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Nutritional quality and bioaccessibility of derivatives from fruits and vegetables by applying thermal and non thermal technologies (pulsed electric fields, high voltage electrical discharges, ultrasound)

Calidad nutricional y bioaccesibilidad de derivados de frutas y vegetales al aplicar tecnologías térmicas y no térmicas (pulsos eléctricos, descargas eléctricas de alto voltaje, ultrasonidos)

Tesis Doctoral Internacional

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CERTIFICAN QUE: la licenciada en Agricultura, desarrollo de la zona de producción agrícola con especialidad en Industria Alimentaria y Procesamiento de los Alimentos, Dña Magdalena Buniowska ha realizado bajo su dirección el trabajo que lleva por título: "Calidad nutricional y bioaccesibilidad de derivados de frutas y vegetales al aplicar tecnologías térmicas y no térmicas (pulsos eléctricos, descargas eléctricas de alto voltaje, ultrasonidos)" para optar al Título de Doctor por la Universitat de Valencia.

Y para que así conste, expiden y firman el presente certificado en Burjassot (Valencia), 16 marzo de 2016.

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ABBREVIATIONS

AA, ascorbic acid/ ácido ascórbico
ANOVA, analysis of variance/ análisis de la varianza
BA, bioaccessibility/ bioaccesibilidad
BHT, butylated hydroxytoluene/ butilhidroxitolueno
BI/ IP, browning index/ índice de pardeamiento
DPPH, 1,1-diphenyl-2-picrylhydrazyl/ 2,2-difenil-p-fenilendiamina
EFSA, European Food Safety/ Autoridad Europea de Seguridad Alimentaria
FAO, Food and Agriculture Organization of the United Nations/ Organización de las Naciones Unidas para la Agricultura y la Alimentación
FDA, Food and Drug Administration/ Administración de alimentos y medicamentos
GAE, gallic acid equivalents/ equivalentes de ácido gálico
H&L, yeast and molds/ hongos y levaduras
HMF, hydroxymethylfurfural/ hidroximetilfurfural
HP/ AP, high pressure/ altas presiones
HPLC, high performance liquid chromatography/ cromatografía líquida de alta eficacia
HTST, high temperature, short time/ alta temperatura, tiempo corto
HVED/ DEAV, high voltage electrical discharges/ descargas eléctricas de alto voltaje
LSD, least significant difference/ diferencia menos significativa
LTLT, long temperature, long time/ temperatura larga, tiempo largo
MO, microwaves/ microondas
NTB, total number of bacteria/ numero total de bacterias
ORAC, oxygen radical antioxidant capacity/ capacidad antioxidante radicales oxígeno
PE, pulsed electric fields/ pulsos eléctricos
PF- Q, physicochemical parameters/ parametros físico-quimicos
PO, organoleptic properties/ propiedades organolepticas

Reb, rebaudioside/ rebaudioside
RSM, response surface methodology/ metodología de superficie de respuesta
SG/ GE, steviol glycosides/ glucósidos de esteviol
SGF, simulated gastric fluid/ fluido gástric simulado
SIF, simulated intestinal fluid/ fluido intestinal simulado
Ste, stevioside/ steviosidos
T, time/ tiempo
Tª, temperature/ temperatura
TA/ AT, total anthocyanins/ antocianinas totales
TAC/ CAT, total antioxidant capacity/ capacidad antioxidante total
TC/ CT, total carotenoids/ carotenoides totales
TEAC, trolox equivalent antioxidant capacity/ capacidad antioxidante trolox equivalente
TI/ IT, turbidity index/ índice de turbidez
TPC, total phenolic compounds/ fenoles solubles totales
T_R, retention time/ tiempo de retención
TT, thermal treatment / tratamiento térmico
UHT, ultra high temperature/ temperatura ultra alta
UK, United Kingdom/ Reino Unido
UPLC, ultra performance liquid chromatography/ cromatografía líquida de alta resolución
USA, United States of America/ Estados Unidos de América
USN, ultrasound/ ultrasonidos
Vit C, vitamin C/ vitamina C

RESUMEN

El consumo de una dieta variada y rica en frutas y hortalizas ayuda a prevenir y a tratar ciertas enfermedades crónicas. La elaboración de nuevas bebidas a base de derivados de frutas y vegetales ricas en compuestos bioactivos, puede ayudar al incremento del consumo de estos alimentos y por tanto contribuir a la prevención de diferentes problemas de salud.

Por otra parte, la industria alimentaria muestra gran interés en la utilización de *Stevia rebaudiana* Bertoni como educolorante natural acalórico, como estrategia nutricional con el fin de disminuir la ingesta de azúcar. Además, la *stevia* contiene sustancias minerales, vitaminas, polifenoles y otros compuestos antioxidantes. En este sentido, las mezclas de bebidas de frutas endulzadas con *stevia* serían ricas en compuestos bioactivos y con una alta capacidad antioxidante.

Sin embargo, durante el procesado de las bebidas a base de frutas y verduras estas propiedades pueden cambiar. Los pulsos eléctricos, descargas eléctricas de alto voltaje así como ultrasonidos son tecnologías no térmicas que están siendo evaluadas como alternativa a las tecnologías térmicas convencionales de conservación. En los últimos años, estas tecnologías tienen un creciente interés debido a su potencial para minimizar pérdidas nutricionales en términos de valor nutritivo y funcional. Por lo tanto, es importante proporcionar y optimizar las condiciones de tratamiento, para minimizar los cambios no deseados que se produzcan en la composición del producto.

Por todo ello el objetivo del presente trabajo es evaluar el impacto de la aplicación de las diferentes tecnologías térmicas (pasterización y esterilización) y de tecnologías no convencionales (pulsos eléctricos, descargas de alto voltaje y ultrasonidos) sobre los componentes nutricionales, compuestos bioactivos y los parámetros fisicoquímicos en

derivados de frutas y verduras. También, se evalúa la bioaccesibilidad de los mismos mediante un proceso de digestión *in vitro* simulada ya que durante la digestión suceden cambios que es necesario conocer.

Los resultados obtenidos en el presente trabajo muestran que las condiciones de los tratamientos y la concentración de *stevia* tienen una influencia significativa en los valores de los compuestos analizados. Los resultados difieren según la tecnología aplicada. Los tratamientos no térmicos en la mayoría de los casos tienen un efecto positivo en el contenido de los compuestos bioactivos y en la capacidad antioxidante total, y un aumento de la bioaccesibilidad de los compuestos bioactivos y del poder antioxidante total en los productos estudiados, dependiente de la intensidad aplicada. Respecto a la bioaccesibilidad de los batidos de fruta y verdura analizados se observa un efecto positivo de la temperatura en la liberación y micelarización.

Por lo tanto, la optimización de los condiciones de los tratamientos térmicos y no térmicos es necesaria para el desarrollo de nuevos productos de frutas y vegetales con un alto contenido en compuestos bioactivos y propiedades antioxidantes mejoradas.

Palabras claves: Pulsos electricos (PE), descargas de alto voltaje (DEAV), ultrasonidos (USN), esterilización, pasterización, bioaccesibilidad, *Stevia rebaudiana* Bertoni, compuestos bioactivos, capacidad antioxidante.

RESUM

El consum amb una dieta variada i rica en fruites i hortalisses ajuden a prevenir i a tractar certes malalties cròniques.

L'elaboració de noves begudes a partir de derivats de fruites i vegetals rics en compostos bioactius, pot ajudar a l'increment del consum d'aquests aliments i per tant, contribuir a la prevenció de diferents problemes de salut.

D'altra banda, últimament la indústria alimentària mostra un gran interés en la utilització de *Stevia rebaudiana* Bertoni com a edulcorant natural acalòric, com a estratègia nutricional amb la finalitat de disminuir la ingesta de sucre. A més, la *stevia* conté substàncies minerals, vitamines, polifenols i altres compostos antioxidants. En aquest sentit, les mescles de begudes de fruites endolcides amb *stevia* serien riques en compostos bioactius i amb una elevada capacitat antioxidant.

No obstant això, durant el processament de les begudes amb fruites i verdures poden canviar les seues propietats. Els polsos elèctrics, descàrregues elèctriques d'alt voltatge així com ultrasons són tecnologies no tèrmiques que estan sent avaluades com a alternativa a les tecnologies tèrmiques convencionals de conservació. En els últims anys, aquestes tecnologies tenen un creixent interés a causa del seu potencial per a minimitzar pèrdues nutricionals en termes de valor nutritiu i funcional. Per tant, és important proporcionar i optimitzar les condicions de tractament, per a minimitzar els canvis no desitjats que es produïsquen en la composició del producte.

Per tot açò, l'objectiu del present treball és avaluar l'impacte de l'aplicació de les diferents tecnologies tèrmiques (pasteurització i esterilització) i de tecnologies no convencionals (polsos elèctrics, descàrregues d'alt voltatge i ultrasons) sobre els components nutricionals, compostos bioactius i els paràmetres físic-químics en derivats de fruites i verdures. També, s'avalua

la bioaccesibilitat dels mateixos mitjançant un procés de digestió *in vitro* simulada, ja que durant la digestió succeeixen canvis que és necessari conéixer.

Els resultats obtinguts en el present treball mostren que les condicions dels tractaments i la concentració de *stevia* tenen una influència significativa en els compostos analitzats. Els resultats difereixen segons la tecnologia aplicada. Els tractaments no tèrmics en la majoria dels casos tenen un efecte positiu en el contingut dels compostos bioactius i en la capacitat antioxidant total, i un augment de la bioaccesibilitat dels compostos bioactius i del poder antioxidant total en els productes estudiats, dependent de la intensitat aplicada. Respecte a la bioaccesibilitat dels batuts de fruita i verdura analitzats s'observa un efecte positiu de la temperatura en l'alliberament i micelarització.

Per tant, l'optimització de les condicions dels tractaments tèrmics i no tèrmics és necessària per al desenvolupament de nous productes de fruites i vegetals amb un alt contingut en compostos bioactius i propietats antioxidants millorades.

Paraules claus: Polsos elèctrics (PE), descàrregues d'alt voltatge (DEAV), ultrasons (USN), esterilització, pasterització, bioaccesibilitat, *Stevia rebaudiana* Bertoni, compostos bioactius, capacitat antioxidant.

SUMMARY

The consumption of a varied diet, rich in fruit and vegetables, helps prevent and treat certain chronic diseases. The development of new beverages based on derivatives from fruit and vegetables rich in bioactive compounds, can help increase the consumption of these foods and therefore contribute to the prevention of various health problems.

Furthermore, the food industry shows great interest in the use of *Stevia rebaudiana* Bertoni as a natural non-caloric sweetener, as a nutritional strategy to reduce sugar intake. Moreover, *stevia* contains minerals, vitamins, polyphenols and other antioxidant compounds. In this respect, mixtures of fruit beverages sweetened with *stevia* would be a rich in bioactive compounds and have a high antioxidant capacity.

However, during the processing of the fruit and vegetable based beverages, these properties may change. Pulsed electric fields, high voltage electrical discharges as well as ultrasound are non-thermal technologies which are being evaluated as an alternative to conventional thermal technologies of conservation. In recent years, these technologies have had an increasing interest due to their potential to minimize nutrient losses in terms of nutritional and functional value. Therefore, it is important to provide and optimize treatment conditions to minimize unwanted changes that occur in the product composition.

In conclusion, the objective of this study is to evaluate the impact of the application of different thermal technologies (pasteurization and sterilization) and non-conventional technologies (pulsed electric fields, high voltage electrical discharges and ultrasound) on the nutritional components, bioactive compounds and physiochemical parameters of derivatives from fruit and vegetables. In addition, the bioaccessibility of these are also evaluated through a process of *in vitro* simulated digestion, as during digestion changes occur which are necesary to know about.

The results obtained in this work show that the conditions of treatments and *stevia* concentration have a significant influence on the values of the compounds analysed. The results differ depending on the technology applied. Non-thermal treatments in most cases have a positive effect on the content of bioactive compounds and total antioxidant capacity, and an increase of bioaccesibility of bioactive compounds and total antioxidant capacity in the products studied, depending on the energy inputs applied. With regard to the bioaccessibility of fruit and vegetable smoothies analyzed, a positive effect of temperature on the liberation and micellarisation is observed.

Hence, the optimization of the conditions of thermal and non-thermal treatment is necessary for the development of new fruit and vegetables products with a high content of bioactive compounds and improved antioxidant properties.

Keywords: Pulsed electric fields (PEF), high voltage electrical discharges (HVED), ultrasound (USN), sterilization, pasteurization, bioaccessibility, *Stevia rebaudiana* Bertoni, antioxidant capacity.

INTRODUCTION

1. INTRODUCTION

1.1 DESARROLLO DE BEBIDAS A BASE DE FRUTAS Y VEGETALES

La industria alimentaria atrae la atención de los consumidores a través de alimentos y bebidas funcionales que son fáciles de preparar y consumir (Wootton-Beard & Ryan, 2011). Durante las últimas décadas, hay una creciente demanda de alimentos y bebidas saludables en todo el mundo así como de alimentos funcionales, ricos en fitoquímicos como las frutas y verduras que se han relacionado con la prevención de ciertas enfermedades, siendo necesario un estudio de las características y beneficios atribuidos a estos compuestos (Dembitsky et al., 2011).

Varios estudios epidemiológicos sugieren que los compuestos bioactivos tienen efectos beneficiosos sobre la salud y están involucrados en la reducción de enfermedades degenerativas como el cáncer de pulmón y del tracto digestivo (Al-Juhaimi, 2014). Algunos estudios demuestran que el consumo de frutas y hortalizas reduce la incidencia y mortalidad de estas enfermedades (Boeing et al., 2012; Wang et al., 2014) y hasta donde se conoce, este efecto protector está determinado por la capacidad antioxidante de estos alimentos, debido principalmente a los polifenoles así como la vitamina C, los carotenoides y la vitamina E (Sikora et al., 2008).

En la actualidad hay un incremento en el desarrollo de nuevas bebidas que son combinaciones de zumos de frutas exóticas y tradicionales e incluso con verduras. Estas frutas son buena fuente de compuestos bioactivos que han demostrado que contribuyen a la capacidad antioxidante (Ribeiro da Silva et al., 2014; Shofian et al., 2011).

Por otra parte, el consumo de productos derivados de mango y papaya como zumos, purés o frutas cortadas ha aumentado, en respuesta a la

demanda de los consumidores de alimentos saludables, de alto valor nutritivo (Rawson et al., 2011a; Reddy et al., 2010).

La papaya es apreciada por su sabor, calidad nutricional y propiedades digestivas. Tiene un alto contenido en carotenoides principalmente β-caroteno (452 μg/100 g) (Jeffery et al., 2012), potasio, fibra, acido ascórbico, elevada capacidad antioxidante (Gayosso-García et al., 2011; Ribeiro da Silva et al., 2014) y taninos condensados con un contenido de 977.16 μg GA/ g (Morillas-Ruiz & Delgado-Alarcón, 2012).
El consumo de papaya mejora el proceso digestivo, debido a la enzima proteolítica "papaína", cuyo contenido es más alto en las frutas maduras (Boshra & Tajul, 2013).

El mango destaca por su sabor, su alto contenido de fibra (2 g/100 g), vitamina C (19-34 mg/100 g) (Ma et al., 2011), β-caroteno (4982 μg /100 g) (Jeffery et al., 2012), vitamina E, y compuestos fenólicos principalmente taninos condensados, flavonoles (971 μg GA/ g), pero depende de la variedad de la fruta (Morillas-Ruiz & Delgado-Alarcón, 2012). Todo ello hace que tenga una alta capacidad antioxidante y calidad nutricional (Kim et al., 2010; Siddiq et al., 2013), que son vitales para el crecimiento, el desarrollo y la salud.

La zanahoria es una verdura rica en carotenoides, α-caroteno (2744 μg/100 g) y β-caroteno (4982 μg/100 g) (Jeffery et al., 2012). También es buena fuente de compuestos fenólicos y fibra (Chantaro et al., 2008). Los distintos colores de las diferentes variedades de zanahorias dependen de la composición de fenoles y carotenoides, siendo el licopeno responsable del color rojo y las antocianinas del color purpura (Arscott & Tanumihardjo, 2010). Cerca de 28 millones de toneladas de zanahorias y nabos se produjeron en todo el mundo en el año 2009. La producción

anual de zanahorias en Europa es superior a 6 millones de toneladas (FAO, 2011).

La calabaza es una buena fuente de fibra y sustancias bioactivas como los carotenoides y polifenoles. El color rojo y naranja indica un elevado contenido de carotenoides (Dietrich et al., 2013), entre 1.4 y 7.6 mg/100 g, incluyendo mayor contenido de luteína y β-caroteno (Kreck et al., 2006). Diferentes estudios indican que el consumo de carotenoides reduce el riesgo de enfermedades degenerativas y cardiovasculares, cataratas, y ciertos tipos de cáncer (Provesi et al., 2011).

La naranja es una buena fuente de compuestos bioactivos, tales como carotenoides, compuestos fenólicos, vitamina C y poder antioxidante total (Al-Juhaimi & Ghafoor, 2013). Numerosas propiedades terapéuticas han sido atribuidas a las frutas cítricas, reducen el riesgo de cáncer, antiviral, antitumoral, tienen actividades anti-inflamatorias, y efectos sobre la fragilidad capilar, así como una capacidad de disminuir la agregación plaquetaria (Karoui & Marzouk, 2013).

Durante los procesos de elaboración de los zumos se producen subproductos de frutas que tienen un elevado contenido de compuestos bioactivos. En la última década se ha incrementado al interés por los subproductos de frutas por su contenido de compuestos bioactivos, entre otros la piel de naranja (subproducto de la industria citrícola) que es buena fuente de compuestos bioactivos como compuestos fenólicos, carotenoides y vitamina C, que pueden ser utilizados para su adición a los alimentos y/o nutracéuticos (González-Gómez et al., 2014; Jwanny et al., 2012), también tienen un uso potencial para obtención de fibra dietética (Wang et al., 2015).

El desarrollo de nuevas bebidas basadas en mezclas de zumos de frutas exóticas y naranja con otros ingredinetes como leche, bebida de avena y bebida de soja han recibido una buena acogida por parte de los consumidores siendo un vehículo para la ingesta de vitaminas, proteínas y minerales (Cilla et al., 2012; Morales-de la Peña et al., 2011).

Existe un consenso general de que las frutas y vegetales son un factor clave para una dieta equilibrada. De acuerdo con los resultados de la EFSA (2010) el consumo de frutas y verduras en Europa es de 386 g mientras que se recomienda 400 g por día. La ingesta diaria de estos alimentos promueve la salud y reduce el riesgo de varias enfermedades crónicas (Boeing et al., 2012). Una forma de aumentar el consumo de frutas y verduras, son los smoothies. Se conoce como "smoothies" las bebidas mezclas de frutas trituradas, pulpa de fruta, zumos de vegetales, yogur o leche (Teleszko & Wojdylo, 2014).

Al utilizar fruta o verdura triturada, los smoothies contienen fibra además de compuestos nutritivos y bioactivos. Por otra parte la digestion de estos smoothies es más sencilla que la fruta ya que con la batidora se pueden triturar las piezas más duras y resistentes. Ello hace que se absorban mejor los nutrientes de los componentes de la bebida.

También hay un aumento de la demanda de productos bajos en energía lo cual conlleva la búsqueda de sustancias naturales que puedan sustituir el sabor dulce del azúcar sin cambios en la apreciación organoléptica y sin efectos negativos en la salud humana. En la elaboración de alimentos y bebidas se está continuamente trabajando para proporcionar calidad sensorial a estos productos y responder a las espectativas de los consumidores (Porto & Andre, 2007). Por eso la industria alimentaria ha mostrado gran interés en el potencial educolorante de algunas plantas y su adición a los productos alimenticios, sobre todo en el uso de *Stevia rebaudiana* Bertoni (Chaturvedula et al., 2011; Korir et al., 2014). Es una planta herbácea dulce cuyas hojas contienen glucósidos diterpénicos, con

un alto poder edulcorante (sin calorias) (Tavarini & Angelini, 2013). La FDA (Food and Drug Administration) autorizó su comercialización en el año 2008 y más recientemente, en Noviembre del 2011, la Comisión Europea ha aprobado los glucósidos de esteviol como nuevo aditivo alimentario (E-960) (EFSA, 2010). Los esteviosidos tienen un gran potencial en la industria alimentaria como una estrategia para reducir el consumo de azúcar (Tavarini & Angelini, 2013; Wölwer-Rieck, 2012). Se emplea en panadería, pastelería, salsas, yogurt, helados, zumos, etc.

Los principales glucósidos de esteviol que se encuentran en la *stevia* son el estevióside (4-13 %) y el rebaudiósido A (2-4 %). Otros glucósidos de esteviol también conocidos son: dulcósido A (0.3 %), rabaudiósido B, C (1-2 %), D, F y esteviolbiósido (Geuns, 2003; Tavarini & Angelini, 2013). La composición de los extractos de *stevia* purificada dependen principalmente del método de producción utilizado en la fabricación y de los factores ambientales y genéticos de la planta original.

Los extractos de *stevia* tienen capacidad para reducir la diabetes, candidiasis, presión arterial elevada y abrasiones de la piel (Alupului et al., 2009). Además de ser un educolorante acalórico, las hojas de *stevia* tienen una alta cantidad de compuestos fenólicos, vitamina C, carotenoides y clorofilas (Abou-Arab et al., 2010; Barba et al., 2014; Lemus-Mondaca et al., 2012; Ramaya et al., 2014; Tadhani et al., 2007). También presenta capacidad antimicrobiana, antifúngica y propiedades antioxidantes (Abou-Arab et al., 2010; Belda-Galbis et al., 2014; Criado et al., 2014; Gamboa, 2012) que hace que el interés de la *stevia* aumente entre los tecnólogos y entre la industria alimentaria. Pero hasta el día de hoy hay pocos datos disponibles con respecto a los esteviol glucósidos y su estabilidad en distintas matrices alimentarias (Nehir El & Simsek, 2012).

1.2 CARACTERISTÍCAS ESTRUCTURALES Y NUTRICIONALES DE LOS COMPUESTOS BIOACTIVOS

1.2.1 ÁCIDO ASCÓRBICO

El ácido ascórbico (AA, vitamina C) es uno de los compuestos solubles en agua, generalmente presente en muchas frutas y plantas.
En el término vitamina C se engloban todos los compuestos que presentan la actividad biológica del ácido L-ascórbico (ácido 2,3-enediol, L-gulónico). Es un compuesto químicamente sencillo, aunque presenta una estructura inusual cuya fórmula empírica es $C_6H_8O_6$; es un derivado lactónico del ácido hexurónico y se corresponde con una forma oxidada de la glucosa; en concreto es una α-cetolactona de 6 átomos de carbono que muestra un anillo lactona de cinco miembros y un grupo de enediol bifuncional con un grupo carbonilo adyacente (Figura 1).

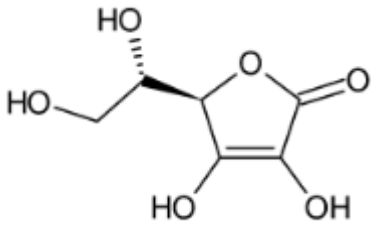


Figura 1. Estructura química del ácido ascórbico.

De acuerdo con Louarme & Billand (2012), el ácido ascórbico es un antioxidante natural y un aditivo alimentario común utilizado para mejorar la calidad del producto a través de la prevención del pardeamiento y la mejora de la vida útil. El ácido ascórbico funciona como antioxidante y cofactor enzimático, además juega un papel fundamental no sólo en los diversos procesos biológicos de las plantas, sino también en el mantenimiento de la salud humana, tales como la reducción de riesgo de

sufrir enfermedades crónicas, ayuda a la formación de colágeno y al desarrollo normal de los huesos, y en el tratamiento del cáncer (Olmos et al., 2006). El ácido ascórbico se encuentra principalmente en frutas y verduras, algunas tienen alto contenido como: pimientos, fresas, limones, papaya, mango, naranjas, uvas, limas (García-Closas et al., 2004; Lee & Kader, 2000).

1.2.2 CAROTENOIDES

Los carotenoides se encuentran en frutas y verduras, se les pueden dividir en dos grupos en función de su composición química. Desde el punto de vista nutricional las xantofilas son la luteína, zeaxantina y β-cryptoxantina y los carotenos más relevantes: α-caroteno, β-caroteno y licopeno. En general, los principales carotenoides en los vegetales son: luteína, β-caroteno, neoxantina y violoxantina, mientras que las xantofilas suelen encontrarse en mayor proporción en las frutas. Las Figuras 2-3 muestran las estructura de varios carotenos y xantofilas.

Los carotenoides forman una de las clases más importantes de pigmentos vegetales y desempeñan un papel crucial en la definición de los parámetros de calidad de las frutas y hortalizas (Van den Berg et al., 2000).

Los carotenoides son responsables de los colores rojo, naranja y tonalidades amarillas de hojas de plantas, frutas, así como del color de las flores. Fiedor & Burda (2014) en un estudio epidemiológico han demostrado una asociación entre el consumo de alimentos ricos en carotenoides con una reducción en el riesgo de podecer varias enfermedades crónicas. La investigación sugiere que los alimentos que contienen carotenoides pueden proteger contra el cáncer de pulmón, boca y cáncer de garganta (Wiesman, 2008).

CAROTENOS

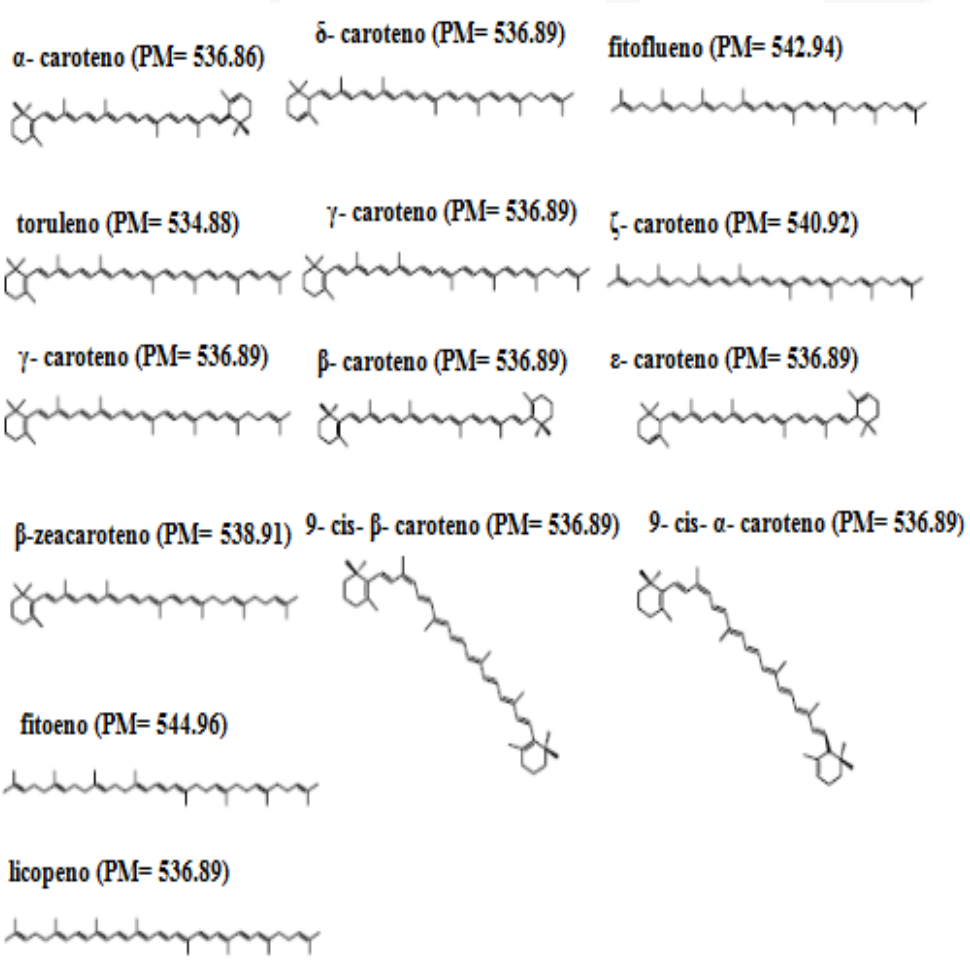


Figura 2. Estructura química de los carotenos.

XANTOFILAS

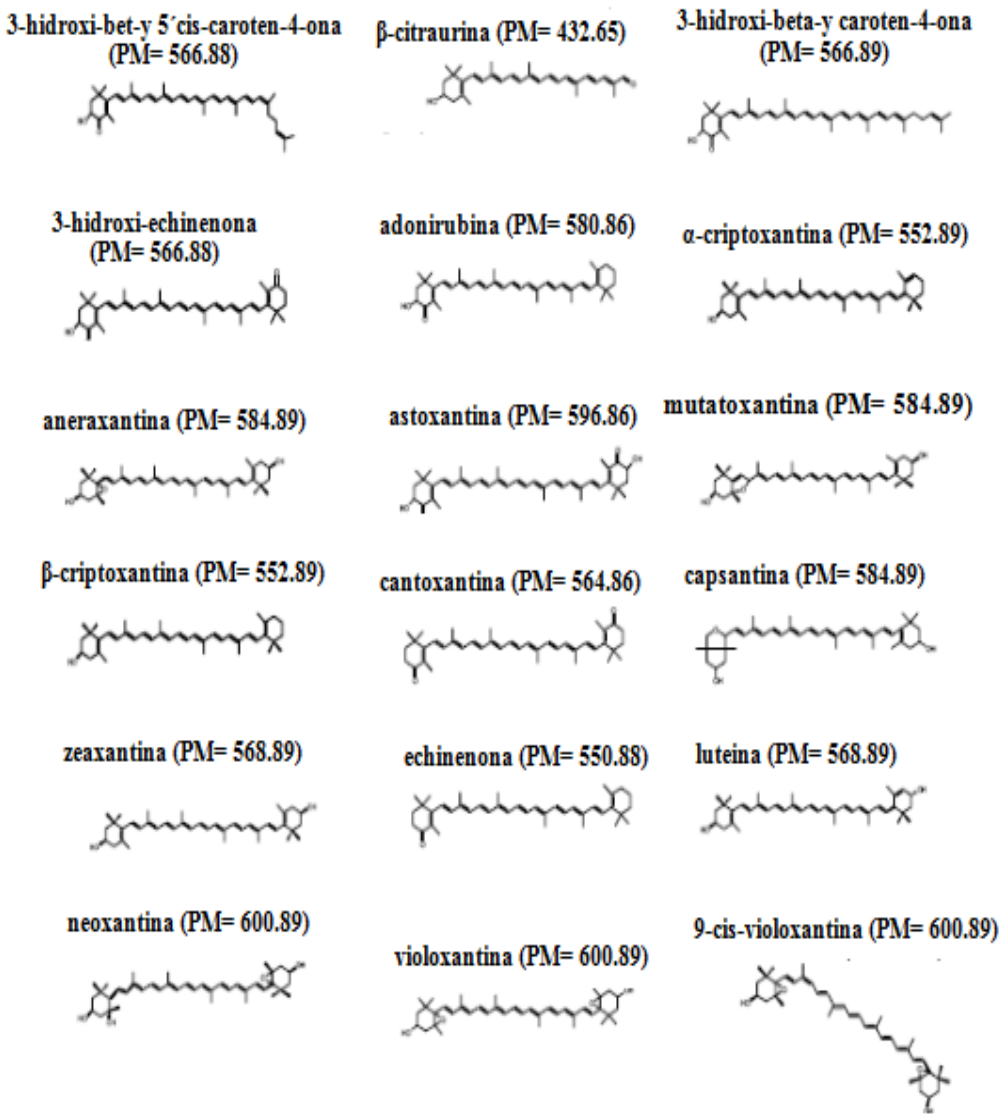


Figura 3. Estructura química de las xantofilas.

1.2.3 COMPUESTOS FENÓLICOS

Los compuestos fenólicos se encuentran en todas las plantas como metabolitos secundarios, por lo tanto, están presentes también en la dieta. Incluyen fenoles simples, ácidos hidroxicinámicos o hidroxibenzoicos, flavonoides, cumarinas y taninos entre otros, con distinta estructura química y actividad.

Químicamente, los compuestos fenólicos son sustancias que poseen un anillo aromático y un ácido benzoico de grupos funcionales incluyendo derivados (ésteres, ésteres metílicos, como glucósidos, etc.) (Tsimidou, 1998). Todos los compuestos fenólicos flavonoides comparten una estructura básica que consta de dos anillos de benceno unidos a través de un anillo heterocíclico. En contraste, los compuestos fenólicos no flavonoides incluyen un grupo más heterogéneo de compuestos desde los más simples como los ácidos fenolicos (C_6-C_1) e hidroxicinamicos (C_6-C_3), y compuestos más complejos como estilbenos (C_6-C_2-C_6), lignanos (C_6-C_3)$_4$, taninos condensados, galotaninos y elagitaninos, cuyo principal componente es el ácido gálico (Bravo, 1998). La Tabla 1 muestra los principales compuestos fenólicos que se pueden encontrar en los alimentos y su estructura química básica.

Últimamente se han llevado a cabo numerosos estudios epidemiológicos que han demostrado la existencia de una asociación significativa entre el consumo de alimentos y bebidas ricas en compuestos fenólicos, y la reducción del riesgo de padecer enfermedades crónicas tales como cáncer, diabetes, enfermedades cardiovasculares, enfermedades inflamatorias e infecciones virales (Agcam et al., 2014; Klimczak et al., 2007).

También juegan un importante papel en la calidad organoléptica y comercial del producto, ya que influyen en los atributos sensoriales del mismo (color, amargor y astringencia) y están involucrados en procesos de oxidación enzimática y no enzimática (Abad-García et al., 2007).

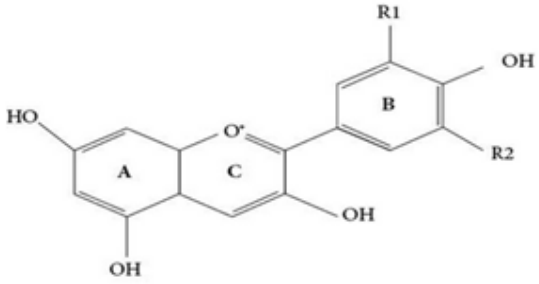
Tabla 1. Clasificación de los compuestos fenólicos. Fuente: Harborne (1989).

ESQUELATO CARBONADO	CLASIFICACIÓN
C_6	Fenoles simples, benzoquinonas
C_6-C_1	Ácidos fenólicos
C_6-C_2	Ácidos fenilacéticos, acetofenonas
C_6-C_3	Ácidos hidroxicinámicos, fenilpropanos, cumarinas, isocumarinas
C_6-C_4	Naftoquinonas
C_6-C_1-C_6	Xantonas
C_6-C_2-C_6	Estilbenos, antraquinonas
C_6-C_3-C_6	Flavonoides, isoflavonoides, neoflavonides
$(C_6$-$C_3)_2$	Lignanos, neolignanos
$(C_6$-C_3-$C_6)_2$	Biflavonoides
$(C_6$-$C_3)_n$	Ligninas
$(C_6)_n$	Melanoidinas
$(C_6$-C_3-$C_6)_n$	Taninos condensados

1.2.4 ANTOCIANINAS

Las antocianinas son un grupo de pigmentos de color rojo, hidrosolubles, ampliamente distribuidos en el reino vegetal. Se encuentran en todos los tejidos de la planta, incluyendo hojas, tallos, raíces, flores y frutos. Estas sustancias protegen a las plantas del ataque de insectos y son responsables de la mayoría de los colores rojo, azul y púrpura (Manach et al., 2004). El color de las antocianinas depende de varios factores intrínsecos, como son los sustituyentes químicos que contenga y la posición de los mismos en el grupo flavilio, si se aumentan los hidroxilos del anillo fenólico se intensifica el color azul, por otra parte la introducción de metoxilos da lugar al color rojo (Kong et al., 2003).

Las antocianinas son glucósidos de antocianidinas, pertenecientes a la familia de los flavonoides, compuestos fenólicos, formados por dos anillos aromáticos A y B. La estructura de las antocianinas en frutas y vegetales puede verse en la Figura 4.



Tipo de Antocianidina	R_1	R_2
Cianidina	OH	H
Delfinina	OH	OH
Malvidina	OCH_3	OCH_3
Peonidina	OCH_3	H
Petunidina	OH	OCH_3

Figura 4. Estructura química de las antocianinas. Fuente: De Pascual & Sánchez-Ballesta (2008).

El interés por los pigmentos antociánicos y la investigación científica se han incrementado en los últimos años, debido no solamente al color que confieren a los productos que las contienen sino a su probable papel en la reducción de las enfermedades coronarias, cáncer, diabetes, a sus efectos antiinflamatorios y mejora de la agudeza visual y comportamiento cognitivo.

1.2.5 ESTEVIÓSIDOS

Las hojas de *stevia* son conocidas por su sabor dulce y acalóricas, contienen una mezcla de glucósidos diterpenos, también conocidos como glucósido de esteviol (Tavarini & Angelini, 2013). El esteviosido es 110-270 veces y el rebaudiosido A es 150-320 veces más dulce que la sacarosa (Abou-Arab et al., 2010). En la Tabla 2 se muestra la estructura y el poder educolorante de los glucósidos de esteviol.

El estevióside es una molécula de esteviol en la cual el átomo de hidrógeno inferior se sustituye por una molécula de β-D-glucosa, y el hidrógeno superior se sustituye por dos moléculas de β-D-glucosa (Figura 5-6).

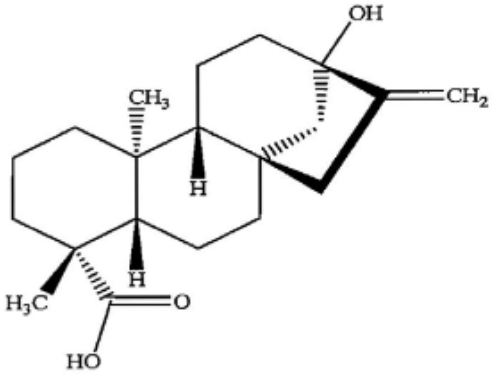


Figura 5. Estructura química de glucósido de esteviol. Fuente: Caracostas et al. (2008).

a) b)

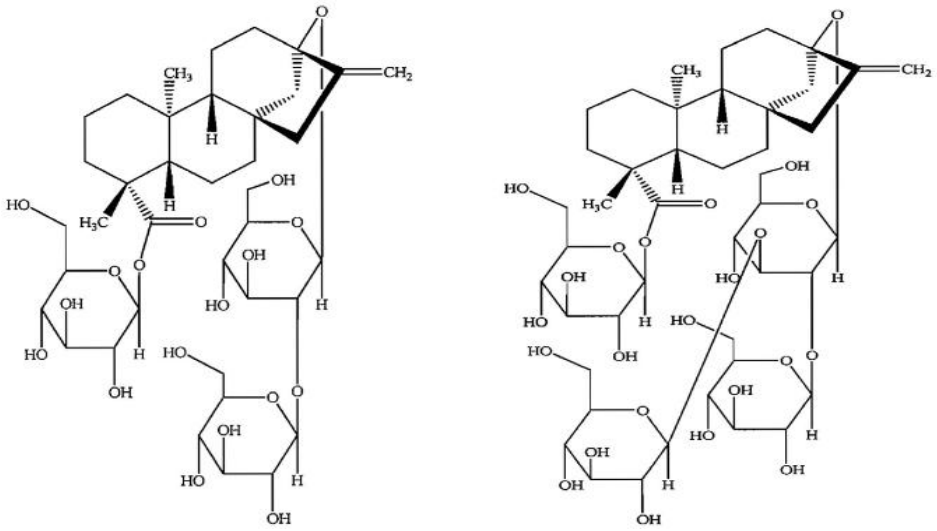


Figura 6. Estructura química de a) esteviosido y b) rebaudiosido A. Fuente: Caracostas et al. (2008).

Tabla 2. Estructura química de los glucósidos de esteviol y su potencia edulcorante. Fuente: Urban et al. (2015).

Nombre de compuseto	R1	R2	Potencia edulcorante
Esteviosido	β-Glc	β-Glc- β-Glc (2->1)	150-250
Rebaudiosido A	β-Glc	β-Glc- β-Glc (2->1)	200-300
Rebaudiosido B	H	β-Glc (3->1)	150
		β-Glc- β-Glc (2->1)	
Rebaudiosido C	β-Glc	β-Glc (3->1)	30
		β-Glc- α-Rha (2->1)	
Rebaudiosido D	β-Glc- β-Glc (2->1)	β-Glc (3->1)	221
		β-Glc- β-Glc (2->1)	
Rebaudiosido E	β-Glc- β-Glc (2->1)	β-Glc (3->1)	174
Rebaudiosido F	β-Glc	β-Glc- β-Glc (2->1)	200
		β-Glc- β-Xyl (2->1)	
Rebaudiosido X	β-Glc- β-Glc (2->1)	β-Glc (3->1)	200-350
		β-Glc- β-Glc (2->1)	
Dulcósido A	β-Glc (3->1)		
Rebaudiosido		β-Glc (3->1)	
Estevilobiosido	β-Glc	β-Glc- α-Rha (2->1)	30
	β-Glc	β-Glc	114
	H	β-Glc- β-Glc (2->1)	90

Esteviol (R1 = R2 = H) es la aglicona de los glicósidos de esteviol. Glc, Rha y Xyl representan fracciones de la glucosa, ramnosa y xilosa.

1.3 TRATAMIENTOS DE CONSERVACIÓN TRADICIONALES

Tradicionalmente los tratamientos térmicos se aplican en la elaboración de alimentos para su procesado, siendo uno de los métodos más efectivos para la inactivación de los microorganismos indeseables en los alimentos (Jabbar et al., 2014). Los diferentes procesos térmicos (pasterización, esterilización) dependen de la intensidad del calor y tiempo aplicado.

1.3.1 Pasterización

La pasterización ha sido el tratamiento térmico más extensamente usado para la conservación de los alimentos en el siglo XX (Moraga et al., 2011), para prolongar la vida útil de los zumos, por la inactivación de microorganismos y enzimas. Sin embargo se ha observado la pérdida de compuestos que confieren sabor, color y vitaminas a los alimentos (Galaverna & Dall'Asta, 2014). Es ampliamente utilizada en alimentos líquidos que son un buen sustrato para el desarrollo microbiano como la leche, sin embargo produce cambios nutricionales ó sensoriales, los cuales dependen de la temperatura y el tiempo del tratamiento (Lang et al., 2010). Los métodos para la pasterización que se aplican actualmente se diferencian por la temperatura, el tiempo y forma de proceso industrial en que se usa. Existen dos grandes grupos de tecnologías de pasterización:

- LTLT (Low Temperature - Long Time): Es el más utilizado en la industria láctea. Se aplica temperaturas bajas de pasterización (62.5-65 ºC) durante mucho tiempo, 30 min (Lewis & Heppel, 2000).
- HTST (High Temperature - Short Time): Utiliza altas temperaturas (75 ºC-90 ºC) con tiempos cortos, 15 s (Pereda et al., 2007).

1.3.2 Esterilización

La esterilización requiere de temperatura superior a 100 ºC. Existen algunos factores que pueden influir en el tiempo de tratamiento, por ejemplo: pH del producto, resistencia térmica de los microorganismos o enzimas, condiciones de calentamiento, tamaño del recipiente, estado físico de los alimentos y condiciones de almacenamiento después del tratamiento térmico.

Las industrias de zumos, derivados lácteos, helados etc, suelen aplicar el tratamiento UHT (Ultra High Temperature) llamado ultrapasterización. Consiste en aplicar temperaturas altas 135-140 ºC durante un tiempo de 2-10 s (Lewis & Heppell, 2000). Los alimentos después de aplicar este método se envasan asépticamente, ya que no necesitan refrigeración para su almacenamiento, y su vida útil es de 3 a 4 meses. El proceso de esterilización puede afectar considerablemente a la calidad de los productos alimenticios, por ejemplo, cambiando su color, sabor, aroma y viscosidad. También puede influir en la degradación de los nutrientes o el cambio de textura (Duarte et al., 2009).

Aunque los tratamientos térmicos son los métodos más comunes para la conservación de productos alimenticios, pueden tener un efecto negativo en su valor nutricional (Rattanathanalerk et at., 2009; Rawson et al., 2011a; Zepka & Mercadante, 2009).

La temperatura alta utilizada para su elaboración y conservación logra una mejor estabilización, inactivación de enzimas y destrucción de microorganismos para minimizar los cambios indeseables en sus características organolépticas (sabor, color, textura) pero por otra parte a menudo exigen pérdidas de sus compuestos bioactivos como vitamina C, polifenoles, antocianinas (Kalisz et al., 2013; Verbeyst et al., 2013), así como disminuyen la capacidad antioxidante (Abid et al., 2014a; Rawson et al., 2011a).

1.4 NUEVOS TRATAMIENTOS DE CONSERVACIÓN DE ALIMENTOS

Los consumidores hoy en día exigen alimentos de alta calidad, que sean seguros y que promuevan la salud física y el bienestar. La alta calidad por lo general se asocia con la naturalidad y frescura (Bigliardi & Galati, 2013; Khan et al., 2013).

La industria alimentaria está buscando nuevos técnicas no térmicas para elaborar alimentos seguros, saludables y no perecederos; los consumidores son conscientes de los beneficios para la salud y los riesgos asociados con el consumo de alimentos, y tienden a optar por alimentos lo más semejantes al fresco que han sido sometidos a tratamientos no severos.

Además, durante los últimos años, la industria alimentaria ha mostrado un creciente interés en la búsqueda de nuevas estrategias de preservación que eliminarían la necesidad de emplear aditivos. Nuevas técnicas seguras y efectivas para evaluar este potencial para el procesamiento y la conservación de los alimentos. En esta linea tecnológica se encuentran los pulsos eléctricos (PE), descargas eléctricas de alto voltaje (DEAV) y ultrasonidos (USN) entre otros, que se han aplicado de manera efectiva en la conservación de alimentos líquidos (Kentish & Feng 2014; Toepfl et al., 2007; Zulueta et al., 2013). Además, estas tecnologías pueden combinarse con las convencionales y mejorar sus efectos letales o inhibidores sobre los microorganismos. La utlilización de estas técnicas no térmicas puede mejorar la calidad y la vida útil de los zumos de fruta y se han convertido en una alternativa potencial a los métodos convencionales (Rupasinghe & Yu, 2012).

1.4.1 Pulsos eléctricos (PE)

El uso de la tecnología de pulsos eléctricos en la industria alimentaria se inició en 1960 (Hamilton & Sale, 1967). Es una de técnica suave de preservación de alimentos que se ha estudiado durante las últimas décadas como una alternativa al tratamiento térmico (pasterización, esterilización) sin comprometer las propiedades sensoriales y nutricionales de los alimentos (Hartyáni et al., 2011; Vervoort et al., 2011).

Los pulsos eléctricos generalmente se aplican en los alimentos fluidos, semi-fluidos o sólidos que pueden tolerar altas intensidades de campo, teniendo poca conductividad eléctrica y que no formen burbujas. Consiste en la aplicación intermitente de campos eléctricos de alta intensidad (1-80 kV/cm) con una corta duración (μs) al material que está colocado entre dos electrodos (Noranizan & Benchamaporn, 2007). Los parámetros de procesado más importantes en estos tratamientos son la intensidad de campo eléctrico aplicada, la energía especifíca, así como el tiempo de tratamiento (Vilkhu et al., 2008). Algunos de los estudios indican que la aplicación de pulsos eléctricos con la intesidad de campo eléctrico más alta mejora el contenido de compuestos bioactvios (Delsart et al., 2014).

En los estudios realizados se observa que los sistemas difieren en las posiciones de los electrodos así como en la forma de las cámaras de tratamiento. El sistema básico de pulsos eléctricos consiste en un generador de impulsos de alta tensión, generador de pulsos, bomba de control y cámara de tratamiento (Figura 7). Los pulsos pueden ser aplicados por lotes o de forma continua, variando en esta última, la configuración de las cámaras de tratamiento y aplicado de forma intermitente o continua.

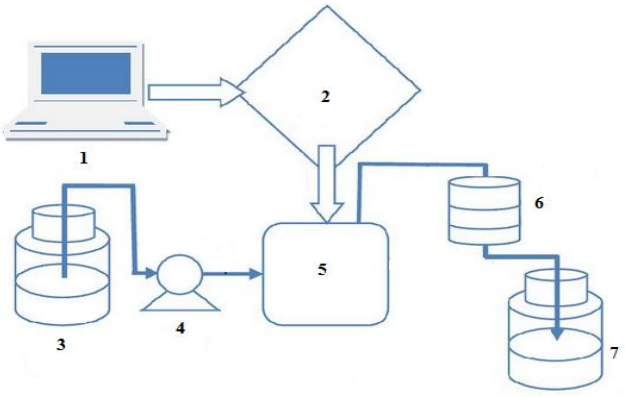


Figura 7. Esquema del sistema de pulsos eléctricos (PE): sistema de control – ordenador (1); generador de pulsos (2); producto (3); bomba de control (4); cámara de tratamiento (5); enfriamiento (6); producto tratado (7) Fuente: Maged et al. (2012).

Para lograr buenos resultados, es importante que la distribución del campo eléctrico y por consiguiente la distribución de temperatura en la cámara de tratamiento sean lo más homogéneas posible (Knoerzer et al., 2012; Meneses et al., 2011). El campo eléctrico afecta a las membranas de las células y puede causar la rotura irreversible de la misma, la alteración en el transporte de iones, y los cambios en la estructura de las enzimas sin efectos adversos significativos sobre el flavor, el color y los nutrientes (Zulueta et al., 2010).

También el procesamiento de PE se aplica en la industria alimentaria para mejorar el transporte de masa, aumentando el rendimiento de metabolitos secundarios y la producción de zumo, así como, para la pasterización de alimentos líquidos sin aplicación de calor (Knorr et al., 2011; Saldana et al., 2010). Además, los métodos asistidos por energía eléctrica pulsada pueden permitir el aumento del rendimiento y la calidad de los compuestos extraídos, disminuyendo así el tiempo y la temperatura de las operaciones de extracción, siendo una alternativa a los cambios

indeseables generados por la pasterización (Cholet et al., 2014; Knorr et al., 2011; Luengo et al., 2013).

Una gran ventaja de la tecnología de PE es que el impacto térmico en los alimentos puede ser reducido, dando lugar a productos frescos con color, textura, sabor más natural, y mayor concentración de compuestos bioactivos, mientras que la vida útil obtenida es la misma (Reineke et al., 2015).

Los tratamientos con pulsos eléctricos no solo pueden mejorar los productos líquidos si no que también puede ser tecnologías útiles para la valorización de los residuos de alimentos y subproductos (Parniakov et al., 2014a). La revisión bibliográfica sobre los efectos de tecnologías de pulsos eléctricos en las diferentes matrices puede verse en la Tabla 3.

1.4.2 Descargas eléctricas de alto voltaje (DEAV)

Esta tecnología se basa en el tratamiento de alimentos líquidos colocados en una cámara entre un electrodo de aguja y un electrodo plano, usando pulsos cortos (Figura 8).

La aplicación de DEAV se basa en el fenómeno de la ruptura eléctrica en el agua. La ruptura eléctrica se produce con un número de fenómenos secundarios tales como alta amplitud de ondas de choque de presión, cavitación de burbujas y creación de turbulencia del líquido. La energía se inyecta directamente en una solución acuosa a través de un canal de plasma formado por una alta corriente de descarga eléctrica de alta tensión entre dos electrodos sumergidos (Boussetta & Vorobiev, 2014).

En los trabajos realizados por diversos investigadores, se observa que la aplicación de DEAV tiene buenas perspectivas para aplicaciónes industriales y mejorar la recuperación de compuestos valiosos de residuos como los polifenoles (Boussetta et al., 2015; Parniakov et al., 2014a). La revisión bibliográfica sobre los efectos de DEAV en las diferentes matrices puede verse en la Tabla 3.

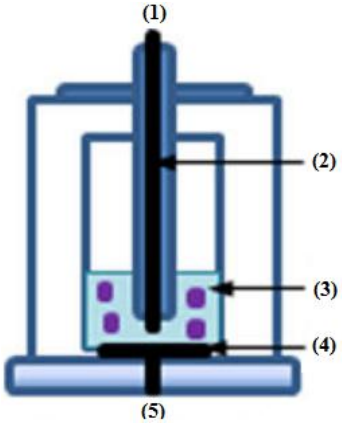


Figure 8. Esquema del sistema de descargas eléctricas de alto voltaje (DEAV): 1) generador; 2) electrodo de aguja; 3) suspensión; 4) electrodo plano; 5) cámara de tratamiento. Fuente: Boussetta et al. (2011).

Tabla 3. Revisión bibliográfica de aplicación de tecnologías PE y DEAV en diferentes matrices.

Tratamiento y condiciones	Muestra	Parámetros	Resultados	Referencia
PE: 4, 20 kV/cm 0.25-6 µs DEAV: 40 kV/cm 1-4 µs	Vino dulce	▪ Inactivación microbiana	- PE y DEAV: Inactivación máxima 3 y 4 log	Delsart et al. (2015)
PE: 5-30 kV/cm 6-12 µs	Arándanos	▪ AT	- Aumento de AT - Reduce la temperatura y el tiempo de extracción	Zhou et al. (2015)
PE: 1-4 kV/cm 20 µs	Puré de zanahoria	▪ Color ▪ CT ▪ Azúcar	- Aumento de a* relacionado con aumento de CT - Aumento de la concentración de azúcar con condiciones: 1 kV/cm/20 µs	Aguiló-Aguayo et al. (2014)
PE: 21.50-25.26 kV/cm 1033.9–1206.2 µs TT: 90 ºC/10-20 s	Zumo de naranja	▪ CFT ▪ CT	- PE: aumento de CFT - PE y TT: aumento de CT	Agcam et al. (2014)
PE: 4, 20 kV/cm 200 µs	Uva/ vinos	▪ AT ▪ Flavonoides ▪ PO	- Aumento de AT y flavonoides: taninos 34 % - Cambio de la PO de la uva con condiciones: 20 kV/cm/200 µs	Delsart et al. (2014)
PE: 35 kV/cm 750 µs	Zumo de naranja	▪ Vit C ▪ CT ▪ Flavonoides ▪ CAT	- Disminución de Vit C y CT - Sin cambios de flavonoides y CAT	(Galaverna & Dall´Asta, 2014)
PE: 40 kV/cm DEAV: 40 kV/cm nº de pulsos: 400	Piel de papaya	▪ CFT ▪ Proteínas ▪ Carbohidratos ▪ CAT	- DEAV: aumento de CFT, proteínas y carbohidratos - PE: aumento de CAT	Parniakov et al. (2014a)

PE: pulsos eléctricos. DEAV: descargas eléctricas de alto voltaje. TT: tratamiento térmico. AT: antocianinas totales. CT: carotenoides totales. CFT: compuestos fenólicos totales. CAT: capacidad antioxidante total. Vit C: vitamina C. PO: propiedades organolepticas. Parametros de color: L*, a*, b*

Tabla 3. Continuación

Tratamiento y condiciones	Muestra	Parámetros	Resultados	Referencia
PE: 0.1-1 kV/cm 5–75 Hz	Puré de zanahoria	▪ CT	- Aumento de la extracción de CT con condiciones: 1 kV/cm/5 Hz - Sin cambios de la extracción de CT con condiciones: 1 kV/cm/10 Hz	Roohinejad et al. (2014)
DEAV: 10 kJ/kg PE: 50 kJ/kg USN: 1010 kJ/kg	Sarmiento de uva	▪ CFT ▪ Proteínas	- DEAV, PE y USN: aumento de CFT y proteínas - DEAV: aumento mayor de CFT y proteínas	Rajha et al. (2014)
PE: 20-40 kV/cm 0.5 Hz, nº pulsos: >1000	Semillas/ Piel de uva	▪ CFT	- PE: aumento de la extracción de CFT	Boussetta et al. (2013)
PE: 1-7 kV/cm 15-150 µs nº pulsos: 5-50	Piel de naranja	▪ Naringina ▪ Hesperidina ▪ CAT	- Aumento de naringina y hesperidina con condiciones: 5 kV/cm/150 µs - Aumento de CAT hasta 192 % con condiciones: 1-7 kV/cm/150 µs	Luengo et al. (2013)
PE: 30 kV/cm nº pulsos: 25	Zumo de mango	▪ *E. Coli*	- Disminución de 4.0 log a 1.0 log	Ait-Ouazzou et al. (2013)
PE: 35 kV/cm 1500 µs 100 Hz	Zumo de tomate	▪ CAT ▪ Licopeno ▪ CT	- Aumento de CAT - Aumento de licopeno 63-65 % - Aumento de CT 14-16 %	Vallverdú-Queralt et al. (2013)
PE: 36 kV/cm 100 µs AP: 600 Mpa 5 min/42 ºC	Zumo de arándanos	▪ Vit C ▪ Color ▪ CFT ▪ CAT	- AP y PE: disminución de Vit C < 5 % - PE: disminución de Vit C durante almacenamiento - AP y PE: sin cambios significativos de color - PE: disminución de CFT después de 7 días - AP: sin cambios significativos de CAT durante 56 días	Barba et al. (2012a)

PE: pulsos eléctricos. DEAV: descargas eléctricas de alto voltaje. USN: ultrasonidos. AP: altas presiones. CT: carotenoides totales. CFT: compuestos fenólicos totales. Vit C: vitamina C. CAT: capacidad antioxidante total. *E. Coli: escherichia coli.* Parametros de color: L*, a*, b

Tabla 3. Continuación

Tratamiento y condiciones	Muestra	Parametros	Resultados	Referencia
TT: 15 s/72 ºC PE+TT: 34 kV/cm 60 s/60 µs/55 ºC	Batidos	▪ NTB ▪ Color ▪ F-Q	- TT y PE+TT: disminución de NTB - TT y PE+TT: disminución de L*, b* - PE+TT: sin cambios significativos de parametros F-Q	Walkling-Ribeiro et al. (2012)
DEAV: 40 kV/cm 0–800 kJ/kg 30 % etanol en agua 30 min/60 ºC	Uva	▪ CAT ▪ CFT	- Aumento de CAT y CFT con la energía ≤ 80 kJ/kg - Disminución de CFT con la energía > 80 kJ/kg - La combinación de etanol junto con la temperatura mejoran la extracción	Boussetta et al. (2011)
PE: 35 kV/cm 800-1400 µs 200 Hz TT: 60 s/90 ºC	Zumo de fruta con bebida de soja	▪ Luteina ▪ Zeaxantina ▪ β-cryptoxantina ▪ CFT	- PE y TT: disminución de luteína, zeaxantina, β-cryptoxantina - PE: sin cambios significativos de CFT	Morales-de la Peña et al. (2011)
PE: 28 kV/cm 20-27 ºC nº pulsos: 50 AP: 600 Mpa 10 min/4-15 ºC	Zumo a base de cítricos	▪ F-Q ▪ Vit C ▪ Color	- AP y PE: sin cambios significativos de parametros F-Q - AP y PE: sin cambios significativos de Vit C - PE: mayor b* y menor a* - AP: mayor L* y menor a*	Hartyáni et al. (2011)
PE: 15-40 kV/cm 40-700 µs TT: 15-30 s/88-99 ºC	Zumo de naranja con leche	▪ Vit C	- PE: retención de Vit C superiores al 90 % - PE: menor degradación inicial de Vit C - PE: aumento de la Vit C durante de almacenamiento	Zulueta et al. (2010)

PE: pulsos eléctricos. DEAV: descargas eléctricas de alto voltaje. TT: tratamiento térmico. AP: altas presiones. F-Q: parámetros físico-químicos. CFT: compuestos fenólicos totales. CAT: capacidad antioxidante total. Vit C: vitamina C. NTB: numero total de bacterias. Parametros de color: L*, a*, b*

Tabla 3. Continuación

Tratamiento y condiciones	Muestra	Parametros	Resultados	Referencia
PE: 35kV/cm 1000 µs/40 ºC 50-250 Hz TT: 30-60 s/90 ºC	Zumo de fresa	▪ AT ▪ Vit C ▪ CAT	- PE: retención de AT 83-102 % - PE: retención de Vit C 98 % - TT: disminución de la Vit C - PE: maxíma retención de CAT con condiciones 35 kV/cm/1000 µs/40 ºC/50 Hz	Odriozola-Serrano et al. (2009a)
PE: 35 kV/cm 1500 µs 100 Hz TT: 30-60 s/90 ºC	Zumo de tomate	▪ Licopeno ▪ α-caroteno ▪ β-caroteno ▪ CFT	- TT: dismunción de licopeno, α-caroteno y β-caroteno - PE: sin cambios significativos en CFT - TT: dismunción de CFT - PE: aumento de licopeno y α-caroteno	Odriozola-Serrano et al. (2009b)
PE: 35 kV/cm 1500 µs/40 ºC TT: 30-60 s/90 ºC	Zumo de zanahoria	▪ β-caroteno ▪ Vit C ▪ CAT	- TT y PE: aumento de β-caroteno - TT y PE: disminución de Vit C - TT y PE: sin cambios significativos en CAT	Quitáo-Teixeira et al. (2009)
PE: 35 kV/cm 1700 µs TT: 30-60 s/90 ºC	Zumo de fresa	▪ Color ▪ HMF ▪ IP	- PE: disminución de a*, b* y aumento de L* - TT: disminución de HMF - TT: aumento de IP, con condiciones: 60 s/90 ºC	Aguiló-Aguayo et al. (2009)
PE: 750 kV/cm 1000 µs/5 s/20 ºC DEAV: 40 kV/cm 0.5 Hz/120 s/20-60 ºC	Piel de uva (Chardonnay)	▪ CFT ▪ Catequina ▪ pH, ºBrix	- PE y DEAV: aumento de CFT - DEAV: aumento de catequina - DEAV: aumento de ºBrix y pH	Boussetta et al. (2009a)
PE: 30-35 kV/cm 50-2050 µs	Zumo de sandía	▪ Licopeno ▪ Vit C ▪ CAT	- Aumento de licopeno - Disminución de Vit C al aumentar el tratamiento - Retención de CAT	Oms-Oliu et al. (2009)

PE: pulsos eléctricos. DEAV: descargas eléctricas de alto voltaje. TT: tratamiento térmico. AT: antocianinas totales. CFT: compuestos fenólicos totales. CAT: capacidad antioxidante total. Vit C: vitamina C. HMF: hidroximetilfurfural. IP: ínice de pardeamiento. Parametros de color: L*, a*, b*

1.4.3 Ultrasonidos (USN)

Los ultrasonidos son una de las tecnologías innovadoras aplicadas ultimamente en la industria alimentaria (Abid et al., 2014b). La sonicación es una técnica que ha sido estudiada, para mejorar la conservación de alimentos (Bhat et al., 2011; Kiani et al., 2013; Li et al., 2007; Rawson et al., 2011b; Tao et al., 2014).

Entre los beneficios del uso de ultrasonidos es el reducido tiempo de procesamiento, consumo de energía, y son respetuosos con el medio ambiente (Chemat et al., 2011; Vardanega et al., 2014). Además, en contraste con algunas de las tecnologías de procesamiento convencionales, los ultrasonidos son rápidos, no destructivos, y precisos (Dolatowski et al., 2007).

La tecnología de ultrasonidos se usa como una opción alternativa en la inactivación de muchas enzimas y microorganismos en condiciones de temperaturas suaves. También los técnicas de USN se puede combinar con técnicas convencionales para mejorar su efectividad así como las propiedades nutricionales de los alimentos procesados (Ross et al., 2003).

En la Figura 9 puede verse un equipo de ultrasonidos.

Figura 9. Equipo de ultrasonidos.

Este método utiliza frecuencias de sonidos entre 20 kHz y 1 MHz (Mason & Lorimer, 2002), dividiendose en dos grupos, (1) ultrasonidos de baja potencia, baja amplitud y alta frecuencia (100-1000 kHz) y (2) ultrasonidos de alta potencia, alta amplitud y baja frecuencia (20-100 kHz).

Estos métodos han sido utilizados con éxito como una monitorización no invasiva en el procesado de alimentos y pueden tener un gran potencial de desarrollo futuro en la industria alimentaria.

Varios autores han reportado mejoras en la calidad de zumos de tomate, fresa y yogur después de la termosonicación (Cruz et al., 2008; Riener et al., 2009) y en zumos de manzana y zanahoria tratados por ultrasonidos a baja temperatura (Abid et al., 2014a; Jabbar et al., 2014). Numerosos estudios van dirigidos a la mejora de la extración de distintos componentes alimentarios utlilizando esta tecnología, así como la mejora de la extración de los compuestos bioactivos.

La revisión bibliográfica sobre los efectos de los ultrasonidos en las diferentes matrices puede verse en la Tabla 4.

Tabla 4. Revisión bibliográfica de aplicación de tecnología USN en diferentes matrices.

Tratamientos y condiciones	Muestra	Parámetros	Resultados	Referencia
24 kHz/400 W 2-10 min amplitud: 7, 22 µm	*Stevia rebaudiana* Bertoni	▪ Esteviosidos ▪ Reb A ▪ CFT	- Aumento de esteviosidos y Reb A con condiciones: 24 kHz/400 W/10 min/22 µm - Aumento de CFT, máximo a los 6 min	Šic Žlabur et al. (2015)
USN: 20 kHz/750 W 25-65 ºC/10 min TT_1: 65 ºC/10 min TT_2: 95 ºC/2 min	Zumo de pera	▪ pH, ºBrix ▪ Vit C ▪ CAT ▪ CFT ▪ Flavonoides ▪ Enzimas ▪ Microorganismos	- TT y USN: sin cambios significativos en pH y ºBrix - USN: mejores resultados en las retenciones de Vit C, CAT y CFT con condiciones: 20 kHz/750 W/65 ºC/10 min - TT_2: disminución de Vit C, CFT, CAT y flavonoides - TT_1 y TT_2: disminución en la actividad de las enzimas y en la inactivación de microorganismos	Saeeduddin et al. (2015)
25 kHz 0-60 min/20 ºC amplitud: 70 µm	Zumo de manzana	▪ CFT ▪ CT ▪ Viscosidad ▪ AT ▪ Conductividad	- Aumento de CFT, CT y viscosidad - Sin cambios significativos en AT y en conductividad	Abid et al. (2014b)
USN+TT: 20 kHz 5 min/15 ºC amplitud: 70 µm 100 ºC/4 min	Zumo de zanahoria	▪ pH, ºBrix ▪ Vit C ▪ CFT ▪ CAT	- Sin cambios signficativos en pH y ºBrix - Disminución de Vit C, CFT y CAT	Jabbar et al. (2014)
28 kHz/600 W 20 ºC/30-90 min	Zumo de pomelo	▪ pH, ºBrix ▪ Vit C ▪ Color ▪ CAT	- Aumento de CAT, Vit C - Sin cambios significativos en pH y ºBrix - Sin cambios significativos en color - Calidad de zumo de mejorada	Aadil et al. (2013)

USN: ultrasonidos. TT: tratamiento térmico. AT: antocianinas totales. CT: carotenoides totales. CFT: compuestos fenólicos totales. CAT: capacidad antioxidante total. Vit C: vitamina C. Reb A: rebaudiosido A. Parametros de color: L*, a*, b*

Tabla 4. Continuación

Tratamientos y condiciones	Muestra	Parámetros	Resultados	Referencia
USN: 20 kHz/400 W 60-70 ºC/10-15 min TT_1: 60 ºC/40 min TT_2: 95 ºC/5 min	Zanahoria	▪ Vit C ▪ PO	- TT_1 y USN 60-70 ºC: retención de Vit C menor al 4 % - TT_2: retención de Vit C en el rango 37.5-85 % - USN: sin cambios significativos en PO	Gamboa-Santos et al. (2013)
20, 490, 986 kHz 56 ºC/ 0-30 min	Puré de frambuesa	▪ CAT ▪ AT ▪ CFT	- Aumento de CAT 17 % y AT 12 % con condiciones: 20 kHz/10 min/56 ºC - Aumento de CFT al 10 % con condiciones: 20 y 986 kHz/30 min/56 ºC - Sin cambios significativos en AT y CAT con condiciones: 986 kHz/30 min/56 ºC	Golmohamadi et al. (2013)
21 kHz/300 W 30 min/T[a] ambiente	Zumo de zanahoria	▪ Color	- Sin cambios significativos en color	Sakowski & Janiszewska, (2013)
USN: 40 kHz/130 W 25 ºC/ 15-60 min TT: 90 ºC/ 30,60 s	Zumo de mango	▪ CFT ▪ pH ▪ Vit C ▪ CT ▪ Color ▪ NTB	- USN aumento de CT 4-9 % y de CFT 30-35 % con las condiciones: 40 kHz/130 W/15-30 min/25 ºC - USN y TT: sin cambios significativos en pH - USN y TT: disminución de NTB - USN: disminución de Vit C y color	Santhirasegaram et al. (2013)
20 kHz 20-60 °C/3-9 min amplitud: 60-120 µm TT: 80 ºC/2 min	Zumo y néctar de manzana	▪ Compuestos aromáticos	- USN: formación de nuevos compuestos aromáticos - TT: desarollo de compuestos aromáticos	Šimunek et al. (2013)

USN: ultrasonidos. TT: tratamiento térmico. T[a]: temperatura. AT: antocianinas totales. CT: carotenoides totales. CFT: compuestos fenólicos totales. CAT: capacidad antioxidante total. Vit C: vitamina C. NTB: numero total de bacterias. PO: propiedades organolépticas. Parametros de color: L*, a*, b*

Tabla 4. Continuación

Tratamientos y condiciones	Muestra	Parámetros	Resultados	Referencia
USN: 20 kHz/25 °C/10 min TT: 98 ºC/180 s	Zumo de zanahoria y manzana	▪ CAT ▪ NTB ▪ Color ▪ pH ▪ IT ▪ β-caroteno	- USN y TT: sin cambios significativos en CAT y NTB - USN y TT: sin cambios significativos en color, pH, CAT y β-caroteno durante almacenamiento - Sin cambios significativos en IT durante almacenamiento	Gao & Rupasinghe, (2012)
20, 24 kHz 25-45 ºC/10 min amplitud: 4,61 µm	Batidos de fruta	▪ CFT ▪ CAT ▪ Color	- Disminución de CFT y CAT con condiciones: 20 y 24 kHz/10 min/61 µm - Disminución de color	Keenan et al. (2012)
20 kHz/247.4 W 40 ºC/2.7 min	Zumo de piña	▪ Azúcares ▪ CFT ▪ Vit C	- Mejora el contenido de azúcares, CFT y Vit C	Nguyen & Le, (2012)
20 kHz 25-45 ºC/10 min amplitud: 4-61 µm	Zumo de sandia	▪ Color ▪ Licopeno ▪ CFT ▪ Vit C	- Retención de Vit C y licopeno 94 % en las condiciones: 20 kHz/10 min/25 ºC - Disminución de Vit C, CFT y licopeno con condiciones: 20 kHz/10 min/45 ºC/61 µm - Aumento de L*, a* ,b* con condiciones: 20 kHz/45 ºC/10 min/ 61 µm	Rawson et al. (2011b)
20 kHz 25 ºC/10 min amplitud: 4-61 µm	Zumo de tomate	▪ pH ▪ ºBrix ▪ Acidez ▪ Color ▪ Vit C	- Sin cambios significativos de pH, ºBrix, acidez - Disminución de color - Disminución de Vit C máxima 32 % con condiciones: 20 kHz/25 ºC/10 min/61 µm	Adekuente et al. (2010)

USN: ultrasonidos. TT: tratamiento térmico. CFT: compuestos fenólicos totales. CAT: capacidad antioxidante total. Vit C: vitamina C. NTB: numero total de bacterias. IT: índice de turbidez. Parametros de color: L*, a*, b*

Tabla 4. Continuación

Tratamientos y condiciones	Muestra	Parámetros	Resultados	Referencia
20 kHz/500 W 4-10 ºC/2-10 min	Zumo de naranja con calcio	▪ Color ▪ Vit C ▪ H&L	- Disminución de color, Vit C y H&L	Gómez-López et al. (2010)
25 kHz/150 W/40 ºC 30-60 min	Piel de naranja	▪ CFT ▪ Flavonoides ▪ CAT	- Aumento de flavonoides - Aumento de CFT 35-40 % y de CAT 30-40 % con condiciones: 25 kHz/150 W/40 ºC/60 min	Khan et al. (2010)
20 kHz/1500 W 5-30 ºC/0-10 min	Zumo de naranja	▪ Vit C	- Disminución de Vit C 15 %	Valdramidis et al. (2010)
24 kHz/20-100 W T[a] ambiente/2-15 min	Bebida a base de soja	▪ pH ▪ Viscosidad	- Aumento de pH con el tiempo, independientemente de la energía aplicada - Aumento de la viscosidad con el aumento de la energía	Bosiljkov et al. (2009)
25 kHz/150 W 15-40 ºC/1-8 h	Piel de naranja	▪ CFT ▪ ácido cinámico ▪ ácido benzoico	- Disminución de CFT a las 1.5 h - Ácido benzoico mas estable que ácido cinámico - Aumento de CFT con aumento del tiempo	Ma et al. (2009)
20 kHz 25-45 ºC/0-10 min amplitud: 24.4-61µm	Zumo de arándanos	▪ Color ▪ AT	- Aumento de L*, con la amplitud más alta - Aumento de a*, con el tiempo más largo - Retención de AT significativo > 94 con condiciones: 20 kHz/10 min/45 ºC/ /61µm	Tiwari et al. (2009)
20 kHz 25-39.9 ºC/2-10 min amplitud: 24.4-61µm	Zumo de fresa	▪ Vit C ▪ AT ▪ Color	- Disminución de Vit C 11 % - Disminución de color al aumentar el nivel de amplitud - Aumento ligero de AT 1-2 % con condiciones: 20 kHz/ 2 min/25 ºC/24.4 µm - Dismiunción de AT	Tiwari et al. (2008)

T[a]: temperatura. CFT: compuestos fenólicos totales. Vit C: vitamina C. AT: antocianinas totales. CAT: capacidad antioxidante total. H&L: hongos y levaduras. Parametros de color: L*, a*, b*

1.5 BIODISPONIBILIDAD/ BIOACCESIBILIDAD DE COMPUESTOS BIOACTIVOS

Antes de concluir sobre cualquier efecto potencial de los compuestos bioactivos en la salud, es importante analizar si el proceso de digestión afecta a los compuestos bioactivos y su estabilidad, así como sus posibles efectos beneficiosos. La biodisponibilidad y bioaccesibilidad son características críticas para evaluar el papel de estos compuestos en la salud humana (Rodriquez-Roque et al., 2013). Existen distintas definiciones de biodisponiblidad pero en general este concepto se refiere a la integración de los diversos procesos mediante los que una fracción de un nutriente ingerido está disponible para la digestión, absorción, transporte utilización y eliminación (Harro et al., 2006; Hurrell & Egli 2010).

Por otra parte la bioaccesibilidad es la fracción de un compuesto que es liberado de una matriz alimentaria en el tracto gastrointestinal y en consecuencia se convierte en disponible para su asorbción (Parada & Aguilera, 2007).

Hoy en día la industria alimentaria se apoya más en tecnologías implementadas en los modelos de digestión *in vitro,* que simulan la digestión gastrointestinal, imprescindibles para conocer la bioaccesiblidad y biodisponiblidad de los compuestos activos presentes y/o incorporados en sus productos.

Gracias a estas metodologías se puede comprobar que cantidad de los compuestos bioactivos presentes en diferentes matrices (alimentos) puede ser potencialmente absorbida por el sistema digestivo (bioaccesibilidad) y cuales de ellas son realmente absorbidas y metabolizadas por el organizmo, llegando al plasma sanguíneo y produciendo el efecto buscado (biodisponiblidad).

Los métodos *in vitro* que simulan los procesos de digestión son ampliamente utilizados para estudiar el comportamiento gastrointestinal de los alimentos o productos farmacéuticos (Minekus et al., 2012), y para estudiar los cambios estructurales, la digestibilidad y la liberación de componentes de los alimentos en condiciones gastrointestinales simuladas. Los métodos de digestión simulados incluyen la fase oral, gástrica e intestinal (Guerra et al., 2012) (Figura 10).

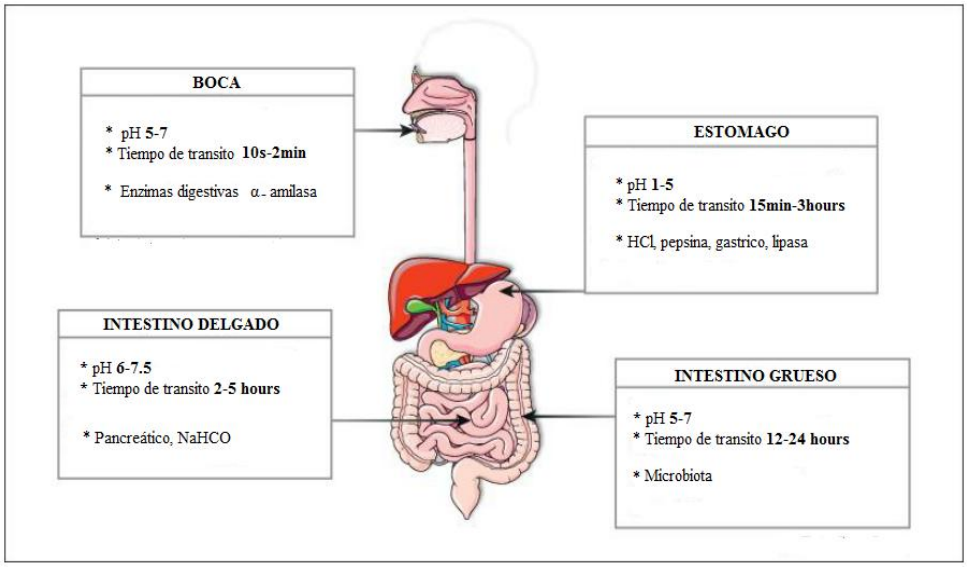


Figura 10. Proceso de la digestión. Fuente: Guerra et al. (2012).

Existen diversos estudios que concluyen que los tratamientos para la conservación de los alimentos influyen en la biodisponiblidad de los compuestos. La revisión bibliográfica sobre los efectos del tratamiento térmico y no térmico en la biodisponiblidad de los compuestos bioactivos puede verse en la Tabla 5.

Tabla 5. Revisión bibliográfica de los efectos del tratamiento térmico y no térmico sobre la bioaccesibilidad de los compuestos bioactivos.

Tratamiento	**Muestra**	**Compuestos bioactivos**	**BA (%)**	**Metodo digestion *in vitro***	**Referencia**
USN: 24 kHz/400 W 15-60 min amplitud: 100 µm	Pulpa de tomate con/sin aceite	• Licopeno	• Disminución de la BA del licopeno sin la adición de aceite • Aumento de la BA del licopeno con la adición de aceite	Hedrén et al. (2002) Colle et al. (2010)	Anese et al. (2015)
TT_1: 70 ºC/80L/h TT_2: 90 ºC/90L/h	Zumo de naranja	• CT • Vit C	• TT_1: sin cambios de la BA de CT • TT_2: aumenta la BA de CT 9-11 % • TT_2: aumenta la BA de Vit C 78 %	Schweiggert et al. (2012) *con algunas modificaciones*	Aschoff et al. (2015)
TT_1: 30 ºC/3 min TT_2: 97 °C/30 s MO: 1000 W/340 s	Pureé de kiwi	• CT	• Sin cambios de la BA	Bouayed et al. (2011)	Benlloch-Tinoco et al. (2015)
TT_1: 65-95 ºC/10 min TT_2: 95-125 ºC/25 min	Zanahoria Tomate	• CT • Licopeno • β-caroteno	• TT_1: disminuación de la BA de β-caroteno y de licopeno • TT_2:aumento de la BA de β-caroteno • Aumento de la BA de CT a mayor intensidad del tratamiento	Lemmens et al. (2009) Palmero et al. (2013)	Palmero et al. (2014)
TT_1: 70 ºC/2 min TT_2: 90 ºC/10 min TT_3: 117 ºC/7 min	Zanahoria cortada	• CT • β-caroteno	• TT_1 y TT_2: aumento de la BA de CT • TT_3: aumento de la BA de β-caroteno	Lemmens et al. (2009) Hedrén et al. (2002)	Lemmens et al. (2013)
AP: 20,50,100 Mpa/4 ºC	Pulpa de tomate	• α-caroteno • Licopeno • Luteína	• Disminución de la BA del licopeno, α-caroteno y luteina con la presión > 50 MPa	Moelants et al. (2012)	Panozzo et al. (2013)

AP: altas presiones. BA: bioaccesibilidad. TT: tratamiento térmico. USN: ultrasonidos. MO: microondas. CT: carotenoides totales. Vit C: vitamina C.

Tabla 5. Continuación

Tratamiento	Muestra	Compuestos bioactivos	BA (%)	Metodo digestion *in vitro*	Referencia
TT: 99 ºC/ 15 s	Zumo de naranja	• CT	• Disminución de la BA de CT 39 %	Garret et al. (1999) Liu et al. (2004)	Stinco et al. (2013)
TT: 90 ºC/10 min AP: 10- 100 MPa/2 ºC	Puré de zanahoria con aceite/ sin aceite	• β-caroteno	• AP: aumento de la BA de β-caroteno con la adición de aceite • BA del β-caroteno al 17 %, sin adición de aceite • TT: aumento de la BA de β-caroteno • AP: aumento de la BA de β-caroteno a presiones < 50 MPa • AP: disminución de la BA de β-caroteno con presiones > 50 MPa	Hedrén et al. (2002)	Knockaert et al. (2012a)
AP: 10 Mpa/ 4 ºC TT_1: 60 ºC/1 min TT_2: 90 ºC/10 min TT_3: 121 ºC/2.5 min	Puré de tomate con aceite/ sin aceite	• Licopeno • β-caroteno	• AP: aumento de la BA del licopeno β-caroteno con la adición de aceite • TT_2: sin cambios de BA • TT_3: dismiunción de BA	Hedrén et al. (2002)	Knockaert et al. (2012b)
TT_1: 70 ºC/2 min TT_2: 90 ºC/10 min TT_3: 121 ºC/3 min	Zanahoria cortada	• β-caroteno	• TT_1, TT_2 y TT_3: aumento de la BA de β-caroteno	Hedrén et al. (2002)	Knockaert et al. (2011)
TT_1: 80 ºC/10 min TT_2: 100 ºC/10 min	Zanahoria cortada	• CT • β-caroteno	• TT_1: aumento de la BA de CT • TT_2: aumento de la BA de β-caroteno	Garret et al. (1999)	Netzel et al. (2011)
TT: 100 ºC/3-25 min	Zanahoria cortada	• β-caroteno	• Disminución de la BA de β-caroteno con el aumento del tamaño de partícula	Hedrén et al. (2002)	Lemmens et al. (2010)
TT_1: 60 ºC/40 min TT_2: 90 ºC/4 min	Tomate	• Licopeno	• TT_1: aumento de la BA del licopeno • T^a < 60 ºC no mejora la BA	Hedrén et al. (2002) Garret et al. (1999)	Svelander et al. (2010)

BA: bioaccesibilidad. TT: tratamiento térmico. AP: altas presiones. CT: carotenoides totales.

Tabla 5. Continuación

Tratamiento	Muestra	Compuestos bioactivos	BA (%)	Metodo digestion *in vitro*	Referencia
TT_1: 90 °C/5–50.2 min TT_2: 100 °C/0-20 min TT_3: 110 ºC/0-14 min	Zanahoria cortada	• β-carotono	• Aumento de la BA de β-caroteno al aumentar temperatura y tiempo	Hedrén et al. (2002) *con algunas modificaciones*	Lemmens et al. (2009)
TT_1 : 95 °C/8 min TT_2: 100 °C/20 min	Tomate	• CT • Licopeno	•TT_2: aumento de la BA de CT •Sin cambios de la BA del licopeno	Hedrén et al. (2002) *con algunas modificaciones*	Tibäck et al. (2009)
TT: 100 ºC/10 min MO: 50 s/800 W	Tomate Pimiento Calabacín	• CT • β-caroteno	•TT y MO: disminución de la BA del β-caroteno en todas las verduras •TT y MO: aumento de la BA de CT	Garrett et al. (1999) *con algunas modificaciones*	Ryan et al. (2008)
USN: 24 kHz/105 W 15- 60 min amplitud: 100 µm	Pulpa de tomate	• Licopeno	•USN: disminución de la BA del licopeno •Disminución de la BA con el aumento de la viscosidad	Moelants et al. (2012) Hedrén et al. (2002)	Anese et al. (2013)
TT: 60-90 ºC/30 min	Pulpa de tomate	• Licopeno	•Aumento de la BA del licopeno al aumentar la temperatura	Hedrén et al. (2002)	Colle et al. (2010)
TT: 95 ºC/8 min	Puré de tomate con/sin aceite Puré de zanahoria	• Licopeno	•La BA del licopeno sin aceite disminuye •La BA es más baja en tomate que en zanahoria	Hedrén et al. (2002)	Moelants et al. (2012)

BA: bioaccesibilidad. TT: tratamiento térmico. USN: ultrasonidos. CT: carotenoides totales. MO: microondas.

OBJECTIVE AND WORK PLAN

El objetivo general de este trabajo es:

Obtener nuevos derivados de frutas y vegetales ricos en compuestos bioactivos tras la aplicación de differentes tecnologías térmicas (pasterización, esterilización) y no térmicas (PE, DEAV, USN) y evaluación del contenido de compuestos bioactivos y esteviósidos y de la bioaccesibilidad por digestión gastrointestinal simulada.

Para conseguir este objetivo general se proponen los siguientes objetivos parciales:

- Estudiar los efectos del tratamiento térmico y diferentes concentraciones de *stevia* en una bebida a base de papaya, mango mezclada con zumo de naranja, avena, acaí y *stevia.*

- Estudiar los efectos de los tratamientos no térmicos (PE, DEAV y USN) en la variación de compuestos bioactivos y la capacidad antioxidante en la bebida a base de papaya, mango y *stevia* así como su bioaccesbilidad.

- Estudiar los efectos de los tratamientos no térmicos (PE y DEAV) en la piel de naranja y la variación de los compuestos bioactivos, la capacidad antioxidante y su bioaccesibilidad.

- Estudiar los efectos de los tratamientos térmicos y no térmico (USN) sobre los carotenoides en los batidos de frutas y verduras y su bioaccesibilidad.

Para alcanzar los objetivos propuestos se plantea el siguiente plan de trabajo:

1. Elaboración de una bebida a base de papaya, mango mezclada con zumo de naranja, avena, acaí y *stevia*

- Predicción de los parametros físico-químicos, glucósidos de esteviol y compuestos bioactivos después de aplicar tratamientos térmicos usando la metodología de superficie de respuesta.

2. Elaboración de una bebida a base de papaya y mango educolorada con *stevia*

- Comparación del efecto de tratamientos de conservación no térmicos (PE, DEAV y USN) con distintas energías sobre los parámetros físico-químicos, compuestos bioactivos y capacidad antioxidante total.
- Evaluación de la bioaccesibilidad *in vitro* de compuestos bioactivos y capacidad antioxidante total.

3. Extración de compuestos bioactivos de la piel de naranja

- Evaluación del efecto de tecnologías no térmicas (PE, DEAV) con distintas energías en el contenido de compuestos bioactivos y capacidad antioxidante total.
- Evaluación de la bioaccesibildad *in vitro* de compuestos bioactivos y capacidad antioxidante total de la piel de naranja tratada.

4. Elaboración de smoothies a base de frutas y vegetales ricos en carotenoides

- Comparación del efecto de tratamientos térmicos (pasterización, esterilización) con la tecnología no térmica (USN), desde el punto de vista de seguridad alimentaria, calidad y preservación de compuestos nutritivos (carotenoides).
- Evaluación de la bioaccesibildad *in vitro* de compuestos nutritivos (carotenoides) en smoothies.

En la Figura 11 se presenta el plan de trabajo desarollado.

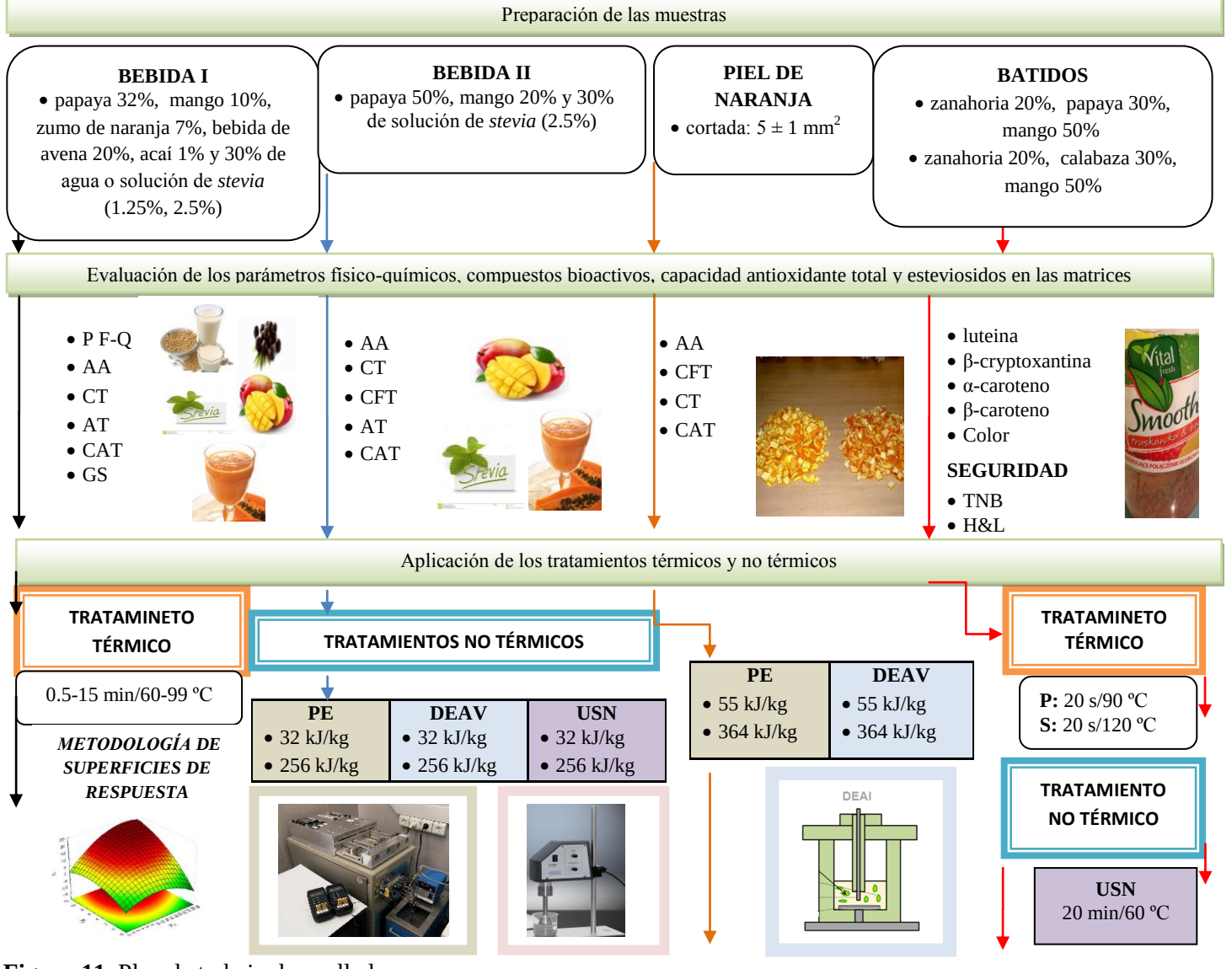
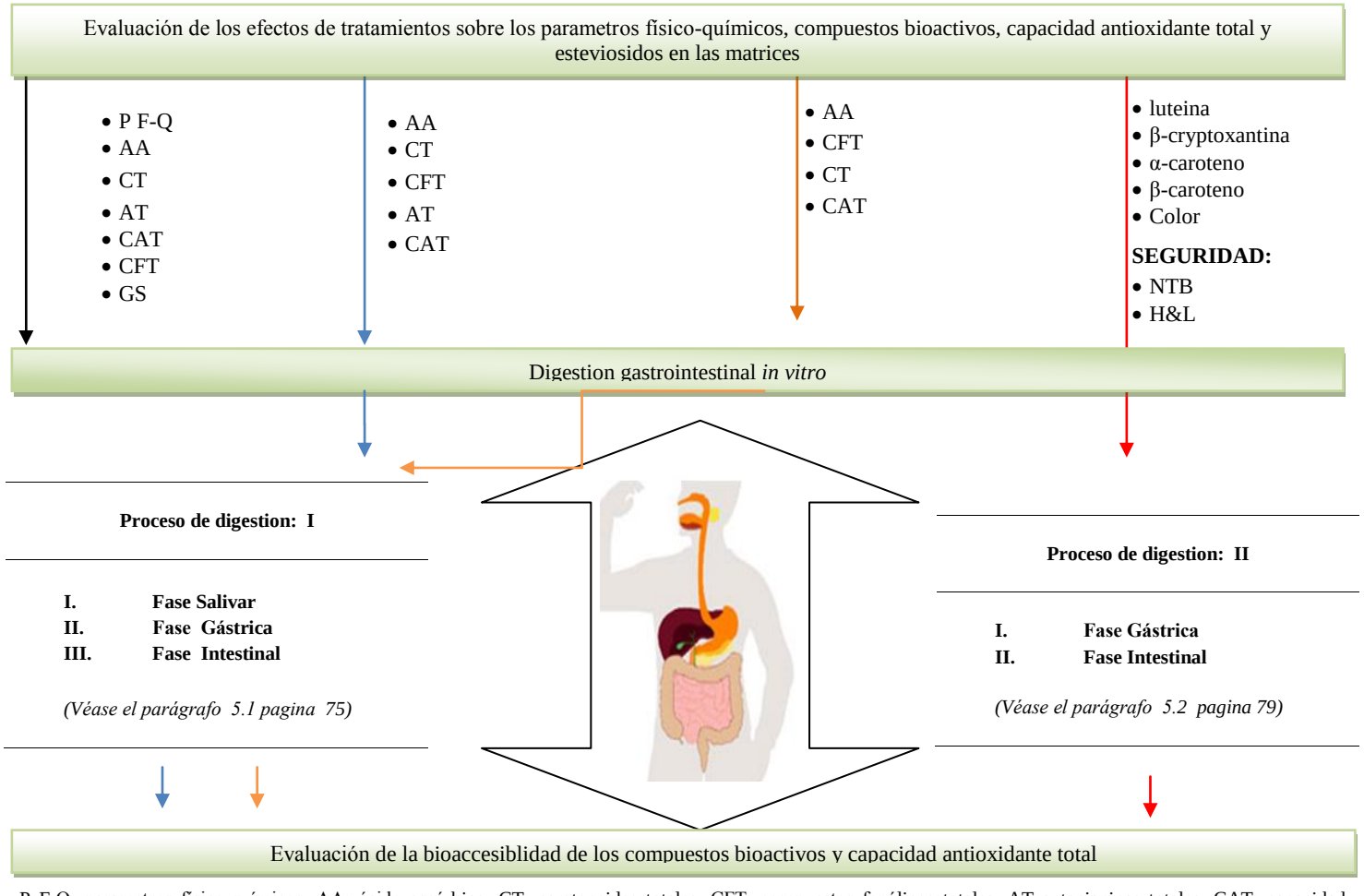


Figura 11. Plan de trabajo desarollado.



P F-Q: parametros físico-químicos. AA: ácido ascórbico. CT: carotenoides totales. CFT: compuestos fenólicos totales. AT antocianinas totales. CAT: capacidad antioxidante total. GS: glucósides de esteviol. NTB: numero total de bacterias. H&L: hongos y levaduras. PE: pulsos eléctricos. DEAV: descargas eléctricas de alto voltaje. USN: ultrasonidos. P: pasterización. S: esterilización.

Figura 11. Continuación

The overall objective of this work is:

To obtain new derivatives from fruits and vegetables rich in bioactive compounds after the application of different thermal technologies (pasteurization, sterilization) and non-thermal (PEF, HVED, USN) and evaluation of the content of bioactive compounds and steviosides and the bioaccessibility by simulated gastrointestinal digestion.

To achieve this overall objective the following partial objectives are proposed:

- To study the effects of thermal treatments and different concentrations of *stevia* in a beverage based on papaya, mango mixed with orange juice, oats, acaí and *stevia.*

- To study the effects of non-thermal treatments (PEF, HVED and USN) on the variation of bioactive compounds and antioxidant capacity in the beverage based on papaya, mango and *stevia* and their bioaccessibility.

- To study the effects of non-thermal treatments (PEF and HVED) on orange peel and the variation of bioactive compounds and antioxidant capacity and their bioaccessibility.

- To study the effects of thermal and non-thermal (USN) treatments on carotenoids in fruit and vegetable smoothies and their bioaccessibility.

To achieve these objectives the following work plan is proposed:

1. Elaboration of a beverage based on papaya, mango mixed with orange juice, oats, acaí and *stevia*

- Prediction of physicochemical parameters, steviol glycosides and bioactive compounds after thermal treatments are applied, using response surface methodology.

2. Elaboration of a beverage based on papaya and mango sweetened with *stevia*

- Comparison of the effect from non-thermal conservation treatments (PEF, HVED and USN) with different energies on the physicochemical parameters, bioactive compounds and total antioxidant capacity.
- Evaluation of the *in vitro* bioaccessibility of bioactive compounds and total antioxidant capacity.

3. Extraction of bioactive compounds from orange peel

- Evaluation of the effect of non-thermal technologies (PEF, HVED) with different energies applied in the content of bioactive compounds and antioxidant capacity.
- Evaluation of the *in vitro* bioaccessibility of bioactive compounds and total antioxidant capacity from treated orange peel.

4. Elaboration of smoothies based on fruits and vegetables rich in carotenoids

- Comparison the effect of thermal treatment (pasteurization, sterilization) with the non-thermal technology (USN), from the point of

view of food safety, quality and preservation of nutritional compounds (carotenoids).

- Evaluation of the *in vitro* bioaccessibility of nutritional compounds (carotenoids) in smoothies.

Figure 12 shows the developed work plan.

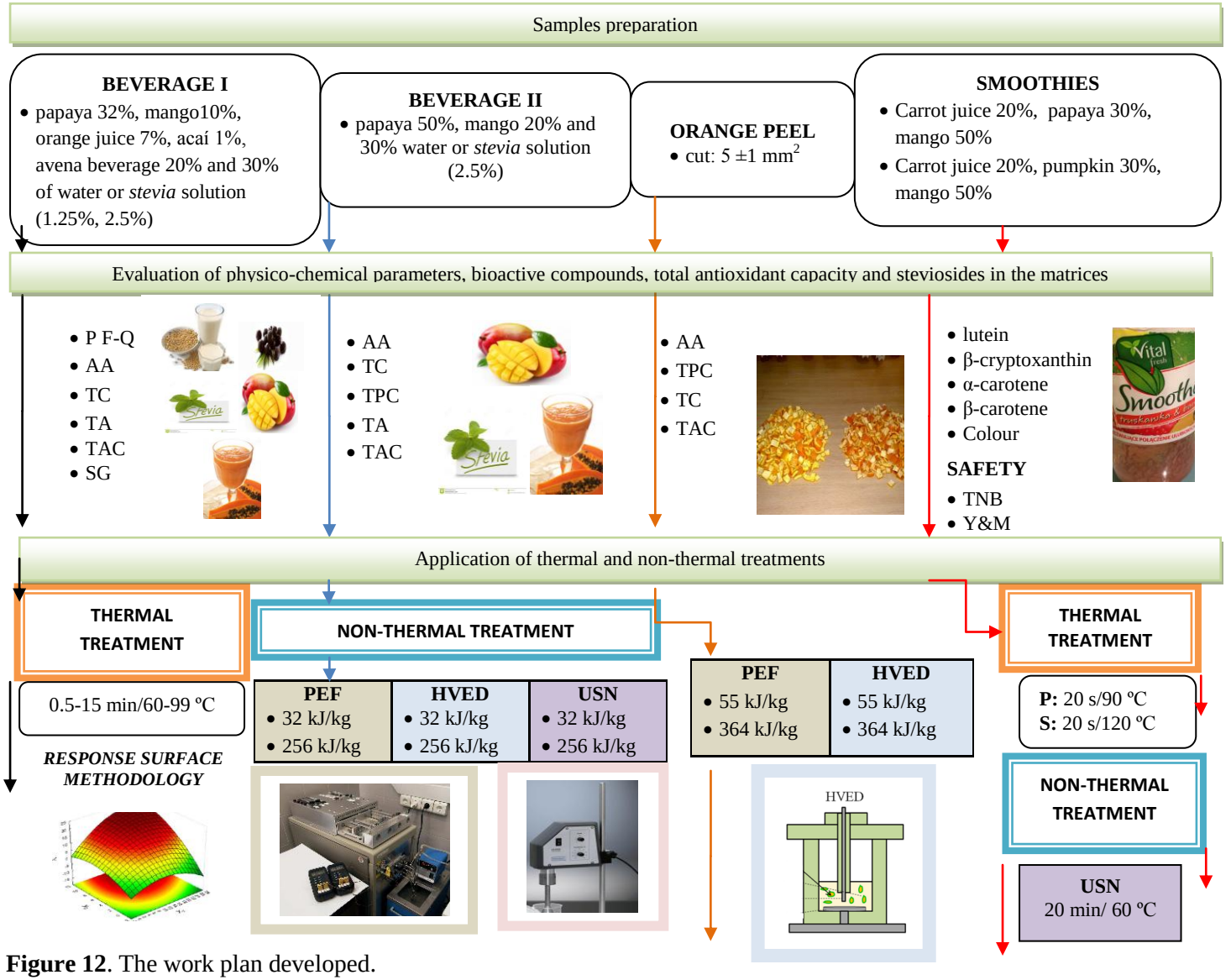
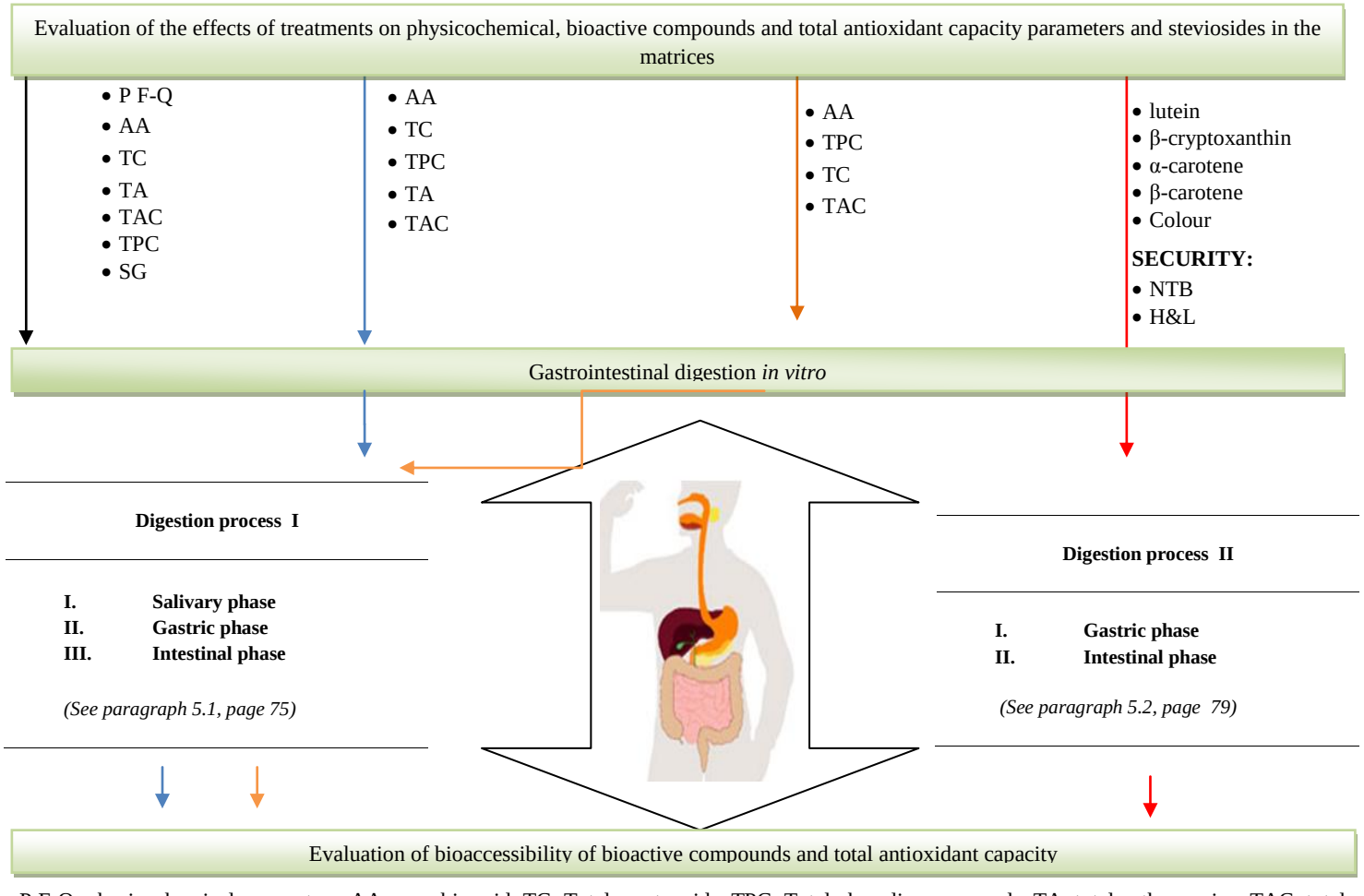


Figure 12. The work plan developed.



P F-Q: physicochemical parameters. AA: ascorbic acid. TC: Total carotenoids. TPC: Total phenolic compounds. TA: total anthocyanins. TAC: total antioxidant capacity. SG: steviol glycosides. TNB: Total number of bacteria. Y&M: yeast and molds. PEF: pulsed electric fields. HVED: high voltage electrical discharges. USN: ultrasound. P: pasteurization. S: sterilization.

Figure 12. Continuation

EXPERIMENTAL WORK

3. EXPERIMENTAL WORK

3.1 SAMPLES

3.1.1 Beverage based on papaya, mango mixed with orange juice, oats, acaí and *stevia*

Papaya *(Carica papaya),* mango *(Mangifera indica),* oranges *(Citrus sinensis)* and oats beverage (Santiveri, Lérida, Spain) were purchased from a local supermarket (Valencia, Spain). Papaya, mango and orange juices were extracted after appropriate washing and hygenization of the fruits. To remove the pulp, before being mixed, the juices were filtered by means of a mesh sieve (pore size: 0.297 mm). Acaí provided by Nature's Way Products Inc. (Utah, USA) (containing 450 mg of acaí berries extract, with 10 % of polyphenols) was added to the beverage. *Stevia* leaves were supplied by company Anagalide, S.A. (Barbastro, Huesca, Spain) and stored at room temperature. A stock solution (8.33%, w/v) of *stevia* was prepared in order to formulate the beverage. For this propose, 100 mL of bottled water at 100 ºC were added on the dried leaves (8.33 g) and were kept for 30 min. The infusion was vacuum filtered using filter Whatman nº 1 (Whatman International Ltd., UK) and the filtrate obtained was stored for the duration of the experiment at 4 ºC. The beverage was prepared by mixing 32 % (v/v) of papaya, 10 % (v/v) of mango juice, 7 % (v/v) of orange juice, 20 % of oats beverage, 1 % of acaí powder (w/v) and water to 100 %. To obtain final *stevia* concentrations of 1.25 % and 2.5 % (v/v), different volumes of *stevia* stock solution (30 and 60 mL) were added to prepare 200 mL of beverage instead of water. The maximum *stevia* concentration (2.5 %, v/v) was selected, taking into account the sucrose concentration of commercial fruit-based beverages and the sweetness equivalence of *stevia.*

3.1.2. Beverage based on papaya, mango and *stevia*

Papaya (*Carica papaya*), mango (*Mangifera indica*) were purchased from a local supermarket (Valencia, Spain). The fruits were washed, dried and chopped separately using a household blender. To remove the pulp, before being mixed, the juices were filtered by means of a mesh sieve (pore size: 0.297 mm). Dried stevia leaves were supplied by company Anagalide, S.A. (Barbastro, Huesca, Spain) and stored at room temperature. A stock solution was prepared as previously *(See section 3.1.1 - page 57).*
The fruit juice mixture was prepared by mixing 50 % (v/v) of papaya, 20 % (v/v) of mango and 30 % (v/v) of *stevia* infusion 2.5 % (v/v).

3.1.3. Orange peel

The orange peel were obtained from orange fruits *(Citrus sinensis, Navel lane-late)* purchased from a local supermarket (Castellon, Spain). The orange peel were removed from the pulp and chopped into square pieces of 5 ± 1 mm^2.

3.1.4. Smoothies

Pumpkin *(Hokkaido),* papaya *(Carica papaya)*, mango *(Mangifera indica),* were purchased from a supermarket Tesco, (Rzeszow, Poland), carrot juice was supplied by company Witmar S.A. (Rzeszow, Poland). Papaya, mango, pumpkin were washed, dried and chopped in a blender for 5 min. Two different smoothies were prepared:

- Smoothie 1: carrot juice 20 %, papaya 30 % and mango 50 % (v/v)
- Smoothie 2: carrot juice 20 %, pumpkin 30 % and mango 50 % (v/v)

All smoothies samples were transferred to 250 mL bottles and stored en refrigerated at 4 ºC. After that, samples were analysed in Institute of Food Science “Agroscope” in Wädenswil (Switzerland) before 24 hours.

4. DETERMINATION OF PARAMETERS FOR ESTIMATING THE FOOD QUALITY

4.1. pH

pH was determined by potentiometric measurement according with A.O.A.C (1997).

Laboratory equipment

- pH-meter Crison micro pH 2001 (Barcelona, Spain).

Method

To measure pH, the sample solution was poured into a measuring beaker so that the level of the sample was always above the junction of the electrode. Then, the sample was gently stirred and dipped the pH electrode into the solution.

4.2. ºBrix

ºBrix was determined by refractometry according with A.O.A.C (1997).

Laboratory equipment

- Digital refractometer ATAGO (ATC-1) (Tokyo, Japan).

Method

To measure ºBrix a drop of the sample solution was placed on a prism, the result was observed through an eyepiece.

4.3. Turbidity index

The turbidity index was determined using method described by Krop & Pilnik (1974).

Laboratory equipment

- Spectrophotometer UV/Vis, Perkin-Elmer Lambda 2.
- Centrifuge Eppendorf 5810 R.

Method

To measure the turbidity index, 3 mL of sample were centrifuge (1500 rpm/10 min/20 ºC). After that, 1.5 mL of the supernatant was taken and the absorbance at 660 nm was measured.

4.4. Browning index

The browning index was determined using method described by Meydav et al. (1977).

Reagents

- ✓ Ethanol, J.T.Baker (Deventer, The Netherlands).

Laboratory equipment

- Spectrophotometer UV/Vis, Perkin-Elmer Lambda 2.
- Centrifuge Eppendorf 5810 R.

Method

To measure the browning index, 4 mL of sample were centrifuged (2000 rpm/20 min/18 °C). After that, the supernatant was taken and diluted with ethanol (1:1 v/v). Then, the mixture was filtered with Whatman nº42 filter (Whatman International Ltd., UK) and the absorbance at 420 nm was measured.

4.5. Colour

The Colour was determined according with Commision International d`Eclairage LAB system (CIELab).

Laboratory equipment

- Spectrophotometer CM-2002, Konica Minolta (Tokyo, Japan).
- Hunter Labscan II spectrophotometric colourimeter (Hunter Associates Laboratory Inc., Reston, VA., U.S.A) controlled by a computer that calculates colour ordinates from the reflectance spectrum.

Method

To measure the colour, the sample solution was poured into a measuring beaker and put to the colorimeter which from the reflectance value, provides the coordinates of uniform CIELab colour system: L* (lightness) = from black (0) to white (100), a* = color from red (positive) to green (negative) and b * = colour from yellow (positive) to blue (negative) values were used to calculate that total colour differences ($\Delta E^*=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$). In Figure 13 the colour diagram and the respective steps defined are shown.

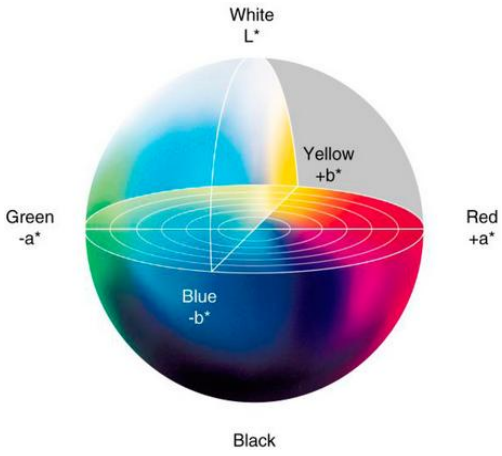


Figure 13. CIELab chromaticity diagram with colour location

4.6. Hydroxymethylfurfural

Hydroxymethylfurfural content was determined using method described by IFFJP (1984).

Reagents

- ✓ Barbituric acid, Merck (Darmstadt, Germany).
  Barbituric acid solution (5 mg/ mL) was prepared by dilution of 500 mg barbituric acid in 70 mL of distilled water. To dissolved it, the mixture was placed in water bath with mild heating. After cooling,

mixture was filled up 100 mL with distilled water. The solution is storage in the fridge under dark conditions.

- ✓ Hydroxymethylfurfural, Fluka (Steinheim, Germany).
  Hydroxymethylfurfural (500 mg/L), should be prepared just before use.
- ✓ Acetic acid glacial 99.7 %, Panreac (Barcelona, Spain).
- ✓ Isopropanol 99.7 %, Panreac (Barcelona, Spain).
- ✓ p-toluidine, Sigma-Aldrich (Steinheim, Germany).
  p-toluidine solution was prepared by dilution of 5 mg p-toluidine in 5 mL of acetic acid glacial and filled up 50 mL with isopropanol. The solution is storage in the fridge under dark conditions.

Laboratory equipment

- Spectrophotometer UV/Vis, Perkin-Elmer Lambda 2.
- Centrifuge Eppendorf 5810 R.

Method

To measure the hydroxymethylfurfural the sample first was filtered with Whatman nº 42 filter (Whatman International Ltd., UK) and centrifuged (4300 rpm/20 min/20 ºC). After that, 1 mL of supernatant was taken to two glass tubes and mixed with 2.5 mL of p-toluidine reagent. Then, 0.5 mL of distilled water to first tube is added and 0.5 mL of barbituric acid to the second one. Both tubes were shaken. The addition of reagents was carried out successively in 1-2 min between samples and absorbance at 550 nm was measured. Hydroxymethylfurfural reacts with barbituric acid and p-toluidine addition, forming a red-coloured compound.

4.7. Ascorbic acid

Ascorbic acid was determined using method described by Esteve et al. (1995).

Reagents

- ✓ Ascorbic acid, Merck (Darmstadt, Germany).
  Ascorbic acid stock solution (1 mg/mL) was prepared by diluting 100 mg of ascorbic acid in 100 mL of oxalic acid 1 % (w/v). The solution is storage in the fridge under dark conditions.
- ✓ Oxalic acid, Panreac (Barcelona, Spain).
- ✓ Trichloroacetic acid, J.T. Baker (Deventer, The Netherlands).
- ✓ Sodium sulphate, J.T. Baker (Deventer, The Netherlands).
- ✓ Acetic acid, Panreac (Barcelona, Spain).
- ✓ Sodium acetate, Panreac (Barcelona, Spain).
- ✓ Acetic acid/sodium acetate 2 M buffer, pH= 4.8.
  Extraction solution (oxalic acid 1 % (w/v); trichloroacetic 2 % (w/v); sodium sulphate 1 % (w/v)) was prepared by dilution 14 g of oxalic acid, 20 g of trichloroacetic acid and 1 g of sodium sulphate in 1 L of distilled water.

Laboratory equipment

- Metrohm polarography 746/647 V.A. Stand Trace Analyzer, coupled with Dosimat Metrohm 685, Metrohm (Herisau, Switzerland).
- Metrohm multi-mode electrode operated in the dropping mercury mode, Metrohm (Herisau, Switzerland).
- A platinum wire counter electrode and a saturated calomel reference electrode, Metrohm (Herisau, Switzerland).

Method

To measure ascorbic acid content, 5 mL sample were diluted in 25 mL of extraction solution (oxalic acid 1 %, w/v; trichloroacetic acid 2 %, w/v; sodium sulphate 1 %, w/v). The solution was shaken and filtered through a folded filter Whatman nº 1 filter (Whatman International Ltd., UK).

Then, 9.5 mL of oxalic acid 1 % (w/v) and 2 mL of acetic acid/sodium acetate 2 M buffer (pH= 4.8) were added to an aliquot of 0.5 mL of filtrate and the solution was transferred to the polarographic cell.

Determinations were carried out using the peak heights and standard additions method. The ascorbic acid concentration was calculated by the method of addition 25 µL of ascorbic acid (1 µg/mL) to the sample and after each addition a record of a polarographic curve was made.

4.8. Total carotenoids

Total carotenoids content was determined using method described by Lee and Castle (2001).

Reagents

- ✓ Hexane, J.T.Baker (Deventer, The Netherlands).
- ✓ Acetone, J.T.Baker (Deventer, The Netherlands).
- ✓ Ethanol, J.T.Baker (Deventer, The Netherlands).

  Extraction solution: hexane/acetone/ethanol (50:25:25 v/v).

Laboratory equipment

- Spectrophotometer UV/Vis, Perkin-Elmer Lambda 2.
- Centrifuge Eppendorf 5810 R.

Method

To measure total carotenoid content 2 mL of sample was homogenized with 5 mL of the extraction solution (hexane/acetone/ethanol (50:25:25, v/v)) and centrifuged (4000 rpm/5 min/5 °C). The top layer of hexane 5 mL was recovered and transferred to a volumetric flask and then volume was adjusted to 25 mL with hexane. Total carotenoid determination was carried out on an aliquot of the hexane extract by measuring the absorbance at 450 nm. Total carotenoids were calculated in accordance with Ritter and Purcell (1981) using an extinction coefficient of β-carotene, $E^{1\%} = 2505$.

4.9 Total phenolic compounds

Total phenolic compounds was determined using method described by Georgé et al. (2005).

Reagents

- ✓ Sodium Carbonate, J.T.Baker (Deventer, The Netherlands).
  Sodium carbonate solution 2 % (w/v). The solution is storage at room temperature under dark conditions.
- ✓ Folin-Ciocalteu, Sigma-Aldrich (Steinheim, Germany).
  Folin-Ciocalteu reagent (50 %, v/v). The solution is stable for one month in the fridge under dark conditions.
- ✓ Gallic acid, UBC (Brussels, Belgium).
  Gallic acid stock solution was prepared by dilution 250 mg of gallic acid in 20 mL of water and 5 mL of ethanol (to dissolved it well). The solution is stable for one month in the fridge under dark conditions.
  The working solution (1 mg/mL) was prepared daily, underwent ultrasonic treatment for 1 min and then, 1 mL of this was taken out and made up to 10 mL with distilled water.

Laboratory equipment

- Spectrophotometer UV/Vis, Perkin-Elmer Lambda 2.
- Centrifuge Eppendorf 5810 R.

Method

To determine total phenolic content, 100 µL of diluted sample was mixed with 3 mL of sodium carbonate solution 2 %. Then, 100 µL of Folin-Ciocalteu 50 % (v/v) was added, mixed by inversion and allowed to stand for 1 hour in darkness at room temperature. After this time, the absorbance at 765 nm was measured. The quantitative determination was made by interpolation of the values on a calibration curve prepared with gallic acid in the range of concentrations comprised between 0.1 and 1.0 mg/mL. Samples with higher concentration were diluted with distilled water.

4.10 Total anthocyanins

Total anthocyanins, monomeric and polymeric anthocyanins contents were determined using a modified method described by Mazza et al. (1999).

Reagents

- ✓ Hydrochloric acid 37 %, J.T.Baker (Deventer, The Netherlands). Hydrochloric acid 5 % (v/v).
- ✓ Sodium metabisulfite, J.T. Baker (Deventer, The Netherlands). Sulphur dioxide 5 % (w/v) was prepared by dilution 0.043 g of sodium metabisulfite in 500 mL of distilled water. The solution is stable for one month at room temperature under dark conditions.

Laboratory equipment

- Spectrophotometer UV/Vis, Perkin-Elmer Lambda 2.

Method

To determine total anthocyanins (A^{ta}) a 10-fold diluted sample of 100 μL of was mixed by inversion with 1700 mL of distilled water and 200 μL of hydrochloric acid 5 % (v/v), and allowed to stand in darkness at room temperature for 20 min. After this time the absorbance at 520 nm in a 10 mm cuvette was measured.

Calculations of total anthocyanins were based on cyanidin-3-glucoside (molar absorptivity 26 900).

4.11. Total antioxidant capacity

TEAC assay

The method described by Re et al. (1999) and modified by Zulueta et al. (2009) based on the capacity of a sample to inhibit the ABTS radical (ABTS •+) compared with a reference antioxidant standard (Trolox) was used.

Reagents

- ✓ Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid), Sigma-Aldrich, (Steinheim, Germany).

Trolox 5 mM stock solution was prepared by dilution 31.25 mg of trolox in 25 mL of ethanol. The solution is stable for about 2 months frozen at - 20°C.

- ✓ Ethanol 99.5 %, J.T.Baker (Deventer, The Netherlands).
- ✓ Potassium persulfate, Panreac (Barcelona, Spain).
  Potassium persulfate 140 mM was prepared by dilution of 0.96 g of potassium persulfate in 25 mL of distilled water.
- ✓ 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Sigma-Aldrich, (Steinheim, Germany).
  ABTS (7 mM) wa prepared by dilution 0.096 g of ABTS in 25 mL of distilled water.
  The radical $ABTS^{\bullet+}$ was prepared by mixing 25 mL of ABTS (7 mM) with 440 µl of potassium persulfate (140 mM) and allowed to stand in darkness at room temperature for 12-16 h (the time required for formation of the radical). The $ABTS^{\bullet+}$ radical is stable for two days at room temperature under dark conditions.
  The working solution was prepared by diluting a stock solution in ethanol until the absorbance at 734 nm, 0.70 ± 0.02 at 30 °C (usually is a dilution 1:100).

Laboratory equipment

- Spectrophotometer Perkin–Elmer UV/Vis Lambda 2 connected to a Julabo UC 5B thermostated bath at 30 ºC (Julabo, Seelbach/Black Forest, Germany).

Method

To measure TEAC method based on application of ABTS decolourisation assay was used. The reaction of inhibition radical $ABTS^{+\bullet}$ took place directly in the measuring cuvette. For this purpose, 2 mL of the $ABTS^{+\bullet}$ radical were added and the absorbance (A_0) at 734 nm was measured. Then, 0.1 mL of the standard solution were added and the absorbance at 60 min

was measured (A_f). The percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition} = (1-A_f/A_0)*100$$

Where A_0 is the absorbance at initial time (after addition of the standard) and A_f is the absorbance obtained when recorded. The sample must be diluted so that the value obtained is placed into the calibration curve. For this purpose, different dilutions of the samples in distilled water (1/10, 1/25, 1/50, 1/75, 1/100) were previously studied, as there may be variation in the amount of antioxidants present.

Trolox calibration curve was prepared for a concentration range of 0-250 mM, and the inhibition percentage obtained for the samples was interpolated to calculate the concentration in trolox equivalents (mM TE).

ORAC assay

The method described by Ou et al. (2001) and modified by Zulueta et al. (2009) was used.

Reagents

- ✓ Fluorescein, Sigma-Aldrich (Steinheim, Germany).
  Fluorescein (78 nM) was prepared by dilution 44 mg of fluorescein in 100 mL of phosphate buffer. The stock solution is stable for one week in the fridge under dark conditions.
  The working solution was prepared daily, taking 0.167 mL of the stock solution and filling it with 25 mL of phosphate buffer.
- ✓ Sodium dihydrogen phosphate, Panreac (Barcelona, Spain).
- ✓ Potassium dihydrogen phosphate, Scharlau (Barcelona, Spain).
- ✓ Phosphate buffer (75 mM, pH 7.0) was prepared by dilution 28.4 g of sodium dihydrogen phosphate in 250 mL of distilled water, and 27.7 g of potassium dihydrogen phosphate in 250 mL of distilled water. Then, from this 61.6 mL of sodium di-hydrogen phosphate

and 38.9 mL of potassium dihydrogen phosphate was diluted in 1L of distilled water. The solution is storage in the fridge.

- ✓ Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid), Sigma-Aldrich (Steinheim, Germany).

  Trolox stock solution (1 mM) was prepared by dilution of 12.5 mg of trolox in 50 mL of phosphate buffer. The solution is stable for about 2 months frozen at -20°C.

  The working solution 20 µM was prepared daily, taking 1 mL of stock solution and filling it with 50 mL of phosphate buffer.

- ✓ AAPH, azobis (2-amidinopropane) dihydrochloride 97 %, Sigma-Aldrich (Steinheim, Germany).

  AAPH (221.25 mM): was prepared daily by dilution 600 mg of AAPH in 10 mL of phosphate buffer.

Laboratory equipment

- Wallac 1420 VICTOR^2 multilabel counter Perkin-Elmer (California, USA) with fluorescence filters, for an excitation wavelength of 485 nm and emission wavelength of 535 nm.
- 96 white well flat bottom wells (Stone, United Kingdom).

Method

In each well of the plate 50 µL of fluorescein (78 nM) and 50 µL of sample, blank (phosphate buffer), or standard (trolox 20 µM) were placed, and then 25 µL of AAPH (221 mM) were added. Variations in measurement between one well and another can arise because of the low conductivity of the polypropylene plates. To avoid this problem, the plate was heated to 37 ºC for 15 min prior to the addition of AAPH. The fluorescence was measured immediately after the addition and measurements were then taken every 5 min until the relative fluorescence intensity (FI %) was less than 5 % of the value of the initial reading.

The ORAC values, expressed as μM trolox equivalent (μM TE) were calculated by applying the following formula:

$$\text{ORAC (μM TE)} = \frac{C_{TROLOX} \text{ x } (AUC_{SAMPLE} - AUC_{BLANK}) \text{ x K}}{(AUC_{TROLOX} - AUC_{BLANK}) \text{ x } 1000}$$

where C_{TROLOX} is the concentration (μM) of trolox (20 μM), K is the sample dilution factor, and AUC is the area below the fluorescence decay curve of the sample, blank and trolox, respectively, calculated by applying the following formula:

$$AUC = (0.5 + f_5/ f_0 + f_{10}/ f_0 + \ldots + f_{n+5}/f_0) \text{ x } 5$$

where f_0 is the initial fluorescence and f_n is the fluorescence at time n.

DPPH assay

The method described by Brand-Williams et al. (1995) was used.

Reagents

- ✓ Trolox (6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid), Sigma-Aldrich, (Steinheim, Germany).
  Trolox 2 mM was prepared by dilution 5 mg of trolox in 1 mL of methanol to obtain a solution of 20 mM. Then, 100 μL of solution 20 mM was taken and diluted with 1 mL of methanol to obtain a 2 mM trolox solution.
- ✓ DPPH (2,2-diphenyl-1-picrylhydrazyl), Sigma-Aldrich (Steinheim, Germany).
  Radical DPPH 0.06 mM was prepared by dilution 2.3 mg of DPPH in 100 mL of methanol. The solution is stable for 12 h in darkness at room temperature.
- ✓ Methanol, J.T.Baker (Deventer, The Netherlands).

Laboratory equipment

- Spectrophotometer UV/Vis, Perkin-Elmer Lambda 2.

Method

To measure DPPH 1.45 mL (0.06 mM) of DPPH radical was added to the measuring cuvette and the absorbance was measured at 515 nm (A_1). Then, 50 µL of sample (appropriately diluted) was added and the absorbance value recorded at 30 min (A_2). The final absorbance value is a result from the difference between A_2-A_1. The result can be expressed as mmol trolox/L which interpolated in a calibration curve or as % inhibition compared to the target using the following expression:

$$\% \text{ inhibition} = [(A_m - A_b / A_b) * 100]$$

where A_m is the sample absorbance and A_b is the absorbance of the blank. The value obtained in the calibration curve to determine Trolox equivalents (mmol TE/L) is interpolated.

4.12. Quantification of Carotenoids

For quantification of carotenoids method described by Reif et al. (2012) with some modification was used. The method was applied during Scientific Internship, Institute of Food Science Agroscope (Wädenswil, Switzerland).

Reagents

- ✓ Methanol, Chemie Brunschwig (Basel, Switzerland).
- ✓ Dichloromethane, Merck (Darmstadt, Germany).
- ✓ Acetonitrile, Carlo Erba Reagenti SpA (Rodano, Italy).
- ✓ Ammonium acetate, Fluka (Buchs, Switzerland).
  Ammonium acetate 0.05 mmol/L was prepared by dilution 0.963 g in 250 mL of distilled water.
- ✓ Acetone, Carlo Erba Reagenti SpA (Rodano, Italy).
- ✓ Butylated Hydroxy Toluene (BHT), Sigma-Aldrich (Buchs, Switzerland).

Extraction solvent: methanol: acetone (1:1) (v/v) with BHT 0.01 % (w/v) addition.

- ✓ β-carotene, α-carotene, β-cryptoxanthin, lutein, Carote Nature GmbH (Ostermundigen, Switzerland).

Laboratory equipment

- UPLC Acquity H-Class Bio (Agilent Technologies, California, USA).
- Van Guard C18 column 1.8 µm, 2.1 x 5 mm (Baden-Dättwil, Switzerland).
- Van Guard C18 pre-column 1.8 µm, 2.1 x 5 mm (Baden-Dättwil, Switzerland).
- Centrifuge Heraeus Multifuge 3RS+, Thermo Scientific.
- Polytron PT 3100 (Zug, Switzerland).

Method

To quantified carotenoids 1 g of sample was mixed with 50 mL of extraction solvent (methanol:acetone 1:1 containing 0.01 % BHT) and transferred into brown 50 mL glass bottle, covered with nitrogen gas and homogenized at full speed for 30 s with Polytron blender under constant nitrogen supply. Then, a vortex briefly was used and ultrasonic bath for 30 min in orden to increase extraction yield. Extract are transferred to 50 mL volumetric flask, the original bottle is rinsed with a small amounts of extraction solvent and the volumetric flask is filled up to the mark with extraction solvent. The volumetric flask is shaken vigorously and left to sediment for 5 min. An aliquot was taken with a plastic Pasteur pipette, transferred into a 10 mL plastic syringe and filtered through a 0.22 µm syringe filter directly into brown UPLC tube. The mobile consist of two solvents: Solvent A, 25 % 0.05 mol/L ammonium acetate and 75 % of mixture acetonitrile/dichloromethane/methanol (75:10:15), Solvent B: acetonitrile/dichloromethane/methanol (60:10:30). Carotenoids were eluted under 0.45 mL/min flow rate and the temperature was set at 35 ºC.

Chromatograms were recorded at 450 nm. Peak identification was achieved by the individual injection of each standards and by comparing their spectra.

4.13. Quantification of steviol glycosides

For quantification of steviol glycosides method of Joint FAO/WHO Expert Committee and Food Additives (JECFA, 2010) with various modification was used.

Reagents

- ✓ Sodium phosphate, Panreac (Barcelona, Spain).
  Sodium phosphate buffer 10 mmol/L pH 2.6 was prepared by dilution of 1.38 g sodium phosphate in 1 L of distilled water.
- ✓ Methanol, Panreac (Barcelona, Spain).
- ✓ Acetonitrile HPLC, Panreac (Barcelona, Spain).
- ✓ Rebaudioside A, stevioside hydrate and steviol hydrate, Sigma-Aldrich (Steinheim, Germany).
- ✓ Rebaudioside C, rebaudioside F, Wako (Osaka, Japan).

Laboratory equipment

- Chromatograph HPLC 1220 COMPACT LC system, Agilent Technologies (California, USA).
- Kromasil 100 C18 column 5 μm, 250 x 4.6 mm, Scharlab (Barcelona, Spain).
- Kromasil 100 C18 precolumn (guard column) 5 μm, 250 x 4.6 mm, Scharlab (Barcelona, Spain).

Methods

To quantify steviol glycosides, samples were filtered through a cartridge Sep-Pak (a reverse-phase C-18 cartridge; Millipore, MA, USA) which retains steviol glycosides. The cartridges were previously activated with 10 mL of methanol and 10 mL of water. Every 10 mL of sample was eluted with 2 mL of methanol, and all methanolic fractions were collected, filtered

through a 0.45 μm membrane filter Millex-HV13 (Millipore, MA, USA) and then analysed by liquid chromatography. The mobile phase consisted of two solvents: Solvent A, acetonitrile and Solvent B, 10 mmol/L sodium phosphate buffer (pH = 2.6) (32:68, v/v). Steviol glycosides were eluted at 1 mL/min flow rate and the temperature was set at 20 ºC. Chromatograms were recorded at 210 nm. The identification of steviol glycosides were obtained out by using standards and by comparing the retention times, while quantification was performed by external calibration with standards.

4.14. Microorganisms

Total viable count as well as yeast and molds were determined using method described by Ranalli & Howell (2002).

Reagents

- ✓ Physiological saline with peptone, BTL, (Łódź, Poland).
- ✓ Plate Count Agar (PCA), BTL, (Łódź, Poland).
- ✓ Chloramphenicol Agar (YGC), BTL, (Łódź, Poland).

Method

To measure the total viable count as well as yeast and molds 10 g of sample were mixed with 90 mL of peptone solution. Serial dilutions from 10^{-1} to 10^{-9} were carried out, and the microbial status was evaluated by means of plate counts. For total viable count PCA agar plate was prepared with sample dilution and incubated at 30 ºC over 72 h, next grown bacterial colonies were counted. To measure yeast and mold YGC agar plate was prepared with sample dilution and incubated at 25 ºC over 5 days. All measurements were expressed in cfu/mL (colony forming units per mL of sample).

5. DIGESTION METHODS

5.1. Digestion method I

This *in vitro* gastrointestinal digestion was carried out in three sequential phases: salivary, gastric and intestinal digestion (including dialysis), as described by Rodríguez-Roque et al. (2013) with some modifications.

Reagents

- ✓ Sodium chloride, Baker (Deventer, The Netherlands).
- ✓ Potassium dihydrogen phosphate, Scharlab (Barcelona, Spain).
- ✓ Hydrogen chloride, Panreac (Barcelona, Spain).
- ✓ Sodium dihydrogen phosphate, Panreac (Barcelona, Spain).
- ✓ Sodium hydroxide, Panreac (Barcelona, Spain).
- ✓ Sodium hydrogen carbonate, Backer (Deventer, The Netherlands).
- ✓ Sodium dodecyl sulfate, Merck (Darmstadt, Germany).
- ✓ Ethylenediaminetetraacetic acid disodium salt dihydrate, Sigma-Aldrich (Steinheim, Germany).
- ✓ α-amylase from bacillus, Sigma-Aldrich (Steinheim, Germany).
- ✓ Pepsin from porcine from stomach, Sigma-Aldrich (Steinheim, Germany).
- ✓ Mucin, Sigma-Aldrich (Steinheim, Germany).
- ✓ Pancreatin from porcine pancreas, Sigma-Aldrich (Steinheim, Germany).
- ✓ Bile Extract porcine, Sigma-Aldrich (Steinheim, Germany).

Laboratory equipment

- Wather bath, Stauart SBS 40.
- Dialysis tubing cellulose membrane, Sigma-Aldrich (Steinheim, Germany).

Method

Figure 14 shows short description of method applied. First saliva solution was prepared by dilution of 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, 8 g NaCl, 100 mg of mucin and α-amylase (200 U/L of enzyme activity) in 1 L of distilled water. Then, 50 mL of sample was transferred to an erlenmeyer flask and 5 mL of saliva (pH 6.75±0.2) were added. This mixture was kept in a shaking water bath (90 rpm/10 min/37 °C). After this time, salivary digested aliquots were taken for analysis. Afterwards, 13.08 mg of pepsin from porcine stomach was added and pH was adjusted to 2 by addition of HCl (12 M). This mixture was incubated in darkness in a shacking water bath (90 rpm/2 h/37 °C). At the end of the gastric digestion, aliquots were taken for analysis and 20 mL were used for titration with NaOH (0.5 M) to pH 7.5 after adding 5 mL of pancreatin (4 g/L) – bile (25 g/L) mixture.

Dialysis membrane was conditioned with 0.01 M EDTA Na_2, 2 % $NaHCO_3$ and 0.1% sodium dodecyl sulfate at boiling point, rinsed with distilled water and cut into segments of 30 cm. Dialysis membrane segments were filled with 25 mL of water-$NaHCO_3$ mixture, with the amount of $NaHCO_3$ (0.5 N) used in the previous titration. 20 mL of the gastric digest were placed into a beaker and the dyalisis membrane was immersed in that digest until reaching pH 5.0. This process allows gradual pH adjustment, mimicking intestinal conditions. After 30 min, 5 mL of pancreatin (4 g/L)-bile (25 g/L) mixture was added and the incubation continued (90 rpm/2 h/37 °C). The dialysed intestinal fraction (fraction inside the dialysis membrane), consisting of soluble compounds of low molecular weight, and the non-dialysed intestinal fraction (fraction outside the dialysis membrane), consisting of soluble and insoluble compounds of low and high molecular weight, were collected and placed in a cold water bath for 10 min to stop intestinal digestion.

Bioaccessibility (%), referred to the percentage of tested compound remaining in the dialysed intestinal fraction related to the original non-digested sample was determined according to Eq. (1).

$$Bioaccessibility\ (\%) = \frac{Dialysed\ \ fraction\ \ x\ 100}{Non-digested\ \ content} \quad (1)$$

where dialysed and non-digested corresponded the bioactive compounds concentration mg/100 g in dialysed fraction and non-digested samples respectively.

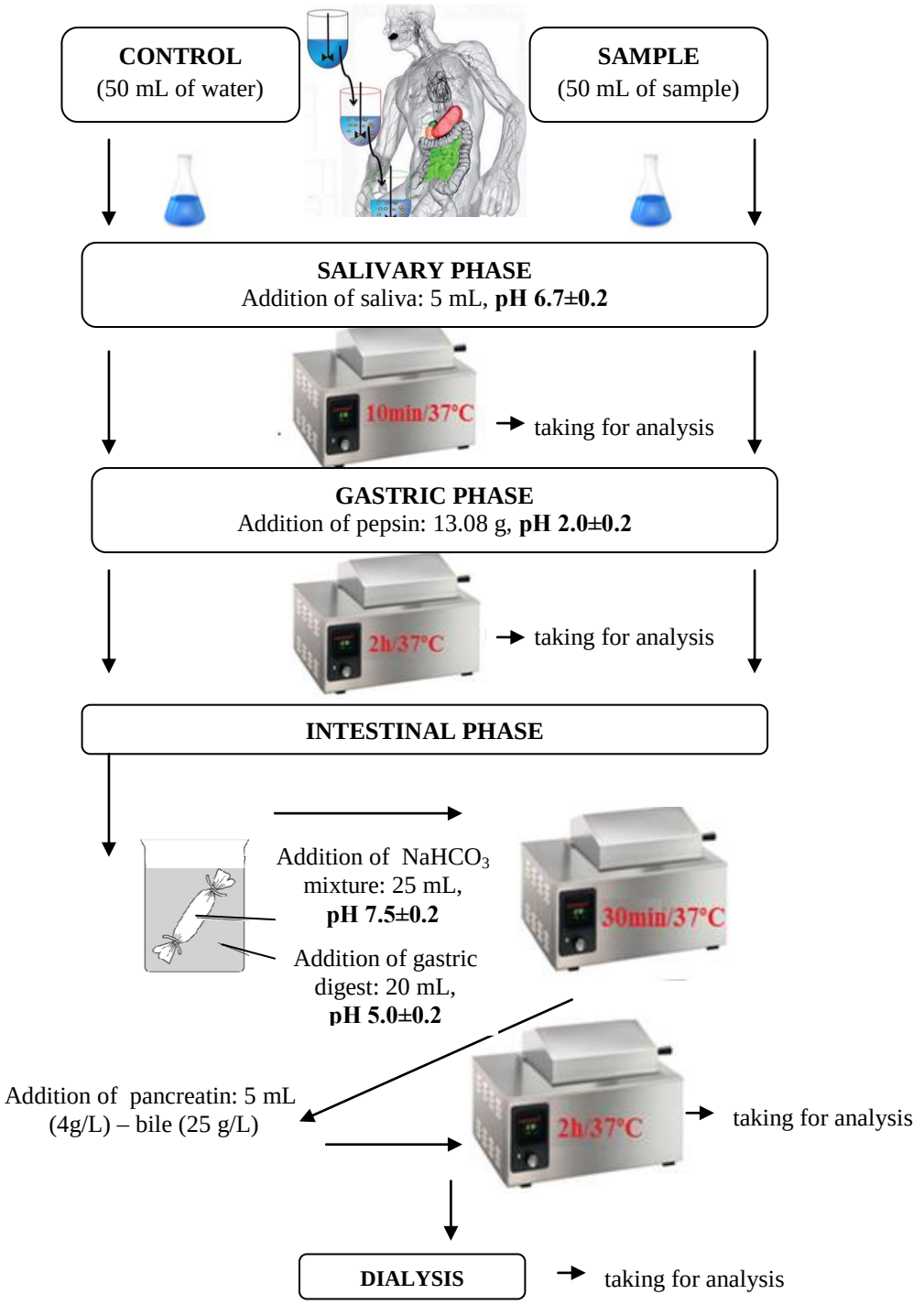


Figure 14. Diagram of *in vitro* digestion method I.

5.2. Digestion method II

The *in vitro* gastrointestinal digestion was carried out in two sequential phases: gastric and intestinal digestion as described by Minekus et al. (2012) with some modification from Kopf-Bolanz et al. (2012). The method was applied during Scientific Internship, Institute of Food Science Agroscope (Switzerland, Wädenswil).

Reagents

- ✓ Potassium chloride, Fluka (Buchs, Switzerland).
- ✓ Potassium dihydrogen phosphate, Sigma-Aldrich (Buchs, Switzerland).
- ✓ Sodium hydrogen carbonate, Sigma-Aldrich (Buchs, Switzerland).
- ✓ Sodium chloride, Fluka (Buchs, Switzerland).
- ✓ Magnesium chloride hexahydrate, Fluka (Buchs, Switzerland).
- ✓ Sodium hydroxide, Carlo Erba Reagenti SpA (Rodano, Italy).
- ✓ Hydrogen chloride, Burdick & Jackson (Seelze, Germany).
- ✓ Calcium chloride hexahydrate, Fluka (Buchs, Switzerland).
- ✓ Methanol, Carlo Erba Reagenti SpA (Rodano, Italy).
- ✓ Acetone, Carlo Erba Reagenti SpA (Rodano, Italy).
- ✓ Butylated Hydroxytoluene (BHT), Sigma-Aldrich (Buchs, Switzerland).
- ✓ Pepsin from porcine from gastric mucosa, Sigma-Aldrich (Buchs, Switzerland).
- ✓ Pancreatin from porcine pancreas, Sigma-Aldrich (Buchs, Switzerland).
- ✓ Bile from bovine and ovine, Sigma-Aldrich (Buchs, Switzerland).

Laboratory equipment

- Polytron PT 3100 (Zug, Switzerland).
- Freeze Dryer (Hoganas, Sweden).

Method

Figure 15 shows short description of digestion method applied. Before digestion process two different fluids were prepared: simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). To prepare SGF: 4.6 g of KCl, 6.8 g of KH_2PO_4, 8.4 g of $NaHCO_3$, 12 g of NaCl, 3 g of $MgCl_2(H_2O)_6$, were mixed with 100 mL of water then pH was adjusted with HCl 37 % to 1.3±0.2.

To prepare SIF: 9.2 g of KCl, 13.6 g of KH_2PO_4, 16.8 g of $NaHCO_3$, 24 g of NaCl, 6 g of $MgCl_2(H_2O)_6$, were mixed with 200 mL of water then pH was adjusted with NaOH 37 % to 8.2±0.2. Fluids such prepared are stable in frozen condition (-20 ºC) for about 1 month. Just before digestion process to SGF fluid 39.2 mg of pepsin porcine and 15 µl of $CaCl_2(H_2O)_6$ were added and dissolved by magnetic stirring.

Then, around 5 g of samples were weight into 50 mL brown bottles, 10 mL of dissolved SGF was added and pH adjusted to 3.0±0.2 if necessary. The sample were covered with nitrogen and incubated (90 rpm/2 h/37 ºC). In this time SIF fluid preparation was continued by addition 3.34 mg of bile and 30 µl of $CaCl_2(H_2O)_6$ and dissolved by means magnetic stirring. Finally, 2.2 g of pancreatin was added, maximum 15 min before the intestinal step and dissolved by magnetic stirring.

After 2 h the pH of samples was checked and the values recorded. In the next step 20 mL of SIF juice was added and pH adjusted if necessary to 7.0 ± 0.2. Then samples were covered with nitrogen and incubated (90 rpm/2 h/37 ºC). After 2 h digested samples were transferred to 50 mL tubes and centrifuged for 4495 rpm/10 min/4 °C to separate solids and non-micellarized lipids from the aqueous phase. After centrifuged stage, the middle phase of the second centrifugation to a glass tube was removed and them used for quantifications:

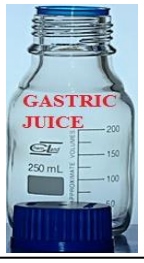
- ✓ To quantify liberation: 5 mL of sample into a 20 mL pear shaped flask were transferred.
- ✓ To quantify micellarisation: 1 mL of sample through a nylon syringe filter 0.22 μm into a 10 mL glass tube were filtered.

Afterwards all samples in liquid nitrogen under constant rotation were frozen. Finally lyophilization (freeze the samples until dryness).

Liberation (%) referred to the percentage of tested compound transferred from the test sample to the aqueous micellar fraction after *in vitro* digestion.

Micellarisation (%) referred to the percentage of tested compound transferred from the digestate to micelles after ultra-centrifugation.

GASTRIC PHASE
5 g of sample with addition of SGF 10 mL, **pH 3.0±0.2**



INTESTINAL PHASE
Addition of SIF: 20 mL, **pH 7.0±0.2**

After incubation samples were centrifuged (4495 rpm/10 min/4 ºC)

To quantify liberation
5 mL of sample into a 20 mL pear shaped flask were transferred.

To quantify micellarisation
1 mL of sample through a nylon syringe filter 0.22 µm into a 10 mL glass tube were filtered.

All samples in liquid nitrogen were frozen and put to lyophilization (until dry).

EXTRACTION FOR CAROTENOIDS QUANTIFICATION

methanol:acetone (1:1) (v/v) containing 0.01% BHT (w/v) was added to freeze dried samples, to dissolved it water bath for 30 min was used.

Samples were filtered and injected a UPLC.

SGF: simulated gastric fluid, SIF: simulated intestinal fluid

Figure 15. Diagram of *in vitro* digestion method II.

RESULTS

6.1. Physicochemical properties, steviol glycosides and bioactive compounds behaviour after thermal processing of a beverage based on papaya, mango mixed with orange juice, oats, acaí and stevia

Nowadays, new products with functional properties based on exotic and innovative ingredients have appeared in the European and North American market, with a good consumer acceptance and a high nutritional value, largely due to its high content in bioactive compounds and antioxidant capacity. Demand for these products is growing, and is therefore necessary a thorough study on the characteristics and benefits attributed to such ingredients (Dembitsky et al., 2011).

Fruit-based beverages have higher healthy properties compared to soft drinks. However, sometimes the use of these fruits for the preparation of various processed products becomes limited mainly due to high acidity, astringency and bitterness of some of these fruits. Therefore, the combination of two or more fruit juices can be a possible strategy in order to prepare new ready to serve beverages. Some previous studies in the published literature have reported that blending of fruit juice helps improving flavor, taste, nutritive values and reduces the cost of production. Fruit juices added with other plant food products are gaining importance in the market in the form of fruit drinks/squashes/appetizers and some different healthy drinks (Bhardwaj & Pandey, 2011). Recently, there has been an increasing interest in the use of natural sweeteners, known as steviol glycosides (200 times sweeter than sucrose), obtained from the leaves of the plant called *Stevia rebaudiana* Bertoni. These compounds can be a nutritional strategy in order to replace or substitute sugar energy content with one or more ingredients of low-calorie content (Chaturvedula et al., 2011).

For instance, *stevia* has attracted economic and scientific interests due to the sweetness and the supposed therapeutic benefits of its leaf. FDA approved

stevia for commercialization in 2008 and more recently, in November 2011, the European Commission (EU) has approved steviol glycosides as a new food additive (E 960). In recent years, food industry is developing an array of new products based on *stevia* plant extracts in order to satisfy the demand of consumers concerned with healthier eating. Many of these new low-sugar products are not just the old standbys like diet sodas and sugarless gum, but foods and drinks like cereals, fruit juices, cookies, bread, ice cream, flavored milk, pasta sauce and even bottled water (Food Consulting, 2010). In Europe, the recent green light will probably lead to wide-scale use (Stoyanova et al., 2011). So far, little data has been available regarding the practical applications in foods and the effects of processing on steviol glycosides stability in real food matrices (Nehir El & Simsek, 2012).

Thermal processing is the most widely used technology to extend the shelf-life of liquid foods by the inactivation of microorganisms and enzymes. However, heat causes irreversible losses of nutritional compounds, undesirable changes in physicochemical properties, and alteration of their antioxidant (Cortes et al., 2008; Torregrosa et al., 2006). A few inadequate conditions during thermal treatment of the juice reflect on an increase of the concentration of the different derivatives of furfural, formed by the degradation of reducing sugars or by Maillard's reaction, and, in addition, changes in colour. Moreover, in presence of high temperatures (e.g. heating, baking) substantial degradation of steviol glycosides might take place (EFSA, 2010). Therefore, there is a need to optimize the processing conditions to obtain healthier food products.

In previous studies, the stability of steviol glycosides in several food matrices (semi skimmed milk, soy drink, fermented milk drink, mock beverage, ice cream, full-fat and skimmed set yogurt, dry biscuits, and jam) was investigated (Jooken et al., 2012; Prakash et al., 2011).

These authors did not find any sign of decomposition of steviol glycosides after processing and subsequent storage. However, more recently, it was

investigated the degradation kinetics of rebaudioside A in various buffer solutions, observing an important degradation of this compound when neutral pH was used (Gong & Bell, 2013). Therefore, at this stage of development there is a need to investigate the behavior of steviol glycosides in real food beverages after thermal processing.

Moreover, exotic fruits such as mango, papaya and acaí contain a large quantity of bioactive compounds such as ascorbic acid, phenolic compounds and carotenoids that have been shown to be good contributors to the total antioxidant capacity of foods (Barba et al., 2013; Zulueta et al., 2009). In addition, *stevia* water extracts and oats also have antioxidant activity mainly due to its high content in these compounds.

6.1.1. Materials and methods

6.1.1.1. Samples

Composition and preparation of the samples is described in the paragraph 3.1.1 on page 57.

6.1.1.2. Physicochemical parametrs

- pH *(See section 4.1 - page 59)*
- ºBrix *(See section 4.2 - page 59)*
- Turbidity index *(See section 4.3 - page 59*)
- Browning index *(See section 4.4 - page 60)*
- Colour *(See section 4.5 - page 60)*
- Hydroxymethylfurfural *(See section 4.6 - page 61)*

6.1.1.3. Nutritional parameters

- Ascorbic acid *(See section 4.7 - page 63)*
- Total carotenoids *(See section 4.8 - page 64)*

- Total anthocyanins *(See section 4.10 - page 66)*

6.1.1.4. Total antioxidant capacity

- TEAC, ORAC *(See section 4.11 - page 66, 68)*

6.1.1.5. Quantification of steviol glycosides *(See section 4.13 - page 73)*

6.1.1.6. Treatments

The experiments were carried out in a plate and frame heat exchanger equipped with nominal 66 s hold time tube (FT74X/HTST/UHT, Armfield, Inc.). In order to achive the appropriate treatment conditions (60-99 ºC, for 0.5-15 min), the beverage was placed in a feed tank and pumped through the heat exchanger. The temperature of 60 ºC was chosen because it is the temperature usually associated with blanching of fruits and vegetables (Gamboa-Santos et al., 2010). Similary, heating liquid foods to 88-99 ºC for 15-30 s is standard commercial practice in order to pasterize this kind of products (Braddock, 1999; Irwe & Olson, 1994). All the treatments were applied in duplicate, with three bottels per treatment. Immediately after heating, the samples were cooled in an ice/water bath (FT 61, Armfield, Inc.) and then stored under refrigeration (4±1 ºC) until needed for analysis.

6.1.1.7. Statistical analysis

A face-centred central composite response surface analysis was used to determine the effect of temperature (ºC) (*T*), time (minutes) (*t*) and *stevia* (%, v/v) concentration (X_3) on the steviol glycosides, bioactive compounds and physicochemical properties (pH, ºBrix, hydroxymethylfurfural, colour, turbidity and browning index) of the beverage. The independent variables of the RSM were: temperature (from 60 to 99 ºC), time (from 0.5 to 15 minutes) and *stevia* concentration (from 0 to 2.5 %, v/v). Three (maximum, minimum and central) values of each factor were considered, leading to 16

experiments (Table 6). The experimental design was performed twice, resulting in two blocks of experiments. The combinations included thermal processing-*stevia* conditions with an intermediate level (central point) of the three variables replicated 4 times, which was used to determine inherent variance in the technique.

Table 6. Experimental design matrix of the thermal treatments-*stevia* processing.

Run	Temperature (ºC)	Time (min)	*Stevia* (%)
1	99	8	1.25
2	80	15	1.25
3	80	0.5	1.25
4*	80	8	1.25
5	99	0.5	2.5
6	60	0.5	0
7	99	0.5	0
8	60	8	1.25
9	60	15	0
10	80	8	2.5
11	60	0.5	2.5
12*	80	8	1.25
13	80	8	0
14	99	15	2.5
15	99	15	0
16	60	15	2.5

*Central point

Experiments were randomized to minimize the systematic bias in the observed responses due to extraneous factors and to increase precision.
Experimental data were fitted to a polynomial response surface. The second-order response function was predicted by the following equation:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{i=i+1}^{3} b_{ij} X_i X_j$$

where Y represents a response variable, b_0 is the centre point of the system, b_i , b_{ii} , and b_{ij} , are coefficients of the linear, quadratic and interactive effect, respectively; X_i, X_i^2, and $X_i\ X_j$ represent linear, quadratic and interactive effect of the independent variables, respectively.

The non-significant terms were deleted from the second-order polynomial model after an ANOVA test, and a new ANOVA was performed to obtain the coefficients of the final equation for better accuracy. In the present study, desirability functions were developed in order to obtain the beverage with the highest levels of steviol glycosides, bioactive compounds and the best physicochemical properties. Subsequently, an ANOVA of three factors (temperature, time, and *stevia* concentration) was applied, and in the parameters for which significant differences were obtained with more than two levels, Tukey's test was applied to ascertain the range of values in which the differences were located. Finally, a study was conducted with the aim of determining whether there were correlations between a pair of variables (Pearson's test). All statistical analyses were performed using SPSS® (Statistical Package for the Social Sciences) v.20.0 for Windows (SPSS Inc., Chicago, USA). The results obtained by applying each test are shown in Annex *(See section 9 -page iii).*

6.1.2. Results and discussion

Impact of thermal processing and stevia concentration on physicochemical properties of the beverages

The value of pH in the untreated beverage sweetened with 0, 1.25 and 2.5 % (v/v) was 4.35±0.10 in all cases. After application of the different thermal treatments, non-significant changes in pH values were observed. Moreover, the values of °Brix were 7.7±0.1, 8.7±0.1 and 9.7±0.1 for the untreated beverages with 0, 1.25 % (v/v) and 2.5 % (v/v) of *stevia*, respectively.

Non-statistically significant changes ($p > 0.05$) were obtained for °Brix values 8.2±0.1 when the various thermal treatments were applied in these beverages.

The results obtained for turbidity index (TI), browning index (BI), colour parameters (a*, b* and L*), hydroxymethylfurfural in the untreated and thermally-treated samples and steviol glycosides in the untreated samples are shown in Tables 7-8. As can be expected, among the studied parameters, *stevia* percentage had the greatest effect on the quality parameters of the beverages.

The turbidity index (TI) of the untreated beverages was 2.427±0.005, 2.614±0.004 and 2.802±0.002 for the samples with 0 %, 1.25 %, 2.5 % (v/v) *stevia* added, but it increased significantly ($p < 0.05$) in all the thermally-treated samples with 1.25 % (v/v) *stevia* and in the thermally-processed (60 ºC/ 0.5-15 min) samples without *stevia* added. In addition, the opposite trend was obtained after applying thermal processing when *stevia* percentage was 2.5 % (v/v). The reduced regression model presented in the equation below allowed for prediction of the effects of independent variables on the TI values:

$$TI = 2.4746 - 0.0036 \cdot t + 0.2321 \cdot stevia + 0.0029 \cdot t*stevia - 0.0674 \cdot stevia^2$$

where *t* is treatment time (minutes) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverage.

Compared to the untreated samples, higher BI values were obtained for the thermally-treated samples when 0 and 1.25 % (v/v) *stevia* percentages were used, however a significant decrease in BI was observed after applying thermal processing (90 ºC, 0.5-15 min) in the samples with 2.5 % *stevia*. The regression model was accurate enough ($R^2 = 96.56$, $p < 0.05$, standard error = 0.199).

The regression equation describes the following model:

$$BI = -2.1803 - 0.0083 \cdot T + 0.3633 * stevia + 0.0067 \cdot T * stevia - 0.0040 \cdot stevia^2$$

where *T* is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverage.

With regard to the CIELAB parameters, statistical analysis of the obtained results immediately after processing showed significant difference ($p < 0.05$) for the a* (redness), b* (blueness) and L*(lightness) values among the untreated and thermally-treated beverages. Compared to the untreated beverages, non-significant changes in L* values were found in the samples without *stevia* and treated at 60 ºC. However higher L* values, which indicated a lightening of juice surface colour, were found for the samples without *stevia* thermally-treated (80-99 ºC) and for the samples with 1.25 and 2.5 % (v/v) of *stevia*.

In addition, the response surface equation obtained in the present study described the experimental data adequately ($R^2 = 98.02$ $p < 0.05$, standard error = 2.602). The equation was as follows:

$$L^* = 83.9959 - 0.0703 \cdot T - 5.0675 \cdot stevia + 0.0013 \cdot T^2 - 0.0442 \cdot T * stevia + 0.0538 * stevia$$

where *T* is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverage.

Table 7. Steviol glycosides and physicochemical properties of untreated beverage based on papaya, mango mixed with orange juice, oats, acaí and *stevia.*

Parameters	Beverage with *stevia* (%)		
	0	**1.25**	**2.5**
	Steviol glycosides (mg/100 mL)		
Rebaudioside A	-	171.5±0.8	286.9±8.4
Rebaudoside C	-	30.1±0.2	63.6±0.1
Stevioside	-	363.8±2.3	637.5±3.0
Rebaudioside F	-	7.5±0.1	14.6±0.1
	Physicochemical properties		
HMF (mg/L)	0.054±0.001	0.096±0.006	0.221±0.029
Turbidity index	2.427±0.005[a]	2.614±0.004[b]	2.802±0.002[c]
Browning index	0.081±0.002[a]	1.145±0.002[b]	2.412±0.003[c]
Lightness (L*)	58.6±0.1[a]	41.2±0.2[b]	33.8±0.1[c]
Redness (a*)	11.7±0.1[a]	8.8±0.1[b]	9.0±0.2[b]
Blueness (b*)	38.6±0.1[a]	31.6±0.2[b]	30.5±0.1[c]

[a-c] Different letters in the same file indicate significant statistical differences in function of the *stevia* percentage HMF: hydroxymethylfurfural.

Table 8. Experimental design matrix in terms of variables and the average values of the response for the experiment on the effects of combined thermal treatments-*stevia* processing on the physicochemical parameters.

Run	T^a (ºC)	Time	*% stevia*	TI	BI	L*	a*	b*	HMF (mg/L)
1	99.0	8.0	1.25	2.670±0.006	1.155±0.003	46.2±0.1	8.3±0.1	32.7±0.1	0.763±0.029
2	80.0	15.0	1.25	2.703±0.004	1.233±0.004	45.1±0.1	8.2±0.2	31.7±0.2	0.804±0.088
3	80.0	0.5	1.25	2.619±0.002	1.170±0.003	43.2±0.1	8.3±0.1	31.1±0.1	0.221±0.029
4*	80.0	8.0	1.25	2.659±0.003	1.158±0.003	43.1±0.1	8.2±0.1	31.3±0.2	0.783±0.059
5	99.0	0.5	2.5	2.573±0.004	2.353±0.004	38.5±0.1	8.5±0.2	32.1±0.1	0.388±0.029
6	60.0	0.5	0.0	2.477±0.001	0.090±0.002	58.6±0.1	11.7±0.2	38.3±0.2	0.096±0.016
7	99.0	0.5	0.0	2.470±0.008	0.123±0.002	59.8±0.1	10.6±0.1	37.9±0.1	0.388±0.029
8	60.0	8.0	1.25	2.647±0.004	1.163±0.004	42.6±0.2	8.6±0.1	31.4±0.1	0.179±0.029
9	60.0	15.0	0.0	2.457±0.009	0.075±0.002	58.6±0.1	11.0±0.1	37.7±0.1	0.200±0.059
10	80.0	8.0	2.5	2.709±0.006	2.438±0.004	36.3±0.2	8.1±0.1	30.5±0.2	0.721±0.029
11	60.0	0.5	2.5	2.663±0.004	2.440±0.002	35.7±0.1	8.2±0.2	29.4±0.1	0.304±0.029
12*	80.0	8.0	1.25	2.661±0.003	1.159±0.003	43.1±0.2	8.2±0.1	31.2±0.1	0.783±0.059
13	80.0	8.0	0.0	2.417±0.005	0.094±0.002	59.0±0.1	10.4±0.2	37.2±0.1	0.408±0.059
14	99.0	15.0	2.5	2.656±0.008	2.392±0.002	40.3±0.1	7.9±0.1	33.2±0.1	0.825±0.059
15	99.0	15.0	0.0	2.412±0.006	0.256±0.006	60.1±0.1	10.3±0.2	38.6±0.1	0.804±0.029
16	60.0	15.0	2.5	2.711±0.003	2.454±0.003	36.5±0.2	8.3±0.1	29.5±0.2	0.346±0.029

Central point. T^a: treatment temperature. TI: turbidity index. BI: browning index. Colour parameters: L,a*,b*. HMF: hydroxymethylfurfural.

The increase in CIE L* values were similar to the results found by Genovese et al. (1997) when they studied the effects of thermal pasteurization (65-70 ºC/15-20 s) in cloudy apple juice and Lee & Coates (1999) for thermally-treated (91 ºC/10 s) grapefruit juice. They attributed it to partial precipitation of unstable, suspended particles in the juice.

With regard to b* values, the behavior was different depending on temperature applied. The reduced regression model presented in the equation below allowed for prediction of the effects of independent variables on the b* values:

$$b^* = 60.1495 - 0.6760 \cdot T - 2.6667 \cdot stevia + 0.0045 \cdot T^2 + 0.0283 \cdot stevia^2$$

where T is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverage.

The b* values of the samples treated at 60-80 ºC were similar or even lower to those found in the untreated samples. However, a significant increase in b* values was observed in the samples treated at 99 ºC, independently of the *stevia* percentage used in the formulation of the beverage. These results were in line with those reported by Lee & Coates (2003) after thermal pasteurization of orange juice (90 ºC/30 s), Cortés et al. (2008) for pasteurized orange juice (90 ºC/20 s), Barba et al. (2010) for thermally-treated (90 ºC for 15, 21 s and 98 ºC for 15, 21 s) vegetables and Barba et al. (2012b) after applying thermal treatment (90 ºC for 15, 21 s and 98 ºC for 15, 21 s) in an orange juice-milk beverage.

The redness (a* values) of untreated samples were 11.7±0.1, 8.8±0.1 and 9.0±0.2 when 0, 1.25 and 2.5 % (v/v) of *stevia* was added. Overall, after thermal processing, a* values were lower to those obtained in untreated samples independently of the *stevia* percentage used (see Table 7).

$$a^* = 35.7949 - 0.2992 \cdot T - 1.0494 \cdot stevia + 0.0018 \cdot T^2 + 0.0148 \cdot stevia^2$$

where *T* is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverage.

This can be explained by the isomeric changes on carotenoids. In this line, Lee & Coates (2003) and Gama & de Sylos (2007) reported that the highest thermal losses in orange juice were from 5,6-epoxide carotenoids. Nevertheless, β-carotene, which is mainly responsible for the bright orange colour of orange juice and total carotenoid pigment content loss, was not significant after thermal treatments. The results obtained in this study are in accord with those found by Patras et al. (2009) for thermally processed (70 ºC, 120 s) strawberry and blackberry purées and Barba et al. (2012a) after applying thermal processing (90 ºC for 15, 21 s and 98 ºC for 15, 21 s) in an orange juice-milk beverage.

The total colour difference (ΔE) indicates the magnitude of colour difference between thermally-treated and unprocessed samples (Figure 16). Difference in perceivable colour can be classified analytically as not noticeable (0 - 0.5), slightly noticeable (0.5 - 1.5), noticeable (1.5 - 3.0), well visible (3.0 - 6.0), and great (6.0 - 12.0) (Cserhalmi et al., 2006). Total colour change (ΔE) was significantly ($p < 0.05$) different in thermally-processed sample at (99 ºC, 0.5-15 min) from unprocessed samples when *stevia* used in formulation was 1.25 % and 2.5 % (v/v). In Figure 16 we can see the effects of temperature and time on total ΔE of the beverage with different percentage of *stevia*.

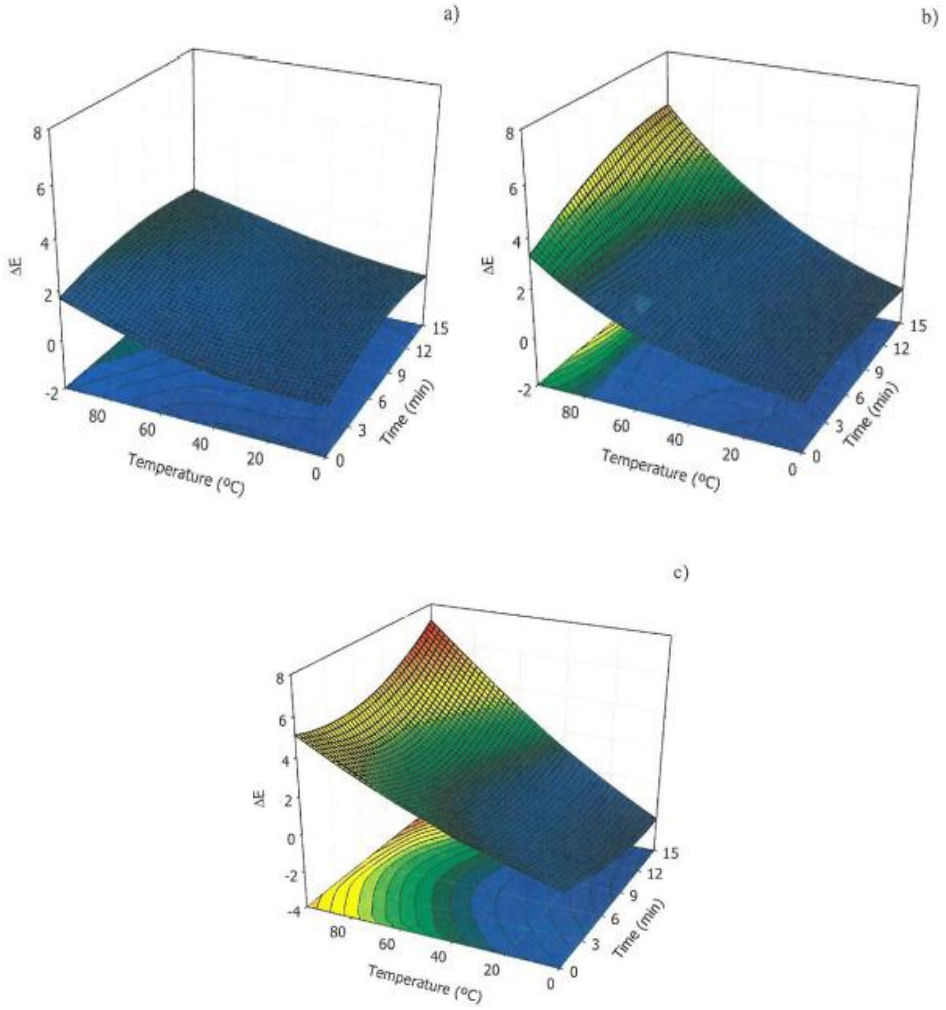
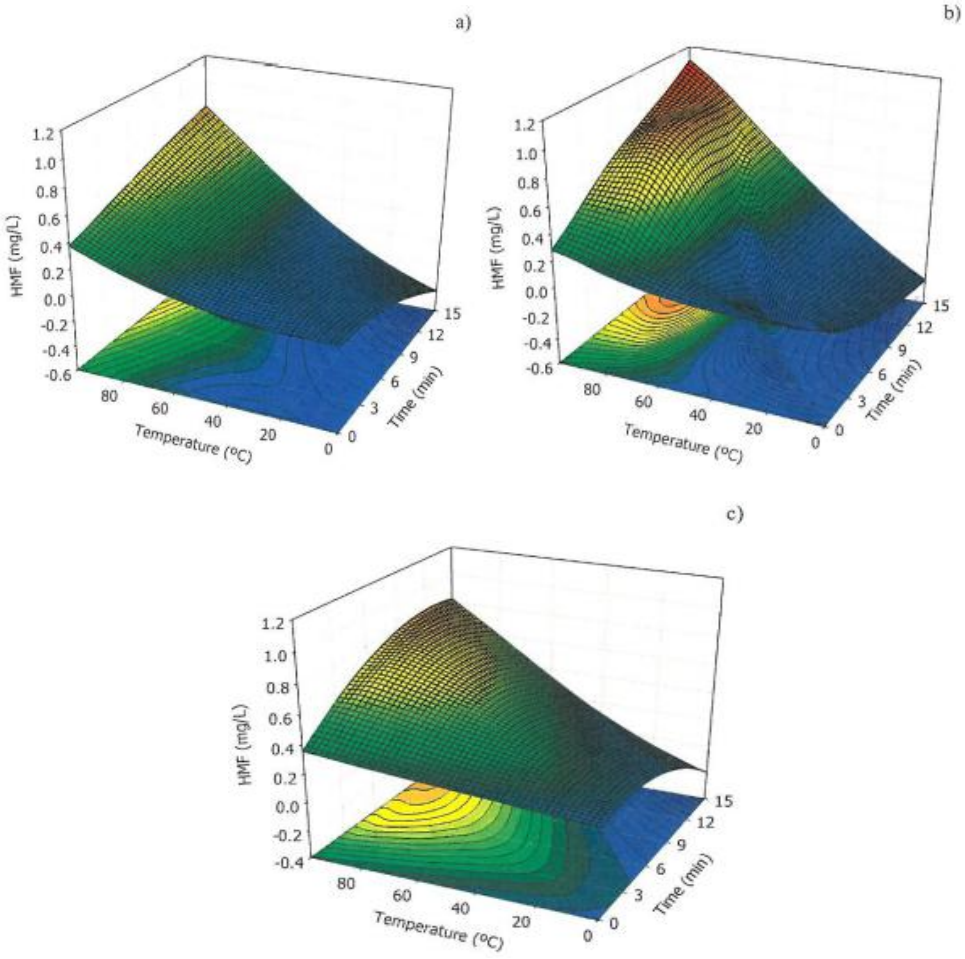


Figure 16. Effects of temperature and time on total colour differences (ΔE) of the beverage based on papaya, mango mixed with orange juice, oats, acaí and different *stevia* concentration*:* a) 0 %, b) 1.25 %, c) 2.5 % (v/v).

With regard to hydroxymethylfurfural (HMF), the results obtained for the three-way ANOVA showed that treatment temperature applied and *stevia* %

had a significant influence ($p < 0.05$) on the HMF content of the beverage analyzed in the present study, obtaining a higher increase in HMF after applying higher temperatures (Table 8, Figure 17).



HMF: hydroxymethylfurfural

Figure 17. Response surface plots for hydroxymethylfurfural content (mg/L) of the beverage as affected by thermal processing at different temperatures (60-99 ºC), times (0.5-15 min) and *stevia* concentration: a) 0 %, b) 1.25 %, c) 2.5 % (v/v).

The regression equation describes the following model:

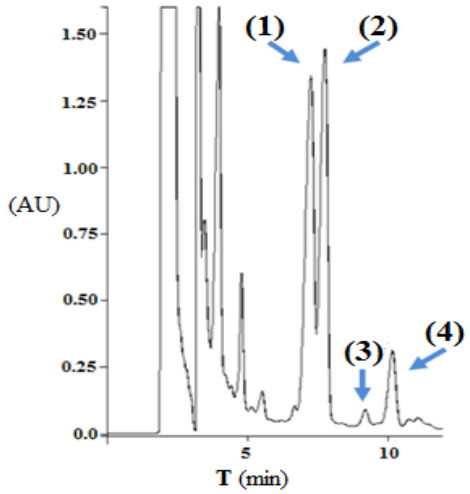
$$HMF\ (mg/L) = 2.42294 + 0.0083 \cdot T - 0.0872 \cdot stevia + 0.0018 \cdot T^{*}stevia$$

where HMF is the hydroxymethylfurfural content (mg/L), *T* is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverage.

But in all cases, the values did not reach the tolerable limit after processing (5 mg/L of HMF) (AIJN 1996). These results were consistent with the findings in the literature (Cortés et al., 2008; Zulueta et al., 2012a).

Impact of thermal processing and stevia concentration on steviol glycosides

Four different steviol glycosides; rebaudioside A (Reb A), rebaudioside C (Reb C), rebaudioside F (Reb F), and stevioside (Ste) in untreated sample were detected (Table 7, Figure 18) with the high-performance liquid chromatography (HPLC).



1: Rebaudioside A. 2: Stevioside. 3: Rebaudioside F. 4: Rebaudioside C.

Figure 18. Chromatography HPLC analysis of steviol glycosides in a beverage based on papaya, mango mixed with orange juice, oats, acaí and *stevia*.

Reb A content in the untreated beverage sweetened with 1.25 and 2.5 % (v/v) of *stevia* was 171.5±0.8, and 286.9±8.4 mg/100 mL, respectively. Three-way ANOVA showed a significant influence ($p < 0.05$) of temperature processing on Reb A contents. The analysis of variance showed that the regression model was accurate enough ($R^2 = 99.71$, $p < 0.05$, standard error = 6.519). The relationship between the independent variables and Reb A can be described by the following equation:

$$\text{Reb A} = -91.4139 - 1.1313 \cdot T + 30.8882 \cdot \textit{stevia} + 0.905003 \cdot T^{*}\textit{stevia} - 0.447197 \cdot \textit{stevia}$$

where Reb A is the concentration of rebaudioside A (mg/100 mL), *T* is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* used in formulation of the beverage.

The analysis of variance indicated a decrease (up to 14 %) in Reb A values after thermal processing when temperature was higher than 60 ºC, independently of the *stevia* percentage used (Figure 19). These findings were in accordance with several other publications that reported a significant effect of thermal processing on steviol glycosides degradation.
According to previous studies found in published literature, Chang & Cook (1983) obtained a degradation (- 32 %) of the original Reb A content after heating this steviol glycoside in an aqueous solution (6.5 mg/mL) at 100 ºC for 8 hours. These authors linked these losses to degradation products such as Reb B and glucose, indicating that the C-19 ester linkage appeared to be the most heat-labile bond in Reb A. Moreover, in a study conducted in a model solution by the Panel on Food Additives and Nutrient Sources added to Food (ANS) (EFSA 2010), they found that the stability of the Reb A was temperature- and time-dependent. The Panel also noted in this study that the extent of degradation of Reb A ranged from a few percent up to 63 % under different storage (pH and temperature) and food production conditions.

In the present study, Reb C content of the untreated beverages with 1.25 % (v/v) and 2.5 % (v/v) *stevia* was 30.1±0.2 and 63.6±0.1 mg/100 mL, respectively (Table 7). The ANOVA results indicated a decrease in Reb C values when temperature and time were increased, independently of the *stevia* percentage used. Experimental data were fitted by a second-order polynomial model:

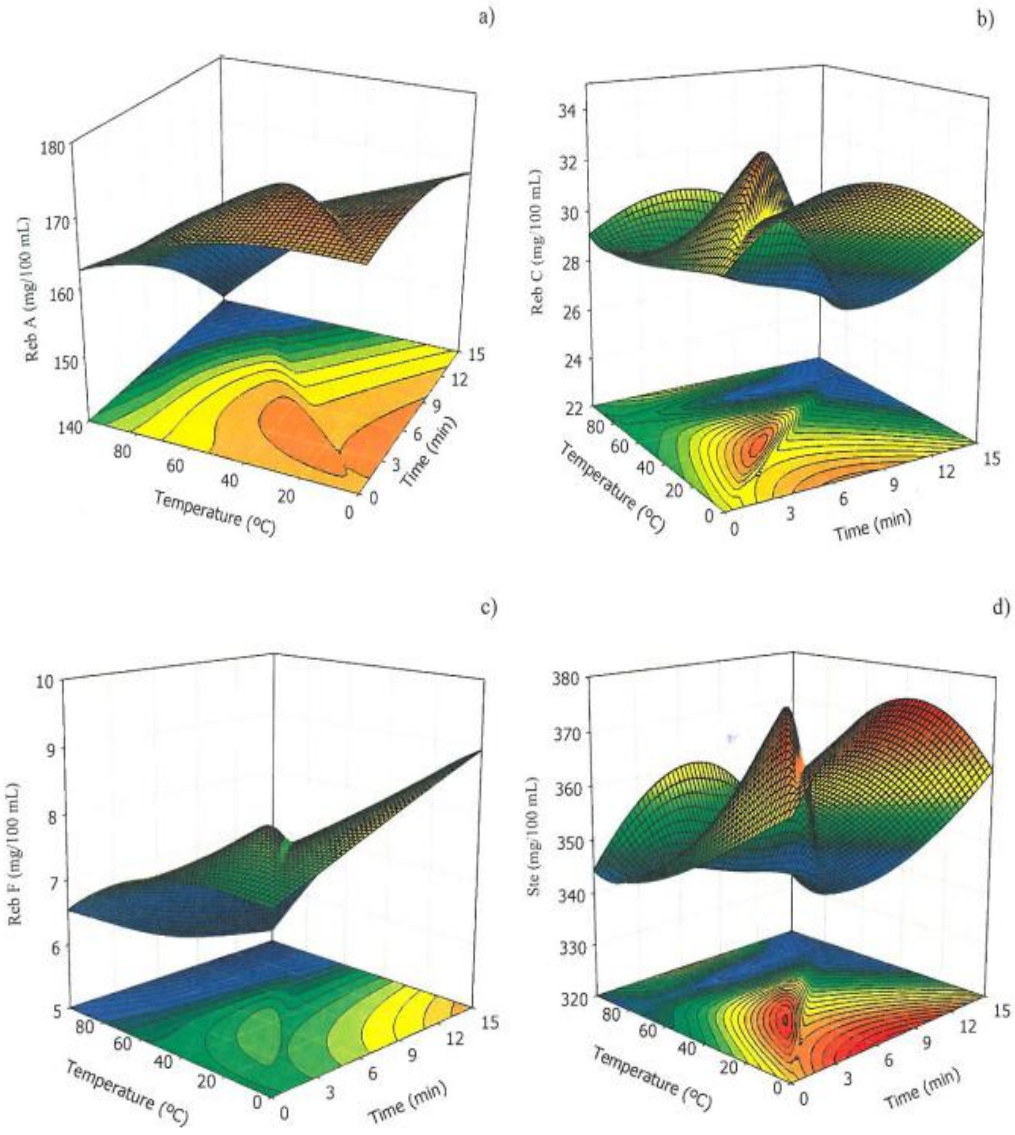
$$\text{Reb C} = 750.2840 - 0.6281 \cdot T - 24.8720 \cdot \textit{stevia} + 0.5025 \cdot T^{*}\textit{stevia}$$

where Reb C is the concentration of rebaudioside C (mg/100 mL), *T* is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverage.

Moreover, Reb F concentration in the beverage with 1.25 % (v/v) *stevia* was 7.5±0.1 mg/100 mL. As can be expected, Reb F concentration was higher when the untreated beverage was sweetened with 2.5 % (v/v) *stevia* (14.6±0.1 mg/100 mL). In addition, the regression analysis test showed that a second-order model fits well the Reb F content after applying thermal processing. The determination coefficient was $R^2 = 98.49$, $p < 0.05$, standard error = 0.718).
Experimental data were fitted by a second-order polynomial model:

$$\text{Reb F} = 19.3731 - 0.08371 \cdot T - 0.5438 \cdot \textit{stevia} + 0.0670 \cdot T^{*}\textit{stevia}$$

where Reb F is the concentration of rebaudioside F (mg/100 mL), *T* is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* in the formulation of the beverage.



a) Reb A: Rebaudioside A. b) Reb C: Rebaudioside C. c) Reb F: Rebaudioside F. d) Ste: Stevioside.

Figure 19. Response surface plots for steviol glycosides content of the beverage (*stevia* 1.25 % (v/v)) as affected by thermal processing at different temperatures (60-99 ºC) and times (0.5-15 min).

On the other hand, stevioside content in the untreated beverage sweetened with 1.25 and 2.5 % (v/v) was 363.8±2.3, and 637.5±3.0 mg/100 mL, respectively. The three-way ANOVA showed that temperature and *stevia* % had a significant effect ($p < 0.05$) on stevioside content after applying thermal treatment.

The determination coefficient for stevioside was ($R^2 = 95.48$, $p < 0.05$, standard error = 58.024). The existence or non-existence of interactions between the two factors evaluates the contribution of each factor to the dependent variable.

The regression equation describes the following model:

$$Ste = - 1012.4000 - 2.1194 \cdot T + 103.2200 \cdot stevia + 1.6955 \cdot T * stevia - 1.12249 \cdot stevia^2$$

where Ste is the concentration of stevioside (mg/100 mL), *T* is treatment temperature (ºC) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverage.

In the present study, stevioside was stable at 60 ºC. However some losses occurred on heating to a temperature of 80 ºC (up to 9 %) and 90 ºC (up to 10 %), independently of the *stevia* percentage used in the formulation of the beverages (Figure 19). Other published data (Kroyer 1999; Kroyer 2010) on the stability of stevioside in an aqueous solution (0.5 g/L) demonstrated that stevioside was stable within a pH range of 2-10 over 2 hours at 60 ºC and losses that occurred on heating to a temperature of 80 ºC were up to 5 %. Similarly, losses up to 5 % of stevioside were observed after 4 hours incubation of tea or coffee at 80 ºC. Chang & Cook (1983) also reported data on the stability of stevioside. Prolonged heating at 100 ºC of stevioside in an aqueous solution (6.5 mg/mL) resulted in a decrease in the stevioside concentration. These authors identified steviolbioside and glucose as

degradation products and the C-19 ester linkage appeared to be the most heat-labile bond in stevioside.

Bioactive compounds after thermal processing

Table 9 shows the content of bioactive compounds and antioxidant capacity after the treatments.

Table 9. Content of bioactive compounds and antioxidant capacity of the beverage affected by thermal processing at different temperatures (60-99 ºC), times (0.5-15 min) and *stevia* concentration (0-2.5 % (v/v)).

Run	T^a (ºC)	t (min)	% *Stevia*	AA (mg/100 mL)	TC (µg/100 mL)	TA (mg/L)	TEAC (mM TE)	ORAC (mM TE)
1	60.0	0.5	0.0	24.9±0.5	499.1±35.3	21.2±1.3	6.4±0.7	4.6±0.1
2	60.0	0.5	2.5	24.3±0.1	424.2±35.3	26.2±0.8	33.2±2.0	36.0±0.1
3	60.0	8.0	1.25	24.1±0.1	411.7±17.7	24.5±1.1	17.3±1.9	22.6±0.1
4	60.0	15.0	0.0	23.5±0.2	461.6±17.7	18.3±1.1	7.1±0.2	4.4±0.1
5	60.0	15.0	2.5	23.5±0.1	436.7±17.6	30.7±1.1	32.1±5.1	36.1±0.1
6	80.0	0.5	1.25	23.6±0.1	411.7±17.7	26.1±1.6	15.2±0.3	21.6±0.2
7	80.0	8.0	0.0	23.1±0.1	499.0±70.6	23.5±0.8	6.5±0.3	7.2±0.1
8*	80.0	8.0	1.25	23.2±0.1	486.6±17.6	24.5±0.6	19.7±1.1	24.1±0.1
9	80.0	8.0	2.5	23.3±0.1	499.1±35.3	29.7±0.8	30.6±6.4	37.3±0.1
10	80.0	15.0	1.25	22.8±0.1	436.7±53.0	23.1±0.3	18.0±1.8	25.0±0.2
11	99.0	0.5	0.0	22.4±0.2	511.5±52.9	26.3±1.3	7.5±0.6	7.5±0.1
12	99.0	0.5	2.5	22.4±0.2	361.8±17.7	31.7±0.3	26.2±1.5	23.0±0.1
13	99.0	8.0	1.25	21.9±0.1	436.7±17.6	29.7±0.8	16.4±0.7	26.6±0.2
14	99.0	15.0	0.0	21.2±0.2	436.7±17.6	19.2±0.8	6.7±1.2	6.9±0.3
15	99.0	15.0	2.5	21.5±0.3	374.3±35.3	15.7±0.3	28.8±1.2	34.2±0.2
16*	80.0	8.0	1.25	23.3±0.3	480.6±20.5	24.8±0.7	20.0±0.9	24.3±0.3

*Central point. T^a: treatment temperature. *t:* treatment time. AA: ascorbic acid. TC: total carotenoids. TA: total anthocyanins. TEAC: trolox equivalent antioxidant capacity. ORAC: oxygen radical antioxidant capacity.

The modifications in ascorbic acid after applying thermal treatments in the beverage were analysed. Ascorbic acid content in the untreated beverage without *stevia* added was 24.8±0.2 mg/100 mL.

Non-significant modifications were observed when the untreated samples were sweetened with 1.25 and 2.5 % (v/v) of *stevia*. In addition, the three-way ANOVA showed that both temperature and time had a significant effect ($p < 0.05$) on ascorbic acid content after applying thermal treatment (Table 9, Figure 20). The existence or non-existence of interactions between the two factors evaluates the contribution of each factor to the dependent variable. The reduced regression model presented in the equation below allowed for prediction of the effects of independent variables on the ascorbic acid values:

$$\text{AA (mg/100 mL)} = 24.7555 + 0.0306 \cdot T - 0.0712 \cdot t - 0.0005 \cdot T^2$$

where T is the treatment temperature (ºC) and t is the treatment time (minutes).

These findings were in close agreement with several other publications that reported a significant ($p < 0.05$) decrease in ascorbic acid content of fruit and vegetables juices when treatment temperature and time were increased.

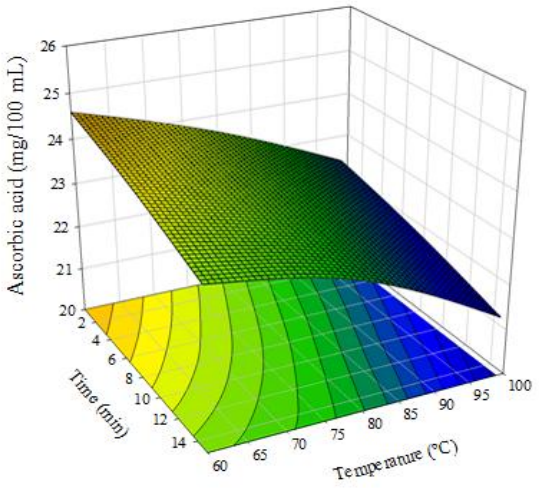


Figure 20. Ascorbic acid degradation at different treatment temperatures (60-99 ºC) and times (0.5-15 min) in a beverage based on papaya, mango mixed with orange juice, oats, acaí and *stevia.*

In this line Barba et al. (2010, 2012b) observed losses of ascorbic acid content ranging from 15 to 18 % and 6 to 12 % in thermally-treated (90 and 98 ºC for 15 and 21 s) orange juice-milk and in a vegetable beverage respectively, as treatment temperature increased. Moreover, Torregrosa et al. (2006) also found losses of 17 % in thermally-treated (98 ºC, 21 s) orange-carrot juice. In addition, Dhuique-Mayer et al. (2007) studied the thermal degradation of ascorbic acid at 50-100 ºC in citrus juices. They also established a first-order model for explaining ascorbic acid degradation during thermal pasteurization at 75-100 ºC until 120 minutes of treatment. Taking into account this model, losses of ascorbic acid content at 75-100 ºC during 0.5 minutes can be considered negligible. However, losses ranged from 1 to 6 % after thermal treatment (75-100 ºC for 5-15 minutes).

Total carotenoid content in the untreated samples without *stevia* was 436.7±17.6. Three-way ANOVA did not show a significant influence of *stevia* percentage in carotenoid content of the untreated samples. In addition, the existence or non-existence of interactions between the two factors evaluates the contribution of each factor to the dependent variable. The regression equation describes the following model:

$$\text{Total carotenoids (µg/100 mL)} = 404.7310 + 0.6427 \cdot T - 13.4029 \cdot t - 26.11 \cdot stevia - 0.5141 \cdot T*stevia - 0.8935 \cdot t^2 + 20.6237 \cdot stevia^2$$

where T is the treatment temperature (ºC), t is the treatment time (minutes) and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverages.

Total anthocyanins content of the untreated samples without *stevia* was 22.0±1.3 mg/L. Acaí was the main contributor to anthocyanins content of the samples, because it can be considered a good source of anthocyanins compared to other known red fruits such as strawberry and raspberry (De Rosso et al., 2008). Moreover, a significant increase in total

anthocyanins content was found when *stevia* at 1.25 % (27.8±1.3 mg/L) and 2.5 % (29.7±0.3 mg/L) was added to the fruit complex mixture. In the line of the results reported in the present research, Muanda et al. (2011) found values of 0.35 mg total anthocyanins/g dry matter when they studied the chemical composition of water extracts from *Stevia rebaudiana* Bertoni. The behaviour of total anthocyanins after applying thermal processing can be explained by the following expression:

$$\text{Total anthocyanins (mg/100 mL)} = -14240.5 + 91.9 \cdot T - 557.7 \cdot t + 2432.2 \cdot stevia - 0.1 \cdot T^2 + 1.3 \cdot T*t - 1.1 \cdot T*stevia + 24.4 \cdot t*stevia - 286.0 \cdot stevia^2$$

where T is the treatment temperature (ºC), t is the treatment time (minutes), and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverages.

Total antioxidant capacity after thermal processing

Total antioxidant capacity measured as TEAC values in the beverage without *stevia* was 6.4±0.3 mM TE. TEAC values were higher when the fruit complex mixture blended with oats was sweetened with *stevia* at 1.25 % (20.3±2.2 mM TE) and 2.5 % (30.4±0.7 mM TE). With regard to antioxidant capacity measured with ORAC assay, the sample without *stevia* showed an antioxidant capacity value of 5.1±0.1 mM TE. The antioxidant capacity values measured with ORAC assay were significantly higher ($p < 0.05$) for the samples with *stevia* at 1.25 % (23.5±0.1 mM TE) and 2.5 % (36.1±0.1 mM TE) than those obtained for the sample without *stevia*. It should be noted that antioxidant capacity measured with TEAC and ORAC methods increased significantly according to the percentage of the *stevia*, in a dose-dependent manner, reaching a maximum at 2.5 % *stevia*, although the results revealed significant differences between samples from

different origin and were not comparable as the based chemical reactions, and the parameters being determined varied considerably (Prior, 2005).
The results obtained for the three-way ANOVA showed that temperature, time and *stevia* percentage had a significant influence ($p < 0.05$) on the total antioxidant capacity of the beverages measured as ORAC values. However, when TEAC assay was used, only *stevia* percentage had a significant effect. Experimental data were fitted by a second-order polynomial model:

$$TEAC\ (mM\ TE) = 6.5347 + 9.3760 \cdot stevia$$

$$ORAC\ (mM\ TE) = 3.72516 + 0.107837 \cdot T - 0214214 \cdot t + 18.4918 \cdot stevia - 0.000930571 \cdot T^2 + 0.00992742 \cdot T*t - 0.0536859 \cdot T*stevia - 0.0413384 \cdot t^2 + 0.203373 \cdot t*stevia - 2.04702 \cdot stevia^2$$

where T is the treatemet temperature (ºC), t is the treatment time (minutes), and *stevia* is the percentage of *Stevia rebaudiana* used in the formulation of the beverages.

The determination coefficient were $r = 0.970$, $p < 0.05$, standard error = 2.220) and 94.405, ($p < 0.05$, standard error = 2.397) for TEAC and ORAC methods, respectively.
Upon comparing the antioxidant capacity results obtained with the TEAC and the ORAC method, a strong correlation was found ($r = 0.948$, $p < 0.05$). Barba et al. (2010) and Zulueta et al. (2009) also found good correlations for ORAC and TEAC methods in vegetables beverage ($r = 0.823$, $p < 0.05$) and orange juice ($r = 0.955$, $p < 0.05$), respectively.

Process optimization

Optimum conditions of thermal treatment for enhancing each of the antioxidant compounds were slightly different. A number of combinations of variables produced maximum ascorbic acid, antioxidant capacity and

total carotenoids while still achieving good colour. As a result, emphasis was placed on optimizing the bioactive compounds (ascorbic acid, total anthocyanins content, total carotenoids) and antioxidant capacity. Optimum thermal treatment conditions for maximizing bioactive constituents are depicted in Table 10.

The multi-response analysis of response surface design using the desirability approach was used to optimize treatment temperature and time. The desirability function is an approach for solving the problem of optimizing several responses and is applied when various responses have to be considered at the same time. This function is first constructed for each individual response, and then it is possible to obtain the overall desirability. Multiple response optimization indicated that bioactive compounds in the sample could be maximized by treating a sample containing 2.5 % *stevia* for 6.2 min at 80 °C. This is a promising finding as the temperature is lower than the maximum used for thermal pasteurization. The response values predicted under these conditions by the multiple response optimization and after applying a validation experiment for confirming the values obtained are detailed in Table 10.

Table 10. Response values of the antioxidant compounds predicted under the optimized conditions and the validation experiment.

PARAMETERS	OPTIMUM	EXPERIMENTAL
AA (mg/100 mL)	23.27	23.2±0.2
TC (μg/100 mL)	450.98	455.6±14.8
TA (mg/L)	28.28	28.50±0.37
ORAC (mM TE)	34.28	32.34±0.25
TEAC (mM TE)	29.93	29.53±0.57

AA: ascorbic acid. TC: total carotenoids. TA: total anthocyanins. TEAC: trolox equivalent antioxidant capacity. ORAC: oxygen radical antioxidant capacity.

Under these conditions, the microbiological assays performed on the treated samples show that the microbial load after the thermal treatment is always < 1 log CFU/mL. The mean contents were compared by a *t* test, and the results showed that there were no significant differences ($p>0.05$) between antioxidant compounds values after applying the optimized method and the experimental values.

6.1.3 Conclusions

RSM using a central composite design was demonstrated to be a useful tool for optimizing thermal treatment conditions to improve bioactive compounds phytochemical constituents in fruit complex mixtures blended with *stevia*. From the response surface plots, treatment temperature was found to have the most significant effect on ascorbic acid content of the beverages. The high coefficients of determination of the variables at a 95 % confidence level for the six mathematical models indicated that second-order polynomial models may be used to predict critical phytochemical parameters of the beverages. Nevertheless, substantial degradation of steviol glycosides might take place at high temperatures. These findings may be applied in the development of new functional foods based on fruit complex mixtures and sweetened with non-caloric sweeteners from *Stevia rebaudiana* Bertoni.

6.2. Bioaccessibility of bioactive compounds and antioxidant capacity after non-thermal processing of beverage based on papaya, mango and stevia

During the last decades, a growing demand for "healthy" food and beverages has been observed worldwide and the diffusion of functional foods throughout the market has given support to the role of foods rich in phytochemicals such as fruits and vegetables in disease prevention. Beverages based on exotic fruits have been designed in response to consumer´s demand for highly nutritious foods as well as innovative tastes and flavours. Between them, mango and papaya juices can be considered dietary sources of bioactive compounds, such as vitamin C, carotenoids and phenolic compounds, which have shown to be good contributors to total antioxidant capacity of foods. Furthermore, an upsurge of interest in the therapeutic potential of plants and their addition to food products has been observed, with an increased interest in the use of the natural sweetener *Stevia rebaudiana* Bertoni (Korir et al., 2014). *Stevia* leaves have been shown to exert antioxidant properties, which stem from their high levels of flavonoids and phenolic compounds (Ramya et al., 2014).
Although thermal processes are the most common methods for preserving food products, they can have a negative effect on nutritional value of foods, so new safe and effective techniques have recently been investigated to evaluate their potential for safe processing and food preservation. In this line, electrotechnologies such as high voltage electrical discharges (HVED), pulsed electric fields (PEF) and ultrasound (USN) have been effectively applied in liquid food preservation (Kentish & Feng, 2014; Timmermans et al., 2014). When these technologies are combined with plant origin preservatives such as infusion of *stevia* leaves, lower power consumption and less impact on the food properties is observed (Sango et al., 2014).

However, before concluding on any potential health effect, it is important to analyse whether the digestion process affects bioactive compounds and their stability, as this, in turn, will affect their bioavailability and their possible beneficial effects. Nutritional efficacy of food products may be ensured by the determination of bioaccessibility, defined as the quantity or fraction which is released from the food matrix in the gastrointestinal tract and becomes available for absorption (Heaney, 2001). In this context, *in vitro* digestion procedures are generally used in the assessment of food compounds bioaccessibility and constitute an easy and fast approach which enable the prediction of *in vivo* trials. A study carried out by Aschoff et al. (2015) assessed bioaccessibility of carotenoids, flavonoids and vitamin C in orange juices and observed that thermal pasteurization increased carotenoid and vitamin C bioaccessibility, while bioaccessibility of flavonoids remained the same as in the fresh orange juice. By contrast, Stinco et al. (2012) obtained a reduction of carotenoid bioaccessibility when orange juices were pasteurized. However, regarding non-thermal processing technologies, there is a lack of available information concerning their impact on bioactive compounds bioaccessibility and their correlation with the corresponding antioxidant capacity under an *in vitro* simulated digestion.

6.2.1. Materials and methods

6.2.1.1. Samples

Composition and preparation of the samples is described in the paragraph 3.1.2 on page 58.

6.2.1.2. Nutritional parameters

- Ascorbic acid *(See section 4.7 - page 63)*
- Total carotenoids *(See section 4.8 - page 64)*

- Total phenolic compounds *(See section 4.9 - page 65)*
- Total anthocyanins *(See section 4.10 - page 66)*

6.2.1.3. Total antioxidant capacity

- TEAC, ORAC, DPPH *(See section 4.11 - page 66, 68, 70)*

6.2.1.4. Digestion method I

A three-stage model digestion based on the procedure described by Rodríguez-Roque et al. (2013) with some modification was applied *(See section 5.1 - page 75).*

6.2.1.5. Treatments

Electric treatments (PEF, HVED)

Electrical treatments in PEF and HVED modes were done using a high voltage pulsed power 40 kV–10 kA generator (Tomsk Polytechnic University, Tomsk, Russia). The initial temperature before PEF or HVED treatment was 20 °C and the temperature elevation after electrical treatment never exceeded 35 °C. Beverage temperature was controlled by a K-type thermocouple (±0.1 K), connected to the data logger thermometer Center 305/306 (JDC Electronic SA, Yverdon-les-Bains, Switzerland). PEF treatments were carried out in a cylindrical batch treatment chamber between two plate electrodes of 9,5 cm^2. The distance between electrodes was fixed at 2 cm, with a corresponding electric field strength of 25 kV/cm. Total treatment duration ($t_t = n \times t_i$) was changed by increasing the number of pulses (n) from 50 to 400. For HVED treatments, the 1 L treatment chamber (inner diameter = 10 cm, wall thickness = 2.5 cm) was equipped with needle-plate geometry electrodes. The diameters of stainless steel needle and the grounded disk electrodes were of 10 and 35 mm, respectively, and the distance between them was of 5 mm. Total

treatment duration was changed by increasing the discharge number n from 50 to 400, with an initial voltage peak amplitude (U) of 40 kV and a pulse repetition rate of 0.5 Hz. Exponential decay of voltage $U_{\propto}$ exp (-t/t_i) with effective decay time $t_i \approx 10.0 \pm 0.1$ μs and damped oscillations with total duration t_i of ≈ 10 μs were observed in PEF and HVED treatment modes, respectively. Total specific energy input (W, kJ/kg) was chosen as a parameter to describe the treatment intensity. Two energy inputs were applied, corresponding to samples PEF_1 and $HVED_1$ (32 kJ/kg) and PEF_2 and $HVED_2$ (256 kJ/kg). The specific energy input (*W*) of PEF and HVED treatments was calculated as shown in Eq. (1).

$$W = \frac{\sum_{i=1}^{n} W_{PEF}}{m} \quad (1)$$

where W_{PEF} is the pulse energy (kJ/pulse), *n* is the number of pulses and *m* is the product mass (kg). W_{PEF} was determined from Eq. (2).

$$w_{PEF} = \int_0^t UxIxdt \quad (2)$$

where *U* is the voltage (V) and *I* is the current strength (A).

Ultrasound (USN)

Ultrasound (USN) treatments were carried out in an ultrasonic processor UP 400 S (Hielscher GmbH, Germany) which operates at 400 W and 24 kHz frequency. Amplitude was fixed at 100 % and cycle was fixed at 1. The titanium sonotrode H14 with a diameter of 14 mm and a length of 100 mm was used to transmit ultrasound inside the sample. The sample was submerged in a cooling bath to avoid the heating induced by USN irradiation. The energy input of USN treatment was calculated as follows (Eq. 3):

$$W_{USN} = \frac{POWER \; x \; t_{USN}}{m} \quad (3)$$

where t_{US} is the total treatment duration (s), *m* is the product mass (kg) and the generator power (400 J/s). The USN duration (t_{USN}) was of 20 and 160 s in order to obtain equivalent energy inputs to the other treatments applied, corresponding to samples USN_1 (32 kJ/kg) and USN_2 (256 kJ/kg).

6.2.1.6. Statistical analysis

An analysis of variance (ANOVA) was applied to the results obtained in order to verify whether there were significant differences in the parameters studied in relation to sample analysed, and to ascertain possible interactions between factors (differences at $p < 0.05$ were considered significant). Where there were differences, an LSD test was applied to indicate the samples in which differences were observed. A multiple regression analysis was performed to study the influence of bioactive compounds to antioxidant capacity (the results are shown in the significant cases, $p < 0.05$). Finally, a study was conducted with the aim of determining whether there were correlations between a pair of variables (Pearson´s test). All statistical analyses were performed using Statgraphics® Centurion XVI (Statpoint Technologies Inc., USA). The results obtained by applying each test are shown in Annex *(See section 9 - page viii)*

6.2.2. Results and discussion

Changes in bioactive compounds (ascorbic acid, total carotenoids, total phenolic compounds and total anthocyanins) and antioxidant capacity due to High Voltage Electrical Discharges (HVED), Pulsed Electric Fields (PEF) and Ultrasound (USN) treatments of a beverage based on papaya, mango and *stevia* were studied. Table 11 shows the contents of the various bioactive compounds and total antioxidant capacity obtained after applying the non-thermal treatments (HVED, PEF and USN) at two equivalent energy inputs (32 kJ/kg and 256 kJ/kg) to the untreated

beverage. Ascorbic acid content was significantly lower ($p < 0.05$) in the HVED, PEF and USN treated beverage in comparison with the untreated (17-91 %). Total carotenoids increased significantly after PEF_1 treatment (18 %), while $HVED_2$ and USN technology caused a significant decrease (28-45 %) ($p < 0.05$) in total carotenoid content, compared to the untreated beverage.

Total phenolic content after $HVED_2$ treatment was significantly higher than that of untreated, while non-significant differences were obtained after the beverage was treated by $HVED_1$, PEF and USN technology when compared with untreated. The analysis of total anthocyanins showed no significant differences between untreated and PEF, HVED and USN treated beverage.

The obtained antioxidant capacity data measured with TEAC and DPPH method evidenced a noticeable improvement ($p < 0.05$) after $HVED_2$ processing. When ORAC method was used to measure antioxidant capacity, PEF and USN treatments resulted in an increase (16-21.4 %) with regard to the untreated beverage. These results are in accordance to those found by other previous studies which have reported the feasibility of PEF and USN to preserve bioactive compounds and antioxidant capacity when they are used for liquid food preservation (Galaverna & Dall'Asta, 2014; Mena et al., 2014; Sango et al., 2014).

Table 11. Content of bioactive compounds and antioxidant capacity of the beverage based on papaya, mango and *stevia* before and after different treatments applied.

	AA (mg/100mL)	**TC** (μg/100mL)	**TPC** (mg/L)	**TA** (mg/L)	**TEAC** (mM TE)	**ORAC** (mM TE)	**DPPH** (mM TE)
Untreated	18.5±0.4^{a}	676.1±3.5^{a}	2685.6±18.4^{ab}	11.8±1.4^{ab}	23.9±0.9^{a}	30.8±0.9^{ab}	24.3±0.5^{ab}
$HVED_1$	4.3±0.6^{b}	591.3±10.9^{a}	2646.6±36.7^{a}	12.0±0.3^{ab}	23.7±1.7^{a}	29.1±1.3^{a}	24.4±0.7^{ab}
$HVED_2$	3.2±0.3^{c}	371.8±67.0^{b}	2805.6±4.6^{c}	13.0±0.5^{a}	26.4±0.6^{b}	31.7±1.3^{b}	26.1±0.1^{c}
PEF_1	15.4±0.2^{d}	800.9±3.5^{c}	2685.6±55.1^{abc}	11.9±0.4^{ab}	23.6±0.2^{a}	37.4±1.9^{c}	24.4±0.4^{ab}
PEF_2	14.8±0.2^{d}	633.7±49.4^{a}	2744.0±73.4^{bc}	12.8±0.3^{a}	25.6±0.3^{ab}	36.8±1.5^{c}	25.5±0.7^{ac}
USN_1	16.9±0.2^{e}	429.1±28.2^{bd}	2701.8±22.9^{ab}	11.2±0.4^{ab}	24.9±0.3^{ab}	35.8±0.1^{c}	23.3±0.7^{b}
USN_2	15.5±0.4^{d}	486.5±17.6^{d}	2721.3±41.3^{abc}	11.9±0.1^{ab}	25.5±0.7^{ab}	37.1±0.1^{c}	24.7±0.4^{abc}

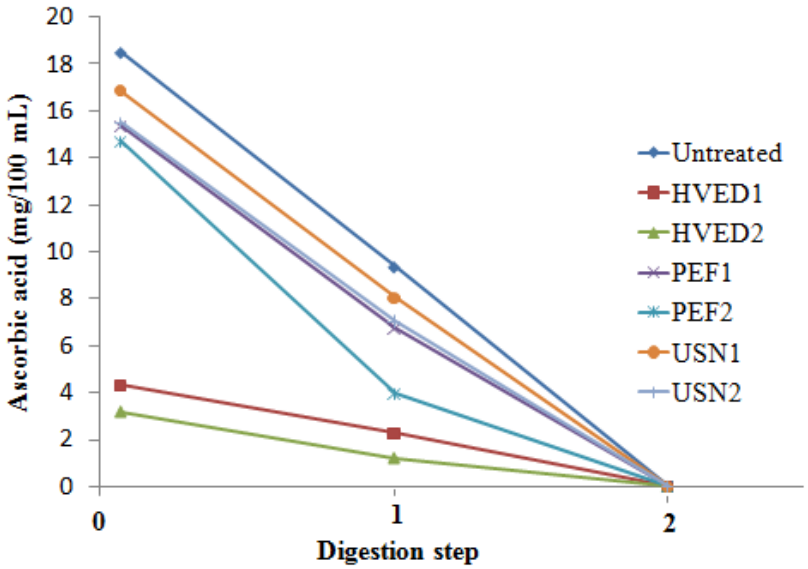
$^{a–d}$ Different letters in the same column indicate a significant difference in function of the samples analysed ($p < 0.05$).

AA: ascorbic acid. TC: total carotenoids. TPC: total phenolic compounds. TA: total anthocyanins. TEAC: trolox equivalent antioxidant capacity. ORAC: oxygen radical antioxidant capacity. DPPH: 2,2-diphenyl-1-picrylhydrazyl. $HVED_1$: high voltage electrical discharges, 32 kJ/kg. $HVED_2$: high voltage electrical discharges, 256 kJ/kg. PEF_1: pulsed electric fields, 32 kJ/kg. PEF_2: pulsed electric fields, 256 kJ/kg. USN_1: ultrasound, 32 kJ/kg. USN_2: ultrasound, 256 kJ/kg.

Digestibility of the beverage based on papaya, mango and stevia treated by HVED, PEF and USN

An *in vitro* gastrointestinal digestion mimicking the physiological processes (transit time, pH and enzymatic conditions) occurring in the gastrointestinal tract of the human digestive system was carried out and bioactive compounds and total antioxidant capacity were measured after each digestion step.

Ascorbic acid content in the beverage treated by HVED, PEF and USN processing during the simulated gastrointestinal digestion is shown in Figure 21.



$HVED_1$: high voltage electrical discharges at 32 kJ/kg. $HVED_2$: high voltage electrical discharges at 256 kJ/kg. PEF_1: pulsed electric fields at 32 kJ/kg. PEF_2: pulsed electric fields at 256 kJ/kg. USN_1: ultrasound at 32 kJ/kg. USN_2: ultrasound at 256 kJ/kg. 0: non-digested sample. 1: gastric digesta. 2: non-dialysed intestinal fraction.

Figure 21. Ascorbic acid content during simulated gastrointestinal digestion of the beverage based on papaya, mango and *stevia* after different treatments applied.

Three-way ANOVA showed that the preservation treatment applied, energy input level and the digestion phase had a significant influence on the values of ascorbic acid ($p < 0.05$).

During gastrointestinal digestion, ascorbic acid content was significantly affected, decreased independently of the treatment used. Regarding untreated sample after gastric conditions, the recovery of ascorbic acid was 50.5 %. Concidering treated sample the recovery was in the range of 26.5 % (PEF_2) to 53.1 % ($HVED_1$).

After the intestinal phase, no ascorbic acid was detected neither in the dialysed nor in the undialysed fraction, showing the lability of this vitamin to the high temperature (ºC) to which samples are subjected during simulated gastrointestinal digestion. Aschoff et al. (2015), reported a bioaccessibility of vitamin C of 78 % in thermally pasteurized orange juice, but temperature of their *in vitro* digestion model did not exceed 10 ºC and pH was not adjusted to gastric and intestinal pH. They did not obtain significant differences when compared to fresh orange juice. Nevertheless, the absence of ascorbic acid in the dialysed intestinal fraction does not mean that none of the ascorbic acid present in the beverage is absorbed by human subjects, as absorption is a much more complex process which involves not just diffusion mechanisms but also active transport which is not considered in the simulated digestion. At doses normally ingested (≤ 180 mg), bioavailability of vitamin C ranges from 80 % to 100 % (Davey et al., 2000). A study carried out by Malo & Wilson, (2000) showed that vitamin C is absorbed all along the small intestine by specific sodium-dependent co-transporters and other studies (Cocate et al., 2014) relate the intake of fruits and other vitamin C sources with the increase of vitamin C plasmatic concentration.

Changes in the total carotenoids, total phenolic compounds and total anthocyanins content due to *in vitro* gastrointestinal digestion of the beverage and processed by different treatments are shown in Table 12.

Table 12. Bioactive compounds during simulated gastrointestinal digestion of the beverage based on papaya, mango and *stevia*, before and after different treatments applied.

	Non-digested	Gastric	%	Non-dialysed	%	Dialysed	%
TC (µg/100mL)							
Untreated	676.1±3.5^{a}	551.6±34.9^{a}	***81.6***	226.4±19.4^{a}	***41.0***	85.8±4.9^{a}	***37.9***
$HVED_1$	591.3±10.9^{a}	510.5±86.3^{a}	***86.3***	398.0±77.6^{b}	***77.9***	202.4±4.9^{bc}	***50.9***
$HVED_2$	371.8±67.0^{b}	178.4±19.4^{b}	***48.0***	164.7±9.7^{c}	***92.3***	140.7±4.9^{d}	***85.4***
PEF_1	800.9±3.5^{c}	518.7±11.6^{a}	***64.8***	439.1±19.4^{b}	***84.6***	223.0±43.7^{b}	***50.8***
PEF_2	633.7±49.4^{a}	376.0±3.9^{a}	***59.3***	229.9±4.9^{a}	***61.1***	133.8±14.6^{de}	***58.2***
USN_1	429.1±28.2^{bd}	403.4±58.2^{a}	***94.0***	428.8±4.9^{b}	***106.3***	161.2±14.6^{cd}	***37.6***
USN_2	486.5±17.6^{d}	389.7±23.3^{a}	***80.1***	367.1±14.6^{b}	***94.2***	92.6±14.6^{ae}	***25.2***
TPC (mg/L)							
Untreated	2685.6±18.4^{ab}	2675.8±10.1^{a}	***99.6***	1685.7±113.6^{ab}	***63.0***	818.1±9.5^{a}	***48.5***
$HVED_1$	2646.6±36.7^{a}	2397.4±20.2^{b}	***90.5***	1587.6±6.3^{a}	***66.2***	905.1±82.0^{bc}	***57.0***
$HVED_2$	2805.6±4.6^{c}	1987.0±25.2^{c}	***70.8***	1190.6±82.0^{c}	***59.9***	467.9±6.3^{d}	***39.3***
PEF_1	2685.6±55.1^{abc}	2347.5±0.1^{b}	***87.4***	1768.3±3.2^{b}	***75.3***	889.5±3.2^{ab}	***50.3***
PEF_2	2744.0±73.4^{bc}	2925.6±91.9^{d}	***106.6***	1750.4±116.7^{ab}	***59.8***	1016.6±6.3^{e}	***58.0***
USN_1	2701.8±22.9^{ab}	2336.8±25.2^{b}	***86.5***	1308.8±9.5^{c}	***56.0***	956.4±9.5^{bce}	***73.1***
USN_2	2721.3±41.3^{abc}	2600.8±5.0^{a}	***95.6***	1659.0±18.9^{ab}	***63.7***	976.5±12.6^{ce}	***58.8***
TA (mg/L)							
Untreated	11.8±1.4^{ab}	11.0±1.2^{ab}	***93.2***	8.1±0.2^{a}	***73.6***	1.5±0.2^{ab}	***18.5***
$HVED_1$	12.0±0.3^{ab}	11.1±0.5^{ab}	***92.5***	8.5±0.4^{a}	***76.6***	3.7±0.4^{c}	***43.5***
$HVED_2$	13.0±0.5^{a}	11.9±1.4^{a}	***91.5***	10.8±0.2^{b}	***90.8***	1.2±0.6^{a}	***11.1***
PEF_1	11.9±0.4^{ab}	10.9±0.5^{ab}	***91.6***	8.4±1.4^{a}	***77.0***	1.3±0.4^{ab}	***15.4***
PEF_2	12.8±0.3^{a}	11.7±0.2^{ab}	***91.4***	10.1±0.4^{b}	***86.3***	2.0±1.4^{b}	***19.8***
USN_1	11.2±0.4^{b}	10.7±0.2^{b}	***95.5***	7.9±0.2^{a}	***73.8***	1.2±0.2^{a}	***15.2***
USN_2	11.9±0.1^{ab}	11.4±0.3^{ab}	***95.8***	8.5±0.4^{a}	***74.6***	1.5±0.2^{ab}	***17.6***

TC: total carotenoids. TPC: total phenolic compounds. TA: total anthocyanins. $HVED_1$: high voltage electrical discharges at 32 kJ/kg. $HVED_2$: high voltage electrical discharges at 256 kJ/kg. PEF_1: pulsed electric fields at 32 kJ/kg. PEF_2: pulsed electric fields at 256 kJ/kg. USN_1: ultrasound at 32 kJ/kg. USN_2: ultrasound at 256 kJ/kg.

The results of the ANOVA showed that treatment applied was a significant factor in carotenoids content through the simulated *in vitro* digestion. After gastric conditions, the recovery of carotenoids was in the range of 48.0 % ($HVED_2$) to 94.0 % (USN_1). Although carotenoids are unlikely to be destroyed by the enzymes used in the present digestion model (Li et al., 2014), they have been shown to be sensitive at acidic pH (Zulueta et al., 2010). Rodríguez-Roque et al. (2014) obtained carotenoid recoveries of 54.0 % and 56.8 % in gastric digesta of a blended fruit juice-milk beverage and a blended fruit juice, respectively.

One-way ANOVA did not show a significant influence of the different treatments in carotenoid content in the gastric digesta, except for $HVED_2$, with a significantly lower content of total carotenoids. In general, total carotenoids content of all treated beverage were reduced under intestinal conditions, except for USN_1, displaying a recovery range from 61.1 % in the case of PEF_2 mixture up to 106.3 % in the USN_1 treated sample, in comparison with their respective gastric digesta. Greater losses of total carotenoids were observed in the dialysed intestinal digesta, being recovered between 25.2 % (USN_2) and 85.4 % ($HVED_2$), with respect to the non-dialysed intestinal fraction.

Regarding total phenolic compounds (TPC) lower contents of TPC were detected after the gastric step for all samples (70.8 – 99.6 %) except for PEF_2 treated mixture, with an increase (106.6 %) in the release of TPC. Amounts of TPC detected after the intestinal phase diminished significantly, with a recovery of 63.0 % in the untreated beverage with regard to the gastric digesta. PEF_1 mixture showed the highest stability of TPC (75.3 %) while USN_1 displayed the greatest losses (56.0 %) in the non-dialysed intestinal fraction. By contrast, the amount of released total phenolic compounds increased stepwise from gastric to intestinal digesta for all apple varieties in a study carried out by Bouayed et al. (2011). Additionally, greater losses of TPC where observed when the dialysed

fraction was compared to the non-dialysed fraction, up to 48.5 % in the untreated beverage. Differently from carotenoids digestibility, TPC in $HVED_2$ treated beverage exhibited the lowest recovery, 39.3 % compared to the non-dialysed fraction.

The pattern of release of anthocyanins was similar to that of TPC. High recoveries of anthocyanins were obtained after the gastric digestion, 91.4-95.8 %, with non-significant differences between the different treatments applied. However, anthocyanins were reduced in a 73.6 % in the untreated beverage after the intestinal digestion in comparison with the gastric digesta. This was also observed by other authors, who attributed it to their low stability in the alkaline conditions of small intestine, as it is generally accepted that anthocyanins are stable at low pH values (between 1 and 3) (Kosinska-Cagnazz et al., 2014; Stanisavljevic et al., 2014). Low recovery of anthocyanins (11.1 %) was obtained in the dialysed fraction of $HVED_{2.}$

Three different antioxidant assays (TEAC, ORAC and DPPH) were used to analyse *in vitro* gastrointestinal digestion, as there is no an official method to determine total antioxidant capacity (TAC) in foodstuff. The scavenging capacities of the HVED, PEF and USN treated beverage under simulated *in vitro* digestion model system are shown in Table 13.

With reagard to untreated sample values of TEAC, ORAC and DPPH assays were 23.9, 30.7 and 24.3 (mM) respectively.

Table 13. Total antioxidant capacity during simulated gastrointestinal digestion of the beverage based on papaya, mango and *stevia* before and after different treatments applied.

	TEAC (mM)	%	ORAC (mM)	%	DPPH (mM)	%
Untreated						
Non-digested	23.9±0.6		30.7±0.6		24.2±0.3	
Gastric digesta	21.1±3.6	***88.2***	27.9±0.7	***90.8***	20.5±0.8	***84.7***
Non-dialysed fraction	11.3±0.0	***53.5***	22.3±1.4	***79.9***	10.5±0.1	***51.2***
Dialysed fraction	6.1±0.1	***53.9***	8.1±0.4	***36.3***	5.4±0.0	***51.4***
$HVED_1$						
Non-digested	23.7±1.7		29.1±1.3		24.4±0.7	
Gastric digesta	15.2±0.7	***64.1***	29.4±3.2	***101.1***	14.4±1.6	***59.0***
Non-dialysed fraction	12.3±0.3	***80.9***	23.3±0.5	***79.2***	9.9±1.6	***68.7***
Dialysed fraction	8.5±0.4	***69.1***	8.4±0.8	***36.1***	7.8±0.1	***78.7***
$HVED_2$						
Non-digested	26.4±0.6		31.7±1.3		26.1±0.1	
Gastric digesta	13.1±0.6	***50.0***	22.9±0.8	***72.2***	12.3±0.3	***47.1***
Non-dialysed fraction	10.6±0.1	***80.9***	22.6±2.5	***98.6***	7.3±0.8	***59.3***
Dialysed fraction	5.5±0.2	***51.8***	5.4±0.5	***23.8***	4.5±1.6	***61.6***
PEF_1						
Non-digested	23.6±0.2		37.4±1.9		24.4±0.4	
Gastric digesta	15.0±1.8	***63.6***	34.2±1.8	***91.4***	13.5±0.1	***55.3***
Non-dialysed fraction	13.5±0.9	***90.0***	26.2±2.4	***76.6***	12.6±1.9	***93.3***
Dialysed fraction	6.8±0.1	***50.3***	9.1±0.9	***34.7***	5.8±0.3	***46.0***
PEF_2						
Non-digested	25.6±0.3		36.8±1.5		25.5±0.7	
Gastric digesta	22.2±0.4	***86.7***	34.0±1.8	***92.3***	21.0±0.9	***82.3***
Non-dialysed fraction	14.8±0.1	***66.6***	24.1±0.9	***70.8***	13.9±0.3	***66.1***
Dialysed fraction	7.5±0.1	***50.6***	9.7±0.4	***40.2***	6.0±2.0	***43.1***
USN_1						
Non-digested	24.9±0.3		35.8±0.1		23.3±0.7	
Gastric digesta	14.1±1.1	***57.0***	33.7±0.1	***94.1***	12.9±0.1	***55.3***
Non-dialysed fraction	13.0±0.6	***92.1***	28.6±0.9	***84.8***	10.4±0.6	***80.6***
Dialysed fraction	5.9±0.2	***45.3***	9.4±0.1	***32.8***	4.0±0.9	***38.4***
USN_2						
Non-digested	25.5±0.7		37.1±0.1		24.7±0.4	
Gastric digesta	14.7±0.9	***57.6***	34.3±0.5	***92.4***	13.4±1.3	***54.2***
Non-dialysed fraction	13.3±0.4	***90.4***	25.5±0.3	***74.3***	12.4±1.6	***92.5***
Dialysed fraction	6.3±0.1	***47.3***	8.9±0.2	***34.9***	5.0±0.6	***40.3***

TEAC: trolox equivalent antioxidant capacity. ORAC: oxygen radical antioxidant capacity. DPPH: 2,2-diphenyl-1-picrylhydrazyl. $HVED_1$: high voltage electrical discharges at 32 kJ/kg. $HVED_2$: high voltage electrical discharges at 256 kJ/kg. PEF_1: pulsed electric fields at 32 kJ/kg. PEF_2: pulsed electric fields at 256 kJ/kg. USN_1: ultrasound at 32 kJ/kg. USN_2: ultrasound at 256 kJ/kg.

After the gastric conditions, total antioxidant capacity in the untreated sample diminished 11.6 %, 9.2 % and 15.3 % measured with TEAC, ORAC and DPPH assay, respectively. Higher losses were obtained for HVED, PEF and USN treated beverage when TEAC and DPPH were employed.

By contrast, the decrease of the total antioxidant capacity measured with ORAC method in the treated beverages was lower in comparison with the untreated sample, expect for $HVED_2$ treatment. Even a slight increase in ORAC was observed in $HVED_1$ (101.1 %) treated beverage. This was also found by He et al. (2014) under thermal processing conditions, where concentrations of catechin and chlorogenic acid model systems exhibited higher ABTS values at pH 3.7 than at pH 6.8, demonstrating the effects of pH adjustment in the antioxidant properties of foods. A significant correlation was found between TEAC and DPPH values ($p=0.0001$). TEAC, ORAC and DPPH values in the non-dialysed fraction of untreated beverage with recovery of 53.5 %, 79.8 % and 51.2 %, respectively was detected, in comparison to that obtained in the gastric digesta. Furthermore, a significant correlation was found between ORAC values and total carotenoids ($p = 0.0092$) and between TPC and TEAC and DPPH values ($p = 0.0323$ and $p = 0.0014$, respectively). TEAC and DDPH values were also found to be correlated ($p = 0.0009$). However, greater losses of total antioxidant capacity were obtained when the dialysed fraction was compared to the non-dialysed fraction (up to 53.9 %, 36.3 % and 51.4 % in the untreated beverage, for TEAC, ORAC and DPPH assay). Non-significant differences were observed when the beverage was treated by PEF, and USN. However, $HVED_2$ led to significant higher losses for TEAC and DPPH method, compared to the non-dialysed fraction, while a lower decrease was obtained for ORAC method in the $HVED_2$ treated beverage. A significant correlation was also obtained between TEAC and DPPH values ($p = 0.0005$) in the dialysed fraction.

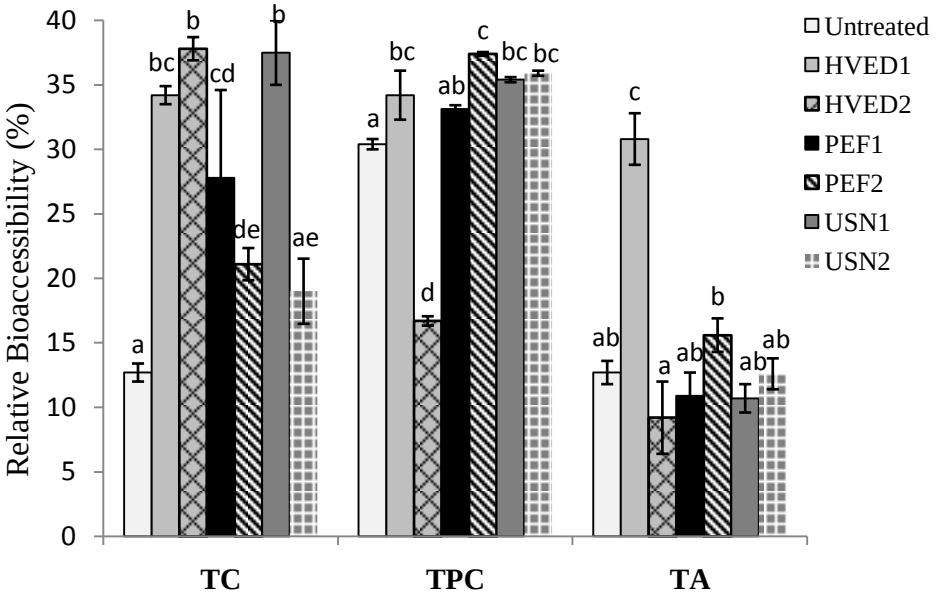
Bioaccessibility of the beverage based on papaya, mango and stevia treated by HVED, PEF and USN technologies

In order to estimate the relative bioaccessibility of bioactive compounds, the ratio between the mean levels of each bioactive compound in the beverage treated by different technologies and after the *in vitro* digestion process was calculated. Results are shown in Figure 22-23.

The bioaccessibility of carotenoids in the untreated beverage was of 12.7 %. As was expected, significantly higher values ($p < 0.05$) were obtained when the beverage was treated by HVED, PEF and USN, food processing can cause cell membrane disruption, leading to the release of bioactive compounds, thus increasing the bioaccessibility of these compounds. Our findings are in agreement with those of a previous study carried out by Aschoff et al. (2015), who obtained an increase in carotenoid bioaccessibility of a thermally pasteurized orange juice in comparison with the fresh orange juice. On the contrary, Stinco et al. (2013) obtained a higher bioaccessibility of individual carotenoids, except for lutein, in fresh orange juice in comparison with ultrafrozen orange juices thawed at room temperature and refrigeration temperature, but lower than orange juice thawed in microwave. However, these same authors in a different study (Stinco et al., 2012) observed that pasteurization reduced carotenoid bioaccessibility in a 39 % compared to fresh squeezed orange juice. As discussed before (Table 12), the processing of the beverage did not increase the release of total carotenoids into the food matrix. Previous studies (Breithaupt et al., 2007) have shown that porcine pancreatic lipase possesses the activity of hydrolysing esters and convert xanthophyll esters to their corresponding free forms, which are the forms detected in plasma after an intake of food rich in esters (Pérez-Galvez et al., 2003). Therefore, the increase of the bioaccessibility percentage might be due to changes in the rheological properties of the

beverage, affecting this way the action of digestive enzymes and thus bioaccessibility. According to Fernández-García et al. (2012), the degree of food processing is significant for micellarisation efficiency of carotenoids, as a high processing degree can maximise the amount of compound that is made soluble from the matrix. In this line, we would assume that higher energy inputs of the processing techniques would lead to higher bioaccessibility but this only occurred in the case of HVED technology (38 %). On the other hand, when increasing the energy input from 32 to 256 kJ/kg in PEF and USN treatment, the bioaccessibility percentage decreased in a 21.1 % and 19 %, respectively. Optimal energy inputs of these techniques which maximise the absorption of carotenoids must be assayed.

With regard to total phenolic compounds, the relative bioaccessibility of the untreated beverage was of 30.5 %. Similarly to the results obtained in the present work, Rodríguez-Roque et al. (2014) obtained TPC bioaccessibility values in the range of 10.9-19.7 % in fruit juice-milk beverages, while TPC bioaccessibility ranged from 79.1 % to 96.4 % in the study carried out by Helal et al. (2014) in cinnamon beverages. $HVED_1$ treatment led to a significant increase ($p < 0.05$) in TPC bioaccessibility in comparison with the untreated sample. However, increasing the energy input to 256 kJ/kg caused a drastically decrease of the TPC bioaccessibility to 16.7 %. Although this technique has been applied for extraction of polyphenols from different by-products (Boussetta et al., 2013), studies are still required to study the effects that this technology has on the digestibility and bioaccessibility of these compounds. HVED may cause electrolysis and formation of highly reactive chemicals during the electrical discharge, so at high energy inputs this technique can be unsuitable for storage or digestion processes where the food compounds present in the matrix are exposed to these reactive chemicals.



TC: total carotenoids. TPC: total phenolic compounds. TA: total anthocyanins. $HVED_1$: high voltage electrical discharges at 32 kJ/kg. $HVED_2$: high voltage electrical discharges at 256 kJ/kg. PEF_1: pulsed electric fields at 32 kJ/kg. PEF_2: pulsed electric fields at 256 kJ/kg. USN_1: ultrasound at 32 kJ/kg. USN_2: ultrasound at 256 kJ/kg.

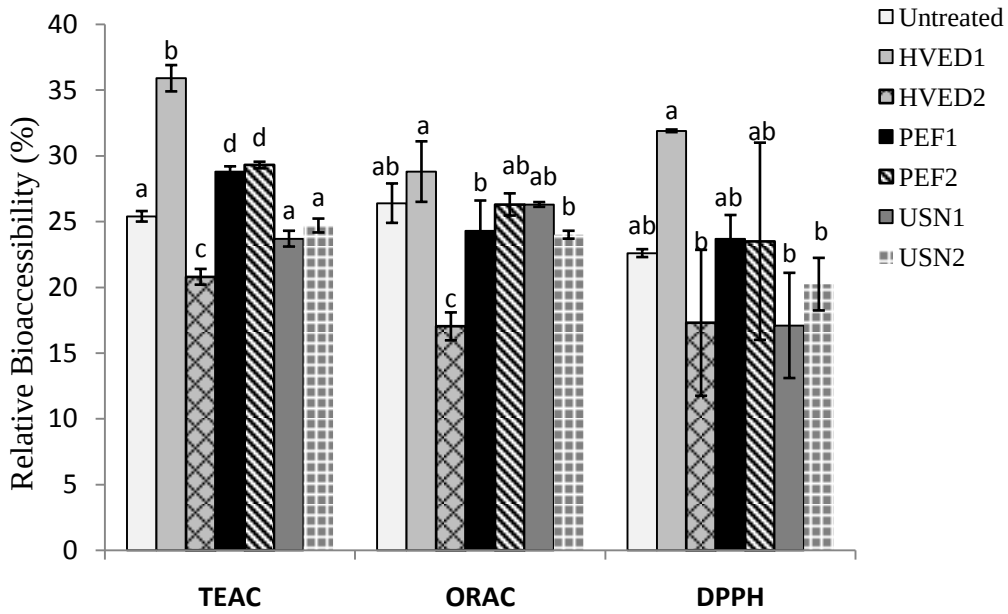
Figure 22. Relative bioaccessibility (%) of total carotenoids, total phenolic compounds and total anthocyanins of the beverage based on papaya, mango and *stevia* before and after different treatments applied.

Moreover, PEF_2, USN_1 and USN_2 treatment resulted in a significant increase in TPC bioaccessibility, as this techniques may promote the release of phytochemicals from the food matrix with no formation of electrolysis products reported up to date.

Meanwhile, bioaccessibility of total anthocyanins ranged from 9.2 % ($HVED_2$) up to 31.0 % ($HVED_1$). Bioaccessibility of anthocyanins has been demonstrated to be lower than that of other flavonoids (Yang et al., 2011). $HVED_1$ beverage exhibited the highest anthocyanins bioaccessibility. However, higher energy input (256 kJ/kg) led to a significant decrease ($p < 0.05$) in the anthocyanin bioaccessibility, as

occurred with bioaccessibility of phenolic compounds, probably because of the formation of highly reactive chemicals. By contrast, higher energy inputs in PEF and USN technology caused an increase in the anthocyanins bioaccessibility, although this was not significant. Results demonstrate that the different technologies applied to a same food matrix in which bioactive compounds are contained can affect their degree of digestibility and thus, their bioaccessibility.

The relative bioaccessibility (%) of TAC values after the gastrointestinal digestion process was also determined in the beverage untreated and treated by HVED, PEF and USN. Results are shown in Figure 23.



TEAC (trolox equivalent antioxidant capacity). ORAC (oxygen radical antioxidant capacity). DPPH (2,2-diphenyl-1-picrylhydrazyl). $HVED_1$: high voltage electrical discharges at 32 kJ/kg. $HVED_2$: high voltage electrical discharges at 256 kJ/kg. PEF_1: pulsed electric fields at 32 kJ/kg. PEF_2: pulsed electric fields at 256 kJ/kg. USN_1: ultrasound at 32 kJ/kg. USN_2: ultrasound at 256 kJ/kg.

Figure 23. Relative bioaccessibility (%) of total antioxidant capacity of the beverage based on papaya mango and *stevia* before and after different treatments applied.

Note that the highest TAC bioaccessibility was obtained in the $HVED_1$ sample, independently of the method used to measure TAC values and was for ORAC (29 %), TEAC (36 %) and DPPH (32 %) respectively. By contrast, applying HVED at an energy input of 256 kJ/kg ($HVED_2$) made TAC bioaccessibility diminish significantly and was in the range (17-20.8 %). Thus it can be concluded that HVED may constitute a promising technology which guarantees the increase of the total antioxidant capacity of the beverage after an *in vitro* digestion process, but depending on the energy input applied.

Further studies evaluating the influence of the energy input of this technology on total antioxidant bioaccessibility are required. By contrast, non-significant differences were found between the different energy inputs applied for PEF and USN treatment, independently of the method used to analyse TAC bioaccessibility.

6.2.3. Conclusions

Despite recent developments in non-thermal technologies applications and particularly their positive effect for enhancing bioactive compounds and total antioxidant content in different food matrixes, bioaccessibility of these compounds must be assayed in order to assure their beneficial effects once ingested. The results obtained in the present work highlight that electric technologies (HVED and PEF) and USN can be seen as promising technologies which enhance the release of bioactive compounds and consequently total antioxidant capacity after a simulated *in vitro* gastrointestinal digestion, promoting health and protecting against several diseases, although energy input must be controlled. Nonetheless, *in vitro* limitations should be taken into account, as these methods enable an approach, but will not absolutely predict how much of a specific bioactive compound a human will absorb and utilise. *In vivo* assays evaluating the

effects of the energy input level of HVED, PEF and USN technology on bioaccessibility of bioactive compounds and total antioxidant capacity are required.

6.3. Bioaccessibility of bioactive compounds and antioxidant capacity from orange peel after pulsed electric fields and high voltage electrical discharges

Traditionally, food wastes have been considered as a problem. However, they could be a great source of valuable nutraceuticals which can be used to deal with the prospects of feeding fast growing population in 21st century. Perspectives originate from the enormous amounts of food related materials that are discharged worldwide and the existing technologies, which promise the recovery, recycling and sustainability of high-added value ingredients inside food chain.

In this line, a great amount of citric wastes and by-products are generated each year in Europe. Orange peels, which are by-products of orange processing, are a good source of bioactive compounds, such as phenolic content, carotenoids, and vitamin C, which can be used as food additives and/or nutraceuticals (De Moraes et al., 2012; González-Gómez et al., 2014; Jwanny et al., 2012; Karoui & Marzouk et al., 2013). Several epidemiological studies suggest that bioactive compounds have beneficial effects and have been involved in the reduction of degenerative diseases such as cancers of the lungs and alimentary tract, being this effect mainly attributed to their antioxidant capacity (Abdullah et al., 2012; Al-Juhaimi, 2014).

Conventional extraction methods used for the recovery of bioactive compounds are based on maceration and heat extraction at temperatures > 60ºC alone and/or combined with different solvents, which can be toxic (i.e., hexane, acetone, methanol, etc.). Moreover, the use of high temperatures can promote nutritional losses (Odriozola-Serrano et al., 2013; Parniakov et al., 2014b).

At this stage of development, there is a need to develop new extraction methods that can reduce the extraction time, temperature and solvent

consumption and contribute to higher extraction efficiency and lower energy consumption as compared to conventional extraction techniques.

For instance, pulsed electric fields (PEF) and high voltage electrical discharges (HVED) can be useful tools to recover bioactive compounds from fruit by-products. Recent scientific and practical efforts have shown full correspondence of pulsed electric fields (PEF) techniques with green extraction concept (Vorobiev & Lebovka, 2010). This concept assumes using renewable plant resources and alternative solvents (water or agro-solvents (ethanol, methyl esters of fatty acids of vegetable oils)), reduction of energy consumption and unit operations, production of high quality and purity of extracts (non-denatured and biodegradable) and extracts co-products instead of wastes (Chemat et al., 2012). Moreover, methods assisted by pulsed electric energy can allow the increase of the yield and quality of the extracted compounds, thus decreasing the time and temperature of extraction operations (Boussetta & Vorobiev, 2014; Cholet et al., 2014; Donsi et al., 2010; Knorr et al., 2011; Luengo et al., 2013; Martin-Belloso & Soliva-Fortuny, 2011; Vorobiev & Lebovka, 2006).

Some previous studies have evaluated the effectiveness of pulsed electric fields on antioxidant compounds recovery from orange peel (Luengo et al., 2013) but there are no studies evaluating the impact of non-thermal treatments (PEF and HVED) upon the bioaccessibility of orange peel thorought a simulated *in vitro* digestion.

Bioaccessibility has been defined as the fraction of a compound released from the food matrix in the gastrointestinal tract and thus available for intestinal absorption (Tagliazucchi et al., 2010). This parameter provides valuable information in order to select the appropriate dosage and source of food matrices as bioactive compounds.

6.3.1. Materials and methods

6.3.1.1. Sample

Composition and preparation of the samples is described in the paragraph 3.1.3 on page 58.

6.3.1.2. Physicochemical parameters

- pH *(See section 4.1- page 59)*
- ºBrix *(See section 4.2 - page 59)*

6.3.1.3. Nutritional parameters

- Ascorbic acid *(See section 4.7 - page 63)*
- Total carotenoids *(See section 4.8 - page 64)*
- Total phenolic compounds *(See section 4.9 - page 65)*

6.3.1.4. Total antioxidant capacity

- TEAC, ORAC *(See section 4.11 - page 66, 68)*

6.3.1.5. Digestion method I

A three-stage digestion model based on the procedure described by Rodriguez Roque et al. (2013) with same modification was applied *(See section 5.1 - page 75).*

6.3.1.6. Treatments

Treatments were obtained as previously reported *(See section 6.2.1.5 - page 113),* although with different energy inputs applied: 55 kJ/kg for $PEF_1/HVED_1$ and 364 kJ/kg for $PEF_2/HVED_2$.

6.3.1.7. Statistical analysis

An analysis of variance (ANOVA) was applied to the results obtained in order to verify whether there were significant differences in the parameters studied in relation to sample analysed, and to ascertain possible interactions between factors (differences at $p < 0.05$ were considered significant). Where there were differences, an LSD test was applied to indicate the samples in which differences were observed. A multiple regression analysis was performed to study the influence of bioactive compounds to antioxidant capacity (the results are shown in the significant cases, $p < 0.05$). Finally, a study was conducted with the aim of determining whether there were correlations between a pair of variables (Pearson´s test). All statistical analyses were performed using Statgraphics® Centurion XVI (Statpoint Technologies Inc., USA). The results obtained by applying each test are shown in Annex *(See section 9 - page xviii)*

6.3.2. Results and discussion

Content of bioactive compounds after pulsed electric fields (PEF) and high voltage electrical discharges (HVED)

Changes in bioactive compounds such as ascorbic acid, total carotenoids, total phenolic compounds as well as antioxidant capacity due to high voltage electrical discharges (HVED), pulsed electric fields (PEF) in orange peel were studied. To better compare the effects of non-thermal treatments on bioactive compounds stability and bioaccessibility through an *in vitro* simulated digestion, equivalent energy inputs of 55 kJ/kg (PEF_1/$HVED_1$) and 364 kJ/kg (PEF_2/$HVED_2$) were used.

Undigested samples, immediately after PEF, HVED treatments

Table 14 shows the content of bioactive compounds and total antioxidant capacity obtained immediately after applying the non-thermal treatments (PEF, HVED) at two equivalent energy inputs (55 and 364 kJ/kg). As can be observed all factors such as type of treatment as well as energy input had a significant influence on changes, however, the extent of improvement or degradation was different.

The analysis of ascorbic acid showed a noticeable decrease after HVED treatment which can be explained by the formulation of gaseous cavitation bubbles, as well as shocked of waves of high pressure (Boussetta & Vorobiev, 2014). A higher decrease was observed after HVED treatment with higher energy applied, which can be explained by the increase in temperature at high energy levels. Furthermore, we also observed a significant decrease of ascorbic acid after applying PEF treatment with higher energy input.

Table 14. Content of bioactive compounds and antioxidant capacity from orange peel after different treatments applied.

	PEF_1	**PEF_2**	**$HVED_1$**	**$HVED_2$**
AA (mg/100 mL)	24.6±0.9	18.3±0.9	14.5±0.9	11.9±0.9
TPC (mgGAE/100 mL)	95.8±0.1	456.1±0.0	184.2±5.5	692.1±1.1
TC (µg/100 mL)	-	-	369.3±7.1	286.9±3.5
TEAC (mM TE)	1.3±0.1	3.5±0.6	1.2±0.1	4.7±0.1
ORAC (mM TE)	1.3±0.0	3.5±0.2	1.5±0.1	5.7±0.1

TPC: total phenolic content. TC: total carotenoids. TEAC: trolox equivalent antioxidant capacity. ORAC: oxygen radical antioxidant capacity. PEF_1: pulsed electric fields (55kJ/kg). PEF_2: pulsed electric fields (364 kJ/kg). $HVED_1$: high voltage electrical discharges (55 kJ/kg). $HVED_2$: high voltage electrical discharges (364 kJ/kg).

On the other hand a significant increase in total phenolic content was found when energy input was augmented for both technologies (364 kJ/kg). The highest phenolic content recovery was obtained after applying HVED at 364 kJ/kg. Moreover, the recovery of phenolic content after PEF

was significantly lower compared to HVED at equivalent energy inputs. The values obtained for PEF were in the range of those previously reported by Luengo et al. (2013) who evaluated the effectiveness of PEF on antioxidant compounds applying PEF (1-7 kV/cm) treatments combined with pressing (5-30 min) in orange peel.

Total carotenoids decreased significantly when $HVED_2$ was used at 364 kJ/kg (286.9 μg/100 mL) in comparison with $HVED_1$ (369.3 μg/100 mL). This fact can be attributed to the formation of chemical products of electrolysis and free reactive radicals, which are able to reduce nutritional quality of high-added value compounds when HVED is applied at high energy inputs (Boussetta & Vorobiev, 2014; Parniakov et al. 2014a).

Surprisingly, PEF treatments caused a total degradation in carotenoid content. A possible explanation for this phenomenon is that PEF does not affect small cell compartments such as chromoplasts, in which carotenoids are mainly found (Schoenbach et al., 2004).

To estimate total antioxidant capacity in orange peel, two different antioxidant assays; trolox equivalent antioxidant capacity (TEAC) and oxygen radical antioxidant capacity (ORAC) were used. The analysis of antioxidant capacity using both methods (TEAC, ORAC) showed an improvement in antioxidant capacity when higher energy input was applied.

In the case of physicochemical properties, there was no difference observed in pH and ºBrix after different treatments were applied.

Digestibility of the orange peel extracts treated by two different treatments (PEF, HVED) at equivalent energy inputs.

In order to better compare the effects of (PEF and HVED) treatments on bioactive compounds and antioxidant capacity, bioaccessibility thorough an *in vitro* simulated digestion was analysed. Three-way ANOVA analysis was performed to evaluate the influence of type of treatment, energy input

and step of *in vitro* digestion on changes in content of bioactive compounds and antioxidant capacity. The analysis regarding ascorbic acid content in orange peel treated by HVED and PEF processing during the simulated gastrointestinal digestion is shown in Table 15.

Table 15. Content of ascorbic acid from orange peel during simulated gastrointestinal digestion after different treatments applied.

	Ascorbic acid (mg/100 mL)
PEF_1	
Non-digested	24.6±0.9[a]
Gastric	4.8±0.9[b]
Non-dialysed fraction	-
Dialysed fraction	-
PEF_2	
Non-digested	18.3±0.9[a]
Gastric	13.2±0.9[b]
Non-dialysed fraction	-
Dialysed fraction	-
$HVED_1$	
Non-digested	14.5±0.9[a]
Gastric	4.7±0.9[b]
Non-dialysed fraction	-
Dialysed fraction	-
$HVED_2$	
Non-digested	11.9±0.9[a]
Gastric	9.9±0.9[b]
Non-dialysed fraction	-
Dialysed fraction	-

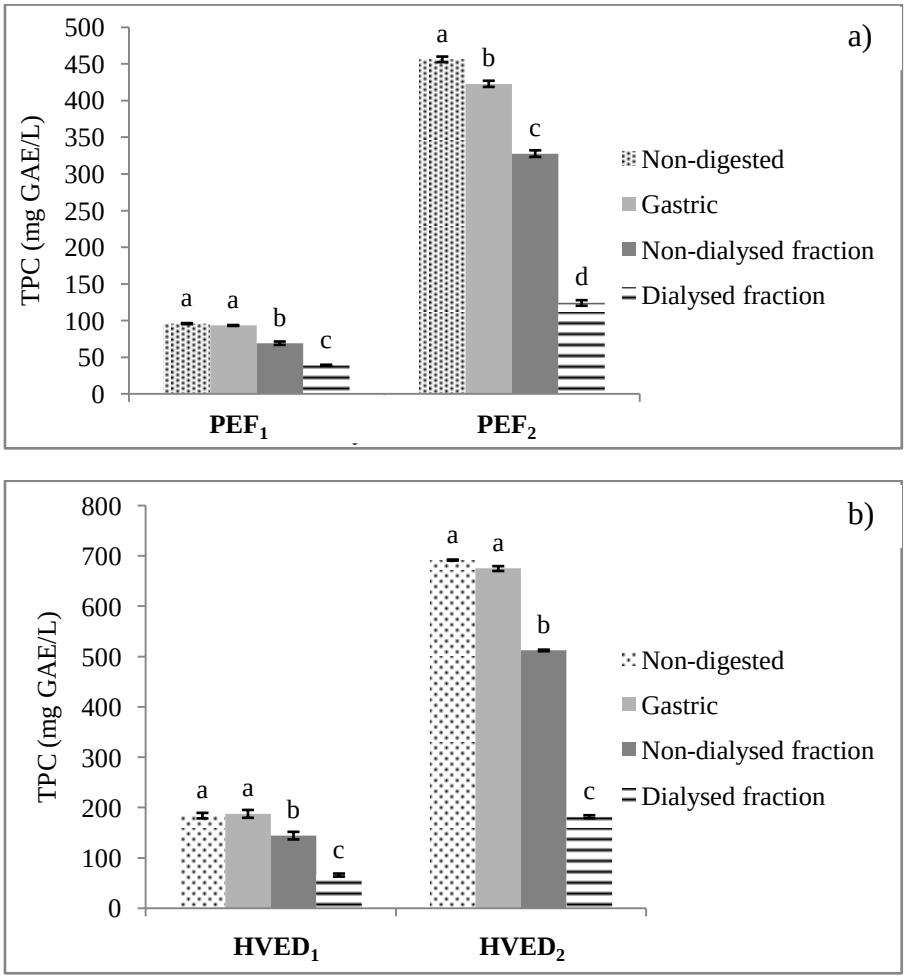
PEF_1: pulsed electric fields (55 kJ/kg). PEF_2: pulsed electric fields (364 kJ/kg). $HVED_1$: high voltage electrical discharges (55 kJ/kg). $HVED_2$: high voltage electrical discharges (364 kJ/kg).

During *in vitro* gastrointestinal digestion, there was a significant decrease in the ascorbic content observed when treatment with lower energy input (55 kJ/kg) was applied. After gastric conditions, the recovery of ascorbic acid was in the range of 19.5 % (PEF_1) to 83.3 % ($HVED_2$).

After the intestinal phase, as well as in the dialyzed fraction, there was no ascorbic acid detected, showing the liability of this vitamin, susceptible as it is to factors such as pH, colour, light and temperature, to which samples are subjected during simulated *in vitro* gastrointestinal digestion.

Changes in total phenolic content (TPC) during *in vitro* gastrointestinal digestion are shown in Figure 24. TPC content was significantly higher in both cases (PEF, HVED) when energy input was augmented. In addition, the gastric phase revealed non-significant modifications in TPC content after gastric digestion in HVED compared to undigested samples. These results are in close agreement to those found by other authors who found that some specific phenolic content from apples appears quite stable after acid hydrolysis in the stomach (Bouayed et al., 2012; Manach et al., 2004; Tenore et al., 2013). After gastric conditions, the recovery of TPC was in the range of 92.7 % (PEF_2) to 102 % ($HVED_1$). Regarding intestinal phase, the application of $HVED_1$ led to a difference in TPC in the non-dialysed fraction in comparison to the undigested sample. Moreover, a significant decrease was found in TPC of the non-dialysed fraction when energy input was augmented in PEF_2, $HVED_2$. This fact can be explained by two reasons: some modifications in the specific compounds were extracted when energy input was augmented, and/or the formation of hydroxyl radicals during water photodissociation caused by electrical discharges (Boussetta & Vorobiev, 2014), which can reduce the content of these compounds during the intestinal phase. The losses which were observed in the non-dialysed intestinal fraction were being recovered between 35.5 % ($HVED_2$) to 56.4 % (PEF_1) with respect to the dialysed fraction. From our analysis, PEF_2 (37.9 %) and $HVED_2$ (35.5 %) obtain

the lowest recovery compared to the non-dialysed fraction. Also, Boussetta et al. (2011) observed a negative effect of electrical discharges when energy values of 80-800 kJ/kg were applied.

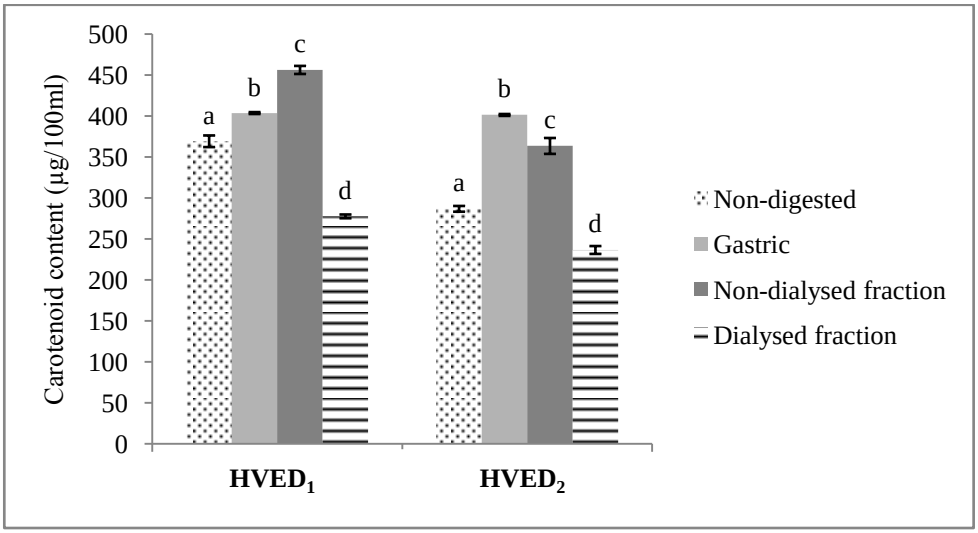


TPC: total phenolic compounds. PEF_1: pulsed electric fields (55 kJ/kg). PEF_2: pulsed electric fields (364 kJ/kg). $HVED_1$: high voltage electrical discharges (55 kJ/kg). $HVED_2$: high voltage electrical discharges (364 kJ/kg).

Figure 24. Total phenolic compounds during simulated gastrointestinal digestion of orange peel after a) pulsed electric fields and b) high voltage electrical discharges.

On the other hand, as can be observed in Figure 25, a significant increase in carotenoids content during gastric digestion and the non-dialysed fraction for both HVED treatments compared to non-digested samples were found. The recovery of carotenoids was 109.3 % for $HVED_1$ and 139 % for $HVED_2$.

These results were in close agreement to those reported by Courrad et al. (2013) and Rich et al. (2003), they observed that lutein and β-carotene were completely retained or even increased a significantly in β-carotene at the end of digestion. These authors attributed this fact to an improved yield promoted by the different processes involved in matrix disruption, thus facilitating the carotenoid extraction from the food matrix. In the non-dialysed fraction we observed a stable recovery for $HVED_1$ (60.9 %) and for $HVED_2$ (65.1 %), with respect to the dialysed fraction.



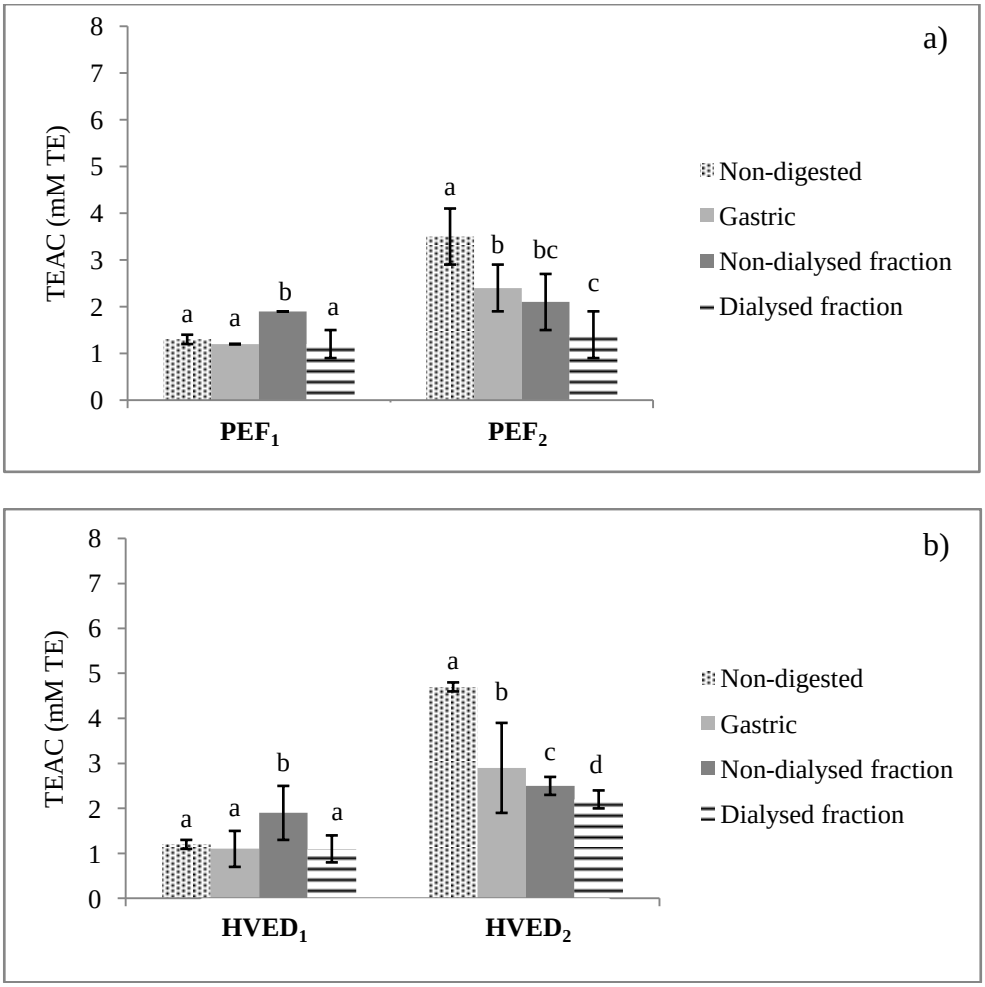
$HVED_1$: high voltage electrical discharges (55 kJ/kg). $HVED_2$: high voltage electrical discharges (364 kJ/kg).

Figure 25. Total carotenoids content during simulated gastrointestinal digestion of orange peel after high voltage electrical discharges.

For instance, in another study, it was reported that particle size had a significant influence in carotenoid bioaccessibility from carrot- and tomato-derived suspensions, with an increase observed in the bioaccessibility of these compounds when particle size was smaller (Moelants et al. 2012). As it was previously reported, HVED is a technology which is based on the fragmentation of the food matrix, thus facilitating carotenoid bioaccessibility. Moreover, Moelants et al. (2012) also attributed the increase in bioaccessibility of carotenoids to interactions between compounds in the complex food matrix.

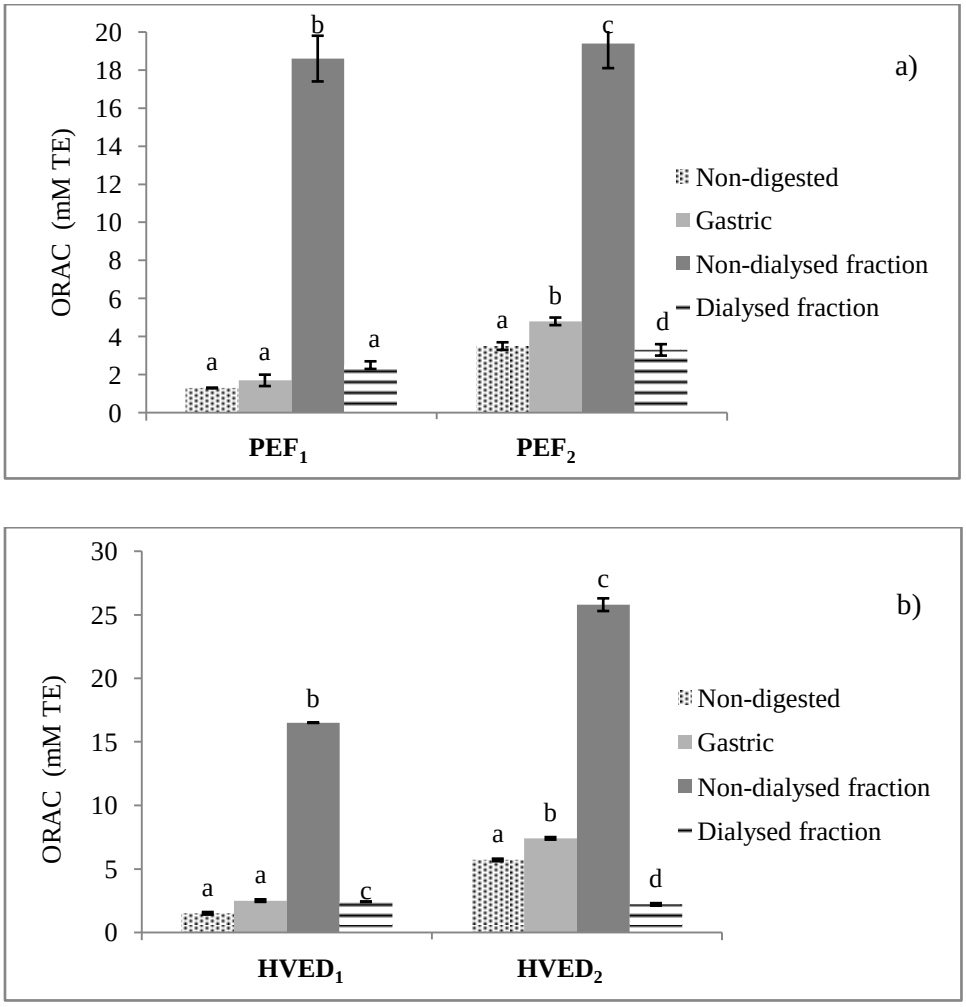
The antioxidant capacity (TAC) of orange peel after PEF and HVED treatments was measured by two different methods (TEAC, ORAC).

Regarding TEAC measurements (Figure 26) a significant increase in the intestinal phase (non-dialysed fraction) in PEF_1 and $HVED_1$ treated samples was observed compared to undigested samples. After gastric conditions a recovery of total antioxidant capacity measured with TEAC methods was in range (61.7 %- 92.3 %). In non-dialysed intestinal digesta recovery was lowest 88 % for the $HVED_2$ sample. Figure 27 shows the contents of the total antioxidant capacity measured with ORAC assay obtained during gastrointestinal digestion after applying two different treatments (PEF, HVED) at two equivalent energy inputs (55 and 364 kJ/kg). It shows a significant enhancement of total antioxidant capacity in non-digested samples when both treatments (PEF and HVED) with higher energy input were applied. However, $HVED_2$ shows the highest value. The ANOVA analysis shows a significant increase ($p < 0.05$) in the intestinal tract after simulated digestion when ORAC methods were used. In all treated samples the highest value was detected after treatments with higher energy input applied (364 kJ/kg).



TEAC: trolox equivalent antioxidant capacity. PEF_1: pulsed electric fields (55 kJ/kg). PEF_2: pulsed electric fields (364 kJ/kg). $HVED_1$: high voltage electrical discharges (55 kJ/kg). $HVED_2$: high voltage electrical discharges (364 kJ/kg).

Figure 26. Antioxidant capacity (TEAC) during simulated gastrointestinal digestion of orange peel after a) pulsed electric fields and b) high voltage electrical discharges.

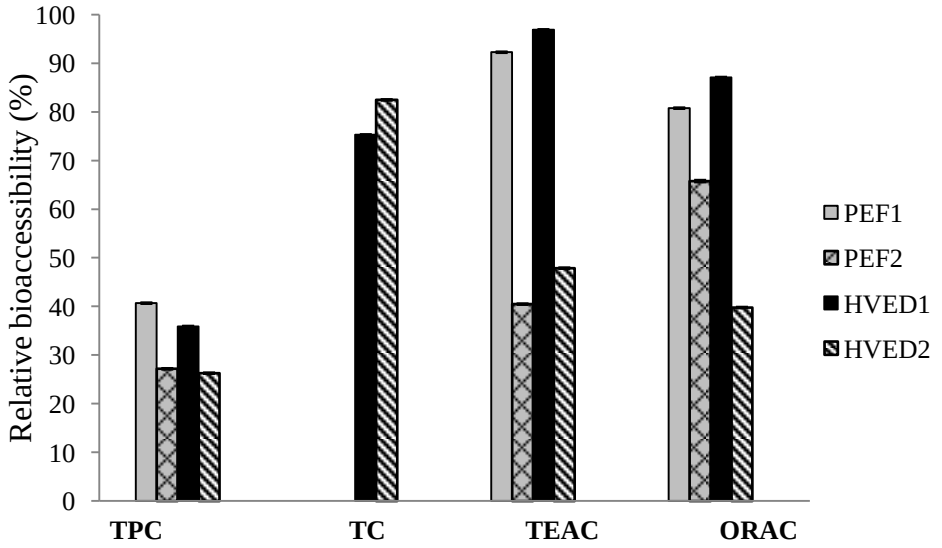


ORAC: oxygen radical antioxidant capacity. PEF_1: pulsed electric fields (55 kJ/kg). PEF_2: pulsed electric fields (364 kJ/kg). $HVED_1$: high voltage electrical discharges (55 kJ/kg). $HVED_2$: high voltage electrical discharges (364 kJ/kg).

Figure 27. Antioxidant capacity (ORAC) during simulated gastrointestinal digestion of orange peel after a) pulsed electric fields and b) high voltage electrical discharges.

Bioaccessibility of orange peel treated by two different treatments (PEF, HVED) at equivalent energy inputs.

The relative bioaccessibility of bioactive compounds and antioxidant capacity is shown in Figure 28.



TPC: total phenolic content. TC: total carotenoids. TEAC: trolox equivalent antioxidant capacity. ORAC: oxygen radical antioxidant capacity. PEF_1: pulsed electric fields (55 kJ/kg). PEF_2: pulsed electric fields (364 kJ/kg). $HVED_1$: high voltage electrical discharges (55 kJ/kg). $HVED_2$: high voltage electrical discharges (364 kJ/kg).

Figure 28. Relative bioaccessibility (%) of bioactive compounds and antioxidant capacity of orange peel treated by pulsed electric fields and high voltage electrical discharges.

The bioaccessibility of carotenoids after $HVED_1$ was 75.3 %. On the other hand when increasing the energy input from 55 to 364 kJ/kg in HVED the bioaccessibility percentage increased to 82.5 %. Similarly, Bengtsson et al. (2010) found that the extraction of β-carotene from plant structure and bioaccessibility can be enhanced by treatment. Moreover, Palmero et al. (2014) observed that the intensity of the treatment plays a determined role

in terms of obtaining a higher fraction of β-carotene that is available for absorption.

Regarding TPC bioaccessibility, the highest value was observed when PEF at lower energy input was applied (40.7 %). However, increasing the energy input to 364 kJ/kg led to a decrease of TPC independently of treatment applied. This phenomenon could be explained by the formation of highly reactive chemicals.

Bioaccessibility of TAC using two different assays (TEAC, ORAC) was also determined. As can be noted, the highest bioaccessibility was obtained in the samples treated by $HVED_1$ and PEF_1 independently of the method used. By contrast, when higher energy input was applied, bioaccessibility decreased significantly for PEF_2 (40.5 %), $HVED_2$ (47.9 %) according to the TEAC method and PEF_2 (65.8 %), $HVED_2$ (39.8 %) for the ORAC method.

6.3.3. Conclusions

From the results obtained in this study it is possible to conclude that PEF and HVED can be used as a useful tool to recover antioxidant compounds from orange peel. Although HVED led to the higher TPC, carotenoids and TEAC, ORAC yields, PEF treatments at equivalent energy inputs showed the higher TPC bioaccessibility. So, at this stage of development, there is a need to optimize processing conditions and further studies dealing this topic are needed.

6.4. Liberation and micellarisation of carotenoids from different smoothies treated by thermal and ultrasound

Yellow, orange, and red colouration of fruits and vegetables are usually caused by a large group of fat-soluble pigments called carotenoids. Low consumption of carotenoids is negatively related to a higher risk of cardiovascular diseases, cancer, cataracts, and age-related macular degeneration (Nagao, 2009). It has been hypothesized that this inverse relationship is caused by the antioxidant capabilities of carotenoids. Moreover, a growing body of research indicates that there is a strong correlation between carotenoids intake and reduced risk in some diseases such as cancer, coronary and cardiovascular diseases (Fiedor & Burda, 2014). It is important to obtain more information about carotenoids bioaccessibility from foods in order to have a better understanding of their potential benefits.

One way of increasing fruit and vegetable intake is to drink smoothies, blended beverages made of fruit, fruit pulp, fruit juices vegetables, yogurt and milk. In order to extend their shelf-life, smoothies are often thermally processed. Many studies have demonstrated that this process can affect the colour of foods (Sikora et al., 2008). Bioactive compounds like carotenoids must be released from the matrix and reach their site of action to exert their biological effects, so bioaccessibility and bioavailability are critical features in assessing the role of these compounds in human health (Rodríguez-Roque et al., 2013). Bioaccessibility represents the maximum amount of carotenoids released from the food that are available for absorption in the enterocytes while the fraction of the dose entering the systematic circulation to participate in physiological function is called bioavailability (Gleise et al., 2012). Nowadays, there is no clear consensus on the best approach to estimate carotenoid bioaccessibility. Application

of *in vitro* digestion and assimilation procedures is necessary to gain information about factors that modulate different steps of carotenoid liberation and micellarisation (Fernandez-García et al., 2012).

Liberation is defined as the percentage of carotenoids transferred during digestion from the test smoothies to the aqueous micellar fraction and has been of recent research interest. In contrast to liberation, micellarisation describes only the percentage of carotenoids transferred during *in vitro* digestion to micelles after ultracentrifugation. Micellarisation is therefore a part of accessibility, which indicates how efficiently a released carotenoid is incorporated into mixed micelles (Aschoff et al., 2015).

There are many studies available, which have investigated *in vitro* absorption of carotenoids using *in vitro* digestion methods. The liberation and micellarisation of carotenoids can be affected by a variety of factors including the amount ingested, food source, matrix, amount and type of processing, amount and type of fat co-ingested, carotenoid interactions, and other dietary components such as fiber (Edwards et al., 2002; Hedrén et al., 2002; Tyssandier et al., 2001).

Conventional thermal processing of fruit and vegetables smoothies remains the most widely adapted technology for shelf-life extension and preservation of these products. However, consumer demand for fresh and nutritious juices has led to interest in non-thermal technologies to apply in the processing of fresh vegetable smoothies to avoid the deleterious effects that heat has on the flavour, colour and nutrients (Tiwari et al., 2009). Non-thermal methods such as ultrasound treatment have been proposed as alternatives to thermal pasteurization so that the changes to flavour and nutritional value can be minimized during processing.

6.4.1. Materials and methods

6.4.1.1. Samples

Composition and preparation of the samples is described in the paragraph 3.1.4 on page 58.

6.4.1.2. Nutritional parameters

- Quantification of carotenoids *(See section 4.12 - page 71)*

6.4.1.3. Microorganisms

- Yeast and molds *(See section 4.14 - page 74)*
- Total viable count *(See section 4.14 - page 74)*

6.4.1.4. Digestion method II

A two-stage digestion model based on the procedure described by Minekus et al. (2014) and slight modified from Kopf-Bolanz et al. (2012) was applied *(See section 5.2 – page 79).*

6.4.1.5. Treatments

For different thermal treatments applied, temperature of 90°C for pasteurization and 120 ºC for sterilization were tested at a holding time of 20 s using autoclave units Autester 18B. For ultrasound treatment an ultrasonic Bracket bath with a continuous wave of 60 °C, during 20 min was used.

6.4.1.6. Statistical analysis

Results were expressed as mean ± standard deviation of determination. An analysis of variance (ANOVA) of the results was carried out in order to determine significant differences ($p < 0.05$) between the different

treatments and different carotenoids. All data were examined with SPSS 17.0 for Windows (SPSS Inc., Chicago, III., U.S.A). The results obtained by applying each test are shown in Annex *(See section 9 -page xxii).*

6.4.2. Results and discussion

For this study three different treatments (sterilization, pasteurization and ultrasound) were applied. In reference to sterilization the temperature was 120 ºC during 20 s. Concerning pasteurization the temperature was 90 ºC also during 20 s. Regarding ultrasound the temperature was 60 ºC during 20 min. All treated samples were compared to the untreated smoothie. To compare the effects of thermal and non-thermal treatment on carotenoid bioaccessibility, *in vitro* simulated digestion was applied. For this purpose a two-stage *in vitro* digestion model has been used. *In vitro* liberation and micellarisation were calculated as the percentages of the respective nutrient transferred from the test food to the supernatant obtained after centrifugation and to the micellar phase obtained by microfiltration of the supernatant, respectively, as described by Schweiggert et al. (2012). Before the study of carotenoids content and their bioaccessibility, a microbial analysis was also carried out to ensure the safety of treated products.

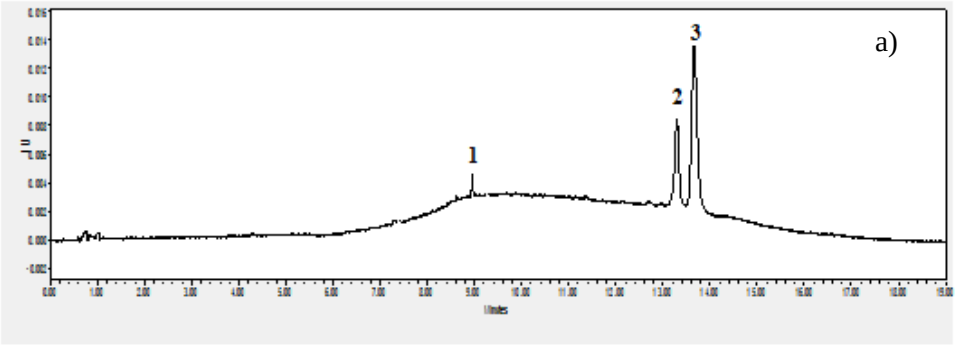
Microbial inactivation

Liquid foods have traditionally been preserved by thermal treatments which are the used method to extend the shelf-life of liquid foods preventing microorganism spoilage and contamination with pathogens. However, this treatment leads to the loss of nutritional compounds and undesirable changes in sensory properties of food (Cortes et al., 2008; Odriozola-Serrano et al., 2013). For our study, total viable counts and yields and molds counts were analysed for all samples immediately after treatments were applied and compared with untreated samples.

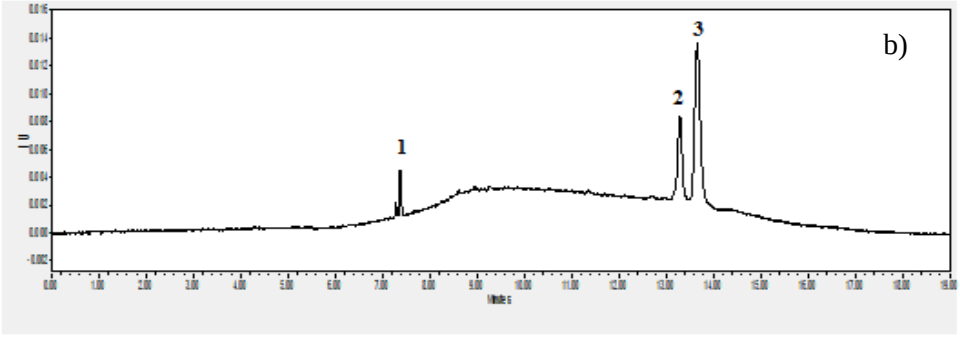
Both smoothies, characterized by an initial microbial concentration of approximately 10^4 cfu/mL, after ultrasound treatments. For both types of smoothie after sterilization no effect of bacterial growth was observed, nor did not detect any bacterial colonies grown after pasteurization treatments.

Undigested samples, immediately after thermal and ultrasound treatments

For each sample three different carotenoids were quantified. The resulting chromatography are presented in Figure 29. In smoothie 1 based on carrot juice-papaya-mango, β–cryptoxanthin, α–carotene and β–carotene were detected. In smoothie 2 based on carrot juice-pumpkin-mango, lutein, α–carotene and β–carotene were found.



1: β–cryptoxanthin; 2: α–carotene; 3: β–carotene



1: lutein; 2: α–carotene; 3: β–carotene

Figure 29. Chromatography of carotenoids content in two different smoothies: a) smoothie 1 b) smoothie 2.

Much research has studied the effects of different thermal and non-thermal methods on the levels of carotenoids in food; some report substantial losses, others no change, while others found increased carotenoid content (D´ Evoli et al., 2013; Provesi et al., 2011).

Table 16 shown the individual carotenoid content in smoothies treated by thermal and ultrasound treatments in comparison with untreated samples. As we can observe there was a different carotenoids content depending on composition of samples detected. The range of three identified carotenoids was for β-cryptoxanthin (0.10-0.21 mg/100 g), α-carotene (1.66-1.98 mg/100 g) and β-carotene (2.24-2.74 mg/100 g) for smoothie 1 and lutein (0.15-0.44 mg/100 g), α-carotene (1.53-1.86 mg/100 g) and β-carotene (2.09-3.02 mg/100 g) for smoothie 2. In both smoothies β-carotene was the most abundant carotenoid.

Table 16. Carotenoids content of different smoothies before and after different treatments applied.

SMOOTHIE 1	**β-cryptoxanthin**	**α-carotene**	**β-carotene**
UNTREATED	0.21±0.01^{a}	1.98±0.16^{a}	2.74±0.24^{a}
STERILIZATION	0.10±0.01^{b}	1.66±0.19^{a}	2.24±0.25^{a}
PASTEURIZATION	0.15±0.00^{ab}	1.88±0.03^{a}	2.53±0.05^{a}
ULTRASOUND	0.18±0.01^{a}	1.92±0.13^{a}	2.42±0.10^{a}
SMOOTHIE 2	**Lutein**	**α-carotene**	**β-carotene**
UNTREATED	0.44±0.01^{a}	1.83±0.09^{a}	2.82±0.16^{a}
STERILIZATION	0.15±0.01^{b}	1.53±0.18^{b}	2.09±0.21^{a}
PASTEURIZATION	0.27±0.01^{ab}	1.80±0.04^{a}	2.39±0.05^{a}
ULTRASOUND	0.38±0.01^{ab}	1.86±0.08^{a}	3.02±0.09^{b}

$^{a-c}$ Different lowercase letters in the same column indicate significant statistical difference in the function of the applied treatment ($p > 0.05$).

With regard to all treatments a significant difference after sterilization in the content of β–cryptoxanthin and lutein was observed. According to a study performed by Lee & Coates (2003) losses after pasteurization (90 ºC, 30 s) in the content of the most labile xanthophylls were detected. However, in our study no significant changes were observed after the pasteurization of both smoothies. On the other hand, no significant changes were observed after sterilization and pasteurization treatments in β-carotene. The results are in agreement with those found by Patras et al. (2009) for thermally processed (70 °C, 120 s) strawberry and blackberry purées and by Barba et al. (2012a) who applied thermal processing (90 °C for 15, 21 s and 98 °C for 15, 21 s) in an orange juice-milk beverage. The concentration of α-carotene decreases during the sterilization process in both smoothies, although only in smoothie 2 changes are significant.

Moreover, there were not significant changes observed in carotenoids after ultrasound treatment except in β-carotene content in smoothie 2 where a slight increase after ultrasound treatment was detected. This phenomena can be explained by the ability of ultrasound to enhance the disruption of cell walls, which might have facilitated the release of bound carotenoid content. In this line, Abid et al. (2014a) found an improvement in carotenoid content after ultrasound treatment in apple juices. Moreover, Rawson et al. (2011b) found a slight increase in lycopene content after ultrasound treatment in watermelon juice.

Colours analysis

Carotenoids are known as pigments responsible for many colours of leaves, fruits, and flowers in plants. The colour depends on their growth, maturity and concentration of carotenoid isomers. The presence of various pigments like α-carotene, β-carotene, β-cryptoxanthin, lycopene, and unresolved mixtures of pigments is responsible for the colour of fruits and

vegetables. Carotenoids are heat stable in systems with minimum oxygen content. However, high carotenoid foods may change colour under the influence of thermal processing because heat induces cis-trans isomerization reactions. Colorimetric measurements of smoothies prior to both thermal and ultrasound treatment yielded for smoothie 1: 63.02, 34.13 and 47.02 for L* (lightness), a* (redness) and b* (blueness) parameters respectively. For smoothie 2 a higher value of b* parameters 54.51 was observed (Table 17). With regard to CIELAB parameters, statistical analysis of the results obtained after sterilization showed a significant difference ($p < 0.05$) for the b* values compared to untreated samples. This finding is supported by investigation from Walkling-Ribeiro et al. (2012) who found a significant decrease and b* parameters when mild pasteurization (72 ºC, 15 s) in fruits smoothies was applied. Compared to the untreated smoothies, non-significant changes in a* values were found in both smoothies after each treatment was applied. Moreover, no-significant change after ultrasound treatment in all parameters was observed. Additionally, a slight increase in L* in smoothie 2 was observed but non-significant. This is in agreement with prior investigations when Sengun et al. 2004 observed an increase in lightness (L*) but a decrease in blue yellow (b*) in ultrasound-treated grape juice compared with non-treated juice. Furthermore, Zenker et al. 2003 observed an increase in lightness of ultrasound-treated orange juice. Moreover, Pala et al. 2015 did not find significant changes in the redness (a*) of the juice after the ultrasound treatment at an amplitude of 75 and 100 % for up to 24 and 12 min, respectively. These results suggest that ultrasound treatment has a potential uses to be as an alternative non-thermal technique for traditional thermal pasteurization processes for maintaining the quality of smoothies from fruit and vegetables.

Table 17. Colour changes of different smoothies before and after different treatments applied.

	L*	a*	b*
SMOOTHIE 1			
UNTREATED	63.02±0.03^{a}	34.13±0.11^{a}	47.02±0.02^{a}
STERILIZATION	60.08±0.13^{a}	32.04±0.05^{ab}	34.11±0.15^{b}
PASTEURIZATION	59.33±0.07^{ab}	34.15±0.07^{a}	47.14±0.05^{a}
ULTRASOUND	59.66±0.05^{a}	33.60±0.41^{a}	44.50±0.28^{ab}
SMOOTHIE 2			
UNTREATED	63.02±0.03^{a}	32.14±0.14^{a}	54.51±0.44^{a}
STERILIZATION	59.08±0.13^{a}	31.81±0.18^{a}	50.10±0.09^{b}
PASTEURIZATION	59.33±0.07^{a}	32.17±0.18^{a}	51.78±0.01^{ab}
ULTRASOUND	64.66±0.05^{ab}	31.22±0.29^{a}	55.20±0.12^{a}

L*: lightness. a*: redness. b*: yellowness. $^{a-c}$ Different lowercase letters in the same column indicate significant statistical difference in the function of the applied treatment ($p > 0.05$).

Liberation and micellarisation of carotenoids content from different smoothies subjected to thermal processing and ultrasound treatment

In order to determine the nutritional efficacy of the recovered compounds, quantification directly from foodstuff is not enough for the prediction of potential *in vivo* effects. Therefore, determination of valuable compound bioaccessibility is necessary. In the present study, we estimate the liberation and micellarisation of carotenoids from different smoothies to obtain an effective tool for the initial screening of carotenoid bioaccessibility.

The analysis regarding carotenoid content during the simulated gastrointestinal *in vitro* digestion are shown in Figure 30-33.

As shown below in Figure 30, a significant difference in carotenoid liberation after applying different treatments was observed but percentages were widely. According to experimental results achieved, all treatments

led to an increase in carotenoid liberation in smoothie 1 in comparison with untreated sample.

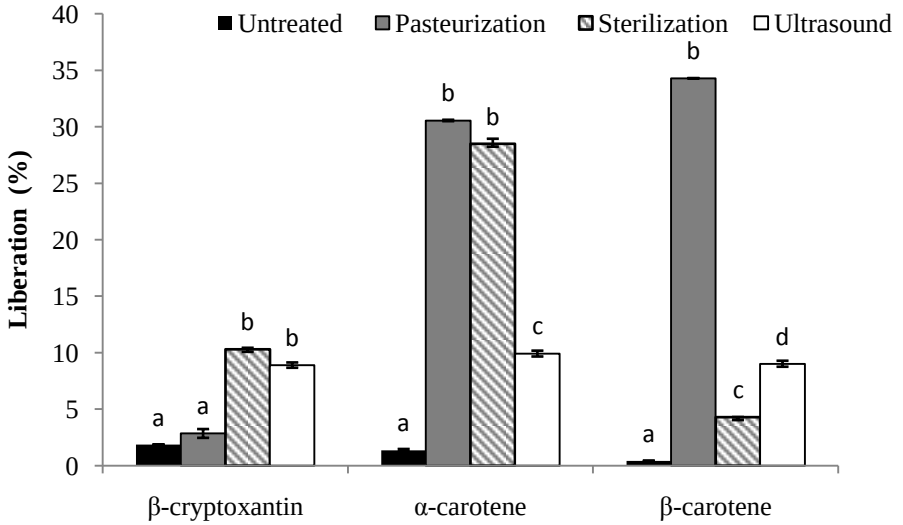


Figure 30. Efficiency of liberation (%) in carotenoids after *in vitro* digestion of smoothie based on carrot juice, papaya and mango (smoothie 1).

This increased in carotenoid liberation after technical processes can be explained by disruption of the natural matrix during food processing. β-cryptoxanthin liberation was in the range 3 %, 10.3 %, 9 % for pasteurization, sterilization and ultrasound treatment respectively. The highest liberation (%) was obtain for β-carotene after pasteurization treatment (34.2 %). This result is in line with the study performed by Lemmens et al. (2009) who found an improvement in β-carotene accessibility after thermal treatments. In this way, Knockaert et al. (2011) revealed that the pasteurization processes cause an increase in β-carotene liberation in carrot juice. Moreover, α-carotene liberation was enhanced significantly after both thermal treatments in comparision with untreated sample. Furthermore, Aschoff et al. (2015) observed a higher carotenoid

liberation in pasteurized orange juice 53.9 % in comparision with freshly squeezed juice 44.9 %. Other author, Stinco et al. (2012) observed difference in carotenoid liberation between fresh orange juice where he detected 30 % and industrially squeezed and pasteurized juice which liberation value up to 52 %. With regard to ultrasound treatment, liberation of all carotenoids was in the range of 9±0.2 %. This may indicate that ultrasound waves during cavitation destroy plant cell walls and cause the extraction of more bioactive compounds which enhance liberation. However, enhancement was not so high, this fact can be explained by treatment condition (temperature 60 ºC over 20 min). In one study Bengtsson et al. (2010) an improvement of β-carotene after grinding treatments applied was observed.

Regarding micellarisation (%), a significant difference after applying different treatments was observed. The results are shown in Figure 31.

Generally, all cases shown higher liberation value compered to bioaccessibility, is in accordance with previous study which reported significantly lowered carotenoid levels after microfiltration of the aqueous supernantant (Bengtsson et al., 2009; Schweiggert et al., 2012). In our study micellarisation (%) was in the range (0.4-11.09 %) As we can observed values of β-carotene after micellarisation stage was limited in the unprocessed sample (below 0.5 %). A smoothie subjected to sterilization process showed significantly higher β-cryptoxanthin and α-carotene micellarisation as compared to the raw unprocessed smoothie. Although carotenoid retention may be negatively affected by thermal treatments, this does not necessarily affect their transfer to micelles. According to Fernández-García et al. (2012), the degree of food processing is significant for micellarisation efficiency of carotenoids, as a high processing degree can maximise the amount of compound that is made soluble from the matrix.

On the other hand, in the case of β-carotene there was no significant difference observed in micellarisation between different treatment applied.

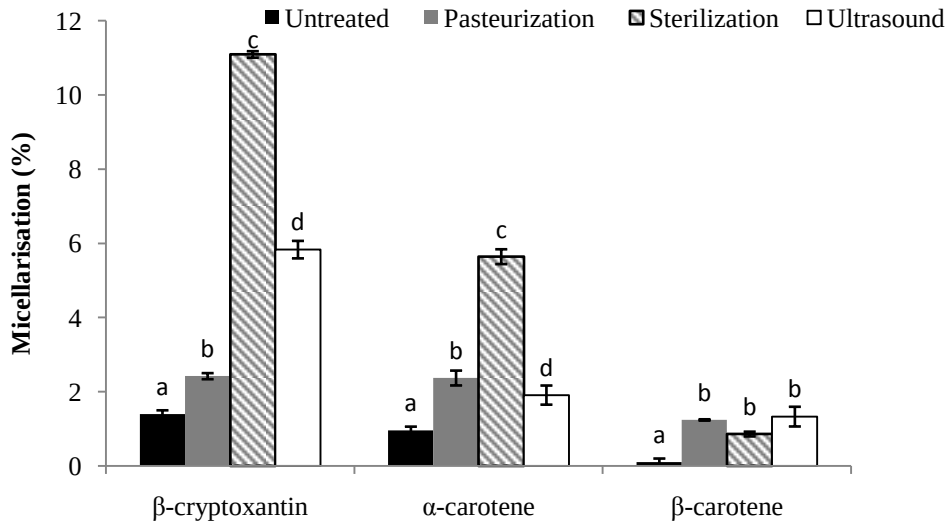


Figure 31. Efficiency of micellarisation (%) in carotenoids after *in vitro* digestion of smoothie based on carrot juice, papaya and mango (smoothie 1).

Our results are similar to those reported by Gupta et al. (2011) who detected that micellarized β-carotene did not differ significantly between the treatments applied. In terms of smoothie 2 (Figure 32), liberation (%) in all carotenoids after different treatments applied was in the range from 5.8 % to 73 %. Surprisingly, no significant difference was found between the quantities of lutein in the digesta compared with different treatments. Moreover, all three methods applied enhanced lutein micellarisation (Figure 33). Regarding liberation, a significant increase was observed when sterilization treatment was used, in range 66 %, 73 % for α-carotene and β-carotene, respectively. This would imply that at the chromoplast level, different matrices with different compositions,modified differently upon processing depending on their structural characteristics.

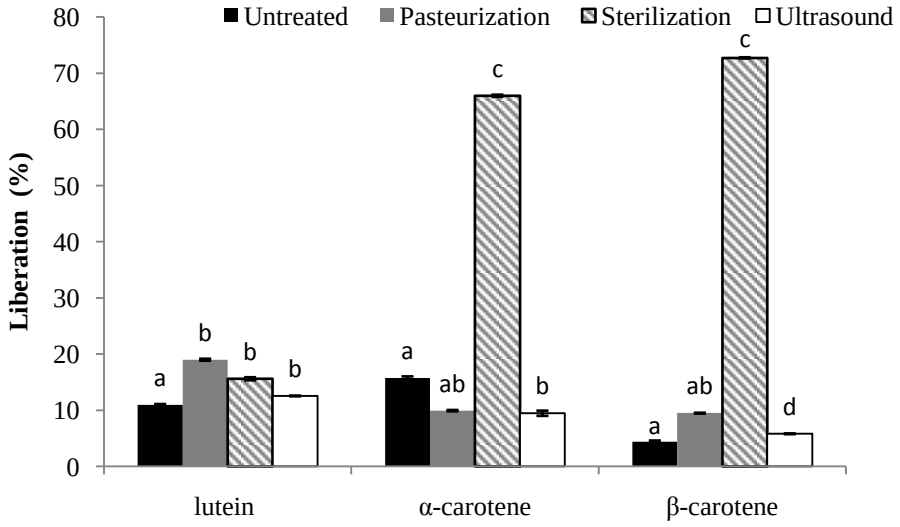


Table 32. Efficiency of liberation (%) in carotenoids after *in vitro* digestion of smoothie based on carrot juice, pumpkin and mango (smoothie 2).

Micellarisation in smoothie 2 (Figure 33) was significantly lower for α-carotene and β-carotene in comparison with smoothie 1 and was in range 1.1-5.4 % and 0.2-3.1 %, respectively. Pasteurization generally reduced ($p < 0.01$) β-carotene micellarisation. Efficiency of micellarisation may be dependet on the type of food matrix and/or the composition the final digesta in which they are contained (Grando-Lorencio et al., 2007). Only 0.5 % of β-carotene in untreated sample was observed in the micells. Moreover, after pasteurization and ultrasound a low percentage of α-carotene (1.1 and 2.4 %) micellarisation was observed. Furthermore, lower percentage for β-carotene (0.2 and 0.5 %) micellarisation was detected. Lutein micellarisation was in the range of 26.1 %, 35.2 % and 18.9 % for pasteurization, sterilization and ultrasound treatment respectively. In comparison with other carotenoids, lutein showed a greater extent in the digesta from smoothies after treatment applied.

Generally, α-carotene and β-carotene were statistically less efficiently transferred to the micelles than lutein.

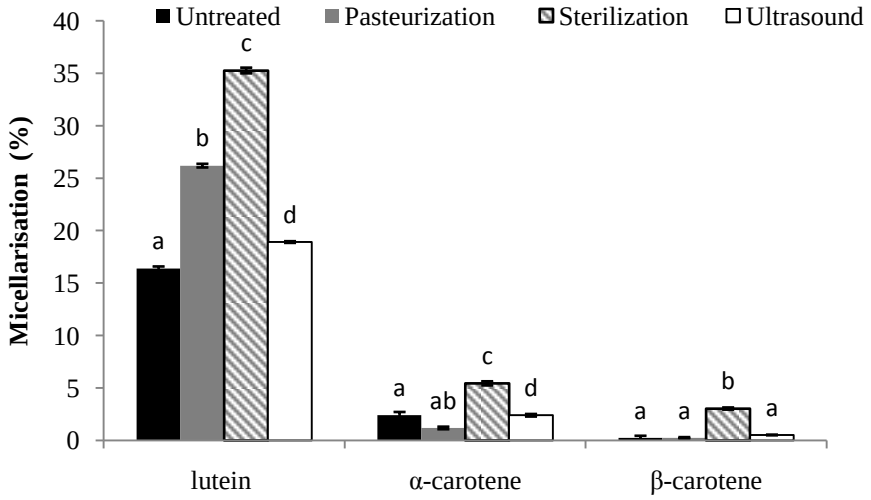


Table 33. Efficiency of micellarisation (%) in carotenoids after *in vitro* digestion of smoothie based on carrot juice, pumpkin and mango (smoothie 2).

This fact can be explain it by lower lipophilicity of lutein (Van het Hof et al., 2000). In relation with our results, other authors Ryan et al. (2008) also observed higher micellarisation (%) in lutein after different thermal treatments were applied. Accordance with Rich et al. (2003) study a big difference between lutein and other carotenoid micellarisation, is that a lutein in vegetables is more soluble than β-carotene in the micelle fraction.

6.4.3 Conclusions

An improved microbiological shelf-life was achieved in a fruit smoothie using both thermal treatments. The assessment of colour was found to be slightly better for ultrasound smoothies than thermal treated smoothies. Although thermal treatment reduced the content of carotenoids in foods, a positive effect of liberation, micellarisation and therefore bioaccessibility was found.

CONCLUSIONS

Los resultados permiten establecer las siguientes conclusiones:

1. Al aplicar diferentes tratamientos térmicos en una bebida a base de papaya y mango mezclada con zumo de naranja, avena, y acaí, adicionando distintas concentraciones de *stevia* se obtiene que:

- La temperatura aplicada y la concentración de *stevi*a influyen en los parámetros fisicoquímicos y la concentración de ácido ascórbico. Mientras que carotenoides totales y antocianinas totales dependen de las tres variables (temperatura, tiempo y *stevia*).

- La capacidad antioxidante depende de la concentración de *stevia* adicionada cuando se determina mediante el método TEAC. Sin embargo, cuando se aplica el método ORAC los resultados dependen de la temperatura y del tiempo de tratamiento.

- Los glicósidos de esteviol (rebaudiosido A, C y F, y esteviosido) varían con la temperatura y tal como era de esperar, con la concentración de *stevia*.

- Al aplicar 80ºC durante 6.2 min y adición de *stevia* al 2.5 % (v/v) a la bebida objeto de estudio, se máximiza el contenido de glicósidos de esteviol, compuestos bioactivos y capacidad antioxidante.

2. La energía (32 kJ/kg y 256 kJ/kg) de los tratamientos no térmicos estudiados (pulsos eléctricos de alta intensidad, descargas eléctricas de alto voltaje y ultrasonidos) en la bebida a base de papaya, mango y *stevia* al 2.5 % es la misma, sin embargo los resultados obtenidos difieren según la tecnología aplicada, destacando que:

- El tratamiento por descargas eléctricas de alto voltaje permite obtener el mayor contenido de compuestos fenólicos totales a 256 kJ/kg. Mientras que, los pulsos eléctricos de alta intensidad incrementa la concentración de carotenoides totales a 32 kJ/kg y los ultrasonidos minimizan la degradación del ácido ascórbico.

- Al comparar con la bebida no tratada, la bioaccesibilidad de los carotenoides totales y compuestos fenólicos totales aumenta después de cada tratamiento no térmico, excepto en los compuestos fenólicos totales que disminuyen significativamente al aplicar las descargas eléctricas de alta intensidad a 256 kJ/kg. Sin embargo, únicamente se observan cambios significativos en la bioaccesibilidad de las antocianinas totales en la bebida tratada por descargas eléctricas de alta intensidad a 32 kJ/kg.

3. Las electrotecnologías aplicadas (PE y DEAV) a la piel de naranja permiten una mejor extracción de ácido ascórbico, compuestos fenólicos totales y mayor capacidad antioxidante del extracto obtenido, independientemente del tratamiento. Los carotenoides totales no se detectan cuando se aplican los PE.

4. Respecto al porcentaje de la bioaccesibilidad de los compuestos fenólicos totales y capacidad antioxidante total (ORAC y TEAC), del extracto de piel de naranja obtenido al aplicar PE y DEAV, siempre es menor a la máxima intensidad independientemente del tratamiento. En el caso de los carotenoides totales, es ligeramente superior a la máxima intensidad aplicada.

5. Cuando se preparan smoothies de frutas y vegetales y se aplican tecnologías térmicas (esterilización y pasterización) y no térmicas (ultrasonidos) el contenido de los distintos carotenoides estudiados (β-cryptoxantina, luteína, α-caroteno y β-caroteno) es menor a mayor temperatura aplicada. Sin embargo, se observa un efecto positivo de la temperatura en la liberación de los carotenoides y su micelarización.

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ANNEX

IMPACTO DEL TRATAMIENTO TÉRMICO, TIEMPO, % *STEVIA* EN LAS CARACTERISTICAS DE LA BEBIDA BASE DE PAPAYA, MANGO MEZCALADA CON ZUMO DE NARANJA, BEBIDA DE AVENA, ACAÍ Y *STEVIA* TRATADA A DIFERENTES TIEMPOS Y TEMPERATURAS

Análisis de varianza (ANOVA) de tres factores para los valores de **pH**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	0.000513746	1	0.000513746	2.67	0.1156
A:Time (min)	0.00589544	3	0.00196515	10.23	0.0002
B:% *Stevia*	0.0914232	2	0.0457116	237.89	0.0000
INTERACCIONES					
AB	0.00482476	6	0.000804127	4.18	0.0055
RESIDUOS	0.00441959	23	0.000192156		
TOTAL (CORREGIDO)	0.149089	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de **ºBrix**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	0.803387	1	0.803387	20.09	0.0002
A:Time (min)	0.532784	3	0.177595	4.44	0.0133
B:% Stevia	18.0086	2	9.0043	225.12	0.0000
INTERACCIONES					
AB	0.398615	6	0.0664359	1.66	0.1759
RESIDUOS	0.919946	23	0.0399977		
TOTAL (CORREGIDO)	22.4489	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de **índce de turbidez**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	0.00601959	1	0.00601959	12.63	0.0017
A:Time (min)	0.00767618	3	0.00255873	5.37	0.0060
B:*% Stevia*	0.423925	2	0.211963	444.69	0.0000
INTERACCIONES					
AB	0.0492106	6	0.00820177	17.21	0.0000
RESIDUOS	0.010963	23	0.000476652		
TOTAL (CORREGIDO)	0.498001	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de **índce de pardeamiento**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	0.000672473	1	0.000672473	0.34	0.5633
A:Time (min)	0.0129946	3	0.00433152	2.22	0.1135
B:% Stevia	28.4073	2	14.2036	7264.83	0.0000
INTERACCIONES					
AB	0.00886662	6	0.00147777	0.76	0.6115
RESIDUOS	0.0449678	23	0.00195512		
TOTAL (CORREGIDO)	31.7531	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de **hydroxymethylfurfural**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	0.837876	1	0.837876	54.02	0.0000
A:Time (min)	0.877111	3	0.29237	18.85	0.0000
B:% *Stevia*	0.157344	2	0.0786721	5.07	0.0150
INTERACCIONES					
AB	0.14632	6	0.0243866	1.57	0.2002
RESIDUOS	0.356747	23	0.0155107		
TOTAL (CORREGIDO)	2.73682	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de **color: a***

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	0.147811	1	0.147811	1.30	0.2664
A:*% Stevia*	43.7728	2	21.8864	192.11	0.0000
B:Time (min)	0.554639	3	0.18488	1.62	0.2115
INTERACCIONES					
AB	1.53475	6	0.255791	2.25	0.0750
RESIDUOS	2.62035	23	0.113928		
TOTAL (CORREGIDO)	56.1707	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de color: **b***

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	13.6041	1	13.6041	28.02	0.0000
A:Time (min)	13.2588	3	4.4196	9.10	0.0004
B:*% Stevia*	331.983	2	165.991	341.94	0.0000
INTERACCIONES					
AB	3.45624	6	0.576039	1.19	0.3479
RESIDUOS	11.1653	23	0.485446		
TOTAL (CORREGIDO)	401.182	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de color: **L***

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperature (ºC)	5.82067	1	5.82067	56.18	0.0000
A:Time (min)	0.792118	3	0.264039	2.55	0.0819
B:% Stevia	2592.95	2	1296.48	12513.56	0.0000
INTERACCIONES					
AB	3.22182	6	0.536969	5.18	0.0019
RESIDUOS	2.27933	22	0.103606		
TOTAL (CORREGIDO)	3336.37	34			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de contenido en **ácido ascorbico**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	23.4621	1	23.4621	589.09	0.0000
A:Time (min)	11.7308	3	3.91025	98.18	0.0000
B:% *Stevia*	0.159823	2	0.0799113	2.01	0.1573
INTERACCIONES					
AB	0.61574	6	0.102623	2.58	0.0466
RESIDUOS	0.916038	23	0.0398277		
TOTAL (CORREGIDO)	45.2396	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de contenido de **fenoles totales**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	3382.25	1	3382.25	0.03	0.8663
A:Time (min)	2.04009E6	3	680031.	5.83	0.0041
B:*% Stevia*	9.2806E7	2	4.6403E7	397.53	0.0000
INTERACCIONES					
AB	3.16018E6	6	526697.	4.51	0.0037
RESIDUOS	2.68474E6	23	116728.		
TOTAL (CORREGIDO)	1.14882E8	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de contenido de **carotenoides totales**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	2473.39	1	2473.39	1.74	0.2005
A:Time (min)	17475.4	3	5825.12	4.09	0.0182
B:% *Stevia*	14731.8	2	7365.92	5.17	0.0140
INTERACCIONES					
AB	15646.0	6	2607.67	1.83	0.1371
RESIDUOS	32750.2	23	1423.92		
TOTAL (CORREGIDO)	85681.2	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Comparación de desviaciones estándar métodos TEAC-ORAC

	ORAC (mM TE)	*TEAC (mM TE)*
Desviación Estándar	12.0958	9.98853
Varianza	146.309	99.7708
Gl	35	35

Razón de Varianzas= 1.46645

Intervalos de confianza del 95.0%
Desviación Estándar de ORAC (mM TE): [9.8107; 15.7783]
Desviación Estándar de TEAC (mM TE): [8.10151; 13.0294]
Razones de Varianzas: [0.747774; 2.87584]

Prueba-F para comparar Desviaciones Estándar
Hipótesis Nula: sigma1 = sigma2
Hipótesis Alt.: sigma1 <> sigma2
F = 1.46645 valor-P = 0.262274
No se rechaza la hipótesis nula para alfa = 0.05.

Análisis de varianza (ANOVA) de tres factores para los valores de **Reb A**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	392.64	1	392.64	8.05	0.0093
EFECTOS PRINCIPALES					
A:Time (min)	494.567	3	164.856	3.38	0.0355
B:*% Stevia*	403205.	2	201602.	4132.41	0.0000
INTERACCIONES					
AB	2466.72	6	411.12	8.43	0.0001
RESIDUOS	1122.07	23	48.7856		
TOTAL (CORREGIDO)	462186.	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de **esteviosidos**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	51.3324	1	51.3324	0.09	0.7718
EFECTOS PRINCIPALES					
A:Time (min)	8058.85	3	2686.28	4.51	0.0125
B:*% Stevia*	2.19271E6	2	1.09635E6	1839.93	0.0000
INTERACCIONES					
AB	7004.54	6	1167.42	1.96	0.1137
RESIDUOS	13704.9	23	595.867		
TOTAL (CORREGIDO)	2.47397E6	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de **Reb F**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	23.1163	1	23.1163	18.41	0.0003
EFECTOS PRINCIPALES					
A:Time (min)	21.0844	3	7.02815	5.60	0.0049
B:*% Stevia*	918.296	2	459.148	365.69	0.0000
INTERACCIONES					
AB	40.3275	6	6.72125	5.35	0.0014
RESIDUOS	28.8779	23	1.25556		
TOTAL (CORREGIDO)	1182.54	35			

Todas las razones-F se basan en el cuadrado medio del error residual

Análisis de varianza (ANOVA) de tres factores para los valores de **Reb C**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
COVARIABLES					
Temperatura (ºC)	84.4107	1	84.4107	7.39	0.0123
EFECTOS PRINCIPALES					
A:Time (min)	44.7747	3	14.9249	1.31	0.2963
B:*% Stevia*	17624.2	2	8812.12	771.40	0.0000
INTERACCIONES					
AB	148.574	6	24.7624	2.17	0.0839
RESIDUOS	262.742	23	11.4235		
TOTAL (CORREGIDO)	20313.9	35			

Todas las razones-F se basan en el cuadrado medio del error residual

IMPACTO DEL TRATAMIENTO PE, DEAV y USN Y LA INTENSIDAD DE LA ENERGÍA APLICADA A COMPUESTOS BIOACTIVOS EN LA BEBIDA A BASE DE PAPAYA, MANGO Y *STEVIA*

Análisis de varianza (ANOVA) de dos factores para los valores de contenido en **carotenoides**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:Tratamiento	198163.	3	66054.2	56.53	0.0000
B:Intensidad	24111.1	1	24111.1	20.63	0.0027
INTERACCIONES					
AB	49856.4	3	16618.8	14.22	0.0023
RESIDUOS	8179.85	7	1168.55		
TOTAL (CORREGIDO)	289133.	14			

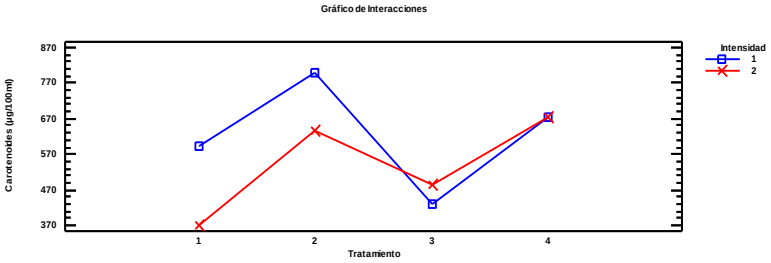
Todas las razones-F se basan en el cuadrado medio del error residual

Método: 95.0 porcentaje Tukey HSD

Tratamiento	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
3	4	457.835	17.092	X
1	4	481.537	17.092	X
4	3	676.174	20.9334	X
2	4	717.315	17.092	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	-235.778	79.8796
1 - 3		23.7023	79.8796
1 - 4	*	-194.637	89.3081
2 - 3	*	259.481	79.8796
2 - 4		41.1413	89.3081
3 - 4	*	-218.34	89.3081

* indica una diferencia significativa.



Análisis de varianza (ANOVA) de dos factores para los valores de contenido en **Vitamina C**

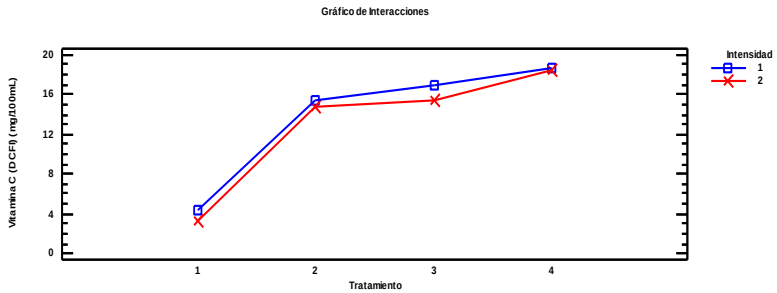
Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:Tratamiento	480.827	3	160.276	1128.87	0.0000
B:Intensidad	2.18405	1	2.18405	15.38	0.0057
INTERACCIONES					
AB	0.780773	3	0.260258	1.83	0.2291
RESIDUOS	0.99385	7	0.141979		
TOTAL (CORREGIDO)	495.097	14			

Todas las razones-F se basan en el cuadrado medio del error residual

Tratamiento	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
1	4	3.77	0.1884	X
2	4	15.075	0.1884	X
3	4	16.1925	0.1884	X
4	3	18.55	0.230742	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	-11.305	0.880488
1 - 3	*	-12.4225	0.880488
1 - 4	*	-14.78	0.984416
2 - 3	*	-1.1175	0.880488
2 - 4	*	-3.475	0.984416
3 - 4	*	-2.3575	0.984416

* indica una diferencia significativa.



Análisis de varianza (ANOVA) de dos factores para los valores de contenido en **Fenoles totales**

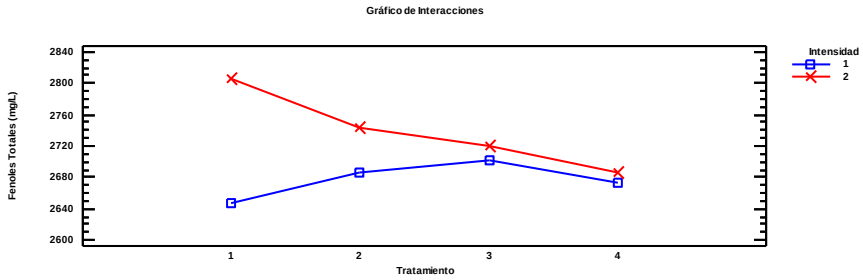
Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:Tratamiento	3685.61	3	1228.54	0.70	0.5833
B:Intensidad	13867.2	1	13867.2	7.86	0.0264
INTERACCIONES					
AB	12737.2	3	4245.73	2.41	0.1529
RESIDUOS	12356.3	7	1765.19		
TOTAL (CORREGIDO)	45147.9	14			

Todas las razones-F se basan en el cuadrado medio del error residual

Tratamiento	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
4	3	2679.08	25.7283	X
3	4	2711.52	21.0071	X
2	4	2714.77	21.0071	X
1	4	2726.12	21.0071	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2		11.355	98.1765
1 - 3		14.599	98.1765
1 - 4		47.041	109.765
2 - 3		3.244	98.1765
2 - 4		35.686	109.765
3 - 4		32.442	109.765

* indica una diferencia significativa



Análisis de varianza (ANOVA) de dos factores para los valores de contenido en **Antocianinas totales**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:Tratamiento	2.64879	3	0.88293	2.31	0.1636
B:Intensidad	1.87534	1	1.87534	4.90	0.0625
INTERACCIONES					
AB	0.203564	3	0.0678545	0.18	0.9085
RESIDUOS	2.68095	7	0.382993		
TOTAL (CORREGIDO)	7.62953	14			

Todas las razones-F se basan en el cuadrado medio del error residual

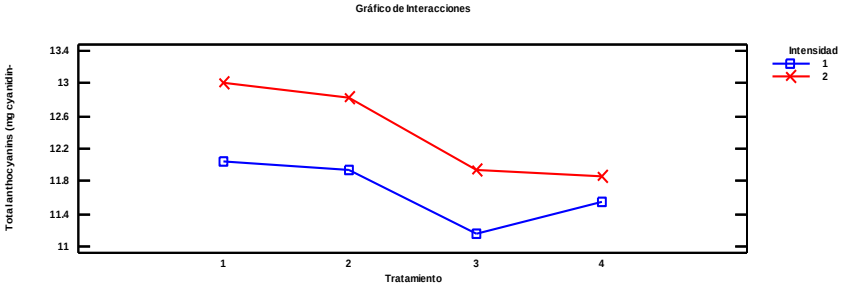
x

Método: 95.0 porcentaje Tukey HSD

Tratamiento	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
3	4	11.5575	0.309432	X
4	3	11.705	0.378975	X
2	4	12.385	0.309432	X
1	4	12.53	0.309432	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2		0.145	1.44613
1 - 3		0.9725	1.44613
1 - 4		0.825	1.61682
2 - 3		0.8275	1.44613
2 - 4		0.68	1.61682
3 - 4		-0.1475	1.61682

* indica una diferencia significativa.

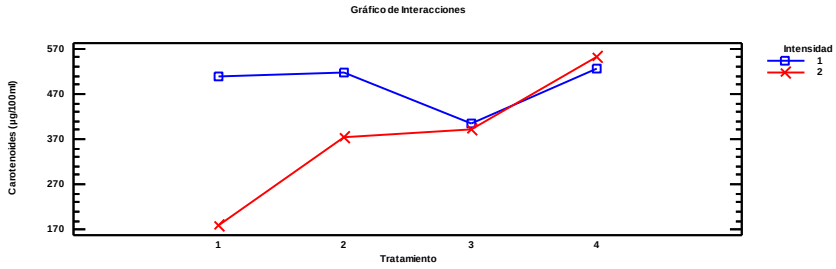


TRATAMIENTO: 1: DEAV; 2: PE; 3:USN; 4: no tratado
INTENSIDAD: 1: 32 kJ/kg; 2: 256 kJ/kg

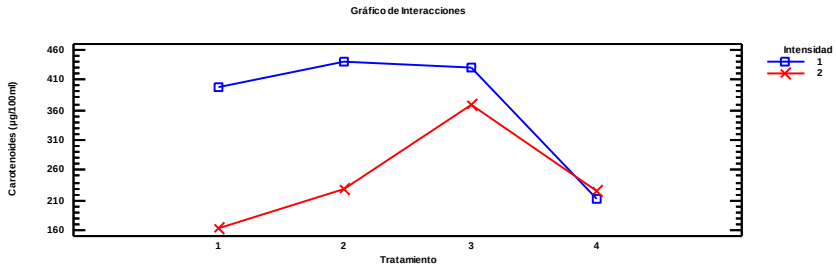
IMPACTO DEL TRATAMIENTO PE, DEAV y USN Y LA INTENSIDAD DE LA ENERGÍA APLICADA A COMPUESTOS BIOACTIVOS DURANTE 3 ETAPAS DE DIGESTION

CAROTENOIDES

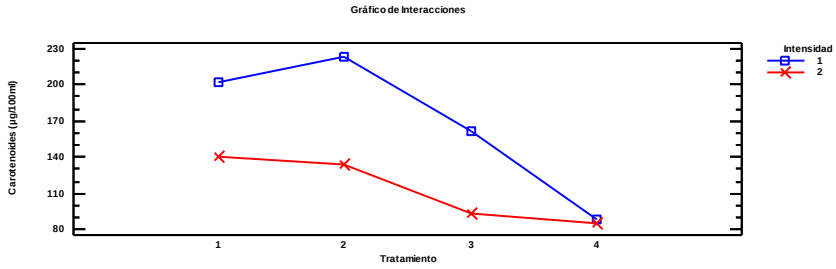
I ETAPA: **GASTRIC**



II ETAPA: **INTESTINAL**



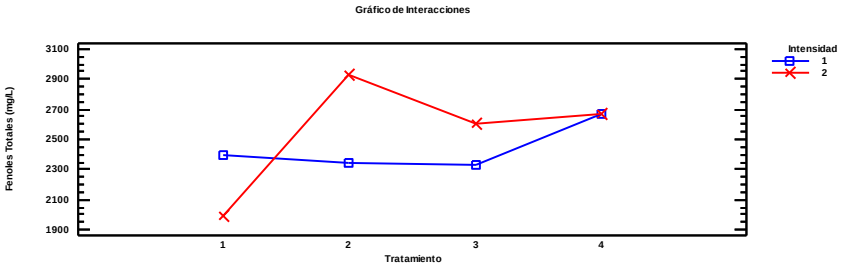
III ETAPA: **DIALIZADO**



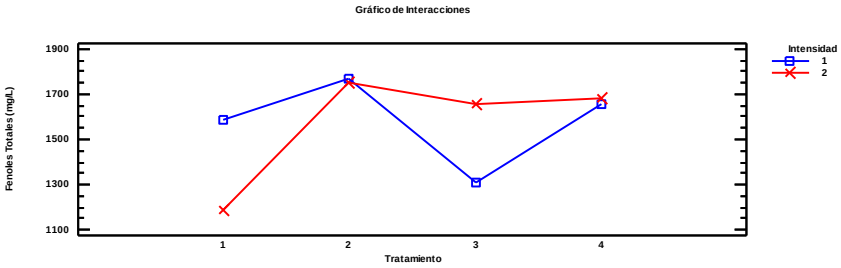
TRATAMIENTO: 1: DEAV; 2: PE; 3:USN; 4: no tratado
INTENSIDAD: 1: 32 kJ/kg; 2: 256 kJ/kg

FENOLES TOTALES

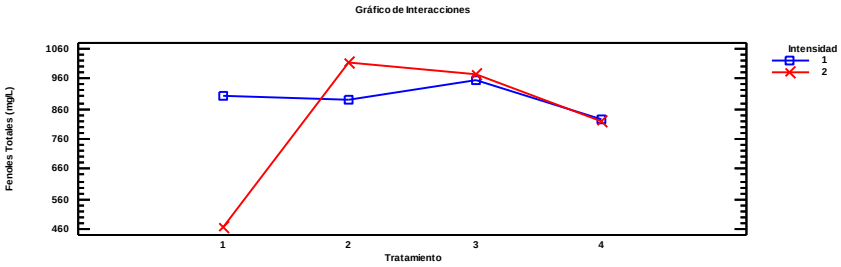
I ETAPA: **GASTRIC**



II ETAPA: **INTESTINAL**



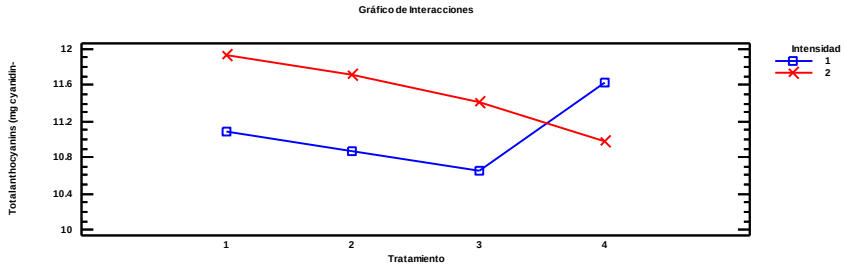
III ETAPA: **DIALIZADO**



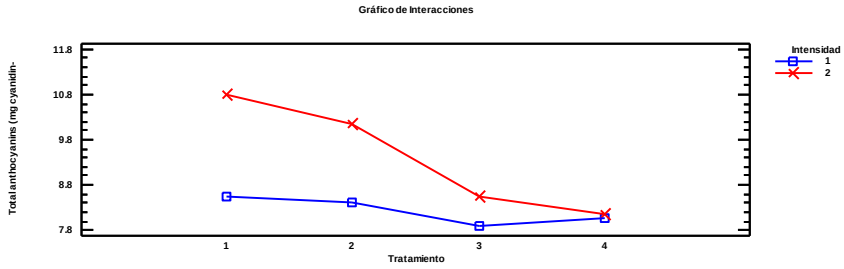
TRATAMIENTO: 1: DEAV; 2: PE; 3:USN; 4: no tratado
INTENSIDAD: 1: 32 kJ/kg; 2: 256 kJ/kg

ANTOCIANINAS TOTALES

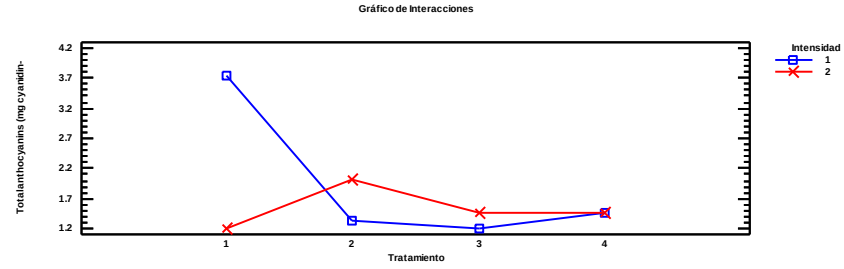
I ETAPA: **GASTRIC**



II ETAPA: **INTESTINAL**



III ETAPA: **DIALIZADO**



TRATAMIENTO: 1: DEAV; 2: PE; 3:USN; 4: no tratado
INTENSIDAD: 1: 32 kJ/kg; 2: 256 kJ/kg

BIOACESIBILIDAD DE LA BEBIDA A BASE DE PAPAYA, MANGO Y *STEVIA* TRATADA POR PEF, DEAV Y USN

CAROTENOIDES TOTALES

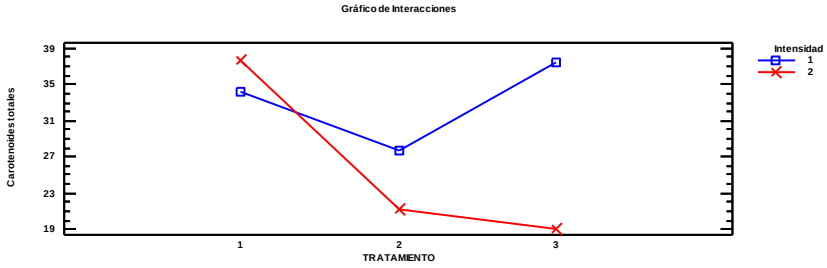
Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:TRATAMIENTO	212.932	2	106.466	5591.71	0.0000
B:Intensidad	130.968	1	130.968	6878.57	0.0000
INTERACCIONES					
AB	198.81	2	99.4051	5220.86	0.0000
RESIDUOS	0.0952	5	0.01904		
TOTAL (CORREGIDO)	645.353	10			

Todas las razones-F se basan en el cuadrado medio del error residual

TRATAMIENTO	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
2	4	24.375	0.0689928	X
3	4	28.245	0.0689928	X
1	3	35.875	0.0844985	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	11.5	0.354948
1 - 3	*	7.63	0.354948
2 - 3	*	-3.87	0.317475

* indica una diferencia significativa.



FENOLES TOTALES

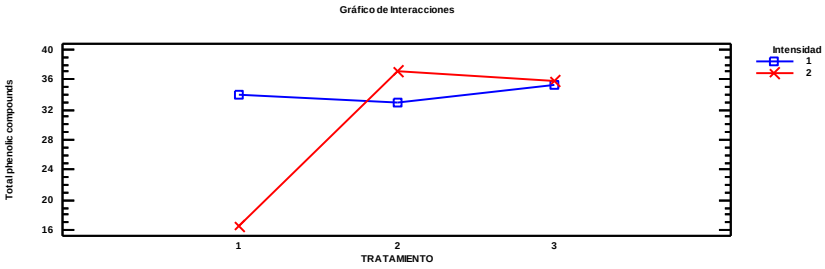
Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:TRATAMIENTO	201.406	2	100.703	14386.16	0.0000
B:Intensidad	47.1779	1	47.1779	6739.69	0.0000
INTERACCIONES					
AB	201.156	2	100.578	14368.30	0.0000
RESIDUOS	0.035	5	0.007		
TOTAL (CORREGIDO)	585.102	10			

Todas las razones-F se basan en el cuadrado medio del error residual

TRATAMIENTO	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
1	3	25.275	0.0512348	X
2	4	35.075	0.041833	X
3	4	35.525	0.041833	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	-9.8	0.215219
1 - 3	*	-10.25	0.215219
2 - 3	*	-0.45	0.192498

* indica una diferencia significativa.



TRATAMIENTO: 1: DEAV; 2: PE: 3: USN
INTENSIDAD: 1: 32 kJ/kg; 2: 256 kJ/kg

ANTOCIANINAS TOTALES

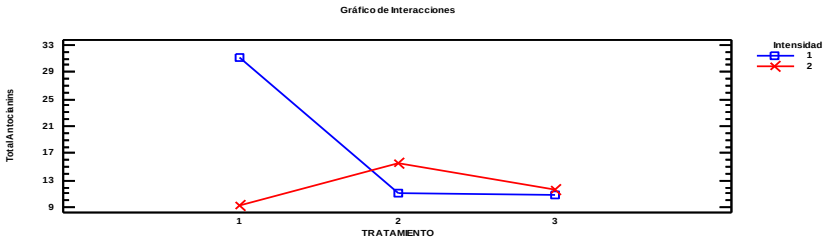
Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:TRATAMIENTO	134.017	2	67.0084	6436.92	0.0000
B:Intensidad	78.8738	1	78.8738	7576.73	0.0000
INTERACCIONES					
AB	308.699	2	154.349	14827.03	0.0000
RESIDUOS	0.05205	5	0.01041		
TOTAL (CORREGIDO)	389.585	10			

Todas las razones-F se basan en el cuadrado medio del error residual

TRATAMIENTO	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
3	4	11.1725	0.0510147	X
2	4	13.275	0.0510147	X
1	3	20.135	0.06248	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	6.86	0.262456
1 - 3	*	8.9625	0.262456
2 - 3	*	2.1025	0.234748

* indica una diferencia significativa.



Comparaciónes de desviaciones estándar métodos: ***TEAC-ORAC***

	TEAC	*ORAC*
Desviación Estándar	4.20035	4.11955
Varianza	17.6429	16.9707
Gl	10	10

Razón de Varianzas= 1.03961

Intervalos de confianza del 95.0%
Desviación Estándar de TEAC: [2.93485; 7.37132]
Desviación Estándar de ORAC: [2.8784; 7.22953]
Razones de Varianzas: [0.279706; 3.86401]

Prueba-F para comparar Desviaciones Estándar
Hipótesis Nula: sigma1 = sigma2
Hipótesis Alt.: sigma1 <> sigma2
F = 1.03961 valor-P = 0.952234
No se rechaza la hipótesis nula para alfa = 0.05.

Comparaciónes de desviaciones estándar métodos: ***TEAC-DPPH***

	TEAC	*DPPH*
Desviación Estándar	4.20035	4.50107
Varianza	17.6429	20.2596
Gl	10	10

Razón de Varianzas= 0.87084

Intervalos de confianza del 95.0%
Desviación Estándar de TEAC: [2.93485; 7.37132]
Desviación Estándar de DPPH: [3.14497; 7.89907]
Razones de Varianzas: [0.234299; 3.23673]

Prueba-F para comparar Desviaciones Estándar
Hipótesis Nula: sigma1 = sigma2
Hipótesis Alt.: sigma1 <> sigma2
F = 0.87084 valor-P = 0.831176
No se rechaza la hipótesis nula para alfa = 0.05.

Comparaciónes de desviaciones estándar métodos: ***ORAC-DPPH***

	ORAC	*DPPH*
Desviación Estándar	4.11955	4.50107
Varianza	16.9707	20.2596
Gl	10	10

Razón de Varianzas= 0.837662

Intervalos de confianza del 95.0%
Desviación Estándar de ORAC: [2.8784; 7.22953]
Desviación Estándar de DPPH: [3.14497; 7.89907]
Razones de Varianzas: [0.225372; 3.11342]

Prueba-F para comparar Desviaciones Estándar
Hipótesis Nula: sigma1 = sigma2
Hipótesis Alt.: sigma1 <> sigma2
F = 0.837662 valor-P = 0.784848
No se rechaza la hipótesis nula para alfa = 0.05.

TRATAMIENTO: 1: DEAV; 2: PE; 3:USN
INTENSIDAD: 1: 32 kJ/kg; 2: 256 kJ/kg

EFECTO DE LOS TRATAMIENTOS TÉRMICOS (PE, DEAV) EN LA PIEL DE NARANJA

Análisisde la varianza (ANOVA) dedos factores para los valores de contenido en **carotenoides totales**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
A:TRATAMIENTO	169625.	1	169625.	40709.90	0.0000
B:Intensidad	2396.3	1	2396.3	575.11	0.0002
INTERACCIONES					
AB	2396.3	1	2396.3	575.11	0.0002
RESIDUOS	12.5	3	4.16667		
TOTAL (CORREGIDO)	171631.	6			

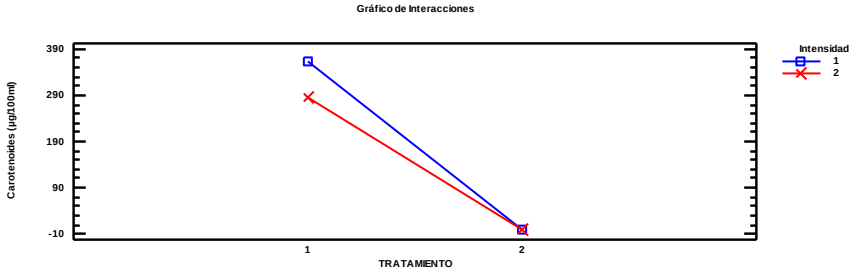
Todas las razones-F se basan en el cuadrado medio del error residual

Método: 95.0 porcentaje Tukey HSD

TRATAMIENTO	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
2	4	0	1.02062	X
1	3	325.6	1.25	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	325.6	5.13566

* indica una diferencia significativa.



Análisisde la varianza (ANOVA) dedos factores para los valores de contenido en **acido ascórbico**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
A:TRATAMIENTO	105.625	1	105.625	455.94	0.0002
B:Intensidad	27.889	1	27.889	120.38	0.0016
INTERACCIONES					
AB	4.761	1	4.761	20.55	0.0201
RESIDUOS	0.695	3	0.231667		
TOTAL (CORREGIDO)	164.337	6			

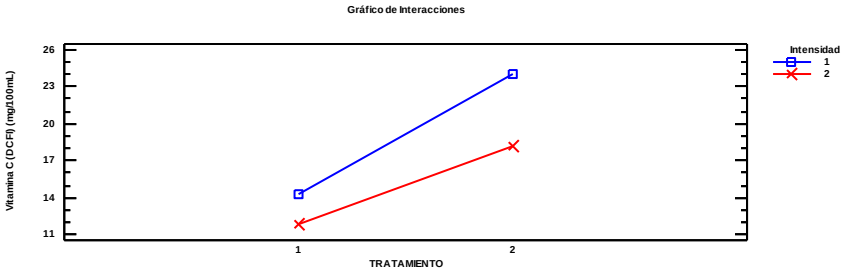
Todas las razones-F se basan en el cuadrado medio del error residual

TRATAMIENTO: 1: DEAV; 2: PE
INTENSIDAD: 1: 55 kJ/kg; 2: 364 kJ/kg

TRATAMIENTO	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
1	3	12.975	0.294746	X
2	4	21.1	0.240659	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	-8.125	1.21097

* indica una diferencia significativa.



Análisisde la varianza (ANOVA) de dos factores para los valores de contenido en **fenoles totales**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:TRATAMIENTO	43099.2	1	43099.2	100620.76	0.0000
B:Intensidad	298910.	1	298910.	697843.24	0.0000
INTERACCIONES					
AB	8265.62	1	8265.62	19297.18	0.0000
RESIDUOS	1.285	3	0.428333		
TOTAL (CORREGIDO)	404821.	6			

Todas las razones-F se basan en el cuadrado medio del error residual

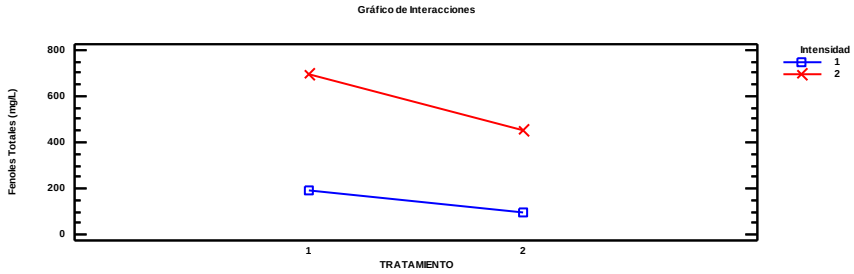
Método: 95.0 porcentaje Tukey HSD

TRATAMIENTO	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
2	4	275.925	0.327236	X
1	3	440.05	0.40078	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	164.125	1.64662

* indica una diferencia significativa.

TRATAMIENTO: 1: DEAV; 2: PE
INTENSIDAD: 1: 55 kJ/kg; 2: 364 kJ/kg



Análisis de la varianza (ANOVA) dedos factores para los valores de **TEAC**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:TRATAMIENTO	0.4	1	0.4	3.33	0.1654
B:Intensidad	13.456	1	13.456	112.13	0.0018
INTERACCIONES					
AB	0.784	1	0.784	6.53	0.0835
RESIDUOS	0.36	3	0.12		
TOTAL (CORREGIDO)	15.9143	6			

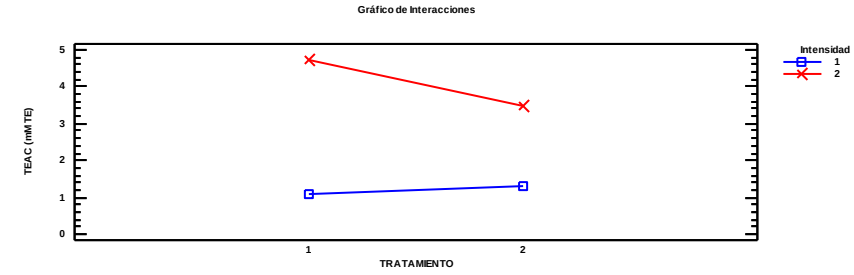
Todas las razones-F se basan en el cuadrado medio del error residual

Método: 95.0 porcentaje Tukey HSD

TRATAMIENTO	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
2	4	2.4	0.173205	X
1	3	2.9	0.212132	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2		0.5	0.87155

* indica una diferencia significativa.



TRATAMIENTO: 1: DEAV; 2: PE
INTENSIDAD: 1: 55 kJ/kg; 2: 364 kJ/kg

Análisis de la varianza (ANOVA) de dos factores para los valores de **ORAC**

Fuente	*Suma de Cuadrados*	*Gl*	*Cuadrado Medio*	*Razón-F*	*Valor-P*
EFECTOS PRINCIPALES					
A:TRATAMIENTO	2.401	1	2.401	288.12	0.0004
B:Intensidad	15.625	1	15.625	1875.00	0.0000
INTERACCIONES					
AB	1.369	1	1.369	164.28	0.0010
RESIDUOS	0.025	3	0.00833333		
TOTAL (CORREGIDO)	21.9886	6			

Todas las razones-F se basan en el cuadrado medio del error residual

Método: 95.0 porcentaje Tukey HSD

TRATAMIENTO	*Casos*	*Media LS*	*Sigma LS*	*Grupos Homogéneos*
2	4	2.4	0.0456435	X
1	3	3.625	0.0559017	X

Contraste	*Sig.*	*Diferencia*	*+/- Límites*
1 - 2	*	1.225	0.229673

* indica una diferencia significativa.

Comparación de desviaciones estándar ***TEAC-ORAC***

	TEAC (mM TE)	*ORAC (mM TE)*
Desviación Estándar	1.62861	1.91436
Varianza	2.65238	3.66476
Gl	6	6

Razón de Varianzas= 0.723753

Intervalos de confianza del 95.0%
Desviación Estándar de TEAC: [1.04947; 3.58631]
Desviación Estándar de ORAC: [1.2336; 4.21554]
Razones de Varianzas: [0.124361; 4.21207]

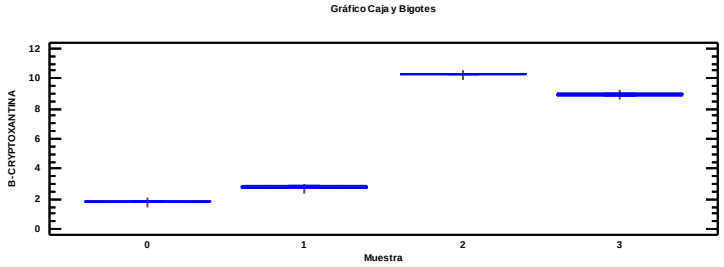
Prueba-F para comparar Desviaciones Estándar
Hipótesis Nula: sigma1 = sigma2
Hipótesis Alt.: sigma1 <> sigma2
F = 0.723753 valor-P = 0.704621
No se rechaza la hipótesis nula para alfa = 0.05

TRATAMIENTO: 1: DEAV; 2: PE
INTENSIDAD: 1: 55 kJ/kg; 2: 364 kJ/kg

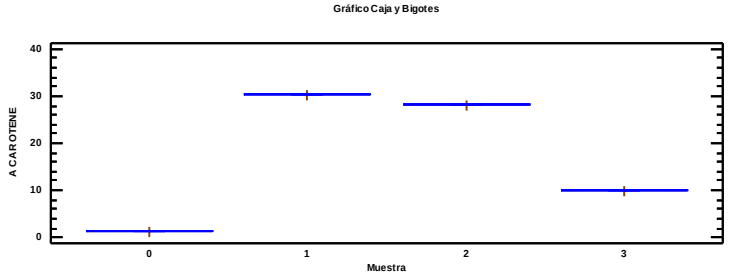
IMPACTO DE TRATAMIENTOS TERMICOS (ESTERILIZACIÓN, PASTERIZACIÓN), Y NO TERMICOS (ULTRASONIDOS) A LOS CAROTENOIDES EN DOS DIFERENTES SMOOTHIES

SMOOTHIE 1 – ETAPA I: LIBERACIÓN

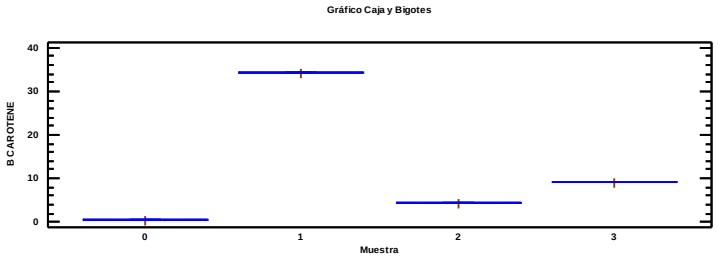
β- cryptoxantina



α- caroteno



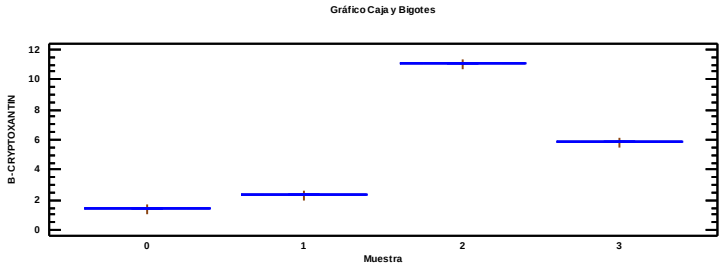
β- caroteno



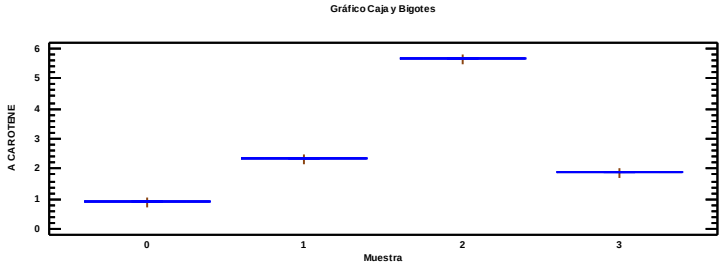
MUESTRA: 1: no tratada; 2: pasterización; 3: esterilización; 4: ultrasonidos

SMOOTHIE 1 – ETAPA II: MICELARIZACIÓN

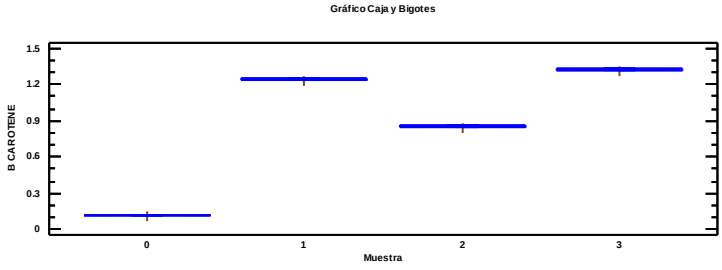
β- criptoxantina



α-caroteno



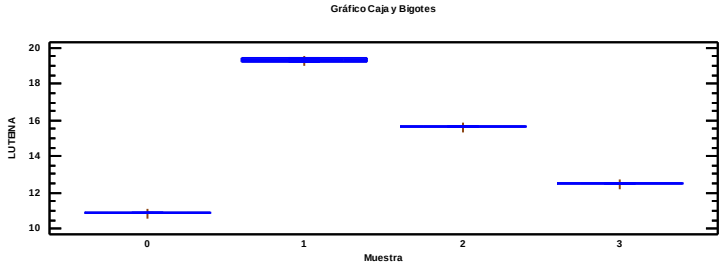
β- caroteno



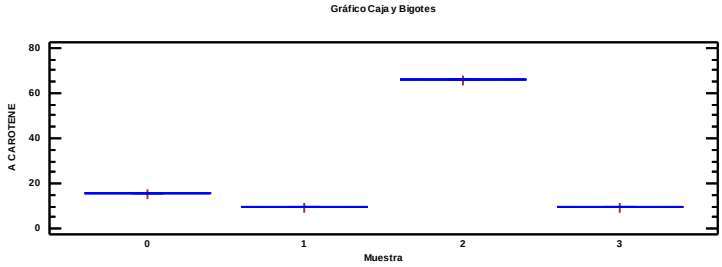
MUESTRA: 1: no tratada; 2: pasterización; 3: esterilización; 4: ultrasonidos

SMOOTHIE 2 – ETAPA I: LIBERACIÓN

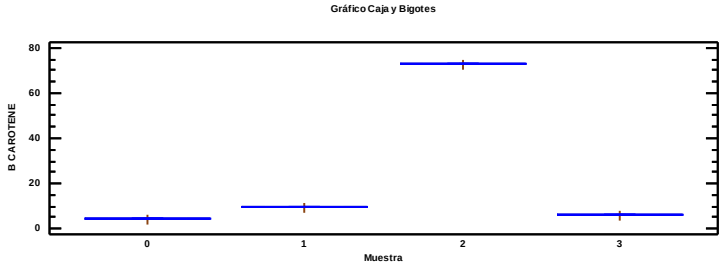
luteina



α-caroteno



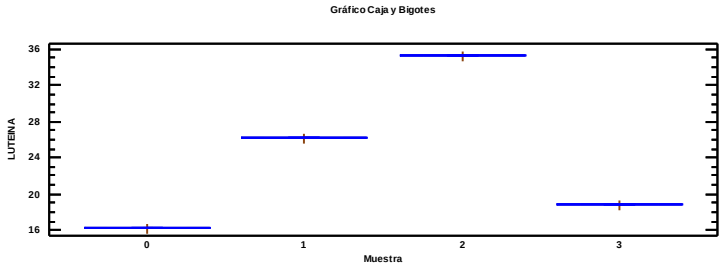
β- caroteno



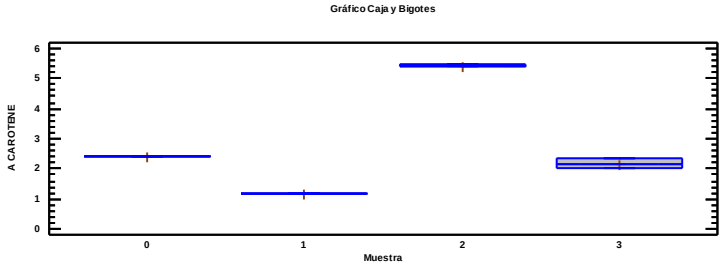
MUESTRA: 1: no tratada; 2: pasterización; 3: esterilización; 4: ultrasonidos

SMOOTHIE 2 – ETAPA II: MICELARIZACIÓN

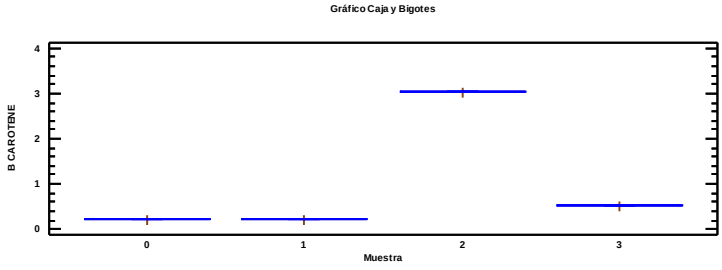
luteina



α-caroteno



β- caroteno



MUESTRA: 1: no tratada; 2: pasterización; 3: esterilización; 4: ultrasonidos