dominance of creamy and higher dominance rate for adhesive and mouth coating sensations in cream-added and extra milk powder-added L-samples seemed to elicit significantly higher RES values. On the other hand, thick, creamy, gummy and adhesive were the dominant attributes that defined the TDS curves of high-concentration HPMC samples which elicited the highest values of RES. Cream and extra milk powder additions contributed differences in the dominance rate and duration of some of these sensations. Brunstrom et al. (2011) demonstrated a link between

expected satiety and the actual satiety that is experienced that can persist well into the inter-meal interval. In the present study, thicker products required more laborious in-mouth handling than their thinner, lighter versions; the analysis of actual consumption times for the different samples corroborated this statement. In consequence H samples, had longer orosensory exposition that might elicit stronger satiating power perception and addition of cream and extra milk powder actively contributed differences in satiety expectations.

Conclusions

Pre-absorptive signals coming from the cognitive and sensory systems contribute expectations about the satiating power of food products. In previous research, a sensation of thickness has been shown to elicit higher perceptions of satiating capacity in beverages and semi-solid food than for thinner versions. However, the majority of hydrocolloids used as thickeners do not present simple flow behaviour, i.e. the more I add the thicker the product will be. Instead, they usually show a complex viscoelastic behaviour that in turn is responsible for a range of in-mouth sensations which could interact to elicit different expected satiating capacities. The addition of other components such as proteins, fat or other carbohydrates can strongly modulate that perception. Another aspect that should not be neglected is the distinctive eliciting of sensations which appear as the product undergoes oral processing during the consumption time. The type of oral processing depends, in turn, on the different characteristics of the product which determine its oral trajectory.

In short, adding thickeners to elicit stronger satiating sensations is not straightforward. A number of factors that can modulate these sensations have to be taken into account: the presence of other components, their interaction, and their influence on the temporality of the in-mouth perception are only few of such possible factors.

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Capítulo 2:

Diseño de yogures con alta capacidad saciante

How is an ideal satiating yogurt described? A case study with added-protein yogurts

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Abstract

Protein is recognized as the macronutrient with the highest satiating ability. Yogurt can be an excellent basis for designing satiating food as it is protein-based food product. Five different set-type yogurts were formulated by adding extra skim milk powder (MP), whey protein concentrate (WPC), calcium caseinate (CAS) or a blend of whey protein concentrate with calcium caseinate (CAS-WPC). A control yogurt without extra protein content was also prepared. Differences in sensory perceptions (through CATA questions) were related to the consumers' expected satiating ability and liking scores (of several modalities). In addition, an "ideal satiating yogurt" was included in the CATA question to perform a penalty analysis to show potential directions for yogurt reformulation and to relate sensory and non-sensory yogurt characteristics to satiating capacity.

Keywords: yogurt, protein, expected satiating ability, CATA question, ideal, penalty analysis.

1. Introduction

Yogurt is one of the most popular dairy products because of its good nutritional value and its healthcare function (Han, Fu, and Zhao, 2015). This milk derivative contributes considerably to the intake of nutrients such as proteins, vitamins B2 and B12, and also certain minerals, mainly calcium, magnesium, and zinc. Knowledge of the beneficial effects of milk derivatives has led manufacturers to produce a wide range of yogurts with different flavors, textures, and consistencies in response to consumer preferences (Luis, et al., 2015). Yogurt contains high levels of protein, which is recognized as the macronutrient with the highest satiating capacity (Blundell, Lawton, Cotton and Macdiarmid, 1996; Benelam, 2009). Consequently, yogurt could be an excellent basis for designing a satiating product (Morell, Hernando, Llorca, and Fiszman, 2015a) that offers the pleasure and satisfaction associated with low-energy /healthier versions of foods without consumers feeling 'deprived' (Hetherington, et al., 2013). However, it is difficult to reformulate since the new constituents can affect the energy density, palatability and texture, and a number of other factors that are involved in eating episodes (Varela and Fiszman, 2013).

In yogurt production, the solids content of milk is usually increased, as milk powder is traditionally used to enrich the yogurt milk before fermentation. However, new milk and whey fractionation technologies produce a wide range of dairy proteins of increased quality and availability, such as whey protein concentrates (WPCs) and Na- or Ca-caseinates, that may provide a cost-effective alternative to skim milk powder (Sodini, Montella, and Tong, 2005) and help to bring new products with added protein onto the market. These dairy proteins have different properties and can be used separately or blended. The effect of caseinates and WPCs has been compared (Damin, Alcântara, Nunes, and. Oliveira, 2009; Guzmán-González, Morais, Ramos, and Amigo, 1999; Akalin, Unal, Dinkci, and Hayaloglu, 2012) but there are few references to the effect of blends of caseinates and WPCs on yogurt properties (Guzmán-González, Morais, and Amigo, 2000; Remeuf, Mohammed, Sodini, and Tissier, 2000).

The effect of the replacement of milk powder with WPC on the textural and physical properties of yogurts has been widely studied, and positive effects on yogurt firmness and viscosity have been reported (Salvador and Fiszman, 2005; Cheng, Augustin, and Clarke, 2000; Puvanenthiran, Williams, and Augustin, 2002). In general, yogurts with sodium caseinate added have also been found to be firmer and have less syneresis than yogurts that have the same protein level due to whey protein-based ingredients (Peng, Serra, Horne, and Lucey, 2009; Marafon, Sumi, Granato, Alcântara, Tamime, and de Oliveira, 2011; Isleten, and Karagul-Yuceer, 2006). Comparison of the effects of caseinate and whey proteins on the sensory properties of yogurt has not been reported.

The firming effect of added proteins could be advantageous in the formulation of yogurts with enhanced expectations in satiating capacity, since it is recognized that textural characteristics play an important role in eliciting these sensations (Morell, Ramírez-López, Vélez-Ruiz, and Fiszman, 2015b). A number of techniques have been used to quantify 'expected satiating capacity' (Brunstrom and Rogers, 2009): rating fullness after showing food images (Forde, van Kuijk, Thaler, de Graaf, and Martin, 2013; de Graaf, Stafleu, Staal, andWijne, 1992), or after tasting a mouthful of food (Green and Blundell, 1996); a comparison method with images (constant stimuli) was also reported (Brunstrom, Shakeshaft, and Scott-Samuel, 2008) to estimate the expected satiety of a number of common foods. These measures are remarkably good predictors of the energy content individuals self-select and ultimately consume (Wilkinson, Hinton, Fay, Ferriday, Rogers, and Brunstrom, 2012).

Formulating yogurts with addition of different proteins could lead to distinctive structural arrangements of the casein or whey protein in the yogurt protein network which would be closely related to its texture sensory sensations and elicitation of different expectations of satiating capacity. *Check-all-that-apply* (CATA) is a sensory technique that has been used to obtain rapid product profile (Meyners and Castura, 2014) where checked terms are considered by the consumers to be perceived as appropriate for describing the sample. An "ideal" product could be included in CATA

questions to be evaluated after all the real samples have been presented; this way a penalty analysis is possible based on the gaps between the real products and the ideal and the impact on liking scores.

The aim of the present work was to relate the sensory (especially texture) characteristics of yogurts with added extra milk powder, whey protein concentrate, and calcium caseinate to their expected satiating capacity. A CATA question including a hypothetical "ideal satiating yogurt" was used for understanding the sensory features related to yogurts' satiating capacity and the potential cues for reformulation according to consumers.

2. Materials and methods

2.1. Ingredients

The ingredients used in the preparation of the yogurts were skim milk powder (kindly supplied by Central Lechera Asturiana, Siero, Spain), whey protein concentrate (AVONLAC 482, Glanbia Nutritionals Ltd., Kilkenny, Ireland), calcium caseinate (Fonterra Co-operative Group Ltd, Reference 385, Palmerston North, New Zealand), freeze-dried lactic culture (Natural Occidental Yogurt N11091 Streptococcus thermophilus, Lactobacillus bulgaricus and Lactobacillus lactis, Genesis Laboratories Ltd., Sofia, Bulgaria), sucralose (EPSA Aditivos Alimentarios, Valencia, Spain) and distilled water.

2.2. Sample preparation

Five different set-type yogurts were formulated: control (C), double skim milk powder (MP), added whey protein concentrate (WPC), added calcium caseinate (CAS), and a blend (50:50 protein basis) of added whey protein concentrate and calcium caseinate

(CAS-WPC). The milk for sample C was prepared with 500 mL of distilled water and 50 g of skim milk powder; whereas 100 g of skim milk powder (instead of 50g) was used to prepare 500 mL of milk for sample MP; sample WPC was prepared by adding 22.08 g of whey protein concentrate to the control milk; sample CAS was prepared by adding 18.28 g of caseinate to the control milk; and sample CAS-WPC was prepared by adding 11.09 g of whey protein concentrate and 9.14 g of calcium caseinate to the control milk. These additions were equivalent to doubling the protein content of sample C.

The skim milk powder, distilled water and whey protein concentrate or calcium caseinate as applicable were placed in glass beakers (1L) and heated in a batch (Precisterm, JP Selecta S.A, Abrera, Spain) at 82-85ºC for 30 min (Morell et al., 2015b). The samples were cooled to the incubation temperature recommended for the culture used (42–43ºC) (digital thermometer, VWR International, Radnor, PA, USA) and inoculated with the lactic culture at 0.5g/100g of milk. Sucralose was added after cooling, to a total concentration of 0.0072g/ 100g of milk. The samples were placed in glass yogurt jars (125 mL) and placed in a yogurt-maker (YG523, Jata Electro, Abadiano, Spain). After a period of 6 h, the samples reached pH values of 4.5-4.6 (PH BASIC 20, Crison Instruments, S.A., Alella, Spain). The jars were individually covered and stored at 4-5 ºC for 48h.

2.3. Physicochemical properties

2.3.1. Instrumental firmness

The firmness of the yogurt samples was measured using a TA.XT-Plus texture analyzer (Stable Microsystems, Godalming, UK) equipped with a 5 kg load cell and a 12 mm diameter flat-ended cylindrical probe. Triplicate yogurt samples in glass containers were used. The samples were kept at 4–5 °C in a refrigerator until they were measured. The crosshead speed was set at 10 mm s^{-1} and the penetration distance at 10 mm. The

firmness of the yogurt was defined as the maximum force measured during sample penetration (Salvador and Fiszman, 2004) expressed in N.

2.3.2. Syneresis

The level of whey that separated from the collapsed gels as a result of centrifugal force was measured. After 48h of storage, approximately 20 g of yogurt was transferred into a 50 mL Falcon® conical polypropylene centrifuge tube. The sample was then centrifuged (Sorvall Super T 21) at 3300× g for 15 min at 4°C. The separated whey was decanted and weighed. The syneresis was expressed as the percentage weight of the whey separated from the yogurt over the initial weight of the yogurt (Amatayakul, Sherkat, and Shah, 2006b).

2.4. Sensory analysis

2.4.1. Consumers

A total of 116 consumers participated in the test (untrained, 59 women and 57 men, aged 18-65 years, mean age 22.9 years). All of them were recruited among the staff and student population of the Polytechnic University of Valencia and the students and employees of the Institute of Agrochemistry and Food Technology (IATA-CSIC). All of them were consumers of dairy products and declared no food allergies or lactose intolerance. All the experiments were performed in compliance with the national legislation, and according to the institutional framework and practices established by CSIC ethics Committee.

2.4.2. Samples

The 5 samples were coded with random three-digit numbers and presented to the consumers in a balanced rotation order, following Williams' design (MacFie and Thomson, 1988). Consumers were provided with 80 ml white plastic cups filled in with 30 g of each sample and plastic spoons. The consumers were instructed to rinse their mouths with water between samples. The yogurts were served at eating temperature (8 to 10ºC).

2.4.3. Generation and selection of terms for the CATA (*check-all-that-apply*) question

A panel of ten assessors, skilled in quantitative descriptive analysis, evaluated the five samples to select the attributes that would be included in the CATA questionnaire. They were first given a number of samples, a brief outline of the procedure and a list of potential attributes taken from the literature (FIL, 1997). They were then asked to choose and write down the most appropriate attributes to describe the sensory properties of the samples and/or to suggest new ones. At the end of a one and a half hour session, including a round table discussion, a consensus on the list of sensory attributes was reached (Stone and Sidel, 2004). The question included 21 attributes, including sensory and non-sensory terms (use and attitude attributes; see below).

2.4.4. CATA sensory profiling

Check-all-that-apply (CATA) questions, a technique that has been applied increasingly in food research, consist of a multiple-choice list of words or phrases from which respondents have to select those they consider apply to the samples they are tasting. The main advantage of this type of questionnaire is that it allows multiple options to be selected instead of limiting respondents to only one answer or forcing consumers to

focus their attention on specific attributes to be evaluated (Smyth, Dillman, Christian, and Stern, 2006). The lists of words or phrases in the CATA questionnaire can include terms related to both sensory and non-sensory characteristics, such as occasions when used, product positioning, feelings, etc. (Varela and Ares, 2012).

The final questionnaire included 15 sensory (dense, gritty, creamy, thick, sweet, acid, rough, artificial/strange flavor, aftertaste, mouth-coating, homogeneous, grainy, fluid, compact, and whey separation) and 6 non-sensory terms (It fills me a lot, It only fills me a little, I would eat it as a mid-morning snack, I would eat it as an afternoon-snack, I would eat it as a dessert, and It is a healthy snack). The instructions given to the participants were: "Please check all the attributes that apply to the yogurt you are tasting". The order in which the 21 attributes were presented was randomized within the two groups (sensory and non-sensory), between products and across consumers.

After assessing the 5 yogurts on the CATA questionnaire, the consumers were asked to describe their ideal satiating yogurt with the same CATA terms. The consumers were asked: "Now, imagine your 'ideal satiating yogurt'. You would feel well after eating it. It would help you to feel full for some time." The consumers had a real pot of yogurt (white plastic, no label, 125 mL) in their booths so that they could imagine the volume they would eat.

2.4.5. Expected satiating capacity intensity and Liking assessment

To assess the expected satiating capacity of the samples, the participants (n=116, the same as in the CATA task) were asked to eat a spoonful of the different samples with the following instructions: "Please taste the sample. How satiating do you think this yogurt is? Imagine eating a whole jar of this yogurt. How full do you think it would make you feel?" (a normal-size 125-mL plastic pot was presented to the panelists for size estimation purposes). Then, they were asked to score how satiating each one was using a 9-box structured scale (from 1 = It does not fill me to 9 = It fills me a lot).

For each sample, the participants were also asked to rate their liking for its flavor, thickness, and globally on three separate 9-box structured hedonic scales (from 1 = I dislike it very much to 9 = I like it very much).

2.5. Data analysis

Analysis of variance was performed on the instrumental firmness and syneresis values, expected satiating scores and liking assessment scores using the Statgraphics Plus 5.1 software package (Statistical Graphics Co., Rockville, Md., U.S.A.). Fisher's least significant difference (LSD) test was used to evaluate mean value differences ($p < 0.05$).

A Correspondence Analysis (CA) was performed on the frequency of selection of the sensory and non-sensory terms as active variables. This provided a concise representation that considers all the information together, linking it to sample positioning (Varela and Ares, 2012; Escofier and Pagès, 1994). The liking scores and satiating ability scores were superimposed on the resulting perceptual space and related to the samples as supplementary variables. The ideal satiating yogurt was added as a supplementary observation based on the CATA frequency. The analysis was performed using R v.3.1.0 (R Development Core Team 2007, Vienna, Austria). The chi-square test was used to study differences in the consumers' perception of the yogurts based on the CATA responses: for each yogurt, the frequency of selection of each term was measured by counting the number of consumers that checked that term to describe each sample. Cochran's Q test (Manoukian, 1986) was carried out on the CATA results in order to identify significant differences between samples for each of the attributes.

Finally, penalty analyses on the CATA data (Ares, Dauber, Fernandez, Gimenez and Varela, 2014) were performed for the global liking to determine the drop in the global liking associated with a deviation of each product from the ideal for each attribute. As recommended by Meyners, Castura, and Carr (2013), penalty analyses can identify 'must have', 'nice to have', and 'must not have' attributes. A first analysis based on

incongruence in which the attribute is missing in the real but not the ideal product allows identifying the 'must have' attributes. For each attribute, mean drops in liking between the two situations are calculated and their significances tested. The same analysis is done based on the incongruence in which the attribute is missing in the ideal but not the real product. When these attributes lead to an increase in liking compared to when they are missing, these are 'nice to have' attributes, but when they affect the liking negatively, they are 'must not have' attributes. The Cochran's Q test and the penalty analyses were performed using XLSTAT statistical software (version 2015, Addinsoft ®, Barcelona, Spain).

3. Results and discussion

3.1. Physicochemical properties of yogurt

Firmness and syneresis are important quality parameters for set yogurt products (Walstra, Geurts, Noomen, Jellema, and van Boekel, 1999). In fact, one reason for the industry's supplementing the milk with dairy ingredients is to increase the total solids content, resulting in firmer products with lower syneresis levels. The firmness results in the present study showed a statistically significant ($p < 0.05$) effect of the dairy ingredient source (WPC>CAS-WPC>MP=CAS>C) (Table 1). Oliveira, Sodini, Remeuf, and Corrieu (2001) reported that the nature of milk supplements significantly affected the firmness of the product, among other factors. The CAS and MP samples showed significantly lower firmness values than WPC and CAS-WPC, but higher ones than the control sample ($p < 0.05$). Sodini, et al., (2005) found that WPC supplementation produced firmer yogurts than the addition of skim milk powder. Protein aggregates formed by the interaction between casein micelles and denatured whey proteins via intermolecular disulfide bonds (coating the micelle) appeared during the first steps of coagulation, enhancing the cross-linking in the gel (Amatayakul, Halmos, Sherkat, and Shah, 2006a). Bhullar, Uddin, and Shah (2002) obtained similar

results, which they attributed to the increased water-binding capacities of the denatured whey proteins in the sample. However, Guzmán-González, et al., (1999) and Damin, et al., (2009) reported that casein-based products (usually sodium caseinate) tended to produce firmer gels with less syneresis than yogurt supplemented with whey protein. According to these authors, when milk was supplemented with caseinate a stronger, positive correlation was observed between protein levels and firmness than for skim milk powder and WPC supplementation.

Table 1. Firmness and syneresis values (and SD) of the control and the yogurts formulated with extra protein.

Sample	Firmness (N)	Syneresis (g/100g)
C	0.20^{d} (0.01)	24.0^{a} (1.6)
MP	0.66^{c} (0.06)	20.7^{b} (1.3)
WPC	1.07^{a} (0.08)	14.4^{c} (0.8)
CAS	0.68^{c} (0.08)	21.9^{b} (1.5)
CAS-WPC	0.77^{b} (0.02)	21.0^{b} (1.2)

*Different superscript letters in the same column indicate statistical differences ($p < 0.05$) among the samples.

*C: control; MP: added milk protein; WPC: added whey protein concentrate; CAS: added calcium caseinate, CAS-WPC: added blend of whey protein concentrate and calcium caseinate.

The level of syneresis is shown in Table 1. The yogurts made with added WPC had significantly the lowest level of whey separation ($p < 0.05$), while the highest was for sample C ($p < 0.05$), with intermediate values for samples MP, CAS and CAS-WPC. Puvanenthiran, et al., (2002) suggested that an increase in the compactness of the yogurt structure due to the reduction in the caseinate- whey protein ratio led to a high level of immobilization of the free water in the yogurt gel. It may be pointed out that the centrifugation results would be influenced by factors such as the rigidity and rheological properties of the gels (Amatayakul, et al., 2006a).

3.2. Sensory analysis

3.2.1. Expected satiating ability

An examination of the expected satiating scores of the 5 samples, measured on a 9-point scale (from not at all satiating to very satiating), showed that significantly the highest score was given to sample MP (Table 2). In the following order, yogurts CAS, CAS-WPC, and WPC were rated as significantly less satiating than sample MP ($p < 0.05$) though CAS and WPC were not significantly different from CAS-WPC ($p > 0.05$). Sample C had significantly ($p < 0.05$) the lowest expected satiating ability score.

3.2.2. Liking assessment

Table 2 shows that sample MP received significantly the highest score for global liking ($p<0.05$), followed by CAS-WPC, C, and WPC with no significant differences between these last three ($p>0.05$). Finally, CAS was the significantly least liked ($p<0.05$).

Regarding flavor preferences, MP, C, and CAS-WPC had significantly higher values though C did not significantly differ from the other two ($p>0.05$). With significantly lower scores, whey protein and caseinate addition (samples WPC and CAS, respectively)

did not differ much from each other. For thickness, CAS-WPC and WPC had significantly the highest values ($p<0.05$), followed by MP, then by CAS, and finally sample C showed the lowest liking value for thickness. In sum, sample CAS was the one that scored consistently low in all three liking scales, possibly due to its dominating grainy, gritty, and rough texture.

Table 2. Satiating ability and global, thickness, and flavor liking mean ratings (and SD) on a 9-box scale of the experimental yogurts.

Sample	Satiating Ability	Liking		
		Global	Thickness	Flavor
C	4.3^{d} (1.9)	5.2^{b} (1.8)	3.0^{d} (1.9)	5.5^{ab} (2.0)
MP	6.8^{a} (1.5)	6.1^{a} (2.1)	6.6^{b} (1.9)	6.0^{a} (2.2)
WPC	5.6^{c} (1.8)	4.9^{b} (1.9)	7.3^{a} (1.5)	4.6^{c} (2.0)
CAS	6.2^{b} (2.0)	4.1^{c} (2.1)	5.3^{c} (1.7)	4.2^{c} (2.2)
CAS-WPC	6.0^{bc} (1.8)	5.3^{b} (1.9)	7.4^{a} (1.1)	5.3^{b} (2.1)

*Different letters in the same column indicate statistical differences ($p < 0.05$) among the samples.

*C: control; MP: added milk protein; WPC: added whey protein concentrate; CAS: added calcium caseinate, CAS-WPC: added blend of whey protein concentrate and calcium caseinate.

3.2.3. CATA Sensory Profiling

Of the 21 terms listed in the CATA questionnaire, 19 presented significant differences between the samples (all except for acid and aftertaste). When the Ideal sample was included in the calculation ($p<0.05$), 20 terms (all except acid) presented differences. These results indicate that the consumers perceived differences in the sensory and non-sensory characteristics of the yogurts, since they selected these characteristics differently to describe the different samples (Table 3).

Table 3. Number of selections of each attribute for the 5 samples formulated with extra protein and the "ideal satiating yogurt", and p values from Cochran's Q test results.

Attribute	Experimental sample*					p-values (Cochran) (between the 5 samples)	Sample "ideal"	p-values (Cochran) (between the 5 samples plus the "ideal")
	C	MP	WPC	CAS	CAS-WPC			
Sensory								
Acid	35	27	24	27	25	0.4	27	0.558
Gritty	6	4	3	75	9	<0.0001	1	<0.0001
Artificial/ strange flavor	20	19	44	51	32	<0.0001	0	<0.0001
Rough	12	10	13	65	16	<0.0001	0	<0.0001
Compact	6	43	79	50	65	<0.0001	41	<0.0001
Creamy	48	88	28	18	46	<0.0001	72	<0.0001
Sweet	40	43	11	6	18	<0.0001	80	<0.0001
Thick	1	25	57	51	53	<0.0001	15	<0.0001

Dense	6	57	64	65	70	<0.0001	38	<0.0001
Fluid	94	15	7	3	8	<0.0001	25	<0.0001
Grainy	5	1	10	71	16	<0.0001	2	<0.0001
Homogeneous	49	64	60	12	52	<0.0001	86	<0.0001
Mouth-coating	33	49	20	43	38	<0.0001	66	<0.0001
Aftertaste	36	37	39	40	35	0.945	17	0.006
Whey separation	78	39	26	28	23	<0.0001	14	<0.0001
Use and attitudes								
It fills me a lot	10	47	33	40	33	<.0001	40	<.00001
It only fills me a little	31	6	16	9	16	<.0001	6	<0.0001
I would eat it as a mid-morning snack	6	21	13	15	19	0.008	46	<0.0001
I would eat it as an afternoon snack	13	33	18	18	16	0.001	62	<0.0001
I would eat it as a dessert	52	57	42	24	48	<0.0001	78	<0.0001
It is a healthy snack	31	10	17	9	20	<0.0001	31	<0.0001

*C: control; MP: added milk protein; WPC: added whey protein concentrate; CAS: added calcium caseinate, CAS-WPC: added blend of whey protein concentrate and calcium caseinate.

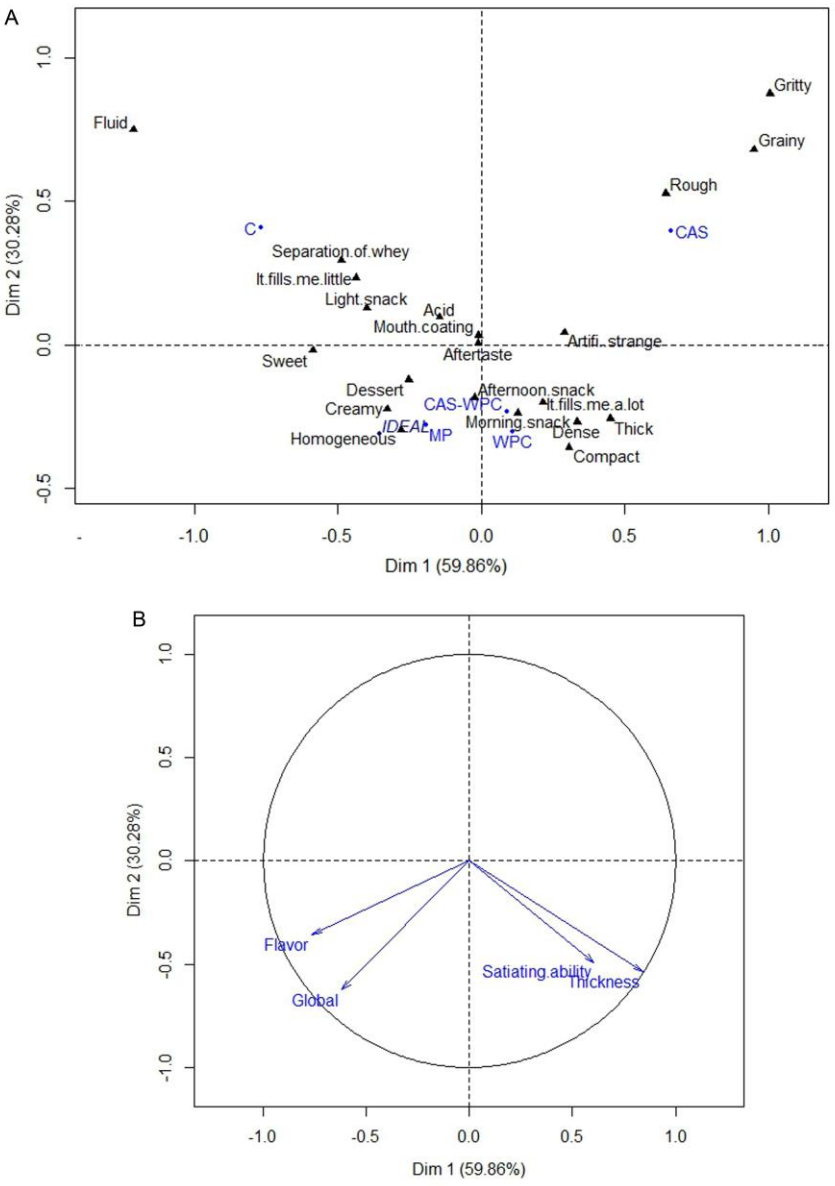

Figure 1. A) Representation of the CATA terms selected for the five samples formulated with extra protein in the first two CA dimensions. Ideal satiating yogurt was considered a supplementary observation. B) Expected satiating ability and Liking (several modalities) were superimposed as supplementary variables.

The CA plots of the frequency of selection of the CATA questionnaire terms helped to bring out the differences and similarities between the five samples (Figure 1A). The first two factors accounted for 90.2% of the data variability (59.9% and 30.3% respectively). As a supplementary observation to find out more about the descriptors that the consumers would attribute to an ideal satiating yogurt, the Ideal sample was positioned on the map by considering the frequency of mention of the attributes the consumers selected for it. The scores for satiating ability and the liking related to the three different modalities were projected on the resulting perceptual space, providing a better understanding of which descriptors were responsible for the satiation expectations and hedonic responses of the consumers (Figure 1B).

The first dimension seemed to oppose samples in terms of smoothness, samples C and CAS being located in the left and right extremes of the plot, respectively (Figure 1A).

Sample C was particularly described as being fluid, and it seems that consumers perceived whey separation. Regarding taste attributes, it was also characterized by being acid. Regarding non-sensory attributes, consumers also described it as being a healthy snack, probably because consumers relate fluid yogurt with a light product, with a poor satiating effect (hence its association with It only fills me a little, see also Figure 1B). It can also be seen from Figure 1B that this product was one of the least liked in terms of thickness, as already described above (see Section 3.2.2).

Sample CAS was positioned opposite the global- and flavor-liking vectors (Figure 1B). It was associated with artificial/strange flavor and aftertaste and with the negative texture attributes listed above (gritty, grainy, and rough). Undesirable textural changes because of protein supplementation of yogurt have been reported previously by several authors (Akalin, et al., 2012; Lee and Lucey, 2010; Frøst and Janhøj, 2007). This sample received the second highest scores for satiating ability, which suggests that its grainy, gritty, and rough mouth-feel lead to longer orosensory exposure, which is related to stronger satiating sensations (Hogenkamp and Schiöt, 2013).

Samples MP, WPC, and CAS-WPC were positioned closer to the center along Dimension 1, but in the negative half of Dimension 2, opposed to C and CAS. MP was characterized by being creamy and quite filling (It fills me a lot) and it also received the highest score for satiating ability (Table 2). This sample was the best liked (globally and for its flavor), most probably due to its high perceived sweetness and creaminess. In addition, this sample was more related to the eating occasions a mid-morning snack, and an afternoon snack than the other samples.

CAS-WPC and WPC were particularly perceived as compact, thick, and dense. The vector corresponding to thickness liking was found near these two samples and the one of satiating ability as well, indicating that consumers related their texture with higher expectations of satiation. Note, however, that sample MP was the one that was expected to be the most satiating (Table 2); therefore, the vector is only indicating relative information. It was evident from these results that the addition of WPC palliated the negative texture features of the added-caseinate-only sample.

The Ideal sample's position was closer to sample MP than to any of the others, being characterized by terms like homogeneous, creamy, mouth coating, and sweet. Like MP, the ideal satiating yogurt would be eaten as an afternoon snack and as a mid-morning snack, but also as a dessert. Creaminess is not a primary sensory property but an integrated sensory perception derived from combined sensations of visual, olfactory, gustatory, and tactile cues, among which smoothness has been suggested as the most important sensory texture-related feature (Chen and Eaton, 2012). It is worth noting that density, thickness, and compactness/ firmness are attributes that consumers generally tend to associate with a product's satiating ability (in case of semi-liquid products). This has been partially demonstrated in the description of the real products in this study. When having to define an ideal satiating yogurt, clearly a grainy or gritty texture is not really desirable (imagined) for contributing filling sensations, whereas other attributes such as sweetness and creaminess are (more) important, apart from some density and thickness. This seems to suggest that if consumers are asked to

describe their ideal satiating product they base their attribute-choosing to a larger extent on their liking than solely on the functional aspects.

3.2.4. Penalty analyses

The penalty analyses helped identifying which attributes are essential, nice to have, and to-be-avoided in each protein-enriched yogurt to be highly accepted compared to an ideal satiating product (Meyners, et al., 2013).

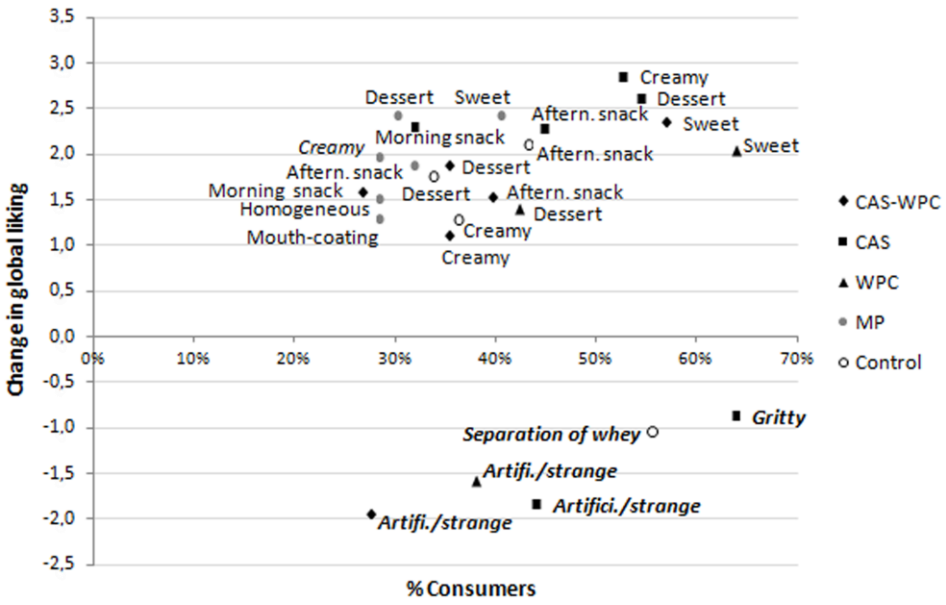

Figure 2. Results of the penalty analysis conducted for the global liking. Only significant attributes are shown. The attributes in regular font are 'must have' attributes. The attributes in italic regular font are those attributes 'nice to have'. Those in italic bold font are 'must not have' attributes.

Figure 2 summarizes for each product which of their attributes are penalizing (that is, which of their present attributes decrease the global liking) or would significantly increase their global liking if included (at least at $p < 0.05$). Note that the global liking of the ideal satiating yogurt was asked, in case it was not 9 (Meyners, et al., 2013), and the mean was 8.7. This means that for a few people, their ideal satiating yogurt does not need to be extremely liked.

3.2.4.1. "Must have" attributes

The "must have" attributes are those that have been highly selected by a large number of consumers for the ideal satiating yogurt but not for the real samples and that would have a high significant positive impact on the global liking (Figure 2, in regular font).

Sweetness was a "must have" attribute, which has been highly selected in the ideal product but missing in the WPC and CAS-WPC samples by 65 and 55% of the consumers, respectively. Missing this attribute is affecting the liking in between 2-2.5 points for these products. In this regard, it has been reported that whey protein induces astringency under acidic conditions (Sano, Egashira, Kinekawa, and Kitabatake, 2005); several mechanisms have been proposed for suppression, or decrease, of perceived astringency due to different sucrose levels including cognitive ones (Ishikawa and Noble, 1995). In the present case, it could be that particularly sample WPC's astringency attenuated the sweetness perception, since they all had the same sweetener content.

These results showed a clear direction for reformulation. Sweetness is also a "must have" attribute for sample MP, although in this case only 40% of the people selected it for the ideal but not for the sample. In contrast, this attribute was not a "must" in the CAS sample. Although sample CAS was the least sweet, and sweetness would increase its liking by 1.5 points, this impact was not significant ($p=0.278$).

Creamy was a "must have" for sample CAS, which would significantly increase its overall liking by nearly 3 points, probably because it was particularly perceived as gritty, grainy, and rough (see Figure 1A), and consequently not creamy (Table 3). Creamy was also a "must have" for CAS-WPC sample, but only for around 35% of consumers, indicating that the mixture with whey proteins palliated lack of creaminess in CAS samples. As expected, creaminess was a "must have" for sample C since it had half the protein content than the rest of the samples; in the same line, creamy, homogeneous, and mouth-coating were "must have" attributes for the MP sample.

Finally, almost all the samples have been penalized for not being felt appropriate as a dessert, or as a snack (both morning- or afternoon-snack). These attributes were highly mentioned for the ideal but not for most of the samples, which suggests that an ideal satiating yogurt should be ideal for a snack, which is not surprising, but also have a pleasurable aspect to it and be perceived/ appropriate as a dessert. In sum, in the consumers' mind, it should appropriate to be eaten in almost every eating occasion.

3.2.4.2. "Nice to have" attributes

The "nice to have" attributes are those that were selected in the real product but not in the ideal with a significant positive impact on the global liking (Figure 2, in italic regular font). Creamy was the only attribute that was more selected for a real sample (MP) than for the Ideal, and was nice to have since it influenced liking positively (around 2 points).

3.2.4.3. "Must not have" attributes

The "must not have" attributes are those that were selected in the real product but not in the ideal and which had a significant negative impact on the global liking (Figure 2, in italic bold font).

Artificial or strange flavor was a "must not have" attribute in the samples CAS, CAS-WPC, and WPC. Probably some different flavor notes derived from caseinate and whey concentrate were perceived in these samples by the consumers.

Gritty was a "must not have" attribute for CAS, as seen also in the CA plot (Figure 1A), an attribute which was selected by more than 60% of the consumers for this sample and penalized its overall liking around 1 point.

Finally, separation of whey was a "must not have" attribute present in sample C and not for the ideal, for 56% of the panel, which also penalized liking by 1 point.

4. Conclusion

The samples that elicited higher expectation of satiating ability were MP followed by CAS, CAS-WPC, and WPC. Satiating ability seemed to be driven by a number of sensory characteristics: from sweetness and creaminess in the case of MP, through grittiness and graininess in the case of CAS, and a general high density and thickness, presented in all these samples. CAS was perceived as having negative flavor attributes (artificial, strange), which could be improved with the use, for example, of some ingredients like starches that are able to confer creaminess and smooth texture and bland flavor. Another solution would point to partial replacement of CAS with WPC to improve, or at least palliate, those negative sensory features.

In contrast, MP was globally the most liked and the nearest to the ideal satiating yogurt. Creaminess, homogeneity, and sweetness seem to be the most advisable attributes to pursue for orosensory liking of any type of yogurt (satiating or not), though some additional density and mouth-coating textural features apparently contributed to the perceived satiating benefit.

Sensory characterization combined with penalty analysis showed to be complementary paths to follow when reformulating yogurt, since not only should the final products be

liked, they should also elicit specific expectations of satiating ability when placed in the mouth. Considering the results as a whole, a combination of skim milk protein supplementation with precisely adjusted amounts of caseinate or whey protein concentrate could open up new ways to design satiating protein-supplemented yogurts.

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Yogurts with an increased protein content and physically modified starch: rheological, structural, oral digestion and sensory properties related to enhanced satiating capacity

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Abstract

Protein is the most effective food macronutrient providing a satiating effect. Thus, formulating dairy foods with increased protein contents can help to modulate food intake. Oral perception cues also contribute an increased perception of satiating capacity when the oral residence time and handling are longer and more laborious. In the present work, yogurts were prepared with double skimmed milk powder (MP) and whey protein (WP), as well as a control (C) without extra protein. Three more samples were prepared by adding 2% of a physically modified starch to each (CS, MPS and WPS, respectively), in order to increase the consistency and impart creaminess. Rheological tests were used to characterize the flow and viscoelastic properties of the samples before and after saliva treatment, and their microstructure was observed. Finally, the differences in sensory perceptions elicited by the samples were related to consumers expected satiating capacity and liking scores. Before *in vitro* oral digestion, MP showed denser areas than C; in WP, two protein networks could be distinguished. In the samples with added starch, starch granules were embedded in the protein networks. After *in vitro* oral digestion, the protein tended to aggregate; the starch granules maintained their structure indicating that they were not broken down by the saliva. These observations were related to the samples' rheological behavior. The sensory graininess, lumpiness and grittiness detected in the WP samples could be linked to the aggregation of whey protein and the formation of two different protein networks. All the added-starch samples elicited creamier and denser sensations than their counterparts without starch. MP with starch was scored as the most satiating and best-liked yogurt sample.

Keywords: added-protein yogurt; physically modified starch; *in vitro* oral digestion; yogurt microstructure; flash sensory profiling; satiating ability.

1. Introduction

Yogurt could be an excellent basis for designing satiating food products. Dairy components are known to suppress short-term food intake and stimulate the mechanisms known to signal satiation and satiety (Aziz and Anderson, 2007). Controlled energy intake in association with a moderately elevated protein intake may represent an effective and practical weight-loss strategy. Yogurt contains high levels of protein, which is recognized as the macronutrient with the highest satiating capacity. In yogurt production, the solids content of milk is traditionally increased. The options for achieving the desired protein and solids contents are removal of water from the milk by membrane filtration or evaporation under vacuum, or adding a dried milk protein (skimmed milk, whey protein concentrates, whey protein isolates, caseinates, etc.) (Damin, Alcântara, Nunes and Oliveira, 2009). Unfortunately, protein addition may result in undesirable textural changes such as chalkiness or grittiness (Lee and Lucey, 2010) and in reduced liking for the mouthfeel (Frøst and Janhøj, 2007), which have to be taken into account during the formulation design step.

The rise in yogurt consumption has been associated with a shift in preference toward extra-thick. Nevertheless, increasing the fat content to enhance the sensation of creaminess is not an option because the consumers' awareness of the link between diet and health could be a factor influencing their preferences. However, the partial or total removal of fat globules from yogurts decreases the overall quality perceived (Folkenberg and Martens, 2003), due to changes in texture, the appearance of syneresis (Kilcast and Clegg, 2002; Houzé, Cases, Colas, and Cayot, 2005), and changes in the flavor profile (Nongonierma, Springett, Le Quere, Cayot, and Voilley, 2006).

Modified starches are widely used to modify texture or replace fat in yogurts (Cui, Lu, Tan, Wang, and Li, 2014). Their functionality and applications could be varied to include stabilization, emulsification and structuring abilities (Kett, Chaurin, Fitzsimons, Morris, O'Mahony, and Fenelon, 2013). However, clean labelling trends are moving the industry to use fewer additives. Physically modified starches (PMS) are not considered additives.

The processes most often investigated for obtaining PMS are, among others, annealing (Wang, Wang, Yu, and Wang, 2014) and heat-moisture treatments (HMT) (Kim and Huber, 2013), which can be used in a great variety of operating conditions (Haghayegh, and Schoenlechner, 2011). Applications of PMS in milk systems have scarcely been studied. The mechanisms, interactions and synergistic effects of using PMS to give creamy, luxurious textures are worth studying, especially in relation to enhancing satiating capacity cues. Increased viscosity promotes longer orosensory exposure, which is known to contribute to the control of energy intake (Hogenkamp and Schiöt, 2013). *In vitro* oral digestion would therefore be a helpful tool to assess the performance of the starch granules during the course of salivary α-amylase action (Morell, Fiszman, Varela, and Hernando, 2014). When two complex systems such as starch and milk ingredients are mixed, the physicochemical properties of the mixed system depend mainly on their relative concentrations and physicochemical properties. A thorough understanding of the interactions between starch and milk ingredients paves the way for manipulating the composition and processing of foodstuffs to tailor their textural consistency and, as a result, their sensory characteristics (Considine, Noisuwan, Hemar, Wilkinson, Bronlund, and Kasapis, 2011).

Typically, evaluating yogurts involves rheological measurements (Mortazavian, Rezaei, and Sohrabvandi, 2009; Ciron, Gee, Kelly, and Auty, 2012), but these are often insufficient to describe the texture of yogurt, which results from both the acid aggregation of casein micelles and the production of exopolysaccharides by dairy lactic acid bacteria during incubation (Beal, Skokanova, Latrille, Martin, and Corrieu, 1999), which improve the yogurt's consistency by avoiding gel fracture and syneresis (Cerning, 1995). Sensory descriptive analysis is one of the most powerful and most extensively used tools in sensory science, as it allows correlations with other kinds of parameter (Stone and Sidel, 2004), such as rheological or microstructural data. Free profiling tasks are alternative sensory methodologies based on the assessors' construction of their own vocabulary. Flash profiling is an original combination of free-choice term selection with

a ranking method based on simultaneous presentation of the whole product set (Dairou and Sieffermann, 2002). In the present work, in order to develop yogurts with a satiating capacity and reduced fat content, rheology, microstructure, and sensory characterization were combined and used as powerful tools for assessing the viability of new technological approaches (Ciron et al., 2012).

The aims of the present work were 1) to analyze the effect of adding extra milk powder, whey protein isolate and a physically modified starch on the microstructural and rheological properties of yogurts before and after *in vitro* oral digestion, and to compare the results with a control sample (with no additions); 2) to characterize the sensory properties of the yogurts through flash profiling; and 3) to relate the rheological, microstructural and sensory features to the yogurts' expected satiating capacity.

2. Materials and methods

2.1. Yogurt ingredients

The ingredients used in preparing the yogurts were skimmed milk powder (MP, kindly supplied by Central Lechera Asturiana, Siero, Spain), physically modified starch (PMS, 10267-47, Ingredion, kindly provided by Univar Ibérica, Chiva, Spain), whey protein isolate (WP, BestProtein SL, El Prat de Llobregat, Spain), freeze-dried lactic culture (Natural Occidental Yogurt N11091 Streptococcus thermophilus, Lactobacillus bulgaricus and Lactobacillus lactis, Genesis Laboratories Ltd., Sofia, Bulgaria), aspartame and acesulfame K (both from EPSA Aditivos Alimentarios, Valencia, Spain), and distilled water.

2.2. Artificial saliva

Artificial saliva was prepared according to the method described by Mishellany-Dutour, Peyron, Croze, François, Hartmann, Alric, and Woda (2011), with some modifications. All the reagents were of analytical grade. The components were sodium bicarbonate (5.208 g/L), potassium phosphate dibasic trihydrate (1.369 g/L), sodium chloride (0.877 g/L), potassium chloride (0.477 g/L), calcium chloride dehydrate (0.441 g/L), mucin from porcine stomach type II (PGM) (Sigma, M2378) (2.16 g/L), α-amylase type VI-B from porcine pancreas (Sigma, A3176) (8.70 g/L or 200.000 units), and HPLC grade double-distilled water. To perform the *in vitro* oral digestion, the ratio of saliva to sample was1:4 (Morell et al., 2014).

2.3 Starch pasting properties

The pasting properties of the PMS were measured using a Rapid Visco Analyser (RVA) (Newport Scientific model 4-SA, Warriewood, Australia). PMS (5.0 g) was suspended in distilled water (20 mL) and the suspension was thoroughly stirred in the RVA at 960 rpm for 10 sec, and then at 160 rpm for the remainder of the test. The temperature was first maintained at 50°C for 1 min, for equilibration, and then raised to 95°C at a rate of 12°C/min. The sample was kept at 95°C for 2.5 min, cooled to 50°C at 12°C/min, and finally maintained at 50°C for 2 min. The experiments were conducted in triplicate. The viscosity was registered during a heating-cooling cycle. Two tests were carried out, with water or water plus artificial saliva as the dispersal medium. In the latter, a saliva:water ratio of 1:4 was used (Morell, et al., 2014).

2.4 Sample preparation

Six different stirred yogurts were formulated: samples C (control), MP (adding skimmed milk powder) and WP (adding whey protein), and these three formulations with 2% of

starch, respectively giving samples CS, MPS and WPS. Milk was prepared with 100 g of skimmed milk powder /1000 mL of distilled water. An additional 100 g of skimmed milk powder /1000 mL was added to samples MP and MPS, and 18.89 g of whey protein isolate /1000 mL was added to samples WP and WPS. These additions were equivalent to doubling the protein content of sample C.

Skimmed milk powder, distilled water (and WPI when necessary) were placed in glass beakers (1L) and heated in a bath (Precisterm, JP Selecta S.A, Abrera, Spain) at 82-85ºC for 30 min (Fiszman, Lluch, and Salvador, 1999). When starch was required, it was added after removing the glass beaker from the bath. The samples were then cooled to the incubation temperature recommended for the culture used (42–43ºC) and inoculated (0.5 g /100 g of milk), and the sweeteners were added (2:1 aspartame:acesulfame K, total concentration 0.011 g /100 g of milk). The mix was poured into glass yogurt jars (125 mL) and placed in a yogurt-maker (YG523, Jata Electro, Abadiano, Spain). After a period of 6 h, the samples reached pH values of 4.5-4.6 (PH BASIC 20, Crison Instruments, S.A., Alella, Spain). The individual jars were covered and stored at 4-5ºC for 48h. Before performing the tests, the yogurts were stirred individually (RZR 1, Heidolph Instruments GmbH and Co., Schwabach, Germany, 2-blade propeller) at 280 rpm for 1 min.

2.5 Microstructure

2.5.1 Low temperature scanning electron microscopy (Cryo-SEM)

A JSM5410® SEM microscope (JEOL, Tokyo, Japan) was used with a Cryo CT-500® unit (Oxford Instruments, Witney, UK) for the Cryo-SEM observation. The samples (1-mm thick) were placed in the holder, fixed with nitrogen slush (T≤−210 °C), transferred frozen to the cryo unit, fractured, etched (−90 °C), and gold-coated (10−2 bar and 40 mA). They were then transferred to the microscope and examined at 15 kV and −130°C at a working distance of 15 mm (Fiszman, et al., 1999).

2.5.2 Light Microscopy (LM)

A Nikon ECLIPSE 80i (Nikon Co., Ltd., Tokyo, Japan) light microscope (LM) was used. For the starch observation, 5 g of PMS were suspended in 20 mL of water and stained with lugol, then 20 µL were observed on a slide (magnification 20x). The same suspension was observed by LM under polarized light before and after heating (15 min, 90ºC) and after *in vitro* oral digestion. For the yogurt observation, 20 µL of yogurt on a slide were observed at 20x magnification (objective lens 10x/0.30 □/0.17 WD 16, Nikon, Tokyo, Japan). A camera (ExWaveHAD, model no. DXC-190, Sony Electronics Inc, Park Ridge, New Jersey) was attached to the microscope and connected to the video entry port of a computer. A 5µL aliquot of saliva was added to each sample for *in vitro* oral digestion. Lugol for starch and toluidine blue (0.1%) for proteins were used as staining agents. The images were captured and stored at a resolution of 1280 x 1024 pixels (interfaced NIS-Elements F, Version 4.0 software, Nikon, Tokyo, Japan).

2.6 Rheological properties

An ARG2 controlled stress rheometer (TA Instruments, New Castle, DE, USA, monitored by ARES Software version V5.7.0) with serrated parallel-plate geometry (40-mm diameter; 2-mm gap) was used for the rheological measurements. A temperature of 10±1°C was selected as representative of the usual consumption temperature of yogurts. It was maintained by an F30-C circulating water bath (Julabo GmbH, Seelbach, Germany) during the measurements. A fresh sample was loaded for each run. To carry out *in vitro* oral digestion, each sample was blended with the artificial saliva in a glass beaker and gently mixed with a spoon. All the samples were allowed to rest for 5 min in the rheometer cell before each measurement (Morell, et al., 2014).

2.6.1 Flow behavior

Flow behavior was measured by recording the shear stress values when shearing the samples at linearly increasing shear rates from 1 to 200 s-1 over 60 s. The data were fitted to the Herschel-Bulkley model (equation 1) using ARES Software version V5.7.0 (T.A. Instruments, New Castle, DE, USA):

$$\tau = \tau_0 + K \cdot \dot{\gamma}^n \qquad \text{(equation 1)}$$

where τ (Pa) is the shear stress, τ_0 (Pa) is the yield stress, K (Pa s^n) is the consistency index, $\dot{\gamma}^n$ is the shear rate (s^{-1}) and n is the flow behavior index.

In time-dependent, non-Newtonian shear-thinning products, perceived thickness is difficult to predict with rheological parameter values since flow in the mouth is a combination of shear and elongational flow (van Vliet, 2002). However, some authors have found that oral thickness correlates well with different rheological indices. According to Cutler and Morris (1968), apparent viscosity at a shear rate of 50 s^{-1} has practical utility as a possible instrumental index of perceived thickness in semisolid foods. It has been used by other authors (Cook, Hollowood, Linforth, and Taylor, 2003). Consequently, the apparent viscosity values at a shear rate of 50 s^{-1} (η_{50}) were also calculated as follows:

$$\eta_{50} = K \cdot \gamma^{n-1} \qquad \text{(equation 2)}$$

2.6.2 Viscoelastic properties

The linear viscoelastic region (LVR) of each sample was determined. Stress sweeps were then performed, applying a stress wave amplitude within the LVR over the frequency range from 10 to 0.01 Hz, and plotting the storage modulus (G') and loss modulus (G") values as a function of the frequency (Rheology AdvantageTM software, version V5.7.0 (T.A. Instruments, New Castle, DE, USA).

2.7 Sensory analysis

Flash profiling – a combination of free-choice term selection with a ranking method – was applied. The simultaneous presentation of the six samples allowed direct sensory comparison (Dairou and Sieffermann, 2002).

2.7.1 Panel

Flash profiling was carried out by 21 assessors (16 women and 5 men, aged 21-45 years) recruited among students and employees of the Institute of Agrochemistry and Food Technology (IATA-CSIC) and the Polytechnic University of Valencia who had experience in the sensory description of dairy products. A total of 134 sensory terms were generated, 44 of which were semantically different. Between 4 and 9 attributes were generated by each panelist.

2.7.2 Procedure

Flash profiling (FP) does not demand a specific participant training stage; it was performed in two steps. Prior to the evaluation session, the researcher explained the procedure to each panelist and gave him/her a printed example of a descriptive ranking of apples for several attributes. In the second session, after tasting the samples, each panelist generated his/her own list of attributes to describe the differences among the six yogurts. No indication was given regarding the number of attributes that should be proposed. The panelists then ranked each sample on an ordinal scale for each attribute they had individually proposed (ties were allowed). The panelists were asked to focus on the descriptive terms, not on the hedonic ones. The evaluation was individual and each panelist was presented with the whole sample set simultaneously. The samples (30 mL) were served at consumption temperature (10.0 ± 0.5ºC) in small white plastic cups

coded with random three-digit numbers. Mineral water was provided for rinsing the mouth between samples.

2.7.3 Liking and satiating capacity assessment

A total of 121 consumers participated in the test. All were recruited from the staff and student population of the Polytechnic University of Valencia, were consumers of dairy products and declared no food allergies or lactose intolerance. For each sample, the participants were asked to rate their liking for its flavor, for its consistency and global liking on 9-box structured hedonic scales (from 1= "I dislike it very much" to 9= "I like it very much").

The participants were then asked to score each sample on how satiating it was, using 9-box structured scales (from 1= "It does not fill me" to 9= "It fills me a lot"). They were asked to eat a spoonful of the sample and score how satiating it would be if they ate a whole pot of it (an empty 125 mL plastic pot was presented to the panelists for normal-size estimation purposes).

2.8 Data analysis

Analysis of variance (ANOVA) was performed on the rheological data using the Statgraphics Plus 5.1 software package (Statistical Graphics Co., Rockville, Md., U.S.A.). Fisher's least significant difference (LSD) test was used to evaluate mean value differences ($p<0.05$) regarding the composition of the samples.

GPA (Generalized Procrustes Analysis) (Gower, 1975) was used for the flash profile data. GPA reduces the scale usage effects, delivers a consensus configuration and allows comparison of the proximity between the terms used by different assessors to describe the products (Moussaoui and Varela, 2010). To identify the samples and terms most

closely related to the expected satiating capacity of the yogurts and the liking for them, a multifactorial analysis (MFA) was performed using "expected satiating capacity" as a supplementary variable. All the analyses were performed with XLSTAT statistical software (version 2010.5.02, Microsoft Excel®, Barcelona, Spain).

3. Results and discussion

3.1 Light microscopy observation of starch

Observation under polarized light showed two populations of different starch granule size (Figure 1A), which was in line with the suppliers' information that this commercial product is a mixture of potato and waxy maize starches. The waxy maize starch granules were smaller and exhibited a Maltese cross, characteristic of radial symmetry about the intersection point of the cross, while the potato granules were bigger, seemed to be partially disrupted (probably by incipient gelatinization), and had virtually no observable Maltese cross.

Light microscope observations of iodine-treated starch suspended in water are illustrated in Figure 1B. The images show some purple-colored starch components outside the starch granules (light purple background), bigger, slightly-colored potato granules and smaller, intensely purple-colored waxy maize granules, typical of amylopectin stained by iodine (Obanni and BeMiller, 1996). After heating the starch-water suspension (15 min, 90ºC), all the starch granules showed some swelling but the granules preserved their integrity (Figure 1C). This would seem to show that the physical treatment (heating) of the starch allowed the forces bonding the starch molecules organized inside the granule to remain intact.

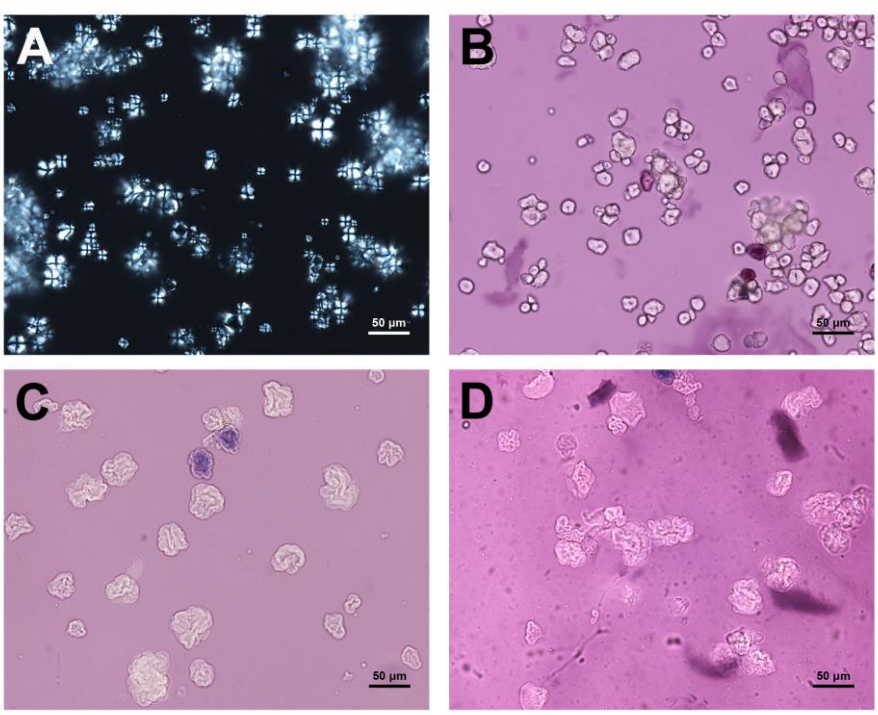

Figure 1. Light microscopy micrographs of the physically modified starch. A) Polarized light image; B) Bright field image: starch dispersed in cold water; C) Bright field image: starch dispersed in water and heated for 15 min at 90ºC; D) Bright field image: starch dispersed in water, heated for 15 min at 90ºC and subjected to *in vitro* oral digestion. Lugol staining. Magnification: 20x.

Figure 1D shows the starch granules after heat treatment of the suspension in water (15 min, 90ºC) and addition of artificial saliva, showing no breakdown of the granules through the action of the α-amylase. Shi and Trzasko (1997) stated that as a consequence of physical modification of the starch under preferred conditions of thermal treatment, linear chains within granules realign themselves in a more orderly manner, thus making it more difficult for the amylase to attack, and that the resulting granular, high-amylose starch showed a peak gelatinization temperature greater than 110°C. High hydrostatic

pressure treatments also lead to increased crystalline perfection in the granules (Ashogbon and Akintayo, 2014). In the present study, the starch was added to the milk during the heating step (85ºC) prior to cooling and inoculation and no higher temperatures were applied.

3.2 Starch pasting properties

The pasting curve of the PMS used in this study (data not shown) showed that the viscosity of the starch in aqueous solution remained both extremely low (at the limit of detection) and unchanged during the heating cycle. When the temperature fell from 95 to 50°C, its viscosity hardly increased. The starch suppliers describe the starch used as a "specialty starch derived from potato and waxy maize", and as a "clean label ingredient" that will create a thick, creamy texture when added to yogurt. The pasting properties of this PMS recalled the behavior of some chemically modified starches, such as oxidized starch, that exhibit low viscosity in their pasting profile. According to Halal, Colussi, Pinto, Bartz, Radunz, Carreño, Dias, and Zavareze, (2015), the reduction in the capacity of oxidized starch to bind water is due, in part, to the structural depolymerization of the starch, while amylopectin, which is primarily responsible for the binding capacity of starch, could undergo hydrolysis at the high temperatures of the oxidizing treatment.

3.3 Microstructure

3.3.1 Light microscopy images of yogurts

Light microscopy images of the control yogurt (C) (Figure 2) showed a grey-colored protein network, stained with toluidine blue. Sample MP had denser areas than sample C (Figure 2, first column) owing to the addition of extra milk protein. In the WP sample, two protein networks could be distinguished: a grey-colored one described above and a

faint blue-colored network that corresponds to the whey protein. In the added-starch samples (CS, MPS and WPS), violet-stained starch granules could be observed embedded in the protein networks (Figure 2, third column).

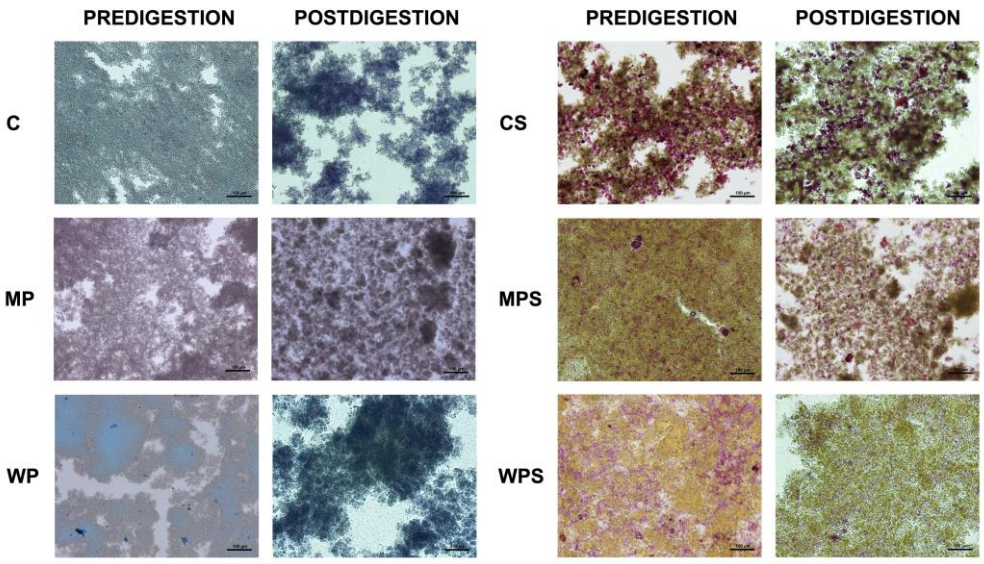

Figure 2. Light microscopy micrographs of the six yogurt samples before and after *in vitro* oral digestion. Lugol (only added-starch samples) and toluidine blue staining. Magnification: 10x.

After *in vitro* oral digestion, a channel-like spread of the water content of the saliva was observed in all the samples. The protein network tended to aggregate, forming some dense and opaque areas (Figure 2, second column). Sano, Egashira, Kinekawa, and Kitabatake (2005) suggested that the aggregation of whey proteins was due to the pH being raised on mixing with saliva.

In the samples with starch (CS, MPS and WPS) after *in vitro* oral digestion (Figure 2, fourth column) the images show the granules embedded in the protein network. This means that the PMS maintained its granular structure resisting digestion by α-amylase.

The protein network in the added-starch samples underwent less aggregation than in those formulated without starch, resulting in a more continuous structure. The structures observed could be related to some sensory features of the different yogurt formulations that will be discussed in section 3.5.

3.3.2 Cryo-SEM images of yogurts

Cryo-SEM images (Figure 3) showed a cell matrix formed after the sublimation of superficial frost known as eutectic artefact or solute aggregation phenomenon, which is inherent to the technique. Soluble solutes accumulate during sample etching, leaving dark spots in the matrix (Llorca, Hernando, Pérez-Munuera, Quiles, Larrea, Fiszman, and Lluch, 2005).

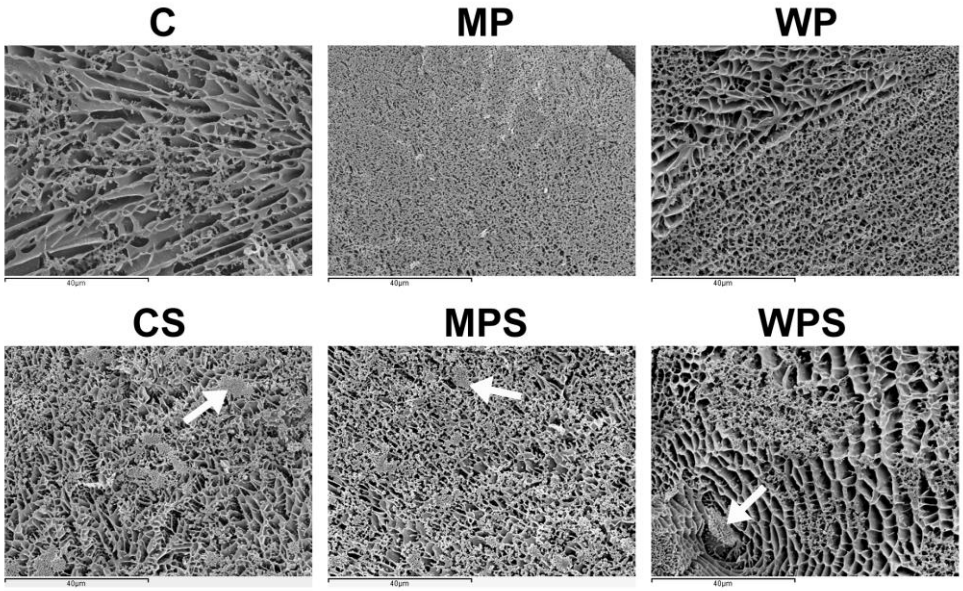

Figure 3. Cryo-SEM micrographs of the six yogurt samples. Magnification: 1500x.

In sample C, a network mainly composed of casein particles linked together in clusters and chains was observed in some areas, as has been described previously (Modler and Kalab, 1983). Sample MP showed a denser cell structure than C as the extra amount of protein contributed more interconnected chains (Figure 3). The casein micelles were observed to be more extensively fused together and finer protein floccules occurred, which became a patently coarse network. Sample WP showed two different phases, consistent with the LM images (Figure 2). One explanation of this phenomenon could be that acid protein gels made from heated milk involved two-step coagulation (O'Kennedy and Kelly, 2000). Gelation of whey protein occurs as the pH reaches an approximate value of 5.2 (Cayot and Lorient, 1998), then, when the pH falls below 4.6, the gelation of casein particles begins, leading to some rearrangements and local stresses in the protein network, and to the appearance of small fractures in the gel. Such fractures would cause small grains to appear in the yogurt after stirring (Lucey and Singh, 1998). Puvanenthiran, Williams, and Augustin (2002) reported that the addition of whey protein to yogurts led to a structure where micelles appeared in the form of individual entities surrounded with finely flocculated protein and linked with small whey protein aggregates; this increase in the number of bonds between particles would explain the dense and finely branched network in sample WP. In the added-starch samples (Figure 3, second row), the cell matrix became denser compared to the equivalent samples without starch.

Observed at a higher magnification (Figure 4), the casein network in sample WP became finer, the size of aggregates smaller and the network of cross-links denser and highly branched. The presence of denatured whey protein on the surface of casein micelles would restrain the approach of other casein particles sterically, reduce the formation of dense clusters (Lucey, Teo, Munro, and Singh, 1998) and retard micelle fusion because of the higher degree of micelle solvation. Remeuf, Mohammed, Sodini, and Tissier (2003) stated that the effect of heating on yogurt microstructure is more pronounced when whey proteins are added to milk. In contrast, the yogurts with extra skimmed milk

powder had a coarse structure with a low degree of casein micelles solvation (Figure 4) and consequently exhibited a coarse and compact structure of relatively large globular aggregates with thick casein chains, as described by Puvanenthiran, et al., (2002).

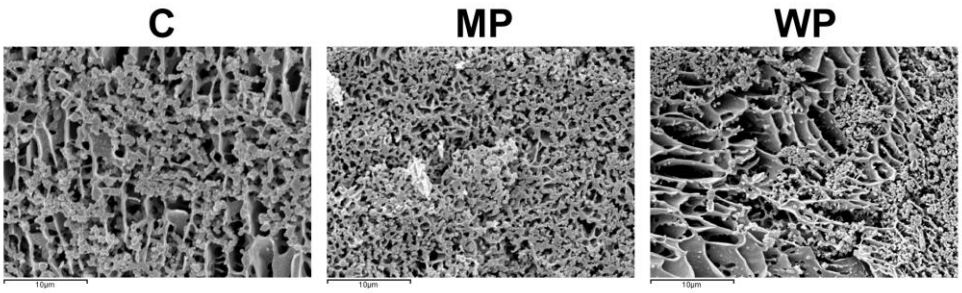

Figure 4. Cryo-SEM micrographs of the three yogurt samples without starch. Magnification: x3500.

3.4. Rheological properties

3.4.1 Flow behavior

Texture of stirred yogurt, one of the most essential properties that determine its quality, is the result of both the acid aggregation of casein micelles and the production of exopolysaccharides during incubation by ropy strains that improve yogurt characteristics (Cerning, 1995). During industrial production of a stirred yogurt the gel structure is broken up by agitation. In terms of rheology, stirred yogurt is a viscoelastic and thixotropic (time-dependent) product (Beal et al., 1999).

Regardless of oral digestion, all the samples showed pseudo-plastic behavior ($n<1$) (Figure 5A and 5B). Sample C showed the least time-dependence both without and with oral digestion; comparing the samples without starch (C, MP, and WP), WP presented the highest viscosity, followed by MP and C. This result is in line with the microscopy

observations, where WP presented a double network that would lead to a reinforced structure while C presented the weakest, loosest structure.

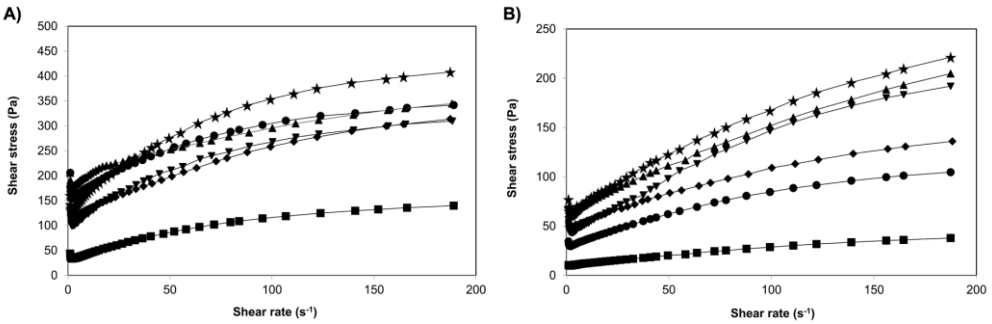

Figure 5. Flow behaviour of the six yogurt samples. A) without *in vitro* oral digestion; B) after *in vitro* oral digestion. C (squares): control sample; MP (diamonds): double skimmed milk powder; WP (inverted triangles): whey protein added; CS (circles): C with starch; MPS (triangles): MP with starch; WPS (stars): WP with starch.

All the yogurts made with starch (CS, MPS, and WPS) showed higher viscosity than their counterparts without added starch (Figure 5A and 5B); swollen starch granules could be thought to act as fillers in the protein network, thus strengthening the protein network properties. Sample WP presented a significantly ($p<0.05$) higher consistency index (Table 1) than all the other samples and the least Newtonian flow behavior (lowest n value). The addition of starch to all the samples significantly increased the K values while not significantly affecting the n values. According to Lankes, Ozer, and Robinson (1998), increased protein interactions and protein–protein bonds increase the elastic character of the gel, making the yogurt less susceptible to rupture. No significant differences ($p>0.05$) were found between the K or n values for samples MP and C: the denser networks seem not to have been sufficient to enhance the flow consistency. Sample C showed the significantly lowest $\sigma 0$ value ($p<0.05$), which represents the

minimum stress value for detecting deformation of the material (Prentice, 1992), and the lowest η_{50}, which is taken as an instrumental index of perceived thickness in the mouth for semisolid foods, while sample WP showed the significantly highest η_{50} value ($p<0.05$), so this sample could be expected to elicit the perception of greatest thickness. Again, the addition of starch to all the samples significantly increased both the $\eta_{50}$and σ_0 values.

The water in the saliva was found to have had a significant dilution effect (Figure 5B), as expected, causing all the systems to present lower K and higher n values (Table 1). The flow curve behavior pattern was the same as when no saliva was added, but the values were lower for the whole set of samples. The fact that the K, η_{50} and σ_0 values after *in vitro* oral digestion were higher in the samples containing starch than in the corresponding yogurts without starch indicated that the starch granules were not broken down by the α-amylase in the saliva.

Table 1. Mean flow behavior values (n=3) of yogurts with and without *in vitro* oral digestion: consistency index (K), flow behavior index (n), yield stress (τ_0), viscosity at 50 s^{-1} (η_{50}).

Sample	Predigestion				Postdigestion			
	K (Pa·s^n)	n	η_{50} (Pa·s)	τ_0 (Pa)	K (Pa·s^n)	n	η_{50} (Pa·s)	τ_0 (Pa)
C	6.6^a (0.7)	0.56^b (0.02)	58^a (2)	23^a (3)	0.62^a (0.03)	0.74^b (0.01)	11.2^a (0.2)	8.6^a (0.3)
CS	11.1^b (1.2)	0.56^b (0.01)	99^{bc} (8)	170^c (35)	2.45^{bc} (0.43)	0.65^a (0.01)	31.2^b (4.6)	24.1^b (1.9)
MP	5.6^a (0.9)	0.57^b (0.04)	79^b (8)	97^b (9)	1.91^b (0.24)	0.75^b (0.03)	35.8^b (1.4)	43.1^c (2.6)
MPS	11.5^b (3.6)	0.56^b (0.01)	101^{bc} (26)	188^c (48)	2.44^{bc} (0.7)	0.71^b (0.04)	48.2^c (8.9)	57.0^d (0.6)
WP	18.2^c (4.3)	0.49^a (0.03)	123^c (19)	82^b (3)	3.0^c (1)	0.70^b (0.06)	59.3^d (5.6)	38.9^c (3.4)
WPS	24.3^d (0.6)	0.49^a (0.01)	164^d (8)	90^b (22)	3.4^c (0.6)	0.72^b (0.03)	67.7^d (6.4)	57.7^d (3.7)

*Different letters in the same column indicate statistically significant differences ($p<0.05$). Values between parentheses are the standard deviations. C: control; MP: double skimmed milk powder; WP: whey protein added; CS, MPS and PS: respectively C, MP and WP samples plus 2% starch.

3.4.2 Viscoelastic properties

Yogurt is a viscoelastic material, so its rheological behavior can be described through the storage (G') and loss (G") moduli (Figure 6). Regardless of oral digestion, all the yogurt samples showed moduli values with a slight frequency dependence, and G'

predominated over G" throughout the frequency range studied that corresponds to a weak gel with elastic characteristics.

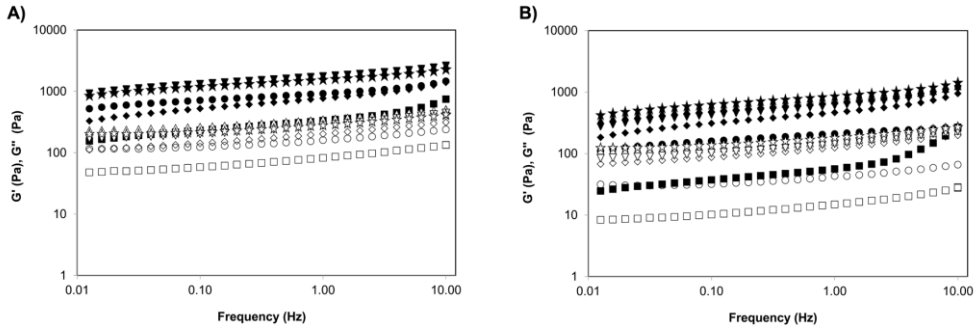

Figure 6. Mechanical spectra of the six yogurts. C (squares): control sample; MP (diamonds): double skimmed milk powder; WP (inverted triangles): whey protein added; CS (circles): C with starch; MPS (triangles): MP with starch; WPS (stars): WP with starch. G' (full symbols), G" (empty symbols).

Comparison of the mechanical spectra and the G' and G" values at 1 Hz (Table 2) of the samples without starch before *in vitro* oral digestion showed that WP showed significantly the highest G' and G" values ($p<0.05$). Starch addition increased the moduli values of all the samples, again indicating a reinforcement of the structure. According to Cayot, Schenker, Houzé, Sulmont-Rossé, and Colas, 2008) the fat globules recovered by proteins in stirred yogurt contribute to the protein gel network. In the present case, the presence of swollen starch granules likewise contributed to the network structure.

Table 2. Mean viscoelastic parameter values (n=3) of yogurts with and without *in vitro* oral digestion: storage modulus (G'), loss modulus (G") and tangent delta (tan δ).

Sample	Predigestion			Postdigestion		
	G' (Pa)	**G'' (Pa)**	**tan δ**	**G' (Pa)**	**G'' (Pa)**	**tan δ**
C	249[a] (16)	62[a] (4)	0.250[d] (0.002)	40[a] (9)	11[a] (2)	0.270[e] (0.005)
CS	752[bc] (76)	130[b] (9)	0.170[a] (0.005)	168[b] (25)	33[b] (4)	0.200[a] (0.004)
MP	566[bc] (128)	151[b] (33)	0.270[e] (0.002)	337[c] (28)	93[c] (7)	0.280[e] (0.001)
MPS	1415[d] (166)	277[d] (34)	0.200[b] (0.001)	559[d] (46)	124[d] (9)	0.220[c] (0.001)
WP	943[c] (74)	212[c] (15)	0.230[c] (0.002)	457[e] (36)	108[e] (10)	0.240[d] (0.003)
WPS	1285[d] (207)	228[c] (33)	0.180[a] (0.009)	677[f] (17)	144[f] (5)	0.210[b] (0.006)

*Different letters in the same column indicate statistically significant differences ($p<0.05$). Values between parentheses are the standard deviations. C: control; MP: double skimmed milk powder; WP: whey protein added; CS, MPS and PS: respectively C, MP and WP samples plus 2% starch.

After *in vitro* oral digestion, the viscoelastic parameter (Table 2) indicated that dilution by the saliva water was the only effect, as the viscoelastic behavior patterns were the same as without *in vitro* digestion.

The relative changes in the G' and G" of the yogurts were mirrored by the tan δ values that compares the amount of energy lost during a test cycle with those stored during this time. All the yogurt samples had tan δ values of less than one, confirming that elastic properties predominated over viscous ones (Figure 6). The tan δ values decreased

significantly in the added-starch samples in comparison with their no-starch counterparts, indicating an increase in the relative contribution of the elastic component to the viscoelasticity of the system. This more solid-like behavior may be attributed to reinforcement by starch granules embedded in the protein network.

3.5. Sensory analysis

3.5.1. Flash profiling

Figures 7A and 7B show the biplots obtained by GPA from the FP data. The first two principal axes accounted for 80.87% of the variability (50.80% and 30.07% respectively). The six samples were placed in 3 groups, perfectly differentiated (Figure 7B) by the type and amount of protein: C and CS, WP and WPS, and MP and MPS.

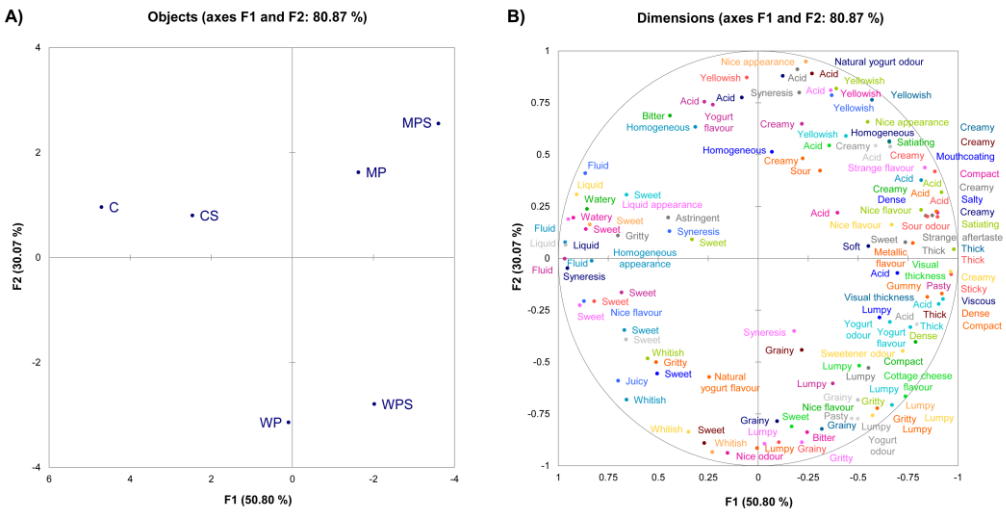

Figure 7. Representations of the terms used to describe the samples (a), and of the six yogurt samples (b) along the first two dimensions of the general Procrustes analysis (GPA) of data from the ultra-flash profile.

The samples with starch were located close to their counterparts, and always on the right. Sample C (Figure 7A) was characterized by its "liquid", "watery" and "fluid" texture (on the left half of the map). Sample CS was located at a little distance (to the right) from sample C. The X axis opposed samples C and CS to samples MP and MPS, which were "creamy", "thick" and "dense" (on the right half of the map). Other attributes placed near these last samples were "compact", "thick", "visual thickness", "mouthcoating", and "satiating". Considering this first dimension, the added-starch samples would be perceived always as creamier, denser, and thicker than their no-starch counterparts. Some flavor attributes were also correlated with the first dimension, which opposed "sweet" (left half) to "acid" and "sour" (right half), while some other flavor attributes with lower numbers of mentions were "strange aftertaste", "strange flavor", "metallic taste", or "salty", all of them on the right half of the map and associated with the MP samples.

The second dimension opposed a series of terms like "lumpy", "grainy", and "gritty" (on the bottom half of the map, close to samples WP and WPS) with "homogeneous texture" and "nice appearance" (Figure 7A). These attributes the panelists perceived in the WP samples could be linked to the presence of the two phases detected in the microscope observations. Terms like "pasty", "dense", and "gummy" also appeared close to the WP samples, showing that they elicited some unpleasant perceptions. Finally, the second dimension also opposed a yellowish color (top half) with a whitish one (bottom half); an extra amount of milk powder seemed to have communicated a pale-yellow color, since both MP and MPS were placed in the right half of the X axis and also shared an area of positive values on the Y axis.

3.5.3. Liking assessment

A clear trend in liking was C>MP>WP. Sample CS received significantly the highest scores for global, texture and flavor liking ($p<0.05$) (Table 3); the only sample with no

significant difference in flavor liking scores was C. These results indicated that adding starch improved the consumers' perception of consistency and their global liking. Based on the first dimension of the FP attribute map, starch contributed a perception of this sample's having a creamier, thicker consistency, causing the CS sample to be preferred over the rest.

Table 3. Hedonic liking scores and intensity of satiating capacity (n = 121) of the yogurts.

Sample	Global	Flavor	Texture	Satiating capacity
C	5.3^{b}	5.7^{cd}	4.0^{b}	3.8^{a}
CS	6.3^{c}	6.0^{d}	6.7^{d}	5.5^{b}
MP	5.0^{b}	4.8^{b}	5.5^{c}	5.5^{b}
MPS	5.3^{b}	5.4^{c}	5.9^{c}	7.1^{d}
WP	3.4^{a}	4.1^{a}	2.9^{a}	5.5^{b}
WPS	3.4^{a}	4.0^{a}	3.2^{a}	6.6^{c}

*Different letters in the same column indicate statistically significant differences ($p<0.05$). C: control; MP: double skimmed milk powder; WP: whey protein added; CS, MPS and PS: respectively C, MP and WP samples plus 2% starch

In the MP samples, a not always significant trend towards higher values for all three liking modalities was observed when starch was added. Finally, the WP samples scored the worst and starch addition had no effect on the liking scores, probably because consumers did not like its gritty, heterogeneous texture and this dominated their liking scores (Table 3).

These results seemed to indicate that in a weaker, more open protein network such as that of the C sample, the inclusion of starch (which retained its granular form) improved the appreciation of its consistency and, consequently, its global liking score. In contrast, adding starch to the MP and WP samples did not improve the consumers' perceptions of them, probably because these protein networks were denser and tighter enough for the structural reinforcement due to the added starch granules to make a perceptible difference. A combination of extra skimmed milk powder (though a smaller amount than in the present study) and PMS to confer creaminess could be a way to achieve good sample liking results.

3.5.4. Satiating capacity

The results on expected satiating capacity showed that MPS was the sample with significantly the highest score ($p<0.05$), followed by WPS. CS, MP and WP did not differ significantly among each other ($p>0.05$). C was expected to be the least satiating sample (Table 3).

These results were related to the attributes chosen by the consumers in the sensory flash profiling exercise. MPS was described as "dense", "compact", "thick", and "creamy", which seemed to trigger expectations of satiating capacity among the consumers, although they did not like this sample the most. This was in line with previous results on HPMC milk-based desserts (Morell, Ramírez-López, Vélez-Ruiz, and Fiszman, 2015), where a thicker, denser texture elicited the perception of higher satiating capacity.

Finally, it is worth noting that adding starch to sample C significantly increased the perception of its satiating capacity, so using starch to reinforce satiating capacity could be a useful tool in this kind of food product.

Conclusions

The relationships between the structure, rheology, and sensory properties of yogurt were investigated intensively to gain a better understanding of how the type and amount of protein, among other factors, can influence perceptions of texture and flavor. The effect of adding a physically modified starch was also studied. In the development of yogurts with enhanced satiating capacity, a combination of rheological, microstructural, and sensory properties has proved to be a powerful tool for assessing the viability of new technological approaches. *In vitro* oral digestion showed that the physically modified starch granules remain unaltered after α-amylase attack giving place to thick, dense and creamy yogurts that could lead to an extended orosensory exposure. Texture seems to be the sensory feature most closely related to eliciting the perception of satiating capacity. Starch addition contributed positively to these sensations, and doubling the protein content with skimmed milk powder achieved a better sensory quality score than using whey proteins.

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The role of starch and saliva in tribology studies and the sensory perception of protein-added yogurts

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Abstract

Increasing the protein content of yogurts would be a good strategy for enhancing their satiating ability. However, the addition of protein can affect product palatability, contributing astringency or an inhomogeneous texture. Increasingly, studies mimicking oral tribology and oral lubrication have been attracting interest among food researchers because of their link with oral texture sensations. In the present study, four double-protein stirred yogurts were prepared by adding extra skimmed milk powder (MP) or whey protein concentrate (WPC) and by adding a physically modified starch to each (samples MPS and WPCS, respectively) to increase the consistency of the yogurts. The lubricating properties of the four yogurts were examined by tribological methods with the aim of relating these properties to the sensory perception described by flash profiling. Samples were also analysed after mixing with saliva.

The tribology results clearly showed that addition of starch reduced the friction coefficient values regardless of the type of protein. Saliva addition produced a further decrease in the friction coefficient values in all the samples. Consequently, adding saliva is recommended when performing tribology measurements of foods in order to give a more realistic picture. The sensory results confirmed that the addition of starch reduced the astringent sensation, especially in the sample WPC, while the MP and MPS samples were creamier and smoother. On the other hand, the astringency of sample WPC was not explained by the tribology results. Since this sample was described as "grainy", "gritty", "rough", "acid" and "sour", further studies are necessary to investigate the role of the number, size, shape and distribution of particles in yogurt samples, their role in astringency perception and their interaction with the perception of the tastes mentioned. Oral tribology has shown itself to be an *in vitro* technique that may aid a better understanding of the dynamics of in-mouth lubrication and the physical mechanisms underlying texture and mouthfeel perception.

1. Introduction

Yogurt is one of the more appreciated functional food matrix primarily consumed (Batista, et al., 2015; Cruz, et al., 2013). It contains high levels of protein, which is known to be the macronutrient with the highest satiating capacity, so it could be an excellent basis for designing satiating food products. However, it is difficult to reformulate yogurt with this aim since adding proteins affects the palatability and texture of the final product (Morell, Piqueras-Fiszman, Hernando, and Fiszman, 2015), particularly in low-fat or fat-free products to keep up with consumer demand and healthy market trends.

Whey protein has been reported to be inherently astringent in an acidic medium such as yogurt, which would affect the acceptance of the product's texture and flavour (Andrewes, Kelly, Vardhanabhuti, and Foegeding, 2011; Beecher, Drake, Luck, and Foegeding, 2008; Childs and Drake, 2010; Sano, Egashira, Kinekawa, and Kitabatake, 2005; Vardhanabhuti, Kelly, Luck, Drake, and Foegeding, 2010). Astringency is normally described as a group of complex sensations that involves perceptions of dryness and roughness of oral surfaces (Ye, Zheng, Ye, and Singh, 2012). According to Andrewes, Kelly, Vardhanabhuti and Foegeding, (2011) astringency in acidic dairy products has been related to whey protein-saliva interactions, so potentially it could be assessed by studying the dynamic behaviour of the whey protein-saliva mixture in the mouth during ingestion.

It has also been shown that an increase in particle size occurs when whey protein is added to yogurt (Puvanenthiran, Williams, and Augustin, 2002). Beaulieu, Pouliot and Pouliot (1999) showed that the binding sites of κ-casein were covered when using a high level of whey proteins, thus forming whey protein aggregates of sizes which are sensed by the tongue, palate and other surrounding soft mouth tissues (Engelen and Van Der Bilt, 2008). The presence of hard, irregularly-shaped particles affects the texture and thus the sensory perception of the yogurt (Engelen, et al., 2005). Greater sensations of roughness and graininess have been reported in whey protein-added yogurt (Cayot, Schenker, Houzé, Sulmont-Rossé, and Colas, 2008; Hahn, Sramek, Nöbel, and

Hinrichs, 2012), affecting the lubrication effects (de Wijk, Prinz, and Janssen, 2006; Krzeminski, et al., 2013). Adding milk solids to yogurt (in the form of milk powder) has been reported as contributing mouthfeel and creaminess, but also graininess and chalkiness (Cliff, et al., 2013). Understanding the mechanisms and factors affecting the perception of graininess, astringency and other related sensations could contribute to improving the strategies for adding protein to acidic dairy products.

Oral tribology and lubrication are little-studied scientific topics in relation to food texture and oral sensation (Chen and Stokes, 2012). De Wijk and Prinz (2005) postulated that astringent sensations may be related to reduced lubrication (that is to say, increased friction). Polyphenols, the compounds most studied in relation to astringency, showed different responses: some increased the friction coefficient with increasing astringency while others were perceived as astringent but did not alter the lubrication properties. These studies imply that astringency is unlikely to be a purely tactile perception caused by a rise in friction. In consequence, direct relationships between sensory perceptions and friction for all conditions and food matrices cannot be found. However, a very recent study on the wine astringency and the lubrication of wine/ saliva mixture showed that a positive correlation exists between the perceived sensory feature and the friction coefficient obtained within a certain range of sliding speeds, between the boundary regime and mixed regime (Brossard, Cai, Osorio, Bordeu, and Chen, 2016).

The mechanical and rheological properties of a wide range of food products have been studied extensively in order to understand and describe in-mouth flow properties and in turn, to relate them to sensory perception (Prakash, Tan, and Chen, 2013). However, these approaches, essentially based on bulk destruction and shear deformation, are not applicable to some sensations detected in the mouth such as creaminess, slipperiness and smoothness, which are perceived by rubbing and squeezing with an upward/ downward movement along with horizontal movements of the tongue against the palate (Prakash, et al., 2013; Prinz, De Wijk, and Huntjens, 2007). Such actions create both normal and shearing forces in the mouth and, when food products (or a food–saliva

mixture) are being processed, generate a sensation of friction/ lubrication between palate and tongue that could become dominant in relation to the food texture and mouthfeel perceptions of liquid and semi-solid food products. For this reason, tribology is an emerging technique for food texture studies that measure the lubricating properties of food (Chen, Liu, and Prakash, 2014).

Liquid or semi-solid food has a shorter oral contact time than solid food, with only a few seconds of retention in the mouth (Engelen and de Wijk, 2012), which limits oral handling activities. Simple up and down movements of the tongue are mainly used to assess thickness. The same movement combined with horizontal movements along the palate is used to assess creaminess (de Wijk, Engelen, and Prinz, 2003; de Wijk, Terpstra, Janssen, and Prinz, 2006), with afterfeel sensations elicited by what remains after the food is swallowed.

According to De Vicente, Stokes and Spikes (2006), the initial behaviour of food material in the mouth may be seen as moving from right to left along the friction curve (also known as Stribeck curves). Initially, the behaviour of the material is governed by its bulk rheological properties when the product film between surfaces is relatively thick. However, as the product is sheared and broken down into a much thinner film during consumption, negligible fluids are entrained into contact and the load is carried by the contacting asperities and is dependent on the surface and interfacial film properties at the molecular scale. The lubrication property of this film is usually described by measuring the friction between two surfaces (Baier, et al., 2009; Joyner, Pernell, and Daubert, 2014; Malone, Appelqvist, and Norton, 2003; Sonne, Busch-Stockfisch, Weiss, and Hinrichs, 2014). By changing the relative speeds between the two rubbing surfaces, the lubrication properties of a food material can be analysed as a layer being squeezed between oral surfaces and as a thin film remaining on the tongue surface, which, in addition, is responsible for the afterfeel in the mouth. Some researchers have considered that friction is involved in the mechanism underlying astringency (Green, 1993; Prinz

and Lucas, 2000), that has been shown to be a trigeminal percept in human subjects (Schöbel, et al., 2014).

Flash profiling is a combination of free-choice term selection with a ranking method based on simultaneous presentation of the whole product set (Dairou and Sieffermann, 2002). In the present study, in order to develop yogurts with higher expectations of satiating capacity and good palatability, tribology and sensory characterization were combined and used as potent tools for assessing the viability of new added-protein yogurt formulations.

The aims of the present work were 1) to analyse the effect of adding extra protein (extra milk powder or whey protein concentrate) on the tribological properties of yogurt, 2) to analyse the effect of adding a physically modified starch to the higher-protein samples, and 3) to characterize the sensory properties of the yogurts through flash profiling in order to relate them to tribological features with the aim of designing satiating yogurts.

2. Materials and methods

2.1. Ingredients

The ingredients used in the preparation of the yogurts were skim milk powder (33,4 g protein/ 100 g) (reference 385, kindly provided by Fonterra Co-operative Group Ltd, Palmerston North, New Zealand), whey protein concentrate (78,12 g protein/ 100g) (Glanbia Nutritionals Ltd., Kilkenny, Ireland), physically modified starch (PMS, Indulge 10267-47, kindly provided by Ingredion, Hamburg, Germany), freeze-dried lactic culture (Yo-Mix 883, Lyo 50 DCU, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, Danisco, Brabrand, Denmark) and distilled water.

2.2. Sample preparation

Four different stirred yogurt samples were formulated: MP (extra skimmed milk powder added), WPC (whey protein concentrate added), and MPS and WPCS (respectively MP and WPC with 2% starch added). Milk was prepared with 50 g of skimmed milk powder/ 500 mL of distilled water. An additional 50 g of skimmed milk powder/ 500 mL was added to samples MP and MPS, and 21.38 g of whey protein concentrate/ 500 mL to samples WPC and WPCS. These additions were equivalent to doubling the original protein content.

Skimmed milk powder and distilled water (and WPC as appropriate) were placed in a glass beaker (1L) and heated in a water bath (DF-101S, Yingyu Yuhua Instrument Factory, Gongyi, China) at 82-85 ºC for 30 min (following the procedure of Morell, Hernando, Llorca and Fiszman (2015)). For the samples with starch, this ingredient was added after removing the glass beaker from the bath. The samples were then cooled to the incubation temperature recommended for the culture used (42–43 ºC) (digital thermometer, VWR International, Radnor, PA, USA) and inoculated with the starter (0.01 g/ 100 g of milk). The mixture was poured into 125-mL glass yogurt jars and placed in a yogurt-maker (YG523, Jata Electro, Abadiano, Spain). After a period of 7 h, the samples reached pH values of 4.5-4.6 (Mettler Toledo FE20 Desktop pH meter, Mettler Toledo International Inc., Shanghai, China). The individual jars were covered and stored at 4-5 ºC for 24 h. Before performing the tests, the yogurts were stirred individually (Ultra-Turrax IKA T25 digital, Werke Staufen, Germany) at 3000 rpm for 3 min.

2.3. Tribological measurements

A texture analyser equipped with Exponent software version 3.2 for automatic data recording (both from Stable Micro Systems, Godalming, UK) was used. Additional accessories were especially designed and constructed, including: a stainless steel base with a water circulation device underneath for temperature control and a moving probe

with three stainless steel balls in a triangular arrangement connected to the load cell of the texture analyser (Chen, et al., 2014). The texture analyser was placed on its side on a levelled workbench (Figure 1).

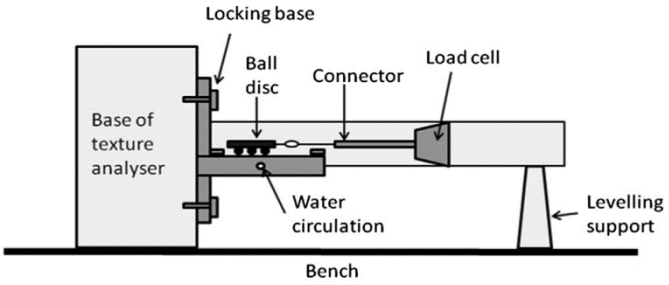

Figure 1. Diagram of the experimental set up for tribological studies using a texture analyser

A shallow round trough, 85 mm in diameter and 4 mm in depth, was placed on top of the platform. A 1 mm thick silicone elastomer (NDA Engineering Equipment Ltd, Kempston, UK) was placed in the trough as the lower substrate surface for the friction tests. To mimic oral conditions better, the silicone rubber was put into a glass with water and soap the night before and subsequently dried to make the surface more hydrophilic before placing it in the trough. An appropriate amount of the fluid sample (around 1 mL) was transferred carefully to the trough to give full film surface coverage. The weight of the moving probe itself was 27.8 g, taking the load to around 0.27 N. On dragging the moving probe, the balls slid along the sample on the substrate surface. Both the upper and lower substrate surfaces in this study were the same as those used in a previous study by De Vicente et al., (2006) The ball-on-disc arrangement is widely used in many commercial tribometers due to easy control of its surface movements.

The sliding speed was set from 0.1 $mm \cdot s^{-1}$ to 10 $mm \cdot s^{-1}$. This was considered the most useful speed range for mimicking the sensations between the tongue and palate in the present investigation, as it provided the greatest friction discrimination. The frictional

behaviour at slow speeds (usually called the boundary lubrication regime), where friction is independent of speed, is that the load is carried by the contacting asperities and is dependent on the surface and interfacial film properties at the molecular scale (Sonne, et al., 2014). In food tribology, this regime is believed to correlate with various sensory mouthfeel properties, including fatty feel, oral slipperiness, creaminess, smoothness, etc. (Baier, et al., 2009; Debon, Vanhemelrijck, Baier, and Guthrie, 2013; Giasson, Israelachvili, and Yoshizawa, 1997; Joyner, et al., 2014; Kokini, 1987; Kokini and Cussler, 1983; Malone, et al., 2003; Nguyen, Bhandari, and Prakash, 2016; Sonne, et al., 2014). At higher speeds (also called the hydrodynamic lubrication regime), a film of food (lubricant) is entrained to fully separate the solid surfaces. Its thickness depends on its viscosity and entrainment speed. The friction here depends on the rheological properties of the lubricant film which forms in the contact area (De Vicente, et al., 2006).

The friction force acting on the load cell consists of two parts: static and dynamic friction. The average force of the dynamic friction was calculated by the texture analyser software and was used to calculate the friction coefficient versus the total surface load. A temperature of 25 ºC was used in this study. This condition was based on the fact that the skin temperature is always lower than the body temperature. The friction coefficient is defined as the ratio of the friction force to the surface load between the two surfaces in contact (Chen, et al., 2014). Stribeck curves usually represent the friction coefficient (μ) as a function of relative speed between the two surfaces, v_s ($mm \cdot s^{-1}$). The friction coefficient (μ), calculated as the friction force divided by load ($\mu = F_f/W$), was measured 10 times for each speed and averaged (Selway and Stokes, 2013).

2.4. Saliva

2.4.1. Whole human saliva

Mechanically stimulated whole human saliva (WHS) was obtained from a single healthy subject who refrained from eating and drinking (except water) for 2 h prior to expectorating saliva. Before saliva collections, the subject was asked to rinse his mouth with deionized water. To stimulate saliva flow, the subject chewed a piece of parafilm (1 g ± 0.1 g) with the head tilted in order to pool saliva at the front of the mouth, according to the procedure described by Bongaerts, Fourtouni and Stokes (2007). Saliva generated during the first 30 s was discarded (Moritsuka, et al., 2006) and the actual saliva collection followed over a period of 2 min with continuous parafilm chewing. The stimulated fresh saliva was used immediately after collection, without centrifugation. It should be noted that saliva was obtained from only one subject due to the constraints of the saliva collection procedure (Rossetti, Bongaerts, Wantling, Stokes, and Williamson, 2009; Vardhanabhuti, Cox, Norton, and Foegeding, 2011). A 200 μL measure of human whole saliva was placed on the sample (1:4) and the lubrication properties of the resulting saliva–sample mixture were measured over the selected speed range (Selway, et al., 2013).

2.4.2. Artificial saliva (AS)

Artificial saliva was prepared according to the method described by Kong and Singh (2008). All the reagents were of analytical grade. The components were sodium chloride (0.117 g/ L), potassium chloride (0.149 g/ L) and sodium bicarbonate (2.1 g/ L) (all from Kelong Chemical, China), mucin from porcine stomach, type II (PGM) (Sigma, M2378) (1 g/ L), α-amylase from *Bacillus subtilis* (MAYA-9000-90-2, MAYA Reagent, China) (4000U/g) (2 g/ L) and HPLC grade double-distilled water. For the *in vitro* oral digestion, the saliva to sample ratio was 1:4 (Morell, Hernando, et al., 2015).

2.5. Sensory analysis

Flash profiling — a combination of free-choice term selection with a ranking method — was applied. The simultaneous presentation of the complete set of samples allowed direct sensory comparison (Dairou, et al., 2002).

2.5.1. Panel

Flash profiling was carried out by 13 assessors recruited among employees of the Zhejiang Gongshang University of Hangzhou (China) who had experience in sensory description of food products and were regular consumers of dairy products. A total of 17 sensory terms were generated. Between 2 and 12 attributes were generated by each panellist; the mean number of terms generated was 6.25 (± 2.8). They were asked to describe all the (non-hedonic) differences they perceived in the yogurt samples. No restrictions about attribute modalities were introduced.

2.5.2. Procedure

Flash profiling does not demand a specific participant training stage. The single session was divided into two stages using English language. In the first, prior to evaluation, the researcher explained the procedure to each panellist and gave him/ her a printed example of a descriptive ranking of apples according to various attributes that not apply to yogurt (crispy, hard, soft and mealy). In the second or evaluation stage, the complete set of samples was presented simultaneously. After tasting the samples, each panellist generated his/ her own list of attributes to describe the differences among the four yogurts for appearance, aroma, taste, texture, or any other characteristic of each sample preventing hedonic descriptors (Santos, et al., 2013). No indication was given regarding the number of attributes that should be proposed. The panellists then ranked all samples on an ordinal scale for each attribute they had individually proposed (ties were allowed).

The panellists were asked to focus on descriptive terms. Their evaluation was individual. The samples (30 mL) were served at consumption temperature (10.0 ± 0.5 ºC). Since it was not important to assess their colour, the samples were served in small blue-coloured glass cups coded with random three-digit numbers. Mineral water was provided for rinsing the mouth between samples (Morell, Hernando, et al., 2015).

2.6. Data analysis

The Exponent software (version 3.2, Stable Micro Systems Ltd., Godalming, UK) preloaded on the texture analyser was used for automatic data recording of the force, distance, and time, and for statistical analysis of the tribological parameters. Generalized Procrustes Analysis (GPA) was used to analyse the flash profile results (Gower, 1975). GPA reduces the scale usage effects, delivers a consensus configuration and allows comparison of the proximity between the terms used by different assessors to describe the products (Moussaoui and Varela, 2010). Panel discriminating power was analysed by Discriminant Analysis (DA) of the sample coordinates values from GPA. The GPA and DA analyses were performed with XLSTAT statistical software (version 2010.5.02, Microsoft Excel®, Addinsoft, Barcelona, Spain).

3. Results and discussion

3.1. Tribological analysis

Stribeck curves (log μ versus log v_s) exhibiting the friction performance of the yogurt systems were obtained, comparing protein type (Figure 2), starch addition (Figure 3), and saliva mixing (Figure 4).

In general, at low sliding speed values, the friction is in the boundary regime of the Stribeck curve. In this case, the load is carried by the contacting asperities and the

friction is independent of speed but dependent on the surface properties both at the molecular level (surface chemistry) and at the microstructural level (the asperity geometry). As the speed increases ($v_s \geq 1$ mm s^{-1}), fluid is entrained into the contact, creating a pressure which supports the separation of the solid surfaces, causing decreased friction effects. As stated by Sonne, et al., (2014) the greatest friction discrimination is found at speeds below 10 mm s^{-1}. Consequently, friction coefficients obtained in this speed range may be considered useful for understanding the potential relevance of tribological results in elucidating the mechanisms involved in perception of the sensations of in-mouth creaminess and smoothness elicited by the yogurts, which in turn could be related to satiating expectation cues. In addition, once the yogurt is cleared from the oral cavity the surface residue may control lubrication (De Vicente, et al., 2006), which could also be related to sensory characteristics such as aftertaste and mouth-coating.

3.1.1. Effect of protein type

The tribological profiles (Figure 2A) showed that the friction coefficient values of samples MP and WPC were very similar at low speeds (from 0.1 to 1 mm s^{-1}). At higher speed values (between 1-3 mm s^{-1}), a "shoulder" appeared for sample MP; its cause is unknown and would require further investigation. One possible explanation could be related to the findings of De Vicente, et al., (2006) who worked with microgel particle suspensions where an increase in friction with increasing speed was related to some confinement of the swollen microgels between the two surfaces. Since the size of the microgels themselves was measured in microns, the film thickness may be expected to be of similar size. A similar scenario could be found in added-protein yogurt, which could be considered a microgel suspension with a polydisperse particle size distribution that depended on the temperature processing and holding times (Hahn, et al., 2012; Sfakianakis and Tzia, 2014). The Stribeck curve for MP passes through a maximum in the transition into the mixed-regime where μ decreases with increasing entrainment

speed. Differences in particle size in the MP and WPC systems could account for the differences in lubricant properties shown by these two systems at low sliding speed. Lee, Heuberger, Rousset and Spencer (2004) also interpreted the lubricant properties of molten chocolate in terms of particle behaviour in the region surrounding the inlet of the sliding tribo-pairs. It should be pointed out that this is only a function of sliding speed. Factors such as viscosity variation and surface load were not included in their analysis. Regarding the viscosity values of samples MP and WPC, in a previous paper Morell, et al., (2015) reported that they were very similar; the same occurred in the added-starch samples.

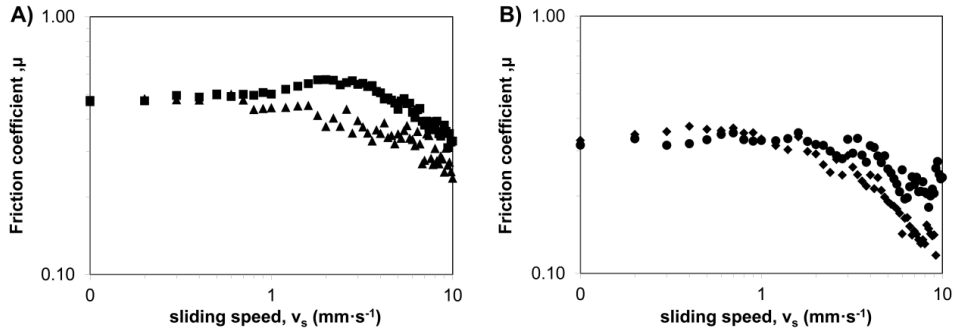

Figure 2. Friction coefficients of yogurts plotted as a function of the sliding speed. A) MP versus WPC and B) MPS versus WPCS. MP (squares), WPC (triangles), MPS (circles), WPCS (diamonds). MP: double skimmed milk powder, WPC: whey protein concentrate added, MPS and WPS: respectively MP and WPC with the addition of 2% physically modified starch.

As expected, as the speed increased the friction coefficient of both the MP and WPC samples decreased as a result of the lubricating film formed between the solid surfaces of the tribo-pairs, so the lubrication effect was only partial (De Vicente, et al., 2006). Sample WPC had lower friction coefficient values than sample MP and exhibited a

generally smoother friction curve. It may be hypothesised that the larger protein particles in sample WPC were probably unable to fit between the asperities of the sliding surfaces, so particle entrainment did not occur and cause lubrication. Sensory graininess is widely reported for yogurt with added whey protein and is usually related to increased particle size (Krzeminski, et al., 2013; Kücükcetin, 2008), while chalkiness is found for yogurts with a high milk powder content (Cliff, et al., 2013).

3.1.2. Effect of starch addition

The addition of physically modified starch caused a considerable reduction in friction over the whole range of sliding speeds studied, for both protein type samples, equalising the friction curves of the two samples with starch (Figures 3A and 3B).

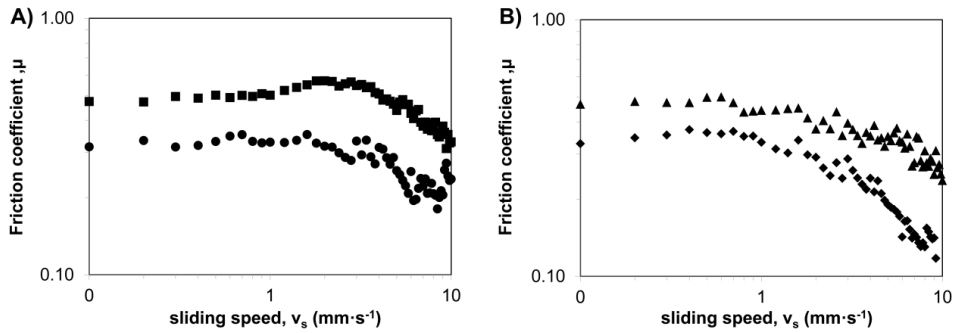

Figure 3. Friction coefficients of yogurts plotted as a function of sliding speed. A) MP versus MPS, B) WPC versus WPCS. MP (squares), WPC (triangles), MPS (circles), WPCS (diamonds). MP: double skimmed milk powder; WPC: whey protein concentrate added; MPS and WPS: respectively MP and WPC with the addition of 2% physically modified starch.

Figure 3A and 3B show the differences between the tribological profiles of the samples prepared with each protein and the corresponding samples with starch. The difference was smaller when starch was added to WPC than when added to MP. The MPS and WPCS samples were very close to each other over the whole speed range measured (Figure 2B), indicating that the effect of the starch is dominant in the lubricating effect of these two samples.

Garrec and Norton (2012) reported that a higher concentration of several hydrocolloids was associated with high viscosity and low friction. Along the same lines, Zinoviadou, Janssen and De Jongh (2008) showed that increasing the concentration of starch in a solution leads to lower friction coefficients, mainly exhibited in the boundary regime.

The physical form of the starch used in the present study was observed under polarized light prior to use (Morell, Hernando, et al., 2015). It included two populations of different starches: the waxy maize starch granules were smaller and exhibited a Maltese cross even after heating at 90 °C for 15 min, while the potato granules were bigger, seemed to be partially disrupted with incipient gelatinization, and had virtually no observable Maltese cross. In other words, the physically modified starch used in the present work provided the system with both intact granules and gelatinized starch. Interestingly, working with an ionic liquid water solvent, Yakubov, et al., (2015) established that a small amount of soluble starch reduced the boundary friction coefficient. This low friction was associated with a thin film formed from the amylose fraction of the starch. In the same study, when intact granules were present in the system the reduction in friction at low speeds was associated with entrainment of particles into the contact and the subsequent change from sliding- to rolling-dominated friction, whereby entrained particles provide a ball-bearing effect. These results would explain the decrease in the friction values of both MPS and WPCS, the yogurt samples with starch (Figure 3A and 3B), where both effects took place. Deposition of a thin amylose film on the rubber surface might lower the friction by changing the surface property in

the boundary regime; the sensory perception of a smoother and creamier product would then be expected.

3.1.3. Effect of saliva

The mechanisms described for perceiving astringency normally involve saliva effects (Vardhanabhuti, et al., 2010), therefore the samples were mixed with saliva in the present study.

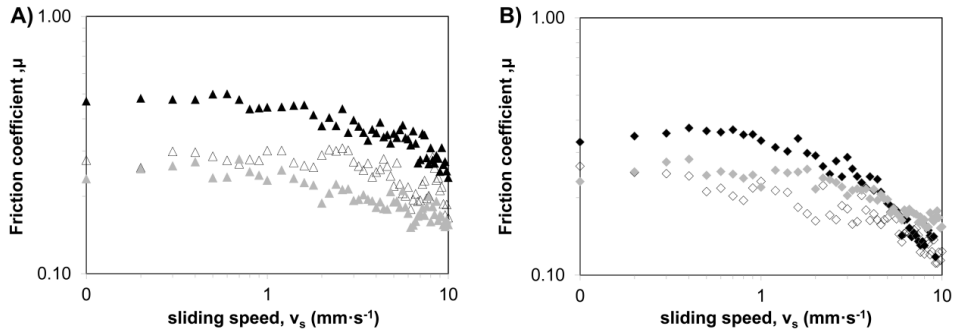

Figure 4. Friction coefficients of yogurts plotted as a function of sliding speed. A) WPC, WPC mixed with AS, and WPC mixed with WHS; B) WPCS, WPCS mixed with AS, and WPCS mixed with WHS. Sample alone: black full symbols; sample + AS (grey full symbols), sample + WHS (empty grey symbols). WPC: whey protein concentrate added; WPCS: WPC with 2% physically modified starch added; AS: artificial saliva; WHS: whole human saliva.

The presence of saliva led to a reduction in friction coefficient values (Figure 4A). This effect was greater in WPC than in WPCS, the sample with starch (Figure 4B). The same trend was observed in samples MP and MPS when saliva was added (data not shown).

This smoothing effect almost disappeared in the samples with starch at higher sliding speeds ($v_s \geq 3$ mm·s^{-1}). In a previous study, Morell, et al., (2015) stated that α-amylase did not attack the intact granules of this physically modified starch after saliva addition, as shown by light microscopy images, whereas the solubilised starch did disappear. Shi and Trzasko (1997) stated that as a consequence of physical modification of the starch under preferred conditions of thermal treatment, linear chains within granules realign themselves in a more orderly manner, thus making it more difficult for the amylase to attack. High hydrostatic pressure treatments also lead to increased crystalline perfection in the granules (Ashogbon and Akintayo, 2014).

The number of intact granules in yogurt with added starch degraded by saliva appeared to be sufficient to maintain low friction values. As mentioned above, owing to their shape and size the starch granules may be entrained between the two surfaces even at low speeds, keeping the surfaces apart and lowering the friction coefficient. This lowering of friction can take place when the particle size is comparable to or larger than that of the roughness of the surfaces involved. Entrainment of particles might then lead to a smothering effect on the surface (Zinoviadou, et al., 2008).

Figure 4 shows that AS vs WHS behave in a very similar fashion in altering the lubrication of yogurt. The dilution effect of mixing with saliva was minimal, since dilution would have the effect of reducing viscosity, so increasing the friction coefficients, but this was not observed in the present measurements.

The added-starch samples mixed with saliva showed the lowest friction values, indicating that these samples spread more easily on the PDMS rubber. As Zinoviadou, et al., (2008) showed, this results in easier wetting of the surfaces, even at low speeds, promoting the confinement of granules remaining in the contact zone. This circumstance supported the idea that in the presence of saliva, a thin layer forms in the mouth between two surfaces such as the tongue and the palate due to the higher degree of interaction between the sample and the surface (Zinoviadou, et al., 2008). Joyner, et al., (2014) also found that adding saliva to several stirred glucono-δ-lactone-acidified

dairy protein gels resulted in a slight decrease in the friction coefficient compared to gels to which water had been added for the purpose of comparison.

3.2. Sensory analysis

The number of samples in the present study (four) could be considered a limitation since the use of low number of samples in a multivariate factor technique as GPA can lead to an unstable perceptual space. The discriminating power of the panel was tested by discriminant analysis of the sample coordinates for the thirteen assessors. The obtained values ($F_{6,86}$ = 116.87, $P < 0.0001$) indicated a good discriminant power.

Figures 5A and 5B show the biplots obtained by GPA from the flash profiling data. The first two principal axes accounted for 85.38% of the variability in the results (56.56% and 28.82% respectively). The four samples were placed in two different halves of the map (Figure 5B). Both the samples containing WPC were placed on the left side of the biplot, whereas the two samples containing MP were placed on the right side. Sample WPCS was far apart from sample WPC but samples MP and MPS were placed very close together.

The X-axis seemed to oppose textural attributes "roughness", "grittiness", "graininess", and "astringency", on the left half of the map, and "smoothness" and "creaminess" on the right half of the map. In a study performed with several types of plain yogurts (Ares, Giménez, and Gámbaro, 2008) it was found that consumers mentioned "too fluid", "gummy texture", "gritty texture", "rough" as sensory defects that were drivers of disliking and of negative intention to try, whereas "creamy", "thick", and "soft" were mentioned as the sensory characteristics of the tried yogurts.

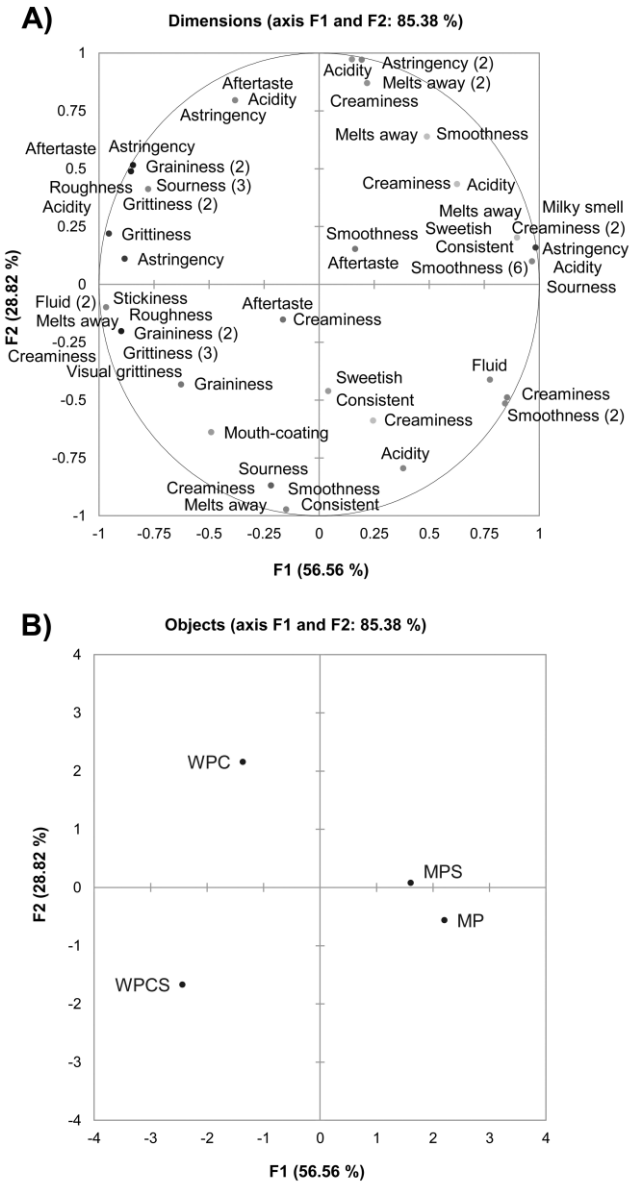

Figure 5. A) Representations of the terms used to describe the samples; the number in parentheses following an attribute is the times the attribute appeared superimposed in the same place in the original GPA output; and B) Representation of the samples, along the first two dimensions of the GPA of data from the ultra-flash profile.

De Wijk, van Gemert, Terpstra and Wilkinson (2003), working with custards, have also previously shown the existence of a Rough-Creamy axis (in the present case running from negative to positive values along the X axis). In their study, food samples were ordered along this dimension according to their fat content, with low-fat samples located near the rough end of the spectrum and high fat samples near the creamy end. Since fat performs many functions, including lubrication, de Wijk, Rasing, and Wilkinson (2003) suggested that the underlying physical mechanism of the Rough-Creamy axis could be friction-related. Particularly the attribute creaminess seemed to be spread for through 3 quarters of the plot; this would be explained because creaminess is a complex percept for non-trained assessors: several texture characteristics such as smoothness, softness or thickness would be involved in its evaluation (Antmann, et al., 2011).

Sample WPC was characterized by terms such as "astringency", "sourness", "acidity", "aftertaste", "graininess" and "grittiness" (on the left half of the map in Figure 5A). Sample WPCS was placed apart from sample WPC (in the upper and lower zones of the left-hand half of the map, respectively). Although terms such as "graininess" and "grittiness" appeared close to the WPCS sample, others such as "melts away", "creaminess", "consistent" and "mouth-coating" were also found near to it (Figures 5A and 5B). It is noteworthy that the addition of physically modified starch turned WPCS into a sample that was perceived as non-astringent and less acidic.

The size of the particles is perceived by the tongue, palate and other surrounding soft mouth tissues (Engelen, et al., 2008). At sufficiently high concentrations, particles of 5 μm in diameter can be detected, as they give rise to sensations of roughness and dryness (de Wijk, et al., 2005). Both samples showed "grittiness" and "graininess" but the panellists only perceived the "astringency" attribute in the WPC sample.

Samples MP and MPS were placed close together on the right half of the map (Figure 5A), near to "creaminess" but mostly with the attribute of "smoothness". These samples

were also characterized by attributes such as "consistent", "melts away" and "sweetness".

Considered as a whole, the perceptual map indicated that both the MP samples would always be perceived as creamier and smoother than the samples with added whey protein.

4. Conclusion

In the present study, the application of tribology measurements to the different yogurt samples designed to be more satiating, aided the interpretation of some of their sensory features. The lubricating properties of samples WPC and MP did not account for the difference in sensory astringency described by consumers. This result implied that astringency in yogurt seemed unlikely to be a purely tactile perception caused by a rise in friction. Since the samples containing WPC were described as gritty and grainy, it was postulated that the differences in particle characteristics — which were not investigated in the present study — could play a role in these perceptions.

The addition of physically modified starch clearly reduced the friction coefficient values of yogurts made with both type of proteins. This effect was attributed to both the intervention of amylose polymer, forming a film on the surfaces, and/ or the presence of intact granules that contribute to a rolling action. In the presence of saliva, the number of intact starch granules was probably sufficient to keep the friction low. The combination of the higher viscosity provided by the soluble starch polymers and the preservation of granules in the starch was regarded as a determining factor in the sensory response. This finding could be of important practical interest for the formulation of yogurt using these kinds of physically modified starches with both gelatinized and no gelatinized fractions.

Finally, saliva enhanced the lubrication properties of all the samples, diminishing the friction values of WPC and MP and further reducing those of the yogurts containing starch. This could indicate that when performing tribology measurements, the presence of saliva would render the scenario more realistic.

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Resumen de resultados

La tesis abarca distintas estrategias para el estudio y diseño de alimentos con capacidad saciante.

En un primer bloque se estudian distintos ingredientes con capacidad de modificar la consistencia y estructura de diversas matrices lácteas y, en consecuencia, su trayectoria oral y percepción sensorial. En este primer bloque, se determinó que los hidrocoloides utilizados para aumentar la viscosidad y consistencia de los batidos desempeñan un papel importante aumentando las expectativas de saciedad que despiertan. La caracterización reológica de los batidos reveló patrones de comportamiento muy diferentes dependiendo del tipo de hidrocoloide. La exposición a la saliva demostró ser de gran interés para aprovechar los cambios reológicos que se asociaron a percepciones relacionadas con las expectativas de saciedad; éste fue el caso del almidón modificado (fosfato dialmidón hidroxipropilado) que siendo el más consistente, tras la digestión, presentó valores de consistencia más bajos que los batidos con gomas. Esta pérdida de pseudoplasticidad y consistencia debido a la digestión parcial de los gránulos de almidón por el ataque de la α-amilasa presente en la saliva, hizo que los batidos se percibieran como cremosos, homogéneos y consistentes en boca, características que hicieron que fueran considerados además como los más saciantes. La percepción de la capacidad saciante parece estar más relacionada con la sensación de cremosidad cuando el batido entra en la boca del consumidor, y en el caso del almidón modificado, con la pérdida de estructura que tiene lugar durante el procesamiento oral, lo que provoca una sensación conocida como "fundente".

Las técnicas utilizadas en el estudio fueron herramientas útiles para investigar más en profundidad la percepción de saciedad esperada. Los factores que determinen la saciedad no pueden ni deben limitarse a la viscosidad aparente del producto sin consumir.

El segundo estudio del bloque se realizó con un postre lácteo adicionado con un mismo hidrocoloide (HPMC) pero en concentraciones crecientes y se investigaron otras variaciones: con el doble de leche en polvo y con adición de nata. Los resultados

experimentales mostraron que una mayor concentración de HPMC originó un sistema más estructurado. El hidrocoloide desarrolla en la muestra un comportamiento viscoelástico que es responsable de una serie de sensaciones temporales complejas en boca produciendo percepción de saciedad. Además del efecto dominante del HPMC, la presencia de glóbulos de grasa (nata) o sólidos de la leche en polvo también desempeñaron un papel importante modulando de forma importante dicha percepción.

A partir del análisis de las curvas TDS se observó que las muestras con baja concentración de HPMC mostraban como sensaciones dominantes "ligero" y "fundente", y fueron las muestras que produjeron valores de saciedad esperada relativa (RES) más bajos. Con adición de nata o leche en polvo el predominio fue de "cremosidad", "adhesividad" y "recubrimiento oral" con valores de RES más altos. Por otro lado, las curvas de TDS de las muestras con alta concentración de HPMC mostraron como atributos dominantes "espeso", "cremoso", "gomoso" y "adhesivo", y consecuentemente obtuvieron mayores valores de RES que las muestras con baja concentración de hidrocoloide. La adición de nata y leche desnatada en polvo contribuyeron aumentando la tasa de dominancia y duración de algunas de estas sensaciones.

En resumen, el efecto de la adición de hidrocoloides con capacidad espesante para provocar sensación saciante es complejo. Existe una serie de factores que puede modular estas sensaciones: la presencia de otros componentes, su interacción y su influencia sobre la temporalidad de la percepción oral son sólo algunos.

El segundo bloque en el que se divide los resultados de la tesis se centra en el diseño de yogures con proteína añadida. Se estudia cómo afecta la adición de distintas proteínas lácteas a la microestructura, reología y percepción sensorial de cada una de las muestras. Además, de acuerdo a los resultados obtenidos en el primer bloque se añadieron también hidrocoloides con el fin de mejorar la consistencia y las propiedades

organolépticas de las muestras y evitar sensaciones como la astringencia producida por la adición de proteínas en medio ácido.

Se añadió a los yogures el doble de la cantidad de proteína original, pero obtenida de distintas fracciones proteicas de la leche. La muestra con el doble de leche en polvo fue globalmente la muestra más aceptada y la más cercana al yogur saciante ideal. La cremosidad, la homogeneidad y el dulzor fueron los atributos más recomendables para formular yogur (saciante o no), aunque algunas características adicionales de textura y recubrimiento oral contribuyeron a la sensación de saciedad esperada.

Dependiendo del tipo de proteína añadida a cada muestra, la capacidad de saciar parecía estar impulsada por una serie de características sensoriales distintas. Por otro lado, a la muestra con adición de caseinato se le atribuyeron atributos como "granuloso" y "arenoso", pero a la vez, fue percibida por atributos negativos de sabor como "artificial" o "extraño" que le perjudicaron. La sustitución parcial de caseinato con proteína del suero disminuyó esas características sensoriales negativas, ya que la muestra que combinaba la adición de ambas proteínas tuvo valores altos de capacidad saciante sin presentar tantos atributos negativos como la muestra con adición de caseinato.

La caracterización sensorial combinada con el análisis de penalización, resultaron complementarios para estudiar la reformulación del yogur, ya que no sólo los productos finales deben ser aceptables, sino que también deben despertar en boca expectativas específicas de capacidad saciante. En resumen, la suplementación con leche desnatada en polvo y cantidades precisas de caseinato o concentrado de proteínas de suero podría abrir nuevas posibilidades en el diseño de yogures saciantes.

En una segunda etapa se evaluaron yogures con adición de distintas proteínas lácteas y almidón físicamente modificado. Este último puede mejorar la percepción de textura cremosa y permite obtener un etiquetado limpio del producto.

Al examinar la estructura de las muestras antes de la digestión oral *in vitro* mediante microscopía se pudo estudiar las diferencias entre proteínas y se pudo analizar el papel

de los gránulos de almidón absorbidos en las redes de proteínas. La digestión oral *in vitro* mostró que los gránulos de almidón permanecen inalterados después del ataque de la α-amilasa presente en la saliva, dando lugar a yogures consistentes y cremosos que podrían conducir a una exposición orosensorial prolongada. Estas características se relacionaron con el comportamiento reológico de las muestras. Cada muestra de almidón añadido mantuvo valores de consistencia más altos y, a su vez, se percibía más saciante que su homólogo sin almidón.

La relación entre los resultados obtenidos mediante la reología, la microestructura y las pruebas sensoriales ayudan a entender mejor cómo el tipo y la cantidad de proteína pueden influir en la percepción de textura y en el sabor y cómo se ve modulado por la presencia de almidón modificado físicamente. La textura parece ser la característica sensorial más estrechamente relacionada con la obtención de la percepción de la capacidad saciante. La adición de almidón contribuyó positivamente a estas sensaciones, y duplicar el contenido de proteína con leche desnatada en polvo logró una mayor puntuación en la calidad sensorial que el uso de proteínas de suero.

La adición de proteínas en este tipo de alimentos desarrolla cierto grado de astringencia. En consecuencia, se pensó que el estudio de la tribología de estos sistemas podría ayudar a entender mejor el desarrollo de estas sensaciones y aportar soluciones con el fin de desarrollar sistemas de alta palatabilidad.

Los estudios tribológicos ayudaron a la interpretación de algunas de sus características sensoriales. Las propiedades lubricantes de algunas muestras no reflejaron la diferencia de astringencia sensorial descrita por los consumidores, indicando que la astringencia no era una percepción puramente táctil causada por un aumento en la fricción. Dado que algunas muestras se describieron como arenosas y granulosas, se estableció que las diferencias en las características de las partículas - que no fueron investigados en el presente estudio - podrían desempeñar un papel relevante en estas percepciones. La adición de almidón modificado físicamente redujo significativamente los valores de los coeficientes de fricción de los yogures. Este efecto se atribuyó tanto a la intervención

del polímero de amilosa, formando una película sobre las superficies, como a la presencia de pequeños gránulos intactos de almidón que contribuyeron a acciones de rodamiento. En presencia de saliva, el número de gránulos de almidón intactos era probablemente suficiente para mantener la fricción baja. La combinación de la mayor viscosidad proporcionada por los polímeros de almidón solubles y la conservación de gránulos en el almidón se consideró como un factor determinante en la respuesta sensorial. Finalmente, la saliva aumentó las propiedades de lubricación de todas las muestras, y se consideró que la presencia de saliva haría que el escenario para mediciones tribológicas fuera más realista. Estos resultados podrían ser de un interés práctico importante para la formulación de yogur usando este tipo de almidones físicamente modificados con fracciones gelatinizadas y sin gelatinizar.

Conclusiones

- Las expectativas de saciedad que despiertan los alimentos semisólidos en los consumidores varían con la viscosidad. En este sentido, los hidrocoloides, aún empleados para producir la misma viscosidad inicial en batidos lácteos, generaron sensaciones de saciedad esperada diferentes dependiendo de su comportamiento reológico.

- En la formulación de postres lácteos, además de la viscosidad del producto, la presencia de distintos componentes como grasa o sólidos lácteos modulan la aparición y duración de las sensaciones en boca y, en consecuencia, su percepción oral y capacidad saciante esperada.

- La adición de proteínas lácteas de diversos orígenes, produce yogures de diferentes características. Combinaciones adecuadas de cada una de ellas o sus mezclas abre nuevas posibilidades en el diseño de yogures saciantes.

- El uso de nuevas metodologías sensoriales, como los cuestionarios CATA y el análisis de penalización muestran ser herramientas válidas en el diseño y ajuste de formulaciones de yogures saciantes de alta aceptabilidad por parte del consumidor.

- El uso de almidones físicamente modificados en los que algunos gránulos permanecen en parte inalterados después del ataque de la amilasa (saliva), da lugar a yogures consistentes y cremosos que contribuyen a una exposición orosensorial prolongada y, en consecuencia, a mejorar las expectativas de saciedad.

- El estudio tribológico de la astringencia detectada por los consumidores en yogures con alto contenido en proteína no es causada por un aumento en la fricción, es decir no corresponde a una percepción puramente táctil. La adición de almidón físicamente modificado y la presencia de saliva reduce significativamente los valores de los coeficientes de fricción.

- El uso combinado de técnicas reológicas, microestructurales y sensoriales ha demostrado ser una herramienta poderosa para evaluar la viabilidad de productos lácteos con alta capacidad saciante y para comprender el efecto del tipo y la cantidad de proteína, así como de otros componentes en formulación de nuevos productos.

Las estrategias aplicadas en la presente tesis permitirían abordar la formulación de cualquier alimento con altas expectativas de capacidad saciante, ya que abarcan todos los aspectos de un nuevo desarrollo: composición, estructura, comportamiento físico y propiedades sensoriales.