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CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Instituto de Agroquímica y Tecnología de Alimentos

IMPACTO DE LA GRASA Y DEL AZÚCAR EN LAS PROPIEDADES FÍSICAS Y SENSORIALES DE DIFERENTES TIPOS DE MATRICES ALIMENTARIAS

TESIS DOCTORAL

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Hacen constar que:

La memoria titulada "**Impacto de la grasa y del azúcar en las propiedades físicas y sensoriales de diferentes tipos de matrices alimentarias**" que presenta Dª Carla Arancibia Aguilar para optar al grado de Doctor por la Universidad Politécnica de Valencia, ha sido realizada en el Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC) bajo su dirección y que reúne las condiciones para ser defendida por su autora.

Valencia, 20 de septiembre de 2013

Fdo: Dra. Elvira Costell Ibáñez

Fdo: Dra. Sara Bayarri Torres

Queda prohibido no sonreír a los problemas,
no luchar por los que quieres, abandonarlo todo por miedo,
no convertir en realidad tus sueños.
(Pablo Neruda)

A mis padres y hermana
A mi tía Rosalía

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Impacto de la grasa y del azúcar en las propiedades físicas y sensoriales de diferentes tipos de matrices alimentarias

Las características de cada matriz alimentaria, la composición y estructura de los ingredientes, así como el efecto de las interacciones entre ellos, pueden modificar el impacto de la grasa y del azúcar en la estructura y en las propiedades físicas y sensoriales del alimento y en su aceptación por el consumidor. Para diseñar nuevos alimentos más saludables es necesario conocer y entender el efecto de estos componentes en las características del producto y las repercusiones que su reducción o eliminación pueden tener en la calidad del mismo. En este contexto, la investigación realizada en esta tesis se ha llevado a cabo en diferentes tipos de matrices alimentarias semisólidas (lácteas, emulsiones agua/aceite y proteicas) y se ha centrado en tres temas: a) analizar los efectos de la modificaciones en la composición de la matriz en sus características físicas y estructurales; b) establecer las conexiones entre dichos cambios, la liberación en la boca de los estímulos odoríferos y sápidos y la percepción del sabor, del dulzor y de la textura y c) explorar la utilización de nuevas metodologías para investigar las percepciones de los consumidores y favorecer la optimización de la calidad sensorial de nuevos productos.

En la primera parte se analizó la influencia del contenido en grasa y de la adición de diferentes concentraciones de dos espesantes de distinta estructura (carboximetilcelulosa y almidón) en la estructura y en las propiedades físicas de postres lácteos y de emulsiones agua/aceite, y la influencia de la adición de los mismos hidrocoloides y del contenido en proteína, en las de los postres de soja. Tanto el contenido en grasa o en proteína, como el tipo y concentración de hidrocoloide modificaron de forma distinta las características físicas y estructurales de las diferentes matrices. En las matrices lácteas, el color se relacionó principalmente con el contenido en

grasa; el comportamiento de flujo dependió principalmente del tipo de hidrocoloide y la viscoelasticidad, de la concentración de este ingrediente. En las emulsiones agua/aceite, tanto la concentración de CMC como la de almidón tuvieron un efecto importante en el comportamiento de flujo, en el que también influyó la concentración de las gotas de grasa. Sin embargo, en las emulsiones con CMC, el contenido en grasa fue el factor más influyente en su viscoelasticidad mientras que, en las emulsiones con almidón, lo fue la concentración de este espesante. Estas diferencias estuvieron bien relacionadas con las observadas en la microestructura y en la distribución del tamaño de partícula de las distintas muestras. En los postres de soja, el efecto de las interacciones, proteína-CMC y proteína-almidón, fue significativo en los valores de todos los parámetros reológicos y el efecto de ambos hidrocoloides, tanto en el comportamiento de flujo como en la viscoelasticidad, dependió de la concentración de proteína.

Para analizar el efecto que los cambios en la composición, estructura y comportamiento reológico tenían en las características sensoriales de diferentes matrices, se realizaron dos estudios. Uno en postres lácteos y otro, en emulsiones, ambos con sabor a cítrico. Se estudió el efecto de la composición (contenido en grasa, tipo y concentración de espesante) en la liberación del sabor y en la percepción sensorial del sabor a limón, dulzor y textura. La liberación *in vivo* de los volátiles responsables del sabor fue cualitativamente similar en ambos tipos de matrices. El contenido en grasa afectó claramente a la liberación del volátil más lipofílico (linalool) pero no, a la del más hidrofílico (*cis*-3-hexen-1-ol). La disminución del contenido en grasa, no solo influyó en la intensidad del olor y sabor, sino que modificó cualitativamente las sensaciones percibidas al variar la secuencia de liberación de los volátiles desde la matriz, en función de la polaridad de cada uno de ellos. En ambos tipos de matrices la liberación de volátiles fue mayor en los productos que contenían almidón que en los que contenían CMC. En

los postres lácteos, la intensidad del sabor cítrico percibida dependió solo del contenido en grasa, mientras que en las emulsiones, la intensidad dependió principalmente del tipo de hidrocoloide. Independientemente del contenido en grasa y de la concentración de espesante, las muestras con almidón se percibieron como más dulces que las muestras con CMC. Ello parece indicar que las diferencias estructurales entre CMC (polimérica) y el almidón (globular) influyeron en el comportamiento de cada matriz durante su transformación en la cavidad bucal modificando la liberación de las moléculas de sacarosa desde la matriz y su posterior contacto con los receptores. Además del impacto en el sabor y en el dulzor, tanto el contenido en grasa como el tipo de hidrocoloide influyeron significativamente en la textura percibida en ambos tipos de matrices.

Conseguir de los consumidores información válida y que tenga utilidad práctica para la formulación de nuevos productos o para incrementar su aceptabilidad, no es tarea fácil. En la tercera parte de esta tesis se exploró la posibilidad de combinar determinadas metodologías para obtener información sobre como perciben y describen los consumidores las diferencias entre muestras (descripción entrecruzada + perfil de libre elección + análisis de Procrustes Generalizado) y también, sobre como las preferencias individuales pueden ayudar a identificar los motivos de los cambios en la aceptación de un producto y facilitar su reformulación (método de superficie de respuesta + escalas JAR *(Just About Right*)). En la primera experiencia se determinó hasta que punto un grupo de consumidores era capaz de percibir y describir diferencias en el color y en la textura de una serie de muestras de bebidas lácteas de distinta composición que diferían en sus propiedades ópticas y reológicas. La metodología propuesta resultó útil, no solo para comprobar que los consumidores eran capaces de detectar las diferencias sino también, que lo eran para identificarlas y para obtener información preliminar sobre la importancia relativa de cada una de ellas. En

la segunda experiencia se estudió la relación entre la aceptabilidad y las variables de composición de un postre de soja con alto contenido en proteína. Se optimizó la aceptabilidad del producto para toda la población de consumidores consultada y para dos subgrupos de dicha población con distintos criterios de preferencia. Mientras que para uno de estos subgrupos los cambios en la composición de las muestras no influyó claramente en su aceptación, para el otro, dichos cambios explicaron satisfactoriamente las diferencias en aceptación detectadas entre las muestras. La información obtenida con las escalas JAR permitió identificar los atributos que debían modificarse para obtener postres de soja más aceptables.

Impacte del greix i del sucre en les propietats físiques i sensorials de diferents tipus de matrius alimentàries

Les característiques de cada matriu alimentària, la composició i estructura dels ingredients, així com l'efecte de les interaccions entre ells, poden modificar l'impacte del greix i del sucre en l'estructura i en les propietats físiques i sensorials de l'aliment i en la seua acceptació pel consumidor. Per a dissenyar nous aliments més saludables és necessari conéixer i entendre l'efecte d'estos components en les característiques del producte i les repercussions que la seua reducció o eliminació poden tindre en la qualitat del mateix. En este context, la investigació realitzada en esta tesi s'ha dut a terme en diferents tipus de matrius alimentàries semisòlides (làcties, emulsions aigua/oli i proteiques) i s'ha centrat en tres temes: a) analitzar els efectes de la modificacions en la composició de la matriu en les seues característiques físiques i estructurals; b) establir les connexions entre els dits canvis, l'alliberament en la boca dels estímuls odorífers i sàpids i la percepció del sabor, de la dolçor i de la textura i c) explorar la utilització de noves metodologies per a investigar les percepcions dels consumidors i afavorir l'optimització de la qualitat sensorial de nous productes.

En la primera part es va analitzar la influència del contingut en greix i de l'addició de diferents concentracions de dos espessants de distinta estructura (carboximetilcelulosa i midó) en l'estructura i en les propietats físiques de postres làcties i d'emulsions aigua/oli, i la influència de l'addició dels mateixos hidrocoloides i del contingut en proteïna, en les de les postres de soja. Tant el contingut en greix o en proteïna, com el tipus i concentració de hidrocoloide van modificar de forma distinta les característiques físiques i estructurals de les diferents matrius. En les matrius làcties, el color es va relacionar principalment amb el contingut en greix; el comportament de flux va dependre principalment del tipus de hidrocoloide i la viscoelasticitat, de

la concentració d'este ingredient. En les emulsions aigua/oli, tant la concentració de CMC com la de midó van tindre un efecte important en el comportament de flux, en el que també va influir la concentració de les gotes de greix. No obstant això, en les emulsions amb CMC, el contingut en greix va ser el factor més influent en la viscoelasticitat mentres que, en les emulsions amb midó, ho va ser la concentració d'este espessant. Estes diferències van estar ben relacionades amb les observades en la microestructura i en la distribució de la grandària de partícula de les distintes mostres. En les postres de soja, l'efecte de les interaccions, proteïna-CMC i proteïna-midó, va ser significatiu en els valors de tots els paràmetres reològics i l'efecte d'ambdós hidrocoloides, tant en el comportament de flux com en la viscoelasticitat, va dependre de la concentració de proteïna.

Per a analitzar l'efecte que els canvis en la composició, estructura i comportament reològic tenien en les característiques sensorials de diferents matrius, es van realitzar dos estudis. Un en postres làcties i un altre, en emulsions, ambdós amb sabor a cítric. Es va estudiar l'efecte de la composició (contingut en greix, tipus i concentració d' espessant) en l'alliberament del sabor i en la percepció sensorial del sabor a llima, dolçor i textura. L'alliberament *in vivo* dels volàtils responsables del sabor va ser qualitativament semblant en ambdós tipus de matrius. El contingut en greix va afectar clarament l'alliberament del volàtil més lipofílic (linalool) però no, a la del més hidrofílic (*cis*-3-hexen-1-ol). La disminució del contingut en greix, no sols va influir en la intensitat de l'olor i sabor, sinó que va modificar qualitativament les sensacions percebudes al variar la seqüència d'alliberament dels volàtils des de la matriu, en funció de la polaritat de cada un d'ells. En ambdós tipus de matrius l'alliberament de volàtils va ser major en els productes que contenien midó que en els que contenien CMC. En les postres làcties, la intensitat del sabor cítric percebuda va dependre només del contingut en greix, mentres que en les emulsions, la intensitat va dependre

principalment del tipus de hidrocoloide. Independentment del contingut en greix i de la concentració d'espessant, les mostres amb midó es van percebre com més dolços que les mostres amb CMC. Això pareix indicar que les diferències estructurals entre CMC (polimérica) i el midó (globular) van influir en el comportament de cada matriu durant la seua transformació en la cavitat bucal modificant l'alliberament de les molècules de sacarosa des de la matriu i el seu posterior contacte amb els receptors. A més de l'impacte en el sabor i en la dolçor, tant el contingut en greix com el tipus de hidrocoloide van influir significativament en la textura percebuda en ambdós tipus de matrius.

Aconseguir dels consumidors informació vàlida i que tinga utilitat pràctica per a la formulació de nous productes o per a incrementar la seua acceptabilitat, no és tasca fàcil. En la tercera part d'esta tesi es va explorar la possibilitat de combinar determinades metodologies per a obtindre informació sobre com perceben i descriuen els consumidors les diferències entre mostres (descripció entrecreuada + perfil de lliure elecció + anàlisi de Procrustes Generalitzat) i també, sobre com les preferències individuals poden ajudar a identificar els motius dels canvis en l'acceptació d'un producte i facilitar la seua reformulació (mètode de superfície de resposta + escales JAR *(Just About Right*)). En la primera experiència es va determinar fins que punt un grup de consumidors era capaç de percebre i descriure diferències en el color i en la textura d'una sèrie de mostres de begudes làcties de distinta composició que diferien en les seues propietats òptiques i reològiques. La metodologia proposada va resultar útil, no sols per a comprovar que els consumidors eren capaços de detectar les diferències sinó també, que ho eren per a identificar-les i per a obtindre informació preliminar sobre la importància relativa de cada una d'elles. En la segona experiència es va estudiar la relació entre l'acceptabilitat i les variables de composició d'un postre de soja amb alt contingut en proteïna. Es va

optimitzar l’acceptabilitat del producte per a tota la població de consumidors consultada i per a dos subgrups de la dita població amb distints criteris de preferència. Mentres que per a un d’estos subgrups els canvis en la composició de les mostres no va influir clarament en la seua acceptació, per a l’altre, els dits canvis van explicar satisfactòriament les diferències en acceptació detectades entre les mostres. La informació obtinguda amb les escales JAR va permetre identificar els atributs que havien de modificar-se per a obtindre postres de soja mes acceptables.

Impact of fat and sugar on the physical and sensorial properties of different food matrices

The impact of fat and sugar on the structural, physical and sensory properties of food, and its consequent acceptance by consumers, can be modified by the characteristics of each food matrix, the composition and structure of ingredients, as well as interactions between them. When designing new healthier foods, one must recognize and understand how these components affect the product's characteristics, and also the impact their reduction or elimination may have on final product quality. Within this context, the thesis reported here was performed on different kinds of semi-solid food matrices (milk-based, water/oil emulsions and protein-based) and focused on three topics: a) to analyze the effects of matrix composition modifications on physical and structural characteristics; b) to make connections between these changes, in-mouth release of odorous and sapid stimuli, and the perception of flavour, sweetness and texture; c) explore the use of new methods to investigate consumer perceptions and facilitate sensory quality optimization for new product development.

In the first part, the influence of fat content and the addition of different concentrations of two thickeners with different structure (carboxymethyl cellulose and starch) on the structure and physical properties of dairy desserts and of water/oil emulsions; was studied. The influence of protein and thickener content of soy desserts was also studied. Findings indicated that fat and protein content, as well as hydrocolloid type and concentration, modified the physical and structural characteristics of the different studied matrices. In dairy matrices, colour was mainly related to fat content, whereas flow behaviour depended mostly upon the type of hydrocolloid, and viscoelasticity on the concentration of this ingredient. In water/oil emulsions,

both starch and CMC concentrations had a significant effect on flow behaviour, which was also influenced by the concentration of fat droplets. However, fat content was the most influential factor on viscoelasticity in CMC emulsions, while thickener concentration exerted the greatest influence on this parameter in starch emulsions. These differences were closely related to the variations observed in microstructure and particle-size distribution of the samples. In soy desserts, protein-CMC and protein-starch interactions had significant effect on all rheological parameter values, while the effects exerted by both hydrocolloids on flow behaviour and viscoelasticity, depended on protein concentration.

Two studies were conducted to analyze the effect that changes in composition, structure and rheological behaviour had on sensory characteristics in the different food matrices. One was performed on dairy desserts and the other on emulsions, both citrus flavoured. A study was made to assess the effect of composition (fat content, thickener type and concentration) on flavour release and sensory perception of lemon flavour, sweetness and texture. The *in vivo* release of volatiles responsible for flavour was qualitatively similar in both food matrix types. Fat content clearly affected the release of the most lipophilic volatile (linalool) but not the most hydrophilic (*cis*-3-hexen-1-ol). Reducing fat content influenced not only the intensity of odour and flavour, but also qualitatively changed the sensations perceived by varying the sequence in which volatiles were released from the matrix, in function on the polarity of each. In both types of matrices, volatiles were released at higher rates in starch-containing products than in those containing CMC. In dairy desserts, perceived citrus-flavour intensity depended only on fat content, whereas in emulsions, intensity depended mainly on hydrocolloid type. Regardless of fat content and thickener concentration, samples with starch were perceived as sweeter than those with CMC. This would suggest that the structural differences between CMC

(polymer) and starch (globular) influenced the way in which each matrix acts during processing in the oral cavity, changing the release of sucrose molecules from the matrix and their subsequent contact with taste receptors. Besides the impact on flavour and sweetness, both fat content and hydrocolloid type significantly influenced perceived texture in both types of food matrix.

It is not easy to obtain information from consumers that can provide valid and practical information to develop new products or to improve their acceptability. Thus, the third part of this thesis explored the possibility of combining certain methodologies to obtain information about how consumers perceive and describe differences between samples (Repertory Grid Method + Free-Choice Profiling + Generalized Procrustes Analysis), and also how information on their individual preferences can be used to help identify changes in product acceptance, and thus facilitate its reformulation (Response Surface Methodology + *Just About Right* scales). The first work was designed to determine the extent to which a consumer group was able to perceive and describe colour and textural differences in a series of milk-beverage samples with different compositions, and which differed in optical and rheological properties. This methodology was useful, not only to check that consumers were able to detect these differences, but that they could also identify them; moreover, it provided preliminary information on the relative importance of each difference. In the second research, the relationship between acceptability and composition variables of a high-protein soy dessert was studied. Product acceptability was optimized for overall consumer population consulted, and for two subgroups with different preference criteria. While changes in sample composition did not clearly influence its acceptance for one of these subgroups, for the other one these changes satisfactorily explained the differences in acceptance detected between samples. The information obtained with the JAR scales identified

the attributes that should be modified to obtain more acceptable soy-based desserts.

ÍNDICE GENERAL

INTRODUCCIÓN

El concepto tradicional de nutrición cuyo objetivo principal es aportar suficientes nutrientes para satisfacer los requerimientos metabólicos de los individuos ha evolucionado hacia el concepto de "nutrición óptima". Ésta se entiende como la optimización de la ingesta diaria de nutrientes y compuestos bioactivos que ayuden a prevenir ciertas enfermedades y favorezcan un estilo de vida más saludable. Dejando aparte el indudable interés que tiene el diseño y fabricación de alimentos destinados a sectores de la población con unos requerimientos nutricionales específicos por su intolerancia a algún componente alimentario (gluten, lactosa, etc.) o por padecer determinadas enfermedades (hipertensión, diabetes, etc.), está comprobado que las dietas inadecuadas o mal equilibradas y los estilos de vida sedentarios aumentan la incidencia de determinadas enfermedades tanto en los países desarrollados como en los que están en vías de desarrollo. Esta situación ha hecho que varias agencias nacionales o supranacionales diseñen estrategias y propongan recomendaciones para disminuir la prevalencia de las enfermedades relacionadas con la dieta y para que se invierta la actual tendencia a la obesidad sobre todo, en los niños y en los adolescentes. La reducción de la ingesta de ácidos grasos saturados y ácidos grasos trans, de azúcar o de sal y el incremento del consumo de frutas y hortalizas, recomendados ya en 2003 por WHO/FAO, constituyen la base de varios programas, como los propuestos en Estados Unidos (USDA, 2010) y en Europa (WHO, 2008), para modificar algunos hábitos alimentarios. En este contexto, la industria alimentaria está llamada a ocupar un papel muy importante proporcionando al consumidor alimentos con menor contenido calórico o un mejor perfil nutritivo (Nehir y Simsek, 2012). Para ello, la modificación de la composición de algunas materias primas, de los procesos de transformación o de la formulación de nuevos productos, pueden ser líneas de investigación interesantes para obtener alimentos más saludables (Fogliano y Vitaglione, 2005). El conseguirlos sin disminuir su calidad sensorial es un reto adicional porque, en última instancia, ésta influye en su

aceptación y puede condicionar su posterior éxito en el mercado. Este hecho es especialmente importante en la aceptación o rechazo de algunos tipos de alimentos (bajos en grasa, en azúcar o en sal) que se presentan ante el consumidor como una posible alternativa a los alimentos convencionales (Urala y Lähtennmäki, 2004; Verbeke, 2006).

Cuando se pretende optimizar la calidad sensorial de los alimentos, la complejidad de la mayoría de las matrices alimentarias hace difícil establecer relaciones directas entre la composición y estructura del producto, y las sensaciones que el hombre experimenta al consumirlo. Cuando se trata de alimentos bajos en grasa o en azúcar, la primera cuestión es analizar y entender al impacto de ambos componentes y también, el de su reducción o eliminación, en la estructura y en las propiedades físicas y sensoriales del alimento.

1. Estrategias para formular alimentos semisólidos bajos en grasa o en azúcar

Tradicionalmente se ha considerado que el principal efecto de la eliminación o reducción de los contenidos en grasa o en azúcar en la calidad de un alimento era una textura defectuosa o la disminución del dulzor. Una de las estrategias más frecuentes para obtener alimentos saludables con características sensoriales similares a las de los productos convencionales es el uso de ciertos ingredientes como los sustitutos de grasa o de azúcar (Kremer *et al.,* 2009; Bayarri *et al.,* 2010; Fujimaru *et al.,* 2012; Gwak *et al.,* 2012; Cadena *et al.,* 2012). Actualmente se considera que la modificación de la estructura del producto, a nivel macroscópico o microscópico, puede ser una estrategia alternativa para obtener alimentos bajos en grasa con nuevas texturas (Simo *et al.,* 2012) o para favorecer la liberación en la cavidad bucal

de las moléculas de sacarosa responsables de la percepción del dulzor (Mosca *et al.,* 2010; Busch *et al.,* 2013).

1.1 Ingredientes para sustituir la grasa y/o el azúcar

Los ingredientes que se utilizan para sustituir a la grasa se pueden clasificar en dos grandes grupos según su composición química y sus propiedades: sustitutos de grasa e imitadores de grasa (Sandrou y Arvanitoyanis, 2000). El primer grupo incluye compuestos que tienen unas propiedades físicas similares a las de la grasa, como los comerciales Olestra o Salatrim, y se obtienen por reacciones químicas entre azúcares y ácidos grasos o por modificación de triglicéridos. Suelen tener unas características sensoriales y térmicas similares a las de la grasa pero no se hidrolizan por las lipasas gástrica o pancreática y, por tanto, no se absorben en el tracto gastrointestinal. Además, su uso tiene ciertas restricciones legales debidas a posibles efectos adversos en la salud de algunas personas (Bimal y Zhang, 2006). El segundo grupo está formado por ingredientes capaces de imitar una o varias de las funciones de la grasa relacionadas con las propiedades físicas o sensoriales del alimento. Normalmente son compuestos de proteínas, de carbohidratos o mezclas de ambos. Los ingredientes de carácter proteico parecen, en principio, una alternativa interesante por su capacidad emulsionante y estabilizante. Por un lado, compensan algunos de los defectos de textura que provoca la falta de grasa y, por otro, enriquecen el valor nutricional del alimento. Su principal inconveniente reside en su influencia en el sabor. No solo reducen la intensidad del sabor propio del producto sino que pueden dar lugar a olores y sabores extraños. Cuando las proteínas sustituyen a la grasa, el alimento sufre una importante modificación en su composición y estructura y, además, surgen interacciones entre la proteína y los compuestos volátiles que son diferentes a las que se producen entre estos compuestos y la grasa. Por ello, el sabor del producto se

puede alterar de forma importante (Overbosch *et al.*, 1991; Kühn *et al.*, 2006). En el segundo grupo, se incluyen un gran número de carbohidratos (celulosas, dextrinas, maltodextrinas, gomas, fibras, almidones, etc.) que se utilizan como sustitutos de grasa en diferentes tipos de alimentos. Debido a su capacidad de retener agua, de reducir la sinéresis o de espesar, gelificar o estabilizar deferentes tipos de matrices son una de las mejores opciones para sustituir la grasa en varios tipos de alimentos. Además, aparte de sus características fisicoquímicas, algunos de ellos actúan también como fibras dietéticas lo que puede mejorar el perfil nutricional del producto. Con este tipo de ingredientes, es posible, aunque no siempre es fácil, igualar el comportamiento reológico de productos líquidos y semisólidos bajos en grasa con el de los productos convencionales, aunque ello no garantice que se iguale la textura que se perciba al consumirlos. A excepción del caso de los alimentos líquidos con un comportamiento de flujo de carácter newtoniano, no se puede considerar que los parámetros reológicos que identifican el flujo o la viscoelasticidad de un producto sean los estímulos responsables de la viscosidad o consistencia percibidas sensorialmente (Costell y Durán, 2000) aunque, en casos concretos, algunos de ellos estén bien relacionados con las citadas sensaciones (van Vliet, 2002; Tárrega *et al.*, 2007). Además, hay que tener en cuenta que la viscosidad o consistencia no configuran por si solos la textura de estos productos. Otros atributos, como la cremosidad, la sensación grasa o la suavidad que dependen principalmente de la microestructura y de las propiedades superficiales del producto, contribuyen decisivamente a la sensación de textura (De Wijk *et al.*, 2003; Weenen *et al.*, 2005; González-Tomás *et al.*, 2009). Al contrario de lo que ocurre con los sustitutos de grasa de tipo proteico, el efecto de la adición de carbohidratos en el sabor del producto es solo cualitativo, similar al que produce la simple reducción del contenido en grasa, porque la mayoría de ellos no tienen ningún tipo de afinidad específica con los componentes volátiles (Cayot *et al.*, 2000; Rega *et al.*, 2002). En cualquier

caso, la utilización de sustitutos de grasa considerando como único criterio el mimetizar la textura de los productos con grasa, puede no ser suficiente para obtener productos aceptables para el consumidor. Además de influir en la textura, la grasa también influye en el color y, sobre todo, en el sabor. El efecto de la reducción de grasa en el sabor es complejo y depende, en cada caso, de la volatilidad y del grado de hidrofobia de los componentes volátiles que configuran el olor de cada producto y de las características de los compuestos no volátiles responsables del gusto (Metcalf y Vickers, 2002; Bayarri y Costell, 2009). En general, cuando el contenido en grasa disminuye, aumenta la intensidad del sabor pero también se puede modificar el carácter del mismo (de Roos, 1997).

El azúcar es el producto de origen natural más utilizado para dar sabor dulce a alimentos y bebidas. Obtenido principalmente de la caña de azúcar y de la remolacha azucarera es, prácticamente, sacarosa. Por las características del dulzor que proporciona y por sus propiedades funcionales y tecnológicas, se considera que es el edulcorante universal por excelencia y su dulzor se utiliza como referencia para evaluar otros ingredientes capaces de generar dulzor. Actualmente, existe una amplia gama de este tipo de ingredientes que, al menos teóricamente, pueden simplificar la sustitución de la sacarosa en la formulación de productos con contenido calórico reducido. De una forma general, los edulcorantes distintos a la sacarosa se pueden clasificar en cuatro grandes grupos: 1) Otros carbohidratos; 2) Polialcoholes; 3) Edulcorantes intensos y 4) Edulcorantes naturales de gran potencia (Marie y Piggott, 1991). A pesar del gran número de opciones, la sustitución de la sacarosa para reducir el contenido calórico de los alimentos plantea algunos problemas. La sacarosa tiene un dulzor limpio, uniforme y una duración corta en la boca, mientras que algunos edulcorantes sintéticos intensos, como la sacarina o el acesulfamo-K, presentan sabores asociados no agradables como el amargo o el metálico y, en general, todos ellos dan lugar a una

permanencia excesivamente larga de la sensación de dulzor en la boca. Por lo demás, su efecto equilibrante en otros gustos (ácido, salado o amargo) o el sinérgico con determinados compuestos volátiles es, generalmente, distinto al del azúcar. A excepción del aspartamo y la estevia, la mayoría de los edulcorantes naturales intensos (proteínas, péptidos o de tipo terpenoide) están en fase de experimentación y todos ello presentan ciertas limitaciones de uso por su inestabilidad frente a ciertos pHs y temperaturas o por su rápido deterioro durante el almacenamiento. Además, los edulcorantes intensos y los naturales de gran potencia no contribuyen a la textura del producto como lo hace el azúcar, lo que implicaría la necesidad de incluir en la formulación otros ingredientes que permitieran corregir este aspecto. Otra cuestión importante es que, aunque los edulcorantes sintéticos o los naturales de gran potencia se han considerado como ingredientes inertes, investigaciones recientes sugieren que algunos de ellos tienen efectos biológicos que pueden influir en la salud del consumidor (Schiffman, 2012). De esta situación se deduce que el edulcorante ideal no existe, la sacarosa aporta demasiadas calorías a la dieta y contribuye a la aparición de caries y, el resto de los edulcorantes, tal como se ha comentado, presentan gustos ajenos al dulzor, importantes limitaciones tecnológicas o pueden ser peligrosos para la salud. Sin embargo, la combinación de dos o tres edulcorantes, incluyendo o no la sacarosa en la mezcla, puede reducir o prácticamente eliminar los problemas asociados al uso de un solo edulcorante (Hutteau *et al.*, 1998; Schiffman *et al.*, 2000).

1.2. Restructuración de matrices

En los últimos años se ha incrementado notablemente el estudio de las bases estructurales de las propiedades de los alimentos debido, en parte, a la disponibilidad de técnicas analíticas mas sensibles y potentes (Aguilera *et al.*, 2000) y al interés en el desarrollo de nuevos sistemas estructurados

capaces de encapsular componentes bioactivos y asegurar su biodisponibilidad (McClements *et al.*, 2009), de formar películas biodegradables o comestibles (Gennadios *et al.*, 1997; Hernández-Izquierdo *et al.*, 2008) o más recientemente, la creación de estructuras que permitan el desarrollo de nuevos productos con menor contenido en grasa (Mao y McClements, 2012; Wu *et al.*, 2013) o la reducción del contenido en estímulos odoríferos y sápidos en el alimento sin reducir la intensidad del sabor o del dulzor percibido (Moritaka y Naito, 2002; Malone *et al.*, 2003; Holm *et al.*, 2009; Mosca *et al.*, 2010; Busch *et al.*, 2013).

La creación de estructuras alimentarias se basa en el ensamblaje de distintos ingredientes de los alimentos como proteínas, polisacáridos, lípidos, azúcares, emulsionantes, minerales y agua. Entre ellos, los lípidos, las proteínas y los polisacáridos ocupan el lugar más importante. La mayoría de los estudios realizados se han basado en el desarrollo de nuevas estructuras de emulsiones. Estas estructuras, consisten básicamente en la unión de dos líquidos inmiscibles, generalmente agua y aceite, con uno de ellos disperso en el otro en forma de pequeñas gotas esféricas (McClements, 2005). Además de las dispersiones simples, aceite/agua o agua/aceite es posible preparar otros tipos de emulsiones múltiples como las de aceite/agua/aceite o agua/aceite/agua. Controlando la composición y la microestructura de estos sistemas se pueden diseñar emulsiones con distinta estructura y diferente comportamiento reológico. La incorporación de biopolímeros, especialmente de proteínas y de hidrocoloides es una de las vías más comunes para estabilizar las emulsiones alimentarias. Las proteínas son capaces de estabilizar las gotas de aceite y los hidrocoloides, de estabilizar el sistema incrementando la viscosidad o gelificando la fase dispersante (Dickinson, 2009). Diversas proteínas, como la caseína, la proteína de suero, la gelatina, o las proteínas de soja, y un elevado número de polisacáridos, como el almidón, los carragenatos, las pectinas, y diversas gomas como la xantana,

guar o la gelana, ofrecen una amplia gama de posibilidades para la creación de nuevas estructuras con distinta capacidad funcional (Benichou *et al.*, 2002; Norton y Frith, 2001; Foegeding, 2006; Mao y McClements, 2012; Wu *et al.*, 2013). Las posibilidades se incrementan, aunque también el estudio se complica, cuando se diseñan sistemas mixtos proteína-hidrocoloide. Durante los últimos 20 años, se han estudiado muchos sistemas mixtos de este tipo que han puesto de manifiesto la existencia de sinergias muy interesantes entre distintas proteínas y diferentes hidrocoloides (Turgeon *et al.*, 2007). La información analizada indica que en muchas ocasiones una variación mínima en el tipo y concentración de los polisacáridos en los sistemas mixtos proteína/polisacárido es suficiente para obtener matrices con diferentes microestructuras (de Jong *et al.*, 2007). En el área de los alimentos, los criterios básicos en los que se ha basado la selección de los ingredientes a utilizar en la creación o mejora de la estructura de un producto han sido los de obtener una determinada textura o incrementar la estabilidad de esta y la del sistema. Recientemente, la idea de incluir en la selección de ingredientes información sobre la relación composición-estructura-comportamiento reológico-textura bucal-percepción del sabor y del gusto, ha abierto una prometedora vía para el desarrollo de nuevos productos con mejores características sensoriales (Renard *et al.*, 2006; Ghosh *et al.*, 2008, Holm *et al.*, 2009; Sala *et al.*, 2010; Foegeding *et al.*, 2010; Sala *et al.*, 2010; de Jongh, 2011; Stieger y van de Velde, 2013).

2. La liberación y percepción del sabor y del dulzor

La percepción del sabor comienza cuando las moléculas sápidas y olorosas se liberan de la matriz alimentaria y se desplazan hasta los receptores del gusto y del olfato. Los compuestos no volátiles son transportados por la saliva hasta los receptores del gusto localizados en la boca y los volátiles, son arrastrados por el aire hasta los receptores del olor, localizados en la

nariz (Taylor, 2002). Entonces se inicia la transducción de las señales detectadas por los receptores hasta el cerebro y se genera la sensación de sabor (Overbosch *et al.*, 1991; Taylor y Roberts, 2004).

La mayoría de los estudios realizados sobre la liberación del sabor de los alimentos se han centrado en analizar la liberación de compuestos volátiles desde la matriz. Existe abundante información sobre los coeficientes de partición de diferentes volátiles en distintas matrices obtenida generalmente por análisis del espacio de cabeza por CG-MS, utilizando técnicas estáticas o dinámicas. Las bases teóricas de los fenómenos de transporte que gobiernan la liberación de los estímulos químicos desde la matriz, como el transporte másico y la difusión, están bien establecidas y los métodos utilizables para controlarlos han sido descritos recientemente por Cayot *et al.* (2008). Desafortunadamente toda esta información no está directamente relacionada con el sabor percibido sensorialmente cuando se consume un alimento. Las condiciones experimentales utilizadas en los análisis cromatográficos son muy diferentes de las existentes en la cavidad bucal durante la masticación y deglución de los alimentos y, por ello, la variación del contenido en determinados componentes volátiles o la de su concentración, no siempre produce el cambio perceptible deseado en el sabor de un alimento. Las características estructurales de la matriz y sus propiedades fisicoquímicas juegan también un papel importante en el proceso de liberación. Desde un punto de vista práctico, está comprobado que para una matriz concreta, cuanto más viscosa o más consistente sea menos intenso es su sabor (Clark, 2002; Boland *et al.*, 2006). Se considera que la disminución de la intensidad de los gustos fundamentales (dulce, amargo, salado, ácido y umami) cuando se aumenta la viscosidad o la textura de un producto, se debe a una demora o a una inhibición parcial del transporte del estímulo dentro de la propia matriz y desde la matriz hasta los receptores. Este proceso está parcialmente gobernado por la velocidad de difusión del estímulo dentro de la matriz, por

el comportamiento reológico del alimento y, en algunos casos, por enlaces entre las moléculas sápidas y las de otros componentes del alimento (Durán y Costell, 1999). Los mecanismos por los que las modificaciones de la viscosidad o de la textura influyen en la liberación del sabor no están totalmente explicados (Hollowood *et al.*, 2002, de Roos, 2003; Bayarri *et al.*, 2007) y, hasta la fecha, ninguno de los modelos teóricos propuestos para predecir el efecto de las características de la matriz en la liberación de los estímulos, está totalmente validado (Taylor, 2002).

La segunda fase del proceso de percepción se desarrolla durante el tiempo que el alimento permanece en la boca hasta su deglución. Durante este periodo, la forma y el tamaño inicial del alimento se transforma hasta alcanzar las características adecuadas para ser tragado y los estímulos químicos se liberan en la boca y son transferidos a los receptores bucales y nasales. Mientras el alimento está en la boca, el área del producto en contacto con la saliva y las concentraciones de los estímulos volátiles y no volátiles en las fase acuosa y en la gaseosa, se modifican continuamente debido a los movimientos bucales durante la masticación y la deglución (Engelen y van der Bilt, 2008; Chen 2009; Stieger y van de Velde, 2013). Para investigar el efecto de estas acciones en la liberación de los volátiles se han desarrollado en los últimos años nuevas técnicas para evaluar “in vivo” la liberación del aroma en la nariz y en la boca, midiendo los volátiles exhalados tras el consumo del alimento por CG-MS (Denker *et al.*, 2006); o durante el consumo del alimento utilizando para ello la espectrometría de masas en combinación con la ionización química a presión atmosférica (APCI-MS) (Taylor y Linforth, 2003; Bayarri *et al.*, 2006; González-Tomás *et al.*, 2007) o con la reacción de transferencia de protones (PTR-MS) (van Ruth *et al.*, 2006; Mestres *et al.*, 2005 y 2006). Con estas técnicas se obtienen datos reales sobre los estímulos que llegan a los receptores nasales por vía retronasal. No obstante hay que tener en cuenta que, a pesar de estos

avances y de la gran importancia en el sabor de los estímulos olorosos percibidos por vía retronasal, también juegan un papel decisivo en el sabor de un producto los olores percibidos por vía ortonasal, los estímulos no volátiles responsables del gusto y las sensaciones trigeminales. Respecto a la percepción del dulzor, su intensidad no solo depende de la concentración de edulcorante que exista en el alimento sino también, de la cantidad del mismo que se libera en la boca durante su consumo que es la que llega a estimular los receptores (Çakir *et al.,* 2012). La textura del producto y su modificación durante la ingestión, gobiernan prácticamente este proceso. En este contexto, algunos autores han propuesto determinadas modificaciones en la estructura de diferentes sistemas modelo para incrementar la intensidad del dulzor percibido sin aumentar la concentración de azúcar, como por ejemplo, la distribución no homogénea de la sacarosa en la matriz (Holm *et al.* 2009; Mosca *et al.,* 2010 y 2012) o el que se incremente la liberación de la parte acuosa del sistema durante la masticación (Sala *et al.,* 2010; de Jongh, 2011).

La tercera fase se inicia cuando las señales detectadas por los receptores viajan por el sistema nervioso central hasta determinadas regiones del cerebro. Durante el consumo de un alimento, el cerebro recibe diferentes señales sensoriales (visuales, olfatorias gustativas, táctiles, trigeminales) y transforma toda esta información en la sensación de sabor (Prescott, 2004). Delwiche (2004) ha revisado como interaccionan todas estas sensaciones tanto a nivel físico como perceptual, discutiendo el impacto que cada una de ellas puede tener en la evaluación sensorial del sabor. Cada sensación responde no solo a una señal sensorial sino a todas las señales que se perciben simultáneamente. Aunque todas estas señales pueden influir en la sensación de sabor, el efecto de la interacción entre el gusto y el olor es tan importante que, prácticamente, configura la sensación de sabor de un alimento.

Por lo tanto, se puede concluir que en los alimentos, aunque las sensaciones del sabor y del gusto están directamente relacionadas con el contenido y concentración de determinados compuestos volátiles y no volátiles, cada matriz y su transformación durante el proceso en la cavidad bucal, van a influir definitivamente en la intensidad del sabor y del gusto percibido. Variando la estructura de la matriz alimentaria y teniendo en cuenta como se modifica por acción de la temperatura y de los movimientos bucales y de la saliva, puede ser posible disminuir la concentración de azúcar en un alimento sin disminuir la intensidad de su dulzor. Lógicamente, para cada aplicación y objetivo, es necesario seleccionar con cuidado las características del ingrediente o ingredientes a utilizar para obtener una estructura con la funcionalidad deseada.

3. El papel del consumidor en la optimización de la calidad sensorial de alimentos saludables

El fin último de los alimentos es que sean aceptables para el consumidor y que éste los incorpore a su dieta. Cuando un consumidor elige y consume un alimento, su respuesta no solo se explica por las características sensoriales del producto ni por su propio estado fisiológico sino que está influido por otros factores. La información previa que tiene sobre el producto, sus experiencias anteriores y sus actitudes y creencias juegan un papel importante en el rechazo o en el consumo continuado de cada alimento (Costell *et al.*, 2010). En general, la aceptación de los productos bajos en grasa o en azúcar está lejos de ser incondicional. Es cierto que su carácter saludable puede suponer un valor añadido pero éste no suele ser suficiente para anular la importancia de sus características sensoriales para asegurar su éxito en el mercado (Siró *et al.*, 2008; Tuorila y Monteleone, 2009). Por ello, el diseño y elección de la formulación de este tipo de productos requiere tener en cuenta, durante diferentes etapas de su desarrollo, las percepciones y

preferencias de la población a la que van dirigidos (van Kleff *et al.*, 2002). Cuando se formula un nuevo alimento y se estudian las relaciones composición-estructura-propiedades físicas-propiedades sensoriales, surgen dos cuestiones críticas: ¿Cómo perciben los consumidores las diferencias entre distintas muestras? ¿Hasta qué punto las diferencias percibidas sensorialmente influyen en la aceptación de un producto?

La descripción y cuantificación de los atributos sensoriales suele hacerse con técnicas analíticas convencionales como los perfiles descriptivos (Deliza *et al.*, 2005). Estas técnicas requieren la selección y definición de una serie de descriptores consensuados y la evaluación de la intensidad de los correspondientes atributos por un panel previamente seleccionado y entrenado (Meilgaard *et al.*, 1999; Hersleth *et al.*, 2003). Por el contrario, los consumidores suelen describir sus sensaciones con palabras comunes, fácilmente entendibles pero que suelen ser totalmente personales y difíciles de interpretar por otras personas. Por otro lado, es difícil que los consumidores generen suficientes descriptores y que estos sean útiles para describir todas sus percepciones sensoriales. Una posible solución para obtener información directa sobre las sensaciones que experimentan los consumidores al ingerir un alimento es utilizar conjuntamente la técnica de la descripción entrecruzada (*Repertory Grid Method*) con la del perfil de libre elección (*Free Choice Profile*) (Jahan *et al.*, 2005; Jaeger *et al.*, 2005; González-Tomás y Costell, 2006). Con la descripción entrecruzada, los consumidores son capaces de crear sus vocabularios personales para describir como perciben un grupo de muestras (Gains, 1994). El perfil de libre elección difiere de los convencionales en que cada consumidor evalúa las muestras con su hoja de cata personal en vez de utilizar todos ellos una hoja de cata común. Los participantes no necesitan entrenamiento y es suficiente con que sean objetivos y capaces de detectar las diferencias perceptibles entre las muestras y cuantificarlas utilizando su propio

vocabulario. Con estas dos técnicas se puede obtener información directa de las percepciones de los consumidores e identificar las dimensiones perceptuales comunes a todo el grupo (Russell y Cox, 2003). Sin embargo, son poco útiles cuando las diferencias entre las muestras son muy pequeñas (Guerrero *et al.,* 1997). Otra cuestión a tener en cuenta es que los datos obtenidos con el perfil de libre elección no pueden analizarse con los métodos estadísticos tradicionales debido a que las matrices individuales tienen dimensiones distintas. Es necesario comparar las configuraciones obtenidas para cada consumidor y obtener una configuración media de todos ellos utilizado el análisis de Procrustes generalizado (*Generalized Procrustes Analysis*) (Gower, 1975; Dijksterhuis y Gower, 1991 y 1992).

Para obtener información sobre la incidencia que los cambios en la formulación de un producto pueden tener en la aceptación del mismo por el consumidor, una de las técnicas más útiles es la de Superficie de Respuesta *(Response Surface Methodology*) (Gacula, 1993). Con ella, previa elección de la variabilidad de determinados ingredientes, se puede seleccionar la formulación con máxima aceptabilidad entre todas las evaluadas (Villegas *et al.,* 2010; Granato *et al.,* 2010; Felberg *et al.,* 2010; Arcia *et al.,* 2011; Chattopadhyay *et al.,* 2013). Para interpretar correctamente y poder utilizar la información obtenida con esta técnica para reformular un producto, hay que considerar dos cuestiones. La primera, que la validez de la relación obtenida entre la composición y la aceptación del producto queda limitada al intervalo de variación de la concentración de los ingredientes utilizado en el ensayo. La segunda, que es muy difícil, con esta información, identificar las causas que originan los cambios en la aceptación porque no existe una conexión directa entre las variables independientes (ingredientes) y las dependientes (aceptación). Tradicionalmente, una manera de subsanar este último problema ha sido identificar la relación entre la intensidad de los atributos sensoriales analizada por un panel entrenado y la aceptación del

alimento evaluada por consumidores e identificar los atributos son los responsables del cambio en aceptación (Ligget *et al.*, 2008; Bayarri *et al.*, 2011). Otra opción puede ser obtener información de la opinión de los consumidores sobre la idoneidad de la intensidad de algunos atributos sensoriales utilizando las escalas diseñadas para ello, conocidas como JAR (*Just About Right).* Los datos obtenidos con estas escalas, que combinan información sobre intensidad y aceptación, pueden ayudar a entender como el consumidor percibe un producto (Gacula *et al.*, 2007) y también, dar una idea sobre la proporción de consumidores que perciben las muestras de una determinada forma (Costell *et al.,* 2010). Quizá el uso combinado de ambas herramientas (superficie de respuesta y escalas JAR) incremente la aplicabilidad de la información suministrada por los consumidores en los procesos de diseño de nuevos productos o de reformulación de los ya existentes.

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OBJETIVOS

El objetivo general de la tesis fue contribuir al conocimiento del impacto que tienen la grasa y el azúcar en las propiedades físicas y sensoriales de matrices alimentarias con distinta composición. Para cumplir con ello, se establecieron tres unidades temáticas:

1. Efecto de la composición de la matriz en sus características físicas y estructurales

   Objetivos específicos:

   - Analizar la influencia del contenido de grasa y de la adición de diferentes espesantes en la microestructura y propiedades físicas de sistemas modelos de emulsiones.
   - Determinar el efecto de la interacción proteína-espesante en las características reológicas de matrices alimentarias con alta concentración proteica.
   - Estudiar el impacto de la grasa y su reducción en las propiedades ópticas y en el comportamiento reológico de sistemas lácteos semisólidos.

2. Efecto de la modificación de la composición en la liberación del sabor y percepción sensorial

   Objetivos específicos:

   - Determinar el efecto de la composición, estructura y comportamiento reológico en la liberación *in vivo* de los volátiles responsables del sabor y en las características sensoriales de emulsiones alimentarias.
   - Estudiar la influencia del contenido en grasa y de la concentración y tipo de espesante en la liberación *in vivo* de los volátiles

responsables del sabor y en las características sensoriales de matrices lácteas.

- Analizar efecto de los espesantes sobre la consistencia y dulzor percibido de matrices lácteas con diferente contenido en grasa y estudiar las relaciones entre los datos sensoriales y los instrumentales.

3. Análisis y propuesta de nuevos enfoques metodológicos aplicables al estudio de la opinión del consumidor para el desarrollo de nuevos productos.

   Objetivos específicos:

   - Obtener información sobre como perciben y describen los consumidores los postres lácteos con diferente composición.
   - Optimizar la aceptabilidad de un nuevo postre de soja con alto contenido proteico teniendo en cuenta la opinión de los consumidores sobre la idoneidad de sus características sensoriales.

PRESENTACIÓN DE TRABAJOS

De acuerdo a los objetivos planteados, esta tesis se desarrolló en tres partes:

a) Estudio del efecto de la composición sobre las propiedades ópticas, reológicas y estructurales (capítulos 1, 2 y 3).

b) Estudio del efecto de la composición sobre la liberación del sabor y en la percepción del sabor, del dulzor y de la textura (capítulos 3, 4 y 5).

c) Estudio de nuevos enfoques metodológicos aplicables a la investigación de la opinión del consumidor para el desarrollo de nuevos productos (capítulos 6 y 7).

Los resultados obtenidos han dado lugar a las siguientes publicaciones:

Capítulo 1

Arancibia, C., Bayarri, S. y Costell, E. 2013. Comparing carboxymethyl cellulose and starch as thickeners in oil/water emulsions. Implications on rheological and structural properties. *Food Biophysics 8, 122-136.*

Capítulo 2

Arancibia, C., Bayarri, S. y Costell, E. Flow properties and viscoelasticity of high-protein soy desserts. Effect of hydrocolloid. Enviado a: *Food and Bioprocess Technology.*

Capítulo 3

Arancibia, C., Castro, C., Jublot, L., Costell, E. y Bayarri, S. Fat-thickener interactions on dairy systems: effects on colour, rheology, flavour release and sensory perception. Enviado a: *LWT- Food Science and Technology.*

Capítulo 4

Arancibia, C., Jublot, L., Costell, E. y Bayarri, S. 2011. Flavor release and sensory characteristics of o/w emulsions. Influence of composition, microstructure and rheological behavior. *Food Research International 46 (6), 1632-1641*.

Capítulo 5

Arancibia, C., Bayarri, S. y Costell, E. 2013. Impact of structural differences on perceived sweetness in semisolid dairy matrices. *Journal Texture Studies, 44, 346-356.*

Capítulo 6

Arancibia, C., Costell, E. y Bayarri, S. 2011. Fat replacers in low-fat carboxymethyl cellulose dairy beverages. Color, rheology and consumer perception. *Journal of Dairy Science 94, 2245-2258.*

Capítulo 7

Arancibia, C., Bayarri, S. y Costell, E. Optimizing acceptability of a high protein soy dessert combining Surface Response Methodology and JAR scales. Enviado a: *Journal of Sensory Studies.*

CAPÍTULO 1

COMPARING CARBOXYMETHYL CELLULOSE AND STARCH AS THICKENERS IN OIL/WATER EMULSIONS. IMPLICATIONS ON RHEOLOGICAL AND STRUCTURAL PROPERTIES

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Abstract

The study reported here aims to obtain information on how thickener type and concentration, and oil content influence rheology, particle size, particle charge and microstructure in o/w model emulsions. Emulsions were prepared at two oil concentrations (5 and 30% wt/wt), each with three CMC concentrations (0.2, 0.3, and 0.4% wt/wt), or three starch concentrations (2, 3, and 4% wt/wt). For each oil concentration, a sample without any added thickener was prepared as reference. Both CMC and swollen starch granules showed a dominating effect on emulsion flow behavior, although the presence and concentration of fat droplets also played an important role. Viscoelasticity of CMC-based emulsions mainly depended of oil concentration whilst in starch-based emulsions the most influential ingredient was starch. A similar situation was detected in terms of particle size distribution; CMC effect was dependent on oil content and starch effect was mainly related to the volume occupied by swollen granules. Differences in microstructure and particle size distribution between CMC and starch emulsions were related to their rheological behavior. Apart from enabling the acquisition of food emulsions with different composition but with similar rheological behavior by adding different hydrocolloids, here we consider thickener effect on other properties in order to obtain food emulsions with adequate characteristics.

Keywords: Food emulsion, carboxymethyl cellulose, starch, rheology, particle characteristics, microstructure

Introduction

Basically, food emulsions are the result of a homogenization process applied to two immiscible liquids, usually oil and water, to obtain a biphasic system with the fat droplets dispersed in an aqueous phase (oil-in-water emulsions) or with water droplets dispersed in an oil phase (water-in-oil emulsions).

Stability and physicochemical and sensory properties of emulsion-based food products are strongly influenced by concentration and characteristics of the droplets that they contain [1-4] and also by the effects of other food ingredients and the interactions among them [5-8]. Due to the practical interest in obtaining food emulsions that remain stable during a determined time period, the identification and description of different instability mechanisms and different ways to assess and control emulsions have been the objective of numerous studies during recent years [9-11]. Selecting the process conditions [12, 13] and the type and concentration of emulsifier and/or stabilizer [14-18] can provide food emulsions with the desired stability but which do not always have the physicochemical and sensory properties required.

Although some hydrocolloids with surface activity can act as emulsifiers in oil-in-water emulsions, the principal role of most of these biopolymers is that they act as structuring, thickening or gelling agents in the aqueous phase. Therefore they modify the rheological behavior of the continuous phase and also contribute to matching the densities of oil and aqueous phases [19]. Besides the influence of hydrocolloids on food emulsion stability, they also can have an important effect on food emulsion appearance, texture and flavor [20-22]. Thus, to analyze the changes in emulsion structure and rheology due to the addition of different hydrocolloids may be important to understanding their effects on stability and on physicochemical and sensory properties of emulsion-based food products.

Starch and carboxymethyl cellulose are two of the most widely used thickeners in the food industry, and some hydrophobically modified starches and cellulose derivates have been proposed as ingredients with surface activity and hence, with both emulsifying and stabilizing properties [23-25] although, as commented by Dickinson [26], there has been no definitive demonstration that the surface activity of hydrocolloids is directly correlated with its emulsification capacity. Furthermore, the relatively high molecular weights of these modified hydrocolloids can limit their emulsification performance [19].

In general and depending on starch type, its concentration and thermo-mechanical treatments during processing, starch-based food systems will behave as a viscous fluid or a gel [27]. Recently, there has been an increase in the use of some types of modified starches (crosslinked, waxy, etc.), instead of native starches, as they show higher thermo mechanical resistance and stability. In food emulsions, if starch granules remain whole after heating, the resultant system may be an emulsion whose rheological behavior depends mainly on the granules' volumetric fraction and on their rigidity or deformability [28], unlike fat droplets, which also play an important role in the flow behavior of starch-based emulsions [29]. Nowadays, CMC is being used as an alternative thickener to starch in many formulated foods because it may act as a viscosity-increasing agent, moisture binder, emulsion stabilizer and improves the texture of a wide range of food products [30]. Its thickening properties mainly depend on the degree of chain polymerization and of substitution of the basic glucose blocks in cellulose with glycopyranose. It has also been observed that adding a small amount of CMC to an oil-in-water emulsion reduces stability and leads to faster creaming by increased droplets flocculation, and that larger concentrations can increase emulsion stability [31]. Besides this, CMC is particularly useful to formulate dietetic foods since, as a dietary fiber, it is physiologically inert

and has beneficial effects on lowering glycemic and cholesterol levels [32, 33]. However, less attention has been paid to the study of CMC-based food emulsions than to starch-based food emulsion systems.

To select the more appropriate hydrocolloid to be added as emulsion thickener in each case, it is important to consider not only their effects on rheology of the system, but also to analyze the effects of their possible interactions with emulsion oil content on emulsion structural characteristics. In this context, the main objective of this work was to analyze and compare the effects of adding different CMC and starch concentrations on the rheological behavior and structural characteristics of oil-in-water emulsions with different fat contents.

Materials and Methods

Composition and Preparation of Oil/Water (O/W) Emulsions

Emulsions were prepared with sunflower oil (Coosol, COOSUR S.A., Jaén, Spain), carboxymethyl cellulose (CMC) (Akucell AF3265 Akzo Nobel, Amersfoort, The Netherlands), medium crosslinked modified tapioca starch (C* Creamtex 75720, Cargill Ibérica S.L., Barcelona, Spain), mineral water (3.00 mg/L calcium, <0.50 mg/L magnesium, 2.44 mg/L sodium, <10 mg/L bicarbonate and 0.94 mg/L chloride content) (Bezoya, Grupo Leche Pascual S.A., Burgos, Spain), sucrose stearate (E-473) (Sisterna SP70, Zeus Química, Barcelona, Spain) and commercial sucrose.

Oil-in-water emulsions were prepared at two oil concentrations (5 and 30% wt/wt), each with three CMC concentrations (0.2, 0.3, and 0.4% wt/wt) or three starch concentrations (2, 3, and 4% wt/wt). For each oil concentration, a sample without any added thickener was prepared as reference or control sample. The amounts of sucrose (7% wt/wt) and sucrose stearate (1% wt/wt) remained fixed in all the samples. Sucrose stearate was chosen as emulsifier

because of its ability to form stable emulsions over a wide range of oil volume fractions [34].

The aqueous phase was prepared by dispersing sucrose stearate and sugar in mineral water, at room temperature for 30 min, with the help of a propeller stirrer (Heidolph RZR 1, Schwabach, Germany). Oil was added slightly to the aqueous phase and the mixture was shaken using a high-performance dispersing instrument at 3000 or 5000 rpm (samples with 5% or 30% oil, respectively) (Ultra Turrax, IKA, T50-basic, Staufen, Germany) for 15 min in a water bath at 10ºC to avoid warming of the sample. The coarse emulsion was then passed through a two-stage high-pressure valve homogenizer (Manton-Gaulin, 15M8TBA, Everett, Massachusetts, USA) at a pressure of 300 kg/cm^2 five times. Emulsions were stored at 4±1ºC overnight to equilibrate. The following day, CMC or starch was added to the o/w emulsion. CMC was added to the o/w emulsion and mixed (Heidolph RZR 1, Schwabach, Germany) at room temperature for 30 min. Starch-based samples were prepared in batches of 800 g as follows: starch and emulsion were weighed in a flask and mixed by magnetic stirrer (Ared, Velp Scientifica, Usmate, Italy) for 10 min at room temperature. Then, the flask was placed in a water bath at 97±1ºC and stirred (Heidolph RZR 1, Schwabach, Germany) constantly at around 200 rpm for 30 min, to be cooled subsequently in a water bath at 10ºC for 10 min. The amount of water that had evaporated in the process was added. All samples were transferred to a closed flask and stored under refrigeration (4±1ºC; 24 h) prior to measurements. At least two batches of each composition were prepared.

Rheological measurements

Flow behavior of emulsions without added thickener and those with the lowest starch concentration (2%) were measured in a Haake VT 550

viscometer (ThermoHaake, Karslruhe, Germany) using a concentric cylinder sensor MV2 (radii ratio= 1.14, length= 60 mm, gap width= 2.6 mm). Flow and viscoelastic behavior of the remaining samples were measured in a controlled stress rheometer RS1 (ThermoHaake, Karlsruhe, Germany), using parallel-plates geometry (60 mm diameter; 1mm gap). At least, two batches of each composition were measured in duplicate at a controlled temperature of 10±1ºC. Samples were allowed to rest for 10 min before measurement and a fresh sample was loaded for each measurement.

Flow behavior

Flow behavior was measured by recording shear stress values when shearing the samples at linearly increasing shear rates from 1 to 200 s^{-1} through 60 s and down in reverse sequence for the same time [35]. Data from the upward curve of the shear cycle were fitted to the Ostwald-de Waele model (Eq. 1)

$$\sigma = K \dot{\gamma}^{n} \quad (1)$$

where σ (Pa) is the shear stress, $\dot{\gamma}$ (s^{-1}) is the shear rate, K (Pa s^{n}) is the consistency index and n is the flow behavior index. These calculations were performed using the Rheowin Pro software (version 3.61, Haake).

Viscoelastic behavior

Viscoelastic properties were measured using small amplitude oscillatory shear tests. First, stress sweeps were made between 0.02 and 300 Pa, at a frequency of 1Hz, in all the systems studied to determine the linear viscoelasticity zone. After that, frequency sweeps at 0.05 Pa were performed from 0.01 to 10 Hz. The oscillatory rheological parameters used to compare the viscoelastic properties of the samples were storage modulus (G'), loss

modulus (G"), complex dynamic viscosity (η*) and loss tangent (tan δ) at 1 Hz [36].

Particle Size Analysis

The droplet-size distributions of each emulsion were measured using a Malvern Mastersizer 2000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Worcestershire, UK). A refractive index value of 1.472 was used for the disperse phase (sunflower oil) and of 1.33 for the continuous phase (water). Particle size calculations were based on the Mie Scattering theory. Volume-weighted mean diameter values ($D_{4,3}$) and the percentage of volume corresponding to each observed population were calculated with the software provided with the equipment (Mastersizer 2000 V5.40). Measurements were made in duplicate for each sample.

Particle Charge Analysis

The ζ-potential of each emulsion was determined using a particle electrophoresis instrument (Zetasizer Nano ZS series, Malvern Instruments, Worcestershire, UK). Particle charge data were collected over 30 continuous readings. All measurements were made in duplicate with fresh samples and ζ-potential measurements were reported as the mean and standard deviation of two separate measurements.

Microstructure

Fluorescence microscopy (Nikon Eclipse 90i, Kanagawa, Japan) was used to analyze the microstructure of the emulsions. A drop of sample was placed on a slide with a cover slip and observed using a magnification of 40x and identical exposure time (1.5 s). The photomicrographs were acquired with a

digital camera (Nikon DS-5Mc, Kanagawa, Japan). The excitation wavelength used was 488 nm. Samples were stained with Nile Red (Sigma-Aldrich Química S.A., Madrid, Spain) to allow for the visualization of the oil phase. Solution of fluorescent staining was prepared by dissolving 0.1 g/L Nile Red in polyethylene glycol (Sigma-Aldrich Química S.A., Madrid, Spain), and was then added to the emulsions at a concentration of 1.5 mL/g. Samples were stained 10 min before analysis.

Statistical Analysis

A two-way ANOVA (oil concentration and thickener concentration) with interaction was applied to the rheological data. Tukey's test ($\alpha = 0.05$) was used to calculate the minimum significant difference. All calculations were carried out with XLSTAT Pro software, version 2007 (Addinsoft, Paris, France).

Results and Discussion

Flow Behavior

The flow curves obtained for emulsions with 5% and 30% oil are shown in Figures 1 and 2. Control emulsions, without added thickener, showed Newtonian flow behavior, whilst emulsions with added CMC or starch exhibited non-Newtonian flow. In general, CMC and starch emulsions showed a shear-thinning and time-dependent flow, and shear and time dependence increased with thickener and oil concentrations.

Considering that the hysteresis loop area represents the energy needed to destroy the structure of a material, the experimental data obtained for CMC-based emulsions (Figure 1a and 1b) showed that increased CMC

concentration produced a more structured system, which partially broke down with increasing shearing time.

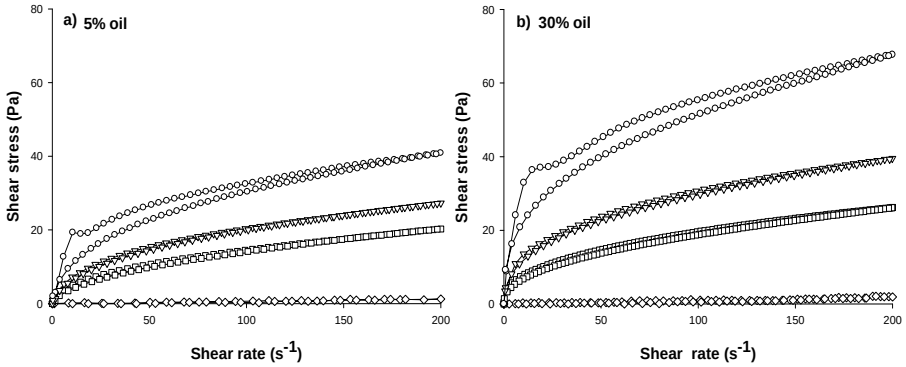

Figure 1. Flow curves of o/w emulsions with 5% oil (a) and 30% oil (b) and different CMC concentrations (◇ = 0%, □ = 0.2%, ▽ = 0.3% and ○ = 0.4%).

By comparing the shear stress versus shear rate profiles of CMC emulsions at low shear rates, it can be observed that samples with the highest CMC content (0.4%) showed an overshoot, which was more pronounced for the 30% oil emulsion. The overshoot may reflect the resistance to deformation of a more structured system, which partially broke down with increasing shear rates. It can be considered that maximum stress in the overshoot represents the yielding transition since the continuous network changes to a discontinuous state at this point [37]. At the lowest CMC concentrations (0.2 and 0.3%) it can be seen that the system is relatively deformable by shearing and the flow curve does not show the overshoot. Similar behavior was observed by Bayarri and Costell [38] on studying the influence of CMC concentration and milk fat content on flow behavior of dairy products. They observed that shear stress versus shear rate profiles of the whole-milk systems with the highest CMC content showed an overshoot and concluded that not only molecular CMC interactions but also fat droplets contributed to the higher resistance to flow of these samples. A similar fact was detected by

Lizarraga *et al.* [39] on studying the flow of whey protein concentrate and λ-carrageenan aqueous mixtures. They interpreted that the overshoot in flow curves at low shear rates, when WPC concentration increases, was due to the effect of interactions between the two biopolymers. The experimental upward curves of all CMC-based emulsions fitted well to the Ostwald-de Waele model with R^2 values ranging between 0.94 and 0.99. ANOVA of flow parameter values showed that the effect of the interaction between oil concentration and CMC concentration was significant on consistency index values, but not on flow index values (Table 1). This interaction indicated that the effect of CMC concentration on the consistency of samples differed depending on oil concentration.

Table 1. Two-way ANOVA of flow and viscoelastic parameters in o/w emulsions with different carboxymethyl cellulose and oil concentrations[a]. F and P values

	Main effects				Interactions	
	A: CMC concentration		B: Oil concentration		A × B	
	F	*p*	*F*	*p*	*F*	*p*
Flow parameters						
K (Pa s^n)	578.75	<0.01	242.05	<0.01	77.92	<0.01
n	242.33	<0.01	6.06	0.04	0.01	1.00
Viscoelastic parameters[b]						
G' (Pa)	131.4	<0.01	4096.69	<0.01	94.02	<0.01
G" (Pa)	514.12	<0.01	5457.53	<0.01	207.05	<0.01
tan δ	30.85	<0.01	4067.58	<0.01	49.00	<0.01
η* (Pa s)	146.50	0.04	4133.85	<0.01	97.29	0.02

[a] *K = consistency index, n= flow index, G = storage modulus, G"= loss modulus, tan δ= loss tangent and η* = complex dynamic viscosity. Viscoelastic parameters values at 1 Hz.*

[b] *Only data of emulsion with added CMC were included. Viscoelastic properties of emulsions without CMC were not measured, due to their fluidity.*

As expected, K values increased significantly with CMC concentration both for emulsions with 5% and 30% oil (Table 2), probably caused by an increase in resistance to flow due to particle-particle interaction. For very low CMC concentrations, the CMC chains are in their most extended conformation. At higher polymer concentrations, extended CMC chains start to overlap and entangle, resulting in a transient network structure [40]. In the 30% oil emulsions, higher oil content seemed to strengthen the system compared with the 5% oil emulsions, particularly at the higher CMC concentrations, giving rise to higher K values.

Table 2. Mean values and significant differences of rheological parameter values for o/w emulsions with different carboxymethyl cellulose and oil concentrations[1].

CMC concentration (% wt/wt)	Oil concentration (% wt/wt)	K (Pa s^n)	n	G' (Pa)	G" (Pa)	tan δ	η* (Pa s)
0	5	0.01^{e}	1.01^{a}	-	-	-	-
0.2	5	1.65^{d}	0.47^{b}	2.00^{d}	2.67^{e}	1.34^{a}	0.53^{d}
0.3	5	3.12^{c}	0.42^{bc}	3.22^{d}	3.94^{e}	1.22^{b}	0.81^{d}
0.4	5	6.02^{b}	0.35^{bc}	6.49^{d}	6.33^{d}	0.98^{c}	1.45^{d}
0	30	0.01^{e}	0.97^{a}	-	-	-	-
0.2	30	2.70^{cd}	0.42^{bc}	77.56^{c}	16.08^{c}	0.21^{d}	12.61^{c}
0.3	30	5.74^{b}	0.37^{bc}	93.58^{b}	21.86^{b}	0.24^{d}	15.30^{b}
0.4	30	12.72^{a}	0.31^{c}	131.64^{a}	32.50^{a}	0.25^{d}	21.58^{a}

[1] K = consistency index, n= flow index, G' = storage modulus, G"= loss modulus, tan δ= loss tangent and η = complex dynamic viscosity. Viscoelastic parameters values at 1 Hz.*

[a-e] Means within a column with common superscripts did not differ significantly (P<0.05).

With regard to flow index, the addition of CMC to the emulsions significantly decreased n values, which ranged from ≈ 1 for emulsions without CMC to 0.35 and 0.31 for emulsions with the highest CMC content (0.4%) and with 5% and 30% oil content, respectively, although this decrease was not significantly affected by oil or CMC concentrations. The increase in flow pseudoplasticity due to CMC addition to the emulsions can

be explained by the orientation of the CMC macromolecules, as they align in the direction of the shearing force [41].

Figure 2a and 2b shows the flow curves obtained for emulsions made with different oil and starch concentrations. As commented previously, control samples for both oil concentrations showed Newtonian flow and, in this case, the 5% oil emulsion with the lowest starch concentration (2%) also showed Newtonian flow behavior. In this sample, the volume fraction of swollen starch granules was so low that there was almost no contact between starch granules; therefore, the flow behavior remained practically unaltered. The other emulsions exhibited a non-Newtonian flow. Samples made with 5% oil and over 2% starch, and 30% oil samples with added starch showed shear thinning behavior; and flow of the emulsions with the highest starch concentration (4%) was also time-dependent.

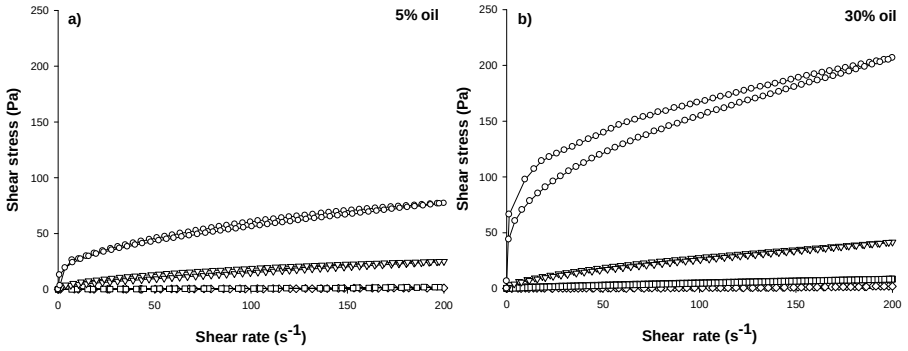

Figure 2. Flow curves of o/w emulsions with 5% oil (a) and 30% oil (b) and different starch concentrations (◇ = 0%, □ = 2%, ▽ = 3% and ○ = 4%).

For comparative purposes the experimental upward curves of all samples fitted to the Ostwald-de Waele model ($0.93<R^2<0.99$). Results from a two-way ANOVA showed that both oil concentration and starch concentration effects were significant ($P<0.05$) on flow parameter values, and that there

was only a significant interaction between the two factors on K values (Table 3).

Table 3. Two-way ANOVA of flow and viscoelastic parameters in o/w emulsions with different oil and starch concentrations[1]. F and P values

	Main effects				Interactions	
	A: Starch concentration		B: Oil concentration		A × B	
	F	*p*	*F*	*p*	*F*	*p*
Flow parameters						
K (Pa s^n)	225.54	<0.01	67.02	<0.01	49.65	<0.01
n	102.81	<0.01	16.15	<0.01	2.05	0.19
Viscoelastic parameters[2]						
G' (Pa)	1354.36	<0.01	796.30	<0.01	549.12	<0.01
G" (Pa)	60.70	<0.01	80.93	<0.01	17.43	0.01
tan δ	59.37	<0.01	0.30	0.62	0.07	0.81
η* (Pa s)	1587.12	<0.01	967.97	<0.01	638.73	<0.01

[1] *K = consistency index, n= flow index, G'= storage modulus, G"= loss modulus, tan δ= loss tangent and η* = complex dynamic viscosity. Viscoelastic parameters values at 1 Hz.*

[2] *Viscoelastic properties of emulsions without and with 2% starch could not be measured, due fluidity of the samples.*

Consistency index values of 5% oil emulsions increased significantly only when the highest starch concentration (4%) was added, whilst in the emulsions made with 30% oil, this effect could be detected at starch concentrations up to 3% (Table 4). With regard to the n value, similar trends were observed for samples with either oil concentration: pseudoplasticity increased with starch concentration and these increases were only slightly dependent on oil content of emulsions. No significant differences on pseudoplasticity were detected between 30% and 5% oil emulsions at the same starch concentration (Table 4). These results indicate that flow differences among starch-based emulsions were greater at higher starch concentrations, and also for higher oil contents. This is in agreement with

that observed about the effect of starch and fat/oil concentrations on consistency of different food matrices such as water-starch pastes [42], starch-milk model systems [43, 44] or mixed colloidal dispersions [29].

Table 4. Mean values and significant differences of rheological parameter values for o/w emulsions with different oil and starch concentrations[1].

Starch concentration (% wt/wt)	Oil concentration (% wt/wt)	K (Pa s^n)	n	G' (Pa)	G" (Pa)	tan δ	η* (Pa s)
0	5	0.01^{c}	1.01^{a}	-	-	-	-
2	5	0.01^{c}	1.00^{ab}	-	-	-	-
3	5	0.65^{c}	0.70^{bc}	3.39^{c}	1.81^{b}	0.54^{a}	0.62^{d}
4	5	9.99^{b}	0.39^{d}	103.62^{b}	15.61^{b}	0.15^{b}	16.68^{b}
0	30	0.01^{c}	0.97^{a}	-	-	-	-
2	30	0.31^{c}	0.81^{ab}	-	-	-	-
3	30	2.51^{b}	0.54^{cd}	39.27^{c}	20.21^{b}	0.52^{a}	7.06^{c}
4	30	27.94^{a}	0.35^{d}	490.90^{a}	65.87^{a}	0.14^{b}	78.85^{a}

[1] *K = consistency index, n = flow index, G' = storage modulus, G" = loss modulus, tan δ= loss tangent and η* = complex dynamic viscosity. Viscoelastic parameters values at 1 Hz.*

[a-e] *Means within a column with common superscripts did not differ significantly (P<0.05).*

It can be concluded that, apart from the dominating effect on emulsion of adding both CMC macromolecules and swollen starch granules, the presence and concentration of fat droplets also played an important role in flow behavior. Increased oil content raised emulsion consistency or viscosity, although its impact on flow pseudoplasticity was negligible. As result, some emulsions differing in composition had similar consistency index values such as emulsions with 5% oil and 0.3% CMC, with 30% oil and 0.2% CMC (Table 2) or with 30% oil and 3% starch (Table 4).

Viscoelastic Properties

The viscoelastic properties of control samples and those containing 2% starch could not be measured due to their fluidity. The mechanical spectra obtained for emulsions with CMC are shown in Figure 3. In general, the viscoelastic characteristics of these emulsions ranged from fluid-like to a weak-gel-like, depending on emulsion composition.

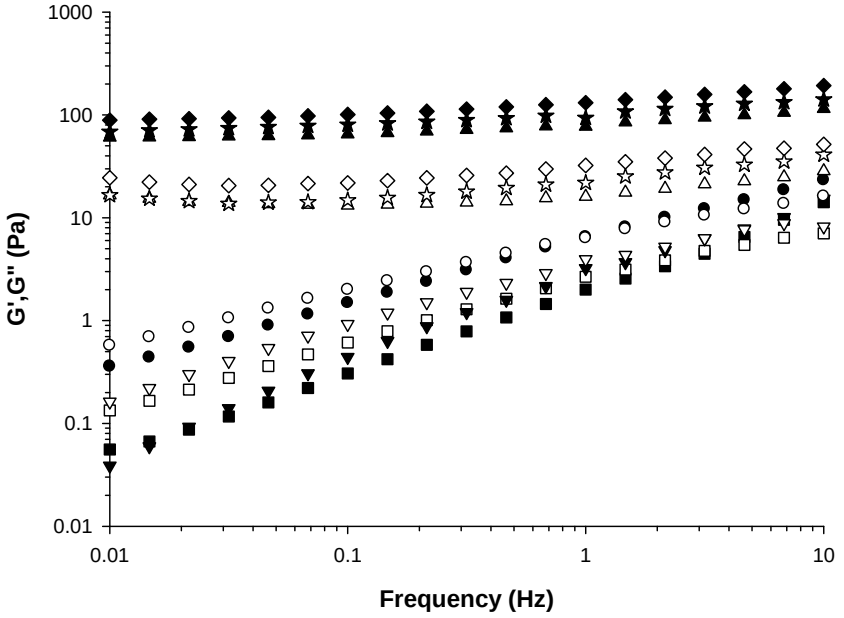

Figure 3. Mechanical spectra of o/w emulsions at different CMC and oil concentrations (□ = 5%oil-0.2%CMC, ▽ = 5%oil-0.3%CMC, ○ = 5%oil-0.4%CMC, △ = 30%oil-0.2%CMC, ☆ = 30%oil-0.3%CMC and ◇ = 30oil-0.4%CMC) (G': *filled symbols* and G": *empty symbols*).

Emulsions with 5% oil containing 0.2% or 0.3% CMC showed a strong frequency dependence on dynamic moduli (G' and G") with the viscous modulus exceeding the elastic one, indicating a fluid-like behavior. When CMC concentration increased, the mechanical spectra changed significantly, indicating the 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 one. Emulsion with 5% oil and the highest CMC concentration (0.4%) presented lower frequency-dependence and G' and G" had similar values. Samples with 30% oil exhibited a weak gel-like behavior and slight variation in dynamic moduli with oscillation frequency and with the elastic response predominating over the viscous one. A similar trend has been observed for aqueous CMC solutions [36, 45, 46]. Kulicke *et al.* [45] studied the characterization of aqueous CMC solutions in terms of their molecular structure and its influence on rheological behavior, they observed that when CMC concentration increases, so too does the number of entanglements (density of the entanglement network), causing the value of the moduli to rise. An ANOVA of two factors with interactions was run to analyze how oil content and CMC concentration influenced G', G'', η* and tan δ values at 1 Hz. Results showed a significant interaction effect between the two factors considered on all viscoelastic parameter values (Table 1). In contrast with the results obtained for flow parameters, oil concentration was the most influential factor on viscoelastic parameter values, revealing differences between samples, as reflected by the higher F values in the ANOVA. For the same CMC concentration, emulsion made with 30% oil exhibited significantly higher values of G', G'' and η* and lower values of tan δ than that made with 5% oil (Table 2). In 5% oil emulsions, the CMC concentration only had a significant effect on G" and tan δ values, whilst in 30% oil emulsions G', G'' and η* values were significantly increased by increasing CMC concentration, although tan δ values remained unaffected. Changes in oil content of CMC-based emulsions clearly altered their viscoelastic properties. The increase in G', G'' and η* values and the decrease in tan δ values when oil content increased from 5% to 30% could indicate some structural changes in the emulsion, related with concentration

and with the physical state of fat droplets. We can hypothesize that the increase in G' average values from 3.90 to 100.92 Pa and of η* values from 2.72 to 49.49 Pa s, when the oil concentration increased from 5% to 30%, could be influenced by an aggregation process or by partial crystallization of fat droplets. As commented by McClements [10], when fat droplets are aggregated or partially crystallized, there is an increase in effective particle size and effective volume fraction, thereby reinforcing emulsion structure. These structural changes may also explain the variation in tan δ values from 1.16 (fluid-like behavior) to 0.23 (weak-gel behavior), when emulsion oil content is increased.

The starch-based emulsions, except those containing 2% starch, exhibited viscoelastic properties usually observed in weak-gel systems: the elastic response (G') predominated over the viscous one (G") and both dynamic moduli showed only a slight variation in oscillation frequency. The frequency dependence of dynamic moduli became parallel and the higher the starch concentration the lower the frequency-dependency of viscoelastic moduli values (Figure 4). A two-way ANOVA with interactions, considering oil content and starch concentration as factors, was performed to analyze the influence these effects on G', G'', η* and tan δ values at 1 Hz. Results showed a significant effect of oil concentration-starch concentration interaction on all viscoelastic parameters values, except on tan δ values that only were dependent on starch concentration (Table 3).

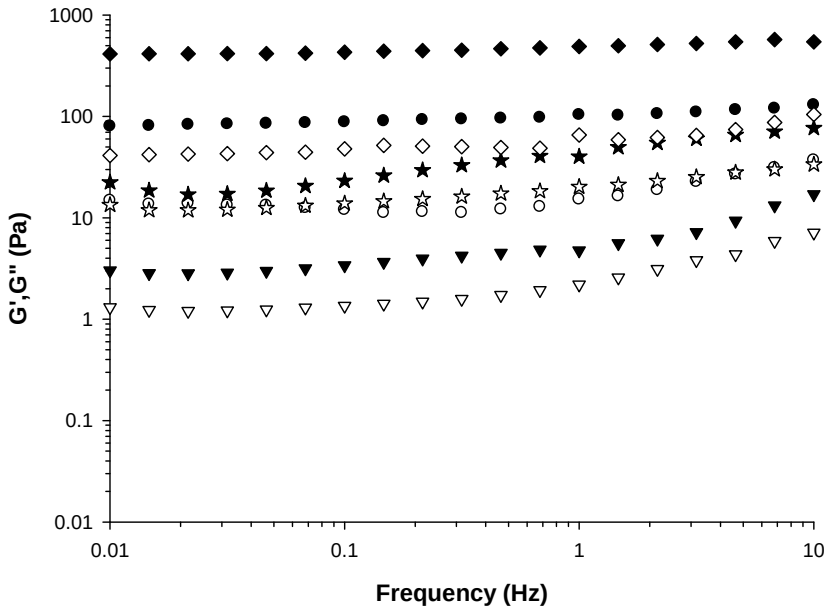

Figure 4. Mechanical spectra of o/w emulsions at different starch and oil concentrations (▽ = 5%oil-3%starch, ○ = 5%oil-4%starch, ☆ = 30%oil-3%starch and ◇ = 30oil-4%starch) (G': *filled symbols* and G": *empty symbols*).

A similar and clear structuring effect was observed due to the increase in starch concentration for emulsions with different oil concentration. At both oil contents, tan δ values decreased, approximately from 0.5 to 0.15, when starch concentration added to emulsions increased from 3% to 4% and there were no significant differences between tan δ values between emulsions with the same starch concentration and different oil content (Table 4). As expected, G', G" and η* values increased with both oil and starch concentrations, although the oil effect was dependent on sample starch concentration. No significant differences on G' and G" values between 5% and 30% oil content in emulsions with 3% starch were detected, whilst significant differences between them could be observed for samples with 4% starch. The effect of oil and starch concentrations on η* values was similar.

Increasing the starch concentration increased complex viscosity values for both oil concentrations, and η* values of 5% oil emulsion were significantly lower than those of 30% oil emulsion for each starch concentration. It can then be concluded that, although the structure and viscoelastic behavior of starch-based emulsions depended mainly on the volumetric fraction occupied by the swollen starch granules and on granule rigidity or deformability [28], the changes in oil content clearly altered their rheological properties. Such an effect could be attributed to changes in the emulsion structure due to an increase in particle concentration [29], to the highest concentration of fat droplets in the voids between the granules and also, to the increase in starch granule rigidity due to possible interactions between starch and fat [47].

Results obtained showed clear differences between CMC and starch in terms of the influence of thickener and oil content on viscoelastic behavior of emulsions. In CMC-based emulsions, the most influential factor was oil concentration, whilst in starch-based emulsions the dominating ingredient was starch, although oil content also contributed to system viscoelasticity. Among samples analyzed, only emulsion with 30% oil and 0.3% CMC and emulsion with 5% oil and 4% starch showed similar values of complex viscosity (Tables 2 and 4).

Particle Size Distribution

Figure 5 shows the droplet size distribution of emulsions based on volume percent of droplets as a function of droplet diameter. Control emulsions, without added CMC, presented a monomodal distribution with an average particle size ($D_{4,3}$) of 0.22±0.01 μm for samples with 5% oil, and of 0.42±0.01 μm for emulsions containing 30% oil. When CMC was added to emulsion made with 5 % oil it gave rise to a bimodal particle size

distribution due to the appearance of new small populations with higher particle size (Figure 5c, 5e and 5g), which may have been due to an incipient association of fat droplets. For each of the three CMC-based emulsions (0.2, 0.3 and 0.4% CMC) with 5% oil, we observed large populations (97.6%, 97.7% and 94.4% of total volume) corresponding to small droplet diameters with average particle sizes ranging from 18.77±0.13 μm to 9.21±0.01 μm, and small populations (2.4%, 2.3% and 5.6% of total volume) of larger particles with average particle sizes of 293±14 μm, 336±28 μm and 229±34 μm (Figure 5c, 5e and 5g). An increase in particle size due to droplet aggregation may have occurred because of droplet flocculation or coalescence [2]. It has been shown that the presence of low concentrations of some anionic polysaccharides in the aqueous phase reduces emulsion stability [19, 26, 31]. In the case of samples with 30% oil, all emulsions presented a monomodal particle size distribution (Figure 5b, 5d, 5f and 5h). As in the emulsions with 5 % oil, CMC addition increases average particle size. However, in this case, by increasing CMC concentration from 0.2 to 0.4% the average particle size decreased from 5.72±0.49 to 0.44±0.01 μm. The emulsion with the highest CMC concentration (Figure 5h) practically reached the average particle size of emulsion without added thickener (0.42±0.01 μm). Differences in the effect of adding theoretically similar CMC concentrations in 5% and 30% oil emulsions may have been due to the higher effective concentration of CMC in the aqueous phase of the latter. An increase in viscosity of the aqueous phase when CMC concentration increases can minimize droplet mobility delaying collision frequency and reducing droplet coalescence.

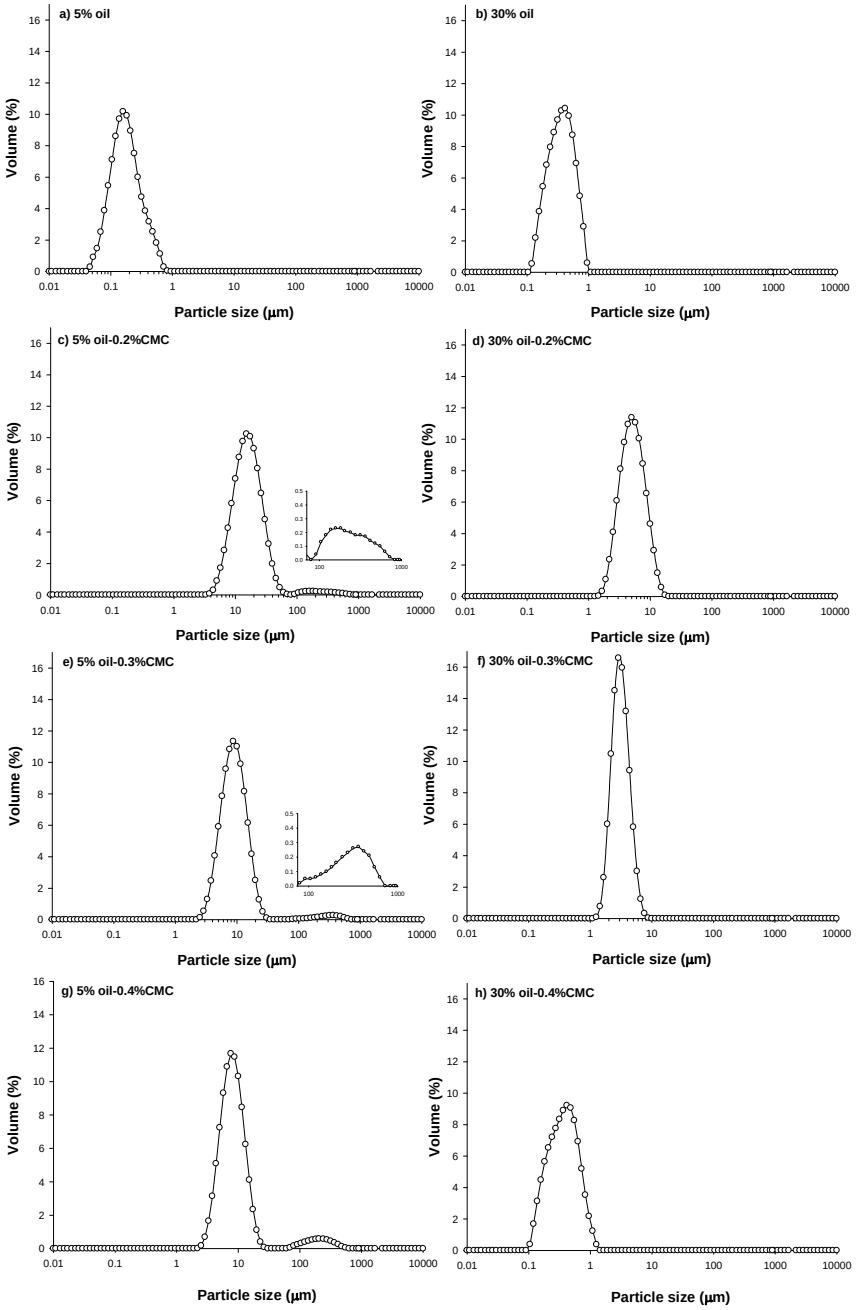

Figure 5. Particle size distribution of o/w emulsions with 5% oil (a, c, e and g) and 30% oil (b, d, f and h) at different CMC concentrations (0, 0.2, 0.3 and 0.4%).

Particle size distribution in starch-based emulsions is shown in Figure 6. In general, and as in the CMC emulsions, starch addition also increased average particle size of emulsions, although the effect differed depending on oil content. Emulsions with 5% oil showed differences in particle size distribution depending on starch concentration. At the lowest starch concentration (2%) (Figure 6c) particle size ranging from 1.90 to 120.23 μm and two peaks were detected. The first peak was located at a particle size of ~6.61 μm and could correspond to a population of small oil droplets, representing the greater volume of the total population. The second peak was located around a particle size of 37.24 μm and could be considered to represent a second population, formed by the dispersed swollen starch granules. Similar average particle size has been observed in other starch-based products like dairy desserts [48] and emulsions [22, 29]**.** In any case, the particle size data corresponding to gelatinized starch granules should only be treated as an estimate, since starch granules are actually non-spherical particles whose refractive index depends on the degree of swelling [29]. Emulsion with the intermediate starch concentration (3%), also showed a particle size distribution with two peaks (Figure 6e), but the higher percentage of total population corresponded to swollen starch granules. At the highest starch concentration, 5% oil emulsion showed a clear bimodal particle size distribution with a relatively small population of oil droplets (6.60% of total volume) with particle sizes between 1.91 and 6.61 μm ($D_{4,3}$=4.46±0.12 μm) and a larger population of starch granules (93.40% of total volume) ranging from 7.59 to 79.43 μm ($D_{4,3}$=36.44±0.24 μm) (Figure 6g).

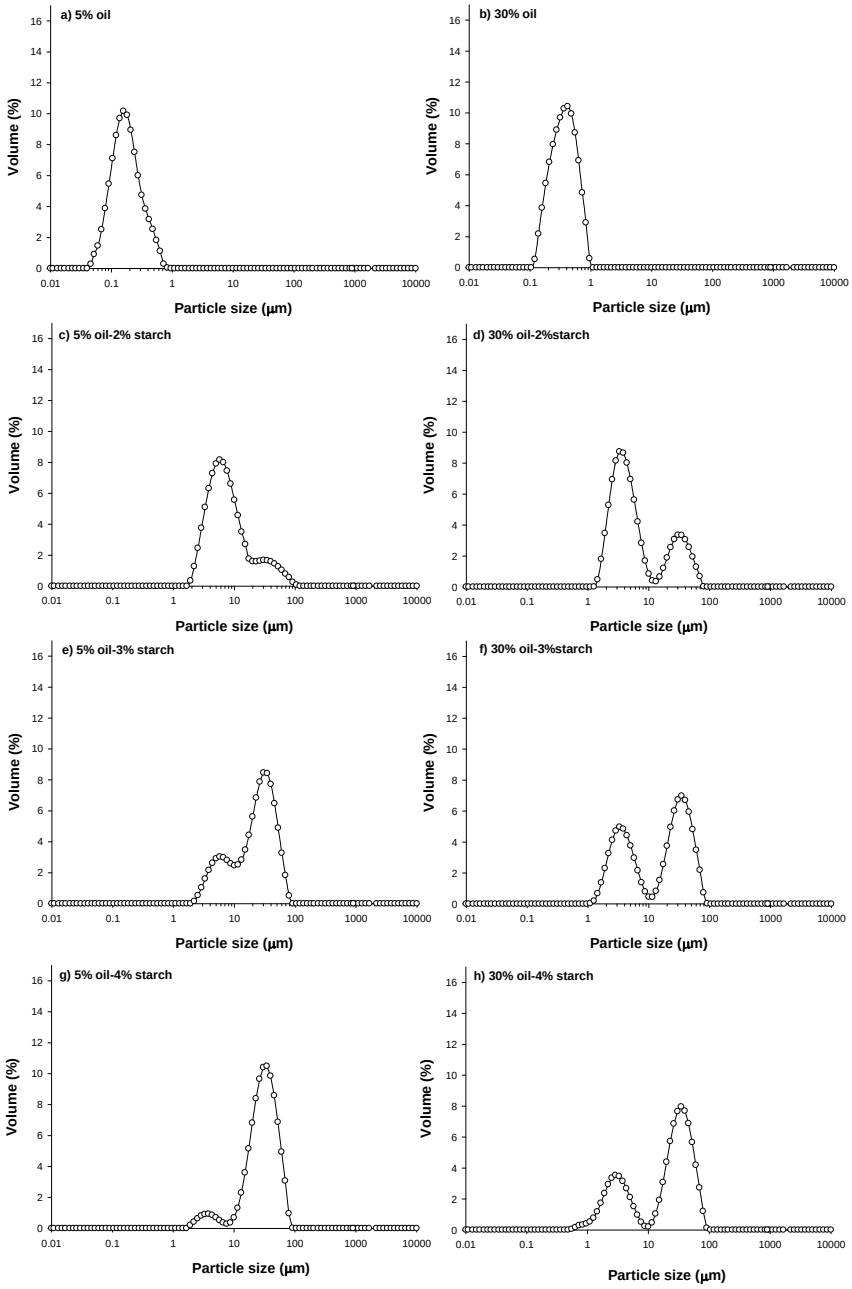

Figure 6. Particle size distribution of o/w emulsions with 5% oil (a, c, e and g) and 30% oil (b, d, f and h) at different starch concentrations (0, 2, 3 and 4%).

When starch was added to 30% oil emulsions, a bimodal particle size distribution was observed in all samples (Figure 6d, 6f and 6h). The average particle size ($D_{4,3}$) of each population was maintained practically stable across the starch concentrations. This fact suggests that fat droplets were distributed within the continuous phase separated by starch granules, reducing their mobility and avoiding contact among them. The first population, which corresponded to fat droplets, showed an average particle size of 4.40±0.11 μm, 4.18±0.07 μm and 3.44±0.02 μm for samples containing 2, 3 and 4% starch, respectively. The second population of swollen starch granules exhibited an average particle size of 37.15±0.07 μm, 38.94±0.18 μm and 39.17±0.08 μm. By increasing starch concentration, the relative volume of the first population decreased from 74.39 to 32.44% and the relative volume of the second population increased from 25.61 to 67.56%.

The effect of CMC and starch on particle size distribution of o/w emulsions with different oil content could principally be due to their water binding capacity and ionic charge, and to the thickener concentration in the continuous phase. CMC effect was dependent on oil content, while starch effect was mainly related to the volume occupied by swollen granules.

Particle Charge

Figure 7a shows ζ-potential values of emulsions as a function of CMC concentration. Emulsions without added CMC showed a different electrical charge of the particles. Control emulsion with 5% oil exhibited high and negative values of ζ-potential while, those with 30% oil showed values near to zero and positive charge. This difference between ζ-potential values can be related to the effect of sucrose stearate as emulsifier. A greater absolute magnitude of ζ-potential could indicate that the amount of sucrose stearate in

5% oil emulsions was sufficient for complete coverage of the fat droplets and to avoid flocculation. Conversely, a value close to zero of ζ-potential in 30% oil emulsions may be a sign of a poor coverage of fat droplets and could indicate droplet flocculation, since droplets can be in closer contact with each other. CMC addition to emulsions had an important effect on ζ-potential values, for both oil concentrations (Figure 7a). In general, all emulsions exhibited a negative charge when CMC was present and increasing CMC concentration increased the magnitude of the negative charge of ζ-potential values; with this effect being more evident in emulsions with 5% oil. This effect of CMC on the electrical characteristics of emulsion droplets could be due the presence of the anionic groups in the polymer chain of CMC and its surface properties.

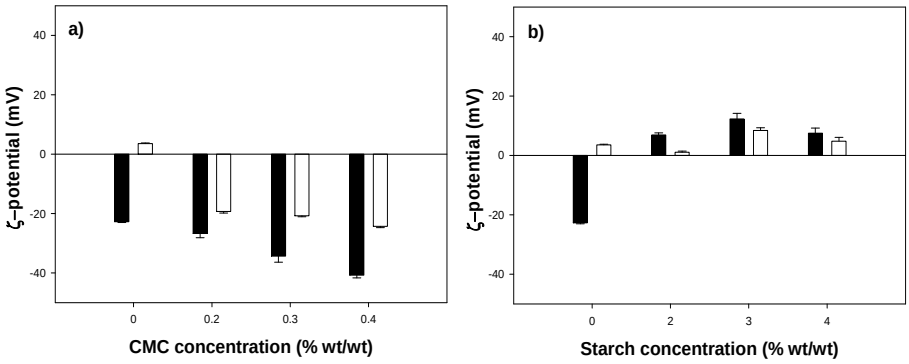

Figure 7. ζ-potential of o/w emulsions with 5% oil (■) and 30% oil (□), at different CMC (a) and starch (b) concentrations (mean values of 3 replicates and standard deviations).

Figure 7b shows the electrical characteristics of control and starch-based emulsions. As commented previously, the oil content of control emulsions had a great influence on ζ-potential values. A minor difference between ζ-potential values was found in starch-based emulsions with different oil

concentration and the same starch concentration. Starch concentration seems to have an effect on the charge of emulsions, but did not have a clear influence on the absolute magnitude of droplet charge. All emulsions with starch showed a positive charge and ζ-potential values varied with the starch concentrations between 7 and 12 mV, and between 2 and 7 mV, for emulsions containing 5% oil and 30% oil, respectively.

It is considered that the larger ζ-potential values indicate a higher electrostatic repulsion between the droplets that can increase emulsion stability. Regarding the ζ-potential values obtained (Figures 4 and 9) better stability of CMC emulsions can be expected. However on visual observation, CMC emulsions showed a gravitational separation over a storage period of 10 days and starch emulsions did not show clear signs of gravity creaming during the 40 days of storage. Other authors have pointed out that ζ-potential by itself could not predict emulsion stability [17]). As commented by Dickinson [26], the unreliability of the ζ-potential as an indicator of relative emulsion stability partially arises because the classical double-layer theory used to estimate ζ values assumes a solid charged particle which moves on a well defined plane of shear. In some hydrocolloid-coated emulsions, the plane of shear can be ill-defined and the droplets cannot be considered as simple charged spheres.

Microstructure

Micrographs of control samples in Figures (8a and 8b) show how the oil concentration affected fat droplet distribution across the continuous phase. In absence of thickeners, the sample with 5% oil exhibited small fat droplets separated and distributed across the image, being apparently stable to the instability mechanisms; whilst the oil droplets in the sample with 30% oil were more concentrated and closer.

When CMC was added to emulsions made with 5% oil, the fat droplets were closely associated with one another and clumped together in certain regions (lighter zone) (Figure 8c, 8e and 8g). By increasing CMC concentration the volume occupied by the CMC and oil droplet aggregates increased, giving rise to a more structured system. These observations are in accordance with particle size results, since CMC addition to 5% oil emulsions led to an increase in particle size due to aggregation of fat droplets. When CMC was dispersed in 30% oil emulsions, the formation of a network-like structure could be observed with part of the fat droplets distributed among the CMC chains and another part dispersed in the continuous phase (Figure 8d, 8f and 8h).

Figure 9 shows the microstructure of control and starch-based emulsions. When starch was added to the emulsions, a globular structure was detected (darker zone). Emulsions with 5% oil and starch exhibited floccules of fat droplets closely clumped together (lighter zone) with starch granules among them (Figure 9c, 9e and 9g). In the case of 30% oil emulsions, the addition of the highest starch concentrations (3 and 4%) had an effect on fat droplet distribution (Figure 9f and 9h). Fat droplet floccules decreased and droplet distribution across the continuous phase was more homogenous. Finally, and as expected, the amount of isolated and swollen starch granules increased with starch concentration at both oil concentrations.

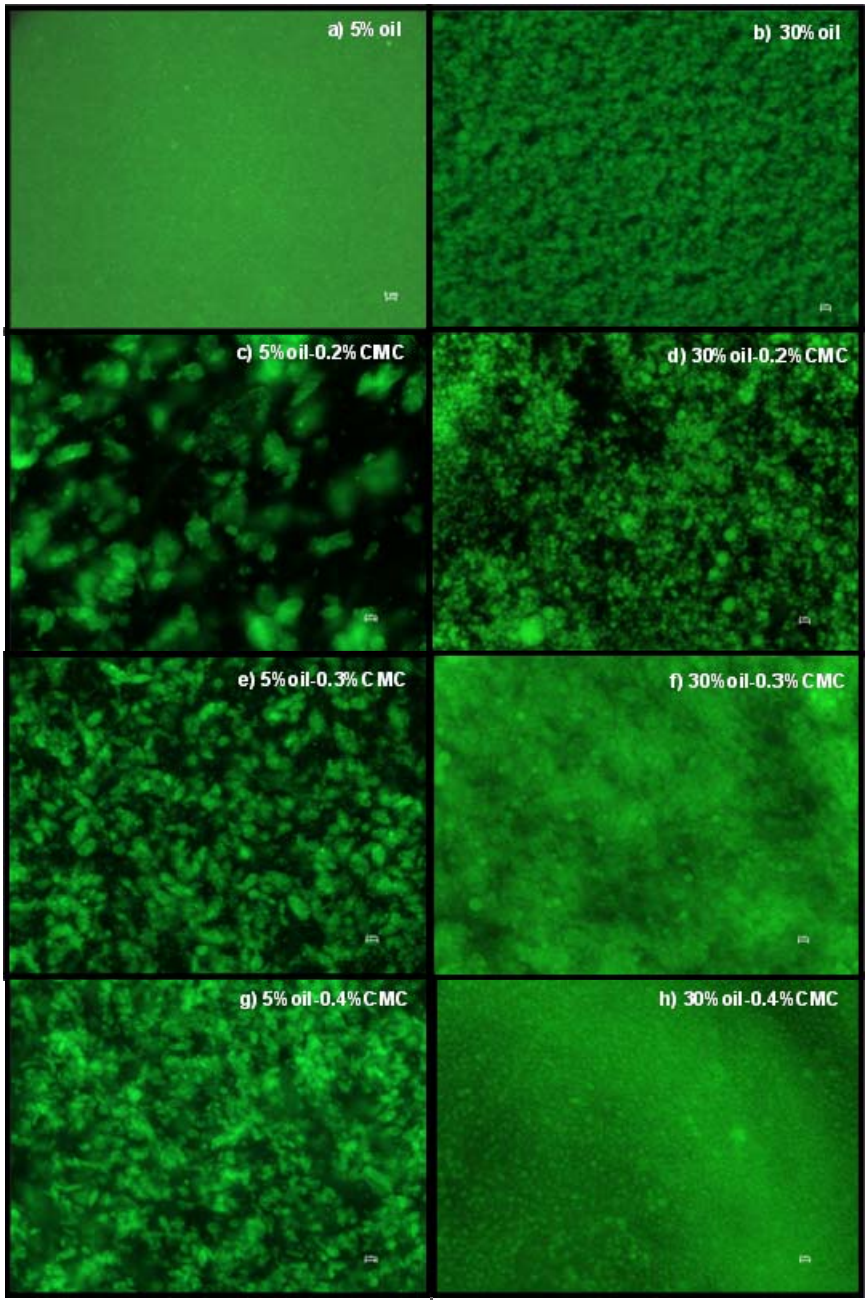

Figure 8. Microscopy images of o/w emulsions with 5% oil (a, c, e and g) and 30% oil (b, d, f and h) at different CMC concentrations (0, 0.2, 0.3 and 0.4%). Micrographs taken at 40x magnification, 10ºC, scale bars to 5 μm, and stained with Nile red.

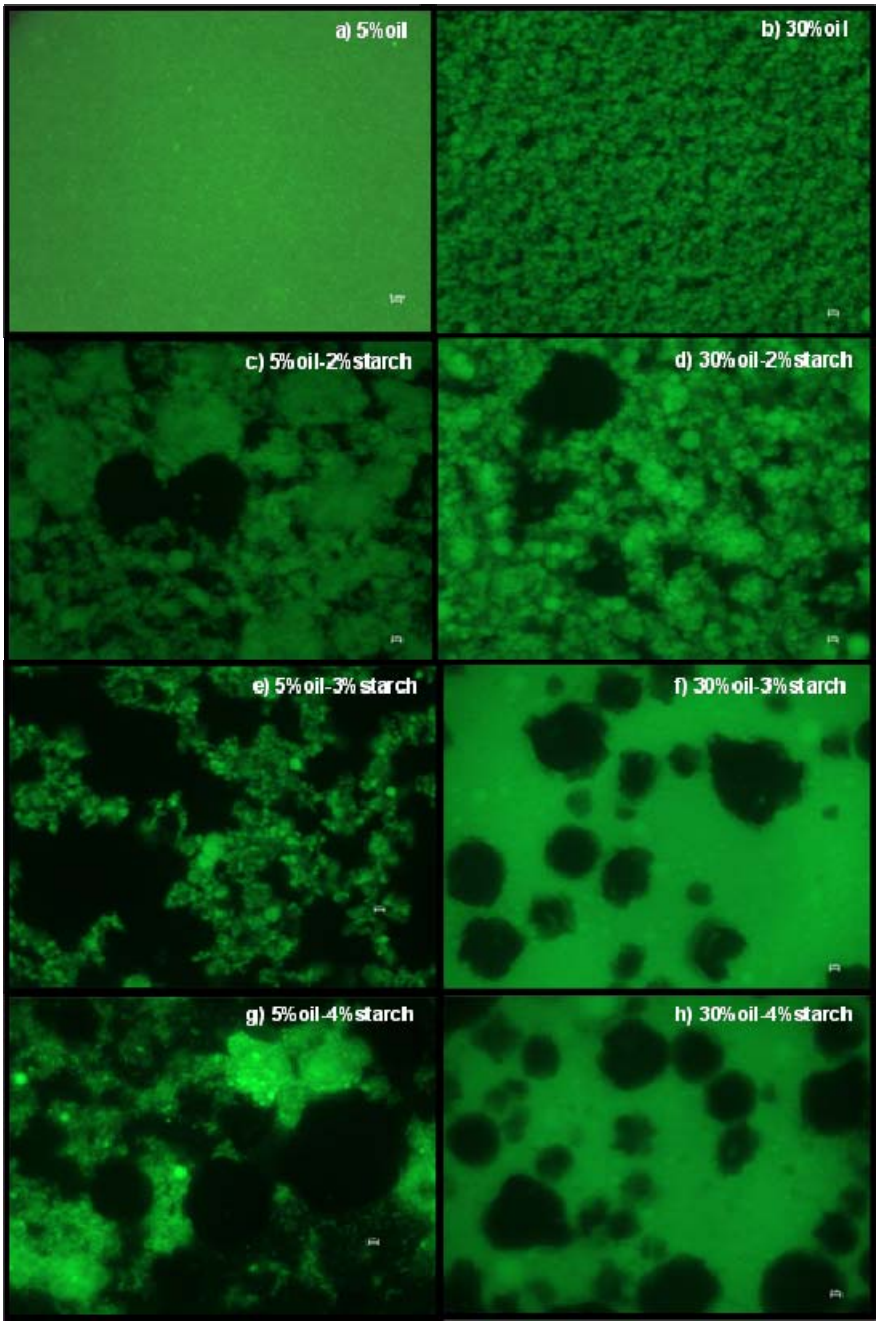

Figure 9. Microscopy images of o/w emulsions with 5% oil (a, c, e and g) and 30% oil (b, d, f and h) at different starch concentrations (0, 2, 3 and 4%). Micrographs taken at 40x magnification, 10ºC, scale bars to 5 μm, and stained with Nile red.

Conclusions

It can be concluded that, despite the possibility of obtaining food emulsions of similar rheological behavior by adding different hydrocolloids, it is necessary to analyze thickener effect on other emulsion properties in order to obtain food emulsions with adequate stability and characteristics. The results obtained in this work indicate that not only is the effect of the addition of different thickeners relevant but also the effect of oil content and of the interaction between them. In general both CMC macromolecules and swollen starch granules showed a dominating effect on emulsion flow behavior, although concentration of fat droplets also played an important role. A different situation was observed in emulsion viscoelasticity. In CMC-based emulsions, the most influential factor was the oil concentration, whilst in starch-based emulsions viscoelasticity mainly depended on starch concentration. A similar situation was detected related to particle size distribution; CMC effect was dependent on oil content and starch effect was mainly related to the volume occupied by swollen granules. Differences observed between microstructure and particle size distribution in CMC and starch emulsions were clearly related to their rheological behavior. More information about the relationships between composition, structure and physical properties of emulsions is required to design new and better food emulsions.

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CAPÍTULO 2

FLOW PROPERTIES AND VISCOELASTICITY OF HIGH-PROTEIN SOY DESSERTS. EFFECT OF HYDROCOLLOID

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Abstract

The objective of this work was to study the influence of two thickening agents with different structural characteristics, a modified starch and carboxymethyl cellulose (CMC), on the rheological properties -flow behaviour and viscoelasticity- and on two instrumental indices of oral thickness of high-protein desserts. Different formulations were prepared at two soy protein isolate (SPI) concentrations (6 and 8% w/w), each with four starch concentrations (2, 2.5, 3 and 3.5% w/w) or four CMC concentrations (0.3, 0.5, 0.7 and 0.9% w/w). Two more samples without added thickener were prepared as control samples. The amounts of sugar (4% w/w), citrus flavour (0.01% w/w) and colorant (0.026%) remained fixed. Experimental flow data for the control sample (without added thickener) were fitted to the Ostwald-de Waele model ($0.98 < R^2 < 0.99$), and the flow curves of samples with thickener were fitted to the Herschel-Bulkley model ($R^2 > 0.99$). The flow curves of all the systems showed a typical shear-thinning behaviour and observable hysteresis loops. The viscoelastic properties of the systems ranged from fluid-like to a weak gel, depending on thickener and SPI concentration. The effects of the interaction between protein content and hydrocolloid concentration on flow and viscoelastic parameter values were significant. Soy protein concentration showed a dominating effect on flow time dependence, yield stress value and storage modulus, although hydrocolloid concentration also played an important role. Classification of samples according to their thickness may be dependent on the instrumental index used.

Keywords: soy desserts, carboxymethyl cellulose, starch, flow properties, viscoelasticity

1. Introduction

The inclusion of soy products as part of the regular diet provides many health benefits. Apart from the reduction in the risk of coronary heart disease and breast and prostate cancers (Barnes, 2006; Xiao, 2008), there is growing general interest in products with protein from vegetable sources in order to obtain a more balanced ratio of vegetable/animal protein intake (Russell et al., 2006). In addition to the health benefits that they afford, soy beverages and desserts can also be an interesting alternative for consumers who are lactose intolerant or allergic to milk or dairy products. The main problem in the acceptance of these products is related to their flavour and to some undesirable textural characteristics (Chambers et al., 2006). To increase their acceptability one must gain a better understanding of their chemical, physical and sensory characteristics and of how each of these factors influences the final response of the consumer. To improve acceptance of soy products several authors have tried to solve flavour and taste drawbacks using different approaches (Yuang & Chang, 2007; Endo et al., 2004; Granato et al., 2010; Chattopadhyay et al., 2013). Less attention has been paid to producing texturally attractive soy products, although various stabilizers, such as gelatin, pectin or gum acacia, have been proposed to improve this attribute (Mepha et al., 2006; Potter et al., 2007).

The nutritional and textural characteristics of soy desserts favour their consumption by several groups of consumers, such as children or older people. Basically they are formulated with soy milk or protein isolates, hydrocolloids, sucrose, aroma and colorants, and the characteristics of each ingredient, their concentrations and the effects of the interactions among them influence the product structure to different extents and also modify its rheological, mechanical and textural attributes.

The structural, physical and sensory features of soy desserts mainly depend on their protein content and on the characteristics and concentrations of the

hydrocolloids added. With regard to protein content, most currently available commercial soy products are formulated with soy protein contents (less than 3g/100g) lower than the amount recommended by various public institutions. For example, the FDA requires at least 6.25 g/serving of soy protein to state a health claim on its label (Walker et al., 2010). Therefore, taking adequate protein content into account can be important when designing soy protein dessert formulations to increase the present range of commercial soy desserts. An important function of hydrocolloids in protein matrices is that they can act as structuring, thickening and stabilizing agents. Starch is one of the most widely used thickeners in the food industry, although carboxymethyl cellulose (CMC) is also being used as an alternative to starch in food products because of its technological and nutritional advantages (Engelen et al., 2005; Bayarri et al., 2010). When starch is heated in water the starch granules swell, and after cooling a viscous paste is formed, with a biphasic structure consisting of swollen starch granules in a continuous phase. Modified starch shows higher thermomechanical resistance, and, if the granules remain whole after pasting, the resultant system is an aqueous dispersion whose rheological behaviour mainly depends on the granules' volumetric fraction and on their rigidity or deformability (Tattiyakul & Rao, 2000; Nayouf et al., 2003). In mixed biopolymer systems, soybean protein could interact with amorphous regions of starch granules, favouring a mutual exclusion effect or modifying water availability for starch (Colombo et al., 2011). Sodium carboxymethyl cellulose (CMC) is a linear, long-chain, water-soluble, anionic polysaccharide widely used in the food industry because it is tasteless, odourless, and forms clear solutions without cloudiness or opacity. It dissolves rapidly in both hot and cold aqueous systems, acts as a moisture binder, emulsion stabilizer and thickener, and improves the texture of a wide range of food products. When cellulose gum is added to systems containing protein, it can cause unexpected effects on their rheological properties, texture and stability (Syrbe et al., 1998). In

mixed protein-polysaccharide systems, associative electrostatic interactions can lead to coacervation or soluble complex formation, depending on the solution conditions (pH and ionic strength) (Dickinson, 2003). Considering the structural differences observed in CMC and starch solutions (Ferry et al., 2006) and break-up mechanisms under shear (Desse et al., 2011), the two thickeners would be expected to affect the physical and sensory characteristics of food matrices differently. This hypothesis seems to be confirmed in a recent work (Arancibia et al., 2013a) about the effects of adding different CMC and starch concentrations on the rheological behaviour of oil-in-water emulsions with different fat contents. We observed that both swollen starch granules and CMC macromolecules showed a dominating effect on emulsion flow behaviour, although the concentration of fat droplets also played an important role. A different situation was observed in emulsion viscoelasticity. In CMC-based emulsions, the most influential factor was the oil concentration, while in starch-based emulsions viscoelasticity mainly depended on starch concentration.

In order to gain a better understanding of the influence of different hydrocolloids on the rheological properties of enriched soy protein desserts, the aim of this work was to study the effects of starch and CMC concentrations on the flow behaviour and viscoelasticity of soy protein desserts formulated with two soy protein isolate concentrations.

2. Material and Methods

2.1 Composition and preparation of samples

Soy-based desserts were prepared with powdered soy protein isolate (SPI) (90% protein; PROSOL 90-GF NT, Cargill, Barcelona, Spain) and a medium crosslinked modified tapioca starch (C* Creamtex 75720, Cargill Ibérica SL, Barcelona, Spain) or carboxymethyl cellulose (CMC) (Akucell AF3265

Akzo Nobel, Amersfoort, The Netherlands) as thickeners. Deionized water, commercial sucrose, citrus flavour (Naranja 1594A, Lucta, Barcelona, Spain) and yellow-orange colorant (NC2 SX WS mct, CHR Hansen S.A., Barcelona, Spain) were also used.

Different formulations were prepared at two SPI concentrations (6 and 8% w/w, corresponding to 6.75 and 7.9 g protein/125 g product), each at four starch concentrations (2, 2.5, 3 and 3.5% w/w) or four CMC concentrations (0.3, 0.5, 0.7 and 0.9% w/w). Two more samples without added thickener were prepared as control samples. The amounts of sugar (4% w/w), citrus flavour (0.01% w/w) and colorant (0.026% w/w) remained fixed.

Soy-based desserts were prepared in batches of 800 g. Soy protein isolate powder was dispersed in deionized water and mixed for 15 min, with the help of a propeller stirrer (Heidolph RZR 1, Heidolph, Schwabach, Germany). The dispersion was placed in a water bath at 90 ± 1 °C and was stirred for 20 min. Then it was cooled in a bath at 10 °C for 10 min and stored at 4 ± 1 °C overnight to ensure complete hydration of soy proteins. The amount of water evaporated in the heating process was replaced gravimetrically. The following day, starch or CMC was added to the SPI dispersion. Starch samples were prepared as follows: starch, sugar, colorant and soy protein dispersion were mixed with the help of a propeller stirrer in a flask for 10 min at room temperature. Afterwards, the flask was placed in a water bath at 90 ± 1 °C and stirred constantly for 25 min. Samples were cooled in a water bath at 10 °C for 10 min. Any water evaporated was replaced gravimetrically. Citrus flavour was added and samples were stirred for 5 min. For CMC sample preparation, a blend of CMC and sugar was added to the SPI dispersion with the colorant and stirred for 35 min at room temperature. Five minutes before the end, citrus flavour was added. All samples were transferred to a closed flask and stored (4 ± 1 °C; 24 h) prior to

rheological measurements. At least two batches of each composition were prepared.

2.2 Rheological measurements

Rheological measurements were carried out in a controlled stress rheometer RS1 (Thermo Haake, Karlsruhe, Germany), using parallel-plate geometry (60 mm diameter; 1 mm gap) and monitored with the RheoWin Job software package (version 3.61, Thermo Haake, Karlsruhe, Germany). A sample temperature of 10 ± 1 °C was maintained during measurements by means of a Phoenix P1 Circulator device (Thermo Haake, Karlsruhe, Germany). Each batch was measured at least in duplicate, using a fresh sample for each measurement. After loading the sample, it was allowed to stand for 10 min to stabilize and reach the desired temperature. After carefully placing the sample between the plates, excess material was wiped off with a spatula.

2.2.1 Flow properties

Sample flow was measured by recording shear stress values when the samples were sheared at linearly increasing shear rates from 1 to 200 s^{-1} for 60 s and down in reverse sequence for the same time (González-Tomás & Costell, 2006). Experimental data from descending flow curves of control samples were fitted to the Ostwald-de Waele model (Eq. 1) and data of samples with thickener added were fitted to the Herschel-Bulkley model (Eq. 2). These calculations were performed using RheoWin Pro Data software (version 3.61, Thermo Haake, Karlsruhe, Germany).

$$\sigma = K\dot{\gamma}^{n} \qquad \text{Eq. 1}$$

$$\sigma = \sigma_0 + K\dot{\gamma}^{n} \qquad \text{Eq. 2}$$

where σ (Pa) is the shear stress, $\dot{\gamma}$ (s^{-1}) is the shear rate, σ_0 (Pa) is the yield stress, K (Pa s^n) is the consistency index and n is the flow index. These parameters were used to characterize the flow behaviour of the samples. Since parameter K units depend on n values, apparent viscosity values at 1 s^{-1} (η_1) were used to compare sample consistency statistically (Yanes et al., 2002).

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 the RheoWin Pro software (Thermo Haake). In view of the influence of the loop contour and of the shear resistance of the sample on the hysteresis area, the percentage of relative hysteresis area (A_R) (Eq. 3) was calculated because it allows a better comparison of flow time dependence behaviour of different samples (Dolz et al., 2000).

$$A_R = \frac{A_{up} - A_{down}}{A_{up}} \times 100 \qquad \text{Eq. 3}$$

In time-dependent and non-Newtonian shear-thinning products, perceived thickness is difficult to predict with rheological parameter values because flow in the mouth is probably a combination of shear and elongational flow (van Vliet, 2002). However, some authors have found that oral thickness correlates with different rheological indices. It has been confirmed that one of them, apparent viscosity at a shear rate of 50 s^{-1}, suggested by Wood in 1968, has practical utility as a possible instrumental index of thickness perceived in semisolid foods (Cook et al., 2003; Arancibia et al., 2013b). Consequently, apparent viscosity values at a shear rate of 50 s^{-1} (η_{50}) were also calculated.

2.2.2 Viscoelasticity

Prior to mechanical spectrum measurements, it was necessary to determine the linear viscoelastic region (i.e. the range where G' and G'' are independent of the stress/strain of the oscillation). Stress sweeps were run between 0.02 to 300 Pa at a frequency of 1 Hz in all systems. The frequency sweeps were performed from 0.01 to 10 Hz at 0.05 Pa. The oscillatory rheological parameters used to compare the viscoelastic properties of the samples were storage modulus (G'), loss modulus (G"), loss angle (Tan δ) and complex dynamic viscosity (η*) at 1 Hz. This frequency, as stated by Lapasin & Pricl (1995) would appear to be a reasonable compromise between measuring very fast, when intermolecular entanglements could contribute to solid-like responses, and measuring at extremely low frequencies, when loss of precision and reliability in the measurements could be a problem. For semisolid products with viscoelastic behaviour, some authors have obtained a good correlation between oral thickness and small deformation measurements at an oscillatory frequency of 50 rad s^{-1} (Richardson et al., 1989; Hill et al., 1995, Tárrega & Costell, 2007). Consequently, complex dynamic viscosity values were determined at 8 Hz, equivalent to 50 rad s^{-1} (η_{8Hz}).

2.3 Experimental design and data analysis

An experimental design with two factors, soy protein isolate concentration (two levels) and hydrocolloid concentration (five levels), was used. The effects of the two factors and the interaction between them on the flow and on the viscoelastic parameter values were analysed by a two-way ANOVA. Significant differences between individual samples were determined by the Tukey test ($\alpha = 0.05$). To obtain information about possible sample groups with different thickness levels, Cluster Analysis (Clustering Ward Method)

(Vigneau & Qannari, 2002) was applied to apparent viscosity values at a shear rate of 50 s^{-1} and to complex dynamic viscosity values at 8 Hz of all samples with thickener added. All these calculations were carried out with XLSTAT Pro software, version 2007 (Addinsoft, Paris, France).

3. Results and discussion

3.1 Flow behaviour

All samples showed hysteresis loops when they were sheared during a complete cycle, indicating that the sample flow was time-dependent (Figure 1). The variation of the shear stress values with the shear rate indicated a non-Newtonian shear-thinning flow. For the samples made with SPI at any concentration, as the starch concentration increased the thixotropic area and the consistency of the system also increased. This behaviour was in accordance with what was observed by other authors when studying the effect of starch concentration on time-dependent behaviour of water-starch pastes (Tattiyakul & Rao, 2000) and on starch-milk model systems (Abu-Jdayil et al., 2004; Tárrega et al., 2005). This could be explained by taking into account the fact that the structure of the system is affected by an increase in the volumetric fraction of the dispersed phase partially constituted by swollen starch granules. A greater difference in both form and magnitude of the hysteresis loops was observed among samples containing 8% SPI (Figure 1b). Assuming that hysteresis loop area is an index of the energy required to destroy the structure responsible for flow-time dependence (Halmos & Tiu, 1981), the results showed that when starch concentration was increased to 2%, 8% SPI samples needed a higher energy for breakdown of this structure than 6% SPI samples (Figure 1a). This seems to indicate that for these samples a different structure could be formed, reflecting changes in the two system phases which have different biopolymer

compositions (McClements et al., 2009). Moreover, samples with 8% SPI and starch content over 2% showed an evident overshoot in the stress-rate curves, indicating that the system structure broke down partially as a result of increasing shearing time (Figure 1b).

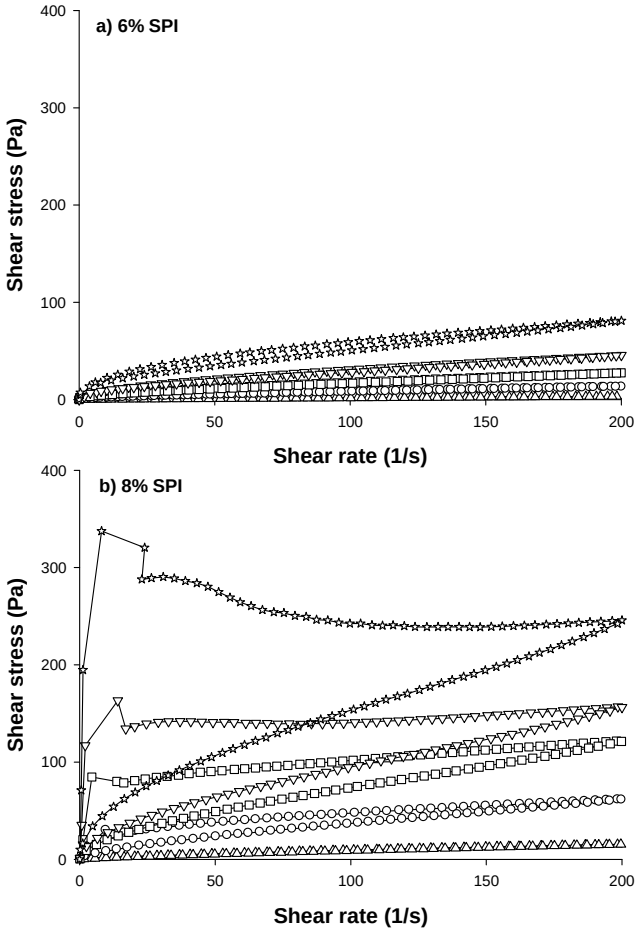

Figure 1. Flow curves of soy-based desserts containing 6% (a) or 8% (b) of soy protein isolate (SPI) and different starch concentrations (△:0%, ○:2%, □: 2.5%, ▽: 3% and ☆: 3.5%)

The maximum in the stress may represent the yielding transition from a continuous network to a discontinuous state at this point (Mujumdar et al., 2002). Similar flow behaviour was observed by Lizarraga et al. (2006) using whey protein concentrate and λ-carrageenan aqueous mixtures. They interpreted that the overshoot in flow curves at low shear rates, when WPC concentration increases, might be due to the effect of interactions between the two biopolymers. Another possibility is that the starch addition effect could be related to a mutual exclusion between starch and SPI that affects the water partition between the two phases, increasing the effective concentration of both biopolymers (Colombo et al., 2011). At the lowest SPI or starch concentrations, the soy dessert samples were relatively deformable by shearing and the flow curves did not show any overshoot. This behaviour could indicate that the two biopolymers behave as individual molecules that are distributed throughout the one-phase system (McClements et al., 2009).

The flow curves of control samples fitted well to the Ostwald-de Waele model ($0.98 < R^2 < 0.99$). Samples with added starch showed shear thinning with an initial resistance to flow consistent with the Herschel-Bulkley model ($R^2 > 0.99$). ANOVA results showed a significant effect ($P < 0.05$) of interaction between SPI and starch concentration on flow parameter values (Table 1). This interaction indicated that the starch concentration effect differed, depending on the SPI content. As expected, σ_0 and η_1 values increased significantly with starch concentration, this variation being higher in samples prepared with 8% SPI (Table 2). Except for the control sample with 6% SPI ($n = 0.90$), the flow index values varied between 0.68 and 0.78, indicating a clear pseudoplastic behaviour. In general, an increase in the starch concentration slightly decreased n values, while changes in SPI concentration had a significant effect on pseudoplasticity only when starch was present. The effect of SPI content on A_R values was more evident than the effect of starch concentration, as reflected by the corresponding F values

(Table 1). The control sample with 8% SPI showed significantly higher A_R values than its counterpart prepared with 6% SPI, and the addition of starch to the soy dispersion had a more noticeable effect at the higher SPI concentration.

Table 1. Two-way ANOVA of rheological parameters[1] of soy-based desserts containing different concentrations of soy protein isolate (SPI) and starch. F and P values

	Main effects				Interactions	
	A: SPI concentration		B: Starch concentration		A × B	
	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
Flow parameters						
σ_0 (Pa)	1186.3	<0.01	161.31	<0.01	148.59	<0.01
n	448.18	<0.01	268.95	<0.01	40.91	<0.01
η_1 (Pa s)	1741.8	<0.01	1996.4	<0.01	288.83	<0.01
A_R (%)	1851.9	<0.01	240.95	<0.01	86.39	<0.01
Viscoelastic parameters						
G' (Pa)	10972	<0.01	4674.0	<0.01	3670.8	<0.01
G" (Pa)	570.23	<0.01	212.41	<0.01	98.83	<0.01
Tan δ	296.50	<0.01	155.19	<0.01	36.77	<0.01
η* (Pa s)	10506	<0.01	4428.7	<0.01	3286.7	<0.01

[1] σ_0 = yield stress, n= flow index, η_1 = apparent viscosity at 1 s^{-1}, A_R = relative hysteresis area, G'= storage modulus, G"= loss modulus, Tan δ= loss angle and η= complex dynamic viscosity.*

Table 2. Mean values and significant differences of rheological parameters[1] of soy-based desserts containing different concentrations of soy protein isolate (SPI) and starch

SPI concentration (% w/w)	Starch concentration (% w/w)	*Flow parameters*				*Viscoelastic parameters*[2]			
		σ_0 (Pa)	n	η_1 (Pa s)	A_R (%)	G' (Pa)	G" (Pa)	Tan δ	η* (Pa s)
6	0	-	0.90^{a}	0.03^{g}	1.29^{f}	-	-	-	-
6	2	0.61^{f}	0.76^{b}	0.88^{g}	1.29^{f}	1.04^{e}	1.20^{e}	1.15^{a}	0.25^{f}
6	2.5	2.04^{e}	0.74^{cde}	2.52^{f}	3.78^{ef}	3.00^{e}	2.68^{e}	0.90^{c}	0.64^{ef}
6	3	4.82^{d}	0.75^{cd}	5.51^{e}	6.98^{de}	5.06^{e}	4.73^{de}	0.94^{bc}	1.10^{e}
6	3.5	11.06^{c}	0.74^{cde}	12.08^{c}	11.16^{d}	12.15^{d}	9.70^{cd}	0.80^{cd}	2.48^{d}
8	0	-	0.78^{b}	0.24^{g}	5.35^{ef}	-	-	-	-
8	2	1.87^{e}	0.72^{de}	3.19^{f}	22.82^{c}	4.79^{e}	5.16^{de}	1.08^{ab}	1.12^{e}
8	2.5	5.01^{d}	0.72^{ef}	7.14^{d}	30.39^{b}	17.31^{c}	11.78^{c}	0.69^{d}	3.33^{c}
8	3	12.53^{b}	0.70^{fg}	16.58^{b}	32.25^{b}	78.47^{b}	28.38^{b}	0.36^{e}	13.29^{b}
8	3.5	20.05^{a}	0.68^{g}	26.08^{a}	42.57^{a}	178.7^{a}	48.66^{a}	0.27^{e}	29.48^{a}

[a-g] Means within a column with common superscripts did not differ significantly (P<0.05)

[1] σ_0 = yield stress, n= flow index, η_1 = apparent viscosity at 1 s^{-1}, A_R = relative hysteresis area, G'= storage modulus, G"= loss modulus, Tan δ= loss angle and η= complex dynamic viscosity*

[2]Viscoelastic properties of soy-based desserts without starch could no be measured, due fluidity of the samples

The flow curves of the CMC-based systems also exhibited a time-dependent and shear thinning with yield stress behaviour (Figure 2). As previously mentioned, control samples for both SPI concentrations did not show yield stress. Measurements of increasing and decreasing shear rate showed hysteresis loops in most samples, indicating thixotropic behaviour. Samples with the higher SPI concentration needed higher energy to break its structure as compared with their counterparts formulated with the lower SPI content (Figures 2a and 2b, respectively).

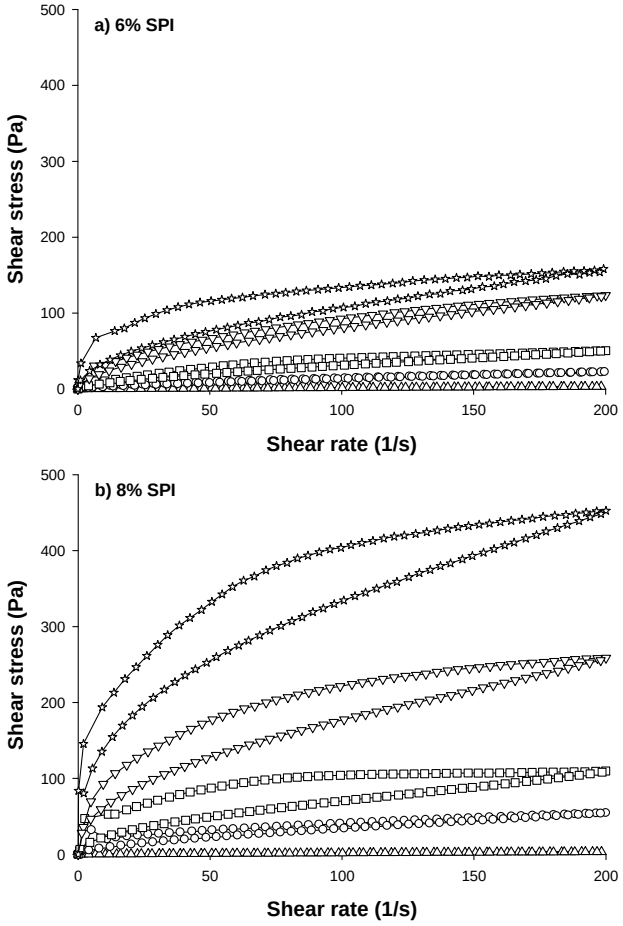

Figure 2. Flow curves of soy-based desserts containing 6% (a) or 8% (b) of soy protein isolate (SPI) and different CMC concentrations (△:0%, ○:0.3%, □: 0.5%, ▽: 0.7% and ☆: 0.9%)

This seems to indicate that the flow behaviour of these systems was affected by both SPI and CMC concentrations without the influence of any qualitative structure change or phase separation. In mixed biopolymer systems that contain a protein and an anionic polysaccharide, their possible association is strongly dependent on pH. In this case, the pH of the soy-based samples, which ranged between 6.25 and 6.37, was clearly above the isoelectric point of soy protein (pH ~ 4.5). In this situation, the protein and the polysaccharide have a negative charge and an electrostatic repulsion between them prevents their molecular assembly (McClements et al., 2009). Flow data of different CMC-based samples were fitted well to the Herschel-Bulkley model, with R^2 values >0.99. Two-way ANOVA results showed that both CMC and SPI concentrations, as well as their interaction, had a significant effect on all flow parameters (Table 3). As expected, σ_0, η_1 and A_R values increased with CMC and SPI concentration, while n values decreased, indicating an increase in the shear-thinning behaviour of the samples. With respect to σ_0, it was observed that the CMC concentration effect was different depending on SPI content. In samples with 6% SPI, σ_0 values varied slightly with CMC concentration, while in samples with 8% SPI an increase in CMC concentration significantly increased σ_0 values. Flow index values decreased significantly with CMC concentration from 0.9 to 0.5 and from 0.78 to 0.45 in samples with 6% and 8% SPI, respectively (Table 4). These results are in agreement with the hypothesis that these systems behave as molecular dispersions of the protein and CMC molecules without any further association between them. The increase in flow pseudoplasticity can be mainly explained by the orientation of the CMC macromolecules and how they align in the direction of the shearing force (Rozema & Beverloo, 1974). Samples with the lowest CMC concentrations (under 0.5%) did not significantly differ for η_1 values among most of the samples, regardless of SPI content, but when CMC concentration was

increased (over 0.5%) η_1 values increased significantly, showing clear differences between different soy dessert samples with different SPI content. This may be due to the increase in the protein volumetric fraction of the dispersed phase and to the distribution and interaction of CMC chains. At lower CMC concentrations, the CMC chains are in their most extended conformation and at higher polymer concentrations extended CMC chains start to overlap and could become entangled, increasing the plasticity and the consistency of sample flow.

Table 3. Two-way ANOVA of rheological parameters[1] of soy-based desserts containing different concentrations of soy protein isolate (SPI) and carboxymethyl cellulose (CMC). F and P values

	Main effects				Interactions	
	A: SPI concentration		B: CMC concentration		A × B	
	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
Flow parameters						
σ_0 (Pa)	587.67	<0.01	211.06	<0.01	116.67	<0.01
n	432.47	<0.01	900.91	<0.01	14.24	<0.01
η_1 (Pa s)	2629.34	<0.01	1904.76	<0.01	843.40	<0.01
A_R (%)	441.04	<0.01	222.85	<0.01	7.93	<0.01
Viscoelastic parameters						
G' (Pa)	1101.3	<0.01	749.23	<0.01	373.26	<0.01
G" (Pa)	293.73	<0.01	284.56	<0.01	67.89	<0.01
Tan δ	682.48	<0.01	489.06	<0.01	53.89	<0.01
η* (Pa s)	959.09	<0.01	679.01	<0.01	317.87	<0.01

[1] *σ_0 = yield stress, n= flow index, η_1 = apparent viscosity at 1 s^{-1}, A_R = relative hysteresis area, G'= storage modulus, G"= loss modulus, Tan δ= loss angle and η*= complex dynamic viscosity.*

Table 4. Mean values and significant differences of rheological parameters[1] of soy-based desserts containing different concentrations of soy protein isolate (SPI) and carboxymethyl cellulose (CMC)

SPI concentration (% w/w)	CMC concentration (% w/w)	*Flow parameters*				*Viscoelastic parameters*[2]			
		σ_0 (Pa)	n	η_1 (Pa s)	A_R (%)	G' (Pa)	G" (Pa)	Tan δ	η^* (Pa s)
6	0	-	0.90^{a}	0.03^{e}	1.29^{i}	-	-	-	-
6	0.3	0.12^{e}	0.78^{b}	0.46^{e}	8.56^{gh}	1.03^{d}	1.62^{e}	1.59^{a}	0.31^{e}
6	0.5	1.15^{de}	0.67^{c}	2.55^{e}	11.29^{fg}	6.13^{d}	5.49^{e}	0.89^{b}	1.31^{e}
6	0.7	3.55^{de}	0.61^{d}	8.41^{d}	13.52^{ef}	25.35^{c}	14.80^{cd}	0.58^{c}	4.67^{d}
6	0.9	4.37^{cd}	0.53^{e}	13.51^{c}	17.66^{de}	43.61^{c}	24.17^{c}	0.55^{c}	7.94^{c}
8	0	-	0.78^{b}	0.24^{e}	5.35^{hi}	-			
8	0.3	1.61^{de}	0.68^{c}	3.01^{e}	18.62^{cd}	6.34^{d}	5.20^{e}	0.82^{b}	1.31^{e}
8	0.5	8.38^{c}	0.63^{d}	12.07^{c}	22.28^{bc}	33.59^{c}	15.41^{cd}	0.46^{cd}	5.88^{cd}
8	0.7	17.16^{b}	0.55^{e}	30.04^{b}	24.54^{ab}	96.97^{b}	33.58^{b}	0.35^{de}	16.34^{b}
8	0.9	31.51^{a}	0.45^{f}	69.19^{a}	28.49^{a}	251.9^{a}	70.80^{a}	0.28^{e}	41.64^{a}

[a-e] Means within a column with common superscripts did not differ significantly ($P<0.05$)

[1] σ_0 = yield stress, n= flow index, η_1 = apparent viscosity at 1 s^{-1}, A_R = relative hysteresis area, G'= storage modulus, G"= loss modulus, Tan δ= loss angle and η^*= complex dynamic viscosity

[2] Viscoelastic properties of soy-based desserts without CMC could no be measured, due fluidity of the samples

3.2 Viscoelastic properties

The viscoelastic characteristics of the control samples could not be measured because of their fluidity. As expected, the viscoelastic properties of the remaining samples depended on SPI and thickener concentration. Mechanical spectra obtained for samples with starch are shown in Figure 3. In general, the viscoelastic behaviour ranged from fluid-like to weak-gel-like, depending on sample composition.

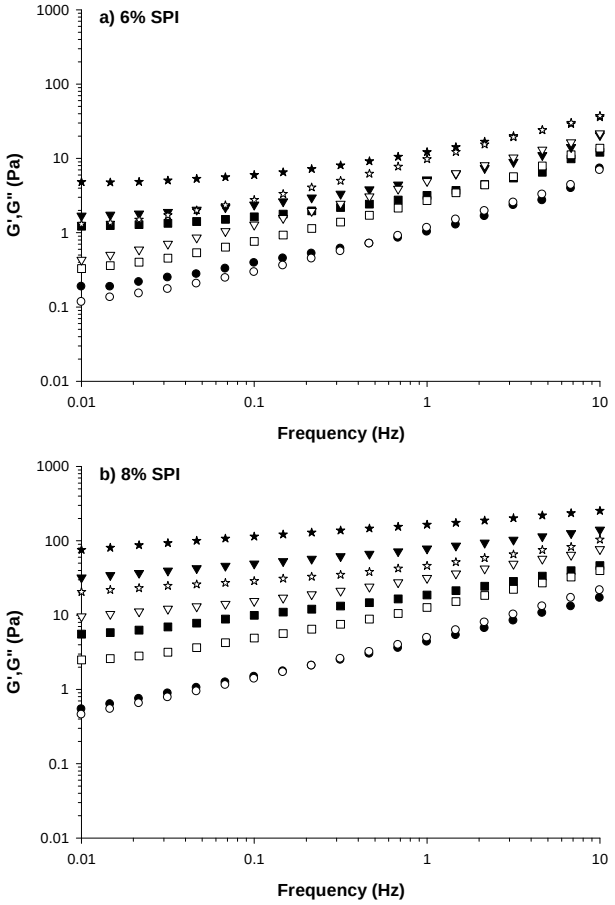

Figure 3. Mechanical spectra of soy-based desserts containing 6% (a) or 8% (b) of soy protein isolate (SPI) and different starch concentrations (○:2%, □ : 2.5%, ▽: 3% and ☆: 3.5%)

For both SPI concentrations, samples with the lowest starch concentration (2%) showed a strong frequency dependence on dynamic moduli with loss modulus (G") over storage modulus (G'), indicating a fluid-like behaviour. When the starch concentration was increased, the mechanical spectra changed markedly, depending on the SPI concentration. Samples with 6% SPI at the intermediate starch concentrations (2.5 and 3%) showed a concentrated polymeric solution behaviour and a lesser frequency dependence. The sample with 6% SPI and the highest starch concentration (3.5%) and most of the 8% SPI samples exhibited viscoelastic properties usually observed for weak-gel systems: elastic response (G') predominated over viscous response (G") and both dynamic moduli showed a slight variation with oscillation frequency. These results suggest that the viscoelastic behaviour of the starch-based soy protein desserts depended not only on the volumetric fraction occupied by the swollen starch granules but also on the SPI concentration. Results obtained from ANOVA showed that both factors, as well as their interaction, had a significant effect ($P < 0.05$) on all parameters studied (Table 1). This interaction indicated that the starch concentration effect on viscoelastic parameters was different, depending on SPI content. In general, G', G" and η* values increased with starch concentration, and this increase was more evident in samples with the higher SPI concentration (Table 2). At the lower starch concentrations (<3.5%), samples with 6% SPI did not show significant differences in viscoelastic parameter values among them, while in samples with 8% SPI, an increase in the starch concentration significantly increased the viscoelastic parameter values, except for tan δ that decreased. At the lowest starch concentration (2%), viscoelastic parameter values of samples with different SPI concentrations did not differ significantly, except complex dynamic viscosity values. However, at starch concentrations over 2.5%, samples with 8% SPI had G', G" and η* values that were significantly higher than their counterparts made with 6% SPI. The differences observed in viscoelastic

behaviour due to starch and protein contents are in agreement with what was observed in the sample flow behaviour and could be due to the same structural changes as commented previously.

The mechanical spectra obtained for CMC-based samples are shown in Figure 4. As in the case of the starch-based samples, samples with CMC showed a viscoelastic behaviour that varied from fluid-like to weak-gel-like. Most of the samples containing 8% SPI, except the sample with the lowest CMC concentration, showed a response typical of weak gels, with a storage modulus (G') higher than loss modulus (G"), and slight frequency dependence. The sample with the lowest CMC concentration (0.3%) exhibited a behaviour typical of an entangled polymer solution, with similar values of G' and G" values. Samples with 6% SPI showed different behaviours, depending on starch concentration. At the lowest CMC concentration, they behaved as a fluid-like system. When the CMC concentration increased, the mechanical spectra changed significantly, indicating the transition from an entanglement polymer solution (samples with 0.5% CMC) to a structured system (samples with 0.7 and 0.9% CMC), and the dynamic moduli became less frequency-dependent and the elastic contribution gradually prevailed over the viscous one. ANOVA results showed a significant interaction effect (P<0.05) between the two factors considered (CMC and SPI concentration) on all parameter values (Table 3). For the same CMC concentration, samples made with 6% SPI exhibited significantly lower values of G', G" and η* and higher values of Tan δ than their counterparts made with 8% SPI, except for the lowest CMC concentration (0.3%) (Table 4). In general, raising the CMC concentration increased the G', G" and η* values and decreased Tan δ, although this effect was more evident in samples with the higher SPI content.

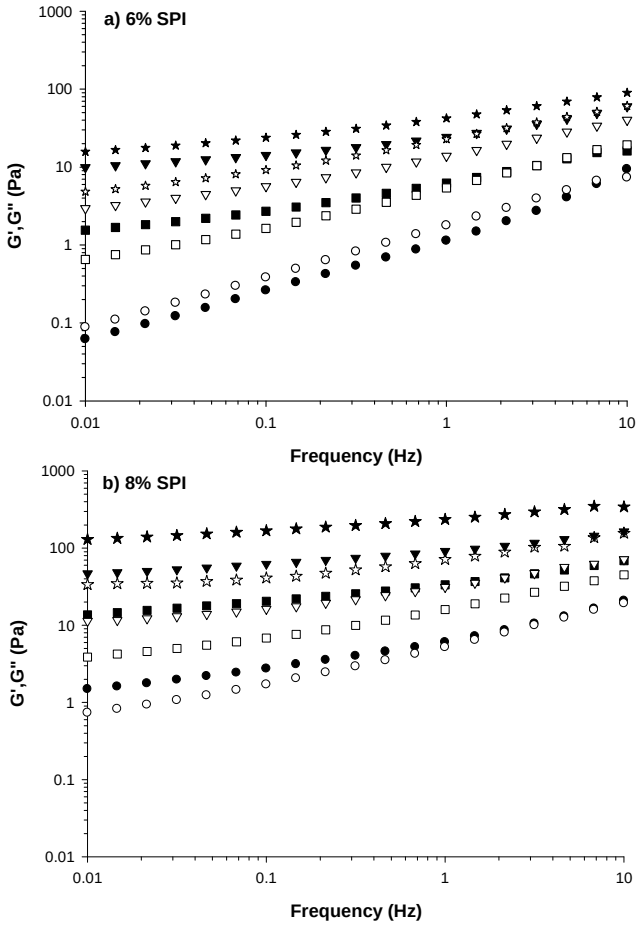

Figure 4. Mechanical spectra of soy-based desserts containing 6% (a) or 8% (b) of soy protein isolate (SPI) and different CMC concentrations (○:0.3%, □: 0.5%, ▽: 0.7% and ☆: 0.9%)

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3.3 Influence of composition on instrumental indices of oral thickness

According to the rheological results obtained, the two hydrocolloids used – starch and CMC – could be used in the formulation of high-protein soy desserts to obtain products with similar rheological behaviour. However, this does not guarantee that it will match the texture perceived. This is logical if one bears in mind that, as commented previously, flow in the mouth is

probably a combination of shear and elongational flow during ingestion and swallowing (van Vliet, 2002), and that perceived thickness depends not only on the shear forces in the mouth but also on saliva effect and mouth temperature. Therefore, rheological information alone may not be enough to explain the textural differences perceived, particularly when products with different composition and structure are being compared (González-Tomás & Costell, 2006). To obtain preliminary information about the possible influence of composition on oral thickness of soy desserts, their effect on two instrumental index values was analysed. One of them was related with their flow properties (η_{50}, apparent viscosity at 50 s^{-1}) and the other one with their viscoelastic behaviour (η_{8Hz}, complex dynamic viscosity at 8 Hz). A two-way ANOVA was used to study the combined effects of SPI content and thickener concentration on these index values. In starch-based samples, the interaction between these factors was significant for both parameters (η_{50}: $F = 211.14$, $P < 0.01$ and η_{8Hz}: $F = 679.60$, $P < 0.01$), indicating that the effect of SPI content was different, depending on starch concentration. When the variation in the two thickness index values was compared, a similar trend was observed. In general, η_{50} and η_{8Hz} increased with starch concentration for both SPI concentrations, but this increase was more evident in samples with the higher SPI concentration (Figures 5a and 6a). In these samples (with 8% SPI), a higher effect of starch on η_{8Hz} values was observed at concentrations over 2.5%. With respect to the CMC-based samples, similar results were obtained. The interaction between the two effects was also significant in the values of the two thickness indices (η_{50}: $F = 1310.6$, $P < 0.01$ and η_{8Hz}: $F = 296.5$, $P < 0.01$). In the case of the η_{8Hz} parameter, at the lowest CMC concentration no significant differences were found between samples with the same CMC concentration and different SPI contents, but when the CMC concentration was increased over 0.3% significant differences were observed (Figures 5b and 6b). The good correlation obtained between the two index values ($R^2 = 0.92$) seems to

confirm the utility of these instrumental measurements to predict perceived consistency or thickness in this type of semisolid product (Cook et al., 2003; Arancibia et al., 2013b).

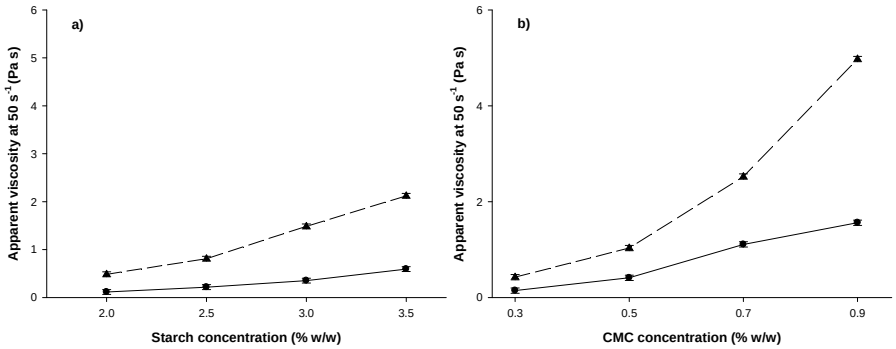

Figure 5. Apparent viscosity at 50 s^{-1} values of soy desserts with starch (a) or CMC (b) containing 6% (○) or 8% (△) of soy protein isolate (SPI)

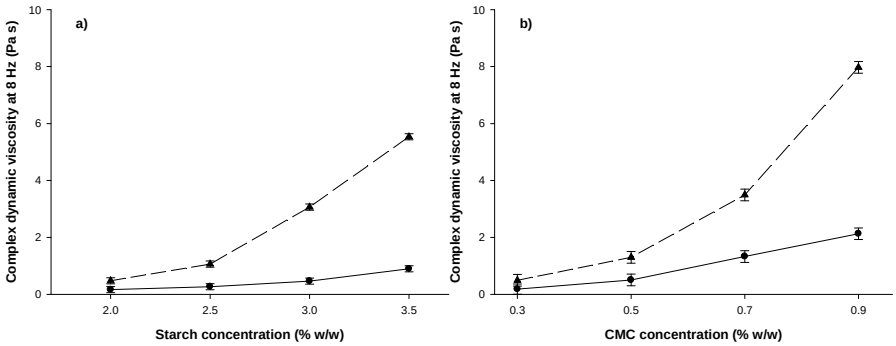

Figure 6. Complex dynamic viscosity at 8 Hz values of soy desserts with starch (a) or CMC (b) containing 6% (○) or 8% (△) of soy protein isolate (SPI)

From a practical point of view, an important role that these instrumental index values could play during soy dessert design and formulation would be to obtain quick preliminary information about possible changes in thickness

level due to compositional variations. By applying Cluster analysis to the two oral thickness indices a different classification of samples was obtained. According to the apparent viscosity values at 50 s^{-1} (Figure 7), samples were classified into three groups: a) high thickness, containing only one sample (8% SPI and 0.9% CMC), with $\eta_{50} = 4.97$ Pas; b) medium thickness, containing six samples, with η_{50} values ranging from 1.03 to 2.52 Pas; and c) low thickness, containing nine samples, with η_{50} values ranging from 0.11 to 0.81 Pas.

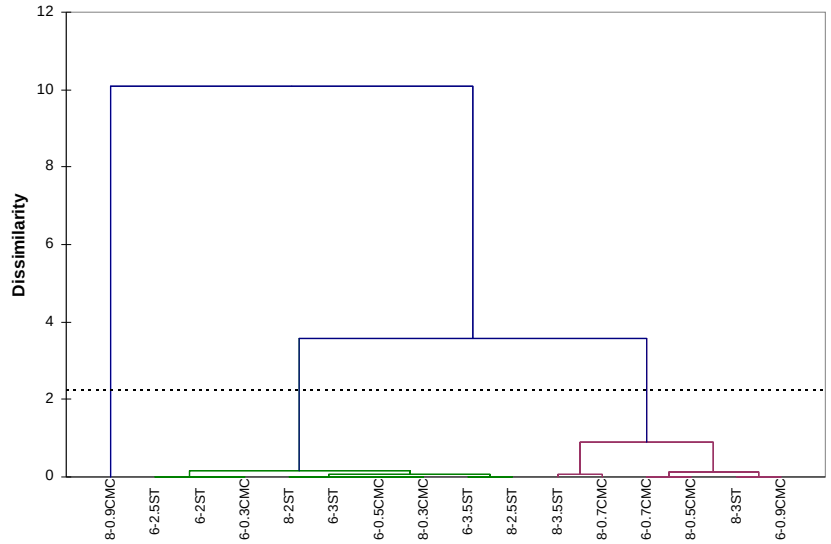

Figure 7. Sample classification according apparent viscosity at 50 s^{-1} values obtained by Cluster Analysis

When the samples were classified into three groups using the complex dynamic viscosity values at 8 Hz (Figure 8), different results were obtained. The high thickness group consisted of the two 8% SPI samples with the highest thickener concentrations (3.5% starch and 0.9% CMC) and with η_{8Hz} values of 5.54 and 7.97 Pas, respectively. The medium thickness group also consisted of two samples. Both of them had the higher SPI content (8%) and 3% starch or 0.7% CMC and η_{8Hz} values of 3.07 and 3.49 Pas, respectively.

Finally, the remaining samples were classified as having low thickness level, with η_{8Hz} values that ranged from 0.17 to 2.13 Pas. These results indicate that the classification of a set of samples according to their thickness level could be dependent on the instrumental index used. To obtain valid information about the thickness level of a set of samples with different compositions using instrumental indices it is necessary to select them previously according to their relationship with the corresponding sensory data.

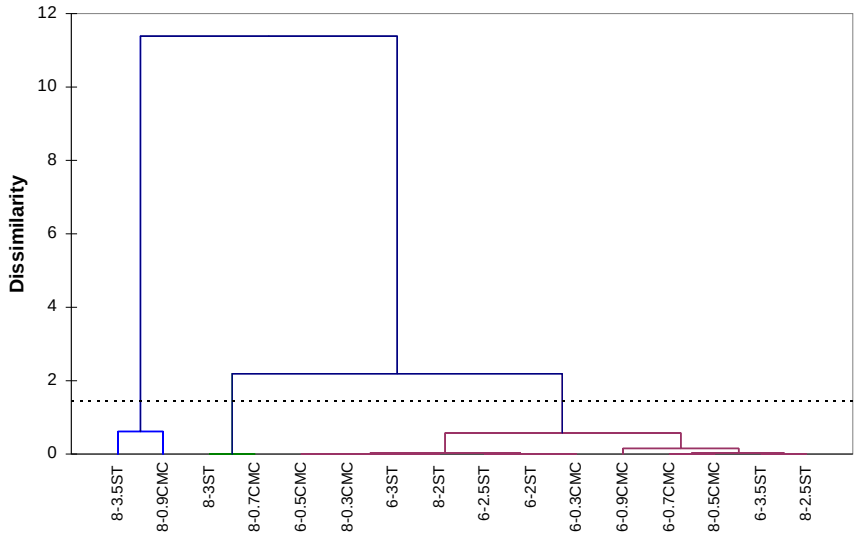

Figure 8. Sample classification according complex dynamic viscosity at 8 Hz values obtained by Cluster Analysis

4. Conclusions

It can be concluded that the addition of different hydrocolloids modifies the rheological behaviour of soy desserts in different ways, and also that it is possible to obtain high-protein soy desserts of similar rheological behaviour by adding different hydrocolloids at different concentrations. When one of the proposed instrumental indices of oral thickness is used to classify a set of

samples according to their possible thickness, the results may be dependent on the instrumental index considered. More information about the effects of different hydrocolloids on product stability and on perceived flavour and texture and about instrumental and sensory relationships are needed to design soy desserts that provide better quality.

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CAPÍTULO 3

FAT-THICKENER INTERACTIONS ON DAIRY SYSTEMS: EFFECTS ON COLOUR, RHEOLOGY, FLAVOUR RELEASE AND SENSORY PERCEPTION

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Abstract

This study aims to obtain information about influence of interactions between thickener type and concentration and fat content on colour, rheological behaviour, *in-vivo* aroma release and sensory characteristics of lemon flavoured dairy desserts. Eight formulations were prepared varying in the thickener type and concentration: carboxymethyl cellulose (1.1% and 1.3%) and modified starch (3.5% and 4.0%) and the milk type (whole or skimmed). The amounts of the other dessert ingredients were fixed. Ingredient interactions affected the physical and sensory characteristics in different extent. Significant interactions among fat content and thickener type and concentration on colour and rheology were detected. Colour was mainly related with fat content, flow behaviour with thickener type and viscoelasticity with thickener concentration. No significant interactions among ingredients were detected on *in-vivo* aroma release although thickener type and concentration and fat content affected significantly flavour release. The delivery to the nasal cavity of the most lipophilic compound (linalool) mainly depended of fat content while thickener type and concentration affected mainly the release of the least lipophilic compound (*cis*-3-hexen-1-ol). Differences perceived in colour, texture and flavour attributes were also affected for ingredient variations in different ways and most of them could be related with those observed on instrumental data.

Keywords: Dairy desserts, thickeners, fat content, physical properties, flavour release, sensory attributes

1. Introduction

Variations on ingredient concentrations or on ingredient characteristics can modify the rheological and sensory properties of the semisolid dairy desserts influencing the consumer response (De Wijk, van Gemert, Terpstra, & Wilkinson, 2003; González-Tomás & Costell, 2006; Verbeken, Bael, Thas, & Dewettinck 2006; Arltoft, Madsen, & Ipsen, 2008). Milk fat content and thickener type and concentration are the most important compositional factors affecting both flavour release and perception and the texture and colour perceived in dairy desserts.

Flavour is not only linked to those food components capable of stimulating the senses of taste and smell when present in a product at a certain concentrations. It depends of the amount of aroma and taste compounds released during food consumption (Overbosch, Afterof, & Haring, 1991; Durán & Costell, 1999; Taylor, 2002). This amount is partially governed by the composition and structure of the food matrix and by its transformation during oral food processing (Buettner, Beer, Hannig, Settles, & Schieberle, 2002; Engelen & van der Bilt, 2008; Chen, 2009; Gierczynski, Guichard, & Laboure, 2011). During mastication and deglutition, matrix structure breakdown and mixing with saliva play an important role in flavour perception (Bayarri, Durán & Costell, 2003; Seuvre, Philippe, Rochard, & Voilley, 2006; Koliandris, Lee, Ferry, Hill, & Mitchell, 2008) although to study these processes *in situ* in the mouth is not easy. To overcome this problem some techniques have been developed to mimic or evaluate aroma release in the nose or mouth (van Ruth & Roozen, 2000; Rabe, Linforth, Krings, Taylor, & Berger, 2004; Salles et al., 2007; Ghosh, Peterson, & Coupland, 2008). Among these, spectrometric methods like atmospheric pressure chemical ionisation (APCI-MS) (Taylor & Linforth, 2003; Lethuaut, Weel, Boelrijk, & Brossard, 2004; Bayarri, Taylor, & Hort, 2006) or proton transfer reaction (PTR-MS) (van Ruth, Floris, & Fayoux, 2006;

Mestres, Kieffer, & Buettner, 2006; Arancibia, Jublot, Costell, & Bayarri, 2011a) could provide direct information about the odour stimuli that reach the olfactory receptors (Linforth, Cabannes, Hewson, Yang, & Taylor, 2010).

Previous studies have shown the influence of milk fat content and of the aroma compound lipophilicity on flavour release of dairy desserts (Van Ruth, de Witte, & Rey Uriarte, 2004; González-Tomás, Bayarri, Taylor, & Costell, 2007; Keršienė, Adams, Dubra, De Kimpe, & Leskauskaitė, 2008) and all of them showed greater retention of lipophilic compounds on increasing fat content. However the influence of thickener type and concentration on aroma and taste release and perception is not so clear. It is generally considered that the more viscous a fluid food or the harder a solid food are, the less flavour is perceived on eating them. Also, the addition of food thickeners is believed to result in a decrease in aroma and taste intensity (Clark, 2002; Boland, Delahunty, & van Ruth, 2006) although this effect seems to be dependent on thickener type (Baines & Morris, 1987; Guinard & Marty, 1995; Hollowood, Linforth, & Taylor, 2002; Bayarri, Rivas, Izquierdo, & Costell, 2007). Starch and carboxymethyl cellulose (CMC) are the most widely used thickeners in dairy desserts. Some authors have been informed about the structural differences observed in CMC and starch solutions (Ferry et al., 2006) and others have been detected that starch and CMC showed different break-up mechanisms under shear (Desse, Mitchell, Wolf, & Budtova, 2011). According with these results, would be expected that both thickeners could affect the physical and sensory characteristics of dairy desserts differently. In this context, a preliminary study showed that the addition of starch and carboxymethyl cellulose in lemon-flavoured dairy desserts not only influenced its rheological behaviour but also its flavour delivery profile (Arancibia, Castro, Jublot, Ziere & Bayarri, 2010).

This work aims to analyse the effect of milk fat content and thickener type (starch and carboxymethyl cellulose) and concentration and of the interactions among them on colour, rheology as well as flavour release and perception in lemon-flavoured dairy desserts.

2. Materials and Methods

2.1 Sample composition and preparation

Samples were prepared with carboxymethyl cellulose (CMC) (Akucell AF3265 Akzo Nobel, Amersfoort, The Netherlands), medium crosslinked modified tapioca starch (C* Creamtex 75720, Cerestar Ibérica, Barcelona, Spain), commercial whole milk powder or skimmed milk powder (Central Lechera Asturiana, Siero, Spain), mineral water (Font Vella, Barcelona, Spain), sugar, colorant (T-PT8-WS, CHR Hansen S.A., Barcelona, Spain) and a lemon flavour mixture composed of two volatile compounds with different hydrophobicity level: linalool (LogP = 2.94) and *cis*-3-hexen-1-ol (LogP = 1.61) (SAFC-Sigma Aldrich, Madrid, Spain).

Eight formulations of dairy desserts were prepared varying in the milk type (whole and skimmed), thickener type (CMC and starch) and thickener concentration (low: 1.1 g/100g CMC or 3.5 g/100g starch; high: 1.3 g/100g CMC or 4 g/100g starch). The thickener concentrations were selected according to previous experiences (González-Tomás et al., 2007; Bayarri, Chuliá, & Costell, 2010). Fixed amounts of sugar (10 g/100g), colorant (37.5 mg/Kg), aroma volatiles (linalool: 90 mg/Kg and *cis*-3-hexen-1-ol: 30 mg/Kg) and the weight of rehydrated milk (80 g/100g) were employed.

Whole and skimmed milk were prepared to obtain a final fat content of 3.5 g/100g and 0.14 g/100g respectively as described previously (Arancibia, Costell, & Bayarri, 2011b). For CMC-based samples, a blend of sugar with CMC was added to the rehydrated milk with the colorant and was stirred

(Heidolph RZR 1, Schwabach, Germany) at room temperature for 35 min. Five min before the end aroma volatiles were added. Starch-based samples were prepared in batches of 800 g as follows: starch, sugar and rehydrated milk were weighed in a flask and mixed under magnetic stirring for 10 min. The flask was placed in a water bath at 96 ± 1 ºC and stirred constantly with a propeller stirrer for 25 min. Samples were cooled in a water bath at 10 ± 1 ºC for 10 min. Any evaporated water was replaced gravimetrically. Aroma volatiles were added and the sample was stirred for 5 min. At least, two batches of each composition were prepared. Each sample was transferred to a closed flask and stored (4 ± 1 ºC; 24 h) prior to colour, rheological, aroma release and sensory measurements.

2.2 Instrumental measurement of colour

Colour was measured in a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Business Technologies, Inc., Osaka, Japan) as described previously (Arancibia, Costell, & Bayarri, 2011b). *L** (brightness), *a** (red component), *b** (yellow component), C* (chroma) and h* (hue) values were obtained. Two measurements were performed on each batch.

2.3 Rheological measurements

Rheological measurements were performed in a controlled stress rheometer RS1 (ThermoHaake, Karlsruhe, Germany), using parallel-plates geometry (60 mm diameter; 1mm gap). Measurements were made at 10 ± 1 ºC, selected as representative of the usual consumption temperature of dairy desserts. Temperature was kept constant by means of a Phoenix P1 Circulator device. A resting period of 10 min was applied after loading the sample. Each batch was measured twice, using a fresh sample for each measurement.

2.3.1 Flow behaviour. Flow behaviour was measured by recording shear stress values when shearing the samples at an increasing shear rate from 1 to 200 s^{-1} for a period of 60 s and in reverse sequence for the same time, as selected previously (González-Tomás & Costell, 2006). The relative thixotropic area (A_R) (Eq. 1) was calculated rather than the magnitude of the thixotropic area, because it allows a better comparison of the time dependence behaviour of different samples (Dolz, González, Delegido, Hernández, & Pellicer, 2000).

$$A_R = \frac{A_{up} - A_{down}}{A_{up}} \times 100 \quad (1)$$

Descending flow curves were fitted to Herschel-Bulkley model (Eq. 2) using Rheowin Pro software (version 3.61),

$$\sigma = \sigma_0 + K\dot{\gamma}^n \quad (2)$$

where σ (Pa) is the shear stress, $\dot{\gamma}$ (s^{-1}) is the shear rate, σ_0 (Pa) is the yield stress, K (Pa s^n) is the consistency index and n (adimensional) is the flow behaviour index.

2.3.2 Viscoelastic behaviour. To determine the linear viscoelastic zone, stress sweeps were performed at a frequency of 1Hz and between 0.02 and 300 Pa. A stress of 0.05 Pa, which is within the linear viscoelastic region for all samples, was selected. Frequency sweeps were then performed from 0.01 to 10 Hz. Storage modulus (G'), loss modulus (G''), complex dynamic viscosity (η^*), and loss angle ($\tan \delta$) values at 1 Hz, were compared.

2.4 In vivo aroma release measurements

Nose breath analyses performed with a High Sensitivity Proton Transfer Reaction-Mass Spectrometer (PTR-MS) (Ionicon, Innsbruck, Austria) was used to measure *in-vivo* aroma release from each sample. Using acetone as a marker for breathing pattern, protonated ions from acetone (*m/z* 59), linalool (*m/z* 137) and *cis*-3-hexen-1-ol (*m/z* 83) were monitored in single ion monitoring mode with dwell time of 100ms. Eight panellists consumed all eight samples at 10 ºC in a single session, with a break of 5 min between each sample. Plain crackers and water were used as palate cleansers. Panellists were asked to breathe in, sip 5 mL of sample from a spoon, close their mouths, swallow the sample and then exhale and continue to breathe normally while resting their nose on the PTR-MS nasal sampling tube. Nose breath was sampled into the PTR-MS at 35 mL min^{-1} and breathing pattern of panellists was monitored for 1 min after sample consumption. From raw data two parameters: the maximum aroma intensity (Imax) and the cumulative area under the 1 min release profile (AUC) were extracted.

2.5 Sensory evaluation

A group of 28 assessors, with previous experience (more than three years) in evaluating sensory differences in dairy products, and familiarized with the attributes under test, evaluated the perceptible differences on the 28 possible pairs of eight samples by a pairwise ranking test (Meilgaard, Civille, & Carr, 1999).

Sensory evaluation was carried out in 14 sessions and each assessor evaluated two pairs of samples per session. The assessors were asked to indicate which sample, within each pair, had a higher intensity of the following attributes: yellowness, lemon flavour, milk flavour, consistency, creaminess and smoothness. Each pair of samples (30mL) was presented at

10 ± 1°C in covered white plastic vessels and labelled with a random three-digit code. First, panellists evaluated intensity differences in yellowness. Secondly, panellists tasted the same volume of each sample from a spoon (5 mL) and evaluated the differences in flavour and texture attribute intensity. White bread, without salt and mineral water, was provided to the assessors for mouth rinsing between each pair of samples. All sessions were carried out under normal white fluorescent illumination in a standardized test room (ISO, 2007). Compusense *five*, release 5.0 (Compusense Inc., Guelph, ON, Canada) was used to data acquisition.

2.6 Experimental design and data analysis

The effects of milk type, thickener type and thickener concentration and their binary interactions on colour, rheological and *in-vivo* aroma release data were analysed by a three-way ANOVA. Experimental design included three two-level factors: milk type (whole and skimmed); thickener type (CMC and starch) and thickener concentration (low and high). Tukey's test (α=0.05) was used to calculate the minimum significant difference. The average values of colour and rheological parameters for all samples were also analysed by Principal Component Analysis (PCA). All calculations were carried out with XLSTAT Pro software v. 2007 (Addinsoft, Paris, France).

Sensory data were analysed according to Meilgaard et al. (1999). The first step in the Friedman analysis is to compute the rank sum for each sample. Then, the Friedman's *T* statistic was obtained with Equation 3.

$$T = \left(\frac{4}{p \times t} \right) \times \sum_{i=1}^{t} R^2 - \left(9 \times p \times \left[t - 1 \right]^2 \right) \quad (3)$$

where p is the number of assessors, t is the number of samples and R is the rank sum for each sample. Experimental T values were compared to the critical values of χ^2 with (t-1) degree of freedom. Significant differences among samples were determined with the Tukey's (HSD) test. The HDS values (α=0.05) were calculated for each attribute with the following equation:

$$HSD = q_{\alpha,t,\infty}\sqrt{pt/4} \quad (4)$$

where $q_{\alpha,t,\infty}$ is the upper-α critical value of the studentized range distribution with ∞ degrees of freedom, p is the number of assessors and t is the number of samples. Principal Component Analysis (PCA) was also applied to the rank sum values of sensory attributes of all samples.

3. Results and Discussion

3.1 Instrumental Colour Measurements

Reflectance spectra in the visible region (400 to 700 nm) for the eight dairy desserts analysed are shown in Figure 1. Whole milk samples showed clearly higher percentages of reflectance light than skim milk samples. Whole-milk samples displayed maximum reflectance values at wavelengths between 500 and 700 nm and, skimmed-milk samples showed a slight decrease in the reflectance percentage values at wavelength values above 600 nm. This difference between samples with different fat content was more evident in CMC-based dairy desserts.

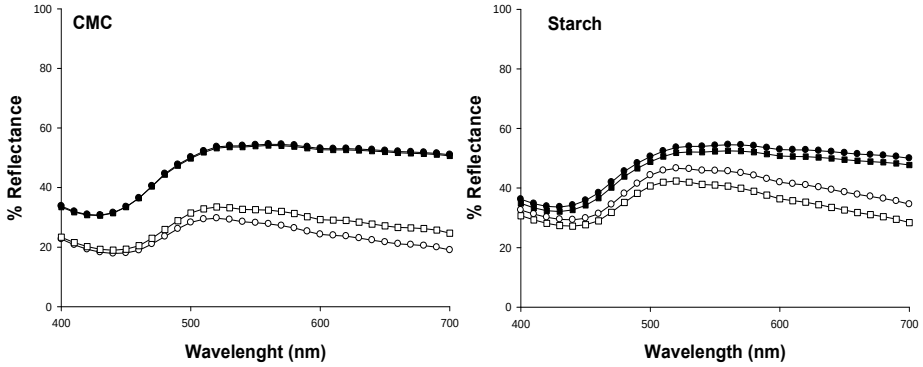

Figure 1. Spectral reflectance curves of CMC (a) and starch (b) dairy desserts with different milk type (filled symbols: whole; empty symbols: skimmed) and thickener concentration (○: low and □: high) (low: 1.1g/100g CMC or 3.5 g/100g starch; high: 1.3 g/100g CMC or 4.0 g/100g starch).

A three-way ANOVA with binary interactions was applied (Table 1). The thickener concentration-thickener type interaction was significant on all colour parameters, indicating that thickener type affected colour parameters differently, depending of their concentration. The milk type-thickener type interaction also had a significant effect on most colour parameters. No significant differences were observed among whole-milk samples with different thickener in L^*, a^* and h* values, while among skimmed-milk samples these colour parameter values differed depending of thickener (Table 2). By increasing CMC levels in skimmed-milk samples, L^*, b^* and C* values increased and a^* and h* values decreased. However, when starch level increased in skimmed-milk samples, L^*, b^* and C* values decreased and a^* and h* values increased.

Table 1. Three-way ANOVA of instrumental color parameters in dairy desserts with different milk type, thickener type and texture level. F and P values

	Colour parameters									
	*L**		*a**		*b**		C*		h*	
	F	P	F	P	F	P	F	P	F	P
Main effects										
A: Milk type	2738.36	<0.01	611.27	<0.01	256.48	<0.01	59.64	<0.01	1710.89	<0.01
B: Thickener type	444.68	<0.01	5.72	0.04	32.08	<0.01	31.86	<0.01	1.49	0.27
C: Texture level	1.17	0.31	0.17	0.70	1.64	0.24	1.09	0.33	1.30	0.29
Binary Interactions										
A x B	526.58	<0.01	0.08	0.78	19.50	<0.01	15.37	<0.01	5.65	0.05
A x C	4.39	0.07	0.22	0.65	2.51	0.15	1.35	0.28	5.28	0.05
B x C	79.12	<0.01	20.45	<0.01	30.72	<0.01	9.27	0.02	139.79	<0.01

Table 2. Mean values and significant differences of instrumental colour parameters in dairy desserts with different milk type, thickener type and concentration

Thickener type	Thickener concentration (% w/w)	Milk type	Colour parameters				
			*L**	*a**	*b**	C*	h*
CMC	1.1	Whole	77.52^{e}	-6.13^{c}	18.86^{c}	19.83^{c}	108.00^{a}
	1.1	Skimmed	58.63^{a}	-9.93^{a}	12.02^{a}	15.59^{a}	129.61^{c}
	1.3	Whole	77.22^{e}	-6.07^{c}	18.64^{c}	19.60^{c}	108.05^{a}
	1.3	Skimmed	62.36^{b}	-8.88^{ab}	15.56^{b}	17.91^{bc}	119.70^{b}
Starch	3.5	Whole	77.45^{e}	-5.59^{c}	16.34^{b}	17.19^{ab}	108.98^{a}
	3.5	Skimmed	71.99^{d}	-8.60^{b}	14.56^{b}	16.91^{ab}	120.57^{b}
	4.0	Whole	76.33^{e}	-5.88^{c}	16.26^{b}	17.37^{ab}	109.81^{a}
	4.0	Skimmed	68.71^{c}	-9.64^{ab}	12.41^{a}	15.72^{a}	127.86^{c}

$^{a\text{-}e}$ *Means within a column with common superscripts did not differ significantly ($P < 0.05$)*

On applying PCA analysis to the average values of the instrumental colour parameters corresponding to the eight samples (Figure 2), the first two components accounted for 97.32% of the total variability. The first component, which explained the higher percentage of variability (86.96%), clearly separated the samples according to milk type. Whole-milk samples, in the negative part of the first component, showed higher b* and a* values and lower h* values than skimmed-milk samples, which were in the positive part. A similar effect of milk fat content on colour parameter values was previously observed in other dairy products. On lowering the fat content, milk was perceived as less white (Phillips et al., 1995) and vanilla ice cream was perceived as more yellow (Guinard et al., 1997). The second component, which accounted for only a small percentage of variability (10.36%), was related in its positive part with the variation in C* value and in its negative part with L* value separating the samples according to thickener type. CMC-based samples were in the upper part of the second

component and the starch-based samples in lower part. The results showed that, as expected, milk fat content was the factor that most affected colour parameter values, although both types of milk and thickener interacted to modify colour of dairy systems evaluated.

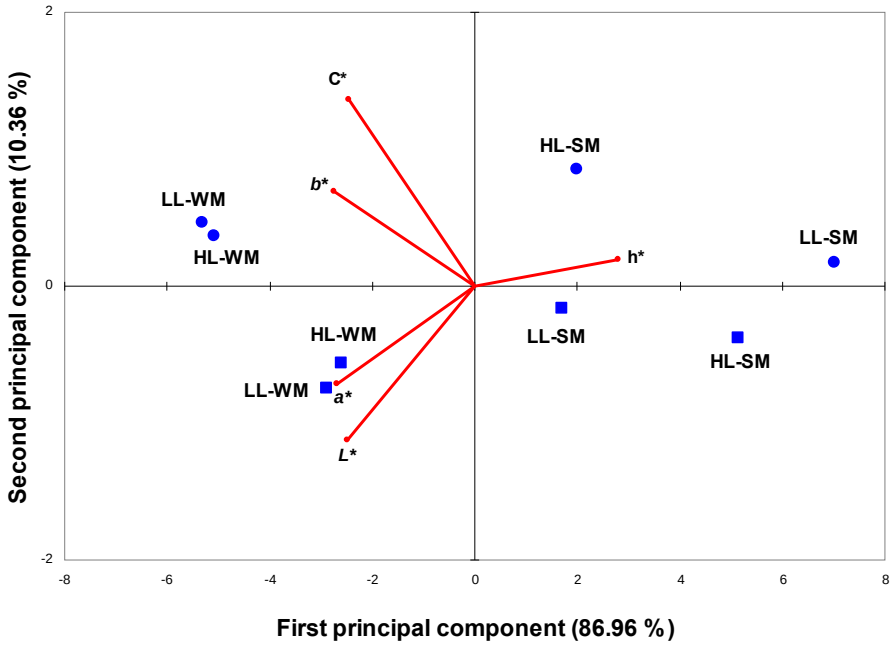

Figure 2. Principal component analysis bi-plot of carboxymethyl cellulose (circle) and starch (square) dairy desserts with different thickener concentration (LL: low and HL: high) and milk type (WM: whole milk and SM: skimmed milk) for instrumental colour parameters: *L** (brightness), *a** (red component), *b** (yellow component), C* (chroma) and h* (hue) (LL: 1.1 g/100g CMC or 3.5 g/100g starch, HL: 1.3 g/100g CMC or 4.0 g/100g starch).

3.2 Rheological properties

3.2.1 Flow behaviour. The eight samples showed hysteresis loops when they were sheared during a complete cycle. Whole-milk samples with CMC at the two concentrations considered showed greater hysteresis loops than their counterparts formulated with skimmed-milk, while slight differences were detected between whole and skimmed-milk samples of each concentration for starch-based desserts. A difference was observed in the form of the hysteresis loop obtained for the CMC-based sample formulated with whole-milk at the higher CMC concentration compared to the loop for the other CMC samples. Its upward curve showed an overshoot with a maximum stress value. Similar results were obtained in a previous work (Bayarri & Costell, 2011). The authors observed that increasing the concentration of CMC up to 1.25%, produced a more structured system that partially broke down with increasing shear rates. For the starch-based samples made with each milk type, the thixotropic area increased on increasing starch concentration. This behaviour can be explained taking into account that the structure of these systems is mainly affected by the increase in the volumetric fraction of the dispersed phase, constituted by swollen starch granules (Abu-Jdayil & Mohameed, 2004). Flow curves of all samples showed a shear thinning with yield stress behaviour, and the experimental data of descending rheograms of all samples were successfully fitted to the Herschel-Bulkley model ($0.998 < R^2 < 0.999$). An analysis of variance with interactions was applied to each flow parameter. Results revealed a significant effect of the three single factors (milk type, thickener type and texture level) as well as of most binary interactions (Table 3).

Table 3. Three-way ANOVA of flow parameters in dairy desserts with different milk type, thickener type and texture level. F and P values

	Flow parameters							
	K (Pa s^n)		n		σ_0 (Pa)		Ar (%)	
	F	P	F	P	F	P	F	P
Main effects								
A: Milk type	366.78	<0.01	40.26	<0.01	391.85	<0.01	86.46	<0.01
B: Thickener type	1035.59	<0.01	1600.12	<0.01	4215.73	<0.01	40.48	<0.01
C: Texture level	687.53	<0.01	49.31	<0.01	2257.73	<0.01	35.22	<0.01
Binary Interactions								
A x B	405.21	<0.01	47.42	<0.01	104.19	<0.01	41.26	<0.01
A x C	95.91	<0.01	148.43	<0.01	0.03	0.88	9.06	0.02
B x C	401.75	<0.01	3.28	0.11	945.92	<0.01	11.89	<0.01

For each texture level, CMC-based systems showed higher consistency and pseudoplasticity than starch-based desserts; however, samples with starch gave the highest yield stress values (Table 4). The whole-milk samples with CMC gave higher values on the consistency index, yield stress, and relative hysteresis area and lower ones on the flow index, than the equivalent samples made with skimmed milk. The higher fat content seemed to strengthen the system particularly at the higher CMC concentrations. The effect of milk type on flow parameter values was not as clear in starch-based samples. Only the variation in yield stress values was significant between whole and skimmed starch-based samples for each concentration (Table 4).

Table 4. Mean values and significant differences of flow parameters in dairy desserts with different milk type, thickener type and concentration

Thickener type	Thickener concentration (% w/w)	Milk type	Flow parameters			
			K (Pa s^n)	n	σ_0 (Pa)	Ar (%)
CMC	1.1	Whole	4.85^{b}	0.62^{b}	5.09^{b}	9.84^{bcd}
	1.1	Skimmed	1.78^{a}	0.67^{c}	2.22^{a}	2.06^{a}
	1.3	Whole	15.70^{c}	0.52^{a}	9.42^{d}	13.37^{d}
	1.3	Skimmed	5.98^{b}	0.59^{b}	7.05^{bc}	6.98^{b}
Starch	3.5	Whole	1.33^{a}	0.78^{d}	16.92^{e}	12.02^{cd}
	3.5	Skimmed	1.35^{a}	0.78^{d}	9.06^{cd}	8.72^{bc}
	4.0	Whole	2.20^{a}	0.81^{d}	39.64^{g}	11.13^{cd}
	4.0	Skimmed	2.50^{a}	0.80^{d}	30.11^{f}	11.85^{cd}

$^{a\text{-}f}$ *Means within a column with common superscripts did not differ significantly ($P < 0.05$)*

3.2.2 Viscoelastic properties. All samples exhibited weak-gel behaviour: the elastic response predominated over the viscous one (G'>G"). Storage (G') and loss modulus (G") values were dependent on the frequency for all the systems studied (Arancibia et al., 2010). For comparative purposes G', G", η*, tan δ values at a frequency of 1 Hz were considered. Analysis of

variance showed a significant effect of all binary interactions on G' and η* values (Table 5). For the other two parameters (G" and tan δ) not all the binary interactions were significant. As expected, viscoelastic parameter values increased with the thickener concentration in both milk types, except tan δ values, which decreased. As in the flow parameter values, the effect of milk type on viscoelastic parameters was higher in CMC samples than in starch-based ones (Table 6). Considering that in this type of system, CMC molecules do not adsorb on the droplet surface at almost neutral pH (6.4) and the surface is covered only by adsorbed protein molecules (Damianou & Kiosseoglou, 2006), the differences observed in the G´, G´´ and η* values between whole and skimmed milk samples may be explained by CMC adsorption on the droplet surfaces through protein-polysaccharide interaction, as well as the thickening and gelation of the continuous phase caused by the CMC itself (Bayarri, González-Tomás, & Costell, 2009).

Table 5. Three-way ANOVA of viscoelastic parameters in dairy desserts with different milk type, thickener type and texture level. F and P values

	Viscoelastic parameters							
	G' (Pa)		G" (Pa)		η* (Pa s)		tan δ	
	F	P	F	P	F	P	F	P
Main effects								
A: Milk type	726.04	<0.01	391.39	<0.01	713.64	<0.01	735.27	<0.01
B: Thickener type	200.83	<0.01	487.82	<0.01	236.24	<0.01	1.28	0.29
C: Texture level	2024.66	<0.01	3554.56	<0.01	2247.43	<0.01	440.96	<0.01
Binary Interactions								
A x B	187.99	<0.01	158.69	<0.01	193.12	<0.01	3.39	0.10
A x C	71.20	0.04	0.07	0.79	63.79	<0.01	105.40	0.02
B x C	5.49	<0.01	4.82	0.06	6.08	0.04	20.48	<0.01

Table 6. Mean values and significant differences of viscoelastic parameters in dairy desserts with different milk type, thickener type and concentration

Thickener type	Thickener concentration (% w/w)	Milk type	Viscoelastic parameters			
			G' (Pa)	G" (Pa)	η* (Pa s)	tan δ
CMC	1.1	Whole	83.39^{c}	34.62^{c}	14.73^{c}	0.42^{bc}
	1.1	Skimmed	23.92^{ab}	17.44^{b}	4.71^{ab}	0.73^{e}
	1.3	Whole	225.08^{f}	72.60^{f}	37.64^{f}	0.32^{a}
	1.3	Skimmed	98.67^{cd}	51.85^{e}	17.74^{d}	0.53^{d}
Starch	3.5	Whole	39.19^{b}	17.54^{b}	6.83^{a}	0.45^{c}
	3.5	Skimmed	14.04^{a}	11.22^{a}	2.86^{a}	0.80^{e}
	4.0	Whole	141.80^{e}	49.06^{de}	23.88^{e}	0.35^{ab}
	4.0	Skimmed	106.44^{d}	46.96^{d}	18.52^{d}	0.45^{c}

$^{a\text{-}f}$ *Means within a column with common superscripts did not differ significantly ($P < 0.05$)*

To study the joint variability of rheological parameters Principal Component Analysis was applied to their mean values (Figure 3). The first component accounted for 62.38% of total variability and was strongly associated with viscoelastic parameters and with consistency index. This component separated the samples according to thickener concentration. Samples with higher concentrations, in the positive part of the first dimension, showed higher G', G" and η* values and lower tan δ values than samples with the lower concentrations, which were in the negative part of the first dimension. This component clearly separated the whole milk sample with the highest CMC concentration as the most structured system from skimmed milk samples with the lower concentration of both thickeners (starch and CMC). The second component, which accounted a 29.14% of total variability, correlated well with most of flow parameters and separated samples according thickener type. The flow of starch-based desserts, located in the upper half, showed greater time dependence and more initial resistance to flow than the CMC-based systems, which were in the lower half of the

second component. Differences in flow behaviour observed for both starch and CMC dairy desserts may be because their particular structures provide a different resistance to flow. The granular structure of starch samples is related with the presence of swollen starch granules as dispersed phase. The polymeric structure of CMC samples is defined by a network formed by the entanglement of chains.

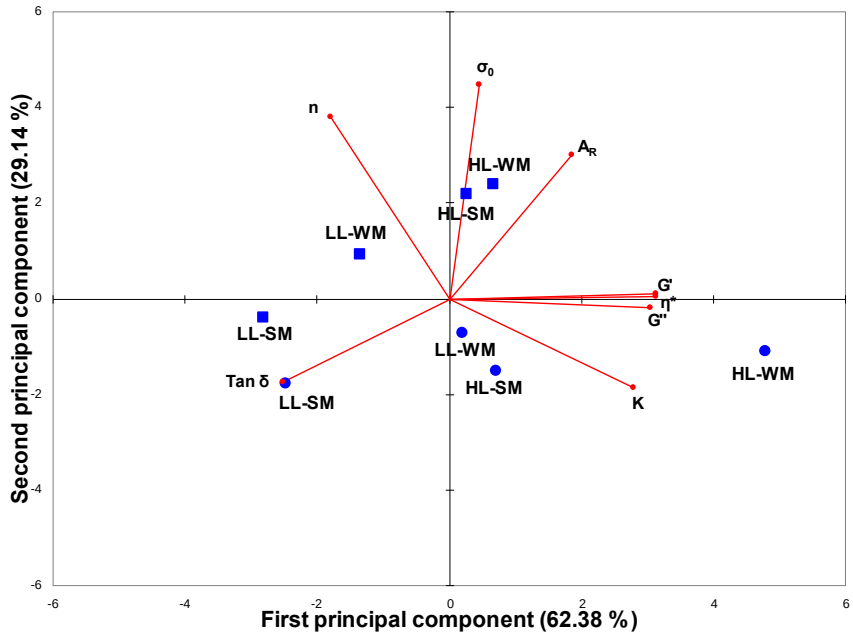

Figure 3. Principal component analysis bi-plot of carboxymethyl cellulose (circle) and starch (square) dairy desserts with different thickener concentration (LL: low and HL: high) and milk type (WM: whole milk and SM: skimmed milk) for rheological parameters: A_R (relative thixotropic area), n (flow index), σ_0 (yield stress), K (consistency index), G' (storage modulus), G" (loss modulus), η* (complex dynamic viscosity) and tan δ (loss angle) (LL: 1.1 g/100g CMC or 3.5 g/100g starch, HL: 1.3 g/100g CMC or 4.0 g/100g starch).

3.3 In-vivo aroma release measurements

A three-way ANOVA with binary interactions was used to study the combined effects of the milk type and thickener type and concentration on the variation of the maximum aroma intensity (Imax) and the cumulative area under the 1 min release profile (AUC) (Table 7). The binary interactions were not significant for either parameter. For the skimmed-milk samples, Imax and AUC values of linalool were higher than that obtained for the whole-milk samples, for both thickeners. However, Imax and AUC values of *cis*-3-hexen-1-ol for starch-based samples were higher than for CMC-based samples (Table 8). An "aroma release map" for each volatile compound was drawn up, representing Imax versus AUC values obtained (Figure 4a and b). These maps facilitate observation of the impact of ingredient variation on *in vivo* aroma release of each volatile compound. Sample position on the maps shows the differences between their Imax and AUC relationships, depending on volatile polarity and thickener type. As expected, the greater retention of linalool on higher fat content samples decreased their release, whereas skimmed fat samples with both thickeners gave better aroma release (Figure 4a). Release of *cis*-3-hexen-1-ol is practically unaffected by sample fat content and depends mainly on the thickener type. The results obtained confirmed the better aroma release from modified starch (Figure 4b) and were in agreement with other authors that showed a significant reduction in mouth flavour release due to the presence of CMC (Kühn, Delahunty, Considine, & Singh, 2009) and a good flavour release from starch in different food matrices (Cayot, Pretot, Doublier, Meunier, & Guichard, 2004; Arancibia, Jublot, Costell & Bayarri, 2011a). Differences in thickener structure (polymeric or globular) can influence their behaviour during oral processing (resistance to break and capacity to mix with the saliva) modifying the release of chemical stimuli to the receptors.

Table 7. Three-way ANOVA of aroma release parameters for each aroma compounds of dairy desserts with different milk type, thickener type and texture level. F and P values

	Aroma release parameters							
	Linalool				*cis*-3-hexen-1-ol			
	Imax[1]		AUC[2]		Imax[1]		AUC[2]	
	F	P	F	P	F	P	F	P
Main effects								
A: Milk type	25.65	<0.01	8.61	0.01	0.25	0.62	0.84	0.37
B: Thickener type	8.92	<0.01	26.51	<0.01	29.44	<0.01	44.21	<0.01
C: Texture level	0.34	0.56	0.01	0.97	0.01	0.98	0.15	0.70
Binary Interactions								
A x B	0.19	0.67	0.23	0.63	1.49	0.23	3.66	0.06
A x C	1.16	0.29	1.25	0.27	1.27	0.27	2.42	0.13
B x C	1.89	0.18	2.92	0.09	0.40	0.53	2.52	0.12

[1] *Imax: maximum aroma intensity*

[2] *AUC: cumulative area under the 1 min release profile*

Table 8. Mean values and significant differences of aroma release parameters in dairy desserts with different milk type, thickener type and concentration

Thickener type	Thickener concentration (% w/w)	Milk type	Aroma release parameters			
			Linalool		*cis*-3-hexen-1-ol	
			Imax[1]	AUC[2]	Imax[1]	AUC[2]
CMC	1.1	Whole	903.8^{ab}	42133.7^{ab}	1060^{a}	22497.5^{a}
	1.1	Skimmed	2205.0^{abc}	63711.1^{abc}	1708.8^{abc}	33263.7^{ab}
	1.3	Whole	627.5^{a}	29303.7^{a}	941.3^{a}	21491.2^{a}
	1.3	Skimmed	2021.3^{abc}	53937.4^{ab}	1401.3^{ab}	21413.7^{a}
Starch	3.5	Whole	1513.8^{ab}	74714.8^{bc}	2733.7^{abc}	56926.1^{bc}
	3.5	Skimmed	2532.5^{bc}	77697.3^{bc}	3132.5^{bc}	53042.4^{abc}
	4.0	Whole	1503.8^{ab}	72906.1^{bc}	3560^{c}	78718.6^{c}
	4.0	Skimmed	3682.5^{c}	103133.5^{c}	2692.5^{abc}	52346.^{1abc}

[a-c] Means within a column with common superscripts did not differ significantly (P < 0.05)
[1] Imax: maximum aroma intensity
[2] AUC: cumulative area under the 1 min release profile

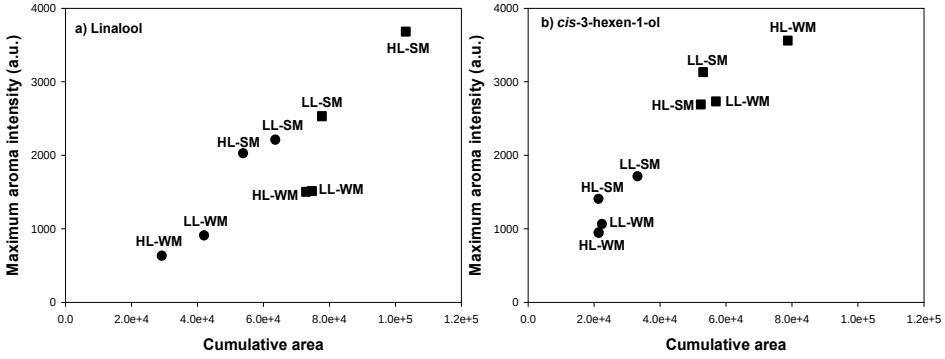

Figure 4. Aroma release map for linalool and *cis*-3-hexen-1-ol of arboxymethyl cellulose (circle) and starch (square) dairy desserts with different thickener concentration (LL: low and HL: high) and milk type (whole milk: filled symbols and skimmed milk: empty symbols). (LL: 1.1 g/100g CMC or 3.5 g/100g starch, HL: 1.3 g/100g CMC or 4.0 g/100g starch)

3.4 Sensory evaluation

Significant differences among samples were found for all attributes evaluated (Table 9). The calculated Friedman F values for yellowness (498.3), lemon flavour (287.6), milk flavour (328.1), consistency (375.0), creaminess (246.9) and smoothness (316.4) were higher than the theoretical value ($\chi^2 = 14.1$; $\alpha = 0.05$). The results showed that yellow colour intensity were perceived significantly higher in skimmed-milk samples than in whole-milk samples. CMC-based samples were perceived as having greater colour intensity than their starch-based counterparts. Lemon flavour depended only of milk type; samples with skimmed milk were perceived as having significantly higher flavour intensity than samples with whole milk. These results are in accordance with those obtained from *in-vivo* aroma release measurements, indicating that fat content and aroma compound lipophilicity are key factors affecting flavour release and perception. As expected, milk-flavour intensity was perceived as significantly higher in whole-milk samples than skimmed-milk samples. Besides, significant differences were found between different thickeners. Starch-based samples were perceived to have greater milk flavour intensity than their counterpart made with CMC. Additionally to the impact on flavour attributes, both milk and thickener type also affected the texture perceived. CMC-based samples made with whole milk were perceived as more consistent (thicker) than skimmed-milk samples. While no significant differences were detected in this attribute between starch-based samples with different fat content. Creaminess and smoothness were affected by both milk type and thickener type. As expected, whole-milk samples were evaluated as creamier than skimmed-milk samples; however these samples did not differ in smoothness. CMC-based samples were perceived as less creamy and smooth than samples with starch.

Table 9. Rank sum values and significant differences of sensory attributes in dairy desserts with different milk type, thickener type and concentration.

Thickener type	Thickener concentration (% w/w)	Milk type	Sensory attributes					
			Yellow colour	Lemon flavour	Milk flavour	Consistency	Creaminess	Smoothness
CMC	1.1	Whole	259^{c}	263^{b}	310^{b}	305^{c}	285^{c}	264^{c}
	1.1	Skimmed	376^{a}	347^{a}	228^{e}	213^{e}	223^{d}	273^{c}
	1.3	Whole	262^{c}	247^{b}	315^{b}	375^{a}	285^{c}	224^{c}
	1.3	Skimmed	374^{a}	334^{a}	239de	299^{c}	255^{cd}	243^{c}
Starch	3.5	Whole	206^{d}	248^{b}	358^{a}	252^{d}	322^{b}	368^{a}
	3.5	Skimmed	307^{b}	342^{a}	264^{cd}	241de	287^{c}	326^{b}
	4.0	Whole	237^{cd}	241^{b}	364^{a}	338^{b}	364^{a}	337^{ab}
	4.0	Skimmed	331^{b}	330^{a}	274^{c}	329^{bc}	331^{b}	317^{b}

$^{a-e}$ Means within a column with common superscripts did not differ significantly ($P < 0.05$).

PCA analysis of rank sum values of sensory attributes showed that the first component accounted for 63.43% of the data variability and up to 90.33% was explained by the first two components (Figure 5). The first component was strongly associated with the colour, flavour and creaminess attributes, and clearly separated the samples by fat content. Whole-milk samples fell in the positive part of this component, showing higher milk flavour intensity and creaminess while in the negative part were skimmed-milk samples, which were perceived as having greater lemon flavour and yellow colour intensity. The second component accounted for 26.90% of variability, and was positively correlated to the most of texture attributes. This component separated the samples according to thickener type. CMC-based samples, located in the upper half, were perceived to have greater consistency, except the skimmed-milk sample containing 1.3% CMC. Starch-based samples, located in the lower half, were considered to have the highest smoothness.

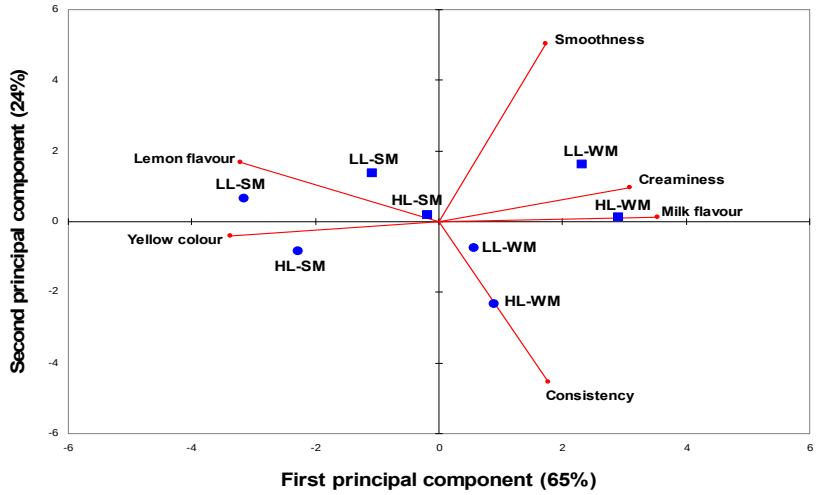

Figure 5. Principal component analysis bi-plot of carboxymethyl cellulose (circle) and starch (square) dairy desserts with different thickener concentration (LL: low and HL: high) and milk type (WM: whole milk and SM: skimmed milk) for sensory attributes (LL: 1.1 g/100g CMC or 3.5 g/100g starch, HL: 1.3 g/100g CMC or 4.0 g/100g starch).

4. Conclusions

This study has shown that, besides the individual effect of the milk fat content and of the type and concentration of thickener, interactions among them can modify the physical and structural characteristics of semisolid dairy products giving rise to clearly perceptible changes in colour, flavour and texture. Effects of these interactions influence optical properties, rheological behaviour and also the *in vivo* flavour release and sensory perception mechanisms of semisolid dairy systems in different ways. Despite the direct influence of milk fat in flavour release and perception and of the effect of thickener on rheological behaviour and on texture perceived in dairy food matrices, the influence of the interactions between them must be also considered in order to develop products with a good sensory quality.

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CAPÍTULO 4

FLAVOR RELEASE AND SENSORY CHARACTERISTICS OF O/W EMULSIONS. INFLUENCE OF COMPOSITION, MICROSTRUCTURE AND RHEOLOGICAL BEHAVIOR

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Abstract

Influence of fat content and thickener type on rheology, structure, stability, *in-vivo* aroma release and sensory perception was studied in lemon flavored o/w model emulsions. Six formulations were prepared by varying the oil content (5 and 30% w/w) and the matrix composition (carboxymethyl cellulose, modified starch and no thickener). The effect of the interaction between fat content and matrix type was significant on most of the flow and viscoelastic parameters but not on flow index or loss modulus values. There were differences in the creaming index due to oil content and thickener type. Higher emulsion stability was obtained with the starch-based emulsions. Fat content affected the delivery to the nasal cavity of the most lipophilic compound (linalool) but did not affect the delivery of the least lipophilic (*cis*-3-hexen-1-ol) while both thickeners influenced the cumulative area under the aroma release profiles from nose breath analyses. Differences in perceived flavor and texture attributes were affected for both fat content and matrix type, which could be explained by instrumental data in most cases.

Keywords: O/w emulsion, thickeners, structure, rheology, flavor release, sensory perception

1. Introduction

Emulsions represent an important category of food products, including ice creams, margarines, sauces, spreads or some dairy drinks. Food emulsions are normally composed by two immiscible liquids, usually oil and water, with one of them being dispersed in the other one as small spherical droplets (McClements, 2005). Technologically, one of the greatest problems is that emulsions are thermodynamically unstable and tend to break during certain processing operations (heat treatment, mechanical deformation, freezing, etc.) or during storage. The incorporation of biopolymers, especially proteins and hydrocolloids represents one of the most common strategies used to stabilize food emulsions. Proteins have absorption properties at oil-water interfaces to form layers around oil droplets and can act as emulsifiers and stabilizers. In general hydrocolloids act by increasing the viscosity or forming a gel network within the dispersing phase; thus delaying the instability processes (McClements, 2007; Dickinson, 2009). Changing emulsion composition by varying fat and stabilizer, both in type and concentration, leads to emulsions with different physical and sensory properties, (Kostyra & Barylko-Pikielna, 2007; Vingerhoeds, de Wijk, Zoet, Nixdorf, & van Aken, 2008) which influences consumers' acceptance.

Type and concentration of fat modify the physical properties of foods and have been reported to influence the perception of flavor both in terms of flavor release and textural changes (Daget, Joerg, & Bourne, 1987; Brauss, Linforth, Cayeux, Harvey, & Taylor, 1999; Guinard, Wee, McSunas, & Fritter, 2002; Malone, Appelqvist, & Norton, 2003; Bayarri, Taylor, & Hort, 2006; Bayarri, Smith, Hollowood, & Hort, 2007). The effect of fat on flavor release and perception is complex, and affects both the release of stimuli from the food matrix and also the release during oral processing of foods. Fat has an effect on aroma release, modifying the qualitative, quantitative and temporal flavor profile of foods (de Roos, 1997). For instance, flavor

release of lipophilic aroma compounds was reported to decrease with increasing lipid levels in the food matrix (van Ruth, King, & Giannouli, 2002; Miettinen, Tourila, Piironen, Vehkalathi, & Hyvönen, 2002; Weel, Boelrijk, Burger, Jacobs, Gruppen, Voragen, & Smit, 2004; González-Tomás, Bayarri, Taylor, & Costell, 2007; Linforth, Cabannes, Hewson, Yang, & Taylor, 2010). Fat has also an influence on the emulsion texture, impacting on creaminess, smoothness or fattiness perception which may modify how flavor is perceived in emulsions during their oral processing (Buettner, Beer, Hannig, Settles, & Schieberle, 2002; Chen, 2009; Bayarri & Costell, 2009).

As commented previously, an important function of hydrocolloid ingredients in oil-in-water emulsions is that they act as a structuring, thickening and stabilizing agent in the aqueous medium (Dickinson, 2009). This effect can be accomplished with structural and rheological changes (Wendin & Hall, 2001; Dolz, Hernández, & Delegido, 2006), which affect the perceived intensity of some sensory attributes. It is generally assumed that increasing viscosity through the addition of thickeners, results in a decrease in flavor and taste intensity. However, the decrease seems to be dependent on thickener type (Baines & Morris, 1987; Hollowood, Linforth, & Taylor, 2002). Starch is one of the most widely used thickeners in the food industry although, carboxymethyl cellulose (CMC) is also being used as an alternative to starch in food products due to its technological and nutritional advantages (Engelen, de Wijk, van der Bilt, Prinz, Janssen, & Bosman, 2005; Jellema, Janssen, Marjolein, Terpstra, de Wijk, & Smilde, 2005; van Ruth, de Witte, & Uriarte, 2004; Bayarri, Chuliá, & Costell, 2010). Considering the structural and rheological differences in the behavior of CMC and starch solutions, both thickeners would be expected to affect the physical and sensory characteristics of emulsions differently. Ferry, Hort, Mitchell, Cook, Lagarrigue, and Valles Pamies (2006), studied the effect of

hydrocolloid type on flavor perception in aqueous systems and observed that starch systems showed much less flavor and saltiness inhibition than hydroxypropylmethyl cellulose systems. The maximum flavor perceived was obtained for starches that maintained granular integrity. They suggested that both flavor perception and mouthfeel can be related to the mixing efficiency of the thickened solutions with water. Polymeric as opposed to granular solution structures mixes less efficiently as a result of entanglements between chains, leading to different mouthfeel attributes and reduced release of tastants to the receptors. A recent study (Desse, Mitchell, Wolf, & Budtova, 2011), has shown that the starch suspension droplets break up more easily than HPMC solution droplets although physico-chemical parameters (viscosity ratio, interfacial tension, capillary number) suggest the opposite. The reason lies in the difference of droplet microstructure inducing different break-up mechanisms.

This information suggests the convenience of considering not only the stabilizing and thickening functions of hydrocolloid added to oil-in-water emulsions if we are to obtain food emulsions with adequate flavor and texture. In this context the objective of this work was to study the influence of fat content (5% and 30%) and thickener type (starch and carboxymethyl cellulose) on rheological behavior, structure, stability, *in-vivo* aroma release and sensory perception in lemon flavored o/w model emulsions.

2. Materials and Methods

2.1 Composition and preparation of oil/water (o/w) emulsions

Emulsions were prepared with sunflower oil (Coosol, COOSUR S.A, Jaén, Spain), sucrose stearate as emulsifier (E-473) (Sisterna SP70, Zeus Química, Barcelona, Spain), carboxymethyl cellulose (CMC) (Akucell AF3265 Akzo Nobel, Amersfoort, The Netherlands), medium crosslinked modified tapioca

starch (C* Creamtex 75720, Cerestar Ibérica, Barcelona, Spain), mineral water (3.00 mg/L calcium, < 0.50 mg/L magnesium, 2.44 mg/L sodium, < 10 mg/L bicarbonate and 0.94 mg/L chloride content) (Bezoya, Grupo Leche Pascual S.A., Burgos, Spain), sucrose and a lemon flavor mixture composed of linalool (LogP = 2.94) and *cis*-3-hexen-1-ol (LogP = 1.61) (SAFC-Sigma Aldrich, Madrid, Spain).

Six different formulations of o/w emulsions were prepared varying in the fat content (5 and 30% w/w oil) and in the matrix type: without thickener (WT), 0.3% w/w CMC (CMC), and 3% w/w starch (ST). Sucrose (7% w/w), sucrose stearate (1% w/w) and volatiles (linalool: 250 ppm and *cis*-3-hexen-1-ol: 50 ppm) were kept constant in all the samples. Sucrose stearate was chosen as emulsifier because of its ability to form stable emulsions over a wide range of oil volume fractions and for its low odor and taste characteristics (Miettinen, Tourila, Piironen, Vehkalathi, & Hyvönen, 2002).

Sucrose stearate and sugar were dispersed in mineral water and stirred (Heidolph RZR 1, Schwabach, Germany) at room temperature for 30 min. A coarse emulsion was prepared by mixing oil and the aqueous phase using a rotor-stator type mixer at 3000 or 5000 rpm (samples with 5% or 30% oil, respectively) (Ultra Turrax, IKA, T50-basic, Staufen, Germany) for 15 min in a water bath at 10ºC to avoid the warming of the sample. The coarse emulsion was then passed through a two-stage high-pressure valve homogenizer (Manton-Gaulin, 15M8TBA, Everett, Massachusetts, USA) at pressure of 300 Kg/cm^2 for 5 passes. Emulsions were stored at 4 ± 1ºC overnight to equilibrate.

The following day, CMC or starch was added to the o/w emulsion. CMC was added to the o/w emulsion and gently mixed (Heidolph RZR 1, Schwabach, Germany) at room temperature for 35 min; at minute 30, volatiles were added. Starch samples were prepared in batches of 800 g as follows: starch and emulsion were mixed with the help of a magnetic stirrer

in a flask for 10 min at room temperature. The flask was placed in a water bath at 99 ± 1ºC and stirred (Heidolph RZR 1, Schwabach, Germany) constantly at around 200 rpm for 30 min, ensuring a period of at least 15 min at 86 ± 1ºC, to be finally cooled in a bath at 10ºC during 10 min. Any evaporated water was replaced gravimetrically. Volatiles were added and the sample was stirred for 5 min. At least, four batches of each composition were prepared. All samples were transferred to a closed flask and stored (4 ± 1ºC; 24 h) prior to rheological, sensory, aroma release and structural measurements. For creaming index measurements, emulsions were stored in 10 mL capped test tubes at 37ºC.

2.2 Rheological measurements

Emulsions without thickener were measured in a concentric-cylinder viscometer Haake model VT 550 (ThermoHaake, Karslruhe, Germany), using a cylinder sensor MV2 (radii ratio = 1.14, length = 60 mm, gap width = 2.6 mm). Emulsions with thickener were measured in a controlled stress rheometer RS1 (ThermoHaake, Karlsruhe, Germany), using parallel-plates geometry (60 mm diameter; 1mm gap). A sample temperature of 10 ± 1ºC was kept during measurements by means of a Haake circulating water bath. Two batches of each composition were prepared and each batch was measured twice, using a fresh sample for each measurement. After loading the sample, a resting period of 10 min was used to allow the sample to recover and reach the desired temperature. After placing the sample between the plates carefully, the excess material was wiped off with a spatula.

2.2.1 Flow behavior

Sample flow was measured by recording shear stress values when shearing the samples at an increasing shear rate from 1 to 200 s^{-1} for a period of 60 s

and in reverse sequence for the same time (González-Tomás, Bayarri, Coll-Marqués, & Costell, 2009). Data from the upward curve of the shear cycle were fitted to the Ostwald-de Waele model (Eq. (1))

$$\sigma = K\dot{\gamma}^{n} \quad (1)$$

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

2.2.2 Viscoelastic behavior

Stress sweeps were made between 0.02 and 300 Pa, at a frequency of 1Hz, in all the systems studied to determine the linear viscoelasticity zone. Frequency sweeps at 0.05 Pa, which is within the linear viscoelastic region, were then performed from 0.01 to 10 Hz. The oscillatory rheological parameters used to compare the viscoelastic properties of the samples were storage modulus (G'), loss modulus (G"), complex dynamic viscosity (η^*) and loss angle ($\tan \delta$) at 1 Hz.

2.3 Structural analysis and physical stability of o/w emulsions

2.3.1 Fluorescence microscopy. Samples were placed in slides with a cover slip and observed under a fluorescence microscope (Nikon Eclipse 90i, Kanagawa, Japan) using a magnification of 40x and an identical exposure time (1.5 s). The photomicrographs were acquired with a digital camera (Nikon DS-5Mc, Kanagawa, Japan). Solution of fluorescent staining was prepared by dissolving 0.1 g/L Nile Red (Sigma-Aldrich Química S.A., Madrid, Spain) in Polyethylene glycol (Sigma-Aldrich Química S.A., Madrid, Spain). Samples were stained 10 min before analysis. The excitation wavelength used was 488 nm.

2.3.2 Particle size distribution

Emulsions particle size distributions were measured using a Malvern Mastersizer 2000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Worcestershire, UK). The refractive index value of sunflower oil (1.472) was used to take measurements. Particle size calculations were based on the Mie Scattering theory. Volume mean diameter values (D[4,3]) and the percentage of volume corresponding to each observed population were calculated using the software (Mastersizer 2000 V5.40). Measurements were made in triplicate for each sample.

2.3.3 Emulsion stability

Emulsions (10 mL) were placed in a transparent test-tube, gently agitated to make sure it was initially homogeneous, and left for 40 days at 37ºC to study gravitational separation. The susceptibility of emulsions to creaming was characterized by a creaming index (CI, Eq. (2)) (McClements, 2007)

$$CI = 100 \times \frac{H_S}{H_E} \tag{2}$$

where, H_E is the total height of the emulsion and H_S is the height of the serum layer.

2.4 In vivo aroma release measurements

In-vivo aroma release from each flavored o/w emulsion was measured from nose breath analyses with a High Sensitivity Proton Transfer Reaction-Mass Spectrometer (PTR-MS) (Ionicon, Innsbruck, Austria). The ionization was performed at E/N ratio of 106 Td (Gouw & Warneke, 2007) (drift voltage: 500V, ionization pressure: 2.43mbar, ionization temperature: 70ºC) and protonated ions from acetone (*m/z* 59), linalool (*m/z* 137) and *cis*-3-hexen-1-

ol (*m/z* 83) were monitored in single ion monitoring mode with dwell time of 100ms. Acetone was used as a marker for breathing pattern. Nose breath was sampled into the PTR-MS at 35 mL/min via a tee-piece connected to the PTR-MS transfer line heated at 160ºC. Seven assessors consumed all six samples at 10ºC in a single session, with a break of 5 min between each sample. The panelists were asked to breathe in, sip 5 mL of sample emulsion from a spoon, close their mouths swallow the sample, and then exhale and continue to breathe normally while resting their nose on the PTR-MS nasal sampling tube. Breathing pattern of panellists was monitored for 1 min after sample consumption. Plain crackers and water were used as palate cleansers. Raw data were analyzed to extract two parameters: the maximum aroma intensity (Imax) and the cumulative area under the 1 min release profile (AUC).

2.5 Sensory evaluation

2.5.1 Descriptor selection and training

An initial list of terms was prepared with the information obtained from bibliography (Kilcast & Clegg, 2002; Dresselhuis, de Hoog, Cohen Stuart, Vingerhoeds, & van Aken, 2008; Hollowood, Bayarri, Marciani, Busch, Francis, Robin, Taylor, & Hort, 2008; Benjamins, Vingerhoeds, Zoet, de Hoog, & van Aken, 2009). Ten assessors with previous experience (more than two years) in evaluating sensory differences in various semi-solid products were asked to evaluate the suitability of these descriptors to describe the sensory characteristics of the samples according to the checklist method (Damasio & Costell, 1991). The initial list, composed of 19 terms regarding the odor, flavor, and texture of the samples, was finally reduced to 14 after the training sessions with the assessors. The final list of descriptors, their end terms, and definitions are shown in Table 1.

Table 1. End terms and definitions of the odor, flavor and texture descriptors used in the sensory evaluation of the o/w emulsions

Category	Descriptor	End terms	Definition
Odor	Odor intensity	Weak-Intense	Magnitude of the odor perceived
	Citrus odor	Not perceived-Intense	Odor associated with general impression of citric fruits
	Grassy odor	Not perceived-Intense	Odor associated with fresh cut grass
	Oily odor	Not perceived-Intense	Odor associated with sunflower oil
Flavor	Flavor intensity	Weak-Intense	Magnitude of the flavor perceived
	Sweetness	Weak-Intense	Elemental taste produced by aqueous solutions of sugar and different sweeteners
	Citric flavor	Weak-Intense	Flavor associated with general impression of citric fruits
	Grassy flavor	Weak-Intense	Flavor associated with fresh cut grass
	Oily flavor	Weak-Intense	Flavor associated with sunflower oil
	After taste	Weak-Intense	Flavor perceived when the product has been swallowed; different from the one perceived when it was in the mouth
Texture	Consistency in mouth	Thin-Thick	Mechanical properties perceived when compressing the product between the month and the palate
	Creaminess	Weak-Intense	Combined perception of fat, smoothness, and viscosity
	Dispersing	Slow-Fast	How quickly the sample breaks down/dissolves in the mouth ready for swallowing
	Mouth coating	Not perceived-Intense	The mouthfeel of the product, once swallowed, consists in the perception of a fat thin layer covering the palate

Assessors were trained in 3 sessions according to the guidelines of the ISO 8586-1 (1993). The first session was held with the panel leader and all of the assessors and was intended to select and define the descriptors, to determine the sample evaluation procedures, and establish the definitive scorecard. In the other 2 sessions, each assessor evaluated the intensity of the 14

previously selected attributes in separate booths on 3 different samples. At the end of these sessions, the panel leader and assessors discussed the individual results obtained to establish consensus criteria for evaluation.

2.5.2 Descriptive profile

Descriptive analysis of the 6 samples was carried out in duplicate over 4 sessions and each assessor evaluated 3 samples per session (Meilgaard, Civille, & Carr, 1999). Each sample (30mL) was presented at 10 ± 1°C in duplicate in two glass cups, fitted with a lid, and labeled with the same random three-digit code. For each sample, odor attributes were evaluated first from one of the glass cups. Then, using the sample contained in the other glass cup, assessors were asked to evaluate the flavor, and finally, texture attributes. Panelists tasted the same volume of each sample from a spoon (5 mL). White bread without salt and soured-up water (0.2 g L^{-1} citric acid) (Vingerhoeds, de Wijk, Zoet, Nixdorf, & van Aken, 2008) were used as palate cleanser. The intensity of each attribute was scored on a nonstructured 10-cm scale, with the corresponding end terms (Table 1). To reduce the influence of serving order, the samples evaluated in each session were served following a Williams design for three samples. All sessions were carried out in a standardized test room (ISO 8589, 2007) with separate booths under normal white fluorescent illumination in morning sessions (11:00 to 13:00 h). Experimental design, data acquisition, and analysis were performed using Compusense five, release 5.0 (Compusense Inc., Guelph, ON, Canada).

2.6 Statistical analysis

A two-way ANOVA (fat content and matrix type) with interaction was applied to the rheological and *in-vivo* aroma release data. Fisher's test

($\alpha = 0.05$) was used to calculate the minimum significant difference. A two-way ANOVA (samples and assessors) with interaction was applied to the sensory data obtained for each attribute. Individual differences between assessors were analyzed by a fixed model, considering assessors as fixed factor. When a significant interaction between assessors and sample was observed for a descriptor, a mixed model ANOVA, considering assessors as random effect, was performed (Carlucci & Monteleone, 2001). F_{sample} values were then recalculated taking the average square of the interaction as denominator. Minimum significant differences were calculated by the Fisher's test ($\alpha = 0.05$). Finally, another two-way ANOVA with interaction was applied to the sensory data to determine the effect of the fat content and matrix type on odor, flavor, and texture attributes. Minimum significant differences were calculated by the Fisher's test ($\alpha = 0.05$). Principal Component Analysis (PCA) with Varimax rotation was applied to the average values of flow and in-vivo aroma release parameters and to the mean values of attribute intensity. All calculations were carried out with XLSTAT Pro software, version 2007 (Addinsoft, Paris, France).

3. Results

3.1 Rheological properties

The flow curves in Figure 1a show that emulsions without thickener presented Newtonian flow behavior with flow index (n) values of around 1 (Table 2). Emulsions with both CMC and starch showed shear thinning (with n values ranging from 0.4 to 0.6) and slightly time-dependent behavior. Viscoelastic properties of emulsions without thickener could not be measured, due to the fluidity of the samples. Most of the samples with thickener had a structure in between those of a concentrated polymer solution and a weak gel (G' and G" had very similar values and were

dependent on frequency). However, CMC-based emulsions with 30% oil showed a gel-like behavior with storage modulus (G') values higher than loss modulus (G") and almost independent of the frequency (Figure 1b).

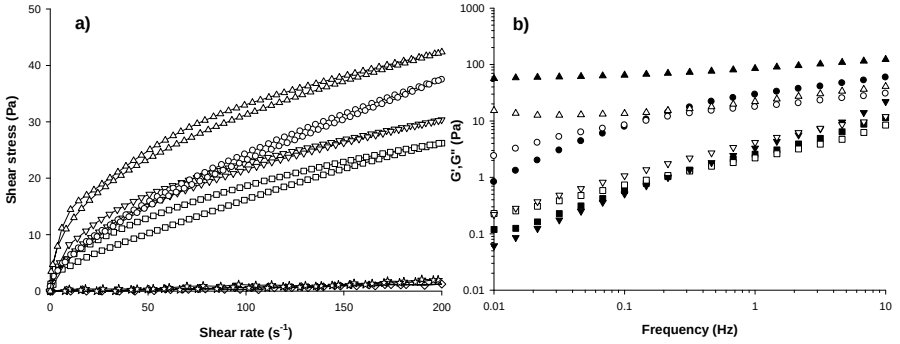

Figure 1. Rheological behaviour of o/w emulsions with different matrix type (WT: without thickeners, CMC: carboxymethyl cellulose and ST: starch) and fat content: 5% oil (◇: 5WT, ▽: 5CMC and □: 5ST) and 30% oil (☆: 30WT, △: 30CMC and ○: 30ST). a) Flow curves and b) Mechanical spectra (G': filled symbols and G": empty symbols).

Analysis of variance of flow parameters and of viscoelastic parameters at 1Hz values showed that the effect of the interaction between fat content and matrix type was significant on K, G', tanδ and η* ($P < 0.01$) but not on n ($P = 0.95$) and G" ($P = 0.15$) values. This interaction indicated that the effect of fat content on the variables considered different depending on the matrix type. Consistency index values increased with oil concentration in both CMC and starch-based emulsions, but remained constant in emulsions without thickener. With respect to G', η* and tanδ, the interaction was due to the higher increase (considering G' and η*) or decrease (considering tanδ) of these parameters with oil content in CMC than in starch systems. In addition, while in 5% oil emulsions CMC systems showed higher tanδ values than starch systems; in 30% oil emulsions the opposite was observed. Fat content and matrix type main effects were significant ($P < 0.01$) on all

variables considered, with the exception of tan δ ($P = 0.26$) and n ($P = 0.16$) parameters that were not affected by the matrix type or by fat content, respectively.

As commented above, the fat content of the emulsions only had an effect on flow behavior when thickener was present, *i.e.*, flow parameters of samples without thickener and different oil content showed no significant differences (Table 2). However, consistency increased with oil concentration when thickener was present. For each fat content, CMC-based systems showed higher consistency than starch-based emulsions. The 5% oil CMC-based emulsion did not differ statistically ($P < 0.05$) from 30% oil starch-based emulsion for flow parameters. With respect to the viscoelasticity of thickened systems, emulsions with 5% oil and with either CMC or starch showed a very similar viscoelastic behavior with no statistical differences on G' and η* values at 1Hz. Similar behavior was observed for 30% oil emulsions with starch (Figure 1b) but with higher G', G'' and η* values. A more structured system was observed in the case of the emulsion made with 30% oil and CMC which presented the highest viscoelastic modulus values and the lowest tanδ values. Tan δ, which is defined as the ratio of the loss modulus (G') to the storage modulus (G'), is a measure of the viscous properties relative to the elastic properties of a viscoelastic material (Shoemaker, Nantz, Bonnans, & Noble, 1992), therefore 5% oil emulsions showed higher viscous properties with tan δ values around 1.

Table 2. Mean values and significant differences of rheological parameters of o/w emulsions with different matrix type (WT: without thickener, CMC: carboxymethyl cellulose and ST: starch) and fat content (5% and 30% oil)

Rheological parameters	5% oil			30% oil		
	WT	CMC	ST	WT	CMC	ST
K (Pa s^n)	0.01^{d}	2.82^{b}	1.53^{c}	0.01^{d}	5.37^{a}	2.92^{b}
n	1.00^{a}	0.44^{cd}	0.57^{b}	0.97^{a}	0.39^{d}	0.53^{bc}
G' (Pa)	-	3.53^{a}	2.60^{a}	-	85.76^{c}	30.74^{b}
G" (Pa)	-	4.32^{b}	1.55^{a}	-	21.34^{d}	19.36^{c}
tan δ	-	1.23^{d}	0.91^{c}	-	0.25^{a}	0.63^{b}
η* (Pa s)	-	0.89^{a}	0.49^{a}	-	14.07^{c}	5.79^{b}

$^{a-d}$ *Means within a row with common superscripts did not differ significantly ($P < 0.05$)*

The results showed that both fat and thickener interact to modify rheological characteristics of o/w emulsions. This is in line with results described by Mela, Langley and Martin (1994); Bayarri, Smith, Hollowood and Hort (2007) and Daget, Joerg, and Bourne (1987). It has been reported that there is more packing of oil droplets at higher oil concentrations than at lower oil concentrations, forming a higher level network structure. As oil concentration decreases, the mean distance between droplets is greater; thus lower rheological parameter values are observed (Ma & Barbosa-Cánovas, 1995).

3.2 Structural properties and physical stability

3.2.1 Particle-size distribution

Particle-size distribution of different o/w emulsions is shown in Figure 2. The droplet size distribution was found to be monomodal for o/w emulsions without thickener, ranging from 0.1 to 0.6 μm with an average particle size of 0.31 ± 0.02 μm ($D_{4,3}$) for samples containing 5% oil, and from 0.2 to 2.2 μm ($D_{4,3}$ = 0.46 ± 0.05 μm) for emulsions with 30% oil.

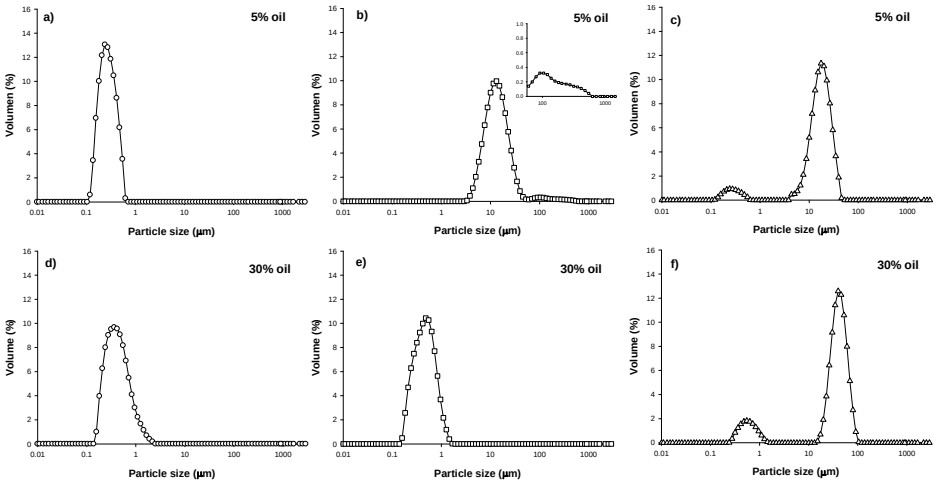

Figure 2. Particle size distribution of o/w emulsions with different fat content (5% and 30% oil) and matrix type (○: without thickener, □: carboxymethyl cellulose and △: starch).

With respect to CMC-based emulsions, the sample with 30% oil and CMC showed a similar monomodal distribution to the sample with the same oil content and without thickener ($D_{4,3}$ = 0.47 ± 0.06 µm, ranging from 0.2 to 2.2 µm). However in the sample with 5% oil, CMC addition resulted in a bimodal distribution with a pronounced increase in particle size: a population of particles ranging from 3.3 to 60.3 µm ($D_{4,3}$ = 13.2 ± 0.4 µm, 97% of volume) and a small population of larger particles ranging from 69.2 to 549.5 µm ($D_{4,3}$ = 105.2 ± 1.9 µm, 3% of total volume). An increase in particle size due to droplet aggregation may have occurred because of droplet flocculation or coalescence (McClements, 2007). Regarding the starch-based emulsions a bimodal distribution was observed for both fat contents: in addition to the previously observed fat droplet population in the emulsions without thickener, a large population of particles was observed in emulsions with both 5% oil (4.4 - 45.7 µm, 93% of total volume) and 30% oil (15.1 - 104.7 µm, 86% of total volume), which can be explained by the presence of starch-granule in these samples.

3.2.2 Microstructure

The droplet-size distribution of the prepared emulsions was well demonstrated by their corresponding droplet morphology observed under the fluorescence microscope (Figure 3).

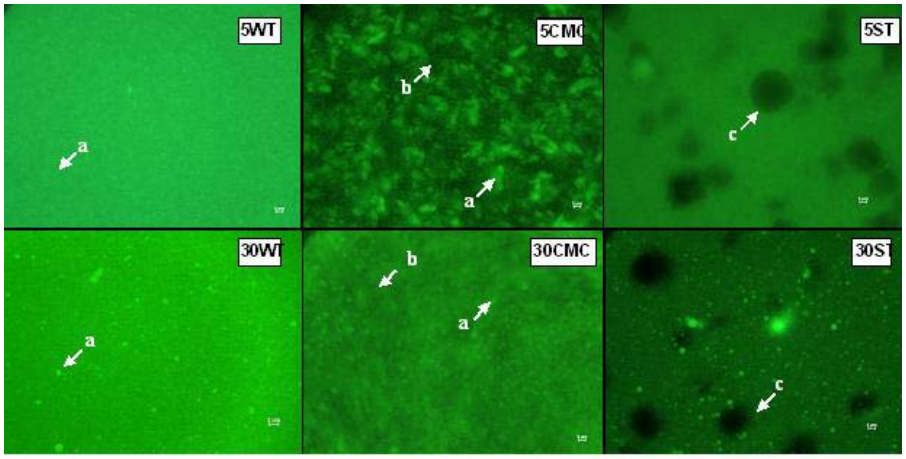

Figure 3. Microscopy images of o/w emulsions with 5% oil (5WT, 5CMC and 5ST) and 30% oil (30WT, 30CMC and 30ST), showing fat droplets (a), carboxymethyl cellulose (b) and starch granules (c) (WT: without thickeners, CMC: carboxymethyl cellulose and ST: starch). Micrographs taken at 40x magnification, 10ºC, scale bars to 5 μm, and stained with Nile red.

The fat droplets in the emulsions without thickener were clearly separated and evenly distributed across the image, thus we assumed that the emulsions were non-flocculated. A similar image was observed for 30% oil emulsions with CMC. However, in the case of 5% oil sample with CMC, the droplets were closely associated with one another and clumped together in certain regions. Isolated and swollen starch granules, as well as fat globules, can be observed in starch-based emulsions, this type of structure has previously been observed in custards made with modified starch (Bayarri, González-Tomás, Hernando, Lluch, & Costell, 2011).

3.2.3 Creaming index

Creaming is one of the most common instability mechanisms in O/W emulsions and its visual observation is the simplest method to obtain information about this gravitational separation. Practical information about the extent of creaming in emulsions can be obtained with the creaming index values and with their evolution during storage. In this work emulsions without thickener showed no obvious signs of gravity creaming over a period of 15 days, *i.e.*, there was no discernible serum layer at the bottom of the tube, nor a distinct cream layer at the top (Figure 4). However, there were substantial differences in creaming behavior of the two polysaccharides and for the two oil contents. CMC addition to emulsion with 5% oil accelerated destabilization. The instability of CMC-based emulsions with 5% oil is indicative of an oil droplet flocculation mechanism, thus confirming the above results observed. Considering 5% oil emulsions, we observed that, while emulsion containing CMC is still less stable than the original emulsion (no added polysaccharide), the presence of starch prevented serum separation over the observation period. With respect to 30% oil emulsion, CMC addition reduced creaming index, which became zero when starch was added. Excellent stability was shown by the starch-based emulsion with no phase separation. However, the values of creaming index depend on the initial droplet concentration and on the packing of the droplets and consequently, one should be careful when to compare stability of emulsions with different composition using creaming index values.

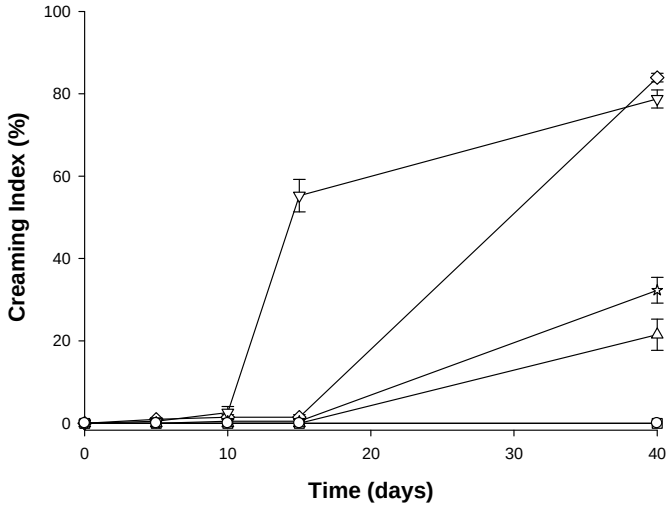

Figure 4. Creaming index of different o/w emulsions with different matrix type (WT: without thickeners, CMC: carboxymethyl cellulose and ST: starch) and fat content: 5% oil (◇: 5WT, ▽: 5CMC and □: 5ST) and 30% oil (☆: 30WT, △: 30CMC and ○: 30ST).

3.3 In-vivo aroma release

The emulsion samples were consumed by seven panelists and the intensity of both linalool and cis-3-hexen-1-ol released was monitored for 1 min after sample consumption. Two parameters were extracted from the aroma release profile of each compound: the maximum aroma intensity (Imax) and the cumulative area under the 1 min release profile (AUC). Analysis of variance showed that fat content affected delivery of the most lipophilic compound (linalool; $P < 0.01$) to the nasal cavity (Imax and AUC), but did not affect the delivery of the least lipophilic compound (*cis*-3-hexen-1-ol; $P > 0.25$). Matrix type had a significant effect on AUC values for both linalool and *cis*-3-hexen-1-ol ($P < 0.05$), but not on Imax values ($P > 0.17$). The interaction between the main effects was not significant ($P > 0.69$). As expected, Imax values of linalool were significantly lower ($\alpha = 0.05$) when panelists

consumed the emulsion with 30% oil compared to the emulsions with 5% oil content (Figure 5a). A reduction of the AUC values of linalool with fat content was also observed, but in this case, matrix type also played a role: within each oil contents, AUC values of starch-based systems were higher (Figure 5c). With respect to the delivery of *cis*-3-hexen-1-ol, Imax was not affected by fat content (P = 0.24) or matrix type (P = 0.17) (Figure 5b), but AUC was higher for starch systems (Figure 5d).

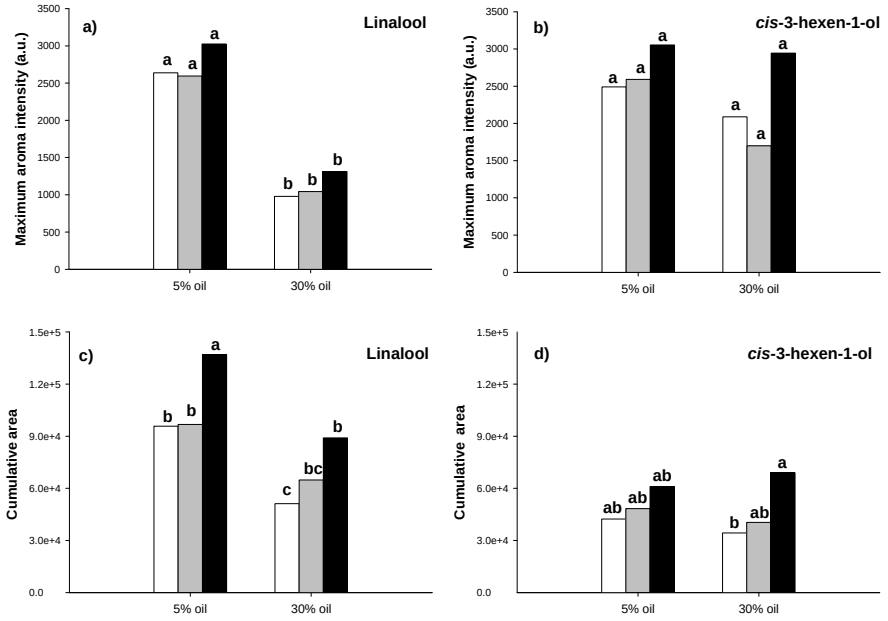

Figure 5. Maximum aroma intensity (a, b) and cumulative area (c, d) of linalool and *cis*-3-hexen-1-ol in vivo of o/w emulsions with different fat content (5% and 30% oil) and matrix type (□: without thickener, ▒: carboxymethyl cellulose and ■: starch).

3.4 Sensory Properties

A fixed model of two-way ANOVA (samples and assessors) with interaction (Table 3) was applied to the sensory scores obtained for the 14 attributes evaluated. Significant differences between samples ($\alpha = 0.05$) were detected except for one attribute: after taste. Assessors were also a significant source of variation in all cases. The significance of the effect of sample × assessor interaction provides information about the influence of assessor's variability on the estimation of sample differences (González-Tomás, Bayarri, & Costell, 2009). In this case, with regard to the 13 attributes for which the panel found significant perceptible differences ($\alpha = 0.05$) among samples, the effect of the interaction sample × assessor was not significant ($P > 0.05$) for eight of them, indicating a good level of concordance among the panel members on their evaluations. For the remaining five attributes (citrus odor, grassy odor, oily odor, grassy flavor and oily flavor), the sample × assessor interaction was significant ($P \leq 0.05$) indicating a certain lack of concordance within the panel. In spite of this, the main sample effect for these attributes remained significant when a mixed model of ANOVA was applied considering assessors as random effect (Table 3), except for two attributes (grassy odor and grassy flavor). Grassy odor/flavor note is conferred by C_6 alcohols and aldehydes, typically trans-2-hexenal and *cis*-3-hexenol (Taylor & Linforth, 2010). Samples with different fat content and matrix type were perceived with a similar grassy odor and flavor, which is in agreement with the aroma release results, where no differences among samples in the maximum intensity of *cis*-3-hexenol were observed.

Table 3. Two-way ANOVA (sample and assessor) with interaction of the sensory attributes (10 assessors, 6 samples, 2 replicates). F values and statistical signification

	Attribute	S:	A:	S x A	Sample[2]
Odor	Odor intensity	15.30*	5.49*	1.51^{NS}	–
	Citrus odor	18.59*	8.73*	2.21*	8.42*
	Grassy odor	4.65*	2.55*	3.83*	1.21^{NS}
	Oily odor	9.61*	7.92*	2.53*	3.79*
Flavor	Flavor intensity	13.78*	12.42*	1.31^{NS}	–
	Citrus flavor	12.53*	3.55*	1.40^{NS}	–
	Grassy flavor	3.79*	3.79*	1.79*	2.11^{NS}
	Oily flavor	18.85*	17.15*	3.58*	5.26*
	Sweetness	12.02*	5.56*	1.19^{NS}	–
	After taste	1.11^{NS}	9.16*	1.73*	
Texture	Thickness	67.10*	3.42*	0.77^{NS}	-
	Creaminess	76.86*	7.00*	1.50^{NS}	-
	Dispersing	57.95*	5.99*	0.92^{NS}	-
	Mouth coating	23.13*	5.98*	1.57^{NS}	-

[1]*Calculated using the mean square error as denominator*
[2]*F-values recalculated using the mean square of the interaction as denominator*
**Significant at P < 0.05; NS = not significant*

To determine the effects of fat content and of matrix type on odor, flavor, and texture attributes, a new two-way ANOVA with interaction was applied to the 11 attributes for which the panel found significant differences among samples (Table 4). The mean values of the 11 sensory attributes for each sample and the significant differences among them are given in Table 5. The effect of the fat content × matrix type interaction was significant only for two attributes, odor intensity and citrus odor; indicating that the effect of fat content on these attributes differed depending on matrix type. Considering emulsions without thickeners and CMC-based emulsions, both odor intensity and citrus odor were perceived significantly higher in 5% oil emulsions than in 30% oil emulsions, but starch-based samples with different fat content

showed a similar odor intensity and citrus odor. Perceptible differences in oily odor were due only to matrix type. Starch-based emulsions were perceived to have higher oily odor intensity than both CMC-based and without thickener emulsions.

Table 4. Two-way ANOVA (fat content and matrix type) with interaction of the sensory attributes scores. F values and statistical significations

Attribute	A: Fat content	B: Matrix type	A x B
Odor			
Odor intensity	36.21*	1.64^{NS}	0.01*
Citrus odor	37.00*	0.48^{NS}	3.29*
Oily odor	2.64^{NS}	9.59*	0.26^{NS}
Flavor			
Flavor intensity	23.14*	4.59*	0.86^{NS}
Citrus flavor	38.38*	1.24^{NS}	2.64^{NS}
Oily flavor	11.60*	7.76*	0.74^{NS}
Sweetness	33.91*	3.88*	0.10^{NS}
Texture			
Thickness	14.21*	142.47*	3.20^{NS}
Creaminess	27.41*	100.04*	1.37^{NS}
Dispersing	5.90*	101.22*	2.31^{NS}
Mouth coating	15.57*	27.13*	0.82^{NS}

**Significant at P < 0.05; NS = not significant.*

Differences perceived in all flavor attributes were affected for both fat content and matrix type, except citrus flavor that was not affected by matrix type. Five percent o/w emulsions were perceived to have more flavor intensity than 30% o/w emulsions, with CMC-based emulsions being perceived to have the lowest flavor intensity. A significant decrease in citrus flavor scores was observed when oil concentration was increased. This fact responds to the diminished release of linalool observed previously for *in-vivo* aroma release measurements. As expected, 30% oil emulsions were

perceived to have a more oily flavor than 5% oil emulsions; especially when thickeners were added. Sweetness intensity was significantly enhanced by addition of oil and decreased by addition of thickeners.

Table 5. Mean values of sensory attributes scores and corresponding Fisher's significant difference of o/w emulsions with different matrix type (WT: without thickener, CMC: carboxymethyl cellulose and ST: starch) and fat content (5% and 30% oil)

Attribute[1]	5 % oil			30% oil		
	WT	CMC	ST	WT	CMC	ST
Odor						
Odor intensity	6.47^{a}	6.55^{a}	5.31^{ab}	4.44^{b}	2.79^{c}	4.36^{b}
Citrus odor	5.43^{a}	4.64^{ab}	3.94^{bc}	1.79^{d}	1.56^{d}	2.85^{cd}
Oily odor	1.28^{c}	1.45^{c}	3.09^{ab}	1.54^{c}	2.31^{bc}	4.03^{a}
Flavor						
Flavor intensity	7.21^{a}	6.27^{abc}	6.83^{ab}	5.40^{c}	3.77^{d}	5.57^{bc}
Citrus flavor	6.41^{a}	4.49^{bc}	5.7^{ab}	2.28^{d}	2.86^{d}	3.19^{cd}
Oily flavor	1.04^{c}	1.92^{c}	2.42^{bc}	1.82^{c}	3.38^{ab}	4.45^{a}
Sweetness	4.10^{bc}	3.27^{c}	3.02^{c}	6.66^{a}	5.43^{ab}	5.21^{b}
Texture						
Thickness	0.61^{a}	6.11^{c}	4.87^{b}	0.86^{a}	7.21^{d}	7.05^{cd}
Creaminess	0.65^{a}	5.49^{b}	4.53^{b}	1.63^{a}	7.42^{c}	6.81^{c}
Dispersing	8.84^{c}	3.19^{a}	5.08^{b}	8.62^{c}	2.77^{a}	3.17^{a}
Mouth coating	0.98^{a}	4.71^{c}	3.11^{b}	2.15^{ab}	6.34^{d}	5.67^{cd}

[a-d] Means within a row with common superscripts did not differ significantly ($P < 0.05$).
[1]Attributes evaluated on a 10-cm, linear, semi-structured scale.

With regard to texture attributes, both fat content and matrix type had a significant effect on all of them. Samples with thickeners were perceived to have more thickness, creaminess and mouth coating than samples without thickener for all fat contents tested. As observed previously, the addition of thickeners increases the consistency of emulsions, which has been found to be the predominant factor influencing the perception of both thickness and creaminess (Akthar, Murray, & Dickinson, 2006; van Aken, de Hoog,

Nixdorf, Zoet, & Vingerhoeds, 2006). Panelists did not perceive significant differences in the texture attributes between thickeners in the 30% oil emulsions. However, in 5% oil emulsions perceptible differences in thickness, dispersing and mouth coating were observed between CMC and starch samples.

4. Discussion

Besides the different rheological behavior observed among samples, fat content and matrix type also affected the structural properties and physical stability of o/w emulsions. In the present work, it was not investigated if the amount of sucrose stearate (1%) added as emulsifier to emulsions was sufficient for complete coverage of the droplets. However both 5% and 30% oil emulsions without thickener added did not show signs of poor coverage of the droplets. These samples were stable during the storage time at least of 15 days. A similar consideration was made by Miettinen, Tourila, Piironen, Vehkalathi, & Hyvönen, 2002, when comparing the effect of 1% sucrose stearate addition on stability of both 5 and 50% fat-containing emulsions. The most remarkable effect was that in the case of 5% oil sample with CMC, the fat droplets were closely associated, thus accelerating emulsion destabilization. In this experiment, the emulsions with higher fat contents (30% oil) also contained slightly higher concentrations of CMC in the aqueous phase (0.71% w/w) compared to the 5% oil emulsions (0.53% w/w), thus increasing the viscosity of the emulsion aqueous phase. This minimized droplet mobility that delayed collision frequency and hence reduced droplet partial/full coalescence. It was believed that in the 5% oil emulsion, the concentration of CMC was not enough to saturate all the surfaces of the droplets thus favoring emulsion destabilization. With respect to cellulose distribution, when oil droplets are small, as occurs in 30% oil emulsions,

cellulose particles are dispersed relatively evenly to give the same concentration of cellulose throughout the volume. When the oil droplets are large or closely associated, as shown in 5% emulsions, the cellulose particles are relegated to regions around the large oil droplet, which prevents their even distribution throughout the volume (Imai, Shimichi, Maruyama, Inoue, Ogawa, Hatae, & Shimada, 1997). An excellent stability, with no phase separation, was shown by the starch-based emulsions, with higher thickener concentrations in the aqueous phase than CMC emulsions (3.2% and 4.3% w/w of starch for 5% oil and 30% oil emulsions, respectively). The different behavior between thickeners could be due to the different thickener concentrations on aqueous phase, water binding capacity, ionic charge, and structure (granular for modified starch vs. polymer for CMC).

Results of i*n-vivo* aroma release confirm that the effect of fat content on the release of aroma compounds is strongly dependent on polarity. Previous studies have shown that the fat content and the aroma compounds lipophilicity are key factors affecting flavor release and all studies show greater retention of lipophilic compounds with increasing fat content (Roberts, Pollien, Antille, Lindinger, & Yeretzian, 2003; Miettinen, Hyvönen, Tuorila, H., 2003; Miettinen, Hyvönen, Linforth, Taylor, & Tuorila, 2004; Philippe, Seuvre, Colas, Langendorff, Schippa, & Voilley, 2006). However, the relationship between thickener type and aroma release is not so clear. On the one hand, previous studies showed no effect of different texturizing agents (CMC, carrageenan, starch, pectin and gelatine) on maximum intensity and/or cumulative area data obtained from *in vivo* monitoring of flavor release (Lethuaut, Weel, Boelrijk, & Brossard, 2004; Mie, Reineccius, Knighton, & Grimsrud, 2004; Aprea, Biasioli, Gasperi, Märk, & van Ruth, 2006). On the other hand, some studies have shown a significant reduction of in-mouth flavor release due to the presence of CMC (Kühn, Delahunty, Considine, & Singh, 2009) and better flavor release

properties from modified starch, which may be due to a salting-out effect (Cayot, Pretot, Doublier, Meunier, & Guichard, 2004; González-Tomás, Bayarri, Taylor, & Costell, 2007). As stated by Ferry, Hort, Mitchell, Cook, Lagarrigue, and Valles Pamies (2006) differences in some thickener structure solutions (polymeric or globular) can also influence mouthfeel attributes and modify the release of chemical stimuli to the receptors. The better flavor release properties observed in this study for starch-based systems were not reflected in a higher flavor intensity or citrus flavor perception.

Although it is generally accepted that sweetness perception decreases with thickener addition (Baines & Morris, 1987; Hollowood, Bayarri, Marciani, Busch, Francis, Robin, Taylor, & Hort, 2008), the relationship between oil content and perceived sweetness is not so clear. Metcalf and Vickers (2002) compared the taste intensities of oil water emulsions with different fat content to the intensities of oil-free samples. They detected an increase in sweetness with fat content that could be produced by the relatively higher concentration of sucrose in the aqueous phase of emulsions. When samples with the same aqueous sucrose concentration were compared they observed that fat did not affect perceived sweetness intensity. Some researchers have observed sweetness enhancement (Tuorila, Sommardahl, Hyvönen, Leporanta, & Mirimaa, 1993; Hollowood, Bayarri, Marciani, Busch, Francis, Robin, Taylor, & Hort, 2008) whereas others have observed sweetness suppression (King, Lawler, & Adams, 2000; Bayarri, Smith, Hollowood, & Hort, 2007) with oil concentration. In this study sweetness intensity was significantly enhanced by oil addition and decreased with thickeners addition. This can be explained in terms of emulsion structure such in that an increase in oil content raised the concentration of sucrose in the continuous aqueous phase; therefore more tastant is available to reach the taste receptors (Hollowood, Bayarri, Marciani, Busch, Francis, Robin, Taylor, & Hort,

2008). Additionally to the impact on aroma and flavor attributes, both the fat content and matrix type also affected the texture perceived. Increasing oil content resulted in an increase in thickness, creaminess, and mouth coating and in a reduction in dispersing, although the oil effect was not as marked as that observed for thickener effect. Compared to samples without thickener, emulsions with thickener were perceived as thicker, creamier, with a greater perception of the thin fat layer when swallowed (mouth coating), which slowly dissolved in the mouth. This fact could be explained by taking into account that emulsion with thickener will drain more slowly from oral surfaces, which may lead to a more persistent coating of these surfaces by the emulsions (van Aken de Hoog, Nixdorf, Zoet, & Vingerhoeds, 2006). When comparing systems with different thickener at the same oil content, some textural differences were observed. Differences in rheological behaviour observed in 30% oil emulsions between ST and CMC systems were higher than in 5% o/w emulsions. However, differences in the textural properties perceived between CMC and ST systems were higher in 5% than in 30% oil emulsions. Shearing of emulsions between tongue and palate can lead to droplet coalescence, and emulsions with a considerable degree of coalescence in the mouth revealed higher scores of fat-related sensory attributes like fattiness, creaminess and thickness (Dresselhuis, de Hoog, Cohen Stuart, Vingerhoeds, & van Aken, 2008). The partial coalescence observed previously in 5% oil emulsions with CMC could be the reason for the significantly higher scores for thickness and mouth coating observed in this sample.

To study the joint variability of the sensory attributes for which significant differences were found as well as of rheological and *in-vivo* aroma release parameters; Principal Component Analysis was applied to their mean values (Fig. 6). Viscoelastic parameters were not included because they were not available for all the emulsions studied. The first component accounted for

52% of total variability and separated the samples mainly by flow behavior parameters and in-mouth texture attributes. Emulsions with thickener, *i.e.*, both starch and CMC-based emulsions, in the positive part of the first dimension, showed higher consistency index, higher thickness, more creaminess, higher mouthcoating and greater oily odor and oily flavor intensity. Emulsions without thickener, in the negative part of the first dimension, showed higher flow behavior index and were evaluated to have faster dissolution in the mouth.

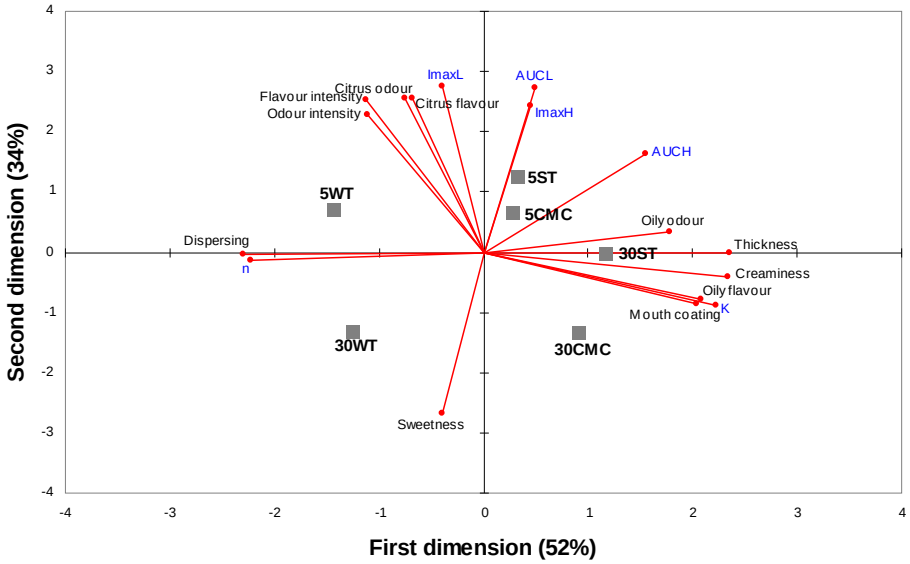

Figure 6. Principal component analysis bi-plot of o/w emulsions with different matrix type (WT: without thickeners, CMC: carboxymethyl cellulose and ST: starch) and fat content: 5% oil (5WT, 5CMC and 5ST) and 30% oil (30WT, 30CMC and 30ST), for *in-vivo* aroma release parameters (ImaxH: maximum aroma intensity of *cis*-3-hexen-1-ol; ImaxL: maximum aroma intensity of linalool; AUCH: cumulative area of *cis*-3-hexen-1-ol; and AUCL: cumulative area of linalool), flow parameters (K: consistency index and n: flow behaviour index), and sensory attributes.

The second component explained 34% of total variability and was defined mainly by aroma release parameters and flavor attributes. Emulsions with

the lower oil content (5%), which were distributed on the positive side, were evaluated to have stronger citrus odor and citrus flavor and greater flavor and odor intensity. These samples showed higher values for *in-vivo* aroma release parameters than samples with 30% oil, which were in the negative part of the second dimension and were evaluated as sweeter.

It can be concluded that, despite their stabilizing effect on o/w emulsions, the addition of different hydrocolloids modifies not only their rheological behavior but also flavor release and sensory perception mechanisms in different ways. More information about the effects of different emulsifiers and stabilizers on perceived flavor and texture of food emulsions is needed to design food emulsions that provide a better sensory quality.

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CAPÍTULO 5

IMPACT OF STRUCTURAL DIFFERENCES ON PERCEIVED SWEETNESS IN SEMISOLID DAIRY MATRICES

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ABSTRACT

The objective of this work was to explore the possibility of obtaining dairy desserts of similar consistency and with different sweetness intensity. Eight formulations were prepared varying in the thickener type and concentration: carboxymethyl cellulose (1.1% and 1.3% w/w) and modified starch (3.5% and 4.0% w/w) and the milk type (whole or skimmed). The amounts of sugar (10% w/w), colorant (37.5 mg/kg) and lemon flavour (120 mg/kg) were fixed. Changes in composition and structure provide products with similar values of three instrumental indices of thickness ($\eta_{10,}$ η_{50} and Kokini shear stress (OSS)). The relationship between each of these instrumental indices and the sensory consistency fitted well to Psychophysical Stevens' Law. No direct relationship was detected between OSS values and sweetness. Some structural differences observed between thickeners can influence product behaviour during oral processing modifying in-mouth delivery of sucrose molecules. It could explain sweetness differences among samples with similar consistency.

PRACTICAL APLICATIONS

Decreasing calorie content of processed foods without modifying their quality and acceptance is important for consumers and the food industry. In dairy desserts, a strategy to compensate or reduce the sensory quality problems associated with calorie reduction is to design novel textures for optimising the delivery of sweet taste stimuli to taste receptors. The results of this work have shown that it is possible to obtain semisolid dairy desserts with similar texture and with different sweetness and also, with different texture and similar sweetness using different thickeners. More studies are needed to understand the effect of oral processing in dairy desserts thickened

with different hydrocolloids to explain the observed variations in sweetness perceived.

KEYWORDS

Starch, carboxymethyl cellulose, texture, microstructure, sweetness

INTRODUCTION

Improving the nutrient composition of processed foods by decreasing calorie content, without modifying their quality and acceptance, is an important issue for consumers and the food industry (Nehir and Simsek 2012). The elimination or reduction of fat, sugar or salt in foods can diminish the prevalence of diet-related diseases and increases consumers´ health and wellness; however, it can also modify food composition and structure. These modifications can change the effects of each food component and the interactions among them, mostly giving rise to clearly perceptible changes in colour, flavour, and texture and lowering consumer acceptance. A common strategy to compensate or reduce the sensory quality problems associated with calorie reduction in foods, is the use of certain ingredients, such as fat, sugar or salt replacers, to obtain healthy products with similar sensory characteristics to conventional foods (Sandrou and Arvanitoyannis 2000; Bayarri *et al.* 2010; Dotsch *et al.* 2009; Kremer *et al.* 2009, Fujimaru *et al.* 2012; Gwak *et al.* 2012; Cadena *et al.* 2012). An alternative strategy is based on modifying product structure to design novel textures for manufacturing reduced fat foods (Simo *et al.* 2012) or optimising the delivery of taste stimuli to taste receptors. In the latter strategy, taste stimuli content is decreased without reducing the strength of the perceived sensation (Moritaka and Naito 2002; Holm *et al.* 2009; Mosca *et al.* 2010; Busch *et al.* 2012).

It is well known that sweetness is not only linked to sweetener concentration present in a product. It depends on the amount of sweet stimulus released in the mouth during food consumption and of the effects of some physicochemical and cognitive interactions (Taylor 2002; Tournier *et al.* 2009). This release may be partially governed by the rate of diffusion of the stimulus within de food, by the rheological behaviour of the product and, in some cases, by binding of sweet compounds with other food components. The influence of food texture on sweetness is often of special interest. It has been reported that the more viscous a fluid food, or the harder a solid food, the less sweetness is perceived during eating (Morris 1995, Clark 2002). Apart from this qualitative trend on the effect of texture on sweetness, there is also a quantitative effect of the type and concentration of thickener or sweetener on sweetness intensity (Walker and Prescott 2000; Costell *et al.* 2000; Moritaka and Naito 2002; Lethuaut *et al.* 2003; Bayarri *et al.* 2007 a, b). Broadly speaking, texture-related sweetness suppression may be attributed to the delay or partial inhibition of sweet stimulus compound transport within the food matrix, and from the matrix itself to the taste receptors. The differences in sweetness intensity perceived in products with different types and concentrations of thickener or sweetener may not only be due to the texture of each food matrix, but also to its structural modification during the process by which a food inside mouth is converted in a product suitable for swallowing (Çakir *et al.* 2012a). Most of the information concerning the role of oral processing on sensory perception focuses on its influence on texture perception, mainly in solid foods (soft or hard). Oral processing of solid foods requires a complex physiological treatment, from the first bite to bolus formation and swallowing (Lucas *et al.* 2002; Chen 2009; Foegeding *et al.* 2011; Çakir *et al.* 2012b). There is also information about how some aspects of breakdown behaviour (rupture strain, deformability modulus, brittleness or melting) influence perceived taste during the in-mouth residence time of solid foods (Costell *et al.* 2000;

Moritaka and Naito 2002; Bayarri *et al.* 2003; Sala and Stieger 2013). Recently, some authors have proposed structure modifications, at macroscopic or microscopic levels, to enhance sweetness intensity in gels, by a inhomogeneous distribution of sucrose (Holm *et al.* 2009; Mosca *et al.* 2010, 2012) or by increasing serum release during mastication (Sala *et al.* 2010; de Jongh 2011).

Oral processing mechanisms are quite different for semi-solid foods and solid foods because the former are practically suitable for swallowing, thus oral processing is dominated by the effects of tongue movements (Prinz *et al.* 2007; de Wijk *et al.* 2011). Semisolids are processed in the mouth by squeezing between tongue and palate, and during in-mouth time residence, they are subject to shear forces and diluted with saliva. Working with model dairy desserts, Lethuaut *et al.* (2003) compared the effect of different types of carrageenan and observed that samples with λ-carrageenan were perceived as sweeter and as having a smoother texture than samples with κ and ι-carrageenan. Bayarri *et al*. (2010) and Arancibia *et al.* (2011a) showed that dairy desserts with the same rheological behaviour but different fat contents were perceived as having similar thickness, creaminess and smoothness. Fat substitution by λ-carrageenan or inulin influenced both perceived sweetness and flavour. These results indicate the possibility to design semisolids food structures with different composition and structure but similar texture, as a way to modulate sweetness perception.

In this context, the main objective of this work was to explore the possibility of obtaining dairy desserts with similar texture but having different compositions and structures and with different sweetness intensities. The particular objectives were to: (1) study how two different thickeners (carboxymethyl cellulose (CMC) and starch) at different concentrations influenced flow behaviour, microstructure and consistency as well as sweetness perceived in dairy desserts with different fat contents; (2)

establish the possible relationships existing between instrumental and sensory data.

MATERIALS AND METHODS

Sample Composition and Preparation

Dairy desserts were prepared with carboxymethyl cellulose (CMC) (Akucell AF3265 Akzo Nobel, Amersfoort, The Netherlands) and medium crosslinked modified tapioca starch (C* Creamtex 75720, Cerestar Ibérica, Barcelona, Spain) as thickener, commercial whole milk powder (25% w/w protein, 39% w/w carbohydrate, 26% w/w fat and 1.2% w/w calcium) or skimmed milk powder (34% w/w protein, 52% w/w carbohydrate, 1% w/w fat and 1.2%w/w calcium) (Central Lechera Asturiana, Siero, Spain), mineral water (35.5 mg/L calcium, 8.6 mg/L magnesium, 11.9 mg/L sodium, 144 mg/L bicarbonate and 13 mg/L chloride content) (Font Vella, Barcelona, Spain), sugar, colorant (T-PT8-WS, CHR Hansen S.A., Barcelona, Spain) and lemon flavour (mixture 3:1 of linalool and *cis*-3-hexen-1-ol) (SAFC-Sigma Aldrich, Madrid, Spain).

Eight different formulations were prepared varying in milk type (whole or skimmed), thickener type (CMC or starch) and thickener concentration (low: 1.1 and 1.3% w/w CMC or high: 3.5 and 4% w/w starch). The amounts of sugar (10% w/w), colorant (37.5 mg/kg), lemon flavour (120 mg/kg) and rehydrated milk weight (80% w/w) were fixed for all samples. Sample composition is given in Table 1.

Table 1. Identification and composition of dairy desserts with different milk types, thickener types and concentrations[1]

Sample code	Thickener types	Thickener Concentrations (%w/w)	Milk types	Fat Contents %(w/w)
A	CMC	1.1	Whole	2.8
B	CMC	1.1	Skimmed	0.1
C	CMC	1.3	Whole	2.8
D	CMC	1.3	Skimmed	0.1
E	Starch	3.5	Whole	2.8
F	Starch	3.5	Skimmed	0.1
G	Starch	4.0	Whole	2.8
H	Starch	4.0	Skimmed	0.1

[1] *Sugar (10% w/w), colorant (37.5 mg/kg) and lemon flavour (120 mg/kg) remained constant in all samples*

Whole and skimmed milks were prepared by dissolving 13.5% w/w milk powder in mineral water to obtain a final fat content of 3.5% w/w and 0.14% w/w, respectively. Milk powder was dispersed in mineral water, at 250 rpm and 85 ºC for 10 minutes with the help of a magnetic stirrer and a hot plate (Ared, Velp Scientifica, Usmate, Italy) and stored at 4 ± 1 ºC overnight to ensure complete hydration of milk proteins.

For CMC-based sample preparation, a blend of sugar with CMC was added to the rehydrated milk with the colorant, and was stirred (Heidolph RZR 1, Schwabach, Germany) for 35 minutes at room temperature. Five minutes before the end of stirring time, lemon flavour mixture was added. Starch-based samples were prepared in batches of 800 g as follows: starch, sugar and rehydrated milk were weighed in a flask and mixed under magnetic stirring (Ared, Velp Scientifica, Usmate, Italy) for 10 min. The flask was placed in a water bath at 96 ± 1 ºC and stirred constantly with a propeller stirrer (Heidolph RZR 1, Schwabach, Germany) for 25 min. Samples were

cooled in a water bath at 10 ± 1 ºC for 10 min. Any evaporated water was replaced gravimetrically. Lemon flavour mixture was added and the sample was stirred for 5 min. At least, two batches of each composition were prepared. All samples were transferred to a closed flask and stored at 4 ± 1ºC for 24 h, prior to rheological and sensory measurements.

Rheological Measurements

Rheological measurements were performed with a controlled stress rheometer RS1 (ThermoHaake, Karlsruhe, Germany), using parallel-plate geometry (60 mm diameter; 1mm gap) and monitored with the RheoWin Job software package (version 3.61, ThermoHaake). Measurements were run at 10 ± 1 ºC, selected as representative of the usual consumption temperature of dairy desserts (Engelen *et al.* 2003a). Temperature was kept constant at by means of a Phoenix P1 Circulator device (ThermoHaake Karlsruhe, Germany). After loading the sample, a resting period of 10 minutes was used to allow the sample to recover and reach the desired temperature. Each sample batch was measured twice, using a fresh sample for each measurement.

Flow Behaviour. Sample flow was measured by recording shear stress values when shearing the sample at an increasing shear rate from 1 to 100 s^{-1} in 30 s. Experimental data were fitted to the Ostwald-de Waele model (Eq. 1) using RheoWin Pro Data software (v. 3.61, Haake).

$$\sigma = K \dot{\gamma}^{n} \quad (1)$$

where σ (Pa) is the shear stress, $\dot{\gamma}$ (s^{-1}) is the shear rate, K (Pa s^{n}) is the consistency index and n is the flow behavior index. Three possible instrumental indices of thickness perceived were calculated based on

previous works. Shama and Sherman (1973) stated that the stimulus associated with the perception of viscosity in semi-solid products could be the shear stress developed at a constant rate of 10 s^{-1}. Wood (1968) suggested that apparent viscosity at a shear rate of 50 s^{-1} represents the in-mouth shear rate. Thus, apparent viscosity values at a shear rate of 10 and 50 s^{-1} were calculated (Eq. 2).

$$\eta_{(10,\,50)} = K\dot{\gamma}^{n-1} \tag{2}$$

Kokini *et al.* (1977) proposed a thickness physical index based on the change of in-mouth shear stress during product ingestion. The flow parameters (K and n) obtained with the Ostwald-de Waele model (Eq.1) were used to calculate the Kokini oral shear stress (τ, OSS) according to Eq. 3 (Elejalde and Kokini 1992):

$$\tau = KV^{n}\left[\frac{1}{h_0^{(n+1)/n}} + \left(\frac{F}{R^{n+3}} \times \frac{n+3}{2\pi K}\right)^{1/n} \times \frac{(n+1)t}{2n+1}\right]^{n^2/(n+1)} \tag{3}$$

where τ is the Kokini oral shear stress (OSS), V is the velocity of tongue (2 cms^{-1}), h_0 is the initial plug height (0.2 cm), F is the normal force (1 N), R is the radius of plug (2.5 cm) and t is time (1 s).

Microstructure

The microstructure of the different dairy desserts was examined using fluorescence microscopy (Nikon Eclipse 90i, Kanagawa, Japan). The photomicrographs were acquired with a digital camera (Nikon DS-5Mc, Kanagawa, Japan). Samples were observed using 40x magnification and an identical exposure time (1.5 s). A drop of sample was placed on a slide with a coverslip prior to analysis. Samples were stained with Nile Red (150 μg

Nile Red/g samples) (Sigma-Aldrich Química S.A., Madrid, Spain) to enable visualization of fat globules. The excitation wavelength used was 488 nm.

Sensory Evaluation

A group of 28 assessors, with previous experience in evaluating sensory differences in dairy products (> 3 years), were recruited from the IATA staff. They evaluated the perceptible differences in sweetness and consistency using a pairwise ranking test, comparing the 28 possible pairs of samples. This test measures the relative intensity of each attribute within each pair of samples and provides a numerical indication of the differences among samples on a scale (Meilgaard *et al.* 1999).

Samples were evaluated in 14 sessions and each assessor evaluated two pairs of samples per session. All sessions were carried out in a standardized test room (ISO, 2007) in morning sessions (11:00 to 13:00 h). Each pair of samples (30 mL) was presented at 10 ± 1°C in plastic cup and labelled with a random three-digit code. The panelists tested the same volume of each sample with a spoon (5 mL) and were asked to judge which sample was sweetest and which was the most consistent. White bread without salt and mineral water were provided to the assessors for mouth rinsing between each pair of samples. Data acquisition was performed using Compusense *five*, release 5.0 (Compusense Inc., Guelph, ON, Canada).

Data Analysis

The effects of milk type (whole or skimmed), thickener type (CMC or starch) and concentration (low or high) and their binary interactions on rheological data were analysed by a three-way ANOVA. Fisher's test ($\alpha = 0.05$) was used to calculate the minimum significant difference. All

calculations were carried out with XLSTAT Pro software v. 2007 (Addinsoft, Paris, France).

Sensory data were analyzed according to Meilgaard *et al.* (1999). The first step in the Friedman analysis is to compute the rank sum for each sample. In this case, the rank sums were obtained by adding the sum of row frequencies (less intensity of the attribute) to twice the sum of the column frequencies (greater intensity of the attribute). Then, the Friedman's *T* statistic was obtained with Eq. 4

$$T = \left(\frac{4}{p \times t} \right) \times \sum_{i=1}^{t} R^2 - \left(9 \times p \times \left[t - 1 \right]^2 \right) \tag{4}$$

where p is the number of assessors, t is the number of samples and R is the rank sum for each sample. Experimental T values were compared to the critical values of χ^2 with (t - 1) degree of freedom. Significant differences among samples were determined with the Tukey's (HSD) test.

The relationship between the magnitude of consistency perceived and the variation of each instrumental index of sensory viscosity (η_{10}, η_{50} and OSS) was fitted to the Psychophysical Stevens' Law (Stevens 1975) (Eq. 5).

$$\psi = k \, \phi^{\beta} \tag{5}$$

where ψ is the sensory magnitude, ϕ is the instrumental index value, k is a constant that depends on the measurement units and the exponent β represents the curvature of the power function. $\beta = 1$ indicates that perceived sensation varies in a linear form with the stimulus intensity*;* $\beta > 1$ indicates that sensation magnitude increases more rapidly than stimuli intensity and $\beta < 1$ that the sensation increases less rapidly than the stimuli.

RESULTS AND DISCUSSION

Flow Properties

All samples showed non-Newtonian and shear-thinning flow and fitted well to the Ostwald-de Waele model ($0.98 \geq R^2 \geq 0.99$) (Figure 1). Analysis of variance of flow parameters showed a significant effect of the interaction between milk type and thickener type on consistency index (K), apparent viscosities at 10 s^{-1} (η_{10}) and 50 s^{-1} (η_{50}) and Kokini oral shear stress (OSS) values (Table 2). This interaction indicated that the milk type effect was different depending of the thickener type.

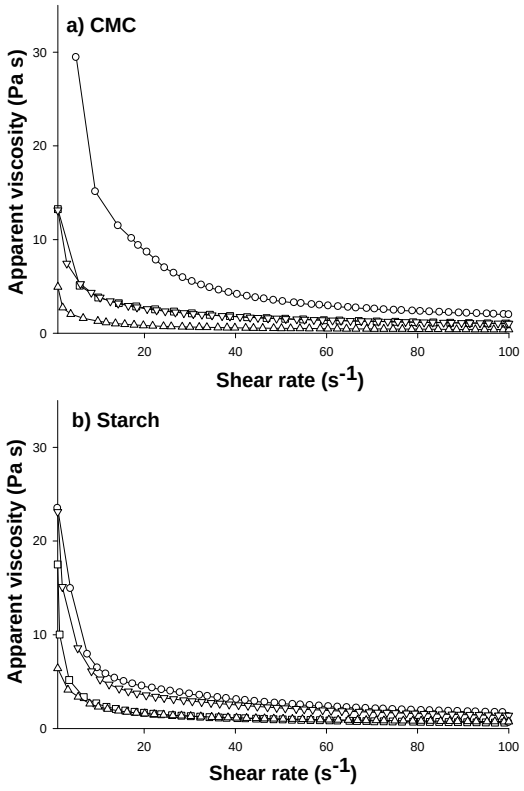

Figure 1. Variation of apparent viscosity with shear rate for dairy desserts with different concentrations of CMC (a) and starch (b): □ = 1.1% CMC or 3.5% starch and whole milk, △ = 1.1% CMC or 3.5% starch and skimmed milk, ○ = 1.3% CMC or 4% starch and whole milk and ▽ = 1.3% CMC or 4% starch and skimmed milk.

Table 2. Three-way anova of flow parameters in dairy desserts with different milk types, thickener types and concentrations. F and p values

	Flow parameters[1]									
	K (Pa s^n)		n		η_{10} (Pa s)		η_{50} (Pa s)		OSS (Pa)	
	F	P	F	P	F	P	F	P	F	P
Main effects										
X: Milk type	59208.3	<0.01	2168.8	<0.01	80.5	<0.01	25.5	<0.01	592.3	<0.01
Y: Thickener type	1905.6	<0.01	0.015	0.91	4.8	0.06	0.6	0.45	4.4	0.07
Z: Thickener concentration	34854.9	<0.01	52.6	<0.01	223.6	<0.01	81.5	<0.01	180.1	<0.01
Interactions										
X x Y	10806.6	<0.01	1.2	0.31	63.7	<0.01	23.7	<0.01	55.0	<0.01
X x Z	4808.4	<0.01	0.1	0.73	17.2	<0.01	3.7	0.09	14.5	0.01
Y x Z	1892.9	<0.01	7.8	0.02	2.2	0.18	0.04	0.95	2.0	0.20

[1] *K = consistency index, n = flow index, η_{10} = apparent viscosity at 10 s^{-1}, η_{50} = apparent viscosity at 50 s^{-1} and OSS = Kokini oral shear stress index.*

In general, whole-milk samples exhibited significantly higher values of K than their equivalent samples made with skimmed milk, except for starch-based samples with the highest starch concentration (samples G and H) (Table 3).

Table 3. Mean values and significant differences of flow parameters of Oswald-de Waele model in dairy desserts with different milk types, thickener types and concentrations

Sample[1]	Flow parameters[2]				
	K (Pa s^n)	n	η_{10} (Pa s)	η_{50} (Pa s)	OSS (Pa)
A	13.04^{c}	0.47^{ab}	3.84^{c}	1.63^{c}	45.72^{c}
B	4.19^{f}	0.50^{a}	1.33^{d}	0.60^{d}	21.64^{e}
C	62.37^{a}	0.28^{d}	11.96^{a}	3.79^{a}	120.20^{a}
D	13.55^{c}	0.43^{bc}	3.67^{c}	1.47^{c}	43.56^{cd}
E	10.92^{d}	0.38^{c}	3.84^{c}	0.98^{cd}	33.27^{cde}
F	6.27^{e}	0.52^{a}	2.65^{cd}	0.96^{cd}	29.61^{de}
G	26.86^{b}	0.40^{c}	6.68^{b}	2.53^{b}	70.80^{b}
H	26.84^{b}	0.39^{c}	6.62^{b}	2.49^{b}	70.18^{b}

[a-f] Means within a column with common superscripts did not differ significantly ($P < 0.05$)

[1] CMC-based samples: A, B, C and D. Starch-based samples: E, F, G and H. Sample composition is given in Table 1

[2] K = consistency index, n = flow index, η_{10} = apparent viscosity at 10 s^{-1}, η_{50} = apparent viscosity at 50 s^{-1} and OSS = Kokini oral shear stress index

The effect of this interaction on $\eta_{10,}$ η_{50} and OSS values was the same: milk type has a significant effect on CMC-based samples, but not on starch-based samples (Figure 2a). The milk type-thickener concentration interaction was only significant on K, η_{10} and OSS values (Table 2). For all samples, increasing the thickener concentration increased the effect of milk type (Figure 2b). The thickener type-thickener concentration interaction only was significant on Ostwald parameters (K and n) (Table 2). As expected, consistency and pseudoplasticity increased significantly with thickener

concentration in most samples; although the effect was more evident for CMC-based samples.

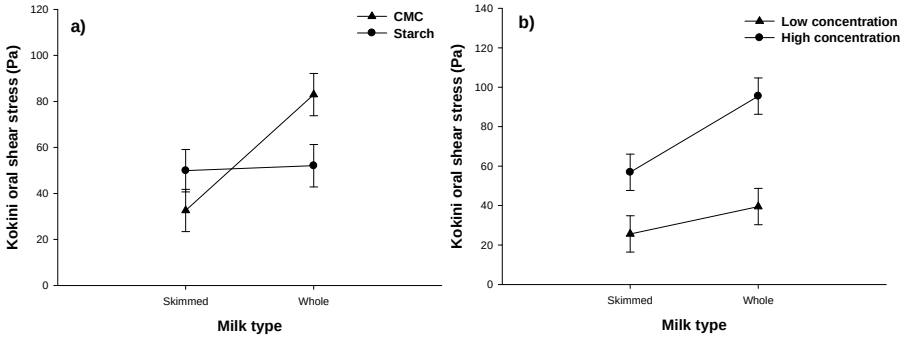

Figure 2. Effect of interaction between milk type and thickener type (a), and between milk type and thickener concentration (b) on Kokini oral shear stress index.

By comparing the three possible thickness index values obtained for all samples, some samples differing in composition gave the same values. No significant differences in $\eta_{10,}$ η_{50} and OSS values were detected among samples A, D and E, between samples B and F, and between samples G and H (Table 3). Composition of samples A, D and E differs in milk type and thickener type and concentration; samples B and F only differ in thickener type and samples G and H in milk type. These results indicate that the different effects of compositional factors and of the interactions among them on flow behaviour of dairy desserts provide products with different fat and calorie contents and equivalent thickness.

Microstructure

Figure 3 shows the micrographs of whole-milk samples observed by fluorescent microscopy. Differences in fat globules distribution across of continuous phase were depended of thickener type and concentration. CMC-

based samples (A and C) presented a coarse stranded structure that increased with CMC concentration. In the image of sample A, there is a lighter zone formed by the CMC agglomerates with fat globules partially embedded and a dark zone corresponding to the dispersant phase. When CMC concentration increased (sample C), it was observed the formation of a more homogeneous structure like a network with fat globules distributed within CMC matrix. The volume occupied by the CMC agglomerates increased and the dark zone had diminished.

Starch-based samples (E and G) exhibited a globular structure with isolated and swollen starch granules, as well as fat globules, dispersed in a continuous phase. When starch concentration increased, fat globules were more concentrated across dispersant phase. These types of structures have previously been observed in dairy desserts prepared with modified starch (Bayarri *et al.* 2011) and also in o/w emulsions made with each of these two thickeners (Arancibia *et al.* 2011b).

As commented previously, consistency and flow pseudoplasticity of samples increased with thickener concentration, although the effect was more evident for CMC-based samples. This can be related with the structural differences observed between CMC-based samples and starch-based samples. By increasing CMC concentration, their extended chains start to overlap and entanglement, giving rise to a transient network structure that shows more resistance to flow. While in the case of starch-based samples when starch concentration increase the flow behaviour is only dominating by swollen starch granules and by their resistance to align in the direction of the shearing force.

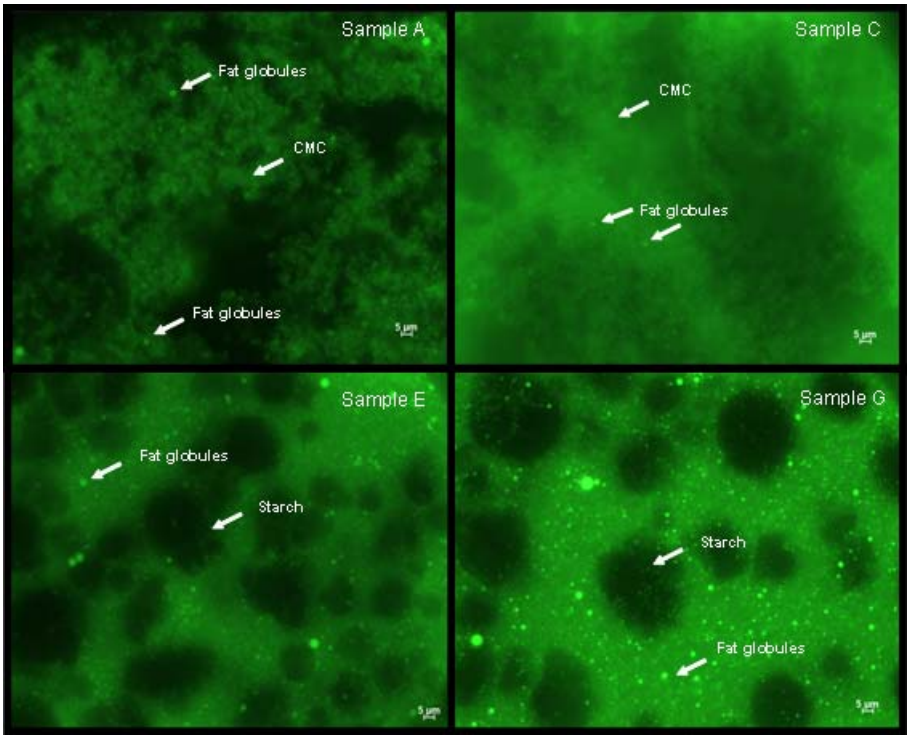

Figure 3. Microscopy images of whole-milk dairy desserts with different thickener type and concentration. Micrographs taken at 40x magnification, 10ºC, scale bars to 5 μm, and stained with Nile red. Identification of samples is given in Table 1.

Sensory Analysis

The results obtained from a pairwise ranking test of eight samples were analysed using the Friedman's *t*-test. The calculated Friedman F values for sweetness and consistency, 181 and 375 respectively, were higher than the theoretical value for $\alpha = 0.05$ ($\chi^2 = 14.1$). Significant differences among samples were found for the two attributes evaluated. The results showed that the differences perceived in sweetness were significantly affected by thickener type. Irrespective of milk type and thickener concentration, all starch-based samples were perceived to have higher sweetness intensity than the CMC-based samples (Figure 4).

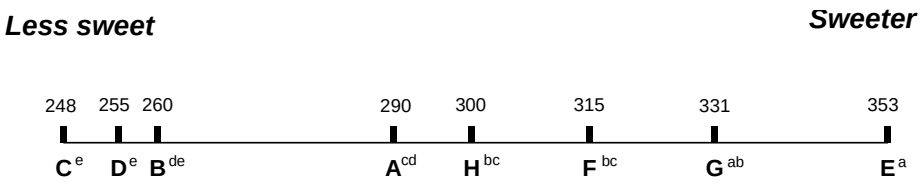

Figure 4. Line diagram of the rank sum scores for sweetness obtained by Pairwise ranking test for dairy desserts with different milk types, thickener types and concentrations. Values above the line are the actual rank sum for each sample, different superscript letters denote significant differences between samples ($\alpha = 0.05$). Identification of samples is given in Table 1.

This result was in agreement with that obtained by others authors about the good flavour and taste release capacity of starch (Cayot *et al.* 2004; González-Tomás *et al*. 2007, Arancibia *et al.* 2011b). An explanation of the good taste release of starch foods during consumption may be the thickness reduction due to the action of saliva enzymes, mainly α-amylase, on amylose chain breakdown during oral processing. The effect of amylase on the breakdown of starch-based foods has been reported by Prinz *et al.* (2007) in an *in vitro* simulation of oral processing. They observed a dramatic reduction in the viscosity of commercial starch-based custards by addition of saliva, and also a 10 s delay before the onset of the effect of saliva on viscosity reduction. The short in-mouth residence time of custard desserts during consumption (around 4s) can explain the limited effect of saliva on thickness perceived in starch-based dairy desserts, as observed by Engelen *et al.* (2003b) and by de Wijk *et al.* (2011). Thus, the enzymatic breakdown of starch-based dairy desserts during consumption cannot fully explain the higher sweetness intensity perceived in starch-based samples. Other structural features, as the different ability of starch and CMC samples to mix with saliva (Desse *et al.* 2011) or the variation in water mobility in each matrix type (de Jongh 2011) can also modify the tastant mass transfer across the saliva layer to the taste receptors. Milk type had a slight effect on

sweetness intensity. For each thickener and concentration, whole-milk samples were perceived as sweeter than their skimmed-milk counterparts. This effect could be due to the relatively higher concentration of sucrose in the aqueous dispersing phase when fat content increases (Metcalf and Vickers 2002).

Besides thickener type effect on consistency, milk type and thickener concentration also influenced this attribute (Figure 5). For the same thickener concentration level, whole-milk samples with CMC were perceived as more consistent than skimmed-milk samples. While no significant differences were detected in this attribute between starch-based samples made with different milk types. By observing the significant differences in consistency among samples, similar trends were detected to those observed for the instrumental indices. In this case, no significant differences in consistency were detected among samples B, E and F with clear differences in composition. CMC-based samples (A and D), perceived as having the same consistency, differed in milk type and CMC concentration. Starch-based samples (G and H), also with the same consistency, differed only in milk type. It can be concluded that differences perceived in consistency among the dairy dessert samples correspond to the effect of interactions among product components, and are in accordance with that observed in flow parameters. However, when the relation between consistency and sweetness was analysed, a clear trend was not found. Variations observed in sweetness seem to be more dependent on structural differences due to thickener type and on product structure modification during oral processing.

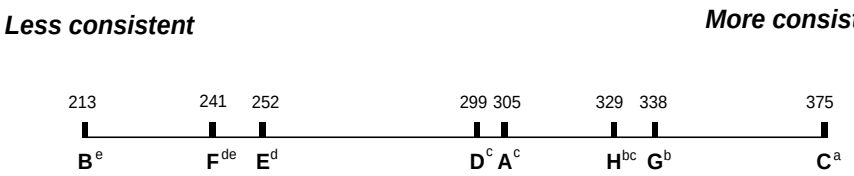

Figure 5. Line diagram of the rank sum scores for consistency obtained by Pairwise ranking test for dairy desserts with different milk type, thickener type and concentration. Values above the line are the actual rank sum for each sample, different superscript letters denote significant differences between samples ($\alpha = 0.05$). Identification of samples is given in Table 1.

Relationships between Instrumental and Sensory Data

To establish the relationship between the magnitude of consistency perceived and the variation in each instrumental index of sensory viscosity ($\eta_{10,}$ η_{50} and OSS), the Psychophysical Stevens' Law was applied. In general, the consistency sensation grew as a power function of the magnitude of the each of the three instrumental sensory indices considered (Figure 6). The exponent β was less than 1 for all three instrumental indices, indicating that the magnitude of the consistency perceived increased less rapidly than the instrumental index values. The fit was good in all cases, although the best correlation coefficients were obtained for η_{50} and OSS (0.98 and 0.94, respectively) (Figures 6b and 6c). These results confirm the practical utility of these instrumental measurements to predict perceived consistency or thickness in this type of products although, as commented by Cook *et al.* (2003), the OSS index has a real meaning in a sensory sense. It is not only based on one shear rate value, it accounts for the shear-thinning flow of product over a range of shear rates. The good relationship observed between the sensory and instrumental measurements of thickness are in agreement with the conclusions drawn by de Wijk *et al.* (2011) regarding the link between different texture attribute perceptions and in-mouth processing time point. They stated that thickness or consistency of custard desserts was a bulk texture attribute, associated with the early stage of oral processing

before in-mouth bolus degradation. They concluded that sensory evaluation only requires the application of compression forces during flow of the relatively intact food in the mouth.

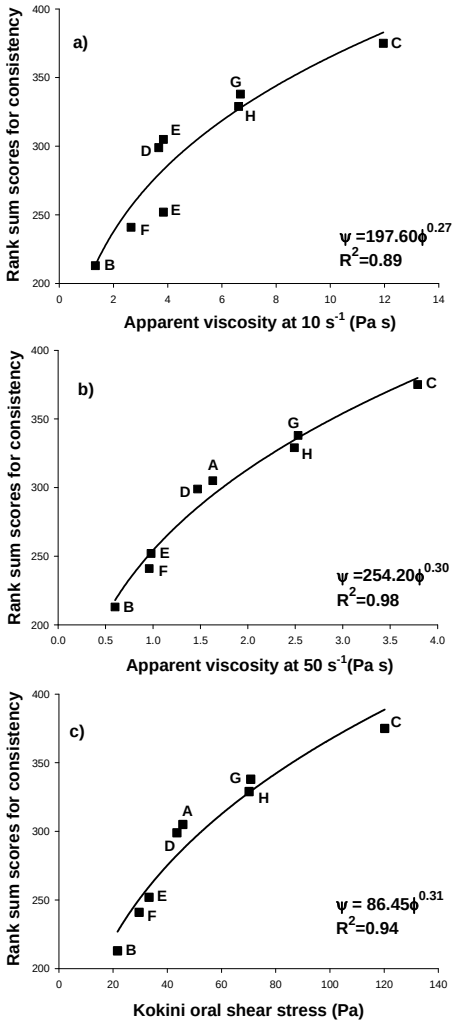

Figure 6. Relationship between perceived consistency and instrumental indices of sensory viscosity in dairy dessert with different milk type, thickener type and concentration, using Psychophysical Stevens' Law: (a) apparent viscosity at 10 s^{-1}, (b) apparent viscosity at 50 s^{-1} and (c) Kokini oral shear stress Identification of samples is given in Table 1.

As commented above, an increase in viscosity is considered to reduce perceived sweetness intensity in liquid and semisolid foods. Cook *et al.* (2003) reported good correlation coefficients for models relating the sensory perception of sweetness to both η_{50} and OSS values, in aqueous viscous solutions with different thickeners. Similar relationships in custard desserts were not so clear mainly due to the different effects of hydrocolloids used as thickeners on perceived sweetness of each semisolid product (Lethuaut *et al.* 2003; González-Tomás *et al*. 2007; Tournier *et al*. 2009). In this work, when the relation between OSS values and perceived sweetness, was explored, no direct relationship was detected. Some samples (A, E and F) with similar OSS values and different compositions were perceived to have different sweetness intensities while other samples with significant differences in OSS values (B, D and C) do not reveal significant differences in sweetness (Figures 4 and 5). It is evident that in the analyzed samples, not only OSS values but variations in composition and structure influence behaviour during oral processing, thus modifying the delivery of sucrose molecules to the taste receptors. As commented previously, structural differences between CMC and starch-based samples and compositional differences due to fat content, can influence sucrose release from the food matrix. Differences in the ease with which the product mixes with saliva (Desse *et al.* 2011), on water mobility (de Jongh 2011) or on the homogeneity of sucrose distribution within product structure (Mosca *et al.* 2010) may explain sweetness intensity differences perceived among samples with similar OSS.

CONCLUSIONS

The results obtained show that it is possible to obtain semisolid dairy desserts with similar texture and different sweetness intensity and also, with different texture and similar sweetness. We can conclude that in semisolid foods, hydrocolloids not only act as stabilisers and textural ingredients but

also could influence food behaviour during ingestion due to their structural characteristics. Novel semisolid structures can be designed using different thickeners, thereby improving in-mouth delivery of sweetener molecules and increasing perceived sweetness intensity. More information is needed to understand the effect of oral processing in dairy desserts thickened with different hydrocolloids to explain the observed variations in sweetness intensity.

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CAPÍTULO 6

FAT REPLACERS IN LOW-FAT CARBOXYMETHYL CELLULOSE DAIRY BEVERAGES. COLOR, RHEOLOGY AND CONSUMER PERCEPTION

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INTERPRETIVE SUMMARY

Decreasing fat content in a food produces changes in a range of food properties which are important in both flavour and texture perception and presumably are responsible for the decrease in acceptability of reduced fat foods. Results of this work showed that both the λ-carrageenan and an inulin blend could be used as fat replacers in carboxymethyl cellulose-based dairy beverages. Instrumental differences among samples due to different fat content and fat replacer composition were sensorially distinguishable by consumers.

ABSTRACT

Low-fat foods are an interesting option for consumers interested in health-related issues or in maintaining adequate body weight; however, fat reduction may influence the consumer's acceptance of the end product. This study aims to obtain information about the effectiveness of λ-carrageenan and a blend (50:50) of short and long-chain inulin as fat replacers in dairy beverages prepared with carboxymethyl cellulose (CMC) and to determine to what extent consumers perceive instrumental color and rheological differences among samples. Results showed that both the λ-carrageenan and the inulin blend could be used as fat replacers in CMC-based dairy beverages and that consumer could distinguish among samples that differed in color and rheological behavior.

Keywords: fat replacers, dairy beverages, physical properties, consumer

INTRODUCTION

Low-fat foods are an interesting option for consumers interested in health-related issues or in maintaining adequate body weight. Furthermore, consumers look for healthy foods with satisfactory sensory characteristics or even for traits similar to those of conventional products (Verbeke, 2006). The elimination or reduction of fat modifies the composition and structure of dairy foods, as well as the expected interactions among components, often giving rise to perceptible changes in color, flavor and texture (Bayarri and Costell, 2009). These changes may influence the consumer's acceptance of the end product.

One of the most common strategies to compensate for fat reduction is the use of fat replacers or fat mimetics (Sandrou and Arvanitoyanis, 2000). Among fat replacers, carbohydrate-based substances, such as starch, cellulose, pectin, inulin, xanthan gum or carrageenan, are of growing interest because they have health-friendly characteristics besides their physicochemical properties (Warrand, 2006). In a previous work on starch-based systems (González-Tomás et al., 2009a), the addition of long-chain inulin (7.5%) gave rise to a low-fat product with the same thickness and creaminess intensity as the full-fat sample; however, it was rougher and consequently its sensory quality was negatively affected. This roughness was probably due to the presence of aggregates of long-chain inulin in the product. One way to avoid this problem is to reduce the long-chain inulin content by using a blend of short and long-chain inulin as fat replacer, as reported in studies into the effect of short and long-chain inulin blends on the rheological and sensory properties of low-fat semisolid starch-based custards (Tárrega et al., 2010). Addition of inulin blends at 7.5% to low-fat desserts did not fully emulate the rheological behavior of the full-fat custard; however, the low-fat sample with the 50:50 inulin blend was perceived to have the same creaminess and thickness as the full-fat sample. A study carried out in our

laboratory (Bayarri et al., 2010) showed that low-fat semi-solid carboxymethyl cellulose (CMC)-based dairy custards with 0.03% λ-carrageenan or with 9% of the inulin blend of short and long-chain inulin displayed similar rheological behavior to the full-fat control sample; that is, no differences were detected in either flow or viscoelasticity. Samples with the same rheological behavior but different fat content were perceived as having similar thickness, creaminess, and smoothness. With respect to flavor perception, the substitution of fat by λ-carrageenan or inulin influences both perceived sweetness and flavor. These studies suggest that blends of short and long-chain inulin or λ-carrageenan can be successfully used as fat replacers in semisolid dairy products, but no information is available for dairy beverages.

In the abovementioned studies, sensory evaluation was carried out by a group of selected panelists with previous experience on sensory analysis of dairy products and working with a previously established vocabulary to examine and describe product characteristics. Sensory evaluation of foods is usually carried out by using conventional techniques such as descriptive analysis, which requires a considerable amount of time to recruit, screen and train assessors, who normally tend to generate complex and scientifically oriented terms. On the contrary, consumer sensory panels generate easily understandable vocabularies that have the disadvantages of being too personal to be interpreted by anyone except the subject, and consumers usually have difficulty in developing a vocabulary to describe the differences between samples. The Repertory Grid Method (RGM) in conjunction with the Free Choice Profile (FCP) is a sensory methodology developed to avoid these disadvantages, allowing researches to obtain direct information about what sensations consumers perceive when eating food (Hersleth et al., 2005; Jahan et al., 2005; Jaeger et al., 2005; González-Tomás and Costell, 2006; Costell et al., 2010). In addition, FCP has some advantages over descriptive

analysis procedures that use a consensus vocabulary: (1) FCP does not require extensive training; (2) vocabulary development is economical in terms of time and effort; and (3) the "consumer-recognizable" terms obtained are useful for marketing. A common problem when working with consumers is to generate sufficient and suitable descriptors to express their sensory perceptions. The RGM seems particularly suited to developing consumer-related vocabulary. With respect to FCP, on the one hand, it differs from conventional profiling in that each consumer develops an individual list of terms to describe the samples rather than using a common scorecard. On the other hand, it is similar in that the assessors must be able to detect differences between samples, verbally describe the perceived attributes and quantify them (Oreskovich et al., 1991). The assessors only have to be objective, and capable of using linear scales and of using the chosen vocabulary consistently (Piggott et al., 1990). One of the advantages of RGM with FCP is that it allows one to gather information about cognitive perception directly from consumers and to identify their common perceptual dimensions (Gains and Thomson, 1990; Russell and Cox, 2003).

The objectives of this work were (1) to obtain information about the effectiveness of λ-carrageenan and a blend (50:50) of short and long-chain inulin as fat replacers in CMC-based dairy beverages; and (2) to determine to what extent consumers perceived instrumental color and rheological differences among samples.

MATERIALS AND METHODS

Sample Preparation and Composition

Samples were prepared with carboxymethyl cellulose (CMC) (Akucell AF3265 Akzo Nobel, Amersfoort, The Netherlands), sucrose, commercial whole milk powder (25% wt/wt protein, 39% wt/wt carbohydrate, 26%wt/wt

fat, and 1.2% wt/wt calcium) or skim milk powder (34% wt/wt protein, 52% wt/wt carbohydrate, 1% wt/wt fat, and 1.2%wt/wt calcium, both from Central Lechera Asturiana, Siero, Spain), vanilla aroma (375 48A Lucta S.A., Barcelona, Spain), yellow-orange colorant (Vegex NC 2c WS mct, CHR Hansen S.A., Barcelona, Spain), λ-carrageenan (Satiagum® ADC 25, Barcelona, Spain) and two types of inulin that differed in average chain length: long-chain (LC, ≥ 23 monomers; Frutafit® TEX!) and short-chain inulin (SC, 7-9 monomers; Frutafit® CLR), both of which were provided by Sensus (Brenntag Química, Barcelona, Spain).

Low-fat samples were prepared at three concentrations of CMC: 0.7, 0.9, and 1.1% (wt/wt), each with two λ-carrageenan contents, 0.01 and 0.03 wt/wt, or three concentrations of a 50:50 blend of short and long-chain inulin (SC/LC), 7, 9, and 11% (wt/wt). The identification and composition of the samples are given in Table 1. For each CMC concentration, two more samples without inulin or λ-carrageenan, one with whole-milk (full-fat control: FFC) and the other with skim milk (low-fat control: LFC) were used as controls. The amounts of sugar (6% wt/wt), colorant (0.052% wt/wt), vanilla aroma (0.016% wt/wt), and the weight of rehydrated milk (80% wt/wt) were fixed across treatments.

Whole and skim milk were prepared in advance by dissolving 13.5% (wt/wt) whole and skimmed milk powder, respectively, in mineral water to obtain a final fat content of 3.5% and 0.14%, respectively. Milk powder was dispersed in mineral water at 250 rpm and 85 ºC for 10 min with the help of a magnetic stirrer and a hot plate (Ared, Velp Scientifica, Usmate, Italy) and stored at 4 ± 1ºC overnight to ensure complete hydration of the milk proteins. To prepare control samples, a dry blend of sugar with CMC was added to the rehydrated milk, with the colorant, and stirred (Heidolph RZR 1, Schwabach, Germany) at room temperature for 35 min; after minute 30, vanilla aroma was added. Samples were transferred to a closed flask and

stored (4 ± 1ºC; 24 h) before to measurement. Low-fat samples containing λ-carrageenan were prepared in the same way, except that λ-carrageenan powder was added to the skim-rehydrated milk together with the dry blend of sugar and CMC. Long-chain inulin and short-chain inulin were mixed at the ratio of 50:50 and low-fat samples were prepared by dispersing skim milk powder and the corresponding inulin concentration in mineral water just before the aforementioned heat treatment.

Table 1. Composition of low-fat dairy beverages

Sample code	CMC concentration (% w/w)	Fat replacer type	Fat replacer concentration (% wt/wt)
1	0.7	λ-carrageenan	0.01
2		λ-carrageenan	0.03
3		Inulin	7
4		Inulin	9
5		Inulin	11
6	0.9	λ-carrageenan	0.01
7		λ-carrageenan	0.03
8		Inulin	7
9		Inulin	9
10		Inulin	11
11	1.1	λ-carrageenan	0.01
12		λ-carrageenan	0.03
13		Inulin	7
14		Inulin	9
15		Inulin	11

Instrumental Measurement of Color

Color was measured in a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Business Technologies, Inc., Osaka, Japan). A 3.5 cm thick layer of sample was contained in optical glass cells 3.8 cm high and 6 cm in

diameter to measure diffused reflected light from the cell bottom using an 8 mm diaphragm aperture. Results were given using the CIELAB system for illuminant D 65 and a 10º angle of vision. Registered parameters were: L^* (brightness), a^* (red component), b^* (yellow component), C^* (chroma) and h^* (hue). Two batches of each composition were prepared and two measurements were performed on each batch.

The color difference (ΔE^*) between control samples, FFC and LFC was calculated by using equation [1] (Francis and Clydesdale, 1975):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad [1]$$

where ΔL^*, Δa^* and Δb^* are the difference between the 2 samples in L^*, a^* and b^*, respectively. The perception of the color difference ΔE^* varies according to the observed color and the sensitivity of the human eye. According to Bodart et al (2008), the human eye distinguishes color difference only if ΔE^* is greater than 1 to 3. For some colors, mainly blues, ΔE^* values of 1 can be detected, but for other colors, such as red, the same ΔE^* may not be perceptible.

Rheological Measurements

Rheological measurements were carried out in a controlled stress rheometer RS1 (ThermoHaake, Karlsruhe, Germany), using parallel-plates geometry (60 mm diameter; 1mm gap). A sample temperature of 10 ± 1ºC, selected as representative of the usual consumption temperature of dairy desserts, was maintained during measurements by means of a Haake circulating water bath (ThermoHaake). Two batches of each concentration combination were prepared and each batch was measured twice, using a fresh sample for each

measurement. After loading the sample, a resting period of 10 min was used to allow the sample to recover and reach the desired temperature. After carefully placing the sample between the plates, the excess material was wiped off with a spatula.

Flow Behavior. Sample flow was measured by recording shear stress values when shearing the samples at an increasing shear rate from 1 to 200s^{-1} for a period of 60 s and in reverse sequence for the same time (González-Tomás et al., 2009b). Data from the upward curve of the shear cycle were fitted to the Ostwald-de Waele model (equation [2]):

$$\sigma = K \dot{\gamma}^{n} \qquad [2]$$

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

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 the RheoWin Pro software (v. 3.61, Thermo Haake). Due to the influence of the loop contour and the shear resistance of the sample on the hysteresis area, the percentage of relative hysteresis area (A_R; equation [3]) was calculated because it allows a better comparison of the thixotropic behavior of different samples (Dolz et al., 2000):

$$A_R = \frac{A_{up} - A_{down}}{A_{up}} \times 100 \qquad [3]$$

Viscoelastic Behavior. Stress sweeps were made between 0.02 and 300 Pa at a frequency of 1Hz in all the systems studied to determine the linear viscoelasticity zone. Frequency sweeps at 0.05 Pa, which is within the linear viscoelastic region, were then performed from 0.01 to 10 Hz. The oscillatory rheological parameters used to compare the viscoelastic properties of the samples were storage modulus (G'), loss modulus (G''), complex dynamic viscosity (η*) and loss angle (tan δ) at 1 Hz.

Sensory Evaluation: Free-Choice Profile

A group of 26 consumers, with an age range of 23 to 55, was recruited from staff and postgraduate students at the research centre (IATA, Instituto de Agroquímica y Tecnología de Alimentos, Valencia, Spain) to take part in the Free-Choice Profile (FCP) study. They were selected on the basis of their being dairy-dessert consumers.

The Repertory Grid Method (RGM) was used to generate an individual set of terms for each assessor in one session. Six samples, two FFC containing 0.7 and 1.1% CMC and four low-fat samples (1, 5, 11 and 15) (Table 1), were selected to generate terms according to their composition. First, the consumers were briefed about the concept behind the methodology and given a procedural outline. Then, individual interviews were performed (approximately 45 minutes each) where three sets of three samples (30 mL each) were presented. To form the first triad, three samples were selected at random from the initial group of six. The second triad was formed by randomly selecting one of the samples from the first triad and then combining it with two more samples selected from the initial group of six. Each subject described the similarities and differences they found among samples within each triad in their own terms. This method was repeated until all six samples were tasted (Russell and Cox, 2003). Consumers were asked

to generate terms concerning the appearance, flavor and texture of the samples. The generated terms were listed by the interviewer on individual score sheets.

In FCP, three control full-fat samples and six low-fat samples chosen on the strength of the rheological results (1, 5, 6, 10, 11 and 15, Table 1) were scored in duplicate over six sessions. Each assessor evaluated three samples per session with its own score card that comprised the terms that he or she previously generated individually. The intensity of each attribute was scored using nine-point scales whose end terms were "not perceived" and "intense." Samples (30 mL) were served at 10 ± 1 ºC in covered white plastic vessels coded with three random digit numbers. For each sample, the appearance attributes were evaluated first. Then, the assessors were asked to evaluate flavor by tasting a teaspoonful of the sample, and finally texture by taking a second teaspoonful. Mineral water was provided to the assessors to rinse their mouths between samples. To reduce the influence of serving order, the samples evaluated in each session were served following a Williams' design for three samples. The samples were evaluated monadically and a rest period of 30 s was imposed between consecutive samples. All sessions were carried out in a standardized test room with separate booths (ISO 8589, 2007) in morning sessions (11:00-13:00 h).

Statistical Analysis

Color and Rheological Data Analysis. To analyze the effect of fat content on color and rheological data of both full-fat and low-fat control samples, Student's *t*-test was used. To study the effect of adding a fat replacer to low-fat samples, a two-way ANOVA (CMC concentration and fat replacer concentration) with interaction was applied for each fat replacer (λ-carrageenan and inulin), including low-fat control samples in the ANOVA.

Significant differences between individual samples were determined by the Fisher's test ($\alpha = 0.05$). Principal Component Analysis (PCA) with varimax rotation was also applied to the average values of color and rheological parameters of all samples (full-fat and low-fat samples). All calculations were carried out with XLSTAT Pro software v. 2007 (Addinsoft, Paris, France).

Sensory Data Analysis. The data from the FCP were analyzed as 18 different samples (nine duplicate samples) using the Generalized Procrustes Analysis (GPA) with Senstools Version 3.3 (OP&P & Talcott, Utrecht, the Netherlands). To investigate the differences between subjects, as well as between samples, the Procrustes analysis of variance (PANOVA) was used, determining the differences between individual and consensus configurations. Results of the PANOVA were expressed as a proportion of the total amount of variance, which was previously rescaled to 100.

RESULTS AND DISCUSSION

Influence of Fat Content on Color and Rheological Behavior

Instrumental Measurement of Color. Results showed that milk type had a significant effect ($t_{cal} > t_{tab}$) on all instrumental color parameters of control samples when analyzed by Student's *t*-test (Table 2). Within each CMC concentration, L^*, a^*, b^* and C^* decreased and h^* values increased on decreasing fat content, and a^*, b^*, and C^* increased with CMC concentration for both milk types. However, CMC addition decreased h^* values in low-fat samples, whereas no change was observed in full-fat samples. The L^* parameter was not affected by CMC addition for either type of milk. To study the total color differences between samples with different fat content, the values of ΔE^* were calculated for each CMC concentration.

The value of ΔE^* of samples with 0.7, 0.9, and 1.1% CMC were 14.38, 18.44 and 14.64, respectively. These values indicated that the total color differences between samples with different fat content were noticeable to the human eye (Bodart et al., 2008).

Rheological Properties. Student's *t*-test showed that significant differences existed in the rheological parameters between samples made with different milk types, except tan δ (Table 2). In general, values for all rheological parameters were greater in full-fat samples, except flow index and tan δ. All samples exhibited thixotropic and shear-thinning flow behavior. For all CMC concentrations, full-fat samples exhibited higher consistency, pseudoplasticity, and thixotropy than low-fat samples; this trend also increased with CMC concentration (Bayarri and Costell, 2011). The viscoelastic characteristics of samples depended on both the milk type and CMC concentration. Full-fat samples showed significantly higher dynamic modulus (G' and G'') and complex dynamic viscosity (η*) values and lower tan δ values than low-fat sample, exhibiting a more structured system, probably due to the presence of fat (Abu-Jdayil et al., 2004). By increasing CMC concentration, full-fat and low-fat samples showed a reinforcement of the structure (Bayarri et al., 2009).

Table 2. Mean values and standard deviations of instrumental color and rheological parameters for full-fat and low-fat control samples with different carboxymethyl celloluse (CMC) concentrations

Item	0.7% CMC		0.9%CMC		1.1%CMC		
	Low-fat	Full-fat	Low-fat	Full-fat	Low-fat	Full-fat	t_{cal}[1]
Color parameters[2]							
L^*	60.0±2.0	70.6±0.3	55.4±1.4	70.9±0.2	59.1±0.8	70.0±0.9	7.9
a^*	0.7±0.1	5.4±0.2	1.2±0.3	5.6±0.1	3.0±0.4	6.7±1.6	14.1
b^*	21.7±2.1	30.3±0.3	22.0±1.0	31.1±0.1	27.5±0.9	36.0±4.5	42.8
C^*	21.8±2.2	30.8±0.3	22.0±1.1	31.6±0.1	27.7±1.0	36.6±4.7	45.1
h^*	88.2±2.2	79.9±0.2	86.8±0.6	79.9±0.1	83.8±0.6	79.6±1.2	5.3
Rheological parameters[3]							
K (Pa s^n)	0.2±0.03	0.8±0.1	1.0±0.1	5.4±0.2	3.8±0.4	11.2±1.1	2.1
n	0.8±0.01	0.7±0.01	0.6±0.01	0.5±0.01	0.5±0.01	0.4±0.02	7.4
A_R (%)	3.6±0.2	6.5±0.7	3.6±0.2	8.2±0.2	3.8±0.2	11.0±0.4	3.8
G' (Pa)	0.7±0.02	6.0±0.5	6.9±0.1	24.9±3.7	26.6±1.8	56.5±0.1	2.5
G" (Pa)	1.5±0.1	5.6±0.1	6.7±0.2	16.9±3.9	17.6±1.3	28.0±0.2	4.0
tan δ	2.3±0.2	0.9±0.1	1.0±0.02	0.7±0.1	0.7±0.01	0.5±0.01	1.6
η* (Pa s)	0.3±0.02	1.3±0.1	1.5±0.02	4.8±0.8	5.1±0.3	10.0±0.1	2.7

[1] t_{tab} ($\alpha \leq 0.1$) = 1.886

[2] L*= brightness; a*= red component; b* = yellow component; C* = chroma; and h* = hue

[3] K = consistency index; n = flow behavior index; A_R = relative hysteresis area; G'= storage modulus; G"= loss modulus; tan δ = loss angle; and η* = complex dynamic viscosity

Influence of Fat Replacer Addition on Instrumental Color of Low-Fat Samples

Results of ANOVA showed that for each fat replacer, the concentration of both CMC and fat replacer had a significant effect (P<0.05) on all instrumental color parameters (Table 3). The interaction between the main effects only significantly affected L^* values when λ-carrageenan was added.

Table 3. Two-way ANOVA of instrumental color parameters in low-fat carboxymethyl cellulose (CMC)-based dairy beverages containing λ-carrageenan or inulin blend as fat replacers

Color Parameters[1]	Main effects				Interactions	
	A: CMC concentration		B: Fat replacer concentration		A × B	
	F-value	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*
λ-carrageenan						
L^*	9.30	<0.01	28.60	<0.01	5.48	0.02
a^*	45.29	<0.01	18.24	<0.01	0.72	0.60
b^*	55.46	<0.01	75.51	<0.01	1.38	0.31
C^*	58.25	<0.01	75.20	<0.01	1.38	0.32
h^*	27.35	<0.01	7.99	<0.01	0.67	0.63
Inulin blend						
L^*	12.83	<0.01	14.38	<0.01	0.98	0.48
a^*	63.25	<0.01	25.11	<0.01	1.29	0.33
b^*	53.61	<0.01	37.43	<0.01	1.10	0.42
C^*	54.57	<0.01	37.35	<0.01	1.12	0.41
h^*	44.33	<0.01	15.67	<0.01	1.02	0.46

[1] *L^*= brightness; a^*= red component; b^* = yellow component; C^* = chroma; and h^* = hue*

In general, for each CMC concentration, fat replacer addition to low-fat samples increased L^*, a^*, b^* and C^* and decreased h^* values (Tables 4 and 5). As indicated by the aforementioned interaction, the increase in L^* values due to λ-carrageenan addition depended on CMC concentration. For high

levels of CMC (0.9 and 1.1%), *L** values increased significantly with λ-carrageenan addition, whereas for 0.7% CMC, the values did not change significantly. The effect of CMC concentration was similar to that observed for fat replacer content. Addition of CMC decreased *h** values and increased *a**, *b** and *C** values in low-fat samples. The *L** values increased with CMC concentration in the systems in which the concentration of fat replacer was higher, whereas no changes were observed at lower concentrations of CMC.

Table 4. Mean values and significant differences of instrumental color parameters for low–fat carboxymethyl cellulose (CMC)-based dairy beverages with different λ-carrageenan concentrations

CMC (%wt/wt)	λ-carrageenan (%wt/wt)	Color parameters[1]				
		*L**	*a**	*b**	*C**	*h**
0.7	0	60.0^{b}	0.7^{a}	21.7^{a}	21.8^{a}	88.2^{d}
	0.01	60.5^{b}	1.6^{ab}	26.3^{b}	26.4^{b}	86.6^{bcd}
	0.03	60.7^{bc}	1.9^{bc}	27.5^{b}	27.6^{b}	86.1^{bc}
0.9	0	55.4^{a}	1.2^{ab}	22.0^{a}	22.0^{a}	86.8^{cd}
	0.01	60.7^{bc}	2.5^{cd}	27.9^{b}	28.0^{b}	84.8^{ab}
	0.03	63.4^{d}	3.2^{def}	30.3^{c}	30.5^{c}	84.0^{a}
1.1	0	59.1^{b}	3.0^{de}	27.5^{b}	27.7^{b}	83.8^{a}
	0.01	63.2^{cd}	3.9^{ef}	32.0^{cd}	32.3^{cd}	83.1^{a}
	0.03	65.4^{d}	4.1^{f}	33.0^{d}	33.2^{d}	82.9^{a}
SE[2]		0.18	0.16	0.11	0.11	0.21

[a-f] Means within a column with common superscripts did not differ significantly (P<0.05)

[1] L= brightness; a*= red component; b* = yellow component; C* = chroma; and h* = hue*

[2] Standard error from ANOVA

Table 5. Mean values and significant differences of instrumental color parameters for low-fat carboxymethyl cellulose (CMC)-based dairy beverages with different inulin blend concentrations

CMC (%wt/wt)	Inulin blend [1] (%wt/wt)	Color parameters[2]				
		*L**	*a**	*b**	*C**	*h**
0.7	0	60^{bcd}	0.7^{a}	21.7^{a}	21.8^{a}	88.2^{e}
	7	60.4^{bcd}	1.5^{abc}	26.1^{b}	26.1^{b}	86.7^{de}
	9	61.1^{cd}	1.8^{bc}	26.8^{b}	26.9^{b}	86.2^{d}
	11	62.6^{de}	2.4^{cd}	28.1^{b}	28.2^{b}	85.1^{cd}
0.9	0	55.4^{a}	1.2^{ab}	22.0^{a}	22.0^{a}	86.8^{de}
	7	57.4^{ab}	1.7^{abc}	25.5^{b}	25.6^{b}	86.1^{d}
	9	57.4^{ab}	1.8^{bc}	25.7^{b}	25.8^{b}	86.0^{d}
	11	62.6^{de}	4.0^{f}	31.0^{c}	31.2^{c}	82.6^{ab}
1.1	0	59.1^{bc}	3.0^{de}	27.5^{b}	27.7^{b}	83.8^{bc}
	7	60.9^{cd}	4.0^{ef}	31.5^{c}	31.8^{c}	82.9^{ab}
	9	61.4^{cd}	4.1^{f}	31.8^{c}	32.1^{c}	82.7^{ab}
	11	64.8^{e}	5.3^{g}	34.6^{d}	35.0^{d}	81.4^{a}
SE[3]		0.21	0.65	0.12	0.12	0.15

[a-g] Means within a column with common superscripts did not differ significantly (P<0.05)

[1] Blend (50:50) of short and long-chain inulin

[2] L= brightness; a*= red component; b* = yellow component; C* = chroma; and h* = hue*

[3] Standard error from ANOVA

To study the joint variability of instrumental color parameters, Principal Component Analysis was applied to mean values obtained for six control samples (full-fat and low-fat) and fifteen low-fat samples containing inulin or λ-carrageenan (Figure 1). The first component accounted for 55.02% of total variability and clearly separated the samples according to CMC concentration. Samples with 1.1% of CMC, in the positive part of the first dimension, showed higher *a**, *b** and *C** values and lower *h** than samples with 0.9 and 0.7% of CMC, which were in the central and negative part of the first dimension, respectively. The second component, which accounted

for 42.2% of variability, was related in its positive part with L^* and separated the samples according to milk type. Full-fat samples, in the positive part of the second dimension, showed higher L^* values and lower h^* values than low-fat samples. In general, adding fat replacer to low-fat samples decreased instrumental color differences between low-fat and full-fat samples.

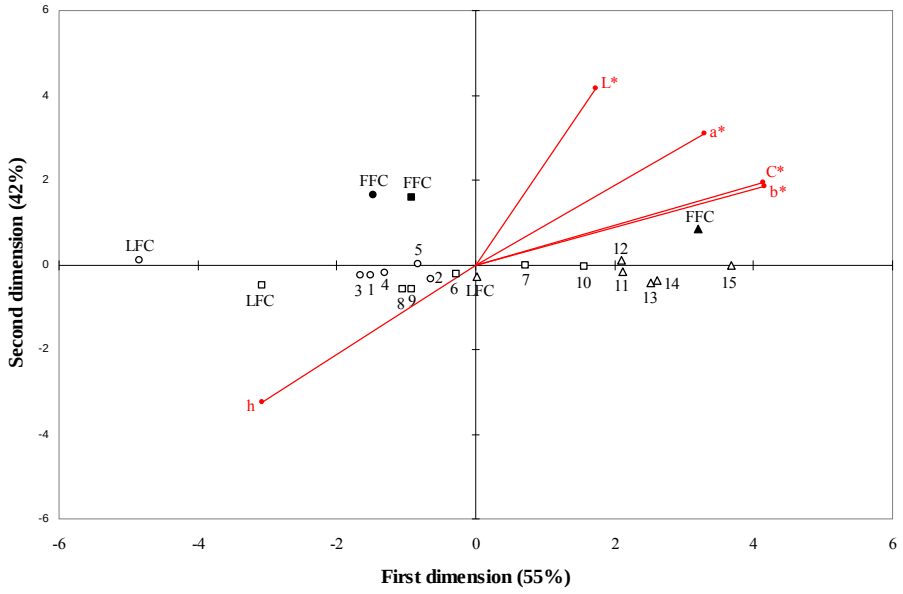

Figure 1. Principal component analysis bi-plot for colour parameters of low-fat (empty symbols) and full-fat (filled symbols) dairy beverages with λ-carrageenan or inulin at different carboxymethyl cellulose concentrations (CMC; circle=0.7%; square=0.9% and triangle=1.1%). Identification of samples is given in Table 1. FFC: full-fat control, LFC: low-fat control.

Agreement exists in the literature regarding the fact that full-fat products are lighter in color than low-fat systems. In a previous work (González-Tomás et al., 2009a), milk type (whole or skimmed) was shown to affect color parameters of starch-based custard desserts. As in the present study, whole milk samples were the lighter-colored ones and showed lower hue values. This fact could be explained by the fact that fat content increases the amount of reflected light in milk (Phillips and Barbano, 1996). However, the effect

of hydrocolloids or inulin on color parameters is not clear, although hydrocolloids have been observed to modify instrumental color in some products. Fernández et al. (2009) studied the quality of mashed potatoes and observed an increase in lightness and yellowness and a loss of greenness on adding xanthan gum or κ-carrageenan. Mittal and Barbut (1993) found that lightness (L^*) was higher in high-fat compared to low-fat raw sausage and that CMC caused a decrease in L^* and an increase in a^*. Different results have also been obtained regarding the effect of inulin addition on color parameters, depending on the average chain length and concentration of inulin and on the product composition (Akalm et al., 2008; De Castro et al., 2008; González-Tomás et al., 2009a; Villegas et al., 2010).

Influence of Fat Replacer Addition on Rheological Properties of Low-Fat Samples

Low-fat samples with different λ-carrageenan concentrations (0, 0.01 and 0.03%) showed observable hysteresis loops when they were sheared during a complete cycle, indicating that the sample flow was time-dependent. The flow curves of all the systems showed a shear thinning behavior with n values from 0.22 to 0.75. The experimental data of ascending rheograms were successfully fitted to the Ostwald-de Waele model ($0.965 < R^2 < 0.999$). The viscoelastic properties of systems ranged from fluid-like to a weak gel, depending on both the λ-carrageenan concentration and CMC concentration. As an example, Figure 2 shows the rheograms and the mechanical spectra obtained for low-fat samples with different λ-carrageenan concentrations, all of which contained 0.9% CMC.

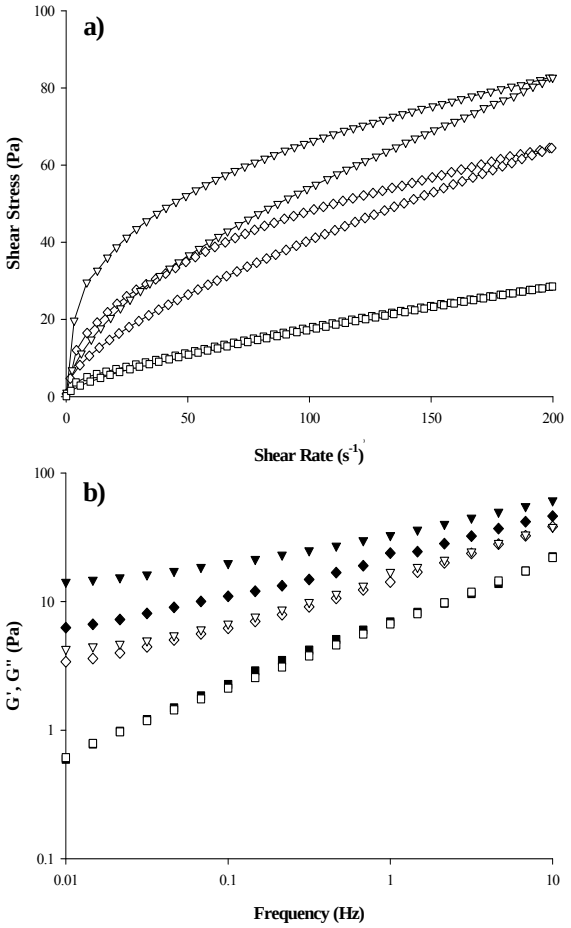

Figure 2. Rheological behaviour of low-fat samples with different λ-carrageenan concentrations (□ = 0%, ◇ = 0.01% and ▽ = 0.03%) containing 0.9% carboxymethyl cellulose: a) upward and downward flow curves and b) mechanical spectra (filled symbols = G', storage modulus; empty symbols = G", loss modulus).

Considering λ-carrageenan as a fat replacer, results of the ANOVA showed that both λ-carrageenan concentration and CMC concentration, as well as their interaction, had a significant effect ($P < 0.05$) on most of the rheological parameters, except on the relative hysteresis area, which was only affected by λ-carrageenan concentration (Table 6).

Table 6. Two-way ANOVA of rheological parameters in low-fat carboxymethyl cellulose (CMC)-based dairy beverages containing λ-carrageenan and inulin blend as fat replacers

Rheological Parameters[1]	Main effects				Interactions	
	A: CMC concentration		B: Fat replacer concentration		A × B	
	F-value	*P-value*	*F-value*	*P-value*	*F-value*	*P-value*
λ-carrageenan						
K (Pa s^n)	652.85	<0.01	786.74	<0.01	210.98	<0.01
n	311.45	<0.01	612.07	<0.01	12.70	<0.01
A_R(%)	1.28	0.32	177.62	<0.01	3.01	0.06
G' (Pa)	458.14	<0.01	142.437	<0.01	28.41	<0.01
G" (Pa)	805.41	<0.01	134.36	<0.01	18.12	<0.01
tan δ	161.10	<0.01	93.67	<0.01	30.91	<0.01
η* (Pa s)	590.85	<0.01	163.04	<0.01	31.36	<0.01
Inulin blend						
K (Pa s^n)	373.58	<0.01	4.80	0.02	2.22	0.11
n	633.92	<0.01	2.36	0.12	1.40	0.29
A_R (%)	1.53	0.26	9.15	<0.01	1.14	0.40
G' (Pa)	1839.81	<0.01	101.75	<0.01	63.23	<0.01
G" (Pa)	1161.71	<0.01	42.96	<0.01	20.82	<0.01
tan δ	618.99	<0.01	9.57	<0.01	4.80	0.01
η* (Pa s)	1642.30	<0.01	83.46	<0.01	49.51	<0.01

[1] *K = consistency index; n = flow behavior index; A_R = relative hysteresis area; G'= storage modulus; G"= loss modulus; tan δ = loss angle; and η* = complex dynamic viscosity*

The increase in sample consistency (K) due to λ-carrageenan addition was greater when CMC content was 1.1% than when it was 0.7% (Figure 3a). The A_R increased with λ-carrageenan content but was not affected by CMC concentration (Figure 3b). Storage modulus, loss modulus and complex dynamic viscosity increased with λ-carrageenan concentration, this increase being larger in general for low-fat samples with 1.1% CMC; G' values are shown (Figure 3c) as an example. Loss tangent values decreased with

carrageenan concentration, especially at the lowest CMC concentration (Figure 3d). A fluid-like behavior was observed for two low–fat samples containing 0.7% CMC and the lowest λ-carrageenan concentrations (0 and 0.01%). The structure of samples with 0.9% CMC and without λ-carrageenan was intermediate between those of a concentrated polymer solution and a weak gel. The remaining samples exhibited weak-gel behavior.

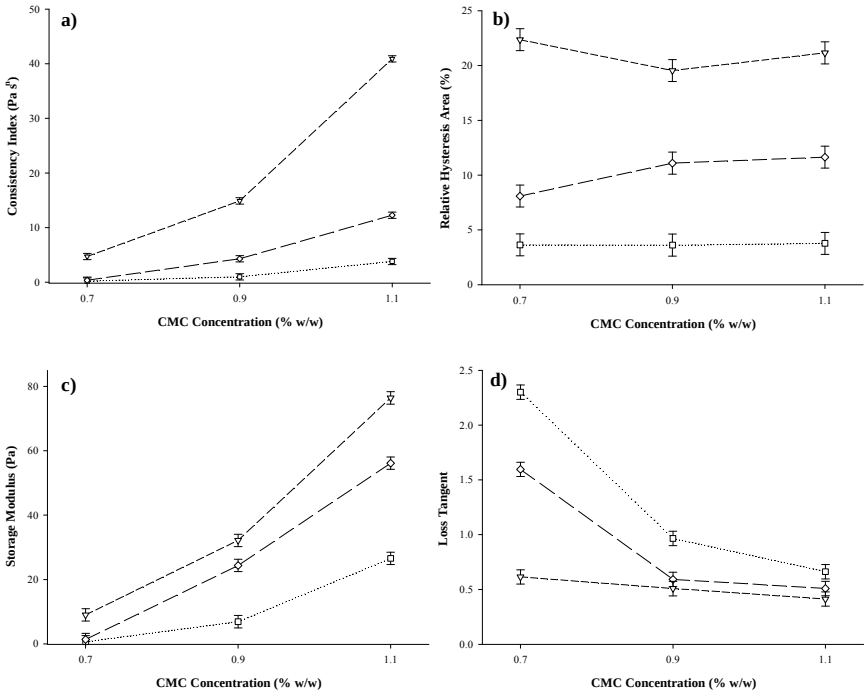

Figure 3. Rheological parameters values: consistency index (a), relative hysteresis area (b), storage modulus (c) and loss tangent (d) of low-fat samples with different λ-carrageenan concentrations (□ = 0%, ◇ = 0.01% and ▽ = 0.03%) containing 0.7, 0.9 y 1.1% carboxymethyl cellulose (CMC).

Rheological properties of low-fat samples showed an increase in the consistency and mechanical strength on increasing λ-carrageenan concentration, probably due to the ability of λ-carrageenan to form gels in the presence of milk by binding with casein micelles (Shchipunov and

Chesnokov, 2003). This increase was greater at the higher CMC concentrations, maybe due to a reduction of interparticle spacing because of the higher CMC concentrations (Vélez-Ruiz and Barbosa-Cánovas, 1997).

The flow curves and the mechanical spectra obtained for some of the samples containing SC/LC inulin blend as a fat substitute, are shown as an example in Figure 4.

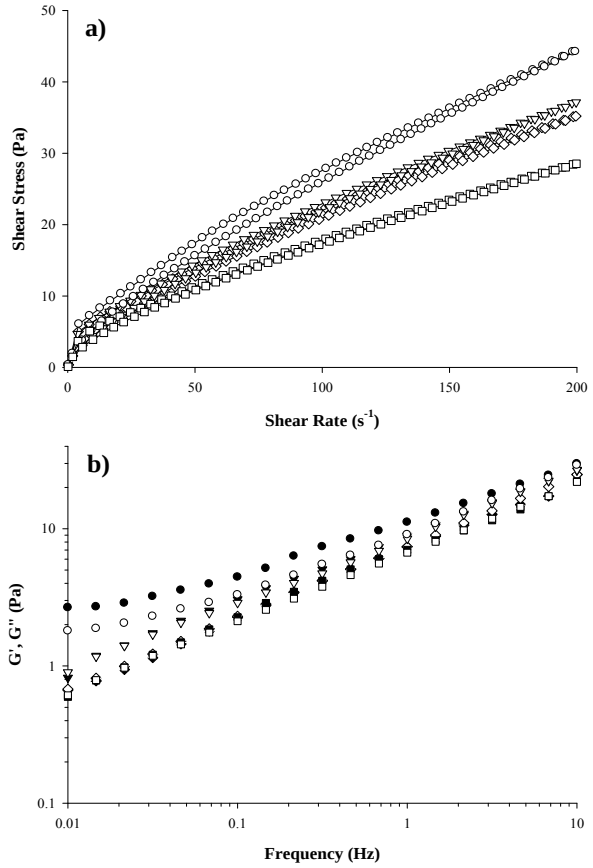

Figure 4. Rheological behaviour of low-fat samples different short-chain/long-chain inulin blend concentrations (□ = 0%, ◇ = 7%; ▽ = 9% y ○ = 11%) containing 0.9% carboxymethyl cellulose (CMC): (a) upward and downward flow curves and (b) mechanical spectra (filled symbols = G', storage modulus; empty symbols = G", loss modulus).

As in the case of samples with λ-carrageenan, the fit to the Ostwald-de Waele model was good for all the samples ($0.998 < R^2 < 0.999$), and showed a pseudoplastic and thixotropic behavior. The effect of CMC concentration was significant ($P < 0.05$) on all variables considered, except on A_R values. Inulin concentration also significantly affected ($P < 0.05$) all rheological parameters except flow index values, which varied from 0.55 to 0.76 (Table 6). In general, a lower effect was observed on adding SC/LC inulin blends to low-fat samples than λ-carrageenan. In the case of the consistency index, an increase was observed only when adding the highest inulin concentration (11%) to systems containing the higher CMC levels (Figure 5a). The relative hysteresis area increased with inulin addition, although this increase was lower than that observed when carrageenan was used as fat replacer (Figure 5b). Because of the significant interaction observed between CMC concentration and inulin blend concentration on viscoelastic parameters (Table 6), systems with the higher CMC concentrations (0.9% and 1.1%) appeared to increase their G', G'' and η* values with the addition of increasing amounts of inulin blend, whereas the lower ones (0.7%) did not (Figure 5c). The tan δ values showed that viscoelastic behavior ranges from fluid behavior (samples with 0.7%) to weak-gel-like (samples with 1.1% CMC), with a concentrated polymer solution structure in between (samples with 0.9% CMC; Figure 5d).

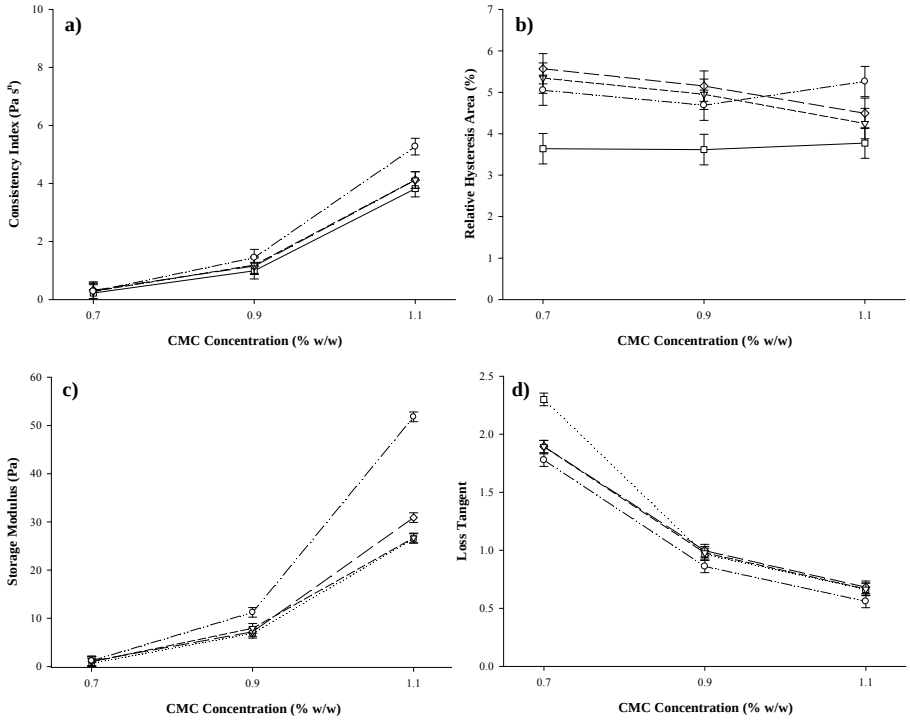

Figure 5. Rheological parameters values: consistency index (a), relative hysteresis area (b), storage modulus (c) and loss tangent (d) of low-fat samples with different short-chain/long-chain inulin blend concentrations (□ = 0%, ◇ = 7%, ▽ = 9% and ○ = 11%) containing 0.7, 0.9 y 1.1% carboxymethyl cellulose (CMC).

Addition of inulin to low-fat dairy beverages has been reported to have different effects on rheological behavior depending on inulin type and concentration, as well as product composition. Villegas and Costell (2007) studied the effects of adding different types of inulin (short-chain, native and long-chain) at different concentrations, on the flow behavior of milk beverages with different fat content. For low-fat milk samples with lower inulin concentrations (2%, 4%, and 6%), the flow was Newtonian and no significant differences in viscosity among the three types of inulin were observed. For the higher inulin concentrations (8 and 10%) samples, the flow was shear thinning and a sharp increase was detected in apparent viscosity of

samples with long-chain inulin. Native inulin at 2% and at 4% increased firmness of low-fat fermented milk (Pinheiro et al., 2009) and the addition of long chain inulin at 5% to low-fat fermented milk significantly increased the apparent viscosity and flow time dependency; however, it did not modify the pseudoplasticity of flow nor the consistency index values (Debon et al., 2010). The observed variations in flow behavior of dairy beverages containing inulin could be explained by different factors according to previous studies: the capacity of inulin to retain water (Soukoulis et al; 2009); the interaction of inulin with milk protein, which can lead to an increase in the molar mass resulting in increased viscosity (Schaller-Povolny and Smith, 2001); and the formation of small aggregates of microcrystals that are able to retain water (González-Tomás et al., 2008).

To compare the rheological behavior of the control full-fat samples and the different low-fat samples, a Principal Component Analysis was applied to study the variability in both flow and viscoelastic parameters (Figure 6). The first component, which accounted for 57.45% of total variability, was related in its positive part with the variation in G', G'' and η^* and in its negative part in tan δ. This component clearly separated the samples according to CMC concentration. Samples with 1.1% of CMC, in the positive part of the first dimension, showed higher values for most of the rheological parameters, whereas 0.7% CMC samples fell in the opposite region, showing the highest tan δ values. The second component accounted for 33.43% of variability and was positively correlated with A_R. This component separated low-fat samples with 0.03% λ-carrageenan that had the highest A_R values. The mapped sample distribution showed that for the highest CMC concentrations (0.9 and 1.1 % CMC), the low-fat sample with 0.01% carrageenan (samples 6 and 11, respectively) or with 11% inulin blend (samples 10 and 15, respectively) were those demonstrating the most similar rheological behavior to that of the whole-milk control sample (FFC with

0.9%CMC and 1.1%CMC), the samples with λ-carrageenan being more similar to full-fat samples in terms of rheological behavior than samples with inulin. In the case of samples with 0.7% CMC, the full-fat sample was clearly separated from all low-fat samples. For each CMC concentration, the full-fat control sample and two low-fat samples containing 0.01% λ-carrageenan or 11% SC/LC inulin blend were selected for sensory analysis.

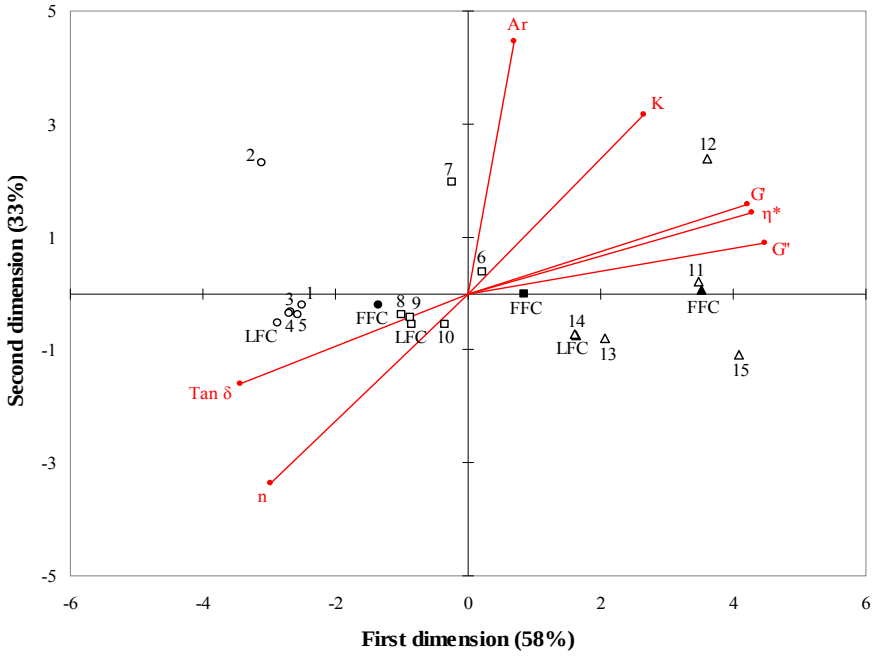

Figure 6. Principal component analysis bi-plot for rheological parameters of low-fat (empty symbols) and full-fat (filled symbols) dairy beverages with λ-carrageenan or inulin at different carboxymethyl cellulose (CMC) concentrations (circle = 0.7%; square = 0.9% and triangle = 1.1%). Identification of samples is given in Table 1. FFC: full-fat control, LFC: low-fat control; K = consistency index; n = flow behavior index; A_R = relative hysteresis area; G'= storage modulus; G"= loss modulus; tan δ = loss angle; and η* = complex dynamic viscosity.

Consumer Perception: Free Choice Profile

Consumers generated 334 terms to describe differences among samples. These terms were subdivided into appearance (123), flavor (93) and texture (118), with individual sets ranging from 6 to 20 descriptors. In spite of the high number of terms, many assessors used identical or similar descriptors. The most frequent terms used by the consumers when describing the desserts were light or strong yellow, consistency and liquid texture. The total amount of real variance, explained by the first 8 dimensions of the average configuration, was 71.15%. Dimensions 1 and 2 accounted for 45.0, and 16.7% of variance, respectively. Further dimensions explained a small proportion of variance (< 5%); therefore, a two-dimensional solution was finally chosen, explaining 61.7% of variance.

The assessors' plot (Figure 7) shows that consumers were randomly distributed along the first and second dimension, indicating that they perceived all the samples in a similar way. This acceptable agreement among consumers was reflected with assessors' residual variance values, which ranged from 0.24 to 0.76. Scaling factors indicate to what extent the initial configuration of an individual has been stretched (values > 1) or shrunk (values < 1), to fit the average configuration. Consumers 17 and 4 had the greatest scaling factor (2.4 and 1.99, respectively), indicating that theses consumers scored over a much narrower range of the scale, thus requiring the configuration to be stretched to obtain closer agreement with the other assessors; consumers 3 and 14 had the smallest scaling factors (0.70 and 0.61) and used a much wider range of the scale (Oreskovich *et al*. 1991).

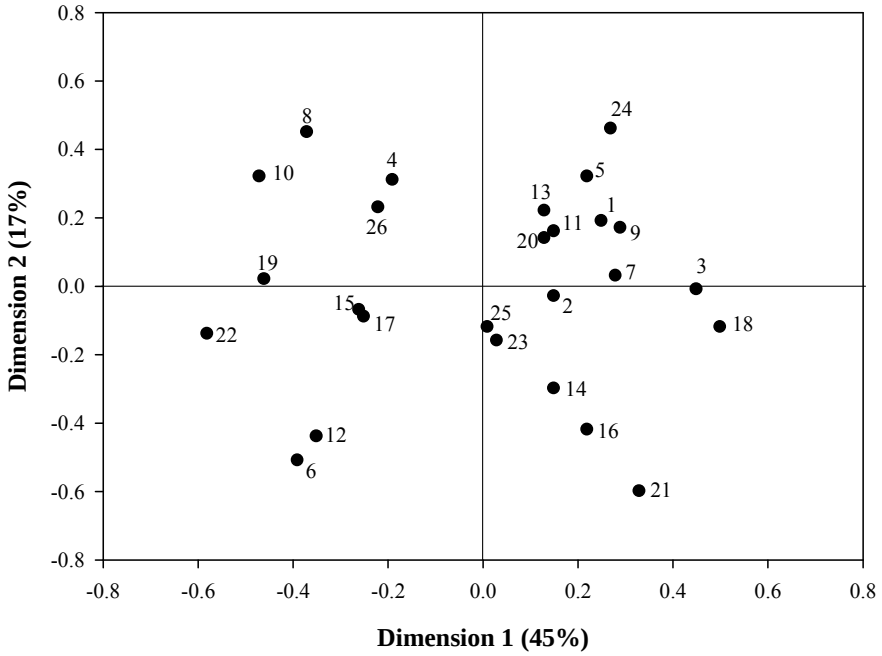

Figure 7. Assessors' plot: first two principal axes obtained by Free Choice Profile.

Figure 8 shows the distribution of the total variance in the samples. Both consensus and residual variance distribution were similar for the two replicates of each sample. A large amount of the total variance is explained by samples 5, 11 and full-fat control with 1.1% CMC, which should consequently be spread over the perceptual sample space. On the contrary, in samples 6 and 10, a low amount of variance was explained; they should be located close to the center of the average sample space. Residuals were very similar among samples, indicating that the consumers' evaluation of the samples agreed.

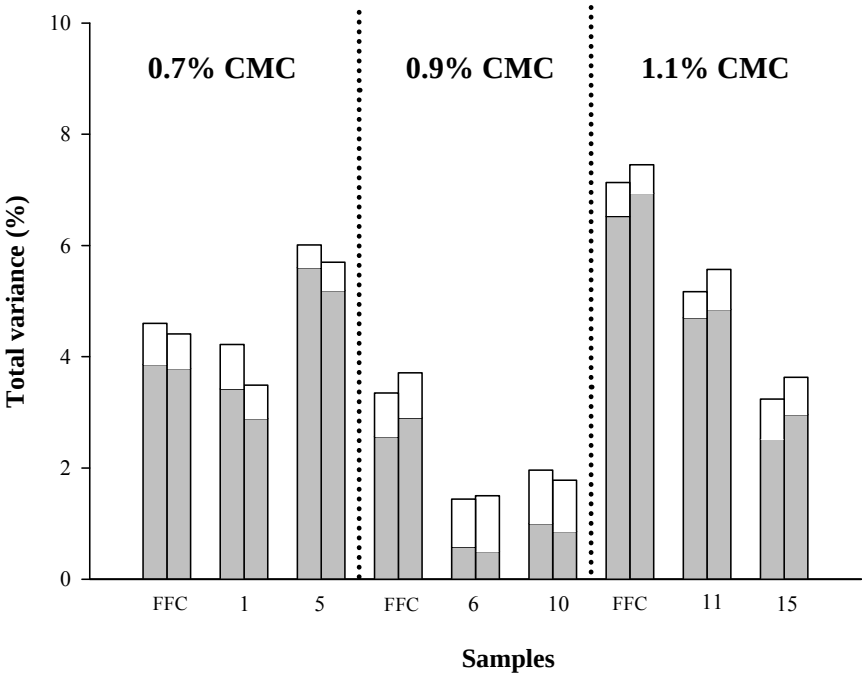

Figure 8. Consensus (grey) and residual variance (white) distributed over the nine samples. Identification of samples is given in Table 1. FFC = full-fat control; CMC = carboxymethyl cellulose.

The average sample space obtained from the GPA is shown in Figure 9. The replicate reproducibility for each sample can be seen by two points, showing they are proximal. Table 7 helps to interpret the dimensions of the average space, showing descriptors with correlations > 0.80. Dimension 1 accounted for 45% of the consensus variance and separated the samples largely by visual consistency (thick visual consistency, thick appearance, fluid appearance, liquid appearance, liquid-visual texture) and in-mouth texture (fluid texture, liquid texture, dense texture, thick texture). This dimension split the samples into two groups. On the left, samples 1, 5, 6, 10 and full-fat control with 0.7% CMC, were mainly described as having a liquid texture. On the right, samples 11, 15 and full-fat control with 0.9% and 1.1% CMC,

were perceived as having a thicker texture. It can be seen that CMC content rather than fat content differentiated samples in terms of consistency. Dimension 2 accounted for 17% of the consensus variance and was mainly related to the flavor and color attributes. On its positive side, it differentiated full-fat samples as having a milky flavor and pale yellow color, while on the negative side, low-fat samples (1, 5, 6, 10, 11, and 15) were evaluated as having a yellow-orange color.

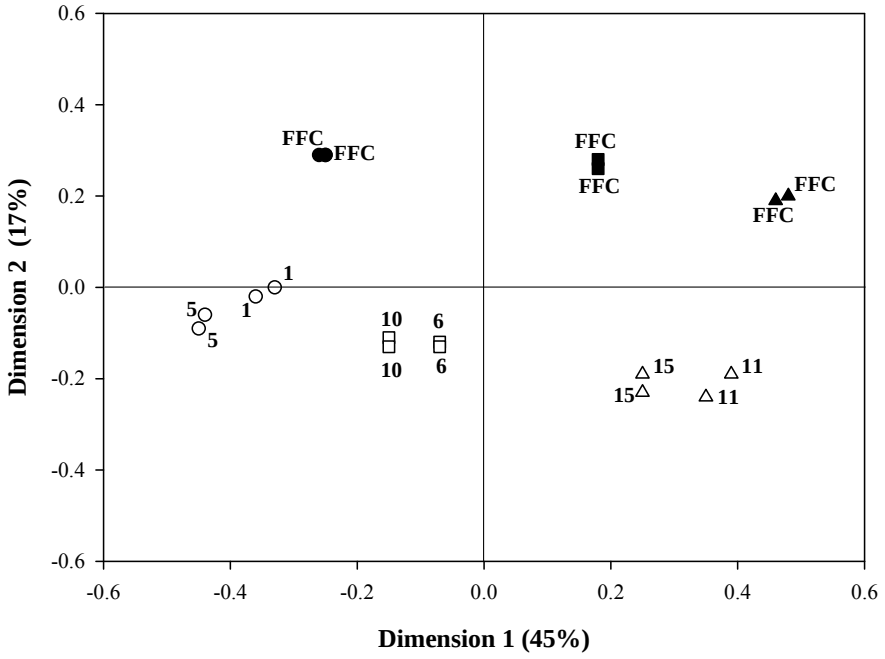

Figure 9. Sample average space for the first two principal dimensions obtained by applying generalized Procrustes analysis to sensory date generated by Free Choice Profile in duplicate. Identification of samples is given in Table 1. Empty symbols = low-fat samples; filled symbols = full-fat control (FFC) samples; carboxymethyl cellulose concentrations: circle = 0.7%; square = 0.9% and triangle = 1.1%.

Table 7. Descriptors having correlations greater than 0.80 with the two dimensions of the average space generated by generalized Procrustes analysis.

Dimension	Correlation	Descriptors[1]
1	+	Dense visual consistency (2), doughy consistency, thick aspect (6), creamy aspect (2), consistent appearance (8), viscous appearance (4), visual consistency (2), viscous visual texture, thick visual consistency, thick visual texture, viscous visual consistency, solid visual consistency, dense appearance, thick texture (9), creaminess, consistent texture (6), viscous texture (9), traditional custard texture, consistency (2), purée consistency, baby food consistency, heavy consistency, lumpy texture, dense texture, custard consistency, béchamel sauce texture, consistency in-mouth.
	-	Liquid appearance (7), light appearance, liquid visual consistency (4), liquid-visual texture, juice-liquid appearance, fluid appearance (2), fluid texture (3), liquid texture (14), light texture (2), milk-shake texture, fluid milk-shake texture, light consistency, vanilla milk-shake texture, liquid milk-shake texture.
2	+	Pale yellow (3), light yellow (4), dull yellow, whitish yellow, soft yellow (3), dead yellow, plastic flavor, whole-milk flavor, milky flavor, whole-milk odor.
	-	Yellow-red colored, strong yellow (2), dark yellow (3), egg yolk color, yellow-orange colored (3), orange-colored (2), artificial-yellow color, bright yellow (2), apricot color.

[1] *In parentheses, number of times that a descriptor had a correlation greater than 0.80.*

CONCLUSIONS

The addition of both λ-carrageenan and an inulin blend, to low-fat CMC-dairy beverages decreased instrumental differences in color and rheological traits between low-fat and full-fat samples; therefore, both could be used as fat replacers in dairy beverages. Moreover, samples with λ-carrageenan added were more similar to full-fat samples in terms of rheological behavior than samples with inulin blend, and slight instrumental differences on color and rheological parameter values were observed among them. Free choice profile results showed that samples differing in color and rheological behavior due to its composition were also sensorially distinguishable by consumers.

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CAPÍTULO 7

OPTIMIZING ACCEPTABILITY OF A HIGH PROTEIN SOY DESSERT COMBINING SURFACE RESPONSE METHODOLOGY AND JAR SCALES

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ABSTRACT

Acceptance of a new high protein (6.75g/serving) citrus flavored soy-based dessert with different concentrations of sucrose (5-17%) and citrus flavor (50-350 ppm) has been optimizing using the Response Surface Method (RSM). Acceptability of each of 16 formulations was evaluated by 130 consumers. Relationships between acceptability scores and composition variables were determined for the overall consumer population and for two subgroups of consumers with different preference criteria, identified by Cluster Analysis. Whilst for subgroup I the composition factors did not show a clear influence on acceptability, for subgroup II variance in acceptability was well explained in terms of sucrose and citrus flavor contents ($R^2 = 94.4$). Results obtained using Just-About-Right (JAR) scales showed that the main differences between both consumer subgroups were related to adequacy of citrus flavor, sweetness and consistency data. Relating JAR percentages and hedonic data provide useful information for explaining differences in hedonic scores.

PRACTICAL APPLICATIONS

The design of soy-based dessert formulations, with adequate protein content and good sensory quality, is important to ensure their market success. It is important to take consumer opinions into account during formulation development to obtain a marketable product. In this respect, considering possible differences in preference criteria among consumer populations and combining information provided by RSM and by JAR scales can be very useful. Such tools can help to improve product acceptance through the modification of product formulation.

Keywords: Product optimization, soy-desserts, response surface, JAR scales

INTRODUCTION

The growing interest in soy-based products as an alternative to dairy products has attracted much attention recently. In addition to their lactose-free and reduced fat content, soy-based products are also a good source of protein and may provide health benefits for the consumer. Studies conducted on the health benefits of soy foods have shown that consumption of soy proteins, as part of regular diet, provide multiple benefits for atherosclerosis, menopausal symptoms and osteoporosis as well as risk reduction of coronary heart disease and of breast and prostate cancers (Messina 1999; Barnes 2006; Xiao 2008). The main problems facing the acceptance of these products are related to their flavor, commonly being referred to as "beany", certain astringency and bitter taste, as well some undesirable textural characteristics (Al Mahfuz *et al.* 2004; Chambers *et al.* 2006; Da Silva *et al*. 2012). Several authors have tried to solve these flavor and taste drawbacks using different approaches such as developing soy varieties with lower lipoxygenase or A-saponins content (Yuang and Chang 2007; Takada *et al.* 2013), modifying process conditions (Endo *et al.* 2004; Suppavorasatit *et al.* 2013) or trying to mask the undesirable flavor and taste by adding flavoring or other ingredients such as fruit juices, chocolate syrup, cocoa powder or coffee (Wang *et al.* 2001; Potter *et al*. 2007; Despande *et al.* 2008; Yuan and Chang 2007; Granato *et al.* 2010; Felberg *et al.* 2010; Chattopadhyay *et al.* 2013). In addition, some authors have described the texture of soy-based products as chalky, dry or oily (Day N'Kouka *et al.* 2004; Potter *et al.* 2007) and some of them have proposed the use of different stabilizers, such a gelatine, pectin or gum acacia, to improve this attribute (Mepha *et al.* 2006; Potter *et al.* 2007). In any case, the sensory problems of soy-based beverages or desserts are also related with soy protein concentration. To avoid this, an important part of currently available commercial soy products are formulated with soy protein contents (less than 3g/100g) that are lower than that

recommended by different public institutions. For example, as commented by Walker *et al.* (2010), the Food and Drug Administration (FDA) requires that a product contain at least 6.25 g/serving of soy protein to state a health claim on its label. Therefore, designing soy protein dessert formulations taking into account both adequate protein content and good sensory quality can be important to ensure their market success.

When consumers select and eat a foodstuff, their response will depend on both the sensory characteristics of the product and on their own physiological and cognitive status (Costell *et al.* 2010). In foods with specific health characteristics, the information supplied regarding their possible health benefits can confer a positive effect on consumers (Walker *et al.* 2010), however, this aspect cannot outweigh their sensory properties (Aikman *et al.* 2006; Verbeke 2006; Siró *et al.* 2008, Bayarri *et al.* 2010; Menezes *et al.* 2011). So, taking consumers' opinions into account during formulation development is an important step in selecting soy-based product formulations.

Several statistical techniques have been developed for consumer-orientated food product optimization (Gacula 1993). Among them, Response Surface Methodology (RSM) has been successfully applied to obtain the best possible formulation that maximizes consumer acceptance given a fixed set of ingredients in different type of foods (Damasio *et al.* 1999; Mendes *et al.* 2001; Chu and Resurrección 2004; Gan *et al.* 2006; Villegas *et al.* 2010; Arcia *et al.* 2011) including soy-based beverages and desserts (Despande *et al.* 2008; Granato *et al.* 2010; Felberg *et al.* 2010; Chattopadhyay *et al.* 2013). Two practical problems arise to interpret RSM information: the first is that the relationship obtained between ingredients and acceptability is limited to the range of ingredient concentrations previously selected; the second it that it is very difficult to identify the causes underlying changes in acceptability due to the possible effects of interactions between ingredients.

Some techniques have been used to analyze to what extent these interactions cause perceptible variations in sensory features and if any such variations affect product acceptability. Ravi and Sucheelamma (2005) have used desirability function analysis to determine the optimum conditions of variables for making high-quality boondi. To do so, they considered the interrelationship between process variables and their effects on product characteristics (instrumental measurements and sensory aspects). Meullenet *et al.* (2008) have studied an ideal density plots method to optimize the formulation of muscadine grape juice, and they concluded that this method represents a simple solution to identifying the location of ideal products in a preference space; and that it can be used as an alternative to RSM. Another option to obtain information about consumer opinion, regarding the adequacy of sensory attribute intensity, is to use the Just About Right (JAR) scales. These scales, which combine both intensity of the attribute and hedonics assessed, can play a diagnostic role in determining how the consumer feels about the product (Gacula *et al.* 2007) and they also provide an idea of the proportion of consumers who perceived each sample in a certain way (Costell *et al.* 2010). Some authors have used both JAR and hedonic scales in consumer testing to provide directional information for food optimization. Hoppert *et al.* (2013) studied consumer acceptance of regular and reduce-sugar yogurt enriched with dietary fiber, and observed that the information provided by the JAR scale might be used to improve product acceptance through product formulation modification. Ares *et al.* (2009) compared the performance of attribute liking questions and JAR scales to evaluate consumers' perceived adequacy of flavor and texture of milk puddings. They concluded that JAR scales were good to measure the adequacy of sensory attribute intensity and that JAR percentages were the best indicators of overall liking scores.

The aims of the current work were to optimize the acceptability of a citrus flavored high protein soy-based dessert using Response Surface Methodology and to obtain complementary information about the sensory features that cause changes in their acceptability relating JAR percentages and hedonic data.

MATERIAL AND METHODS

Materials

Soy-based desserts were prepared with powdered soy protein isolate (90% protein) (SPI) (PROSOL 90-GF NT, Cargill, Barcelona, Spain), carboxymethyl cellulose (CMC) (Akucell AF3265 Akzo Nobel, Amersfoort, The Netherlands), mineral water (35.5 mg/L calcium, 8.6 mg/L magnesium, 11.9 mg/L sodium, 144 mg/L bicarbonate and 13 mg/L chloride content) (Font Vella, Barcelona, Spain), commercial sucrose, citrus flavor (Naranja 1594A, Lucta, Barcelona, Spain) and colorant (NC2 SX WS mct, CHR Hansen S.A., Barcelona, Spain).

Experimental design and sample preparation

For the optimization study, a set of samples was prepared varying sucrose and citrus flavor concentrations. Sample composition was selected according to a two-factor central composite design with replicates of the central point for estimating pure error (Gacula 1993). The design was comprised for 16 points: 4 factorial, 4 axial, and 8 central points (Table 1). Concentration ranges for sucrose (from 5 to 17% w/w) and citrus flavor (from 50 to 370 ppm) were selected based on preliminary assays. The amounts of soy protein isolate (6% w/w, that corresponding to 6.75 g protein in a serving of 125 g

dessert), carboxymethyl cellulose (0.9% w/w) and colorant (0.1% w/w) remained fixed in all samples.

Table 1. Experimental design of soy-based desserts, showing coded and uncoded values of levels

Formulation	Coded level		Uncoded level	
	Sucrose	Citrus flavor	Sucrose (% w/ w)	Citrus flavor (ppm)
1	-1	-1	8	125
2	1	-1	14	125
3	-1	1	8	275
4	1	1	14	275
5	-2	0	5	200
6	2	0	17	200
7	0	-2	11	50
8	0	2	11	350
9-16	0	0	11	200

Soy-based desserts were prepared in batches of 800 g. Soy protein isolate powder was dispersed in mineral water and mixed for 15 min, with the help of a propeller stirrer (Heidolph RZR 1, Schwabach, Germany). The dispersion was placed in a water bath at 90 ± 1ºC and was stirred constantly for 20 min. Then, it was cooled in a bath at 10ºC for 10 min and stored at 4 ± 1ºC overnight to ensure complete hydration of soy proteins. The amount of water that had evaporated in the heating process was replaced gravimetrically. The following day, a blend of CMC with sugar was added to the soy protein dispersion with the colorant, and stirred (Heidolph RZR 1, Schwabach, Germany) for 35 min at room temperature. Five min before the end, citrus flavor was added. All samples were transferred to a closed flask and stored at 4 ± 1ºC for 24 h, prior to sensory evaluation.

Sensory evaluation

The optimization study was performed by 130 consumers recruited by a local consumer association (Association of Valencia Consumers and Users-AVACU) through a short questionnaire sent by mail. The participants were selected according to the following criteria: age, gender, consumers of dairy and/or soy products. Consumers distribution was as follows: 67.7% women (32.3% men), and 38% were between 18-30 years old, 42.3% between 31-45 years old, and 19.2% over 45 years old. About 62.3% of participants consume yogurt at least 2-3 times a week, and 32.2% and 22% of them consume dairy desserts or soy milk 4-5 times a month, respectively. Prior to the test, it was confirmed that participants had no allergies to soy products.

Each consumer first evaluated the overall acceptability of each one of the 16 formulations, using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). The consumers also evaluated the adequacy of citrus flavor intensity, acidity, sweetness, bitterness and consistency using a 5-point just about right (JAR) scale (1 = too weak, 3 = just about right, 5 = too strong). Each consumer evaluated four samples per session over four sessions. Time lapse between evaluations of two consecutive samples was fixed at 30 seconds. To reduce the influence of serving order, in each session samples were presented following a Williams' design for four samples (MacFie *et al*. 1989). At least, one of the samples in each session corresponded to the central point of the design (samples 9 to 16, Table 1).

Sensory evaluation was carried out in a standardized test room (ISO 2007) with separate booths under normal white fluorescent illumination in morning sessions (11:00 to 13:00 h. Samples (30 mL) were presented at 10 ± 1°C in plastic cup and labeled with a random three-digit code. White salt-free bread and mineral water were provided to the consumers for mouth rinsing between samples. Data acquisition was performed using Compusense *five*, release 5.0 (Compusense Inc., Guelph, ON, Canada).

Data analysis

A two-way ANOVA, considering sample and consumer as factors, was applied to the overall acceptability data. Tukey's test ($\alpha = 0.05$) was used to calculate the minimum significant difference.

To identify consumer subgroups with different preferences they were segmented based on acceptability scores of the samples by using Cluster Analysis (Clustering Ward Method) (Vigneau and Qannari 2002). To analyze differences in sample acceptability data between the two subgroups of consumers, Student's *t*-test was used. These calculations were carried out with XLSTAT Pro software, version 2007 (Addinsoft, Paris, France).

Response surface methodology (RSM) was used to analyze optimization study results (Gacula 1993). Acceptability data from the total consumer population and from each of the two subgroups of consumers previously identified by Cluster Analysis were submitted to multivariate regression analysis and fitted to a second order model equation provided in the design:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{22}X_2^2 + B_{12}X_{12} + Error$$

Eq.1

where Y is the overall acceptability; B_0 is the intercept (constant); B_1, B_2 the linear, B_{11}, B_{22} the quadratic and B_{12} the interaction effects; X_1 and X_2 the independent variables (concentration of sucrose and citrus flavor, respectively). In the selected models, only statically significant parameters at $p \leq 0.05$ were considered based on Student's test. ANOVA of the regression equations allowed the calculation of goodness of fit and of significance of the linear, quadratic and interaction effects. Validity of the models obtained was evaluated as a function of their respective coefficients of determination

(R^2) as well as by an analysis of the lack of fit. These analyses were performed with the SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).

Data from JAR scales were analyzed separately for each consumer subgroup and the percentage of consumers who perceive the sample attributes in a certain way was calculated. As rules of thumb, to conclude that a specific attribute is at its optimal level, a minimum of 70% of the responses are usually expected to be in the "just about right" group and to conclude that an attribute is not at its optimal level, a minimum of 20% of consumers is usually needed in the "too weak" or "too strong" categories (Meullenet *et al.* 2007).

RESULTS AND DISCUSSION

Optimization of soy-based citrus flavored dessert

A two-way ANOVA (sample and consumer) was performed on acceptability data of soy-based desserts with different citrus flavor and sucrose concentrations obtained from the overall consumer population. Results showed that sample composition ($F = 27.44$, $p < 0.01$) had a significant effect on acceptability of the different formulations. In general, the degree of liking differed significantly among samples with scores ranging from 3.49 for sample 5 with the lowest sucrose concentration to 5.82 for sample 2 with the highest sucrose concentration. No significant differences in acceptability were detected among replicate samples corresponding to the central point of design (samples 9-16) (Tables 1 and 2).

Table 2. Acceptability of soy-based desserts with different sucrose and citrus flavour concentrations for overall consumers (N = 130) and for subgroups I (N = 44) and II (N = 86)

	Acceptability		
Sample[1]	**Overall**	**Subgroup 1**	**Subgroup II**
1	4.74^{e}	3.98^{b}	5.13^{f}
2	5.82^{ab}	4.30^{ab}	6.59^{a}
3	4.97^{de}	4.32^{ab}	5.30^{ef}
4	5.53^{abc}	4.16^{b}	6.23^{abcd}
5	3.49^{f}	2.91^{c}	3.78^{g}
6	5.99^{a}	5.16^{a}	6.41^{ab}
7	5.20^{cde}	4.23^{b}	5.70^{cdef}
8	5.29^{bcd}	4.57^{ab}	5.65^{def}
9	5.55^{abc}	4.48^{ab}	6.09^{abcd}
10	5.36^{bcd}	3.98^{b}	6.07^{abcd}
11	5.64^{abc}	4.34^{ab}	6.30^{abc}
12	5.63^{abc}	4.43^{ab}	6.24^{abcd}
13	5.36^{bcd}	4.52^{ab}	6.23^{abcd}
14	5.72^{abc}	4.57^{ab}	6.31^{abc}
15	5.37^{bcd}	4.43^{ab}	5.85^{bcde}
16	5.40^{bcd}	4.39^{ab}	5.92^{bcde}

[a-g] Means within a column with common superscripts did not differ significantly (P < 0.05)

[1] Identification of samples is given in Table 1

In order to establish the relationship between acceptability and composition variables (citrus flavor and sucrose concentrations), data were fitted to a second-order model. The regression model obtained was significant (F-ratio = 56.18, $p < 0.01$), explained 91.7% of all variance in data and the lack of fit was not significant ($p > 0.05$) (Table 3). The model included both linear and quadratic terms for sucrose (S, %w/w) and only a quadratic term for citrus flavor (C, %w/w) (Table 4):

$$\text{Acceptability} = 5.54 + 0.55*S - 0.20S^2 - 0.07C^2 \qquad \text{Eq. 2}$$

Table 3. ANOVA of the regression models relating acceptability of soy-based dessert with sucrose and citrus flavor concentrations for overall consumers (N=130) and for subgroups I (N=40) and II (N=86)

Responses	Sources of variation	Df	Sum of squares	Mean square	F-ratio	*P*-value
Acceptability of overall consumers[1]	Model	3	4.77	1.59	56.18	<0.01
	Residual	12	0.34	0.03		
	Total	15	5.11			
Acceptability of subgroup I[2]	Model	1	1.81	1.81	18.54	<0.01
	Residual	14	1.37	0.10		
	Total	15	3.18			
Acceptability of subgroup II[3]	Model	2	6.70	2.23	84.79	<0.01
	Residual	12	0.32	0.03		
	Total	15	7.01			

[1] *R^2 = 93.35%, R^2 adjusted for Df = 91.69%, P-value (lack of fit) = 0.10*

[2] *R^2 = 56.97%, R^2 adjusted for Df = 53.90%, P-value (lack of fit) = <0.01*

[3] *R^2 = 95.50%, R^2 adjusted for Df = 94.37%, P-value (lack of fit) = 0.72*

Table 4. Estimated regression coefficients of the fitted equations obtained for acceptability of overall consumers (N = 130) and for subgroups I (N = 44) and II (N = 86) of the soy-based desserts depending on sucrose and citrus flavor concentrations

	Overall		**Subgroup I**		**Subgroup II**	
Coefficient	**Estimated**	**SE***	**Estimated**	**SE***	**Estimated**	**SE***
B_0	5.54	0.06	4.29	0.08	6.13	0.05
Linear						
B_1	0.55	0.04	0.39	0.09	0.64	0.05
B_2	**	**	**	**	**	**
Quadratic						
B_{11}	-0.20	0.03	**	**	-0.26	0.03
B_{22}	-0.07	0.03	**	**	-0.11	0.03

* *Standard error*

** *No significant coefficient (P-value > 0.05)*

For sucrose, the signification of positive linear and of negative quadratic coefficients indicated that acceptability increased with sucrose concentration until reaching maximum value, and from this sucrose concentration value, a higher amount of sucrose lowered acceptability because of excessive sweetness. With regard to citrus flavor, the low coefficient value of this component indicated that the variation in concentration barely affected acceptability of soy-based desserts. The surface response and contour plot created by the predictive model (Figure 1) shows that highest acceptability values would be obtained for a wide range of sucrose concentrations, between 12 and 17% (w/w), and of citrus flavor concentrations (80 - 320 ppm). Despite the statistical adequacy of the regression model obtained, one must bear in mind that it was obtained from average acceptance scores from the overall consumer population. Its practical validity is based on the assumption that consumer acceptability criteria are homogeneous. If this is not so, the optimal formulation or formulations selected may not have a real meaning and market response predictions may fail (Damasio *et al.* 1999).

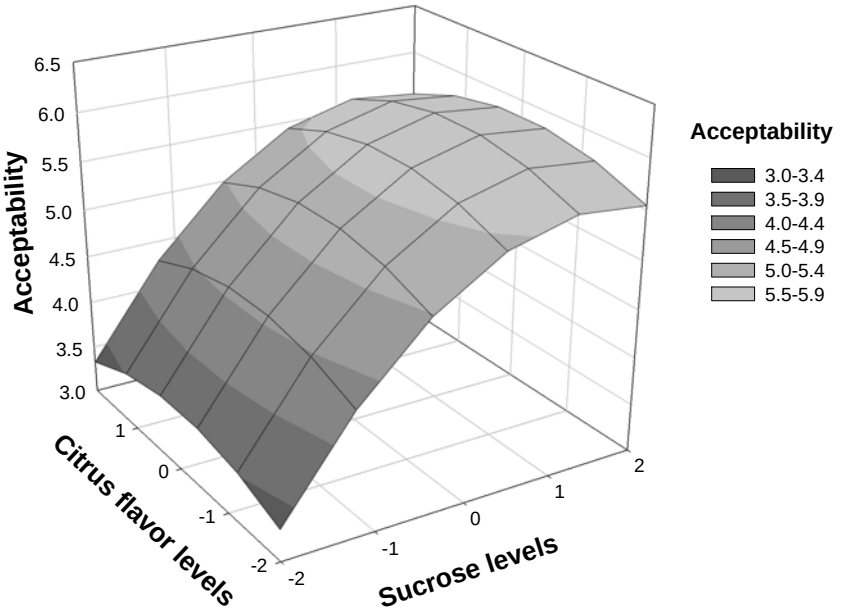

Figure 1. Response surface of acceptability of soy-based desserts for overall consumers (N = 130) as related to sucrose and citrus flavor levels.

In order to detect possible subgroups of consumers with different preference criteria Cluster Analysis was applied to overall acceptability scores. Two subgroups of consumers were identified (Figure 2). A first group (SI, N = 44) representing 33.8% of the total population surveyed and a second one (SII, N = 86) representing 66.2% of consumers.

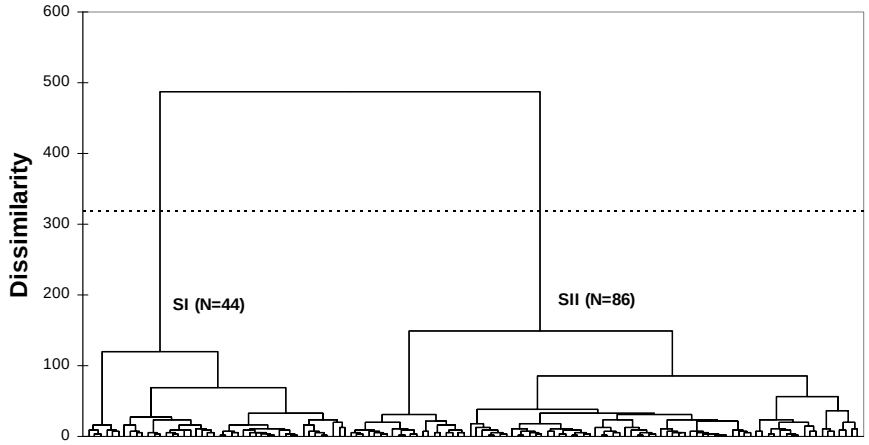

Figure 2. Consumer segmentation (N = 130) by cluster analysis. SI: consumer subgroup I and SII: consumer subgroup II.

The ANOVA of acceptability data for each subgroup of consumers showed that for both consumer groups, sample composition had a significant effect (SI: F-ratio = 6.01, $p < 0.01$; SII: F-ratio = 26.21, $p < 0.01$) on acceptability values of the different samples. When sample acceptability scores from each consumer subgroup were compared using a Student's *t*-test, a significant difference was detected between them (experimental value $t = 14.8$; theoretical value at $\alpha = 0.05$, $t = 1.75$). In general, SI consumers gave lower acceptability scores (from 2.9 to 5.2) than SII consumers (from 3.8 to 6.6) (Table 2). For the SI consumers only the sample with the highest sucrose concentration (sample 6) showed a hedonic score higher than 5, while for SII consumers, only the sample with the lowest sucrose concentration (sample 5) showed a hedonic score lower than 5. It can be concluded that the main difference between the responses to soy-based desserts of the two consumer subgroups was related with hedonic preferences. Consumers in subgroup I did not like this type of product and they gave acceptability score of below 4.6 for most of the samples evaluated. Consumers in subgroup II liked these

type of desserts and their acceptability scores for most samples were higher than 5.6. On analyzing the demographic characteristics of consumers included in each of the two subgroups, a clear relationship could not be observed. Subgroup I comprised 75% women and subgroup II 64% women. With respect to age, the most remarkable difference between the two subgroups was in over 45 year-old consumers. Subgroup I had a higher percentage of these consumers (25%) than subgroup II (16%). With regard to consumption habits, only 16% of consumers in subgroup I (7 people) had soymilk at least 4-5 times per month, whilst 24% of the total population of subgroup II (21 consumers) did so. These differences in consumption frequency could partially explain differences obtained in acceptability scores of soy-based desserts. Several authors have suggested that habitual consumption of a food increases its acceptability. Felberg *et al.* (2010) optimized a coffee-soy-based beverage varying sugar, soymilk power and instant coffee concentrations. They observed that there were three segments of consumers with different soy-beverage and coffee consumption patterns and that a part of the acceptance differences among samples seems to be related with soy beverage consumption habits. Villegas *et al.* (2009) compared acceptability of milk and soymilk vanilla beverages and its relationship with demographic characteristics, consumption frequency and product sensory input. They observed that acceptability differences among samples were more closely related to product sensory characteristics than to consumer conditions (demographic or consumer habits). In spite of this, regular soymilk consumers awarded significantly higher acceptability scores to soy-based beverages than non soymilk consumers.

For subgroup I, the regression model obtained when data were fitted to a quadratic equation was significant and included only the linear term for sucrose (Tables 2 and 3). Acceptability was related to sucrose concentration (S, %w/w) by the following regression equation:

$$\text{Acceptability} = 4.29 + 0.39*S \qquad \text{Eq. 3}$$

The response surface generated when plotting the model showed a directly proportional relationship between acceptability and sucrose concentration with maximum acceptability values for the highest sucrose concentrations, independently of citrus flavor concentration (Figure 3).

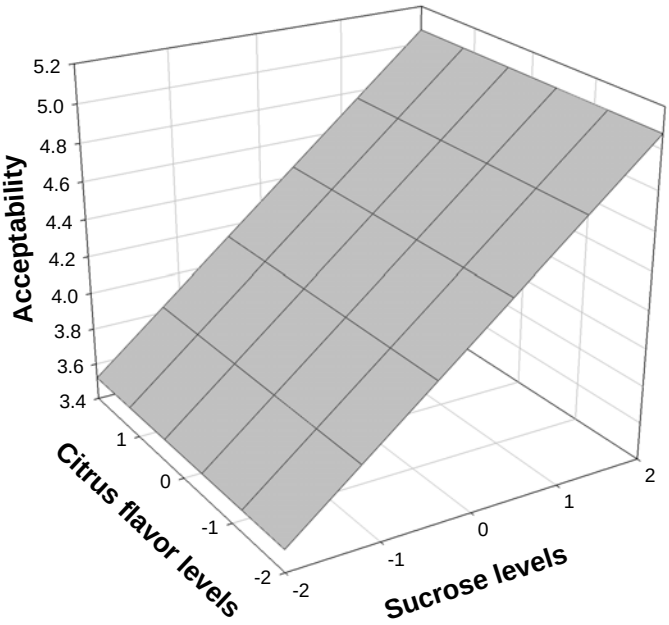

Figure 3. Response surface of acceptability of soy-based desserts for consumer subgroup I (N = 44) as related to sucrose and citrus flavor levels.

In general, for this group of consumers it would seem that an increase in sucrose concentration could slightly increase the acceptability of citrus flavored soy-based desserts. In any case, this result should be considered only approximate, taking into account the low R^2 value ($R^2 = 53.9\%$) and the significant lack of fit for the regression model obtained ($p < 0.01$) (Table 3). The significance of the lack of fit indicated that the model does not fit data

well enough and that it has a limited flexibility to characterize the relationship between acceptability and the composition variables considered.

With regard to subgroup II, the regression model obtained was significant and explained 94.37% of all variance in data. The lack of fit was not significant ($p = 0.74$), and the model included both a linear and quadratic term for sucrose, and a quadratic term for citrus flavor (Table 3). The sucrose-citrus flavor interaction was not significant. Thus, both ingredients are important for this group of consumers. The equation that represents the relationship between acceptability and composition variables (sucrose: S, % w/w) and citrus flavor: C, % w/w) is as follow:

$$\text{Acceptability} = 6.13 + 0.64*S - 0.26S^2 - 0.11C^2 \qquad \text{Eq. 4}$$

As can be seen in the response surface generated by the model (Figure 4), sucrose concentration had a stronger effect on acceptability values than citrus flavor concentration. For sucrose, as in the case with overall consumers, the linear coefficient was positive and the quadratic one negative, which indicated that acceptability increased with sucrose up to a maximum value (over 14% sucrose) and that dessert acceptability decreased when sucrose concentration increased from 15% to 17%. With respect to citrus flavor, the plot shows that variation in citrus flavor affected acceptability values to a lesser degree to the respective changes in sucrose concentration.

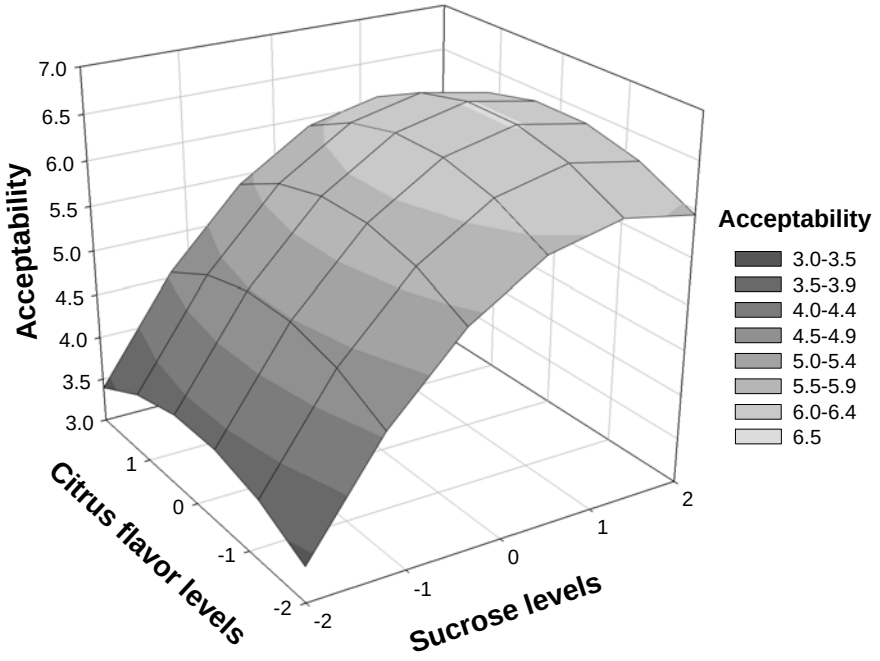

Figure 4. Response surface of acceptability of soy-based desserts for consumer subgroup II (N = 86) as related to sucrose and citrus flavor levels.

These results illustrate the different information that can be obtained considering only the average data of the whole consumer population surveyed or the average data of consumer subgroups with different preference patterns. With the first option, the model indicates that there is a wide range of sucrose and citrus flavor concentrations that obtain an optimal product for the overall consumer population. When acceptability average data provided by each of the two consumer subgroups were considered, different information was achieved. For SI consumers the composition factors considered did not show a clear influence on acceptability, whereas for SII consumers variability on hedonic scores can be explained in terms of sucrose and citrus flavor concentrations. The consumers forming this last subgroup, preferred samples with slightly higher sucrose and moderate citrus

flavor contents (samples 2, 4 and 6) (Tables 1 and 2). It can be considered that for this consumer subgroup the maximum values for soy-based dessert acceptability may be reached at sucrose concentrations of 14% and at citrus flavor concentration of around 125-200 ppm (Figure 4).

Response to JAR scales. Relationship with acceptability

When responses to JAR scales of the two consumer subgroups, SI (soy desserts "dislikers") and SII (soy desserts "likers"), were compared, some similarities and differences between them were detected (Figure 5).

A similar variation trend was observed for adequacy of citrus flavor intensity and of taste attributes (acidity, sweetness and bitterness), and clear differences were detected with respect to consistency adequacy of samples perceived by both consumer subgroups. Regarding citrus flavor (Figure 5a and 5b), samples 6 and 9 were considered to have optimal citrus flavor levels by subgroups I and II, respectively. In both cases the percentage of consumers that considered the cited samples' flavor as "just about right" was over 70%. These samples had the same flavor concentration (200 ppm), but some differences in sucrose concentration (17% and 11%, respectively). Regarding the acidity (Figure 5c and 5d), the JAR response percentage was lower than 45% for the two subgroups and for all samples. Over 50% of consumers in both subgroups considered that samples had "too weak" o "weak" acidity. It suggests the convenience of increasing acidity in this type of product to increase its acceptance. For SI consumers, some of samples evaluated were considered as with "just about right" sweetness intensity (Figure 5e). Only the sweetness intensity of sample 2, with 14% sucrose, was considered as JAR by more than 50% of these consumers (54.6%). SII consumers showed a different opinion. For this subgroup, 73.3% of consumers perceived sample 9 (central point formulation, 11% sucrose) as

having an optimal sweetness level (Figure 5f). With respect to bitterness (Figure 5g and 5h), JAR data showed very similar responses of both consumer subgroups while they did not show a clear relationship between sample composition variation and adequacy of bitterness perception. For both SI and SII consumers, the highest percentage of "too strong" or "strong" responses (around 30%) corresponded to samples 3 and 8, which were the samples with the highest citrus flavor concentration, while sample 9 was considered to have adequate bitterness with 59% of "just about right" responses. As commented previously, consistency results showed differences in the sample evaluation between the two subgroups (Figure 5i and 5j). Over 50% of SI consumers considered that all samples had a "strong" or "too strong" consistency and only between 30-40% of them perceived consistency as adequate. Whilst for subgroup II, all samples were considered as having "just about right" consistency by 45-60% of consumers.

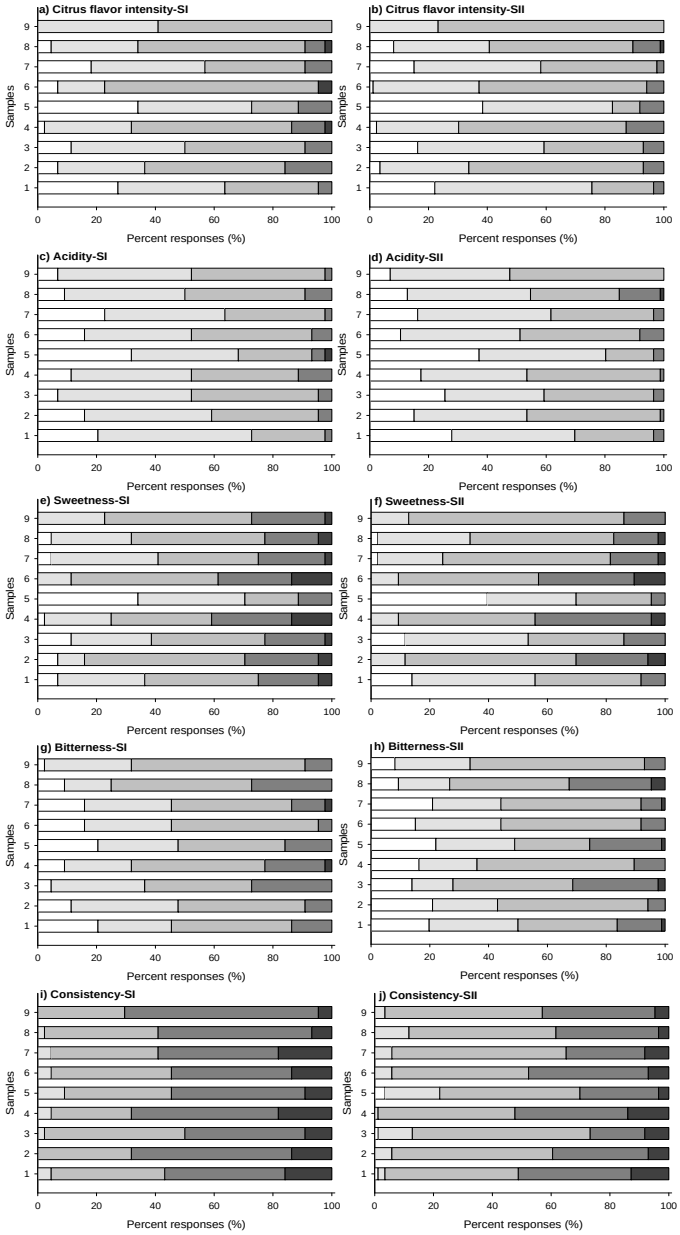

Figure 5. Consumer assessment distribution about the appropriateness of different sensory attributes using the 5-point Just About Right scale: too weak (□), weak (□), just about right (□), strong (□) and too strong (□). SI: data from subgroup I and SII: data from subgroup II. Data of sample 9 is the average of data obtained for samples corresponding to central point of the design (samples from 9 to 16) (Table 1).

Results obtained showed there were no important differences between SI and SII consumers' responses regarding adequacy of acidity and bitterness among samples. Results also showed that the main differences between both consumer subgroups were related to citrus flavor, sweetness and consistency data. These latter results can be illustrated comparing JAR percentages obtained from SI and SII consumers for each sample (Figure 6). Differences in JAR response percentages for citrus flavor (Figure 6a) and for sweetness (Figure 6b) depended on sample composition. For example, similar JAR percentages were obtained for citrus flavor of samples 4 and 7 and for sweetness of samples 1, 6 and 8 by the two consumer subgroups. Meanwhile, clear differences in JAR percentages were observed for citrus flavor of samples 6 and 9 and for sweetness of samples 7 and 9, which could reflect distinct preference criteria of each consumer subgroup respect to "adequate intensity" of these attributes. From a practical point of view, JAR data provide other important information for product formulation related with the effect of odor and tastant crossmodal interactions on perceived flavor and taste intensity. In this case, an interaction between sweetness and citrus flavor could explain why samples 5 and 6 were respectively perceived as "to weak" or "just about right" for citrus flavor (Figure 6a) by both consumer subgroups, despite having the same citrus flavor concentration (200 ppm) but different sucrose concentrations (5% and 17%). Secondly, samples 2 and 4 with the same sucrose content (14%) but with different citrus flavor content (125 and 275 ppm) showed a clear difference for JAR percentage for sweetness (Figure 6b). These results are in accordance with those obtained by other authors with respect to the effect of crossmodal interactions on perceived flavor intensity in different type of foods (Nasri *et al.* 2011; Hoopert *et al.* 2013). Lastly, it seems that differences in JAR response percentages for consistency (Figure 6c) did not depend on sample composition.

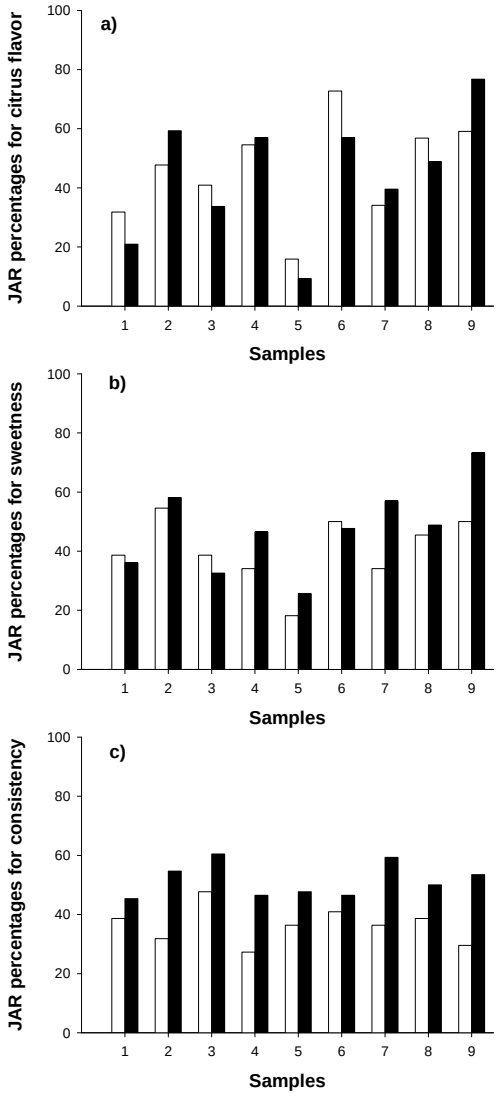

Figure 6. Consumer percentage for each of the two subgroups: subgroup I (□) and subgroup II (■) who indicated that samples were "Just About Right" for citrus flavor intensity (a), sweetness (b) and consistency (c).

For all samples evaluated, JAR percentages obtained for SI consumers were lower than those obtained for SII consumers. Furthermore, by comparing inter-sample variability of JAR percentages for citrus flavor, sweetness and consistency within each consumer subgroup it can be observed that

variability was lower for consistency. As expected, this fact indicates that the change in composition among samples influences sample consistency to a lesser extent than citrus flavor and sweetness intensity. Information obtained about rheological behavior of the evaluated samples (data not shown) confirms that changes in citrus flavor and sucrose concentrations hardly affected their flow behavior.

To obtain information about how sensory characteristics adequacy influenced overall acceptability of the different samples, the percentages of consumers who considered that a specific attribute was JAR, were related with acceptability scores for each of the two subgroups. In general, a similar trend was observed in the relationship between JAR percentages and acceptability for flavor and taste attributes for both subgroups of consumers although, in all cases, samples acceptability scores for subgroup II were higher than that for subgroup I. For example, the relationship between acceptability data and JAR percentages for citrus flavor intensity is shown in Figure 7. Apart from quantitative differences in sample acceptability scores from consumer subgroups comprising "dislikers" and "likers", it can be observed that JAR percentage below 20% correspond to the lowest acceptability scores and that JAR percentages ranging from 60 to 80% correspond to the highest acceptability scores. These results seem to be in accordance with observations by Meullenet *et al.* (2007) about the value of JAR percentages as a practical index of each specific attribute level.

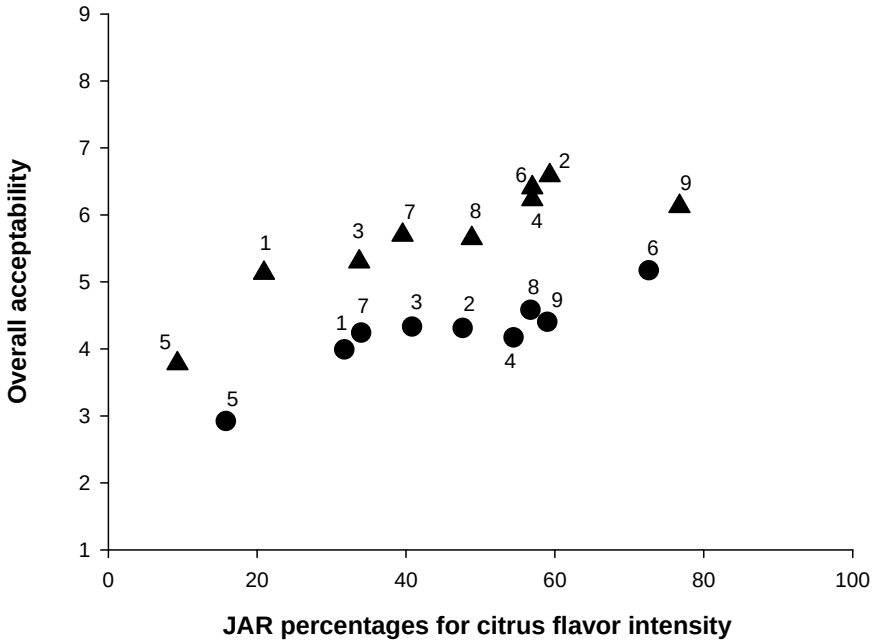

Figure 7. Relationship between JAR percentages for citrus flavor intensity and acceptability data of soy-based desserts for the two consumer subgroups: subgroup I (●) and subgroup II (▲).

With respect to consistency, there is not a direct relationship between acceptability scores and JAR percentages within each consumer subgroup (Figure 8). Sample acceptance was more closely related with the particular consistency preferences of each consumer subgroup than with consistency level of samples. Hedonic scores of SI consumers ranged from 2.9 to 5.3 and it is clear that they did not like sample consistency at all. As commented previously, over 50% of these consumers considered that all samples had a "strong" or "too strong" consistency. Hedonic scores of SII consumers ranged from 3.8 to 6.6 and all samples were considered as having "just about right" consistency by 45-60% of these consumers. These results indicate the convenience of design two types of soy-based dessert with different consistency levels to flavor product acceptance by groups of consumers with different consistency preferences.

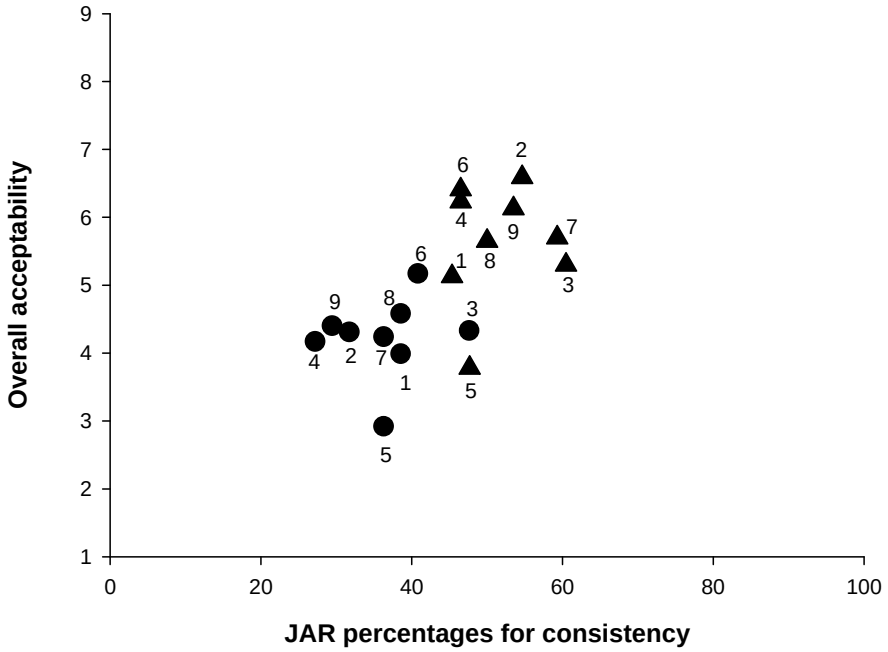

Figure 8. Relationship between JAR percentages for consistency and acceptability data of soy-based desserts for the two consumer subgroups: subgroup I (●) and subgroup II (▲).

CONCLUSIONS

To optimize acceptability of high protein soy-based desserts, Response Surface Methodology (RSM) has proven a valuable tool to obtain the most acceptable formulation given a fixed set of ingredients. The real validity of the selected formulation depends on the homogeneity of consumer acceptability criteria. In this work, we have pointed out that information obtained when considering the mean acceptance scores for the overall consumer population as dependent variable in regression analysis differed from that obtained when using the mean acceptance scores for each of the two consumer subgroups, segmented according to their preference criteria by Cluster Analysis. The predictive model obtained from average acceptance scores from the overall consumer population (R^2 = 91.7) showed that highest

acceptability values would be obtained at a wide range of sucrose concentrations, between 12 and 17% (w/w), and of citrus flavor concentrations (80-320 ppm). For consumer subgroup I representing 33.8% of the population, the composition factors considered did not show a clear influence on acceptability (R^2 = 53.9), although a direct relationship between acceptability and sucrose concentration was detected. For consumer subgroup II representing 66.2% of consumers, variability in hedonic scores can be well explained in terms of sucrose and citrus flavor concentrations (R^2 = 94.4). The maximum values of soy-based desserts acceptability were reached at sucrose concentrations of 14% and at citrus flavor concentration around of 125-200 ppm). It can be concluded that if possible individual differences among consumer responses are ignored; the proposed regression model may poorly fit the data from a considerable number of consumers. Response to JAR scales of the two consumer subgroups showed a similar trend regarding adequacy of citrus flavor intensity and taste attributes (acidity, sweetness and bitterness) and clear differences with respect to consistency adequacy. The relationships between JAR percentages and hedonic scores can be a very useful tool to optimize the sensory quality of foods.

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DISCUSIÓN GENERAL DE LOS RESULTADOS

Para el desarrollo y optimización de alimentos con bajo contenido en grasa y en azúcar que sean aceptables para los consumidores, es necesario, tal como se ha comentado en la Introducción de esta Tesis, analizar y entender no solo la influencia de ambos componentes en la estructura, en las propiedades físicas y sensoriales y en la respuesta de los consumidores, sino también, la del efecto de sus interacciones con los otros ingredientes del alimento. En este contexto, y de acuerdo con el objetivo general de esta tesis, se han realizado una serie de experiencias en diferentes tipos de matrices alimentarias (lácteas, emulsiones agua/aceite y proteicas) cuyos resultados pueden agruparse en tres áreas temáticas: a) relaciones entre las modificaciones de la composición de las matrices alimentarias y sus características físicas y estructurales; b) conexiones entre los cambios en la composición, estructura y propiedades físicas de las matrices y la liberación en la boca de los estímulos responsables del sabor y con la percepción del sabor, del dulzor y de la textura y c) aplicación de nuevas metodologías para favorecer la incorporación de las opiniones y preferencias de los consumidores durante el desarrollo y optimización de nuevos productos.

Relaciones entre las modificaciones de la composición de las matrices alimentarias y sus características físicas y estructurales

Dentro de esta primera temática se analizó la influencia del contenido en grasa y de la adición de diferentes concentraciones de dos espesantes de distinta composición y estructura y con diferentes características tecnológicas (carboximetilcelulosa (CMC) y almidón) en la estructura y en las propiedades físicas de tres tipos de matrices alimentarias semisólidas: emulsiones agua/aceite, postres lácteos y postres de soja. En los dos primeros tipos de matrices se analizó el posible papel de los dos

hidrocoloides como sustitutos de grasa y también, como agentes modificadores de su textura (capítulos 1 y 3). Mientras que, en el caso de los postres de soja se consideró que el principal objetivo de la incorporación de los hidrocoloides a su formulación era la consecución de productos con una determinada textura (capítulo 2). En general, tanto el contenido en grasa o en proteína como el tipo y concentración de espesante modificaron de forma distinta las características físicas y estructurales de las diferentes matrices. El estudio con emulsiones se realizó en muestras con dos concentraciones de aceite (5 y 30%) y diferentes concentraciones de CMC (0.2, 0.3, y 0.4%) o de almidón (2, 3, y 4%). Se observó que tanto la concentración de CMC como la de almidón tuvieron un efecto importante en el comportamiento de flujo, en el que también influyó la concentración de las gotas de aceite. Sin embargo, el efecto en la viscoelasticidad dependió del tipo de hidrocoloide. En las emulsiones con CMC, el contenido en grasa fue el factor más influyente mientras que en las emulsiones con almidón, lo fue la concentración de este hidrocoloide. Estas diferencias estuvieron bien relacionadas con las observadas en la microestructura y en la distribución del tamaño de partícula de las distintas emulsiones. El efecto de la CMC en la microestructura y en la distribución del tamaño de partículas dependió principalmente del contenido en aceite mientras que el del almidón dependió del volumen relativo ocupado por los gránulos de almidón gelatinizados. El estudio en las matrices lácteas semisólidas se realizó en muestras preparadas con leche entera o desnatada, adicionando distintas concentraciones de CMC (1.1 y 1.3%) o de almidón (3.5 y 4.0%). El color de las muestras se relacionó principalmente con el contenido en grasa. La interacción entre el tipo de hidrocoloide y su concentración tuvo un efecto significativo en los valores de la mayoría de los parámetros instrumentales de color. En las muestras elaboradas con leche entera, ni la concentración ni el tipo de hidrocoloide modificó su color. En las muestras fabricadas con leche desnatada, ambos hidrocoloides no solo influyeron en el color de las muestras sino que lo

hicieron de forma distinta. El comportamiento de flujo de los sistemas bajos en grasa, dependió sobre todo del tipo de espesante y la viscoelasticidad, de la concentración. La adición de CMC incrementó claramente la dependencia del tiempo, la seudoplasticidad y la consistencia de las muestras elaboradas con los dos tipos de leche. El efecto de la adición de almidón en las propiedades de flujo no fue tan claro, sólo fueron significativas las diferencias entre los valores del umbral de fluencia entre las muestras con diferente contenido en grasa. La viscoelasticidad de estas muestras, tanto las elaboradas con leche entera como las elaboradas con leche desnatada aumentó con la concentración de hidrocoloide adicionado. El efecto del tipo de leche en la viscolesticidad fue mayor en las muestras con CMC que en las elaboradas con almidón.

Los resultados obtenidos en estos trabajos pusieron de manifiesto que utilizando distintas concentraciones de ambos hidrocoloides se pueden obtener productos con distintos comportamientos reológicos y también, que era posible formular postres lácteos y emulsiones con diferente contenido en grasa y con comportamientos reológicos similares. Esto indica que, en principio, ambos hidrocoloides pueden ser útiles como modificadores de la textura y como sustitutos de grasa en emulsiones aceite/agua y en matrices lácteas. Sin embargo, los resultados también indicaron que era necesario tener en cuenta los cambios que las distintas concentraciones de cada hidrocoloide producían en otras características del producto (color, microestructura, estabilidad, etc.) por su posible incidencia en las características sensoriales y en la calidad final del producto.

Como base para el posterior diseño y formulación de postres de soja con alto contenido en proteínas, se prepararon muestras con dos concentraciones de aislado de proteína de soja (SPI) (6 y 8%) y con diferentes concentraciones de CMC (0.3, 0.5, 0.7 y 0.9 %) o de almidón (2, 2.5, 3 y 3.5%). Tanto la concentración de SPI como la de CMC o la de almidón influyeron

significativamente en el comportamiento de flujo y en las propiedades viscoelásticas de estos postres de soja, aunque la influencia de los espesantes dependió de la concentración de proteína. Los resultados permitieron concluir que la adición de distintos hidrocoloides a distintas concentraciones modificaba el comportamiento reológico de las muestras de forma diferente. Ello puede permitir elaborar postres con distintas texturas y también obtener productos con distinta composición y similar comportamiento reológico. Aunque tal como se ha comentado en el caso de las emulsiones y de las matrices lácteas, para diseñar postres de soja enriquecidos en proteína de buena calidad y aceptables para el consumidor, es necesario obtener más información sobre los efectos de los distintos ingredientes en la estabilidad y en el sabor y textura percibidos en este tipo de productos.

Conexiones entre los cambios en la composición, estructura y propiedades físicas de las matrices y la liberación en la boca de los estímulos responsables del sabor y del gusto, y con la percepción del sabor, del dulzor y de la textura

Como se ha comentado en la introducción de esta tesis, aunque es evidente que la composición química, las propiedades físicas y la estructura de los alimentos son los estímulos primarios de las sensaciones que los consumidores experimentan al ingerirlos, las transformaciones que el alimento sufre durante su permanencia en la cavidad bucal juegan también un papel importante en la liberación de los estímulos químicos desde la matriz y en la percepción de la textura. En este contexto, se ha estudiado primero la influencia del contenido en grasa y del tipo de espesante (CMC y almidón) en la liberación “in vivo” de los compuestos volátiles en matrices lácteas y en emulsiones aceite/agua con sabor a limón (mezclas de linalool y

cis-3-hexen-1-ol) aplicando la técnica de espectrometría de masas con una fuente de ionización de transferencia de protones acoplada. De las curvas obtenidas se extrajeron dos parámetros: la intensidad máxima de cada volátil liberado y el área acumulada del volátil liberado durante el primer minuto. En experiencias posteriores se analizó sensorialmente la intensidad del sabor, del dulzor y de las características de la textura de las muestras correspondientes a cada tipo de matriz (capítulos 3, 4 y 5). La liberación *in vivo* de los volátiles responsables del sabor fue cualitativamente similar en ambos tipos de matrices. El contenido en grasa de cada matriz influyó claramente en la liberación del volátil más lipófílico (linalool), mientras que prácticamente no afectó a la liberación del más hidrofílico (*cis*-3-hexen-1-ol), que dependió principalmente de la consistencia y del tipo de hidrocoloide incluido en cada muestra. La disminución del contenido en grasa, no solo influyó en la intensidad del olor y sabor, sino que también modificó cualitativamente las sensaciones percibidas al variar la secuencia de liberación de los volátiles desde la matriz, en función de la polaridad de cada uno de ellos. En ambos tipos de matrices la liberación de volátiles fue mayor en los productos que contenían almidón que en los que contenían CMC. Es posible que las diferencias estructurales observadas entre los productos elaborados con carboximetilcelulosa (estructuras poliméricas) o con almidón (estructuras globulares) hayan influido en su comportamiento durante su residencia en la cavidad bucal por su diferente resistencia a la rotura y a la deformación o su distinta capacidad para mezclarse con la saliva. Ello podría explicar que el proceso de liberación de los volátiles de cada matriz hasta los receptores dependiera no solo de la consistencia de la muestra sino también, de las características estructurales del hidrocoloide utilizado en su formulación. Al analizar sensorialmente el sabor y la textura de los postres lácteos se observó que la intensidad del sabor a limón dependía del contenido en grasa. Las muestras elaboradas con leche desnatada se percibían con un sabor a limón mas intenso que las elaboradas

con leche entera lo que concordaba con la liberación de los volátiles *in vivo* detectada instrumentalmente. Las muestras con almidón se percibieron con un sabor a leche más intenso que las elaboradas con CMC. Además de su impacto en el sabor, tanto el contenido en grasa como el de hidrocoloide influyeron en la textura de las muestras. Mientras las muestras con CMC y leche entera resultaron claramente más consistentes que las elaboradas con leche desnatada, no se detectaron diferencias en consistencia entre las muestras elaboradas con almidón y con distinto tipo de leche. En las emulsiones aceite/agua, también se observó que las de menor contenido en aceite se percibían con un sabor cítrico más intenso que las que contenían mayor concentración de aceite (30%). Lo que también coincidía con la liberación *in vivo* de los volátiles detectada instrumentalmente. Respecto a las diferencias percibidas en la textura entre las muestras con CMC y con almidón, se observó que eran mayores en las emulsiones con el 5% de aceite que entre las del 30%. Parece que la coalescencia de los gránulos de grasa durante el cizallamiento de la muestra entre la lengua y el paladar puede ser la responsable de las diferencias detectadas. Respecto a la intensidad del dulzor, independientemente del contenido en grasa y de la concentración de espesante, las muestras de postres lácteos con almidón se percibieron como más dulces que las muestras con CMC. Las diferencias estructurales entre ambos hidrocoloides o la distinta movilidad del agua en ambos tipos de productos pueden también influir en el proceso de transferencia de las moléculas de azúcar, a través de la capa de saliva, hasta los receptores. En las emulsiones, el dulzor percibido fue más intenso en las que contenían mayor cantidad de aceite y disminuyó con la adición de hidrocoloides. El aumento de dulzor con la concentración de aceite en la emulsión podría explicarse considerando que al aumentar la cantidad de aceite, la concentración de azúcar se incrementa en la fase acuosa por lo que, durante su consumo se libera en la boca una mayor cantidad de azúcar para estimular los receptores. El que al aumentar la concentración de hidrocoloide

disminuya el dulzor percibido responde al bien conocido efecto supresor de la consistencia en la intensidad del dulzor y la diferencia de la magnitud del efecto entre las muestras elaboradas con cada hidrocoloide puede originarse en las diferencias estructurales entre ambos.

Aplicación de nuevas metodologías para favorecer la incorporación de las opiniones y preferencias de los consumidores durante el desarrollo y optimización de nuevos productos

Como se ha comentado en la introducción de esta tesis, el diseño y elección de la formulación de productos con bajo contenido en grasa o en azúcar requiere tener en cuenta, durante diferentes etapas de su desarrollo, las percepciones y preferencias de los consumidores. Conseguir de los consumidores información válida y que sea útil para formular nuevos productos o para incrementar la aceptación de los ya existentes, no es tarea fácil. Por ello, durante los últimos años, se han propuesto diferentes tipos de metodologías para estudiar y entender el comportamiento del consumidor. En la tercera parte de esta tesis se exploró la posibilidad de combinar distintos métodos para obtener información sobre cómo perciben y describen los consumidores las diferencias entre muestras y también, sobre cómo las preferencias individuales pueden incidir en los resultados cuando se pretende optimizar la calidad sensorial de un producto. Independientemente de la influencia de factores externos en la aceptación de un alimento (precio, marca comercial, hábitos culturales, etc.), cuando se formula un nuevo alimento y se estudian las relaciones composición - estructura - propiedades físicas - propiedades sensoriales, surgen dos cuestiones críticas: ¿Cómo perciben los consumidores las diferencias entre distintas muestras? ¿Hasta qué punto las diferencias percibidas sensorialmente influyen en la aceptación

de un producto? Para poder obtener respuestas a la primera cuestión, se analizó, cómo los consumidores percibían y podían describir las diferencias entre bebidas lácteas de distinta composición y propiedades físicas, utilizando la combinación de distintas técnicas: descripción entrecruzada, perfil de libre elección y análisis de Procrustes Generalizado (capítulo 6). El estudio se realizó en una serie de muestras de bebidas lácteas con diferente contenido en grasa y adicionando distintos ingredientes como sustitutos de grasa. La metodología propuesta resultó útil para comprobar que los consumidores eran capaces de detectar las diferencias en color y en textura que podrían ser esperables por los resultados obtenidos al medir instrumentalmente el color de las muestras y caracterizar su comportamiento reológico. Pero además, permitía obtener información sobre cómo los consumidores describían las diferencias percibidas sensorialmente e incluso, sobre la importancia relativa de cada una de ellas. Esta información puede resultar de gran utilidad práctica durante las primeras etapas de desarrollo de nuevos productos. Es evidente que no todas variaciones sensoriales, cualitativas o cuantitativas, percibidas por los consumidores influyen en la aceptación de un determinado alimento. Para obtener información sobre la incidencia que los cambios en la formulación de un producto pueden tener en la aceptación del mismo por el consumidor, una de las técnicas más conocidas y utilizadas es la de Superficie de Respuesta. Con ella, previa elección de la variabilidad de determinados ingredientes, se puede seleccionar la formulación con máxima aceptabilidad. La utilidad práctica del método puede verse reducida por la dificultad para identificar las variaciones sensoriales que dan lugar a cambios en la aceptación del producto. Además, la validez real del resultado obtenido puede estar limitada por el efecto de una variabilidad no detectada en los criterios de preferencia de la población de consumidores encuestada. En esta situación, se diseñó una experiencia para optimizar la calidad sensorial de postres de soja con alto contenido en proteína (capítulo 7), combinando la información obtenida con

diferentes técnicas: Detectar posibles subgrupos de consumidores con diferentes criterios de preferencia segmentándolos con un análisis de cluster; Obtener, para cada subgrupo de consumidores, la formulación con máxima aceptación y finalmente, identificar los cambios sensoriales que modifican la aceptación del producto utilizando la información obtenida con las escalas JAR *(Just About Right*). Los resultados obtenidos pusieron de manifiesto que utilizando conjuntamente las tres técnicas se pueden identificar los atributos que deben modificarse para obtener postres de soja más aceptables y obtener una información mas precisa sobre la posible respuesta de los consumidores ante este tipo de productos.

CONCLUSIONES

1. La adición de hidrocoloides con distintas características estructurales y fisicoquímicas, influye de forma diferente en la estructura y en las propiedades físicas y sensoriales de las matrices alimentarias debido a los efectos de las interacciones entre ellos y los otros componentes de cada matriz.

2. En las emulsiones aceite/agua, tanto la concentración de las macromoléculas de carboximetilcelulosa como la de los gránulos de almidón fueron los factores que más influyeron en su comportamiento de flujo. Sin embargo, en el comportamiento viscoelástico y en la distribución del tamaño de partículas, el efecto fue distinto: en las emulsiones con CMC, el factor más importante en estas características fue el contenido en aceite y en las emulsiones con almidón, lo fue el volumen ocupado por los gránulos de almidón.

3. En las matrices lácteas, el color dependió solo del contenido en grasa; el comportamiento de flujo, del hidrocoloide adicionado y el comportamiento viscoelástico dependió principalmente de la concentración de hidrocoloide. Variando la concentración y el tipo de hidrocoloide se pueden obtener productos bajos en grasa con un comportamiento reológico similar al de los productos con grasa.

4. En las matrices de proteína de soja, tanto el comportamiento de flujo como el viscoelástico, dependieron del efecto de la interacción entre la proteína y el hidrocoloide.

5. La liberación de los volátiles responsables del sabor hasta la cavidad nasal, dependió principalmente del contenido en grasa, tanto en las emulsiones como en las matrices lácteas. En ambas, la grasa influyó en la liberación del compuesto más lipofílico pero no, en la del menos lipofílico. El aumento de la concentración de los hidrocoloides no influyó en la intensidad máxima de los volátiles exhalados pero sí en la cantidad total de aroma liberado.

6. Aparte del efecto directo de los hidrocoloides en la estructura, comportamiento reológico y en la textura percibida en emulsiones y en matrices lácteas, también influyeron en la liberación y percepción del sabor y en la percepción del dulzor. En ambos tipos de matrices, los sistemas con almidón liberaron los volátiles con más rapidez que los sistemas con CMC y se percibieron con un sabor y dulzor más intensos.

7. Para obtener productos bajos en grasa con sabor similar al de los productos con grasa no solo es necesario igualar su textura sino también, rediseñar la mezcla de volátiles a adicionar. Respecto a la intensidad del dulzor es posible obtener productos que con la misma concentración de azúcar tengan textura similar y diferente intensidad o diferente textura y similar dulzor. Ello implica diseñar texturas que se modifiquen de forma distinta durante el periodo en el que el producto permanece en la cavidad bucal, antes de su deglución.

8. La utilización conjunta de la descripción entrecruzada, del perfil de libre elección y del análisis de Procrustes Generalizado resulta útil para conocer las sensaciones que experimentan los consumidores al ingerir un alimento y

permite obtener una información preliminar sobre la importancia relativa de cada una de ellas.

9. Teniendo en cuenta la posible existencia, en la población de consumidores de subgrupos de personas con distintos criterios de preferencia y combinando la información obtenida con la metodología de la superficie de respuesta y con las escalas JAR, se pueden optimizar las formulaciones de nuevos productos, identificar los atributos que deben modificarse para hacerlos más aceptables y obtener una información mas precisa sobre la posible respuesta de los consumidores en el mercado