La publicación de este número contó con el apoyo financiero
del Comité para el Desarrollo de la Investigación –CODI–
Universidad de Antioquia

Vitae

REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS

Universidad de Antioquia. Medellín, Colombia
ISSN 0121- 4004 – ISSNe 2145-2660
Volumen 22 número 2
Mayo – Agosto, 2015

La Revista Vitae es el órgano difusor de la Facultad de Ciencias Farmacéuticas y Alimentarias de la Universidad de Antioquia. Está dirigida a profesionales y estudiantes interesados en la ciencia y tecnología farmacéutica y alimentaria. Contempla información derivada de investigaciones y revisiones relacionadas con los medicamentos, los cosméticos, los alimentos y los productos naturales.

La responsabilidad por los juicios, opiniones y puntos de vista expresados en los artículos publicados corresponde exclusivamente a sus autores.

PERIODICIDAD
Tres números al año

PRECIO DE SUSCRIPCIÓN ANUAL
Colombia: $ 120.000
Estudiantes: $ 65.000
Exterior: US $ 70
EUR $ 55

PRECIO PUBLICACIÓN ARTÍCULO
Colombia $440.000
Exterior: US $ 220
EUR $ 180

TIRAJE
300 ejemplares
revistavitae@udea.edu.co
http://aprendeenlinea.udea.edu.co/revistas/index.php/vitae
http://www.udea.edu.co/vitae

Indexada en:

- **ISI Web of Science:** Thomson Scientific.
  Factor de impacto año 2013: 0.259
- **SciVerse SCOPUS/Elsevier B.V.**
- **EMBASE:** Biomedical Answers.
- **PUBLINDEX:** Índice Nacional de Publicaciones Seriadas, Científicas y Tecnológicas de Colombia. Colciencias. Categoría A1.
- **LILACS:** Índice de la Literatura Latinoamericana en Ciencias de la Salud.
- **LATINDEX:** Índice Latinoamericano de Revistas Científicas y Tecnológicas.
- **CUIDEN:** Base de datos, Granada (España).
- **CAS:** Chemical Abstracts.
- **SciELO:** Scientific Electronic Library Online.
- **OJS:** Open Journal System.
- **DOAJ:** Directory of Open Access Journals.
- **e-revistas:** Plataforma Open Access de Revistas Electrónicas Españolas y Latinoamericanas.
- **REDALYC:** Red de Revistas Científicas de América Latina, El Caribe, España y Portugal.
- **SIIC Data Bases:** Sociedad Iberoamericana de Información Científica (SIIC).
- **EBSCO Host.**

CANJE
Universidad de Antioquia.
Departamento de Bibliotecas Sección Canje
canjebc@biblioteca.udea.edu.co
Apartado Aéreo 1226 Medellín – Colombia.
Telefax 57(4) 219 59 92 ó 219 59 93

CORRESPONDENCIA Y SUSCRIPCIÓN
Edificio de Extensión Universidad de Antioquia
Calle 70 No. 52-62 Piso 3 Oficina 303
Teléfono: 57(4) 219 84 55

Vitae

MISIÓN

La Revista Vitae tiene como misión la difusión del conocimiento derivado de la investigación y de las revisiones bibliográficas relativas a los medicamentos, los cosméticos, los alimentos y los productos naturales, mediante publicaciones que tienen cobertura tanto a nivel nacional como internacional.

MISSION

Journal Vitae's mission is the diffusion of the knowledge derived from researches and bibliographic reviews related to medicines, cosmetics, food and natural products, through publications of both national and international coverage.

OBJETIVO

Divulgar los resultados de investigaciones relativas a los medicamentos, los cosméticos, los alimentos, los productos fitoterapéuticos y demás insumos sanitarios; obtenidos con una adecuada rigurosidad científica, tecnológica y académica, evaluados por pares académicos expertos en los diferentes temas, y que contribuyan al avance y desarrollo de las ciencias farmacéuticas y de los alimentos.

OBJECTIVE

Journal Vitae's objective is to disclose the results of researches related to medicines, cosmetics, food, phytotherapeutic products and other sanitary supplies, obtained with adequate scientific, technological and academic rigor. These results are evaluated by academic partners who are experts in the different subjects, and contribute to the advance and development of the pharmaceutical and food sciences.

Vitae

REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
Universidad de Antioquia. Medellín, Colombia
ISSN 0121- 4004 – ISSNe 2145-2660
Volumen 22 número 2 Mayo – Agosto de 2015 págs. 65-166

CONTENIDO

Vitae

REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
Universidad de Antioquia. Medellín, Colombia
ISSN 0121- 4004 – eISSN 2145-2660
Volume 22 Issue 2 May to August 2015 pages 65-166

CONTENT

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 73
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a01

EDITORIAL

Freeze-drying: perceptions and challenges for drying foodstuffs and plant extracts

La liofilización: percepciones y desafíos para el secado de productos alimenticios y extractos vegetales

Constain SALAMANCA[1*], John ROJAS[2]

One of the most important features of foodstuffs and plant extract-based products is related to their fragile stability, especially when exposed to several ambient factors such as air, light radiation, heat and water vapour. The latter may contribute to the increase of free or bound water associated to the product. In this scenario, these variables may lead to different phenomena such as self-oxidation and hydrolysis of functional compounds and thus, a considerable inconsistency of products where these substances are part of occurs. On the other hand, these factors affect more drastically the product stability, especially when they possess a high intrinsic surface area and thus, are more likely to absorb a significant amount of water. Furthermore, in liquid products oxidation, hydrolysis and precipitation problems are more probable to happen. Nevertheless, the common methods for water removal by heat treatment cannot be used in this class of products. This is explained by the potential degradation and hence activity loss of their functional constituents. This phenomenon is even more critical when volatile compounds play a substantial role in the organoleptic properties of the finished product. Therefore, the water removal process results in a complex and expensive task that cannot be accomplished with conventional methodologies.

The above mentioned environmental factors have led to the search for alternative processes which support the isolation, manufacture and storage of this class of products under very specific and strict conditions. These processes include mainly freeze-drying, which is also known as lyophilization. Thus, freeze-drying corresponds to a process where water is removed through a phase transition known as sublimation. In this case, the thermodynamic direct transition from the solid to the gas phase occurs. For this reason, an intermediate transformation to the liquid phase is by-passed. Technically, water must be submitted to a very strict conditions of temperature and pressure; usually, below -20°C and less than 4.58 Torr (610.5 Pa), respectively. Under these conditions, equilibrium between the solid and gas phases takes place. Therefore, in this transition a low amount of heat flows spontaneously from the environment to the frozen product preventing the product from overheating and degradation. Nowadays, freeze-drying shows a great benefit for drying unstable materials since it increase their stability significantly. However, this process exhibits some drawbacks. First, the infrastructure required for the process is expensive and energy-consumption is considerable. On the other hand, a freeze-drier is difficult to operate and careful training must be taken into account for adequate standardization and validation of the drying process. Failure to follow these conditions leads to a high variability in the external morphology of the dried material. The freeze-drying conditions affect significantly the resulting product and hence, its subsequent processing and packaging. Therefore, it is imperative to control the morphological characteristics of the solids while employing this process.

The paradox of drying unstable and labile substances without affecting their functional properties is the choice for the optimal conditions in terms of cost and quality benefits. As a result, the production, development and storage conditions when these labile substances are included into finished products is eased, and help to fulfil their required quality requirements established by the regulatory agencies.

[1] Pharmaceutical Physical-chemistry lab. Natura Investigation group, Pharmaceutical Chemistry Program, Department of Chemical Sciences, School of Natural Sciences, Icesi University, Cali, Colombia.
[2] Department of Pharmacy, School of Pharmaceutical and Food Sciences, University of Antioquia. Medellín, Colombia.
[*] Corresponding Author: chsalamanca@icesi.edu.co

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 75-86
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a02

PHYSICAL CHARACTERIZATION OF FREEZE-DRIED FOAM PREPARED FROM *ALOE VERA* GEL AND GUAR GUM

CARACTERIZACIÓN DE ESPUMAS LIOFILIZADAS PREPARADAS A PARTIR DE GEL DE *Aloe vera* Y GOMA GUAR

Verónica SANTACRUZ-VÁZQUEZ[1*], Claudia SANTACRUZ-VÁZQUEZ[1], José Oscar LAGUNA CORTÉS[2]

Recibido: Agosto 26 de 2014 Recibido: Septiembre 21 de 2015

ABSTRACT

Background: Foams are colloidal dispersions of a gas suspended in a dispersing phase, which consisting of a semi-freeze-dried or viscous liquid phase. The physical properties of food foams are the result of the bubble characteristics and their spatial arrangement. **Objectives:** The aim of this work was to obtain foams of *A. vera* gel and guar gum and describe the changes in their physical properties and microstructure during freeze-drying using the fractal dimension concept and image analysis techniques. **Methods:** The porosity, density, and volume expansion factor of the fresh foams that were based on the *A. vera* foams were determined. The kinetics of foam texture, color, porosity and microstructure of the freeze-dried foams were obtained. The fractal texture dimension of surface (FD_{SDBC}) and microstructure (FD_{ESEM}) of the foams were determined as indicators of structural changes after freeze-drying. The guar gum concentrations used to obtain the *A. vera* prefoam were expressed in w/w as F1 (control sample without gum), F2 (2%), F3 (4%) and F4 (6%). **Results:** We obtained stable freeze-dried foams of *Aloe vera* gel and guar gum. The porosity, density and volume expansion factor of the fresh and freeze-dried foams were affected by the addition of the guar gum. Changes in the topology of the freeze-dried foam surface during the drying process resulted in a high rugosity compared with the original smooth surface. The microstructure of the dried foam samples suggested a relationship between the gum concentration of the prefoam *A. vera* gel mixture and the physical properties before and after freeze-drying, such as an increase in the microstructural alterations and surface roughness during freeze-drying. The roughness of the freeze-dried foam surface, described by the FD_{SDBC} represented the macroscopic physical changes of the samples and correlated with the changes in the foam microstructure, which were described by the fractal dimension of the Environmental Scanning Electron Microscopy ESEM microphotographs (FD_{ESEM}). **Conclusions:** The digital analysis of the structure and porosity of the freeze-dried foam can be used to quantify the effect of gum concentrations on the morphological features and physical properties of foams during freeze-drying.

Keywords: Food property, foaming capacity, freeze-drying, *A. vera* gel foam, fractal analysis

[1] Benemérita Universidad Autónoma de Puebla, Facultad de Ingeniería Química, Ciudad Universitaria, 72570, Puebla, Pue., México.

[2] Instituto Tecnológico de Puebla, Av. Tecnológico No. 420. 72220, Puebla, Pue., Méxic o.

[*] Corresponding Author:: versanva@gmail.com

RESUMEN

Antecedentes: Las espumas son dispersiones coloidales de un gas en una fase líquida viscosa. Las propiedades físicas de las espumas alimentarias son el resultado de las características de sus burbujas y su disposición espacial. **Objetivos:** El objetivo de este trabajo fue obtener espumas de gel de *A. vera* y goma guar y describir los cambios en sus propiedades físicas y su microestructura durante el secado por liofilización utilizando el concepto de dimensión fractal y las técnicas de análisis de imagen. **Métodos:** Se determinó la porosidad, densidad, factor de expansión volumétrico de las espumas frescas de *A. vera*. Así como la cinética de liofilización, textura, isotermas de sorción, color, porosidad y la microestructura las espumas liofilizadas. La dimensión fractal de la textura (FD_{SDBC}) y microestructural (FD_{ESEM}) de las espumas de gel de *A. vera* y goma guar liofilizadas se determinó como un indicador de los cambios estructurales después de la liofilización. Las concentraciones de goma de guar utilizados para obtener la solución de clara de huevo preespuma se expresaron en w/w como F1 (muestra de control sin goma), F2 (2%), F3 (4%) y F4 (6%). **Resultados:** Fue posible obtener espumas liofilizadas estables de gel de *A. vera* y goma guar. La porosidad, densidad, factor de expansión volumétrico de las espumas se vieron afectadas con la adición de goma guar. Los cambios en la topología de la superficie de la espuma liofilizada durante todo el proceso de secado dieron lugar a alta rugosidad en comparación con la superficie lisa original. La microestructura de las muestras de espuma secas sugirió una relación entre la concentración de goma de las espumas de *A. vera* y las propiedades físicas antes y después de la liofilización como un aumento en las alteraciones microestructurales y rugosidad de la superficie durante el secado por congelación. La rugosidad de la superficie de la espuma liofilizada, se describió por la relación FD_{SDBC} que representa los cambios físicos macroscópicos de las muestras y se correlacionó con los cambios en la microestructura de espuma, que fueron descritos por la dimensión fractal de las micrografías ESEM (FD_{ESEM}). **Conclusiones:** El análisis digital de la estructura y la porosidad de la espuma liofilizada se puede utilizar para cuantificar el efecto de las concentraciones de goma guar en las características morfológicas de las espumas durante el secado por congelación.

Palabras clave: Propiedades de los alimentos, capacidad espumante, secado por liofilización, espumas de *A. vera*, análisis fractal.

INTRODUCTION

Foams are colloidal dispersions of a gas or gas mixture suspended in a viscous liquid phase (1). The physical properties of food foams (e.g., texture, density and stability) are the result of the bubble characteristics (e.g., size) and their spatial arrangement (2, 3). The most important factors contributing to the stabilization of foams and the avoidance of destabilization mechanisms are a high viscosity of the liquid phase and the presence of resistant and elastic adsorbed protein films (3). Commonly used stabilizers include natural hydrophilic and modified hydrophilic starches, gums, modified celluloses, pectin, gelatin and other proteins. Particularly, gums are often used to increase the viscosity of solutions and suspensions to form foams, and they act as thickeners in the aqueous dispersion phase. Gums may produce a particular texture, which is used to stabilize the dispersed phase or to reduce the formation of sugar or ice crystals in foams (4).

Aloe vera gel has wide applications in the cosmetic, nutraceutical and therapeutic industry. The gel of *A. vera* is used as an ingredient in cosmetics and medical products because its composition contains mannose polymers with sugars, including glucose and acemannan polymers, which are the active ingredients in the scar removal process. *A. vera* gel has vitamins, minerals, enzymes, proteins and phytosterol (5). This gel has therapeutic potential as an antidiabetic, anti-inflammatory, antioxidant, immunomodulatory, anti-ulcer, hepatoprotective, moisturizing agent, among other applications (6). However, thermal processing techniques affect the functionality of the chemical structures of *A. vera* gel, resulting in thermal damage and a denaturation process (7, 8). There are several treatments for the conservation of the gel, including convective drying and drum drying; however, these methods have disadvantages such as decreasing the antioxidant capacity of the gel when using a drying temperature of 50 to 90°C (9). Spray drying is another method

widely used to preserve Aloe extracts because it provides less damage to the biological activity of the final products compared with convective drying with forced air; this is due to the short processing times.

Moreover, the freeze-drying method has been extensively used in the pharmaceutical and food industries to obtain dehydrated materials that are easily degraded (10). Freeze-drying is often used because the process enables the retention of the physical and chemical properties of the products, such as the color, the vitamins, the antioxidant contents and other sensorial properties, including the original aroma and flavor. Here, freeze-drying was used for the dehydration of fresh *Aloe vera* foams (10, 11).

Guar gum is a galactomannan obtained from the endosperm of the Cyamopsis tetragonolobus seed. This polysaccharide is formed from galactose and mannose molecules and is used as a food additive in various foods products for stabilization. This additive is natural and economical. Its main property is to change in behavior of the water present in food. Guar gum swells and dissolves in polar solvents on dispersion and forms strong hydrogen bonds. The hydration rates are reduced in the presence of dissolved salts and other water-binding agents, such as sucrose (12).

The most significant characteristic of guar gum is its ability to form a viscous colloidal dispersion that is dependent on the time, temperature, concentration, pH and the time of agitation. However, guar gum was employed in this research because it is derived from a food instead of a bacterial exopolysaccharide, and it is not produced using common food allergens (13).

Recently, it was demonstrated that the microstructure of materials plays an important role in the physical properties of foodstuffs. Examples of these influences include the deformation and shrinkage that occurs during drying because microstructural characteristics of materials have an effect on the diffusion mechanisms and other parameters associated with moisture transfer (14). Kerdpiboon *et al.* (15) identified a relationship between the microstructural changes of carrot cubes and their physical alterations during air drying using fractal and image analysis. The concept of fractal texture was introduced to describe the rough or heterogeneous texture in the images and has been employed to quantify surface irregularity. According to Quevedo *et al.* (16), the texture of the images is usually called the texture feature (TF), and it is an important tool used in pattern recognition to characterize the arrangement of the basic constituents of a surface material. TF is useful in the analysis and description of natural scenes and environments and in the classification or categorization of pictorial data (17, 18). One method for determining texture features is the analysis of the surface intensity (SI), which is obtained by plotting the (x, y) pixel coordinates versus the gray level of each pixel (z axis) (19, 20). When the SI is characterized using the fractal dimension (FD), the TF is called the fractal texture of the image. If the FD is evaluated using the shifting differential box-counting method algorithm (SDBC), the FD is denoted as FD_{SDBC} (21). Furthermore, Pentland (22) showed that the fractal dimension of a surface dictates the fractal of the image intensity surface, which should be similar to the fractal dimension of the physical surface. Fractal texture has been reported to be sufficiently sensitive to allow for the detection of the surface roughness of products such as chocolate, bread, potatoes and other foods (23 - 27). The aim of this work was obtain foams of *A. vera* gel and guar gum and describe the changes in their physical properties and microstructure during freeze-drying using the fractal dimension concept and image analysis techniques.

MATERIALS AND METHODS

Selection and collection of the plants and the mucilage gel of the *A. vera* leaves

A. vera plants were collected from a herbarium of the Benemérita Universidad Autónoma de Puebla in Puebla, Mexico. The selected *A. vera* plants were 30 - 35 months-old with bright green leaves and without any visible spots. These plants had grown in controlled conditions in the herbarium in an area with partial shade and wide ventilation. The temperature inside the herbarium was 20±1°C. The specimens were planted with a spacing of approximately 0.50 m between plants. After cutting the leaves of the plants, they were washed with water to remove the dirt and dust to avoid contamination. Once the leaves were clean, we proceeded to separate the epidermis from the parenchyma fillet with a sterilized knife.

Preparation of the fresh foams

An ultrasonic homogenizer (UH-300, SMT, Japan) was used to disperse guar gum (Guarcel^{R}, México CAS no. 9000-30-0) in fresh *A. vera* gel (100 g) for 6 min at 20°C (1500 r min^{-1}). The guar gum concentrations used to obtain the prefoam solution of *A. vera* are expressed in w/w as F1 (control sample without gum), F2 (2%), F3 (4%) and F4 (6%).

Fresh foam density and volume expansion factor

The fresh foam density was determined by measuring the mass of a fixed volume of the foam. This determination was performed carefully to avoid destroying the foam structure and to ensure that there were no voids created while filling the foam into the measuring beaker. The density experiments were performed in triplicate (28).

To determine the physical structure of the four different foams, including the fresh *A. vera* gel without gum F1 (control sample), F2 (2%), F3 (4%) and F4 (6%), the foaming potential was measured with a volumetric procedure (28) using the following equation:

$$X=M/m \quad (1)$$

where M is the mass of a strictly determined volume of the mixture prior to foaming (g), and m is the mass of the same volume of the mixture after foaming (g).

The foam volume expansion factor, Vo, was determined according to the following equation:

$$Vo=\frac{V\,p}{V1} * 100\ (\%) \quad (2)$$

where Vp is the foam volume immediately after foam preparation (cm^3), and $V1$ denotes the volume of the solution (cm^3) used for foaming.

The porosity of the fresh foams was evaluated according to the following equation:

$$\varepsilon\,(\%) = \left(1-\frac{\rho_p}{\rho_n}\right) * \ 100 \quad (3)$$

where ρ_p is the density of the sample after bubble aeration, and ρ_n is the density of the sample prior to bubble aeration.

The foam stability factor was determined according to the following equation:

$$T = \left(\frac{A\ V}{A}\right) * \ 100\ (\%) \quad (4)$$

where A is the volume of the liquid from which the foam was made (cm^3), and V is the liquid leakage volume (cm^3) obtained during 15 min of foam storage.

Preparation of the freeze-dried foams

A similar procedure for preparation of the fresh foams was employed to obtain the freeze-dried foams. The prepared fresh foams were weighed, frozen and sublimated in a freeze-drying system Labconco 1 lt. (Labconco, Kansas City, MO, USA). Sample temperatures were reduced from 5 to −40°C at a constant cooling rate (−0.5 K min^{-1}), and the frozen samples were subsequently lyophilized at −20°C for 40 h. Then, the final weight and moisture content of the freeze-dried *A. vera* sample was measured (28). The moisture content (db) of the freeze-dried foams was evaluated according to AOAC method 32.1.03 (29). Each experimental freezing-drying run was conducted in triplicate, and the measurements of the corresponding physico-chemical properties of the *A. vera* foams were also repeated thrice.

Freeze-dried foam texture

A TAXT2 texture analyzer (Stable Micro Systems, Ltd., Godalming, Surrey, UK) was used to determine the firmness of the freeze-dried foams. The sample was placed on a hollow planar base (30), and a direct force was applied to the sample using a 5 mm spherical probe at a constant crosshead speed of 2 mm s^{-1}. A plot of the force *vs*. distance travelled by the plunger during the test was prepared. The hardness was defined as the maximum force in the force–deformation curve, and the crispness was characterized by the number of peaks and the slope of the first peak. Foam samples were tested, and the average values for hardness and crispness are reported (31).

Color measurement

A CIELAB colorimetry system was used to determine the foam colors using a WR10 8 mm Color Meter CIELAB (Graigar). The evaluated parameters, L (luminosity), a (red–green), and b (blue–yellow), were determined using the Hunter scale. Color was measured directly on the product surface. The total color difference (ΔE) was determined using the equation:

$$\Delta E=((L_t-L_0)^2+(a_t-a_0)^2+(b_t-b_0)^2)^{1/2} \quad (5)$$

where a_t is the Hunter scale coordinate indicating the redness or greenness of the foams at the final freeze-drying time, t, and a_0 is the same coordinate before the freeze-drying process, t = 0. The values, b_t and b_0, are the Hunter scale coordinates indicating the blueness or yellowness of the foams at the final freeze-drying time, t, and before the freeze-drying process at t = 0, respectively. L_t and L_0 re the Hunter scale coordinates indicating lightness of the foams at the final freeze-drying time, t, and before the freeze-drying process at t = 0, respectively, while ΔE is the total color difference (32).

Microstructure of the freeze-dried foams

The microstructure of the dried samples was examined using an Environmental scanning electronic microscope (ESEM) (LEO 1420VP, Carl Zeiss SMT AG, Oberkochen, Germany). Triplicate samples of the freeze-dried foam were viewed at 5000X magnification, and images were stored on a PC (AMD Turion X2, 500 GB, 500 MHz) as grayscale bitmaps with brightness values between 0 and 255.

Porosity of the freeze-dried foams

The image analysis software, ImageJ 1.34s (National Institutes of Health, NIH, USA), was used to quantify the characteristics of the porous freeze-dried foam, including the pore diameter and pore area. The image analysis was performed on the Image J software, and the algorithm used by this software was an automatic thresholding function, which is an iterative procedure based on the isodata algorithm (33). This algorithm divides the image into objects and background by taking an initial threshold. Then, the averages of the pixels at or below the threshold and pixels above are computed, and the averages of those two values are computed. The threshold is incremented, and the process is repeated until the threshold is larger than the composite average. There are many more methods for setting an automatic threshold, such as Otsu's method, entropy method, triangle method, k-means clustering, (33); however, in this study we used the cited algorithm because Image J was available for analysis of the image data.

Each pixel of an ESEM micrograph was assigned a gray intensity value (0 – 255). A threshold-based segmentation technique was employed to distinguish the pore from the freeze-dried phase using an appropriate gray level threshold. A binary image was then generated. The pixels with gray levels less than the selected threshold were assigned as pores and appeared black in a binary image, whereas the pixels with gray levels greater than the selected threshold were identified as the freeze-dried phase and appeared white in a binary image. Assuming a spherical shape, the pore diameter was estimated based on the known pore area. The pore area was determined by counting the number of pixels filled in the specified space (25).

Evaluation of the fractal texture dimension (FD_{SDBC}) of the freeze-dried foam surfaces

The lighting system used to obtain the images of the foam surfaces during freeze-drying included two lamps (15 V, 150 W, D 417053). A color digital camera Power Shot A620 digital camera (Canon Inc., Tokyo, Japan) was located vertically over the sample at a distance of 25cm and the angle between the camera axis and the light source was 45°. Considering that ambient illumination is very critical for reproducible imaging, the lighting system and the camera was put inside a wooden box whose internal walls were painted black to avoid the external light and reflections. The iris was operated in manual mode with a lens aperture of f = 8, speed setting of 1/6, and both zoom and flash were turned off to achieve repeatability.

Images were acquired of two sides of the foams, and were taken with no zoom, no flash, and a maximum resolution of the camera (2048*1536 pixels) and storage in JPEG format. *A. vera* foams images were acquired every 4 h during the drying period and they were saved on a PC (AMD Turion X2, 500 GB, 500 MHz) and then transformed from an RGB format to a black and white format as grayscale bitmaps. The brightness values were between 0 and 255 before calculating the fractal texture dimension (FD_{SDBC}).

The box-counting method was used to evaluate the fractal texture dimension (FD_{SDBC}) in the surface view images of the dried foams. In this study, the shifting differential box-counting method was employed to compute the fractal texture for the 2-D grayscale images of the foam surface. For this purpose, ImageJ 1.34s software (National Institute of Health, NIH, USA) and the codec proposed by Per Henden (Version 1.0, 2006-02-05) were used.

The fractal texture dimension (FD_{SDBC}) calculation was performed using an algorithm designed in MatlabTM Software (version 6.5) where FD_{SDBC0} and FD_{SDBC} are the fractal dimension texture of the foam surface images of the fresh sample and the sample at any given interval during freeze-drying, respectively.

A similar procedure was applied to obtain the evaluation of the fractal texture dimension in the ESEM microphotographs (FD_{ESEM}) of the freeze-dried foam. The microphotographs were saved on a PC (AMD Turion X2, 500 GB, 500 MHz) and then transformed from an RGB format to a black and white format as gray scale bitmaps. The brightness values ranged between 0 and 255 prior to calculating the fractal texture dimension (FD_{SDBC}), which was obtained by applying the previously mentioned procedure for the fractal texture dimension (FD_{SDBC}) of the freeze-dried foam surfaces (25).

All statistical analyses were performed using Minitab 13.1 software (Minitab Inc., Philadelphia State College, USA). A two-way analysis of variance ANOVA was applied to evaluate the effect of gum concentration in experimental data obtained from fractal texture of ESEM photomicrographs (FD_{ESEM}) and the fractal texture dimension (FD_{SDBC}) of the freeze-dried foam surfaces. Statistical analysis of Multiple Comparison Procedures were applied on the FD_{SDBC} values and FD_{ESEM} values using the Fisher LSD test to determine the presence of significant differences among FD_{SDBC} values and FD_{ESEM} values.

RESULTS

Fresh foam density

During the whipping process, air was brought into the *A. vera* gel prefoam and trapped in the liquid as bubbles. The foam density decreased as the whipping time increased, resulting in a minimum density after 3 min of whipping. As the concentration of gum increased in the F2, F3 and F4 samples, the foam density increased relative to foam F1. The initial foam density of approximately 0.93 g cm^{-3} decreased to 0.19, 0.26, 0.32 and 0.35 g cm^{-3} after 3 min of whipping for F1, F2, F3 and F4, respectively. After 5 min of whipping, an increase in the density was evident (0.27, 0.35, 0.60 and 0.68 g cm^{-3} for F1, F2, F3 and F4, respectively).

During the first 3 minutes of the whipping process, air was brought into the *Aloe vera* gel and entrapped in the liquid as bubbles (Fig 1). This led to a decrease in the foam density as the whipping time increased. The curves of the fresh *Aloe vera* gel foam densities during the whipping are similar to those of other foods that are high in viscosity, such as tomato paste foam (34). Beyond the five minutes, the foam density increased rapidly and was attributed to more thinning of the liquid film, more mechanical deformation and more bubble wall rupture during the extended whipping (31, 32). As the concentration of the guar gum increased to 2%, 4% and 6% (w/w), the foam density increased because the movement of the foaming agent from the aqueous phase towards the air–aqueous interface was limited and it was insufficient for the reduction in surface tension, which enhanced the foam formation (32).

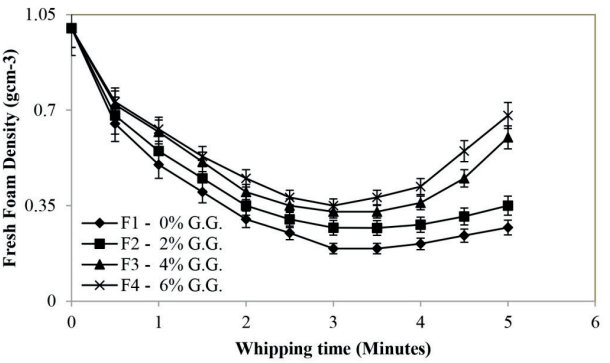


Figure 1. Fresh *A. vera* gel foam density as a function of the gum concentration during the whipping process

The gum concentration significantly affected the density of the fresh foams (ANOVA, $P<0.05$) based on the magnitudes of the F values ($F=2.91$). The results showed that the control sample (F1) exhibited the best foaming properties relative to the F2, F3 and F4 samples (Table 1). The samples with 4 and 6% guar gum concentrations in the *A. vera* gel foam showed much lower foaming parameters than F2, although the best results were obtained for the *A. vera* gel without gum (F1). This result indicates that the addition of guar gum substantially decreases the foaming potential of the *A. vera* gels. Similar results were obtained by Karim (30) using methyl cellulose in starfruit foams.

Porosity and mass density are important parameters of fresh foams. These two parameters are correlated because an increase in density is accompanied by a decrease in porosity. The foam with guar gum showed the lowest porosity (64.30% F4) and the highest density (0.35 g cm^{-3}) compared to products without gum.

Table 1. Comparison of the foaming potential of the fresh *A. vera* gel and the mixtures with guar gum.

	Foaming potential			
	Foam volume expansion factor Vo (%)	**Foaming potential X**	**Foam Stability factor T (%)**	**Porosity (%)**
Fresh Aloe vera gel, F1	162±2.5	1.18±0.03	43.00±2.8	78.22±2.4
Fresh Aloe vera gel with guar gum at 2% (w/w), F2	136.0±1.6	1.12±0.02	25.60±3.5	70.66±3.6
Fresh Aloe vera gel with guar gum at 4% (w/w), F3	118.0±1.8	1.11±0.02	17.80±2.4	65.46±2.8
Fresh Aloe vera gel with guar gum at 6% (w/w), F4	112.0±2.2	1.11±0.02	15.70±2.8	64.30±3.5

Kinetics of the freeze-dried foam under different drying conditions

The initial moisture content of the *A. vera* gel foams was 6.690.15 kg/kg db. The drying kinetics of F1, F2, F3 and F4 are presented in Fig. 2. The overall moisture content was reduced from 6.69, 5.32, 4.42 and 3.80 kg/kg db for F1, F2, F3 and F4, respectively, to 0.39 kg/kg db. Two different stages were observed in the freeze-drying curve of every tested formulation. The first stage extended over the first 20 h of drying (Fig. 2), the second stage also lasted 20 h, which corresponded to the equilibrium stage.

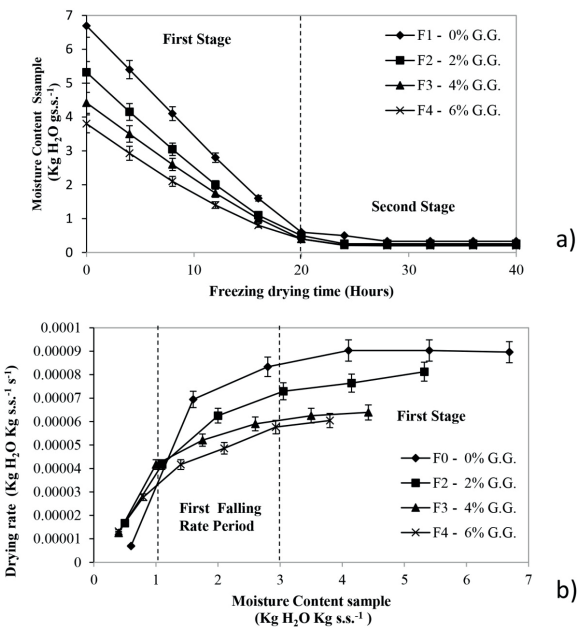


Figure 2. a) Moisture Content of *A. vera* gel foams. b) Drying rate of the *A. vera* gel foam during freeze-drying process.

A similar observation was reported by Lim *et al.* (35). The drying rate data that is presented in Fig. 2b shows a higher drying rate at the early stage of drying (first stage) because the moisture content of the *A. vera* gel foam decreased from the initial moisture content to approximately 3.0 kg/kg db.

The falling rate period could be divided into two periods according to the change in the drying rate curves. The first falling rate period (I) occurred between a moisture content of 3.0 and 1.0 kg/kg db, and it corresponded to the loss of free water. This water was freely available around the foam and could easily sublimate (36). The second rate period (II) exhibited a very low drying rate that decreased gradually with the decreasing moisture content, which was possibly due to the small amount of free water available given that the diffusion of bound water is the main mechanism controlling the water transport. In the last stage, the internal diffusion rate of moisture was found to decrease with time (Fig 2b).

Texture analysis of the freeze-dried foams.

The texture of the dried foams was evaluated using a compressive test (Fig. 3a). The maximum force of rupture is defined as the hardness, and the slope of the first peak was used to describe the crispness of the samples (37).

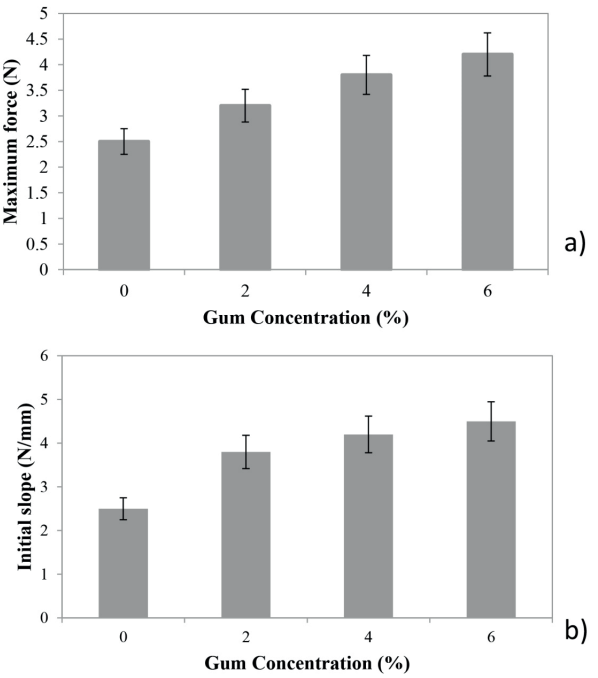


Figure 3. a) Maximum force of rupture. b) Initial slope of the first peak of dried *A. vera* gel foam with different gum concentrations.

The texture of the dried foams was strongly affected by the sample pore structure. F1 had a density of 0.19 g cm^{-3} and was characterized by larger pores and a limited number of smaller pores. F1 presented a lower density structure and lower strength values with respect to F2, F3 and F4. The maximum rupture forces of the samples were 2.52±0.23 N for F1, 3.28±0.22 N for F2, 3.80±0.40 N for F3 and 4.26±0.44 N for F4. The statistical analysis showed that the texture was significantly ($P<0.05$) affected by the gum concentration, as shown in Fig. 3a. Regarding the crispness, the F3 and F4 samples were spongy and were not crispy. These textural properties were similar to those reported for freeze-dried apple chips, which have a very porous structure (38). As shown in Fig. 3b, the gum concentration influenced the hardness and spongy textural features of the samples. Because the higher gum concentration in the fresh foam reduced the ability of water to act as a plasticizer in the dried foams, it caused a significant increase in the T_g and textural properties. This behavior was also observed for mango gel with maltodextrin, immature West Indian cherry with maltodextrin or gum Arabic and date palm with maltodextrin (36).

Color sample after freeze-drying

The total color difference, ΔE, of the *A. vera* foams before and after freeze-drying was obtained. The ΔE between the fresh *A. vera* foam and the freeze-dried samples were significantly different for F1 (ΔE=20), F2 (ΔE=25.5), F3 (ΔE=28.7) and F4 (ΔE=32.1). The color foams significantly changed with the freeze- drying process because the final dehydrated foams had a significantly lower luminosity than the fresh foam. The addition of the gum into the foam formulation produced a yellowish product whose intensity depended on the gum concentration.

Microstructure analysis of the freeze-dried foam tissue

Porosity measurement

The pore shapes of the *A. vera* gel foams before drying were spherical (not shown here). When the samples were freeze-dried, the pore shape changed. The pore size is shown in Fig. 4a.

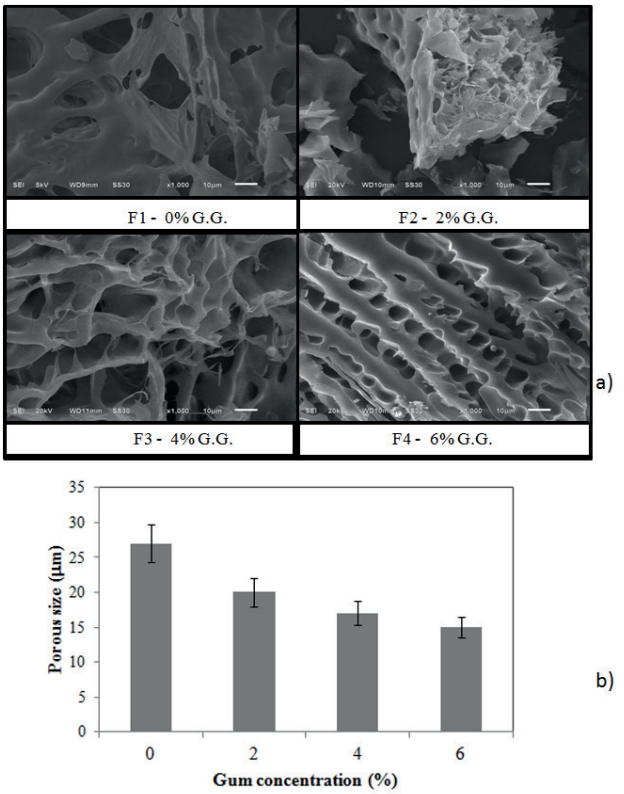


Figure 4. a) ESEM microphotographs and b) Pore size in the ESEM microphotographs of freeze-dried *A. vera* gel foams with different gum concentrations (1000X magnification, 20kV).

The pore diameter was estimated from the known pore area by assuming the spherical shape that was reported for F1. The foam density was found to affect the pore size of the *A. vera* gel foams. The F1 sample had an initial foam density of 0.19 g cm^{-3} and more large pores in the range of 15 - 20 μm than the samples with higher gum concentrations and higher foam densities. This large pore assembly might be generated by the coalescence of adjacent bubbles and the removal of ice crystals from the foam structure. However, the number of small pores that ranged from 5 – 10 μm was lower compared with the F3 and F4 samples. The pore size of foams F1, F2, F3 and F4 were 27±3.2, 20±2.5, 17±1.8 and 15±1.7μm, respectively. As shown in Fig. 4b, the F2 and F3 samples had few pores with diameters larger than 20 μm. The increases in the void area fraction of the samples during freeze-drying for F1, F2, F3 and F4 were 35.68, 33.06, 21.49 and 14.95%, respectively. As shown in Fig. 4a, the pore structure was significantly different among the samples with different gum concentrations.

The roughness of the freeze-dried foam microstructure (Fig. 5a), as described by the FD_{ESEM} values, represented the microscopic physical changes of the samples during the freeze-drying process. The foams without gum (F1) or with a low gum concentration (F2) exhibited higher FD_{ESEM} values than the other samples (Fig. 5).

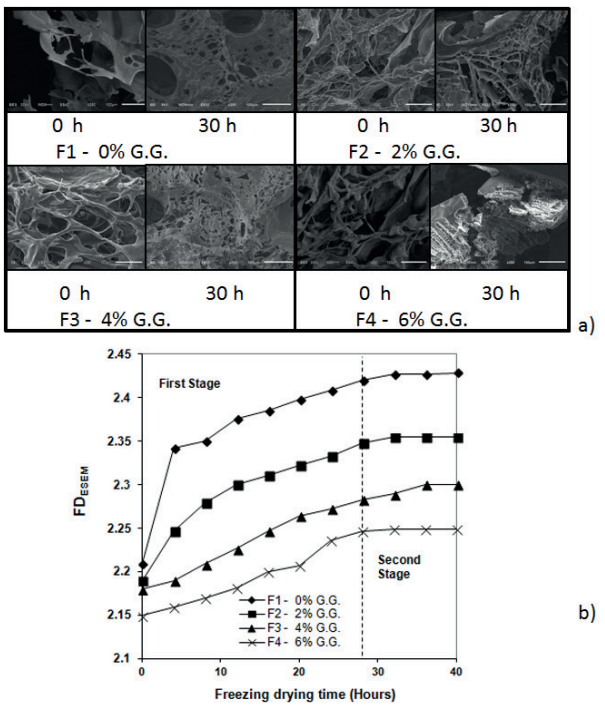


Figure 5. a) ESEM microphotographs of the freeze-dried foam (250X, 5kV) and b)FD_{ESEM} values of A. *vera* foams during freeze-drying at different gum concentrations

Under these conditions, the freeze-dried foam microstructure became rougher as a consequence of rapid water ice loss during freeze-drying, resulting in microstructural deformation of the pores. The maximum FD_{ESEM} values of the samples were 2.438 for F1, 2.361 for F2, 2.303 for F3 and 2.253 for F4. The multiple comparison analysis showed that the gum concentration in the foam formulations produced microstructural modifications with significant differences in the $FD_{ESEM.}$

The roughness of the freeze-dried foam surface (Fig. 6a), as described by the FD_{SDBC} values, represented the macroscopic physical changes of the samples and was correlated with changes in the freeze-dried foam texture and microstructure, which were described by the fractal dimension of the ESEM microphotographs(FD_{ESEM}).

Fig. 6b shows the variation of the FD_{SDBC} as a function of the freeze-drying time for the foams with different gum concentrations. The foam surface was mostly porous at the beginning of the freeze- drying with a FD_{SDBC} value of 2.220. However, during freeze-drying, the macroporous surface irregularity increased as a result of moisture loss, which was reflected by the increase in the FD_{SDBC}. Two different stages were observed in this curve. The first stage extended over the first 28 h of drying (Fig. 2) and exhibited a significant increase in the FD_{SDBC} compared to the second stage. The second stage lasted 12 h in which changes to the freeze-dried foam macroporous surface were less pronounced as the moisture content approached an equilibrium value (Fig. 6b).

The FD_{SDBC} values from the images of the dried freeze-dried foam were close to 2.24 to 2.35 during the last stage of drying, and neither further dehydration nor deformation occurred for the F1 formulation. Samples F3 and F4 exhibited a lower macroporous roughness and lower FD_{SDBC} values of the foam images (39).

Statistical analysis showed that the FD_{SDBC} value was significantly ($P<0.05$) affected by the gum concentration, as shown in Fig. 6b.

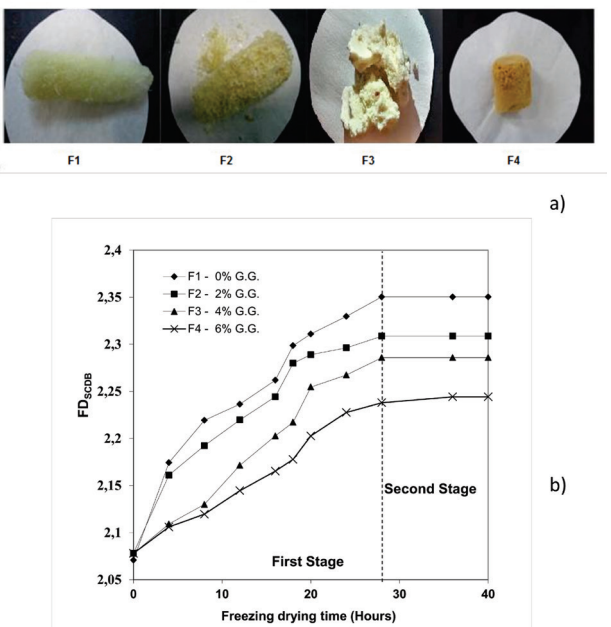


Figure 6. a) Images of the freeze-dried foam with different gum concentrations. b) FD_{SCDB} values of A. *vera* foams during freeze-drying at different gum concentrations.

DISCUSSION

The lowest density value for the F1 formulation was attributed to the fact that the movement of the foaming agent from the aqueous phase towards the air–aqueous interface was limited (30), and it was insufficient for a reduction in the surface tension, which enhances foam formation. As shown in Fig. 1, an increase in the gum concentration from 0.0% to 6.0% led to a greater than 50% increase in the foam density. The foam density curves during the whipping process presented a trend similar to those of other foods, such as tomato paste foam (30); i.e., the foams had a low density during the first 3 min, whereas there was an increase in the foam density at 5 min.

The drying rate data presented in Fig. 2b show that at the initiation of freeze-drying the sublimation front (moving boundary) is situated at the outer surface of the fresh foam sample.

During the freeze-drying process, the drying rates decreased with decreasing moisture content, signaling the beginning of the falling rate period (second stage). At this time, the sublimation front recedes from the outer surface of the foam towards the center of the sample resulting in an increased diffusion path for the sublimed moisture that was originated at the sublimation (ice) front; this renders an increased resistance to the internal diffusion of water vapor inside the sample (40, 41). The shape of the curves in Fig 2a and the drying rate in Fig 2b indicate that the internal diffusion of water vapor inside the sample is controlling the freeze-drying process. These results demonstrate the importance of the gum concentration on the moisture movement. The diffusion of water through the low-density foam was less constrained in the F1 formulation and decreased when the gum concentration in the *A. vera* gel foam increased in F2, F3 and F4.

The ESEM microphotographs showed that the freeze-dried foams exhibited a clearly deteriorated microstructure in which the removal of water ice was evident. At the high gum concentrations of F2, F3 and F4, dense products were obtained in the freeze-dried foam structure (Fig. 6a). F1 presented a more irregular microstructure pattern compared to the F2 and F3 foams in which the deformation of the structures was less pronounced. A heterogeneous foam structure was observed during freeze-drying in the absence of the gum.

The highest gum concentration (6%) produced the lowest irregularity in FD_{SDBC} and, consequently, the lowest FD_{SDBC} values. Under these conditions (F4), the deformation of the freeze-dried foam generated less roughness on the freeze-dried foam macroporous surface. At the intermediate gum concentrations, the deformation process was assisted by the high moisture transfer from the interior to the exterior of the freeze-dried foam. The kinetics of the variation of the deformation of the macroporous freeze-dried foam (FD_{SDBC}) was a function of the gum concentration and density, which also affected the overall quality of the final product. The freeze-dried foam without gum experienced a higher deformation, and the FD_{SDBC} values were higher than those obtained at higher gum concentrations.

The roughness of the freeze-dried foam surface, described by the FD_{SDBC} value, represented the macroscopic physical changes of the samples and correlated with changes in the foam microstructure, which were described by the fractal dimension of the ESEM microphotographs(FD_{ESEM}).

CONCLUSIONS

We obtained stable freeze-dried foams of *Aloe vera* gel and guar gum. The porosity, density and volume expansion factor of the fresh and freeze-dried foams were affected by the addition of guar gum. The microstructure of the dried foam samples suggested a relationship between the gum concentration in prefoam *A. vera* gel mixture and the physical properties before and after freeze-drying. Digital analysis of the structure and porosity of the freeze-dried foam was used to quantify the effect of gum concentrations on the morphological features of the foams during freeze-drying. The microstructure of the various samples suggested a relationship between the gum concentration and the density of the *A. vera* gel foams, which increased during freeze-drying. A simple relationship between the microstructural changes (FD_{ESEM}) of the freeze-dried foams and their gum concentration during freeze-drying was observed using combined fractal techniques and image analysis.

The addition of guar gum at concentrations of 2%, 4% and 6% produced high-density *Aloe vera* foams of 0.35, 0.60, and 0.68 g cm^{-3}, respectively, at an optimum whipping time of 3 min. The freeze-dried foams formed during the falling rate period with higher drying rates. An increased amount of

guar gum (6%) produced freeze-dried porous *Aloe vera* foams with increased amounts of hardness and crispness, low densities and smaller pore sizes. The analysis of the SEM microphotographs of the freeze-dried *Aloe vera* foams demonstrated that the addition of guar gum produced a pronounced a stable structure, which was also associated with the low values of the FD_{SDBC}. To produce a stable freeze-dried *Aloe vera* foam an addition of guar gum at 6% concentration is recommended.

ACKNOWLEDGMENTS

We acknowledge financial support from the CONACYT Project 132983.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ABBREVIATIONS

A = Volume of the liquid from which the foam was made (cm^3).

FD_{SDBC} = Fractal dimension texture of the foam surface images during the freeze-drying process.

D_{SDBC0} = Fractal dimension texture of the foam surface images prior to the freeze-drying process.

FD_{ESEM} = Fractal dimension texture of the freeze-dried foam determined from the ESEM photomicrographs.

FD_{ESEM0} = Fractal dimension texture of the fresh foam determined from the ESEM photomicrographs.

M = Mass of a strictly determined volume of the mixture before foaming (g).

m = Mass of the same volume of the mixture after foaming (g).

t = Freeze-drying time (min).

T = The foam stability factor.

V = Liquid leakage volume (cm^3) obtained during 15 min of foam storage.

$V1$ = Foam volume immediately after foam preparation (cm^3).

$V1$ = Foam volume of the solution (cm^3) used for foaming.

X= Foaming potential of the *A. vera* gel.

ε(%)= Porosity of the fresh foams.

ρ_p = Density of the sample after bubble aeration ($g\ cm^{-3}$).

ρ_n = Density of the sample before bubble aeration ($g\ cm^{-3}$).

REFERENCES

1. Prins, A. Advances in Food Emulsions and Foams, Dickinson E, Stainsby G. Eds. New York, U.S.A: Elsevier Applied Science; 1988. Principles of foam stability; 91-122.
2. Germain JC, Aguilera JM. Identifying industrial food foam structures by 2D surface image analysis and pattern recognition. J Food Eng. 2012 July; 111:440-448.
3. Sankat CK, Castaigne F. Foaming and drying behaviour of ripe bananas. LWT-Food Sci. Technol. 2004 August; 37: 517-525.
4. Mleko S, Kristinsson HG, Liang Y, Gustaw W. Rheological properties of foams generated from egg albumin after pH treatment. LWT-Food Sci. Technol. 2007 June; 40: 908-914.
5. Shabnam Javed, Atta-ur-Rahman. Chapter 9 – Alo vera Gel in Food, Health Products, and Cosmetics Industry. Stud. Nat. Prod. Chem. 2014, 41: 261-285.
6. Anirban RS, Dutta Gupta, Sampad Ghosh, Shashaank M, Aswatha Bibek Kabi. Chemometric studies on mineral distribution and microstructure analysis of freeze-dried *Aloe vera L.* gel at different harvesting regimens. Ind. Crop. Prod. 2013 November; 51: 194-201.
7. Cervantes-Martínez CV, Medina-Torres L, González-Laredo RF, Calderas F, Sánchez-Olivares G, Herrera-Valencia EE, Gallegos-Infante JA, Rocha-Guzman NE, Rodríguez-Ramírez J. Study of spray drying of the *Aloe vera* mucilage (*Aloe vera barbadensis miller*) as a function of its rheological properties. LWT-Food Sci. Technol. 2014 March; 55: 426-435.
8. Gulia A, Sharma HK, Sarkar BC, Upadhyay A, Shitandi A. Changes in physico-chemical and functional properties during convective drying of *Aloe vera* (Aloe *barbadensis*) leaves. Food Bioprod. Process. 2010 June; 88: 161-164.
9. Jafar F, Farid M. Analysis of Heat and Mass Transfer in Freeze-drying. Dry Technol. 2003 February; 21: 249 - 263.
10. Chakraborty R, Bera M, Mukhopadhyay P, Bhattacharya P. Prediction of optimal conditions of infrared assisted freeze-drying of *A. vera* (Aloe barbadensis) using response surface methodology. Sep. Purif. Technol. 2011 July; 80: 375-384.
11. Rey L, May JC. Freeze-Drying/ Lyophilization of Pharmaceutical and Biological Products. New York, U.S.A: Marcel Dekker Inc.; 2010. 580 pp.
12. Deepak M, Sheweta B, Bhupendar Singh K. Deep Guar gum: processing, properties and food applications—A Review. J Food Sci Technol. 2014 March; 51(3): 409-418.
13. Prabhanjan H, Gharia, MM, Srivastava HC. Guar gum derivatives. Part I:Preparation and properties. Carbohyd Polym. 1989 January; 11(4): 279–292.
14. Panyawong S, Devahastin S. Determination of deformation of a food product undergoing different drying methods and conditions via evolution of a shape factor. J. Food Eng. 2007 January; 78: 151-161.
15. Kerdpiboon S. Thesis Summary: Use of Fractal Analysis to Evaluate Physical Changes of Foods during Drying. Dry Technol. 2007 June; 25: 1131.
16. Quevedo R, López GR, Aguilera JM, Cadoche L. Description of food surfaces and microstructural changes using fractal image texture analysis. J. Food Eng. 2002 August; 53: 361-371.
17. Santacruz-Vázquez C, Santacruz-Vázquez V, Chanona-Perez J, Jaramillo-Flores M E, Welti-Chanes J, Gutierrez-Lopez G F. Encyclopedia of Agricultural, Food, and Biological Engineering, 2nd ed. Boca Raton, Florida U.S.A: CRC Press; 2010 Oct 21. Fractal Theory Applied to Food Science; 1-12 p.

18. Santacruz-Vázquez V, Santacruz Vázquez C, Laguna-Cortés JO, Toxqui-López S. Cambios en la textura de la superficie de esferas de melón (Cucumis melo cantalupensis) durante el secado por fluidización. Vitae. 2013 November; 20(3): 161-171.
19. Valous NA, Drakakis K, Sun DW. Detecting fractal power-law long-range dependence in pre-cooked pork ham surface intensity patterns using Detrended Fluctuation Analysis. Meat Sci. 2010 October; 86(2): 289 - 297.
20. Valous NA, Mendoza F, Sun DW, Allen P. Texture appearance characterization of pre-pork ham images using fractal metrics: Fourier analysis dimension and lacunarity. Food Res. Int. 2009 April; 42: 353 - 362.
21. Wen-Shiung Ch, Shang-Yuan Y, Chih-Ming H. Two algorithms to estimate fractal dimension of gray-level images. Opt. Eng. 2003 August; 42(8): 2452 – 2464.
22. Pentland AP. Fractal based description of natural scenes. IEEE T Pattern Anal. 1984 November; 6: 661- 674.
23. Kerdpiboon S, Devahastin S, Kerr WL. Comparative fractal characterization of physical changes of different food products during drying. J. Food Eng. 2007 December; 83: 570 -580.
24. Dàvila E, Parés D. Structure of heat-induced plasma protein gels studied by fractal and lacunarity analysis. Food Hydrocolloid. 2007 March; 21: 147 – 153.
25. Kerdpiboon S, Kerr WL, Devahastin S. Neural network prediction of physical property changes of dried carrot as a function of fractal dimension and moisture content. Food Res. Int. 2006 December; 39: 1110 - 1118.
26. Pedreschi F, Aguilera JM, Brown CA. Characterization of food surfaces using scale-sensitive fractal analysis. J. Food Process. Eng. 2000 January; 23: 127-143.
27. Xu P, Mujumdar AS, Yu B. Fractal Theory on Drying: A Review. Dry Technol. 2008 May; 26: 640-650.
28. Thuwapanichayanan R, Prachayawarakorn S, Soponronnarit S. Drying characteristics and quality of banana foam mat. J. Food Eng. 2008 June; 86: 573 -583.
29. Association of Official Analytical Chemists International, AOAC. Official methods of analysis; 16th Ed.; Arlington, Virginia, USA, 1995.
30. Karim AA, Wai CC. Characteristics of foam prepared from starfruit (Averrhoa carambola L.) puree by using methyl cellulose. Food Hydrocolloid. 1999 May; 13: 203 -210.
31. Haihong Zhang, Guiying Xu, Teng Liu, Long Xu, Yawen Zhou. Foam and interfacial properties of Tween 20- bovine serum albumin systems. Colloid surface A. 2013 January; 416: 23-31.
32. Karim AA, Wai CC. Foam-mat drying of starfruit (*Averrhoa carambola L.*) puree. Stability and air drying characteristics. Food Chem. 1999 February; 64: 337-343.
33. Ridler TW, Calvard S. Picture thresholding using an iterative selection method, IEEE T Syst Man CY C, SMC-8 1978: 630-632.
34. Cooke RD, Breag GR, Ferbe CEM, Best PR, Jones J. Studies of mango processing I. The foam-mat drying of mango (Alphonso cultivar) puree. Int. J. Food Sci. Tech. 1976 October; 11: 463- 473.
35. Lim LT, Tang J, He J. Moisture sorption characteristics of freeze-dried blueberries. J. Food Sci. 1995 July; 60: 810 -814.
36. Hamdami N, Monteau JY, Le Bail A. Transport properties of a high porosity model food at above and sub-freezing temperatures. Part 2: Evaluation of the effective moisture diffusivity from drying data. J. Food Eng. 2004 May; 62: 385 – 392.
37. Vickers Z, Bourne MC. Crispness in food -A review. J. Food Sci. 1976, 41: 1153 -1157.
38. Sham PWY. Scaman CH, Durance TD. Texture of vacuum dehydrated apple chips as affected by calcium pretreatment, vacuum level, and apple variety. J. Food Sci. 2001 November; 66: 1341 -1347.
39. Santacruz-Vázquez C, Santacruz-Vázquez V. The spatial distribution of ß-carotene impregnated in apple slices determined using image and fractal analysis. J. Food Sci. Tech. 2013 July. Doi 10.1007/s13197-013-1097-5.
40. Rahman MS, Al-Amri OS, Al-Bulushi IM. Pores and physico-chemical characteristics of dried tuna produced by different methods of drying. J. Food Eng. 2002 August, 53: 301-313.
41. Rahman MS, Toward prediction of porosity in foods during drying: A brief review. Dry Technol. 2001, 19: 1-13.

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 87-100
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a03

MODIFICATION OF NUTRITIONAL VALUE DURING STORAGE OF INFANT FORMULAS ELABORATED WITH DIFFERENT INTACT AND PARTIALLY HYDROLYZED PROTEINS AND CARBOHYDRATES

MODIFICACIÓN DEL VALOR NUTRICIONAL DURANTE EL ALMACENAMIENTO DE FÓRMULAS INFANTILES ELABORADAS CON DIFERENTES PROTEÍNAS INTACTAS, PARCIALMENTE HIDROLIZADAS Y CARBOHIDRATOS

J. Contreras-CALDERÓN, Ph.D.[1*], E.J. GUERRA-HERNÁNDEZ, Ph.D[2], B. García-VILLANOVA, Ph.D[2]

Recibido: Agosto 31 de 2014. Aceptado: Septiembre 21 de 2015.

ABSTRACT

Background: Human milk is considered the best source of nutrition for young infants. However, if mothers cannot provide adequate breast milk or if infants are premature or have a low birth weight, breast-feeding must often be replaced or complemented with infant formulas (IFs). The interactions between infant formula components (proteins, fats, carbohydrates, vitamin and minerals) mainly affect carbohydrates and proteins (Maillard reaction, MR), but those involving proteins are especially important in products used in infant feeding because of the high protein requirements of infants. On the other hand, fatty acids (FAs) are considered important in infant development. **Objectives:** The aim of the present work was to test the stability of IFs made with different ingredients, analyzing the available lysine losses (for protein stability) and the FAs content and the peroxide value (for fat stability) during stored at normal and adverse conditions and to propose a faster control of that stability. **Methods:** Available lysine analyzed by high-performance liquid chromatography (HPLC), lipid oxidation by titrimetric method and FAs profile by gas chromatography (GC) were determined in four types of IFs prepared with intact and partially hydrolyzed proteins and different carbohydrates (lactose or maltodextrins) during storage at 4, 20 and 30 ºC for 24 months at normal water activity (Aw=0.1-0.4), and at Aw of 0.65 at 20 and 30 ºC for 4 weeks. These IFs were prepared twice (IF_1 and IF_2) in different batches by a Spanish dietary product company. **Results:** At 30ºC, available lysine losses were 40-50% in all IFs analyzed. The behavior and percentage lysine loss between 1 and 4 weeks of storage at 30 °C with Aw=0.65 was similar to those obtained after 24 months of storage at 30 °C. No significant changes were observed in fatty acid profile during storage. Oxidation was only observed in opened packs and after 4 weeks/30 °C/Aw=0.65. **Conclusions:** The losses of available lysine increase to higher time and storage temperatures. The FAs shows a good stability for any storage condition; however peroxide values prove more sensitive than FAs changes for evaluating fat oxidation during the storage of IFs.

Keywords: Infant formulas, available lysine, milk protein, fatty acids, peroxides

[1] Departamento de Alimentos, Facultad de Ciencias Farmacéuticas y Alimentarías, Universidad de Antioquia, Medellín, Colombia

[2] Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Granada, Campus Universitario de Cartuja, 18012 Granada, España

[*] Corresponding author: jose.contrerasc@udea.edu.co

RESUMEN

Antecedentes: La leche materna es la mejor fuente nutricional para niños recién nacidos. Sin embargo, cuando las madres no pueden proveer leche, o si los niños son prematuros o tienen bajo peso al nacer, la lactancia debe ser reemplazada o complementada con fórmulas infantiles (IFs). La interacción entre los componentes de las IFs (proteínas, grasas, carbohidratos, vitaminas y minerales) principalmente afecta carbohidratos y proteínas (Reacción de Maillard, MR), pero los que implican proteínas son especialmente importantes en alimentación infantil debido a los altos requerimientos de proteína de los lactantes. Por otra parte, los ácidos grasos (FAs) son considerados importantes en el desarrollo de los niños. **Objetivos:** El objetivo fue evaluar la estabilidad de IFs elaboradas con diferentes ingredientes, analizando lisina útil (para estabilidad de proteínas), contenido de FAs y valor de peróxidos (para estabilidad de grasas) durante el almacenamiento bajo condiciones normales y adversas, y proponer un control más rápido de la estabilidad. **Metodos:** Los análisis de lisina útil por cromatografía liquida de alta resolución (HPLC), oxidación lipídica por el método de titulación y el perfil de FAs por cromatografía de gases (GC) fueron determinados en cuatro tipos de IFs preparadas con proteínas intactas y parcialmente hidrolizadas con diferentes carbohidratos (lactosa y maltodextrinas) durante el almacenamiento a 4, 20 y 30 ºC por 24 meses a actividad de agua normal (Aw=0,1-0,4), y a Aw de 0,65 a 20 y 30 ºC por 4 semanas. Estas IFs fueron preparadas dos veces (IF_1 y IF_2) en baches diferentes por una empresa Española de productos dietarios. **Resultados:** A 30ºC, las pérdidas de lisina útil fueron de 40-50% en todas las IFs analizadas. El comportamiento y porcentaje de pérdidas de lisina entre la 1 y 4 semana de conservación a 30 °C con Aw=0,65 fue similar a las obtenidas tras 24 de almacenamiento a 30 °C. No se observaron cambios significativos en el perfil de FAs durante el almacenamiento. Oxidación solo se observo en los envases abiertos y después de 4 semanas/30 °C/Aw=0.65. **Conclusiones:** Las pérdidas de lisina útil incrementan a mayor tiempo y temperatura de almacenamiento. Los FAs muestran una buena estabilidad para cualquiera de las condiciones de almacenamiento, sin embargo el valor de peróxidos evidencia mayor sensibilidad que los cambios en FAs para evaluar la oxidación de las grasas durante el almacenamiento de IFs.

Palabras clave: Formulas infantiles, lisina útil, proteína láctea, ácidos grasos, peróxidos

INTRODUCTION

Infant formulas (IFs) are designed as substitutes for maternal milk when breast feeding is not possible. Generally, they contain a source of protein such as milk protein, milk protein hydrolysate or soybean protein. Lactose, starch, maltodextrins, sucrose and triglycerides are used as energy sources (1). There are two types of formula for normal feeding, adapted formula suitable for infants under 4 months old and follow-up formula for those over 4–6 months old. IFs prepared with partially hydrolyzed proteins are also used in certain cases, e.g., to prevent or treat cow's milk allergies (2)

Production, handling and storage warrant greater attention during the manufacture of IFs compared with other food formulations, because they are more subject to reactions and interactions and their physical properties and nutrient availability can undergo major changes during storage. The storage temperature and relative humidity are two important factors in the control of physico-chemical properties and microbial quality of infant foods. Several types of physico-chemical damage can occur during storage, e.g., lactose crystallization, the Maillard reaction (MR), lipid peroxidation, caking, and interactions and reactions between proteins and polysaccharides, etc (1). The MR involves amino acids and reducing carbohydrates and can lead to loss of nutritional value (3). IFs containing reducing carbohydrates (lactose or dextrinomaltose) and proteins or free amino acids are prone to non-enzymatic browning as a result of the MR.

Loss of lysine availability and decrease of protein digestibility are the main nutritional consequences of the MR during the manufacture and storage of IFs (4, 5). This nutritional damage is of particular interest in IFs because they are frequently the only protein source during early infancy. The nutritional requirement for lysine of babies aged 0 to 6 months is around 107 mg/kg /day (6).

On the other hand, fatty acids (FAs) are considered important in infant development. The main FAs in human milk are palmitic (20%), oleic (38%) and linoleic (15%) (7). Unsatured FAs are highly susceptible to oxidation; lipid oxidation is the main cause of deterioration of lipid-containing IFs. The reaction between atmospheric oxygen and unsaturated lipids produces a wide range of hydroperoxides (8). Lipid oxidation has received much attention because of its undesirable implications for human health and its contribution to a decrease in the nutritional value of foods. Thus, peroxide values indicate the initial products of autoxidation and can only be used to study peroxide formation in the early stage of oxidation. During the course of oxidation, peroxide value may reach a peak and then decline (9).

There have been several studies on the lysine loss caused by heat treatment and storage of powered IFs (10-12) and on the influence of water activity during storage of powdered milk-like systems (13). However, there are no data on the effects of adverse storage conditions (temperature and Aw) that usually occur in the European Mediterranean areas, African, American, and Asian countries who consume these products or on comparisons between formulas made with different intact proteins and those made with protein hydrolysates prepared with different carbohydrates. Moreover, there are no comparative studies available on products with the same formulation but different batches of ingredients.

The purpose of this study was to determine the loss of available lysine and assess lipid oxidation by peroxide level and fatty acid profile during the storage of IFs prepared by industry with intact or hydrolyzed proteins under normal (with the original product water content) and under abnormal humidity conditions (water activity, Aw=0.65), using different ingredients (protein and carbohydrates) and using different batches of the same ingredients, to discover whether the IFs still complied with established requirements and European regulations after storage and to propose a faster control of that stability.

MATERIALS AND METHODS

Samples and reagents

The study was performed using four powdered IFs prepared with different carbohydrates (maltose-dextrin (dextrose equivalent (DE)=17), sucrose or lactose) and milk proteins (calcium caseinate (90% of protein), whey protein milk low in lactose (76% of protein), whey protein milk (64% of protein), and partially hydrolyzed (molecular weight of 8000–20,000 Da) whey protein milk low in lactose (82% of protein)). Two IFs had intact protein (A and B) and two had partially hydrolyzed whey protein (C and D) (Table 1). IFs A, C and D are adapted formulas and infant formula B is a follow-up formula. Each formula was prepared twice (IF_1 and IF_2) under the same conditions and their composition was given by the Spanish dietary product company (Table 1). Although the formulation was the same in both plants, the ingredients were from different batches. The infant formula D only was prepared once, with the same batch protein ingredient used in infant formula C2.

Ingredients were mixed with deionized water at <60 ºC under agitation to obtain 40% total solids (approximately 3 hours). Oil was added at 90-95 ºC for 22 sec. Spray-drying was performed at 215-230 ºC and the temperature of exit air was 85-95 ºC. There was a final amino acid addition stage in dry, partially hydrolyzed formulas; the added amino acids were L-Arginine, L-Phenylanaline, L-Histidine, L-Trytophan, and L Tyrosine. The final product was packed under nitrogen atmosphere in a 400g aluminium bottle.

Acetic acid, ethanol (HPLC grade), hexane, methanol, sodium thiosulfate, starch sulphuric acid and potassium iodide were obtained from Panreac (Barcelona, Spain). N-ε-2,4-DNP-L-lysine-HCl, butyl hydroxy anisol (BHA), chloroform, FAs methyl esters, sodium methylate, hexane, margaric acid (C:17) and derivative reagent 1-Fluoro-2,4-dinitrobenzene (FDNB) solution were purchased from Sigma-Aldrich (Madrid, Spain).

Table 1. Final composition of IFs.

Formulas	% Content	
A	Prepared 1	Prepared 2
Carbohydrates	54.9	
Sucrose	24.7	
Maltose-dextrin	30.2	
Proteins	11.33	11.56
Whey protein milk low in lactose	5.38	5.49
Calcium caseinate	5.90	6.10
Fat	27.7	
Linoleic acid	4.20	
Minerals	3.1	
Water content	3.3	
B	**Prepared 1**	**Prepared 2**
Carbohydrates	55.2	
Lactose	55.2	
Proteins	11.94	12.10
Whey protein milk	5.73	5.81
Calcium caseinate	6.21	6.29
Fat	27.3	
Linoleic acid	5.50	
Minerals	2.90	
Water content	3.00	
C	**Prepared 1**	**Prepared 2**
Carbohydrates	55.9	
Sucrose	8.4	
Maltose-dextrin	47.5	
Proteins	13.14	13.29
Partially hydrolyzed whey protein milk low in lactose	13.14	13.29
Fat	25.35	
Linoleic acid	4.10	
Minerals	2.90	
Water content	2.60	
D	**Prepared 1**	
Carbohydrates	55.90	
Sucrose	8.4	
Lactose	47.5	
Proteins	13.5	
Partially hydrolyzed whey protein milk low in lactose	13.5	
Fat	25.35	
Linoleic ccid	4.1	
Minerals	2.9	
Water content	2.6	

Storage conditions

Formulas, packed under nitrogen atmosphere in a 400g aluminum bottle were introduced into an oven at 20 or 30 ºC or cold chamber at 4 ºC and analyses were performed after 3, 6, 12, 15 and 24 months of storage (useful life of an IF). Storage at 4 ºC was not performed at 3 months. A different bottle was used for each storage time and temperature and was removed at the corresponding time. Another study was realized at Aw=0.65, samples of formula for each storage time and temperature were placed in open Petri dishes in desiccators with sodium nitrite saturate solution to obtain an Aw of 0.65 (14), which were then placed in an oven at 20 or 30 ºC during 1, 2, 3 and 4 weeks of storage. All IFs were tested at baseline (month 0 at room temperature). After the corresponding storage period, samples were kept at -20 ºC until their analysis.

Available lysine determination

ε-NDP-lysine was determined by HPLC, following the method used for IFs by Contreras-Calderón et al., 2009 (2). A sample containing approximately 4 mg of protein was derivatized by addition of FDNB solution in 3% v/v, ethanol. The FDNB derivative was hydrolyzed with HCl. HPLC was performed using a Perkin–Elmer Model 250 fitted with a Waters 717 automatic injector and a Perkin–Elmer 235 UV diode array detector, using a Model 1020 Perkin–Elmer Nelson integrator-computer. Fifty microliters of filtered solution were separated in a Novapack reverse-phase C_{18} HPLC column (4 μ, 150x3.9 mm, Waters, Milford, MA) operating at room temperature. ε-DNP-lysine was determined by the external standard method. The stock standard solution was 100 mg/ml of N-ε-2.4-DNP-L-lysine-HCl in methanol:water (1:4). Working standard solutions (2-10 mg/ml) were prepared by diluting stock standard solutions in 0.01 M sodium acetate (pH 5). A regression coefficient (r^2) of 0.9903 was obtained. The linear regression equation used was Y = 42082X + 2188, where Y is the peak area and X the ε- NDP-lysine concentration (mg/l). Duplicate analyses of duplicate samples were carried out (n = 4).

Fatty acid determination

Fat extraction was performed following the method described by Folch et al., 1957 (15). Two ml of IFs_1 (1.2 g/10 ml bi-distilled water) was added to 20 ml chloroform: methanol solution (2:1), 2 ml of BHA 1500 mg/l and 200 μl of internal standard C_{17} (20 mg/ml), shaking for 30 min in an amber Erlenmeyer flask with rubber stopper, then centrifuging the solution at 4500 rpm for 10 min to obtain three layers. The chloroform layer was evaporated under vacuum. Subsequently, 6 ml of sodium methylate was added and then boiled for 5 min. Following the method, 6 ml of a 3% (v/v) solution of sulphuric acid in methanol was added and then boiled for a

further 5 min. Finally, after 5 min of cooling, 4 ml of hexane was added, shaken gently, and water was added to bring the hexane layer to the neck of the flask. Finally, 2 μl were injected into a Perkin Elmer autosystem GC equipped with flame ionization detector (FID) (16) coupled to a Perkin-Elmer integrator. An SP-2330 capillary column (30 m x 0.75 mm) (Teknokroma, Barcelona, Spain) was used. Operating conditions were: injector 250 ºC, detector 300 ºC, oven at 50 ºC heated at 15 ºC/min to 190 ºC and held at 190 ºC for 5 min and then heated at 5 ºC/min to 230 ºC and held at 230 ºC for 5 min. Nitrogen at a flow rate of 1.43 ml/min was utilized as carrier gas. The split used was 1:35. Fatty acid methyl esters (FAMEs) were identified by comparison with relative retention times of standards. FAs concentrations were calculated according to the integrated peak area relative to the internal standard using the following correction factors for the individual FAMEs (two standard mixtures, each injected 3 times): 1.0527(C8), 0.989(C10), 0.913(C12), 0.958(C14), 0.908(C16), 0.947(C18), 0.914(C18:1), 0.997(C18:2), 1.13(C18:3), 1.015(C20), 1.061(C22). Range of FAMEs concentrations was 0.00094 - 0.015 mg/ml for C12, C16 and C18 and 0.0005-0.008 mg/ml for C6, C10, C14, C18:1, C18:2, C18:3, C20 and C22. Duplicate analyses of duplicate samples were carried out (n = 4).

Peroxide value

Fat extraction was performed following the method described by Folch et al., 1957 (15). Peroxide value was determined only in IFs_1 according to the peroxide value of oils and fats (17); 4 ml acetic acid-chloroform solution (3:1) was added to the fat obtained (0.25 g approximately) and dissolved by shaking. Subsequently, 1 ml of saturated potassium iodine solution was added, swirled for one min and stored in darkness for 5 min, followed by the addition of 10 ml of water. It was slowly titrated with 0.01 N sodium thiosulphate standard solution, vigorously shaking until the yellow color almost disappeared. Then, 0.5 ml of 1% starch solution was added and titration by vigorous shaking continued to release all iodine from the chloroform layer until the blue disappeared. Samples were analyzed in duplicate.

Statistical analysis

Statgraphics 5.1software was used for the statistical analysis. The two-Way ANOVA test was used to compare the storage times and the temperatures effect on available lysine (LSD test was used). The one-way ANOVA test was used to compare the storage times effect on FAs and peroxide value, and level of significance was set at 95%.

RESULTS

Available lysine determination

Precision of the entire available lysine procedure, including acid hydrolysis, sample preparation and RP-HPLC analysis, was evaluated using a commercial follow-up infant formula (n=8). The mean available lysine was 3.69 mg/100g of protein with a coefficient of variation of 4.6%. The detection limit (LOD) (three times the signal-to-noise ratio) was 7.25×10^{-4} mg/100g of protein, and the quantification limit (LOQ) (ten times the signal-to-noise ratio) was 2.42×10^{-3} g/100 mg of protein (2).

Normal storage

Available lysine contents during storage are shown in Table 2. During storage at normal conditions significant ($p < 0.05$) effects of time and temperature on lysine losses were observed in all IFs analyzed, except in the time for IFs B_2 and D.

Table 2. Available lysine content (mg/100 g of protein, dry weight), and percentage (%) of loss, during normal storage of IFs.

Time (months)	Formula A_1						Formula A_2					
	4ºC	%	20ºC	%	30ºC	%	4ºC	%	20ºC	%	30ºC	%
0	4993±133^{a}	0	4993±133^{a}	0	4993±133^{a}	0	5870±41.0^{a}	0	5870±41.0^{a}	0	5870±41.0^{a}	0
3	-		4777±21.0^{b1}	4	4642±10.0^{b2}	7	-		4977±20.0^{b1}	15	4940±124^{b1}	16
6	4822±2.00^{b1}	3	4334±10.0^{c2}	13	4435±7.00^{c3}	11	4998±165^{b1}	15	4813±31.0^{c1}	18	4764±93.0^{b1}	19
15	4308±82.0^{c1}	14	4282±41.0^{c1}	14	4032±93.0^{d2}	19	4854±21.0^{b1}	17	4650±10.0^{d2}	21	4143±155^{c3}	29
24	-		-		2533±93.0^{e}	49	-		-		2817±0.00^{d}	52
Time (months)	**Formula B_1**						**Formula B_2**					
	4ºC	**%**	**20ºC**	**%**	**30ºC**	**%**	**4ºC**	**%**	**20ºC**	**%**	**30ºC**	**%**
0	6438±183^{a}	0	6438±183^{a}	0	6438±183^{a}	0	5951±30.0^{a}	0	5951±30.0^{a}	0	5951±30.0^{a}	0
3	-		5630±143^{b1}	13	5369±61.0^{b2}	17	-		5387±409^{b1}	9	5267±901^{a1}	11
6	5272±143^{b1}	18	5047±41.0^{c2}	22	4857±195^{c2}	25	5090±162^{b1}	14	4937±429^{b2}	17	4612±225^{a2}	23
15	4761±82.0^{c1}	26	4852±7.00^{d1}	25	4437±51.0^{d2}	31	4614±193^{c1}	22	4344±82.0^{c1}	27	4038±7.00^{b2}	32
24	-		-		3730±133^{e2}	42	-		-		3238±338^{c}	46
Time (months)	**Formula C_1**						**Formula C_2**					
	4ºC	**%**	**20ºC**	**%**	**30ºC**	**%**	**4ºC**	**%**	**20ºC**	**%**	**30ºC**	**%**
0	5694±235^{a}	0	5694±235^{a}	0	5694±235^{a}	0	5307±92.0^{a}	0	5307±92.0^{a}	0	5307±92.0^{a}	0
3	-		5491±7.00^{a1}	4	5307±10.0^{b2}	7	-		4597±153^{b1}	13	4568±113^{b1}	14
6	5568±9.00^{a1}	2	5501±21.0^{a2}	3	5100±9.00^{c3}	10	4174±31.0^{b1}	21	4045±92.0^{c1}	24	4024±110^{c1}	24
15	4366±7.00^{b1}	23	4306±93.0^{b1}	24	4198±1.00^{d2}	26	4225±41.0^{b1}	20	3984±102^{c2}	25	3727±62.0^{d3}	30
24	-		-	-	3389±218^{e}	40	-		-	-	2977±21.0^{e}	44
Time (months)	**-**						**Formula D**					
	-	**-**	**-**	**-**	**-**	**-**	**4**	**%**	**20**	**%**	**30**	**%**
0	-	-	-	-	-	-	5295±173^{a}	0	5295±173^{a}	0	5295±173^{a}	0
3	-	-	-	-	-	-	-		5252±92.0^{a1}	1	5331±41.0^{a1}	0
6	-	-	-	-	-	-	5206±6.00^{a1}	2	5283±31.0^{a1}	0	5321±21.0^{a1}	0
15	-	-	-	-	-	-	5196±71.0^{a1}	2	5008±41.0^{b2}	5	5104±62.0^{b2}	4
24	-	-	-	-	-	-	-		-		3354±238^{c}	37

n = 4. Different letters indicate significant differences between storage times (columns) and different numbers between temperatures (rows).

Formulas with intact proteins

Formula A. For the IFs A, showed significant losses ($p < 0.05$) respect to the control sample (0 months of storage), from the third month for all temperatures tested, and were higher at elevated temperature and storage time. Available lysine losses were between 3% after 6 months at 4ºC for IF A_1 and 52% after 24 months at 30 ºC for IF A_2.

Formula B. Losses respects to the control sample (0 months of storage) in IFs B were significant ($p < 0.05$) after 3 months for all temperatures tested, except in IF B_2 at 30°C. Available lysine loss after 24 months at 30 ºC ranged between 42 and 46% for IF B_1 and IF B_2 respectively (Table 2).

Formulas with partially hydrolyzed proteins

Formulas C and D. Losses in available lysine respect to the control sample (0 months of storage) in the IFs C samples were significant ($p < 0.05$) from 3 months in all IFs, except in IF C_1 at 4 and 20°C. Available lysine losses were between 20 and 30% after 15 month for the three temperatures and between 40 and 44% after 24 months at 30 ºC for IF C_1 and IF C_2 respectively (Table 2).

IF D (with lactose), with the same protein ingredient as IF C_2, and prepared only once, showed a significant losses ($p < 0.05$) only after 15 months of storage at 20 and 30 °C, with a loss of available lysine at the end of the storage of around 37% (Table 2).

Storage at Aw = 0.65

The available lysine contents during storage at 20 and 30 °C are shown in Table 3. Losses in available lysine in all IFs were significant ($p < 0.05$) at the two temperatures tested from the 1 week respect to the control sample (0 weeks of storage). The effect of time and temperature on lysine losses was significant ($p < 0.05$) in all IFs.

Formulas with intact proteins

Formulas A and B. Available lysine losses from formulas A and B after 1 and 4 weeks of storage were statistically significant ($p < 0.05$) at the two temperatures tested (20 and 30 °C) (Table 3). At 30 °C, losses ranged from 42 to 62% after 4 weeks. After 4 weeks of storage at 20 °C, losses of lysine were almost the same as at 30 °C after 1 week for all prepared product (A and B), except for IF B_1 where losses were lower at 20 °C. For the same storage times, significant losses ($p < 0.05$) were observed between 20 and 30 °C except in IF B_2 after 4 weeks.

Formulas with partially hydrolyzed proteins

Formulas C and D. At both 20 and 30 °C, there were significant lysine losses ($p < 0.05$) from these IFs, prepared with hydrolyzed proteins, at 1 week and from 1 to 4 weeks of storage, except for formula C_2 and D at 30 °C between 1 and 4 weeks (Table 3). After 4 weeks at 30 °C, losses were 42% and 34% for IF C_1 and C_2 respectively, lower than those found from the IFs prepared with intact proteins (42 to 62%). After 4 weeks of storage at 20 °C, available lysine losses were almost the same as those at 30 °C after 1 week for both preparations (1 and 2) of formulas C and D. For the same storage times, significant losses ($p < 0.05$) were observed between 20 and 30 °C except in IFs C_2 and D after 4 weeks.

Table 3. Available lysine content (mg/100 g of protein, data expressed in dry weight) and percentage (%) of loss during storage at Aw 0.65 of IFs.

Time (weeks)	A_1				A_2			
	20°C	**%**	**30°C**	**%**	**20°C**	**%**	**30°C**	**%**
0	4933 ± 133^{a}	0	4933 ± 133^{a}	0	5870±41.0a	0	5870 ± 41.0^{a}	0
1	3091 ± 42.0^{b1}	38	2604 ± 2.00^{b2}	48	4813 ± 21.0^{b1}	18	3895 ± 32.0^{b2}	34
4	2640 ± 31.0^{c1}	47	1903 ± 11.0^{c2}	62	3837 ± 42.0^{c1}	35	2541 ± 53.0^{c2}	57
Time (weeks)	**B_1**				**B_2**			
	20°C	**%**	**30°C**	**%**	**20°C**	**%**	**30°C**	**%**
0	6438 ± 183^{a}	0	6438 ± 183^{a}	0	5951 ± 30.0^{a}	0	5951 ± 30.0^{a}	0
1	4642 ± 42.0^{b1}	28	3833 ± 41.0^{b2}	40	4391 ± 51.0^{b1}	26	3542 ± 10.0^{b2}	40
4	4313 ± 63.0^{c1}	33	3065 ± 0.00^{c2}	52	3523 ± 41.0^{c1}	41	3461 ± 31.0^{c2}	42
Time (weeks)	**C_1**				**C_2**			
	20°C	**%**	**30°C**	**%**	**20°C**	**%**	**30°C**	**%**
0	5694 ± 235^{a}	0	5694 ± 235^{a}	0	5307 ± 92.0^{a}	0	5307 ± 92.0^{a}	0
1	4412 ± 95.0^{b1}	23	3740 ± 54.0^{b2}	34	4590 ± 63.0^{b1}	14	3715 ± 43.0^{b2}	30
4	3549 ± 42.0^{c1}	38	3286 ± 33.0^{c2}	42	3431 ± 32.0^{c1}	35	3491 ± 21.0^{b1}	34
Time (weeks)	**-**				**D**			
	-	**-**	**-**	**-**	**20°C**	**%**	**30°C**	**%**
0	-	-	-	-	5295 ± 173^{a}	0	5295 ± 173^{a}	0
1	-	-	-	-	4159 ± 32.0^{b1}	21	3309 ± 42.0^{b2}	38
4	-	-	-	-	3306 ± 21.0^{c1}	38	$3308 \pm 5\ .00^{b1}$	38

n = 4. Different letters indicate significant differences between storage times (columns) and different numbers between temperatures (rows).

Fatty acid determination

The total Fatty acid content in IFs during storage ranged between 18.2 and 23.4 g/100 g of sample, being the oleic acid (C18:1 cis9) the major fatty acid present in the IFs (Table 4). No significant ($p < 0.05$) changes were observed in fatty acid profile during storage.

Peroxide value

Table 5 shows the behavior of peroxide values, expressed in meq O_2/kg, during storage of IFs. No oxidation was observed after 6 months of storage at 30 °C or after 15 months at 20 °C in closed retail packs (Table 5).

No oxidation was observed after 1 week of storage in opened pack at 30 °C/Aw=0.65%. The significant increases in the peroxide values were significant ($p < 0.05$) from 12 months in opened pack at 30 °C in all IFs and after 30°C/4week Aw=0.65% in IFs A and B respect to the control sample (0 months and 0 weeks of storage respectively). Peroxide values ranged from 10.6 to 12.6 meq O_2/kg after 15 months in opened pack at 30 °C and from 0 to 4.89 meq O_2/kg after 4 weeks in opened pack at 30 °C/Aw=0.65.

Table 4. Fatty acid content in IFs during storage (g/100 g of sample).

Fatty Acids	A1			
	Baseline	**30°C/15 months**	**30°C/1week/Aw0.65%**	**30°C/4week/Aw0.65%**
C8	0.63±0.00	0.62±0.01	0.64±0.05	0.64±0.09
C10	0.52±0.03	0.48±0.01	0.48±0.05	0.48±0.05
C12	3.22±0.06	3.22±0.05	3.20±0.51	3.23±0.17
C14	1.33±0.01	1.33±0.02	1.38±0.16	1.44±0.01
C16	1.86±0.01	1.75±0.01	1.75±0.06	1.84±0.06
C18:0	0.80±0.03	0.73±0.03	0.73±0.10	0.80±0.31
C18:1	9.73±0.02	8.98±0.32	8.99±0.12	9.86±0.40
C18:2	4.35±0.04	3.91±0.09	3.92±0.15	4.22±0.31
C18:3	0.43±0.02	0.37±0.01	0.42±0.03	0.43±0.03
C20	0.07±0.06	0.07±0.01	0.07±0.01	0.08±0.00
C22	0.15±0.03	0.14±0.02	0.13±0.03	0.16±0.02
Total	23.1±0.30	21.6±0.60	21.7±1.30	23.2±1.40
Fatty Acids	**B1**			
	Baseline	**30°C/15 months**	**30°C/1week/Aw0.65%**	**30°C/4week/Aw0.65%**
C8	0.47±0.02	0.43±0.07	0.57±0.01	0.58±0.10
C10	0.34±0.01	0.41±0.01	0.42±0.00	0.40±0.06
C12	2.62±0.03	2.91±0.16	2.94±0.01	2.91±0.33
C14	1.29±0.06	1.32±0.07	1.32±0.01	1.29±0.13
C16	1.84±0.07	1.90±0.02	1.80±0.05	1.76±0.08
C18:0	0.86±0.03	0.84±0.01	0.83±0.05	0.81±0.01
C18:1	9.40±0.64	10.3±0.07	9.50±0.52	9.27±0.15
C18:2	4.37±0.29	4.56±0.09	4.45±0.23	4.25±0.08
C18:3	0.46±0.02	0.43±0.02	0.44±0.03	0.44±0.01
C20	0.11±0.06	0.09±0.00	0.07±0.01	0.09±0.00
C22	0.19±0.01	0.19±0.00	0.18±0.02	0.17±0.01
Total	22.0±1.20	23.4±0.50	22.5±1.00	22.0±0.90
Fatty Acids	**C1**			
	Baseline	**30°C/15 months**	**30°C/1week/Aw0.65%**	**30°C/4week/Aw0.65%**
C8	2.01±0.07	2.26±0.10	2.43±0.25	2.41±0.21
C10	1.99±0.12	2.01±0.26	2.04±0.24	2.02±0.02
C12	1.85±0.21	2.18±0.17	2.18±0.30	2.20±0.01
C14	0.64±0.29	0.74±0.23	0.91±0.12	0.92±0.03
C16	1.21±0.09	1.41±0.08	1.38±0.02	1.42±0.13
C18:0	0.54±0.10	0.59±0.09	0.55±0.09	0.55±0.07
C18:1	5.87±0.36	6.60±0.19	6.04±0.37	6.21±0.56
C18:2	3.54±0.31	3.97±0.43	3.89±0.25	3.99±0.32
C18:3	0.37±0.02	0.41±0.06	0.42±0.02	0.43±0.03
C20	0.07±0.01	0.07±0.01	0.06±0.01	0.06±0.00
C22	0.11±0.10	0.12±0.03	0.11±0.01	0.09±0.00
Total	18.2±1.70	20.3±1.70	20.1±1.70	20.3±1.40

n = 4

Table 5. Peroxide value in IFs during storage (meq O_2/kg).

	A_1	B_1	C_1
Pto 0	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
30ºC/6 month (closed pack)	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
30ºC/12 month (opened pack)	8.67 ± 0.20^b	5.96 ± 0.10^b	6.38 ± 0.30^b
30ºC/15 month (opened pack)	12.1 ± 0.1^c	10.6 ± 0.2^c	12.6 ± 0.10^c
30ºC/1week Aw 0.65%	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
30ºC/4week Aw 0.65%	4.89 ± 0.10^d	4.23 ± 0.30^d	0.00 ± 0.00^a

n = 2. Different letters indicate significant differences between storage times.

DISCUSSION

Available lysine determination

Normal storage

Formulas with intact proteins

Formula A. The results (Table 2) show that the temperature and storage time had a significant effect on lysine losses. Differences in lysine losses between the two formulas (A_1 and A_2) derived from differences in the batches of proteins used, since their treatments were identical. It was also observed during the processing (2) that the initial content of available lysine differed between the two formulas. The linear inverse correlation between storage time and available lysine content was > 0.93 (r^2) and statistically significant ($p < 0.05$) at 30 ºC storage temperature for both formulas (Table 6).

Formula B. The linear inverse correlation obtained between storage time and available lysine content was > 0.92 (r^2) for formula B_2 at the three temperatures (Table 6). The correlation was statistically significant ($p < 0.05$) for both B formulas at 30 ºC but only for IF B_2 at 20 ºC. No significant differences in IF B_2 after 3 month at 30°C may be due to the high standard deviation of this sample (Table 2).

Table 6. Inverse linear correlations (r^2) between normal storage time and available lysine.

Temperature (ºC)	Time (months)	Formulas						
		A_1	A_2	B_1	B_2	C_1	C_2	D
4	15	0.973	0.764	0.891	0.924	0.899	0.606	0.730
20	15	0.734	0.614	0.732	0.929★	0.927★	0.670	0.867
30	24	0.934★	0.949★	0.859★	0.939★	0.998★	0.895★	0.756

★ $p < 0.05$

4 ºC. n = 3

20 ºC. n = 4

30 ºC. n = 5

For both types of formula made with complete proteins (A and B), losses were similar after 24 months of storage at 30 °C. These formulas were prepared with different types of carbohydrate (maltodextrins versus lactose), and with different kinds of whey protein. These data are consistent with lysine losses measured during the manufacture of both formulas (2). While there was always loss of lysine during storage at the temperatures studied, losses were smallest at 4 ºC, and it may be desirable to keep the formulas routinely under refrigeration. With long-term (15 months) storage, losses were similar at 4 and 20 °C. The latter temperature should certainly not be exceeded during storage, because changes are much higher at 30 °C.

Guerra-Hernández et al., 2002 (12) reported available lysine losses of 8.9% in IF elaborated with milk protein and lactose and stored for 3 months at

20 °C and of 88.3% in IF stored for 3 months at 55 °C. The IF stored at 20 °C showed slightly lower losses than those observed in the present study. Ferrer et al., 2003 (4) stored adapted and follow-up IFs elaborated with milk protein and lactose at 20 and 37 °C for 24 months. Initial available lysine values were 8430 and 7900 mg/100 g of protein respectively, and they found storage losses for adapted IF of 20% at 20 °C and 24% at 37 °C and for follow-up IF of 48% and 49% at 20 and 37 °C, respectively. In the present study, initial levels of available lysine in the IFs were lower, but the losses were similar. Chávez-Servín et al., 2008a (18) store powder IF based on milk proteins and lactose at 20 and 37 °C for 12 months. The initial available lysine values were ranged between 6010 and 6040 mg/100 g of protein, similar to obtained in the present study and an average value accepted for human milk (6.6 g/100 g of protein) (19), however these authors only obtain losses when the storage is at 37 °C. In dried milk, El and Kavas, 1997 (20) found lysine losses of around 17% when skim milk powder was stored for 18 months at room temperature (20 – 30 °C). These same authors found that the available lysine of milk powders decreases during the drying process and storage (6 months) around 14 and 15% respectively.

The nutritional requirements of lysine for babies aged 0 to 6 months are around 107 mg/kg/day (6). A baby of 1-2 weeks weighing 3.3 kg therefore requires 353 mg of lysine/day, and the daily consumption of IF is 77.4 g (3 measures of 4.3 g per bottle for 6 bottles in 24 h according to label recommendations). For a baby aged 3 months weighing 5 kg, consumption of formula is around 150 g/day (7 measures of 4.3 g per bottle for 5 bottles in 24 h), and the need for lysine is approximately 535 mg/day. Lysine contents of IF A_1 and IF A_2 were 5000 and 5870 mg/100g of protein, respectively, exceeding the minimum estimated needs of infants, who require IF to provide a minimum of around 3200 mg of lysine per 100 g protein. This was the case for all storage times and temperatures, except for 24 months at 30 °C (Table 2). Recommended dietary allowances of lysine for infants aged 7 to 12 months are around 89 mg/kg/day (6). For a baby aged 12 months weighing 9 kg, consumption of formula is around 125 g/day (5 measures of 8.33 g per bottle for 3 bottles in 24 h according to label recommendations), and the need for lysine is approximately 801 mg/day. Lysine contents of IF B_1 and IF B_2 were 6440 and 5950 mg/100g of protein, respectively, more than covering the needs of infants, who require IF to provide a minimum of around 5300 mg of lysine per 100 g protein. Likewise, the stored IF B samples would cover infant needs up to 3 months of storage at all three temperatures tested (Table 2). However when children use follow–up formulas must consume other foods that can provide lysine. At present, there is no legal maximum content of blocked lysine; however, the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) recommend that the amount of blocked lysine for preterm infants should be kept to a minimum (21).

Formulas with partially hydrolyzed proteins

Formulas C and D. The loss of available lysine with time was nearly linear ($r^2 = 0.90$), and was significant ($p < 0.05$) for IF C_1 at 20 and 30 °C and for IF C_2 at 30 °C (Table 6). No significant differences in IF C_1 after 3 month at 4 and 30°C may be due to the high standard deviation of the control sample (0 months of storage) (Table 2).

In our study, the reduction of lysine in IF D was not significantly time-dependent (Table 6). At 30 °C lysine losses were similar in IF D, containing lactose, to those in IF C_2 with maltodextrins, as observed during their processing (2).

Available lysine losses after storage for 24 months at 30 °C were slightly lower in IFs prepared with hydrolyzed proteins (C and D) than in those prepared with intact proteins (A and B), probably more amino acids are available to react in IFs made with partially hydrolyzed proteins (22), proportionally reducing the amount of lysine that reacts. Similar lysine losses were observed during long-term IF storage at 4 and 20 °C, suggesting that product refrigeration is unlikely to be beneficial in these cases. These similar losses are due to the advance of MR which decreases the amount of reactants (sugars and amino acids) until the reaction is stabilized.

Storage at Aw = 0.65

It is important to study the stability of these formulas under the non-optimal storage conditions that can be found in some geographic areas, e.g., on the Mediterranean coast, where relative humidity is high and summer temperatures exceed 30 °C. Additionally, it is also useful to provide the industry with a tool for evaluating possible lysine losses after shorter periods of storage.

Formulas with intact proteins

Formula A and B.

At 30 °C and Aw=0.65, only 4 weeks were needed to achieve the same available lysine losses as those obtained after 24 months of storage at normal Aw. The time-dependency of available lysine on storage time was non-significant, probably due to the small data numbers.

Malec et al., 2002 (13) found losses of around 60% in a lactose-casein model system stored at 37 °C for 800 hours (around 1 month) with Aw=0.69. These losses were similar to those from IF A (prepared with sucrose and maltose-dextrin) and higher than those from IF B (prepared with lactose) after 4 weeks of storage at 30 °C/Aw = 0.65% (Table 4). Gen Pen and Melton, 2007 (23) found losses of around 4% in a lactose-sodium caseinate dry model system heated at 60 °C for 96 hours with relative humidity (RH) between 65 - 75%.

Formulas with partially hydrolyzed proteins

Formulas C and D.

The IFs prepared with hydrolyzates and stored at 30 °C and Aw=0.65 only required between 1 and 4 weeks to achieve the same loss as that obtained after 24 months of storage with normal Aw. IFs C_2 and D, with the same proteins but different carbohydrate (maltodextrins vs. lactose) had a similar initial content of lysine and loss during storage. No major difference in available lysine loss was observed between weeks 1 and 4, with all formulas showing the highest losses during the first week (30 - 48%), when humidity reached maximum values (around 9% in some products) before stabilizing (unpublished data).

The results show a clear dependence of time and temperature of storage on the content of available lysine in IFs. High temperature and long-term storage periods increase the loss of available lysine.

Fatty acid determination

The major fatty acid present in the IFs in quantitative terms was oleic acid (C18:1 cis9) with around 42% of total FAME in formulas elaborated with intact protein and 32% in formulas elaborated with hydrolyzed protein. Likewise the oleic acid content reported for human milk is the range of 24-40% (24). The level of saturated fatty acids (SFA) in IFs was around 36% in formulas elaborated with intact protein and 46% in formulas elaborated with hydrolyzed protein (also within the range found in human milk of 35-50%). The content in medium-chain FAs (MCFA C10:0-C14:0) was around 20% in IFs elaborated with intact protein and around 25% in formulas elaborated with hydrolyzed protein and these contents were much higher than that in human milk, especially due to the high content of C12:0 (approximately 12% in all IFs vs. 3-8% in human milk). Linoleic acid (LA C18:2 cis9-cis12) was the most abundant fatty acid of the PUFA fraction, with values of approximately 4% (91% of PUFA), which are lower than those present in human milk (8-18%) but within the range established by European regulations (25) (between 1.53 and 6.15) (Table 7). The content of alpha linolenic acid (ALA) (C18:3 cis9-cis12-cis15) account for approximately 0.4% and within that established by European regulations (25) (> 0.25) (Table 7). The LA/ALA ratio of the studied formulas range from 9.5-10.1, which falls almost with the ratio in human milk and that established by European regulations (25) (between 5 and 15) (Table 7). The FAs content were similar to reports by other authors for IFs (26-29).

Table 7. Comparison between recommendations for the fatty acid composition of IFs and values obtained.

FAs	Recommendations*	Values obtained
Lauric and myristic acids	<20% of total FAs	13.7-19.7
Linoleic acid	1.53-6.15 g/100 g of sample	3.54-4.37
α- linolenic acid	>0.25 g/100 g of sample	0.37-0.46
Linoleic/ α- linolenic	5-15	9.5-10.1

* European Union: Commission Directive 2006/141/EC on infant formulae and follow-on formulae and amending Directive 1999/21/EC (25).

No significant changes ($p < 0.05$) in FAs composition were observed during the storage of any of the three IFs under study. The results obtained by other authors are contradictors, Roumeu-Nadal et al., 2007 (27) found significant losses in supplemented formulas with LC-PUFA stored at 25 ° C for 15 months but not in unsupplemented formulas, as happens in our case. However, Rodriguez-Alcala et al., 2007 (30) found significant oleic and linolenic acid losses in adapted and follow-up formulas stored at room

temperature for 4 years. Chavez-Servin et al., 2009 (8) found significant linoleic losses in formulas with LC-PUFA supplemented stored at 25 and 40 °C for 18 months, but did not find significant losses for the same acid in the 55% of commercial formulas stored in open package at 25°C for 70 days (31). Manglano et al., (2005) (32) also found greatest significant loss of linoleic acid from storage month 15 onwards in IFs at 22 and 37 °C. Neither, Kus, Aued-Pimentel, & Mancini-Filho (2011b) (33) found significant losses in PUFA in 20% commercial formulas analyzed after 8 months of storage at room temperature. Rufian-Henares et al., 2005 (16) studied the FAs profile during storage of liquid enteral formula prepared with similar ingredients, and found statistically significant ($p < 0.01$) losses in oleic (C18:1), linoleic (C18:2) and linoleic (C18:3) acids after all time periods assayed (1-4 weeks at 32 and 55 °C) but there were no losses in the other FAs. A different behavior was observed during long-term storage (36 weeks): at 20 and 30 °C, there were statistically significant ($p < 0.01$) losses in all of the FAs analyzed, whereas at 4 °C, significant losses were only observed in the unsaturated FAs. The greater stability of infant versus enteral formulas may be due to their different states (powder vs. liquid), that is a different heat treatment: pasteurization vs sterilization. These results show that the analysis of FAs profiles can be used to establish the composition of three IFs but not to know the influence of storage conditions. On the other hand, this analysis can lacks of the sensitivity necessary for evaluating the stability lipid in the IFs.

Peroxide value

Peroxide values were higher in formulas elaborated with intact proteins (IF A_1 and IF B_1) than formula elaborated with hydrolyzed protein (IF C1). The content of unsaturated FAs in formulas A and B was higher than obtained in formula C (64 vs 54%). The peroxide value is a good indicator of the quality of fat. Freshly refined fats should have hydroperoxide levels of less than 1 meq O_2/kg (34). The limiting peroxide value specified by Joint FAO/WHO in 1989 (35) standards for refined oil is 10 meq O_2/kg. Roumeu-Nadal, Chávez-Servín, Castellote, Rivero, and López-Sabater (2007) (27) studied the oxidation stability of the lipid fraction in milk powder formulas and reported initial values of 0.52, 0.85 and 0.98 meq O_2/kg, respectively, for non-supplemented formula (NSF) and supplemented formulas (SFA and SFB) with different levels of n-3 and n-6 LC-PUFA. After 15 months of storage at 25 °C, NSF showed a hydroperoxide level of only 1.48 meq O_2/kg. In contrast, these levels increased slowly in SFA at 25 and 37 °C to be 4.5 and 10.5 fold higher than initial values, respectively. The hydroperoxide value of formulas without supplementary PUFA was very low or zero when stored at room temperature (20 – 25 °C) and in closed retail packs, similar results to those obtained by us. These same authors (Chávez-Servín, Castellote, Martin, Chifré, & López-Sabater, 2009) (8) studied the stability during storage of two IFs supplemented with PUFA stored at 25° and 40 °C for 18 months, the initial peroxide values ranged between 0.34 and 1.26 meq O_2/kg and reached values between 48.56 and 54.97 meq O2/kg after 18 months of storage at 40°C. In these IFs the oxidation occurred before storage, different to observed in the IFs of the present study where peroxides were not observed at the beginning of storage. Manglano et al., (2005) (32) also found no oxidation in all newly manufactured IFs with values between 1.3 and 65.1 meq O2/kg in IFs stored at 22 and 37 °C during 17 months. This same authors found significant differences ($p = 0.000$) with respect to storage time, though not depending on the storage temperature.

The peroxide values in the IFs under study were within permitted values for refined oils except for IFs stored at 30 °C for 15 months in opened packs. No oxidation was observed in formulas in closed retail packs, and oxidation was only observed in opened packs after > 6 months of storage at 30 °C. These small changes were not observed in the FAs determination probably because this analysis lacked the necessary sensitivity for evaluating lipid stability in IFs. Therefore peroxide values can serve as indicators of lipid oxidation and food damage.

CONCLUSIONS

Our study suggests that the losses of available lysine increased with higher storage temperatures up to 37 - 52%, according to the IF in question, after storage at 30 °C for 24 months. Similar losses are obtained when using maltodextrin instead of lactose as a carbohydrate. Percentage losses between 1 and 4 weeks of storage at 30 °C with a water activity of 0.65 were similar to those after 24 months of storage at 30 °C at normal water activity. This

may offer a useful method for rapidly estimating possible lysine losses in long-term storage. The rate of available lysine loss was higher in IFs prepared with intact protein than in those prepared with hydrolyzed proteins, which may be because other amino acids besides lysine take part in the MR. The lysine requirements of infants could be met by IFs stored at 4, 20 and 30 °C for 15 months but not by those stored at 30 °C for 24 months. The FAs values obtained were within ranges established by European regulations. FAs composition showed no significant changes during storage for any time period at any temperature or Aw. Peroxide values proved more sensitive than FAs changes for evaluating fat oxidation during the storage of IFs.

ACKNOWLEDGEMENTS

This work was supported by a predoctoral fellowship obtained from the Junta de Andalucía and by a research project from Commission Interministerial de Ciencia y Tecnología (Project AGL 2010 2977). The authors would like to thank to the Committee for the Development of Research -CODI- University of Antioquia and its strategy for Sustainability of Research Groups 2014-2015. The authors would like to thank Professor Oscar Vega Castro for assisting with the statistical analysis.

CONFLICT OF INTERESTS

Authors have declared no conflict of interests.

REFERENCES

1. Nasirpour A, Scher J, Desobry S. (2006). Baby foods: formulations and interactions [A Review]. Crit Rev Food Sci Nutr. 2006; 46: 665-681.
2. Contreras-Calderón J, Guerra-Hernández E, García-Villanova B. Utility of some indicators related to the Maillard browning reaction during processing of infant formulas. Food Chem. 2009; 114: 1265-1270.
3. Henle T, Walter H, Klostermeyer H. Evaluation of the extent of the early Maillard reaction in milk products by direct measurement of the Amadori product lactuloselysine. Z Lebesm Unters For. 1991; 193: 119-122.
4. Ferrer E, Alegría A, Farré R, Abellan P, Romero F, Clemente G. Evolution of available lysine and furosine contents in milk- based infant formulas throughout the shelf-life storage period. J Sci Food Agric. 2003; 83: 465–472.
5. Puig C, Perez MD, Ros L, Sanchez L, Calvo M. Effect of processing on the composition of infant fórmulas. Milchwissenschaft—Milk Sci Int. 2003; 58: 476-480.
6. DRIs. Dietary reference intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (macronutrients). The National Academy of Sciences. USA; 2002.
7. García-Onieva M. Lactancia artificial: técnica, indicaciones, fórmulas especiales. Pediatria integral. 2007; 11: 318-326.
8. Chávez-Servín JL, Castellote AI, Martin M, Chifré R, López-Sabater MC. Stability during storage of LC-PUFA-supplemented infant formula containing single cell oil or egg yolk. Food Chem. 2009; 113: 484-492.
9. Liang JH. Kinetics of fluorescence formation in whole milk powders during oxidation. Food Chem. 2000; 71: 459-463.
10. Ferrer E, Alegría A, Farré R, Abellán P, Romero F. Effects of thermal processing and storage on available lysine and furfural compounds contents of infant formulas. J Agric Food Chem. 2000; 48: 1817-1822.
11. Ferrer E, Alegría A, Farré R, Abellán P, Romero F, Clemente G. Evolution of available lysine and furosine contents in milk- based infant fórmulas throughout the shelf-life storage period. J Sci Food Agric. 2003; 83: 465-472.
12. Guerra-Hernández E, Leon C, Corzo N, García-Villanova B, Romera JM. Chemical changes in powdered infant fórmulas during storage. Int J Dairy. Technol. 2002; 55: 171–176.
13. Malec LS, Pereyra AS, Naranjo GB, Vigo M.S. Influence of water activity and storage temperature on lysine availability of a milk like system. Food Res Int. 2002; 35: 849-853.
14. Salmarch M, Labuza TP. Influence of relative humidity on the physicochemical state of lactose in spray-dried sweet whey powder. J Food Sci. 1980; 45: 1231-1235.
15. Folch J, Lees M, Stanley, GHS. A simple method for the isolation and purification of lipids from animal tissue. J Biol Chem. 1957; 226: 497-509.
16. Rufian-Henares JA, Guerra-Hernandez EJ, García-Villanova B. Evolution of fatty acid profile and lipid oxidation during enteral formula storage. Jepen-Parente Enter. 2005; 29: 204-211.
17. AOAC. Oficial Methods of Análisis of the Association of Oficial Analytical Chemists. Helrich, K (Ed), 15 th edn. Method 965.33. Arlington, Virginia, USA; 1990.
18. Chávez-Servín JL, Castellote AI, López-Sabater MC. Evolution of available lysine and lactose contents in supplemented microencapsulated fish oil infant formula powder during storage. Int J Food Sci. Tech. 2008a; 43: 1121-1128.
19. WHO. Energy and protein requirements. In: 7.3 Quality and digestibility of dietary proteins. Geneva: World Health Organization Technical Report Series 724. 2000. Available at: http://www.fao.org/DOCREP/003/AA040E/AA040E00.htm.
20. El SN, Kavas A. Available lysine in dried milk after processing. Int J Food Sci Nutr. 1997; 48: 109-111.
21. Bremer HJ. et al. Nutrition and feeding of preterm infants – Committee On Nutrition of the Preterm Infant, European Society of Paediatric Gastroenterology and Nutrition. Acta Paediatr Scand. 1987; 336: 3-14.
22. Penndorf I, Biedermann D, Maurer S.V, Henle, T. Studies on N-terminal glycation of pepdides in hypoallergenic infant formulas: Quantification of alpha-N-(2-furoylmethyl) amino acids. J Agric Food Chem. 2007; 55: 723-727.
23. Gen Pan G, Melton LD. Nonenzymatic browning of lactose and caseinate during dry heating at different relative humidities. J Agric Food Chem. 2007; 55: 1036-1042.
24. Jensen RG, Ferris AM, Lammi-Keefe CJ, Henderson RA. (1990). Lipids of bovine and human milks: a comparison. J of Dairy Sci. 1990; 73:223-240
25. European Union: Commission Directive 2006/141/EC on infant formulae and follow-on formulae and amending Directive 1999/21/EC. Official Journal of the European Union L 401, 30 December 2006, p. 1-33.
26. Satchithanadam S, Fritsche J, Rader JI. Gas chromatographic analysis of infant formulas for total fatty acids, including trans fatty acids. J AOAC Int. 2002; 85/1(86-94): 1060-3271.
27. Roumeu-Nadal M, Chávez-Servín JL, Castellote AI, Rivero M, López-Sabater MC. (2007). Oxidation stability of the lipid fraction in milk powder formulas. Food Chem. 2007; 100: 756-763.

28. Ren D, Wang D, Huo G. (2010). Fatty acid composition of breast milk and infant formula in the region of Harbin, China. Milchwissenschaft. 2012; 65 (2): 165-168.
29. Kus MMM, Silva SA, Aued-Pimentel S, Mancini-Filho J. (2011a). Nutrition facts of infant formulas sold in Sao Paulo state: assessment of fat and fatty acid contents. Revista de Nutricao-Brazilian J Nutr. 2011a; 24 (2): 209-218.
30. Rodriguez-Alcala LM, Garcia-Martinez MC, Cachon F, Marmesat S, Alonso L, Marquez-Ruiz G, Fontecha, J. Changes in the lipid composition of powdered infant formulas during long-term storage. J Agric Food Chem. 2007; 55 (16): 6533-6538.
31. Chávez-Servín JL, Castellote AI, López-Sabater MC. Volatile compounds and fatty acid profiles in commercial milk-based infant formulae by static headspace gas chromatography: evolution after opening the packing. Food Chem. 2008b; 107: 558-569.
32. Manglano P, Lagarda MJ, Silvestre MD, Vidal C, Clemente G, Farré, R. Stability of the lipid fraction of milk-based infant formulas during storage. Eur. J. Lipid Sci. Technol. 2005; 107: 815–823.
33. Kus MMM, Aued-Pimentel S, Mancini-Filho J. Stability of polyunsaturated fatty acids in commercial infant formulas. Brazilian J Food Tech. 2011b; 14 (2), 145-153.
34. Rosell JB. (1989). Measurement of rancidity. In JC Allen & RJ Hamilton (Eds.), Rancidity in foods. London: Elsevier Applied Science; 1989. 23-52 p.
35. FAO/WHO. Food Standard Program Recommended International Standards, London; 1989.

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 101-110
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a04

EVALUACIÓN SENSORIAL E INSTRUMENTAL DE TEXTURA DE SALCHICHONES TIPO ESTÁNDAR QUE CONTIENEN UN EXTENSOR CÁRNICO DE PASTA DE POLLO

MECHANICAL PROPERTIES AND SENSORY EVALUATION OF SALCHICHON STANDARD EXTENSION CONTAINING AN EXTENSOR OF MECHANICALLY DEBONED CHICKEN MEAT

Diana Marcela GONZÁLEZ RODRÍGUEZ M.Sc.[1*], Elizabeth GIRALDO LOPERA IQ[2], Diego Alonso RESTREPO MOLINA M.Sc.[3]

Recibido: Enero 26 de 2014 Aceptado: Septiembre 21 de 2015.

RESUMEN

Antecedentes: La carne de pollo mecánicamente deshuesada (CMD) o pasta de pollo es uno de los ingredientes principales utilizado en la industria cárnica para aportar proteína en la formulación de embutidos, normalmente se importa, pero se busca obtener extensores de esta materia prima con el fin de contribuir al desarrollo de la industria nacional. **Objetivos:** El objetivo de este trabajo fue determinar la mejor mezcla entre fibra, cuero y carragenina, mediante la evaluación de las propiedades microbiológicas, bromatológicas, reológicas y sensoriales de un salchichón tipo estándar fabricado con pasta de pollo y un extensor que reemplaza un 35% y 50% de esta en la formulación de la matriz cárnica. **Métodos:** Se realizó un diseño experimental que contempló la combinación de tres extensores en diez puntos de mezcla, cada uno compuesto por fibra, cuero de cerdo y carragenina respectivamente. Se llevó a cabo la experimentación para niveles de reemplazo de un 35% y 50%, cada uno de los salchichones fue sometido a evaluación de Análisis de Perfil de Textura (TPA), elasticidad y firmeza en el campo instrumental y el respectivo análisis sensorial. **Resultados:** La dureza y la masticabilidad en el análisis instrumental de textura disminuyeron con la aplicación de los extensores, sin embargo no hay diferencias estadísticamente significativas entre la aplicación de los tres extensores para la dureza. Respecto al análisis sensorial se encontró que la mezcla entre fibra y cuero de cerdo favorece las propiedades sensoriales, adicionalmente una interacción entre fibra y cuero de cerdo mejoró la masticabilidad, la elasticidad aumentó en los salchichones extendidos y una interacción entre los tres extensores para un reemplazo de 35% aumenta la elasticidad con respecto al testigo. La firmeza, a un reemplazo del 35%, disminuye para cualquier salchichón extendido, pero se encontró una posible interacción de los tres extensores, caso contrario para un reemplazo del 50%. **Conclusión:** Según el análisis instrumental de textura, para reemplazos del 35% y 50%, no se encontró una mezcla que exhibiera comportamientos similares al de la pasta de pollo en un salchichón estándar. Sin embargo de acuerdo a los resultados sensoriales una combinación entre fibra y cuero de cerdo da como resultado salchichones extendidos con aceptabilidad entre los consumidores.

Palabras claves: Alimentos, ácidos grasos, Carragenina, Proteínas Musculares.

[1] Facultad de Ciencias Agrarias, Universidad Nacional de Colombia – Sede Medellín. Medellín, Colombia.

[2] Facultad de Minas, Universidad Nacional de Colombia – Sede Medellín. Medellín, Colombia.

[3] Profesor Asociado. Universidad Nacional de Colombia - Facultad de Ciencias Agrarias - Departamento de Ingeniería Agrícola y de Alimentos. A.A. 1779, Medellín, Colombia.

[*] Autor de correspondencia: dmgonzalr@unal.edu.co

ABSTRACT

Background: Mechanically separated chicken (MSC) or chicken paste, is a finely textured chicken meat, and one of the principal ingredients used in the meat industry as a protein supplement in the production of processed meats; normally, it is imported; nevertheless, other meat extenders are being used in order to contribute to the development of the domestic industry. **Objectives:** This study aimed to determine the best mixture of fiber, pig skin, and carragennan, through an evaluation of the microbiological, bromatological, rheological and sensorial properties of standard sausages produced with chicken paste and extenders that replaced 35% and 50% of the paste inside a meat matrix. **Methods:** An experimental design that combined three extenders composed of fiber, pigskin, and carragennan in ten mixture points was used. The experiment was carried out with 35% and 50% replacement levels. Each sausage was subjected to a Texture Profile Analysis (TPA), elasticity, and firmness analysis, using instruments and a sensorial evaluation. **Results:** The firmness and chew ability in the instrumental analysis of the texture decreased with the application of the extenders; however, there were no statistically significant differences between the applications of the three extenders for firmness. The sensory analysis showed that the fiber and pigskin mixture favored the sensorial properties. Furthermore, the interaction between the fiber and pigskin improved the chew ability. The elasticity increased in the sausages with the extenders, and an interaction between the three extenders at the 35% replacement increased the elasticity, as compared to the control. The firmness, at the 35% replacement, decreased in the extended sausages, but there was a possible interaction between the three extenders that was not seen at the 50% replacement. **Conclusion:** According to the instrumental analysis of the texture, at 35% and 50% replacements, there was not a mixture that exhibited a behavior similar to that of chicken paste in a standard sausage. However, according to the sensory results, a combination of the fiber and pigskin resulted in a sausage with extenders that was acceptable for consumers.

Keywords: Food, fatty acids, Carragennan, Muscular Proteins.

INTRODUCCIÓN

Es un reto para la industria y para la investigación, desarrollar nuevos ingredientes que sean competitivos y funcionales, que permitan diseñar alimentos que sean menos costosos y cumplan con los requerimientos de nutrientes básicos. Enmarcados en políticas ambientales, saludables, de eficiencia del proceso y de reducción de costos, la búsqueda está orientada a ingredientes que satisfagan los requerimientos de proteína en la nutrición humana, que tengan propiedades funcionales como capacidad de retención de agua, capacidad de formar geles, capacidad de formar emulsiones, que en su proceso de obtención generen el mínimo de residuos para el medio ambiente y que todo esto se obtenga al mínimo costo (1,2).

Dentro de las opciones de ingredientes funcionales que podrían reemplazar la proteína cárnica en algún porcentaje, se encuentran los derivados de la soya, la proteína extraída del rumen del bovino (3), los aislados, texturizados y concentrados de proteínas de cereales, los hongos, las fibras de frutas y hortalizas, entre otros (4–6).

Un extensor cárnico no es un ingrediente que sustituye la carne o proteína de origen animal en un 100%, es un producto o combinación de ingredientes como las fibras, el plasma sanguíneo, las gomas, las carrageninas, el cuero de cerdo, los aceites de origen vegetal, entre otros, que hacen parte de una formulación cárnica, con el objetivo de disminuir la grasa, la proteína y la sal, para obtener productos más económicos y saludables. Adicionalmente el extensor debe tener buenas propiedades funcionales como, alta capacidad de retener agua, capacidad de formar emulsiones y geles, entre otras; lo anterior debido a que estas propiedades son las que confieren la textura, la jugosidad y la aceptabilidad del producto por parte de los consumidores (7–11).

El objetivo de este trabajo fue determinar la mejor mezcla entre fibra, cuero y carragenina, mediante la evaluación de las propiedades microbiológicas, bromatológicas, reológicas y sensoriales de un salchichón tipo estándar fabricado con pasta de pollo y un extensor que reemplaza un 35% y 50% de esta en la formulación de la matriz cárnica

MATERIALES Y MÉTODOS

Materias primas

La carne de bovino (10 % de grasa), la grasa de cerdo y la pasta de pollo fueron adquiridas en mercados locales, y mantenidas en refrigeración (2 ±2 °C) durante 24 horas para su utilización. El resto de ingredientes de la formulación como almidón de papa, proteína aislada de soya, nitral sal curante (mezcla comercial de nitrito de sodio y cloruro de sodio), Condimento Completo Tecnas (mezcla comercial de especias), Colorante Natural y los extensores fibra, cuero de cerdo y carragenina fueron suministrados por la empresa Tecnas S.A.

Diseño experimental

El trabajo experimental tuvo una duración de 12 semanas. En la figura 1. Se puede observar la región experimental, la cual consiste en un diseño de mezclas (12), a partir del cual se elaboraron veintiún formulaciones de salchichón estándar siguiendo lo establecido en la Norma Técnica Colombiana NTC 1325 (13). Una formulación sin extensión de pasta de pollo (Testigo para todos los reemplazos – 0%), diez formulaciones obedecen a un reemplazo del 35% de la pasta de pollo con los extensores y las otras diez de acuerdo con un reemplazo del 50%. En las tablas 1 y 2 se observan cada uno de los tratamientos para los reemplazos del 35% y 50 % respectivamente, los cuales corresponden a la forma como se distribuyen los tres ingredientes en la totalidad del extensor cárnico mencionado en las formulaciones de la tabla 3.

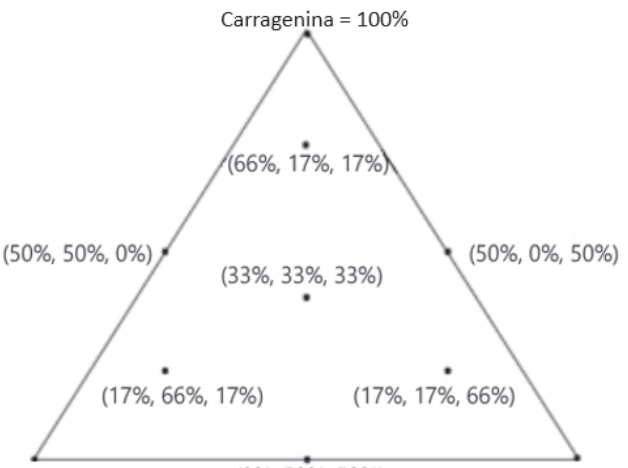


Figura 1. Región experimental, diseño mezclas (Carragenina, Cuero y Fibra)

Tabla 1. Diseño experimental reemplazo del 35%.

#	Reemplazo del 35%					
	% en extensor			gramos		
	A	B	C	A	B	C
1	16,7	16,7	66,7	18,7	18,7	74,7
2	33,3	33,3	33,3	37,3	37,3	37,3
3	100,0	0,0	0,0	112,0	0,0	0,0
4	0,0	100,0	0,0	0,0	112,0	0,0
5	0,0	0,0	100,0	0,0	0,0	112,0
6	50,0	50,0	0,0	56,0	56,0	0,0
7	66,7	16,7	16,7	74,7	18,7	18,7
8	50,0	0,0	50,0	56,0	0,0	56,0
9	0,0	50,0	50,0	0,0	56,0	56,0
10	16,7	66,7	16,7	18,7	74,7	18,7

Tabla 2. Diseño experimental reemplazo del 50%.

#	Reemplazo del 50%					
	% en extensor			Gramos		
	A	B	C	A	B	C
1	16,7	16,7	66,7	26,7	26,7	106,7
2	33,3	33,3	33,3	53,3	53,3	53,3
3	100,0	0,0	0,0	160,0	0,0	0,0
4	0,0	100,0	0,0	0,0	160,0	0,0
5	0,0	0,0	100,0	0,0	0,0	160,0
6	50,0	50,0	0,0	80,0	80,0	0,0
7	66,7	16,7	16,7	106,7	26,7	26,7
8	50,0	0,0	50,0	80,0	0,0	80,0
9	0,0	50,0	50,0	0,0	80,0	80,0
10	16,7	66,7	16,7	26,7	106,7	26,7

A: Fibra, B: Cuero de cerdo, C: Carragenina

Tabla 3. Formulaciones cárnicas para la elaboración de los salchichones.

INGREDIENTE	g/4 kg según la extensión		
	0%	35%	50%
Res 90/10 3MM	520	520	520
CDM	1920	1248	960
Extensor cárnico (fibra, cuero, carragenina)	0	112	160
Grasa de cerdo	320	420	464,8
Proteína Aislada de Soya	80	80	80
Almidón de papa	200	200	200
Sal Curante (Nitral al 6%)	13,2	13,2	13,2
Condimento completo tecnas	146	146	146
Colorante natural para embutidos	16	16	16
Agua	784,8	1244,8	1440

Preparación de los salchichones

La elaboración de los salchichones se realizó con base en la información expuesta en las tablas 1, 2 y 3, en la planta piloto de la Fundación INTAL (Instituto de Ciencia y Tecnología Alimentaria), bajo la supervisión del equipo de innovación y desarrollo de Tecnas S.A (Itagüí, Colombia).

La carne y la grasa a temperatura de refrigeración fueron cortadas en trozos y luego pasadas por un molino con discos de tamaño 3mm (Torrey®, Referencia M12FS), por separado, luego fueron pesadas en las cantidades exactas de cada formulación y se llevaron a refrigeración para su posterior uso.

Se dispuso la pasta de pollo, la carne y el nitral en un cutter (Mainca®, modelo CM-14) para comenzar con la etapa de homogenización, luego de aproximadamente 1 minuto de mezclado se adicionó la mitad del agua y el colorante, seguido de las demás proteínas y la segunda porción de agua incluyendo el extensor, y por último se adicionó la grasa, el almidón y los condimentos, todo el proceso llevado a cabo a una temperatura inferior de 5°C. Posteriormente se embutió la pasta obtenida en una funda de PVC (cloruro de polivinilo) cero mermas, impermeable al vapor de agua, obteniendo salchichones de aproximadamente 400g. Luego se llevaron a una marmita que contiene agua a una temperatura de 80°C durante una hora, hasta que el producto alcanzó una temperatura interna superior a los 73°C. Cumplido el tiempo de cocción se realizó un proceso de enfriamiento de los salchichones con agua a temperatura ambiente hasta que el producto alcanzara una temperatura interna de mínimo 30°C y disponerlo posteriormente en un cuarto frío a temperatura de refrigeración entre 0 y 4 °C para su posterior análisis.

Proteína y grasa

Para cuantificar el contenido de proteína y grasa se seleccionaron las formulaciones con un porcentaje de fibra superior al 20% y cantidad de carragenina por debajo del 50%. El análisis se realizó para los salchichones que contenían los extensores fibra y cuero de cerdo en alguna combinación o en un porcentaje del 100% cada uno. Para cada uno de estos se evaluaron los contenidos de proteína y grasa, los cuales se determinaron por el método Kjeldahl (NTC 4657(14)) y por extracción soxhlet (NTC 668(15)) respectivamente.

Análisis microbiológico

Para el análisis microbiológico se evaluaron salchichones en los tiempos 8 y 31 días después de la elaboración, almacenados a temperaturas entre 0 y 4°C. Para el análisis de aerobios mesófilos se siguió el procedimiento descrito en la norma NTC 4519 (16), mientras que para el recuento de coliformes fecales y totales, se utilizó el procedimiento establecido en la norma NTC 4516 (17). Las determinaciones de *Staphylococcus aureus*, esporas de *Clostridium* sulfito reductoras y *Salmonella sp*, se llevaron a cabo siguiendo las NTC 4779(18), NTC 4834(19) y NTC 4574(20) respectivamente. Todos estos análisis fueron realizados por duplicado.

Medición instrumental de la textura

El análisis de perfil de textura (TPA) fue realizado en un texturómetro TA-XT Plus (Stable Micro Systems®). Tres muestras de salchichón por tratamiento fueron analizadas, se tomaron bocados de 20 mm de diámetro y 25 mm de alto, a temperatura ambiente fueron comprimidas axialmente al 70% de su altura original. Las curvas fuerza de deformación-tiempo se obtuvieron utilizando una celda de carga de 30 kg y una velocidad de preensayo, ensayo y post ensayo de 2,0 mm/s. Durante el análisis fueron determinados los parámetros dureza, elasticidad, cohesividad, gomosidad, masticabilidad y resilencia; los cuales se obtuvieron mediante el uso de un Software Exponent Stable Micro System, versión 3.0.5.0. Los valores registrados para cada parámetro corresponden a la media de 3 mediciones, de los cuáles únicamente se analizaron Dureza y Masticabilidad. Con una metodología desarrollada por la empresa Tecnas, se evaluaron las propiedades de firmeza y elasticidad, siendo estas cuatro variables de respuesta las de interés para el análisis instrumental de textura.

Evaluación sensorial

A partir de una evaluación descriptiva se realizó la evaluación sensorial de los salchichones bajo las normas NTC 3932(21) y NTC 5328(22). Se seleccionaron y evaluaron seis descriptores, dureza, masticabilidad, cohesividad, sabor característico, apariencia y calidad general de los salchichones. Para la prueba sensorial, las muestras fueron cortadas en porciones de 20 mm de ancho y 10 mm de alto, e identificadas con números aleatorios de tres cifras. La evaluación sensorial fue realizada por 5 jueces pertenecientes al panel sensorial de la Fundación

INTAL (Instituto de Ciencia y Tecnología Alimentaría, Itagüí, Colombia) los jueces son entrenados durante un mes siguiendo las metodologías establecidas en las referencias: GTC 245 (23), GTC 232 (24), NTC 4489 (25), NTC 3915 (26) y NTC 3929 (27); a quienes se les suministró un formato con una escala de intensidad no estructurada de 10 puntos donde 0 = no hay percepción y 10 = intensidad fuerte. No se realizaron repeticiones, ya que se tenían datos de tres réplicas auténticas.

Análisis estadístico

Los datos de cada medición en los diferentes tratamientos fueron tratados mediante un análisis de varianza de una vía (P≤0,1) para el modelo de regresión cúbico propuesto sin intercepto y restando la media de la variable respuesta a la misma ya que permite analizar la significancia de los efectos de los tratamientos en la tabla Anova y para los parámetros estimados en el modelo de regresión. A partir de estos modelos de regresión se realiza un análisis de superficie de respuesta Simplex {3,2} con 4 puntos en el centro. Todos los análisis fueron realizados usando el programa estadístico R versión 2.15.1 (2012-06-22) - "RoastedMarshmallows" Copyright (C) 2012.

RESULTADOS

Contenido de grasa y proteína

En la tabla 4, se observan los resultados del contenido de grasa y proteína, el # indica la mezcla a la cual se le realizaron los análisis, las cuales se encuentran en la tabla 1 y 2.

Tabla 4. Contenido de grasa y proteína

#	Nivel de reemplazo*			
	35%		50%	
	Grasa	Proteína	Grasa	Proteína
Testigo	13,72d	12a	13,72e	12a
2	13,72d	10,5b	14,36b	10,8d
3	13,72d	10,5b	14,36b	10,8d
4	15,27a	10d	14,43a	10,7e
6	14,68b	10,5b	13,14f	10,9c
7	13,67e	10,4c	11,9g	11b
8	13,81c	10,4c	13,79d	11b
10	12,56f	10,5b	14,16c	10,8d

* En cada columna, valores con letra diferente indican que existen diferencias estadísticamente significativas según la prueba de Tukey (P≤0,05).

Análisis microbiológico.

Luego de analizar los salchichones pasados 8 y 31 días de almacenamiento para determinar la presencia de diferentes microorganismos tales como mesófilos, coliformes totales, *Stapylococcus aureus, Clostridium* sulfito reductoras y *Salmonella sp.,* se encontró que los valores de los mismos no exceden los límites permitidos en la normatividad.

Análisis instrumental de la textura.

En la tabla 5 se presentan los resultados de las variables de respuesta dureza, masticabilidad, firmeza y elasticidad.

Tabla 5. Parámetros estudiados del Análisis perfil de textura (TPA) de salchichones tipo estándar elaborados con diferentes extensores.

Mezcla	Reemplazo del 35%*			
	Dureza	Masticabilidad	Firmeza	Elasticidad
0	4743 ± 70 a	734 ± 152 a	289 ± 30 a	64,9 ± 0,4 a
1	3326 ± 628 b	482 ± 160 ab	241 ± 30 ab	64,4 ± 1,4 a
2	2897 ± 382 b	404 ± 134 b	208 ± 14 b	65,9 ± 1,4 a
3	3245 ± 438 b	377 ± 102 b	237 ± 16 ab	65,2 ± 1,2 a
4	3202 ± 392 b	492 ± 90 ab	257 ± 40 ab	65,5 ± 1 a
5	3447 ± 488 b	441 ± 148 ab	248 ± 36 ab	63,8 ± 2 a
6	3369 ± 564 b	614 ± 154 ab	272 ± 20 ab	64,9 ± 0,4 a
7	3475 ± 634 b	414 ± 178 b	241 ± 56 ab	65,1 ± 1,2 a
8	3264 ± 424 b	455 ± 74 ab	234 ± 46 ab	63,8 ± 1,4 a
9	3073 ± 448 b	417 ± 128 ab	279 ± 22 ab	63,9 ± 1,6 a
10	2980 ± 498 b	410 ± 108 b	239 ± 16,2 ab	65,9 ± 0,6 a
	Reemplazo del 50%			
0	4424 ± 630 a	834 ± 342 a	282 ± 50 a	64,3 ± 1,2 a
1	2425 ± 374 b	372 ± 196 b	190 ± 20 b	66,1 ± 1,2 b
2	3018 ± 194 b	371 ± 50 b	224 ± 18 ab	66,8 ± 1,2 ab
3	2381 ± 424 b	371 ± 90 b	224 ± 56 ab	63,7 ± 2,2 ab
4	2859 ± 650 b	302 ± 96 b	246 ± 46 ab	65 ± 1,8 ab
5	2656 ± 184 b	355 ± 112 b	215 ± 11,2 ab	65,3 ± 1 ab
6	2754 ± 408 b	419 ± 138 b	228 ± 30 ab	67 ± 2,2 ab
7	2881 ± 310 b	371 ± 40 b	245 ± 30 ab	66,4 ± 1,8 ab
8	2730 ± 492 b	393 ± 78 b	227 ± 54 ab	66,3 ± 1 ab
9	3095 ± 472 b	407 ± 120 b	240 ± 52 ab	66,4 ± 1,4 ab
10	3013 ± 380 b	362 ± 100 b	223 ± 12 ab	66,3 ± 1,8 ab

*En cada columna, valores con letra diferente indican que existen diferencias estadísticamente significativas según la prueba de Tukey (P≤0,05).

Evaluación Sensorial

En la tabla 6, se pueden observar los resultados obtenidos para cada uno de los descriptores definidos en el análisis sensorial.

Tabla 6. Valores obtenidos para las variables de respuesta en la evaluación sensorial.

	Variables de respuesta					
	Reemplazo 35%*					
	Dureza	**Masticabilidad**	**Cohesividad**	**Calidad General**	**Apariencia**	**Sabor Característico**
Test	5,68 ± 0,32 ab	6,01 ± 0,38 a	5,92 ± 0,4 a	6,53 ± 0,44 a	6,98 ± 0,82 a	6,18 ± 0,72 a
1	4,39 ± 0,74 bc	5,37 ± 0,86 ab	5,26 ± 1,06 a	4,43 ± 0,26 bc	5,52 ± 0,4 a	4,85 ± 0,54 b
2	4,47 ± 0,4 bc	5,06 ± 0,54 ab	4,51 ± 0,62 a	4,64 ± 0,36 bc	6,08 ± 0,94 a	5,39 ab ± 0,3 ab
3	4,75 ± 0,38 bc	4,71 ± 0,24 b	4,97 ± 0,18 a	4,77 ± 0,22 bc	5,96 ± 0,42 a	5,72 ± 0,28 ab
4	4,47 ± 0,44 bc	4,58 ± 0,5 b	4,77 ± 0,46 a	3,84 ± 0,72 c	5,79 ± 0,86 a	4,71 ± 0,82 b
5	4,18 ± 0,36 c	4,65 ± 0,38 b	4,61 ± 0,38 a	4,68 ± 0,04 bc	6,02 ± 0,12 a	5,08 ± 0,74 ab
6	5,28 ± 0,58 abc	5,10 ± 0,1 ab	4,95 ± 0,94 a	4,57 ± 0,22 bc	6,35 ± 0,7 a	4,77 ± 0,36 b
7	5,26 ± 0,5 abc	5,02 ± 0,32 ab	5,4 ± 0,2 a	5 ± 0,1 bc	6,55 ± 0,36 a	5,79 ± 0,44 ab
8	6,17 ± 0,5 a	5,40 ± 0,2 ab	5,13 ± 0,52 a	5,32 ± 0,86 ab	6,28 ± 0,38 a	5,68 ± 0,18 ab
9	5,08 ± 0,3 abc	5,02 ± 0,22 ab	4,79 ± 0,38 a	4,72 ± 0,4 bc	6,41 ± 0,62 a	4,87 ± 0,1 b
10	5,22 ± 0,9 abc	5,23 ± 0,54 ab	5,38 ± 0,22 a	4,89 ± 0,58 bc	5,98 ± 0,64 a	5,56 ± 0,58 ab
	Reemplazo 50%					
Test	5,82 ± 0,08 a	5,68 ± 0,34 a	5,09 ± 0,16 a	6,02 ± 0,26 a	5,75 ± 0,44 a	5,88 ± 0,14 a
1	4,09 ± 0,48 b	4,29 ± 0,14 b	4,77 ± 0,1 a	4,35 ± 0,34 b	5,77 ± 0,38 a	4,98 ± 0,54 a
2	4,64 ± 0,16 b	4,85 ± 0,78 ab	4,88 ± 0,76 a	4,57 ± 0,36 b	5,67 ± 0,14 a	4,5 ± 0,76 a
3	4,29 ± 0,44 b	4,42 ± 0,04 b	4,57 ± 0,48 a	4,77 ± 0,78 b	5,7 ± 0,26 a	5,03 ± 0,46 a
4	4,3 ± 0,24 b	4,27 ± 0,38 b	4,05 ± 0,56 a	4,76 ± 0,48 b	5,39 ± 0,26 a	5,14 ± 0,44 a
5	4,68 ± 0,28 b	4,5 ± 0,4 b	4,74 ± 0,62 a	4,91 ± 0,24 ab	5,59 ± 0,28 a	4,95 ± 0,42 a
6	4,9 ± 0,88 ab	4,43 ± 0,7 b	4,76 ± 0,5 a	5,07 ± 0,42 ab	6,08 ± 0,44 a	5,22 ± 0,6 a
7	4,74 ± 0,4 ab	4,47 ± 0,34 b	4,8 ± 0,2 a	4,48 ± 0,54 b	5,61 ± 1,06 a	4,81 ± 0,38 a
8	5,1 ± 0,5 ab	4,83 ± 0,14 ab	4,88 ± 0,52 a	4,92 ± 0,1 ab	5,85 ± 0,46 a	5 ± 0,6 a
9	5,19 ± 0,54 ab	4,97 ± 0,36 ab	5,12 ± 0,64 a	4,95 ± 0,54 ab	5,54 ± 1,52 a	5,1 ± 0,92 a
10	4,93 ± 0,22 ab	4,77 ± 0,14 ab	4,99 ± 0,2 a	4,73 ± 0,4 b	6,05 ± 0,36 a	4,87 ± 0,58 a

* En cada columna, valores con letra diferente indican que existen diferencias estadísticamente significativas según la prueba de Tukey (P≤0,5).

A continuación se encuentra el modelo empírico que arrojó diferencias significativas entre los diferentes tipos de reemplazo con un R^2 ajustado de 0,0247.

$$Y= -0,0008932A+0,0391174B-0,0002101C+2,0543644W-0,0014934AB-0,0003578BC-0,0181060AW-0,0454684BW+0,0014094ABW \quad \text{Ecuación 1}$$

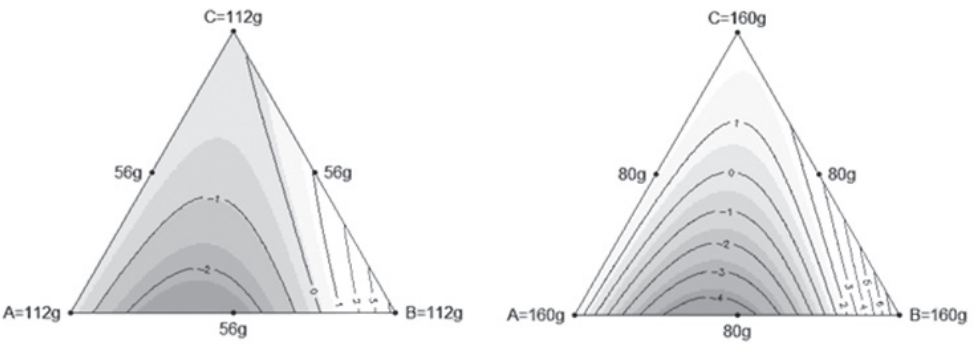


Figura 2. Superficie de respuesta general estimada para reemplazos del 35% (izquierda) y 50% (derecha) sensorial respectivamente.

Tabla 7. Regiones óptimas de la superficie de respuesta.

Evaluación sensorial						
Nivel de reemplazo						
	Modelo General					
	35%			50%		
	Óptimo	Mín.	Max	Óptimo	Mín.	Max
Fibra	0,237	0,040	2,240	0,095	0,004	6,528
Cuero	111,117	100,904	111,440	159,811	148,725	159,811
Carra-genina	0,647	0	10,993	0,095	0,004	11,237

DISCUSIÓN

Es importante resaltar que para ningún reemplazo, el contenido de proteína se encontró por fuera de la norma, también se destaca el cumplimiento con la normatividad respecto a la ausencia de microorganismos patógenos. Se encontró una disminución generalizada para las propiedades texturales de dureza y masticabilidad, al igual que un sabor a grasa marcado y una disminución en el sabor característico.

Contenido de grasa y proteína

Para cuantificar el contenido de proteína y grasa se seleccionaron las formulaciones con un porcentaje de fibra superior al 20% y cantidad de carragenina por debajo del 50%. Todos los salchichones con reemplazo cumplen con el requerimiento de proteína y grasa establecido en la norma NTC 1325, 10% y 28% respectivamente, para productos cárnicos cocidos estándar (13). El comportamiento observado fue que la proteína disminuyó en las formulaciones extendidas, con respecto al testigo, pero nunca por debajo del límite permitido. Lo anterior se debe a que se sustituyó un 35% y 50% de proteína originaria de la pasta de pollo respectivamente, y dos de los extensores, fibra y carragenina, no son de origen proteico. Sin embargo, en el contenido de grasa se observó que las formulaciones 6, 7 y 10 presentaron contenidos de grasa inferiores al testigo, las dos primeras para un reemplazo del 50%, y las dos últimas para un reemplazo del 35% (tabla 1, 2 y 4). Adicionalmente se puede observar que la formulación número 4, la cual representa una formulación de salchichón estándar que contiene un extensor compuesto solo de cuero de cerdo y carragenina, fue la que presentó los contenidos más bajos de proteína y más altos de grasa, para ambos niveles de extensión, este extensor aporta grasa y colágeno como proteína siendo la relación grasa/proteína mayor. Se conforman un grupo de formulaciones que no presentan diferencias significativas entre sí en sus contenidos de grasa y proteína, entre los cuales no se puede generalizar ningún patrón de comportamiento. Choe *et al*. (28) encontraron resultados similares donde se hacen diferentes reemplazos del contenido de grasa, manteniendo la cantidad de proteína

Desde el punto de vista del alto contenido de grasa y la baja cantidad de proteina, reflejados en los análisis se puede decir que la formulación número 4 será poco recomendable comparada con las demás (tabla 1, 2 y 4).

Análisis microbiológico

Se puede observar que ninguno de los resultados microbiológicos, superó el límite máximo permitido establecido por la NTC 1325, 100,000 UFC/g para mesófilos, 500 UFC/g para coliformes, <100 UFC/g para *Staphylococcus aureus*, <10 UFC/g para esporas de *Clostridium* sulfito reductor y ausencia/25g para *Salmonella sp* (13). No hubo diferencias significativas, ni cambios que puedan atribuirse a la sustitución de la pasta de pollo por los extensores. Sin embargo, se observó un crecimiento en los mesófilos, esto puede deberse a la naturaleza de estos microorganismos, ya que crecen a temperaturas que rondan los 30 °C, y no necesitan condiciones selectivas ni específicas para crecer, entre ellos se pueden encontrar microorganismos patógenos por lo cual se realizaron las pruebas para los otros microorganismos mencionados. La refrigeración debería inhibir el crecimiento de mesófilos, pero a temperaturas entre 0 y 10 °C pueden crecer microorganismos psicrófilos y psicrotolerantes. Los microorganismos psicrotolerantes pueden crecer entre los 0 °C - 40 °C luego de varias semanas de refrigeración, por lo anterior en la prueba de mesófilos los psicrotolerantes se recuperan con los mesófilos (29, 30). Se percibieron olores desagradables en las muestras después de los 31 días de almacenamiento.

Análisis instrumental de la textura.

Para las variables dureza, masticabilidad y firmeza se observó una disminución estadísticamente significativa con un $p \leq 0,5$ en las formulaciones que tienen el extensor con respecto al testigo, lo anterior sucede para los dos niveles de reemplazo trabajados, caso contrario se presentó para la variable elasticidad. Autores reportan resultados contrarios

realizados en salchichas: (28, 31, 32). La calidad de la textura se afecta cuándo se disminuyen las composiciones de proteína y grasa y se aumenta la cantidad de agua, aunque la fibra, la carragenina y el cuero de cerdo tienen la característica de aumentar la dureza, la masticabilidad y la firmeza, por la característica de los geles que forman (33, 34), es posible que las concentraciones utilizadas no hayan tenido los efectos esperados. Adicionalmente en los estudios realizados, la cantidad de proteína cárnica se ha mantenido constante, variando únicamente el contenido de grasa. En este estudio se quiso evaluar la extensión de la proteína y no de la grasa, por lo que es posible decir que los extensores que no son de origen cárnico funcionan bien para extender la grasa, manteniendo la cantidad de proteína animal constante.

La disminución en la dureza, masticabilidad y firmeza que a su vez tienen una relación directa entre sí, se debe a una variación de las propiedades como: capacidad de retención de agua y capacidad emulsificante de la matriz cárnica con reemplazo, debida a la presencia del extensor y al aumento de agua en la formulación (35-37). Cuando se busca reemplazar parte de las proteínas animales con proteínas vegetales o en su defecto con polisacáridos, existen interacciones químicas que se pierden como la de los fosfatos con la proteína animal. Esta interacción que depende del pH y la carga, es causante de una mayor o menor capacidad de retención de agua, capacidad emulsificante y capacidad de formar geles. La baja cantidad de miosina y pocas interacciones proteína–proteína generan la textura blanda (38). Por ejemplo el cuero de cerdo que en su gran mayoría es colágeno tiene propiedades bajas para emulsificar grasas y buenas para ligar agua si tiene condiciones adecuadas de temperatura, por ende una posible explicación de la interacción entre el cuero y la fibra es que la fibra tiene buena capacidad de estabilizar la grasa evitando la separación de fases, exhibiendo propiedades positivas para la extensión (10), luego se complementa con el cuero de cerdo en el momento de retener agua. Estudios como el de Oliveira *et al*. (39) muestran un aumento en las propiedades texturales en salchichas extendidas con geles provenientes de cuero de cerdo y celulosa amorfa para reemplazar porcentajes de grasa del embutido, con ventajas como la reducción de ácidos grasos insaturados.

Es de esperar que existan interacciones entre los tres extensores, pero estas interacciones no alcanzan a ser lo suficientemente fuertes para retener el agua de la formulación y emulsificar las grasas. Lo anterior, ya que la fibra y la carragenina son polisacáridos y el cuero de cerdo es en su gran mayoría colágeno y grasa, siendo el colágeno una proteína de origen animal del tejido conectivo, que proporciona otro tipo de propiedades (40,41).

Evaluación Sensorial

Para las 21 formulaciones establecidas en la tabla 1 y 2 del diseño experimental, se evaluaron seis descriptores sensoriales. Se encontró que las variables, cohesividad y apariencia, no presentaron diferencias estadísticamente significativas, con respecto al testigo ni entre ellas (tabla 6). Por el contrario todas las formulaciones, tanto para el reemplazo del 35% y del 50%, mostraron disminuciones estadísticamente significativas para un $p<0,05$ en la dureza, la masticabilidad, la calidad general y el sabor característico, resaltándose entre ellas la formulación número 4 que presentó la menor calidad general para un reemplazo del 35%. Esta última formulación contiene el extensor que tiene 100% cuero de cerdo. Sin embargo; según el modelo estadístico presentado gráficamente en la figura 2, en la ecuación 1 con sus respectivos óptimos en la tabla 7, muestra que la mejor respuesta sensorial se encuentra cuando se usa un 100% de cuero de cerdo.

Adicionalmente el panel registró texturas blandas y sabores a grasa más marcados, como sensaciones atípicas en todas las formulaciones. Resultados similares reportaron algunos autores, que extendieron sus formulaciones con aceites de origen vegetal, registrando disminuciones en todas las propiedades sensoriales (32). Otras investigaciones trabajaron en reemplazos de grasa en salchichas, por fibra, cuero de cerdo y carragenina, a lo cual no se registraron cambios estadísticamente significativos, lo que llevó a los autores a concluir que estos ingredientes son potenciales extensores de grasa, pero contrastando con la presente investigación no serían potenciales extensores de un extensor con relativo alto porcentaje de proteína de origen animal como lo es la pasta de pollo (28,42).

Contrario a los resultados obtenidos en el presente trabajo, estudios como el de Akweeted *et al.* (43) demuestran que el uso de extensores en rollos de carne con harina de frijol como fuente de proteína, resultaron ser convenientes desde el punto de vista sensorial y de textura, manteniendo las propiedades del testigo, sin embargo a medida

que aumentaron la cantidad de extensor también aumentaron la cantidad de especias para mitigar el cambio de sabor.

Con los resultados de esta investigación podrían recomendarse futuros estudios sobre el comportamiento de un extensor que tenga una cantidad alta de cuero de cerdo, buscando disminuir las cantidades de grasa y conservar las cantidades de proteína. Otro factor importante a estudiar sería la evaluación de la incorporación de diferentes especias que mitiguen la alteración negativa en el sabor característico del salchichón. Adicionalmente es recomendable realizar un estudio nutricional que confirme la disminución en los niveles de ácidos grasos saturados.

CONCLUSIONES

La sustitución de un porcentaje de pasta de pollo en una formulación de salchichón tipo estándar, por un extensor conformado por fibra, cuero de cerdo y carragenina disminuyó considerablemente las propiedades de dureza, masticabilidad y firmeza, y aumentó la elasticidad, situación que se mantuvo para reemplazos del 35% y 50%, sin embargo, cualquiera de las mezclas evaluadas (fibra, cuero y carragenina) como extensores de la pasta de pollo, podrían ser utilizadas con este fin, dado que no se encontraron diferencias estadísticamente significativas en los resultados del análisis instrumental de textura. Así mismo a nivel sensorial se observó un decremento en la mayoría de las propiedades evaluadas, aunque se encontró un modelo empírico general que predijo como mejor combinación de ingredientes, 0,23 gramos de fibra, 111,11 gramos de cuero de cerdo y 0,64 gramos de carragenina, dando como resultado que la formulación conformada en mayor proporción por cuero de cerdo fue la que obtuvo resultados más cercanos al óptimo.

Finalmente la sustitución de proteína de origen animal por extensores obtenidos a partir de fibras, otros polisacáridos y proteínas de tejido conectivo en derivados cárnicos, cambia notablemente las propiedades del producto, por ende, se convierte en un reto tecnológico para la investigación y la industria de alimentos, lograr conseguir extensores con capacidad de retención de agua, y capacidad de emulsificar, similares a las proteínas de origen animal.

AGRADECIMIENTOS

A la Dirección de Investigación de la Universidad Nacional de Colombia (DIME), Sede Medellín y a Tecnas S.A, por la contribución académica y financiera que permitió el desarrollo del proyecto.

CONFLICTO DE INTERESES

Los autores no declaran ningún conflicto de intereses.

REFERENCIAS

1. Hoek AC, van Boekel MAJS, Voordouw J, Luning PA. Identification of new food alternatives: How do consumers categorize meat and meat substitutes? Food Qual Prefer. June 2011; 22(4):371–83.
2. Pérez WAP, Molina DAR, Valencia JUS. Revisión: Uso de Ingredientes no Cárnicos como Reemplazantes de Grasa en Derivados Cárnicos. Rev Fac Nal Agr Medellín. 2011; 64(2):6257–64.
3. Conti-Silva AC, Pinto e Silva MEM, Arêas JAG. Sensory acceptability of raw and extruded bovine rumen protein in processed meat products. Meat Sci. Agosto 2011; 88(4):652–6.
4. Hoek AC, Luning PA, Weijzen P, Engels W, Kok FJ, de Graaf C. Replacement of meat by meat substitutes. A survey on person- and product-related factors in consumer acceptance. Appetite. June 2011; 56(3):662–73.
5. Khan MI, Arshad MS, Anjum FM, Sameen A, Aneeq-ur-Rehman, Gill WT. Meat as a functional food with special reference to probiotic sausages. Food Res Int. Diciembre 2011; 44(10):3125–33.
6. Jiménez F, Sánchezz FJ, Olmedilla B. Design and development of meat-based functional foods with walnut: Technological, nutritional and health impact. Food Chem. Diciembre 2010; 123(4):959–67.
7. Ackroyd A, Sedlis E, McArthur K, Scheiner T, Wekwete B, Ghatak R, . Physical, Textural and Sensory Properties of Chocolate Cup Cakes Prepared Using Prune Puree as a Fat Replacer. J Am Diet Assoc. September 2010; 110(9, Supplement):A73.
8. Deng JW, Bhaduri S, Ghatak R, Navder KP. Effect of Chickpea Puree as a Fat Replacer on the Physical, Textural and Sensory Properties of Cheesecake. J Am Diet Assoc. September 2011;111(9, Supplement):A63.
9. Hurtado S, Saguer E, Toldrà M, Parés D, Carretero C. Porcine plasma as polyphosphate and caseinate replacer in frankfurters. Meat Sci. March 2012; 90(3):624–8.
10. O'Shea N, Arendt EK, Gallagher E. Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. Innov Food Sci Emerg Technol. October 2012; 16:1–10.
11. Candogan K, Kolsarici N. Storage stability of low-fat beef frankfurters formulated with carrageenan or carrageenan with pectin. Meat Sci. June 2003; 64(2):207–14.
12. John A. Cornell. A Primer on Experiments with Mixtures. WILEY; 2011.
13. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Industrias alimentarias. Productos cárnicos procesados no enlatados. No. NTC 1325. Quinta actualización. Bogotá: Icontec; 2008.
14. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Alimento para animales. Determinación del contenido de nitrógeno y cálculo del contenido de proteína cruda. Método kjeldahl. No. NTC 4657. Bogotá: Icontec; 1999.

15. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Alimentos y materias primas. Determinación de los contenidos de grasa y fibra cruda NTC 668 [Internet]. BogotáI: Icontec; 2002 [cited June 1, 2013].
16. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Microbiología de los alimentos para consumo humano y animal. Método horizontal para el recuento de microorganismos. Técnica de recuentode colonias A 30 °C. No. NTC 4519. Primera Actualización. Bogotá: Icontec; 2009.
17. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Microbiología de alimentos y productos de alimentación animal. Método horizontal para la detección yenumeración de coliformes técnica del número más probable. No. 4516. Primera Actualización. Bogotá: Icontec; 2009.
18. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Microbiología de alimentos y alimentos para animales. Método horizontal para el recuento de estafilococos coagulasa positiva (Staphylococcus aureus y otras especies). No. NTC 4779. Primera Actualización. Bogotá: Icontec; 2007.
19. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Microbiologia de alimentos y alimentos para animales. Metodo horizontal para el recuento de clostridium sulfito reductores e identificacion de clostridium perfringens - tecnicas de recuento de colonias. No. NTC 4834. Bogotá: Icontec; 2000.
20. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Microbiología de alimentos y alimentospara animales.Método horizontal para la detección desalmonella SPP. No. NTC 4574. Primera Actualización. Bogotá: Icontec; 2007.
21. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Análisis sensorial. Identificación y selección de descriptores para establecer un perfil sensorial por una aproximación multidimensional. No. NTC 3932. Bogotá: Icontec; 2002.
22. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Análisis sensorial. Directrices para el uso de escalas derespuesta cuantitativas. No. NTC 5328. Bogotá: Icontec; 2005.
23. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Análisis sensorial. Guía general para la selección, entrenamiento y seguimiento de evaluadores. Parte 1: evaluadores seleccionados. No. GTC 245. Primera edición. Bogotá: Icontec; 2013
24. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Análisis sensorial. Metodología. Guía general para el establecimiento de un perfil No. GTC 232. Primera edición. Bogotá: Icontec; 2012
25. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Análisis sensorial. Metodología. Perfil de textura No. NTC 4489. Primera edición. Bogotá: Icontec; 1998
26. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Análisis sensorial. Metodlogía. Método para identificar la sensibilidad del gusto. No. NTC 3915. Primera actualización. Bogotá: Icontec; 2012
27. Instituto Colombiano de Normas Técnicas y de Certificación (ICONTEC). Análisis sensorial. Metodlogía. Métodos del perfil del sabor No. NTC 3929. Primera actualización. Bogotá: Icontec; 2009
28. Choe J-H, Kim H-Y, Lee J-M, Kim Y-J, Kim C-J. Quality of frankfurter-type sausages with added pig skin and wheat fiber mixture as fat replacers. Meat Sci. Abril 2013;93(4):849–54.
29. Madigan MT, Martinko JM, Parker J, Fernández EG, Fernández CR, Pérez MS. Brock Biología de los Microorganismos. 10ª edición. España; 2004
30. Ercolini D, Russo F, Ferrocino I, Villani F. Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. Food Microbiol. Abril 2009;26(2):228–31.
31. Özvural EB, Vural H. Utilization of interesterified oil blends in the production of frankfurters. Meat Sci. March 2008;78(3):211-6.
32. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA. Effect of orange dietary fibre, oregano essential oil and packaging conditions on shelf-life of bologna sausages. Food Control. Abril 2010;21(4):436–43.
33. Thrimawithana TR, Young S, Dunstan DE, Alany RG. Texture and rheological characterization of kappa and iota carrageenan in the presence of counter ions. Carbohydr Polym. Agosto 2010;82(1):69–77.
34. Jiang G, Huang W, Li L, Wang X, Pang F, Zhang Y, Structure and properties of regenerated cellulose fibers from different technology processes. Carbohydr Polym. February 14, 2012;87(3):2012–8.
35. Brenner T, Johannsson R, Nicolai T. Characterization of fish myosin aggregates using static and dynamic light scattering. Food Hydrocoll. March 2009;23(2):296–305.
36. Cao Y, Xia T, Zhou G, Xu X. The mechanism of high pressure-induced gels of rabbit myosin. Innov Food Sci Emerg Technol. October 2012;16:41–6.
37. Raghavan S, Kristinsson HG. Conformational and rheological changes in catfish myosin during alkali-induced unfolding and refolding. Food Chem. March 1, 2008;107(1):385–98.
38. Molina DAR, Mejía CMA, Digiammarco RAR, Campuzano AA. Industria de Carnes. 2001.
39. Miriam de Oliveira Faria a, Tayssa Martins Cipriano a, Adriano Gomes da Cruz b, Bibiana Alves dos Santos c, Marise Aparecida Rodrigues Pollonio c, Paulo Cezar Bastianello Campagnol. Properties of bologna-type sausages with pork back-fat replaced with pork skin and amorphous cellulose Meat Sci. February 2015;104:44–51
40. Messia MC, Di Falco T, Panfili G, Marconi E. Rapid determination of collagen in meat-based foods by microwave hydrolysis of proteins and HPAEC–PAD analysis of 4-hydroxyproline. Meat Sci. October 2008;80(2):401–9.
41. Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocoll. Diciembre 2011;25(8):1813–27.
42. Sampaio GR, Castellucci CMN, Pinto e Silva MEM, Torres EAFS. Effect of fat replacers on the nutritive value and acceptability of beef frankfurters. J Food Compos Anal. June 2004; 17(3–4):469–74.
43. W.Y.Akweteya, I.N.Oduro, W.O.Ellis. Whole cowpea (*Vigna unguiculata*) flour (WCPF) as non-conventional extender in meatloaf . Food BioSci. November 2013; 5: 42-46.

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 111-120
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a05

FABRICATION OF AN ANTIMICROBIAL ACTIVE PACKAGING AND ITS EFFECT ON THE GROWTH OF *PseudomonaS* AND AEROBIC MESOPHILIC BACTERIA IN CHICKEN

ELABORACIÓN DE UN ENVASE ACTIVO ANTIMICROBIANO Y SU EFECTO EN EL DESARROLLO DE *Pseudomonas* Y BACTERIAS AEROBIAS EN POLLO

Odilia Azucena HIGUERA-BARRAZA BEng.[1], Herlinda SOTO-VALDEZ PhD.[1], Evelia ACEDO-FÉLIX PhD.[1†], Elizabeth PERALTA M.Sc.[1]

Recibido: Marzo 31 de 2015 Recibido: Septiembre 21 de 2015

ABSTRACT

Background: One of the aims of food packaging is to protect the product from environmental factors that can cause a reduction in quality. Surface growth of microorganism is one of the leading causes of food spoilage. One option is to use antimicrobial packaging to provide an increased margin of safety and quality. **Objectives:** The aim of this study was evaluate the effect of active packaging with eugenol on growth of Pseudomonas and aerobic mesophilic bacteria in fresh chicken pieces**. Methods:** Three batches of low-density polyethylene (LDPE) film, containing 0, 9.0 and 7.7, mg g^{-1} eugenol (control, AAF1 and AAF2, respectively), were extruded in a pilot-plant scale blown-extrusion machine. The films with eugenol lost 42.7% and 36.8% (AAF1 and AAF2, respectively) of eugenol during processing and absorbed UV-visible light at 300-261 nm. The kinetics of eugenol release from the AAF1 into the air at 5°C and 25ºC displayed Fick's behavior, and a diffusion coefficient of 10^{-8} cm^2 s^{-1} was calculated. **Results:** Eugenol showed antimicrobial activity on *in vitro,* using paper discs with 1.74, 0.87 and 0.36 mg eugenol on 10^8 CFU mL^{-1} of *Pseudomonas fluorescens* in Muller-Hinton agar. Chicken thighs were wrapped in the AAF2 film, and the effects on the growth of *Pseudomonas* and aerobic mesophilic bacteria (AMB) were evaluated after storage for 5 d at 5°C. The AAF2 showed a moderately antimicrobial effect in reducing the growth of *Pseudomonas* (1.1×10^6 CFU g^{-1}) relative to growth in the control film (6.0×10^6 CFU g^{-1}) ($P < 0.05$). The film with eugenol was effective in reducing the growth of AMB (9.0×10^5 CFU g^{-1}) relative to growth in the control film (1.7×10^6 CFU g^{-1}) ($P < 0.05$). **Conclusions:** Despite the high losses of eugenol during the extrusion of the films, they showed an antimicrobial effect during contact with fresh chicken under commercial conditions. This study shows the potential use of eugenol for application in LDPE antimicrobial packaging film.

Keywords: Antimicrobial active packaging, diffusion of eugenol, *Pseudomonas fluorescens*.

[1] Centro de Investigación en Alimentación y Desarrollo, A.C. Apdo. Postal 1735. Hermosillo, Sonora 83304, México.

[†] Deceased.

[*] Autor de correspondencia: eperalta@ciad.mx

RESUMEN

Antecedentes: Uno de los principales objetivos del envasado de alimentos es protegerlo de factores que puedan afectar y causar una reducción en la calidad. El desarrollo de microorganismos en la superficie es uno de las causas principales del deterioro de los alimentos. Una opción es el empleo de envases con propiedades antimicrobianas. **Objetivos:** El objetivo del presente estudio fue evaluar el efecto de un envase antimicrobiano conteniendo eugenol en el desarrollo de *Pseudomonas* y bacterias mesofílicas aerobias (BMA) en piezas de pollo. **Métodos:** Tres lotes de película de polietileno de baja densidad (PEBD) conteniendo 0, 9.0 y 7.7 mg g^{-1} de eugenol (control, AAF1, AAF2, respectivamente) fueron obtenidas por extrusión-soplo utilizando un extrusor a nivel planta piloto. Se calculó la cinética de liberación del eugenol de la AAF1 hacia el aire a 5**°C** y 25**°C.** Se evaluó la capacidad antimicrobiana *in vitro* del eugenol sobre 10^8 UFC mL^{-1} de *Pseudomona fluorescens* utilizando discos de papel conteniendo 1.74, 0.87 y 0.36 mg de eugenol en agar Muller-Hinton. Las piezas de pollo fueron envueltas en la película AAF2 y almacenadas a 5°C evaluando a los 5 días el efecto de la película en el desarrollo de *Pseudomonas* y en BMA. **Resultados:** El eugenol mostró actividad antimicrobiana inhibiendo el crecimiento de *P. fluorescens.* Las películas conteniendo eugenol perdieron durante el proceso de extrusión 42.7% y 36.8% (AAF1 y AAF2 respectivamente) del total añadido mostrando un comportamiento fickiano con un coeficiente de difusión de 10^{-8} cm^2 s^{-1}. Las AAF2 mostraron un efecto moderado en la reducción del desarrollo de Pseudomonas (1.1×10^6 CFU g^{-1}) comparadas con el control (6.0×10^6 CFU g^{-1}) ($P < 0.05$). Las películas con eugenol (AAF2) fueron efectivas al reducir el desarrollo de las BMA (9.0×10^5 CFU g^{-1}) comparadas con la película control (1.7×10^6 CFU g^{-1}) ($P < 0.05$). **Conclusiones:** A pesar de las pérdidas del eugenol durante el proceso de extrusión para la obtención de las películas, estas mostraron un efecto antimicrobiano sobre las piezas de pollo. Por lo tanto, este estudio muestra el uso potencial del eugenol para la aplicación en envases antimicrobianos elaborados a base de PEBD.

Palabras clave: Envases activos antimicrobianos, coeficiente de difusión, eugenol, *Pseudomonas fluorescens*.

INTRODUCTION

In recent years, chicken consumption has maintained a steady growth trend, representing over 43% of meat consumption in México [1]. Global consumption of chicken in American continent is almost three times the global average. In the EU consumption of meat per capita, which reached its lowest level in 2013 in the last 11 years (64.7 kg), it is expected to recover from this year. Thus, in 2023 it is expected that consumption per capita will reach 66.1 kg and projecting a global growth by about 2.8% annually from 2013 to 2022; this growth trend is mainly influenced by an increased demand for white meat, which is low in fat [2-4]. Fresh chicken is commonly consumed due to its nutritional profile, versatility and low price. The increased demand for fresh poultry and the need for transportation to distant markets have increased the necessity for extending the shelf life of poultry products [5].

Chicken is a highly perishable product even when stored under chilled conditions. Chicken products have a high number of spoilage microorganisms and may contain pathogenic bacteria. The normal refrigerated shelf life of fresh chicken is less than 5 days from the time of slaughter [6]. Bacteria from the genus *Pseudomonas* are considered the major producers of the volatile compounds responsible for altered flavors (aldehydes, ketones and esters) in foods. *Pseudomonas fluorescens* (*P. fluorescens*) is one of the most predominantly isolated bacteria associated with the spoilage of fresh poultry [7].

One of the aims of food packaging is to protect the product from environmental factors that can cause a reduction in quality [8]. Efforts have been made to minimize food-package interactions, such as migration, sorption and gas permeability [9]. However, these interactions have also been used in a positive way to improve the protection capacity of the package, such as in active packaging systems. Active packaging can be defined as a type of container that includes additives that help to extend the shelf life of the food by preserving the quality longer than conventional packaging [10]. These materials provide additional functions that in some way enable the package to interact with the food to improve its quality, safety and convenience. The release of volatile compounds from packaging materials is a mass transfer process by which low molecular mass substances initially present in the package are released into the contained product.

The diffusion coefficient (*D*) measures the rate at which the release process occurs, and it is described by Fick's second law [11].

Surface growth of microorganisms is one of the leading causes of food spoilage [12] directly affecting the quality and safety of the food. One option for decreasing the surface spoilage of foods is to incorporate antimicrobial agents into the packaging material to provide an increased margin of safety and quality. Antimicrobial food packaging acts to reduce, inhibit or retard the growth of microorganisms that may be present in the packaged food [13]. A packaging system that allows for the slow release of an antimicrobial agent into the food could significantly increase the shelf life and retain the quality of a variety of foods [14]. Several articles have reported the use of antimicrobial agents to formulate antimicrobial packaging [6, 13–18]. Essential oils and their components have antimicrobial properties against microorganisms (including Gram-positive and Gram-negative bacteria) [13, 14, 16, 19–24]. The use of essential oils (e.g., cinnamon, oregano, clove, eucalyptus, lavender, lemongrass, lemon, lime, orange, peppermint, basil, wintergreen and thyme) as antimicrobial agents, *in vitro* and in foodstuffs, has been reported in several studies [13, 16, 20–26]. The FDA has categorized clove oil as generally recognized as safe (GRAS) for use in dental cement and as a food additive (CFR 184.1257). Eugenol (4-allyl-2-methoxyphenol) is a naturally occurring phenolic compound that is the major essential oil component of cloves (90.1%). Eugenol shows absorption of UV light with a maximum absorption at 265 nm. It is a volatile liquid at room temperature, with moderate water solubility (2.46 mg L^{-1} at 25°C). It has shown antioxidant and antimicrobial properties [27]. It has been reported that clove oil and eugenol can affect *P. fluorescens* growth [28]. Eugenol has shown great potential for use as an active additive in food packaging applications. The diffusion of pure eugenol into plastic films has been previously tested [29], but its release as an additive from packaging has not been evaluated.

When designing antimicrobial active packaging, it is important to consider the effects of the package fabrication process on the active additive. In the case of chicken packaging, the effects of the film extrusion process must be determined. The aim of this work was to manufacture antimicrobial active films (AAFs) using an extrusion-blow process, with eugenol incorporated into the polymeric matrix. An AAF1 was manufactured to measure the kinetics of the release of eugenol at 5°C and 25°C (AAF1). Then, another batch was manufactured under the same conditions as above (AAF2) to determine the effectiveness of the film on fresh chicken pieces (thighs) by following the growth of *Pseudomonas spp.* and aerobic mesophilic bacteria (AMB) during storage at 5°C.

MATERIALS AND METHODS

Materials

Low-density polyethylene LDPE (Certene) was obtained from Muelhstein (Houston, TX, USA) with a 110°C of melting point, 2.3 g/10min of melt index and 0.924 g cm^{-3} of density. Eugenol was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade water and methanol were supplied by JT Baker (Ecatepec, México). Fresh chicken thighs were purchased from a local supermarket in Hermosillo, México.

Organisms and media

P. fluorescens of American Type culture collection (ATCC) 13525 (Manassas, VA, USA) was kept at 5°C in nutrient broth and agar. Mueller-Hinton Agar, Pseudomonas Agar F and Plate Count Agar were obtained from Difco (Becton Dickinson, Franklin Lakes, NJ, USA). NaCl, phosphate buffer (monobasic and dibasic sodium phosphate), Tween 80 and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Film manufacturing

The AAFs were produced on a pilot-plant scale extruder. Two batches of pellets containing the antimicrobial agent were produced by mixing LDPE with 9.0 and 7.7 mg g^{-1} of eugenol (AAF1 and AAF2, respectively). A pilot-plant scale extrusion machine with three heating zones at 130°C, equipped with a filament die and coupled to a pelletizer (Beutlespacher, México, D.F.), was used. Films were obtained from the two formulations by using the blow extrusion process with a pilot-plant sized mono-spindle extruder with four heating zones at 130°C (Beutelspacher, México D.F., México) at the Centro de Investigación en Alimentación y Desarrollo, A.C. campus Hermosillo, México. A control film without eugenol was also prepared under the same conditions. The thickness of the films was

measured with a model DTT micrometer from E.J. Cady & Co. (Wheeling, IL, USA). Ten film specimens were measured, and data were expressed in micrometers (μm). The AAF1 was produced to determine the kinetics of release, immediately after obtaining the film. The AAF2 was produced one month later under the same conditions than AAF1. This film was stored at -20°C until the *in vitro* antimicrobial effect and packaging of chicken pieces were performed.

Extraction and quantification of eugenol in the films

AAF1, AAF2 and control films were extracted using methanol with constant stirring at 40°C for 24 h. The extraction was repeated to ensure complete extraction of eugenol. The extracts were filtered into amber vials with 0.22 μm membrane Durapore® filters (Millipore Corporation, Bedford, MA, USA). The eugenol quantification was performed by reverse-phase high-performance liquid chromatography (HPLC) using a liquid chromatograph (Varian 9012, México) coupled to a fluorescence detector (Varian 9075, México) at an excitation wavelength of 272 nm and an emission wavelength of 298 nm. The liquid chromatograph was equipped with a 150 x 4.6 mm C_{18} ChromSep column (Varian, México) protected with a C_{18} guard column (50 mm). A 10 μL sample volume was injected into the HPLC using a Rheodyne 7125 injector (Rheodyne, Bershire, UK) and was eluted (isocratic flow) with 85:15 methanol:water at 1 mL min^{-1}. Three replicates were performed for each sample. A calibration curve for eugenol was prepared by making solutions ranging from 0.5 to 9.0 μg mL^{-1} of eugenol in methanol. The retention time for eugenol was 2.6 min, and the limit of quantification in methanol (LOQ) was 0.012 μg mL^{-1}.

Release of eugenol from the AAF1 at 5°C and 25°C

Fifty-four film samples of AAF1 (2.5 x 5 cm) were exposed to the environment (both sides). Thirty pieces were exposed at 5°C (recommended temperature for storing fresh chicken), and twenty-four pieces were exposed at 25°C (room temperature). The release of eugenol was monitored periodically by taking sets of three pieces of film during the incubation periods of 20 h at 5°C and 8 h at 25°C. Eugenol was quantified in the films, as described in the previous section.

The release process can be described as the kinetics of the diffusion of the volatile compound in the film, and it is expressed as D (diffusion coefficient). An analytical solution for the equation describing Fick's second law, which describes diffusion in two dimensions and at an infinite volume (environment) [30, 31], was used to determine the value of D.

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{m=0}^{\infty}\frac{1}{(2m+1)^2}\exp\left\{-D(2m+1)^2\pi^2\ t/l^2\right\} \quad \text{(Equation 1)}$$

where M_t/M_∞ is the concentration of eugenol released at time t divided by the concentration of eugenol released at equilibrium; and l is the thickness of the film (cm). D is the diffusion coefficient (cm^2 s^{-1}), which was calculated using Eq. (1) by plotting the ratio of t vs. M_t/M_∞. D was determined by minimizing the sum of the squares of errors (SSE) between the measured and estimated values. The MATLAB program was used to find the best fitting of the data to Eq. (1) by using the non-linear regression (nlinfit) function in MATLAB R2008b (MathWorks, Natick, MA, USA) [32].

In vitro antibacterial activity of eugenol on *P. fluorescens*

The agar diffusion method was used to determine the antibacterial activity of eugenol. Sterile paper discs (5 mm diameter) containing eugenol were prepared for the *in vitro* antibacterial activity test in accordance with the method of [33]. Overall, 10 μL of different eugenol solutions, which were prepared at dilutions of 1:1, 1:5 and 1:10 with Tween 80 (10%) and DMSO (0.5%), were loaded on sterile paper discs. Sterile discs with phosphate buffer and Tween 80 in DMSO (0.5%) were used as negative controls.

The discs were set on the surface of Muller-Hinton agar dishes freshly loaded with 10^8 CFU mL^{-1} of *P. fluorescens*, and the dishes were incubated at 25°C for 24 h. The analysis was carried out using three discs for each dilution, and the diameters of inhibition zones around the discs were measured (mm).

The minimal inhibitory concentration (MIC) of eugenol was determined by the agar diffusion method according the previously described technique. The minimum concentration showing a clear zone of 10 mm or less was taken as the MIC.

The ANOVA test was performed for each eugenol dilutions disc, and significant differences between the mean diameters of inhibition zones around the discs were determined by the Tukey-Kramer test ($P < 0.05$) using Number Cruncher Statistical Systems (NCSS) [34].

Antimicrobial effect of AAF2 on fresh chicken

Four pouches (17 x 8 cm) made of the AAF2 and control films were used for wrapping fresh chicken thighs under hygienic conditions. All treatments were stored for 5 days at 5°C. Samples (duplicates) of all treatments were taken at 0 and 5 days of storage to evaluate *Pseudomonas* and AMB survival. The counting of *Pseudomonas* and AMB was performed in accordance with the procedure established by NOM-092-SSA1-1994 [35], and NOM-110-SSA1-1994 [36]. For *Pseudomonas* counts, serial dilution of the samples was performed in buffered phosphate, and 0.1 mL of each dilution was spread on *Pseudomonas* Agar F. Incubation was carried out at 26 ± 1°C for 24-48 h, and values were reported as CFU g^{-1}.

The ANOVA test was performed, and significant differences between the means of the counts obtained from the antimicrobial active packaging and the control packaging were determined by the Tukey-Kramer test ($P < 0.05$) using NSCC [34].

RESULTS

Thickness of the AAF1 with eugenol

The thicknesses of the AAF1, AAF2 and control films were 27.9 ± 1.85, 26.4 ± 2.79 and 20.8 ± 3.93 μm, respectively. Differences in the thicknesses values of films prepared due to variations in the output (gap) of the extruder die. There were no significant differences in the values of the film elaborated.

The effect of processing conditions on the concentration of eugenol in the AAF and on eugenol release.

The concentrations of eugenol in the AAF1 and AAF2 were 5.16 ± 0.06 mg g^{-1} and 4.87 ± 1.02 mg g^{-1}, respectively. These values are 57.3% and 63.2% of the eugenol originally added before processing respectively.

Release of eugenol from the AAF1 at 5°C and 25°C

Figure 1 shows the release of eugenol in the AAF1 at different temperatures. At 5°C, the concentration of eugenol decreased from 5.16 ± 0.78 mg g^{-1} to 0.28 ± 0.04 mg g^{-1} in 16 h, a time at which equilibrium was reached. At 25 °C, the eugenol decreased from 5.16 ± 0.78 mg g^{-1} to 0.26 ± 0.01 mg g^{-1}, reaching equilibrium within 6 h, 10 hours earlier than at 5°C.

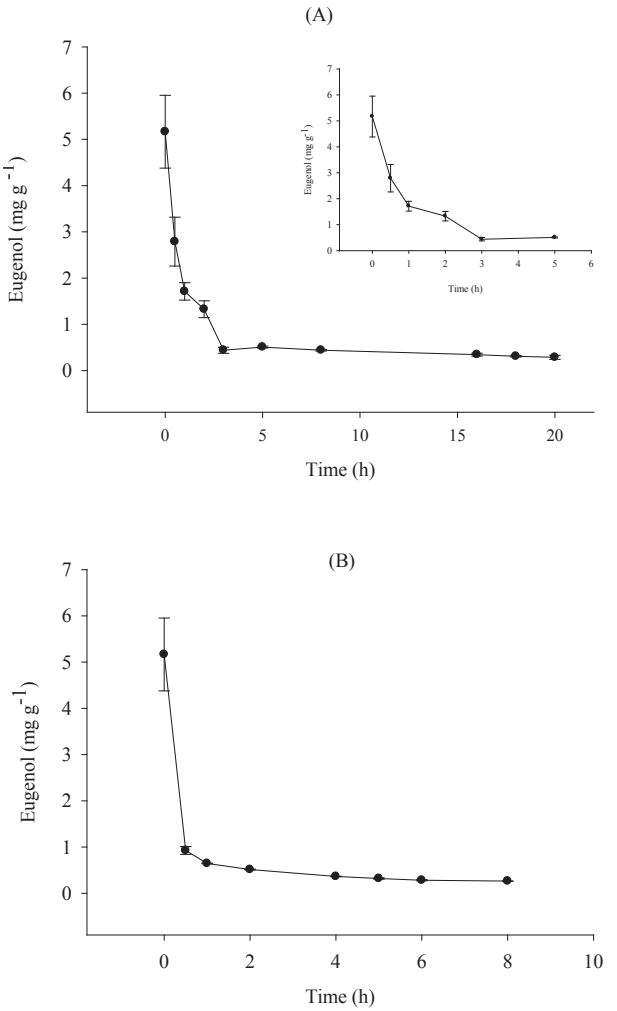


Figure 1. Changes in concentration of eugenol in the AAF1 at 5°C (A) and 25°C (B). Results are means of 3 replicates. Bars indicate standard deviation.

DIFFUSION OF EUGENOL FROM THE AAF1

Figure 2 shows the diffusion graphs, following Fick's second law, for the release experiments carried out at 5°C and 25°C (AAF1). The *D* values were determined according to Eq (1) as 2.04 ± 0.12 x 10^{-8} cm^2 s^{-1} at 5°C and 7.89 ± 0.39 x 10^{-8} cm^2 s^{-1}

at 25°C. These values are within the same order of magnitude, but a higher *D* value was obtained at 25°C.

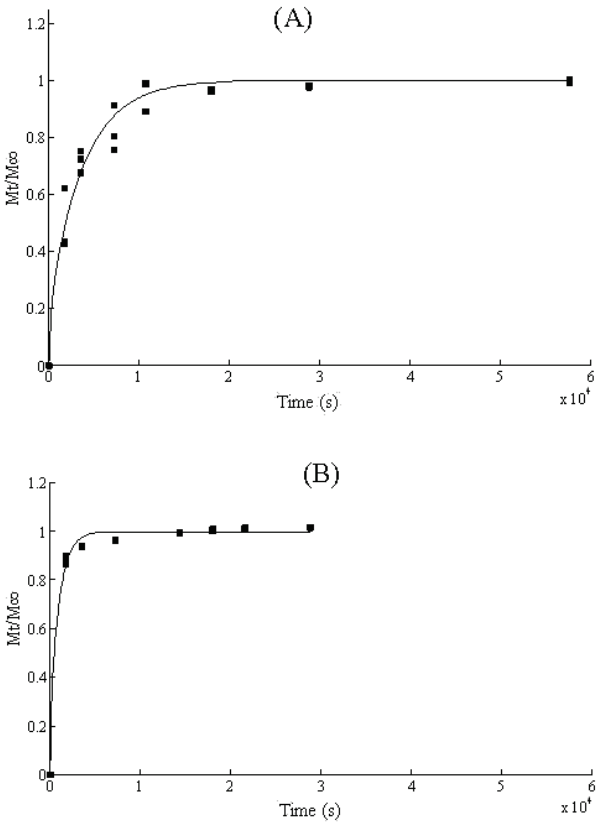


Figure 2. Diffusion of eugenol from AAF1 to the environment at (A) 5°C and (B) 25°C. The y-axes show the mass of eugenol diffused at time *t*, divided by the mass of eugenol diffused at equilibrium (M_t/M_∞). Thickness of the films was 27.9±1.85 μm.

In vitro antibacterial activity of eugenol on *P. fluorescens*

Eugenol inhibited the growth of *P. fluorescens* at all of the dilutions tested. The 1:1 dilution (1.74 mg eugenol per disc) presented the largest inhibition zone (21 mm), followed by 1:5 (0.87 mg eugenol per disc; 16-15 mm) and 1:10 (0.36 mg eugenol per disc; 10 mm considered the MIC). These results are summarized in Figure 3.

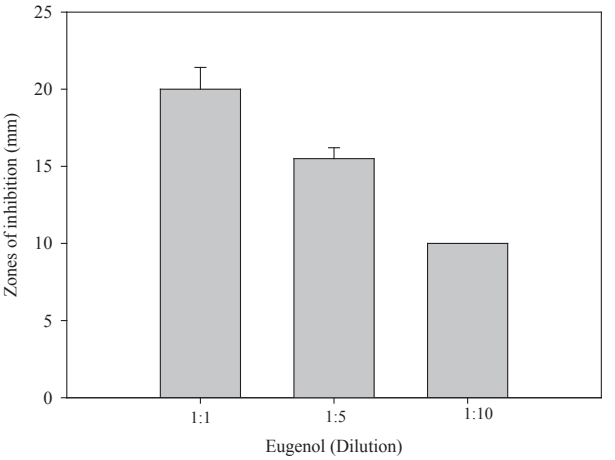


Figure 3. Antimicrobial activity of different dilutions of eugenol in paper discs against *P. fluorescens* (inhibition zones in mm). Bars indicate standard deviation of 2 replicates.

The control disc (without eugenol) presented no inhibition zone. Statistically significant differences ($P < 0.05$) were observed for the different dilutions.

The effect of the AAF2 on fresh chicken pieces

The AAF2 was tested directly on fresh chicken pieces (thighs). The results of microbial counts of *Pseudomonas ssp.* and AMB at 5°C are summarized in Figure 4. The rationale for incorporating antimicrobial compounds into packaging is to prevent surface growth in foods where a high level of spoilage and contamination occurs. The presence of eugenol (4.87 mg g^{-1}) in the AAF2, showed a small reduction of the number of *Pseudomonas* (1.1 x 10^6 CFU g^{-1}) compared to the control film (6.0 x 10^6 CFU g^{-1}), with statistically significant differences ($P < 0.05$) (6.3 log CFU g^{-1} and 6.77 log CFU g^{-1} respectively).

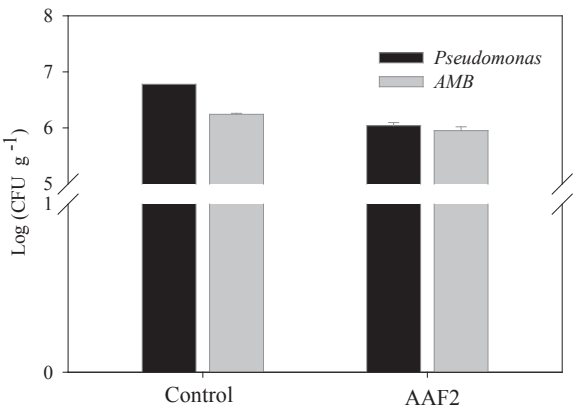


Figure 4. Count of *Pseudomonas* and AMB in chicken pieces packed in the AAF2 and control films for 5 days at 5°C. Bars indicate standard deviation of 2 replicates.

Thus, the presence of eugenol in the AAF2 caused it to be more effective than the control film. These results are consistent with those of the *in vitro* test, in which *P. fluorescens* was sensitive to the presence of eugenol (at concentrations as low as 0.36 mg eugenol per disc).

The effect of the AAF2 on the growth of AMB in fresh chicken thighs was better to reduce the microflora, from 1.7 x 10^6 CFU g^{-1} in the control film and 9.0 x 10^5 CFU g^{-1} in the film with eugenol ($P < 0.05$) (5 days at 5°C) (6.2 log CFU g^{-1} and 5.9 log CFU g^{-1} respectively). These bacterial counts found in the present work, are within the limits established by NOM-034-SSA1-1993 for 5 x 10^6 CFU g^{-1} in México [37].

DISCUSSION

The high losses of eugenol during the manufacture of films were due to the high processing temperatures, which caused the rapid evaporation or decomposition of the eugenol. In addition, the losses may have been caused by the chemical instability of eugenol in the presence of air, light, oxygen and other environmental factors. Suppakul *et al*., [38] reported higher losses of antimicrobial agents, such as linalool and methylchavicol from LDPE and ethylene vinyl acetate (EVA) than those observed in the present study; after the blowing process, the authors observed a concentration of 0.34 g/100 g, down from an initial concentration of 1.0 g/100 g. Galloto *et al.,* [39] reported that nearly 30% of the thymol initially included in a formulation of antimicrobial low density polyethylene (LDPE) film remained after the extrusion process. Yuwono *et al*. [27], reported that the vapor pressure of eugenol increases from 0.01 mm Hg at 20°C to 20 mm Hg at 138.7°C; therefore, losses of eugenol were influenced by evaporation into the environment due to the high volatility of eugenol at processing temperatures.

At the end of the diffusion experiments, close to 94% of eugenol had been released from both films. As the temperature increased, the time needed to cause the release of eugenol was diminished due to the fact that an increased temperature favored the evaporation of eugenol from the polymer matrix into the environment. These results suggest that the antimicrobial release began within the first minutes of the exposure of the films to the environment and quickly reached equilibrium. The results agree with those reported by Dhoot *et al*. [32], who reported that the steady state of the diffusion of eugenol through a linear low-density polyethylene (LLDPE) film was reached after 8.3 h at 16°C. The release of eugenol from the AAFs depends on factors such as temperature and polymer type. If exposed to the air, a vapor pressure of 0.03 mm Hg at 25°C [27] indicates that eugenol will exist solely as a vapor in the ambient atmosphere. Vapor-phase eugenol is degraded in the atmosphere by a reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 6 hours, a time that coincides with the release observed at the temperature of this study (25°C). The behavior of the AAFs shows that during their application as food packaging they will release eugenol under refrigerated conditions. In the case of temperature increases, the release rate will rise, increasing the antimicrobial activity, which will be needed due to the increased growth rate of microorganisms at higher temperatures.

Regarding the diffusion of eugenol from the AAF1, Kuorwel *et al.,* [40], reported that increasing the temperature from 15 °C to 35 °C, the diffusion coefficient increased for carvacrol, linalool and thymol. Piringer and Baner [41], reported a *D* value of 1.3 x 10^{-10} cm^2 s^{-1} for eugenol in LDPE films that were in contact with methanol/ethanol at 23°C. This value is lower than the values determined in the present work because they kept the polymer phase in contact with a methanol/ethanol liquid phase. The contact with organic liquids may have decreased the release of eugenol from the film. Diffusion of eugenol in LLDPE films was also determined by Dhoot *et al*. [32], who found a *D* value of 8.86 x 10^{-10} cm^2 s^{-1} at 23°C. However, these authors reported the diffusion, or penetration, of pure liquid eugenol into the LLDPE matrix. Therefore, it seems that pure eugenol penetrates more slowly into a polymer matrix than it is released at a low concentration from a polymer into the environment. The fast migration of eugenol is due to its ability to diffuse through the LDPE chains to rapidly leave the system. It appears that there are no chemical interactions between the eugenol molecule and the non-polar chains of polyethylene. The use of such films could increase the shelf life of a foodstuff under refrigerated conditions by implementing a system with a slow migration of the agent from the packaging material onto the surface of the foodstuff. Encapsulation of eugenol in materials

like ß-cyclodextrin before its incorporation into the films is the next step in this research.

The control disc (without eugenol) presented no inhibition zone. This result confirmed the *in vitro* antimicrobial activity of eugenol, which showed a high inhibitory effect on *P. fluorescens*. Girova *et al*., [28], reported that clove oil, which is composed of 76.8% eugenol, showed antimicrobial activity against *P. fluorescens*. Ouattara *et al*. [29] reported that *P. fluorescens* was affected by a 1:100 dilution of clove oil. In both cases, the antimicrobial effect was attributed to the presence of eugenol. The mechanism of action of the antimicrobial effect of eugenol remains unclear. An important characteristic of eugenol is its hydrophobicity. This property allows it to penetrate lipopolysaccharide layer in the Gram-negative bacterial cell membrane and to disturb cellular structures [25]. It has been suggested that this activity is caused by the presence of phenolic compounds and monoterpenes [20]. The phenolic components weaken the phospholipid bilayer of the cell membrane, causing an increase in permeability and leakage of the intracellular components [17]. Pandima *et al*., [42] reported that primary mechanism of action of eugenol is disruption of the cytoplasmic membrane, which increases its non-specific permeability. This hyperpermeability is followed by leakage of ions and extensive loss of other cellular contents, including the intracellular proteins and ultimately results in cell death.

Han and Floros [43], reported that during an extrusion, the high temperature conditions in the extruder affected the chemical stability of incorporated antimicrobial substances and reduced their residual antimicrobial activity. The results of the present study showed that the AAF2 films showed moderately reduction of growth of *Pseudomonas* and AMB when they were used to package chicken thighs. Eugenol, by its nature, is highly soluble in the fatty chicken skin, which promotes its diffusion and action on the surface of the chicken pieces. Another possible explanation is the effect of differences between the diffusion rate of the antimicrobial agent and the growth rate of the microorganisms. The antimicrobial agent could be depleted and lose the antimicrobial activity of the film before the growth of the microorganisms increases. Also due to the release of eugenol from the AAF2, perhaps the remaining amount was actually below the MIC, insufficient to inhibit growth of the bacteria studied. Based on *P. fluorescens* growth curve (not shown), the log phase started after 7.5 hours of incubation (26°C). By that time, most of the eugenol had already been released from the AAF (25°C). Therefore, the antimicrobial activity of AAF depends on the relationship between the release rate of eugenol from the film and the growth rate of bacteria.

Despite the losses of eugenol during the processing of the AAF, and the rapid release from the film at 5°C and 25°C, AAF2 was effective in retarding the growth of *Pseudomonas* and AMB in chicken thighs. The release of the antimicrobial agent was directed towards the food surface, which allowed relatively low concentrations of eugenol in the LDPE film to maintain the microbial quality of chicken.

In order to minimize the losses of eugenol during the production of the AAFs, research on the encapsulation of eugenol in ß-cyclodextrin and its incorporation into multilayer films are currently under way. Future studies will also evaluate the effect of this antimicrobial agent on the sensory properties of chicken.

CONCLUSIONS

LDPE films supplemented with 9.0 and 7.7 mg g^{-1} eugenol were manufactured, and 42.6% and 36.7% of the eugenol, respectively, were lost during the production process. The release of eugenol from the films into the environment showed Fick's behavior, with D values of 10^{-8} cm^2 s^{-1} at 25°C and 5°C (same order of magnitude). At 25°C the equilibrium was reached within 6 h, 10 h earlier than 5°C. Despite the losses of eugenol during the extrusion of the films, they showed an antimicrobial effect during contact with fresh chicken by reducing the growth of *Pseudomonas* and AMB. Therefore, the addition of eugenol to LDPE showed a potential to maintain the chicken quality in food packaging applications.

AKNOWLEDGEMENTS

We gratefully acknowledge Q.B. Rosalva *Pérez-Morales* for technical assistance with the microbiological studies. This study was supported by funds from/CONACYT/FOMIX (SON-2008-CO3-106207) and Qualyplast, S.A. de C.V.

CONFLICT OF INTERESTS

The authors declare that no conflict of interest exist for the present work.

REFERENCES

1. SAGARPA. *Situación y perspectiva de la producción de la carne en México*. Disponible en: http://www.sagarpa.gob/ganaderia.
2. Avicultura.com. La producción de carne de ave crecerá en la UE hasta las 13,6 millones de toneladas en 2023. Disponible en: http://www.avicultura.com/2014/02/24/la-produccion-de-carne-de-ave-crecera-ue-hasta-las-millones-de-toneladas-en-2023/.
3. Tendencias Avícolas Mundiales: El consumo de pollo en América casi triplica el promedio mundial. http://www.elsitioavicola.com/articles/2043/tendencias-avacolas-mundiales-el-consumo-de-pollo-en-amarica-casi-triplica-el-promedio-mundial/#sthash.EWK4GlbJ.dpuf.
4. Tendencias Avícolas Mundiales: Mayor consumo de pollo fuera de la UE. Disponible en: http://www.elsitioavicola.com/articles/2471/tendencias-avacolas-mundiales-2013-mayor-consumo-de-pollo-fuera-de-la-ue/#sthash.19cGIVqg.dpuf Tendencias avícolas mundiales. 2013.
5. Jiménez SM, Salsi MS, Tiburzi, MC, Rafaghelli RC, Tessi MA, Coutaz VR. Spoilage microflora in fresh chicken breast stored at 4°C: Influence of packaging methods. J Appl Microbiol. 1997; 83: 613-618.
6. Shin J, Harte B, Ryser E, Selke S. Active packaging of fresh chicken breast, with allyl isothiocyanate (AITC) in combination with modified atmosphere packaging (MAP) to control the growth of pathogens. Food microbiology and safety. J Food Sci. 2010; 75: 65-71.
7. Arnaut-Rollier I, Vauterin L, De Vos P, Massart DL, Devriese LA, De Zutter L, Van Hoof J. A numerical taxonomic study of the *Pseudomonas* flora isolated from poultry meat. J Appl Microbiol. 1999; 87: 15-28.
8. Hotchkiss JH. Current and future trends in active food packaging. In Proceedings of the II Food Packaging International Congress RISEA-2000. Editor H. Soto-Valdez. Hermosillo, Sonora, México. 2000. 39-51 p.
9. Ahvenainen R, Hurme E. Active and smart packaging for meeting consumer demands for quality and safety. Food Addit Contam. 1997; 14**:** 753-763.
10. Vermeiren L, Devlieghere F, van Beest M, de Kruijf N, Debevere J. Developments in the active packaging of foods. Trends Food Sci Tech. 1999; 10: 77-86.
11. Crank J. The mathematics of diffusion (2nd ed.). Oxford, UK: Oxford Science Publications. 1975.
12. Cooksey K. Utilization of antimicrobial packaging films for inhibition of selected microorganism. In ACS symposium series. Food packaging. Washington, DC: American Chemical Society. 2000. 17-25 p.
13. Jayasena DD, Cheorun J. Essential oil as potential antimicrobial agents in meat and meat products: A review. Trends Food Sci Tech. 2013; 34: 96-108.
14. Ramos M, Jiménez A, Peltzer m, Garrigós MC. Characterization and antimicrobial activity studies of polypropylene films with carvacrol and thymol for active packaging. J Food Eng. 2012; 109:513-519.
15. Irkin R, Esmer OK. Novel food packaging system with natural antimicrobial agents. J Food Sci Technol. 2015. DOI 10.1007/s13197-015-1780-9
16. Sanla N, Jangchud A, Chonhenchob V, Suppakul P. Antimicrobial activity of cinnamaldehyde and eugenol and their activity after incorporation into cellulose-based packaging films. Packag Tech Sci. 2012; 25:7-17.
17. Ouattara B, Simard RE, Piette G, Bégin A, Holley RA. Diffusion of acetic and propionic acid form chitosan-based antimicrobial packaging films. Food chemistry and toxicology. J Food Sci. 2000; 65: 768-773.
18. Sung SY, Lee TS, Tee TT, Bee ST, Rahmat AR, Rahman WAWA, Tan, Ach, Vikhraman M. Antimicrobial agents for food packaging applications. Review. Trends Food Sci Tech. 2013; 33: 110-123.
19. Celikel N, Kavas G. Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech J Food Sci. 2008; 26: 171-181.
20. Friedman M, Henika P, Mandrell R. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes* and *Salmonella enterica*. J Food Protect. 2002; 65: 1545-560.
21. López P, Sánchez C, Batlle R, Nerín C. Development of flexible antimicrobial films using essential oils as active agents. J Agr Food Chem. 2007; 55: 8814-8818.
22. Becerril R, Gómez-Lus P, Goñi P, López P, Nerín C. Combination of analytical and microbiological techniques to study the antimicrobial activity of a new active food packaging containing cinnamon or oregano against *E. coli* and *S. aureus.* Anal Bioanal Chem. 2007; 388: 1003-1011
23. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essentials oils. BMC Complem Altern M. 2006; 6: 39-46.
24. Tippayatum P, Chonhenchob V. Antibacterial activities of thymol, eugenol and nisin againts some food spoilage bacteria. Kasetsart Journal (Nat. Sci.) 2007; 41: 319-323.
25. Burt S. Essential oils: Their antibacterial properties and potential applications in food- a review. Int J Food Microbiol. 2004; 94: 223-253.
26. Saïdana D, Mahjoub S, Boussaada O, Chria J, Mahjoub MA, Chérif I, Helal AN. Antibacterial and antifungal activities of the essentials oils of two saltcedar species form Tunisia. J Am Oil Chem Soc. 2008; 85: 817-826.
27. Yuwono M, Siswandono Hafid AF, Poernomo AT, Agil M, Indrayanto G, Ebel S. Eugenol. Analytical profiles of drug substances and excipients. London, UK. Academic Press. Vol. 27. In H. Brittain (Ed.). 2002; 149–177 p.
28. Girova T, Gochev V, Jirovetz L, Buchbauer G, Schmidt E, Stoyana A. Antimicrobial activity of essential oils form spices against psychrotrophic food spoilage microorganism. Biotechnol Biotec Eq. Special Edition. 2010; 547-552.
29. Ouattara B, Simard RE, Holley RA, Piette GJP, Bégin A. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int J Food Microbiol. 1997; 37: 155-162.
30. Manzanarez-López F, Soto-Valdez H, Auras R. Peralta E. Release of α-tocopherol from poly(lactic acid) films, and its effect on the oxidative stability of soybean oil. J Food Eng. 2011; 104: 505–517.
31. Soto-Valdez H, Auras R, Peralta E. Fabrications of poly(lactic acid) films with resveratrol and diffusion to ethanol. J App Polym Sci. 2011; 121: 970-978.
32. Dhoot G, Auras R, Rubino M, Dolan K, Soto-Valdez H. Determination of eugenol diffusion through LLDPE using FTIR-ATR Flow Cell and HPLC Techniques. Polymer. 2009; 50: 1470-1482.
33. Mann CM, Markham JL. A new method for determining the minimum inhibitory concentration of essential oils. J Appl Microbiol. 1998; 84: 538-544.
34. Hintze JL. NCSS. Quick start and self help manual. Statistical system for windows. Kaysville, UT: NCSS. 2007.
35. NOM-092-SSA1-1994. Norma Oficial Mexicana. Bienes y servicios método para la cuenta de bacterias aerobias en placa.
36. NOM-110-SSA1-1994. Norma Oficial Mexicana. Bienes y servicios. Preparación y dilución de muestras de alimentos para su análisis microbiológico.

37. NOM-034-SSA1-1993. Norma Oficial Mexicana. Bienes y servicios. Productos de la carne, carne molida y carne molida moldeada. Envasadas. Especificaciones sanitarias.
38. Suppakul P, Sonneveld K, Bigger SW, Miltz J. Loss of AM additives from antimicrobial films during storage. J Food Eng. 2011; 105: 270-276.
39. Galotto MJ, Valenzuela X, Rodríguez F, Bruna J, Guarda A. Evaluation of the effectiveness of a new antimicrobial active packaging for fresh atlantic salmon (*Salmo Salar* L.) Shelf life. Packag Technol Sci. 2012; 25(6): 363-372.
40. Kuorwel KK, Cran MJ, Sonneveld K, Miltz J, Bigger SW. Migration of antimicrobial agents from starch-based films into a food simulant. LWT Food Sci Tech. 2013; 50: 432-438.
41. Piringer OG, Baner AL. (Eds.). Plastics packaging materials for food. Weinheim, Germany: Wiley-VCH. 2000.
42. Pandiva DK, Arif NS, Sakthivel R, Karutha PS. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. J of Ethnopharmacol. 2010; 130: 107–115.
43. Han JH, Floros JD. Modeling antimicrobial activity loss of potassium sorbate againts Baker´s yeast after heat process to develop antimicrobial food packaging materials. Food Sci Biotechnol. 1999; 8: 11-14.

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 121-129
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a06

ESTUDIO DEL PROCESO DE CLARIFICACIÓN DE HIDROLIZADOS DE ALMIDÓN DE YUCA UTILIZANDO MEMBRANAS CERÁMICAS

STUDY OF CLARIFICATION PROCESS OF CASSAVA STARCH HYDROLYSATES USING CERAMIC MEMBRANES

Maria V. ACEVEDO-ESTUPIÑAN, IQ[1]; Carlos O. PARRA-ESCUDERO, IQ.[1];
Carlos J. MUVDI-NOVA, IQ, PhD.[2*]

Recibido: Agosto 15 de 2014 Aceptado: Septiembre 21 de 2015

RESUMEN

Antecedentes: La etapa de clarificación de hidrolizados de almidón para la producción de jarabes de glucosa es la principal causa de su producción por lotes, además de acarrear altos costos al utilizar filtros rotatorios al vacío, carbón activado y tierras diatomeas que aseguren la calidad del producto. **Objetivos:** Evaluar la etapa de clarificación de hidrolizados de almidón de yuca obtenidos por vía enzimática utilizando tecnología con membranas, a diferentes temperaturas, diámetros de poro de membrana y presiones transmembrana. **Métodos:** La clarificación se realizó utilizando un piloto compuesto por un tanque enchaquetado, donde se llevó a cabo la reacción de hidrólisis. La filtración se realizaba conectando el tanque a una bomba que permitía enviar el fluido al módulo membranario; la presión transmembrana fue fijada con una válvula a la salida del módulo. Los experimentos se llevaron a cabo usando membranas con diferentes tamaños de poro en un diseño factorial 2^2, evaluando dos niveles de temperatura (50 y 70°C) y dos niveles de presión transmembrana (0,15 y 0,30 MPa). Se midieron los caudales obtenidos para el retenido y el permeado. Las muestras obtenidas fueron analizadas para evaluar la calidad del permeado (turbidez, cantidad de proteína retenida, contenido de materia seca y °Brix). El análisis estadístico se llevó a cabo con el software Statgraphic Centurion XVI.I®. **Resultados:** Los resultados obtenidos mostraron que las presiones transmembrana (0,15 y 0,30 MPa) y temperaturas (50 y 70 °C) evaluadas no influyen significativamente sobre la permeabilidad ni calidad del permeado (p-valores > 0,05) permitiendo trabajar con la menor presión tranmembrana (menor desgaste de equipos y menor gasto energético), y a la temperatura de hidrólisis, lo cual permitiría trabajar bajo condiciones de producción en continuo. Además, se observó que es posible utilizar membranas con diámetro de poro de hasta 0,8 μm; aumentado hasta en 5 veces la productividad y disminuyendo la turbidez en un 99%. **Conclusiones:** Es posible utilizar membranas cerámicas de microfiltración de hasta 0,8 μm, en condiciones moderadas de presión transmembrana y a la temperatura de hidrólisis para la clarificación de hidrolizados de almidón de yuca sin disminuir la calidad del permeado.

Palabras clave: Filtración, membranas, hidrolizado, almidón, yuca.

[1] Ingeniero Químico. Escuela de Ingeniería Química. Universidad Industrial de Santander (UIS), Bucaramanga, Colombia.

[2] Ingeniero Químico. Escuela de Ingeniería Química. Universidad Industrial de Santander (UIS), Bucaramanga, Colombia.

[*] Autor de correspondencia: cjmuvdi@uis.edu.co

ABSTRACT

Background: Clarification step of starch hydrolysates for the production of glucose syrups is the main responsible for batch production; in addition, it causes high costs when using rotary vacuum filters, activated carbon and diatomaceous earth to ensure product quality. **Objective**: To evaluate the clarification step of hydrolyzed cassava starch obtained enzymatically, using membrane technology at different temperatures, membrane cut-off and transmembrane pressures. **Methods**: Clarification was conducted using a pilot composed by a jacketed tank, where the hydrolysis reaction was carried out. The filtration was performed by connecting the tank to a pump to send the fluid to the membrane carter; transmembrane pressure was fixed with a valve placed at the end of the module. The experiments were carried out using membranes with different cut-off sizes in a factorial design 2^2, two temperatures (50 and 70°C) and two transmembrane pressure levels (0.15 and 0.30 MPa) were evaluated. The flows obtained for the retentate and permeate were measured. The obtained samples were analyzed to assess the permeate quality (turbidity, amount of retained protein, dry matter content and °Brix). Statistical analysis was performed with software Statgraphic Centurion XVI.I®. **Results**: The results showed that the pressures (0.15 to 0.30 MPa) and temperatures (50 and 70°C) evaluated did not influence significantly the permeability, or the quality of the permeate (p-values > 0.05), it can allow to work with lower pressure (less wear on equipment and less energy), at hydrolysis temperature, it would allow to work under conditions of continuous production. Moreover, the possibility to use membranes with pore diameters up to 0.8 μm was observed; it allow to increase up to 5 times the productivity and decrease the turbidity by 99%. **Conclusions**: It is possible to use ceramic microfiltration membranes up to 0.8 μm in moderate transmembrane pressure, at hydrolysis temperature for clarification of cassava starch hydrolysates without decreasing the quality of the permeate flow.

Keywords: Filtration, membranes, hydrolysate, starch, cassava.

INTRODUCCIÓN

Los jarabes de glucosa son soluciones líquidas altamente viscosas de glucosa concentrada (2,140 cP a 75% de materia seca; 53,7 equivalente dextrosa y 26,7°C) que pueden contener maltodextrinas (1). Su poder edulcorante está entre 40 y 79; siendo 100 el poder de dulzura de la sacarosa (2). Estos jarabes son muy apreciados en la industria de alimentos ya que proporcionan dulzor, suavidad y brillo a los productos. Además ayudan a la formación de cristales más pequeños, facilitan la masticación de las gomas, y disminuyen la actividad del agua prolongando la duración del producto, entre otros beneficios. Aparte son precursores para otros sacáridos como la fructosa, cuyo poder edulcorante es mayor (80-130). Estas características permiten su utilización en productos como: chocolates, helados y dulces en general (3-6).

La materia prima para la producción de jarabes de glucosa es el almidón, polisacárido formado por unidades de glucosa, que generalmente está compuesto aproximadamente por 80% p/p de amilopectina y 20% p/p de amilosa en base seca. La yuca es una de las principales fuentes de almidón conteniendo hasta un 87,7% p/p en base seca; superando en más del 10% a otras fuentes de almidón como maíz, papa y plátano (7).

La producción de jarabes de glucosa comienza con la hidrólisis, generalmente, enzimática del almidón que permite obtener ED (Equivalentes Dextrosa) superiores a 95 (8). Consta de dos etapas: licuefacción y sacarificación. En la etapa de licuefacción se produce una solución de dextrinas por la acción de las α-amilasas que atacan los enlaces α-1,4 del almidón (9–12), obteniendo hidrolizados de 15-20 ED (6). En la etapa de sacarificación, las glucoamilasas atacan los enlaces α-1,4 y α-1,6 para transformar las dextrinas en azúcares de bajo peso molecular (glucosa, maltosa o mezclas) (9-13). Al terminar esta etapa se puede obtener un hidrolizado con un contenido final de 93-96 ED.

Una vez finalizada la sacarificación, el hidrolizado es clarificado para remover sólidos suspendidos e impurezas insolubles. Para ello, tradicionalmente se utiliza carbón activado con el fin de remover color y sabor, seguido de una filtración frontal mediante filtros prensa o filtros rotatorios al vacío con tierras diatomeas como auxiliar filtrante (9, 14-17). Estos procesos toman mucho tiempo para alcanzar un

óptimo de calidad al pasar por varios "purificadores". Además, no son económicamente rentables al utilizar varios auxiliares filtrantes por cochada, sin mencionar el impacto medio ambiental al desechar, por ejemplo, las tierras diatomeas. Una alternativa más eficiente para realizar la clarificación es el uso de membaranas. Con esta tecnología se ahorraría en otras operaciones unitarias para asegurar la calidad del producto como intercambio iónico, decoloración y uso de carbón activado para retirar sabores. La incorporación de membranas de micro o ultrafiltración permiten obtener una calidad superior en términos de turbidez, color y contenido microbiológico (8). Uno de los grandes problemas de los procesos con membranas es la colmatación. Este fenómeno tiene como efecto la pérdida del rendimiento de la membrana; evidenciada en la disminución del flujo en el permeado. La colmatación depende del tipo de fluido a tratar, del material membranario y de las condiciones de operación. Varios autores han implementado membranas en el proceso de producción de jarabes de glucosa, la mayoría de ellos han trabajado con membranas de ultrafiltración entre 3 y 15 kDa (17-21); por lo tanto, los bajos flujos de permeado alcanzados no son económicamente rentables. Además, estos mismos estudios han utilizado principalmente membranas orgánicas de fibra hueca. Por el material utilizado, estas membranas no pueden ser utilizadas bajo condiciones de temperatura de hidrólisis del proceso, lo cual redundaría en tiempos de parada y mayores costos (22,23). Igualmente, no se ha realizado a la fecha estudios comparativos que busquen analizar el efecto del tamaño de poro sobre la calidad del permeado.

El objetivo de este trabajo fue evaluar la influencia de la temperatura, la presión transmembrana y el diámetro de poro de membranas cerámicas de microfiltración y ultrafiltración en el mejoramiento de la productividad de la etapa de clarificación, sin disminuir la calidad del producto clarificado y bajo condiciones que permitirían el estudio del proceso bajo una configuración en continuo.

MATERIALES Y MÉTODOS

Protocolo de limpieza de membranas

Al final de cada filtración se llevó a cabo el protocolo de limpieza; en el cual se utiliza una solución de NaOH 0,5 N y 1% de NaClO, a 0,1 MPa y 60°C y 1 h de filtración. Posteriormente se realiza un lavado con agua destilada hasta neutralidad y se verifica la permeabilidad al agua de la membrana.

Materiales e insumos.

Almidón de yuca, Almiyuca® suministrado por Almidones de Sucre S.A.S. Sucre, Colombia. Enzimas α-amilasa (Liquozyme SC DS®) y glucoamilasa (Spirizyme Fuel®) suministradas por Novozymes, North America Inc, Franklinton, N.C. United States. Membranas cerámicas monocanal de α-alumina con diámetro de 0,007 m, TAMI®, Nyons, Francia. Bomba de desplazamiento positivo: Bomba rotatoria SI-5009 Tuthill®, Burr Ridge, IL, United States, acoplada con un motor Siemens de 1 HP y 1090 rpm con variador de frecuencia CFW-08 (WEG®, Bogotá, Colombia). HCl al 37%, azul de coomasie G-250, etanol al 99,9% y albúmina de suero bovino (BSA) Merck®, Bogotá, Colombia; ácido ortho fosfórico al 85% suministrado por Yequim Ltda. Bucaramanga, Colombia. Hipoclorito de Sodio al 13% suministrado por Multiquímicos Ltda. Bucaramanga, Colombia, y NaOH en escamas suministrado por Suquin Ltda. Bucaramanga, Colombia.

Producción de hidrolizados de almidón de yuca

Los hidrolizados de almidón utilizados en las pruebas de ultrafiltración y microfiltración, se prepararon a partir de 6 L de una solución almidón de yuca-agua al 20%p/v, bajo agitación (30 rpm). Los parámetros de operación de la reacción de hidrólisis enzimática son los reportados por Ruiz (26) para licuefacción (pH 5, 80°C, tiempo de reacción 15 min y relación enzima/sustrato 0,028%p/p) y para sacarificación (pH 4,5 , 70°C, tiempo de reacción 45 min y relación enzima/sustrato 0,063%p/p). La temperatura de hidrólisis se controló con un baño termostatado y el pH se reguló con NaOH y HCl 2N. Las reacciones de hidrólisis se detuvieron bajando el pH a 2,5 y manteniéndolo durante 1 h. Los hidrolizados fueron almacenados a una temperatura de -20°C.

Proceso de filtración membranaria

La clarificación de los hidrolizados se llevó a cabo en un piloto de acero inoxidable con capacidad de 10 L, representado en la Figura 1. Éste consiste en un tanque de almacenamiento enchaquetado, el cual asegura la alimentación del hidrolizado al sistema.

Una bomba de desplazamiento positivo impulsa la alimentación hacia el módulo membranario, en donde se lleva a cabo la filtración. La presión transmembrana (ΔP_{TM}) se fija con una válvula globo ubicada en la corriente del retenido a la salida del módulo membranario. El retenido es recirculado al tanque de almacenamiento, mientras el permeado se recupera. El caudal de permeado se midió cada 5 min; al final de las pruebas se midió el caudal del retenido y se tomaron muestras de ambos para sus posteriores análisis fisicoquímicos. Las pruebas de filtración membranaria tuvieron una duración de 30 min.

En la primera etapa de la experimentación se analizó el efecto de la temperatura y la presión transmembrana sobre la ultrafiltración del hidrolizado obtenido en la etapa de sacarificación. Se trabajó con una membrana de 15 kDa (membrana que aseguró una retención de enzima superior al 95%). El diseño de experimentos fue de tipo factorial 2^2 (25). Los niveles evaluados para la temperatura fueron: $T_{máx}$ de 70°C, y $T_{mín}$ de 50°C. Los niveles de temperatura se fijaron teniendo en cuenta tanto el rango de actividad de la enzima glucoamilasa (24), como el rendimiento de la bomba de desplazamiento positivo: a menor temperatura, mayor viscosidad y mejor rendimiento (17, 20, 27, 28). Para la presión transmembrana los niveles evaluados fueron 1,5 y 3 MPa. Es de esperar que a una mayor presión haya una mayor permeabilidad debido a que la fuerza impulsora es la diferencia de presión transmembrana; el nivel máximo de presión evaluado estuvo condicionado a la capacidad del piloto. La filtración del hidrolizado se realizó recirculando el retenido al tanque de alimentación.

En la segunda etapa de la experimentación, se evaluó el efecto del diámetro de poro con el objetivo de estudiar la posibilidad de aumentar la productividad del proceso de clarificación, sin disminuir por tanto, la calidad del producto clarificado. Para esto se utilizaron membranas cerámicas monocanal, cada una con diferentes diámetros de poro: 150 y 300 kDa, y 0,3; 0,45; 0,8 y 1,4 μm. La clarificación se realizó utilizando las mejores condiciones de presión transmembrana y temperatura encontradas en la etapa anterior.

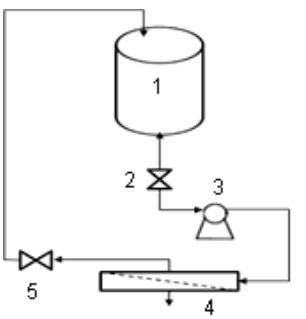
Métodos experimentales.

El porcentaje de retención de proteínas en las membranas fue calculado al comparar la cantidad de proteína medida en el permeado con la del retenido, Ecuación 1; la cantidad de proteína en las muestras fue determinada mediante el método de Bradford a 595 nm (24), usando un espectrofotómetro Genesys 20 ThermoSpectronic®.

$$\%\text{Retención} = \frac{\text{1-cantidad de proteína en el permeado}}{\text{Cantidad de proteína en el alimento}} * 100$$

Ecuación 1. *Cálculo del porcentaje de retención de enzima*

La turbidez de los hidrolizados clarificados y el retenido fue determinada utilizando un turbidímetro Hach 2100Q® según USEPA 180.1. Los caudales de permeado y retenido se determinaron por gravimetría, pesando el permeado/retenido obtenido en un tiempo determinado con una balanza analítica OHAUS, la permeabilidad de la membrana se calculó al dividir el caudal del permeado en el área superficial de la membrana (diámetro * longitud*π) (16), el porcentaje de pérdida de permeabilidad fue calculado al comparar la permeabilidad a los 10 minutos y a los 30 minutos. El porcentaje de materia seca fue determinado según la norma A.O.A.C. 1980. Para determinar los °Brix se utilizó un refractómetro Fisher No. 13 964 70C de 0-90%. El análisis estadístico se llevó a cabo con el software Statgraphic Centurion XVI.I®. El diseño de experimentos fue de tipo factorial 2^2 (25).



No	Elemento
1	Tanque de alimentación enchaquetado
2	Válvula
3	Bomba de desplazamiento positivo
4	Módulo membranario
5	Válvula reguladora de presión

Figura 1. *Piloto de filtración membranaria.*

RESULTADOS

Efecto de la ΔP_{TM} y de la temperatura en la filtración del hidrolizado obtenido en la etapa de sacarificación.

La Figura 2 muestra los resultados obtenidos para las permeabilidades de la membrana de 15 kDa a las condiciones de operación propuestas para sacarificación. En esta figura se puede observar, que para las dos temperaturas estudiadas (50 y 70 °C), el cambio en la permeabilidad al inicio (10 min) y final

(30 min) del proceso de filtración, para la ΔP_{TM} de 0,3 MPa, provoca una caída en la permeabilidad de la membrana del 29,4% a 50 ºC y del 27,7% a 70 ºC.

Sin embargo, con el mismo procedimiento, a 0,15 MPa las pérdidas de permeabilidad están por debajo del 2,8 % Esto puede explicarse por la colmatación de la membrana inducida en el sistema debido a las condiciones de diseño del piloto utilizado; el cual requiere el cierre de la válvula para alcanzar la ΔP_{TM} requerida, haciendo que disminuya la velocidad del retenido, y por lo tanto la velocidad de arrastre sobre la superficie membranaria.

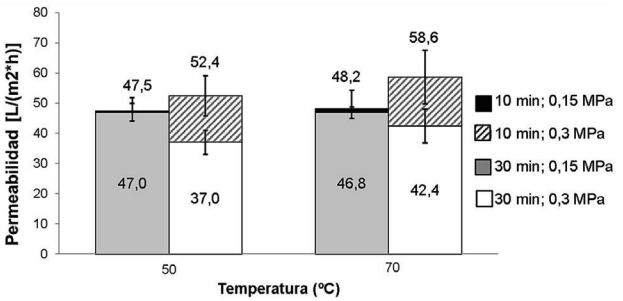


Figura 2. Permeabilidades a los 10 y 30 min de filtración después de la etapa de sacarificación con membrana de 15 kDa; hidrolizado de 17,2 ºBrix; pH de 2,5; concentración de glucoamilasa de 0,063%p/p; ΔP_{TM} de 0,15 y 0,3 MPa y temperatura de 50 y 70°C.

La Figura 3 muestra los resultados del % de materia seca y los ºBrix obtenidos para esta etapa. En la figura se observan valores menores para los permeados con respecto al hidrolizado alimentado en la filtración, tanto para los °Brix (en promedio 9,9%) como para el % de materia seca (en promedio 28,2%). Esto muestra la capacidad de la membrana para retener sólidos (suspendidos y solubles) no deseados en el clarificado, permitiendo el paso de los azúcares solubles. Adicionalmente, se cuantificó la cantidad de proteína, es decir, la cantidad de enzima presente en los permeados obtenidos en esta etapa con la membrana de 15 kDa, y se encontró que en promedio el porcentaje de retención de enzima fue del 97,56% ± 0,76%

Las mejores condiciones de operación encontradas, teniendo en cuenta las condiciones de operación del piloto, fueron ΔP_{TM} 0,15 MPa y 70°C puesto que permiten un menor desgaste de la bomba, al no utilizar la máxima presión, además de menores pérdidas de permeabilidad, y trabajar a la temperatura de hidrólisis.

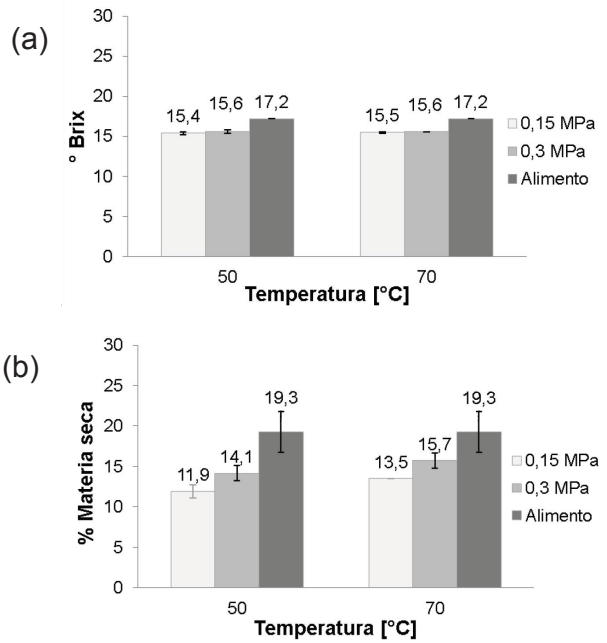


Figura 3. Resultados de los análisis fisicoquímicos realizados a los permeados obtenidos después de sacarificación, para: (a) °Brix. (b) %Materia seca. Con membrana de 15 kDa; hidrolizado de 17,2 ºBrix; pH de 2,5; concentración de glucoamilasa de 0,063%p/p; ΔP_{TM} de 0,15 y 0,3 MPa y temperatura de 50 y 70°C. (a) °Brix. (b) %Materia seca.

Efecto del diámetro de poro en la calidad y cantidad de permeado obtenido en la etapa de sacarificación.

La Figura 4 muestra los resultados de las permeabilidades obtenidas utilizando membranas con diferentes diámetros de poro, evaluados a los 10 y 30 min para las mejores condiciones de operación encontradas previamente, ΔP_{TM} 0,15 MPa y 70°C.

En la figura se pueden observar pérdidas de permeabilidad en todas las membranas. Los porcentajes de pérdida de permeabilidad, calculados al comparar la permeabilidad determinada del retenido con la del concentrado, para las membranas de 150 kDa; 300 kDa; 0,2 μm; 0,45 μm; 0,8 μm y 1,4 μm, son respectivamente 11,52%, 9,43%, 17,16%, 14,46%, 45,06% y 57,71%. Se observa una tendencia de aumento de las pérdidas de permeabilidad con el aumento del diámetro de poro, evidenciando una mayor colmatación de las membranas al aumentar su diámetro de poro.

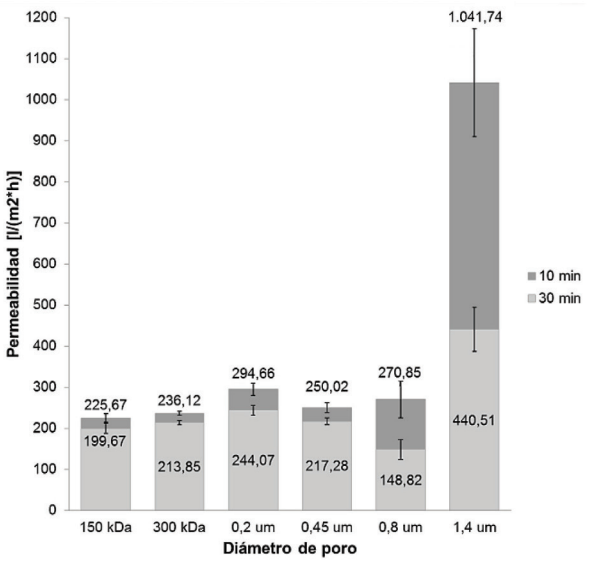


Figura 4. Comparación de las permeabilidades de hidrolizados sacarificados a los 10 y 30 min para las membranas con diámetros de poro de 150 y 300 kDa, y 0,2; 0,45; 0,8 y 1,4 μm, realizadas a 70°C con ΔP_{TM} de 0,15 MPa, pH 2,5 y alimentación a 17,2 °Brix.

La Figura 5 muestra los resultados obtenidos al evaluar la turbidez de los permeados obtenidos con diferentes diámetros de poro para las mismas condiciones de la Figura 4. En esta figura se aprecia que la clarificación obtenida con los diferentes diámetros de poro es efectiva, pues se alcanzó una disminución de más del 98,9% en todas las membranas (excluyendo la de 1,4 μm (89%)), con respecto al alimento.

La Figura 6 muestra los resultados obtenidos al evaluar el % de retención de enzima para todas las membranas evaluadas. En esta se evidencia un % de retención de enzima cercano al 90% (excepto para la membrana de 1,4 μm (7,58%)).

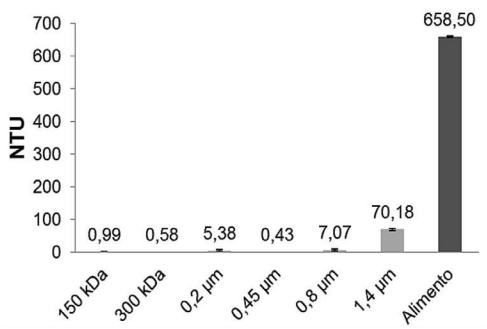


Figura 5. Turbidez de muestras de alimento y de permeado obtenidos con membranas de 150, 300 kDa y 0,2; 0,45; 0,8 y 1,4 μm a 70°C, con ΔP_{TM} de 0,15 MPa, pH 2,5 y alimentación a 17,2 °Brix.

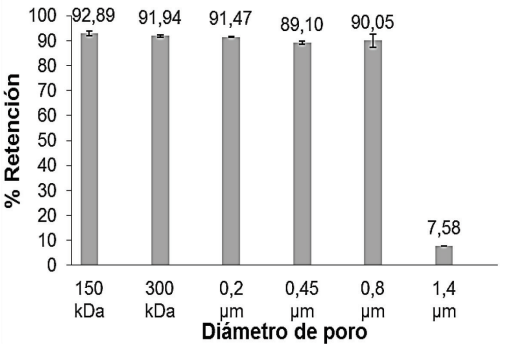


Figura 6. Porcentaje de retención para los permeados obtenidos con membranas de 150, 300 kDa y 0,2; 0,45; 0,8 y 1,4 μm a 70°C, con ΔP_{TM} de 0,15 MPa, pH 2,5 y alimentación a 17,2 °Brix.

La Figura 7 muestra los análisis fisicoquímicos para los permeados obtenidos con las diferentes membranas, para °Brix y % de materia seca. Se observa que estas dos variables de respuesta no presentan variación significativa con el diámetro de poro.

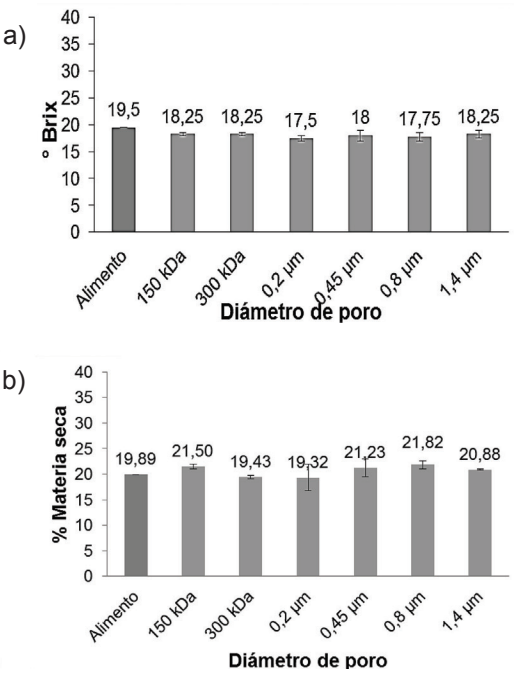


Figura 7. Resultados de los análisis fisicoquímicos realizados para las muestras de alimento y permeado de las membranas con diámetros de poro de 150 y 300 kDa, y 0,2; 0,45; 0,8 y 1,4 μm. (a)°Brix. (b) %Materia seca. Experimentos realizados a 70°C, ΔP_{TM} 0,15 MPa, pH 2,5 y alimentación a 17,2 °Brix.

DISCUSIÓN

Los resultados encontrados indican que en las condiciones de operación evaluadas, el efecto de la presión transmembrana y la temperatura no son significativos (los promedios se encuentran dentro de las desviaciones obtenidas); se podría decir, por ejemplo, que a una temperatura de 80°C se puede trabajar tanto con una presión de 0,15 MPa o 0,3 MPa sin obtener una mejora considerable en la permeabilidad. Esto se corroboró mediante un análisis estadístico con el Software Statgraphic Centurion XVI.I®, en el cual los datos fueron tratados por análisis ANOVA mostrando valores p mayores a 0,05. Los resultados anteriormente señalados indican que es posible trabajar con condiciones más suaves de operación sin afectar el rendimiento del proceso, lo cual redundaría en una reducción de costos y facilidad de operación. Estos resultados son contrarios a lo encontrado por Grześkowiak-Przywecka & Słomińska (21), quienes trabajaron con membranas cerámicas Tami® tubulares de 3 canales y diámetros de poro de 3, 5, 8 y 15 kDa para producir jarabes de maltosa a partir de almidón de papa sacarificado a 55°C y presión transmembrana de 0,4-0,6 MPa. Ellos encontraron que la permeabilidad se incrementa linealmente con la presión, su comportamiento lo describen mediante la ecuación y = 44,9x - 14,1 (x: presión transmembranaria, y: flujo de permeado) en un rango de 0,4-0,7 MPa. Sin embargo, la máxima permeabilidad que alcanzaron (a 0,7 MPa) fue de 17 L/m^2.h, muy por debajo del flujo de permeado alcanzado en el presente trabajo con la membrana de 15 kDa a 50°C y 0,15 MPa (37 L/m^2.h). Igualmente, Gaouar *et al*. (29) también obtuvieron mayores permeabilidades a mayor presión. Ellos trabajaron con membranas tubulares Carbosep® (ZrO_2 soportada en carbón poroso) de 10, 50 y 150 kDa para producir jarabes de maltosa a partir de almidón de yuca sacarificado. Este trabajo muestra que para mantener un flujo constante de permeado de 20 L/m^2.h durante 6 h, con una membrana de 50 kDa y temperaturas de operación entre 60 y 68°C, era necesario aumentar la presión transmembrana de 0,2 a 0,44 MPa. Sin embargo, Mameria *et al*. (18) encontraron que para bajas velocidades lineales, el flujo de permeado es independiente a la presión transmembrana aplicada (al trabajar con presiones mayores a 0,2 MPa a 50°C). Esto lo comprobaron usando una membrana Carbosep M5® de 10 kDa (ZrO_2 soportada en carbón poroso) en un biorreactor membranario para sacarificar los residuos sólidos de la producción de aceite de oliva. Los resultados de Mameria *et al*. (18) concuerdan con los encontrados por Kazemi *et al*. (30, 31) quienes hallaron que la presión tiene un punto máximo después del cual no se incrementa el flujo de permeado. Estos resultados explican el comportamiento de los encontrados en el presente artículo.

Los datos obtenidos en este trabajo señalan la posibilidad de llevar a cabo el proceso de filtración bajo condiciones moderadas de presión transmembrana, que permitan un menor desgaste de los equipos y menores gastos energéticos. Igualmente, el análisis estadístico muestra que las variables de entrada, en los rangos utilizados, no afectan de forma significativa los °Brix y el % de materia de seca (valores p mayores a 0,05), indicadores de la calidad del producto. Es decir que ni la presión transmembrana ni la temperatura afectan significativamente las características del permeado. Estos resultados son esperados en cuanto a calidad, debido a que la selectividad depende únicamente del tipo de membrana, en este caso la exclusión se realiza por tamaño de poro: solo se permite el paso de sustancias más pequeñas que el poro de la membrana; por lo tanto, estas dos variables no deberían afectar la cantidad de sólidos retenidos por la membrana.

En las pruebas utilizando membranas de micro y ultrafiltración, los resultados muestran una tendencia general de aumento de la colmatación (ensuciamiento) al aumentar el diámetro de poro de la membrana. Esto es debido a la mayor presencia de partículas que pueden pasar parcialmente a través de los poros, impidiendo el paso de otras sustancias. Gaouar *et al.* (29) encontraron que en todos los casos, después de un periodo transitorio inicial, el flujo de permeado disminuye con el tiempo y esta disminución es más importante cuanto mayor es el tamaño de poro. La pérdida de permeabilidad fue evaluada en tres de membranas 10, 50 y 150 kDa y sus pérdidas de flujo aproximadas son de 10%, 33% y 53%, respectivamente.

En las pruebas de calidad de permeado con membranas cerámicas de diferente tamaño de poro, los resultados obtenidos indican que es posible mejorar la productividad de esta técnica al utilizar una membrana con un diámetro de poro de hasta 0,8 μm. Esto aumentaría el flujo de permeado hasta en 5,2 veces lo obtenido con una membrana de 15 kDa, que es la membrana más utilizada por varios

autores (17, 19, 20, 21, 22), sin que ello conlleve a una pérdida significativa de la calidad del permeado. Sobre este tema trabajaron Singh & Cheryan (8) utilizando membranas cerámicas Ceramem® de 0,2 μm a 0,17 MPa y 60ºC. Ellos evaluaron el grado de clarificación de los hidrolizados sacarificados de almidón de maíz comparando el color del permeado y del retenido. Sus resultados coinciden con lo obtenido en el presente trabajo y muestran que el hidrolizado clarificado fue consistentemente menor en color a través de todo el proceso; estos resultados confirman que la microfiltración puede ser efectivamente usada para la clarificación del almidón hidrolizado.

CONCLUSIONES

Este trabajo permitió el análisis de la influencia de la temperatura y presión transmembrana en la clarificación de hidrolizados de almidón de yuca, así como la comparación con respecto al flujo de permeado y calidad del permeado utilizando membranas con distintos diámetros de poro. Los resultados de este estudio contribuyen al mejoramiento del proceso de producción de jarabes de glucosa en cuanto a tiempos, calidad y costos.

Específicamente, se encontró que: la presión transmembrana y la temperatura no tuvieron un efecto significativo en la operación de ultrafiltración (bajo las condiciones evaluadas), mostrando que se pueden obtener permeabilidades similares a 1,5 y 3 MPa a 70°C; lo que permite trabajar la hidrólisis bajo condiciones de presión moderadas y a la temperatura de hidrólisis.

Igualmente, los resultados obtenidos evidencian la posibilidad de trabajar con membranas de microfiltración con diámetro de poro de hasta 0,8 μm, sin reducir la calidad del permeado y aumentando la productividad hasta en 5,2 veces; mejorando la eficiencia del proceso de clarificación. Este trabajo permitió comprobar las ventajas del uso de membranas cerámicas de α-alúmina en la etapa de clarificación de hidrolizados de almidón de yuca, entre ellas: resistencia térmica, mecánica y química; ya que las membranas pudieron ser utilizadas en diferentes condiciones de temperatura y presión, además de permitir el uso de hipoclorito de sodio como agente limpiador, según el protocolo de limpieza (32). Otras ventajas como baja colmatación y alta selectividad se ven reflejadas a través de las variables permeabilidad en el tiempo, porcentaje retención y turbidez.

Los resultados obtenidos evidencian la posibilidad de configurar el sistema en continuo para minimizar los tiempos de parada, maximizar los volúmenes producidos y la posible recuperación de enzimas retenidas que podrían ser usadas en reacciones sucesivas, mientras se remueven continuamente los productos a través de la membrana, al mismo tiempo que se clarifica el hidrolizado obtenido.

Limitaciones y recomendaciones

En un trabajo posterior es importante aumentar la superficie membranaria del piloto utilizado, con el fin de establecer estudios para la producción de hidrolizados de almidón de yuca en continuo.

AGRADECIMIENTOS

Los autores agradecen a la Vicerrectoría de Investigación y Extensión de la Universidad Industrial de Santander, a Promitec S.A.S y al Departamento administrativo de Ciencia, Tecnología e Innovación – COLCIENCIAS, por el apoyo brindado para la realización del presente trabajo, que hace parte del proyecto titulado "Aumento de la productividad de concentrados de jarabes de glucosa obtenidos a partir de hidrolizados de almidón de yuca por métodos no convencionales" (cód. VIE9417).

REFERENCIAS

1. Hobbs L. Sweeteners from starch; production, properties and uses. Starch: Chemistry and technology. 2009;799-821.
2. Uribe JP. Obtencion de jarabe fructosado a partir de almidon de platano [Tesis]. Morelos, México: Instituto Politecnico Nacional; 2004.
3. Arango M. Jarabes de Maiz. Revista de Alimentos.2008;22.
4. Promitec. Promitec soluciones biotecnologicas naturales (sitio en internet). Promitec S.A.S. Disponible en:http://promitec.com.co/images/documentos/192Ficha%20t%C3%A9cnica%20Jarabe%20de%20Glucosa%20-%20Nat%20Bio%20080.pdf. Acceso el 25 de Agosto 2012.
5. Tate & Lyle. *Tate & Lyle Home (sitio en internet). Disponible en:* http://www.tateandlyle.co.uk/NR/rdonlyres/et5lc6vlrt6ftayeubuywmqlxnyd65dcpe3v7fzp6muzg6awn5kseo5gzullqfrgol6vgzketyee3pguit2bwjbgzbb/Espanol_Edulcorantes_Liquid.pdf. Acceso el 28 de Julio 2012.
6. Kearsley MW, Dziedzic SZ. *Handbook of starch hydrolysis products and their derivates.* Londres: Blackie Academic & Professional; 1995.
7. Aya WA, Pineda J, Sanchez O, Cardona C. Análisis comparativo de diferentes materias primas amiláceas para la obtención de alcohol carburante. 2006. *Memorias del congreso Colombiano de Ingenierìa Quìmica*, ISSN: 1692-925X.
8. Singh N, Cheryan M. (1998). Properties and Composition of Concentrates and Syrup Obtained by Microfiltration of saccharified Com Starch Hydrolysate. J Cereal Sci. *27*:315-320.
9. Henrissat. A classification of glycosyl hydrolases based on amino-acid sequence similarities. *Biochemical.* 1991; 309-316.

10. Latorre L. *Análisis estructural y modificación funcional de la glucoamilasa de Saccharomises Cerevisae Var Diasticus [Tesis doctora]. Departamento de Bioquímica y Biología molecular.* Valencia, España: Universidad de Valencia. 2008.
11. Sánchez CP, Mejía CE, Figueroa C, Esquivia M, Agudelo LM, Zapata N. Bioprospección de microorganismos nativos amilolíticos. *Facultad de Ciencias Agropecuarias. Universidad de Antioquia*. 2004; 2(1):8-17.
12. Rutz, Janssen. *Biofuel Technology Handbook*. Munich: WIP Renewable Energies. 2007.
13. Amutha R., Gunasekaran P.. Production of ethanol from liquiefied cassava starch using co-immobilized cells of zymomonasmobilis and saccharomyces diastaticus. J Biosci Bioeng. *2001. 92*, 560-564.
14. Basso A. Vacuum filtration using filteraids. *Chemical Engineering. 1982. 89(8)*, 159-160.
15. Kuske E, Bonnett D. Versatile filter aid helps assure precise quality for corn syrups. *Food Engineering. 1977. 49(11)*, 92-94.
16. Mera, I., & Carrera, J. (2005). Obtencion de glucosa a paritr de almidon de yuca (Manihot Sculeta). *Revista de Biotecnologia Universidad del Cauca. 2005; 3(1):* 54-63.
17. Slominska L, Niedbach J. Study on the influence of the so-called filtration enzyme action on the membrane filtration process of wheat starch hydrolysates. *Desalination. 2009;* 296-301.
18. Mameria N et al. Enzymatic saccharification of olive mill solid residue in a membrane reactor. J Memb Sci. *2000; 178*, 121-130.
19. Grześkowiak-Przywecka A., Słomińska L. Continuous pótato starch hydrolysis process in a membrane reactor with tubular and hollow-fiber membranes. *Desalination. 2005; 184*, 105-112.
20. López, Ulibarri R, Hall G. Saccharification of cassava flour starch in a hollow fiber membrane reactor. Enzyme Microb Technol. *1997*; 398-404.
21. Grześkowiak-Przywecka A., Słomińska L. Saccharification of pótato starch in an ultrafilration reactor. J Food Eng. *2007; 79*:539-545.
22. Andrić P., Meyer A.S., Jensen P.A., Dam-Johansen K. Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis. II. Quantification of inhibition and suitability of membrane reactors. Biotechnol Adv. *2010; 28*, 407-425.
23. Sulej-Chojnacka J., Konował E. , Prochaska K.. Contiuous recycle membrane reactor for enzymatic hydrolysis of dual modified potato starch. *Desalination. 2005; 184*, 105-112.
24. Bradford. M. A rapid and senstive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 1976. 72, 248-254.
25. Galbiati J. *Diseño de experimentos factoriales con aplicaciones a procesos industriales.2007.*
26. Ruiz M. *Obtencion de jarabes de glucosa a partir de almidon de yuca por medio de hidrólisis enzimática, para ser usados como sustrato en la producción de bioetanol [Tesis].* Bucaramanga, Colombia. Universidad Industrial de Santander. Escuela de Química, 2009
27. Chen Y., Huang S., Tang Z., Chen X., Zhang ZStructural changes of cassava starch granules hydrolyzed by a mixture of a-amylase and glucoamilase. Carbohydr Polym. *2011*; 272-275.
28. Morales S, Alvarez H, Sanchez C. Dynamic models for the production of glucose syrup from cassava starch. *Food and Bioproducts Processing. 2008*; 25-30.
29. Gaouar O, Zakhia N, Aymard C, Rios GM. Production of maltose syrup by bioconversion of cassava starch in an ultrafiltration reactor. Ind Crops Prod. 1998; 7, 159-167.
30. Kazemi M.A, Soltanieh M., Yazdanshenas M. Mathematical modelng of crossflow microfiltration of diluted malt extract suspension by tubular ceramic membrane. J Food Eng. 2013. 116:926-933.
31. Kazemi M.A, Soltanieh M., Yazdanshenas M., Fillaudeau L. Influence of crossflow microfiltration on ceramic membrane fouling and beer quality. Desalination. 2013. 51:4302-4312.
32. Acosta. J., Rios, S. Aplicación de las técnicas con membranas de microfiltración y ultrafiltración para la clarificación y fraccionamiento de sangre de bovino cruda e hidrolizada. Tesis para optar al título de Ingeniero Químico. Universidad Industrial de Santander. Bucaramanga. 2013.

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 130-139
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a07

OPTIMIZACIÓN EXPERIMENTAL DE UNA FORMULACIÓN DE PULPA DE UCHUVA (*Physalis peruviana*) PARA MEJORAR SU PROCESAMIENTO EN EL SECADO POR ATOMIZACIÓN

EXPERIMENTAL OPTIMIZATION OF CAPE GOOSEBERRY PULP (*Physalis peruviana*) FORMULATION TO IMPROVE ITS PROCESSING BY SPRAY DRYING

Misael CORTÉS R. Ph.D.[1*]; Gustavo HERNÁNDEZ S., MSc.[2]; Héctor J. CIRO V., Ph.D. [1]

Recibido: Junio 22 de 2014 Aceptado: Abril 26 de 2015

RESUMEN

Antecedentes: En Colombia, el fruto de la uchuva (*Physalis peruviana*) está identificado como un fruto promisorio de exportación, por lo que la mejora de su procesamiento representa contribuir a una mayor competitividad de la cadena. **Objetivos:** el objetivo de este trabajo fue optimizar en función de parámetros fisicoquímicos y reológicos la formulación de una suspensión a base de pulpa de uchuva mezclada con los aditivos maltodextrina (MD) y goma arábiga (GA) con fines de ser utilizada en secado por atomización. **Métodos:** La optimización se realizó aplicando un diseño experimental central compuesto mediante superficie de respuesta con dos factores (MD y GA) y variables de respuesta (densidad, ^{o}Brix, a_w, pH, y parámetros reológicos). **Resultados**: La caracterización reológica de las suspensiones mostró un comportamiento tipo adelgazante con esfuerzo de cedencia (Modelo de Hershel-Bulkley). La suspensión que mejor se ajustó a los criterios de optimización para fines de secado por atomización fue para la formulación basada en MD (24%) y GA (0%) con caracterización reológica de Herschel-Bulkley tipo adelgazante con índice de consistencia ($K= 0{,}119\pm0{,}017$ Pa.s^n), índice de potencia ($n=0{,}75\pm0{,}03$) y esfuerzo de cedencia ($\tau_o = 0{,}092\pm0{,}069$ Pa). Los resultados para las propiedades fisicoquímicas en el punto optimizado fueron densidad = $1{,}167\pm0{,}005$ g/mL, °Brix = $35{,}5\pm0{,}5$, $a_w = 0{,}974\pm0{,}002$. El pH y τ_o no fueron estadísticamente significativos ($p>0{,}05$) en la formulación de las suspensiones. **Conclusiones**: La optimización experimental representa una herramienta importante que permitió obtener una formulación de uchuva con MD y GA apta para secado por aspersión, representando un ahorro en tiempo y dinero para el investigador y la industria.

Palabras clave: *Physalis peruviana* L., goma arábiga, maltodextrina, reología, optimización experimental.

ABSTRACT

Background: In Colombia, the Cape gooseberry fruit (*Physalis peruviana*) is identified as a promising export fruit, so improving its processing is contributing to greater competitiveness of the chain. **Objectives:** The aim of this study was to optimize suspension of cape gooseberry pulp mixed with gum arabic (GA) and maltodextrin (MD) with the purpose of being used in spray drying. **Methods:** The optimization was performed using a central composite design with response surface of two factors (MD and

[1] Departamento de Ingeniería Agrícola y Alimentos, Universidad Nacional de Colombia. Medellín, Colombia.

[2] Facultad de Ingeniería, Universidad de Córdoba. Montería, Córdoba.

[*] Autor de correspondencia: mcortesro@unal.edu.co

GA) and response variables (density, ° Brix, water activity (a_w), pH, and rheological parameters). **Results:** The rheological characterization of the suspensions showed a shear thinning behavior with yield stress (Herschel-Bulkley Model). The results indicated that best suspension to be used in spray drying would be formulated with MD (24%) and GA (0%) showing a rheological response of Herschel-Bulkley fluid with consistency index (K=0.119±0.017 Pa-s^n), power index (n=0.75±0.03) and yield stress (τ_o=0,092±0,069 Pa). To the optimized condition, the results for the physicochemical properties were density (1.167 ± 0.005 g/mL), °Brix (35.5±0.5), a_w (0.974±0.002). Additionally, the pH and τ_o of Herschel Bulkley model was not statistically significant (p>0.05) in the suspension formulation. **Conclusions:** The experimental optimization is an important tool that allowed us to obtain a formulation of gooseberry with MD and GA suitable for spray drying, representing a saving in time and money for research and industry.

Keywords: *Physalis peruviana* L., gum arabic, maltodextrin, rheology, experimental optimization.

INTRODUCCIÓN

Physalis es un importante género de la familia *Solanaceae* contando con cerca de 120 especies que se distribuyen a través de las regiones tropicales y subtropicales del mundo, encontrándose principalmente en América, con un pequeño número de especies en Europa y en los países del suroriente y centro de Asia. *Physalis peruviana* L., denominada comúnmente en Colombia como uchuva (Cape gooseberry) es una de las especies más conocidas de este género y es ampliamente utilizada en la medicina tradicional como anticancerígeno, es cultivada en zonas alpinas y subparamos, entre 2300 y 3000 m.s.n.m y temperaturas entre 6 y 17ºC, con cacarterísticas fisicoquímicas como pH DE 3,5 A 3,7; °Brix entre 13 y 15 y acidez alrededor de 1,6 a 2,0% (1-7). La uchuva es unos de los frutos de mayor tasa de exportación desde Colombia con niveles de exportación alrededor de 28 millones de dólares y 5.200 toneladas (8), siendo Europa el destino principal, destacándose Alemania y Holanda con más del 60% de la demanda (9).

La técnica de secado por atomización es la más utilizada para obtener productos en polvo y micropartículas (10). Consiste en agregar el compuesto que se requiere recuperar en una solución de un polímero que lo vehiculice y forme una cavidad protectora. Para el secado de pulpas de frutas, el polímero se mezcla con la pulpa, formando una suspensión, que luego es bombeada a una cámara de atomización. El fluido es atomizado por un disco rotatorio o boquilla de presión y mediante la circulación del aire a temperaturas de entrada superiores a 100 ºC e inferiores a 250 °C, se produce la eliminación del agua y la formación de las micropartículas (10-11). Entre los materiales auxiliares de secado más frecuentemente usados para el secado por atomización se encuentran las gomas (arábiga, carragenina); proteínas (gelatina, caseinato); almidones modificados y maltodextrinas (12-16). La goma arábiga (GA) es un biopolímero obtenido del exudado del árbol acacia, de la familia *Leguminosae,* originaria de Egipto de las especies *senegal y seyal*, es uno de los materiales formadores de película más efectivo para microencapsular; sin embargo, el costo y su limitada disponibilidad comercial, han restringido su uso a nivel industrial (13-16). Por su parte, la maltodextrina (MD) es un polisacárido no edulcorante, con un aporte nutricional de 4 calorías por gramo, obtenido de la hidrólisis parcial, ácida y/o enzimática del almidón de papa, arroz o maíz obteniendo entre 2 y 20 equivalentes de dextrosa (DE). La aplicación de la MD en el proceso de encapsulación está asociada a la baja viscosidad, a altos contenidos de sólidos, a la buena solubilidad, a la capacidad de formar películas y a su bajo costo (17-19).

La reología representa una ciencia de mucho interés en el campo de los alimentos, ya que estudia la deformación y flujo de las materias primas sin procesar, de los productos intermedios o semielaborados y los productos finales de la industria alimentaria (20-21). Se ha encontrado que el aumento de las concentraciones de MD conduce a la producción de tamaños de partículas mayores, si se mantiene constante la presión de atomización o la velocidad de rotación del disco; esto puede estar relacionado con la viscosidad de la alimentación, la cual aumenta de manera exponencial con la concentración de MD. Algunos autores también encontraron que a mayor viscosidad disminuye el rendimiento de sólidos cuando la presión de atomización o las RPM del disco atomizador son constantes (22-24). Los modelos reológicos representan una relación matemática que permite caracterizar la naturaleza física

de un fluido, estudiando la viscosidad en función de la deformación y/o el tiempo. Para ello, se utilizan normalmente los modelos constitutivos donde se relacionan el esfuerzo cortante con la razón de deformación y/o tiempo (25-27).

Actualmente el nivel de industrialización de la uchuva está en ascenso concentrándose particularmente en el uso del producto fresco (fruta), mermeladas, gelatinas, bocadillos, aromáticas, producto liofilizados, pulpas concentradas o productos mínimamente procesados. Sin embargo, procesos industriales de aprovechamiento de la pulpa usando tecnología de secado por aspersión con el objetivo de desarrollar productos en polvo diferenciados no se han reportado en Colombia, productos que podrían generar expectativas a nivel industrial con características de comercialización nacional o internacional.

Así, el objetivo de este trabajo fue optimizar la formulación de una suspensión a base de pulpa de uchuva mezclada con GA (1,14 - 13,86%) y MD (9,51 - 26,49%), en función de parámetros reológicos y fisicoquímicos, y con fines de ser utilizada en secado por atomización.

MATERIALES Y MÉTODOS

Se utilizaron uchuvas frescas (UF) ecotipo Colombia provenientes de fincas productoras del municipio de la Unión, Departamento de Antioquia (2.500 m.sn.m y temperatura promedio de 13°C). Las uchuvas se seleccionaron libres de daños externos y con una madurez comercial en la escala de color 3 a 4, según la norma técnica colombiana, NTC 4580 (28). Frutos de uchuva fueron desintegrados inicialmente en una licuadora industrial a 3.500 rpm, luego esta pulpa desintegrada se despulpó mecánicamente a 3.500 rpm con un cedazo de 1000 μ (despulpadora, Comek S.A.); y finalmente, este producto se refinó manualmente en una malla de 500 μ para obtener la pulpa final, que se almacenó a 4°C. Se utilizó como aditivo MD (Shandong Boalingbao Biotechnology Co Ltd®) con DE entre 19 a 20, solubilidad 98%, pureza 96,4%. Adicionalmente, la GA (Caragun International – Additifs Alimentaires®) de solubilidad 98% y pureza 96%. La suspensión de alimentación al secador por aspersión (pulpa más aditivo) se homogenizó utilizando un Ultraturrax T25, Janke & Kunkel IKA – Labortechnik a 8000 rpm durante 2 minutos.

Análisis fisicoquímicos y reológicos.

Se determinó la actividad de agua (a_w) con un higrómetro de punto de rocío a 25 °C AquaLAB Decagon serie 3TE (AOAC 978.19B(c) 1998) (29), sólidos solubles o °Brix con un refractómetro Leica auto ABBE (AOAC 932.12, 1998) (29), pH con un potenciómetro Schott CG840B (AOAC 981.12, 1998) (29) y la densidad del líquido por el método del picnómetro a 20 °C (AOAC 945.06, 1998) (29). Se utilizó un reómetro Brookfield modelo DV-III Ultra y geometría cilindros concéntricos, con una separación de 0,9 mm, temperatura controlada de 20 °C, velocidad de deformación de 10^{-2} a 10^{2} s^{-1} y un tiempo total de ensayo de 1000 s. Las pruebas reológicas rotacionales de las cuales se obtuvieron las respectivas curvas de flujo y reogramas, se realizaron aplicando una rampa de descenso y ascenso, tomando está última curva para determinar el ajuste de los modelos reológicos independientes del tiempo. Los datos experimentales se ajustaron a los modelos de Bingham (ecuación 1), Casson (ecuación 2), Herschel-Bulkley (ecuación 3) y Ley de potencia (ecuación 4):

$$\tau = \tau_o + K\,\dot{\gamma} \qquad \textbf{Ecuación 1.}$$

$$\sqrt{\tau} = \sqrt{(\tau_o)} + \sqrt{K}\,\sqrt{(\dot{\gamma})} \qquad \textbf{Ecuación 2.}$$

$$\tau = \tau_o + K\,(\dot{\gamma}^n) \qquad \textbf{Ecuación 3.}$$

$$\tau = K\,\dot{\gamma}^n \qquad \textbf{Ecuación 4.}$$

La viscosidad aparente (μ_a) en Pa.s., está dada por (20):

$$\mu_a = \frac{\tau}{\dot{\gamma}} \qquad \textbf{Ecuación 5.}$$

En las expresiones anteriores τ es el esfuerzo cortante aplicado en Pa, γ es la tasa de deformación por cortante en s^{-1}, K, τ_o y n son constantes provenientes de la regresión para cada modelo respectivo.

Optimización de la suspensión

Para el proceso de optimización de la suspensión se aplicó un diseño experimental de superficie de respuesta central compuesto con dos factores (%MD y %GA) y variables de respuesta (densidad, °Brix, a_w, pH y parámetros reológicos), utilizando

el software Design Expert 6.0. Los parámetros reológicos para la optimización fueron seleccionados de acuerdo al mejor modelo según la bondad de ajuste en la regresión. La región de análisis se fijó para límites inferior y superior como sigue: MD (9,51 – 26,49%) y GA (1,14 -13,86%).

La tabla 1 muestra los 11 experimentos (3 puntos centrales y 8 no centrales) definidos por el diseño experimental, generando el siguiente modelo:

$$Y= \mu + \alpha*MD + \beta*GA + \gamma*MD*GA + \delta*MD^2 + \varepsilon*GA^2. \textbf{ Ecuación 6}$$

Tabla 1. Diseño experimental para las suspensiones de Uchuva.

Factores		
Nº	**MD (%)**	**GA (%)**
1	26,49	7,50
2	12,00	3,00
3	24,00	3,00
4	12,00	12,00
5	18,00	7,50
6	18,00	1,14
7	9,51	7,50
8	18,00	7,50
9	24,00	12,00
10	18,00	13,86
11	18,00	7,50

Las restricciones para el proceso de optimización fueron establecidas maximizando los sólidos solubles medidos en °Brix y la actividad de agua (a_w) y minimizando el pH, índice de consistencia (K) e índice de flujo (n) (22-23).

RESULTADOS

Caracterización reológica

La figura 1 presenta las curvas de fluidez de las 11 formulaciones del diseño experimental para las suspensiones de uchuva.

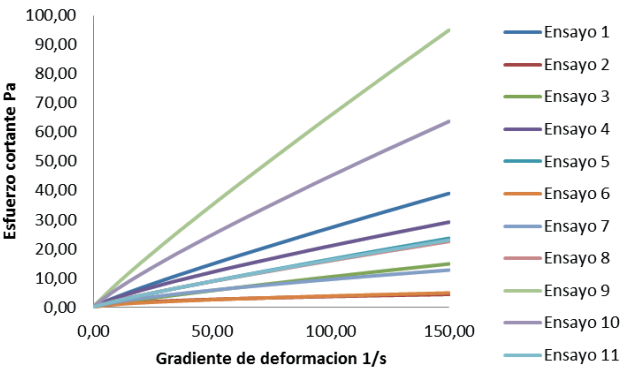


Figura 1. Curva de fluidez, esfuerzo cortante vs gradiente de deformación.

La tabla 2, presenta los resultados para los modelos reológicos que presentaron la mejor bondad de ajuste (Casson y Herschel-Bulkley) determinada por un alto coeficiente de determinación (R^2). De los modelos ajustados, fue el modelo de Bingham el que presentó la menor bondad de ajuste (R^2<0,7), seguido por la ley de potencial ($R^2 \approx$ 0,91). En general, se puede observar un mejor ajuste con el modelo de Herschel-Bulkley con relación al modelo de Casson, lo cual se puede explicar al hecho de que el modelo de Casson no presenta la habilidad de expresar la disminución de la viscosidad con el incremento tasa de corte (la viscosidad es constante para este tipo de modelo), comportamiento típico presentado en las suspensiones preparadas a partir de MD y GA

Tabla 2. Parámetros reológicos para los Modelos de Casson y Herschel-Bulkley.

Ensayo	Casson				Herschel Bulkley			
	K x10^{-1}	**n**	**τ_ox10^{-1}**	**R^2**	**Kx10^{-1}**	**n**	**τ_ox10^{-1}**	**R^2**
1	2,69±0,67	0,50	1,98±0,79	0,98	4,50±0,85	0,89±0,04	1,72±0,65	0,999
2	0,47±0,12	0,50	6,80±1,17	0,93	4,69±0,95	0,45±0,15	0,02±0,05	0,919
3	0,95±0,14	0,50	1,04±0,55	0,95	1,80±0,45	0,88±0,07	1,22±0,72	0,983
4	1,67±0,26	0,50	4,07±1,47	0,96	5,03±0,76	0,81±0,03	0,98±0,18	0,999
5	1,73±0,81	0,50	1,30±0,26	0,93	2,58±0,88	0,90±0,08	2,28±1,91	0,956
6	0,40±0,04	0,50	2,90±0,83	0,95	2,83±0,98	0,57±0,09	0,04±0,02	0,906
7	0,71±0,07	0,50	4,20±0,99	0,95	3,64±0,43	0,71±0,02	0,20±0,16	0,959
8	1,37±0,83	0,50	1,94±0,59	0,96	3,02±0,34	0,86±0,04	1,46±0,77	0,998
9	5,87±1,16	0,50	2,67±0,49	0,97	9,94±0,94	0,91±0,02	0,63±0,24	0,998
10	3,76±1,00	0,50	4,48±0,51	0,97	8,56±0,32	0,86±0,01	0,75±0,14	0,999
11	1,44±1,01	0,50	1,56±0,79	0,94	2,94±0,15	0,87±0,02	0,88±0,25	0,951

La tabla 3, presenta los efectos asociados a los factores de estudio con sus interacciones, para las variables respuesta índice de consistencia (K) e índice de flujo (n). El ANOVA usando un modelo de regresión de segundo orden fue significativo ($p<0,05$) mostrando que los factores %MD y %GA con sus interacciones son influyentes en el rango de formulación, mientras la variable respuesta esfuerzo de cedencia (τ_o) no fue significativa ($p>0,05$) en el rango de estudio.

Tabla 3. Coeficientes del modelo estadístico para los parámetros reológicos de Hershel-Bulkley.

Efecto	Índice de consistencia ($Pa.s^n$)	Índice de flujo (n)
μ	0,28	0,88
α	0,040	0,098
β	0,21	0,10
γ	0,20	-0,083
Δ	0,073	-0,037
ε	0,15	-0,080
Modelo ($p<0,05$)	0,0001	0,0022
Falta de ajuste ($p<0,05$)	0,2343	0,1218
R^2	98,97	95,49

La figura 2, muestra la superficie de respuesta para los parámetros reológicos K y n del modelo de Herschel-Bulkley, en función del % MD y %GA utilizados en la suspensión.

Caracterización fisicoquímica.

Las tablas 4 y 5, indican los parámetros fisicoquímicos de densidad de suspensión, °Brix, a_w y pH, y los efectos asociados a los factores de estudio con sus interacciones, respectivamente. El ANOVA, mostró efectos significativos ($p>0,05$) con los factores de %MD y %GA excepto para el pH ($p<0,05$), siendo su valor promedio 3,36 ±0,03. Estos resultados son similares a los reportados por Dak *et al.* (34-35).

Tabla 4. Caracterización fisicoquímica de las suspensiones de uchuva.

Factores			Parámetros fisicoquímicos			
Nº	MD (%)	GA (%)	Densidad (g/mL)	°Brix	a_w	pH
1	26,49	7,50	1,195±0,002	42,20±0,02	0,969±0,002	3,37±0, 01
2	12,00	3,00	1,125±0,002	27,40±0,04	0,978±0,003	3,35±0, 01
3	24,00	3,00	1,170±0,001	37,00±0,02	0,973±0,002	3,38±0, 02
4	12,00	12,00	1,152±0,002	34,00±0,09	0,974±0,004	3,36±0, 01
5	18,00	7,50	1,160±0,002	35,00±0,07	0,973±0,001	3,38±0, 02
6	18,00	1,14	1,134±0,001	30,00±0,02	0,977±0,002	3,32±0, 01
7	9,51	7,50	1,123±0,001	29,00±0,01	0,978±0,003	3,33±0, 03
8	18,00	7,50	1,155±0,001	34,40±0,02	0,974±0,002	3,34±0, 01
9	24,00	12,00	1,187±0,003	42,00±0,05	0,970±0,004	3,41±0, 02
10	18,00	13,86	1,171±0,005	39,60±0,03	0,973±0,002	3,41±0, 01
11	18,00	7,50	1,156±0,002	35,00±0,02	0,975±0,001	3,33±0, 04

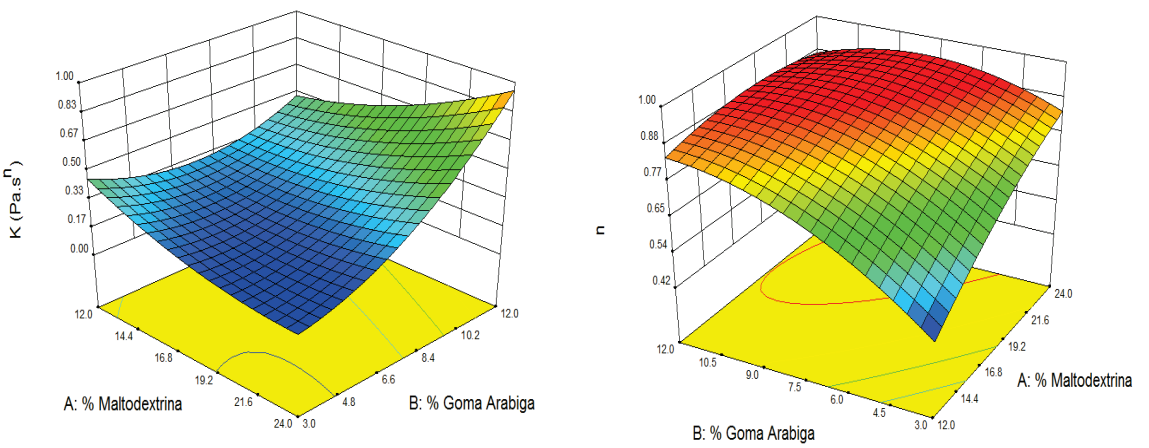


Figura 2. Superficie de respuesta de los parámetros reológicos de Herschel-Bulkley: índice de consistencia (2a); índice de flujo (2b).

Tabla 5. Análisis de varianza para las variables fisicoquímicas de las suspensiones de uchuva.

Efecto	Densidad (g/mL)	°Brix	a_w
μ	1,16	34,80	0,970
α	0,023	4,53	-0,0027
β	0,012	3,15	-0,0016
γ	$-0,0025^{NS}$	$-0,40^{NS}$	$0,00025^{NS}$
Δ	$0,0016^{NS}$	$0,38^{NS}$	$-0,00037^{NS}$
ε	$-0,0015^{NS}$	$-0,025^{NS}$	$0,00037^{NS}$
Modelo ($p<0,05$)	0,0002	<0,0001	0,0031
Falta de ajuste ($p<0,05$)	0,2078	0,3756	0,5901
R^2	98,22	99,64	94,81

NS: no significativo

La figura 3, presenta las superficies de respuesta de los parámetros fisicoquímicos (densidad, °Brix y a_w) en función de los factores de estudio (%MD y %GA).

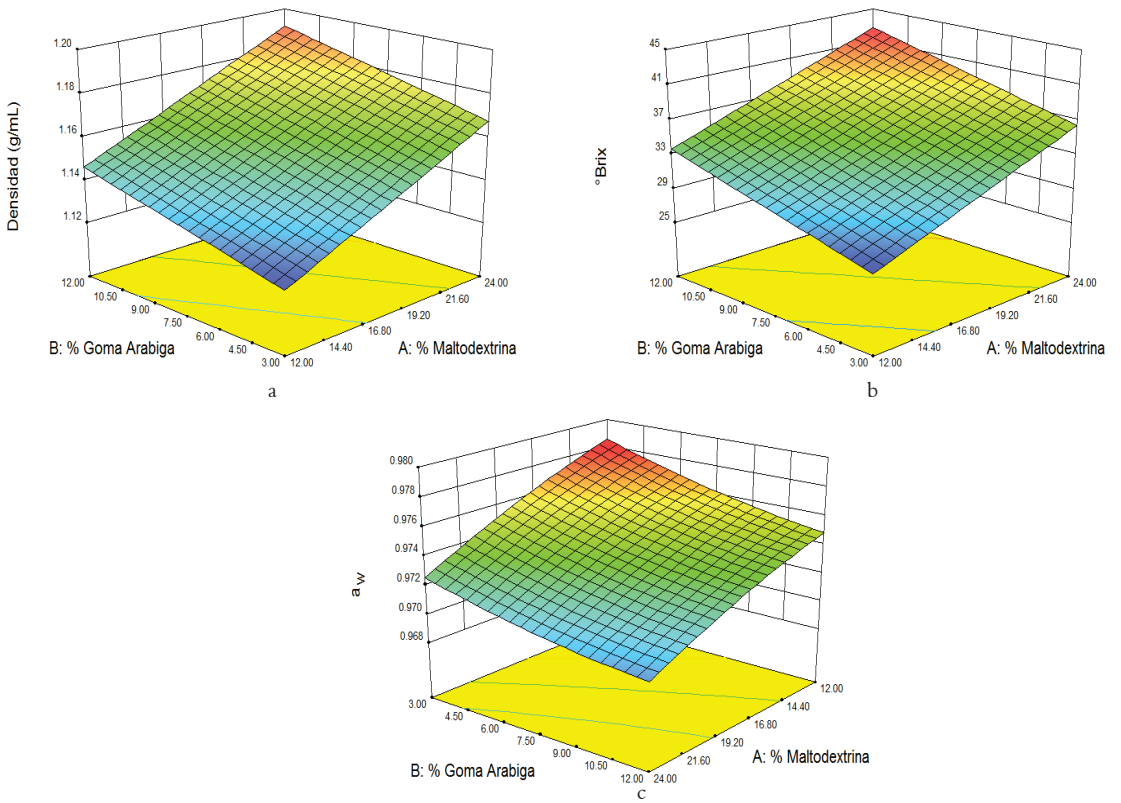


Figura 3. Superficie de respuesta de los parámetros fisicoquímicos para suspensiones de uchuva: a) densidad; b) °Brix y c) a_w.

Optimización de la suspensión

Para la optimización del proceso, las variables (pH=3,36±0,03) y esfuerzo de cedencia (τ_o=0,092±0,069 Pa) al no presentar efecto estadístico ($p>0,05$), no se tuvieron en cuenta en las restricciones del proceso de optimización. Adicionalmente, el modelo seleccionado para la optimización fue el Herschel-Bulkley, ya que los parámetros reológicos presentaron la mejor bondad de ajuste.

Los resultados de la optimización se indican en la tabla 6, en la cual la optimización I es dada teniendo presente la formulación basada con MD y GA. Debido a que el % GA es en proporción mucho menor al % MD en la formulación y que esta supera en costos a la MD en un relación de 5 a 6 veces su precio, se decidió generar una segunda optimización de la suspensión considerando la ausencia de la GA (Optimización II).

Tabla 6. Resultados del proceso de optimización para suspensiones de uchuva.

Optimización	MD (%)	GA (%)	Variables de respuesta optimizadas				
			Densidad (g/mL)	oBrix	aw	K (Pa.sn)	n
I	22,17	3,0	1,160±0,007	35,2±0,7	0,974±0,003	0,143±0,012	0,83±0,08
II	24,00	0	1,167±0,005	35,5±0,5	0,974±0,002	0,119±0,017	0,75±0,03

DISCUSIÓN

Caracterización reológica

Los gráficos de la figura 1 presentan una tendencia no lineal a medida que crece el gradiente de deformación, lo cual identifica un comportamiento no newtoniano, el cual se define como aquellas sustancias cuyo índice de flujo (n) es menor o mayor a 1 (21).

La tabla 2, muestra que los modelos de Casson y Herschel-Bulkley, describen adecuadamente el comportamiento de un fluido no newtoniano, tipo adelgazante con esfuerzo de cedencia ($n<1$ y $\tau_o>0$). Comportamiento reológico para concentraciones acuosas entre 10-30 %GA han sido similares a los encontrados en este trabajo (30) con lo que se refuerza el efecto del nivel de concentración de los sistemas de alto peso molecular en la respuesta reológica para las suspensiones acuosas. Para la mayoría de las matrices alimentarias y productos de naturaleza biológica, los modelos de Casson y Herschel-Bulkley generalmente presentan un mejor ajuste a los datos experimentales con respecto a los modelos de ley de potencia y Bingham (31). Estos modelos han sido utilizado para describir el comportamiento reológico de diversos productos como pulpa de mango (32-35), guayaba (36), cereza de las indias (37) jugo de zanahoria pasteurizado (38) y miel de abeja (39).

Para el modelo de Herschel-Bulkley, el índice de consistencia (K) presentó valores en el intervalo de 0,180 a 0,994 $Pa.s^n$, los cuales son menores con respecto a otras pulpas como la de mango a 16,6°Brix de 3,41 a 4,57 $Pa.s^n$, en el intervalo de 10 a 60 °C (32) y en la pulpa de papaya de 12 a 51°Brix de 0,7 a 15,3 $Pa.s^n$, en el intervalo de 25 a 55 °C (40). Adicionalmente, los valores del índice de comportamiento de flujo (n) para las pulpas de mago y papaya (0,154 y 0,240 respectivamente), fueron menores a los obtenidos para la pulpa de uchuva con MD y GA, donde los aditivos no generaron efectos significativos en la matriz alimenticias evaluada (32-35). Estas diferencias son debidas a la diferente naturaleza del producto y características fisicoquímicas y estructurales de la pulpa y los aditivos estudiados (37-41). Desde el punto de vista industrial, la disminución en los índices de consistencia, índice de flujo y la baja magnitud del esfuerzo de cedencia facilitaría el transporte por bombeo, el intercambio de calor y de masa durante el procesamiento por secado por aspersión disminuyendo también las pérdidas de cabeza de presión durante el transporte de flujo, resultando en poca demanda de energía para el proceso (27).

Los modelos descritos en la tabla 3, pueden ser usados de forma predictiva (falta de ajuste no significativa) para estimar los parámetros reológicos del modelo Herschel-Bulkley en función del %MD y %GA. Para la variable respuesta índice de consistencia (K), los efectos asociados a los factores de estudio con sus interacciones son positivos, lo que muestra que un incremento en los valores de formulación dará como resultado un mayor valor de K. En la variable índice de flujo (n), el efecto individual de cada factor es positivo, pero la combinación de los factores tiene un efecto negativo en el valor esperado de la variable respuesta.

La figura 2a, muestra que altos %MD con bajos %GA generan bajos valores de K, mientras que altos %MD con altos %GA generan los mayores valores para el índice de consistencia (K). Además, se observa un efecto positivo de cómo incrementa el valor de K cuando él %MD es mayor y se aumenta progresivamente con él %GA, lo que refuerza el efecto que la adición de sistemas de alto peso molecular contribuyen a los efectos espesantes. Se observa el efecto espesante con el incremento de la concentración de goma arábiga, situación similar ha sido encontrada en otros productos tales como suspensiones de mango y piña (25). Adicionalmente, la figura 2b, muestra la relación entre los aditivos usados y el índice de flujo (n) obtenido, indicando que altos %MD y %GA incrementan su valor. Por otro lado, menores valores de n se obtienen para bajos %MD y %GA y con ello menor efecto viscoso.

En las superficies de repuestas de la figura 2, se nota la alta correlación entre los aditivos, principalmente en los parámetros reológicos mostrando puntos máximos y mínimos, indicando que en estos puntos se obtiene la máxima interacción de la pulpa de uchuva con la MD y GA (33-42). Esta interacción pulpa-aditivo sobre los parámetros reológicos es explicada por sus estructuras, donde los aditivos empleados presentan amplia cantidad de puentes de hidrógenos, y diferencias conformacionales, donde la MD posee una conformación de cadena lineal a diferencia de la GA, que es ramificada (32-42).

Caracterización fisicoquímica.

Los resultados de las tablas 4 y 5, indicaron una relación lineal entre las variables de respuesta con respecto a los %MD y %GA. Adicionalmente, los altos coeficientes de regresión obtenidos (R^2>0,94) y falta de ajuste no significativa (p>0,05), indican que los modelos obtenidos son de naturaleza predictiva en el rango de los % de aditivos estudiados.

Los valores de densidad y °Brix aumentaron con los %MD y %GA y como consecuencia también aumentaron el índice de consistencia, aunque la a_w mostró tendencia a disminuir con el porcentaje de los aditivos. Este comportamiento ha sido reportado en diversos productos como pulpa de mango (32-35), guayaba (36), cereza de las indias (37) jugo de zanahoria pasteurizado (38) y miel de abeja (39). La pulpa uchuva mezclada con MD y GA obtenida presentó mayor contenido de solidos solubles a las obtenidas en mango por Vidal *et al*. (32) (°Brix = 16,6, densidad = 1,112 g/mL y a_w = 0,981); y que las obtenidas en la pulpa de papaya por Telis *et al*. (40) (°Brix = 12, densidad = 1,232 g/mL en el intervalo de 25°C a 55°C y a_w = 0,973).

En la figura 3, se observan que los cambios son más apreciables en los °Brix, debido al aporte de sólidos solubles de la MD como un polisacárido moderadamente dulce; mientras que la densidad incrementa desde 1,123 a 1,195 g/mL debido al peso molecular de ambos aditivos (19). Pedroza *et al.* (16), evidenciaron el efecto significativo de la MD y la GA sobre la densidad, aunque sus interacciones no fueron significativas, situación evidenciada en esta investigación. Los °Brix para las suspensiones estudiadas incrementaron de 27,4% a 42,2%, donde el %MD tiene un efecto mayor que él %GA. Sin embargo, la interacción de estos componentes no fue significativa, lo que es atribuible a que los °Brix son el mismo % de sólidos solubles, por lo que no hay más componentes sólidos en la mezcla que refracten la luz en ángulo diferente a un azúcar. Este aumento de los °Brix se considera benéfico, ya que incrementa el tamaño de partícula (37-41). La a_w presenta una tendencia a disminuir con respecto a los %MD y %GA, siendo estos cambios observados mínimos (0,969 a 0,978), aunque el modelo consideró la incidencia del %MD y %GA como un efecto significativo. Las interacciones para los aditivos no presentaron efectos significativos; aun así, ambos hidrocoloides confirieron un ligero efecto depresor de la a_w en la suspensión con respecto a pulpa de uchuva (0,969 ± 0,005 a 0,985 ± 0,004, respectivamente).

El pH no presentó diferencias significativas con respecto a los aditivos empleados, siendo su variación en el rango de 3,32 a 3,41. No obstante, se observa una mayor influencia por efecto del incremento del %GA, ya que en solución su pH varía entre 4 y 6 (42), y que se hace sinérgico con el incremento del %MD.

Optimización de la suspensión.

Un análisis entre las dos optimizaciones de la tabla 6, no muestra diferencias apreciables entre las variables evaluadas. En la optimización II los valores de K y n son menores con relación a la Optimización I, lo que favorece un menor consumo de energía de bombeo y atomización, un incremento del rendimiento en la recuperación de sólidos de la pulpa mediante un procedimiento de secado por atomización, y una disminución de los costos, lo cual es respaldado por Lopera *et al*. (42), Tonon *et al.* (22) y Jinapong *et al*. (23).

Las restricciones consideradas se consideran importantes, debido a que a mayor densidad y °Brix, se obtiene un mayor tamaño de partícula en el polvo y el proceso de secado se realiza a menores costos; a menor a_w se da mayor interacción entre el aditivo y el agua por lo que se requiere mayor energía para evaporar el agua, en el secado por aspersión. Por otro lado, si la suspensión tienen un menor índice de flujo (n<<1), requerirá menor potencia de bombeo y de atomización, y al ser relacionado con un índice de consistencia menor, se facilita una mejor transmisión de calor desde el aire hasta la gota atomizada, generando un secado rápido, obteniéndose un producto en polvo homogéneo y estable (22-24) (44).

CONCLUSIONES

Las suspensiones evaluadas presentaron un comportamiento reológico de fluidos no newtoniano y adelgazante con el esfuerzo, siendo los modelos reológicos de Hersey Bukley y Casson los que presentaron el mejor ajuste de regresión. El conocimiento de los factores reológicos y fisicoquímicos permitió obtener la combinación óptima de las materias primas que admitan aumentar al máximo los sólidos, con una viscosidad apta para alimentar al secador por aspersión. Este diseño logra, con pocas réplicas, optimizar las variables del proceso, lo que significa ahorro en tiempo y dinero para el investigador y la industria. La combinación de MD y la pulpa de uchuva (°Brix = 35,5%±0,5), sin GA y con parámetros reológicos de acuerdo al modelo de Herschel-Bulkley (K = 0,119±0,017 y n = 0,75±0,03), permitieron obtener una mínima viscosidad aparente logrando el equilibrio entre las variables básicas que permiten el bombeo de la suspensión y favoreciendo el rendimiento del producto en un proceso posterior de secado por aspersión.

Conflicto de intereses.

Los autores manifiestan que no se tiene ningún interés comercial o asociativo que represente un conflicto de intereses en relación con el trabajo presentado.

Limitaciones del presente estudio.

A pesar de que el esfuerzo de cedencia se obtuvo por ajuste matemático (modelo de regresión), debió de realizarse una validación del valor encontrado usando el método de aspas y/o dinámica oscilatoria, técnicas instrumentales que no se tenían disponibles. Por lo tanto, se sugiere en fututos estudios que impliquen un análisis reológico con esfuerzos de cedencia su validación experimental. Adicionalmente, en este estudio se evaluó la caracterización reológica y fisicoquímica a temperatura constante. Sin embargo, sería importante evaluar el efecto de la temperatura para las variables físicoquímicas y reológicas, ya que a nivel industrial para algunas aplicaciones se debe de calentar la suspensión para ser alimentada al secador por atomización.

REFERENCIAS

1. Wu SJ, Tsai JY, Chang SP, Lin DL, Wang SS, Huang SN, Nq LT. Supercritical carbon dioxide extract exhibits enhance antioxidant and anti-inflammatory activities of Physalis peruviana. J. Ethnopharmacol. 2006; 108(3): 407-413.
2. Bastos GN, Santos AR, Ferrerira VM, Costa AM, Bispo CI, Silveira AJ, et al. Antinociceptive effect of the aqueous extract obtained from roots of Physalis angulata L. on mice. J. Ethnopharmacol. 2006; 103(2): 241-245.
3. Wu SJ, Ng LT, Huang YM, Lin DL, Wang SS, Huang SN, *et al*. Antioxidant activities of *Physalis peruviana*. J. Biol Pharm Bull. 2005; 28(6):963-936.
4. Pérez-Castorena AL, García M, Martínez M, Maldonado E. Physalins from *Physalis solanaceus*. J. Biochem Syst Ecol. 2004; 32(12):1231-1234.
5. Wu SJ, Ng LT, Lin DL, Huang SN, Wang SS, Lin CC. *Physalis peruviana* extract induces in human Hep G2 cells through CD95/CD95L system and the mitochondrial signaling transduction pathway. J. Cancer Lett. 2004; 215(2):199-208.
6. Mayorga H, Duque C, Knapp H, Winterhalter P. Hydroxyester disaccharides from fruits of cape gooseberry (*Physalis peruviana*). J. Phytochemistry. 2002; 59(4): 439-445.
7. Ahmad S, Malik A, Yasmin R, Ullah N, Gul W, Khan PM. *et al*. Whitanolides from *Physalis Peruvian,* J. Phytochemistry. 1999; 50(4):647-651.
8. Ministerio de agricultura y desarrollo rural de Colombia y CCI. Sistema de inteligencia de mercados: información de monitoreo internacional [Internet]. 2006. [consulta: febrero de 2008.]. No. 13. Bogotá. 12. Disponible en: http://www.agronet.gov.co/www/docs_agronet/2006427154348_uchuvamarzo.pdf;
9. CCI - Corporación Colombia Internacional. Perfil del producto (uchuva). Boletín 10, 2002.
10. Barbosa M, Borsarelli C, Mercadante A. Light stability of spray-dried bixin encapsulated with different edible polysaccharide preparations. J. Food Res Int. 2005; 38(8-9): 989-994.
11. Vila, Jato JL. Tecnología Farmacéutica. Madrid, 1997.
12. Kanakdande D, Bhosale R, Singhal RS. Stability of cumin oleoresin microencapsulated in different combination of gum arabic, maltodextrina and modified starch. J. Carbohyd Polym., 2007; 67(4): 536-541.
13. Yadav MP, Igartuburu JM, Yan Y, Nothnagel NA. Chemical investigation of the structural basis of the emulsifying activity of gum arabic. J. Food Hydrocolloid. 2007; 21(2):297-298.
14. Ramakrishnan A, Pandit N., Badgujar M., Bhaskar C., Rao M., Encapsulation of endoglucanase using a biopolymer gum Arabic for its controlled release. J. Bioresource Technol. 2007; 98(2): 368-372.
15. Phillips G, Williams P. Studies on acacia exudates gums. J. Food Hydrocolloid. 2005; 19(4):661-667.
16. Pedroza-Islas R, Vernon-Carter EJ, Durán C, Trejo-Martı□nez S. Using biopolymer blends for shrimp feedstuff microencapsulation I. Microencapsule particle size, morphology and microstructure. J. Food Res Int. 1999; 32(5): 367-374.
17. Madene A, Jacquot M, Scher J, Desobry S. Flavour encapsulation and controlled release a review. J. Int Food Sci Tech. 2006; 41 (1); 1-21.
18. Avaltroni F, Bouquerand PE., Normand V. Maltodextrin molecular weight distribution influence on the glass transition temperature and viscosity in aqueous solutions, J. Carbohyd Polym. 2004; 58(3):323-324.
19. Pedroza-Islas R, Alvarez- Ramirez J, Vernon-Carter EJ. Using biopolymer blends for shrimp feedstuff microencapsulation. II: dissolution and floatability kinetics as selection criteria. J. Food Res Int. 2000; 33(2):119-124.
20. Briggs J y Steffe J. Using Brookfield data and the Mitschka method to evaluate power law foods. J. Texture Studies. 1997; 28(5): 517-522.
21. Barbosa-Cánovas GV, Kokini JL, Mal Ibarz A. The rheology of semiliquid foods. J. Food Nutr Res. 1996; 39:1–69.
22. Tonon R, Brabet C, Hubinger M. Influence of process conditions on the physicochemical properties of acai (Euterpe oleraceae Mart.) powder produced by spray drying. J. of Food Engineering. 2008 Oct; 88(3):411–418.

23. Jinapong N, Suphantharika M., Jamnong P. Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. J. of Food Engineering. 2008; 84(2): 194–205.
24. Keogh MK, Murray CA., O'kennedy BT. Effects of ultrafiltration of whole milk on some properties of spray-dried milk powders. J. International Dairy Journal. 2003; 13(12):995–1002.
25. Pelegrine D, Vidal J, Gasparetto C. Study of aparent viscosity of mango (Keitt) and pineapple (Pérola) pulps. J. Ciência e Tecnología de Alimentos. 2000; 20(1):128-131.
26. Sánchez C, Oria R, Sánchez G. Efecto de la temperatura en las propiedades reológicas de purés de guayaba (Spidium guajava L). [PhD Thesis]. España: Universidad de Zaragoza, 2006.
27. Haminiuk C, Sierakowski M, Izidoro D y Masson M. Rheological Characterization of Blackberry Pulp. Braz. J. Food Technol. 2006; 9(4): 291-296.
28. ICONTEC. Instituto Colombiano de Normas Técnicas y Certificación. Frutas frescas: Uchuva. Especificaciones. Norma Técnica Colombiana NTC 4580. 15, 1999.
29. AOAC. Official methods of analysis. Washington D.C: Association of Analytical Chemists; 1998.
30. Tanaka S, Fang Y, Nishinari K. Anomalous Rheological Behaviour of Gum Arabic Solutions. J. Foods and Food Ingredients. 2006; 211 (3):216-221.
31. Holdsworth SD. Rheological models used for the prediction of the flow properties of foods products: a literatura review. J. Transactions of the Institution of Chemical Engineers, Part C. 1993; 71: 139-179.
32. Vidal J, Pelegrine D, Gasparetto C. Effect of the rheological behavior of mango pulp (Magífera indica L-Keitt). J. Ciência e Tecnologia de Alimentos. 2004; 24(1):39-42.
33. Branco IG, Gasparetto CA. Response surface methodology applied to the study of temperature effect on the rheological behavior of ternaries mixtures with mango pulp and orange and carrot juices. Ciênc. Tecnol. Aliment. 2003; supl: 166–171.
34. Dak M., Verma RC., Sharma GP. Flow characteristics of juice of "Totapuri" mangoes. J. Food Eng. 2006; 76(4): 557-561.
35. Dak M, Verma RC, Jaaffrey SNA. Effect of temperature and concentration on rheological properties of "Kesar" mango juice. J. Food Eng. 2007; 80(4): 1011-1015.
36. Sánchez C, Oria R, Sánchez-Gimeno AC. Efecto de la temperatura en las propiedades reológicas de purés de guayaba (*Psidium Guajaba* L.). Simposio Poscosecha. Orihuela, Valencia, España. 2006.
37. Da Silva F, Guimaraes D, Gasparetto. C. Rheology of acerola juice: effects of concentration and temperature. Ciênc. Tecnol. Aliment. 2005; 25(1): 121- 126.
38. Vandresen S, Quadri M, De Souza J, Hotza D. Temperature effect on the rheological behavior of carrot juices. J. Food Eng. 2009 Jun; 92 (3): 269-274.
39. Pereira E, Queiroz A, Feitosa R. Comportamento reológico de mel da abelha urucu (*Melípona scutellaris*, L.). Rev. ciênc. exatas nat. 2003; 5(2): 179-186.
40. Telis J, Guizzo L, Telis V. Rheological Properties of Rehydrated Papaya. Braz. J. Food Technol. 2003; 6 (2): 221-227.
41. Ferreira G., Melo A., Silvestre R., Gasparetto C., Efeito da temperatura no comportamento reológico das polpas de caju e goiaba. J. Ciencias Exatas e Naturais. 2002; 4 (2): 175-184.
42. Lopera S, Guzmán C, Cataño C, Gallardo C. Desarrollo y caracterización de micropartículas de ácido fólico formadas por secado por atomización, utilizando goma arábiga y maltodextrina como materiales de pared. J. Vitae. 2009; 16(1): 55-65.
43. Luh BS, Tropical fruit beverage. Fruit & vegetable juices processing technology. 3ª ed. (Eds. p.e. nelson and d.k. tressler). Connecticut: AVI, 344 – 435, 1980.
44. Toneli J, Park KJ, Murr F, Negreiros A. Spray drying optimization to obtain inulin powder. In: Proceedings of the 15th International Drying Symposium (IDS 2006). Budapest, Hungary.

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 140-147
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a08

PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHANGES IN COMMERCIAL TILAPIA (*Oreochromis niloticus*) DURING COLD STORAGE

CAMBIOS FISICO-QUÍMICOS Y MICROBIOLÓGICOS EN TILAPIA COMERCIAL (*Oreochromis niloticus*) DURANTE ALMACENAMIENTO REFRIGERADO

Nelson GUTIÉRREZ GUZMÁN PhD.[1*], Isabel FERNÁNDEZ SEGOVIA PhD.[2], Ana FUENTES LOPEZ PhD.[2], María RUIZ RICO MsC.[2], José Manuel BARAT BAVIERA PhD.[2]

Recibido: Julio 24 de 2014 Aceptado: Septiembre 21 de 2015

ABSTRACT

Background: The microbiological and chemical processes are the main responsible for deterioration of fresh fish. Therefore, it is essential to avoid these processes by applying good manufacturing practices during fish handling, distribution and storage. **Objective:** The aim of this paper was to evaluate the physico-chemical and microbial changes in commercial tilapia (*Oreochromis niloticus*) during cold storage in order to establish the shelf life since its arrival at the supermarket. **Methods:** 27 aquacultured tilapia specimens were analyzed at 0, 2, 4, and 7 days of storage at 4ºC. Measurements of texture, color, water holding capacity, total volatile basic nitrogen (TVB-N), thiobarbituric acid index, ATP-related compounds, as well as microbial analyses were carried out. **Results:** The TVB-N content was high at the beginning of the study, remaining stable during the storage. Lipid oxidation of samples was minimum, so this process did not contribute to the fish spoilage. It was observed the breakdown of inosine-5'- monophosphate (IMP) into Ino (inosine), and Ino into Hx (hypoxantine). The texture parameters and colour underwent changes as a consequence of the fish spoilage. Low microbial counts were observed at day 0, but *Enterobacteriaceae* and mesophilic counts gradually increased throughout storage. **Conclusions:** The K_1-value showed the progressive spoilage of the fish during the cold storage. The decrease of hardness and firmness confirmed the loss of quality throughout the time of study. The low microbial counts at the beginning of the study demonstrated the good quality of the tilapia; however, the increase of the mesophilic counts at the end of the studied period showed that tilapia was not fit for human consumption at day 7.

Keywords: Tilapia, quality, shelf-life, cold storage.

RESUMEN

Antecedentes: Los procesos microbiológicos y químicos son los principales responsables del deterioro del pescado fresco. Por tanto, es esencial evitar estos procesos aplicando buenas prácticas de fabricación durante la manipulación, distribución y almacenamiento del pescado. **Objetivo:** El objetivo de este trabajo fue evaluar los cambios físico-químicos y microbiológicos en tilapia comercial (*Oreochromis niloticus*) du-

[1] Facultad de Ingeniería, Universidad Surcolombiana, Neiva, Colombia.
[2] Departamento de Tecnología de Alimentos, Universidad Politécnica de Valencia, Valencia, España.
[*] Autor de correspondencia: ngutierrezg@usco.edu.co

rante el almacenamiento refrigerado con el fin de establecer la vida útil desde su llegada al supermercado. **Métodos:** 27 especímenes de tilapia fueron analizados a día 0, 2, 4 y 7 de almacenamiento a 4 ºC. Se llevaron a cabo medidas de textura, color, capacidad de retención de agua, nitrógeno básico volátil total (N-BVT), índice del ácido tiobarbitúrico, compuestos relacionados con el ATP, así como análisis microbianos. **Resultados:** El contenido en N-BVT fue alto al principio del estudio, siendo estable durante el almacenamiento. La oxidación lipídica de las muestras fue mínima, por lo que este proceso no contribuyó al deterioro del pescado. Se observó la degradación de inosina-5'- monofosfato (IMP) a Ino (inosina), y de Ino a Hx (hipoxantina). Los parámetros de textura y color sufrieron cambios como consecuencia del deterioro del pescado. Se observaron bajos recuentos microbianos a día 0, pero los recuentos de *Enterobacteriaceae* y de mesófilos aumentaron gradualmente durante el almacenamiento. **Conclusiones:** El valor K_1 mostró el deterioro progresivo del pescado durante el almacenamiento en refrigeración. La disminución de dureza y firmeza confirmó la pérdida de calidad a lo largo del tiempo de estudio. Los bajos recuentos microbianos al principio del estudio demostraron la buena calidad de la tilapia; sin embargo, el aumento de los recuentos de mesófilos al final del periodo estudiado mostraron que la tilapia no era adecuada para el consumo humado a día 7.

Palabras clave: Tilapia, calidad, vida útil, almacenamiento refrigerado.

INTRODUCTION

Aquaculture is expanding in all the continents as far as new areas and species are concerned, and is also intensifying and diversifying the product range in species and product forms to meet consumer requirements. Freshwater fish production has increased dramatically in the last two decades, and has been driven chiefly by the rapid development of tilapia and other species. Tilapia is the third most cultured fish, after carps and salmonids, and the global production of this fish rose to 3.5 million of tons in 2010 (1). Tilapia offers adequate biologic and ecologic features, such as adaptability, fast growth, disease resistance and efficient feed conversion capacity (2), and is one of the fish species with the highest production and distribution worldwide. Aquaculture tilapia production is located mainly in Asia, with 72% of world production, followed by Africa (19%) and America (9%) (1). It is believed that imports of tilapia to Europe will increase significantly in the near future because, owing to environmental concerns, more consumers are looking for suitable alternatives to white fish fillets. The main markets in the European Union (EU) are big cities where large communities of African, Chinese and Asian citizens live. However, tilapia consumption in non-ethnic markets has also increased recently. China is the leading supplier of European countries, and Poland, Spain and Germany are the largest markets in the EU (3).

Freshness is the most important attribute when evaluating fish quality. When fish die, several postmortem changes take place. They are related to the breakdown of the cellular structure and biochemistry, and also to the growth of microorganisms that are either naturally associated with fish or form part of the flora due to contamination during handling (4). These modifications, which affect fish quality and shelf life, are changes in pH, texture, water holding capacity and color, protein and ATP degradation, lipid oxidation, as well as production of undesirable compounds such as molecular low weight volatile bases (TVB-N), which are produced by bacterial or enzymatic action.

The quality of fish products found in supermarkets is highly related to handling, transportation and distribution conditions. Scientific shelf life studies have been carried out to determine changes during chilled storage (5, 6, 7). Some of these studies start at the time of slaughter or capture; however, the fish that usually arrive at retailers do not always present the same freshness. Nowadays, consumers are far away from fish production areas. Although distribution channels have improved in the last few decades, the long distance between producers and consumers means that fish arrive at the supermarkets with different grades of freshness.

The aim of this work was to evaluate the physico-chemical and microbial changes in commercial tilapia (*Oreochromis niloticus*) during cold storage in order to establish the shelf life from its arrival at supermarket.

MATERIALS AND METHODS

Materials

In this work, 27 aquacultured tilapia (*Oreocrhomis niloticus*) specimens were used. Fish were purchased from a local supermarket in Valencia (Spain) and were transported to the laboratory in polystyrene boxes with ice. There was no information available about the background of the fish samples (feed composition, handling or transport conditions) or slaughter dates. Fish were wrapped in plastic film to minimize dehydration and any contact with oxygen during cold storage. Samples were analyzed at 0, 2, 4, and 7 days of storage at 4ºC.

Before the analyses, fish were headed, gutted and filleted, and two fillets per fish were obtained. Samples were taken from the dorsal muscle of the two fillets, except for the compression test, which was the analysis performed on the whole fish.

Analytical Methods

Proximate composition: Moisture, lipid, protein and ash contents were assayed by AOAC methods 950.46, 991.36, 928.08, and 920.153, respectively (8).

Physico-chemical parameters

Measurements of pH were carried out according the method described by Rizo *et al.* (2015) (9). Water holding capacity (WHC) was determined according to the technique described by García *et al.* (2006) (10). For this determination, a portion of 0.3 g of sample was placed between two dry filter papers and two acrylic plates on which a 10 kg pressure was applied for 15 min. The sample was weighed before and after being compressed, as well as the dry and wet filter papers. WHC was expressed as g of water held per 100 g of total water in the fish sample. The total volatile basic nitrogen (TVB-N) content was determined by steam distillation following the method described by Malle and Tao (1987) (11), and was expressed as mg N/100 g of muscle.

The TBA index was determined by a spectrophotometric technique according to the method described by Vyncke (1970) (12) and the results were expressed as mg malonaldehyde (MDA)/kg of fish muscle.

The ATP-related compounds, consisting of inosine-5'- monophosphate (IMP), inosine (Ino), and hypoxantine (Hx), were determined by HPLC according to the method described by Barat *et al.* (2008) (13), with minor modifications. The analysis was conducted in a Hitachi LaChrom Elite liquid chromatography (Hitachi Ltd., Tokyo, Japan®) with a pump (model L-2130), an auto-sampler (model L-2200) and a UV detector (model L-2400). Data acquisition was performed with the EZChrom Elite software (Agillent Technologies, Palo Alto, CA, USA®). Separations were done on a reverse-phase Ultrabase C18 250 x 4.6 mm, internal particle diameter of 5 mm (Análisis Vínicos, S.L., Tomelloso, Spain®). Compounds were identified using retention time comparison of unknowns with those of standards and by standard addition or "spiking". IMP, Ino, and Hx were quantified according to the external standard method, using calibration curves of the peak area of compound *vs* concentration of compound under identical chromatographic conditions. K_1-values were calculated according to Equation (1):

$$K_1(\%) = \frac{[Ino]+[Hx]}{[IMP]+[Ino]+[Hx]} \times 100 \qquad \textbf{Equation1}$$

Where IMP is inosine 5'-monophosphate; Ino, inosine; Hx, hypoxanthine. All chemical reagents were provided by Sigma-Aldrich (St. Louis, MO, USA®).

Texture profile analysis (TPA) and compression test were performed on tilapia using a Texture Analyser TA.XT2® (Stable Micro Systems, Surrey, UK) equipped with a load cell of 250 N. For the TPA analysis, samples were obtained by cutting parallelepiped pieces (40 × 30 mm) from the dorsal muscle of the fillet. In this test, a flat-ended cylindrical plunger (7.5 mm diameter) was employed. The plunger was pressed into the sample at a constant speed of 0.8 mm/s until 50% of the sample height was achieved. Force-distance curves were processed to obtain seven texture parameters: hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience.

For the compression study, the texture analyzer was equipped with a 24.5 mm-diameter flat-ended cylindrical plunger. The plunger was pressed down at a constant speed of 5 mm/s into the sample until compressing 7 mm (14). This analysis was performed on the whole fish behind the dorsal fin.

Instrumental color analyses were performed with a Minolta Chroma Meter CM-3600d

(Minolta, Osaka, Japan®) with a D65 light source and a 10º observer. Data were expressed using the CIE L*a*b* system to represent lightness (L*), redness (a*), and yellowness (b*). Furthermore, the values of chroma (C*ab), which defines saturation of color, and the angle of hue (h*ab) were obtained.

Microbial analyses. Mesophilic counts were done according to the method provided in standard UNE-EN ISO 4833:2003 (15). *Enterobacteriaceae* were enumerated according to the method described by Pascual and Calderón (2000) (16). All the culture media were provided by Scharlau Chemie, S.A. (Barcelona, Spain). All the analyses were performed in duplicate and the results were expressed as log CFU/g.

Statistical analysis. Statistical treatment of the data was performed using the Statgraphics Centurion (Statpoint Technologies, Inc., Warrenton, VA, USA®). An analysis of variance (One-Way ANOVA), in which storage time was the factor, was conducted for each parameter evaluated to test whether there were significant differences throughout cold storage. The LSD procedure (least significant difference) was used to test for differences between averages at the 5% significance level.

RESULTS

Proximate composition. The proximate composition of fresh tilapia is shown in Table 1. These values agree with the data presented by Yanar *et al*. (2006) (17). Regarding the fat content, our results agree with the data reported by Suloma *et al*. (2008) (18); however, lipid content was higher, and subsequently moisture was lower, than those provided in other studies. The protein content data are in accordance with those obtained by other authors for the same fish species (17, 19, 20). The crude protein content in fish muscle can range from11to 24% (wet weight), depending on specie, state of nutrition, the reproductive cycle of the animals, etc. (20).

Table 1. Proximate composition of tilapia (means and standard deviations, n = 9).

Moisture g/100g	74.6 ± 1.3
Protein g/100g	19.1 ± 1.0
Lipid g/100g	2.8 ± 0.9
Ash g/100g	1.5 ± 0.4

Table 2 shows the values of moisture content, pH, WHC, TVB-N and TBA index of tilapia during cold storage. The initial value of pH was 6.06, increasing progressively during the storage time. These results are in accordance with the results obtained by Tome *et al*. (2000) (21) and Khalafalla *et al*. (2015) (22) for the same fish species. Fresh fish pH oscillates between 6.0 and 6.5 (23), depending on different factors such as fish species, season, diet, level of activity or stress during capture, and storage conditions (24).

Table 2. Physico-chemical parameters of tilapia during cold storage (Mean ± Sd. n = 6).

Day	0	2	4	7
Moisture (g/100 g)	73.12 ± 1.32^{a}	73.90 ± 1.77^{ab}	75.59 ± 1.01^{bc}	75.75 ± 1.03^{c}
pH	6.06 ± 0.09^{a}	6.12 ± 0.06^{ab}	6.27 ± 0.05^{ab}	6.25 ± 0.06^{a}
WHC (g water held/100 g total water)	61.93 ± 0.03^{a}	56.58 ± 1.85^{b}	54.40 ± 1.61^{c}	52.28 ± 1.23^{d}
TVBN (g N/100 g fish)	28.75 ± 1.69^{a}	28.08 ± 3.84^{a}	27.41 ± 1.41^{a}	31.14 ± 0.35^{a}
TBA (mg MDA/Kg fish)	0.36 ± 0.02^{a}	0.33 ± 0.04^{a}	0.43 ± 0.04^{b}	0.42 ± 0.01^{b}

WHC: water holding capacity; TVB-N: total volatile basic nitrogen; TBA: thiobarbituric acid index.
Same letters in the same row indicate homogeneous group membership ($p<0.05$)

The WHC values significantly decreased during storage (Table 2), which agree with other studies (10, 25). The decrease in the WHC can be due to a gradual denaturation of proteins because of the microbial activity and pH changes during the storage.

The TVB-N content was high at the beginning of the study (Table 2). The changes of this parameter throughout the storage were non-significant. The TVB-N r is commonly considered a quality index for unprocessed fish products as its increase is related to the activity of spoilage bacteria and endogenous enzymes (26, 27, 28). The action of such enzymes results in the formation of compounds, including ammonia, monoethylamine,

dimethylamine, etc. which give fish a characteristic off-flavour (29).

The TBA index remained low and constant throughout the study, ranging between 0.33 and 0.43 mg MDA/kg (Table 2), which is in accordance with other studies with tilapia (17). Different limit of acceptability values have been reported for this index. According to Connell (1995) (30), TBA values of 1-2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish normally develop an objectionable odor. Ruiz-Capillas and Moral (2001) (31) established that the minimum TBA index value detectable by panellists was 1.44 mg MDA/kg.

ATP-related compounds. Figure 1 depicts the changes of inosine 5'-monophosphate (IMP), inosine (Ino), hypoxanthine (Hx) and the K_1-value during cold storage. The evolution of major adenine nucleotides and their related compounds can provide information about biochemical changes during storage. The ATP catabolism to IMP has been reported to be essentially caused by endogenous enzymes. Nevertheless, the hydrolysis of Ino and Hx formation may also result from bacterial enzymes (32, 33). In addition, several authors have found that loss of sensory quality correlates with increases in the Hx level during storage (34).

In this study, ATP, ADP and AMP were not analyzed because the conversion of ATP into IMP is usually completed in 1 day, is presumed totally autolytic (35), and the main changes during storage occur in IMP, Ino and Hx (9).

The IMP levels decreased from 9.5 to 0.5 μmol/g. The Ino contents remained low during the 7 days of study with a maximum value of 2.0 μmol/g at the end of storage. The initial Hx contents were low but increased during the storage period to reach 12.3 μmol/g.

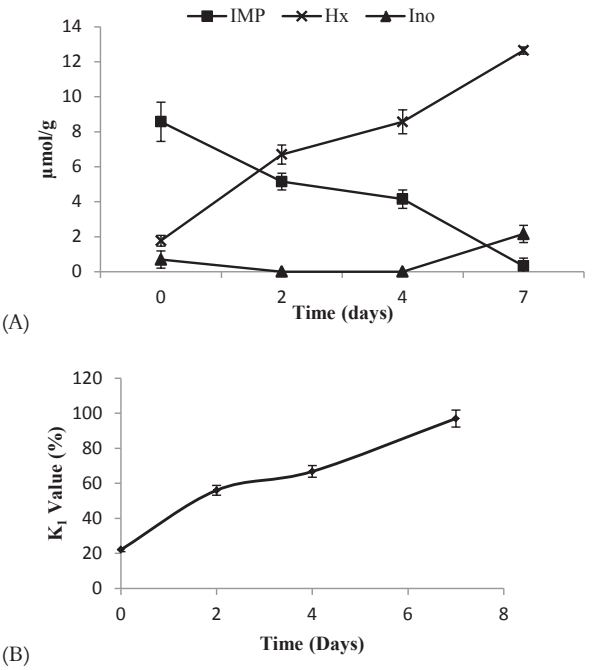


Figure 1. (A) Inosine 5'-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx) (μmol/g), and (B) K_1-value of fresh tilapia (day 0) and samples in cold storage for 2, 4 and 7 days (Mean and Sd, n=6).

Ehira and Uchiyama (1987) (4) have demonstrated that the K_1-value, which measures the extension of IMP degradation, is a good freshness index for a large number of fish species. In this study the initial values of K_1 were over 20%, increasing during the storage period.

Texture. Firmness, hardness, adhesiveness and gumminess significantly changed during storage (Table 3). It is important to note the decrease of firmness and hardness observed throughout the studied period, which indicates changes in the structure of fish.

Table 3. Textural parameters evolution of tilapia during cold storage (Mean ± Sd., n = 6).

Time (Days)	**0**	**2**	**4**	**7**
Compression test (whole fish)				
Firmness (N)	23.85 ± 0.46^a	19.38 ± 2.24^b	17.37 ± 2.01^c	16.60 ± 1.75^c
TPA (fish fillet)				
Hardness (N)	40.70 ± 2.76^a	30.76 ± 6.16^{ab}	26.02 ± 2.62^{ab}	19.44± 6.20^b
Adhesiveness (g·s)	-0.58 ± 0.03^a	-0.27 ± 0.03^b	-0.33 ± 0.03^{ab}	-0.45 ± 0.14^{ab}
Springiness (ratio)	0.51 ± 0.16^a	0.46 ± 0.03^a	0.54 ± 0.05^a	0.42 ± 0.01^a
Cohesiveness (ratio)	0.36 ± 0.02^a	0.33 ± 0.04^a	0.43 ± 0.04^a	0.42 ± 0.01^a
Gumminess (N)	14.85 ± 1.72^a	10.19 ± 0.88^{ab}	11.33 ± 2.27^{ab}	7.67 ± 1.23^b
Chewiness (N)	7.67 ± 3.22^a	4.70 ± 0.38^a	6.16 ± 1.79^a	3.19 ± 0.41^a
Resilence (ratio)	0.13 ± 0.02^a	0.12 ± 0.02^a	0.13 ± 0.01^a	0.12 ± 0.03^a

Same letters in the same row indicate homogeneous group membership ($p<0.05$)

Color. Lightness of tilapia muscle slightly decreased with storage time, while a★ and b★ parameters progressively increased ($p<0.05$) (Table 4). The mechanism which leads to color changes in tilapia is unclear, although some authors have reported that one or the causes is the oxidation of muscle myoglobin (36).

Table 4. Color parameters evolution of tilapia during cold storage.(Mean ± Sd. n = 6).

Time (Days)	0	2	4	7
L★	44.42 ± 0.42^{a}	44.77 ± 1.47^{a}	43.68 ± 0.35^{ab}	42.69 ± 0.34^{b}
a★	-3.46 ± 0.24^{a}	-3.25 ± 0.16^{a}	-2.33 ± 0.14^{b}	-2.13 ± 0.26^{b}
b★	3.59 ± 1.39^{a}	4.29 ± 0.57^{a}	6.15 ± 0.60^{b}	5.99 ± 0.32^{b}
$C^{\star}_{ab}$	5.05 ± 1.09^{a}	5.39 ± 0.49^{ab}	6.57 ± 0.61^{b}	6.36 ± 0.31^{b}
h_{ab}	-0.77 ± 0.19^{a}	-0.91 ± 0.06^{a}	-1.20 ± 0.02	-1.22 ± 0.04^{b}
ΔE	-	2.02 ± 0.98	3.15 ± 1.19	1.38 ± 0.36

Same letters in the same row indicate homogeneous group membership ($p<0.05$)

Microbial analyses. The evolution of mesophilic bacteria and *Enterobacteriaceae* counts are shown in Figure 2. Mesophilic counts gradually increased throughout storage. Limits of 6-7 log CFU/g for mesophilic bacteria have been established for fresh water and marine species fit for human consumption (16). Samples reached this limit of acceptability at day 7 of the study. No *Enterobacteriaceae* were found on days 0 and 2 of storage, but they increased to 1.6 log CFU/g at the end of storage.

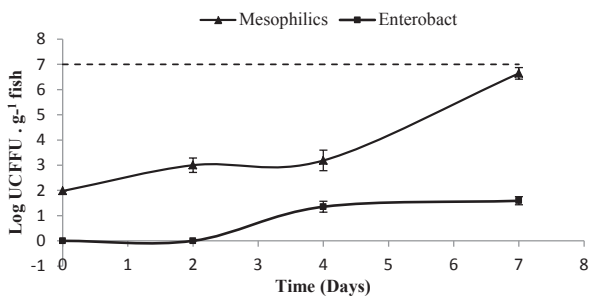


Figure 2. Counts of mesophilic bacteria and *Enterobacteriaceae* (log CFU/g) of fresh tilapia (day 0) and samples in cold storage for 2, 4 and 7 days (Mean and Sd, n=6). Upper areas of the horizontal lines are unacceptable (dashed line for mesophilic).

DISCUSSION

Proximate composition. The chemical composition of tilapia is highly affected by: season, environmental conditions, water quality, state of maturity, feeding conditions or sex (37, 38). Regarding the fat content, Puwasatien *et al*. (1999) (19) reported that tilapia muscle contained 1.8% of fat. Visentainer *et al*. (2005) (39) and Chaijan (2011) (20) reported even lower lipid contents (1.09 and 1.10%, respectively), Younis *et al*. (2015) (38) reported values lower than 1% of fat. According to these results, tilapia can be classified as a lean fish because its fat content is below 2 g fat/100 g. However, in the present study tilapia showed a lipid content of 2.8% and should, therefore, be classified as low-fat fish (total lipid content from 2% to 4%) (40). The protein content found in this study (19.1%) was higher than the values reported in other studies on tilapia (38). However, the moisture content in this work was lower than those found by Younis *et al*. (2015) (38) and Fonseca *et al*., (2013) (41), which could explain the differences found in in lipid and protein contents.

Physico-chemical parameters. The behavior of the TVB-N (high initial value with no significant increase throughout the storage) could not be related to the quality changes of tilapia during the study. Several works have shown a poor correlation of TVB-N with storage time and sensory fish quality (42). Variation in TVB-N of a particular fish species depends on the fish non-protein nitrogen content, which in turn depends on the type of fish feeding, catching season, fish size, and also on other environmental factors (29).

The TBA index remained between 0.33 and 0.43 mg MDA/kg, which can be considered low in comparison with other studies on tilapia, where values about 1 mg MDA/kg were reached at 6 days of storage. Therefore, it can be concluded that in this study lipid oxidation of samples was minimum, so this parameter did not contribute to the fish spoilage.

The changes observed in the IMP, Ino and Hx contents throughout the storage indicate the breakdown of IMP into Ino, and Ino into Hx. Therefore, the K_1-value increased during the storage period. Ehira and Uchiyama (1987) (4) established that in recently captured fish K_1-value should not exceed 10%, a fish of excellent quality should not exceed 20%, and values between 20% and 45% indicate fish of acceptable quality, while values greater than 60% indicate spoiled fish. Based on these categories, at the beginning of the study tilapia fish could be considered moderately fresh and spoiled from day 4 of cold storage.

Fish death triggers autolytic and microbiological processes that make muscle softer and less elastic. Hernández *et al*., (2009) (43) found that hardness values highly correlated with storage time and microbial counts. In this study, noticeable changes were observed especially in hardness and firmness, which underwent an important decrease during the storage time, which agree with other studies on tilapia (44). The decrease in the hardness and firmness could be due to the protein denaturation.

Regarding color changes, it is important to highlight the variability of data found in different studies on tilapia. Oliveira *et al*., (2014) (44) reported higher values of L* and b*, while Fonseca *et al*. (2013) (41) found lower values of L* and higher of a*, compared with this study. The differences in the color could be due to the different environment and different diets, which directly affect the color muscle.

Microbial analyses. *Enterobacteriaceae* was not reached the acceptability limit of 3 log CFU/g established in other studies (26). *Enterobacteriaceae* in the flora of fish appears when fish are obtained from polluted water, or if there is a delay in chilling after capture. Furthermore, *Enterobacteriaceae* may occur due to cross contaminations during post-processing; e.g., filleting. In this sense, aquaculture fish are expected to be of good hygienic quality since they live in a controlled environment and are slaughtered through good practices. Moreover handling, distribution and storage conditions are usually more controlled than in wild fish.

Mesophilic bacteria counts reached the acceptability limit (6-7 log CFU/g) at day 7 of the study. Taking into account this limit, the shelf life of tilapia used in this study would be lower than 7 days. In other studies on tilapia (38, 41, 44), this limit has been reached after a longer period of time. This is due to that in this work the day 0 of study was not the slaughter day, but the day of purchase in the supermarket.

In order to establish properly the shelf life period, a sensory analysis is needed to confirm the results obtained by physico-chemical and microbial analyses.

CONCLUSIONS

The results of the physico-chemical and microbial analyses showed the spoilage of tilapia during cold storage. TVB-N was not a good indicator of the fish spoilage. No oxidation could be observed in the tilapia during the 7 days of storage. The ATP-related compounds and K_1-value showed the progressive spoilage of the fish during the cold storage. The decrease of hardness and firmness confirmed the loss of quality throughout the time of study. The low microbial counts at the beginning of the study demonstrated the good quality of the tilapia; however, the increase of the mesophilic counts at the end of the studied period showed that tilapia was not fit for human consumption at day 7.

It is important to consider that the distance between producers and consumers decisively determines degree of fish freshness in the destination market, for this reason it is essential to apply good manufacturing practices to ensure a good quality of fish when reaching consumers.

REFERENCES

1. Food and Agriculture Organization of the United Nations (FAO). The State of the World Fisheries and Aquaculture. FAO Corporate Document Repository, 2012. [Accessed September 13 of 2012]. Available in: http://www.fao.org/docrep/016/i2727e/i2727e.pdf.
2. Morales G, Blanco L, Arias ML, Chaves C. Evaluación de la calidad microbiológica de tilapia fresca (*Oreocrhomis niloticus*) proveniente de la zona norte de Costa Rica. Arch Latinoam Nutr. 2004 Dec; 54(4): 433-437.
3. Food and Agriculture Organization of the United Nations (FAO). Market Reports: June 2013. [Accessed December 18 of 2013]. Available in: http://www.globefish.org/tilapia-june-2013.html.
4. Ehira S, Uchiyama H. Determination of fish freshness using the k value and comments on some other biochemical changes in relation to freshness. In *Seafood quality determination*, Kramer DE & Liston J (eds.), Elsevier Science Publishers BV, Amsterdam, Nederland 1987, p. 185-207.
5. Zaragoza P, Fuentes A, Fernández-Segovia I, Vivancos J-L, Rizo A, Ros-Lis J, Barat J M, Martínez-Mañez R. Evaluation of sea bream (*Sparus aurata*) shelf life using an optoelectronic nose. Food Chem. 2013 Jun; 138 (2-3): 1374-1380.
6. Zaragozá P, Fuentes A, Ruiz-Rico M, Vivancos J-L, Fernández-Segovia I, Ros-Lis J, Barat J, Martínez-Máñez R. Development of a colorimetric sensor array for squid spoilage assessment. Food Chem. 2015 May; 175: 315-321.
7. Ruiz-Rico M, Fuentes A, Masot R, Alcañiz M, Fernández-Segovia I, Barat JM. Use of the voltammetric tongue in fresh cod (*Gadus morhua*) quality assessment. Innovative Food Science & Emerging Technologies. 2013 18(1): 256-263.
8. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 17th Ed. Arlington, VA. AOAC International. 2002.
9. Rizo A, Máñes V, Fuentes A, Fernández-Segovia I, Barat J M. Physicochemical and microbial changes during storage of smoke-flavoured salmon obtained by a new method. Food Control. 2015 Oct; 56: 195-201.
10. García JA, Núñez FA, Rentería AL, Jiménez JA, Espinosa MR. Calidad de canal y carne de trucha arco iris (*Oncorhynchus mykiss Richardson*), producida en el noroeste del Estado de Chihuahua. Hidrobiológica. 2006 Jun; 16(1): 11-22.

11. Malle P, Tao SH. Rapid Quantitative Determination of Trimethylamine using Steam distillation. J Food Protect. 1987 Sept; 50(9): 756-769.
12. Vyncke W. Direct determination of the thiobarbituric acid value in trichloracetic acid extract of fish as a measure of oxidative rancidity. Fett Wiss Technol. 1970 Dec; 72: 1084-1087.
13. Barat JM, Gil L, García-Breijo E, Aristoy MC, Toldrá F, Martínez-Mañez R, Soto J. Freshness monitoring of sea bream (*Sparus aurata*) with a potentiometric sensor. Food Chem. 2008 Jun; 108: 681–688.
14. Sánchez J, Costell E. Estrategias de alimentación y cultivo de la dorada. Estudio patológico y control de la calidad sensorial. Valencia, España: IATA CSIC; 2002. 108 p.
15. International Organization for Standardization (ISO). ISO 4833, 2003 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 degrees C. 2003.
16. Pascual MR, Calderón V. Microbiología alimentaria: metodología analítica para alimentos y bebidas. Madrid, España: Díaz de Santos; 2000. 464 p.
17. Yanar Y, Celik M, Akamca E. Effects of brine concentration on shelf-life of hot-smoked tilapia (*Oreochromis niloticus*) stored at 4ºC. Food Chem. 2006 Jul; 97: 244–247.
18. Suloma A, Ogata HY, Garibay ES, Chavez D, El-Haroun ER. Fatty acid composition of Nile tilapia *Oreochromis niloticus* muscles: a comparative study with commercially important tropical freshwater fish in Philippines. 8th International Symposium on Tilapia in Aquaculture. Cairo: Egypt 2008; pp. 921-932.
19. Puwasatien P, Judprasong K, Kettwan E, Vasanachitt K, Nakngamanong Y, Bhattacharjee L. Proximate composition of raw and cooked Thai freshwater and marine fish. J Food Compos Anal. 1999 Mar; 12: 9–16.
20. Chaijan M. Physicochemical changes of tilapia (*Oreochromis niloticus*) muscle during salting. Food Chem. 2011 Dec; 129: 1201–1210.
21. Tome E, Iglesias M, Kodaira M, Gonzales A. Efecto de la temperatura de almacenamiento en el rigor mortis y en la estabilidad de la tilapia (Oreocrhomis Ssp.) cultivada. Rev cient FCV-LUZ. 2000 Mar; 10(4): 339-345.
22. Khalafalla F, Ali F, Hassan A-R. Quality improvement and shelf-life extension of refrigerated Nile tilapia (*Oreochromis niloticus*) fillets using natural herbs. University journal of basic and applied sciences. 2015; 4: 33-40.
23. Fennema O R. Química de los alimentos. Zaragoza, España: Ed Acribia; 2000. 1258 p.
24. Ocaño-Higuera VM, Marquez-Ríos E, Canizales-Dávila M. Castillo-Yáñez FJ, Pacheco-Aguilar R, Lugo-Sánchez ME, García-Orozco KD, Graciano-Verdugo AZ. Postmortem changes in cazon fish muscle stored on ice. Food Chem. 2009 Oct; 116: 933–938.
25. Sotelo I, Filomena A, Rodríguez J. Evaluación de las propiedades del Cajaro (Phractocephalus hemiliopterus) como potencial para la obtención de surimi y productos derivados. Rev MVZ Córdoba. 2008 Dec; 13(3): 1456-1463.
26. Fuentes A, Fernández-Segovia I, Barat JM, Serra JA. Influence of sodium replacement and packaging on quality and shelf life of smoked sea bass (Dicentrarchus labrax L.). LWT-Food Sci Technol. 2011 May; 44(4): 917–923.
27. Kykkidou S, Giatrakou V, Papavergou A, Kontominas MG, Savvaidis IN. Effect of thyme essential oil and packaging treatments on fresh Mediterranean swordfish fillets during storage at 4 ºC. Food Chem. 2009 Jul; 11: 169-175.
28. Özogul K, Özyurt G, Kuley E, Özkutuk S. Sensory, microbiological and chemical assessment of the freshness of red mullet (Mullus barbatus) and goldband goatfish (Upeneus moluccensis) during storage in ice. Food Chem. 2009 May; 114: 505-510.
29. Goulas AE, Kontominas MG. Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (Sparus aurata): Biochemical and sensory attributes. Food Chem. 2007; 100: 287-296.
30. Connell JJ. Control of fish quality 4th ed. Oxford, United Kingdom: Ed. Wiley BlackWell; 1995. 256 p.
31. Ruiz-Capillas C, Moral A. Residual effect of CO_2 on hake (Merluccis merluccius L.) stored in modified and controlled atmospheres. Eur Food Res Technol. 2001 Jan; 212: 413-420.
32. Dalgaard P. Freshness, quality and safety in seafoods. Flair-Flow Europe Technical Manual F-FE 380A/00. Danish Institute for Fisheries Research May 2000. [Accessed January 31 of 2012]. Available: http://flairflow4.vscht.cz/seafood00.pdf.
33. Surette ME, Gill TA, LeBlanc PJ. Biochemical basis of postmortem nucleotide catabolism in cod (Gadus morhua) and its relationship to spoilage. J Agr Food Chem. 1988 Jan; 36(1): 19–22.
34. Giménez B, Roncalés P, Beltrán JA. Different packaging conditions to improve shelf-life of filleted rainbow trout. In *Prooceedings of 30th WEFTA Plenary Meeting*, Gudjónsson A, Niclasen O (Eds.), Tórshavn Froya Fródskaparfelag, Faeroe Islands 2000. pp. 43-50.
35. Alasalvar C, Taylor KDA, Öksüz A, Garthwaite T, Alexis MN, Grigorakis K. Freshness assessment of cultured sea bream (Sparus aurata) by chemical, physical and sensory methods. Food Chem. 2001 Jan: 72: 33–40.
36. Venugopal V, Postharvest quality changes and safety hazards. . In: *Seafood processing: Adding value through quick freezing, retortable packing and cook-chilling*. Venugopal V. Editor. (New Jersey): 2006. CRC Press; p. 23-60
37. Saeed S M. Impact of environmental parameters on fish condition and quality in lake Edku, Egypt. J Aquat Biol Fish. 2013; 17 (1): 101-112.
38. Younis E M, Al-Asgah N, Abdel-Warith A, Al-Mutairi A. Seasonal variations in the body composition and bioaccumulation of heavy metals in Nile tilapia collected from drainage canal in Al-Ahsa, Saudi Arabia. Saudi J Biol Sciences. 2015; 22: 442-447.
39. Visentainer JV, de Souza NE, Makoto M, Hayashi C, Franco MR. Influence of diets enriched with flaxseed oil on the □-linolenic, eicosapentaenoic and docosahexaenoic fatty acid in Nile tilapia (*Oreochromis niloticus*). Food Chem. 2005 May; 90 (4): 557-560.
40. Ackman RG. Seafood lipids and fatty acids. Food Rev Int. 1990; 6(4): 617-646.
41. Fonseca G G, Cavenaghi-Altemio A, Silva M de F, Arcanjo V, Sanjinez-Argadoña E J. Influence of treatments in the quality of Nile tilapia (*Oreochromis niloticus*) fillets. Food Science & Nutrition. 2013 March; 1(3): 246-253.
42. Baixas-Nogueras S, Bover-Cid S, Veciana-Nogues MT, Vidal-Carou MC. Effect of gutting on microbial loads, sensory properties, and volatile and biogenic amine contents of European hake (Merluccius merluccius var. Mediterraneus) stored in ice. J Food Protect. 2009 Aug; 72 (8): 1671–1676.
43. Hernández MD, López MB, Álvarez A, Ferrandini E, García-García B, Garrido MD. Sensory, physical, chemical and microbiological changes in aquacultured meagre (*Argyrosomus regius*) fillets during ice storage. Food Chem. 2009 May; 114 (1) : 237– 245
44. Oliveira PR, Oliveira CA, Sobral PJ, Balieiro JC, Natori NN, Viegas MM. How Stunning methods affect the quality of Nile tilapia meat. CyTA Journal of Food. 2014; 13(1): 56-62.

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 22 número 2, año 2015
Universidad de Antioquia, Medellín, Colombia. págs. 148-159
DOI: http://dx.doi.org/10.17533/udea.vitae.v22n2a09

CONTENT OF Hg, Cd, Pb AND As IN FISH SPECIES: A REVIEW

CONTENIDO DE Hg, Cd, Pb Y As EN ESPECIES DE PECES: REVISION

Julián ZULUAGA RODRÍGUEZ[1*], Sara Elisa GALLEGO RÍOS[1], Claudia María RAMÍREZ BOTERO[1]

Recibido: Marzo 15 de 2015 Aceptado: Septiembre 21 de 2015

ABSTRACT

Background: One of the main water contaminants are Hg, Cd, Pb and As. The chain of contamination of these metals and metalloid follows a cyclical order: Industry, atmosphere, land, water, phytoplankton, zooplankton, fish, and humans. Currently, Hg, Cd, Pb and As researches are of interest because consumption of fish with high toxic metal and metalloid concentrations affects human health. **Objective:** Provide information on the characteristics of Hg, Cd, Pb and As in the problematic of fishing resource contamination, their implications on human health, and international evidence on studies conducted on *Caranx, Scomberomorus*, *Epinephelus, Euthynnus, Lutjanus, thunnus and Megalops* fish genera. **Methods**: Database, Science Direct, Pub Med, Escopus, Springer Link, and Scopus, available information was reviewed using the keywords: Heavy metals, water pollution, fish, mercury, cadmium, lead, arsenic, health risk, regulations, biomagnification, and bioaccumulation. **Results**: The metals that pose the highest risks for human health are mercury, cadmium, lead, and arsenic which cause important complications in the nervous system, kidneys, bones, lungs, and cardiovascular system due to their toxicity and possible carcinogenic effect. Fish contents of Hg, Cd, Pb and As varies depending on the zone, environmental conditions, the contamination level of the fishing site, and the characteristics of the fish, being some fish more prone to accumulating higher concentrations of these metals in their muscles; among the species, the metal that showed the highest risk was mercury, being in high concentrations in the largest, most enduring predatory fish. **Conclusions:** Studies submitted in this review, may be used as the base for future comparisons with Hg, Cd, Pb and As concentration values in different fish studies for the *Caranx, Scomberomorus*, *Epinephelus, Euthynnus, Lutjanus, and Megalops* genera in order to be able to determine consumption recommendations and warnings.

Key words: Fishes, Water pollution, Mercury, Cadmium, Lead, Arsenic

RESUMEN

Antecedentes: Uno de los principales agentes contaminantes en las aguas son Hg, Cd, Pb y As. La cadena de contaminación de estos metales sigue un orden cíclico: industria, atmósfera, suelo, agua, fitoplancton, zooplancton, peces y humanos. Las investigaciones en Hg, Cd, Pb y As son de interés en la actualidad ya que el consumo de pescado con altas concentraciones de estos metales y metaloides tóxicos afecta la salud humana. **Objetivo:** Ofrecer información de las características del Hg, Cd, Pb y As en la problemática de contaminación de los recursos pesqueros, sus implicaciones para la salud humana y evidencias internacionales de los estudios realizados en los peces de los generos *Caranx, Scomberomorus*,

[1] Grupo Impacto de los Componentes Alimentarios en la Salud ICAS, Escuela de Nutrición y Dietética, Universidad de Antioquia UdeA, Medellín, Colombia

[*] Corresponding author : julian.zuluagar@udea.edu.co

Epinephelus, Euthynnus, Lutjanus y Megalops. **Métodos**: Se revisó información disponible en las bases de datos, Science Direct, Pub Med, Escopus, Springer Link, Scopus, utilizando las palabras clave: heavy metals, water, fish, mercury, cadmium, lead, arsenic, health risk, regulations, biomagnification, bioacumulation. **Resultados**: Los metales con mayor riesgo para la salud humana son mercurio, cadmio, plomo y arsénico, los cuales causan complicaciones importantes en el sistema nervioso, renal, óseo, pulmonar, cardiovascular debido a su toxicidad y posible efecto carcinogénico. El contenido de Hg, Cd, Pb y As en el pescado varía dependiendo de la zona, las condiciones medioambientales, el grado de contaminación del lugar de pesca y las características del pez, siendo algunos peces más propensos a acumular concentraciones más elevadas de estos metales en el músculo, entre las especies de peces, el metal que mostro mayor riesgo fue el mercurio, estando en altas concentraciones en los peces más grandes, de mayor longevidad y depredadores. **Conclusiones:** Los estudios presentados en esta revisión pueden servir como base para futuras comparaciones con valores de concentración de metales pesados en diferentes estudios de peces para los géneros *Caranx, Scomberomorus*, *Epinephelus, Euthynnus, Lutjanus y Megalops,* con la finalidad de poder establecer recomendaciones y advertencias sobre su consumo.

Palabras Claves: Peces, contaminación del agua; mercurio; cadmio; plomo; arsénico

INTRODUCTION

Currently, chemical products coming from sources like industrial, urban, and agricultural waste discharges are contaminants of the surfaces and sediments of the waters in the world(1). One of the main contaminant agents in the waters are Mercury (Hg), Cadmium (Cd), Lead (Pb), and Arsenic (As). The chain of contamination of these metals and metalloid follows a cyclical order: Industry, atmosphere, land, water, phytoplankton, zooplankton, fish, and humans (2). In some fish species, contamination levels are so high, they may cause adverse effects on human health. Some countries issue alerts on the risk of contaminated fish consumption; however, they are ignored by public at large who continue consuming without taking into account the serious health consequences (3).

Trace elements are present in every ecosystem in the world (4); nevertheless, the increase of these metals and metalloids in marine systems has become a risk to human health due to their toxic effects (5). The main Hg, Cd, Pb and As contamination factors in fluvial and marine sources are industrial and municipal wastewater discharge, mining, combustion of fossil fuels, deforestation and fertilizers used in agriculture (6–8). Also natural sources such as soil weathering and volcanic activity pollute the environment significantly (9). Trace elements can be soluble in water and react with organic matter forming complexes and chelates, which increase its solubility, availability and dispersal (10).

Once trace elements are released into the waters, they bioaccumulate into aquatic sediments depending on environmental conditions water cycle, seasonal variations, pH, microorganisms, sediment reduction and oxidation potential (11–15); in the sediments takes place the migration of metal compounds from the abiotic environment into aquatic organisms and the subsequent introduction and bioaccumulation in marine food chains (16), predators exhibit highest concentrations (17). Accumulation of metal in the tissues organism depends mainly on water concentrations, bioavailability and fish trophic position (18, 19).

The main metal threats to human health have been mainly associated to Hg, Cd, Pb, and As exposure (20, 21); they get into humans through diet, which poses a risk for populations with fish consumption over 8 – 12 ounces per week, exceeding the United States Environmental Protection Agency (U.S. EPA) recommendations (22, 23).

Hg has a series of chemical transformations in the environment, appearing as zero oxidation state (Hg^0), mercurous state (Hg^+), and mercuric state (Hg^{+2}); additionally, it may form organic compounds, being methylmercury (MeHg), the most important form in terms of toxicity and effects on health (5). MeHg causes damages at the central nervous system level and its neurotoxic effect is attributed to mitochondrial damage via glutathione reduction (GSH), which reduces ATP synthesis and increases lipid, protein, and DNA peroxidation; MeHg, due to its high affinity with the sulfhydryl group, it forms complexes with N-acetylcysteine and cysteyne, important precursors of GSH, which increases free radical production and reduces the oxidation defense systems and the imbalance between both processes produces the un-

controlled release of calcium from the mitochondrion, disturbing intracellular calcium levels (24, 25).

Cd accumulates in the human body for long periods of time causing damages to the nervous system, kidneys, bones, lungs, and cardiovascular system (26, 27), Cd cytotoxicity induces cellular dysfunctions, including cell death, reducing DNA breathing, and increasing mutagenesis. It has been classified as carcinogenic for humans (Group 1) (28), in spite of it not being directly mutagenic. DNA damages that take place after exposure to cadmium seem to be mainly mediated by the indirect production of free radicals, partly due to the inhibition of cellular antioxidants and oxidative stress increase (29).

Pb is neurotoxic and, due to its capacity to join erythrocytes, it may be widely distributed to the human body organs where it concentrates and generates damage. Lead toxicity is due to its ability to substitute itself with other divalent cations, specially calcium and zinc, affecting their functions into the cell, mainly in the cellular organelles like the mitochondrion, where its concentration affects the energetic metabolism and favors the generation of free radicals. One of the reasons why lead may substitute itself with diverse cations in the cell is its ability to form stable interactions with oxygen and sulfur, common components in the sites where proteins join the metal, and, besides, due to its longer ionic range and higher electronegativity lead is more related to these proteins than other cations like calcium and zinc; however, lead charge distribution is irregular due to the presence of two inert electrons in its electron cloud, which alters the structure and function of the joined protein, inhibiting its functionality (30, 31).

Chronical exposure to As, has been associated to skin injury, peripheral neuropathy, encephalopathy, hepatomegaly, cirrhosis, hematite metabolic alterations, bone marrow depression, diabetes, and renal failure. The action mechanisms of this metal depend on the way they present. In its pentavalent form, as arsenate (As^{+5}), it may replace phosphorus in multiple biochemical reactions having as the main consequence ATP depletion. In its trivalent form, arsenite (As^{+3}), it has a great affinity with the thiol groups, turning it into a powerful GHS inhibitor, and thioredoxin reductase, which may alter the cellular oxide reduction mechanisms generating cytotoxicity. As^{+3} it is also a pyruvate dehydrogenase (PDH) inhibitor, which ultimately reduces ATP production (32, 33).

There are important precedents on the serious consequences of Hg, Cd, Pb and As contaminated fish consumption has on human health, being worth mentioning: Minamata (Japan) tragedy, where over 900 people lost their lives and two million suffered health problems as a consequence of having eaten Hg contaminated fish, Minamata disease (34), and the event at Jinzu River basin, at Toyama prefecture, where one of the most serious Cd prolonged intake intoxication cases takes place following the mining extractions that contaminated those waters (35). In South America, an anthropogenic source contamination increase has been evidenced in high concentrations of trace elements on fish tissues (36). In Colombia, there are studies on biota metal concentration determination studies, such as those conducted by Olivero and Johnson at Ciénaga Grande de Achi, located on Caribona River basin, Cauca River sub-basin; Ciénaga de Simití, east of San Lucas mountain range, on Magdalena River; and south of Bolívar, Magdalena and Cauca lowland swamps, on species characteristic of these regions (37). However, there are no reports of importance on the Colombian Caribbean, on Hg, Cd, Pb and As contamination fishing resources, like porgy, coastal trevally, jack mackerel, wahoo, skipjack tuna, mud sucker, grouper, tuna and gropers, among others, belonging to the genera *Caranx, Scomberomorus*, *Epinephelus, Euthynnus, Lutjanus, thunnus and Megalops* (38); which are of commercial importance in the market of this region (39). Therefore, it is required to conduct studies to determine fish Hg, Cd, Pb and As content in this region. The objective of this work was to submit a review of the characteristics of Hg, Cd, Pb and As in the contamination problematic of fishing resources, their implications on human health, and international evidence of studies conducted on fish belonging to genera (*Caranx, Scomberomorus*, *Epinephelus, Euthynnus, Lutjanus, Thunnus and Megalops*).

MATERIALS AND METHODS

A bibliographical review of information between 2001 and 2015 was performed along some classical articles about Mercury, Cadmium, Lead, and Arsenic contents in *Caranx, Scomberomorus*, *Epinephelus, Euthynnus, Lutjanus, Thunnus and Megalops* genera that share characteristics with the fish of the same genera found in theColombian Caribbean ; those researches that quantified wet weight (mg/kg o μg

Hg /g wet weight) or if this information was possible to be extracted from the article were selected. These metals were also characterized and articles about the implication of consumption on health were taken into account. The regulations stipulated by Codex Alimentarius and Joint FAO/WHO Expert Committee on Food Additives (JECFA) were considered to analyze the maximum metal content allowable limits in fish and fish existing tolerable provisional intake recommendations to guarantee safe consumption. Documents were selected from databases, Science Direct, Pub Med, Escopus, Springer Link, and Scopus using the following keywords for the search: Heavy metals, water contamination, fish, mercury, cadmium, lead, arsenic, health risk, regulations, biomagnification, and bioaccumulation.

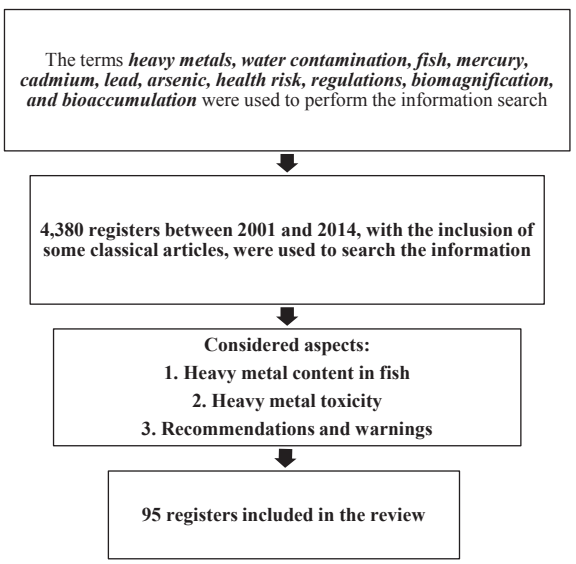


Figure 1. Search flowchart.

RESULTS

Mercury

Hg is a transition element of Group 12 (II B), with an atomic mass of 200.59 amu and it is one of the most toxic metals in aquatic ecosystems. A series of complex chemical transformations allows mercury to be present in the environment in its three oxidation states (5). Atmospheric Hg in the form of mercury vapor (Hg^0), is derived from soil weathering, volcanic activity and ocean evaporation. Also anthropogenic sources like refining and combustion of fossil fuels, gold mining, chlor alkali industry, cement, steel and phosphate production; the release of Hg in the world are estimated in 6000 tons per year, in Colombia it is estimated that each year around 31.26 tons are released into the water (40). In the environment Mercury vapor it is oxidized to a water-soluble inorganic form (Hg^{+2}), the metal may then be reduced back to Hg^0, or it may be methylated by microorganisms present in the freshwater and oceanic sediments, this biomethylation reaction produces MeHg, the mainly form of mercury in aquatic organism. MeHg into aquatic food chains follows the cycle, plankton, herbivorous fish and carnivorous fish (5,41).

Gastrointestinal absorption of Hg^{+2} form compounds in food is around 15%, while MeHg absorption is 90 to 95%; generally, about 80 to 100% of Hg found in fish muscle corresponds to MeHg (41). The biological average life of Hg is estimated at about 44 days (5).

The toxicological profile of Hg varies depending on its form. Exposure to Hg^0 and MeHg produce symptoms in the central nervous system, while Hg mono and divalent forms act mainly in the kidney. MeHg corresponds to the most toxic organic form of Hg (25). It is neurotoxic due to its accumulation in the central nervous system, deteriorating physiological functions through the interruption of the endocrine glands (42). MeHg is classified as carcinogenic for humans, group 2B, mainly related to liver and esophagus cancer (28). In addition, methylmercury can cross the placenta causing neurotoxic effects during human brain development, probably permanent (43, 44).

Some authors have reported the content of Hg in fish *(Scomberomorus cavalla, Caranx ignobilis, Caranx melampygus, Lutjanus bohar, Euthynnus affinis, Lutjanus russelli, Thunnus albacores, Thunnus alalunga, Thunnus obesus, Thunnus Thynnus and Megalops cyprinoids*, Table), in the Gulf of México, Queensland, Australia, southeast of Taiwan, western and central Pacific Ocean, Mediterranean Sea and southwest coast of India (45–49) finding Hg levels in muscle over 0.5 μg Hg/g wet weight for the largest, most enduring predatory fish (*Scomberomorus cavalla* 1.085μg Hg/g wet weight, *Caranx melampygus* 0.681 ± 0,174 μg Hg/g wet weight, see table). According to what has been reported in Queensland, the highest Hg levels are mainly due to biomagnification of the food chain of this metal (46). These findings are congruent with data reported by other researchers on these fish species that have similar characteristics (50–52).

An epidemiologic study conducted in San Francisco was able to demonstrate that the consumption of predatory fish, which may have high Hg levels in its MeHg form entails adverse health effects. The study determined a significant correlation of high levels of mercury on subjects who consumed swordfish 1.94 times a month, in average, using as reference, 150- 277 gram portions. This research analyzed blood Hg levels on 89 participants who consumed more than 30 species of fish and 82 of them showed levels over 5 μg Hg/L in the blood and 16 showed levels over 20 μg Hg/L in the blood (US EPA and the National Academy of Sciences recommend keeping blood mercury levels under 5 μg/L) (53).

Table 1. Hg, Cd, Pb and As content in fish of *Caranx, Scomberomorus, Epinephelus, Euthynnus, Lutjanus,Thunnus and Megalops* genera.

Región	Species	Common name	n	Means ± SD (mg/kg wet weight or μg/g wet weight)				Reference
				Hg	Cd	As	Pb	
Erren River, Taiwan	*Megalops cyprinoids*	Indo-Pacific tarpon	31	0.081 ± 0.026	<0.0125	0.683± 0.175		Chen et al (2004) (54)
Southwest Coast, India	*Lutjanus russelli*	Serra Spanish mackerel	18	0.09 ± 0.11	0.04 ± 0.03		0.21 ± 0.13	Thiyagarajan et al (2012) (55)
Pearl River delta, China	*Lutjanus griseus*	Mangrove Snapper	65		0.03 ± 0.02	0.39± 0.25	0.03 ± 0.01	Leung et al (2014) (56)
	Lutjanus stellatus	Star Snapper	68		0.07± 0.04	1.53 ± 0.99	0.04 ± 0.01	
Rio de Janeiro, Brazil	*Caranx crysos*	Blue runner	12		0.01 ± 0.02	0.6 ± 0.2	0.3 ± 0.3	Medeiros et al (2012) (57)
Gulf of México	*Scomberomorus cavalla*	Sierra	9		0.206 ± 0.08		0,421	Ploetz et al (2007) (11)
			385	1,085				EPA (2003) (45)
Bandar Abbas, Iran (Persian Gulf)	*Scomberomorus commerson*	Spanish mackerel	6		0.078± 0.048		0.223±0.135	Saei-Dehkordi and Fallah (2011) (58)
	Epinephelus Coioides	Grouper	6		0.076±0.023		0.297±0.111	
Queensland, Australia	*Caranx ignobilis*	Lowly trevally	4	0,234 ± 0,250				Denton and Burdon (1986) (46)
	Caranx melampygus	Blue-fined trevally	2	0.681 ± 0,174				
	Lutjanus bohar	Red bass	2	0.223 ± 0.037				
	Euthynnus affinis	Bonito	3	0.075 ± 0.053				
Shalateen, Egypt	*Thunnus albacares*	Yellowfin tuna	4		0,06 ± 0,01		0,32 ± 0,03	El-Moselhy et al (2014) (47)
Baja California Sur, Mexico	*Thunnus albacares*	Yellowfin tuna	37	0.15 ± 0.10				Ordiano et al (2012) (48)
Western and central Pacific Ocean	*Thunnus alalunga*	Albacore	115	0.444 ± 0.148				Chen et al (2014) (49)
	Thunnus obesus	Bigeye tuna	75	0.929 ± 0.668				
Mediterranean Sea	*Thunnus Thynnus*	Bluefin tuna	73	0.20 ± 0.07	0.02 ± 0.01	2.61 ± 1.47	0.10 ± 0.03	Storelli et al (2005) (59)

In Colombia, Hg contamination is mainly associated to mining activity, combustion of fossil fuels, waste incineration, waste disposal, waste water, where the metal is released into the water and atmosphere contaminating fish living in those areas (60). A study conducted on hair samples of 1,328 individuals, located along Cauca and Magdalena rivers, where most mining activities take place, showed that individual hair Hg average was 1.560 $\pm$ 0.060 μg/g, with values oscillating between 0.010 and 20.140 μg Hg/g in hair; 52.0% of the studied population exceeded the 1.0 μg Hg/g in hair recommendation determined by USEPA. The habitants located closer to the mining areas were those who showed the highest Hg level in hair; in this study, 99.8% consumed fish at least once a day

and in some cases the intake was three times a day (61). Another study conducted at Caimito, a town near San Jorge River, located in the northwestern region of Colombia, apparently free from mining activities, hair mercury levels were measured to 94 participants between 15 to 65 year-old, finding an average Hg concentration of 4.910 $\pm$ 0.550 μg Hg /g in hair; no significant differences were found on Hg levels in hair by sex or age but, however, a small but significant correlation was found between fish consumption frequency and Hg levels in hair (62).

Due to Hg intake consequences on health, the Codex Alimentarius determines maximum allowed levels of MeHg in fishing products and fish meat of 0.5 μg MeHg/ g, excepting predatory fish, where the maximum allowed level is 1.0 μg MeHg/ g; additionally, the Codex also counts on an inorganic Hg Provisional Tolerable Weekly Intake of 4 μg/ kg of body weight for humans and MeHg of 1.6μg/ kg of body weight (63). In 2007, JECFA reaffirmed the existing PTWI of 1.6 μg /kg of body weight for MeHg, based on the toxicological critical point (neurotoxicity development) in the human species (64).

Cadmium

Cd is an element of the Group 12 (II B), with an atomic weight of 112.40 amu and it is issued to the ground, water, and air through mining, non-ferrous metal refining, manufacturing and application of phosphate fertilizers, fossil fuel burning, and waste incineration and disposal. There is evidence that about 30,000 tons of cadmium are released into the environment every year with an estimated 4,000-13,000 tons from human activities affecting aquatic organisms,The average level of cadmium in ocean water has been reported between <5 and 110 ng/L (65). The nutrient-like profiles of Cd and correlation whit the phosphate in the ocean indicate its uptake by phytoplankton. Cd is then transferred to zooplankton and upper trophic levels through food chain webs; however the exact mechanisms of Cd uptake and the relationship with phosphates and other nutrients remain unknown (66–68). Human beings absorb Cd 5 to 8%, factor that is favored with low iron, calcium, and protein diets. Cd is transported by blood and distributed mainly to the liver and kidney (5) where it is long-term stored in the organism, having an average biological life of 17 to 30 years in human beings. Cd negatively affects the kidney, inducing tubular kidney failure and chronic kidney failure; in the lungs, it causes fibrosis (65), cardiovascular system risks due to the increment of cholesterol and free fatty acids in the blood, increasing the risk of aortic and coronary atherosclerosis (69), although the mechanisms and Cd relation to dyslipidemia have not been elucidated yet; studies suggest it is mainly due to the reduction of the High Density Lipoproteins (HDL) and to the high triglyceride and HDL proportion. Furthermore, Cd also affects children central nervous system causing neurological disorders, learning problems, and hyperactivity (70, 71). Some studies on adults and children's exposed to cadmium have suggested abnormal behavior and decreased intelligence, however because to the blood- barrier protection the direct toxic effect to occur only with cadmium exposure prior to blood–brain barrier formation or with blood–brain barrier dysfunction (5). It has been classified as carcinogen for group 1 humans and it has been mainly related to lung, prostate, pancreas, kidney, and bladder cancer (28). Several studies conducted in the US, Trinidad, Brazil, China, Iran, India, Egypt, Mediterranean Sea and Taiwan, demonstrated that the Cd concentrations in fish muscle were usually low. Among *S. Commerson, E. Coioides, C. crysos, L. griseus, L. stellatus, T albacares, T thynnus and M. cyprinoids* species, cadmium in muscle was generally lower than 0.1 μg Cd/g of wet weight (46,54–56,58), excepting the Gulf of Mexico, where Cd content in muscle was 0.206 $\pm$ 0.08 μg Cd/g of wet weight in *S. cavalla*, although this study found a significant correlation between the size of *S. cavalla* and liver Cd levels; a correlation between the size of *S. cavalla* and muscle Cd content was not found (11). The study conducted by Saei-Dehkordi and Fallah (58) in the Persian Gulf, found differences between the muscle Cd concentrations in *S. Commerson* during summer and winter seasons, 0.053 $\pm$ 0.035 and 0.102 $\pm$ 0.048 μg Cd/g of wet weight, respectively; the high Cd muscle levels during the winter are attributed to metal precipitation in the water due to the rainy season (58, 72).

Due to health consequences of Cd intake, Codex Alimentarius and JECFA determined a human Provisional Tolerable Monthly Intake (PTMI) of 25 μg Cd/kg of body weight (63, 73).

Lead

Pb is a Group 14 element (IV A), with an atomic weight of 207.2 amu; it exists in the Pb^0, Pb^{+2}, and

Pb^{+4} oxidation states, generally in combination with two or more elements to form compounds (7); it gets to the aquatic system due to the ground superficial erosion and atmospheric deposition (74). Environmental levels have increased over 1,000 times in the last three centuries as result of human activity; the highest increase has taken place between 1950 and 2000 (7). Speciation of lead in marine waters is largely influenced by carbonates, chlorates and organic natural ligands. The proportion of inorganic complexes of lead is largely determined by the pH of the water, the concentration of these complex gradually increase with increasing total metal loading in sediments which suggest a potential threat to benthic organisms and aquatic biota in the marine system (75–77). Once absorbed, it is transported in the bloodstream to other tissues and it is accumulated in high concentrations in bones, teeth, liver, lung, kidney, brain, and spleen going through the blood-brain and placental barrier (5). Pb average biological life may be considerable higher in children than in adults; in the blood, it has an estimated average life of 35 days; in soft tissue, of 40 days; and in bones, 20 – 30 years (78). The main way of absorbed Pb excretion is the urinary tract, in general, with glomerular kidney filtration; it is also excreted with the bile through the gastrointestinal tract (5). The most affected systems by Pb are the nervous, the cardiovascular, hematologic, and renal. Lead poisoning symptoms are headaches, irritability, abdominal pain, and others related to the nervous system (20). Pb chronic toxicity in humans frequently produces apathy, irritability, low attentional capacity, epigastric pain, constipation, vomit, convulsions, coma, and death. In children, it may present encephalopathy with lethargy, mental dullness, vomit, irritability, and anorexia; in the most serious cases, prolonged exposure to Pb may reduce the cognitive function and cause conduct disorders, especially aggression, psychosis, confusion, and mental deficit (7). Pb is one of the contaminants consumed in diet that has been clearly identified as a risk for human health (79), it has been classified as carcinogenic for 2B Group humans while inorganic lead compounds have been classified as carcinogenic for 2A Group humans, mainly related to stomach cancer (28).

Often, high Pb concentrations in fish take place in areas close to mining activities and in areas with a high presence of this metal industry (80). Several studies have examined Pb concentrations in fish in the Gulf of Mexico, Iran, Brazil, China, Egypt, Mediterranean Sea and India; the highest levels have been reported by Ploetz et al. (11) in the Gulf of Mexico (0.421 μg Pb/g wet weight) in *S. cavalla.* Saei-Dehkordi and Fallah (58) in the Persian Gulf found lead concentrations in *S. Commerson and E. Coioides* that oscillate between 0.158 and 0.367 μg Pb/g wet weight; this study measured Pb concentrations in fish both in the winter and in the summer. Both species *S. Commerson and E. Coioides,* showed higher lead levels during the winter, *0.289 and* 0.367 μg Pb/g, respectively. Medeiros et. Al (57), reports levels of 0.3 μg Pb/g wet weight in the species *C. crysos.* The lowest Pb levels were found in fish in the Pearl River delta (China) in the species *L. griseus and L. stellatu,* with 0.03 and 0.04 μg Pb/g wet weight, respectively, in spite of it being a highly contaminated area by human activities (56).

Due to health consequences of Pb intake, Codex Alimentarius determines a maximum limit of Pb in fish of 0.3 mg/kg of wet weight (63). The current PTWI recommendation in humans of 25 μg Pb/kg of wet weight determined by JECFA has been withdrawn; the same committee, after assessing it in 2011, set forth it is not possible to determine a new PTWI that may be considered health protector (81).

Arsenic

As is a Group 15 element (V A), with an atomic weight of 74.922 amu found highly distributed in a natural way on the earth's crust; it may exist in the As^{+3} and As^{+5} oxidation states in a wide number of inorganic and organic forms with different toxicity levels (8). As anthropogenic sources include pesticides, wood and industry preservatives, mining and smelting wastes (32). As dissolves easily in marine waters where is present in trivalent, pentavalent and methylated forms. In anoxic conditions, Arsenate (As^{+5}) is the dominant dissolved species, in surface water the arsenate is uptake by phytoplankton together with phosphate and transferred to arsenite (As^{3+}) and methylarsenate and dimethylarsenate. Other organoarsenic compounds of arsenate and arsenite like monomethylarsonic acid (MMA) dimethylarsinic acid (DMA), arsenobetaine (Ab) are produced by microbiological processes in the sediments (82,83). Arsenobetaine is considered the dominant As form in acuatic organisms including fish where Ab represents more than 95% of As total (84,85).

As compounds are absorbed by the gastrointestinal tract. Ingested inorganic As average biological life is about 10 hours and 50-80% is excreted in about three days, while methylated As has an average life of 30 hours. Ingested As may go through the placental barrier affecting the fetus, it is transported in the blood joined to the red cells and it is distributed throughout the body; once absorbed, it is oxidized and methylated in the liver to form monomethylarsonic acid and dimethylarsinic; this process may lead to the formation of dimethylated As metabolites. Most As is readily excreted in urine as MMA and DMA (5).

As has high affinity with the sulfhydryl groups, rich in keratin and tend to concentrate on the skin but it may be deposited also in bones, teeth, hair, and fingernails, mostly when the exposure is chronic (5). The evident signs of chronic As toxicity are skin changes and wart or callus formation on palms or soles, along interspersed hyperpigmentation areas on the face, neck, and back (86). Chronic exposure may produce neurotoxicity of the central and peripheral nervous system while the signs of As acute toxicity are mainly: vomit, diarrhea, cramps, salivation, fever, cardiovascular disorders, and it may lead to death (5). Arsenic is carcinogenic for group 1 humans and it is mainly related with lung, kidney, bladder, and skin cancer (28). Arsenic compounds affect human immune function. In environmentally exposed children, there is not correlation between total arsenic in urine and superoxide anion production and in adult smelter workers, higher levels of urinary arsenic correlated with increased lipid peroxidation and lower vitamin E levels in blood (5).

According to researches conducted in Brazil, China, Mediterranean Sea and Taiwan, among analyzed toxic metals As concentrations were the highest (54, 56, 59, 57). The highest and lowest observed were 2.61 ± 1.47 μg As/ g wet weight for *T. thynuss* and 0.39± 0.25 μg As/ g wet weight for *L. griseus* in Mediterranean Sea and the Pearl River delta region (China) (56,59). Chen et al, reported that As concentrations in fish muscles varied depending on the capture place which are widely influenced by human activities (54). As concentration reported in Brazil and Taiwan were similar: 0.600 ± 0.200 25 μg As/g wet weight for *C. crysos and* 0.683± 0.175 for *M. cyprinoids* (54, 57) .

PTWI for inorganic As previous recommendations of 2.1 μg As/Kg of body weight determined by JECFA have been withdrawn because they are considered without protective effect (87).

DISCUSSION

This paper develops general and important aspects of pollution of Hg, Cd, Pb and As in some fisheries resources of commercial importance, give an overview of each metal, their natural and anthropogenic sources, cycles and speciations, toxicity and health effects, state regulations and recommendations for tolerable intake. It is important to note that the information shown here provide an overview of the conditions that determine the concentration of Hg, Cd, Pb and As in marine ecosystems.

In the global context, studies on trace elements contents in fish and their health risks are related to factors like the characteristics of the fish, according to Denton et al. Chen et. Al. in their researches, Hg contents in fish muscular tissue and liver tissue are clearly dependent on trophic levels, observing higher mercury levels in largest, most enduring, and predatory fish (46, 54). However, not only the species, the size, and the age of the fish are the determining factors for a higher metal content, as Ploetz et al. point out in their *S. cavalla* study which did not find any correlation between the size of the fish and its muscle cadmium content (11) and Chen et al. did not observe differences in the As concentrations according to the species of the fish (54). Other characteristics are also determinant for biota metalscontent, as pointed out by Velusamy et al in their Mumbay Bay (India) findings, with higher trace metal accumulation in demersal fish followed by neritic and pelagic fish (88). Additionally, aquatic ecosystem environmental conditions like the level of contamination, increase metal concentrations due to fish susceptibility to toxic substances present in water, as stated by Thiyagarajan et al, on their studies in different zones on the southwest coast of India related to high populations and industrial effluents as the main metal contamination sources in coastal waters (55).

Some studies have given importance to the effects of the season on metal concentration in fish, making comparisons between the winter and summer seasons. Saei-Dehkordi and Fallah refer in their Persian Gulf studies conditions as water temperature, fish dietary factors, and the growth as determinants in the fluctuation of metal concentrations. Researchers have found higher

mercury, arsenic, cadmium, and lead levels in fish during the winter in comparison to the summer; these significant differences may be attributed to the precipitation of waste caused by rain (58, 72). In other findings, Marrugo et al. reported contradictions in higher Hg values during the dry season; however, it is mainly associated to the increase of contaminated sources (mining activities) during this season (60).

Trace elements content varies depending on the zone, environmental conditions, the contamination level of the fishing area, and the characteristics of the fish (size, endurance, and diet) constitute relevant factors, being some fish more prone to accumulating higher concentrations of these metals in the muscle. Data taken from researches conducted in the Gulf of Mexico, Iran, Brazil, Australia, China, India, Egypt, western and central Pacific Ocean, Mediterranean Sea and Taiwan, where Hg, Cd, Pb and As contents in *Caranx, Scomberomorus*, *Epinephelus, Euthynnus, Lutjanus, Thunnus and Megalops* fish genera were determined, (Table 1), allows determining safe consumption limits according to Hg, Cd, Pb and As content in fish, using the method proposed by Ikem and Egievor (89), when comparing these data with allowed tolerable intake according to Codex Alimentarius and JECFA international regulation and using the fish consumption recommendation used by FDA and U.S. EPA for 8-12 ounce weekly consumption (23); for instance: If an average 70 Kg adult is considered with an ingest of 360 g/week of *C. melampygus* fish in Queensland (Australia) with a mercury content of 0.804 μg Hg /g wet weight (Table 1) which corresponds to a value of 4.134 μg Hg/kg of body weight per week (0.804 μg Hg/g wet weight x 360 g/70 kg), this consumption would represent possible risks given it is above the 4 μg Hg/ kg of body weight PTWI determined for humans by CODEX and JECFA (63). Taking into account that MeHg contribution to total mercury in fish generally corresponds to between 80% and 100%, using the same calculations for a 360 g/week consumption of *C. melampygus* of the Queensland study, it would represent a weekly ingest of 3.307 μg MeHg/kg of body weight, exceeding the 1.6 μg/kg of body weight PTWI for humans determined by CODEX and JECFA (63, 64). Through this method it is possible to estimate the risk of fish consumption according to metal content in the Cd case data collected in the US, Trinidad, Brazil, China, Iran, India, and Taiwan for *S. Commerson, E. Coioides, C. crysos, L. griseus, L. stellatus and M. cyprinoids* species; none of them represented a health risk because they did not exceed the 25 μg Cd/ Kg of body weight PTMI determined by CODEX and JECFA (63, 73). Currently, there are no availed CODEX and JECFA criteria to determine safe Pb and As ingests (63, 81, 87).

Usually in highly contaminated aquatic habitats the concentrations of metals in the muscle of the fish, particularly mercury, exceed the permissible limits for human consumption and involve serious health threats (46, 49), however some investigators as Kehrig et al. Havelková et al. and Farkas et al. have shown that metals concentration in fish muscle can also be found in areas with low or absent sources of pollution (90–92).

Levels of Hg, Cd, Pb and As in water bodies determine significantly the content of these elements in fish tissues (55, 93), therefore it's not enough with only know characteristics such as species, age and size of the fish, but also take into account these metal releases from natural and anthropogenic sources into marine ecosystems. Although exist reports of the metals concentration in several oceans from the world, more studies about their distribution in the water bodies are required to understand the metals behavior in marine currents, allow the development of models for measuring the relationship between emission sites and reception regions (94, 95).

The limitations of this review lie in the few information about the marine currents effects in the distribution of trace elements across water bodies, which makes it difficult assessing the relation- ship between emission source and receptor region and how this affect the distribution, accumulation and concentration of metals in marine ecosystems, on the other hand it is difficult to conclude one specific cause that determines the content of toxic metals in fish species, since this is not only a single factor but the whole of them . A future revision requires more research that clearly concludes on the ecotoxicology, cycles and speciation of some metals and their effects on the human body, as well as relations between water bodies and the distribution of trace elements.

CONCLUSION

The Hg, Cd, Pb and As content in fish tissue are determine by several aspects that includes both fish characteristics and metal behavior in the marine ecosystem, by which is important take in count

each of these, in any study of this field. Also in important note that metal content in fish represents a public health risk in populations with frequent fish consumption, due to the toxicity it may represent to the different human body systems. Among these metals, mercury outstands due to its high levels of concentration in the muscular tissue in some fish species and the capacity to form more toxic compounds, as well as its high availability in aquatic ecosystems due to constant industrial use, being, particularly, gold mining in the South American case. Some of the species studied in the present paper are migratory fish, that is an important bioindicator of the increase contamination from the research areas. Studies submitted in this review, may be used as the base for future comparisons with Hg, Cd, Pb and As concentration values in different fish studies for the *Caranx, Scomberomorus, Epinephelus, Euthynnus, Lutjanus, Thunnus and Megalops* genera in order to be able to determine consumption recommendations and warnings.

ACKNOWLEDGEMENTS

We would like to thank Antioquia Governor's Office Royalty General System, the financing entity, and Universidad de Antioquia.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

1. Patterson J. Introduction - Comparative dietary risk: Balance the risk and benefits of fish consumption. Comments Toxicol. 2002;8(4-6):337–43.
2. Kadar I, Koncz J, Fekete S. Experimental study of Cd, Hg, Mo, Pb and Semovement in soil-plant-animal systems. krmiva. 2001;43(3):185–90.
3. Burger J, Gochfeld M. A framework and information needs for the management of the risks from consumption of self-caught fish. Environ Res. 2006;101(2):275–85.
4. Kojadinovic J, Potier M, Le Corre M, Cosson RP, Bustamante P. Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean. Environ Pollut. 2007;146(2):548–66.
5. Tokar EJ, Boyd WA, Freedman JH, Waalkes MP. Toxic effects of metals. Casarett and Doull's Toxicology. 8th ed. McGraw-HilL; 2015. p. 933–80.
6. Zhang L, Wong MH. Environmental mercury contamination in China: Sources and impacts. Environ Int. 2007;33(1):108–21.
7. Agency for Toxic Substance and Disease Registry. Toxicological Profile for Lead. Atlanta: U.S. Department of Health and Humans Services, Public Health Service, Centres for Diseases Control; 2007.
8. Agency for Toxic Substance and Disease Registry. Toxicological Profile for Arsenic. Atlanta: U.S. Department of Health and Humans Services, Public Health Service, Centres for Diseases Control; 2007.
9. Henley RW, Berger BR. Nature's refineries - Metals and metalloids in arc volcanoes. Earth-Science Rev. 2013;125:146–70.
10. He ZL, Yang XE, Stoffella PJ. Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol. 2005;19(2-3):125–40.
11. Ploetz DM, Fitts BE, Rice TM. Differential accumulation of heavy metals in muscle and liver of a marine fish, (King Mackerel, Scomberomorus cavalla Cuvier) from the Northern Gulf of Mexico, USA. Bull Environ Contam Toxicol. 2007;78(2):124–7.
12. Chandra Sekhar K, Chary NS, Kamala CT, Suman Raj DS, Sreenivasa Rao A. Fractionation studies and bioaccumulation of sediment-bound heavy metals in Kolleru lake by edible fish. Environ Int. 2004;29(7):1001–8.
13. Cheng-xiu LU, Jie-min C. Speciation of Heavy Metals in the Sediments from Different Eutrophic Lakes of China. Procedia Eng. 2011;18:318–23.
14. Pempkowiak J, Sikora A, Biernacka E. Speciation of heavy metals in marine sediments vs their bioaccumulation by mussels. Chemosphere. 1999;39(2):313–21.
15. Zhang L, Shi Z, Zhang J, Jiang Z, Wang F, Huang X. Spatial and seasonal characteristics of dissolved heavy metals in the east and west Guangdong coastal waters, South China. Mar Pollut Bull. Elsevier Ltd; 2015;95(1):419–26.
16. Nfon E, Cousins IT, Järvinen O, Mukherjee AB, Verta M, Broman D. Trophodynamics of mercury and other trace elements in a pelagic food chain from the Baltic Sea. Sci Total Environ. Elsevier B.V.; 2009;407(24):6267–74.
17. Olmedo P, Pla A, Hernández a. F, Barbier F, Ayouni L, Gil F. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environ Int. Elsevier Ltd; 2013;59:63–72.
18. Qiu Y-W. Bioaccumulation of heavy metals both in wild and mariculture food chains in Daya Bay, South China. Estuar Coast Shelf Sci. Elsevier Ltd; 2015;163:7–14.
19. Gray JS. Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull. 2002;45:46–52.
20. Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003;68:167–82.
21. Agency for Toxic Substance and Disease Registry. Substance Priority List. Department of Health and Humans Services, Public Health Service,Centres for Diseases Control, Atlanta, GA.; 2013.
22. Castro-González MI, Méndez-Armenta M. Heavy metals: Implications associated to fish consumption. Environ Toxicol Pharmacol. 2008;26(3):263–71.
23. United States Environmental Protection Agency. Fish: what pregnant women and parents should know [Internet]. 2014 [cited 2014 Sep 6]. Available from: http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm393070.htm
24. Ceccatelli S, Daré E, Moors M. Methylmercury-induced neurotoxicity and apoptosis. Chem Biol Interact. 2010;188(2):301–8.
25. Carocci A, Rovito N, Sinicropi MS, Genchi G. Mercury toxicity and neurodegenerative effects. Reviews of Environmental Contamination and Toxicology. 2014. p. 1–18.
26. Zhang W-L, Du Y, Zhai M-M, Shang Q. Cadmium exposure and its health effects: A 19-year follow-up study of a polluted area in China. Sci Total Environ. 2014;470–471:224–8.
27. Méndez-Armenta M, Ríos C. Cadmium neurotoxicity. Environ Toxicol Pharmacol. 2007;23(3):350–8.
28. International Agency for Research in Cancer. Monographs on the evaluation on carcinogenics risks to humans [Internet]. 2014 [cited 2014 Sep 11]. Available from: http://monographs.iarc.fr/ENG/Classification/
29. Bertin G, Averbeck D. Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie. 2006;88(11):1549–59.
30. Gwaltney-Brant SM, Haschek WM, Rousseaux CG, Wallig MA. Chapter 41 - Heavy Metals. Haschek and Rousseaux's Handbook

of Toxicologic Pathology (Third Edition). Boston: Academic Press; 2013. p. 1315–47.
31. Garza A, Vega R, Soto E. Cellular mechanisms of lead neurotoxicity. Med Sci Monit. 2006/02/28 ed. Instituto de Fisiologia, Universidad Autonoma de Puebla, Pue, Mexico.; 2006;12(3):57–65.
32. Abernathy CO, Thomas DJ, Calderon RL. Health effects and risk assessment of arsenic. J Nutr. 2003;133((5 Suppl 1)):1536s – 8s.
33. Hughes MF. Arsenic toxicity and potential mechanisms of action. Toxicol Lett. 2002;133(1):1–16.
34. McCurry J. Japan remembers Minamata. Lancet. England; 2006. p. 99–100.
35. Inaba T, Kobayashi E, Suwazono Y, Uetani M, Oishi M, Nakagawa H, et al. Estimation of cumulative cadmium intake causing Itai–itai disease. Toxicol Lett. 2005;159(2):192–201.
36. De Jesus IS, da Silva Medeiros RL, Cestari MM, de Almeida Bezerra M, de Mello Affonso PRA. Analysis of Metal Contamination and Bioindicator Potential of Predatory Fish Species Along Contas River Basin in Northeastern Brazil. Bull Environ Contam Toxicol. 2014;1–6.
37. Olivero J, Johnson B. El lado gris de la minería del oro: la contaminación con mercurio en el norte de Colombia. Colombia: Universidad de Cartagena; 2002.
38. Bustamante C, Olivares F, Jimenez W. Calderos de mayor importancia en la pesca artesanal de Golfo de Urabá. Autoridad nacional de acuicultura y pesca; 2012.
39. SEPEC.
40. Ministerio de Ambiente Vivienda y Desarrollo territorial. Cuantificación de liberaciones antropogénicas de mercurio en colombia. Republica de Colombia; 2010. p. 0–82.
41. EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal . 2012;10:241.
42. Tan SW, Meiller JC, Mahaffey KR. The endocrine effects of mercury in humans and wildlife. Crit Rev Toxicol. 2009;39(3):228–69.
43. Debes F, Weihe P, Grandjean P. Cognitive deficits at age 22 years associated with prenatal exposure to methylmercury. Cortex. 2015 Jun;
44. Karagas MR, Choi AL, Oken E, Horvat M, Schoeny R, Kamai E. Review Evidence on the Human Health Effects of Low-Level Methylmercury Exposure. Env Heal Perspect. 2012;120(6):799–806.
45. Enviromental Protection Agency (EPA). Mercury in Marine Life Database. U.S. Office of Wetlands, Oceans, and Wathersheds. Washington, DC; 2003.
46. Denton GRW, Burdon-Jones C. Trace metals in fish from the Great Barrier Reef. Mar Pollut Bull. 1986;17:201–9.
47. El-Moselhy KM, Othman AI, Abd El-Azem H, El-Metwally ME a. Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. Egypt J Basic Appl Sci. Elsevier Ltd; 2014;1(2):1–9.
48. Ordiano-Flores A, Rosíles-Martínez R, Galván-Magaña F. Biomagnification of mercury and its antagonistic interaction with selenium in yellowfin tuna Thunnus albacares in the trophic web of Baja California Sur, Mexico. Ecotoxicol Environ Saf. 2012;86:182–7.
49. Chen CY, Lai CC, Chen KS, Hsu CC, Hung CC, Chen MH. Total and organic mercury concentrations in the muscles of Pacific albacore (Thunnus alalunga) and bigeye tuna (Thunnus obesus). Mar Pollut Bull. Elsevier Ltd; 2014;85(2):606–12.
50. Lima APS, Sarkis JES, Shihomatsu HM, Müller RCS. Mercury and selenium concentrations in fish samples from Cachoeira do PiriáMunicipality, ParáState, Brazil. Environ Res. 2005;97(3):236–44.
51. Dabeka R, McKenzie AD, Forsyth DS, Conacher HB. Survey of total mercury in some edible fish and shellfish species collected in Canada in 2002. Food Addit Contam. 2004;21(5):434–40.
52. Mendez E, Giudice H, Pereira A, Inocente G, Medina D. Total Mercury Content—Fish Weight Relationship in Swordfish (Xiphias gladius) Caught in the Southwest Atlantic Ocean. J Food Compos Anal. 2001;14(5):453–60.
53. Hightower JM, Moore D. Mercury levels in high-end consumers of fish. Env Heal Perspect. 2003;111(4):604–8.
54. Chen YC, Chen CY, Hwang HJ, Chang WB, Yeh WJ, Chen MH. Comparison of the metal concentrations in muscle and liver tissues of fishes from the Erren River, Southwestern Taiwan, after the restoration in 2000. J Food Drug Anal. 2004;12(4):358–66.
55. Thiyagarajan D, Dhaneesh K V, Kumar TTA, Kumaresan S, Balasubramanian T. Metals in fish along the southeast coast of India. Bull Environ Contam Toxicol. 2012;88(4):582–8.
56. Leung HM, Leung AOW, Wang HS, Ma KK, Liang Y, Ho KC, et al. Assessment of heavy metals/metalloid (As, Pb, Cd, Ni, Zn, Cr, Cu, Mn) concentrations in edible fish species tissue in the Pearl River Delta (PRD), China. Mar Pollut Bull. 2014;78:235–45.
57. Medeiros RJ, dos Santos LMG, Freire AS, Santelli RE, Braga AMCB, Krauss TM, et al. Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. Food Control. 2012;23(2):535–41.
58. Saei-Dehkordi SS, Fallah AA. Determination of copper, lead, cadmium and zinc content in commercially valuable fish species from the Persian Gulf using derivative potentiometric stripping analysis. Microchem J. 2011;98:156–62.
59. Storelli MM, Storelli MM, Storelli A, Storelli A, Marcotrigiano GO, Marcotrigiano GO. Accumulation of mercury, cadmium, lead and arsenic in sword sh and blue n tuna from the Mediterranean Sea: A comparative study. Mar Pollut Bull. 2005;50:1004–7.
60. Marrugo-Negrete J, Benitez LN, Olivero-Verbel J. Distribution of mercury in several environmental compartments in an aquatic ecosystem impacted by gold mining in northern Colombia. Arch Env Contam Toxicol. 2008;55(2):305–16.
61. Olivero-Verbel J, Caballero-Gallardo K, Marrugo Negrete J. Relationship between localization of gold mining areas and hair mercury levels in people from Bolivar, north of Colombia. Biol Trace Elem Res. 2011;144:118–32.
62. Olivero J, Johnson B, Arguello E. Human exposure to mercury in San Jorge river basin, Colombia (South America). Sci Total Environ. 2002;289:41–7.
63. Codex Alimentarius Commission. Joint FAO/WHO food standards programme Codex Committee on contaminants in foods sixth session [Internet]. 2012 [cited 2014 Jun 10]. Available from: ftp://ftp.fao.org/codex/meetings/cccf/cccf6/cf06_INFe.pdf.
64. FAO/WHO Expert Commite on Food Additives. Methylmercury [Internet]. 2011 [cited 2014 Nov 20]. Available from: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3083
65. Agency for Toxic Substance and Disease Registry. Toxicological Profile for Cadmium. Atlanta: U.S. Department of Health and Humans Services, Public Health Service, Centres for Diseases Control; 2012.
66. Auger P a., Machu E, Gorgues T, Grima N, Waeles M. Comparative study of potential transfer of natural and anthropogenic cadmium to plankton communities in the North-West African upwelling. Sci Total Environ. Elsevier B.V.; 2015;505:870–88.
67. Horner TJ, Lee RBY, Henderson GM, Rickaby REM. Nonspecific uptake and homeostasis drive the oceanic cadmium cycle. Proc Natl Acad Sci U S A. 2013;110(7):2500–5.
68. Hendry KR, Rickaby REM, de Hoog JCM, Weston K, Rehkämper M. Cadmium and phosphate in coastal Antarctic seawater: Implications for Southern Ocean nutrient cycling. Mar Chem. Elsevier B.V.; 2008;112(3-4):149–57.

69. Houston MC. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and-myocardial infraction. Altern Ther Heal Med. 13:128–33.
70. Kim K. Blood cadmium concentration and lipid profile in Korean adults. Environ Res. 2012;112:225–9.
71. Thatcher RW, Lester ML, McAlester R, Horts R. Effects of low levels of cadmium and lead on cognitive functions in children . Arch Environ Heal. 37:159–66.
72. Saei-Dehkordi SS, Fallah AA, Nematollahi A. Arsenic and mercury in commercially valuable fish species from the Persian Gulf: Influence of season and habitat. Food Chem Toxicol. 2010;48(10):2945–50.
73. FAO/WHO Expert Committe on Food Additives. Cadmium [Internet]. 2013 [cited 2014 Nov 20]. Available from: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1376
74. Sepe A, Ciaralli L, Ciprotti M, Giordano R, Fumari E, Costantini S. Determination of cadmium, chromium, lead and vanadium in six fish species from the Adriatic Sea . Food Addit Contam. 2003;20:543–52.
75. Chakraborty P, Babu PVR, Sarma V V. A study of lead and cadmium speciation in some estuarine and coastal sediments. Chem Geol. Elsevier B.V.; 2012;294-295:217–25.
76. Woosley RJ, Millero FJ. Pitzer model for the speciation of lead chloride and carbonate complexes in natural waters. Mar Chem. Elsevier B.V.; 2013;149:1–7.
77. Heier LS, Lien IB, Strømseng AE, Ljønes M, Rosseland BO, Tollefsen KE, et al. Speciation of lead, copper, zinc and antimony in water draining a shooting range-Time dependant metal accumulation and biomarker responses in brown trout (Salmo trutta L.). Sci Total Environ. Elsevier B.V.; 2009;407(13):4047–55.
78. Papanikolaou CN, Hatzidaki GE, Belivanis S, Tzanakakis GN, Tsatsakis MA. Lead toxicity update, A brief review. Med Sci Monit. 2005;11:329–36.
79. Liu P, Wang C-N, Song X-Y, Wu Y-N. Dietary intake of lead and cadmium by children and adults – Result calculated from dietary recall and available lead/cadmium level in food in comparison to result from food duplicate diet method. Int J Hyg Environ Health. 2010;213(6):450–7.
80. Eisler R. Compendium of Trace Metals and Marine Biota. Compendium of Trace Metals and Marine Biota. 2010.
81. FAO/WHO Expert Committe on Food Additives. Lead [Internet]. 2011 [cited 2014 Nov 20]. Available from: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3511
82. Matschullat J. Arsenic in the geosphere--a review. Sci Total Environ. 2000;249:297–312.
83. Marlborough SJ, Wilson VL. Arsenic speciation driving risk based corrective action. Sci Total Environ. Elsevier B.V.; 2015;520:253–9.
84. Ruttens a., Blanpain a. C, De Temmerman L, Waegeneers N. Arsenic speciation in food in Belgium. Part 1: Fish, molluscs and crustaceans. J Geochemical Explor. Elsevier B.V.; 2012;121:55–61.
85. Amlund H, Ingebrigtsen K, Hylland K, Ruus A, Eriksen DØ, Berntssen MHG. Disposition of arsenobetaine in two marine fish species following administration of a single oral dose of [14C] arsenobetaine. Comp Biochem Physiol - C Toxicol Pharmacol. 2006;143(2):171–8.
86. Kakkar P, Jaffery NF. Biological markers for metal toxicity. Environ Toxicol Pharmacol. 2005;19(2):335–49.
87. FAO/WHO Expert Commite on Food Aditives. Arsenic [Internet]. 2011 [cited 2014 Nov 20]. Available from: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1863
88. Velusamy A, Satheesh Kumar P, Ram A, Chinnadurai S. Bioaccumulation of heavy metals in commercially important marine fishes from Mumbai Harbor, India. Mar Pollut Bull. 2014;81:218–24.
89. Ikem A, Egiebor NO. Assessment of trace elements in canned fishes (mackerel, tuna, salmon, sardines and herrings) marketed in Georgia and Alabama (United States of America). J Food Compos Anal. 2005;18(8):771–87.
90. Kehrig H a., Malm O, Moreira I. Mercury in a widely consumed fish Micropogonias furnieri (Demarest, 1823) from four main Brazilian estuaries. Sci Total Environ. 1998;213:263–71.
91. Havelková M, Dušek L, Némethová D, Poleszczuk G, Svobodová Z. Comparison of mercury distribution between liver and muscle - A biomonitoring of fish from lightly and heavily contaminated localities. Sensors. 2008;8(7):4095–109.
92. Farkas A, Salánki J, Specziár A. Age- and size-specific patterns of heavy metals in the organs of freshwater fish Abramis brama L. populating a low-contaminated site. Water Res. 2003;37(5):959–64.
93. França S, Vinagre C, Caçador I, Cabral HN. Heavy metal concentrations in sediment, benthic invertebrates and fish in three salt marsh areas subjected to different pollution loads in the Tagus Estuary (Portugal). Mar Pollut Bull. 2005;50(9):998–1003.
94. Aparicio-González A, Duarte CM, Tovar-Sánchez A. Trace metals in deep ocean waters: A review. J Mar Syst. Elsevier B.V.; 2012;100-101:26–33.
95. Kocman D, Horvat M, Pirrone N, Cinnirella S. Contribution of contaminated sites to the global mercury budget. Environ Res. Elsevier; 2013;125:160–70.

EVALUADORES / REVIEWERS

El comité Editorial expresa sus agradecimientos a todas las personas que colaboraron en la evaluación de los manuscritos puestos a consideración para ser publicados en la Revista Vitae Volumen 22 No. 2.

Editorial Board expresses gratitude to reviewers who cooperated with the evaluation process of all the manuscripts published in this volume 22 Nº2 of Revista Vitae.

Amparo Alegría Torán
University of Valencia
Madrid - España

Martha Mesías
Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN)
Madrid -España

Juan Torres
Nutresa
Medellín -Colombia

William Albarracín Hernández
Universidad de Nariño
Nariño – Colombia

Eduardo Rodríguez Sandoval
Universidad Nacional de Colombia
Medellín – Colombia

Raquel Rodríguez Raposo
Universidad de la Laguna
Santa Cruz de Tenerife - España

Dario Tellez
Instituto Politécnico Nacional
Mexico

Gustavo Fidel Gutiérrez López
Instituto Politécnico Nacional
Mexico

John Rojas
Universidad de Antioquia
Medellín - Colombia

Héctor Suarez Mahecha
Universidad Nacional de Colombia
Medellín – Colombia

Paulo Roberto Campagnoli de Oliveira Filho
Universidade Estadual Paulista
Sao Paulo - Brasil

Claudia Ramírez Botero
Universidad de Antioquia
Medellín - Colombia

Claudio Jiménez Cartagena
Universidad de Antioquia
Medellín - Colombia

Hermes Rafael Santis
Politécnico Colombiano Jaime Isaza Cadavid
Medellín - Colombia

Edwin Moncada Acevedo
Instituto Tecnológico Metropolitano
Medellín - Colombia

Marina Arrieta
Universidad Politécnica de Valencia
Valencia - España

Lida Andrea Quinchía Bustamante
Fresenius Kabi Deutschland GMBH
Bad Homburg - Germany

Oscar Vega
Universidad de Antioquia
Medellín - Colombia

INSTRUCCIONES A LOS AUTORES*

Acta 22 de Junio de 2012

Alcance y política de revisión

La Revista VITAE es una publicación científica de la Facultad de Química Farmacéutica de la Universidad de Antioquia, con una periodicidad cuatrimestral, que tiene como misión la divulgación del desarrollo y los avances académicos e investigativos en los diversos campos de las ciencias farmacéuticas, alimentarias y afines. Publica manuscritos originales e inéditos, los cuales son seleccionados por el Comité Editorial y evaluados por pares nacionales e internacionales. La responsabilidad por los juicios, opiniones y puntos de vista expresados en los manuscritos publicados corresponde exclusivamente a los autores. La posición de la Facultad se consigna en la sección Editorial.

Reserva de derechos

El estudio y la selección de los manuscritos enviados por los colaboradores están a cargo del Comité Editorial. La recepción de un manuscrito no implica la aceptación ni publicación del mismo. En los manuscritos aceptados, el Comité Editorial se reserva el derecho de realizar las modificaciones editoriales necesarias para su publicación, al igual que su fecha de aparición en la Revista.

Tipos y Clasificación de los manuscritos

La Revista VITAE publica los siguientes tipos de manuscritos:

- Artículos Resultado de Investigación
- Artículos Cortos
- Revisiones Estructuradas
- Editorial y Comentarios Editoriales
- Cartas al Editor

Los artículos son clasificados en una de las siguientes secciones:

- Alimentos: Ciencia, Tecnología e Ingeniería
- Atención Farmacéutica
- Biotecnología
- Farmacología y Toxicología
- Industrial Farmacéutica
- Productos Naturales

Presentación de manuscritos

La Revista VITAE acepta para evaluación manuscritos en español y/o en inglés. El envío del manuscrito se debe realizar a través de la plataforma Open Journal System, donde la Revista administra los procesos de evaluación y publicación. Para ello, se debe dirigir a la página web: www.udea.edu.co/vitae. De igual manera, se debe adjuntar los documentos solicitados por el equipo editorial, tal como se especifica en la información consignada en dicha página: los formatos (información del manuscrito y de los autores) y la licencia de acceso abierto. En esta página web encontrará una versión amplia de estas instrucciones, donde podrá consultar todo lo relacionado a los parámetros de presentación del manuscrito e información completa acerca de la estructura de cada uno de los tipos de manuscrito y las normas de estilos de los mismos.

Revisión previa al cumplimiento de las normas y políticas editoriales:

Verificación del cumplimiento de las normas editoriales. El Equipo Editorial realiza una revisión en la que se verifica que el manuscrito cumpla con las normas estipuladas en este documento: entrega de la información solicitada, licenciamiento de la obra, estructura completa y adecuada del manuscrito y citación de acuerdo a las normas Vancouver. El autor puede verificar el cumplimiento de los requisitos antes de enviar el manuscrito utilizando la ***Lista de Verificación*** que se encuentra disponible en la página web, en *Author's forms and guidelines*.

Revisión Editorial. Posterior a la verificación del cumplimiento de las normas editoriales, antes de ser enviados a la evaluación por pares, el Comité Editorial realiza una evaluación previa de todos los manuscritos que cumplen las normas editoriales. El propósito de esta pre-revisión es garantizar que la estructura del manuscrito y los contenidos sean claros, pertinentes y reportados adecuadamente, con el fin de facilitar la evaluación por parte de los pares. Como resultado, el manuscrito puede ser enviado a revisión por pares, devuelto a los autores para correcciones o rechazado.

Revisión por pares (*peer-review*):

Una vez el Comité Editorial verifica que el manuscrito cumple con todos los parámetros establecidos por la Revista envía el manuscrito a dos pares, como mínimo, quienes deben emitir su concepto por escrito en el formato establecido para ello, a través de la plataforma *Open Journal System*. El Equipo Editorial revisa y valora las evaluaciones, si es necesario se asesora de personas idóneas y como resultado, **acepta la publicación del manuscrito, lo devuelve a los autores para correcciones, o lo rechaza de forma definitiva**.

En los casos en que se solicitan correcciones, los autores deben enviar la nueva versión a través de la misma plataforma en un plazo máximo de catorce días calendario a partir de la fecha de notificación. En la corrección de pruebas de impresión final, sólo se permite cambios de forma relativos a redacción o estilo.

El manuscrito se publica en línea y de forma impresa, de la cual se envía tres ejemplares al autor principal.

Costos de publicación

El valor a pagar por manuscrito, exceptuando las cartas al editor y los comentarios editoriales, es de trescientos cincuenta mil pesos colombianos ($440.000 COP), para transacciones nacionales, o doscientos dólares ($220 USD), para transacciones internacionales. Este valor se paga cuando se notifica la aceptación para la publicación de la versión definitiva del manuscrito. La impresión de gráficos, figuras o fotografías en color es opcional y tiene un costo adicional por página necesaria de cien mil pesos colombianos ($100.000 COP) para transacciones nacionales o sesenta y cinco dólares ($65 USD) para transacciones internacionales.

Licenciamiento del manuscrito

Los manuscritos publicados en la Revista VITAE quedan disponibles gratuitamente para la consulta pública, tanto en el sitio web como en los diferentes sistemas de indexación y bases de datos a los que está suscrita la Revista, bajo la Licencia Creative Commons, en el modo Attribution-Noncommercial-No Derivative Works aprobada en Colombia y por tanto son de acceso abierto (*Open Access*). Por tanto, los autores ceden, sin derecho a retribuciones económicas, a la Universidad de Antioquia, Revista VITAE, los Derechos sobre la publicación y reproducción en diferentes medios de difusión por el tiempo que establezca la normatividad vigente, mediante el documento de Licencia de Acceso Abierto a la Publicación propuesto para tal fin.

* La documentación requerida para presentar los manuscritos: los formatos, la lista de verificación, la licencia de acceso abierto a la publicación y una copia de estas instrucciones pueden ser descargados del sitio web www.udea.edu.co/vitae

INSTRUCTIONS TO AUTHORS*

Minutes No. 22 of June, 2012

SCOPE AND REVISION POLICIES

The Journal VITAE is a four-monthly scientific publication of the Pharmaceutical Chemistry Faculty of the University of Antioquia, which has the mission of spreading the voice about the development and the academic and research advances in the various fields of pharmaceutical, food and related sciences. The Journal publishes original and novel manuscripts, which are selected by the Editorial Board and evaluated by national and international peers. The responsibility over judgments, opinions and points of view expressed in the published manuscripts lies exclusively on the authors. The statement of the Faculty is recorded in the Editorial section.

RESERVATION OF RIGHTS

The evaluation and selection of the manuscripts submitted by the collaborators are in charge of the Editorial Board. The reception of a manuscript does not imply neither its approval nor publication. For the accepted manuscripts, the Editorial Board reserves the right to perform the necessary editorial modifications for its publication, as well as its release date in the Journal.

TYPES AND CLASIFICATION OF MANUSCRIPTS

The Journal Vitae publishes the following types of manuscripts:

- Articles of research results
- Short articles
- Structured Reviews
- Editorial section and Editorial comments
- Letters to the Editor

The articles are classified in one of the following sections:

- Foods: Science, technology and engineering.
- Pharmaceutical care
- Biotechnology
- Pharmacology and toxicology
- Pharmaceutical Industry
- Natural products

SUBMISSION OF MANUSCRIPTS

The Journal VITAE receives either English or Spanish written articles for evaluation. The submission of the article must be done through the Open Journal System platform, where the Journal manages the evaluation and publication processes. For this, the authors must go to the web page: www.udea.edu.co/vitae. Likewise, the requested documentation by the Editorial team must be attached as it is specified through the information available in the web page: the forms (information about the manuscript and the authors) and the Open access license. In this web page the authors will find a larger version of these instructions, where will be able to find everything related to the submission parameters of the manuscript and complete information about the structure of every type of manuscript and its style rules.

PREVIOUS REVISION TO THE FULFILLMENT OF THE NORMS AND EDITORIAL POLICIES

Verification of the fulfillment of the editorial norms. The Editorial Team performs a revision in which is verified that the manuscript meets the stipulated norms in this document: submission of the requested information, licensing of the work, complete and proper structure of the manuscript and quotation in accordance with the Vancouver rules. The author may verify the fulfillment of the requirements before submitting the manuscript by using the List of verification, which is available in the web page in the Author's forms and guidelines sections.

Editorial revision. After the verification of the fulfillment of the editorial norms, and before being sent to the peers for evaluation, the Editorial Board performs a previous evaluation of all manuscripts that meet the editorial norms. The purpose of this previous revision is to guarantee that the structure of the manuscript and its contents are clear, relevant and properly reported, in order to facilitate the evaluation performed by the peers. As a result, the manuscript could be sent for peer review, returned to authors for corrections or rejected.

PEER REVIEW

Once the Editorial Board verifies that the manuscript meets all the established parameters by the Journal, the manuscript is sent to two peers, at least, who must give a written concept in the established format for this, through the platform Open Journal System. The Editorial Team reviews and assesses the evaluations, taking advice from qualified people if necessary, and as a result may approve the publication of the manuscript, return it to the authors for corrections, of reject it definitively.

In those cases that corrections are requested, the authors must send the new version using the platform within 14 (fourteen) calendar days since the date of notification. In the correction of tests of final printing, only form changes related to redaction and style are allowed.

The manuscript is published online and in printed version, which is sent 3 (three) copies to the main author.

PUBLICATION CHARGES

The amount payable for a manuscript, excluding the letters to editor and the editorial comments, is three hundred and fifty thousand Colombian Pesos ($440.000 COP) for national transactions, or two hundred dollars ($220 USD) for international transactions. This amount is paid when the approval for the publication of the manuscript's final version is notified. The printing of graphics, figures or color photographs is optional and applies extra charge of one hundred Colombian Pesos ($100.000 COP) per required page, for national transactions, or sixty five dollars ($65 USD) for international transactions.

LICENSING OF THE WORK

The manuscripts published in The Journal VITAE remain freely available for public consultation on the web site as on the different indexing systems and data bases that the Journal is subscribed, under the license Creative Commons, in the mode Attribution-Noncommercial-No Derivative Works, adopted in Colombia, and therefore are of Open Access. Hence the authors give, without right to economical retributions, to the University of Antioquia, Journal VITAE, the copyrights on the publication and reproduction through different diffusion media by the time set in the current regulations, by filling the document of Open Access License to the publication proposed for this purpose.

* Requested documentation for the submission of manuscripts: forms, verification list, and the Open access license to the publication. A copy of these instructions can be downloaded from the web site: www.udea.edu.co/vitae

INFORMACIÓN GRUPOS DE INVESTIGACIÓN
Facultad de Química Farmacéutica / Universidad de Antioquia

Grupos clasificados en convocatoria COLCIENCIAS 2013	Coordinador	Objetivo del Grupo
Grupo de Investigación en Sustancias Bioactivas (A1)	Prof. Edison Osorio. Magister en Ciencias Farmacéuticas. Doctor en Farmacia. Profesor área de Fitoquímica. ejosorio48@gmail.com edison.osorio@udea.edu.co	Búsqueda de nuevas alternativas terapéuticas con compuestos activos a partir de fuentes naturales. Investigación en alimentos funcionales y materias primas funcionales útiles para la industria alimentaria, cosmética y farmacéutica.
Productos Naturales Marinos (A)	Prof. Diana Margarita Márquez Fernández Magíster en Ciencias Químicas Doctora en Ciencias Químicas diana.marquez@udea.edu.co	Investigar la biodiversidad colombiana haciendo especial énfasis en los productos naturales marinos y búsqueda de productos naturales funcionales. Además hemisintetizar compuestos bioactivos, realizar marchas fitoquímicas, estandarizar y validar metodologías de análisis y control de calidad de medicamentos y productos afines.
Programa de Ofidismo y Escorpionismo (A1)	Prof. Sebastián Estrada. Magister en investigación y desarrollo de medicamentos. Sebastian.estrada@siu.udea.edu.co	Fortalecer la investigación interdisciplinaria en el campo de la toxinología. • Adelantar investigaciones relacionadas con la clínica, epidemiología y tratamiento específico de las intoxicaciones causados por animales venenosos, plantas y microorganismos. • Realizar proyectos de investigación orientados a la búsqueda de moléculas o productos con aplicación farmacéutica, alimentaría o agrícola. • Evaluar el uso de los venenos de origen natural con potencial aplicación en los campos: farmacéutico, alimentario, cosmético y agrícola. • Consolidar y ampliar las estrategias educativas en el área de la toxinología para beneficio de la sociedad. • Formar estudiantes de pregrado y posgrado en las áreas misionales del programa y facilitar los intercambios colaborativos con sectores productivos y grupos de investigación nacionales e internacionales..
Biodegradación y Bioconversión de Polímeros - BIOPOLIMER (A)	Freimar Segura Sánchez. Magister en Ciencias Farmacéuticas. Doctor en Farmacotecnia y Biofarmacia de la Universidad de Paris Sud-Francia. Profesor del Área Industrial Farmacéutica. Freimar.segura@udea.edu.co freimars@gmail.com	Biodegradar y/o bioconvertir residuos agroindustriales a productos de valor agregado como enzimas, compuestos aromáticos u otros con actividad biológica, utilizando hongos basidiomicetos o sus enzimas ligninolíticas aisladas, para obtener biocombustibles, productos farmacéuticos, alimentos para animales, o nutrientes humanos y estabilizarlos utilizando técnicas de inmovilización. Por medio de nanotecnología desarrollar transportadores inteligentes para medicamentos, cosméticos y alimentos que permitan utilizarlos de forma más segura, eficiente y eficaz.
Diseño y Formulación de Medicamentos, Cosméticos y Afines (A1)	Prof. Oscar Flórez Acosta. Doctor en Ciencias Farmacéuticas y Alimentarias. Profesor Área Industrial Farmacéutica. Oscar.florez@udea.edu.co	Diseño, formulación y reformulación de productos farmacéuticos, cosméticos y afines.
Estudios de estabilidad de medicamentos, cosméticos y alimentos (Reconocido)	Cecilia Gallardo Cabrera. Doctora en Ciencias Químicas. Profesora Área de Producción Farmacéutica. cecilia.gallardo@udea.edu.co,	Contribuir al desarrollo de la industria y al mejoramiento de la salud pública, a través de la investigación e implementación de estudios de estabilidad en medicamentos, cosméticos y alimentos, de acuerdo a consideraciones científicas y regulaciones nacionales e internacionales. Desarrollo de tecnologías viables para la estabilización de dichas matrices.
Promoción y Prevención Farmacéutica (A1)	Prof. Pedro Amariles Muñoz. Magister en Farmacia Clínica y Farmacoterapia. Doctor en Farmacología. Profesor Área de Atención Farmacéutica. grupoppf@udea.edu.co www.udea.edu.co/pypfarmaceutica	Evidenciar la importancia y la contribución del profesional farmacéutico a la utilización, efectiva, segura y económica de los medicamentos, al igual que al mejoramiento de las condiciones de salud de la comunidad en contexto del Sistema General de Seguridad Social de Colombia. En este sentido, el grupo se orienta a: (1) diseñar y realizar trabajos de investigación relacionados con la implementación y valoración del efecto en salud de los servicios de Atención Farmacéutica: Seguimiento Farmacoterapéutico, Dispensación, Indicación Farmacéutica, Farmacovigilancia, Farmacoeconomía y Educación en Salud; (2) diseñar, desarrollar y valorar el efecto de herramientas informáticas sobre la eficacia y eficiencia en la realización de los servicios de Atención Farmacéutica; y (3) realizar labores de extensión y asesoría relacionadas con intervenciones en promoción de la salud; prevención de la enfermedad; y orientación al uso efectivo, seguro y económico de los medicamentos.
Grupo de Nutrición y Tecnología de Alimentos (A1)	Prof. José Edgar Zapata Montoya. Doctor en Biotecnología. Profesor Área de Ingeniería Aplicada edgar.zapata@udea.edu.co jedgar_4@yahoo.com	Desarrollar nuevas propuestas alimentarias basadas en métodos de conservación no térmicos y en procesos biotecnológicos. Revalorar subproductos proteícos por medio de hidrolisis enzimática, modelar biorreactores enzimáticos y fermentativos. Aprovechar excedentes de cosecha de frutas y hortalizas por medio de deshidratación osmótica, secado en lecho fluidizado y secado convectivo. Elucidar rutas metabólicas de microorganismo de interés alimentario y farmacéutico. Evaluar nuevas sustancias de origen natural con actividad antioxidantes.
Biotecnología Alimentaria –BIOALI (A)	Prof. José Contreras Calderón. Doctor en Ciencia y Tecnología de Alimentos. Profesor Área de Ingeniería Aplicada. jose.contrerasc@udea.edu.co jccc78@hotmail.com	Bioconvertir materias primas y residuos agroindustriales en productos de interés alimentario mediante microorganismos. Desarrollar e implementar indicadores que permitan a la industria evaluar, controlar y mejorar la calidad de productos frescos y procesados. Diseñar, formular y estandarizar nuevos productos innovadores, funcionales y con alto valor añadido. Innovar en el desarrollo de empaques alimentarios inteligentes, funcionales y amigables con el medio ambiente. Brindar herramientas a comunidades de bajos recursos para que amplíen sus opciones y tengan acceso a alimentos saludables de bajo costo.
Grupo de Investigación en Análisis Sensorial (B)	Prof. Olga Lucía Martínez. Álvarez. M.Sc. Salud Pública. Especialista en Ciencia y Tecnología de Alimentos. Profesora Área de Ingeniería Aplicada. gruposensorial@udea.edu.co; grupsensorial@gmail.com	Investigar los factores que intervienen en la calidad organoléptica de alimentos, bebidas, cosméticos, productos naturales, farmacéuticos y afines en las etapas de I+D+i . Realizar investigaciones sobre caracterización sensorial de materias primas y productos, incluyendo denominaciones de origen. Investigación y desarrollo ingenieril de sistemas tecnológicos de producción para el sector agroindustrial. Estudiar la correlación fisicoquímica, instrumental y sensorial.
Grupos sin categoría en convocatoria COLCIENCIAS 2013	**Coordinador**	**Objetivo del Grupo**
Grupo de Estudio e Investigaciones Biofarmacéuticas	Prof. Adriana María Ruiz Correa. MSc Ciencias Básicas Biomédicas (énfasis biodisponibilidad y bioequivalencia). Doctora en Ciencias Farmacéuticas. Profesora área Industrial Farmacéutica. amaria.ruiz@udea.edu.co	Profundizar en todos aquellos aspectos que afectan la absorción de los principios activos desde su forma de dosificación y desarrollar las metodologías necesarias para determinar estos efectos. Realizar estudios biofarmacéuticos, tanto *in vivo* como *in vitro*, para verificar si la sustancia activa llega al sitio de acción y de esta manera garantizar la eficacia terapéutica.
Grupo de Investigación en Tecnología en Regencia en Farmacia (creado en 2012)	Prof. Carlos Cataño Rocha. Magister en Ciencias Químicas. Profesor del área de Ciencias Farmacéuticas. Carlos.catano@udea.edu.co	Fortalecer la investigación en el campo de acción del Tecnólogo en Regencia de Farmacia con énfasis en Programas de Atención Primaria en Salud (APS) y en Temas de Terapias Alternativas y/o Complementarias
Grupo de Investigación en Alimentos Saludables -GIAS	Prof. María Orfilia Román Morales. Magíster en Química. Profesora Área de Ingeniería Aplicada. mroman897@gmail.com grupogias@udea.edu.co	Diseñar, desarrollar y evaluar alimentos de alta aceptabilidad, nutritivos e inocuos, acorde con la tendencia actual del desarrollo de la industria alimentaria, adicionados de fibra dietaria, compuestos bioactivos y/o ingredientes funcionales, con el fin de ofrecer a la población colombiana nuevos productos alimentarios con efectos saludables y/o funcionales.

La connaissance doit être universelle
Wissen muss sein universell
El coneixement ha de ser
Conoscenza deve essere
Conhecimento deve ser
El conocimiento debe ser

Knowledge must be UNIVERSAL

Our Journal is ready to make universal the results of your research. From 2012 all the manuscript can be submitted in English or Spanish, or both (bilingual edition). The process will be done with intenational peer reviewers using english forms.

Vitae
REVISTA DE LA FACULTAD
DE QUÍMICA FARMACÉUTICA
UNIVERSIDAD DE ANTIOQUIA
MEDELLÍN, COLOMBIA

more info:
http://www.udea.edu.co/vitae/
vitae@udea.edu.co

Index in:

CAS A division of the American Chemical Society · CUIDEN · SciELO Scientific Electronic Library Online · bvs biblioteca virtual en salud · Publindex Indexación - Homologación · ELSEVIER EMBASE.com · latindex · DOAJ DIRECTORY OF OPEN ACCESS JOURNALS · redalyc · e-revist@s · EBSCO HOST · NEW · PUBLIC KNOWLEDGE PROJECT · SIIC · THOMSON ISI ISI Web of SCIENCE. · SCOPUS refine your research · INDEXED IN ISI Web of Knowledge Current Web Contents

Vitae

REVISTA DE LA FACULTAD DE QUÍMICA FARMACÉUTICA

CUPÓN DE SUSCRIPCIÓN / SUBSCRIPTION COUPON

Nombres y apellidos
(Name and surname)

Cédula o Nit.
(I.D.)

Dirección
(Address)

Correo electrónico
(e-mail)

Teléfono
(Phone Nº)

Ciudad
(City)

País
(Country)

Fecha
(Date)

Firma
(Signature)

Forma de Pago

Banco
(Bank)

Ciudad
(City)

Giro postal o bancario Nº
(Money or banker's order Nº)

Valor de la suscripción anual -tres números-

Colombia.....	$120.000
Estudiantes (Anexar constancia).....	$65.000
Exterior (Incluye transferencia bancaria).....	US$ 70 EUR $ 55

Todo pago debe hacerse a nombre de la Universidad de Antioquia – Revista VITAE.

Para su comodidad usted puede consignar el valor de la suscripción en las cuentas nacionales No. 180-01077-9 del Banco Popular o No. 1053-7037272 de Bancolombia, en cualquier oficina del país, a nombre de la UNIVERSIDAD DE ANTIOQUIA centro de costos 8516. Si paga por este sistema, sugerimos tomar una fotocopia del recibo y enviarnos el original, adjuntando este cupón diligenciado.

Precio publicación por artículo: Colombia $440.00; Exterior US$220 - EUR$180

Correspondencia, canje y suscripciones: *Revista VITAE, Facultad de Química Farmacéutica, Universidad de Antioquia, Edificio de Extensión, Calle 70 No. 52-62 Piso 3 oficina 303. Teléfono: 57(4) 219 84 70. Apartado Aéreo 1226, Medellín, Colombia.* Telefax (574) 219 54 59.

Dirección electrónica: www.udea.edu.co/vitae

Internet: http://aprendeenlinea.udea.edu.co/revistas/index.php/vitae

Esta revista se imprimió en:
L. Vieco S.A.S.
PBX: (574) 448 9610
comercial@lvieco.com
Medellín - Colombia