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INCORPORACIÓN DE EDULCORANTES NO CARIOGÉNICOS Y CON BAJO ÍNDICE GLICÉMICO EN EL PROCESADO DE FRUTA (CÍTRICOS Y SANDÍA) Y MONITORIZACIÓN DE PARÁMETROS A LO LARGO DEL ALMACENAMIENTO

TESIS DOCTORAL

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Valencia, Septiembre 2015

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“La inteligencia consiste no sólo en el conocimiento, sino también en la destreza de aplicar los conocimientos en la práctica” (Aristóteles).

“Reza como si todo dependiera de Dios.
Trabaja como si todo dependiera de ti” (San Agustín).

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RESUMEN

Como decía Hipócrates en la Antigua Grecia: "Que tu alimento sea tu medicina". De acuerdo con este pensamiento se muestra la sociedad actual que manifiesta un interés creciente por el consumo de alimentos sanos, seguros y con una elevada calidad nutricional y funcional, siendo éste el caso de frutas como sandías y cítricos. Sin embargo, su consumo en fresco lleva consigo el inconveniente de ser perecederos. Como alternativa, su procesado consigue extender su vida útil manteniendo ciertas características del producto fresco, utilizando procesos como la deshidratación osmótica y la elaboración de mermeladas y de gelatinas. No obstante, uno de los componentes más importantes en su formulación es la sacarosa, pero su consumo, está unido al desarrollo de enfermedades como diabetes, obesidad, caries, hiperlipemias, etc. Por esta razón, la industria alimentaria, oferta edulcorantes naturales (tagatosa, isomaltulosa, oligofructosa y stevia) como sustitutos de la sacarosa, ofreciendo así, la posibilidad de fabricar productos bajos en calorías o acalóricos, no cariogénicos y funcionales.

Teniendo en cuenta lo mencionado anteriormente, se han planteado en esta tesis doctoral los siguientes objetivos:

A) Cinética de deshidratación osmótica de rodajas de naranja y limón, utilizando edulcorantes naturales (isomaltulosa, tagatosa, oligofructosa y extracto acuoso de stevia).

B) Desarrollo de mermeladas de cítricos, así como, de gelatinas de sandía y de cítricos, con propiedades no cariogénicas y de bajo índice glicémico mediante la sustitución de sacarosa por edulcorantes naturales (tagatosa, isomaltulosa y oligofructosa). Evaluación del efecto de dichos edulcorantes sobre las propiedades físico-químicas, ópticas, antioxidantes, mecánicas y sensoriales en mermeladas y gelatinas, así como su comparación con marcas comerciales. Control de la estabilidad a lo largo del periodo de almacenamiento y análisis microbiológico.

C) Monitorización de mermeladas a lo largo del periodo de almacenamiento, mediante una red de sensores inalámbricos, basada en una arquitectura robusta.

A la luz de los resultados obtenidos en la deshidratación osmótica de rodajas de naranja se puede concluir que los valores de difusividad efectiva obtenidos para el jarabe cuya combinación de edulcorantes saludables fue isomaltulosa-oligofructosa supuso una deshidratación más rápida frente al resto de los jarabes. Sin embargo, los resultados observados en la deshidratación osmótica de rodajas de limón, mostraron que el jarabe cuya combinación presentaba tagatosa, oligofructosa y extracto acuoso de stevia supuso una deshidratación más rápida, puesto que los valores de difusividad efectiva fueron mayores que en el resto.

En cuanto a las mermeladas se elaboraron de tres tipos: naranja, mandarina y limón. La capacidad antioxidante experimentó un aumento tras el periodo de almacenamiento en las mermeladas de naranja y mandarina pero un descenso en las de limón. Por otra parte, las mermeladas de naranja con mayor proporción de oligofructosa presentaron una menor luminosidad. Sin embargo, las mermeladas de mandarina, con mayor contenido en tagatosa aumentaron su luminosidad. Por el contrario, el tono y la luminosidad disminuyeron en las mermeladas de limón, puesto que pardearon con el tiempo. Respecto a la reología, la combinación oligofructosa-tagatosa mejoró la consistencia y componente elástica en las mermeladas de naranja. Las mermeladas de limón elaboradas con los nuevos edulcorantes mostraron una mayor consistencia que la mermelada comercial, pero menos que el control, al contrario de lo que sucedió con las mermeladas de mandarina con mayor contenido en tagatosa, dando lugar a una componente elástica menor. A nivel sensorial, en las mermeladas de mandarina y de limón la muestra mejor evaluada fue la que presentaba un mayor contenido en tagatosa, tanto en la aceptación global como en la intención de compra. Sin embargo, en las mermeladas de naranja, la combinación oligofructosa-tagatosa, presentó mejores valores frente al control. Finalmente, todas las mermeladas fueron estables microbiológicamente durante el tiempo de estudio.

En relación a las gelatinas, se elaboraron de dos tipos: sandía, y cítricos (combinación de naranja, limón y mandarina). En el caso de las gelatinas de sandía, se mejoró la capacidad antioxidante en la combinación de isomaltulosa-tagatosa con valores similares a los del control, pero menores que los de la gelatina comercial. Por el contrario, se produjo un descenso de la capacidad antioxidante en las gelatinas de cítricos con la misma combinación, al final del periodo de almacenamiento. A su vez, las propiedades mecánicas de las gelatinas de sandía resultaron muy similares entre ellas y respecto al control, siendo estables durante el almacenamiento. Sin embargo, en las gelatinas de cítricos cuya combinación presentaba mayor contenido en isomaltulosa, se observó una mayor adhesividad. Asimismo, la combinación isomaltulosa-oligofructosa, mostró una mayor elasticidad frente al resto. Además, ambos parámetros se mantuvieron constantes con el tiempo de almacenamiento. Respecto al color, las gelatinas de ambos tipos, que contenían sólo tagatosa, mejoraron la luminosidad durante el almacenamiento. A nivel sensorial, las gelatinas de sandía y de cítricos con igual proporción de isomaltulosa y tagatosa, recibieron las mejores puntuaciones por parte de los consumidores.

Por último, de la monitorización de mermeladas durante el periodo de almacenamiento se demostró la plena concordancia entre los resultados obtenidos mediante la recopilación de datos suministrados por la red de sensores inalámbricos, basada en la arquitectura EDETA, con los resultados obtenidos de forma paralela en los análisis realizados de forma experimental en el laboratorio, ofreciéndose además, nuevas ventajas en el uso de la red de sensores, pudiéndose citar entre otras la obtención de medidas en tiempo real de forma automática e incluso en remoto.

RESUM

Com deia Hipòcrates en l'Antiga Grècia: "Que el teu aliment siga la teu medicina". D'acord amb aquest pensament es mostra la societat actual que manifesta un interès creixent pel consum d'aliments sans, segurs i amb una elevada qualitat nutricional i funcional, sent aquest el cas de fruites com els melons d'Alger i els cítrics. No obstant això, el seu consum en fresc porta amb si l'inconvenient de ser peribles. Com a alternativa, el seu processat, aconsegueix estendre la seua vida útil mantenint certes característiques del producte fresc, utilitzant processos com la deshidratació osmòtica i l'elaboració de marmalades i de gelatines. Un dels components més importants en la seua formulació és la sacarosa, però el seu consum, està unit al desenrotllament de malalties com a diabetis, obesitat, càries, hiperlipèmies, etc. Per aquesta raó, la indústria alimentària, ofereix edulcorants naturals (tagatosa, isomaltulosa, oligofructosa i stevia) com a substituts de la sacarosa, oferint així, la possibilitat de fabricar productes baixos en calories o acalóricos, no cariogénicos i funcionals.

Tenint en compte el que es menciona anteriorment, s'han plantejat en aquesta tesi doctoral els següents objectius:

A) Cinètica de deshidratació osmòtica de rodanxes de taronja i llima, utilitzant edulcorants naturals (isomaltulosa, tagatosa, oligofructosa i extracte aquós de stevia).

B) Desenrotllament de marmalades de cítrics, així com, de gelatines de meló d'Alger i de cítrics, amb propietats no cariogèniques i de baix índex glicèmic per mitjà de la substitució de sacarosa per edulcorants naturals (tagatosa, isomaltulosa i oligofructosa). Avaluació de l'efecte dels dits edulcorants sobre les propietats fisicoquímiques, òptiques, antioxidants, mecàniques i sensorials en marmalades i gelatines, així com la seua comparació amb marques comercials. Control de l'estabilitat al llarg del període d'emmagatzemament i anàlisi microbiològic.

C) Monitorització de marmalades al llarg del període d'emmagatzemament, per mitjà d'una xarxa de sensors sense fil, basada en una arquitectura robusta.

A la llum dels resultats obtinguts en la deshidratació osmòtica de rodanxes de taronja es pot concloure que els valors de difusivitat efectiva obtinguts per al xarop amb la combinació d'edulcorants saludables dels quals va ser isomaltulosa-oligofructosa va suposar una deshidratació més ràpida enfront de la resta dels xarops. No obstant, els resultats observats en la deshidratació osmòtica de rodanxes de llima, van mostrar que el xarop amb la combinació tagatosa, oligofructosa i extracte aquós de stevia va suposar una deshidratació més ràpida, ja que els valors de difusivitat efectiva van ser majors que a la resta dels xarops.

Per a les marmalades es van elaborar de tres tipus: taronja, mandarina i llima. La capacitat antioxidant va experimentar un augment després del període d'emmagatzemament en les marmalades de taronja i mandarina però un descens en les de llima. D'altra banda, les marmalades de taronja amb major proporció d'oligofructosa van presentar una menor lluminositat. Mentres que les marmalades de mandarina, amb major contingut en tagatosa van augmentar la seua lluminositat. Al contrari, el to i la lluminositat van disminuir en les marmalades de llima, ja que van fosquejar amb el temps. Respecte a la reología, la combinació oligofructosa-tagatosa va millorar la consistència i component elàstica en les marmalades de taronja. Les marmalades de llima elaborades amb els nous edulcorants van mostrar una major consistència que la comercial, però menys que el control, al contrari del que va succeir amb les marmalades de mandarina amb major contingut en tagatosa, donant lloc a una component elàstica menor. A nivell sensorial, en les marmalades de mandarina i de llima la mostra millor avaluada va ser la que presentava un major contingut en tagatosa, tant en l'acceptació global com en la intenció de compra. No obstant això, en les marmalades de taronja, la combinació oligofructosa-tagatosa, va presentar millors valors enfront del control. Finalment, totes les marmalades van ser estables microbiològicament durant el temps d'estudi.

En relació a les gelatines, es van elaborar de dos tipus: meló d'Alger, i cítrics (combinació de taronja, llima i mandarina). En el cas de les gelatines de meló d'Alger, es va millorar la capacitat antioxidant en la combinació d'isomaltulosa-tagatosa amb valors semblants als del control, però menors que els de la gelatina comercial. Per contra, es va produir un descens de la capacitat antioxidant en les gelatines de cítrics amb la mateixa combinació, al final del període d'emmagatzemament. Al seu torn, les propietats mecàniques de les gelatines de meló d'Alger van resultar molt semblants entre elles i respecte al control, sient estables durant l'emmagatzemament. Així i tot, en les gelatines de cítrics la combinació de les quals presentava major contingut en isomaltulosa, es va observar una major adhesivitat. Tanmateix, la combinació isomaltulosa-oligofrcutosa, va mostrar una major elasticitat enfront de la resta. A més a més, els dos paràmetres es van mantindre constants amb el temps d´emmagatzemament. Respecte a les propietats òptiques, les gelatines dels dos tipus, que contenien només tagatosa, van millorar la lluminositat durant l`emmagatzemament. A nivell sensorial, les gelatines de meló d'Alger i de cítrics amb la mateixa proporció d´isomaltulosa i tagatosa van rebre les millors puntuacions per part dels consumidors.

Finalment, de la monitorització de marmalades durant el periode d'emmagatzemament es va demostrar la plena concordança entre els resultats obtinguts per mitjà de la recopilació de dades subministrades per la xarxa de sensors sense fil, basada en l'arquitectura EDETA, amb els resultats obtinguts de forma paral·lela en els anàlisis realitzats de forma experimental al laboratori, oferint-se a més nous avantatges en l'ús de la xarxa de sensors, podent-se citar entre altres l'obtenció de mesures en temps real de forma automàtica i inclús en remot.

ABSTRACT

As Hippocrates said in ancient Greece: "Let your food be your medicine". According to this thought, today's society shows a growing interest in healthy, safe and high quality nutritional and functional foods, such as fruits like watermelons and citrus. However, their fresh consumption carries the disadvantage of being perishable. Alternatively, their processing makes it possible to extend their marketability, maintaining certain characteristics of fresh products, using processes such as osmotic dehydration and elaboration of marmalades and jellies. However, one of the most important components in its formulation is sucrose, but its consumption is linked to the development of diseases such as diabetes, obesity, tooth decay, etc. For this reason, the food industry provides natural sweeteners (tagatose, isomaltulose, oligofructose and stevia) as sucrose replacers, thus offering the possibility of manufacturing low calorie or non-caloric, non-cariogenic and functional products.

Considering the above, in this PhD thesis the following objectives have been raised:

A) Kinetics of osmotic dehydration in orange and lemon slices, using natural sweeteners (isomaltulose, tagatose, oligofructose and aqueous extract of stevia).

B) Development of marmalades, as well as jellies of watermelon and citrus, with non-cariogenic and low-glycaemic properties replacing sucrose with natural sweeteners (tagatose, isomaltulose, oligofructose) instead sucrose. Evaluation of the healthy sweeteners effect on physicochemical, optical, antioxidants, mechanical and sensory properties in marmalades and jellies, as well as their comparison with commercial products purchased at the market. Microbiological analyses over the storage period have been studied.

C) Monitoring of marmalades over the storage period, by means of a Wireless Sensor Network based on a robust architecture EDETA.

According to the results obtained in the osmotic dehydration of orange slices, the values of effective diffusivity obtained for syrup formulated with isomaltulose-oligofructose carry out a faster dehydration over other studies syrups. However, the results observed in the osmotic dehydration of lemon slices showed that the syrup formulated with tagatose, oligofructose and aqueous extract of stevia succeed a faster dehydration, to the rest.

Regarding to marmalades, three types were developed: orange, mandarin orange and lemon. The antioxidant capacity showed an increase in orange and mandarin orange marmalades but a decreased in lemon marmalades, after the storage period. Moreover, orange marmalades with the highest content in oligofructose had the lowest luminosity compared to the rest. Moreover, mandarin orange marmalades, with the highest content of tagatose improved its luminosity. On the contrary, lemon marmalades with the highest proportion of isomaltulose initially presented a higher luminosity compared to the rest, but browning over time. Furthermore, in the mechanical properties, the oligofructose-tagatose combination improved consistency and elastic component of orange marmalade over time. Lemon marmalades made with the new sweeteners showed greater consistency than commercial ones, but less than control, unlike what happened with mandarin orange marmalades formulated with tagatose, given place to a lower elastic component. At the sensory level, mandarin orange and lemon marmalades formulated with the highest content of tagatose showed the best evaluation in both the global acceptance and intention of buying. However, orange marmalades formulated with tagatose and oligofructose presented better scores than control. Finally, all the marmalades were microbiologically stable over storage time.

Concerning jellies two types were developed: watermelon, and citrus (orange, lemon and mandarin orange combination). For watermelon jellies, the antioxidant capacity improved in the mixture of isomaltulose and tagatose with similar values than control jelly, although lower than in commercial jelly. In contrast, in citrus jellies the antioxidant capacity decreased for the same combination at the end of the storage period.

Besides, the mechanical properties of the new watermelon jellies were very similar between them and respect to the control, being stable over the storage period.However, in citrus jellies with the highest content of isomaltulose, a greater adhesiveness was observed. Furthermore, the isomaltulose-oligofructose combination showed the highest springiness. Likewise both parameters remained over time. Regarding colour properties, samples formulated only with tagatose improved the brightness during storage, for both types of jellies. In the case of sensory analysis, watermelon and citrus jellies with equal proportion of isomaltulose and tagatose, received the best scores from consumers.

Finally, about the monitoring of marmalades during storage period, it was observed the full agreement between the results obtained by collecting data supplied with the wireless sensor network based on EDETA architecture, with the results obtained in parallel in the experimental analyses performed in the laboratory; also offering new advantages in the use of the sensor network, may be mentioned inter obtaining real time measurements automatically or even remotely.

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JUSTIFICACIÓN E INTERÉS DEL ESTUDIO

JUSTIFICACIÓN E INTERÉS DEL ESTUDIO

El desarrollo de alimentos saludables, no cariogénicos y con bajo índice glicémico suscita gran interés en la industria agroalimentaria por la preocupación creciente de la sociedad en mejorar su nutrición mediante hábitos alimenticios saludables. El consumo de frutas como cítricos y sandías ofrece múltiples propiedades nutricionales, ya que se encuentra relacionado con la prevención de enfermedades, aunque no siempre es posible consumirlas en fresco por su estacionalidad y/o falta de facilidad en su consumo. Las alternativas que ofrecen la elaboración de mermeladas y gelatinas, así como la deshidratación osmótica, permiten ampliar las posibilidades de consumo de la fruta, ya que mantienen en la medida de lo posible sus características nutricionales, y conjuntamente, alargan su vida útil. No obstante, supone la incorporación de sacarosa en su formulación, dando lugar a productos con un alto potencial cariogénico y aporte calórico elevado, entre otros aspectos negativos, relacionados con la obesidad, la diabetes, las caries, etc.

Considerando todo lo expuesto anteriormente, en esta tesis se estudió la viabilidad de la sustitución de sacarosa, en la formulación de mermeladas, gelatina y fruta osmodeshidratada, por edulcorantes naturales como son tagatosa, isomaltulosa, stevia y oligofructosa, los cuales se pueden encontrar actualmente en el mercado. Se han evaluado las características fisicoquímicas (humedad, sólidos solubles, pH, actividad de agua), capacidad antioxidante, propiedades ópticas y reológicas. Asímismo, se realizó un seguimiento microbiológico durante los respectivos períodos de almacenamiento y se realizaron análisis sensoriales de las muestras de mermeladas y gelatinas. Además, se observó la viabilidad de la sustitución de pectina por agar-agar en la formulación de mermeladas como agente gelificante, ya que actualmente esta propuesta no se encuentra en el mercado.

A su vez, se llevó a cabo la monitorización mediante una red de sensores inalámbricos, de una serie de parámetros (temperatura y humedad) en mermeladas a lo largo de su periodo de almacenamiento, aportando ventajas a los métodos tradicionales.

I. INTRODUCCIÓN

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I.1. Los beneficios de la fruta

Las frutas son alimentos con una gran cantidad de nutrientes y sustancias naturales que proporcionan numerosos beneficios para la salud humana. La Organización Mundial de la Salud recomienda que todas las personas deben comer por lo menos cinco porciones de verduras y/o frutas diferentes al día (OMS, 2010); de este modo se produce un aporte natural de los requerimientos diarios de vitaminas y minerales.

Por un lado, las frutas carecen de grasas (excepto los frutos secos, olivas, aguacates y cocos que aportan aceites beneficiosos para el organismo), y a su vez ofrecen un gran contenido en vitaminas antioxidantes naturales, como la vitamina C, que es la vitamina predominante en las frutas. Esta vitamina, no es sintetizada por el organismo, y debe ser proporcionada en la alimentación. De entre las frutas, los cítricos presentan un gran contenido de esta vitamina.

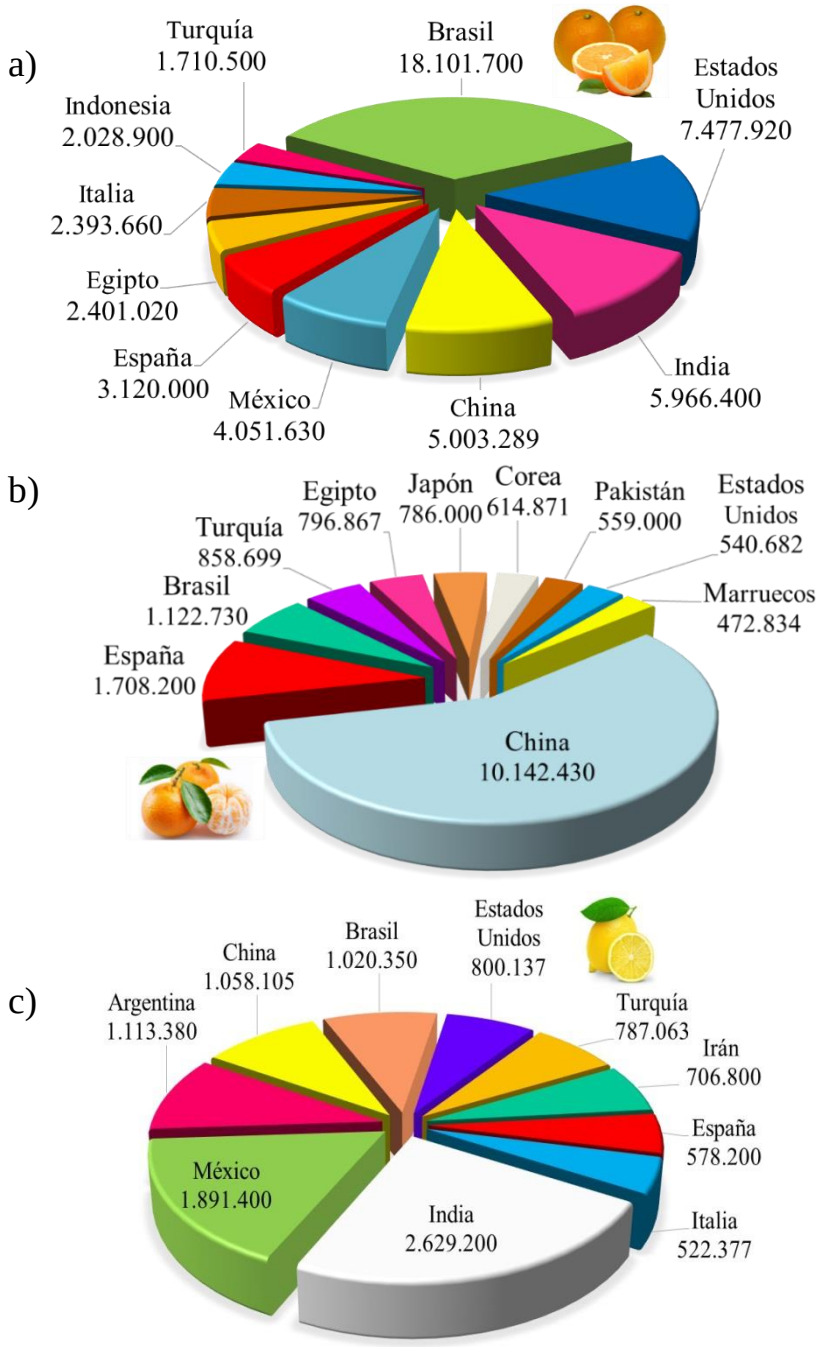
Por otro lado, las frutas proporcionan una rápida hidratación del organismo y ayudan al correcto funcionamiento del aparato digestivo por sus cualidades diuréticas y depuradoras del organismo y a su vez, por la presencia de fibras solubles de origen vegetal. El aporte hídrico viene determinado por el principal componente de la fruta, el agua, generalmente con más del 90%. En el caso de la sandía, el agua representa un 95% de su composición nutritiva.

I.1.1. El Sector de los Cítricos

Los cítricos son frutos estacionales, su producción en España comienza en noviembre y culmina en mayo.

Forman parte de este grupo de frutas: naranjas (*Citrus sinensis*), mandarinas (Citrus *reticulata*), limones (*Citrus limón),* pomelos (*Citrus paradisi)* y limas (*Citrus aurantifolia),* entre otros. Poseen un alto valor nutricional, por su alto contenido en fibra, vitaminas, minerales y ácido ascórbico (vitamina C), así como gran cantidad de antioxidantes como carotenoides, flavonoides y compuesto fenólicos, considerados altamente beneficiosos para la salud humana (Álvarez *et al.,* 2014; Navarro *et al.,* 2011). De igual modo, es recomendable su consumo para curar enfermedades, entre ellas, el escorbuto (carencia de vitamina C), remedio muy conocido por los marineros y hombres de la mar desde el siglo XVII, los cuales navegaban largas travesías sin pisar tierra firme.

En referencia a la producción de cítricos, a nivel mundial, España ocupa el sexto lugar en la producción de naranjas, el segundo en la producción de mandarinas y el noveno lugar en la producción de limones, con una producción total anual en cítricos superior a 5 millones de toneladas durante la última década. Sin embargo, es el mayor productor de cítricos de la Unión Europea. En la Figura I.1., se representan los diez países con mayor producción en cítricos a nivel mundial (FAOSTAT, 2013). A nivel nacional, la Comunidad Valenciana es la región citrícola por excelencia, posee una superficie cultivada de 182.000 ha, que corresponden con un 60% del total nacional y una producción anual de más 3.5 millones de toneladas, lo que representa más del 60% de la producción nacional (CAPAA, 2014). En cuanto a la exportación de cítricos, España es el principal exportador de cítricos a nivel mundial, seguido de Sudáfrica, Turquía, China y EE.UU. En consecuencia, es el primer país exportador a nivel europeo (Figura I.2.) (DATACOMEX, 2014).



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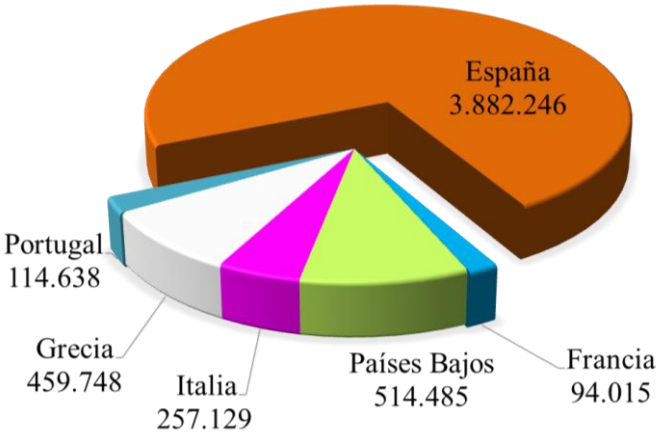

Figura I.2. Principales países exportadores de cítricos a nivel europeo (toneladas) (DATACOMEX, 2014).

Asimismo, a nivel nacional, la Comunidad Valenciana, es la primera región española exportadora de cítricos ya que aporta un 73% del total nacional. De hecho, en el año 2010, se creó la Indicación Geográfica Protegida (IGP) "Cítricos Valencianos" y su consejo regulador que se encarga de proteger e identificar a los mismos (CAPAA, 2010). Destacan las mandarinas con un 52% y las naranjas con un 40% del total de las exportaciones citrícolas valencianas (Tabla I.1.), (IVACE, 2014).

Tabla I.1. Volumen exportación citrícola de la Comunidad Valenciana y su porcentaje respecto al total nacional (% S/T), en el año 2013 (millones de euros) (IVACE, 2014).

PRODUCTO	**EXPORTACIÓN**	**% S/T**
MANDARINAS	1.100	52
NARANJAS	848	40
LIMONES	138	7
POMELOS	16	1
RESTO CÍTRICOS	1	0
TOTAL	**2.104**	**100**

En la Tabla I.2., se muestran los países a los que fueron destinadas las exportaciones citrícolas en el año 2013. Como puede observarse, Alemania es el primer destino, seguido de Francia, Países Bajos y Reino Unido que representan un 61% de las exportaciones de cítricos valencianos.

Tabla I.2. Destinos de las exportaciones citrícolas de la Comunidad Valenciana y su porcentaje respecto al total nacional (% S/T), en 2013 (millones de euros) (IVACE, 2014).

PAÍS	**EXPORTACIÓN**	**% S/T**
ALEMANIA	515	24
FRANCIA	476	23
PAÍSES BAJOS	151	7
REINO UNIDO	144	7
POLONIA	130	6
ITALIA	119	6
BÉLGICA	81	4
SUECIA	55	3
REPÚBLICA CHECA	54	3
ESTADOS UNIDOS	47	2
SUIZA	42	2
RUSIA	38	2
NORUEGA	32	2
AUSTRIA	31	1
SUBTOTAL	1.915	91
TOTAL	**2.104**	**100**

Por otro lado, los excedentes de producción citrícola, que en el caso de la Comunidad Valenciana se sitúan en torno a 15.000 toneladas de cítricos (CAPAA, 2014), ofrecen múltiples posibilidades como es el caso del abastecimiento alimentario en explotaciones ganaderas, donde la dieta del ganado se ve enriquecida por el gran aporte nutricional de los cítricos como son las vitaminas, minerales, fibra, etc. Asímismo, la industria alimentaria transforma los excedentes en zumos, néctares y mermeladas. En cuanto al sector de la cosmética, también son muy apreciados los aceites esenciales que se encuentran en los cítricos para la fabricación de cremas y perfumes, entre otros (Bakkali *et al.*, 2008).

Últimamente, debido al efecto del cambio climático y a la concienciación por parte de la sociedad frente al sector de los hidrocarburos, se está promoviendo el estudio del uso de excedentes cítricos como biocombustible (Conesa *et al.*, 2013).

I.1.2. Sector de la Sandía

La sandía (*Citrullus vulgaris*) es una fruta de verano que proporciona múltiples beneficios saludables. Pertenece a la familia de las cucurbitáceas y es originaria de África. Posee un alto contenido en agua. Por esa razón su pulpa es granulosa y acuosa, con un sabor dulce por la presencia de fructosa y un color rojizo debido a la elevada cantidad de carotenoides presentes en la pulpa y que posteriormente serán transformados por el organismo en vitamina A. Además, posee gran cantidad de licopeno, un antioxidante de acción preventiva frente a tumores, infecciones del aparato respiratorio y que actúa como defensor del sistema inmunológico (Hong *et al.*, 2015).

La composición nutricional de la sandía muestra un bajo contenido en calorías por su bajo porcentaje en hidratos de carbono, pero es rica en vitaminas, minerales y enzimas necesarias para el buen desarrollo del organismo. Además, presenta múltiples propiedades, como

por ejemplo, la función de las antitoxinas del jugo de sandía que ayuda a limpiar la sangre y los tejidos de impurezas. También, es recomendable para enfermos de riñones y vías urinarias, hipertensión y obesos por su acción diurética. Además, es laxante por su contenido en fibra que acelera el tránsito intestinal y por tanto recomendable su consumo en pacientes de gota, artritis, reuma y ciática (Hong *et al.*, 2015).

En cuanto a la producción mundial de sandías, España ocupa el decimosegundo puesto (FAOSTAT, 2013). En la Figura I.3., se muestran los principales países productores a nivel mundial.

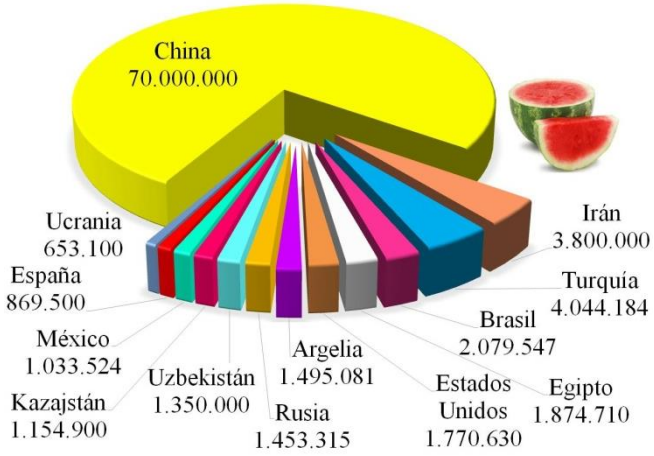

Figura I.3. Principales países productores de sandía a nivel mundial (toneladas) (FAOSTAT, 2013).

Sin embargo, España es el primer país europeo con una producción total anual superior a 869.500 toneladas, y una superficie total de 17.900 hectáreas, como puede observarse en la Tabla I.3., lo que supone unos ingresos de 3.5 millones de euros (FAOSTAT, 2013). La Comunidad Autónoma que mayor producción tiene es Andalucía (3.793 ha), seguida de la Región de Murcia (3.054 ha) y la Comunidad Valenciana, con una producción de 42.923 toneladas y con una superficie total de 2.136 hectáreas (CAPAA, 2014; MAGRAMA, 2012,2014).

Tabla I.3. Principales países productores europeos, porcentaje respecto al total europeo (% S/T), y superficie de cultivo (FAOSTAT, 2013).

PAÍS	PRODUCCIÓN (t)	% S/T	SUPERFÍCIE (ha)
ESPAÑA	869.500	31,26	17900
RUMANIA	574.187	20,64	26018
GRECIA	620.600	22,31	13000
ITALIA	392.527	14,11	11557
HUNGRÍA	183.900	6,61	6300
BULGARIA	64.247	2,31	3049
CROACIA	30.955	1,11	836
SUBTOTAL	45.365	1,63	1753
TOTAL	**2.781.281**	**100**	**80.413**

Respecto al comercio de exportación de sandía, España es el principal exportador a nivel europeo (Figura I.4). En coherencia con el volumen de producción, la Comunidad Valenciana ocupa el tercer lugar en volumen de exportación con un 24.05% de la cuota nacional, precedido por Andalucía (45.20%) y Murcia (26.68%) (MAGRAMA, 2014).

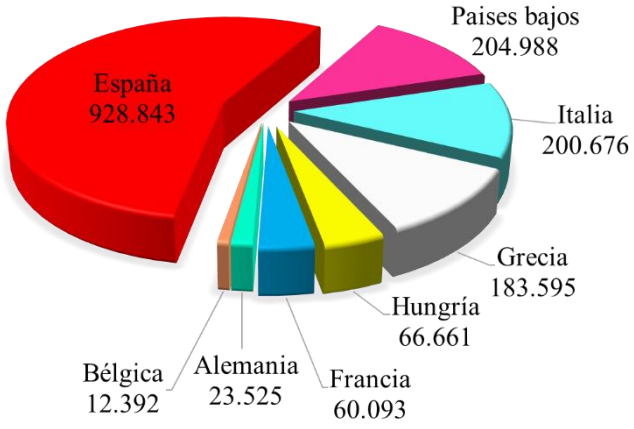

Figura I.4. Principales países exportadores a nivel europeo de sandía (toneladas) (DATACOMEX, 2014).

La sandía es un fruto estacional con carácter estival, con una gran demanda por sus propiedades refrescantes. Cuando se produce un excedente de producción, los productores optan por la venta a la industria agroalimentaria que realiza su procesado en zumos y licuados extraídos de la pulpa de la fruta. Por este motivo, las cáscaras de sandía a priori son desechadas, aunque actualmente se están realizando estudios para extraer un aminoácido llamado citrulina, (que se encuentra en la parte blanca de la cáscara), con numerosas propiedades entre ellas, paliar enfermedades cardiovasculares y dolencias musculares en deportistas (Bahri *et al.,* 2013).

I.1.3. Alternativas de consumo de frutas en fresco

Los consumidores demandan actualmente alimentos con excelentes propiedades de textura, sabor y aroma, pero sin olvidar las características funcionales como un elevado contenido en fibra, vitaminas, minerales, compuestos antioxidantes, unido a un bajo índice glicémico y no cariogénicos, como es el caso de las frutas, las cuales poseen un alto valor nutricional. Sin embargo, su consumo en fresco a veces puede resultar difícil por su carácter estacional y por tanto su perecibilidad y en determinados casos por su tamaño y necesidad de pelado, lo que dificulta su consumo fuera del hogar. Por ello el procesado de la fruta se presenta como alternativa al consumo en fresco, consiguiendo extender su vida útil pero manteniendo algunas de las características del producto fresco. De entre los productos elaborados a base de frutas se encuentran las mermeladas, gelatinas y las frutas deshidratadas osmóticamente.

La deshidratación osmótica es una técnica utilizada ampliamente por la industria para la conservación y diseño de nuevos productos a partir de frutas (Earle, 1988; Peinado *et al.,* 2012). La técnica se basa en reducir la actividad de agua del producto, inhibiendo así el crecimiento microbiano y la actividad enzimática consiguiendo alargar la

vida útil del alimento. La operación consiste en la inmersión de un alimento (entero o segmentado) en una disolución acuosa de elevada concentración en solutos (disolución osmótica) con la finalidad de extraer el agua del alimento (Pointing, 1973; Peinado *et al*., 2012).

Las mermeladas son productos tradicionales, elaborados con fruta troceada, sacarosa y pectina de fruta (agente gelificante) sometidos a un proceso de cocción hasta obtener una textura semifluida o espesa, tal y como se especifica en el R.D. 863/2003 de 4 de julio, por el que se aprueba la Norma de calidad para la elaboración, comercialización y venta de confituras, jaleas, «marmalades» de frutas y crema de castañas. En el proceso se concentra la fruta con sacarosa hasta obtener una cantidad de sólidos solubles en un rango entre 40 y 60ºBrix. Con pH entre 2.8-3.8 se deprime el crecimiento microbiano, que junto con la alta concentración de sólidos solubles hacen posible que el producto sea estable en el tiempo y se conserven ciertas propiedades nutricionales. Según se explicita en el R.D. 863/2003, «Marmalade» es la mezcla, con la consistencia gelificada apropiada, de agua, de azúcares y de uno o varios de los productos siguientes, obtenidos a partir de cítricos: pulpa, puré, zumo, extractos acuosos y pieles. Por este motivo, reciben la denominación exclusiva de "Marmalades", que las distingue del resto de mermeladas elaboradas con otras frutas. En este mismo documento, se expone que la sandía no es una fruta adecuada para la elaboración de confituras y/o mermeladas de calidad «extra», si bien se puede emplear en la elaboración de un postre gelificado (refrigerado), como son las gelatinas.

Los postres de gelatina están elaborados en base a gelatina, que proviene del hidrolizado de colágeno, habitualmente animal (vacuno y cerdo), aunque recientemente se están elaborando gelatinas procedentes de pescado (Karim y Bhat, 2009). El proceso de elaboración de forma tradicional se realiza mediante el mezclado de zumo de fruta, agua, gelatina y sacarosa.

Es necesario la disolución previa de la gelatina en agua, antes de la cocción rápida hasta alcanzar la temperatura de gelificación (Edwards, 2002). A su vez en el proceso de elaboración suelen añadirse colorantes, por lo que unido a su estructura de gel, ofrece un producto muy atractivo para el consumidor. Así mismo, presenta un precio muy asequible y es consumido por un amplio sector de la población, por ser fuente de proteínas.

Como se ha podido observar, de forma habitual en la elaboración de estos productos se utiliza sacarosa, como el azúcar por excelencia, por su textura y sabor característicos. Sin embargo presenta ciertas connotaciones negativas asociadas a su consumo (cariogénesis, aporte calórico elevado, aumento en el índice glicémico, etc…) y por este motivo en algunos productos es reemplazado por edulcorantes naturales o sintéticos.

I.2. Las alternativas al azúcar

Como se ha mencionado anteriormente, el consumo de sacarosa implica ciertos inconvenientes asociados a la salud. Por este motivo, la Organización Mundial de la Salud (OMS), establece nuevas directrices orientadas hacia una reducción del consumo de azúcares simples hasta el 5% de la ingesta calórica total diaria para un adulto con un índice de masa corporal normal (OMS, 2014). Con este objetivo, se pretende reducir las enfermedades como la diabetes tipo II, la obesidad, la caries dental, así como las que afectan a las vías coronarias, que se presentan en niños y adultos.

Este colectivo necesita alternativas a la sacarosa en su alimentación, como la utilización de edulcorantes acalóricos. Por ejemplo para diabéticos, estos edulcorantes no elevan los niveles sanguíneos de glucosa ni provocan la liberación de insulina por el páncreas, de la misma

forma que no aportan energía y se favorece el mantenimiento de su peso corporal (Lina *et al*., 2002; Lu *et al*., 2008).

I.2.1. Edulcorantes clásicos

Los edulcorantes son todas aquellas sustancias, naturales o de síntesis, con sabor dulce y por tanto, con la capacidad de endulzar los alimentos. En relación a los edulcorantes de síntesis o sintéticos se clasifican en dos grupos:

- Edulcorantes de volumen: Pertenecen a este grupo los azúcares y los polialcoholes, siendo el aporte calórico mucho mayor en los azúcares.
- Edulcorantes intensivos: Son acalóricos y su consumo no proporciona energía, pertenecen a este grupo la sacarina, el aspartame, la sucralosa, etc.

Para establecer el poder edulcorante (PE) se toma como referencia a la sacarosa, con un valor de uno (ó 100) (Edwards, 2002). En cuanto al índice glicémico (IG), se evalúa el impacto de la ingesta de glucosa, siendo 100 el valor de referencia (Romero *et al.*, 2002; Wolever, 2006). En consecuencia, el Reglamento Europeo nº 1338/2008 (CE, 2008), expone una lista de edulcorantes permitidos en alimentación. En ella se encuentran aquellos edulcorantes que pueden ocupar el lugar de la sacarosa en la elaboración de productos alimenticios. En la Tabla I.4., se muestran los valores índice glicémico (IG) y poder edulcorante (PE), así como las calorías por gramo que aportan, para los edulcorantes de volumen más importantes.

Tabla I.4. Valores de poder edulcorante, índice glicémico y las calorías por gramo que aportan de edulcorantes de volumen con mayor relevancia (Chattopadhyay *et al.*, 2014).

COMPUESTO	PODER EDULCORANTE (PE)	Cal/g	ÍNDICE GLICÉMICO (IG) (%)
Fructosa	1.5-1.8	4.0	19-23
Azúcar invertido	1.3	4.0	30
Sacarosa	1.0	4.0	61-65
Xilitol	1.0	3.0	7-8
Maltitol	0.5-0.9	3.0	35-52
Glucosa	0.5	4.0	100
Sorbitol	0.6	2.6	9
Manitol	0.5-0.72	1.6	0
Maltosa	0.4	4.0	105
Lactitol	0.35-0.4	2.4	6
Lactosa	0.2-0.4	4.0	46
Galactosa	0.3	4.0	30

I.2.1.1. Edulcorantes de volumen

Los principales edulcorantes de volumen se clasifican en:

A) Monosacáridos

En este grupo se encuentran fructosa, galactosa y glucosa (Figura I.5.) La fructosa se obtiene de forma natural de la achicoria (*Cichorium intybus*) o de las alcachofas de Jerusalén (*Helianthus tuberosus*), aunque también se encuentra en la miel y en algunos jugos de frutas. Puede obtenerse a partir de jarabe de glucosa. La fructosa también se conoce como el azúcar de frutas o levulosa. Es el más dulce de los carbohidratos. Tiene casi el doble de poder edulcorante que el azúcar de mesa (sacarosa). Posee una propiedad que la hace indispensable para su

uso en productos para diabéticos, puesto que tiene un metabolismo independiente de la insulina y en consecuencia muestra un IG entre 19-23 (Tabla I.4.). Por otro lado, se afirma que la fructosa en pequeñas cantidades suaviza el sabor de los edulcorantes intensivos cuando estos se utilizan en productos denominados "sin azúcar", puesto que tiene un PE entre 1.5-1.8, mayor que el de la sacarosa (Tabla I.4.). Presenta, a su vez, la característica de ser muy soluble, siendo altamente higroscópica y por este motivo se presenta en forma de jarabe (Edwards, 2002; Godshall, 2007). Sin embargo, recientemente se ha descubierto que la ingesta prolongada de fructosa en cantidades elevadas provoca un aumento en la resistencia del organismo a la insulina (Botanical, 2012). Este inconveniente estaría relacionado con el origen de la fructosa ya que la proveniente del jarabe de maíz de alta fructosa, de menor precio y calidad, está asociado con una mayor inicidencia de obesidad (Periche *et al*., 2015).

En cuanto a la galactosa, se encuentra presente en la lactosa. Es precisamente en las glándulas mamarias donde este compuesto se sintetiza para formar parte de la leche materna. Tras ser absorbida, se transforma en glucosa en el hígado y por tanto, también constituye una fuente energética (4 calorías/g) y su poder edulcorante es similar al de la lactosa (Tabla I.4) (Wolever, 2006; Godshall, 2007).

La glucosa o dextrosa, es el azúcar más importante dado su abundancia. Se encuentra en frutas dulces, en la miel, el jarabe de maíz y las verduras entre otros. Su estructura molecular, junto a la de la fructosa y a la galactosa se muestra en la figura I.5. Es conocido como "el azúcar de la sangre". La reserva más importante de glucosa en el organismo se encuentra en el hígado y los músculos, en forma de glucógeno, por lo que es importante incluir alimentos que contengan carbohidratos, que el organismo transforma en glucosa, para un adecuado funcionamiento de nuestro cuerpo. El IG, cuantifica el nivel de glucemia (concentración de glucosa) en sangre producido por la ingesta de alimentos. Por ésta razón,

la glucosa es el compuesto que se toma como referencia para cuantificar el índice glicémico, presentando un IG de 100 (Tabla I.4) (Wolever, 2006; Godshall, 2007).

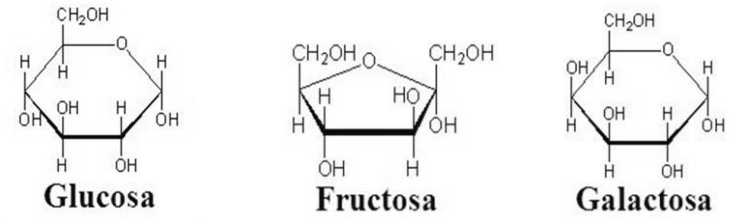

Figura I.5. Estructuras moleculares de fructosa, galactosa y glucosa.

B) Disacáridos

En este grupo se encuentran sacarosa, lactosa y maltosa, y por este orden son los tres azúcares de mayor acción cariogénica (Figura I.6.). Numerosos estudios epidemiológicos han demostrado la asociación entre caries y sacarosa. Esto es debido, a que los azúcares consumidos con la dieta constituyen el sustrato de la microflora bucal dando inicio al proceso de cariogénesis. Además, un consumo elevado también puede desencadenar problemas de obesidad e incluso hipertrigliceridemia (Blanco, 2002).

La sacarosa se extrae tanto de la remolacha azucarera *(Beta vulgaris)* como de la caña de azúcar *(Saccharum officinarum).* El azúcar extraído de la remolacha debe ser purificado, para obtener un sabor agradable, al contrario que ocurre con el azúcar obtenido de la caña de azúcar, que sin ser refinado y blanqueado, es decir, como azúcar moreno, es altamente apreciado por su sabor. Se trata de un disacárido no reductor constituido por dos monosacáridos reductores, glucosa y fructosa, unidos por el enlace glucosídico α-1,2, también conocido como enlace O-glucosídico (Figura I.6.). Es el azúcar más utilizado por los consumidores, presenta numerosas propiedades sensoriales y físico-químicas, puesto que proporciona la estructura y la textura adecuada de

muchos alimentos tradicionales. Tiene un IG entre 61-65 y por ser la sustancia de referencia presenta un PE de 1 (Tabla I.4.) (Edwards, 2002; Godshall, 2007).

En cuanto a la lactosa, se trata del principal azúcar presente en la leche (Figura I.6.). Algunos individuos son incapaces de metabolizar la lactosa, por la carencia de la enzima lactasa, por este motivo se les identifica como "Intolerantes a la lactosa". Actualmente las industrias lecheras presentan ciertas alternativas como productos sin lactosa, donde se ha hidrolizado la lactosa para obtener sus monosacáridos constituyentes (glucosa y galactosa). Presenta un IG de 16 y un PE de 9.2 (Tabla I.4.) (Edwards, 2002; Godshall, 2007).

En referencia a la maltosa, se trata de un disacárido formado por dos unidades de glucosa (Figura I.6.). Se produce por la hidrólisis del almidón, pero también se encuentra en los granos de cereal en germinación. Es utilizada como edulcorante suave en alimentos, debido a su bajo poder edulcorante (Tabla I.4.) (Damodaran, 2008).

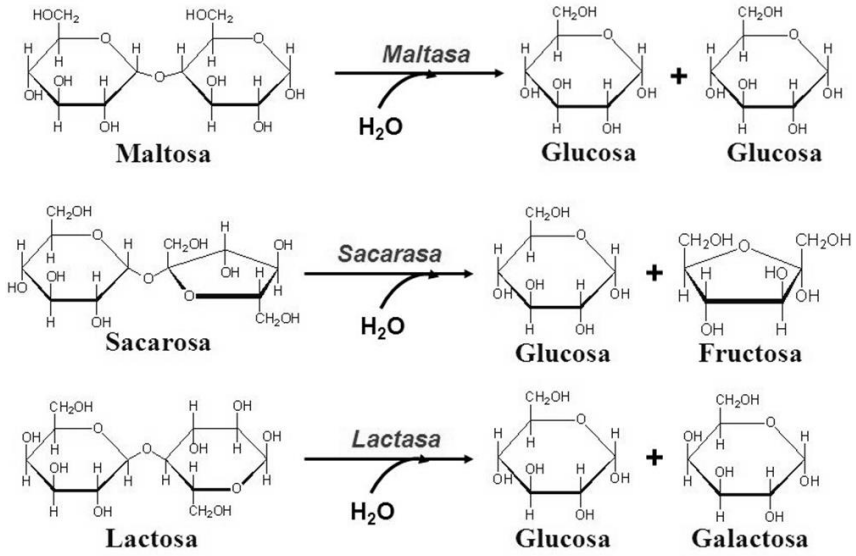

Figura I.6. Estructuras moleculares de maltosa, sacarosa, y lactosa.

C) Jarabe de glucosa

Se obtiene por hidrólisis ácida a partir del almidón de maíz, patata o trigo y su ingrediente principal es la maltosa. El más utilizado en la industria alimentaria es el jarabe con alto contenido en fructosa (42% aproximadamente, aunque puede llegar a cantidades superiores, del 55% y hasta del 90%), lo que obliga a que tras la hidrólisis del almidón, se lleve a cabo una isomerización enzimática, que asegure la transformación de una parte de glucosa en fructosa (Edwards, 2002).

D) Azúcar invertido

Es una solución acuosa de fructosa en proporción variable y glucosa. Se obtiene a partir de la sacarosa por hidrólisis ácida o enzimática. Su empleo mayoritario es en la fabricación de alimentos manufacturados. Por el contrario, el uso de éste compuesto ha disminuido porque el jarabe de glucosa es más barato y presenta mejores propiedades, aunque en su favor se comenta que los residuos que contienen azúcar pueden tratarse para producir azúcar invertido. Como puede observarse en la tabla I.4, muestra un poder edulcorante superior a la sacarosa (Edwards, 2002).

E) Polialcoholes

Constituyen un grupo bastante homogéneo de edulcorantes que poseen en común algunas características. Se encuentran presentes en distintos alimentos pero su extracción no es rentable, por lo que se obtienen por hidrogenación, a partir de diferentes azúcares. Poseen un poder edulcorante inferior, generalmente, al de la sacarosa, siendo muy empleados en pastelería, heladería y en la industria del chicle. Su valor energético es aproximadamente la mitad del de la sacarosa (Edwards, 2002). Además, debido a su menor absorción intestinal hace que se comporten como laxantes osmóticos. Son utilizados también por la microbiota presente en el colon, lo que explica la flatulencia que

provocan, y por tanto limitan su utilización especialmente en niños. No obstante, presentan la característica de ser no cariogénicos y algunos de ellos además de cómo edulcorantes, también son utilizados por la industria alimentaria como diluyentes, humectantes y estabilizantes (Edwars, 2002; Chattopadhyay *et al.,* 2014). Los polialcoholes más importantes son:

- Sorbitol: se encuentra de forma natural en las plantas, como las algas rojas, y también en frutas como peras, manzanas, cerezas y melocotones. Procede de la glucosa, por este motivo, posee un poder edulcorante similar (Tabla I.4.; Figura I.7.). Se utiliza en la industria alimentaria como humectante, agente de carga y/o edulcorante en caramelos y chicles, lo que proporciona sensación de frescor al producto. Se absorbe finalmente en el tubo digestivo, y en consecuencia tiene el mismo valor energético que la glucosa (Edwards, 2002; O'Donnell y Kearsley, 2012).
- Maltitol: se utiliza como sustitutivo de la sacarosa en diversas formulaciones debido a su alto poder edulcorante entorno al 90% y características físico-químicas similares (Tabla I.4.; Figura I.7.) (Edwards, 2002; O'Donnell y Kearsley, 2012).
- Lactitol: se produce por hidrogenación del azúcar de la leche o lactosa. Es poco dulce, como puede observarse en la Tabla I.4., lo que puede suponer una ventaja en determinadas aplicaciones. En la Figura I.7., se muestra su estructura molecular (Edwards, 2002; O'Donnell y Kearsley, 2012).
- Manitol: proporciona casi la mitad de las calorías por gramo que la glucosa (50% PE), como puede observarse en la Tabla I.4. Como inconveniente muestra una mala absorción en el tubo digestivo. Proviene de la manosa, que se encuentra en algas y frutas y se utiliza como agente de carga o espolvoreado en chicles (Edwards, 2002; O'Donnell y Kearsley, 2012).
- Isomaltitol: la mezcla de los isómeros resultantes de la hidrogenación de la isomaltulosa que se obtiene por tratamiento

enzimático de la sacarosa o azúcar común (Figura I.7.). Se utiliza como agente de carga en productos, como los caramelos, en que ha sustituido al azúcar. Su característica principal es que es poco higroscópico, aunque tiene un precio muy elevado lo que hace que se encarezca el producto final (Edwards, 2002; O'Donnell y Kearsley, 2012).

- Eritritol: posee un efecto laxante menor al resto, y un rango de valores de IG entre 7-13 y un PE=1. En la Figura I.7., puede observarse su estructura química. Su uso está muy extendido en la industria de chicles y dentífricos por su sensación refrescante (Edwards, 2002; O'Donnell y Kearsley, 2012).

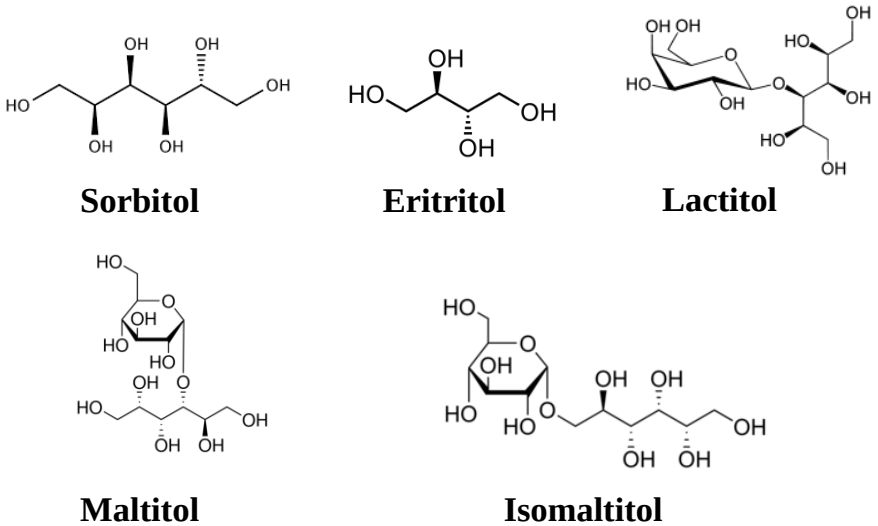

Figura I.7. Estructuras moleculares de sorbitol, maltitol, lactitol, eritritol e isomaltitol.

I.2.1.2. Edulcorantes intensivos

Los edulcorantes intensivos, se designan así por su alto poder edulcorante por lo que su consumo en pequeñas cantidades es suficiente. No obstante, la duración de la impregnación de las papilas gustativas excede a la observada con los edulcorantes naturales, es el denominado efecto "Lingering" o de persistencia. Se pueden considerar acalóricos

porque presentan un bajo contenido en calorías o incluso dependiendo del tipo de edulcorante, puede ser hasta nulo como ocurre con la sacarina. Por este motivo son utilizados como sustitutos del azúcar en las dietas de control de peso (Edwards, 2002; O'Donnell y Kearsley, 2012). En la Tabla I.5., se encuentran los valores de poder edulcorante para los edulcorantes intensivos más destacados, así como su contenido en calorías por gramo.

Tabla I.5. Poder edulcorante de los edulcorantes intensivos más destacados.

COMPUESTO	**PODER EDULCORANTE (PE) (%)**
Sacarina	300-500
Aspartame	160-220
Acesulfame K	200
Ciclamato	30-40
Sucralosa	600

A continuación se detallan las características de los edulcorantes intensivos más destacados:

La sacarina, es la o-sulfimida benzoico, posee un elevado poder edulcorante, pero puede dejar un sabor residual amargo, que se puede evitar si se asocia con ciclamato. No se metaboliza y se excreta por vía renal. Como puede observarse en la Tabla I.5., presenta un poder edulcorante en torno a 300-500. Presenta un comportamiento muy estable, y no aporta ninguna caloría, además no es cariogénico. Su uso está muy extendido en la industria alimentaria. Está aprobado por el Comité de Expertos en Aditivos Alimentarios de la FAO/OMS. La Ingesta Diaria Admisible (IDA) es de 5 mg por kilogramo de peso corporal por día (Weihrauch y Diehl, 2004).

En cuanto al Ciclamato, se trata del ciclohexil sulfamato sódico. Posee un sabor agradablemente dulce (Tabla I.5.) empleándose normalmente asociado a la sacarina en una relación 1:10, una relación sinérgica con la que se obtiene un mayor poder edulcorante y un menor sabor residual amargo. La Ingesta Diaria Admisible (IDA) es de 7 mg por kilogramo de peso corporal por día. No debe utilizarse en la elaboración mermeladas, puesto que no inhibe la proliferación de gérmenes, al igual que pasa con la sacarina y en contraposición a la sacarosa (Chattopadhyay *et al.*, 2014).

El aspartamo, es el éster metílico del aspartil fenilalanina, capaz de provocar un fuerte sabor dulce pero sin ser residual (Tabla I.5.), presentando además una capacidad potenciadora del efecto de otros edulcorantes. Su uso no es recomendable en procesos con altas temperaturas ni en medios ácidos, como en zumos, porque la descomposición de la molécula libera fenilalanina, lo que es desaconsejable para pacientes fenilcetonúricos y debe hacerse constar su presencia en los envases. Reconocida por la FDA desde 1995 (Edwards, 2002; Chattopadhyay *et al.*, 2014).

En relación al acesulfamo K, es un edulcorante que pertenece a la familia de los dióxidos de oxatiozinonas, presenta similitudes estructurales con la sacarina, y deja un sabor residual amargo también como la sacarina. Estable a elevadas temperaturas y presenta un efecto sinérgico con otros edulcorantes intensos. Posee un poder edulcorante de 200 (Tabla I.5.) (Chattopadhyay *et al.*, 2014).

La sucralosa o dulcina, se obtiene a partir de la sacarosa. Presenta gran estabilidad en medio ácido, lo que la hace idónea para su uso en zumos de frutas. Es soluble y fácil de manipular. Como puede observarse en la Tabla I.5., es 600 veces más dulce que el azúcar, pero no es cariogénico y se utiliza en una amplia gama de productos, bebidas refrescantes, mermeladas, frutas enlatadas, lácteos, postres, helados, etc.

Asimismo, presenta una I.D.A. de 15 mg por kilogramo de peso corporal por día (Edwards, 2002; Chattopadhyay *et al.*, 2014).

Sin embargo, estos edulcorante intensivos presentan en la actualidad mucha controversia ya que se está cuestionando su relación con el desarrollo de distintos cánceres y otras enfermedades (Weihrauch y Diehl, 2004; Soffritti *et al*., 2006; Renwick y Nordmann, 2007). Por su parte, la autoridad europea en seguridad alimentaria (EFSA) ha ratificado la seguridad de todos los edulcorantes que se encuentran en la lista positiva de aditivos, así como las dosis recomendadas, aludiendo que no existen evidencias científicas de manifiesten la peligrosidad para el consumo humano por parte de los mismos. Sin embargo, se encuentran en continua evaluación por parte de la Comisión Europea.

Por esta razón, la industria alimentaria ofrece edulcorantes obtenidos de forma natural, metabolizables por el organismo y con ventajas nutricionales y funcionales como la isomaltulosa, la oligofructosa, la stevia, la tagatosa, etc. Consecuentemente, el reto es comprobar su viabilidad en la reformulación de productos tradicionales para mantener o incluso mejorar sus propiedades tecnológicas.

I.2.2. Nuevos edulcorantes

Según la Organización Mundial de la Salud (OMS), se recomienda una reducción del consumo de azúcares y otros carbohidratos de absorción rápida como la sacarosa, así como aumentar la actividad física diaria para frenar la tendencia a la obesidad y a la diabetes del tipo 2 (Lu *et al.*, 2008). Además, dada la problemática de los sustitutos de los azúcares actuales, es importante encontrar alternativas más saludables. A continuación se hará una descripción de los cuatro nuevos edulcorantes estudiados en esta tesis.

La Oligofructosa, es un oligosacárido derivado de la fructosa. Actúa como fibra dietética soluble de carácter prebiótico que regula el tránsito gastrointestinal, favoreciendo el crecimiento de la microbiota gastrointestinal beneficiosa y la absorción del calcio. Además, produce una reducción de los niveles de colesterol y los niveles de azúcar en sangre (Chacón-Villalobos, 2006; Ledur *et al.*, 2013). Su capacidad edulcorante se encuentra aproximadamente entre un 30% y un 60% frente a la sacarosa y es fácilmente hidrolizado por acción de ácidos o enzimas (Coussement, 1999). Por otra parte, posee un bajo contenido calórico, lo que le confiere múltiples beneficios saludables, así como un alto nivel de solubilidad y propiedades tecnológicas muy relacionadas con los de la sacarosa y los jarabes de glucosa. A menudo se suele utilizar en combinación con edulcorantes de alta intensidad y posee al mismo tiempo la ventaja de no comprometer el sabor y la textura, ofreciendo así productos nutricionalmente mejorados (Rao, 2001; Franck, 2002; O'Donnell y Kearsley, 2012).

Otro nuevo edulcorante a tener en cuenta es la Stevia. Su principal compuesto es el esteviósido (Figura I.8). Se trata de un glucósido formado por tres moléculas de glucosa unidas al alcohol (diterpénico esteviol), que está presente en las hojas de Stevia rebaudiana, planta silvestre autóctona de América del Sur (Paraguay), por este motivo, se trata de un edulcorante intensivo vegetal (Goyal, 2010). No aporta calorías. Su dulzor es 300 veces más que el azúcar (Kroyer, 2010). El Comité Mixto FAO/OMS de Expertos en Aditivos Alimentarios revisó los esteviósidos y asignó una Ingesta Diaria Aceptable temporal de 2 mg por kilogramo de peso corporal por día (JECFA, 2008; EFSA, 2011). Posee numerosas propiedades: antiinflamatorias, diuréticas, antihipertensas, antihiperglicémicas, antioxidantes, antimicrobiana (inhibe crecimiento de *Salmonella typhimurum, B. subtilis y S. aureus) y* antifúngica (Gosh, 2008; Chatsudthipong y Muanprasat, 2009; Lemus-Mondaca, *et al.*, 2012).

Por otra parte, en los últimos años está adquiriendo un creciente interés la D-Tagatosa (Figura I.8.) que es un monosacárido (cetohexosa) de origen natural, isómero de la D-galactosa, posee el aspecto de polvo cristalino blanco con un poder edulcorante del 92% respecto a la sacarosa (Oh, 2007; Taylor *et al.,* 2008; Calzada-León et al., 2013). Se encuentra de manera natural en varios alimentos, como queso y yogurt, entre otros. Se produce comercialmente a partir de la lactosa en un proceso que implica la isomerización de la lactosa a D-galactosa y aunque se extrae de la proteína de la leche, en su presentación final no contiene ninguna traza de lactosa, ni tampoco presenta gluten ni fructosa, por lo que puede ser consumida por personas con intolerancias. Presenta propiedades prebióticas lo que resulta beneficioso para el organismo y posee un índice glucémico así como un aporte calórico próximos a cero.

Es adecuada para los diabéticos porque no se metaboliza a través de la sangre, sino que pasa directamente por el aparato digestivo, y por lo tanto no afecta al nivel de glucosa. Posee propiedades tecnológicas similares a la sacarosa como aspecto y sabor. Se considera un ingrediente funcional debido a que se metaboliza parcialmente y ejerce funciones de fibra soluble, favoreciendo la proliferación de bacterias ácidolácticas. Es termoestable y puede emplearse para elaborar repostería. Previene la caries dental (Levin, 2002; Petersen-Skytte, 2006; Oh, 2007; Lu, 2008; Taylor *et al*., 2008; Donner *et al.,* 2010; Petersen-Skytte *et al.,* 2012; Calzada-León *et al.,* 2013; Chattopadhyay et al., 2014). Está reconocida como un producto seguro por la FDA (Food and Drug Administration), la FAO y la OMS (OMS, 2001). Además se encuentra dentro de los nuevos alimentos/ingredientes alimentarios autorizados por la Unión Europea, con arreglo al Reglamento Europeo nº 258/97 (CE, 2005a), con uso autorizado como "nuevo ingrediente alimentario", desde el año 2005.

En cuanto a la Isomaltulosa, es un disacárido reductor, isómero de la sacarosa (Figura I.8), presente en pequeñas cantidades en la caña de azúcar y la miel (Lina *et al*., 2002). Industrialmente se obtiene a partir de la sacarosa (*Schiweck et al.*, 1990). Posee un poder edulcorante de aproximadamente un 42% frente a la sacarosa, y cabe destacar que por su bajo índice higroscópico, proporciona estabilidad a productos como caramelos, chicles y chocolate alargando su vida útil (De Oliva-Neto *et al*., 2009). Su poder calórico es similar a la sacarosa, por lo que puede ser una fuente de energía alternativa a la misma (Lina *et al.*, 2002). Resulta muy interesante su aplicación al desarrollo de alimentos para diabéticos y para deportistas por su lenta liberación de glucosa y fructosa. Proporciona la misma cantidad de energía que el azúcar común, pero con un leve efecto sobre los niveles de azúcar en sangre y de insulina en el cuerpo humano, siendo además totalmente digerible (Lina *et al*. 2002). De acuerdo con el Reglamento Europeo nº258/97 (CE, 2005b), de la Unión Europea, se autorizó su comercialización como nuevo alimento o nuevo ingrediente alimentario en el año 2005.

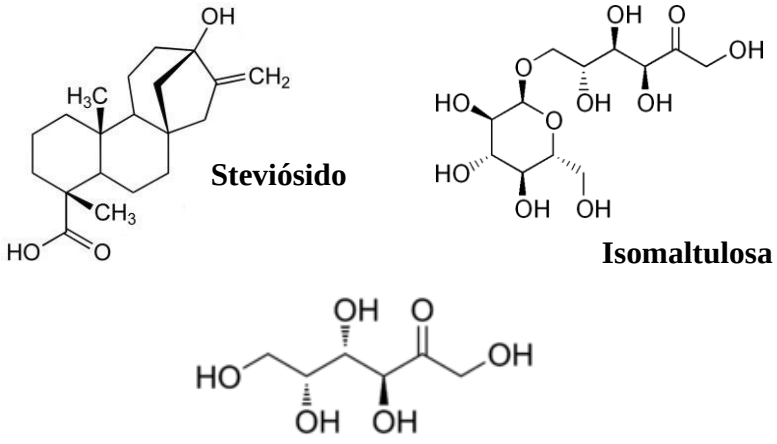

Figura I.8. Estructuras moleculares de tagatosa, isomaltulosa y steviósido de stevia.

Por todas las propiedades mencionadas, estos azúcares/edulcorantes naturales parecen ofrecer buenas expectativas como sustitutivos de los azúcares tradicionales, siendo aptos para cualquier sector de la población, incluso para aquellos que padecen diabetes, obesidad o tienen predisposición a la caries dental. (Coussement, 1999; Chacón-Villalobos, 2006).

I.3. Monitorización mediante redes de sensores inalámbricos

Las Redes Inalámbricas de Sensores (RIS), también conocidas como *Wireless Sensor Networks* (WSN), han sido identificadas como una de las tecnologías más prometedoras, por diversos motivos, entre ellos porque dan respuesta a las exigencias actuales referentes al establecimiento de redes que cubran necesidades de comunicación de forma flexible (tiempo y espacio) y autónoma (autoconfiguración e independencia de una estructura fija) (Bonastre *et al.*, 2012; Capella *et al.*, 2014). De esta forma, gracias al avance de las comunicaciones inalámbricas y las redes de sensores, la tecnología está permitiendo medir parámetros que antes no se podían medir con dispositivos de pequeño tamaño y de bajo coste energético y económico.

Por esta circunstancia se ha propiciado la aparición de redes de sensores, donde pueden colocarse nodos de sensores inalámbricos debido a su bajo coste y consumo para poder realizar la monitorización de forma continua y prolongada en el tiempo, hecho que no era posible con los anteriores dispositivos de medición manual, donde un operario realizaba las mediciones de forma puntual. Asimismo, dichos dispositivos (nodos) son capaces de obtener información del entorno y reenviarla de forma inalámbrica a un centro de coordinación lo que ofrece posibilidades inimaginables en multitud de aplicaciones (Bonastre *et al.*, 2012; Capella *et al.*, 2014). En la mayoría de las aplicaciones se pretende que los nodos no requieran mantenimiento, puesto que una vez desplegados no son recuperables. En este entorno, los mayores desafíos se encuentran en

minimizar dos factores fundamentales: coste y consumo, prolongando al máximo el tiempo de servicio.

En los últimos años se han propuesto numerosos protocolos para RIS. Sin embargo, la nueva arquitectura denominada EDETA (*Energy-efficient aDaptative hiErarchical and robusT Architecture*) se propone como la más apropiada para ésta aplicación y prometedora, enfocada principalmente a reducir el consumo de energía (Capella *et al.*, 2009). Una arquitectura establece la organización lógica de los dispositivos que forman la red, definiendo la operativa de la misma, con el objetivo de proporcionar las mejores características de comunicación, fiabilidad, flexibilidad, etc. Además, la arquitectura propuesta es escalable, auto-configurable, soporta de forma transparente múltiples sumideros, y proporciona características como tolerancia a fallos y tiempos acotados, sin degradar las prestaciones de la red (Capella *et al.*, 2014).

La principal innovación consiste en sustituir sensores de elevada complejidad, de coste elevado y limitados en número, por una infraestructura de comunicaciones con un número mayor de dispositivos sensores, más sencillos y más baratos porque cada nodo está dotado de enlaces de radio de baja potencia de tal modo que el área de cobertura es relativamente pequeña. Se obtienen de éste modo, magnitudes físicas del entorno, que además dan soporte a las comunicaciones de otros nodos como elementos de infraestructura. De esta forma se consigue economizar de forma significativa el consumo de potencia (Capella *et al.*, 2014). Los *nodos* por medio de los sensores, obtienen la información, y posteriormente, la traducen y la transcriben a los *nodos sumideros* utilizando las comunicaciones inalámbricas, éstos a su vez, reciben los datos de todos los nodos sensores de la red, los procesan y los reenvian hacia el sistema de gestión de la información. Esta información, se almacena en la base de datos, y en tiempo real por medio de internet los datos son accesibles desde un móvil (Bonastre *et al.*, 2012; Capella *et al.*, 2014).

La estructura del *nodo sensor*, se muestra en la Figura I.9., donde se puede observar el microcontrolador (interpreta los datos), la RIS (con un corto rango debido a las restricciones energéticas), una pequeña batería y el sensor, que es el que se encarga de obtener los datos que a su vez, serán procesados y transmitidos.

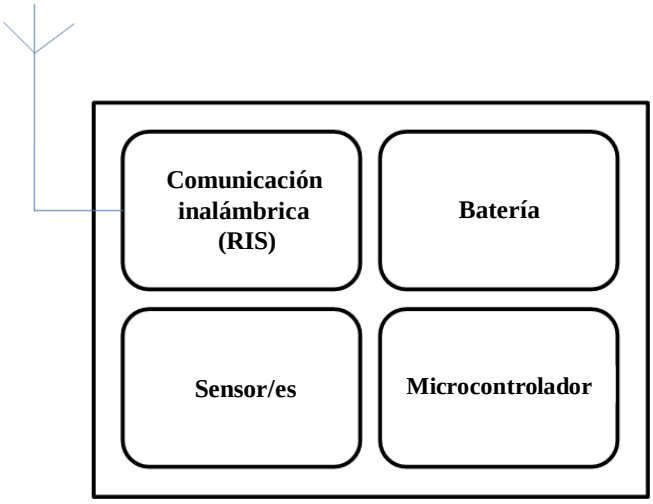

Figura I.9. Estructura del *nodo sensor* (Capella *et al.*, 2014).

La estructura del *nodo sumidero*, está basada en un microcontrolador, como se muestra en la figura I.10. Además de recibir la información, la procesa y detecta las situaciones de alarma generando una respuesta por medio de el sistema de tolerancia a fallos, para corregir los errores en las medidas o cualquier otra eventualidad (Bonastre *et al.*, 2012; Capella *et al.*, 2014). Está formado por un interfaz de sistemas de comunicación inalámbricos (RIS), compatible con los *nodos sensores;* una interfaz LAN/WAN, que recibe la señal de comunicación; un microcontrolador que recoge los datos de los nodos sensores y un sistema de almacenamiento de datos.

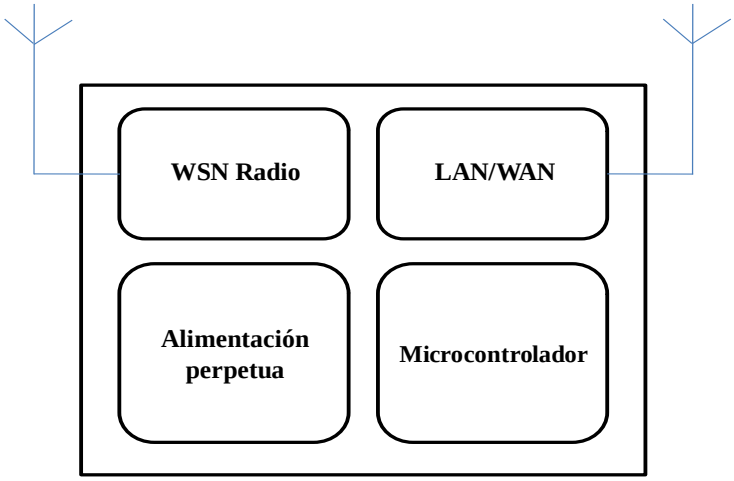

Figura I.10. Estructura del *nodo sumidero* (Capella *et al.*, 2014).

La característica principal de las RIS, que es su capacidad de organización automática. Así, es posible realizar despliegues de sensores en cualquier situación. De esta forma es posible mantener la red en funcionamiento incluso cuando algunos nodos se encuentran fuera de servicio, debido a fallos, modo bajo consumo, agotamiento baterías, etc. En estos casos la red es capaz de reorganizarse y continuar funcionando. A continuación, en la Figura I.11., se puede observar el esquema de la visión global de la arquitectura EDETA.

La evaluación de los mecanismos se ha realizado mediante una doble vía: implementación real y simulación, demostrándose que los mecanismos propuestos consiguen proporcionar robustez en las comunicaciones, tolerancia a fallos y tiempos de respuesta acotados. Esta arquitectura ha sido aplicada con éxito a sistemas reales en diversos campos como la automoción, aplicaciones industriales, entornos inteligentes, identificación de productos, domótica y seguridad, control de consumo energético, estudio de invernaderos, monitorización del medio ambiente, y un sinfín de nuevas aplicaciones entre ellas, en la industria alimentaria (Boukerche, 2009).

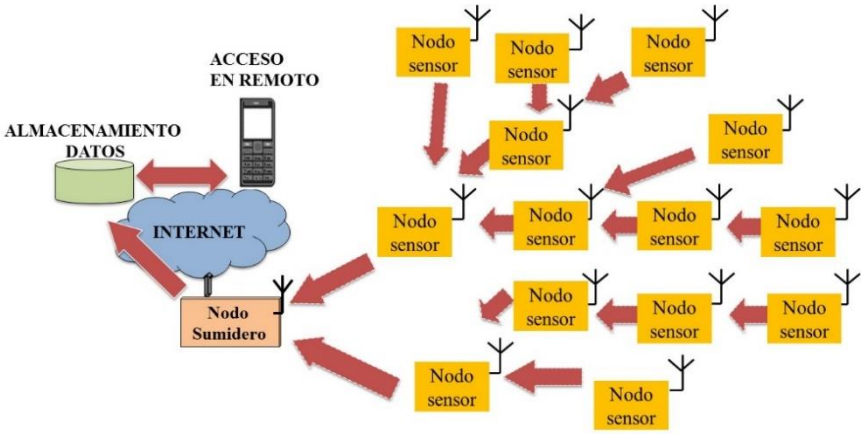

Figura I.11. Esquema de la arquitectura EDETA.

Asimismo, el diseño de una red de sensores, cuando se aplica a la trazabilidad de los alimentos debe tener en cuenta, los siguientes requisitos: recursos limitados, entornos dinámicos, uso del medio de transmisión no fiable, la diversidad de aplicaciones, la privacidad y la seguridad alimentaria. De acuerdo con éste último punto, en el Reglamento Europeo No 1935/2004 y No 450/2009, se establecen los materiales y objetos activos e inteligentes destinados a entrar en contacto con los alimentos en los envases, con el fin de garantizar que no hay efectos negativos que pongan en peligro la seguridad alimentaria y la salud humana.

Anteriores estudios han propuesto sistemas de trazabilidad sobre el control de la temperatura en la cadena alimentaria en condiciones de refrigeración durante el almacenamiento y transporte (Wang *et al.*, 2006; Zhang *et al.*, 2009). En la presente tesis, se propone la monitorización en mermeladas, con el fin de controlar su trazabilidad durante el periodo de almacenamiento, y así, poder observar los cambios producidos, de forma automática y en remoto, proporcionando además características de fiabiliad, a tiempo real alertas automáticas por parte del sistema, entre otras.

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II. OBJETIVOS Y PLAN DE TRABAJO

II. OBJETIVOS Y PLAN DE TRABAJO

II.1. Objetivo general

Se plantean tres objetivos generales en la presente Tesis Doctoral:

II.1.1. Desarrollar productos deshidratados osmóticamente, y estudiar el efecto de edulcorantes naturales como tagatosa, isomaltulosa, extracto acuoso de stevia y oligofructosa, como agentes osmóticos y así poder establecer el efecto de las variables del proceso de formulación sobre la cinética de transferencia de materia y su modelización en rodajas de naranja y limón.

II.1.2. Desarrollar tres tipos de mermeladas (naranja, limón y mandarina) y dos tipos de gelatinas (sandía y cítricos), formuladas con edulcorantes saludables como tagatosa, isomaltulosa, y oligofructosa como sustitutos de la sacarosa. Evaluar el efecto de los nuevos edulcorantes sobre las propiedades físico-químicas, mecánicas, ópticas, antioxidantes y sensoriales y finalmente su estabilidad microbiológica a lo largo del periodo de almacenamiento.

II.1.3. Monitorización de las mermeladas de mandarina a lo largo del periodo de almacenamiento mediante una red inalámbrica de sensores basada en la arquitectura EDETA.

II.2. Objetivos específicos

Los objetivos específicos se han dividido en función de los experimentales realizados en las frutas estudiadas, presentándose en el mismo orden en el que se desarrollan en el capítulo de Resultados.

II.2.1.Cinética de deshidratación osmótica de rodajas de naranja utilizando edulcorantes (isomaltulosa, extracto acuoso de stevia y oligofructosa)

II.2.2. Modelización de la deshidratación osmótica de rodajas de limón utilizando nuevos edulcorantes (tagatosa, isomaltulosa, extracto acuoso de stevia y oligofructosa).

- Realizar un estudio cinético, analizando cambios en la actividad de agua, variación de masa total, de agua y de sólidos solubles, en las rodajas de naranja y limón, en función de la combinación de edulcorantes utilizada.
- Analizar la difusividad efectiva obtenida del estudio cinético y el comportamiento de los edulcorantes como agentes osmóticos.

II.2.3. Influencia de los edulcorantes saludables (tagatosa y oligofructosa) sobre sus características fisicoquímicas en mermelada de naranja.

II.2.4. Caracterización de mermeladas de limón elaboradas con edulcorantes saludables (isomaltulosa y tagatosa).

II.2.5. Efecto de isomaltulosa y tagatosa como sustitutos de la sacarosa en mermeladas de mandarina.

- Desarrollo de mermeladas de naranja, mandarina y limón utilizando edulcorantes de bajo índice glicémico y no cariogénicos, en sustitución de la sacarosa en la formulación. Evaluar la influencia de la sustitución de la sacarosa por edulcorantes no cariogénicos en las mermeladas.
- Analizar los parámetros de composición, capacidad antioxidante, propiedades reológicas y ópticas, al inicio y durante el periodo de almacenamiento (45 días para las mermeladas de naranja, 60 días para las mermeladas de limón y 360 días para las mermeladas de mandarina), y a su vez comparar los parámetros con los obtenidos con mermeladas comerciales formulada con sacarosa.
- Estudiar la estabilidad microbiológica de las mermeladas de naranja durante el periodo de almacenamiento
- Evaluación sensorial mediante un panel de catadores, con el fin de comprobar el nivel de aceptación del consumidor.

II.2.6. Trazabilidad fiable basada en redes de sensores inalámbricos aplicado a una nueva formulación de mermelada de mandarina.

- Diseño de un sistema de trazabilidad, durante la etapa de almacenamiento de los parámetros de humedad y temperatura, en mermeladas de mandarina elaboradas con edulcorantes no cariogénicos y de bajo índice glicémico (isomaltulosa y tagatosa).
- Monitorización con redes de sensores inalámbricos sometidos a EDETA (**E**nergy-efficient a**D**aptative hi**E**rarchical and robus**T** **A**rchitecture), con el fin de garantizar las condiciones óptimas del producto para su consumo.
- Análisis experimental de composición, temperatura y estabilidad microbiana durante todo el tiempo de almacenamiento (360 días).

II.2.7. Caracterización de gelatinas de sandía elaboradas con edulcorantes no cariogénicos (isomaltulosa y tagatosa).

II.2.8. Influencia de los edulcorantes no cariogénicos (oligofructosa, isomaltulosa y tagatosa) sobre sus características fisicoquímicas en gelatinas de cítricos.

- Desarrollo de gelatinas elaboradas con zumo de sandía para las gelatinas de sandía, y con zumo de naranja, mandarina y limón, para las gelatinas de cítricos utilizando edulcorantes de bajo índice glicémico y no cariogénicos en sustitución de la sacarosa, y así poder evaluar su efecto en la formulación.
- Analizar los parámetros de composición, capacidad antioxidante, propiedades ópticas y mecánicas, al inicio y durante el periodo de almacenamiento (15 días para las gelatinas de sandía, y 45 días para las gelatinas de cítricos), y a su vez comparar los parámetros con los obtenidos con la gelatina comercial en el caso de las gelatinas de sandía.
- Estudio de la estabilidad microbiana en el caso de las gelatinas de cítricos durante el periodo de almacenamiento.

- Evaluación sensorial mediante un panel de catadores y así comprobar el nivel de aceptación.

II.3. Plan de trabajo

El plan de trabajo seguido en la presente Tesis Doctoral se estableció en función de la consecución de los objetivos específicos planteados:

II.3.1. Revisión bibliográfica y de legislación vigente, en función de la temática del estudio.

II.3.2. Planificación de experimentales, y puesta a punto de los procesos de deshidratación osmótica y de elaboración de mermeladas y gelatinas.

II.3.3. Obtención de la materia prima (naranjas, limones, mandarinas y sandías), ingredientes (sacarosa y edulcorantes naturales).

II.3.4. Formulación con edulcorantes naturales, no cariogénicos y de bajo índice glicémico como isomaltulosa, tagatosa, oligofructosa y extracto acuoso de stevia, en el caso de la deshidratación osmótica, y para mermeladas y gelatinas con isomaltulosa, tagatosa, oligofructosa.

II.3.5. Caracterización de la composición (contenido en humedad y sólidos solubles, actividad del agua y pH), iniciales y durante el periodo de estudio, de los productos obtenidos en la deshidratación osmótica.

II.3.6. Análisis de la composición, capacidad antioxidante, propiedades reológicas (ensayo estacionario y oscilatorio), ópticas (espacio de color Cie L*a*b*), mecánicas (ensayo TPA de textura) y sensoriales (panel de 30 catadores, escala hedónica 9-puntos), al inicio y durante el periodo de estudio, en mermeladas y gelatinas. Estudio y comparación con marcas comerciales.

II.3.7. Evaluación de la estabilidad microbiológica en mermeladas y gelatinas, al inicio y durante el periodo de almacenamiento.

II.3.8. Tratamiento de datos aplicando técnicas estadísticas (Statgraphics Centurion, Statpoint Technologies, Inc. Warrenton, Virginia, USA).

III. RESULTADOS

III.1.KINETICS OF OSMOTIC DEHYDRATION OF ORANGE SLICES USING HEALTHY SWEETENERS

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ABSTRACT

Orange slices have been osmotically dehydrated using as osmotic agents new healthy sweeteners: isomaltulose, oligofructose and aqueous extract of stevia. A kinetic study was done by analyzing changes in the water activity, total mass, mass of water and mass of soluble solids in orange slices, depending on the combination of sweeteners used in syrups (A: 30% isomaltulose and 70% of water; AS: 30% of isomaltulose, 35% aqueous solution with 1% of stevia and 35% water; B: 20% isomaltulose, 20% oligofructose and 60% water and BS: 20% isomaltulose, 20% oligofructose, 30% aqueous solution with 1% of stevia and 30% water). The results showed that the incorporation of stevia in syrups increased total mass and water mass losses of orange slices. Besides, by fitting second Fick's law effective diffusivities have been obtained in order to estimate the time to reach different concentrations of soluble solids in orange slices.

Keywords: orange, isomaltulose, oligofructose, stevia, osmotic dehydration, kinetics.

INTRODUCTION

Orange is a food with high content of healthy nutrients and it has great tradition and economic importance in Valencia. The development of new orange products would be a good way to promote the consumption of this fruit, improving the nutritional health of society.

In this way, the osmotic dehydration (OD) has been widely used for conservation and design of new products from fruits. OD consists on the introduction of foods in a low water activity solution in order to induce water outflows and inflows of external solutes and therefore allows storing the foods for longer periods improving the stability and quality of products. This treatment depresses the food water activity and improves

the biochemical and microbiological stability whereas producing physical and structural changes during its storage (Cháfer *et al.,* 2001; Park *et al.,* 2002; Gomes Alves *et al.,* 2005; Moura *et al.,* 2005; García-Segovia *et al.,* 2010; Monnerat *et al.,* 2010; Castro-Giráldez *et al.,* 2011).

Nowadays there is an important demand for high quality products in the food market and osmodehydrated oranges with healthy osmotic agents could be very appreciated by consumers. Sugars have been usually used as osmotic agents to obtained osmodehydrated fruits. However, it is widely known that sugars have cariogenic effects and most of them increase the glucose levels in blood and can be related with different diseases (diabetes, obesity, etc…). Fortunately, healthy osmotic agents are already available in the market with beneficial properties for our organisms since they are non-cariogenic and have a low glycemic index (Soto *et al.,* 2002; Goyal *et al.,* 2010). This is the case of isomaltulose, oligofructose and stevia.

Isomaltulose is a reducing disaccharide which is naturally present in honey, and sugar cane juice, and their taste and viscosities of aqueous solutions appearance are similar to sucrose. The physicochemical properties of isomaltulose (Palatinose®) permit the substitution of sucrose in most sweet foods (De Oliva-Neto *et al.,* 2009; Lina *et al.,* 2002; Peinado *et al.,* 2013). It has a sweetening power of approximately 42% compared to sucrose, and also should be noted that because of its low hygroscopic rate, provides stability to products such as candy, gum and chocolate. It can be an alternative source of energy to sucrose, because its caloric power is similar. However, the solubility at room temperature of isomaltulose (30%) is much lower than sucrose (65%) (Kaga and Mizutani, 1985; Schiweck *et al*., 1990; Periche *et al.,* 2014).

Regarding the oligofructose, it is obtained by partial enzymatic hydrolysis of chicory inulin and it is a soluble dietary fiber with prebiotic character to enhance the growth of beneficial gut bacteria and calcium absorption. On the other hand, oligofructose is low in calories, which

gives multiple health benefits (Bosscher *et al.*, 2006; Raschka *et al.*, 2005; Rao, 2001). Nowadays, it is used as ingredient in many food products, and it is known that reduced serum insulin and glucose. Oligofructose helps to decrease hepatic fatty acid and triacylglycerol synthesis and coordinates the activity of all lipogenic enzymes (Franck, 2002; Al-Sherajia *et al.*, 2013).

Stevia is a plant that has been consumed as a food and also used as a medicine in some countries such as Japan and Paraguay (Lemus-Mondaca *et al.*, 2012). This plant has a sweetening power 15 times greater than sucrose, multiple therapeutic properties (anti-oxidant, anti-microbial, anti-fungal activity, anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal and diuretic effects), but it is calorie free (Chatsudthipong *et al.*, 2009). Currently, the use of stevia leaves or extracts from stevia was approved by FDA as a dietary supplement in the US, and under similar classifications in several other countries, it has GRAS status (FDA GRAS Database, 2008). In November 2011, the European Commission approved steviol glycosides as food additives (European Commission, 2011).

Therefore, these new healthy sweeteners could be suitable for any sector of the population, even for those with diabetes, obesity or with predisposition to dental caries. Additionally, citric fruits are a good source of bioactive compounds like ascorbic acid, polyphenols and carotenoids that have been shown to be good contributors to the total antioxidant capacity of foods and have been involved in the prevention of some degenerative diseases (Devalaraja *et al*., 2011; Kim *et al*., 2011).

Thus, the aim of this study was to characterize the kinetic behavior of the dehydrated orange slices using different combinations of healthy sweeteners (isomaltulose, oligofructose and aqueous stevia extract) in order to determine the time needed to obtain different levels of soluble solids concentration in the fruit.

MATERIALS AND METHODS

Preparation of sample

Valencia Late oranges were used as raw material, selected with similar color, size and maturity stage of an agricultural plot from Líria (Valencia). The oranges were peeled and cut into 0.5 cm thick slices using a household slicer (Fagor Delice CF- 150).

Osmotic dehydration treatment

Isomaltulose (Palatinose™ PST-N, Beneo-palatinit), oligofructose (Fructalose® OFP, Sensus) and an aqueous extract with 1% of dry leaves Stevia (*S. rebaudiana Raab*, Vitalfood, Rohrbach, Germany) were used as agents for osmotic dehydration. Table 1 shows the combinations of these three sweeteners used in the four syrups considered and the code assigned. The kinetic study was carried out for 48 hours by analyzing samples at 0, 10, 20, 30, 45, 60, 90, 120, 240, 300 minutes and at 24 and 48 hours. The ratio between syrup and orange slices was 20:1 (*w/w*) in order to not modify the concentration of soluble solids of the syrup, with constant stirring.

Table III.1.1. Percentage of sweeteners in the syrups used in the study of osmotic dehydration of orange slices.

Syrup	Isomaltulose	Oligofructose	Aqueous solution containing 1% of Stevia	Water
A	30%	-	-	70%
AS	30%	-	35%	35%
B	20%	20%	-	60%
BS	20%	20%	30%	30%

Physicochemical analysis

Soluble solids of liquid phase in oranges slices and syrups were measured by a refractometer (Abbe Refractometer, Atago), obtaining the results in °Brix and also expressed as z_s (kg soluble solids/kg liquid phase). Moisture content (x_w*: kg water/kg orange* slices) was analyzed gravimetrically followed an adaption of the method AOAC, (2000). Water activity (a_w) was determined by a hygrometer (Decagon CX-1). All determinations were made in triplicate.

Kinetic study and modeling

Variation of total mass, soluble solids and water mass were calculated for all times considered in this study. Besides that, a Fick's model was used to obtain the effective diffusivity (D_e: m^2/s) of soluble solids (Crank, 1975; Barat *et al.,* 1998; Cháfer *et al.,* 2001) depending on the composition of the syrup used.

RESULTS AND DISCUSSION

Physicochemical analysis

The syrups used for this kinetic study reached 26.5±0.4, 27.7±0.4, 30.0±0.4 and 32.5±0.4 of ºBrix for formulations A, AS, B and BS, respectively. These results show that the highest proportions of solutes in syrups with oligofructose increased the °Brix and the same effect was observed when aqueous extract of stevia was added.

Figure 1 represents the results of a_w and °Brix *versus* time of osmotic dehydration as a function of the syrup employed. As was expected, the longer the time of dehydration higher the concentration of soluble solids in orange slices, especially for AS and BS. Other studies with orange and other fruits (strawberry, apple, apricot) show similar results (Cháfer *et al.*, 2001; Castelló *et al.,* 2006, 2009; İspir *et al.,* 2009). In accordance with this behavior, the water activity decreased, but less in samples

osmodehydrated with syrups containing stevia extracts, indicating the important influence of stevia in this process.

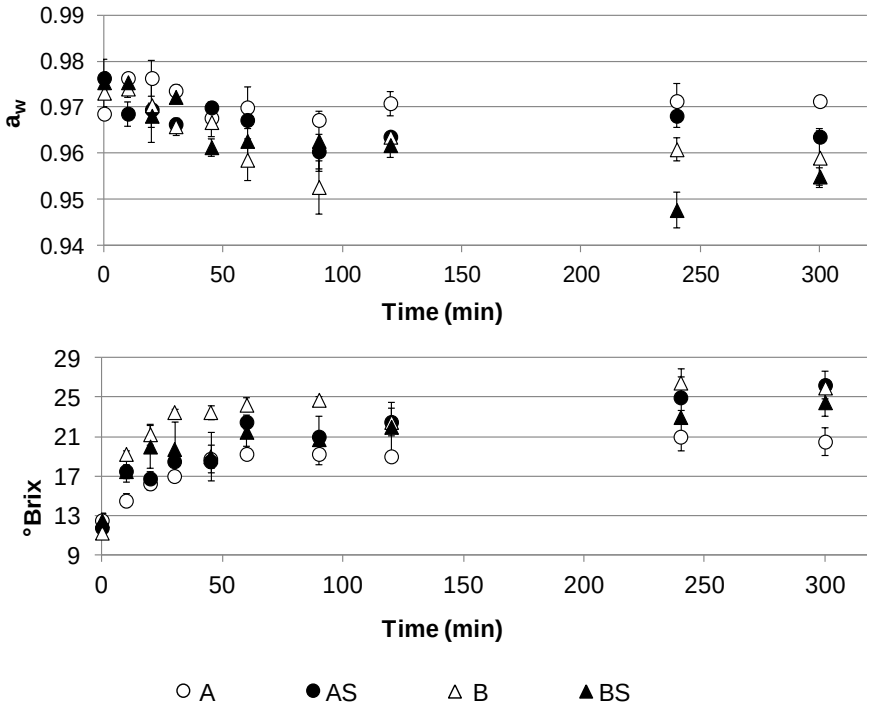

Figure III.1.1. Results in a_w and °Brix dehydration function of time for all the treatments studied.

Kinetic study and modeling

The results of variation of total mass (ΔM), water mass (ΔM_w) and soluble solids mass (ΔM_s) recorded in this study were obtained with the following formulas (Shi *et al.*, 1994; Fito *et al.*, 1996) and are presented in Figure 2.

$$\Delta M = \frac{M^t - M^0}{M^0} \qquad (1)$$

$$\Delta M_w = \frac{M^t x_w^t - M^0 x_w^0}{M^0} \qquad (2)$$

$$\Delta M_s = \frac{M^t x_s^t - M^0 x_s^0}{M^0} \quad (3)$$

Being

M^i: mass of orange slices (kg) at time i (i=0 or t)

M_w^i: mass of water of orange slices (kg) at time i (i=0 or t)

M_s^i : mass of soluble solids of orange slices (kg) at time i (i=0 or t)

x_w^i : mass fraction of water (kg of water/kg of orange slices) at time i (i=0 or t)

x_s^i : mass fraction of soluble solids (kg of soluble solids/kg of orange slices) at time i (i=0 or t)

There was also checked that the experimental total mass loss was similar to the calculated mass loss by using eq. (4).

$$\Delta M = \Delta M_s + \Delta M_w \quad (4)$$

In coherence with the previous results, both the addition of stevia and oligofructose in syrups increased total and water mass losses of oranges slices since their driving forces were greater than when only isomaltulose was used.

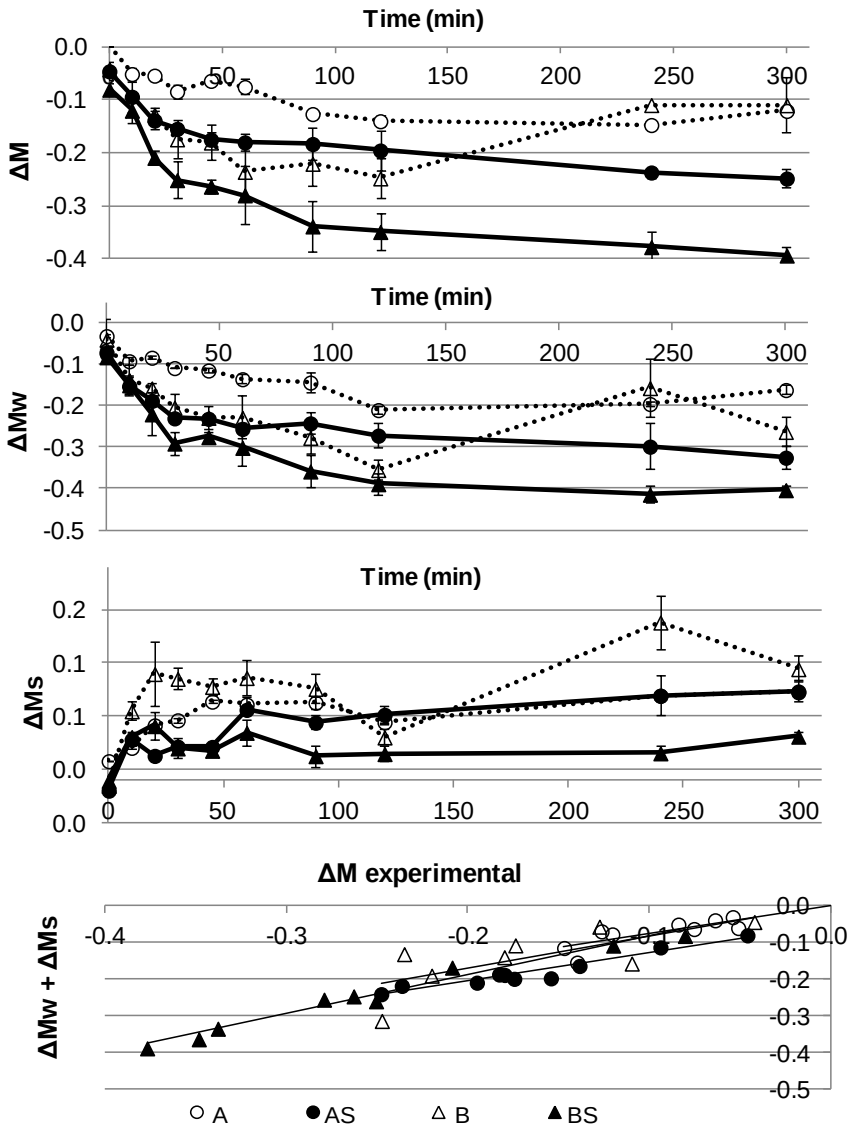

Figure III.1.2. Results of variation of total mass (ΔM), of water mass (ΔM_w) and soluble solid mass (ΔM_s) as well as material balances recorded in this study.

Moreover, the changes in the composition of the liquid phase of orange slices were modeled using the eq. (5).

$$Y_s^t = \frac{(z_s^t - y_s)}{(z_s^0 - y_s)} \quad (5)$$

Where:

Y_s^t: driving force of soluble solids (dimensionless)

z_s^i: soluble solid mass fraction in the liquid phase at time i (i=0 or t)

y_s: soluble solid mass fraction in the osmotic solution used for dehydration.

Therefore the y_s is the maximum soluble solid concentration that the orange slices could reached at equilibrium.

Figure 3 shows the experimental points of $1\text{-}Y_s$ *versus* $t^{0.5}$ to adjust them to a simplified Fickian approach for diffusion in a plane sheet, with only one term of the Fick's second law series solution for short times (Crank, 1975) (equation 6). From the fitting of this model, it is possible to obtain the kinetic parameter of effective diffusivity (D_e) which allows us to predict the process time required to achieve a specific concentration of soluble solids in orange slices (Table 2).

$$1 - Y_s = \left[\frac{4D_e t}{\pi l^2}\right]^{0.5} \quad (6)$$

Where, t is time of processing (s) and $\boldsymbol{l}$ is half thickness of the dehydrated sample (m).

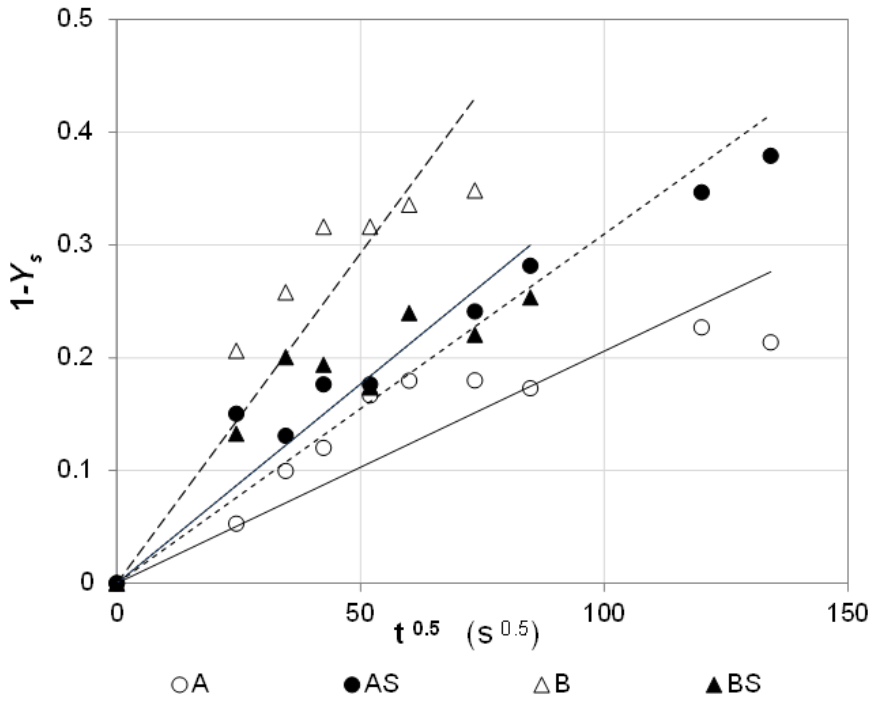

Figure III.1.3. Results *1-Y_s vs.* t $^{0.5}$ (square root of time of dehydration) for all the treatments.

The results of D_e show that the best fitting was observed when AS syrup was used in dehydration of orange slices. Furthermore, B syrup presented a higher slope, which corresponded to the highest value of effective diffusivity. This would corroborate that the combination of oligofructose with isomaltulose implies a faster dehydration of orange slices due to the highest concentration of soluble solids in those osmotic solutions.

Table III.1.2. Results of the effective diffusion coefficient (D_e), slope and correlation coefficient (R^2) of Fick's equation for an infinite sheet (Crank, 1975).

Syrup	D_e (m^2/s)	Slope	R^2
A	$2.16 \cdot 10^{-09} \pm 1.2 \cdot 10^{-10}$	$2.06 \cdot 10^{-3} \pm 0.06 \cdot 10^{-3}$	0.71±0.02
AS	$4.7 \cdot 10^{-09} \pm 3 \cdot 10^{-10}$	$3.20 \cdot 10^{-3} \pm 0.08 \cdot 10^{-3}$	0.90±0.12
B	$1.71 \cdot 10^{-08} \pm 3 \cdot 10^{-10}$	$5.89 \cdot 10^{-3} \pm 0.05 \cdot 10^{-3}$	0.79±0.05
BS	$7.08 \cdot 10^{-09} \pm 1.3 \cdot 10^{-10}$	$3.8 \cdot 10^{-3} \pm 0.03 \cdot 10^{-3}$	0.68±0.16

CONCLUSIONS

This study makes possible to model the osmotic dehydration behavior of orange slices depending on the combination of different new non cariogenic and prebiotic sweeteners (isomaltulose, oligofructose and stevia) used in the osmotic solution. Thus, the different effective diffusivity has been obtained to predict the required times to dehydrate orange slices. Moreover, it has been checked that stevia aqueous extract enhance the concentration of soluble solids in these products. Therefore these new sweeteners could be used to develop healthier osmodehydrated products also from other fruits.

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II.2. MODELLING OSMOTIC DEHYDRATION OF LEMON SLICES USING NEW SWEETENERS

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ABSTRACT

Lemon slices were osmotically dehydrated using the following healthy sweeteners as osmotic agents: tagatose, isomaltulose, oligofructose and aqueous extract of stevia. A kinetic study using a Fickian approach was performed, which also analysed the changes in water activity, total mass, mass of water and mass of soluble solids in lemon slices. The results showed that the greatest value of effective diffusivity (De) in osmodehydrated lemon slices was obtained from a combination of oligofructose and stevia [D_e= (10.2±0.3) x 10^{-9} $m^2 \cdot s^{-1}$]. However, the level of water activity (a_w) reached with this syrup was the highest (a_w=0.978±0.004 after 1440 min) meaning that the product might be less stable. Additionally, isomaltulose favoured the total mass loss, whereas tagatose did the opposite. Finally, the syrup recommended for dehydrating lemon slices would be a combination of tagatose, oligofructose and aqueous extract of stevia since its De was similar to the value obtained when only oligofructose and stevia were used, but a_w values were lower.

Keywords: lemon, tagatose, isomaltulose, oligofructose, stevia, osmotic dehydration, kinetics.

INTRODUCTION

Citrus fruits have played an important role in the economy and dietary habits in Spain. They are a good source of bioactive compounds like ascorbic acid, polyphenols and carotenoids and have been involved in the prevention of some diseases such as diabetes, obesity, cancer and cardiovascular diseases (Devalaraja *et al*., 2011; Kim *et al.,* 2011). Among these citrus fruits, lemon is the product consumed the least due to its high acidity. However, the addition of sweeteners might be an effective way to counteract this feature. Therefore, the development of new sweet lemon products could promote consumption of this fruit, and in turn, improve the nutritional health of society.

Osmotic dehydration (OD) might be a suitable technique for obtaining such products, since it has been widely applied to other fruits, such as oranges (Cháfer *et al*., 2001; Rubio-Arraez, *et al*., 2015), pears (Park *et al.*, 2002), tomatoes (Azoubel & Murr, 2004), apples (Derossi *et al*., 2008; Castelló *et al*., 2009), apricots (İspir & Toğrul, 2009), strawberries (Castelló *et al.*, 2006; Castelló *et al*.,2010), kiwis (Castro-Giráldez *et al.*, 2011) and cherries (Silva *et al.*, 2012). Besides, OD has already been studied as an alternative, which gives uses to lemon by-products (Masmoudi *et al*., 2007). OD consists of placing foods in a low water activity solution in order to induce water outflows and inflows of external solutes, resulting in high quality products that can be stored longer.

However, the common use of sugars in the OD stage leads to an enhancement in the cariogenic property of the final products as well as an increase in their glycemic index, and can also be linked to different diseases (diabetes, obesity, etc.). Fortunately, there are other new sweeteners available in the market which are non-cariogenic and also have other advantages over conventional sugars or sweeteners, such as isomaltulose, oligofructose, stevia and tagatose (Goyal *et al*., 2010). Each one is described below.

Isomaltulose is a reducing disaccharide which is naturally present in honey, and sugar cane juice, and its taste is similar to sucrose. The physicochemical properties of isomaltulose enable it to be used a substitute for sucrose in most sweet foods (Lina *et al*., 2002; Peinado *et al*., 2013). It has a sweetening power of approximately 42% compare to sucrose and it can be used as an alternative to sucrose, because its caloric power is similar (Periche *et al.*, 2014).

Oligofructose is low in calories, meaning it has multiple health benefits. It is obtained by partial enzymatic hydrolysis of chicory inulin and it is a soluble dietary fiber with prebiotic character to enhance the growth of beneficial gut bacteria and calcium absorption (Rao, 2001; Raschka & Daniel, 2005).

Stevia is a plant that has been consumed as a food and also used as a medicine in some countries such as Japan and Paraguay (Lemus-Mondaca *et al.,* 2012). The sweetening power of this plant is 15 times greater than sucrose, and it has multiple therapeutic properties (antioxidant, antimicrobial, anti-fungal activity, anti-hyperglycemic, anti-hypertensive, anti-inflammatory, antitumor, antidiarrheal and diuretic effects), but it is calorie free (Chatsudthipong & Muanprasat, 2009).

Tagatose is a D-galactosa isomer in milk and milk products. In comparison with other sugars, it has numerous health benefits including a lower glycemic index, and low calorie content. It also reduces the symptoms associated with type II diabetes and it is recommended for patients with obesity or heart diseases (Oh, 2007; Lu *et al.,* 2009; Shankar *et al.,* 2013). Tagatose exerts greater osmotic pressure, and hence has less water activity than sucrose at equivalent concentrations (Patra *et al.,* 2009). D-Tagatose is well suited for confectionery products such as chocolate and candies, fudges, caramels, ice cream, soft drinks, and breakfast cereals because tagatose crystallizes easily (FAO/WHO, 2003).

In consideration of all the above, the aim of this work is to study the effect of different combinations of healthy osmotic agents (tagatose, oligofructose, isomaltulose and stevia) on the kinetic behaviour of lemon slices in order to obtain mathematical models following second Fick's law. For this purpose, variation of total mass, soluble solids and water mass changes have been analysed over time.

MATERIALS AND METHODS

Preparation of sample

Eureka Lemons of a similar colour, size and ripeness were selected from an agricultural plot in Llíria (Valencia). The lemons were peeled and cut into 0.5 cm thick slices using a household slicer (Fagor Delice CF- 150).

Osmotic dehydration treatment

Tagatose (Tagatesse®, Damhert) Isomaltulose (Palatinose™ PST- N, Beneo), oligofructose (Fructalose® OFP, Sensus) and an aqueous extract with 1% of dry Stevia leaves (*S. rebaudiana Raab;* Vitalfood, Rohrbach, Germany) were used as agents for OD. Table 1 shows the combinations of these four sweeteners used in the four syrups considered and the code assigned.

The kinetic study was carried out for 48 h by analysing samples at 0, 10, 20, 30, 45, 60, 90, 120, 240, 300 min at 24 and 48 hours. The ratio between syrup and lemon slices was 20:1 (*w/w*) with constant stirring so as not to modify the concentration of soluble solids in the syrup.

Table III.2.1.Percentage of sweeteners in the syrups used in the study of osmotic dehydration of lemon slices.

Syrup	Tagatose (%)	Isomaltulose (%)	Oligofructose (%)	Aqueous solution containing 1% of Stevia (%)	Water (%)
T	30	-	-	-	70
OS	-	-	30	35	35
IT	10	20	-	-	70
ITOS	10	10	10	10	50

Physicochemical analysis

Soluble solids in the liquid phase in lemon slices and syrups were measured by a refractometer (Abbe Refractometer, Atago, Bellevue, WA, USA), obtaining the results in °Brix and also expressed as z_s (kg soluble solids per kg liquid phase). Moisture content (x_w: kg water per kg lemon slices) was analysed gravimetrically following an adaptation of the AOAC method, (2000). Water activity (a_w) was determined by a

hygrometer (Decagon CX-1, Pullman, WA, USA). All determinations were carried out in triplicate.

Kinetic study and modelling

Variation of total mass (ΔM), mass of soluble solids (ΔM_s), and mass of water (ΔM_w) were calculated for all times considered in this study. Additionally, a Fick's model was used to obtain the effective diffusivity (D_e: $m^2 \cdot s^{-1}$) of soluble solids (Crank, 1975; Cháfer *et al.*, 2001) depending on the composition of the syrup used.

Statistical analysis

Statgraphics plus (version 5.1, Statpoint Technologies, Inc., Warrenton, VA, USA) software was used to perform the statistical analyses, the factor taken into account being the composition of the syrup used in the OD.

RESULTS AND DISCUSSION

Physicochemical analysis

The average values of composition and water activity for lemons used in these experiments were the following: 83±4% of water, 15.7±0.4% of soluble solids and 0.986±0.003 of water activity. The ºBrix of the osmotic solutions used were 35.2±0.7 for syrup T, 29.5±0.7 for syrup OS, 36.3±0.7 for syrup IT and 32.9±0.7 for syrup ITOS. According to these results, tagatose would lead to the highest driving force.

Fig. 1 represents the results of water activity (a_w), °Brix and moisture content (x_w) of lemon slices *vs.* time of osmotic dehydration depending on the syrup used. As was expected the longer the time of dehydration the higher the concentration of soluble solids in lemon slices.

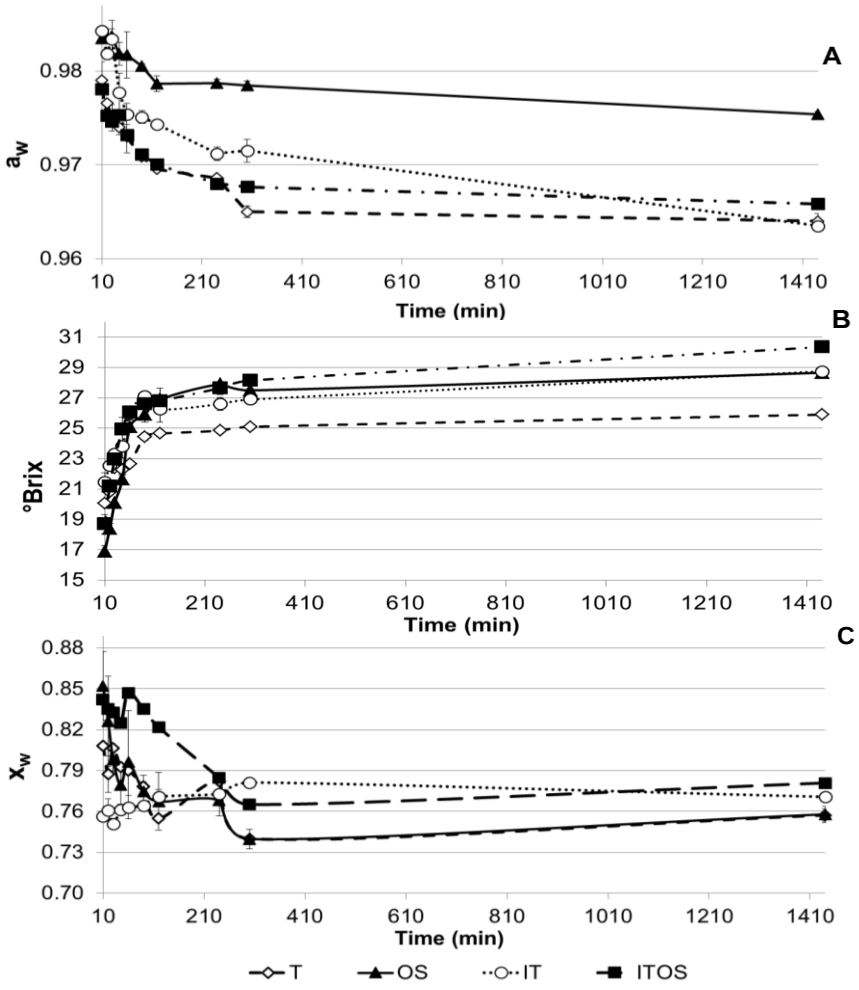

Figure III.2.1. A) Water activity (a_w) *vs.* time B) °Brix *vs.* time and C) x_w *vs.* time for the lemon slices dehydrated with different osmotic solutions.

Other studies with oranges and other fruits (strawberry, apple, apricot) showed similar results (Cháfer *et al.*, 2001; Castelló *et al.*, 2006, 2009; İspir & Toğrul, 2009). In the sweeteners studied, noteworthy was that the samples osmodehydrated with oligofructose and stevia showed the highest levels of water activity, while syrup composed only by tagatose led to the lowest increase in soluble solids. However, no differences were

found in terms of moisture content among the slices treated with the four syrups studied.

Kinetic study and modelling

The results for variation of total mass (*ΔM*), water mass (*ΔM*$_w$) and soluble solid mass (*ΔM*$_s$) recorded in this study were obtained using the following formulas (Shi & Fito, 1994) shown in Figure 2.

$$\Delta M = \frac{M^t - M^0}{M^0} \quad (1)$$

$$\Delta M_w = \frac{M^t x_w^t - M^0 x_w^0}{M^0} \quad (2)$$

$$\Delta M_s = \frac{M^t x_s^t - M^0 x_s^0}{M^0} \quad (3)$$

Where

M^i: M mass of lemon slices (kg) at time i (i=0 or t)

M_w^i: mass of water of lemon slices (kg) at time i (i=0 or t)

M_s^i : mass of soluble solids of lemon slices (kg) at time i (i=0 or t)

x_w^i : mass fraction of water (kg of water/kg of lemon slices) at time i (i=0 or t)

x_s^i : mass fraction of soluble solids (kg of soluble solids/kg of lemon slices) at time i (i=0 or t)

As can be seen in Fig. 2 samples dehydrated with syrups containing isomaltulose (IT and ITOS) showed the greatest losses of total mass, especially when they were combined with oligofructose and stevia. On the contrary, syrup composed by oligofructose and stevia (OS) kept the mass of lemon slices constant while syrup containing only tagatose (T) led to an increase in total mass in all samples for the whole process.

Therefore, tagatose would be more beneficial in the development of osmodehydrated products, as contrary to what is common in this process, it led to an increase in mass. Consistent with the values of moisture content registered during the process, no differences were found with regard to water and soluble solid mass changes.

However, the combination of oligofructose and stevia with or without isomaltulose gave rise to the highest rates of soluble solid mass intake. This behaviour is noteworthy since driving forces recorded for these syrups were lower than in the other cases, and consequently, a lower rate of soluble solid intake would have been expected. It seems that it was more difficult for tagatose molecules to penetrate the structure of the lemon slices, whereas for oligofructose, it was easier to dehydrate this product.

Moreover, the changes in the composition in the liquid phase of lemon slices were modeled using the eq. (4).

$$Y_s^t = \frac{(z_s^t - y_s)}{(z_s^0 - y_s)} \quad (4)$$

Where:

Y_s^t: driving force of soluble solids (dimensionless)

z_s^i: soluble solid mass fraction in the liquid phase at time i (i=0 or t)

y_s: soluble solid mass fraction in the osmotic solution used for dehydration.

The latter (y_s) was considered to be equal to the equilibrium concentration of each syrup, having the values mentioned at the beginning of this section.

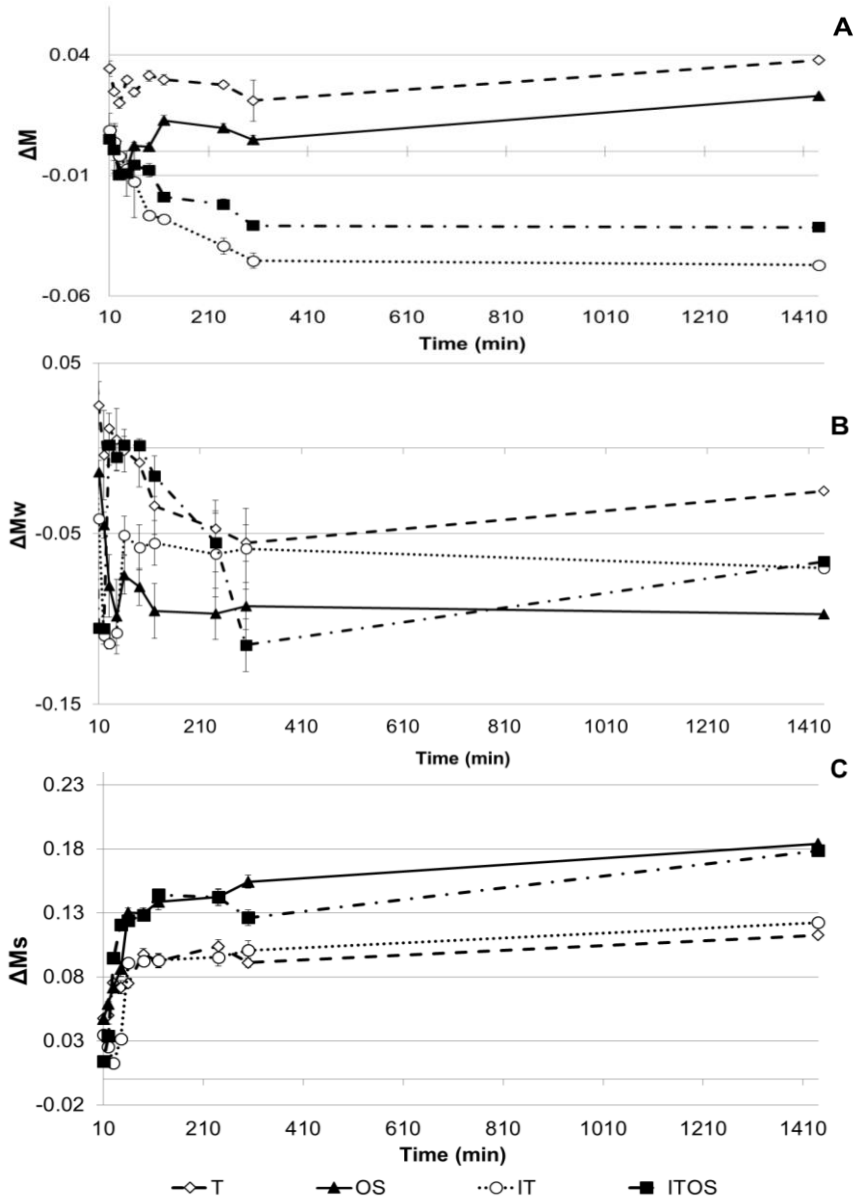

Figure III.2.2. A) Variation of total mass (ΔM), B) Variation of water mass (ΔM_w) and C) Variation of soluble solid mass (ΔM_s) for the lemon slices dehydrated with different osmotic solutions.

Figure 3 shows the experimental points 1-Y_s *vs.* $t^{0.5}$ to adjust them to a simplified Fickian approach for diffusion in a plane sheet, with only one term of the Fick's second law series solution for short times (Crank, 1975) (equation 5).

$$1 - Y_s = \left[\frac{4D_e t}{\pi l^2}\right]^{0.5} \quad (5)$$

Where, t is time of processing (s) and l is half thickness of the dehydrated sample (m).

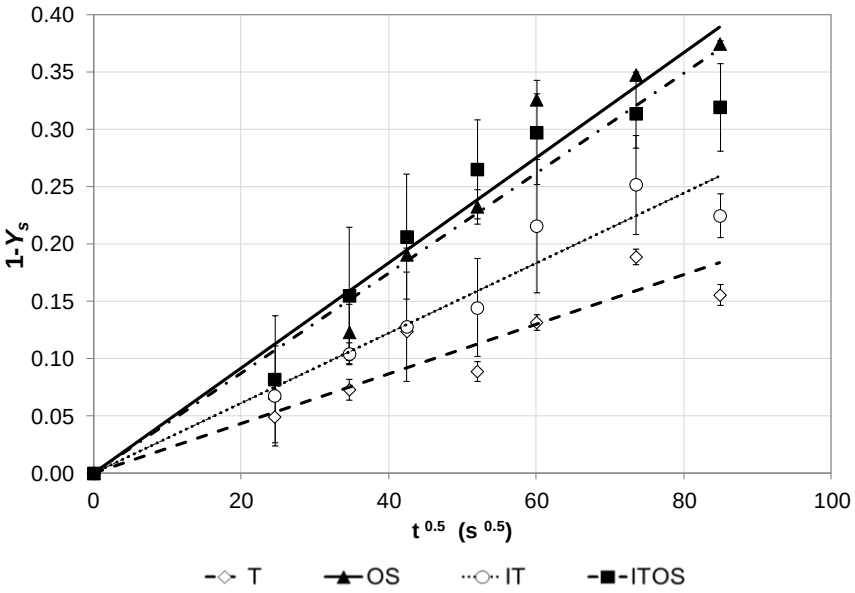

Figure III.2.3. 1-Driving force of soluble solids (Y_s) *vs.* $t^{0.5}$ (square root of time of dehydration) for the lemon slices dehydrated with different osmotic solutions.

In this case, the range of time considered corresponded only to the first 120 min of osmotic treatment. From the fitting of this model, it is possible to obtain the kinetic parameter of effective diffusivity (D_e) which allows us to predict the process time required to achieve a specific concentration of soluble solids in osmodehydrated lemon slices (Table 2).

Table III.2.2. Values of effective diffusion coefficient (D_e) and correlation coefficients (R^2) of Fick's equation for an infinite sheet (Crank, 1975) in lemon slices osmodehydrated with different syrups.

Syrup	D_e x 10^{-9} ($m^2 \cdot s^{-1}$)	R^2
T	2.27 ± 0.15^{a}	0.87±0.05
OS	10.2 ± 0.3^{c}	0.95±0.01
IT	4.7 ± 1.9^{ab}	0.91±0.03
ITOS	9 ± 3^{bc}	0.91±0.02

Equal letters indicate homogeneous groups.

The results of D_e showed that the best fitting was observed when OS syrup was used in the dehydration of lemon slices. Furthermore, syrup composed of oligofructose with stevia extract (OS) presented a higher slope, which corresponded to the highest value of effective diffusivity, whereas syrup made of tagatose showed the longest times of osmodehydration since the effective diffusivity was the lowest. This is consistent with the results relating to water and soluble solid mass changes. Isomaltulose did not significantly affect the values of D_e for the syrups composed of tagatose or oligofructose.

These results give evidence of the potentiality of the use of these new sweeteners to produce healthier osmodehydrated lemon slices. Furthermore, in our previous studies carried out in orange slices (Rubio-Arraez *et al*., 2015); the combination of oligofructose with isomaltulose implied a faster dehydration of samples. However, in that case, tagatose was not used in the formulation of the different syrups used for the osmodehydration of oranges. Thus, the present research opens more possibilities to develop this kind of products.

CONCLUSIONS

According to this study, isomaltulose gave rise to the highest mass loss values in the osmodehydrated lemon slices, in contrast with tagatose. However, the level of concentration of soluble solids reached by tagatose would be lower than in the case of the other sweeteners and would be reached more slowly. Oligofructose combined with stevia would lead to a quicker concentration of soluble solids, but also to the highest level of water activity and therefore would potentially be the least stable. To solve this problem it would be recommendable to combine oligofructose, aqueous extract of stevia and tagatose.

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III.3. INFLUENCE OF HEALTHY SWEETENERS (TAGATOSE AND OLIGOFRUCTOSE) ON THE PHYSICOCHEMICAL CHARACTERISTICS OF ORANGE MARMALADE

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ABSTRACT

Today's society shows a growing interest in healthy, safe and high nutritional quality food. Thus, in this paper sweet orange marmalades have been developed using healthy sweeteners (tagatose and oligofructose) in different proportions. Analyses of Brix, pH, moisture, water activity, antioxidant capacity, optical and rheological properties have been carried out, initially and after 45 days of storage. Microbiological analyses have also been performed to determine their stability. Furthermore, a sensorial assessment has been conducted to find out acceptance of these new orange marmalades by consumers. The results showed that the highest proportion of oligofructose contributed to improve the initial antioxidant capacity of marmalades. The marmalade with the same proportions of oligofructose and tagatose was more consistent and showed a further increase in the elastic component over time. All marmalades had a similar appearance, but oligofructose reduced L*. Finally, orange marmalades made with healthy sweeteners were better scored.

Keywords: marmalade, tagatose, oligofructose, rheology, color, sensory analysis.

Practical Applications

The development of new healthier marmalades offer new alternatives to the conventional ones not only to prevent caries and obesity but also to provide functional features associated with the use of tagatose and oligofructose as sweeteners. However, not always it is possible to replace traditional components by others and that is why is so important to assess their technological influence. In this study, the viability of the use of both sweeteners to prepare orange marmalades has been checked.

INTRODUCTION

Nowadays, owing to the current lifestyle of society, there is an increasing demand for healthy food products such as fruit and vegetables. However, these products are highly perishable with the consequent problems of distribution and shelf life. As an alternative, processing makes it possible to extend their marketability, whilst maintaining some of the characteristics of the fresh products to a certain extent. In this regard, marmalades are a typical example of more stable fruit-derived products.

Among the diverse variety of fruits and the requirements for manufacturing marmalades, oranges may be a good choice in the Mediterranean area as they are readily available at an affordable price, and also because of their high nutritional content. In the case of marmalades, as in many other cases, sugars/sweeteners are crucial for these kinds of products to achieve the right texture. In addition, they are responsible for the high concentration of Brix, the reduction of a_w and consequently the control of the microbial growth in the product. Traditionally, sucrose has been used as the main sugar to manufacture marmalades. However, because of the negative connotations associated with sugar consumption (cariogenesis, high caloric intake, increase in the glycemic index, etc…) it has been replaced by bulk sweeteners (polyols) or high-potency sweeteners (saccharine, aspartame…) depending on the properties required in the product (Edwards 2002; O'Donnell and Kearsley 2012). Nevertheless, these sweeteners also present some drawbacks. Concretely, most of the polyalcohols have a laxative effect.

In the case of high-potency sweeteners, there is a lot of controversy since their relation with the development of different cancers and other diseases is being questioned (Weihrauch and Diehl 2004; Soffritti *et al*. 2006; Renwick and Nordmann 2007).

The World Health Organization (2014) considers reducing the excessive consumption of sugars and other carbohydrates of fast

absorption, such as sucrose an urgent matter, whilst increasing daily physical activity in order to stop the trend towards obesity and diabetes type 2. A fast absorption of sugar may cause glycemic peaks and the excess sugar may be quickly converted into fat in the organism (Lu *et al*. 2008; Lina *et al*. 2002).

In fact, most of the sugars (sucrose, fructose and glucose) have around 4 kcal/g, although their glycemic index (GI) changes. Thus, glucose has the highest GI, with a value of 100, followed by sucrose with 65 and fructose with a GI of 25. Factors such as obesity, diabetes, and the increasing awareness of the need to improve diet, increase the demand for alternative sweeteners to those previously mentioned. Fortunately, the food industry currently offers healthy alternatives, such as tagatose, oligofructose, stevia, isomaltulose, etc… The challenge is to check their viability to reformulate traditional products in order to keep or even improve their technological properties.

One of the alternatives to the traditional sweeteners is D-Tagatose (D-tag) that is a ketohexose bulk sweetener, a stereoisomer of D-galactose, with a texture very similar to sucrose, almost as sweet as sucrose, since its sweetening power is 92% (Oh 2007; Taylor *et al.* 2008; Calzada-León *et al.* 2013) but with only 1.5 kcal/g and it does not cause dental caries (Levin, 2002). D-tagatose received Generally Recognized as Safe status by the Food and Drug Administration in 2001 and entered the US market as a sweetener in 2003 (Donner *et al.,* 2010). It is found naturally in several foods, including cheese and yoghurt. It can also be produced from D-galactose by means of a chemical method using calcium as a catalyst (Oh 2007). Tagatose is very suitable for confectionary products, ice creams, soft drinks and breakfast cereals (Vastenavond *et al*. 2011). It is considered a functional food because it is partially metabolized and the part that is not absorbed (80% of the intake) ferments in the colon, where it performs functions as soluble fiber (Taylor *et al*. 2008) favouring lactic acid bacteria and *Lactobacillus* specie bacteria (Petersen-Skytte 2006).

On the other hand, oligofructose is an oligosaccharide derived from fructose, which acts as dietary fibre regulating intestinal transit. It improves calcium absorption (van den Heuvel *et al*. 1996) and reduces cholesterol and blood sugar levels (Chacón-Villalobos 2006). Moreover, it presents a prebiotic effect because it favours the selective growth of lactic bacteria and bifidobacteria (Ledur *et al*. 2013). Oligofructose has approximately between 30% and 60% of the sweetness of sucrose and it is easily hydrolysed by the action of acids or enzymes (Coussement 1999). It is highly soluble and possesses technological properties that are closely related to those of sucrose and glucose syrups (sweet taste, stability…) because it has free sugars (Pimentel *et al*., 2014). It is often used in combination with high intensity sweeteners. The replacement of carbohydrates by oligofructose offers the advantage of not compromising on taste and texture, while delivering nutritionally enhanced products (Franck 2002).

The aim of this paper focuses on characterising sweet orange marmalades formulated with different combinations of tagatose and oligofructose analysing their moisture content, Brix, pH and antioxidant capacity initially and after 45 days of storage. Besides, their rheological and optical properties have been also registered. Finally, a sensorial analysis has been carried out to assess their acceptability by potential consumers.

MATERIALS AND METHODS

Formulations and Manufacturing Processes of Orange Marmalades

Marmalades were produced using 60% orange pulp (*Citrus sinensis navelate*), 40% sucrose (Azucarera Española, Burgos, Spain) or healthy sweeteners (tagatose or oligofructose) and 1% agar-agar (Roko Agar, Llanera, Asturias, Spain) on the percentage of sucrose or sweeteners. Tagatose was obtained from Damhert Nutrition (Tagatesse, Heusden-

Zolder, Belgium) and according to the information of the label it was composed by 39.9% of tagatose, 39.9% of isomalt, 0.02% of sucralose and 20% of dietary fiber (inulin and oligosaccharides). Oligofructose was obtained from Sensus (Frutalose OFP, Roosendaal, Netherlands).

The following notation was used depending on the combination of sweeteners used: Control marmalade: 100% sucrose, Marmalade A: 50% oligofructose and 50% tagatose, Marmalade B: 30% oligofructose and 70% tagatose, and Marmalade C: 70% oligofructose and 30% tagatose.

Oranges collected directly from crop were peeled and mixed with sucrose or the corresponding combination of healthy sweeteners and the agar-agar in a thermal blender (Thermomix, TM31, Vorwerk, Wuppertal, Germany). After that, the mixture was cooked at 100 C for 20 min at 350 rpm. Then, glass jars previously sterilized in an autoclave at 121 C for 15 min, were filled with the marmalade. These jars were turned over to ensure proper sealing for 1 h. Finally, the marmalade was allowed to cool for 24 h and in that time jellification took place. Three batches of oranges were used to prepare the marmalades. Triplicated analyses were performed for each batch on the first day of storage and after 45 days of storage.

Analytical Determinations

Moisture Content, Brix, pH and Water Activity

Moisture content (x^w), was determined gravimetrically by drying approximately 1 g of marmalade to a constant weight in a vacuum oven at 60 C (method 934.06 AOAC 2000). Water activity (a_w) was determined with a dew point water activity meter Decagon Devices, Inc. (Aqua Lab 4TE, Pullman, WA, USA). Soluble solid content (Brix) was measured with a refractometer at 20 C (Atago 3T, Tokyo, Japan) and pH was registered with a pH-meter (Seven Easy, Mettler Toledo, Barcelona, Spain).

Antioxidant Capacity

The antioxidant activity of marmalades was analyzed on the basis of the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl free radical (Brand-Williams *et al.* 1995; Shahidi *et al*. 2006). One gram of marmalade was mixed with 6 mL of pure methanol for 5 min in a vortex, keeping the supernatant. This mixture was centrifuged at 13.000 rpm for 10 min.

The absorbance of 3.9 mL of the DPPH solution (0.025 mg/mL, prepared in methanol: water (80:20)) was read at 515 nm in a spectrocolorimeter Thermo Fisher Scientific, Inc. (Helios Zeta UV-VIS, Waltham, MA, USA). Then, 0.1 mL of the supernatant was mixed with the methanolic solution of DPPH and absorbance was read again after 30 min. Quantification was performed considering a standard curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed as mg of Trolox equivalent per 100 g of marmalade.

Optical Properties

The colour of orange marmalades placed in 20 mm-wide cuvettes was measured using a spectrocolorimeter Konica Minolta, Inc. (CM-3600d, Tokyo, Japan). CIE-L*a*b* coordinates were obtained using D65 illuminant and 10º observer as reference system.

Rheological Analysis

Rheological properties of studied orange marmalades were obtained using a controlled stress rheometer Thermo Fisher Scientific, Inc. (Haake RheoStress 1, Waltham, MA, USA), at 25 C. Measurements were carried out with plate–plate geometry and a 2.0 mm gap for steady state and oscillatory tests (Sato and Cunha 2009), by means of steady state essays or oscillatory essays to study the pseudoplastic or viscoelastic behavior of marmalades respectively. Firstly, steady state measurements were

performed with a shear rate linearly ranging from 0 to 100 s^{-1}, in 3 sweeps (up, down and up-cycles), in order to eliminate thixotropy. The data obtained in the third sweep were fitted to the Herschel–Bulkley model (eq.1) (Peinado *et al.* 2012).

This model can describe Newtonian and a large group of time independent non-Newtonian fluids. There are three parameters: τ is the shear stress (Pa), τ_0 is the yield stress above which the fluid starts flowing (Pa), γ is the shear rate (s^{-1}), k is the index of consistency ($Pa \cdot s^n$) and n is the index of fluidity (Skelland 1967).

$$\tau = \tau_0 + \kappa \cdot \gamma^n \qquad (1)$$

In second place, an oscillatory assay was carried out following the power-law that described the mechanical spectrum within the linear viscoelastic region in terms of storage (*G'*) and loss (*G"*) modulus as a function of frequency (eqs. 2 and 3) (Subramanian *et al.* 2006; Basua *et al*. 2011):

$$G' = a \cdot \omega^b \qquad (2)$$

$$G'' = c \cdot \omega^d \qquad (3)$$

Where, ω x is the angular speed ($rad \cdot s^{-1}$), a is the low frequency storage modulus (Pa^b); b is the power-law index for the storage modulus (dimensionless); c is the low frequency loss modulus (Pa^d); and, d is the power-law index for the loss modulus (dimensionless).

The value of the shear stress to fulfill the linearity of *G'* and *G"* was obtained in a preliminary trial. In order to do so, an interval of shear τ = 0.1-10 Pa was studied and three fixed frequencies were marked in the range of 0.1-10 Hz. Once these three curves were represented, the lineal zone of viscosity was obtained. Having chosen the value of τ in the linear zone for all the frequencies, the oscillatory assay was performed between 0.1-10 Hz (Peinado *et al.* 2012).

Microbiological Analysis

Serial dilutions were prepared by homogenising 10 g of marmalade with 90 mL of 1% sterile peptone water in a stomacher bag, using sterile techniques. Mesophilic aerobic populations were analysed in Plate Count Agar (Scharlau Chemie, 1-329, Barcelona, Spain) incubating samples for 72 h at 31 C. Yeast and moulds were determined in Sabouraud Chloramphenicol Agar (Scharlau Chemie, 1-166, Barcelona, Spain) plates for 5 days at 31 C. Samples were analysed at 45 days of storage.

Sensorial Analysis

An acceptance test using a 9-point hedonic scale (ISO 4121:2003) was used to evaluate the following attributes: color, aroma, texture, consistency, spreadable capacity, palatability, flavor, sweetness, bitterness, and global preference (ISO 5492:2008) in the three formulations with different combinations of healthy sugars (A, B and C) along with the control marmalade. The possible appearance of sineresis was also assessed. Moreover, the intention of buying was considered. The panel consisted of 30 trained panelists, in the age range of 20-50 years old, who are regular consumers of this kind of marmalades. Samples were presented in jars of 25 mL presented one after another. Three testing sessions were conducted in a sensory evaluation laboratory built according to the international standards for test rooms (ISO 8589: 2007).

Statistical Analysis

Statgraphics plus (version 5.1, Statpoint Technologies, Inc. Warrenton, Virginia, USA,) software was used to perform the statistical analyses. Analyses of variance (multifactor ANOVA) were carried out to discern whether the effect of the formulation or the time of storage on the studied marmalades was significant. The interactions between factors were considered.

RESULTS AND DISCUSSION

Compositional Characterization of Marmalades

Table 1 shows the results of moisture content (x^w), Brix, water activity (a_w), pH and antioxidant capacity. In all cases, the sugar concentration reached in marmalades was 50 Brix and it remained during the storage period considered. Moreover, marmalades B and C showed higher water content than the control and marmalade A. With time, the moisture content of samples B and C reduced, while in marmalade A it increased though in all cases there were slight differences. Regarding water activity, storage time was the factor which implied the most significant influence, showing a small decrease in water activity at 45 days of storage, except for sample A. Focusing on the pH, all marmalades showed values below 3.8, which would ensure proper microbiological stability. For all analyzed products, pH ranged between 3 and 4, in the same magnitude of order as the pH of other jams made using strawberry, peach, plum and apricot (Carbonell *et al.* 1991; García-Martínez *et al*. 2002).

Furthermore, in the control marmalade and sample A, pH decreased after storage as was also observed by Rababah *et al*. (2011) in strawberry jams with sucrose. In terms of antioxidant capacity, initially formulation C showed the highest value probably due to the greater amount of oligofructose in its composition, in contrast with the results of Ścibisz and Mitek (2009) who observed that high bush blueberry jams had the lowest levels of anthocyanins and total phenolics when oligofructose was included in their formulation. In our research, both formulation A and B reported lower antioxidant content than the control, showing that tagatose reacts less than table sugar with free radicals. These results differ from those reported by Zeng *et al*. (2012) who observed that via the Maillard reaction, rare sugars (especially D-tagatose) induced a more remarkable improvement than D-fructose in the radical scavenging activity and oxidation–reduction potential of the hydrolysates of tune backbone.

TABLE III.3.1. Values for moisture content (x^w), Brix, water activity (a_w), pH and antioxidant capacity of orange marmalades initially and after 45 days of storage.

FORMULATION	***TIME*** *(days)*	x^w *(g water/g marmalade)*	***ºBRIX***	a_w	***pH***	***ANTIOXIDANT CAPACITY*** *(mg Trolox/100 g marmalade)*
CONTROL	1	0.443 ± 0.003^{ab}	50.37 ± 0.15^{a}	0.9232 ± 0.0008^{b}	3.707 ± 0.006^{e}	47 ± 4^{c}
	45	0.437 ± 0.006^{a}	50.6 ± 0.2^{a}	0.916 ± 0.003^{a}	3.49 ± 0.010^{a}	81 ± 2^{g}
A	1	0.437 ± 0.006^{a}	50.1 ± 0.2^{a}	0.9227 ± 0.0006^{b}	3.770 ± 0.002^{g}	33.3 ± 0.8^{a}
	45	0.462 ± 0.004^{c}	50.2 ± 0.4^{a}	0.918 ± 0.004^{b}	3.680 ± 0.010^{d}	59.5 ± 0.8^{d}
B	1	0.509 ± 0.004^{e}	49.20 ± 0.10^{b}	0.9204 ± 0.0008^{b}	3.613 ± 0.015^{b}	39 ± 2^{b}
	45	0.4689 ± 0.0019^{c}	51.0 ± 1.10^{a}	0.913 ± 0.003^{a}	3.727 ± 0.006^{f}	65 ± 2^{e}
C	1	0.484 ± 0.0011^{d}	50.47 ± 0.06^{a}	0.918 ± 0.004^{b}	3.663 ± 0.006^{c}	58 ± 3^{d}
	45	0.449 ± 0.004^{b}	50.4 ± 0.4^{a}	0.911 ± 0.006^{a}	3.727 ± 0.006^{f}	76 ± 3^{f}

Equal letters indicate homogeneous groups

Moreover, in our study, the antioxidant capacity of marmalades increased over time, showing possible combinations of components which would imply the appearance of new antioxidants. However, Rababah *et al.* (2011) observed that antioxidant activity of orange marmalades prepared with sucrose decreased significantly after 3 and 4 months, and 5 months, respectively.

Rheological Properties

The rheological properties of the studied marmalades were determined by two tests: steady and oscillatory obtaining the parameters of the models considered in each case. The results obtained in the stationary test are presented in Figure 1. Rheograms indicate that initially there were no differences between samples, except for formulation B which scored slightly higher than the rest. After the storage period, the shear stress exceeded was reduced in all cases although to a lesser extent in marmalade A. Furthermore, in Table 2 the parameters of the Herschel-Bulkley model are shown. As expected, all marmalades showed a shear thinning behavior (n <1). However, the yield stress (τ_0) was similar in marmalade control and formulation A initially and after 45 days of storage whereas in formulations B and C the values of this parameter were significantly lower especially after storage in B. Besides, marmalade A showed the highest level of consistency after storage, giving evidences that the combination of oligofructose and tagatose in same proportions would improve consistency of marmalades during the storage. With respect to the index of fluidity (n), no significant differences were found considering the formulation studied but the time implied a reduction of this index in all cases. In studies carried out by other authors (Peinado *et al*. 2012; Rosa *et al*. 2009) where sugars were also replaced by other sweeteners (in this case isomaltulose) a decrease in consistency and cohesiveness of strawberry jams was observed respect to the sucrose-jams. In our study, only an increase of consistency was observed in the combination of 50% oligofructose and tagatose, but not for the other blends.

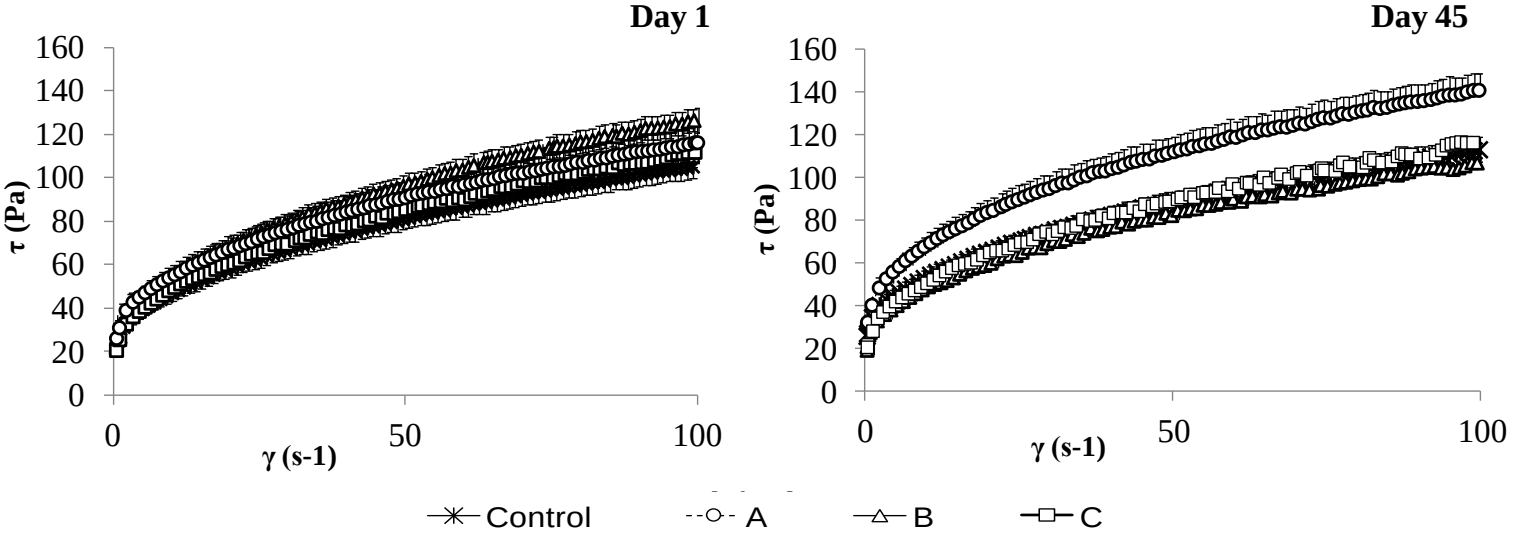

Figure III.3.1. Mean flow curves (rheograms) obtained from the steady assay of orange marmalades at the beginning and at the end of storage. Samples were coded respect to the amount of sugars as: Control (100% sucrose); A (50% oligofructose and 50% tagatose); B (30% oligofructose and 70% tagatose), and C (70% oligofructose and 30% tagatose).

Table III.3.2. Rheological parameters of the Herschel-Bulkley model and parameters of the power-law model for marmalades initially and at the end of storage.

		STEADY TEST- HERSCHEL-BULKLEY MODEL			**OSCILLATORY TEST- POWER LAW**			
					G'		**G"**	
FORMULATION	**TIME** (days)	τ (Pa)	***k***	***n***	***a***	***b***	***c***	***d***
CONTROL	1	18.8±0.9^{c}	9.8±1.2^{a}	0.48±0.02^{c}	390±95^{a}	0.16±0.01^{a}	76±31^{a}	0.28±0.04^{ab}
	45	18.5±0.6^{c}	11.6±0.7^{b}	0.46±0.01^{bc}	386±20^{a}	0.156±0.003^{a}	85±2^{a}	0.207±0.016^{a}
A	1	18.67±0.18^{c}	14±3^{bc}	0.43±0.03^{abc}	388±25^{a}	0.152±0.010^{a}	92±22^{a}	0.17±0.06^{a}
	45	18.21±1.06^{c}	18.6±0.5^{d}	0.41±0.01^{a}	680±37^{c}	0.150±0.003^{a}	146±1^{b}	0.183±0.006^{a}
B	1	15.7±.0.9^{b}	13.5±1,8^{bc}	0.46±0.02^{bc}	411±7^{ab}	0.17±0.01^{a}	90±7^{a}	0.27±0.03^{ab}
	45	11±3^{a}	13.9±1.3^{bc}	0.42±0.01^{a}	347±8^{a}	0.178±0.003^{a}	69±18^{a}	0.35±0.10^{b}
C	1	15±2^{b}	11.6±1.6^{b}	0.46±0.02^{bc}	468±57^{b}	0.167±0.017^{a}	105±17^{a}	0.24±0.02^{a}
	45	14.48±0.09^{b}	13.5±1.4^{bc}	0.44±0.01^{b}	374±36^{a}	0.20±0.07^{a}	92±25^{a}	0.26±0.09^{ab}

Equal letters indicate homogeneous groups

Figure 2 shows the rheological results of the oscillatory assay where the frequency dependence on storage (*G'*) and loss (*G"*) moduli of the orange marmalades formulated with healthy sweeteners are represented. This type of test determines the ratio between the elastic and viscous component of a material and it is useful to quantify to what extent it behaves as a solid or liquid.

Since in all cases *G'* was greater than *G"*, marmalades showed a semi-solid behavior. This is a typical gel characteristic being more elastic than viscous (Peinado *et al*. 2012).

Concerning the formulations studied, at the beginning of storage there were no differences between the control marmalade and the marmalade with the same amount of tagatose and oligofructose (formulation A), for both moduli (*G'* and *G"*). Moreover, marmalades B and C were similar in terms of viscous level, whereas the most elastic character was for marmalade with more oligofructose (C). At the end of storage, the increase in the elastic component of formulation A was noteworthy, unlike what was observed in the other marmalades, giving evidence of the interaction between the analyzed factors as a function of the sweeteners used. Besides in this assay, marmalade B had more similar elastic characteristics to the control marmalade.

In order to quantify in depth the differences between the oscillatory test of the analyzed samples, the values of both the storage (*G'*) and the loss (*G"*) moduli were fitted with respect to the angular speed (ω) with the power-law model as described in materials and methods. The parameters of this model are shown in Table 2. As can be observed, initially there were no significant differences between samples in *a* and *b* parameters of the power-law model for the storage modulus.

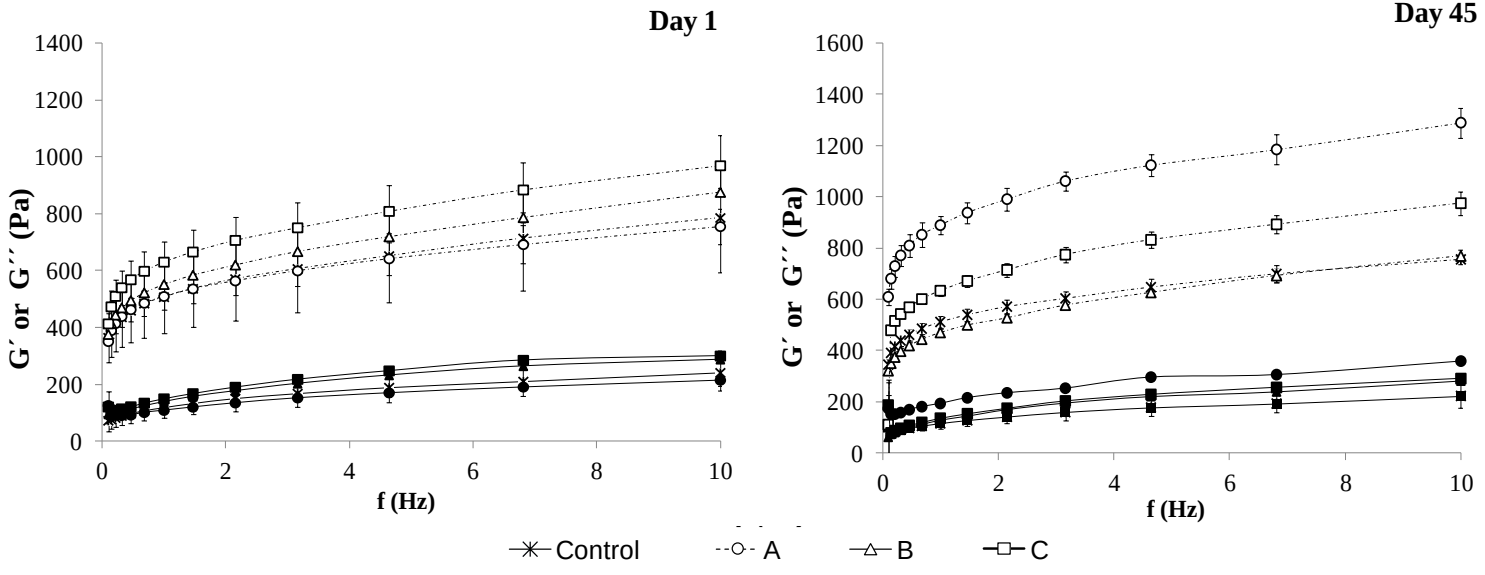

Figure III.3.2. Average frequency curves obtained in the oscillatory test of orange marmalades at the beginning and end of storage. Samples were coded respect to the amount of sugars as: Control (100% sucrose); A (50% oligofructose and 50% tagatose); B (30% oligofructose and 70% tagatose), and C (70% oligofructose and 30% tagatose). Empty symbols refer to values of storage modulus (G') and filled symbols refer to values of loss modulus (G").

However, in marmalade A *a* increased significantly after 45 days of storage, while the time factor did not affect the other formulations. This behavior would be consistent with that observed in Fig. 2, reflecting a more elastic nature of marmalade A at the end of storage. Regarding the terms related to the loss modulus (*c* and *d*) it should also be mentioned that marmalade A had a significantly greater value of *c* in coherence with the position of its curve *G"* versus frequency (ω) above the rest of the samples (Fig. 2). Nevertheless, these differences were much lower than in the case of the parameters related to the storage modulus. In addition, parameter *d* fluctuated more between the cases studied. In fact lower values were only observed in the formulation A with regard to the initial control and at the end of storage and higher values in marmalade B after 45 days.

In contrast to the few differences found in this study, Peinado *et al.* (2012) observed that by replacing sucrose for isomaltulose in the formulation of different strawberry spreadable products resulted in a decrease in parameters *a* and *c* of the power-law model. This decrease was associated with how sugar type influences the availability of water in the mixture of pectin-sugar-acid and therefore in the formation of hydrogen bonds and the possible association of water in the pectin polymer chain. In this study, the gelling agent used was agar-agar instead of pectin and it could have homogenized the rheological properties of the marmalade regardless of the type of sugar used, except in the case of marmalade A.

Optical Properties

Colorimetric coordinates *b** and *a** of the different studied sweet orange marmalades initially and at the end of storage placed in the chromatic plane are shown in Figure 3A. The new marmalades, especially for A and B formulations, increased *b** and *a** coordinates in comparison with the values of the control marmalade. Consequently the chrome ($C^*=(a^{*2}+b^{*2})^{1/2}$) followed the same trend, while the hue

(h*=arctg(b*/a*)) remained very similar to the control giving place to a similar appearance in all cases. Furthermore, values of initial and final luminosity (*L**) of the studied marmalades are also presented in Figure 3B. According to these results, initially, *L** of samples A and especially B was much higher than in the control marmalade unlike what happened to marmalade C. Therefore, high concentrations of oligofructose significantly reduced *L** of marmalades. Considering the time factor in *L**, the most stable formulations were A and C, while *L** in the control and B changed oppositely, increasing in the case of control and decreasing for B.

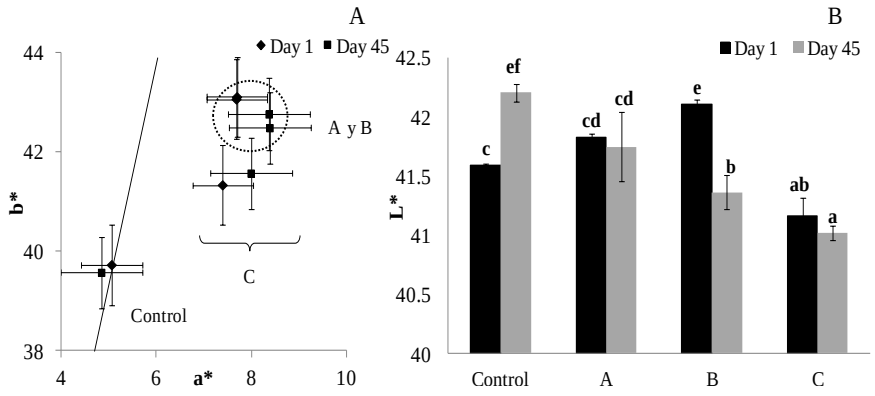

Figure III.3.3. A) Chromatic plane representation (b*-a*) of the studied marmalades initially and after 45 days of storage. Straight line represents the hue of control orange marmalade for day 1 from (0,0). B) Luminosity (L*) of the different formulations of marmalade initially and after 45 days of storage. Samples were coded respect to the amount of sugars as: Control (100% sucrose); A (50% oligofructose and 50% tagatose); B (30% oligofructose and 70% tagatose), and C (70% oligofructose and 30% tagatose). Equal letters indicate homogeneous groups.

In other studies, Peinado *et al*. (2015) showed that strawberry jams formulated with the healthier sugar isomaltulose and different concentrations of citric acid and pectin, darkened with time. Besides that, the colorimetric coordinates of the products containing the sucrose-isomaltulose blend seemed to be influenced by the percentages of pectin and citric acid while the colour of the samples containing the fructose-isomaltulose blend, did not seem to be affected by the different variables.

Therefore the influence of the different ingredients on the food system does not only depend on their concentration or distribution within the different system phases but also on the different component interactions during the studied period (Dervisi *et al.* 2001; Renard *et al.* 2006; Peinado *et al*. 2015).

Microbiological Analysis

There were no colonies of molds and yeast or aerobic mesophillic found in any of the marmalades in this study during the storage period considered.

Sensory Analysis

Figure 4 shows a radial chart of the average scores for each attribute evaluated (color, aroma, texture, consistency, spreadable capacity, palatability, flavor, sweetness and bitterness) besides the global preference and intention of buying of the studied marmalades. As can be seen, A and B formulations showed the highest scores in all attributes, although no significant differences were found in color, aroma, texture and consistency among the samples. Furthermore, A and B, which had higher proportions of tagatose, showed the highest sweetness.

This would be consistent with the recommendations given by the manufacturer of the commercial tagatose (two tablespoons of sucrose provides the same sweetness as one tablespoon of tagatose), although as was mentioned in the introduction, tagatose should have similar sweetening power to sucrose (Oh 2007; Taylor *et al.* 2008; Calzada-León *et al.* 2013).

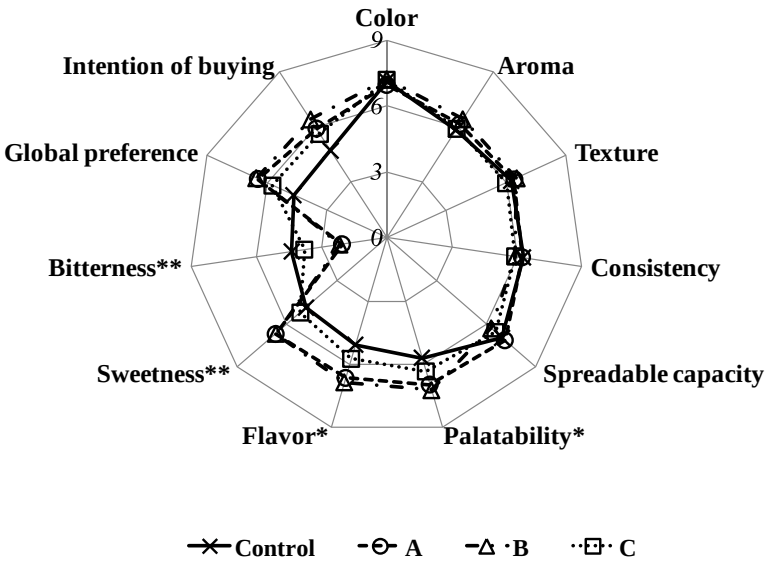

Figure III.3.4. Results of the sensory analysis in the evaluation of the samples coded respect to the amount of sugars as: Control (100% sucrose); A (50% oligofructose and 50% tagatose); B (30% oligofructose and 70% tagatose), and C (70% oligofructose and 30% tagatose).
* p-value <0.05, ** p-value <0.01.

However, the commercial tagatose used in this study was composed also by oligosaccharides, isomalt and sucralose. The higher sweetening power of this combination could be due to the synergic influence between these sweeteners. In fact, according to the Patent EP0946112 B1 (Dörr and Jager 2002), oligosaccharides increase the sweetness and improve the taste of an acesulfame-k/aspartame mixture.

Moreover, although the level of bitterness in the marmalades evaluated in this study was expected to be very low, analyzing the possible interference of the combination of sweeteners used in this property was deemed relevant.

Thus, the control and formulation C had the highest bitterness, showing the great ability of tagatose to hide this taste. Furthermore, no marmalade developed syneresis.

In relation to the rheological properties, the results obtained in the sensorial analysis were in accordance with those registered instrumentally. Finally, attention should be brought to the fact that both the global preference and buying intention of all marmalades formulated with healthy sweeteners were higher than those containing only sucrose.

CONCLUSIONS

The reformulation of orange marmalade with healthy sweeteners such as tagatose and oligofructose is feasible. Only oligofructose improved the antioxidant capacity compared to marmalade prepared with sucrose and also reduced its luminosity. In general, all marmalades had the same appearance.

In terms of rheology, formulation with the same proportion of tagatose and oligofructose improved consistency and elastic component of marmalades over time. Moreover, all of them reported microbiological stability in storage during the storage period considered. Finally, global acceptance and intention of buying of marmalades with healthy sweeteners were higher than for marmalade containing only sucrose.

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III.4. EVALUATION OF LEMON MARMALADE FORMULATED WITH NEW SWEETENERS (TAGATOSE AND ISOMALTULOSE)

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ABSTRACT

Marmalades are an alternative to fresh citrus fruit because they have a longer shelf life and their reformulation with healthier components could enhance their consumption. Thus, the aim of this study was to make lemon marmalades in which sucrose is replaced by sweeteners such as tagatose and isomaltulose, which are non-cariogenic and have a low glycemic index. Analyses of ºBrix, pH, moisture, water activity, antioxidant capacity, optical and rheological properties were carried out on marmalades on their first day of storage, and after 60 days of storage. Microbiological analyses were also performed. Moreover, a sensory evaluation was performed to assess its consumer acceptance as compared to marmalade made with sucrose. The results showed that the antioxidant capacity of the new formulations was lower than in marmalade with sucrose.

Moreover, marmalades made with healthy sweeteners showed lower consistency than those made with sucrose. Lemon marmalades formulated with a higher proportion of isomaltulose initially had high luminosity compared to the other samples, but browned over time. All marmalades were microbiologically stable, and the marmalades made with healthy sweeteners were scored better than those made with sucrose.

Keywords: lemon marmalade, sweeteners, tagatose, isomaltulose, antioxidant capacity, rheology, colour, microbiology, sensory analysis.

INTRODUCTION

Fruits have a short shelf life but high nutritional value. Marmalades are an alternative to fresh fruit, and they also provide an outlet for surplus fruit production, offering a very stable product. Sucrose has traditionally been used as the main sugar in marmalades. Sucrose provides a high energy input for daily activities due to its high glycemic index, but it is high in calories. Excessive consumption of sucrose can cause several diseases such as tooth decay, obesity and diabetes (Edwards, 2002;

O'Donnell and Kearsley, 2012; WHO, 2014). However, the food industry offers other natural sweeteners, which do not lead to such problems. In fact, this research group has already done studies on the reformulation of orange marmalade with a combination of tagatose and oligofructose on their rheological, optical, antioxidant and sensorial properties (Rubio-Arraez et al., 2015).

Tagatose and isomaltulose are two of those non-cariogenic sweeteners that are slowly released into blood. In fact, D-Tagatose (D-tag) it is considered a functional food because it is partially metabolized and the part that is not absorbed (80% of the intake) ferments in the colon, where it performs functions as soluble fiber (Taylor *et al*., 2008) favoring *lactic acid* and *Lactobacillus* bacteria (Petersen-Skytte, 2006). Moreover, it is a stereoisomer of D-galactose, it is found naturally in cheese and yoghurt (Oh, 2007; Lu *et al*., 2008).

Furthermore, it is very suitable for confectionary products, ice creams, soft drinks and breakfast cereals (Vastenavond *et al*., 2011), since it is almost as sweet as sucrose and its texture is very similar to sucrose (Oh, 2007; Taylor *et al.*, 2008; Calzada-León *et al.*, 2013). Additionally, tagatose has only 1.5 kcal/g and it does not cause dental caries (Levin, 2002). Tagatose received Generally Recognized as Safe status by the Food and Drug Administration in 2001 and entered the US market as a sweetener in 2003 (Donner *et al.*, 2010).

On the other hand, isomaltulose has a third of the sweetening power of sucrose and the physicochemical properties of isomaltulose enable it to be used as a substitute for sucrose in most sweet foods (Lina *et al.*, 2002; De Oliva-Neto y Menão, 2009; Peinado *et al.*, 2013). Furthermore, isomaltulose is a reducing disaccharide which is naturally present in honey, and sugar cane juice, its taste and viscosities of aqueous solutions are similar to those of sucrose and it has the same caloric power (Schiweck *et al*., 1990; Periche *et al.*, 2014).

Given the characteristics of these two sweeteners, they could be used to reformulate traditional foods to make them healthier for society. Thus, the aim of this study was to evaluate the potential use of healthy sweeteners (isomaltulose and tagatose) as an alternative to sucrose in lemon marmalades, by analyzing their colour, rheological properties, antioxidant capacity, microbiological stability and sensorial acceptance.

MATERIALS AND METHODS

Lemon Marmalade Formulations and Manufacturing Processes

Marmalades were produced using 50% lemon pulp (*Citrus limon eureka* also known as *Four Seasons*), 50% sucrose (Azucarera Española, Burgos, Spain) or healthy sweeteners (tagatose or isomaltulose) containing 1% agar-agar (Roko Agar, Asturias, Spain).Isomaltulose was obtained from Beneo (Palatinose, Mannheim, Germany). Tagatose was obtained from Damhert Nutrition (Tagatesse, Heusden-Zolder, Belgium), which was composed by 39.9% of tagatose 39.9% of isomalt, 0.02% of sucralose and 20% of dietary fiber (inulin and oligosaccharides). The following notation was used depending on the combination of sweeteners used: Control marmalade: 100 % sucrose, Marmalade A: 60% isomaltulose and 40% tagatose, Marmalade B: 50% isomaltulose and 50% tagatose, and Marmalade C: 30% isomaltulose and 70% tagatose. A commercial lemon marmalade was also characterized (*Ora et Labora*, Lemon Marmalade, Monasterio Santa Paula, Sevilla, Spain).

Lemons were selected and picked fresh. Subsequently, they were peeled and mixed with the corresponding combination of healthy sweeteners/sucrose and the agar-agar in a thermal blender (Thermomix, TM31, Vorwerk, Germany) for 3 min. Afterwards the mixture was cooked at 100 ºC for 20 min at 350 rpm. The glass jars, which had previously been sterilized in an autoclave at 121 ºC for 15 min, were then filled with the marmalade and turned over to ensure proper sealing for 1

hour. Finally, the marmalade was allowed to cool for 24 hours and became jellified. Three batches of lemons were used to prepare the marmalades. Analyses were triplicated on the first day of storage and after 60 days of storage.

Analytical Determinations

Water Activity, pH, Moisture Content and Brix

Water activity (a_w) was determined with a dew point water activity meter made by Decagon Devices, Inc. (Aqua Lab 4TE, Pullman, Washington, USA), at 25 ºC. The pH was registered with a pH-meter (Seven Easy, Mettler Toledo, Barcelona, Spain). Moisture content (x_w) was determined gravimetrically by drying approximately 1 g of marmalade until a constant weight, in a vacuum oven at 60ºC (method 934.06, AOAC 2000). The soluble solids content (Brix) was determined in a refractometer at 20ºC (Atago 3T, Tokyo, Japan).

Antioxidant Capacity

The antioxidant activity of marmalades was analyzed on the basis of the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl free radical (Brand-Williams *et al.* 1995; Shahidi *et al*. 2006). 1 g of marmalade was mixed with 6 mL of pure methanol for 5 min in a vortex, keeping the supernatant.

This mixture was centrifuged at 13000 rpm for 10 min. The absorbance of 3.9 mL of the DPPH solution (0.025 g/L, prepared in methanol: water (80:20)) was read at 515 nm in a spectrocolorimeter manufactured by Thermo Fisher Scientific, Inc. (Helios Zeta UV-VIS, Waltham, Massachusetts, USA). Then, 0.1 mL of the supernatant was mixed with the methanolic solution of DPPH and absorbance was read again after 30 min. Quantification was performed considering a standard curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic

acid) and the results were expressed as mg of Trolox equivalent per 100 g of marmalade.

Optical Properties

The optical properties of lemon marmalades were measured using a spectrocolorimeter manufactured by Konica Minolta, Inc. (CM-3600d, Tokyo, Japan). CIE-L*a*b* coordinates were obtained using D65 illuminant and 10º observer as the reference system. All analytical determinations were performed on sweet lemon marmalades in 20 mm-wide cuvettes.

Rheological Analysis

The rheological properties of the lemon marmalades studied were measured using a controlled stress rheometer manufactured by Thermo Fisher Scientific, Inc. (Haake RheoStress 1, Waltham, Massachusetts, USA), at 25 ºC. Measurements were carried out in triplicate with plate–plate geometry and a 2.0 mm gap for steady state and oscillatory tests (Sato and Cunha 2009), by means of steady state essays or oscillatory essays to study the pseudoplastic or viscoelastic behavior of marmalades, respectively.

Firstly, steady state measurements were performed linearly with a shear rate ranging from 0 to 100 s^{-1}, in 3 sweeps (up, down and up-cycles), in order to eliminate thixotropy. The data obtained in the third sweep were fitted to the Herschel–Bulkley model (equation 1). This model describes Newtonian and a large group of time independent non-Newtonian fluids. There are three parameters: τ is the shear stress (Pa), τ_0 is the yield stress above which the fluid starts flowing (Pa), γ is the shear rate (s^{-1}), k is the index of consistency ($Pa \cdot s^n$) and n is the index of fluidity (Skelland, 1967).

$$\tau = \tau_0 + \kappa \cdot \gamma^n \quad (1)$$

Secondly, an oscillatory assay was carried out based on the power-law describing the mechanical spectrum within the linear viscoelastic region in terms of storage (*G'*) and loss (*G"*) modulus as a function of frequency between 0.1-10 Hz (equations 2 and 3) (Subramanian *et al.* 2006; Basu *et al*. 2011):

$$G' = a \cdot \omega^b \quad (2)$$

$$G'' = c \cdot \omega^d \quad (3)$$

Where, ω is the angular speed (rad·s^{-1}), a is the low frequency storage modulus (Pa^b); b is the power-law index for the storage modulus (dimensionless); c is the low frequency loss modulus (Pa^d); and, d is the power-law index for the loss modulus (dimensionless). The shear stress value required to fulfil the linearity of *G'* and *G"* was obtained in a preliminary trial. For this purpose, an interval of shear τ = 0.1-10 Pa was studied and three fixed frequencies were marked in the range of 0.1-10 Hz. Once these 3 curves were represented, the lineal zone of viscosity was obtained, having chosen the value of τ in the linear zone for all the frequencies (Peinado *et al.* 2012).

Microbiological Analysis

Serial dilutions were prepared by homogenizing 10 g of marmalade with 90 mL of 1% sterile peptone water in a stomacher bag, using sterile techniques. Mesophilic aerobic populations were analyzed in a Plate Count Agar (Scharlau Chemie, 1-329, Barcelona, Spain), by incubating samples for 72 hours at 31ºC. Yeast and molds were determined in Sabouraud Chloramphenicol Agar (Scharlau Chemie, 1-166, Barcelona, Spain) plates kept at for 5 days. Samples were analyzed after 60 days of storage.

Sensorial Analysis

An acceptance test using a 9-point hedonic scale (ISO 4121,2003) was used to evaluate the following attributes: colour, arome, texture, consistency, spreadable capacity, palatability, flavour, sweetness, bitterness, and global preference (ISO 5492,2008) in the three formulations made with different combinations of healthy sugars (A, B and C), as well as the control marmalade. Additionally, the possible appearance of sineresis and intention of buying were assessed.

For this purpose, a panel was formed consisting of 30 trained panelists ranging in age from 20 to 50 years old, who are regular consumers of this kind of marmalades. Testing sessions were conducted in a sensory evaluation laboratory built according to the international standards for test rooms (ISO 8589, 2007).

Statistical Analysis

Statgraphics plus (Centurion, Statpoint Technologies, Inc., Warrenton, Virginia, USA.) software was used to perform the statistical analyses. Analyses of variance (multifactor ANOVA) were performed to study the interactions between the formulation and time of storage on the marmalades.

RESULTS AND DISCUSSION

Compositional characterization of marmalades

Table 1 shows the results reflecting the composition (Brix and moisture content (x_w)), pH, water activity (a_w) and antioxidant capacity of the lemon marmalades studied. It is noteworthy that none of the marmalades prepared with the new sweeteners reached the Brix of the commercial (≈65 Brix) or the control (≈59 Brix) samples. However, the new marmalades do meet the standards of Council Directive 2001/113/EC of 20 December 2001 relating to fruit jams, jellies and

marmalades and sweetened chestnut purée intended for human consumption, since this Directive allows soluble content lower than 60 Brix when sweeteners are used in the formulation for these products, rather than sugars. Over time the values of Brix remained constant. In coherence with the Brix results, marmalades formulated with the new sweeteners showed the highest values for moisture content ($x_{w)}$, whereas the commercial marmalades showed the lowest value, followed by the control sample. However, in this case, moisture content significantly increased after storage, probably due to the condensation of water vapor in the space located in the inner part of the lids.

Accordingly, the water activity of the commercial lemon marmalades was significantly lower than in the other cases. Among the new marmalades, the lowest water activity was registered for marmalade C, which had the highest amount of tagatose. Therefore, tagatose would make the water molecules more compact than isomaltulose. After storage, few changes were observed in a_w. It should also be highlighted that all samples prepared in this study showed lower values of pH than the commercial marmalades. Initially the marmalades B and C showed higher pH values, but all samples reached similar values after storage. Besides, all pH values were lower than 3.5, which would ensure a proper microbiological stability of these products, as was observed in other fruit jams made with strawberry, peach, plum or apricot (Carbonell *et al.*, 1991; García-Martínez *et al*., 2002).

Also noteworthy was that the lemon marmalade formulations A, B and C showed a lower antioxidant content than the control marmalade, although time significantly reduced the antioxidant content in all cases. Furthermore, the commercial sample showed only 0.11±0.04 mg Trolox eq/100 g which would be consistent with the rapid deterioration of antioxidant compounds in which occurs in lemon marmalade. In our previous study (Rubio-Arraez et al., 2015) it was observed that the highest proportion of oligofructose contributed to improve the initial antioxidant capacity of orange marmalades.

Table III.4.1. Values for moisture content (x_w), Brix, water activity (a_w) and pH of lemon marmalades initially and after 60 days of storage. Values for commercial lemon marmalade are also included.

FORMULATION	***TIME*** *(days)*	x_w *(g water/ g marmalade)*	***ºBRIX***	a_w	***pH***	***ANTIOXIDANT CAPACITY*** *(mg Trolox/ 100 g marmalade)*
CONTROL	1	0.374 ± 0.005^{a}	58.93 ± 0.06^{d}	0.8470 ± 0.0005^{a}	2.011 ± 0.011^{a}	80.09 ± 3.38^{c}
	60	0.417 ± 0.007^{c}	58.97 ± 0.06^{d}	0.8439 ± 0.0006^{a}	2.492 ± 0.011^{d}	37.42 ± 1.81^{a}
A	1	0.425 ± 0.002^{d}	55.07 ± 0.12^{a}	0.9024 ± 0.0009^{d}	2.123 ± 0.015^{b}	67.19 ± 3.86^{b}
	60	0.441 ± 0.003^{e}	55.23 ± 0.06^{a}	0.8998 ± 0.0011^{c}	2.553 ± 0.006^{e}	35.52 ± 2.40^{a}
B	1	0.419 ± 0.006^{cd}	55.23 ± 0.06^{a}	0.8992 ± 0.0004^{c}	2.487 ± 0.006^{d}	69.95 ± 11.94^{b}
	60	0.456 ± 0.005^{f}	55.27 ± 0.06^{a}	0.89986 ± 0.00011^{c}	2.557 ± 0.006^{e}	39.14 ± 1.28^{a}
C	1	0.407 ± 0.004^{b}	56.40 ± 0.10^{c}	0.8923 ± 0.0019^{b}	2.467 ± 0.006^{c}	65.10 ± 2.96^{b}
	60	0.447 ± 0.005^{ef}	55.67 ± 0.06^{b}	0.89060 ± 0.0005^{b}	2.577 ± 0.006^{f}	33.04 ± 7.84^{a}
COMMERCIAL	—	0.2986±0.0016	64.57±0.06	0.8058±0.0006	3.21±0.03	0.11±0.04

Equal letters indicate homogeneous groups

Rheological Properties

The rheological properties of marmalades studied were determined using two tests, both steady and oscillatory, to obtain the parameters of the models considered in each case. The results obtained for the stationary test of lemon marmalades, based on the combination of sweeteners used and the storage time, is presented in Figure 1.

The rheograms of lemon marmalades fluctuated, possibly due to the increased presence of lumpy parts. In any case, over the storage time, the curves remained similar. The rheograms of commercial lemon marmalade showed no clear trend due to the high presence of cells and lemon peel. Therefore, the curve is not shown. The parameters of the Herschel-Bulkley model for lemon marmalades studied at the beginning and the end of the period considered are shown in Table 2.

As can be seen, the use of the new sweeteners initially led to lower values of shear stress (τ) than in marmalades prepared with sucrose. However, the consistency index (k) was initially significantly higher in marmalade with the highest amount of tagatose (formulation C). In studies carried out by other authors, (Peinado *et al*. 2012) where sugars were also replaced by other sweeteners (isomaltulose), a decrease in consistency and cohesiveness of strawberry jams was observed.

On the other hand, the storage time caused a compaction in lemon marmalades since the values of shear stress significantly increased. The index of fluidity (n) also increased over time but this increase was only significant in formulation A, which might be due to the fact that the isomaltulose content was higher. However, the index of consistency (k) significantly decreased in formulation C, dropping to half the initial index, despite registering the highest initial values, as previously mentioned. In our previous study (Rubio-Arraez et al., 2015), the orange marmalade with the same proportions of oligofructose and tagatose was more consistent.

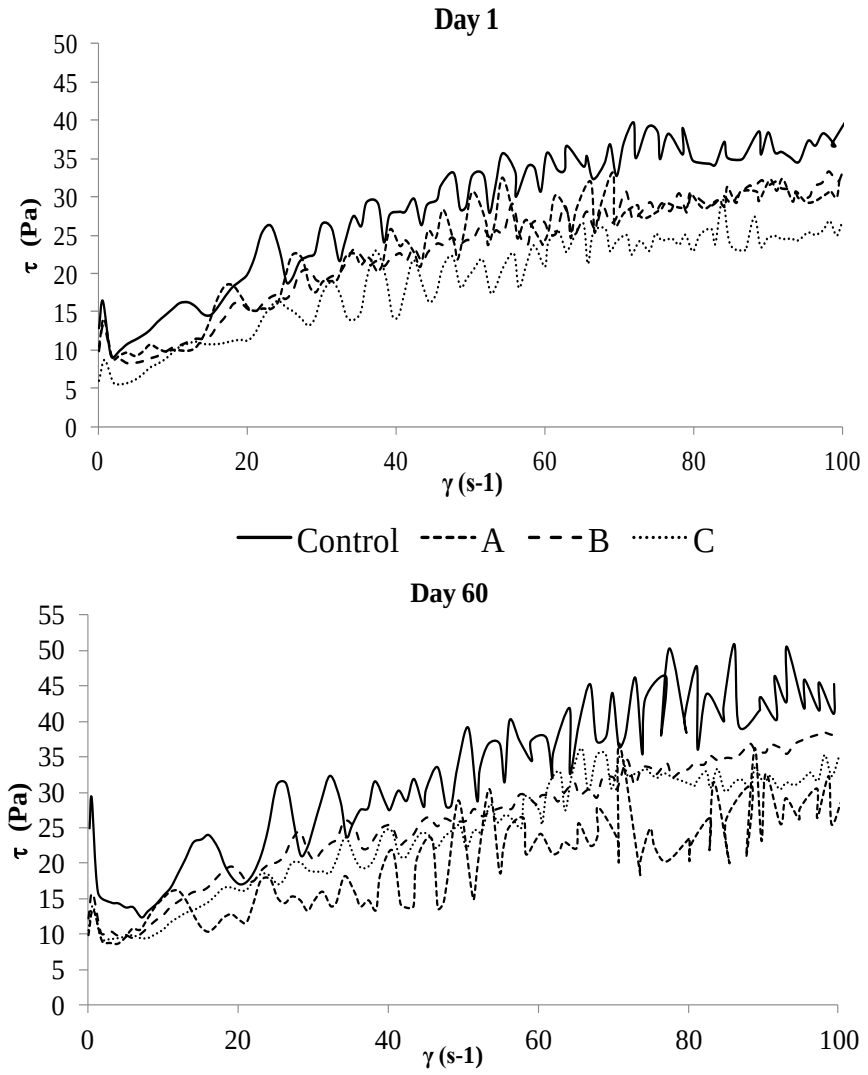

Figure III.4.1. Mean flow curves (rheograms) obtained from the steady assay of lemon marmalades at the beginning and at the end of storage. Samples were coded respect to the amount of sugars as: Control (100% sucrose); A (60% isomaltulose and 40% tagatose); B (50% isomaltulose and 50% tagatose), and C (30% isomaltulose and 70% tagatose).

Table III.4.2. Rheological parameters of the Herschel-Bulkley model and parameters of the Power-Law model for marmalades initially and at the end of storage. Equal letters indicate homogeneous groups.

		STEADY TEST-HERSCHEL-BULKLEY MODEL			
FORMULATION	**TIME** (days)	τ (Pa)	***k***	***n***	η (Pa·s) **(γ=50 s^{-1})**
CONTROL	1	8±2^{c}	1.8±1.0^{ab}	0.629±0.090^{a}	0.59±0.06^{a}
	60	12±2^{c}	1.08±0.87^{a}	0.752±0.169^{ab}	0.60±0.16^{a}
A	1	6.2±0.7^{b}	1.6±0.6^{ab}	0.64±0.09^{a}	0.52±0.08^{a}
	60	10.5±0.6^{c}	0.4±0.2^{a}	0.865±0.117^{b}	0.446±0.107^{a}
B	1	5.8±.0.9^{b}	1.7±0.5^{ab}	0.59±0.07^{a}	0.44±0.04^{a}
	60	9.1±1.6^{a}	0.8±0.3^{a}	0.8±0.1^{ab}	0.47±0.09^{a}
C	1	3.2±0.6^{a}	2.403±1.014^{b}	0.5±0.1^{a}	0.45±0.09^{a}
	60	8.6±2.3^{c}	1.2±0.3^{a}	0.69±0.07^{ab}	0.52±0.16^{a}

(cont.) Table III.4.2. Rheological parameters of the Herschel-Bulkley model and parameters of the Power-Law model for marmalades initially and at the end of storage. Equal letters indicate homogeneous groups.

		OSCILLATORY TEST-POWER LAW			
		G´		G´´	
FORMULATION	**TIME** (days)	***a***	***b***	***c***	***d***
CONTROL	1	449 ± 125^{b}	$0{,}0099\pm0{,}0004^{ab}$	102 ± 27^{b}	$0{,}01123\pm0{,}00102^{a}$
	60	586 ± 35^{c}	$0{,}0099\pm0{,}0002^{ab}$	138 ± 9^{b}	$0{,}0098\pm0{,}0009^{a}$
A	1	194 ± 99^{a}	$0{,}0104\pm0{,}0002^{b}$	65 ± 13^{a}	$0{,}0107\pm0{,}0007^{a}$
	60	289 ± 15^{a}	$0{,}0104\pm0{,}0003^{b}$	70 ± 2^{a}	$0{,}0098\pm0{,}0007^{a}$
B	1	210 ± 29^{a}	$0{,}013\pm0{,}003^{b}$	54 ± 9^{a}	$0{,}014\pm0{,}004^{a}$
	60	268 ± 44^{a}	$0{,}0109\pm0{,}0001^{b}$	63 ± 10^{a}	$0{,}0125\pm0{,}0003^{a}$
C	1	232 ± 18^{a}	$0{,}0083\pm0{,}0016^{a}$	56 ± 5^{a}	$0{,}0089\pm0{,}0010^{a}$
	60	308 ± 32^{a}	$0{,}01077\pm0{,}00015^{a}$	74 ± 6^{a}	$0{,}0113\pm0{,}0006^{a}$

As for the results of the oscillatory test, Figure 2 shows the evolution of the storage (G') and loss (G") moduli *versus* frequency for the lemon marmalades studied. Furthermore, the results of varying both G' and G'' were adjusted for the angular velocity (ω) (rad·s^{-1}) to the model of the Power Law. The resulting values of the parameters of this model are presented in Table 2. This type of test determines the ratio between the elastic and viscous component of a material and quantifies to which the material behaves as a solid or liquid. Specifically, the storage modulus (G') is associated with the elastic component of the material, whiles the loss modulus (G"), is associated with its viscous component. Since in all cases G' was greater than G" moduli the lemon marmalades showed a semi-solid behavior (Peinado *et al.*, 2012).

This characteristic is typical of a gel, since it is more elastic than viscous. The parameters *a* and *c* decreased significantly with new sweeteners, but there were no differences between the combinations studied. The storage time increased these parameters in all cases, but the increase was only significant in the control marmalade. These results are consistent with the curves presented in Figure 2, where the marmalade control curves of G' and G" as a function of frequency are placed above the others, especially at the end of storage. Besides, Peinado *et al.* (2012) observed the same when sucrose was replaced by isomaltulose in the reformulation of different strawberry spreadable products.

This decrease was associated with how the type of sugar influences the availability of water in the mixture of pectin-sugar-acid, and therefore in the formation of hydrogen bonds and the possible association of water in the pectin polymer chain (Peinado et al., 2012). In the present study, the gelling agent used was agar-agar instead of pectin and it could have homogenized the rheological properties of the marmalade regardless of the type of sugar used. However, the parameters *b* and *d* were similar in all marmalades. Besides, in orange marmalade formulated with oligofructose and tagatose as a substitutes of sucrose and agar-agar there was an increase in the elastic component (G') after 45 days of storage (Rubio-Arraez et al., 2015).

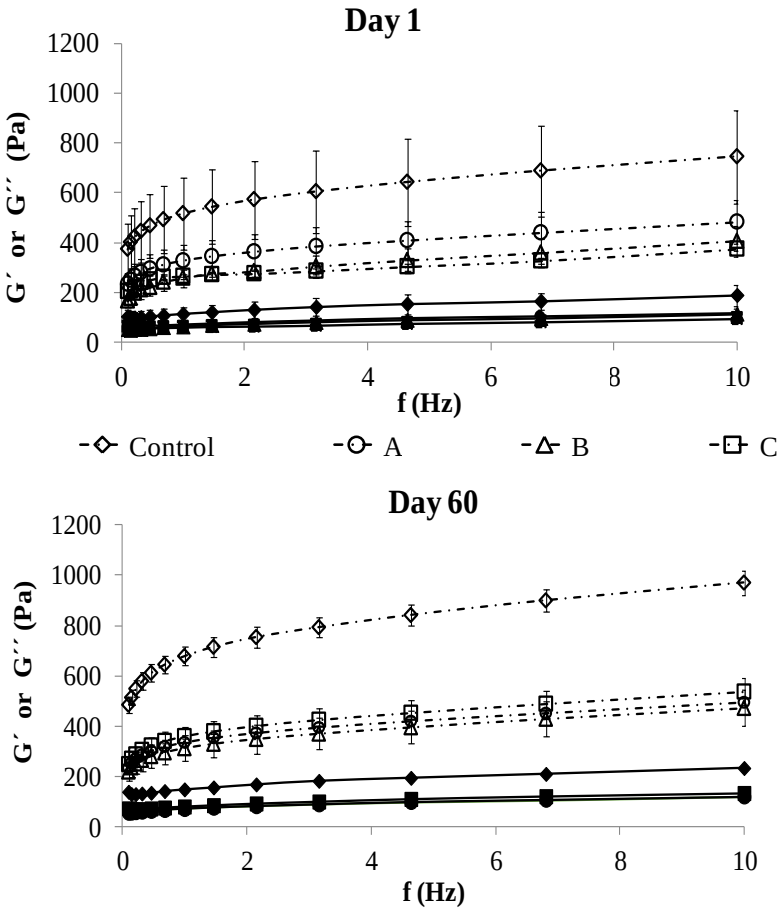

Figure III.4.2. Average frequency curves obtained in the oscillatory test of lemon marmalades at the beginning and end of storage. Samples were coded respect to the amount of sugars as Control (100% Sucrose); A (60% isomaltulose and 40% tagatose); B (50% isomaltulose and 50% tagatose), and C (30% isomaltulose and 70% tagatose). Empty symbols referred to values of G´ and filled symbols referred to values of G´´.

Optical Properties

Figure 3 shows the interaction charts for the colorimetric coordinates L*, a* and b*, chroma ($C^*=(a^{*2}+b^{*2})^{1/2}$) and hue ($h^*=arctg(b^*/a^*)$) of the

different lemon marmalades studied, both initially and at the end of storage. On the other hand, the values for these coordinates of the commercial lemon marmalade were L*=35.03±0.11, a*= 1.57±0.05, b*= 9.7±0.1, chroma=9.81 ± 0.08 and hue=80.80±0.24. It should be pointed out that the storage time for this commercial marmalade was unknown. As can be seen, the *L** of control marmalade and the samples formulated with combination B and C were very similar, while sample A, which had the highest percentage of isomaltulose, initially had a higher luminosity, although luminosity decreased at the end of storage as in the other cases. The orange marmalades formulated with oligofructose and tagatose had a similar appearance, but oligofructose reduced L* and the highest content of tagatose also decreased L*, a* and b* after 45 days of storage (Rubio et al., 2015).

Coordinate a* for the marmalade with formulation B initially showed the highest value. However, after 60 days of storage no significant differences were observed between the new marmalades, although their coordinate a* was higher than in the control marmalade. In contrast, coordinate b* decreased after storage. Consequently, the chroma (C*) and hue (h*) decreased during storage, leading to browning in the marmalades. This browning could also be related to a reduction in polyphenols (antioxidant capacity) over time, which would be also responsible for the previously mentioned increase in pH after storage, which occurred in the samples.

These results are also consistent with those found by Peinado *et al*., (2015) who reported that strawberry jams formulated with the healthy sweetener isomaltulose and different concentrations of citric acid and pectin darkened during storage. Additionally, the colorimetric coordinates of the products containing the sucrose-isomaltulose mixture seemed to be influenced by the percentages of pectin and citric acid, while the color of the samples containing the fructose-isomaltulose mixture did not seem to be affected by the different variables. Therefore the influence of the different ingredients on the food system does not only depend on their concentration or distribution within the different system phases but also on the different component interactions during the period studied (Dervisi *et al.* 2001; Peinado *et al*. 2015).

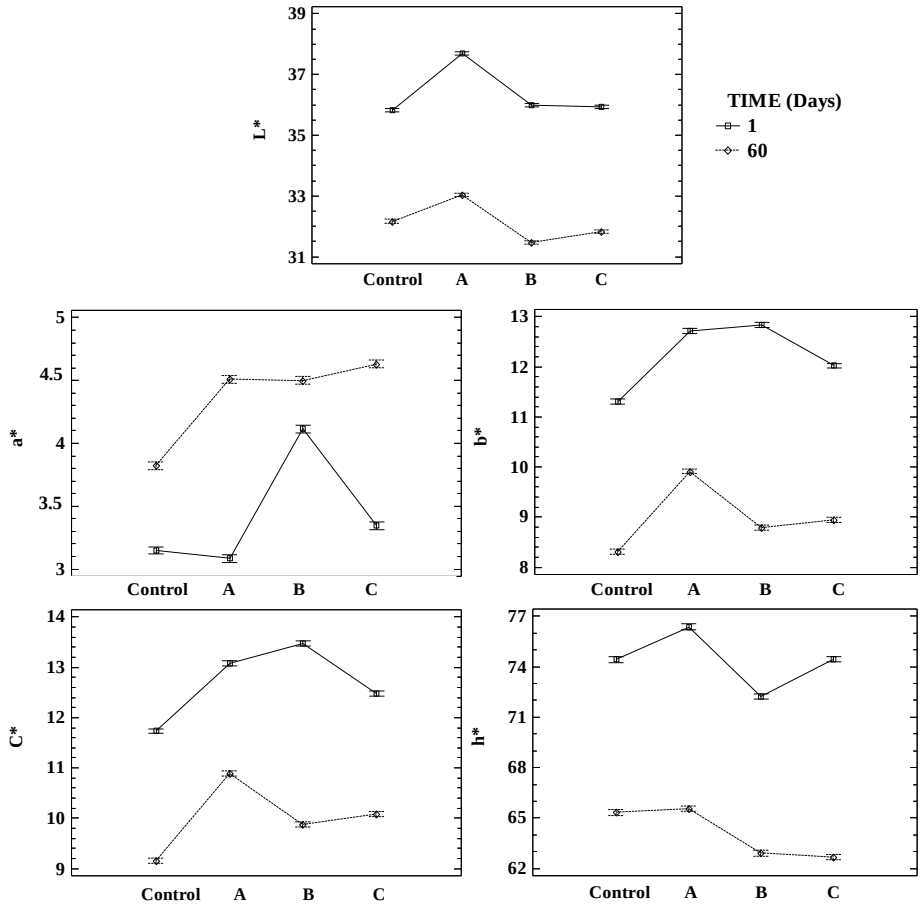

Figure III.4.3. Interaction graphics (significant level of 95%) of colour parameters: L*, a*, b* coordinates, chroma (C*) and hue (h*) of the lemon marmalade as a function of the formulation and storage time. Samples were coded respect to the amount of sugars as: Control (100% Sucrose); A (60% isomaltulose and 40% tagatose); B (50% isomaltulose and 50% tagatose), and C (30% isomaltulose and 70% tagatose).

Microbiological Analysis

There were no colonies of molds and yeast or aerobic mesophillic found in any of the marmalades in this study over the storage period considered.

Therefore, in all cases the products were stable from a microbiological point of view.

Sensory Analysis

Figure 4 shows a radial chart of the average scores for each attribute evaluated. No significant differences in colour, arome, texture, spreadability and consistency were detected. However, new formulations improved palatability with respect to the control marmalade. Moreover, although the bitterness level for the marmalades evaluated in this study was expected to be very high, it seems that the combination of sweeteners reduced this bitterness, due more to the effect of tagatose than to the effect of isomaltulose.

Thus, the control marmalade had the highest bitterness followed by formulation A, C and B. Besides, the lemon marmalades with the highest amounts of tagatose (B and C) were the sweetest, showing that there is a sweetness threshold for a concentration of tagatose of 50% in the proportion of sweeteners used in the formulations. Although tagatose should have a sweetening power similar to sucrose (Oh 2007; Taylor et al. 2008; Calzada-León *et al.* 2013), the sweetening powers were not similar in this case.

This behavior could be due to the fact that the commercial tagatose used in this study was composed also by oligosaccharides, isomalt and sucralose, which increased the sweetening power for their due a synergic effect. In fact, according to the Patent EP0946112 B1 (Dörr and Jager 2002), oligosaccharides increase the sweetness and improve the taste of an acesulfame-k/aspartame mixture. Consequently, the formulation B showed the highest values of acceptance and intention of buying, followed by formulation C, without significant differences between the two marmalades. Additionally, the new lemon marmalades were evaluated as being better than the control. Moreover, in the case of orange marmalades (Rubio-Arraez et al., 2015) those prepared with new healthy sweeteners (tagatose and oligofructose) had better scored than marmalade prepared with sucrose.

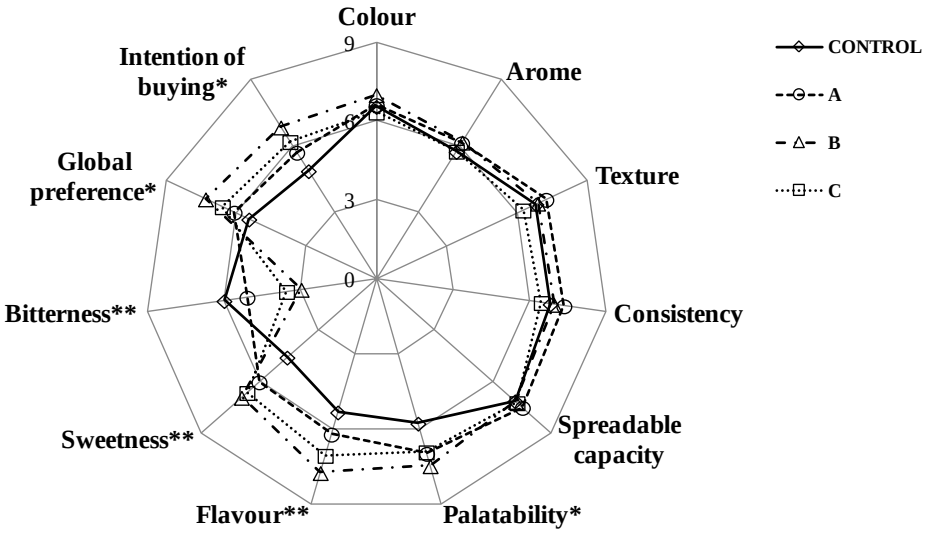

Figure III.4.4. Results of the sensory analysis in the evaluation of the lemon marmalades coded respect to the amount of sugars as: Control (100% Sucrose); A (60% isomaltulose and 40% tagatose); B (50% isomaltulose and 50% tagatose), and C (30% isomaltulose and 70% tagatose).* p-value <0.05, ** p-value <0.01.

CONCLUSIONS

The reformulation of lemon marmalade with non-cariogenic sweeteners such as tagatose and isomaltulose is possible, since although the new marmalades did not reach the same concentration of soluble solids as marmalades made with sucrose, they were microbiologically stable over the storage period considered. More specifically, isomaltulose increased their luminosity and hue. Furthermore, the combination of the new sweeteners did not influence viscoelasticity, although it was lower than in marmalade with sucrose. Finally, tagatose led to the best scores for lemon marmalades, mainly due to its high sweetening power.

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III.5. EFFECT OF REPLACING SUGAR WITH TAGATOSE AND ISOMALTULOSE IN MANDARIN ORANGE MARMALADE ON RHEOLOGY, COLOUR, ANTIOXIDANT CAPACITY, AND SENSORY PROPERTIES

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ABSTRACT

The aim of this study was to make mandarin orange marmalades in which sucrose is replaced by sweeteners such as tagatose and isomaltulose, which are non-carcinogenic and have a low glycemic index. Analyses of rheology, colour, antioxidant capacity, microbiology and sensory properties were carried out on marmalades on their first day of storage, and after 90, 180 and 360 days of storage. The results showed that marmalades made with healthy sweeteners had a less elastic character and were thinner in consistency than those made with sucrose. Luminosity was shown to be highest in mandarin orange marmalades made with tagatose, although colour was stable for 6 months to one year of storage. Tagatose also enhanced the antioxidant activity of these marmalades. All marmalades were microbiologically stable. Finally, marmalades made with tagatose alone scored the highest for global acceptance and intention of buying by consumers.

Keywords: Marmalade, mandarin orange, tagatose, isomaltulose, rheology, colour, sensory analysis.

INTRODUCTION

Mandarin orange fruits (*Citrus reticulata*) have a high nutritional composition (high content of phenolics, ascorbic acid, dietary fiber, etc.) and their consumption prevent diseases mainly due to this fruit's antioxidant activity (Dhuique-Mayer and others 2005; Balasundram and others 2006). Citrus are the most popular fruits within European and North American Consumers and Spain is one of the major producers and exporters of this fruit (Gorinstein and others 2001).

Mandarin oranges are usually consumed as fresh fruit but also as marmalades or juice. Most marmalades are prepared with sucrose. However, this sugar has a high glycemic index and is also high in calories. Consequently, excessive consumption of sucrose can cause several diseases such as obesity, diabetes and tooth decay (Edwards 2002; O'Donnell and

Kearsley 2012; WHO 2014). Nowadays, there are other alternative natural sweeteners available in the market such as tagatose and isomaltulose, whose properties are healthier. These sweeteners are non-cariogenic and are released slowly into blood.

D-Tagatose (D-tag) is a stereoisomer of D-galactose. It can also be produced cheese and yoghurt (Oh 2007; Lu and others 2008) and it has only 1.5 kcal/g (Levin 2002). Because it favors the growth of *Lactobacillus* bacteria (Petersen-Skytte 2006) it is considered a functional food (Taylor and others 2008). Furthermore, tagatose can be used to make products such as ice creams, soft drinks and breakfast cereals (Vastenavond and others 2011) since its texture is very similar to sucrose and it is almost as sweet as sucrose (Oh 2007; Taylor and others 2008; Calzada-León and others 2013). Tagatose received Generally Recognized as Safe status by the Food and Drug Administration in 2001 (Donner and others 2010).

On the other hand, isomaltulose is a reducing disaccharide which is naturally present in honey, and sugar cane juice. Its caloric power, appearance, taste and viscosities of aqueous solutions are similar to those of sucrose (Schiweck and others 1990; Periche and others 2014). Moreover, given the physicochemical properties of isomaltulose, it can be used as a substitute for sucrose in most sweet foods and it has a third of the sweetening power of sucrose (Lina and others 2002; De Oliva-Neto and Menão, 2009; Peinado and others 2013).

Given the properties of these two sweeteners (isomaltulose and tagatose), the aim of this work was to evaluate their potential use as an alternative to sucrose in mandarin orange marmalades. For this purpose, their antioxidant capacity, rheological properties, colour, and sensorial acceptance were analyzed.

MATERIALS AND METHODS

Mandarin orange Marmalade Formulations and Manufacturing Processes

Marmalades were produced using 50% mandarin orange pulp (*Citrus reticulata Clementina*), 50% sucrose (Azucarera Española, Burgos, Spain) or sweeteners (tagatose or isomaltulose) containing 1% agar-agar (Roko Agar, Llanera, Asturias, Spain). Isomaltulose (Beneo, Mannheim, Germany), Tagatose (Tagatesse, Heusden-Zolder, Belgium).

The following notation was used depending on the combination of sweeteners used: Control marmalade: 100% sucrose, Marmalade A: 75% tagatose and 25% isomaltulose, Marmalade B: 50% tagatose and 50% isomaltulose, Marmalade C: 25% tagatose and 75% isomaltulose and Marmalade D: 100 % tagatose. Mandarin oranges were selected and picked fresh. Subsequently, they were peeled and mixed with the corresponding combination of healthy sweeteners/sucrose and the agar-agar in a thermal blender (Thermomix, TM31, Vorwerk, Wuppertal, Germany) for 3 min.

Afterwards the mixture was cooked at 100 ºC for 20 min at 350 rpm. The glass jars, which had previously been sterilized in an autoclave at 121ºC for 15 min, were then filled with the marmalade and turned over to ensure proper sealing for 1 hour. Finally, the marmalade was allowed to cool for 24 hours and became jellified. Three batches of mandarins were used to prepare the marmalades. Analyses were triplicated on the first day of storage and after 90, 180 and 360 days of storage.

Analytical Determinations

Rheological Analysis

The rheological properties of the mandarin orange marmalades studied were analyzed using a controlled stress rheometer manufactured by Thermo Fisher Scientific, Inc. (Haake RheoStress 1, Waltham, Massachusetts, USA), at 25ºC. Measurements were carried out in triplicate with plate–plate geometry and a

2.0 mm gap for steady state and oscillatory tests (Sato and Cunha 2009), by means of steady state essays or oscillatory essays to study the pseudoplastic or viscoelastic behavior of marmalades, respectively.

Firstly, an oscillatory assay was carried out, following the power-law describing the mechanical spectrum within the linear viscoelastic region in terms of storage (*G'*) and loss (*G"*) modulus as a function of frequency ranging from 0.1-10 Hz (equations 1 and 2) (Subramanian and others 2006; Basu and others 2011):

$$G' = a \cdot \omega^b \quad (1)$$

$$G" = c \cdot \omega^d \quad (2)$$

Where, ω is the angular speed (rad·s^{-1}), a is the low frequency storage modulus (Pa^b); b is the power-law index for the storage modulus (dimensionless); c is the low frequency loss modulus (Pa^d); and, d is the power-law index for the loss modulus (dimensionless). The shear stress value required to fulfill the linearity of *G'* and *G"* was obtained in a preliminary trial. For this purpose, an interval of shear τ = 0.1-10 Pa was studied and three fixed frequencies were marked in the range of 0.1-10 Hz. Once these 3 curves were represented, the lineal zone of viscosity was obtained. The value of τ in the linear zone was chosen for all frequencies (Peinado and others 2012).

Secondly, steady state measurements were performed with a shear rate linearly ranging from 0 to 100 s^{-1}, in 3 sweeps (up, down and up-cycles), in order to eliminate thixotropy. The data obtained in the third sweep were fitted to the Herschel–Bulkley model (equation 3). This model describes both Newtonian and a large group of time independent non-Newtonian fluids. There are three parameters: τ is the shear stress (Pa), τ_0 is the yield stress above which the fluid starts flowing (Pa), γ is the shear rate (s^{-1}), k is the index of consistency (Pa·s^n) and n is the index of fluidity (Skelland 1967).

$$\tau = \tau_0 + \kappa \cdot \gamma^n \quad (3)$$

Optical Properties

The Optical properties of mandarin orange marmalades were measured using a spectrocolorimeter Konica Minolta, Inc. (CM-3600d, Tokyo, Japan). All analytical determinations were performed on mandarin orange marmalades in 20 mm-wide cuvettes. CIE-L*a*b* coordinates were obtained using D65 illuminant and 10º observer as reference system.

Antioxidant Capacity

The antioxidant activity of marmalades was analyzed on the basis of the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl free radical (Brand-Williams and others 1995; Shahidi and others 2006). 1 g of marmalade was mixed with 6 mL of pure methanol for 5 min in a vortex, keeping the supernatant. This mixture was centrifuged at 13,000 rpm for 10 min.

The absorbance of 3.9 mL of the DPPH solution (0.025 mg/mL, prepared in methanol: water (80:20)) was read at 515 nm in a spectrocolorimeter Thermo Fisher Scientific, Inc. (Helios Zeta UV-VIS, Waltham, Massachusetts, USA). Then, 0.1 mL of the supernatant was mixed with the methanolic solution of DPPH and absorbance was read again after 30 min. Quantification was performed considering a standard curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed as mg of Trolox equivalent per 100 g of marmalade.

Microbiological Analysis

Serial dilutions were prepared by homogenizing 10 g of marmalade with 90 mL of 1% sterile peptone water in a stomacher bag, using sterile techniques. Sample for analysis were taken on days 1, 90, 180 and 360. Yeast and moulds were determined in Sabouraud Chloramphenicol Agar (Scharlau Chemie, 1-166, Barcelona, Spain) plates for 5 days at 31ºC. Mesophilic aerobic populations were analyzed in Plate Count Agar (Scharlau Chemie, 1-329, Barcelona, Spain) incubating samples for 72 hours at 31ºC.

Sensorial Analysis

An acceptance test using a 9-point hedonic scale (ISO 4121:2003) was used to evaluate the following attributes: colour, arome, texture, consistency, spreadable capacity, palatability, flavour, sweetness, bitterness, global preference (ISO 5492:2008) and intention of buying in the three formulations with different combinations of healthy sugars (A, C and D), as well as the control marmalade. A panel was formed consisting of 30 trained panelists ranging in age from 20 to 50 years old, who are regular consumers of this kind of marmalades.Testing sessions were conducted in a sensory evaluation laboratory built according to the international standards for test rooms (ISO 8589: 2007). The marmalade B, formulated with isomaltulose and tagatose at the same proportion, was not considered to sensorial analysis since the aim of this test was to determine the consumers' preference for tagatose or isomaltulose and the other marmalades had higher amount of each of these sweeteners.

Statistical Analysis

Analyses of variance (multifactor ANOVA) were performed using a multiple comparison test and LSD test (α=95%), with Statgraphics Centurion software (Statpoint Technologies, Inc. Warrenton, Virginia, USA). More specifically, the interactions between factors (time of storage and formulation on the marmalades) were studied.

RESULTS AND DISCUSSION

Rheological Properties

The rheological results of the oscillatory assay, which were based on the evolution of the storage (G') and loss (G") moduli *versus* frequency for the marmalade studied are shown in Figure 1. As can be observed, the marmalades prepared by totally replacing sucrose with tagatose (marmalade D) showed the lowest values of G' and G". Since the storage modulus measures the stored energy, which represents elasticity, and the loss modulus

measures the energy dissipated as heat, which represents viscosity, marmalades D were found to have the lowest elastic and viscous behavior. In contrast, the results for marmalade prepared with the highest amount of isomaltulose (formulation C) were most similar to the results for the control marmalade, most likely due to the analogous chemical structure of the sucrose and isomaltulose molecules. Moreover, the storage modulus of the control marmalade increased over time whereas in the samples formulated with the new blenders of sweeteners, the G' modulus decreased. In terms of G", time did not lead to any relevant changes.

However, in our previous studies (Rubio-Arraez and others 2015) carried out on orange marmalade formulated with oligofructose and tagatose as a substitutes for sucrose, there was an increase in the elastic component (G') after 45 days of storage. Consequently, it can be concluded that depending on the nature of the chemical structure of the sweetener used, the rheological behavior will be different. Besides, changes in the combination could lead to changes in the viscoelastic behavior of the product. In order to better assess the differences between the results of oscillatory tests performed on the analyzed samples, the values of both the storage (G') and the loss (G") moduli were adjusted from the angular speed (ω) to the power-law model, as described in materials and methods. The parameters of this model are shown in Table 1.

According to the results obtained in Figure 1, marmalades formulated with tagatose alone (formulation D) showed the lowest values for the low frequency storage modulus (parameter *a*). These were followed by marmalades made with formulation A, which had the second highest concentration of tagatose. There were no significant differences between the control samples and the marmalades prepared with the highest amount of isomaltulose.

Table III.5.1. Rheological parameters of the power-law model model and parameters of the Herschel-Bulkley for mandarin orange marmalades initially and at the end of storage.

		OSCILLATORY TEST-POWER LAW				HERSCHEL-BULKLEY MODEL			
		G´		G´´					
FORMULATION	**TIME** (days)	***a***	***b***	***c***	***d***	**τ** (Pa)	***k***	***n***	***η*** (Pa·s) **(γ=50 s^{-1})**
CONTROL	1	1079±34^{d}	0.14±0.01^{b}	245±22^{c}	0.14±0.03^{b}	243±7^{c}	59.78±1,01^{c}	0.245±0.004^{a}	2,34±0,07^{c}
	90	1120±27^{d}	0.15±0.01^{b}	214±19^{c}	0.2±0.1^{b}	224.1±20.3^{c}	71±7^{c}	0.231±0.001^{a}	2,24±0.21^{c}
	180	1551±268^{e}	0.16±0.01^{b}	377±80^{d}	0.14±0.01^{b}	236.3±21.2^{c}	72±7^{c}	0.231±0.001^{a}	2.363±0.211^{c}
	360	1172±347^{d}	0.13±0.01^{b}	214±20^{c}	0.19±0.01^{b}	234.91±21.01^{c}	71±7^{c}	0.230±0.001^{a}	2.35±0.21^{c}
A	1	387±30^{ab}	0.16±0.01^{bc}	90±5^{ab}	0.17±0.02^{b}	181.5±12.4^{bc}	39.1±3.3^{b}	0.294±0.004^{b}	1.815±0.124^{a}
	90	234±18^{ab}	0.20±0.02 ^{bc}	52±8^{ab}	0.36±0.04^{b}	169.62±18.73^{b}	36.2±4.1^{b}	0.3±0.1^{b}	1.7±0.2^{a}
	180	328±30^{ab}	0.2±0.1^{c}	114±28^{ab}	0.19±0.04^{b}	203±4^{bc}	38.1±1.1^{b}	0.286±0.001^{b}	2.03±0.04^{b}
	360	308±32^{ab}	0.108±0.002^{a}	74±6^{ab}	0.011±0.001^{a}	197±5^{bc}	38.2±1.1^{b}	0.28±0.01^{b}	1.96±0.05^{b}
B	1	755±117^{c}	0.161±0.002 ^{bc}	168±29^{b}	0.20±0.02^{b}	163±20 ^{ab}	37±34^{b}	0.28±0.01^{b}	1.7±0.2^{a}
	90	844±33^{c}	0.151±0.012^{b}	155±20^{b}	0.26±0.04^{bc}	209±6^{c}	40±3^{b}	0.29±0.01^{b}	2.1±0.1^{b}
	180	944±2^{c}	0.19±0.01^{c}	216±51^{b}	0.22±0.01^{bc}	161±7^{ab}	39.1±1.4^{b}	0.281±0.002^{b}	1.6±0.1^{a}
	360	578±6^{b}	0.19±0.02^{c}	138±52^{b}	0.26±0.03^{bc}	161±7^{ab}	39.2±1.3^{b}	0.281±0.002^{b}	1.6±0.1^{a}

Equal letters indicate homogeneous groups.

(Cont.) Table III.5.1. Rheological parameters of the power-law model model and parameters of the Herschel-Bulkley for mandarin orange marmalades initially and at the end of storage.

		OSCILLATORY TEST-POWER LAW				STEADY TEST			
		G´		G´´		HERSCHEL-BULKLEY MODEL			
FORMULATION	**TIME** (days)	***a***	***b***	***c***	***d***	**τ** (Pa)	***k***	***n***	***η*** (Pa·s) **(γ=50 s^{-1})**
C	1	1036±194^{cd}	0.14±0.01^{b}	241.4±30.3^{bc}	0.09±0.04^{ab}	142.69±13.02^{ab}	26±3^{a}	0.343±0.002^{c}	1.43±0.13^{a}
	90	1088±187^{cd}	0.11±0.01^{a}	238±46^{bc}	0.015±0.001^{a}	156.4±3.4^{ab}	27.3±0.3^{a}	0.336±0.002^{c}	1.564±0.034^{a}
	180	943±2^{c}	0.19±0.01^{c}	216±51^{bc}	0.21±0.01^{b}	155.8±6.2^{ab}	26.7±1.5^{a}	0.336±0,004^{c}	1.558±0.062^{a}
	360	935±24^{c}	0.100±0.002^{a}	200±10^{bc}	0.015±0.002^{a}	153.91±5.53^{ab}	26.6±1.4^{a}	0.333±0.003^{c}	1.5±0.1^{a}
D	1	265±26^{a}	0.21±0.01^{c}	75±7^{a}	0.278±0.002^{b}	135.99±11.64^{a}	29.2±2.7^{a}	0.296±0.004^{b}	1.35±0.12^{a}
	90	234±18^{a}	0.20±0.02^{c}	52±8^{a}	0.36±0.04^{c}	168.56±60.34^{b}	41.3±9.6^{b}	0.251±0.032^{a}	1.686±0.603^{a}
	180	200±2^{a}	0.22±0.01^{c}	40±43^{a}	0.4±0.2^{c}	145±25^{a}	33.93±2.54^{b}	0.271±0.031^{b}	1.45±0.25^{a}
	360	190±1^{a}	0.22±0.01^{c}	40±43^{a}	0.4±0.2^{c}	143±13^{a}	33.91±2.52^{b}	0.27±0.03^{b}	1.430±0.132^{a}
COMMERCIAL	----	2585±1049	0.17±0.03	713±293	0.391±0.431	565.5±376.4	293±158	0.050±0.044	5.655±3.764

Equal letters indicate homogeneous groups.

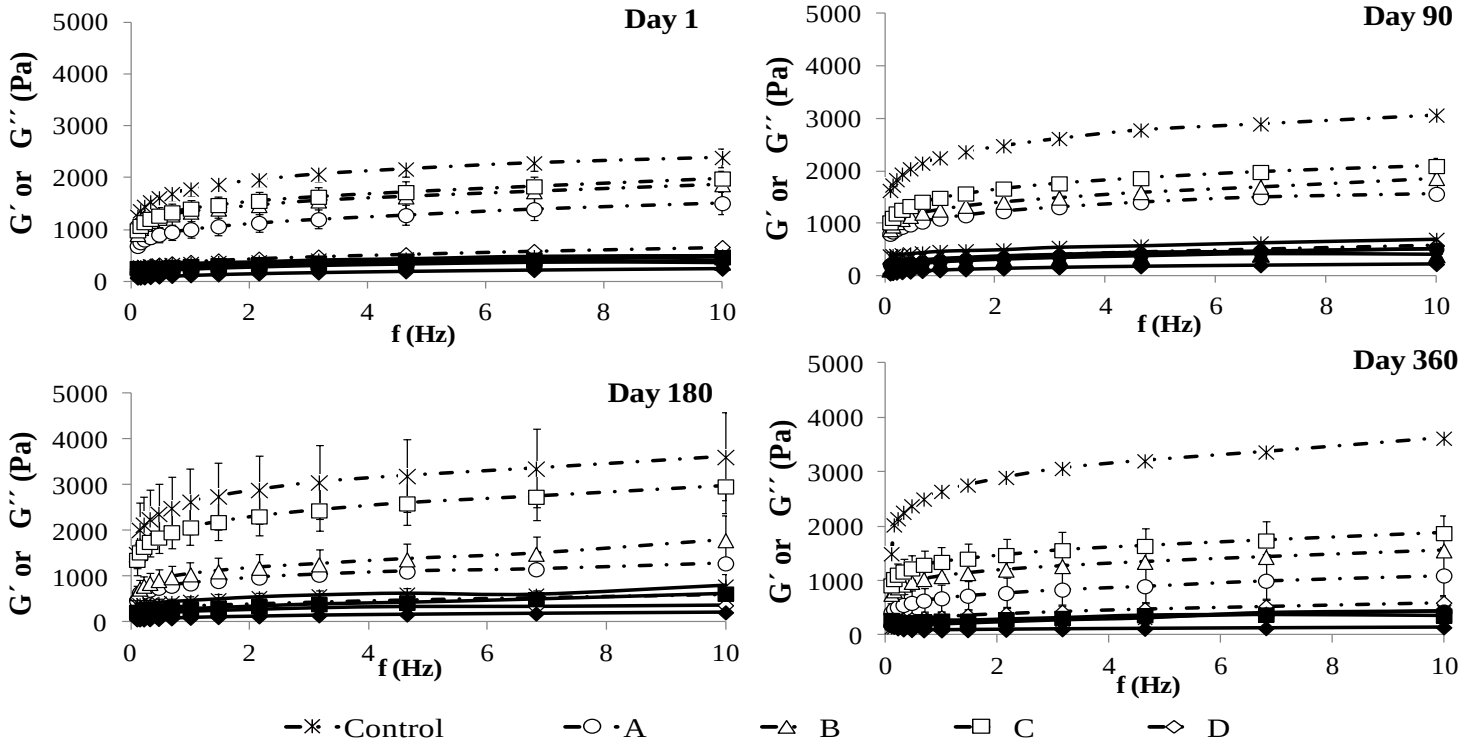

Figure III.5.1. Average frequency curves obtained in the oscillatory test of mandarin orange marmalades over the storage periodst considered. Samples were coded based on the amount of sweeteners as: Control (100% sucrose); A (75% tagatose and 25% isomaltulose), B (50% tagatose and 50% isomaltulose), C (25% tagatose and 75% isomaltulose), and D (100% tagatose). Unshaded symbols refer to values of G´ and shaded symbols refer to values of G´´.

Consistent with the evolution of G' curves over time, the *a* parameter significantly decreased over time only in formulation B, i.e. after 360 days of storage, whereas there was an increase in the control marmalades after 180 days of storage. In terms of parameter b of the power-law of G', marmalade D again differed from the other formulation, but in this case, it showed a higher value. Initially no differences were found among the other formulations.

However there was a significant decrease in formulations A and C after 360 days of storage, with an abrupt fall in the curves shown in Figure 1. In the case of the c and d parameters of G" modulus for power-law, the trends shown were similar to those of the*a* and *b* parameters but with less marked differences. Peinado and others (2012) observed that replacing sucrose with isomaltulose in the formulation of different strawberry spreadable products resulted in a decrease in parameters *a* and *c* of the power-law model, but parameters *b* and *d* were similar in all marmalades. Based on these results the combination of the new sweeteners leads to formulations with less elastic character in comparison to the control marmalade.

On the other hand, the results obtained for the stationary test of mandarin orange marmalades based on the combination of sweeteners used and the storage time, are presented in Figure 2. The rheograms of mandarin orange marmalades for samples formulated with the new sweeteners were below the control samples, but there were only slight differences between them. Moreover, the storage time curves of the control marmalades remained similar but the curves of the new marmalades decreased. The parameters of the Herschel-Bulkley model for the mandarin orange marmalades studied over the period considered are shown in Table 1. Despite the evolution of the curves of the new orange marmalades mentioned above, the parameters of the Herschel-Bulkley model did not differ statistically over time. Nevertheless, the yield stress above which the fluid starts flowing (τ_0) and the index of consistency (k) were the lowest in formulation C, but the index of fluidity (n) was the highest in this formulation.

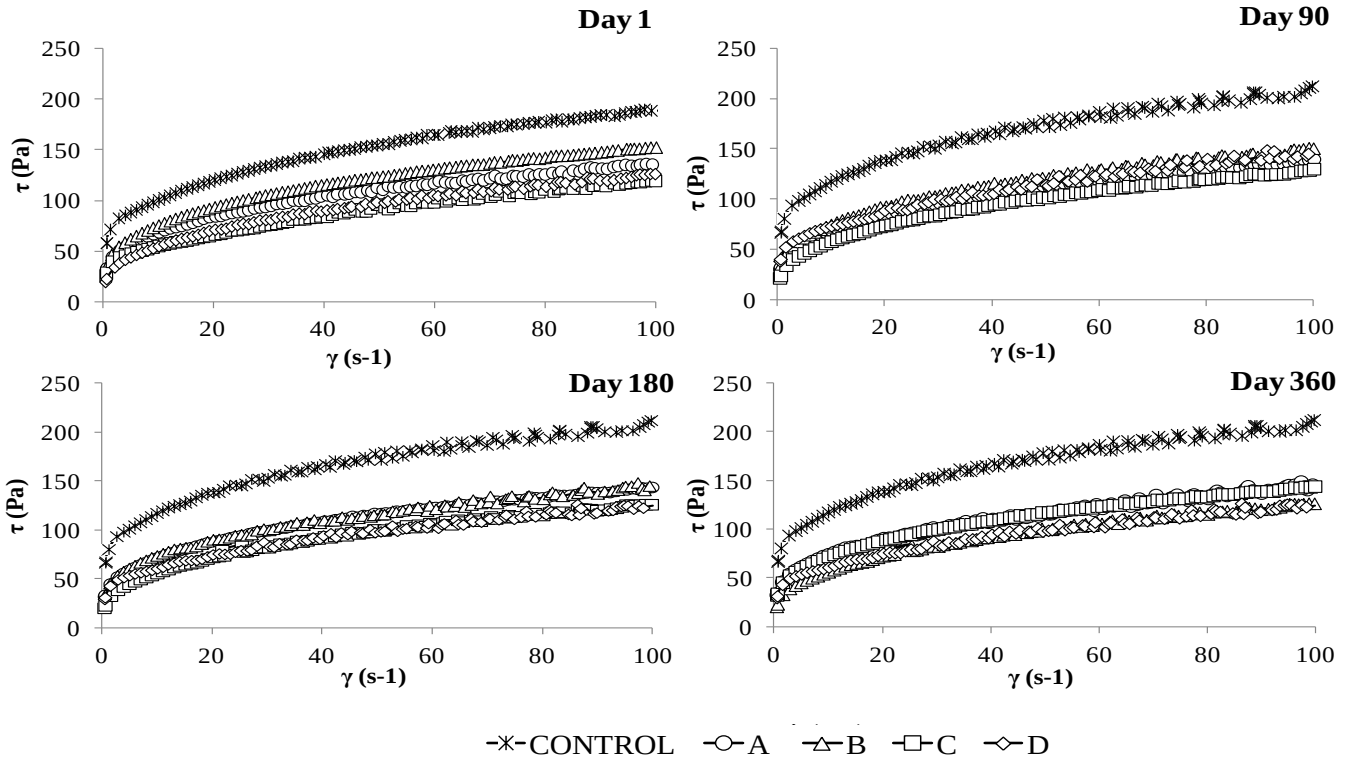

Figure III.5.2. Mean flow curves (rheograms) obtained from the steady assay of mandarin orange marmalades over the storage period considered. Samples were coded based on the amount of sweeteners as: Control (100% sucrose); A (75% tagatose and 25% isomaltulose), B (50% tagatose and 50% isomaltulose), C (25% tagatose and 75% isomaltulose), and D (100% tagatose).

Since formulation C had the greatest amount of isomaltulose, it can be concluded that this sweetener was responsible for the above described behavior. In the other cases, marmalades also showed values of τ_0 and k which were lower than in the control samples but to a lesser extent than in formulation C.

These results were consistent with those obtained by Peinado and others (2012) in jam prepared with osmodehydrated strawberry using isomaltulose as an osmotic agent, who observed a decrease in the consistency and cohesiveness of these jams with respect to those prepared with sucrose. When considering other combinations of sweeteners in our studies on orange marmalade (Rubio-Arraez and others 2015), a blend of oligofructose and tagatose in the same proportions increased the consistency of marmalades during storage. Therefore, it can be concluded that sweeteners with a high amount of fiber (long chain of monosaccharides) would modify the rheological behavior of marmalades (in the stationary test) over time, whereas mixtures of sweeteners with short molecules (isomaltulose and tagatose) would give rise to more stable marmalades from a rheological point of view.

Optical Properties

Interaction charts of the colorimetric coordinates L*, a* and b*, chroma ($C^*=(a^{*2}+b^{*2})^{1/2}$) and hue ($h^*=arctg(b^*/a^*)$) of the different mandarin orange marmalades studied over one year of storage are shown in Figure 3. According to these results, marmalade D (with the highest content of tagatose) had the highest luminosity throughout the entire storage time. This formulation was followed by sample B (equal proportions of isomaltulose and tagatose), whereas marmalades A, C and control were initially very similar. During storage, luminosity decreased for all combinations studied.

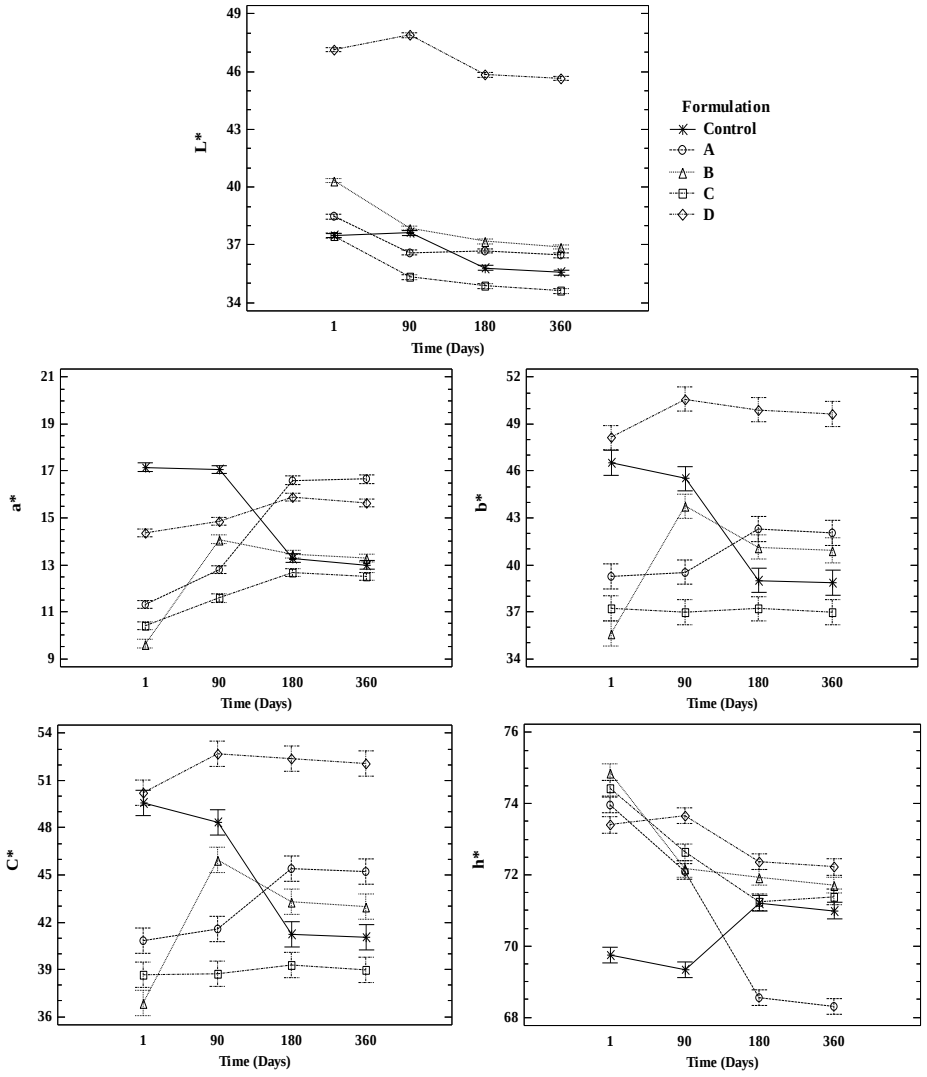

Figure III.5.3. Interaction graphics (significant level of 95%) of colour parameters: L*, a*, b* coordinates, chroma (C*) and hue (h*) of the different formulations of mandarin orange over the storage period . Samples were coded based on the amount of sweeteners as: Control (100% sucrose); A (75% tagatose and 25% isomaltulose), B (50% tagatose and 50% isomaltulose), C (25% tagatose and 75% isomaltulose), and D (100% tagatose).

The control marmalade initially showed the highest value for coordinate a*, but this value decreased after 90 days of storage. Furthermore, in the other combinations, the coordinate a* increased during during the first 90 days of storage rebut then remained constant. Marmalade D had the highest values for coordinate b* and the chrome throughout the whole storage time, whereas these values decreased in the control marmalade after 90 days of storage, as in coordinate a*. In contrast, marmalades C, which had the highest amount of isomaltulose, showed constant values for b* and C* over storage.

Therefore, it can be concluded that tagatose was responsible for the changes registered in both parameters. As a consequence of the changes in a* and b*, the initial hue of the new mandarin orange marmalades was higher than in marmalades prepared with sucrose. Besides, the hue decreased during the first 180 days of storage in the marmalades formulated with the new sweeteners, especially for formulation A, unlike in the case of the control marmalades. Noteworthy is that after 180 days, all marmalades showed similar values of hue except for formulation A.

This pattern was also observed for the other colour parameters in all the mandarin orange marmalades. After 6 months of storage the colour of these marmalades was quite similar regardless of the differences registered before that period. In our previous studies carried out on orange marmalade formulated with different combinations of tagatose and oligofructose (Rubio-Arraez and others 2015) it was observed that marmalades with the highest content of tagatose showed a decrease in L* and an increase in a* and b* coordinates after 45 days of storage. Peinado and others (2015) showed that strawberry jams formulated with isomaltulose and different concentrations of citric acid and pectin jams darkened during storage.

Antioxidant Capacity

The results relating to the antioxidant activity of the mandarin orange marmalades studied are shown in Figure 4. Initially all marmalades showed the same antioxidant activity, but after 3 months of storage, antioxidant activity increases in formulations with the highest content of tagatose. However, no significant differences were found in the other formulations.

After 3 months of storage, the antioxidant activity of all marmalades slightly reduced, but after one year values were similar to those initially obtained, except in formulations with more tagatose, which again showed the highest antioxidant activity. Consequently this new sweetener could enhance the ability of the antioxidants of mandarin orange fruit to scavenger free radicals.

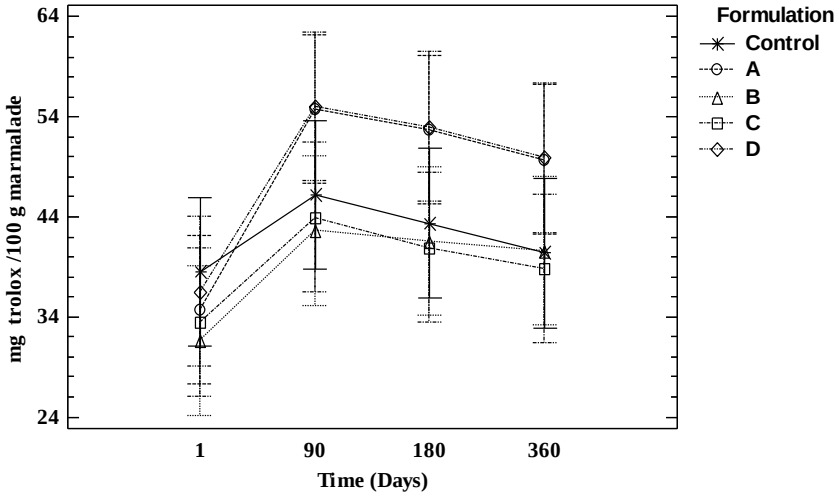

Figure III.5.4. Interaction graphic (significant level of 95%) of antioxidant activity of the different formulations of mandarin orange marmalade over the storage period. Samples were coded based on the amount of sweetners as: Control (100% sucrose); A (75% tagatose and 25% isomaltulose), B (50% tagatose and 50% isomaltulose), C (25% tagatose and 75% isomaltulose), and D (100% tagatose).

Besides, in orange marmalade (Rubio-Arraez and others 2015) with sucrose or new sweeteners (tagatose and oligofructose) there was also an increase in the antioxidant capacity after 45 days of storage, showing possible combinations of components that would lead to the appearance of new antioxidants. However, Rababah and others (2011), observed that the antioxidant activity of orange marmalades prepared with sucrose decreased significantly after 3 months of storage.

Microbiological Analysis

All mandarin orange marmalades were safe from a microbiological point of view since no colonies of moulds and yeast or mesophillic aerobics were found in any of the marmalades studied over the storage period considered.

Sensory Analysis

The results of the sensory analysis of the mandarin orange marmalades formulated with sucrose or new sweeteners are shown in Figure 5. No significant differences were detected in the attributes of colour, aroma, texture, consistency and spreadable capacity.However, the marmalade prepared by replacing sucrose with tagatose alone (formulation D) obtained the best scores for palatability, flavour, global preference and intention of buying. Besides, no significant differences in sweetness were found in formulation D as compared to the control marmalade. Conversely, marmalade with more isomaltulose (formulation C) obtained the lowest scores for sweetness, palatability and flavour, probably due to the low sweetening power of this sugar (Lina and others 2002).

In the case of orange marmalades (Rubio-Arraez and others 2015), those prepared with the new healthy sweeteners (tagatose and oligofructose) scored better than marmalade prepared with sucrose. Therefore, mandarin orange seems to be more sensitive to the different combinations of sweeteners than orange or otherwise, the combination of tagatose and isomaltulose leads to greater differences in the sensorial analysis of marmalades than the combination of tagatose and oligofructose.

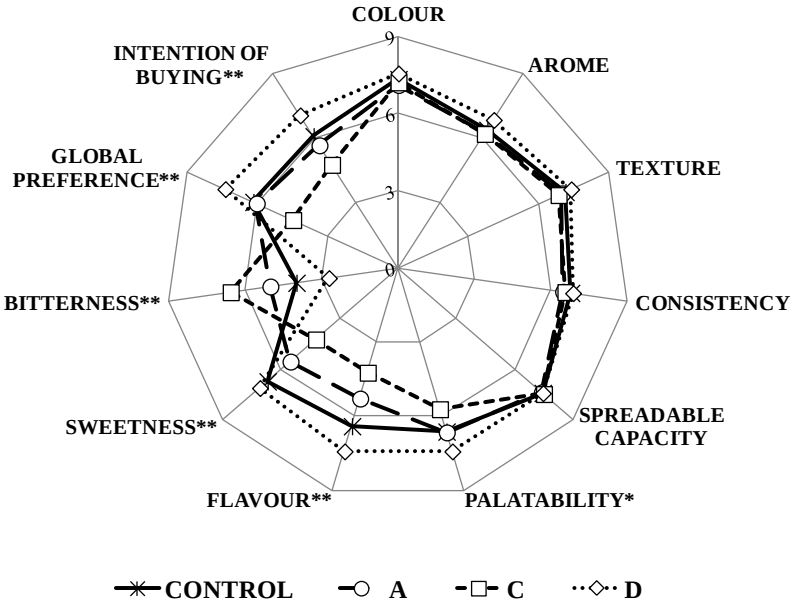

Figure III.5.5. Results of the sensory analysis in the evaluation of the samples coded based on the amount of sweeteners as: Control (100% sucrose); A (75% tagatose and 25% isomaltulose), B (50% tagatose and 50% isomaltulose), C (25% tagatose and 75% isomaltulose), and D (100% tagatose). *p-value <0.05, ** p-value <0.01

CONCLUSIONS

According to these results it is possible to reformulate mandarin orange marmalade with non-cariogenic sweeteners such as tagatose and isomaltulose. However, the complete replacement of sucrose with tagatose leads to a significant difference in the rheological behavior of this type of marmalade, giving rise to a less elastic character. Additionally, tagatose increases the luminosity of marmalades but it improves their antioxidant capacity. In all cases, the colour parameters remained constant after 6 months of storage. Finally, the flavour of tagatose scored better than isomaltulose due to the low sweetening power of the latter.

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III.6. RELIABLE TRACEABILITY BASED ON WIRELESS SENSOR NETWORKS APPLIED TO A NEW MANDARIN ORANGE MARMALADE FORMULATION

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ABSTRACT

This work presents a study and application of the Wireless Sensor Networks (WSN) paradigm to food traceability systems. In this line, an advanced monitoring of stored mandarin orange marmalades applying a robust WSN architecture called EDETA (***E****nergy-efficient a****D****aptative hi****E****rarchical and robus****T*** ***A****rchitecture*) is proposed. The properties of this proposal allow guaranteeing the optimum conditions of these marmalades for human consumption. Furthermore, to apply and demonstrate the features of this approach, in this paper the development of mandarin orange marmalades using non cariogenic and low glycemic index sweeteners, such as tagatose and isomaltulose, instead of sucrose in the formulation is proposed. Temperature, moisture content, pH, solid soluble content, water activity and microbiological analyses have been carried out initially and after 90,180 and 360 days of marmalades storage.

The results showed that marmalade B (equal proportion of tagatose and isomaltulose) presented the lowest scores, over storage time. Regarding temperature, the new marmalades and control showed the same trend over storage time. Besides, all marmalades were microbiologically stable. Furthermore, the proposed WSN approach has been applied to this new marmalade to test their suitability, obtaining excellent results about accuracy, reliability and energy consumption. Also, applying this approach new features are obtained, such as the possibility of continuous monitoring in an automatic manner along the time or automatic and complete analysis of the monitored data.

Keywords: Marmalades, sweeteners, Wirelesss Sensor Networks, robust traceability advanced monitoring.

INTRODUCTION

Traceability is the most important instrument to ensure the quality and food safety in the food industry. The Food and Agriculture Organization of the United Nations (FAO, 2003), the World Health Organization (FAO & WHO, 2003), the Codex Alimentarius Commission (CAC, 2003,2005), and the International Organization for Standardization (ISO, 2005,2007) characterized by the development of international standards and guidelines for food traceability in the food industry (Petersen, 2004). Concretely, the CAC recommended Hazard Analysis Critical Control Point (HACCP) as the most effective system to guarantee the safe food supply (Beulens *et al.*, 2005). According to ISO guidelines, ISO 22000 (2005) includes analyzing methods of food hazards from HACCP and the approach of the management system from ISO 9001, in order to guarantee the safety food until human consumption (FMRIC, 2008). Furthermore, ISO 22005 (2007) defined the principles and objectives of traceability for the design and implementation of a feed and food traceability system. Additionally, there are other private food quality and safety standards such as International Standard for Auditing Food Suppliers (IFS), Eurep-GAP, Safe Quality Food (SQF), and the British Retail Consortium (BRC), etc.

In recent decades a spectacular growth of computer networks and wireless communications, promoted by continuous technological advances have been achieved. Therefore, have appeared increasingly small, powerful and less expensive electronic circuits, also allowing important advances in the field of transducers. All this allows the development of new devices for detection and measurement of any magnitude in a simple way and with great accuracy, all with a small size and low cost. The possibility to implement this low-cost devices and without maintenance, offers the opportunity to obtain information from the environment and forward it wirelessly to coordination center (sink) extending the range of applications to an unimaginable spectrum (Capella *et al.*, 2014). In this line, we propose the application of WSN paradigm with a specific architecture to food monitoring in order to guarantee the quality attributes of obtained food products in the food chain.

In the bibliography, there are studies such as Jedermann *et al.* (2006), proposing a system for fruit logistics. Moreover, Zhang *et al.*, (2009) developed a temperature-managed traceability system for frozen and chilled food. All these proposals do not pay attention to energy consumption, system durability or cost of the monitoring nodes.

Furthermore, in stored food sector, it is necessary to perform an exhaustive and robust monitoring of the elaboration and conservation, in order to guarantee delivery the product in perfect conditions for human consumption. For all these reasons in this work we propose the application of EDETA architecture that provides reliability and energy efficiency to the system. This allows the development of new systems for detection and measurement of any magnitude in a simple way and with great accuracy, all with a small size and low cost (Capella *et al.*, 2014).

On the other hand, owing to the current lifestyle of society, there is an increasing demand for healthy food products such as fruits. Spain is one of the major producers and exporters of various kinds of citrus fruits as mandarin orange (*Citrus reticulata*). Therefore, its consumption as fresh fruit, marmalades or juice due to its higher content of phenolics, dietary fibre, ascorbic acid and trace elements that can prevent diseases mainly of its antioxidant activity (Dhuique-Mayer *et al.*, 2005; Balasundram *et al.*,2006; Rubio-Arraez *et al.*, 2015a, 2015b) Traditionally, the marmalade was prepared with sucrose, however, it shows a great calorie contribution in addition to high glycemic index and their excessive consumption can cause several diseases such as obesity, diabetes and tooth decay (Edwards, 2002; O'Donnell and Kearsley, 2012; WHO, 2014). Currently, food industry present other naturally sweeteners as tagatose and isomaltulose. There are non-cariogenic sweeteners that are slowly released into blood.

Firstly, D-Tagatose (D-tag) it is a stereoisomer of D-galactose, it is found in yoghurt and cheese, (Oh, 2007; Lu *et al*., 2008). Additionally, it has only 1.5 kcal/g (Levin, 2002), beside its texture is very similar than sucrose and it is almost as sweet as sucrose (Oh, 2007; Taylor *et al.*, 2008; Calzada-León *et*

al., 2013). In fact, it is considered a functional food (Taylor *et al*., 2008) favoring *Lactobacillus* bacteria (Petersen-Skytte, 2006).

Secondly, isomaltulose is a reducing disaccharide which is present in sugar cane juice and honey, and its appearance, taste viscosities of aqueous solutions and caloric power are similar to sucrose (Schiweck *et al*., 1990; Periche *et al*., 2014). Furthermore, isomaltulose has a third of the sweetening power of sucrose and its physicochemical properties permit the substitution of sucrose in most sweet foods (Lina *et al.,* 2002; De Oliva-Neto and Menão, 2009; Peinado *et al.,* 2013).

In summary, the principal aim was established a traceability food system according to the robust design of WSN directed by the EDETA architecture, to be applied to the advanced monitoring of food products such as mandarine orange marmalades, along all their storage time, and even from mobile devices. The secondly aim, in accordance with the properties of new sweeteners, was to evaluate the potential use of them as an alternative to sucrose in the development of mandarin orange marmalades.

MATERIALS AND METHODS

Components and formulation of mandarin orange marmalades

Marmalades were produced using 50% mandarin orange pulp (*Citrus reticulata Clemenules*), 50% sucrose (Azucarera Iberia S.L., Madrid, Spain) or sweeteners (isomaltulose or tagatose) containing 1% agar-agar (Roko Agar, Asturias, Spain), Tagatose (Tagatesse, Heusden-Holder, Belgium), Isomaltulose (Beneo, Mannheim, Germany). Samples were coded depending on the combination of sweeteners used: Control marmalade: 100% sucrose, Marmalade A: 75% tagatose and 25% isomaltulose, Marmalade B: 50% tagatose and 50% isomaltulose, Marmalade C: 25% tagatose and 75% isomaltulose and Marmalade C: 100 % tagatose. We also characterized commercial mandarin orange marmalade.

Manufacturing process

Three batches of mandarin oranges were selected and collected directly from the field. Afterward, they were peeled and mixed with the corresponding combination of healthy sweeteners/sucrose and the agar-agar in a thermal blender (Thermomix, TM31, Vorwerk, Wuppertal, Germany) for 3 min. Then, the mixture was cooked at 100 ºC for 20 min at 350 rpm. After that, glass jars previously sterilized in an autoclave at 121ºC for 15 min, were filled with the marmalade and turned over to ensure proper sealing for 1 hour. At last, the marmalade was allowed to cool for 24 hours and in that time jellification took place as can be seen in Figure 1.

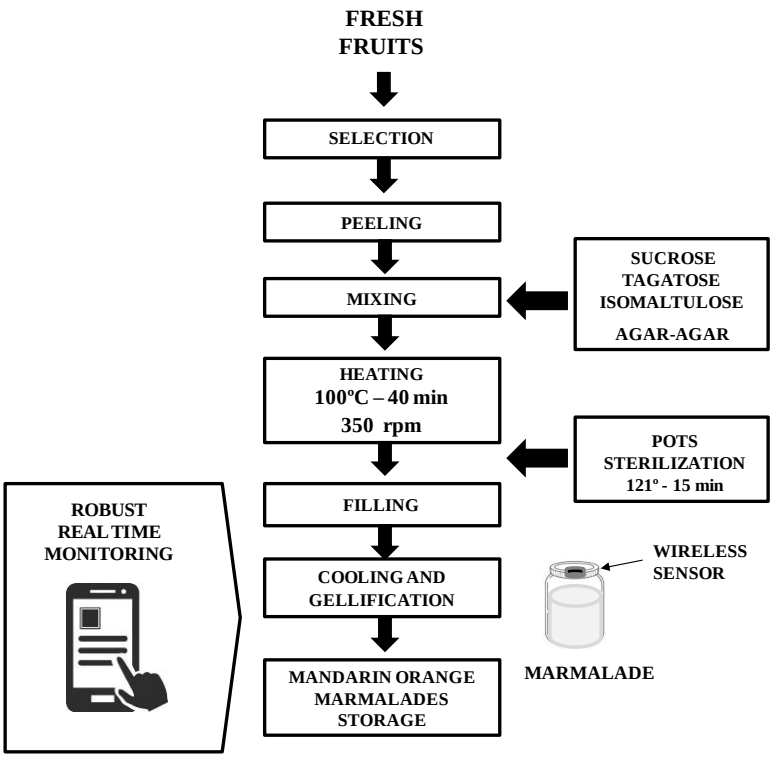

Figure III.6.1. Flow chart of the manufacturing process of mandarin orange marmalade.

WSN monitoring process by EDETA architecture

Once concluded the elaboration and packaging process, continuous monitoring during all storage (360 days), by means of sensor nodes located in the top of the packages must be realized.

The sensor nodes are composed by these pieces (Fig.2A): First the microcontroller, that is a computer on a chip. In this work an ARM Cortex-M4 microprocessor (SMT32F4, STMicroelectronics, Geneva, Switzerland) have been selected. Second the wireless communication network, in order to provide the communication service, being selected a short range technology, due to the energy restrictions. Thirdly, autonomous power energy, based on a small size battery. Finally, the sensors or transducers, which allow the node to obtain data from the process for their later processing and transmission. A Sensirion temperature /humidity sensor (SH1, Sensirion AG, Staefa, ZH, Switzerland) have been used.

On the other hand, a high power microcontroller system or even a computer normally constitutes the gateway node. This special node is the destination of the information obtained by all the sensors and it is in charge of managing the whole WSN. In addition, it is also involved in the data evaluation, be it for the detection of alarm situations, generating the corresponding warnings or for performing fault-tolerance actions, for instance, to discard erroneous measurements, or even ask for their repetition (Bonastre et al., 2012; Capella et al., 2014).

The main pieces of the sink nodes are show in Fig.2B: on one hand a WSN communication interface, compatible with the one utilized by the sensor nodes. LAN (Local Area Network)/WAN (Wide Area Network) interface, by means of which the gateway node is able to provide the users with the information retrieved by the sensor nodes. On another hand a high power microcontroller, that will be in charge of perform all the data management and fault tolerance operations. And the last piece is the data storage system, that will be in charge to guarantee the data and logs durability and persistence.

In this manner, the operation consists on this procedure: the sensor nodes collect the information (by means of the indicated transducers) and could be periodically or when an event occurs (value change, threshold exceeded, alarm activation, etc.). This information is sent to the gateway node using the wireless network.

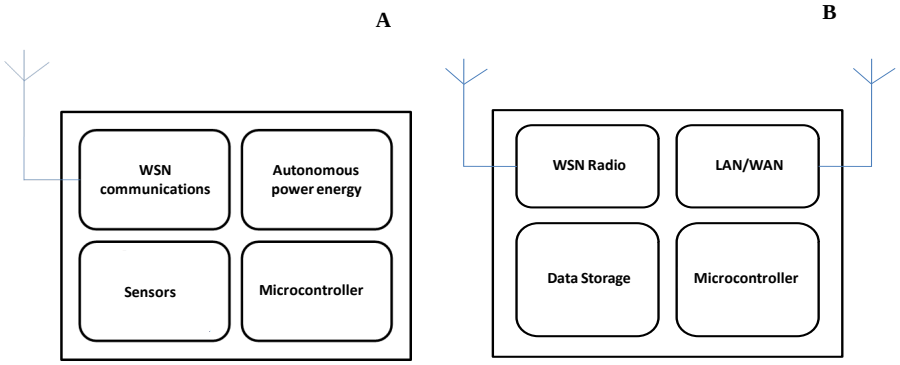

Figure III.6.2. A) Sensor Node structure and **B)** Sink Node structure.

In this stage, it is very important detect possible problems of conservation, in order to guarantee traceability and delivery the product in optimum conditions, being required the proposed features of robust and reliable WSN monitoring. In the design of the sensor network have been considered, due to its application to food monitoring, the following requirements: limited resources, dynamic environments, use of unreliable transmission medium, information privacy and safety food.

In this line, the European Regulations No 1935/2004 and No 450/2009 have been observed, about the material and articles intended to come into contact with food.

Analytical determinations of temperature, moisture and soluble solids content, pH and water activity

Furthermore, we registered by triplicate analyses the experimental values in the laboratory on the first day of storage and after 90,180 and 360 days of storage to corroborate the values obtained with WSN monitoring.

The marmalades were storage in dry ambient box. Temperature was registered with a digital thermometer (Testo AG, Lenzkirch, Germany). Moisture content (x_w*: g water/g marmalade*) was determined gravimetrically by drying approximately 1 g of marmalade to a constant weight in a vacuum oven at 60ºC (method 934.06, AOAC 2000). Soluble solids content (Brix) was determined in a refractometer at 20ºC (Atago 3T, Tokyo, Japan). The pH was registered with a pH-meter (Seven Easy, Mettler Toledo, Barcelona, Spain) previously calibrated with buffered solutions of pH 7.0 and 4.0. Water activity (a_w) was determined with a dew point water activity meter (Decagon Devices, Inc., Pullman, Washington, USA), at 25ºC.

Microbiological Analysis

Serial dilutions were prepared by homogenising 10 g of marmalade with 90 mL of 1% sterile peptone water in a stomacher bag, using sterile techniques. Mesophilic aerobic populations were analysed in Plate Count Agar (Scharlau Chemie, 1-329, Barcelona, Spain) incubating samples for 72 hours at 31ºC. Yeast and moulds were determined in Sabouraud Chloramphenicol Agar (Scharlau Chemie, 1-166, Barcelona, Spain) plates for 5 days at 31ºC. Sample for analysis were taken on days 1, 90, 180 and 360.

Statistical analysis

Statgraphics Centurion software (Statpoint Technologies, Inc., Warrenton, Virginia, USA) was used to perform the statistical analyses. Analyses of variance (multifactor ANOVA) were performed to study the interactions between the formulation and time of storage factors on the marmalades.

RESULTS AND DISCUSSION

WSN monitoring process by EDETA architecture

In order to provide traceability characteristics for safety and food quality maintenance, an approach based on a WSN has been applied to obtain information about the products during production and storage, in a real time and reliable manner. This allows additionally ulterior data processing that can provide extra-information unimaginable without these techniques. It is remarkable that all this monitoring information and data analysis is offered even from mobile devices (Fig. 3). Reliability it is very important in this application, for this reason it is proposed a new version of the EDETA architecture (Capella et al., 2009) with additional mechanisms to ensure reliability, to discard erroneous measurements, or even ask for their repetition, tolerate nodes and routes faults, among others. These features have been tested by the monitorization platform for dependable WSN design, which has been developed. On the other hand, the absence of wires makes the nodes depend on their own energy resources; therefore, multi-hop routing mechanisms are contemplated by EDETA to reduce energy consumption.

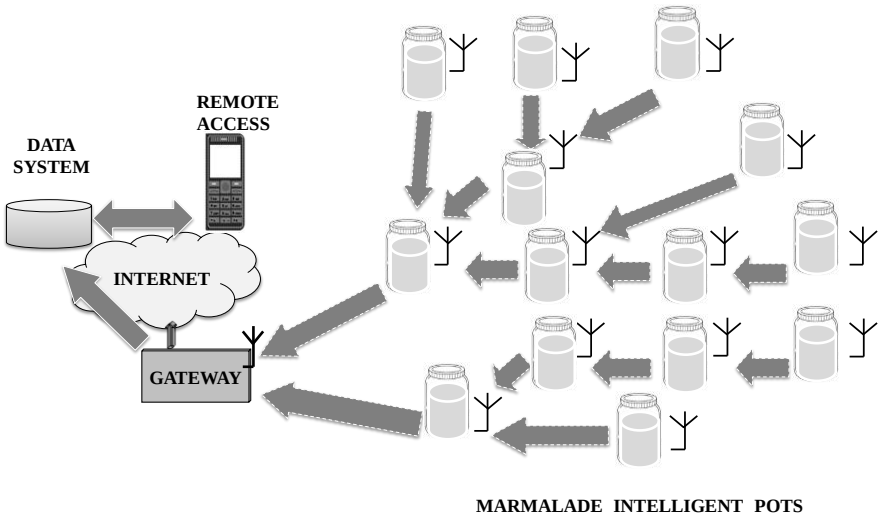

Figure III.6.3. Global WSN marmalade robust monitoring.

Mandarin orange marmalades have been monitored by a WSN based on EDETA architecture (Energy-efficient aDaptative hiErarchical and robusT Architecture) during storage time measuring temperature and moisture content as proof concept. The marmalades were storage in dry ambient box. Small and low cost devices (sensor nodes) that are able to obtain information from the process by means of transducers and transmit it towards a sink node using wireless communications compose WSN´s. The node on a database stores this information, where usually through the Internet it is available for its use, be it in real time or for statistical analysis, even though mobile devices (Capella et al., 2014). An advantage of WSNs consists of its easy deployment. The first function of each sensor node is the automatic setting up of a multi-hop route to the sink node. As an additional advantage, the same procedures needed in the initial setting up may be used to find an alternative route in case of route losses. The information gathered by the sink node is stored on a database, be it local or remote, accessible through communication systems such as Internet, and will allow the real time monitoring and later study of the process (Fig. 3) (Bonastre et al., 2012, Capella et al., 2014).

Furthermore, Figure 4 shows the obtained results from this monitoring. As expected, the temperature and moisture content data collected by the wireless sensor network deployed for monitoring are fully consistent with the analytical determinations, demonstrating that the WSN approach can be used as traceability food system, that also offers extra-features as explained previously.

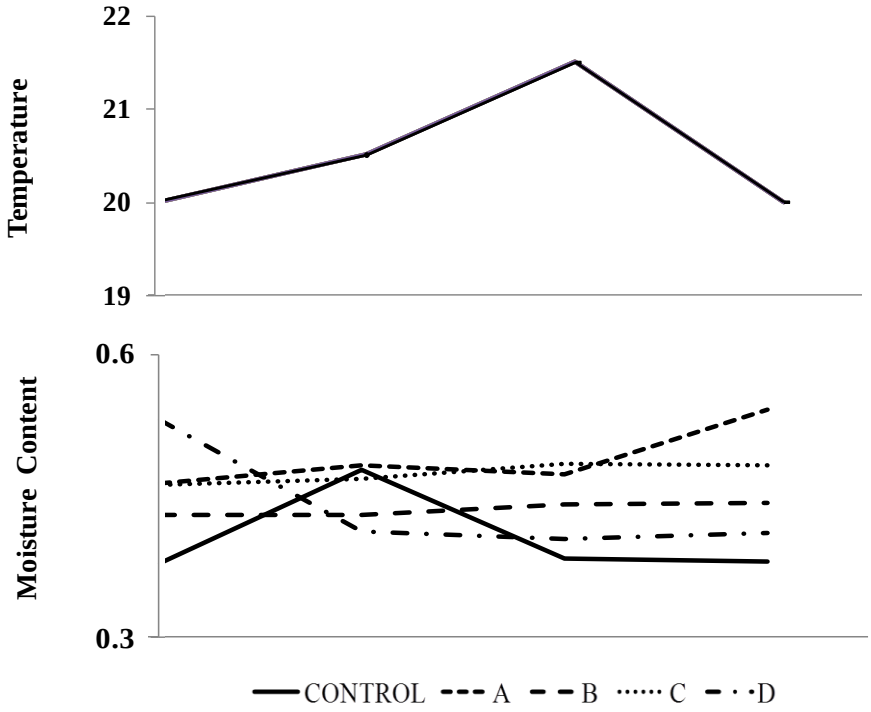

Figure III.6.4. Results obtained to monitoring parameters of temperature and moisture content.

Compositional characterization of marmalades

Table 1 show the results of the composition (Soluble solids and moisture content (xw)), temperature, pH and water activity (aw) of the mandarin orange marmalades studied. In general, the marmalades prepared with the new sweeteners presented slightly lower Brix values than commercial or the control ones. Nevertheless, these new marmalades would be in agreement with the Council Directive 2001/113/EC of 20 December 2001 relating to fruit jams, jellies and marmalades and sweetened chestnut purée intended for human consumption (EC, 2001). The Council Directive allows soluble content lower than 60 Brix when sweeteners, instead of sucrose, are used in the formulation for these products (EC, 2001).

Regarding moisture content (xw), marmalade D (100% tagatose), showed the higher scores than commercial and control ones. In contrast, marmalade B (equal proportion of tagatose and isomaltulose) had the lowest scores between new marmalades, during storage time. In our previous studies (Rubio-Arraez, et al., 2015a) in orange marmalades formulated with oligofructose and tagatose as sweeteners instead sucrose, the marmalades with higher content of tagatose showed the higher moisture content score, in coherence with these results obtained in this work. However, the lowest water activity value was registered for marmalade D, that containing only tagatose, with similar values to the control and commercial marmalades.

Relating to temperature recorded, can mentioned that all samples presented the regular fit over storage time. With regard to the pH, the new marmalades showed higher values than the commercial marmalades, although, the marmalade C (with higher content of isomaltulose) presented similar values to the control. However, all pH values were less 4, which in principle would ensure proper microbiological stability of these products. According to in the same magnitude of order as the pH of other jams made using strawberry, peach, plum and apricot (Carbonell et al., 1991; García-Martínez et al., 2002) and orange marmalades (Rubio-Arraez, et al., 2015a).

Microbiological Analysis

There were no colonies of molds and yeast or aerobic mesophillic found in any of the marmalades in this study during the storage period considered. Therefore, in all cases the products were stable from a microbiological point of view.

Table III.6.1. Experimental values for moisture content (x_w), storage temperature (C), pH, soluble solids content (Brix), and water activity (a_w) of mandarin orange marmalades over storage period. Also values of commercial mandarine marmalade.

FORMULATION	**TIME** (days)	x_w (g water/ g marmalade)	**T (C)**	**pH**	**BRIX**	a_w
CONTROL	1	0.4 ± 0.1^{a}	20 ± 1^{a}	3.62 ± 0.03^{b}	58.8 ± 0.3^{d}	0.897 ± 0.003^{b}
	90	0.47 ± 0.02^{b}	$20,5\pm1^{b}$	3.60 ± 0.01^{b}	59.7 ± 0.6^{e}	0.893 ± 0.001^{b}
	180	0.4 ± 0.1^{a}	$21,5\pm1^{c}$	3.55 ± 0.04^{a}	59.1 ± 0.1^{e}	0.891 ± 0.001^{b}
	360	0.4 ± 0.1^{a}	20 ± 1^{a}	3.5 ± 0.2^{a}	59.0 ± 0.1^{e}	0.890 ± 0.001^{b}
A	1	0.462 ± 0.001^{b}	20 ± 1^{a}	3.95 ± 0.01^{c}	58.3 ± 0.2^{d}	0.916 ± 0.001^{d}
	90	0.481 ± 0.001^{b}	$20,5\pm1^{b}$	3.90 ± 0.01^{c}	58.4 ± 0.2^{d}	0.9184 ± 0.0001^{d}
	180	0.5 ± 0.1^{b}	$21,5\pm1^{c}$	3.86 ± 0.03^{c}	57.5 ± 0.1^{c}	0.923 ± 0.001^{d}
	360	0.542 ± 0.001^{c}	20 ± 1^{a}	3.81 ± 0.02^{c}	57.1 ± 0.1^{c}	0.922 ± 0.001^{d}
B	1	0.43 ± 0.04^{b}	20 ± 1^{a}	3.98 ± 0.01^{d}	57.2 ± 0.3^{b}	0.907 ± 0.001^{c}
	90	0.430 ± 0.002^{b}	$20,5\pm1^{b}$	4.08 ± 0.01^{d}	57.2 ± 0.1^{c}	0.9076 ± 0.0003^{c}
	180	0.440 ± 0.002^{b}	$21,5\pm1^{c}$	4.04 ± 0.01^{d}	57.2 ± 0.1^{c}	0.9054 ± 0.0003^{c}
	360	0.442 ± 0.001^{b}	20 ± 1^{a}	4.03 ± 0.01^{d}	57.1 ± 0.1^{c}	0.9013 ± 0.0002^{c}

Equal letters indicate homogeneous groups (α= 95%)

(Continuous) Table III.6.1. Experimental values for moisture content (x_w), storage temperature (C), pH, soluble solids content (Brix), and water activity (a_w) of mandarin orange marmalades over storage period. Also values of commercial mandarine marmalade.

FORMULATION	**TIME** (days)	x_w (g water/ g marmalade)	**T (ºC)**	**pH**	**BRIX**	a_w
C	1	0.46 ± 0.01^{b}	20 ± 1^{a}	3.63 ± 0.01^{b}	56.1 ± 0.1^{b}	0.9232 ± 0.0011^{d}
	90	0.468 ± 0.002^{b}	$20,5\pm1^{b}$	3.66 ± 0.01^{b}	56.6 ± 0.2^{b}	0.9175 ± 0.0002^{d}
	180	0.483 ± 0.002^{b}	$21,5\pm1^{c}$	3.66 ± 0.01^{b}	55.2 ± 0.1^{a}	0.926 ± 0.001^{d}
	360	0.482 ± 0.001^{b}	20 ± 1^{a}	3.63 ± 0.01^{b}	55.3 ± 0.1^{a}	0.9238 ± 0.0003^{d}
D	1	0.53 ± 0.02^{c}	20 ± 1^{a}	3.41 ± 0.01^{d}	58.2 ± 0.3^{d}	0.885 ± 0.001^{a}
	90	0.512 ± 0.002^{ab}	$20,5\pm1^{b}$	3.92 ± 0.01^{c}	58.7 ± 0.3^{d}	0.888 ± 0.001^{a}
	180	0.505 ± 0.001^{ab}	$21,5\pm1^{c}$	4.01 ± 0.02^{d}	57.3 ± 0.3^{c}	0.889 ± 0.001^{a}
	360	0.511 ± 0.001^{ab}	20 ± 1^{a}	3.99 ± 0.01^{d}	57.1 ± 0.1^{c}	0.889 ± 0.001^{a}
COMMERCIAL	—	0.47±0.24	20±1	3.16±0.01	61.2±0.3	0.8363±0.0003

Equal letters indicate homogeneous groups (α= 95%)

CONCLUSIONS

The wireless sensor networks based on EDETA robust architecture demonstrate a great potential for marmalade quality monitoring, being a high valuable tool for disciplines such as food processing. Also, the increasing number of applications which may need new sensors with the required characteristics to ensure food quality, open a promising research field, where a considerable growth in the near future is to be foreseen. The proposal meets the required characteristics of flexibility, scalability, dependability and easy installation and operation, also provides a perfect adaptation to the necessities of this type of food monitoring applications.

On the other hand, the reformulation of mandarin orange marmalade with non-cariogenic sweeteners such as tagatose and isomaltulose is feasible. Concerning moisture content, the marmalades formulated in equal proportion of tagatose and isomaltulose, presented similar values than commercial or control marmalades, over storage time. Furthermore, all marmalades elaborated for this study presented the regular fit temperature over storage time. Moreover, all of them reported microbiological stability during the storage period considered. As a general conclusion, can be stated that a new formulation of mandarin orange marmalade has been demonstrated as a good product satisfying all required characteristics and that an approach for real time and robust monitoring using WSN based on a new version of EDETA architecture has been successfully applied for the traceability of the mandarin orange marmalade.

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III.7. CHARACTERIZATION OF WATERMELON JELLY WITH NON CARIOGENIC SWEETENERS

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ABSTRACT

The aim of this study was to evaluate the substitution of sucrose with non-cariogenic and low glycemic index (isomaltulose and tagatose) sweeteners in jelly to which watermelon juice has also been added. The results showed that the jelly made with the new sweeteners had lower soluble solids. Moreover, the antioxidant activity increased over storage time in the control sample and in the jelly containing isomaltulose and tagatose, but without reaching the same levels as in the commercial jelly, due to its enrichment with vitamin C. In addition, new sweeteners did not affect the instrumental texture of jelly, and were very stable over time. However, the colour changed, especially in the jelly containing only tagatose. Finally, the jelly formulated with equal proportions of tagatose and isomaltulose achieved the same score in the sensory evaluation as the commercial jelly, evidencing that it is feasible to use these sweeteners to reformulate watermelon jelly.

Keywords: jelly, watermelon, tagatose, isomaltulose, antioxidants, colour, texture and sensory evaluation.

INTRODUCTION

Nowadays the society demands new increasingly healthier products which include fruits and vegetables in their formulations, along with new food processing technologies to make manufacturing these foods possible. Besides, the food industry must meet this challenge at a competitive price. One way of doing so is to add fruit to gelatine desserts. This jelly is usually prepared with traditional sugars (sucrose, glucose, etc…). However, consuming jelly of this type involves drawbacks for health (cariogenesis, high caloric intake, increase in the glycemic index, etc.). In this regard, WHO's current recommendation is that sugars should account for less than 10% of total energy intake per day. The objective of WHO's guideline is to provide recommendations on the consumption of free sugars in order to reduce the risk of non-communicable diseases

(NCDs) in adults and children, with a particular focus on the prevention and control of weight gain, dental caries and diabetes type II (WHO, 2014).

It is widely known that synthetic sweeteners in different foods can replace traditional sugars, especially in beverages. These sweeteners can be classified as bulk sweeteners such as the polyalcohol-type, or as intensive sweeteners such as saccharine, aspartame, etc. However, the fact that there is a laxative effect in the case of many polyalcohols or that intensive sweeteners may be linked to the development of different cancers or other diseases (Weihrauch and Diehl, 2004; Soffritti et al., 2006; Renwick and Nordmann, 2007, Guerrero and Flores, 2014) should be taken into account. Furthermore, these sweeteners do not always have the technological characteristics needed to provide the same texture or flavour as traditional sugars, so they must be combined with other components. Even then it is not always possible to obtain a similar product.

Nowadays, alternative natural sweeteners which are metabolized by the organism and have nutritional advantages, such as isomaltulose, tagatose, oligofructose, stevia, etc., can be found in the market. However, the challenge is to determine if their use is viable in the reformulation of traditional products, which would mean that they conserve or even improve the food's technological properties.

Isomaltulose is a disaccharide for use as a carbohydrate source, which fully or partially replaces sucrose or other highly digestible carbohydrates. Based on its chemical definition, isomaltulose is a sucrose-isomer consisting of glucose and fructose. However, compared to sucrose or glucose, it is less glycemic, less insulinemic and is non-cariogenic given that the linkage between glucose and fructose is more stable than in sucrose (Lina et al., 2002; FDA, 2005). In a solution, isomaltulose offers very low inversion rates and a low tendency to

hydrolize. In 2005 isomaltulose was recognized as being safe (GRAS) (FDA, 2005).

On the other hand, tagatose is a low carbohydrate functional sweetener, which is very similar to galactose in structure. It is naturally occurring and can be found in some dairy products. Tagatose has a physical bulk similar to sucrose or table sugar and is almost as sweet. However, it is metabolized differently, has a minimal effect on blood glucose and insulin levels, and also provides a prebiotic effect (Oh, 2007; Taylor et al., 2008; Calzada-León et al., 2013). Tagatose is especially suitable as a flavour enhancer or as a low carbohydrate sweetener. It can be used in ready-to-eat cereals, diet soft drinks, health bars, frozen yogurt/non-fat ice cream, soft confectionary, hard confectionary, frosting and chewing gum (Vastenavond et al., 2011). Tagatose is only minimally absorbed by the upper gastrointestinal tract. The unabsorbed tagatose is fermented in the colon, where it acts as soluble fibre (Taylor et al., 2008, Kearsley and O'Donnell, 2012). Besides, it does not promote tooth decay and it only provides 1.5 kcal/g to one's diet (Levin, 2002; Li et al., 2013). Tagatose has been approved, given that it is "Generally Recognized as Safe" (GRAS) in 2010 (FDA, 2010).

Watermelon is a seasonal fruit whose surplus production is generally not exploited enough. To change this situation, this fruit could be used to prepare gelatine desserts. Moreover, watermelon plays an effective role in reducing oxidative stress through phytochemical lycopene, is also high in other antioxidants and has been linked to a decreased risk of coronary heart disease (Hong et al., 2015). Therefore an increase in watermelon consumption could lead to a general improvement in the health of a society.

The aim of this study was to evaluate the replacement of sucrose in jelly with non-cariogenic and low glycemic sweeteners (isomaltulose and tagatose) and the addition of fresh watermelon juice in their formulation, by analysing its composition (Brix and moisture content), pH, water

activity, antioxidant capacity, mechanical and optical properties and stability over time. The results were then compared with those obtained for a control sample of jelly (with sucrose and watermelon juice) and with a commercial jelly (with sucrose, flavourings and colourants). A sensorial analysis was also carried out to check their acceptance.

MATERIALS AND METHODS

Components and formulation of watermelon jelly

The jelly was manufactured with watermelon (Citrullus vulgaris) juice, sucrose (Azucarera Iberia S.L., Madrid, Spain), isomaltulose (Beneo Mannheim, Germany), tagatose (40% of purity, Damhert NV/SA, Heusden-Holder, Belgium) and jelly (Junca Gelatines SL, Girona, Spain).

The jelly was prepared using the same proportions of ingredients as in commercial watermelon flavoured jelly powder (Royal, Kraft Foods, Madrid, Spain): 85% of sugars and 9.4% of gelatine. Following the manufacturer's instructions, the content of the power was diluted with 500 g of water, leading to a final composition of 12.6% of sugars and 1.6% of gelatine. In the jelly prepared with watermelon juice, the amount of sugars registered in the juice were taken into account when adding sweeteners in order to maintain the same proportion of sugars and gelatine as in the commercial formula. Furthermore, the water was replaced with 50% of watermelon juice. It is important to point out that commercial jelly also contain vitamin C, acidity regulators (fumaric acid, sodium citrate), flavourings and colorants (E100: curcumine and E120: carminic acid).

Depending on the combination of sweeteners/sugars as compared to the content of sugars, the following notation was used: Control jelly: 100% sucrose; I50T50 jelly: 50% isomaltulose and 50% tagatose; T jelly: 100% tagatose; I jelly: 100% isomaltulose; and commercial jelly (100% sucrose and no watermelon juice).

Manufacturing process of watermelon jelly

Figure 1 shows the flow chart of the stages required to prepare jelly for this study. The amounts of each component were weighed on an analytical scale (Precisa Gravimetrics AG, model BJ 6100D, Dietikon, Switzerland). Juice was extracted using a liquidiser (Molinex, model vitapress, Mayenne, France). For the stages of mixing and blending w a thermal blender (Thermomix, model TM31, Vorwerk, Wuppertal, Germany) was used. Once obtained, the containers were filled with the final product and stored in a refrigerator at 4 ºC.

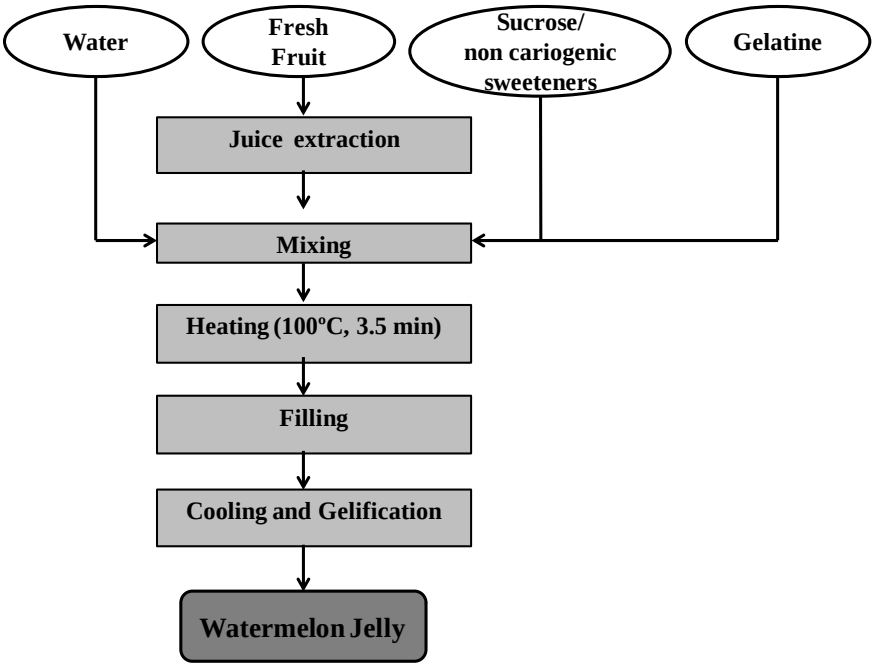

Figure III.7.1. Flow chart of the manufacturing process of watermelon jellies.

Analytical determinations

Analyses of moisture content, Brix, pH, water activity, antioxidant capacity, and optical and mechanical properties were performed for each

formulation of jelly, both initially and after 15 days of storage at 4 ºC in triplicate. Following are the methodologies followed for each case.

Moisture content, soluble solids (Brix), water activity and pH

Moisture content (x_w: kg water/kg watermelon jelly) was analysed gravimetrically following an adaptation of the AOAC method, (2000). Soluble solids of samples were measured by a refractometer (Atago3T, Tokyo, Atago), the results being obtained in Brix. Water activity (a_w) was determined using a hygrometer (Decagon Devices, Inc., model 4TE, Pullman, Washington, USA), at 25ºC. The pH was registered by pH-meter (Mettler Toledo, model SevenEasy, Barcelona, Spain), previously calibrated with buffered solutions of pH 7.0 and 4.0.

Antioxidant capacity

The antioxidant activity of watermelon jelly was analysed following the method described by Shahidi *et al*. 2006 with some modifications. It is based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. One gram of watermelon jelly was mixed with 6 mL of pure methanol for 5 min in a vortex, keeping the supernatant. This mixture was centrifuged at 13,000 rpm for 10 min. The absorbance of 3.9 mL of the DPPH solution (0.025 mg/mL, prepared in methanol: water (80:20)) was read at 515 nm in a spectrocolorimeter Thermo Fisher Scientific, Inc. (Helios Zeta UV-VIS, Waltham, Massachusetts, USA). Then, 0.1 mL of the supernatant was mixed with the methanolic solution of DPPH and absorbance was read again after 30 min. Quantification was performed considering a standard curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed as mg of Trolox equivalent per 100 g of watermelon jelly.

Optical Properties

The colour of watermelon jellies placed in 20 mm-wide cuvettes was measured using a spectrocolorimeter (Konica Minolta, Inc., CM-3600d model, Tokyo, Japan). CIE-L*a*b* coordinates were obtained using D65 illuminant and 10º observer as reference system.

Mechanical Properties

The samples were examined with a Texture Profile Analysis test (TPA) using a TA.XT plus Texture Analyser (Stable Micro Systems, Godalming, U.K.). For this purpose, a load cell of 50 kg and a 45 mm diameter cylindrical probe were used. The test conditions involved two consecutive cycles of 50% compression with 15 s between cycles. The test speed was 1 mm/s. From the resulting force-time curve the following parameters were quantified, and are defined by Bourne (1978) as: hardness (N) (maximum peak force during the first compression cycle), cohesiveness (the ratio of the positive force area during the second compression and the first compression), gumminess (N) (hardness x cohesiveness), adhesiveness (negative force area) and springiness (the distance that the sample recovers till the maximum peak of the first compression cycle divided by the distance from the beginning of the second compression cycle to its maximum peak).

Sensorial Analysis

An acceptance test using a 9-point hedonic scale (ISO 4121:2003 and ISO 5492:2008) was used to evaluate the following attributes in the samples: colour, flavour, texture, sweetness, global preference and intention of buying. The panel consisted of 30 trained panellists (aged from 20 to 50) who are regular consumers of this kind of dessert. Testing was conducted in a sensory evaluation laboratory built according to the international standards for test rooms. In this analysis the watermelon jelly formulated only with isomaltulose in the content of sweeteners (I) was not considered because the other samples of jelly had better quality.

Statistical analysis

Analyses of variance (multifactor ANOVA) were carried out using Statgraphics plus software (Statpoint Technologies, Inc., Centurion, Warrenton, Virginia, USA) to discern whether the effect of formulation or storage was significant on the watermelon jelly studied. Interactions between the factors were also considered.

RESULTS AND DISCUSSION

Compositional characterisation of watermelon jelly

Table 1 shows the results of moisture content (x_w), Brix, pH, water activity (a_w) and the antioxidant capacity of the jelly formulations with sucrose or new sweeteners (tagatose and isomaltulose). In all cases, the jelly reached a concentration of soluble solids higher than 14 Brix, being higher in the commercial and control samples. Consequently, the samples made with new sweeteners showed a greater content of water. Despite the statically significant differences found in terms of water activity in the jelly examined, in all cases the values of a_w were 0.99. Therefore, the type of sweetener had no influence on this parameter. However, the pH was much lower in the commercial jelly due to the acidity regulators (fumaric acid and sodium citrate) used in that formulation. Time did not cause significant changes in any of these parameters, except for the pH, which increased after 15 days of storage.

Antioxidant capacity was initially found to be the highest in the commercial sample due to the presence of vitamin C. On the other hand, the samples of jelly prepared with watermelon juice all initially showed the same antioxidant capacity, but this capacity increased in control samples and in jelly formulated with the combination of isomaltulose and tagatose, unlike in samples formulated with only isomaltulose or tagatose. Thus, the mixture of isomaltulose and tagatose could have a synergetic effect favouring antioxidant concentration.

TABLE III.7.1. Values for moisture content (x_w), Brix, water activity (a_w) and pH of watermelon jellies formulated with sucrose (control and commercial) or with new sweeteners (isomaltulose and tagatose), initially and after 15 days of storage.

FORMULATION	***TIME*** *(days)*	x_w *(g water/ g watermelon jelly)*	***ºBRIX***	a_w	***pH***	***ANTIOXIDANT CAPACITY*** *(mg Trolox/100 g watermelon jelly)*
COMMERCIAL	1	0.852 ± 0.001^{a}	16 ± 0.4^{d}	0.996 ± 0.001^{c}	3.667 ± 0.006^{a}	65 ± 9^{d}
	15	0.850 ± 0.001^{a}	15.7 ± 0.2^{d}	0.994 ± 0.001^{b}	3.80 ± 0.02^{b}	55 ± 5^{e}
CONTROL	1	0.853 ± 0.002^{a}	16.27 ± 0.06^{cd}	0.994 ± 0.0002^{b}	6.337 ± 0.006^{e}	7.9 ± 1.2^{ab}
	15	0.849 ± 0.003^{a}	16.27 ± 0.15^{c}	0.991 ± 0.001^{a}	6.46 ± 0.02^{f}	16.2 ± 0.2^{c}
I	1	0.863 ± 0.001^{b}	14.4 ± 0.2^{a}	0.993 ± 0.001^{ab}	6.203 ± 0.006^{d}	8.8 ± 1.3^{b}
	15	0.864 ± 0.005^{b}	14.73 ± 0.06^{ab}	0.993 ± 0.001^{ab}	6.37 ± 0.04^{f}	2.35 ± 1.13^{a}
T	1	0.860 ± 0.002^{b}	15.03 ± 0.15^{b}	0.992 ± 0.001^{ab}	6.25 ± 0.02^{d}	8.26 ± 0.69^{b}
	15	0.865 ± 0.003^{b}	15.1 ± 0.1^{b}	0.993 ± 0.002^{ab}	6.40 ± 0.03^{f}	3.69 ± 1.49^{ab}
I50T50	1	0.863 ± 0.001^{b}	14.8 ± 0.2^{b}	0.993 ± 0.0005^{b}	6.113 ± 0.013^{c}	9.93 ± 0.49^{b}
	15	0.873 ± 0.006^{c}	14.77 ± 0.21^{b}	0.994 ± 0.002^{b}	6.27 ± 0.04^{e}	15.83 ± 1.16^{c}

Equal letters indicate homogeneous groups

Mechanical properties

Figure 2 shows the average curves of the TPA analysis carried out on the samples of jelly used in this study along with the scheme used to determine the mechanical parameters. As can be seen, the curves obtained for commercial jelly showed more pronounced peaks than the other samples, both at the beginning and at the end of storage. Furthermore, after 15 days of storage, the second peak of the curve shifted to the right, especially in commercial jelly.

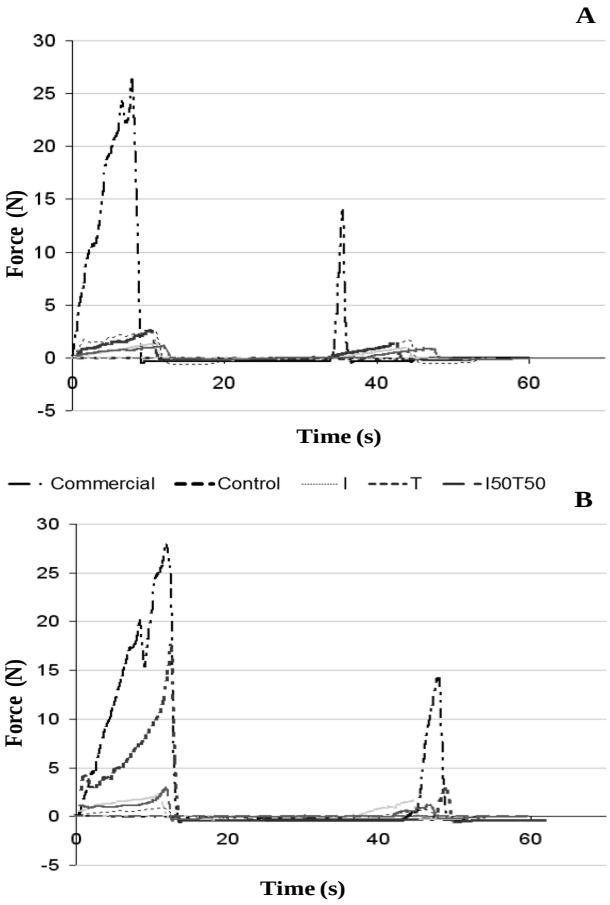

Figure.III.7.2. Representative curves of TPA test for watermelon jellies studied as a function of sweeteners used in its formulation initially (A) and after 15 days of storage (B).

Furthermore, Figure 3 shows the interaction charts (a significant level of 95%) of the mechanical parameters. Initially there were no significant differences in any mechanical parameters between the different samples of jelly, except for the springiness of commercial jelly, which was significantly lower. However, after 15 days of storage, the commercial jelly showed a significant increase in hardness and the gumminess while the control samples showed a decrease in adhesiveness, cohesiveness and springiness. Jelly formulated with non-cariogenic sweeteners maintained all its mechanical properties over time. However, in previous studies (Periche *et al.*, (2014)) carried out in gummy confections, lower values of hardness were observed when isomaltulose was used as compared to samples prepared with sucrose and glucose syrup before being stored.

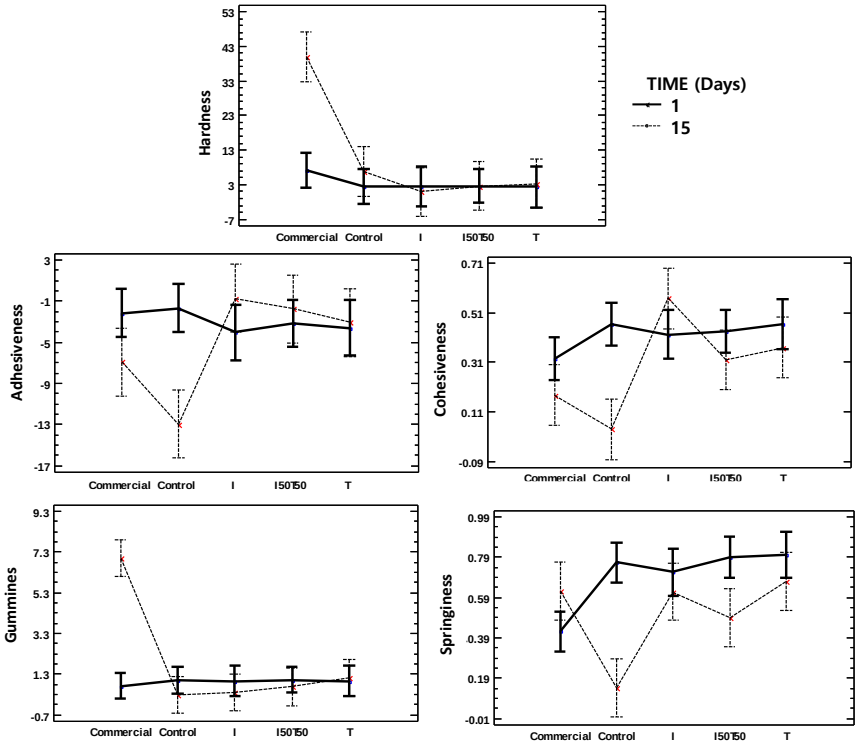

Figure III.7.3. Interaction graphics (significant level of 95%) of hardness, adhesiveness, cohesiveness, gumminess and springiness of watermelon jellies as a function of the formulation and storage time.

Therefore, depending on the product studied the interaction of sweeteners in gel formation has a different effect. According to these results, the new sweeteners used in this study could have a greater ability to maintain the mechanical properties of the gel over time. This behaviour could be attributed to the effect of the acids present in the commercial formulation.

Optical properties

Figure 4 shows the interaction charts of the colorimetric coordinates L*, a* and b*, chrome ($C^*=(a^{*2}+b^{*2})^{1/2}$) and hue ($h^*=arctg(b^*/a^*)$) depending on the formulation used and the storage time. As can be seen, the commercial jelly showed significant differences in coordinates L*, a* and b* as compared to the jelly samples formulated with sweeteners. More specifically, commercial samples showed lower values of luminosity but higher values of a* and b* coordinates. These differences may be attributable to the colourants used in commercial jelly (carminic acid (E-120) and curcumin (E-100)).

Taking in consideration only the jelly formulated with watermelon juice, it was noteworthy that the use of the new sweeteners significantly reduced its luminosity. Besides, L* in formulation with only tagatose significantly decreased over time. Initially there were no significant differences among samples for a* coordinate, but after storage, there was a significant change in the sample of jelly with only tagatose once again, but in this case it was an increase. The b* coordinate followed the same trend as a* coordinate, but in this case the mixture of isomaltulose and tagatose also showed an increase after storage. As a consequence, the chrome (C*) was significantly greater after 15 days in samples that only contained tagatose. Finally, the major values of hue (h*) were recorded in the jelly with the combination of isomaltulose and tagatose.

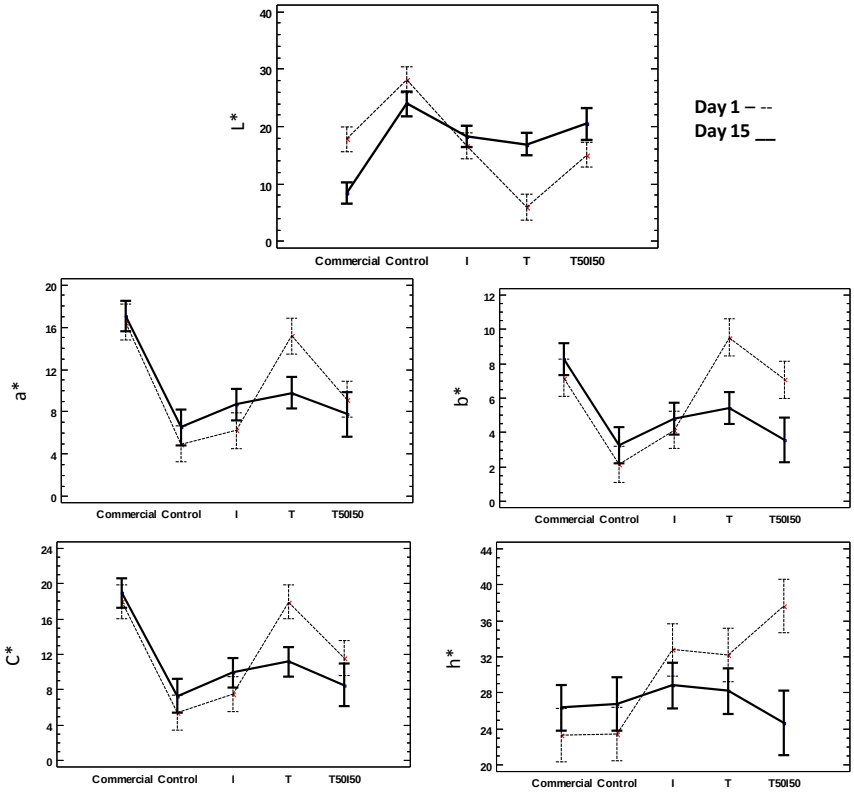

Figure III.7.4. Interaction graphics (significant level of 95%) of colour parameters: L*, a*, b* coordinates, chroma (C*) and hue (h*) of the watermelon jellies as a function of the formulation and storage time.

Sensory analysis

The results of sensory analysis of jelly, depending on their formulation (commercial, control, T, I50T50), are presented in Figure 5. In this case, two ANOVAs were performed in order to assess the influence of the commercial jelly on these results.

One ANOVA was carried out on all formulations (commercial, control, T, T50I50) and in the case of the other ANOVA, the commercial sample (control, T, T50I50) was excluded.

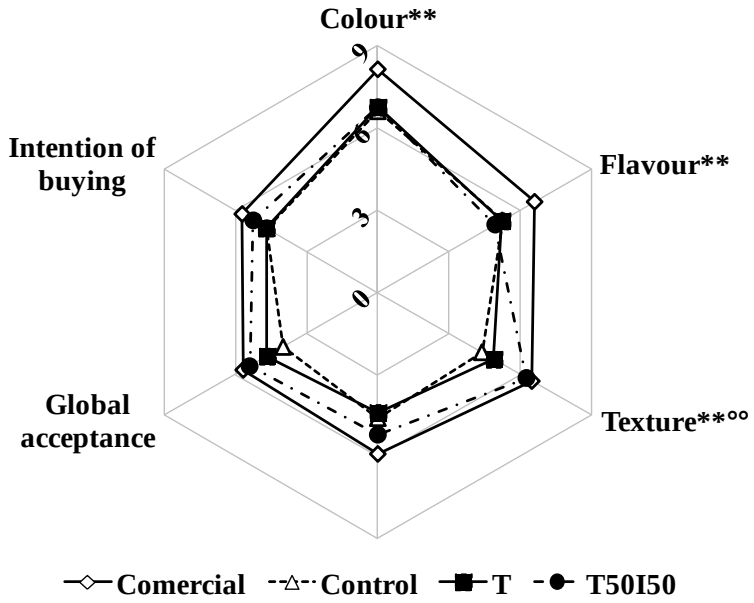

Figure III.7.5. Sensory assessment of watermelon jelly as a function of the formulation. Level of significance (ns) of the ANOVA considering all jellies: *ns: 95%, **ns: 99%. Level of significance (ns) ANOVA without considering the commercial jelly: °°ns: 99%.

From the results obtained in the first ANOVA it was concluded that colour and flavour were better in commercial jelly in comparison to the other samples of jelly due to the addition of colourants and artificial flavours. Moreover, the values for texture of the jelly made with the mixture of isomaltulose and tagatose were similar to those obtained for commercial jelly, being significantly lower in the other two cases. These results were the opposite of those obtained in the instrumental analysis of the mechanical properties, since no significant differences were initially detected in any of the samples studied, except for springiness, which was lower in the commercial sample. Finally, there were no significant differences observed with respect to sweet taste, global acceptance and intention of buying between the samples of jelly studied in the ANOVAs analysed.

Therefore, according to this study, the replacement of sucrose with a mixture of isomaltulose and tagatose in equal proportion would be feasible from a sensory point of view.

CONCLUSIONS

The reformulation of watermelon jelly with the non-cariogenic sweeteners used in this research is viable. The mixture of isomaltulose and tagatose and storage enhanced its levels of antioxidant capacity to values similar to those of jelly with sucrose, although values registered for commercial jelly were not reached.

The mechanical properties of jelly made with the new sweeteners were similar to those prepared with sucrose and were very stable. However, in terms of sensory analysis, texture was a determinant attribute in the evaluation of the jelly, and the two types of jelly scored the best were the jelly containing equal proportions of isomaltulose and tagatose and the commercial jelly. However, further studies should be carried out in order to improve the colour stability of jelly formulated only with tagatose over time.

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III.8. PHYSICOCHEMICAL CHARACTERISTICS OF CITRUS JELLY WITH NON-CARIOGENIC AND FUNCTIONAL SWEETENERS

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ABSTRACT

In this study the effect of sweeteners with low glycemic index and non-cariogenic characteristics (isomaltulose, oligofructose and tagatose) in jelly prepared with citrus juice has been evaluated considering as reference a citrus jelly formulated with sucrose. For that, analyses of soluble solids, moisture content, pH, water activity, antioxidant capacity, optical and mechanical properties of different blenders of these new sweeteners have been carried out, initially and after 15, 30 and 45 days of storage. Besides, mesophilic aerobics and moulds and yeasts have been also counted to determine their stability over time. A sensory evaluation of the citrus jelly has also been done.

The results showed the antioxidant activity decreased over storage time in all formulations. Throughout time tagatose increased luminosity whereas coordinates a*, b* and chrome of all the new formulations were lower than in jellies with sucrose. Moreover, the use of only oligofructose or tagatose or the mixture of isomaltulose and tagatose were the formulations most similar to the control jelly. Finally, the jelly prepared with the combination of isomaltulose and tagatose in equal proportions obtained the best scored in the sensorial analysis.

Keywords: jelly, citrus fruits, sweeteners, antioxidants, mechanical properties and sensory evaluation.

INTRODUCTION

Currently awareness of health-related issues in society has increased the demand of new functional foods and consequently food industry must be constanly innovating to offer consumers new alternative products. Bearing this in mind, the reformulation of jelly desserts with new non-cariogenic sweeteners available in the market could be a good chance to achieve this goal.

Traditionally, jelly desserts are mainly produced with edible gelatine, water, sugar and flavors. Despite the fact that this type of dessert is not considered with a high nutritional value, it is important to point out that this situation might change if natural vitamins and antioxidants provided from fruit juice were included in its formulation instead of the water.

Citrus fruits such as orange, lemon and mandarin orange have many beneficial properties due to their high content of fibre, vitamins, minerals, ascorbic acid and specially high content in antioxidant compounds, such as carotenoids, flavonoids and phenolic compounds (Álvarez *et al.*,2014; Balasundram *et al.*, 2006; Dhuique-Mayer *et al*., 2005; Navarro *et al.*, 2011). Besides, this type of fruits are available thoroughout the year because they are grown in the north and south hemispheres. Concretely, there is a total production of citrus fruits of 136 millions of tons per year divided in 71 millions of tons of oranges, 29 millions of tons tones of mandarin oranges and 15 millions of tons of lemons (FAOSTAT, 2013). As far as we know, a jelly dessert prepared with a mixture of different citrus juices does not exist in the market and it could expand the possibilities of commercialization.

In terms of composition, although jelly desserts have low content of gelatine this type of protein contains 18 different amino acids, including 8 essential amino acids (GME, 2015a, 2015b), being particularly rich in glycine, proline and hydroxyproline. Furthemore, gelatine is a natural colloide with properties of gelling and a stabilizing effect. Therefore, gelatine has a quite high nutritional value but with a low caloric power (17 kJ/ kg or 4 kcal/ g).

Other important components of jelly desserts are sugars. It is widely known that their their excessive consumption is related to tooth decay, diabetes and obesity (Edwards, 2002; Weihrauch & Diehl, 2004; Sloan, 2005; O'Donnell & Kearsley, 2012; WHO, 2014)), among other illnesses. To cope with these issues nowadays there are natural sweeteners such as tagatose, isomaltulose and oligofructose (FDA, 2001, 2005, 2010, 2011)

which need to be studied in order to check their capacity to replace sucrose and other sugars in traditional foods as jelly desserts.

Oligofructose is an oligosaccharide derived from fructose, which acts as dietary fibre regulating intestinal transit. It presents a prebiotic effect because it favours the selective growth of bifidus bacteria (Ledur *et al*., 2013). Besides, it reduces cholesterol and blood sugar levels (Chacon-Villalobos, 2006) and improves calcium absorption (Van den Heuvel *et al*., 1999). Nevertheless, it is highly soluble and possesses technological properties (sweet taste, stability…) analogous to sucrose (Pimentel *et al*., 2015). In 2011, oligofructose was recognized as safe (GRAS) (FDA, 2011).

D-Tagatose (D-tag) is a ketohexose, a stereoisomer of D-galactose and it is found naturally in several foods, including cheese and yoghurt. Its texture is very similar to sucrose and almost as sweet as sucrose, with only 1.5 kcal/g and it does not provoke dental caries (Levin, 2002; Oh, 2007; Taylor *et al.*, 2008; Calzada-Leon *et al.*, 2013). Tagatose is very suitable for confectionary products, ice creams, soft drinks and breakfast cereals (Vastenavond *et al*., 2012). Tagatose is minimally absorbed by the upper gastrointestinal tract. The unabsorbed tagatose is fermented in the intestines, causing a change in the proportions of various short chain fatty acids (Taylor *et al*., 2008). Thus, it is considered a functional food and besides it performs functions as soluble fibre favouring lactic acid bacteria and Lactobacillus specie bacteria (Petersen-Skytte, 2006). D-tagatose received GRAS status by the Food and Drug Administration in 2001 (Levin, 2002; Donner *et al.*, 2010; FDA, 2001, 2010).

Isomaltulose is a reducing disaccharide which is naturally present in honey, and sugar cane juice, and its appearance, taste and viscosities of aqueous solutions are comparable to sucrose (Schiweck *et al.*, 1990; Periche *et al.*, 2014). Based on its chemical definition compared to sucrose or glucose, it is less insulinemic, less glycemic and is non-cariogenic (Lina *et al.*, 2002).

However, it has a third of the sweetening power of sucrose (Lina *et al.,* 2002; De Oliva-Neto & Menão, 2009; Peinado *et al.,* 2012). In 2005, isomaltulose was recognized as safe (GRAS) (FDA, 2005).

In accordance with the properties of these three sweeteners (oligofructose, isomaltulose and tagatose), the aim of this paper was to evaluate their potential use as an alternative to sucrose in the development of jelly dessert along with the addition of fresh citrus juice on composition, antioxidant capacity, mechanical and optical properties, and sensory analysis.

MATERIALS AND METHODS

Components and formulation of citrus jelly

Jelly was manufactured with citrus fruits juice (*Citrus reticulata clementina, Citrus limon eureka, Citrus sinensis navelate*), sugar/sweeteners and gelatine (Junca Gelatines S.L., Girona, Spain). In control jelly sucrose (Azucarera Iberia S.L., Madrid, Spain) whereas in the new jellies the amount of sucrose was replaced by different mixtures of oligofructose obtained from Sensus (Frutalose, Roosendaal, Netherlands), isomaltulose obtained from Beneo (Palatinose, Mannheim, Germany) and tagatose obtained from Damhert Nutrition (Tagatesse, Heusden-Holder, Belgium).

The jelly dessert was prepared using the same proportions of ingredients as in a commercial orange flavoured jelly powder (Royal, Kraft Foods, Madrid,Spain) which were: 85.2% of sugars and 9.5% of gelatine. It is important to point out that commercial jelly also contained vitamin C, acidity regulators (fumaric acid, sodium citrate), flavourings and colourants (E100: curcumine and E120: carminic acid) but these components were not included in the jelly of this study. Following the manufacturer's instructions, the content of the powder was diluted with 500 g of water, leading to a final composition of 12.6% of sugars and 1.6% of gelatine.

In the jelly prepared with citrus juice, the amount of sugars contained in the juice were taken into account when adding sweeteners in order to maintain the same proportion of sugars and gelatine as in the commercial formula. Furthermore, 50% of the amount of water was replaced by citrus juice. The citrus juice was prepared with the following proportionos of each fruit: lemon juice 14%, orange juice 43% and mandarin orange juice 43%. Depending on the combination of sucrose/sweeteners used in jelly, the following notation was used: Control: 100% sucrose; I50T50: 50% isomaltulose and 50% tagatose; T: 100% tagatose; I: 100% isomaltulose; I50O50: 50% isomaltulose and 50% oligofructose, and O jelly: 100% oligofructose.

Manufacturing process of citrus jelly

Figure 1 shows the flow chart of the stages required to prepare jelly for this study. The amounts of each component were weight in an analytical scale (Precisa Gravimetrics AG, model BJ 6100D, Dietikon, Switzerland). Juice was extracted using a liquidiser (Molinex, model vitapress, Mayenne, France). For the stages of mixing and blending, a thermal blender (Thermomix, model TM31, Vorwerk, Wuppertal, Germany) was used. Once the mixture was obtained, containers were filled and stored at refrigeration at 4ºC.

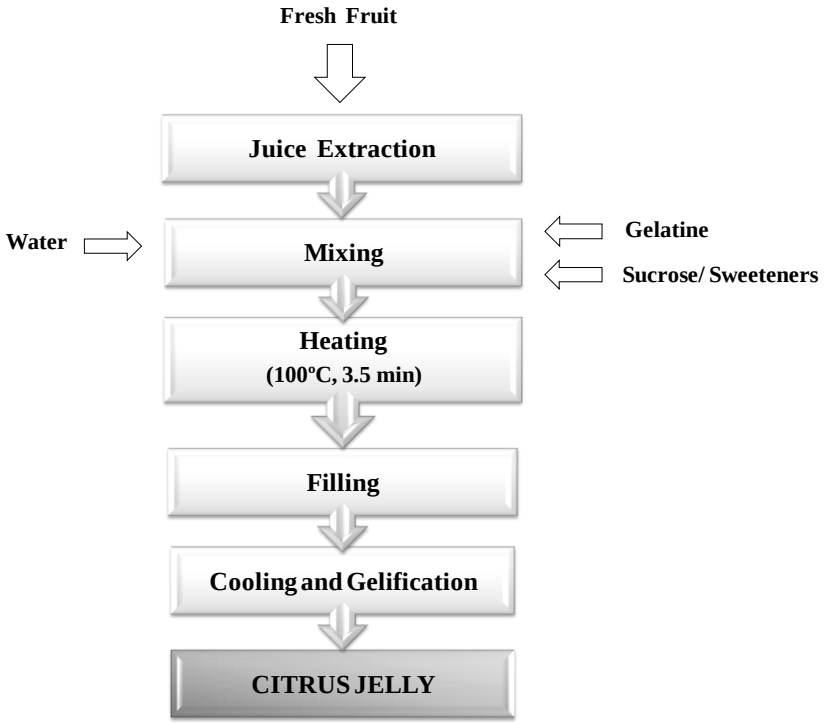

Figure III.8.1. Flow chart of the manufacturing process of citrus jellies

Analytical determinations

Analysis of moisture content, Brix, pH, water activity, antioxidant capacity, optical and mechanical properties and microbiological analysis were performed for each formulation of citrus jelly at 1, 15, 30 and after 45 days of storage at 4 ºC by triplicate. Next the methodologies followed for each case are described.

Moisture and soluble solids content, pH and water activity

Moisture content (x_w: g water/g citrus jelly) was analysed gravimetrically following an adaptation of the AOAC method (2000). Soluble solids content of samples were measured by a refractometer at 20ºC (Atago3T, Tokyo, Japan), the results being obtained in Brix.

pH was registered using a pH-meter (Mettler Toledo, model SevenEasy, Barcelona, Spain), previously calibrated with buffered solutions of pH 7.0 and 4.0. Water activity (a_w) was determined using a hygrometer (Decagon Devices, Inc., model 4TE, Pullman, Washington, USA), at 25ºC.

Determination of antioxidant capacity

The antioxidant activity of citrus jelly was analysed following the method described by Shahidi *et al*., 2006. This method is based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. 1 g of citrus jelly was mixed with 6 mL of pure methanol in a vortex for five minutes, keeping the supernatant. This mixture was centrifuged at 13,000 rpm for 10 minutes.

The absorbance of 3.9 mL of the DPPH solution (0.025 mg/mL, prepared in methanol: water (80:20)) was read at 515 nm in a spectrocolorimeter Thermo Fisher Scientific, Inc. (Helios Zeta UV-VIS, Waltham, Massachusetts, USA). Then, 0.1 mL of the supernatant was mixed with the methanolic solution of DPPH and absorbance was read again after 30 minutes. Quantification was performed considering a standard curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed as mg of Trolox equivalent per 100 g of citrus jelly.

Optical Properties

The optical properties of citrus jelly placed in 20 mm-wide cuvettes was measured using a spectrocolorimeter UV (Konica Minolta Inc., CM-3600d model, Tokyo, Japan). CIEL*a*b* coordinates were obtained using D65 illuminant and a 10º observer as reference system. Registered parameters were: L* (brightness), a* (red component), b* (yellow component), Chroma ($C^*=(a^{*2}+b^{*2})^{1/2}$) and hue ($h^*=arctg(b^*/a^*)$).

Mechanical Properties

The samples were examined with Texture Profile Analysis test (TPA) using a TA.XT plus Texture Analyser (Stable Micro Systems, Godalming, U.K.). For this purpose, a load cell of 50 kg and a 45 mm diameter cylindrical probe were used. The test conditions involved two consecutive cycles of 50% compression with 15 seconds between cycles. The test speed was 1 mm/s. Based on the resulting force-time curve it was possible to measure the following parameters were quantified, and are defined by Bourne (1978) as: hardness (N) (maximum peak force during the first compression cycle), cohesiveness (the ratio of the positive force area during the second compression and the first compression), adhesiveness (negative force area), gumminess (N) (hardness x cohesiveness) and springiness (the distance that the sample recovers till the maximum peak of the first compression cycle divided by the distance from the beginning of the second compression cycle to its maximum peak).

Microbiological analysis

Serial dilutions were prepared by homogenizing 10 g of citrus jelly with 90 mL of 1% sterile peptone water in a stomacher bag, using sterile techniques. Mesophilic aerobic populations were analyzed in Plate Count Agar (Scharlau Chemie, 1-329, Barcelona, Spain) incubating samples for 72 hours at 31ºC. Yeast and molds were determined in Sabouraud Chloramphenicol Agar (Scharlau Chemie, 1-166, Barcelona, Spain) plates for 5 days at 31ºC. Samples were analyzed at 1,15,30 and 45 days of storage. Plates were inoculated by triplicated. Microbial counts were expressed as CFU/g.

Sensorial Analysis

An acceptance test using a 9-point hedonic scale (ISO 4121, 2003; ISO 5492, 2008) was used to evaluate the following attributes in the samples: colour, flavour, texture, sweetness, global preference and intention of buying. The panel consisted of 30 trained panellists (aged from 20 to 50) who are regular consumers of this kind of dessert. Testing was conducted in a sensory evaluation laboratory built according to the international standards for test rooms. In this analysis the citrus jelly formulated using sweeteners containing only isomaltulose (I) and combination isomaltulose-oligofructose (I50O50), were not considered because the other samples of jelly were of a better quality.

Statistical analysis

Analyses of variance (multifactor ANOVA) were carried out by Statgraphics plus software (Statpoint Technologies, Inc., Centurion, Warrenton, Virginia, USA) to discern whether the effect of formulation or storage was significant on the citrus jelly studied. Interactions between factors were also considered.

RESULTS AND DISCUSSION

Compositional characterisation of citrus jelly

Table 1 shows the results of solids soluble content (Brix), moisture content (x_w), and water activity (a_w), pH, and antioxidant capacity of the jelly formulations with sucrose or new sweeteners (tagatose, oligofructose and isomaltulose). Initially, all jelly desserts reached a concentration of soluble solids around 22 Brix, but formulation that contained only oligofructose (O) had the highest values of ºBrix (≈23 Brix) unlike formulations contaning only isomaltulose (I) or tagatose (T) that showed the lowest values of ºBrix (≈21 Brix).

Table III.8.1. Values for, brix, moisture content (x_w), pH, water activity (a_w) and antioxidant capacity of citrus jelly formulated with sucrose (control) or with new sweeteners (isomaltulose, oligofructose and tagatose), initially, 15 days, 30 days and after 45 days of storage.

FORMULATION	***TIME*** *(days)*	x_w *(g water/ g marmalade)*	***ºBRIX***	***pH***	a_w	***ANTIOXIDANT CAPACITY*** *(mg Trolox/ 100 g Jelly)*
CONTROL	1	0.785±0.001^{a}	21.4±0.1^{b}	3.383±0.006^{a}	0.9846±0.0002^{c}	53.2±0.4^{d}
	15	0.785±0.004^{a}	21.8±0.2^{b}	3.543±0.006^{b}	0,9819±0.0001^{a}	30.6±1.4^{bc}
	30	0.911±0.07^{a}	21.5±0.5^{b}	3.523±0.006^{b}	0.9839±0.0002^{b}	26.8±1.1^{b}
	45	0.817±0.002^{a}	21.43±0.12^{b}	3.51±0.02^{b}	0.9848±0.0002^{c}	19.2±0.1^{a}
T	1	0.792±0.004^{a}	21.1±0.1^{b}	3.367±0.006^{a}	0.9894±0.0004^{d}	61.6±1.8^{d}
	15	0.64±0.29^{a}	20.7±0.3^{a}	3.533±0.012^{b}	0.9829±0.0002^{b}	32.1±2.2^{bc}
	30	0.88±0.02^{a}	22.2±0.3^{bc}	3.637±0.006^{d}	0.984±0.0001^{c}	28.8±2.0^{b}
	45	0.804±0.005^{a}	22.8±0.3^{c}	3.64±0.01^{d}	0.9851±0.0001^{d}	19.1±0.2^{a}
I50T50	1	0.78±0.01^{a}	22.5±0.006^{c}	3.677±0.006^{d}	0.9855±0.0002^{c}	57.7±5.9^{d}
	15	0.793±0.003^{a}	21.5±0.1^{b}	3.55±0,01^{b}	0.983±0.0003^{b}	27.4±1.5^{b}
	30	0.91±0.05^{a}	21.3±0.3^{b}	3.62±0.03^{c}	0.984±0.0001^{c}	25.4±1.2^{b}
	45	0.817±0.003^{a}	20.7±0.3^{a}	3.54±0.01^{b}	0.9826±0.0004^{b}	19.6±1.0^{a}

Equal letters indicate homogeneous groups.

(Cont.)Table III.8.1. Values for, brix, moisture content (x_w), pH, water activity (a_w) and antioxidant capacity of citrus jelly formulated with sucrose (control) or with new sweeteners (isomaltulose, oligofructose and tagatose), initially, 15 days, 30 days and after 45 days of storage.

FORMULATION	***TIME*** *(days)*	x_w *(g water/ g marmalade)*	***ºBRIX***	***pH***	a_w	***ANTIOXIDANT CAPACITY*** *(mg Trolox/ 100 g Jelly)*
I	1	0.809±0.004^{a}	21.1±0.1^{b}	3.527±0.006^{b}	0.986±0.0001^{c}	53.9±1.7^{d}
	15	0.804±0.013^{a}	20.6±0.2^{a}	3.487±0.006^{b}	0.9856±0.0004^{c}	36.2±1.2^{cd}
	30	0.88±0.04^{a}	20.5±0.2^{a}	3.53±0.02^{b}	0.9826±0.0002^{b}	34.6±1.0^{cd}
	45	0.818±0.003^{a}	21.2±0.1^{b}	3.52±0.01^{b}	0.9804±0.0003 ^{a}	18.8±0.3^{a}
O	1	0.775±0.003^{a}	23.2±0.1^{c}	3.64±0.01^{d}	0.9886±0.0001^{d}	52.5±1.3^{d}
	15	0.775±0.004^{a}	23.03±0.06^{c}	3.61±0.01^{c}	0.9868±0.0003^{c}	30.9±2.5^{bc}
	30	0.88±0.04^{a}	22.63±0.15^{c}	3.587±0.006^{c}	0.9866±0.0001^{c}	26.6±2.4^{b}
	45	0.82±0.07^{a}	22.57±0.12^{c}	3.593±0.006^{c}	0.9872±0.0003^{c}	17.9±0.2^{a}
I50O50	1	0.811±0.048^{a}	21.37±0.06^{b}	3.543±0.006^{b}	0.9885±0.0001^{d}	54.7±0.1^{d}
	15	0.866±0.143^{a}	21.23±0.06^{b}	3.527±0.006^{b}	0.9829±0.0003^{b}	30.9±2.5^{cd}
	30	0.897±0.005^{a}	21.2±0.3^{b}	3.51±0.02^{b}	0.9831±0.0002^{b}	31.7±0.4^{cd}
	45	0.81±0.02^{a}	21.3±0.2^{b}	3.52±0.02^{b}	0.9826±0.0004^{b}	18.2±0.3^{a}

Equal letters indicate homogeneous groups.

The storage decreased significantly ºBrix of formulation I50T50 but they increased in formulation T, being control and I50O50 the most stable formulations. Consequently, tagatose would be responsible for the variation of the soluble solid content over time. These results are in accordance our previous study carried out with watermelon jelly formulated with isomaltulose and tagatose (Rubio-Arraez et al., 2015), where jelly formulated only with tagatose showed initially around 15 Brix but there was an increase at the end of storage (15 days) remaining constant the soluble solid content (≈16 Brix) in control samples formulated only with sucrose.

In terms of moisture content, there were no significant differences due to formulation and only after 30 days of storage there was a significant increased but moisture content was the same as initially after 45 days in all cases. Besides, values of water activity were always 0.98, although formulation T showed the highest a_w initially. However, over the storage time there was a significant decrease in a_w in almost all cases especially in formulation I. Again, the pH was very similar in all formulations of jelly, but it initially was lower in formulation T and control jelly, althougth all jellies presented similar values after 45 days of storage.

As can be seen in table 1 initially all samples of jelly prepared with citrus juice showed the same antioxidant capacity except for I50T50 and T jellies which had the highest values due to their content in tagatose, which would be responsible for this behaviour. However, in all cases there was a significant reduction of the antioxidant capacity over the storage period considered, reaching similar values after 45 days for all formulations. Nevertheless, in a previous study carried out in watermelon jelly (Rubio-Arraez *et al.,* 2015), isomaltulose and tagatose and storage enhanced its levels of antioxidant capacity.

Optical properties

The interaction charts of the colorimetric coordinates L*, a* and b*, chroma (C*) and hue (h*) of the citrus jellies considering as factors the formulation of sucrose/sweeteners used and the storage time are shown in Figure 2. Initially control jelly desserts had more similarities in terms of luminosity with samples containing tagatose, but for coordinates a* and b* and for chroma formulation I50O50 was closed to control jelly. It was also observed that the citrus jelly formulated with tagatose (T and I50T50) showed an increase of their luminosity after 45 days of storage time in contrast with the decrease observed in formulations with isomaltulose and the combination of isomaltulose with oligofructose (I and I50O50) at the end of storage.

Coordinate a* in jellies containing only oligofructose or isomaltulose was the most stable in time but coordinate b* increased over time in formulation I whereas it decreased in formulation O. At the end of storage a*, b* and C* of the new formulations of jellies were lower than in control jellies, except for a* of formulation I50T50 which was equal to the control jelly. In terms of h*, it was noteworthy that all formulations showed values around the results of the control jelly, being formulation I above control jelly in the whole period of storage and formulation O the most similar to control jelly.

Mechanical properties

Figure 3 shows the average curves of the TPA analysis carried out on the samples of jelly used in this study. Moreover, Figure 4 shows the interaction charts (a significant level of 95%) of the mechanical parameters. As can be seen, initially the curves obtained for O jelly (formulated only with oligofructose), showed more pronounced peaks than the other samples and consequently they had the highest values of hardness without statistical differences respect to citrus jelly formulated with tagatose (T and I50T50), whereas samples prepared with isomaltulose showed the lowest hardness.

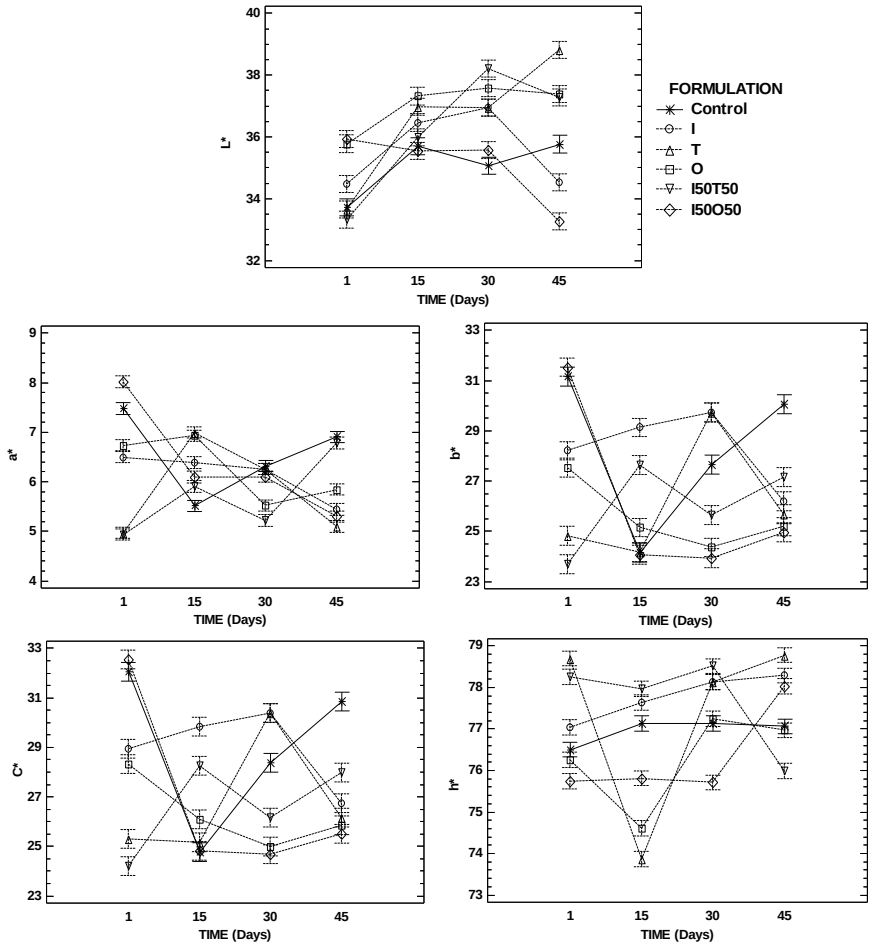

Figure III.8.2. Interaction graphics (95% of significant level) of colour parameters: L*, a*, b* coordinates, chroma (C*) and hue (h*) of the citrus jelly as a function of the formulation and storage time.

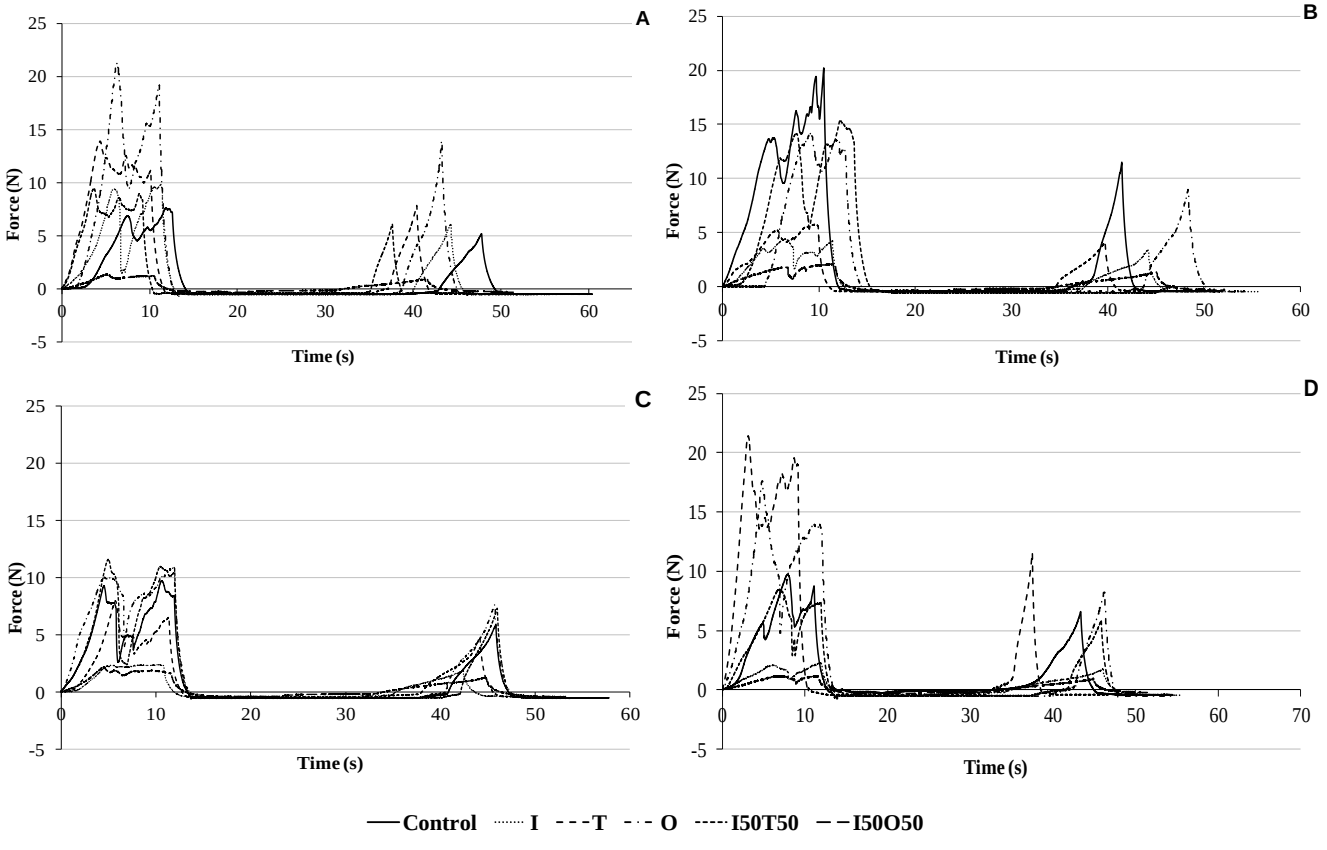

Figure III.8.3. Representative curves of TPA test for citrus jelly studied as a function of sweeteners used in its formulation initially (A), at 15 days (B), at 30 days (C) and after 45 days of storage (D).

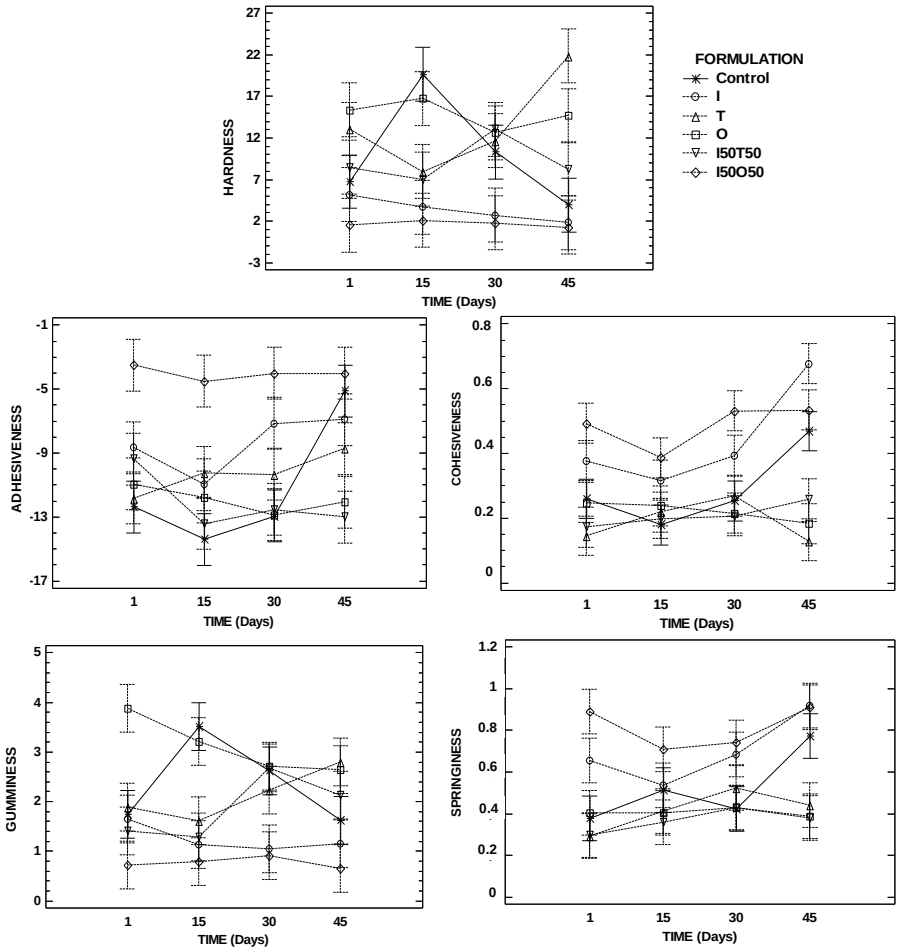

Figure III.8.4. Interaction graphics (95% of significant level) of hardness, adhesiveness, cohesiveness, gumminess and springiness of citrus jelly as a function of the formulation and storage time.

Besides, the second peak of control jelly was placed on the right of the others formulations. After 15 days of storage, the O jelly curve was overcome by the control jelly curve and its second peak was shifted to the right. However at 30 days of storage the second peaks of all formulation were placed together. Additionally, formulations with only tagatose (T) and oligofructose (O) showed highest peaks at the end of the storage (45 days). Even though, factor time did not have a significant effect on most of the mechanical parameters analyzed in these jellies.

However, the formulation composed by isomaltulose and oligofructose (I50O50) showed the highest values of adhesiveness. Besides, cohesiveness and springiness were also higher in that formulation and when there was only isomaltulose in the sweetener content of jelly (I). In contrast, gumminess was very low in formulation I50O50. Therefore, the most similar jellies to control samples were those prepared only with oligofructose (O) or tagatose (T) or the mixture of isomaltulose and tagatose (I50T50).

Microbiological analysis

Microbial counts of mesophilic aerobics, yeasts and moulds were not found in any of the citrus jelly at 1, 15, 30 days during storage. However, at the end of storage (45 days) there were presence of mesophilic aerobics, yeasts and moulds, except for the formulation that only contain oligofructose. According to Pascual & Calderón (2000), the microbial counts must not exceed $5 \cdot 10^2$ CFU/g mesophilic aerobics and $5 \cdot 10^1$ CFU /g yeasts and moulds. Even though, the microbial count was low ($3 \cdot 10^1$ CFU/g mesophilic aerobics and $2 \cdot 10^1$ CFU /g yeasts and moulds) after 45 days in all cases. These results evidence that the product is microbiologically stable for the studied period. The microbiological stability of the samples could be attributed to the acidity of citrus juice which gave place to a low pH (≈3.5) in citrus jellies.

Sensory analysis

The results of sensory analysis of citrus jelly, depending on their formulation (control, T, I50T50, O), are presented in Figure 5. As can be seen, O and I50T50 formulations showed the highest scores in all attributes, although no significant differences were found in colour, aroma and texture among the samples formulated with new sweeteners. Moreover, T and I50T50 formulations showed the highest sweetness, due to their higher content of tagatose. This would be according with the recommendations given by the manufacturer of the commercial tagatose (two tablespoons of sucrose provides the same sweetness as one

tablespoon of tagatose), though as was mentioned in the introduction, tagatose should have similar sweetening power to sucrose (Oh 2007; Taylor et al. 2008; Calzada-León et al. 2013). It is noteworthy that the global preference and intention of buying of jelly formulated with equal proportion of tagatose and isomaltulose (I50T50) presented the better score. These results are in accordance with our others studies carried out in watermelon jelly (Rubio-Arraez *et al*., 2015). Therefore, the replacement of sucrose by a mixture of isomaltulose and tagatose in equal proportion would be feasible from a sensory point of view.

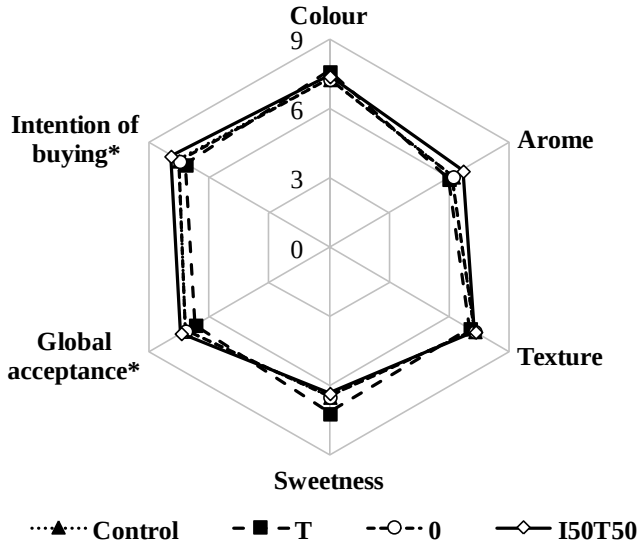

Figure III.8.5. Sensory assessment of citrus jelly as a function of the formulation. Level of significance (ns) of the ANOVA considering all jelly: *ns: 95%.

CONCLUSIONS

The reformulation of citrus jelly with non-cariogenic and low glycemic index sweeteners used in this research is viable. Besides, tagatose favoured the antioxidant capacity of citrus jelly initially, but not differences among all formulations were found after storage. In general, at the end of storage coordinates a*, b* and chrome of the new formulations of jellies were lower than in jellies with sucrose.

From the mechanical point of view the recommended formulations would be oligofructose (O) or tagatose (T) or the mixture of isomaltulose and tagatose (I50T50). In citrus jellies with only oligofructose there was no microbial presence in the considered storage period. According to sensorial analysis, I50T50 was the best scored jelly.

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IV. CONCLUSIONES

IV.CONCLUSIONES

IV.1. Cinética de deshidratación osmótica de rodajas de naranja utilizando edulcorantes saludables.

- Se ha comprobado que el extracto acuoso de stevia aumenta la concentración de sólidos solubles en estos productos.
- Los resultados pusieron de manifiesto que tanto la incorporación de stevia como de oligofructosa en los jarabes aumentó la pérdida de masa total y de masa de agua de las rodajas de naranja.
- El ajuste de la ecuación de Fick fue mejor cuando se utilizó la combinación de isomaltulosa y extracto acuoso de stevia.
- La difusividad efectiva de sólidos solubles fue mayor en las muestras deshidratadas utilizando la combinación de isomaltulosa-oligofructosa, lo que demuestra que la concentración de azúcares sería más rápida.

IV.2. Modelización de la deshidratación osmótica de rodajas de limón utilizando nuevos edulcorantes.

- En las muestras deshidratadas osmóticamente con isomaltulosa se favoreció la pérdida de masa total, sin embargo en las muestras sometidas a la tagatosa como agente osmótico sucedió lo contrario.
- La concentración de sólidos solubles alcanzados por las muestras deshidratadas con tagatosa sería menor que en el caso de los otros edulcorantes y además sería alcanzado más lentamente.
- La combinación de oligofructosa, extracto acuoso de stevia y tagatosa como agentes osmóticos, muestran una mayor concentración de sólidos solubles.
- La difusividad efectiva en rodajas de limón fue mayor en la combinación de oligofructosa y extracto acuoso de stevia.

IV.3. Influencia de los edulcorantes saludables (tagatosa y oligofructosa) sobre sus características fisicoquímicas en mermelada de naranja.

- La reformulación de mermelada de naranja con edulcorantes saludables como tagatosa y oligofructosa es factible.
- La mermelada de naranja formulada con igual proporción de la tagatosa y oligofructosa presentó mayor consistencia y su componente elástica aumentó con el tiempo.
- Las mermeladas elaboradas con oligofructosa mejoraron su capacidad antioxidante en comparación con la mermelada control. A su vez, presentaron ligeros cambios en el color, reduciendo su luminosidad durante el tiempo de almacenamiento.
- Las mermeladas resultaron estables microbiológicamente, durante todo el tiempo de almacenamiento.
- En relación al análisis sensorial, las formulaciones A (con igual proporción de tagatosa y oligofructosa), y B (30% oligofructosa y 70% tagatosa) presentaron las mejores calificaciones en todos los atributos.

IV.4. Caracterización de mermeladas de limón elaboradas con edulcorantes saludables (isomaltulosa y tagatosa).

- La reformulación de mermelada de limón con edulcorantes no cariogénicos, tales como isomaltulosa y tagatosa es posible.
- La capacidad antioxidante de las nuevas formulaciones disminuyó con el almacenamiento.
- La combinación de los nuevos edulcorantes redujo ligeramente los valores de los parámetros reológicos con respecto a las muestras con sacarosa.
- Las mermeladas de limón con una mayor proporción de isomaltulosa en su formulación, inicialmente presentaron alta luminosidad y tono en comparación con el resto, pero con el tiempo pardearon.
- Todas las mermeladas fueron microbiológicamente estables durante el período de almacenamiento considerado.

- Las mermeladas de limón elaboradas con tagatosa obtuvieron mejores puntuaciones en el análisis sensorial, debido a su alto poder edulcorante.

IV.5. Efecto de isomaltulosa y tagatosa como sustitutos de la sacarosa en mermeladas de mandarina.

- Es posible reformular las mermeladas de mandarina con edulcorantes no cariogénicos como isomaltulosa y tagatosa.
- Las muestras elaboradas sólo con tagatosa mostraron diferencias significativas en el comportamiento reológico, dando lugar a un carácter menos elástico y menor consistencia que el control.
- Se produjo una mejora en la luminosidad y la capacidad antioxidante en mermeladas con mayor contenido en tagatosa a lo largo del almacenamiento
- La muestra formulada únicamente con tagatosa obtuvo la mejor puntuación en la aceptación global y la intención de compra de los consumidores.

IV.6. Monitorización de mermeladas de mandarina durante la etapa de almacenamiento, utilizando redes de sensores inalámbricas.

- Las redes de sensores inalámbricas, son una herramienta muy valiosa para disciplinas como la tecnología de alimentos. Se divisa un futuro muy prometedor en la investigación debido al creciente número de aplicaciones que pueden necesitar nuevos sensores cuyo objetivo es asegurar la calidad de los alimentos.
- Se ha propuesto un enfoque basado en redes robustas de sensores inalámbricos con los requisitos específicos de flexibilidad, escalabilidad, confiabilidad y facilidad de instalación y operación, para la vigilancia y monitorización de alimentos almacenados, concretamente en las mermeladas de mandarina.
- Los resultados obtenidos en el análisis experimental coinciden con los obtenidos mediante la monitorización de las mermeladas con la red de sensores inalámbrica basada en la nueva arquitectura EDETA; obteniéndose además numerosas ventajas adicionales, como la

posibilidad de obtener medidas en tiempo real y de forma remota, obtener alarmas automáticas por parte del sistema, así como mucha mayor información sobre el estado de las mermeladas (al obtener más medidas) y un tratamiento automatizado de la misma.

- En cuanto a los parámetros de temperatura, todas las mermeladas muestran la misma tendencia y respecto al contenido en humedad, las mermeladas cuya combinación era en igual proporción tagatosa e isomaltulosa mostraron valores similares al control y al comercial.
- Las mermeladas de mandarina permanecieron estables microbiológicamente durante el tiempo de estudio (360 días).
- En líneas futuras de investigación, son necesarios nuevos transductores y sensores, con las características apropiadas para su integración en una red de sensores (básicamente de reducido tamaño, bajo coste y consumo), que posibiliten la monitorización de nuevos parámteros, permitiendo extender la aplicación de esta propuesta.

IV.7. Caracterización de gelatinas de sandía elaboradas con edulcorantes no cariogénicos (isomaltulosa y tagatosa).

- La capacidad antioxidante de las gelatinas de sandía, aumentó sobre todo en las gelatinas formuladas con sacarosa y con la combinación isomaltulosa-tagatosa durante el tiempo de almacenamiento, pero sin llegar a los mismos niveles que en la gelatina comercial debido a su enriquecimiento con vitamina C.
- Se confirma que los nuevos edulcorantes no afectaron a la textura instrumental de la gelatina, permaneciendo estable en el tiempo al igual que la gelatina control. Sin embargo, el color cambió especialmente en la gelatina que contenía sólo tagatosa.
- En cuanto al análisis sensorial la gelatina con la combinación en igual proporción de isomaltulosa y tagatosa, logró los máximos resultados. Por tanto es viable el uso de estos edulcorantes no cariogénicos en la formulación de gelatinas.

IV.8. Influencia de los edulcorantes no cariogénicos (oligofructosa, isomaltulosa y tagatosa) sobre sus características fisicoquímicas en gelatinas de cítricos.

- La reformulación de la composición de gelatinas de cítricos con edulcorantes de bajo índice glicémico y no cariogénicos utilizados en esta investigación es posible.
- La capacidad antioxidante disminuyó con el tiempo de para todas las muestras, sin embargo, la formulación de isomaltulosa y tagatosa mostró la mejor puntuación entre todas las formulaciones.
- Las gelatinas de cítricos formuladas con tagatosa, mostraron mayor luminosidad a lo largo del tiempo de almacenamiento.
- Las propiedades mecánicas de las gelatinas fueron muy similares, a excepción de la gelatina I50O50 (en igual proporción isomaltulosa y oligofructosa), con mayor adhesividad y gomosidad.
- La estabilidad microbiológica se mantuvo hasta los 45 días, manifestándose la presencia de mesófilos aerobios, levaduras y mohos, en todas las gelatinas, a excepción de la gelatina formulada únicamente con oligofructosa, que continuó inalterada más allá del tiempo de estudio.