# UNIVERSIDAD AUTÓNOMA DE BAJA CALIFORNIA

## Instituto de Ciencias Agrícolas


# HARINA DE LINAZA Y PASTA DE TOMATE PARA EL DESARROLLO DE UN ALIMENTO CÁRNICO FUNCIONAL

## T E S I S

**QUE COMO REQUISITO PARCIAL**
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## PRESENTA


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Las principales causas de muerte en México son las enfermedades cardiovasculares (ECV) y cáncer de diversos tipos. La alta prevalencia de obesidad y sobrepeso, derivada de una mala alimentación, es uno de los factores de riesgo que mayor influencia tiene en el desarrollo de ECV. A pesar de que la carne y sus productos son fuente importante de proteínas de alta calidad, vitaminas y minerales, tienen una imagen negativa en un cierto sector de la población ya que algunos estudios han relacionado su consumo con las ECV. Es necesaria investigación para desarrollar alimentos saludables, nutritivos, seguros y de calidad, que sean opciones de consumo para aquellas personas que buscan cuidar su salud, objetivo que cumplen los productos cárnicos funcionales (PCF). El uso de material vegetal como ingredientes en la formulación de los nuevos productos cárnicos es una buena estrategia para reducir el riesgo de ECV. La linaza y el tomate son ingredientes con potencial para ser incorporados en una matriz cárnica como las hamburguesas. Las evidencias científicas muestran que el consumo de linaza y tomate ha sido asociado a una disminución de ECV y cáncer de colon. Sin embargo, incorporar ingredientes no tradicionales a los productos cárnicos no es tarea fácil, pues su calidad e inocuidad pueden verse comprometida. Mantener una buena calidad en los PCF asegura un mayor éxito en su comercialización. El objetivo del presente trabajo fue desarrollar un producto cárnico tipo hamburguesa utilizando como ingredientes harina de linaza (HL) y pasta de tomate (PT). Se utilizó la técnica de metodología de superficie de respuesta (MSR) para estudiar el efecto de la adición de HL (0 al 20%) y PT (0 al 10%), en las características de calidad de hamburguesas de res. Se evaluó el color (L*, a* y b*), pérdida de peso por cocinado (PPC), pH, análisis de perfil de textura (APT) y análisis sensorial. La

adición de HL redujo los valores L* y a* ($P<0.05$) y disminuyó la PPC ($P<0.05$). Un efecto opuesto se observó cuando se agregó PT ($P<0.05$). Al incrementar los porcentajes de HL y PT en el producto, todos los parámetros del APT se vieron disminuidos. A pesar de que la adición de HL en la formulación de hamburguesas de res afecta las propiedades sensoriales, los panelistas evaluaron el producto con una puntuación aceptable. De acuerdo a los modelos de predicción obtenidos por MSR, los porcentajes óptimos para la formulación de hamburguesas de res fueron 3% de HL y 10.8% de PT. Sin embargo, otras combinaciones resultaron en un producto de calidad aceptable y presuntamente con un mejor perfil nutricional, situación que fue explorada en otro experimento. En éste, los tratamientos evaluados fueron: 1) Testigo = 0% HL+0% PT; 2) T1 = 0% HL+20% PT; 3) T2 = 5% HL+15% PT; 4) T3 = 10% HL+10% PT; 5) T4 = 15% HL+5% PT; y 6) T5 = 20% HL+0% PT. Se evaluaron las propiedades sensoriales, perfil de ácidos grasos y contenido de colesterol. Las propiedades sensoriales para los tratamientos T1 y T2 fueron similares al tratamiento Testigo, mientras que el tratamiento T3 mostró una puntuación aceptable en el análisis sensorial. Las puntuaciones del análisis sensorial tienden a disminuir cuando los niveles de HL superan el 10% (T4 y T5). El tratamiento T3 con una combinación de 10% HL+10% PT resultó con mejor perfil nutricional sin demérito en sus propiedades sensoriales. En esta formulación, el contenido de ácido α-linolénico representó el 43.52% del total de ácidos grasos presentes, razón por la cual la relación AGP/AGS aumentó de 0.24 en el tratamiento Testigo a 2.95, mientras que la relación n-6/n-3 disminuyó de 7.18 en el tratamiento Testigo a 0.29. Hubo una reducción del 45% en el contenido de colesterol en la formulación T3, respecto al Testigo. Por otro lado, desde el punto de vista de inocuidad del producto, la formulación con 10% HL+10% PT resulta más segura, respecto a la formulación óptima (3% HL+10.8% PT). Lo anterior fue explorado en un experimento adicional estableciendo modelos de predicción por MSR, tanto para la inactivación térmica como para el crecimiento en abuso de temperatura de *Escherichia coli* O157:H7. Ante un escenario de contaminación por esta bacteria patógena (7 log UFC/g) y simulando condiciones de cocimiento deficientes al momento de preparar el producto (60°C por 10 min), el modelo estima una reducción de 3.62 log UFC/g cuando se tiene una formulación de 10% HL+10% PT, respecto a la formulación óptima en la que se tiene una reducción de 2.74 log UFC/g. Al simular condiciones de almacenamiento en abuso de temperatura (15°C durante 5 días), y una contaminación inicial de 2.5 log UFC/g, el modelo estima un crecimiento de *Escherichia coli* O157:H7 de 2.91 log UFC/g en la formulación de 10% HL+10% PT, mientras que en la formulación óptima la predicción fue de 3.63 log UFC/g. La HL y PT son alimentos que pueden ser utilizados como ingredientes en la formulación de hamburguesas de res con un mejor perfil nutricional, más seguros y sin demérito en las propiedades de calidad.

Palabras clave: hamburguesa de res, calidad de la carne, alimentos funcionales

**ABSTRACT**

Cardiovascular diseases (CVD) and diverse types of cancer are the main death causes in Mexico. The high prevalence of obesity and overweight, as consequence from a poor diet, is one of the risk factors that has greater influence on the development of CVD. Although meat and meat products are important sources of vitamins, minerals and high quality proteins, they have a negative image in certain sector of the population because some studies have related its consumption to the CVD. For this reason, research is required to develop healthy and safe products as consumption options, objective that is met by functional meat products (FMP). The use of vegetables as ingredients in meat products formulation is a good strategy to reduce the risk of CVD. Flaxseed and tomato are foods with potential to be incorporated as ingredients into a meat product like beef patties. Scientific evidence shows that consumption of flaxseed and tomato has been associated with a decrease in CVD and colon cancer. However, addition of non-traditional ingredients to meat products is not easy because its quality and safety can be compromised. Keeping FMP quality assures a major success of consumer preference. The objective of this study was to develop a new meat product using flaxseed flour (FF) and tomato paste (TP) as main ingredients. The effect of FF (0-10%) and TP (0-20%) on quality parameters of beef patties was studied using response surface methodology (RSM). Color (*L*, a*, b**), cooking loss (CL), pH, texture profile analysis (TPA) and sensory analysis were evaluated. The *L**, *a** and CL values decreased with FF addition ($P<0.05$). An opposite effect was observed when PT was added ($P<0.05$). Incorporation of FF and TP in the product formulation decreased all TPA parameters. Although sensory properties of beef patties were affected by the incorporation of FF, panelists evaluated the product with an acceptable score. According to the prediction models obtained by RSM, the optimal percentages for beef patties formulation were 3% of FF and 10.8% of TP. However, other combinations of FF and TP resulted in a product with acceptable sensory quality and presumably better nutritional profile, situation that was studied in a different experiment. The treatments evaluated for this new experiment were: Control = 0% FF 0% TP; T1 = 0% FF, 20% TP; T2 = 5% FF 15% TP; T3 = 10% FF 10% TP; T4 = 15% FF 5% TP; and T5 = 20% FF 0% TP. Sensory properties, fatty acids profile and cholesterol content were evaluated. Sensory properties for T1 and T2 treatments were similar to the control, while T3 showed an acceptable score in sensory analysis. Sensory analysis scores decreased when FF levels exceeded 10% (T4 and T5). The T3 treatment with a combination of 10% FF and 10% TP resulted with better nutritional profile without demerit in its sensory properties. In this formulation, the α-linolenic acid content represented 43.52% of total fatty acids, reason by which the PUFA/SFA ratio increased from 0.24 in the control to 2.95, while the n-6/n-3 ratio decreased from 7.18 to 0.29. There was a 45% reduction in cholesterol content in T3 with respect to the control formulation. Taking into account microbiological contamination, treatment with 10% FF and 10% of TP is less susceptible to *Escherichia coli* O157:H7 contamination regarding to the optimal formulation (3% FF and 10.8% TP). This was explored in another experiment, establishing prediction models by RSM, for both thermal inactivation and temperature abuse growth of *Escherichia coli* O157:H7. In the scenario of contamination by this pathogenic bacteria (7 log CFU/g) and simulating deficient cooking conditions (60°C for 10 min), the model estimated 3.62 log CFU/g reduction when the product was formulated with 10% FF and 10% TP, while the estimated reduction was 2.74 log CFU/g in the optimal formulation. By simulating conditions of temperature abuse storage (15°C during 5 d), and an initial contamination of 2.5 log CFU/g of *Escherichia coli* O157:H7, the model estimated an increment to 2.91 log CFU/g in the 10% FF 10% TP formulation, while in the optimal formulation the prediction was to 3.63 log CFU/g. The FF and TP are foods that can be used as ingredients in the beef patties formulation with better nutritional profile, safer and without demerit in quality properties.

Key words: beef patties, meat quality, functional foods

# CAPÍTULO 1
# INTRODUCCIÓN GENERAL

La carne es uno de los alimentos más completos de que dispone el ser humano para su sustento y desde la antigüedad, a través de todo su proceso evolutivo, se ha adaptado para consumir cantidades suficientes que lo han ayudado en su desarrollo (Smil, 2002). La carne es una fuente importante de proteínas de alto valor biológico, vitamina $B_{12}$, hierro, zinc y selenio. Es fuente también de ácidos grasos esenciales de importancia como el ácido linoléico conjugado (Williams, 2007). Por su composición nutrimental tan completa, la carne ha sido considerada como un alimento que puede disminuir las deficiencias de muchos de los nutrimentos en la población (Engelmann et al., 1998; Ruxton et al., 2013). Además, su consumo es altamente recomendado en ciertos periodos de la vida como la etapa de crecimiento, embarazo, entrenamiento físico intenso, periodos de enfermedad y vejez (Fruh y Greenblum, 2014). A pesar de todo lo anterior, la carne y sus productos han sido vinculados a enfermedades cardiovasculares (ECV) y cáncer de colon (McAfee et al., 2010), debido a su contenido de grasas saturadas, colesterol y sal, lo que en cierta manera representa un freno en su consumo.

Desarrollar productos cárnicos saludables y seguros sin demérito en su calidad es un reto para el tecnólogo de alimentos y para la industria cárnica misma. Las tendencias en alimentación están cambiando y debemos adaptarnos a ello. Hoy en día el consumidor busca alimentos más saludables, que además de cubrirle sus necesidades básicas de nutrición también lo ayuden a disminuir los problemas de salud. Los alimentos funcionales son una buena opción para una sociedad en las que los hábitos alimenticios están cambiando. Un alimento funcional se definen como aquel alimento que logra demostrar que posee efectos beneficiosos para la salud sobre una o más funciones del organismo, más allá de sus propiedades nutricionales habituales, de modo tal que mejore el estado general de salud o reduzca el riesgo de enfermedad o ambas (Siro et al., 2008). Las estrategias para el desarrollo de productos cárnicos funcionales son diversas, siendo una de ellas la reformulación de los productos cárnicos mediante el uso de ingredientes no tradicionales (Jiménez-Colmenero et al., 2010) o bien, la disminución o sustitución de los tradicionales como las grasas saturadas.

Dos de los alimentos que pueden utilizarse como ingredientes en el desarrollo de productos cárnicos funcionales son la linaza y el tomate. Los principales componentes de la linaza son proteínas (21%), fibra dietética (28%) y grasa (41%). Tiene un alto contenido en ácidos grasos poliinsaturados (73% del total de la grasa) y baja cantidad de ácidos grasos saturados (9%). Del total de los ácidos grasos, el α-linolénico (ALA) constituye alrededor del 55% (Bloedon y Szapary, 2004). El ALA es un ácido graso esencial con múltiples beneficios que se manifiestan al consumirlo como parte de la dieta, destacando una disminución de las ECV (Barceló-Coblijn y Murphy, 2009; Bloedon y Szapary, 2004; Dodin et al., 2008). Debido a estas propiedades, la linaza es una opción viable para incorporarse durante la elaboración de productos cárnicos (Bilek y Turhan, 2009; Singh et al., 2011; Valencia et al., 2008).

El tomate es otro de los alimentos que puede ser considerado como funcional (Canene-Adams et al., 2005). Éste es una excelente fuente de vitaminas, minerales y compuestos fitoquímicos en los que se incluyen carotenoides y polifenoles (Navarro-González et al., 2011). El consumo del tomate ha sido asociado también a una disminución de ECV y cáncer de colon (Canene-Adams et al., 2005), por lo que también puede ser tomado en cuenta para la elaboración de productos cárnicos (Candogan, 2002; García et al., 2009; Østerlie y Lerfall, 2005).

Sin embargo, la formulación de alimentos cárnicos funcionales pueden alterar las características de calidad, propiedades fisicoquímicas y nutricionales del producto, así como aspectos sensoriales e inocuidad. La harina de linaza y pasta de tomate son alimentos que pueden utilizarse como ingredientes en el desarrollo de hamburguesas de res con una connotación saludable, sin demerito en su calidad y más seguras para su consumo. Por ello, el objetivo del presente trabajo fue desarrollar un producto cárnico funcional usando como ingredientes harina de linaza y pasta de tomate. Los objetivos específicos planteados fueron: a) Estudiar el efecto de la incorporación de harina de linaza y pasta de tomate sobre las características de calidad de hamburguesas de res; b) Analizar el perfil nutricional de hamburguesas de res por efecto de la adición de harina de linaza y pasta de tomate; c) Determinar los riesgos microbiológicos a que están sujetos los productos desarrollados.

# CAPÍTULO 2
# REVISIÓN DE LITERATURA

## Importancia de la Carne para Consumo Humano

La carne se define como el tejido animal que ha sido dictaminado como inocuo y apto para el consumo humano o se destinan para este fin, obtenido de canales de animales domésticos como bovinos, porcinos, ovinos, caprinos, aves, o animales de caza (Codex-Alimentarius, 2005). Por su parte, la Norma Oficial Mexicana NOM-009-Z00-1994, define a la carne como la estructura compuesta por fibra muscular estriada, acompañada o no de tejido conjuntivo elástico, grasa, fibras nerviosas, vasos linfáticos y sanguíneos, de las especies animales autorizadas para el consumo humano.

La carne ha sido un componente importante en nuestra dieta desde nuestros orígenes mismos en la que su consumo ha ejercido un papel fundamental en el desarrollo y evolución de la especie humana (Smil, 2002). Por su composición nutricional ha sido considerada uno de los alimentos más completos del que disponemos para nuestra alimentación (Pereira y Vicente, 2013). La carne es una importante fuente de proteínas de alto valor biológico, fuente de aminoácidos esenciales, vitaminas del complejo B, minerales como hierro, zinc y selenio, y ácidos grasos esenciales como el ácido linoleico conjugado (Williams, 2007).

En Estados Unidos y muchos países desarrollados el consumo de carne ha mostrado un aumento sostenido, siendo la carne roja la más consumida con el 58% del total de las carnes, y dentro de este porcentaje, el 22% en forma procesada. Es innegable que la carne forma parte importante en la dieta normal de la población pues contribuye con el 15% de las necesidades energéticas, 40% de las de proteína y 20% de las de grasa (Daniel et al., 2011). A pesar de todas las bondades mostradas por la carne, algunos estudios han asociado su consumo con la aparición de enfermedades cardiovasculares y cáncer de colon

(Cross et al., 2007; Kontogianni et al., 2008; Snowdon et al., 1984), debido a que naturalmente es fuente de ácidos grasos saturados y colesterol. De ahí que muchas de las recomendaciones sean disminuir el consumo de carne y aumentar el de frutas, cereales enteros y verduras (OMS, 2005). La relación entre la alimentación y la salud no es un tema nuevo, pues desde hace más de 2500 años Hipócrates ya había acuñado su hoy célebre frase "deja que el alimento sea tu medicina y la medicina sea tu alimento". Antes de abordar el tema de los alimentos funcionales (AF) es necesario primero incursionar en la problemática que actualmente vivimos en relación a la alimentación y la salud, y como a partir de ésta relación surgen los AF.

## La Alimentación y las Enfermedades Crónicas no Transmisibles

Los alimentos que diariamente consumimos tienen como objetivo principal el aporte de nutrientes suficientes para cubrir los requerimientos metabólicos de un individuo y proporcionar bienestar (Lean, 2015). Sin embargo muchas veces no es posible proveer al organismo de los nutrientes necesarios para un buen funcionamiento, ocasionando con ello una mala nutrición y la aparición de enfermedades. Nuestra sociedad está caracterizada por una población enferma, destacando las enfermedades crónicas no transmisibles como diabetes, cáncer, enfermedades cardiovasculares y enfermedades crónicas respiratorias. La mortalidad, la morbilidad y la discapacidad atribuidas a las enfermedades no transmisibles (ENT) representan actualmente alrededor del 60% de todas las defunciones en el mundo (Alwan, 2011). La situación en México no es muy diferente a lo que ocurre mundialmente, pues las dos principales causas de muerte son las enfermedades cardiovasculares y la diabetes mellitus, siendo uno de los factores de riesgo más importante el sobrepeso y la obesidad (Cervera et al., 2010). De acuerdo a los resultados presentados por la Encuesta Nacional de Salud (ENSANUT, 2012b), el sobrepeso y la obesidad entre los adultos presenta una prevalencia combinada del 71.28% y 28% en los niños en edad escolar.

Este problema ha sido considerado por el gobierno mexicano como una emergencia de salud que afecta de manera importante la productividad de las empresas, el desempeño escolar y el desarrollo económico como país (ANSA, 2010). En 2008 los costos atribuibles a la obesidad en México fueron 42,000 millones de pesos, equivalente a 13% del gasto total en salud y estos costos podrían ascender a 101,000 millones de pesos para el 2017 (ENSANUT, 2012a). Estas cifras indican claramente un reto muy importante para el Sector Salud de nuestro país en términos de promoción de estilos de vida saludables en la población y desarrollo de políticas públicas para disminuir este problema.

Independientemente de las acciones implementadas por nuestro gobierno para revertir estos problemas derivados de una alimentación deficiente, y ante el hecho de un aumento gradual y sostenido de las ENT, ha aumentado también la preocupación entre la población mexicana por cuidar su salud, demandado alimentos con una connotación más saludables. Ante esto, la industria de alimentos se está adaptando a los nuevos cambios y empieza a ofertar productos que el consumidor pide, alimentos con una connotación más saludable (Decker y Park, 2010; Navarro-González et al., 2011). Sin embargo, esto no se logra fácilmente ya que la mejora de la oferta de alimentos debe hacerse sin alterar drásticamente las necesidades del consumidor, tales como la calidad, conveniencia y costos. La investigación y producción de AF es un área aún no explotada en nuestro país y con gran potencial para enfrentar muchos de los problemas de salud que nuestra población padece. Es importante destacar que los AF por sí solos no son la respuesta a los problemas de salud derivado de una mala alimentación, pues ésta como tal es compleja y difícil de acotar, sus límites son extensos ya que se involucran aspectos económicos, políticos, sociales, culturales, religiosos y muchos más.

## Los Alimentos Funcionales

El principal objetivo que perseguimos al tomar nuestros alimentos día a día es incorporar a nuestro cuerpo los nutrientes necesarios para un buen funcionamiento del organismo; esto es a lo que se llama nutrición adecuada. Es claro suponer que con una nutrición adecuada no debería haber enfermedades relacionadas con la alimentación. Sin embargo, la realidad es totalmente diferente ya que muchas de las enfermedades tienen en su etiología factores nutricionales implícitos. Dada la relación existente entre alimentación y salud, el término de alimentación adecuada está siendo sustituido por el de “alimentación óptima”, término que se refiere a la posibilidad de que algunos alimentos mejoren la salud de la población y reduzcan el riesgo de desarrollar determinadas enfermedades. Con el concepto de alimentación óptima nacen los llamados alimentos funcionales; un alimento puede ser considerado AF si logra demostrar científicamente que posee efectos beneficiosos para la salud sobre una o más funciones del organismo, más allá de sus propiedades nutricionales habituales, de modo tal que mejore el estado general de salud, reduzca el riesgo de enfermedad o ambas (Siro et al., 2008).

La primera reseña histórica del uso de los AF viene de Japón donde en la década de los 80´s y gracias a la ayuda del gobierno de ese país se comenzó la investigación y se propuso el término de alimentos saludables para uso específico, en inglés *Foods for Special Health Use* (FOSHU). Este concepto expone por primera vez la existencia de “alimentos con efecto específico sobre la salud” y en consecuencia, la población se puede beneficiar de su consumo. Desde su origen en Japón hace más de 30 años, rápidamente se extendieron a Europa y después a los Estados Unidos, representado actualmente una industria económicamente importante que día a día crece. Las ventas en el mundo de este tipo de alimentos ascendió en el año 2012 a 44 mil millones de dólares, siendo Estados Unidos el país que más ventas registró en este campo, 6.9% mayor al año anterior. En nuestro país el desarrollo de alimentos funcionales es en general muy limitado, probablemente debido a la muy deficiente investigación en el tema. En cuanto a los productos cárnicos, en México no existe un producto catalogado como funcional por lo que el desarrollo y comercialización de éstos representa una ventana de oportunidad para el sector (Cervera et al., 2010).

Es un hecho que la carne y sus productos proyectan una imagen negativa para un sector de la población, de ahí la necesidad profundizar en la investigación que permita el desarrollo de nuevos productos que satisfagan las necesidades que el consumidor actual demanda. La investigación en productos cárnicos funcionales es un área poco explotada en México pero muy necesaria para sentar las bases del desarrollo de los nuevos productos cárnicos y para establecer las regulaciones necesarias para su comercio.

## Estrategias para el Desarrollo de Productos Cárnicos Funcionales

El desarrollo de productos cárnicos funcionales puede lograrse siguiendo varias estrategias, dos de las principales son: 1) suplementación de los compuestos bioactivos desde la dieta a nivel de producción animal, 2) reformulación de los alimentos adicionando ingredientes funcionales (Olmedilla-Alonso et al., 2013). A continuación se describen las estrategias antes mencionadas, los ingredientes bioactivos que pueden incorporarse al alimento y evidencias científicas tomadas como ejemplo en cada una de las categorías.

### Suplementación de ingredientes funcionales a nivel de producción animal

Ácido linoleico conjugado (CLA). El CLA es un término colectivo para designar los isómeros del ácido linoléico, siendo el más estudiado el isómero 9 cis, 11 trans debido a sus efectos biológicos, atribuyéndole propiedades antioxidantes, anticarcinogénicas, antiaterosclerótica, antidiabetogénica y protección del sistema inmune (Hur et al., 2007; Tanaka, 2005). Generalmente la carne y leche de rumiantes posee mayor contenido de CLA que los productos de animales no rumiantes. El CLA se sintetiza en forma natural en el rumen de los animales por biohidrogenación de los ácidos grasos poliinsaturados de la dieta, principalmente ácido linoléico (18:2) y alfa linolénico (18:3) y por la acción de la enzima ∆-9-desaturasa sobre el ácido11 trans octadecanoico (18:1), uno de los metabolitos secundarios formados durante la biohidrogenación de los ácidos grasos polinsaturados (Corl et al., 2001). Como ejemplo de lo anterior se tiene el estudio realizado por Bessa et al. (2007), quienes determinaron la concentración de ácido11 trans octadecanoico y CLA en carne de cordero de animales alimentados con dietas suplementadas con aceite de girasol y aceite de linaza encontrando un aumento de estos compuestos por efecto de la dieta, siendo mayor en carne de aquellos animales alimentados con dietas ricas en aceite de girasol. Por otro lado, Griinari et al. (2000) realizaron una infusión abomasal directa de ácido11 trans octadecanoico y determinaron la síntesis endógena de CLA por acción de la enzima ∆-9-desaturasa, midiendo el contenido de CLA en leche de vacas lactantes, encontrando un aumento del 31% de CLA.

<u>Vitamina E</u>. La vitamina E es un micronutriente esencial para el mantenimiento de la salud y el bienestar de los seres humanos debido a sus propiedades antioxidantes Brigelius-Flohé y Galli (2010). La suplementación de vitamina E en la dieta animal mejora la calidad de la carne obtenida limitando la oxidación lipídica y proteica, además de proveer a la carne de una fuente alterna de vitamina E para el consumidor. Lynch et al. (1999) investigaron el efecto de la suplementación dietaria de vitamina E (dosis de 2000 UI/kg de alimento/d, durante 50 d en reses), sobre la calidad, estabilidad a la oxidación y contenido de alfa tocoferol de la carne obtenida. En su estudio encontraron que la carne de animales sujetos a una dieta alta de vitamina E presentó mayor contenido de alfa tocoferol, además de un mejor color y estabilidad oxidativa después de 7 d de almacenamiento a 4°C. Lauzurica et al. (2005) estudiaron el efecto de la suplementación dietaria de vitamina E (0 a 1000 mg/kg de alimento, durante 37 d) sobre la calidad de la carne de cordero almacenada en atmosfera modificada. Los resultados de este estudio muestran que dietas con dosis altas de vitamina E incrementan los niveles de alfa tocoferol en la carne y alargaron la vida de anaquel de carne empacada en atmosfera modificada hasta por 14 d.

Ácidos grasos n-3. Los ácidos grasos n-3 se componen de una cadena carbonada con átomos de hidrógeno unidos a ella, dobles ligaduras y al final un grupo carboxilo. Toda la familia de ácidos grasos n-3 tienen la primera ligadura entre los carbonos 3 y 4 contados a partir del extremo metilo (Nguemeni et al., 2013). Los ácidos grasos n-3 son importantes para mantener la salud y no pueden ser sintetizados por nuestro organismo por lo que es necesario obtenerlos de la dieta. Los más importantes son el ácido alfa linolénico (ALA, 18:3), ácido eicosapentanoico (EPA, 20:5) y docosahexanoico (DHA, 22:6). Estos están involucrados en el desarrollo adecuado del cerebro e hígado de los infantes y juegan un papel muy importante en la prevención y tratamiento de varias enfermedades (Gorjão et al., 2009). El consumo regular de ácidos grasos n-3 disminuye el contenido de triglicéridos en la sangre y reduce la incidencia de enfermedades cardiovasculares (Olza et al., 2010; Skulas-Ray et al., 2014). Aunque la principal fuente de ácidos grasos n-3 es el pescado, la carne de animales alimentados con dietas ricas en ácidos grasos n-3 es fuente alternativa de estos compuestos bioactivos (Mapiye et al., 2013). Como ejemplo de ello pueden mencionarse los estudios de Nassu et al. (2011), quienes investigaron los cambios en el perfil lipídico de carne de reses alimentadas con linaza, encontrando un aumento de ácido alfa linolénico en los cortes obtenidos; mientras que Albertí et al. (2014), reportaron un aumento en el contenido de ácido alfa linolénico en carne de toretes alimentados con linaza. Por otro lado, Sardi et al. (2006), estudiaron el efecto de la suplementación dietaria de algas marinas (AM), ricas en DHA, sobre el perfil lipídico de carne de cerdo. Concluyen que los animales alimentados con AM presentan un aumento significativo en los niveles de DHA en la carne obtenida.

Selenio. El selenio es un mineral esencial en nuestro organismo, forma parte integral de las selenoproteínas involucradas en regular importantes funciones fisiológicas. Su deficiencia está asociada a una disminución de la función inmune y a un amento a la susceptibilidad al cáncer, enfermedades cardiovasculares, distrofia muscular, diabetes, artritis, cataratas y otras enfermedades (Zhang et al., 2010). La carne es la mayor fuente de selenio en la dieta y de acuerdo a los estudios disponibles, su contenido puede incrementarse alimentando a los animales a base de dietas ricas en selenio. Este aumento de selenio en la carne puede ser una excelente manera de mejorar su consumo, sobre todo en poblaciones deficientes. Algunos ejemplos que evidencian un aumento en el contenido de selenio en la carne se describen a continuación. Taylor et al. (2008), determinaron el valor nutritivo de carne de res alimentados con dietas enriquecidas con selenio (68.1 µg Se/kg peso corporal/d) y reportaron un aumento sustancial de selenio en la carne obtenida, al compararla con el tratamiento testigo. Concluyen que el enriquecimiento de la carne con selenio es un proceso viable y seguro para generar alimentos ricos en selenio para el consumo humano. Por otro lado, Vignola et al. (2009), utilizaron dietas ricas en selenio (0.3 mg/kg) para la alimentación de corderos y reportaron un incremento en los niveles de selenio en la carne obtenido comparado con el tratamiento testigo.

**Formulación de productos cárnicos funcionales**

Suplementación con fibra. La fibra dietaria es un componente de los alimentos vegetales como cereales, frutas, verduras y leguminosas, que no puede ser digerida por el organismo. La fibra dietaria facilita la evacuación, previene y ayuda a eliminar el estreñimiento, ejerce además un efecto hipocolesterolémico al ligar los ácidos biliares y disminuir la absorción del colesterol (Kaczmarczyk et al., 2012). La fibra dietaria se clasifica en fibra insoluble y fibra soluble. La fibra insoluble consiste principalmente en celulosa, hemicelulosa y lignina, este tipo de fibra se encuentra en el salvado de trigo, granos integrales y verduras. La fibra soluble comprende gomas y pectinas y se encuentra en frijol, avena, cebada, manzanas, ciruelas, peras y naranjas (Elleuch et al., 2011). Todos estos son ingredientes potenciales para los nuevos productos cárnicos funcionales, donde además de reducir la grasa imparten otras características. La fibra aumenta la capacidad de retención de agua en el producto y hace que éstos pierdan menos agua por manejo o durante su congelación/descongelación y por lo tanto hace que el riesgo por contaminación microbiana sea menor al no existir agua libre para ello. Además, la pérdida de peso al cocinarlo también es menor, manteniéndose más jugoso y con mejor textura, y liberando más lentamente los compuestos volátiles responsables de su sabor. Schmiele et al. (2015), estudiaron la incorporación de fibra de celulosa amorfa (Z-trim®) como un sustituto de grasa en un modelo cárnico. Sus resultados indican que es posible reducir hasta un 50% de grasa y obtener productos con 1.3 g fibra/100 g producto sin demérito en las características tecnológicas (estabilidad de la emulsión, firmeza, dureza, color y pérdida de peso durante el almacenamiento). Álvarez y Barbut (2013), estudiaron el efecto de la inulina y β-glucano sobre la estabilidad de la emulsión, color y textura de una pasta cárnica baja en grasa. Sus resultados indican que agregar una mezcla de ambos componentes al sistema cárnico mejora su estabilidad, color, textura y reduce la pérdida de peso por cocción.

<u>Suplementación con ácidos grasos n-3</u>. Diversos estudios epidemiológicos, clínicos y bioquímicos han evidenciado que los ácidos grasos poliinsaturados n-3 presentes en los alimentos de manera natural o suplementados, tienen una función protectora contra el cáncer de colon, artritis reumatoide y enfermedades cardiovasculares (Gorjão et al., 2009; Mozaffarian y Wu, 2011). Las fuentes de ácidos grasos n-3 son tanto de origen animal como vegetal. Pueden ser utilizados aceites de algas, pescado y linaza entre otros. Todos ellos son también ingredientes potenciales para el desarrollo de productos cárnicos más saludables. Como ejemplo de ello se tienen los siguientes estudios. Bilek y Turhan (2009) usaron la harina de linaza (HL) como ingrediente funcional en la elaboración de hamburguesas de res. Adicionaron hasta 15% de HL y reportaron una disminución en la relación de ácidos grasos n-6/n-3 (5.76 en el control y 0.36 en los tratamientos con 15% HL), con cambios mínimos en la calidad fisicoquímica y sensorial del producto.

<u>Productos cárnicos con antioxidantes de origen natural</u>. Los antioxidantes son sustancias que previenen o retardan los procesos de deterioro ocasionados por el oxígeno. Desde el punto de vista biológico, son sustancias que disminuyen

significativamente los efectos adversos de las especies reactivas al oxígeno durante las funciones fisiológicas normales del organismo. Algunas de las sustancias antioxidantes naturales más conocidas son el β-caroteno (pro-vitamina A), el licopeno, la vitamina C (ácido ascórbico), la vitamina E (α-tocoferol) y el selenio. Los extractos de fuentes vegetales y aceites esenciales son la principal fuente de antioxidantes naturales con potencial para ser utilizados en la elaboración de los productos cárnicos funcionales (Shah et al., 2014). Selani et al. (2011), utilizaron extractos de cáscara y semilla de uvas como antioxidantes naturales en carne de pollo, sus resultados indican que estos extractos son efectivos en retardar la oxidación lipídica de carne de pollo almacenada en congelación hasta por 9 meses. Los extractos de brócoli (2%) son también efectivos en retardar la oxidación lipídica de nuggets almacenados en refrigeración sin demérito en la aceptabilidad del producto de acuerdo a un estudio realizado por Banerjee et al. (2012).

## La Linaza y el Tomate como Ingredientes Funcionales

Una de las estrategias para el desarrollo de los productos cárnicos funcionales es la adición de nuevos ingredientes que por sí solo tienen una imagen saludable ante el consumidor y de los cuales se tiene suficiente evidencia científica sobre su impacto en la salud. Los estudios disponibles indican que el uso de materia vegetal es una de las mejores alternativas para la elaboración de alimentos funcionales (Rodríguez-Carpena et al., 2012; Vural et al., 2004). Existe una gran gama de ingredientes no tradicionales por probar para la formulación de productos cárnicos entre las que se pueden incluir el uso de linaza y tomate para dotarlos de ácidos grasos insaturados y fitoquímicos.

### Linaza

La linaza ha ganado recientemente la atención como un alimento funcional debido a su excelente perfil nutricional. Los principales componentes de la linaza son proteínas (21%), fibra dietética (28%) y lípidos (41%). Tiene un alto contenido en ácidos grasos poliinsaturados (73% del total de los lípidos) y baja cantidad de ácidos grasos saturados (9%). Del total de los ácidos grasos, el α-linolénico constituye alrededor del 55% (Bloedon y Szapary, 2004). El ácido α-linolénico (ALA) es un ácido graso esencial con múltiples beneficios que se manifiestan al consumirlo como parte de la dieta, destacando una disminución de las ECV (Barceló-Coblijn y Murphy, 2009; Bloedon y Szapary, 2004; Dodin et al., 2008). La linaza es también rica en lignanos (Singh et al., 2011), los cuales ejercen un efecto preventivo en la aparición de diferentes tipos de cáncer (Touré y Xueming, 2010). Debido a estas propiedades, la linaza es una opción viable para incorporarse durante la elaboración de productos cárnicos (Bilek y Turhan, 2009; Singh et al., 2011; Valencia et al., 2008). Algunos de los componentes de la linaza y su efecto en la salud se mencionan a continuación.

Lignanos. Los lignanos son compuestos fenólicos presentes en las plantas, similares a la estructura de las hormonas sexuales endógenas por lo que se ha hipotetizado que actúan *in vivo* alterando el metabolismo hormonal y reduciendo el riesgo de cáncer. La linaza es la mayor fuente del lignano secoisolariciresinol diglucósido (SDG) que se conoce (Thompson et al., 1997) con aproximadamente 0.3% (Landete, 2012). Una vez consumido, el SDG es convertido por la microflora a enterodiol y es a su vez oxidado a enterolactona. Se ha probado que ambos compuestos tienen un efecto protector contra cáncer de mama, colon y próstata (Brooks y Thompson, 2005; Hutchins et al., 2001; Touré y Xueming, 2010).

Los estrógenos son hormonas sexuales esteroideas femeninas producidas principalmente por los ovarios y por la placenta durante el embarazo. Actúan sobre determinados genes regulando la síntesis proteica e induciendo la proliferación celular sobre algunos órganos como el endometrio, mama y ovario, preparando así a la mujer para perpetuación de la especie. Por otro lado, la mayor incidencia de cáncer de mama se da en la etapa postmenopáusica de la mujer, donde la fuente de producción de los estrógenos se da fuera de los ovarios. La presencia de estrógenos en esta etapa de la mujer induce la proliferación celular y la aparición de cáncer de mama. El estrógeno es sintetizado por las enzimas aromatasa y 17β-hydroxiesteroide deshidrogenasa (17β-HSD), las cuales están presentes en el tejido de los senos, por lo que su expresión y actividad está asociada con la aparición de cáncer de mama (Li et al., 1998; Yue et al., 2001). El enterodiol y la enterolactona influyen en la síntesis de estrógenos inhibiendo la actividad de las enzimas aromatasa y 17β-HSD, ejerciendo así un efecto protector contra el cáncer de mama (Brooks y Thompson, 2005).

Ácido α-linolénico (ALA).- El ALA (18:3n-3) es un ácido graso esencial presente en los aceites vegetales. Del total de los lípidos contenidos en la linaza, el ALA es el más abundante. El ALA tiene un efecto protector contra las ECV al interferir con la producción de eicosanoides proinflamatorios y proagregatorios (prostaglandina $E_2$, tromboxano $A_2$ y leucotrieno $B_4$). Para ello, el balance entre los ácidos grasos poliinsaturados n-6 y n-3 es importante en la dieta. El ácido linoléico (AL) (n-6) y el ALA son ácidos grasos esenciales por lo que son adquiridos a través de la ingesta de alimentos. Una vez consumidos, pueden ser convertidos a diferentes ácidos grasos que producen diferentes clases de eicosanoides. Estos a su vez tienen efectos diferentes sobre la inflamación, agregación plaquetaria y vasoconstricción. Por un lado, el AL puede ser convertido a ácido araquidónico (AA) (20:4n-6) a través de etapas de elongación. De la misma manera, el ALA puede ser convertido a ácidos grasos de cadena larga n-3, ácido eicosapentanoico (EPA) (20:5n-3) y ácido docosahexanoico (DHA) (22:6n-3). Estos a su vez producen eicosanoides menos inflamatorios (prostaglandina $I_3$, tromboxano $A_3$ y leucotrieno $B_5$). El metabolismo de los lípidos hacia la producción de n-3 o n-6 requiere de las mismas enzimas, por lo que están en competencia hacia una u otra vía. De ahí que un exceso de una clase de ácidos grasos poliinsaturados puede guiar a una producción específica de eicosanoides. Los eicosanoides producidos por el EPA y DHA están involucrados en la disminución de la agregación plaquetaria, vasoconstricción y trombosis, mientras que los eicosanoides producidos del AA tienen un efecto contrario. En síntesis, el ALA, EPA y DHA pueden reducir la relación n-6/n-3 y así favorecer la biosíntesis de eicosanoides menos inflamatorios.

**Tomate**

Otro alimento que también es considerado como funcional es el tomate (Canene-Adams et al., 2005). Éste es una excelente fuente de vitaminas, minerales y compuestos fitoquímicos en los que se incluyen carotenoides y polifenoles (Navarro-González et al., 2011). El consumo del tomate ha sido asociado también a la disminución de ECV y cáncer de colon (Canene-Adams et al., 2005), por lo que también puede ser tomado en cuenta para la elaboración de productos cárnicos (Candogan, 2002; García et al., 2009; Østerlie y Lerfall, 2005). Algunos componentes del tomate y su impacto en la salud se mencionan a continuación.

Licopeno.- El licopeno es un pigmento vegetal, soluble en lípidos, que aporta el color rojo característico a los tomates, sandías y en menor cantidad a otras frutas y verduras. Pertenece a la familia de los carotenoides como el β-caroteno, sustancias que no sintetiza el cuerpo humano, sino los vegetales y algunos microorganismos, debiéndolo tomar en la alimentación como micronutriente.

Las evidencias epidemiológicas sugieren que el consumo de licopeno contribuye en la reducción del riesgo de cáncer de próstata. El licopeno actúa por diferentes mecanismos en forma cooperativa reduciendo la proliferación de células cancerosas, disminuyendo el daño al DNA y mejorando las defensas contra el estrés oxidativo.

El oxígeno es un componente esencial de todos los organismos vivos y durante el metabolismo aerobio, la generación de especies reactivas al oxígeno (EROs) es inevitable. Una de las consecuencias de las EROs dentro de la célula es que pueden causar la oxidación de lípidos, proteínas y el DNA. El daño oxidativo al DNA puede modificar y modular la expresión de genes implicadas en las etapas de iniciación del cáncer. El licopeno es componente bioactivo que predomina en los tomates y el que más actividad antioxidante presenta (Erge y Karadeniz, 2011) y el consumo de éste protege a la célula evitando la oxidación de lípidos, proteínas y el DNA (Wertz et al., 2004).

Otro mecanismo por el que el licopeno disminuye el riesgo de cáncer de próstata es inhibiendo la interleucina-6 (IL-6). Esta es una citocina que promueve el proceso de inflamación y actúa como factor de crecimiento parácrino y autócrino en células epiteliales de la próstata (Wertz et al., 2004). Smith et al. (2001) estudiaron la relación entre los niveles séricos de IL-6 y la progresión del cáncer de próstata, encontrando una correlación positiva. Evidenciaron que el control de la IL-6 tiene grandes beneficios en pacientes con cáncer de próstata.

## Legislación Mexicana

Hoy en día un importante sector de la población mundial, convencida de la relación entre la alimentación y la salud, está demandando alimentos no solo para satisfacer el hambre sino también para prevenir las enfermedades relacionadas con la nutrición y mejorar su bienestar físico y mental (Siro et al., 2008). Esta situación es aprovechada por las empresas para crear los nuevos productos que satisfagan lo que el consumidor está pidiendo. Sin embargo es muy fácil caer en el engaño porque muchos de los productos ofertados que se hacen llamar funcionales no están sustentados por una base científica (Menrad, 2003).

La legislación en este campo es vital y necesaria para regular los productos funcionales. Los alimentos funcionales como tal iniciaron en Japón y es ese país quien está a la vanguardia en el tema de legislación. Europa y Estados Unidos cuentan también con leyes que regulan el mercado de los alimentos funcionales, sin embargo, en Latinoamérica y particularmente en México la legislación al respecto es aun pobre. La dependencia gubernamental encargada de vigilar cualquier riesgo sanitario en relación a los alimentos en México es la Comisión Federal para la Protección contra Riesgos Sanitarios

(COFEPRIS) (http://www.cofepris.gob.mx). Aunque no está enfocada propiamente a vigilar el cumplimiento de propiedades, venta y distribución de los alimentos funcionales, esta dependencia si regula el etiquetado y la publicidad de los alimentos en general, y es la encargada de vigilar y corregir cualquier anomalía o riesgo a la población. Además, existen Normas Oficiales en relación al etiquetado de los alimentos que deben cumplirse para comercializarlos, tal es el caso de la Norma Oficial Mexicana *NOM-051-SCFI/SSA1-2010*, *Especificaciones generales de etiquetado para alimentos y bebidas no alcohólicas pre envasados, Información comercial y sanitaria*. Dicha norma establece la información comercial y sanitaria que debe contener el etiquetado de los alimentos y bebidas no alcohólicas pre envasados de fabricación nacional o extranjera, así como determinar las características de dicha información. La información contenida en las etiquetas de los alimentos y bebidas no alcohólicas pre envasados debe ser veraz y describirse y presentarse de forma tal que no induzca a error al consumidor con respecto a la naturaleza y características del producto. Otra norma de interés que actualmente se está actualizando es la Norma Oficial Mexicana *NOM-086-SSA1-1994, Bienes y servicios. Alimentos y bebidas no alcohólicas con modificaciones en su composición. Especificaciones nutrimentales.* Los productos adicionados con fibra o ácidos grasos omega-3, y aquellos alimentos en los que se ha modificado el contenido de grasas saturadas, colesterol y sal son regulados por esta norma.

Es todavía mucho el trabajo que se tiene que realizar para evitar acciones fraudulentas contra el consumidor. La industria de alimentos no debe caer en el engaño ante el consumidor con el único aliciente de incrementar sus ventas. Debe probar científicamente que sus productos ofertados tienen un efecto real benéfico en la salud de quien los consume. Debe trabajar en incrementar la confianza en su mercado sustentado con evidencias científicas; estudios que proporcionen las pruebas necesarias sobre los efectos benéficos de estos alimentos en la salud del consumidor pues es la base para una legislación adecuada. Los productos cárnicos funcionales no existen en México como tal y no se consideran una clasificación de los alimentos. La legislación existente se enfoca solo en vigilar la seguridad de los alimentos.

## Evaluación de la Calidad de la Carne y Productos Cárnicos

La calidad de la carne y sus productos siempre ha sido importante para el consumidor, y es un tema especialmente crítico para la industria cárnica actual. Debido a que la demanda de carne y productos cárnicos ha ido en aumento en la mayoría de los países, la industria de la carne constantemente debe producir y suministrar carne de calidad atendiendo aspectos de sabor, nutrición y seguridad, que garanticen su consumo continuo. La calidad de la carne es un concepto difícil de definir porque está determinado por las preferencias de los consumidores, mismas que están relacionadas directamente con los sentidos como el aspecto, el olor, el sabor y sensación al masticar (Joo et al., 2013). Desde el punto de vista científico, la calidad de la carne es evaluada atendiendo los aspectos físicos, químicos, nutricionales, microbiológicos y sensoriales, entre otros. Algunas de las evaluaciones más comunes para evaluar la calidad de la carne y productos cárnicos se mencionan a continuación.

### Color

El color de cualquier objeto, incluyendo la carne, se define como la percepción del patrón espacial de la longitud de onda que emana de tal objeto (Young y West, 2001). El color es uno de los atributos más importantes en los alimentos, ya que éste es el parámetro principal que el consumidor juzga antes de adquirir un producto y está relacionado con la composición química o el grado de desarrollo o de alteración de un alimento. Generalmente el color preferido para la carne de res es el rojo cereza brillante. Éste parámetro está dado principalmente, primero por la concentración del pigmento muscular mioglobina, que da el color característico rojo de la carne, y segundo, la tonalidad del color depende de la proporción de los diferentes estados de oxidación de la mioglobina en la carne.

Los equipos más ampliamente utilizados para la medición del color en la carne y productos cárnicos son los colorímetros, mismos que miden el color en los valores *L**, *a** y *b**. El valor *L** se refiere a luminosidad del alimento y su medición esta dado en la escala de 0 (negro) a 100 (blanco). El valor *a** representa la variación entre el rojo y el verde en una escala de -60 a +60, donde valores positivos reflejan un color rojo y negativos verde. El valor *b** representa la variación de color entre el amarillo y el azul en una escala de -60 a+60, donde valores positivos dan una coloración amarilla y negativos azul.

**Potencial de hidrógeno (pH)**

El pH indica la concentración de iones hidronio que están presentes en determinadas sustancias. Tradicionalmente, el pH se mide usando un voltímetro equipado con un electrodo de vidrio. Matemáticamente el pH puede definirse como el logaritmo negativo de la concentración de iones hidronio y su escala de medición abarca de 0 a 14 (Buck et al., 2002). Probablemente, el pH es el atributo de calidad más comúnmente medido en la carne y productos cárnicos ya que influye en su capacidad tecnológica y estabilidad física, química, microbiológica y sensorial (Byrne et al., 2000). El pH normal de la carne de res oscila en el rango de 5.5 a 5.8 (Silva et al., 1999). Para el caso de las hamburguesas de res, su pH dependerá de los componentes en su formulación, variando éste entre 5.29 a 5.79 (Candogan, 2002; Sánchez-Escalante et al., 2003).

**Actividad de agua ($a_w$)**

La $a_w$ de un alimento es un factor muy importante para la conservación de éstos, ya que influye en las reacciones físicas, químicas, enzimáticas y microbiológicas; por otra parte, también confieren propiedades coligativas, reológicas y de textura a los alimentos (Fennema, 1996). Matemáticamente la $a_w$ está definida como la relación entre la presión de vapor de la solución (p) y la presión de vapor ejercida por el solvente ($p_0$). A medida que aumenta $a_w$ de un alimento, la probabilidad de contaminación y deterioro microbiano también aumenta. La $a_w$ de la carne de res es alta alrededor de 0.99 (Kinsella et al., 2006).

**Análisis de perfil de textura (APT)**

El APT es una importante prueba realizada a los alimentos para medir sus propiedades físicas y reológicas de manera objetiva. Consiste en someter al alimento a una prueba de doble compresión que simula su comportamiento al ser masticado e ingerido (Bourne, 1978). Para esta medición, tradicionalmente se utiliza un texturómetro, donde se realiza una compresión de la muestra y se obtiene una gráfica con la que se calcula el comportamiento reológico del alimento. Las determinaciones más importantes incluyen la dureza, elasticidad, cohesividad y masticabilidad. La dureza se refiere a la resistencia que opone la muestra a ser deformada, es decir, la fuerza del alimento, expresada en gramos o kilogramos. La cohesividad es un parámetro muy importante en los alimentos, ya que indica la tendencia de las partículas del alimento a mantenerse unidas, evaluando la energía mecánica que se necesita para destruir la estructura interna de la muestra a evaluar, y está dada por la relación existente entre el área debajo de la curva correspondiente a la segunda y primera compresión. La masticabilidad es un parámetro relacionado con la dureza, cohesividad y la elasticidad de una muestra y se expresa en g x mm (Nishinari et al., 2013). Todos los parámetros de textura mencionados anteriormente varían en función de la formulación del alimento y de las condiciones de almacenamiento (El-Magoli et al., 1996; Ganhão et al., 2010)

**Propiedades sensoriales**

El análisis sensorial de los alimentos se define como una disciplina científica utilizada para evocar, medir, analizar e interpretar las reacciones a aquellas características de alimentos y otras sustancias, que son percibidas por los sentidos de la vista, olfato, gusto, tacto y oído (Lawless y Heymann, 2010). Éste análisis es de suma importancia para el mejoramiento y optimización de los productos alimenticios existentes. Así mismo, hoy en día esta herramienta es utilizada para realizar investigaciones en la elaboración e innovación de nuevos productos, en el aseguramiento de la calidad y para su promoción y venta, conociendo así los requerimientos y preferencias del consumidor (Resurreccion, 2007).

**Composición química y nutricional**

Determinar la composición química básica de un alimento en términos de su contenido de humedad, grasa, proteína y ceniza, proporciona una idea general de su calidad nutricional. Determinaciones específicas como perfil de ácidos grasos, contenido de colesterol, aminoácidos, vitaminas y minerales, son útiles en el desarrollo de nuevos productos y es información nutricional valiosa para el consumidor (Martin et al., 2013).

**Inocuidad de la carne**

La carne debe estar libre de cualquier microorganismo patógeno que comprometa su seguridad. *Escherichia coli* O157:H7 es considerada uno de los patógenos alimentarios más peligrosos conocidos que se trasmite principalmente por ingerir carne contaminada que ha sido cocinada deficientemente (Kiranmayi et al., 2010). La composición de la carne y la formulación de los productos cárnicos son factores importantes que determinan la inactivación de los microorganismos durante el cocimiento o su crecimiento durante el periodo de almacenamiento.

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# CAPÍTULO 3
# ARTÍCULOS DE INVESTIGACIÓN GENERADOS

Para cumplir con los objetivos planteados en esta investigación, el trabajo se dividió en etapas que a continuación se describen.

En la primera etapa se estudiaron las características de calidad de carne molida para hamburguesas a la que se le incorporó linaza y pasta de tomate. Para ello, se empleó la metodología de superficie de respuesta (MSR) con el fin de establecer las ecuaciones matemáticas que predicen la calidad del producto en cualquier combinación de los ingredientes no tradicionales. Con la ayuda de estas ecuaciones, se seleccionó una formulación con las mejores características de calidad para un producto con connotación funcional, comparado con el tradicional. El producto de este primer experimento fue el artículo:

**Valenzuela-Melendres M**, J.P. Camou N.G. Torrentera-Olivera, E. Álvarez-Almora, D. González-Mendoza, L. Avendaño-Reyes, H. González-Ríos. 2014. Response surface methodology for predicting quality characteristics of beef patties added with flaxseed and tomato. Meat Science (97)54-61

Esta parte del trabajo dio pauta a una estancia de investigación en el Departamento de Agricultura de los Estados Unidos (USDA), cuyo objetivo fue desarrollar un modelo para predecir la reducción de *Salmonella* en un producto tipo jerky de res usando como factores de control la temperatura, pH, actividad de agua y concentración de sorbato de potasio. Cabe resaltar que en este trabajo estuvo vinculada una empresa productora de jerky de los Estados Unidos, misma que buscaba un método alternativo que asegurara la reducción de *Salmonella* en el producto. La experiencia en el manejo de la MSR adquirida en la primera etapa del trabajo de tesis sirvió para proponer el trabajo que se desarrolló en la estancia de investigación. Los resultados de este estudió se presentaron en el siguiente artículo:

Vijay K. Juneja, **Martin Valenzuela-Melendres**, Dilek Heperkan, Derrick Bautista, David Anderson, Cheng-An Hwang, Aida Peña-Ramos, Juan Pedro Camou and Noemi Torrentera-Olivera. Development of a predictive model for *Salmonella* spp. reduction in meat jerky product with temperature, potassium sorbate, pH, and water activity as controlling factors. Journal of Food Microbiology (Enviado)

De suma importancia para el consumidor es contar con información sobre el contenido nutricional de los nuevos productos cárnicos funcionales. Este aspecto fue abordado en la segunda etapa de la investigación donde se determinó el contenido de colesterol, perfil de ácidos grasos y composición química de varias formulaciones de carne molida para hamburguesas adicionadas con harina de linaza y pasta de tomate. Los resultados se enviaron para su publicación a la revista *Journal of Food Science and Technology.*

**Martín Valenzuela-Melendres**, Noemí G. Torrentera-Olivera, Juan Pedro Camou, Leonel Avendaño-Reyes, Daniel González-Mendoza, Enrique Álvarez-Almora, Humberto González-Rios. Physicochemical, sensory and nutritional quality of beef patties added with flaxseed and tomato paste (enviado).

En esta misma etapa se realizó una investigación adicional derivada del curso Alimentos Funcionales, donde se estudió el perfil nutricional de un producto tipo salchicha adicionado con pasta de tomate y pulpa de aguacate. El artículo generado fue:

**Valenzuela-Melendres M.,** N. G. Torrentera-Olivera, G. González-Aguilar, M. Villegas-Ochoa, L. G. Cumplido-Barbeitia and J.P Camou. 2014. Use of avocado and tomato paste as ingredients to improve nutritional quality of pork frankfurter. Journal of Food Research. (3) 132-143.

Otro de los aspectos importantes que se abarcó en esta investigación fue la inocuidad del producto funcional. Todos los productos cárnicos son propensos a contaminarse con microorganismos patógenos que son de interés en salud pública. El comportamiento de estos patógenos depende de muchos factores, siendo la composición del alimento un aspecto importante que determina su proliferación. En la tercera y última etapa de la presente investigación se estudió el comportamiento de *Escherichia coli* O157:H7 simulando un cocimiento inadecuado del producto, y un almacenamiento en refrigeración y abuso de temperatura, utilizando la metodología de superficie de respuesta. Se continúa trabajando en los resultados para su eventual publicación.

## Artículo 1

### Response surface methodology for predicting quality characteristics of beef patties added with flaxseed and tomato paste

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## ABSTRACT

Surface response methodology was used to study the effect of flaxseed flour (FS) and tomato paste (TP) addition, from 0 to 10% and 0 to 20% respectively, on beef patties quality characteristics. The assessed quality characteristics were color (L *, a *, b *), pH and texture profile analysis (TPA). Also, sensory analysis was performed by measuring parameters of color, juiciness, firmness, and general acceptance. FS addition reduced L* and a* values and decreased weight loss of cooked products ($P<0.05$). An opposite effect was observed when TP was added ($P<0.05$). All TPA parameters decreased when percentages of FS and TP were increased in the formulation of beef patties. Furthermore, FS and TP addition adversely affected sensory characteristics of cooked product ($P<0.05$); nevertheless, all sensory characteristics were evaluated with an acceptable score (> 5.6). Results show that FS and TP are ingredients that can be used for beef patties preparation.



**Keywords:** beef patty, functional foods, meat quality, response surface methodology


## Introduction

Meat is an important source of protein and essential nutrients including iron, zinc, vitamin $B_{12}$ and folic acid (Scollan et al., 2006; Schönfeldt and Gibson, 2008). Some studies have shown the benefits of meat consumption (McAfee et al., 2010; Givens et al., 2006; Biesalski, 2005; Valsta et al., 2005). However, a sector of the population perceives meat as a food that is detrimental to their health (Oliveira et al., 2011) because some epidemiological studies have associated meat consumption with cardiovascular diseases (CVD) and colon cancer (Chan and Giovannucci, 2010; Goldhaber, 2010; Paik et al., 2005). It is clear that today's consumers pay more attention to their diet and its relationship to health. This reality cannot be ignored by the meat industry that must face the new changes to suit consumer’s current needs by offering alternative products with a healthy connotation, in which case functional meat products are a good option (Bhat and Bhat, 2011; Arihara and Ohata, 2010; Jiménez-Colmenero et al., 2001).

Functional foods are fortified with special constituents that possess advantageous physiological effects (Hardy, 2000; Kwak and Jukes, 2001; Stanton et al., 2005). This kind of products may improve the general conditions of the body or decrease the risk of some diseases (Siro et al., 2008). Natural ingredients may be added to meat products to reach health-oriented consumers with the objective of increasing antioxidant activity (Yıldız-Turp and Serdaroglu, 2010), fatty acid profile modification (Rodríguez-Carpena et al., 2012; Yılmaz et al., 2002), fiber addition (Fernández-Ginés et al., 2003), amongst others.

Flaxseed and tomatoes are foods that have proven themselves to be effective against colon cancer and CVD (Dodin et al., 2008; Canene-Adams et al., 2005). Flaxseed is high in protein, dietary fiber and polyunsaturated fatty acids, highlighting its α-linolenic acid content (Bloedon and Szapary, 2004), while tomato is an excellent source of phytochemicals, including carotenoids and polyphenols (Navarro-González et al., 2011). Both tomato and flaxseed are natural ingredients with great potential to be incorporated into a meat product.

During the development of functional meat products, incorporation of nontraditional ingredients in the formulation could affect quality properties. It is important to known how these new ingredients affect meat quality. Response surface methodology (RSM) helps us explore variations in meat products quality when ingredients levels have been changed in the formulation (Velioğlu et al., 2010; Sarıçoban et al., 2009). This technique is a mathematical and statistical tool used to test multiple variables in a process, observing its direct effect or interaction and quadratic effect (Gan and Latiff, 2011; Gan et al., 2009). RSM has been used for the simultaneous study of various factors on quality parameters during the development of meat products (Velioğlu et al., 2010; Sarıçoban et al., 2009).

Therefore, the objective of this work was to evaluate the effect of flaxseed and tomato paste addition on the quality of beef patties using RSM.

## Materials and methods

*Experimental design*

The effect of adding flaxseed and tomato paste on the characteristics of quality (pH, color, antioxidant capacity, texture profile analysis and sensory analysis) of beef patties was studied. The characteristics of quality were evaluated using a response surface model with a central composite design (CCD) with two factors (flaxseed and tomato paste). Experimental and coded levels for both factors are presented in Table 1. Thirteen experimental runs in random order, with five replications on the central point and simple runs for the rest of the treatments of the CCD were performed. All determinations of the response variables were carried out in triplicate. The experimental design of this study is presented in Table 2.

*Ingredients acquisition and meat preparation*

Beef inside round (*Semimembranosus*), flaxseed flour (FS) (*Linum usitatissimum L*) and tomato paste (PT) (*Lycopersicum esculentum*) were used and obtained from the local market. All meat cuts were obtained from animals with not more than 5 days of harvest. Meat was used the same day of purchase, cut into pieces no larger than 5 $cm^3$ and ground using a Hobart grinder (model 4152, Troy, Ohio, USA) through a 3/16 " plate. Ground meat was stored in refrigeration (2 °C) until further use.

One-kilogram batches per treatment were used for beef patties preparation. Meat (73% moisture, 6% fat), FS (19% protein, 7% moisture), TP (90% moisture) and spices were mixed according to percentages specified in the experimental runs in Table 2. Ingredients in each batch were manually homogenized for 10 min. Beef patties were formed into 9 cm x 1 cm diameter of thickness to obtain approximately 70 g per unit. An electric skillet (Cook Master Oster, Model 3222-3, Mississauga, Ontario, Canada) was used for cooking the patties 1 min each side and then 15 s per side until reaching 72 °C, measured in the geometrical center with a thermocouple.

*Product quality determinations*

Quality parameters assessed for fresh products were: proximate analysis (moisture, fat, protein and ash), objective color (L*, a*, b*), pH and sensory color; and for cooked products were: texture profile analysis (hardness, elasticity, cohesiveness and chewiness) and sensory analysis (juiciness, taste, firmness and overall acceptability). All determinations were carried out between 24 h after preparation of beef patties, except proximate analysis. Samples for this determination were stored at -18°C until further use.

*Proximate composition*

Moisture, ash, protein and fat content were determined according to AOAC methods (AOAC, 2011). Moisture (g water/100 g sample) was determined by drying a 5 g sample at 100 °C to constant weight. Ash was performed at 550 °C for 4 h (g ash/100 g sample). Protein (g protein/100 g sample) was analyzed according to Kjeldahl method. Factor 6.25 was used for conversion of nitrogen to crude protein. Fat (g fat/100 g sample) was calculated by weight loss after an extraction with petroleum ether in a goldfish apparatus.

*Physicochemical evaluation.*

Beef-patties surface color was measured with a Minolta colorimeter using the D65 illuminant and 10° standard observer (Chroma meter CR-400, Konica Minolta Sensing, Inc. Japan) recording L*, a* and b* values. L* value indicates lightness; a* value indicates redness; and b* value indicates yellowness. For Hydrogen potential (pH) measurement, five grams of ground patties sample was weighed into 100 mL beakers and 45 mL of distilled water were added and homogenized. A portable pH meter (Hanna, Model HI 98140, Woonsocket, RI, USA) equipped with a puncture type combination pH electrode was used, and the reading was taken once stabilized.

*Texture profile analysis (TPA)*

A Texture Analyzer TA-XT2 (Stable Micro Systems, Surrey, UK) was used in raw beef patties. Cubic samples (1 x 1 x 1 cm) were cut from burgers and subjected to a two-cycle compression test. Samples were compressed to 50% of their original height with a 7.5 cm diameter cylindrical probe attached to a 50 kg compression cell with a cross-head speed of 1 mm/s. Texture profile parameters were determined according to Bourne (1978b) and interpreted as follows: Hardness (kg) is the maximum force required to compress the sample; cohesiveness is the extent to which sample could be deformed prior to rupture (A2/A1), being A1 the total energy required for the first compression and A2 the total energy required for the second compression; springiness (cm) is the ability of the sample to recover its original shape after the deforming force is removed, and chewiness (kg x cm) is the work needed to masticate the sample for swallowing (hardness x cohesiveness x springiness).

*Sensory analysis*

Sensory evaluations were conducted by an 8-trained member panel (ISO-8586-1, 1993b) in an environmentally controlled (21 ± 1 °C, 55 ± 5% relative humidity) room partitioned into booths. Three patties from each formulation were cooked as previously described, and maintained warm in an oven until testing within 4-7 min. Square pieces approximately 1.5 x 1.5 cm were cut from the center of patties, and were served at room temperature. Each sample was coded with randomly selected 3-digit numbers. Panelists were instructed to clean their palates between samples using water. Color, flavor, firmness and juiciness of beef patties were scored using a 9 point Hedonic scale, where 1 represented dislike extremely and 9 represented like extremely. Each attribute was discussed and tests were initiated after the panelists were familiarized with scales. At the end of the test, panelists were asked to give a score for product overall acceptability from 0 to 9.

*Statistical analysis*

Experimental data of each determination were adjusted to a second order complete polynomial model, and coefficients were determined for every term of the model. Prediction of the response variable was given by the following model:

$$RV = \beta_0 + \beta_1 * FS + \beta_2 * TP + \beta_{11} * FS^2 + \beta_{22} * TP^2 + \beta_{12} * FS * TP$$

Where:

RV = response variable (pH, color, texture and sensory)

FS = flaxseed flour (%)

TP = tomato paste (%)

$\beta_0$ = regression coefficient for the intercept

$\beta_1$ = linear regression coefficient for FS

$\beta_2$ = linear regression coefficient for TP

$\beta_{11}$ = quadratic regression coefficient for FS

$\beta_{22}$ = quadratic regression coefficient for TP

$\beta_{12}$ = regression coefficient of FS*TP interaction

Analysis of variance (ANOVA), coefficient of determination ($R^2$), and lack of fit of model were determined using the statistical program JMP® version 10.0.0 (SAS, Institute, Inc). Statistical significance of the model and model variables was determined at the 5% probability level ($p<0.05$).

JMP® software was used for simultaneous optimization of the multiple responses. Optimal ingredients levels of flaxseed flour and tomato paste were determined by superimposing the plots for all response variables (Capanzana & Buckle, 1997; Vatsala et al., 2001). The optimum formulations were selected for calculating the predicted values of response variables using the prediction equations derived by RSM.

## Results and discussion

*Proximate analysis*

Table 3 shows results of proximate analysis for all experimental runs, while table 4 presents regression coefficients and the significances for flaxseed and TP addition as well as and their interactions. Moisture content ranged from 70.38 to 77.71 %, value that decreased as the percentage of FS increased ($P<0.05$), possibly due to an increase in dry matter content in hamburger formulation. Fat content ranged from 1.39 to 4.59 %, this value increased with FS addition ($P<0.05$), behavior that was associated to the fat content of FS (30%). Similar results were reported by Elif Bilek and Turhan (2009) in hamburgers formulated with 15 % FS. Addition of TP in the formulation of hamburgers had an opposite effect on moisture and fat percentages ($P<0.05$), probably due to a dilution effect (TP, moisture 90 %). The same behavior was observed for protein values which ranged between 16.06 and 21.35%. Candogan (2002) observed the same trend in hamburgers made with 15% of TP. Examples of increasing flaxseed and tomato content in beef hamburgers formulation and their effect on moisture, fat, and protein content are shown in experimental runs numbers 3 and 5 of table 3.

*Color, pH, and cooking weight loss*

Table 5 presents the regression coefficients, coefficients of determination and significances of the models for the response variables of color, pH, and cooking weight loss. All models to estimate these variables were significant ($P<0.05$), none presented lack of fit ($P>0.05$), and $R^2$ ranged between 0.94 and 0.99 indicating that these models were suitable for predicting the response variable to changes of FS and TP levels in the formulation of beef burgers. In accordance with the obtained results, the incorporation of FS decreased values of L* and a* on the product, while an increase in the amounts of TP had an opposite effect (Figure 1). Color is the first quality taken into account by the consumer when one purchases a meat product. Visual perception of color in the raw product is influenced by the degree or intensity of lightness (L*) and by the balance between red (a*) and yellow (b*) values (Shahiri Tabarestani and Mazaheri Tehrani, 2012). Color of ground meat products like beef hamburgers can be altered by the proportion of non-meat ingredients in the formulation (Whyte, 2006). Therefore, the effects on final-product color due to incorporation of FS and TP can be attributed to natural color of these ingredients and their amount of addition in beef patties formulation. In this study, L* and a* values changed inversely to FS content in hamburgers since they presented lower values to those of fresh ground meat (L* = 44.02, a* = 4.77, and L* = 49.72, a* = 19.59, respectively). On the other hand, when TP was added to hamburgers, L* and a* values increased due to the moisture and red color of tomato paste. According to Candogan (2002), the effect of TP to improve color in beef burgers can be attributed to the red pigment, lycopene, present in TP. Østerlie and Lerfall (2005); Sing et al. (2011) and Yılmaz et al. (2002) also reported the effect of TP on beef-patties color.

Analysis of variance indicated that pH regression model was significant ($P<0.05$); the model explained 98% of the variation in the experimental data, and there was no lack of fit ($P>0.065$). Figure 2 shows the effect of FS and TP incorporation in the pH of hamburgers. The acidic nature of TP (pH = 4.2) caused a decrease of pH in beef patties when TP increased. Similar results were reported by Candogan (2002) in beef burgers made with 10 and 15% TP. Garcia et al. (2009) and Deda et al. (2007) also reported a decrease in pH by adding TP in various meat products. A decrease in pH can affect the capacity of water retention and sensory characteristics of meat products. Deda et al. (2007) found a negative correlation between the loss of water and pH of sausages containing TP; Garcia et al. (2009) reported that the acidity of beef burgers (pH = 5.43) with 6% of dried tomato peel could be one of the reasons for low product acceptability.

Table 5 also shows the effect of FS and TP addition on cooking weight loss. Figure 3 illustrates these effects in a three dimension graph. The addition of FS decreased ($P<0.05$) cooking weight loss, probably due to the increase in dry matter in the formulation and its ability to retain moisture. Similar results were presented by Elif Bilek and Turhan (2009) in beef burgers with 15% addition of FS. Yi et al. (2012) also reported a decrease in cooking weight loss of beef burgers with 2% rice flour. On the other hand, the addition of TP increased cooking weight loss ($P<0.05$) probably due to an increase in the percentage of moisture in product (Table 3) and a decrease in pH. Velioğlu et al. (2010) reported an increase in the loss of moisture by cooking when the amount of water in the formulation of beef burgers was increased. Although Deda et al. (2007) found no significant differences ($P>0.05$) in cooking weight loss between sausages made with 12% of TP and control, the reported values were higher in the treatment with added TP.

*Texture profile analysis (TPA)*

Analysis of variance indicates that cohesiveness, hardness, elasticity and chewiness regression models were significant ($P<0.05$) (Table 6). Figure 4 shows the effects of FS and TP on these variables. Incorporation of FS and TP in beef patties decreased ($P<0.05$) all texture attributes, exerting more effect the addition of FS than TP as shown in table 6 by the regression coefficients. There were no reports on the use of FS in beef patties and its impact on texture. However, several researchers have shown the effect of incorporation of different extenders in the texture of meat products. Yi et al. (2012) reported a reduction in hardness of beef hamburgers with 2% rice flour. Yang et al. (2007) studied the effect of oatmeal on the texture of low fat sausages and reported a reduction in hardness, cohesiveness and chewiness when they incorporated 25% of hydrated oatmeal. In both studies incorporation of rice flour or oatmeal increased moisture retention and improved texture of meat products. In our study, TP addition decreased both hardness (Table 6) and water retention (Table 5), probably due to a weakening of the protein matrix formed during the heating. Kerr, Wang, and Choi (2005) suggested the same explanation when they observed a decrease in hardness of low fat Italian sausage added with 30% hydrated oatmeal. A similar behavior was also shown by Yang et al. (2007) who reported a decrease in hardness of low fat pork sausages added with 25% tofu.

*Sensory evaluations*

Sensory evaluation results are presented in Table 7. Regression models for all evaluated sensory attributes were significant (P<0.05). There was a linear effect (P<0.05) of FS and TP on firmness. An increase in the incorporation of FS and TP decreased firmness of beef hamburgers; these results were consistent with hardness measured by TPA analysis (Figures 4 and 5). Juiciness also decreased with the increase of TP (P<0.05), while FS had no effect (P>0.05). These results were related to the amount of water released when cooking and enhanced by the moisture content in TP (Table 5). According to Velioğlu et al. (2010), water loss during cooking directly affects the appearance and texture of the burgers.

Flavor and color were influenced only by the addition of FS (P<0.05). The effect on flavor may be due to oxidation of polyunsaturated fatty acids of flaxseed oil (Nykter et al., 2006). According to regression coefficients shown in Table 7, both FS and TP had a negative effect on hamburger general acceptance (P<0.05). (Pelser et al. (2007a)) reported a decrease in acceptance of pork sausages added with flaxseed oil. This low acceptance was attributed to the oxidation of polyunsaturated lipids present in flaxseed. Elif Bilek and Turhan (2009) reported a decrease in hamburger sensory attributes with the increase of 0 to 15% flaxseed flour. Garcia et al. (2009), reported a decrease in beef burgers general acceptance when increasing the amount of tomato dried peel from 0 to 6%. In our study, though the negative effect that FS and TP had on the general acceptance of hamburgers, these were evaluated with high scores (> 5.6), results not shown.

*Optimization*

A series of contour plots were generated and superimposed (Figs. 1–5). An area of optimum performance was located for all the response variables in which limits of each response had been established (Capanzana & Buckle, 1997; Gan et al. 2007). The region that satisfied all limits was selected as the optimum point. Based on these superimposed plots, the optimal beef patty formulation was 3% of FS and 10.8% of TP. The predicted values for each of the response variables were as follows: L* = 49.37, a* = 16.46, b* = 15.96, pH = 5.41, cooking loss = 29.21%, instrumental hardness was 1.66 kg, springiness = 0.67, cohesiveness = 0.48 and chewiness was 0.62 kg; sensory score for color was 6.9, flavor was 6.4, firmness 6.5, juiciness was 5.9 and overall acceptability was 6.6.

## Conclusion

Quality and sensory characteristics of beef hamburgers were affected by the addition of FS and TP. Addition of FS decreased L* and a* values, while TP increased them. pH decreased with the addition of TP and increased with addition of FS. Cooking weight loss was reduced by addition of FS and increased with TP. Hardness, elasticity, cohesiveness and chewiness were reduced with FS and TP; of these two FS had a greater effect. With respect to sensory properties, even though FS and TP decreased sensory scores, they all showed well accepted scores. The models developed in this study can be used to estimate desired quality characteristics of beef patties within the limits of FS and TP up to 10% and 20%, respectively. The optimized formula predicted by the models was composed of 3% FS and 10.8% TP.

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Table 1. Experimental levels of two independent variables in terms of actual values

| Variables | Symbols | Coded variables levels | | | | |
|---|---|---|---|---|---|---|
| | | -α (-1.414) | -1 | 0 | 1 | α (1.414) |
| FS (%) | $X_1$ | 0 | 1.5 | 5 | 8.5 | 10 |
| TP (%) | $X_2$ | 0 | 3 | 10 | 17 | 20 |

Flaxseed flour (FS), Tomato paste (TP)

Table 2. Coded and experimental values of response variables for central composite design

| Run | Coded level | | Experimental value | |
|---|---|---|---|---|
| | $X_1$ | $X_2$ | FS (%) | TP (%) |
| 1 | - | - | 1.5 | 3 |
| 2 | - | + | 1.5 | 17 |
| 3 | + | - | 8.5 | 3 |
| 4 | + | + | 8.5 | 17 |
| 5 | -1.414 | 0 | 0 | 10 |
| 6 | 1.414 | 0 | 10 | 10 |
| 7 | 0 | -1.414 | 5 | 0 |
| 8 | 0 | 1.414 | 5 | 20 |
| 9 | 0 | 0 | 5 | 10 |
| 10 | 0 | 0 | 5 | 10 |
| 11 | 0 | 0 | 5 | 10 |
| 12 | 0 | 0 | 5 | 10 |
| 13 | 0 | 0 | 5 | 10 |

Flaxseed flour (FS), Tomato paste (TP)

Table 3. Proximate composition (%) of the different beef patties[1]

| Number* | Moisture | Fat | Protein | Ash |
|---|---|---|---|---|
| 1 | 75.09 ± 0.47 | 2.10 ± 0.07 | 19.74 ± 0.01 | 1.69 ± 0.01 |
| 2 | 77.71 ± 0.23 | 2.14 ± 0.12 | 17.08 ± 0.23 | 1.56 ± 0.01 |
| 3 | 70.38 ± 0.16 | 4.27 ± 0.02 | 21.35 ± 0.18 | 2.02 ± 0.02 |
| 4 | 72.81 ± 0.38 | 3.84 ± 0.17 | 18.65 ± 0.06 | 1.80 ± 0.04 |
| 5 | 77.97 ± 0.57 | 1.39 ±0.02 | 17.37 ± 0.17 | 1.60 ± 0.01 |
| 6 | 70.82 ± 0.01 | 4.59 ± 0.06 | 20.03 ± 0.07 | 1.97 ± 0.01 |
| 7 | 72.25 ± 0.16 | 2.96 ± 0.02 | 20.18 ± 0.57 | 1.81 ± 0.03 |
| 8 | 75.66 ± 0.07 | 3.09 ± 0.11 | 16.06 ± 0.47 | 1.71 ± 0.03 |
| 9 | 73.86 ± 0.17 | 3.12 ± 0.02 | 18.70 ± 0.60 | 1.79 ± 0.01 |
| 10 | 73.74 ± 0.27 | 2.74 ± 0.01 | 18.40 ± 0.02 | 1.79 ± 0.01 |
| 11 | 72.69 ± 1.28 | 3.64 ± 0.01 | 19.83 ± 0.38 | 1.81 ± 0.03 |
| 12 | 74.09 ± 0.31 | 2.88 ± 0.02 | 18.80 ± 0.01 | 1.78 ± 0.02 |
| 13 | 73.77 ± 0.10 | 2.80 ± 0.20 | 18.04 ± 0.13 | 1.80 ± 0.01 |
| Control | 74.80 ± 0.20 | 1.86 ± 0.03 | 19.49 ± 0.49 | 1.74 ± 0.02 |

[1]Average ± standard error
*Run from Table 2

Table 4. Estimated regression coefficients (RC) and analysis of variance of the regression models for proximal composition of beef patties

| | Moisture | | Fat | | Protein | | Ash | |
|---|---|---|---|---|---|---|---|---|
| | RC | SE | RC | SE | RC | SE | RC | SE |
| Intercept | 73.631* | 0.191 | 3.05* | 0.131 | 18.891* | 0.301 | 1.792* | 0.013 |
| FS | -2.465* | 0.151 | 1.048* | 0.103 | 0.874* | 0.238 | 0.137* | 0.010 |
| TP | 1.233* | 0.151 | -0.025 | 0.103 | -1.408* | 0.238 | -0.062* | 0.010 |
| FS*TP | -0.046 | 0.214 | -0.116 | 0.147 | -0.01 | 0.336 | -0.019 | 0.014 |
| FS*FS | 0.033 | 0.162 | -0.009 | 0.111 | 0.171 | 0.255 | -0.003 | 0.011 |
| TP*TP | 0.117 | 0.162 | 0.006 | 0.111 | -0.118 | 0.255 | -0.017 | 0.011 |
| $R^2$ | 0.98 | | 0.94 | | 0.88 | | 0.97 | |
| *P values* | | | | | | | | |
| Regression | <0.001 | | <0.001 | | 0.004 | | <0.001 | |
| Lack of fit | 0.95 | | 0.836 | | 0.484 | | 0.024 | |

Flaxseed flour (FS), tomato paste (TP)
*P<0.05
SE = standard error

Table 5. Estimated regression coefficients (RC) and analysis of variance of the regression models for physicochemical characteristics of beef patties

| | L* | | a* | | b* | | pH | | Cooking loss (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | RC | SE | RC | SE | RC | SE | RC | SE | RC | SE |
| Intercept | 48.53* | 0.165 | 14.49* | 0.164 | 15.80* | 0.105 | 5.437* | 0.004 | 22.400* | 0.282 |
| FS | -1.117* | 0.131 | -2.736* | 0.130 | 0.182 | 0.083 | 0.028* | 0.003 | -11.06* | 0.223 |
| TP | 0.826* | 0.131 | 0.706* | 0.130 | 1.417* | 0.083 | -0.065* | 0.003 | 1.935* | 0.223 |
| FS*TP | 0.389 | 0.185 | -0.006 | 0.184 | -0.371* | 0.117 | 0.008 | 0.005 | -0.500 | 0.316 |
| FS*FS | 0.405* | 0.140 | 1.025* | 0.139 | 0.234* | 0.089 | -0.001 | 0.003 | 0.831* | 0.239 |
| TP*TP | 0.032 | 0.140 | -0.136 | 0.139 | -0.007 | 0.089 | 0.001 | 0.003 | -0.293 | 0.239 |
| $R^2$ | 0.94 | | 0.98 | | 0.97 | | 0.98 | | 0.99 | |
| *P values* | | | | | | | | | | |
| Regression | <0.001 | | <0.001 | | <0.001 | | <0.001 | | <0.001 | |
| Lack of fit | 0.9614 | | 0.9959 | | 0.88 | | 0.065 | | 0.99 | |

Flaxseed flour (FS), tomato paste (TP)
*P<0.05
SE = standard error

Table 6. Estimated regression coefficients (RC) and analysis of variance of the regression model for texture profile analysis of beef patties

| | Hardness | | Springiness | | Cohesiveness | | Chewiness | |
|---|---|---|---|---|---|---|---|---|
| | RC | SE | RC | SE | RC | SE | RC | SE |
| Intercept | 1.294* | 0.048 | 0.612* | 0.011 | 0.448* | 0.004 | 0.354* | 0.045 |
| FS | -0.601* | 0.038 | -0.109* | 0.009 | -0.060* | 0.003 | -0.422* | 0.030 |
| TP | -0.358* | 0.038 | -0.049* | 0.009 | -0.016* | 0.003 | -0.233* | 0.030 |
| FS*TP | 0.012 | 0.054 | -0.015 | 0.012 | 0.022* | 0.005 | 0.225* | 0.050 |
| FS*FS | 0.201* | 0.041 | 0.027* | 0.009 | 0.012* | 0.004 | 0.205* | 0.038 |
| TP*TP | 0.019 | 0.041 | -0.002 | 0.009 | -0.007 | 0.004 | 0.045 | 0.38 |
| $R^2$ | 0.98 | | 0.96 | | 0.97 | | 0.97 | |
| *P values* | | | | | | | | |
| Regression | 0.0001 | | 0.0001 | | 0.0001 | | 0.0001 | |
| Lack of fit | 0.0208 | | 0.418 | | 0.192 | | 0.0016 | |

Flaxseed flour (FS), tomato paste (TP)
*P<0.05
SE = standard error

Table 7. Estimated regression coefficients and analysis of variance of the regression model for sensorial attributes of beef patties

| | Firmness | | Juiciness | | Flavor | | Color | | Overall acceptance | |
|---|---|---|---|---|---|---|---|---|---|---|
| | RC | SE | RC | SE | RC | SE | RC | SE | RC | SE |
| Intercept | 6.202* | 0.132 | 5.908* | 0.087 | 5.908* | 0.133 | 6.480* | 0.100 | 6.092* | 0.132 |
| FS | -0.648* | 0.104 | 0.037 | 0.068 | -0.759* | 0.105 | -0.641* | 0.079 | -0.771* | 0.104 |
| TP | -0.547* | 0.104 | -0.384* | 0.068 | -0.034 | 0.105 | -0.002 | 0.079 | -0.343* | 0.104 |
| FS*TP | 0.012 | 0.147 | 0.422* | 0.097 | -0.107 | 0.149 | 0.090 | 0.111 | -0.195 | 0.147 |
| FS*FS | 0.087 | 0.112 | 0.178* | 0.073 | 0.269* | 0.113 | 0.237* | 0.084 | 0.213 | 0.111 |
| TP*TP | -0.039 | 0.112 | 0.039 | 0.073 | 0.219 | 0.113 | -0.012 | 0.084 | 0.080 | 0.111 |
| $R^2$ | 0.90 | | 0.89 | | 0.89 | | 0.91 | | 0.91 | |
| *P values* | | | | | | | | | | |
| Regression | 0.002 | | 0.003 | | 0.002 | | 0.001 | | 0.001 | |
| Lack of fit | 0.935 | | 0.369 | | 0.955 | | 0.872 | | 0.208 | |

Flaxseed flour (FS), tomato paste (TP)
*P<0.05
SE = standard error

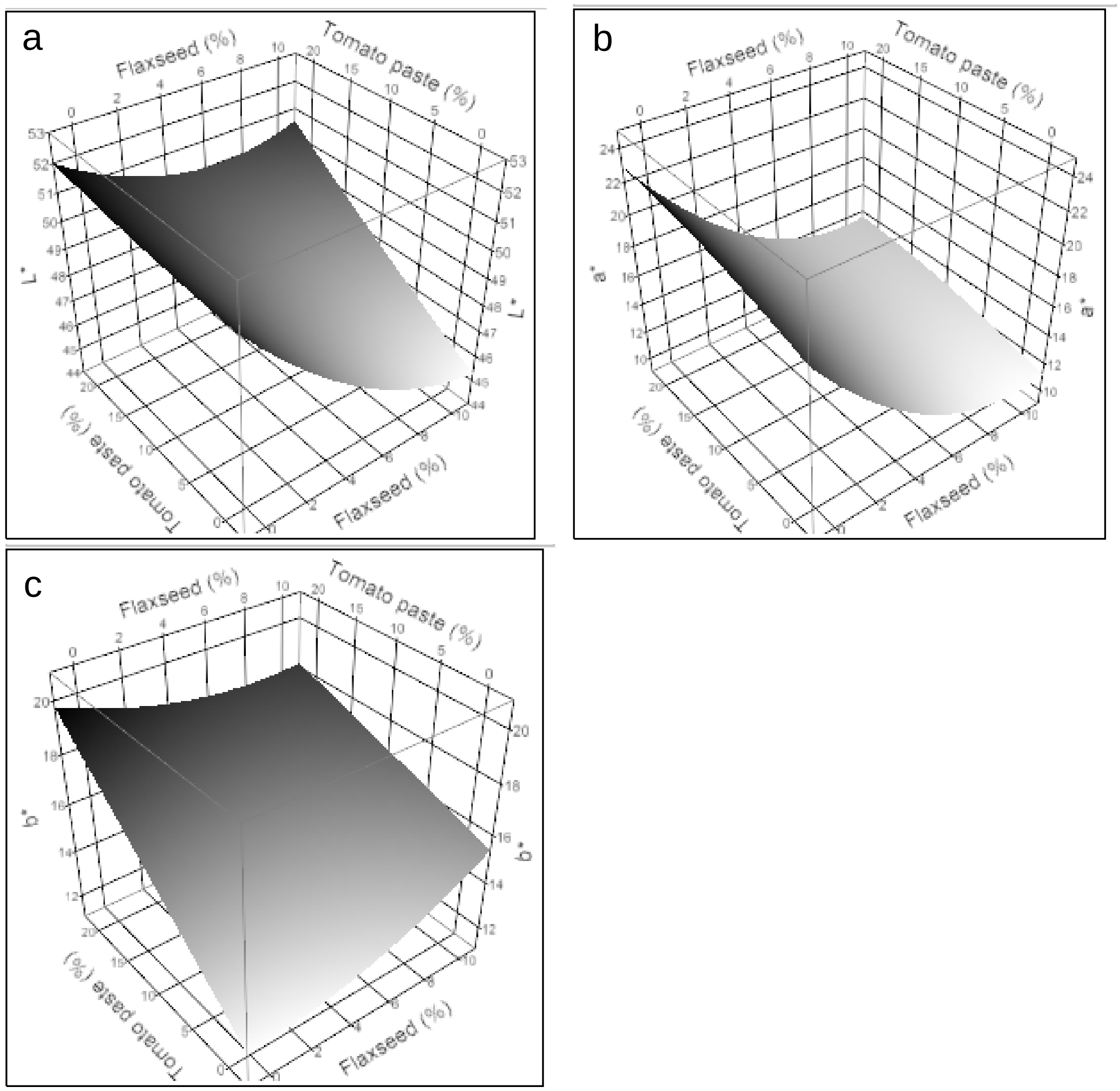


Figure 1. Effects of flaxseed flour and tomato paste on a) lightness (L*), b) redness (a*) and c) yellowness (b*) of beef patties

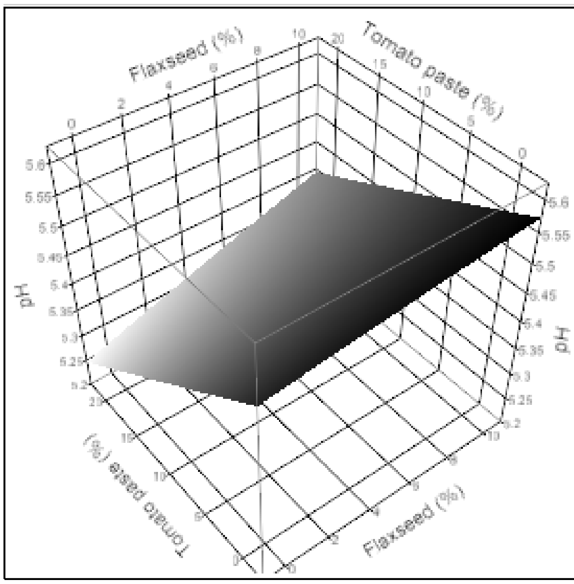


Figure 2. Effects of flaxseed flour and tomato paste on pH of beef patties

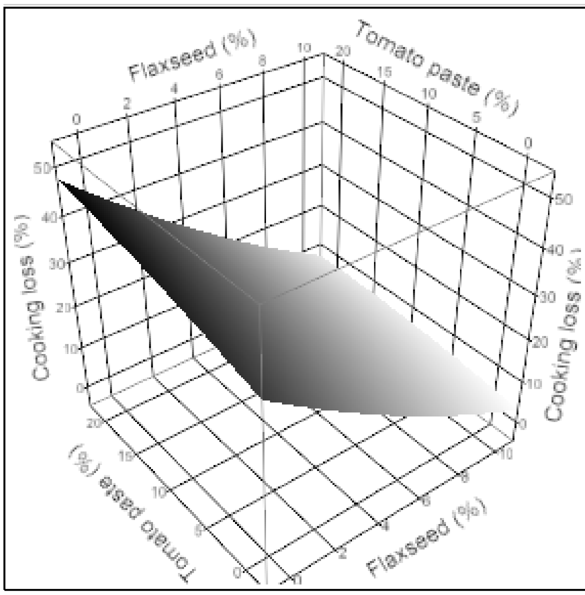


Figure 3. Effects of flaxseed flour and tomato paste on cooking loss (%) of beef patties

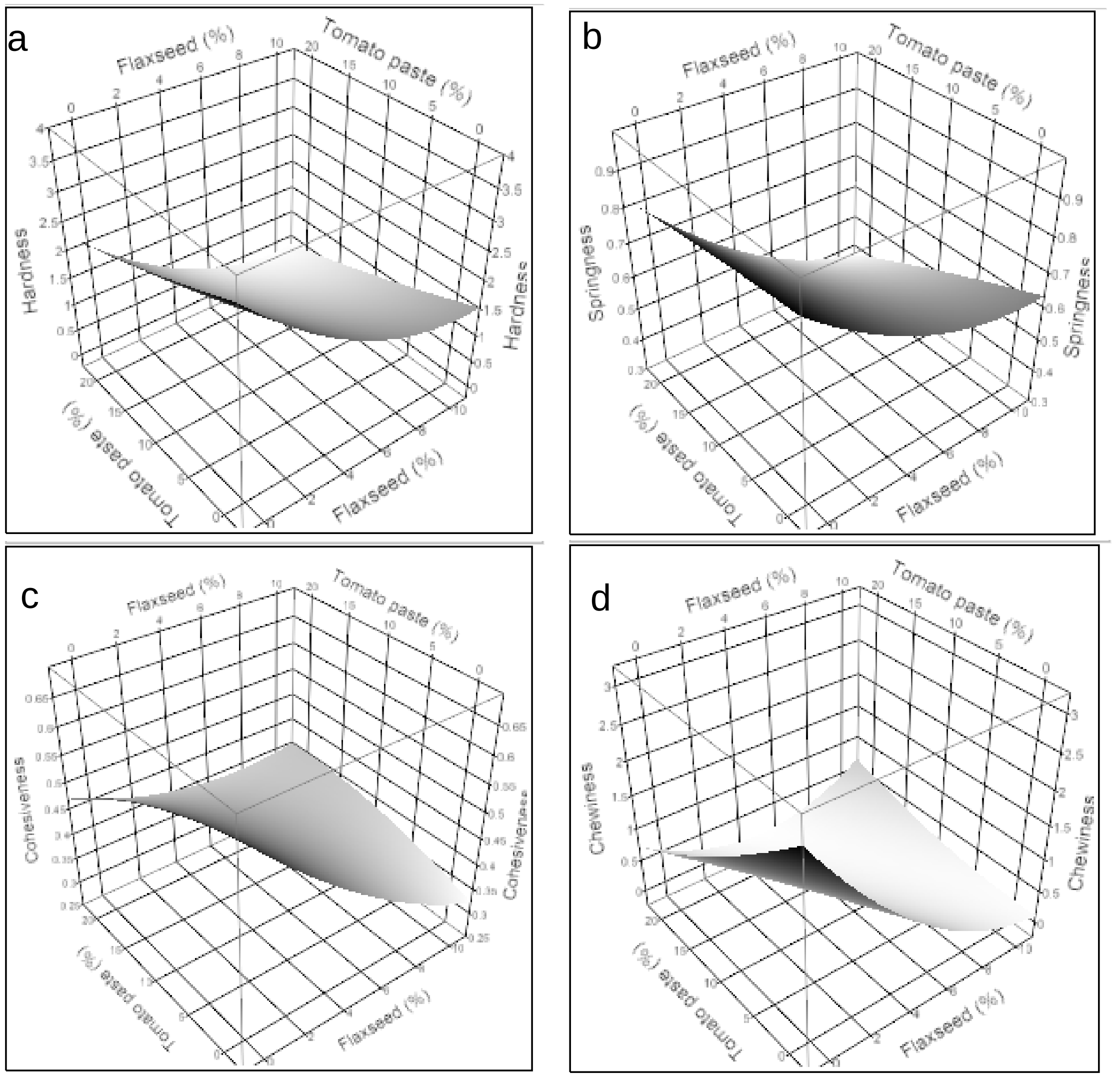


Figure 4. Effects of flaxseed flour and tomato paste on a) hardness, b) springiness, c) cohesiveness and d) chewiness of beef patties

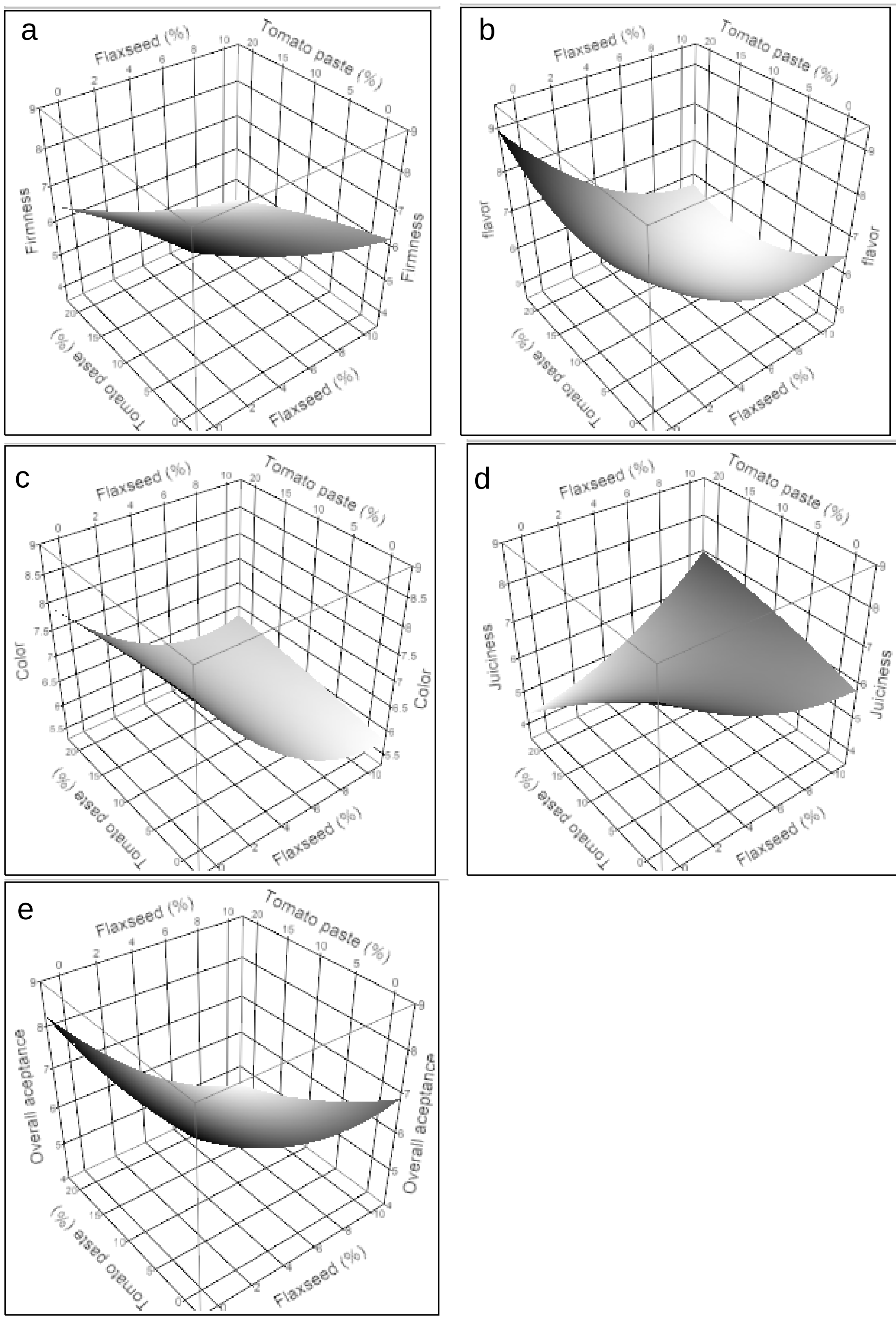


Figure 5. Effects of flaxseed flour and tomato paste on sensory characteristics a) firmness, b) flavor, c) color, d) juiciness and e) overall acceptance of beef patties

**Artículo 2**

# Development of a predictive model for *Salmonella* spp. reduction in meat jerky product with temperature, potassium sorbate, pH, and water activity as controlling factors


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## ABSTRACT

The objective of this study was to develop a predictive model for the inactivation of *Salmonella* spp. in ground beef jerky, as a function of temperature (T), pH, potassium sorbate (PS), and final water activity ($a_w$). Following a central composite design, ground beef was combined with PS (0 to 0.3%, w/w), pH adjusted from 5-7, inoculated with a cocktail of 6 serotypes of *Salmonella* spp. and heat processed at a temperature between 65 to 85°C until final $a_w$ ranging from 0.65 to 0.85 was achieved. Surviving *Salmonella* cells were enumerated on tryptic soy agar overlaid with xylose lysine desoxycholate agar (pre-tempered to 47°C) after incubation for 48h at 30°C. Bacterial inactivation was quantified in terms of logarithmic reductions of *Salmonella* counts (log CFU/g) and inactivation rate (log (CFU/g)/h). The results indicated that pH, PS and T significantly ($p < 0.05$) interacted to inactivate *Salmonella* in beef jerky. Decreasing meat pH significantly ($p < 0.05$) increased the efficacy of PS and T to reduce the levels of *Salmonella* spp. Beef jerky processed at 82°C, pH 5.5, with 0.25% PS to a final $a_w$ of 0.7 resulted in a maximum *Salmonella* logarithmic reduction of 5.0 CFU/g and an inactivation rate of 1.3 log (CFU/g)/h. The predictive model developed can be used to effectively design drying processes for beef jerky under low humidity conditions and thereby, ensuring an adequate degree of protection against risks associated with *Salmonella* spp.

## INTRODUCTION

Beef jerky, a widely consumed ready-to-eat meat product in the United States, has a high nutritional value, is low in fat and high in protein, and does not require refrigeration if a proper processing is applied to destroy microorganisms (USDA/FSIS, 2003a). The product is shelf-stable and does not require re-heating prior to consumption. Nevertheless, inadequate time/temperature treatment during jerky processes, combined with poor handling, cross contamination and unacceptable packaging practices (Cavallo, 2014) have been the cause of

*Salmonella* spp. outbreaks (CDC, 1995) and multiple recalls by the USDA/FSIS in the past few years (USDA/FSIS, 2014; USDA/FSIS, 2003b; USDA/FSIS, 2013; USDA/FSIS, 2011). Beef jerky producers are not only concerned about safety but also about physicochemical quality of the product. Safety, sensory quality, and room temperature stability of beef jerky can be achieved by the interaction of several intrinsic and extrinsic factors i.e., meat pH, marinade composition, added preservatives, final $a_w$, temperature and relative humidity during processing, among others.

Meat pH is an important factor to consider during jerky production because it is related to the drying rate and drying time (Bowser et al., 2009; Thiagarajan et al., 2006). Another factor to consider is the thermal inactivation of microbial pathogens so as to provide an adequate degree of protection (Yoon et al., 2005). Also, addition of preservatives increases the death of microorganisms during the drying process (Harrison et al., 2006). Potassium sorbate is a preservative that exhibits antimicrobial activity against *Salmonella* and other pathogens (To and Robach, 1980; Rice and Pierson, 1982; Lim and Mustapha, 2004; Zare et al., 2014; Zamora and Zaritzky, 1987) and may be used to increase the death rate of *Salmonella* during jerky production. Water activity is another important factor that needs to be controlled during the drying process to achieve shelf stability (Ingham et al., 2006) and prevent undesirable growth of microorganisms. The recommended $a_w$ for beef jerky is 0.85 or lower for stability (USDA/FSIS, 2012).

Slow drying process along with low humidity conditions may result in survival of *Salmonella* in jerky products. As a result, USDA/FSIS has emphasized the need to control relative humidity during the drying process. As such, humidity can be difficult to control with the current drying equipment that are available in small jerky processing facilities. Therefore, these kind of facilities need to implement or control other conditions during the manufacture of beef jerky to decrease the risk of *Salmonella* spp. Processing conditions that can be easily controlled and implemented in small plants to inactivate *Salmonella* spp. in beef jerky are temperature (T), water activity ($a_w$), pH and the addition of chemical preservatives. Therefore, the objective of this study was to develop a predictive model for the inactivation of *Salmonella* spp. in ground beef jerky as a function of T, pH, PS, and final $a_{w}$.

## MATERIALS AND METHODS

### *Materials*

Ground beef (95 % lean, 5% fat) was obtained from a local supermarket and stored frozen until used. Potassium sorbate, phosphoric acid and sodium phosphate were obtained from Sigma (Sigma-Aldrich Co., St. Louis, MO) and commercial salt (Morton® Tender Quick ® salt) was obtained from a local market.

### *Bacterial strains and inoculum preparation*

Six strains of *Salmonella* spp. (*S.* Montevideo*, S.* Typhimurium Copenhagen, *S.* Senftenberg, *S.* Typhimurium, phage type DT-104, *S.* Enteritidis, phage type 4, and *S.* Enteritidis, phage type 13a) isolated from human clinical cases, beef and pork, were used in this study. These cultures were obtained from the Residue Chemistry and Predictive Microbiology Research Unit, Eastern Regional Research Center (Wyndmoor, PA) culture collection. The strains were preserved in vials at -80°C in Brain Heart Infusion (BHI) broth (Becton, Dickinson and Company, Sparks, MD) with 15% (v/v) glycerol (Sigma-Aldrich Co., St. Louis, MO) and individually grown by transferring 0.1 mL of the thawed culture to 10 mL of BHI broth followed by 24 h incubation at 37°C (Lab-Line Instruments, Inc., Melrose Park IL). This procedure was performed twice. Subsequently, 0.1 mL of each culture was inoculated into 50 mL of BHI broth and incubated at 37°C for 24 h with stirring. Cultures were centrifuged (Marathon 21000R, International Equipment Company, Needham Heights, MA) at 5000xg for 15 min, performing a second operation with 9.9 mL of 0.1% peptone water (PW) (Becton, Dickinson and Company) in order to discard metabolic products. The pellet was finally suspended in 2 mL of peptone water. For experimental use, a cocktail was prepared by mixing equal volumes of each activated strain of *Salmonella* spp.

*Experimental Design*

A central composite design (CCD) was performed to determine the effects and interactions of the four independent variables: water activity (0.65, 0.70, 0.75, 0.80 and 0.85), pH (5.0, 5.5, 6.0, 6.5 and 7.0), potassium sorbate (0, 0.075, 0.15, 0.225 and 0.3%) and temperature (65, 70, 75, 80 and 85°C). The experimental design consists of sixteen factorial points, eight axial points at a distance of ± 2.0 from the center and seven replicates of the central point for a total of 31 treatments. An additional 5 runs without PS added at a meat pH of 6.0 and final $a_w$ of 0.8 were performed as controls (Table 1). Logarithmic reduction (LogR; CFU/g) and inactivation rate (IR; (CFU/g)/h) of *Salmonella* spp. were selected as the responses. All experiments were replicated twice. The mathematical model corresponding to the CCD is:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{K} \beta_{ii} X_i^2 + \sum\sum \beta_{ij} X_i X_j + \varepsilon$$

where $y$ is the dependent variable (LogR and IR), $\beta_0$ is the model constant, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ are the model coefficients and $\varepsilon$ is the error. They represent the linear, quadratic and interaction effects of the variables.

*Preparation of jerky*

Ground beef was previously thawed at refrigeration temperature and mixed with 1.5% Morton® Tender Quick salt, using a Kitchen Aid Mixer (Model K5SSDWH, St. Joseph, MI). Potassium sorbate (Sigma-Aldrich Co., St. Louis, MO) was added and pH adjusted with either diluted phosphoric acid (1:3; Sigma-Aldrich Co.) or sodium phosphate (Sigma-Aldrich Co.), according to the central composite design matrix (Table 1). The samples were stored overnight in a sterile bag and pH was readjusted when necessary before jerky formation. Each bag was inoculated with 0.4 mL of bacterial cocktail and mixed manually for 1 min and then, in a stomacher (Stomacher 400 Lab system, Seward, London, UK) for an additional 2 min to ensure a homogeneous distribution. Each treatment was transferred to a sterilized jerky-forming gun (Weston, Strongsville, OH) to form jerky strips directly on a sterile wire mesh panel. Following the manufacturer's instructions, approximately 150 g of inoculated sample treatment was loaded into the gun, and the ‘‘strip’’ attachment was used to form 10 strips, each 1.12 x 0.6 x 11cm. An additional 10 non-inoculated strips were prepared for measurement of $a_w$ during the drying process of each treatment evaluated. Average jerky strip weight was 11 g and initial *Salmonella* concentration was 7-8 log CFU/g.

*Jerky processing and enumeration of surviving bacteria*

Samples of jerky prepared for each treatment were placed in a home style convection oven (1800 W, 47 x 41.3 x 28.6 cm) (Breville USA, Torrance, CA) and heated at temperatures to achieve predetermined final $a_{w,}$ as indicated in the experimental design (Table 1). Temperature during each heating process was monitored with data loggers (Fisher Scientific™, Pittsburgh, PA) equipped with K-type thermocouple probes, suspended in the center of the oven, away from the heating element. The relative humidity (%RH) was monitored with a micropack III humidity data logger (Datatrace, Lakewood, CO) placed in the center of the oven, and was kept at 5% during the drying process. The $a_w$ of duplicate non-inoculated strips was measured at 0.5 h intervals with an AquaLab Series 4 $a_w$ meter (Decagon Devices, Inc., Pullman, WA). After the drying process, the samples were cooled at room temperature for 10 min. Jerky samples were cut into small pieces of approximately of 2 x 2 mm and 3 g were weighed into 10 x 5 cm polyethylene sterile bags (Spiral Biotech, Bethesda, MD). Thereafter, 6 mL of PW was added and homogenized using a stomacher. Serial dilutions were performed by transferring 1 mL of the filtrate to 9 mL of PW and appropriate dilutions were plated on tryptic soy agar plates (TSA; Difco, Sparks, MD) overlaid with selective xylose lysine desoxycholate (XLD; Remel Inc., Lenexa, KS) agar (pre-tempered to 47°C) after 2 h of resuscitation of stressed bacteria. Colonies were enumerated after 48 h incubation at 30°C in an incubator (Labline-460-1CE; Thermo Scientific, USA).

*Data processing and statistical modeling*

The results of the bacterial counts were transformed to $Log_{10}$ colony forming units per gram (CFU/g), and expressed in terms of logarithmic reductions of *Salmonella* counts (log CFU/g) and inactivation rate (log (CFU/g)/h). The response variables were adjusted to a second order complete polynomial model. Analysis of variance (ANOVA), coefficient of determination ($R^2$), and lack of fit of model were determined using the statistical program JMP® version 11.0.0 (SAS Institute, Cary, NC). Statistical significance of the model and model variables were determined at the 5% probability level ($p< 0.05$). JMP® software was used for simultaneous optimization of the variable responses. Optimal processing conditions of $a_w$, pH, PS and T were determined by superimposing the plots for the response variables (Capanzana and Buckle, 1997; Vatsala et al., 2001).

## RESULTS AND DISCUSSION

The interactive effects of $a_w$, pH, PS and T on the inactivation of a six strains mixture of *Salmonella* spp*.* were quantified by estimating the $Log_{10}$ CFU reduction (LogR; CFU/g) and inactivation rate (IR; (CFU/g)/h) in beef jerky under low relative humidity (RH ≤ 5%) drying conditions. Observed LogR and IR are shown on Table 1. The LogR of *Salmonella* ranged from a minimum of 2.75, when jerky was processed at pH 5.0, 0.15% PS, 75°C and dried to $a_w$ 0.75 to a maximum of 5.03 at pH 5.5, 0.225% PS, 80°C and dried to $a_w$ 0.7. IR ranged from a minimum of 0.57, when jerky was processed at pH 5.5, 0.025% PS, 70°C and dried to $a_w$ 0.7 to a maximum of 1.26 when processed at pH 5.5, 0.225% PS, 80°C and dried to $a_w$ 0.7. On the other hand, increasing drying T during beef jerky processing with no added PS at a constant $a_w$ and pH resulted in increased LogR and IR of *Salmonella* spp. For example, jerky processed at pH 6.0, 0.0% PS, and dried to $a_w$ 0.8, the LogR increased from 2.85 log CFU/g at 65°C to 4.34

log CFU/g at 85°C, while IR increased from 0.58 log (CFU/g)/h to 1.12 log (CFU/g)/h at the same conditions, respectively. For all temperatures, higher LogR and IR were obtained on those treatments with the addition of PS. The addition of PS tended to increase *Salmonella* sensitivity to heat, and this effect was particularly observed at higher T and lower pH. For example, at a constant pH of 5.5 and 80°C, LogR and IR increased from 3.84 to 5.03 log CFU/g and from 0.9 to 1.26 log (CFU/g)/h, respectively, with increased PS levels from 0.075 to 0.225%.

As expected, when processing T increased and final product $a_w$ decreased during jerky manufacturing, higher LogR were achieved. For example, in samples with 0.075% PS and pH 5.5, increasing process T from 70 to 80°C and decreasing final product $a_w$ from 0.8 to 0.7, LogR increased from 2.90 to 3.84. The pathogen was also sensitive to pH, and it was evident in the increase of IR from 1.11 to 1.26 when pH of jerky was decreased from 6.5 to 5.5 at the same processing conditions of $a_w$ 0.7, 0.225% PS and 80°C. A similar trend was observed at the same processing conditions except for $a_w$ 0.8.

The regression models are displayed in Table 2. The $R^2$ were 0.92 and 0.95 for LogR and IR, respectively, indicating that there was good agreement between the experimental and predicted values (Figure 1). Also, P value <0.0001 indicated that the predicted and observed values for the models were not significantly different. Non-significant lacks of fit ($P > 0.05$) were observed, indicating that variation between samples was due only to the factors selected for the models and pure error. Based on all these criteria, the models described the experimental data satisfactorily and can be used to estimate LogR and IR as a function of $a_w$, pH, PS and T within the limits tested in the experiment. Figures 2 and 3 show 3D response surface plots of LogR and IR, respectively, as a function of $a_w$, pH, PS and T. The graphical representations were obtained using the developed second-order polynomial equation indicated on Table 2. Based on results shown on Table 2, the factor exerting the greatest effect on increasing LogR and IR was T. According to Saldaña et al. (2012), T affects membrane phospholipids in such a way that bacteria membrane loses its elastic properties as T increases, and therefore renders it more easy to disruption. In our study, increasing T from 65 to 85°C during the drying process, LogR and IR increased from 2.85 to 4.34 log CFU/g and from 0.58 to 1.12 (log CFU/g)/h, respectively, at the same conditions of pH (6.0) and final $a_w$ (0.8). A study by Juneja et al. (2010) also reported the same effect of T on *Salmonella* IR.

Conversely, PS addition decreases heat resistance of *Salmonella* during the drying of beef jerky possibly due to cell membrane disruption (Sofos et al., 1986). The ability of PS to inactivate *Salmonella* is related to meat pH as indicated by the significant (P<0.05) interaction between pH and PS. This interaction indicates that as meat pH decreases, PS is more effective in inactivating *Salmonella*. Meat pH has an inverse effect on bacterial cell damage, i.e., decreasing meat pH results in parallel increase in cell damage (Saldaña et al., 2012); thereby, decreasing the resistance of *Salmonella* and enhancing the effect of PS. The effectiveness of PS at low pH was also observed against *L. monocytogenes* by González-Fandos and Dominguez (2007) and Zare et al. (2014) against *Staylococcus aureus* and *Escherichia coli*. However, increasing meat pH without PS addition tends to increase logR of *Salmonella* during the drying process of beef jerky under low RH conditions. It is well known that meat retains more water at pH levels different to its isoelectric point (pH = 5.1) (Puolanne and Peltonen, 2013) and this retention of water has influence on drying rate and time. Manipulating meat pH at values near the isoelectric point during drying process of beef jerky can increase drying rate and decrease drying time (Thiagarajan et al., 2006; Bowser et al., 2009). Water retained from humidity by the effect of pH keeps the surface of the product moister and prevents unwanted concentration of solutes. It is known that drying beef jerky in a wet environment is more effective to kill bacteria (Pflug et al., 2001). On the contrary, research has demonstrated that bacteria become more heat resistant as product moisture levels decrease or concentrations of solutes increase (Goepfert et al., 1970).

Beef jerky production is a time consuming process, because the meat needs to be dried slowly at a relatively low temperature. Two factors that have significant influence on drying time are meat pH and chamber RH (Bowser et al., 2009; Thiagarajan et al., 2006). Decreasing drying time can be achieved by minimizing the amount of humidity during processing. This is desirable for jerky manufacturers since it reduces production time and costs. However, *Salmonella* inactivation rate should be increased if a short drying process is used during processing to ensure safety of the product as well as to determine compliance with the regulatory performance standards. According to USDA/FSIS (2012), the lethality treatment of meat jerky should achieve at least 5.0 log reduction of *Salmonella* spp. and should also accomplish sufficient reductions of other bacterial pathogens of public health concern. For this reason, we propose mathematical models to predict LogR and IR of *Salmonella* as a function of aw, pH, PS and T as the first step necessary for adequate beef jerky drying process. Based on the predictive models obtained in this research, a series of contour plots were generated and visually compared. Contour plots were superimposed and a combination of $a_w$, pH, PS and T were selected to predict at least 5 log CFU/g reduction and 1.3 log (CFU/g)/h. Results showed that processing beef jerky at 82°C, pH 5.5, with 0.25% PS to a final $a_w$ of 0.7 resulted in a maximum *Salmonella* logarithmic reduction of 5.0 CFU/g and maximum inactivation rate of 1.3 log (CFU/g)/h. This combination of parameters was used to experimentally validate the predicted values of LogR and IR by the model equation. According to the validation of results shown on Table 3, the experimental and predicted values were not significant ($p < 0.05$). Thus, the model can be used with confidence to select a combination of processing conditions to inactivate *Salmonella* spp. to determine compliance with the USDA/FSIS performance standards.

## CONCLUSIONS

Beef jerky process can accomplish thermal inactivation of *Salmonella* spp. at low relative humidity when controlling conditions such as meat pH, addition of PS, low $a_w$ and high temperature. According to the model generated in this study, the process that ensures at least 5 logarithmic reductions of *Salmonella* spp. and higher inactivation rate was: the adjustment of meat pH to 5.5, 0.25% of PS, and drying at 82°C until $a_w$ 0.7 was reached. This predictive model can be used by small processors of jerky as a tool to establish a safe drying process.

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Table 1. Experimental combination of treatments used for central composite design and experimental logarithmic reduction and inactivation rate of *Salmonella* spp.

| | | | | | Log reduction (log CFU/g) | | Inactivation Rate (log (CFU/g)/h) | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Aw | pH | PS | T | Means | SD | Means | SD |
| 1 | 0.7 | 5.5 | 0.075 | 70 | 3.15 | 0.36 | 0.57 | 0.07 |
| 2 | 0.7 | 5.5 | 0.075 | 80 | 3.84 | 0.07 | 0.90 | 0.02 |
| 3 | 0.7 | 5.5 | 0.225 | 70 | 3.84 | 0.22 | 0.77 | 0.04 |
| 4 | 0.7 | 5.5 | 0.225 | 80 | 5.03 | 0.57 | 1.26 | 0.14 |
| 5 | 0.7 | 6.5 | 0.075 | 70 | 4.10 | 0.21 | 0.75 | 0.04 |
| 6 | 0.7 | 6.5 | 0.075 | 80 | 4.78 | 0.05 | 1.01 | 0.01 |
| 7 | 0.7 | 6.5 | 0.225 | 70 | 4.07 | 0.14 | 0.79 | 0.03 |
| 8 | 0.7 | 6.5 | 0.225 | 80 | 5.00 | 0.37 | 1.11 | 0.08 |
| 9 | 0.8 | 5.5 | 0.075 | 70 | 2.90 | 0.02 | 0.68 | 0.00 |
| 10 | 0.8 | 5.5 | 0.075 | 80 | 3.47 | 0.03 | 0.99 | 0.01 |
| 11 | 0.8 | 5.5 | 0.225 | 70 | 3.10 | 0.13 | 0.76 | 0.03 |
| 12 | 0.8 | 5.5 | 0.225 | 80 | 3.77 | 0.34 | 1.26 | 0.11 |
| 13 | 0.8 | 6.5 | 0.075 | 70 | 3.37 | 0.26 | 0.84 | 0.07 |
| 14 | 0.8 | 6.5 | 0.075 | 80 | 3.99 | 0.26 | 1.09 | 0.07 |
| 15 | 0.8 | 6.5 | 0.225 | 70 | 3.51 | 0.01 | 0.86 | 0.00 |
| 16 | 0.8 | 6.5 | 0.225 | 80 | 4.06 | 0.19 | 1.16 | 0.05 |
| 17 | 0.65 | 6 | 0.15 | 75 | 3.93 | 0.21 | 0.75 | 0.04 |
| 18 | 0.85 | 6 | 0.15 | 75 | 3.11 | 0.09 | 0.96 | 0.03 |
| 19 | 0.75 | 5 | 0.15 | 75 | 2.75 | 0.11 | 1.00 | 0.04 |
| 20 | 0.75 | 7 | 0.15 | 75 | 4.41 | 0.34 | 0.95 | 0.07 |
| 21 | 0.75 | 6 | 0 | 75 | 3.77 | 0.07 | 0.82 | 0.02 |
| 22 | 0.75 | 6 | 0.3 | 75 | 4.39 | 0.06 | 0.90 | 0.01 |
| 23 | 0.75 | 6 | 0.15 | 65 | 3.01 | 0.28 | 0.60 | 0.06 |
| 24 | 0.75 | 6 | 0.15 | 85 | 4.50 | 0.49 | 1.14 | 0.12 |
| 25 | 0.75 | 6 | 0.15 | 75 | 4.20 | 0.04 | 0.88 | 0.01 |
| 26 | 0.75 | 6 | 0.15 | 75 | 3.95 | 0.00 | 0.85 | 0.00 |
| 27 | 0.75 | 6 | 0.15 | 75 | 4.02 | 0.18 | 0.85 | 0.04 |
| 28 | 0.75 | 6 | 0.15 | 75 | 3.86 | 0.10 | 0.84 | 0.02 |
| 29 | 0.75 | 6 | 0.15 | 75 | 3.94 | 0.24 | 0.85 | 0.05 |
| 30 | 0.75 | 6 | 0.15 | 75 | 3.63 | 0.02 | 0.78 | 0.00 |
| 31 | 0.75 | 6 | 0.15 | 75 | 3.81 | 0.32 | 0.82 | 0.07 |
| 32 | 0.8 | 6 | 0 | 65 | 2.85 | 0.17 | 0.58 | 0.03 |
| 33 | 0.8 | 6 | 0 | 70 | 3.04 | 0.00 | 0.68 | 0.00 |
| 34 | 0.8 | 6 | 0 | 75 | 3.36 | 0.05 | 0.81 | 0.01 |
| 35 | 0.8 | 6 | 0 | 80 | 3.72 | 0.33 | 0.93 | 0.08 |
| 36 | 0.8 | 6 | 0 | 85 | 4.34 | 0.23 | 1.12 | 0.06 |

Means = average of two replicates, SD = standard deviation

Table 2. Estimated regression coefficient (RC) and analysis of variance of the regression models for logarithmic reduction (LogR, CFU/g) and inactivation rate (IR, (log CFU/g)/h) of *Salmonella* spp. in beef jerky.

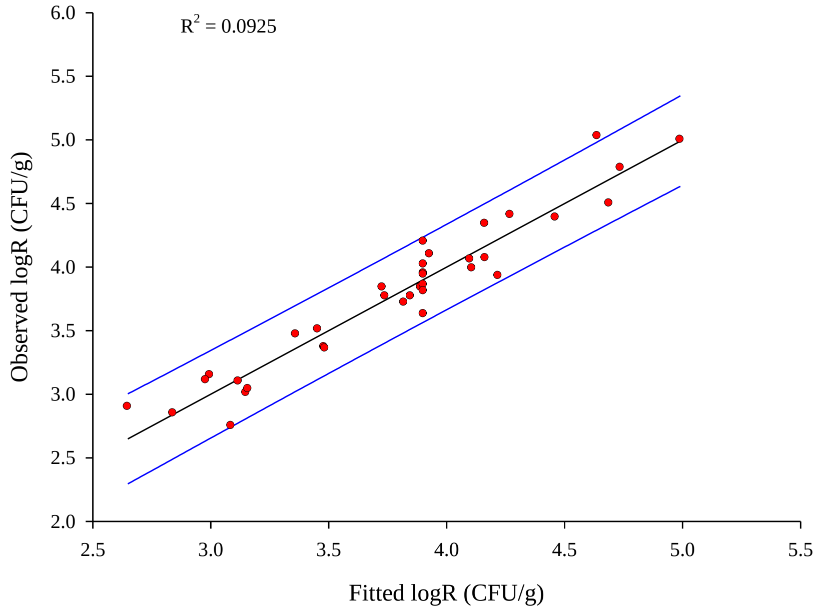
| | LogR | | IR | |
|---|---|---|---|---|
| | RC | SE | RC | SE |
| Intercept | 3.90* | 0.072 | 0.844* | 0.018 |
| Aw | -0.309* | 0.041 | 0.035* | 0.010 |
| pH | 0.296* | 0.042 | 0.013 | 0.010 |
| PS | 0.181* | 0.039 | 0.058* | 0.009 |
| T | 0.384* | 0.040 | 0.163* | 0.010 |
| Aw*pH | -0.025 | 0.052 | 0.006 | 0.012 |
| Aw*PS | -0.065 | 0.046 | -0.009 | 0.011 |
| pH*PS | -0.123* | 0.052 | -0.042* | 0.013 |
| Aw*T | -0.045 | 0.048 | 0.002 | 0.012 |
| pH*T | -0.021 | 0.052 | -0.031* | 0.013 |
| PS*T | 0.004 | 0.035 | 0.019* | 0.009 |
| Aw*Aw | -0.076 | 0.038 | 0.008 | 0.009 |
| pH*pH | -0.056 | 0.038 | 0.040* | 0.010 |
| PS*PS | 0.049 | 0.032 | 0.005 | 0.008 |
| T*T | 0.004 | 0.031 | 0.011 | 0.008 |
| $R^2$ | 0.92 | | 0.95 | |
| *Pvalues* | | | | |
| Regression | <0.0001 | | <0.0001 | |
| Lack of fit | 0.321 | | 0.070 | |

SE = Standard error
*P < 0.05

Table 3. Predicted and experimental values under optimum conditions based on combination of response (LogR and IR)

| Response variable | Predicted value | Experimental value[a] |
|---|---|---|
| LogR (CFU/g) | 5.0 | 4.85 ± 0.56 (ns) |
| IR (CFU/g)/h | 1.3 | 1.23 ± 0.10 (ns) |

[a] Mean of triplicate determination
ns = not significant ($P < 0.05$) between experimental and predicted values
LogR = logarithmic reductions
IR = inactivation rate



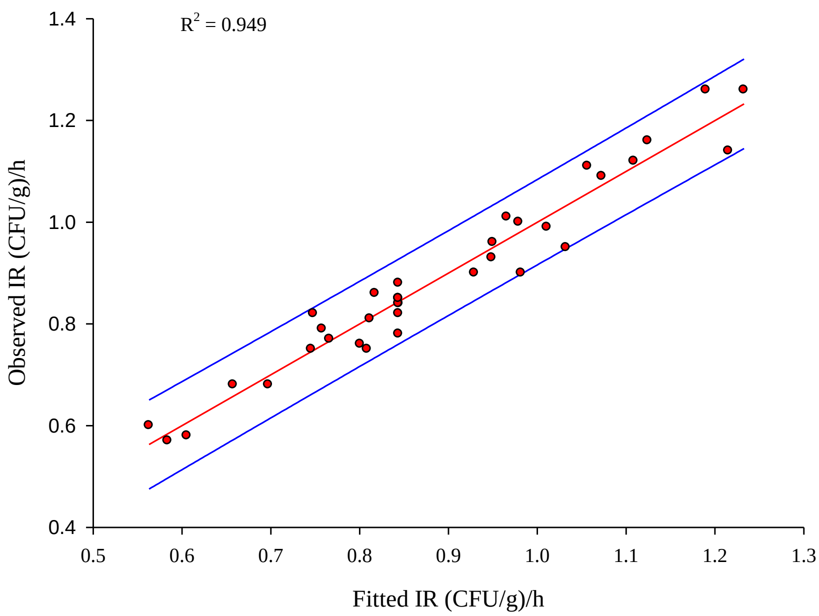




Figure 1. Agreement between observed and fitted logarithmic reduction and inactivation rate of *Salmonella* spp. in beef jerky drying process as a function of $a_w$, pH, PS and T.

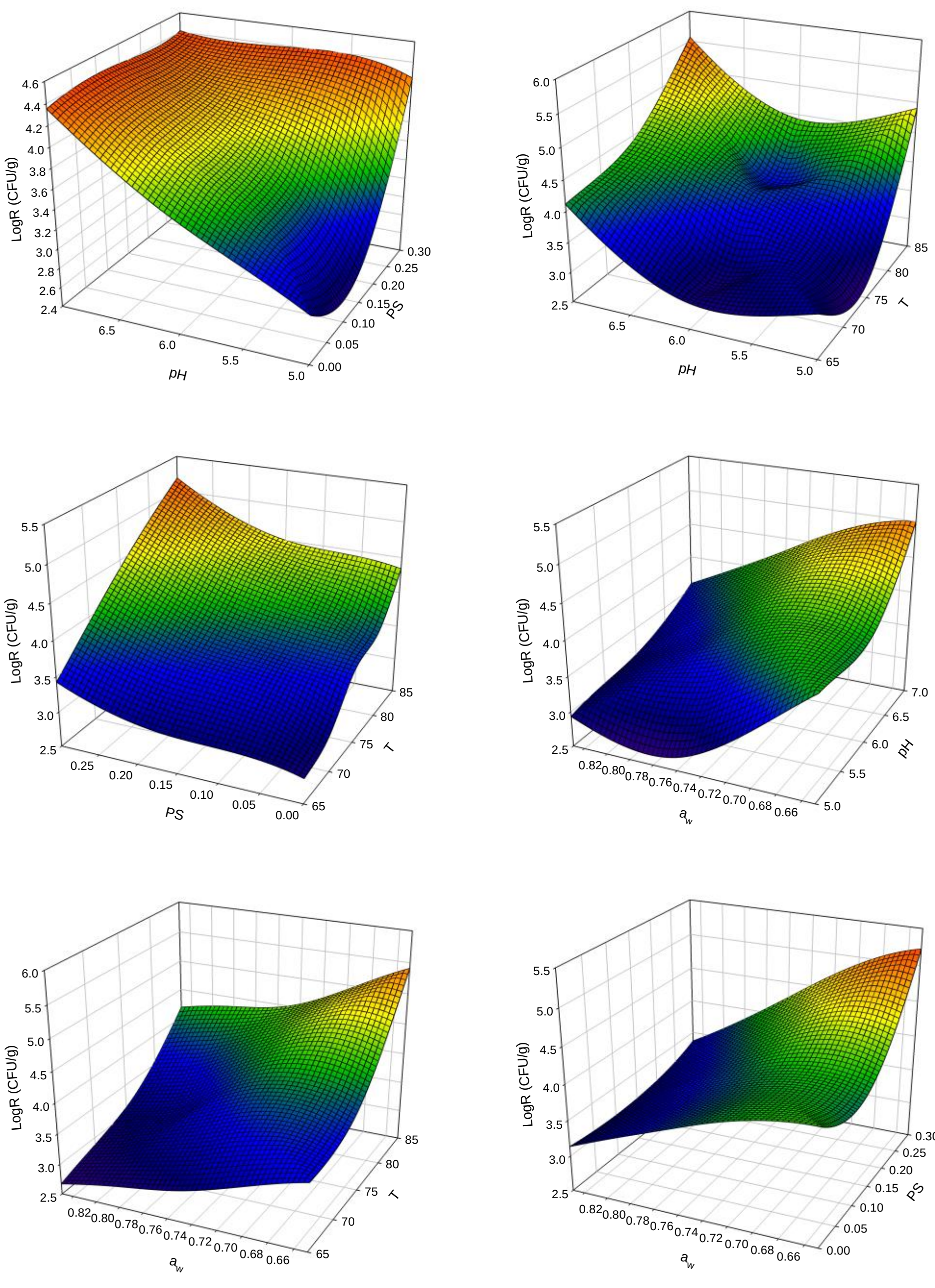



Figure 2. Response surface plots for logarithmic reduction (CFU/g) of *Salmonella* spp. in beef jerky drying process as a function of $a_w$, pH, PS and T

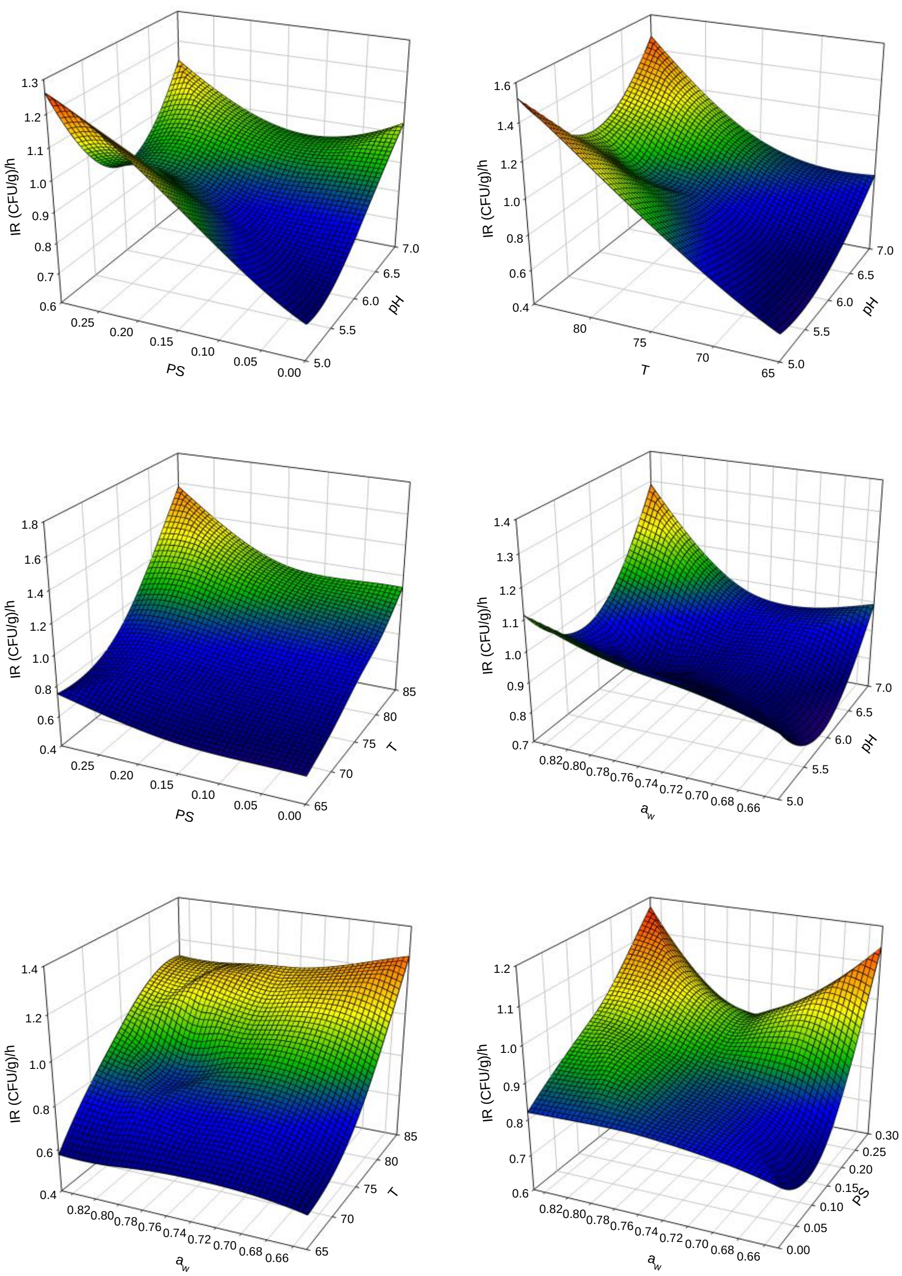



Figure 3. Response surface plots for inactivation rate (CFU/g)/h of *Salmonella* spp. in beef jerky drying process as a function of $a_w$, pH, PS and T

## Artículo 3

# Physicochemical, sensory and nutritional quality of beef patties added with flaxseed and tomato paste

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**Abstract**

The objective of this study was to evaluate the effect of flaxseed flour (FS) and tomato paste (TP) addition on physicochemical, sensory and nutritional quality of beef patties. Treatments were: control=0% FS + 0% TP; T1=0% FS + 20% TP; T2=5% FS + 15% TP; T3=10% FS + 10% TP; T4=15% FS + 5% TP; and T5=20% FS + 0% TP. Protein content increased while saturated fatty acids decreased with FS addition. Objective color, L * and *a** values and texture parameters decreased with FS addition to beef patties formulation. Sensory properties for treatments T1 and T2 were similar to the control treatment, while T3 showed acceptable sensory scores. The α-linolenic acid content of beef patties increased as FS addition increased. The PUFA/SFA ratio increased from 0.24 in the control treatment to 4.49 in T5. The polyunsaturated fatty acids ratio n-6/n-3 decreased from 7.18 in the control treatment to 0.29 in T5. A combination of flaxseed and tomato paste can be used as novel ingredients to develop beef patties with better nutritional profile without demerit of their physicochemical and sensory properties.

**Keywords:** beef patties, functional foods, *Linum usitatissimum L.*, *Lycopersicum esculentum*

**Introduction**

The prevalence of obesity, overweight and non-communicable diseases represent a serious health problem for any region or country. In Mexico the combined prevalence of obesity and overweight is 71.28% (ENSANUT 2012) and it has been considered by the government as a health emergency that significantly affects the productivity of companies, child's academic development and the economy of a country.

Obesity and overweight have been associated to consumption of foods with high content of saturated fats or foods with high caloric content (Hoogerbrugge et al. 2001). An important sector of the population has some restrictions to meat and meat products consumption due to saturated fats, cholesterol, salt contents and its relationship with certain types of cancer (Cross et al. 2007), despite being a good source of protein, vitamins and minerals important for maintenance and development of the organism. Households in Mexico spend 34.1% of their total income in the purchase of food and beverages, and of this total, spend 23.3% for the buying of meat (ENIGH 2014). To maintain or to increase this influence in the national economy is a challenge for the meat industry; for this reason, with the growing concern of consumers for taking care of their health, the meat industry must adapt to these new needs. A good strategy is to change the nutritional profile of traditional products combining them with foods such as fruits, vegetables and cereals, and in this way offer alternatives to those consumers that seek food with a health connotation. With the incorporation of these new ingredients in the formulation of meat products, bioactive compounds are added. Even more this addition may help in reducing saturated fats, cholesterol and certain additives. However, a challenge for the food technologist is to maintain the physicochemical and sensory properties of the product. Among the options of using non-traditional ingredients for the development of new meat products are flaxseed and tomato. Flaxseed is rich in alpha-linolenic acid (55% of total fatty acids), proteins (21%), dietary fiber (28%) and phytoestrogens such as lignans (Bloedon and Szapary 2004). Tomato, on the other hand, is source of vitamins, minerals, and phytochemical compounds which include carotenoids and polyphenols (Navarro-González et al. 2011). Consumption of both foods has been associated with a decrease in cardiovascular disease and certain types of cancer (Barceló-Coblijn and Murphy 2009; Canene-Adams et al. 2005; Dodin et al. 2008).

It has been shown that the incorporation of flaxseed in meat products improves the profile of fatty acids but color and sensory properties are affected when the addition is greater than 5% (Bilek and Turhan 2009; Pelser et al. 2007; Singh et al. 2011). In addition, color and sensory properties of meat products improve if tomato paste is added up to a maximum of 20% in the formulation (Candogan 2002; García et al. 2009; Østerlie and Lerfall 2005). It is expected that with a combination of flaxseed and tomato paste a more acceptable meat product with better fatty acid profile and sensory properties could be obtained.

The objective of this research was to evaluate the physicochemical, sensory and nutritional properties of beef burgers added with combinations of flaxseed and tomato paste.

## Materials and Methods

*Experimental design*

The physicochemical, sensory and nutritional quality of beef patties as affected by flaxseed (FS) and tomato paste (TP) addition were studied. Both ingredients were incorporated into the formulation according to the following treatments: control (without addition of FS and TP); T1=0% FS + 20% TP; T2=5% FS + 15% TP; T3=10% FS + 10% TP; T4=15% FS + 5% TP; and T5=20% FS + 0% TP. Two replicates of the experiments were carried out in different times. In each replication, three patties were sampled and analyzed for their proximate composition, pH, objective color, texture profile analysis, sensory analysis, fatty acids profile and cholesterol content. One-way analysis was performed on quality measurements with Number Cruncher Statistical Systems 2007 (NCSS, Kaysville, UT) software (Hintze 2007). Significant differences were estimated at a probability level in the type I error of 0.05. When significant differences between treatments were found Tukey-Kramer test was performed.

*Ingredient acquisition and meat preparation*

Beef inside round (*Semimembranosus*), FS (*Linum usitatissimum* L) and TP (*Lycopersicum esculentum*) were used and obtained from the local market. All meat cuts were obtained from animals with not more than 5 days of harvest. Meat was used the same day of purchase, cut into pieces no larger than 5 $cm^3$ and ground using a Hobart grinder (model 4152, Troy, Ohio, USA) through a 3/16" plate. Ground meat was stored in refrigeration (2 °C) until further use.

One kilogram batches per treatment were used for beef patties preparation. Meat (73% moisture, 6% fat), FS (7% moisture, 31% fat), TP (80% moisture, 0.4% fat) and spices were mixed according to percentages specified in the experimental design. Ingredients in each batch were homogenized for 10 min. Beef patties were manually formed into 9 cm x 1 cm diameter of thickness to obtain approximately 70 g per unit. An electric skillet (Cook Master Oster, Model 3222-3, Mississauga, Ontario, Canada) was used for cooking the patties 1 min each side and then 15 s per side until reaching 72 °C, measured in the geometrical center with a thermocouple.

*Product quality determinations*

Quality parameters assessed for treatments were: proximate analysis (moisture, fat, protein and ash), objective color (*L*, a*, b**), pH, texture profile analysis (hardness, elasticity, cohesiveness and chewiness), sensory analysis (color, flavor, firmness, juiciness, and overall acceptability), fatty acid profile and cholesterol content. All determinations were carried out between 24 h after preparation of beef patties, except proximate analysis, fatty acid profile and cholesterol content. Samples for this determination were stored at -18°C until further use.

*Proximate composition, objective color and pH*

Moisture, ash, protein (N x 6.25) and fat content were determined on raw beef patties (AOAC 2000). Surface color was measured with a Minolta colorimeter using the D65 illuminant and 10° standard observer (Chroma meter CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) recording *L**, *a** and *b** values. For pH measurement, a portable pH meter (Hanna, Model HI 98140, Woonsocket, RI, USA) equipped with a puncture type combination pH electrode was used, and the reading was taken once stabilized.

*Texture profile analysis (TPA)*

The textural properties of cooked beef patties were evaluated using a Texture Analyzer TA-XT2 (Stable Micro Systems, Surrey, UK). Cubic samples (1 x 1 x 1 cm) were cut from burgers and subjected to a two-cycle compression test.

Samples were compressed to 50% of their original height with a 7.5 cm diameter cylindrical probe attached to a 50 kg compression cell with a cross-head speed of 1 mm/s. Texture profile parameters were determined according to Bourne (1978) and interpreted as follows: Hardness (kg), maximum force required to compress the sample; cohesiveness, extent to which sample could be deformed prior to rupture (A2/A1), being A1 the total energy required for the first compression and A2 the total energy required for the second compression; springiness (cm), ability of the sample to recover its original shape after the deforming force is removed, and chewiness (kg x cm), work needed to masticate the sample for swallowing (hardness x cohesiveness x springiness).

*Sensory evaluation*

Sensory evaluation was conducted by an 8-trained member panel (ISO-8586-1 1993) in an environmentally controlled (21 ± 1 °C, 55 ± 5% relative humidity) room partitioned into booths. Three patties from each formulation were cooked as previously described, and maintained warm in an oven until testing within 4-7 min. Square pieces approximately 1.5 x 1.5 cm were cut from the center of patties, and were served at room temperature. Each sample was coded with randomly selected 3-digit numbers. Panelists were instructed to clean their palates between samples using water. Color, flavor, firmness and juiciness of beef patties were scored using a 9 point hedonic scale, where 1 represented dislike extremely and 9 represented like extremely. Each attribute was discussed and tests were initiated after the panelists were familiarized with scales. At the end of the test, panelists were asked to give a score for product overall acceptability from 1 to 9.

*Lipid profile and cholesterol content*

Lipid extraction of beef patties was carried out by the procedure of Bligh and Dyer (1959) using chloroform:methanol (2:1 vol/vol). Before fatty acid methylation, the solvent was removed from lipid extracts in a water bath at 35 °C under a nitrogen atmosphere. Lipid extracts were transmethylated in presence of boron trichloride according to the method of Park and Goins (1994).

Composition of the fatty acid methyl esters (FAME) was performed in an Agilent (Model 7890 B, Santa Clara, CA, USA) gas chromatograph equipped with an autosampler (Model 7693) and a flame ionization detector (FID). Fatty acids were separated on a 100 m × 0.25 mm (i.d.) fused silica capillary column (SP-2560, Supelco, Bellefonte, PA., USA). The oven temperature was programmed from an initial temperature of 150 °C (20 min) to a final temperature of 220 °C at the rate of 5 °C/min. The injector temperature was set at 250 °C, and the FID temperature at 300 °C. The samples were run using helium at 17 psi pressure as the carrier and make-up gas. Chromatograms were recorded with a computing integrator (ChemStation chromatography manager, Agilent Santa Clara, CA, USA). Fatty acids were identified by comparing retention times with those of fatty acid methyl ester standards (Supelco 37 Component FAME Mix, Bellefonte, PA., USA). Fatty acids were expressed as a percentage of total fatty acids. Total percentages of saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids (PUFA), PUFA:SFA and n-6/n-3 ratios were calculated from these data.

The cholesterol content was estimated by gas chromatography (GC) following the method modified by Thompson and Merola (1992). Cholestan was used as the internal standard. Samples were saponified with potassium methylate in methanol, and then extracted with hexane, and finally derivatizated into trimethylchlorinesilyl ether. Cholesterol derivatives were analyzed by GC (Agilent, Model 7890 B, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a HP 1901 capillary column (0.25 mm x 30m). Sample injection was performed in a pulse split port at 330 °C. Oven temperature was set at 260 °C and FID was held at 330 °C. Helium was used as carrier gas. Cholesterol was identified by the retention time of the standard mixture and quantified by the ChemStation computer program according to the internal standard quantity.

## Results and Discussion

*Proximate composition*

Table 1 shows proximate composition of beef patties. The moisture content increased with the addition of TP and decreased with the incorporation of FS in the product formulation. The moisture level of T1 treatment with 0% FS + 20% TP was 74.89% which was higher ($P<0.05$) than T5 with 20% FS + 0% TP. Addition of FS decreased the moisture content of beef patties due to the increment in dry matter and lipid content ($P<0.05$) Similar results were obtained by Bilek and Turhan (2009) for beef patties formulated with flaxseed flour from 0 to 15%. The protein contents of beef patties increased as the FS content increased and TP decreased in beef parties and ranged from 19.4 (T1) to 27.37% (T5). Likewise, lipid contents of beef patties increased with FS addition. The lipid content was 4.17% in T1 treatment with 0% FS + 20% TP while in T5 treatment with 20% FS + 0% TP was 10.13%. Flaxseed flour has a protein and lipid contents of 19 and 31%, respectively, and a high proportion of α-linolenic acid (Bloedon et al. 2004), attributes that may be attractive to the beef patties consumer at the time of acquiring the product. According to a study carried out by PROFECO (2010), on the nutritional quality of 24 brands of beef patties, protein and lipid contents ranged from 10 to 14.9 % and from 11 to 15.5 %, respectively. In general, the beef patties developed in this research had a better nutritional balance with regard to beef patties sold in Mexico

*Color, pH and texture profile analysis (TPA)*

Table 2 shows the results of color ($L^*$, $a^*$ and $b^*$), pH and TPA of beef patties. Luminosity ($L^*$) and $a^*$ value increased ($P<0.05$) as FS decreased and TP increased in beef patties, with no changes in $b^*$ value. Tomato paste has a typical red color due to lycopene, the main pigment present in the fruit (Arias et al. 2000), maintaining its color during the development of products. This redness was kept at 20 and 15% of TP addition (T1 and T2 treatments). However, *a** value decreased when more than 5% of FS was added to beef patties. Candogan (2002), studied the effect of tomato paste addition (5 to 15%) on quality characteristics of beef patties reporting an increase of *a** value, a decrease in *L** and without changes in the *b** value. Yogesh et al. (2015), studied the incorporation of flaxseed powder in a meat batter at levels of 1 to 5% and reported a decrease of *L** and *a** values as flaxseed powder increased in the formulation.

Flaxseed flour and tomato paste addition affected pH of product in different ways. The lowest pH value was for beef patties with 20 and 15% of TP addition. Flaxseed flour slightly increased pH values, while tomato paste decreased them ($P < 0.05$) due to its acidic nature (pH = 4.2). Yogesh et al. (2015), also reported the same effect of flaxseed flour on pH of meat batter, while Candogan (2002) reported a decrease in the pH of beef burgers made with 10 and 15% of tomato paste. Garcia et al. (2009) and Deda et al. (2007) also reported a decrease in pH by adding tomato paste in different meat products.

Flaxseed flour and tomato paste addition on beef patties reduced all texture parameters; more than 48% hardness reduction occurred at all treatments compared to the control. The T5 treatment with 20% FS + 0% TP showed the lowest values for springiness, cohesiveness and chewiness with 0.47, 0.38 and 1.59, respectively, which was different ($P<0.05$) from the control treatment. Similar results were presented by Yogesh et al. (2015) in a study with meat batter incorporated with different levels of flaxseed flour, and by Doménech-Asensi et al. (2013) in a mortadella type product added with 10% of tomato paste.

*Sensory evaluation*

Sensory evaluation results are presented in Table 3. It was observed that all sensory attributes evaluated increased as tomato paste level increased and flaxseed flour levels decreased in beef patties formulation. For example, treatment T1 with 0% FS + 20% TP had the highest sensory score for juiciness, color and overall acceptance, which were different (P<0.05) from T5 with 20% FS + 0% TP. There was no difference (P>0.05) between T1 and control treatment in all sensory attributes. Similar results were presented by Candogan (2002), who reported that tomato paste incorporation into beef patties up to 15% level increased juiciness and did not result in any adverse effect on flavor characteristics. Some research has shown that tomato paste addition to meat products prevent lipid oxidation, the main cause for low scores on sensory evaluation (Candogan 2002; Doménech-Asensi et al. 2013). On the contrary, incorporation of flaxseed flour into meat products had an adverse effect on sensory attributes (Pelser et al. 2007). For example, T5 with 20% FS + 0% TP had the lowest sensory scores for flavor, juiciness, firmness, color and overall acceptability which were different (P<0.05) to T1 and control treatments. This negative effect of flaxseed flour on sensory attributes of beef patties has been also reported in other studies (Bilek and Turhan 2009; Yogesh et al. 2015). Flaxseed oil may confer to the product a strong flavor, unpleasant odor and flavor, because of either poor seed quality or unfavorable process or storage conditions (Wiesenborn et al. 2005). On the other hand, it is known that addition of flaxseed to meat products increases the proportion of polyunsaturated fatty acid and lipid oxidation (Pelser et al. 2007) affecting the sensory attributes of meat products (Singh et al. 2011). Nevertheless the negative effects on sensory attributes that flaxseed incorporation has in meat products, it also has multiple health benefits (Bloedon and Szapary 2004; Kawakami et al. 2015). On the other hand, because TP addition increased sensory attributes and objective color of beef patties, a balance between TP and FS addition was reached, which it could be attractive to consumers from the sensory and nutritional viewpoint. For example, T2 treatment with 5% FS + 15% TP had similar sensory score (P>0.05) to T1 and control treatment in all evaluated attributes. The 5% FS incorporation in a meat formulation was the optimum addition reported in other studies (Bilek and Turhan 2009; Yogesh et al. 2015), however, incorporation of 10% FS in combination with 10% TP as in T3 could be an alternative formulation with acceptable sensory scores (Table 3) but with higher quantity of polyunsaturated fatty acids and low cholesterol (Table 4).

*Lipid profile and cholesterol content*

Lipid profile of beef patties is shown in Table 4. There were significant differences ($P < 0.05$) in the type and quantity of lipids between the control treatment and those with addition of FS. The largest content of saturated fatty acids (SFA) occurred in control treatment and in those which only contained TP (T1); they had 50% of SFA, and it was reduced to 14.35% when FS was incorporated at a level of 20% (T5). In beef patties formulated with different levels of FS, the predominant SFA were palmitic (C16:0) and stearic (C18:0), while the most abundant monounsaturated fatty acid (MUFA) was oleic acid (C18:1 n9), and the predominant polyunsaturated fatty acid (PUFA) were linoleic acid (C18:2 n6) and α-linolenic acid (C18:3 n3). Similar results were obtained by Bilek and Turhan (2009) in beef patties formulated with different levels of flaxseed and by Novello and Pollonio (2013) in beef patties added with 5% of flaxseed flour. The PUFA were higher in treatments with FS, mainly due to the high content of α-linolenic acid present in the flaxseed flour (Rubilar et al. 2010). The PUFA/SFA ratio ranged from 0.24 in the control treatment to 4.49 in T5 treatment with 20% of FS. As expected, this ratio increased as FS increased in the beef patties formulation. The n-6/n-3 ratio was around 8.0 in the control and T1 treatment with 0% FS + 20% TP, different ($P<0.05$) from treatments with FS added, where n-6/n-3 ratio was around 0.3. Similar trends were observed by Singh et al. (2011) in a low fat chicken meat patties incorporated with 3% of linseed oil and by Pelser et al. (2007) in a Dutch style fermented sausage manufactured with a substitution of pork backfat by different percentages of flaxseed oil.

Cholesterol content was significantly ($P<0.05$) higher in beef patty control than in treatments with FS and TP added (Table 4). The values ranged from 73.03 mg/100g in beef patty control to 26.89 mg/100g in T5 with 20% FS and this represent a reduction of 63% in cholesterol content. An important reduction on cholesterol content was also observed in other treatments with FS and TP addition. For example, in T3 with 10% FS and 10% TP there was reduction of 55% on cholesterol content. Similar trend was reported by Muguerza et al. (2001)

on traditional Spanish fermented sausage where fat was replaced by 30% olive oil pre-emulsified with soy protein isolate.

## Conclusion

The results of the present study showed that the combination of flaxseed flour and tomato paste on the manufacture of beef patties lead to products with better sensory properties, enhanced fatty acid profile and low cholesterol content. Based on evaluated sensory attributes, better scores were obtained with 20 and 15% of TP addition. Nevertheless, incorporation of 10% each, TP and FS, with a balance between nutritional and sensory attributes could be a good alternative for consumers seeking novel meat products.

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Table 1. Chemical composition[1] of beef patties formulated with flaxseed flour and tomato paste.

| | Control | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|
| Moisture | $69.51^{bc}\pm0.70$ | $74.89^{c}\pm0.33$ | $71.80^{c}\pm0.36$ | $65.5^{b}\pm0.26$ | $64.95^{b}\pm5.36$ | $57.31^{a}\pm0.08$ |
| Lipid | $5.72^{b}\pm0.13$ | $4.17^{a}\pm0.03$ | $6.34^{b}\pm0.04$ | $8.82^{c}\pm0.06$ | $8.54^{c}\pm0.44$ | $10.13^{d}\pm0.14$ |
| Protein | $23.3^{b}\pm1.03$ | $19.40^{a}\pm0.46$ | $20.7^{a}\pm1.04$ | $23.65^{bc}\pm3.34$ | $25.5^{cd}\pm0.61$ | $27.37^{d}\pm6.62$ |
| Ash | $1.55^{b}\pm0.13$ | $1.60^{b}\pm0.15$ | $1.57^{b}\pm0.12$ | $1.74^{b}\pm0.02$ | $1.86^{b}\pm0.11$ | $2.20^{a}\pm0.06$ |

Control=0% FS+0% TP; T1=0% FS+20% TP; T2=5% FS+15% TP; T3=10% FS+10% TP; T4=15% FS+5% TP; and T5=20% FS+0% TP

FS = flaxseed flour, TP = tomato paste.

[1]g/100g product

Average of three determinations ± SE. Different letters within the same row, indicate significant differences (P < 0.05)

Table 2. Instrumental color and texture of beef patties formulated with flaxseed flour and tomato paste.

| | Control | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|
| L* | $40.26^{a}$±0.66 | $44.89^{b}$±0.45 | $44.69^{b}$±1.09 | $44.33^{b}$±1.02 | $42.43^{ab}$±0.65 | $41.83^{ab}$±0.26 |
| a* | $15.42^{bc}$±0.6 | $19.06^{d}$±0.26 | $16.85^{cd}$±0.76 | $14.42^{ab}$±0.41 | $13.33^{ab}$±0.54 | $12.26^{a}$±0.39 |
| b* | $10.62^{a}$±0.17 | $14.21^{b}$±0.35 | $15.74^{b}$±0.58 | $16.05^{b}$±0.55 | $14.81^{b}$±0.28 | $16.01^{b}$±0.72 |
| pH | $5.55^{bc}$±0.00 | $5.42^{a}$±0.03 | $5.44^{ab}$±0.01 | $5.55^{bc}$±0.01 | $5.61^{c}$±0.00 | $5.65^{c}$±0.01 |
| Hardness (kg) | $20.52^{b}$±2.4 | $7.97^{a}$±1.25 | $7.93^{a}$±0.58 | $8.77^{a}$±0.52 | $10.56^{a}$±0.55 | $9.02^{a}$±0.57 |
| Springiness (cm) | $0.83^{d}$±0.02 | $0.77^{c}$±0.01 | $0.64^{b}$±0.01 | $0.62^{b}$±0.02 | $0.59^{b}$±0.01 | $0.47^{a}$±0.01 |
| Cohesivity | $0.65^{e}$±0.02 | $0.62^{de}$±0.02 | $0.54^{cd}$±0.02 | $0.47^{bc}$±0.01 | $0.42^{ab}$±0.02 | $0.38^{a}$±0.02 |
| Masticability (kg x cm) | $10.92^{c}$±0.95 | $3.87^{b}$±0.71 | $2.70^{ab}$±0.01 | $2.59^{ab}$±0.27 | $2.65^{ab}$±0.21 | $1.59^{a}$±0.04 |

Control=0% FS+0% TP; T1=0% FS+20% TP; T2=5% FS+15% TP; T3=10% FS+10% TP; T4=15% FS+5% TP; and T5=20% FS+0% TP

FS = flaxseed flour, TP = tomato paste.

Average of three determinations ± SE. Different letters within the same row, indicate significant differences (P < 0.05)

Table 3. Effect of flaxseed flour and tomato paste on sensory attributes of beef patties[1].

| | Control | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|
| Flavor | 7.23$^{c}$±0.23 | 7.14$^{c}$±0.20 | 6.14$^{b}$±0.15 | 5.04$^{a}$±0.21 | 5.03$^{a}$±0.26 | 4.81$^{a}$±0.24 |
| Firmness | 6.88$^{b}$±0.18 | 6.82$^{b}$±0.18 | 6.00$^{ab}$±0.22 | 5.26$^{a}$±0.27 | 5.60$^{a}$±0. 32 | 5.46$^{a}$±0.26 |
| Juiciness | 6.55$^{b}$±0.22 | 6.68$^{b}$±0.16 | 6.41$^{b}$±0.17 | 6.19$^{ab}$±0.22 | 5.59$^{a}$±0.21 | 5.48$^{a}$±0.19 |
| Color | 6.22$^{abc}$±0.29 | 6.90$^{c}$±0.68 | 6.60$^{bc}$±0.55 | 6.54$^{abc}$±0.43 | 5.88$^{ab}$±0.40 | 5.80$^{a}$±0.34 |
| Overall acceptance | 6.71b$^{c}$±0.25 | 6.77$^{c}$±0.16 | 6.31$^{abc}$±0.15 | 5.93$^{ab}$±0.21 | 5.66$^{a}$±0.22 | 5.54$^{a}$±0.21 |

[1]Hedonic escale: 1= dislike extremely, 9 = like extremely

Control=0% FS+0% TP; T1=0% FS+20% TP; T2=5% FS+15% TP; T3=10% FS+10% TP; T4=15% FS+5% TP; and T5=20% FS+0% TP

FS = flaxseed flour, TP = tomato paste.

Average of three determinations ± SE. Different letters within the same row, indicate significant differences (P < 0.05)

Table 4. Effect of flaxseed flour and tomato paste on fatty acid profile[1] and cholesterol content[2] of beef patties.

| | Control | T1 | T2 | T3 | T4 | T5 |
|---|---|---|---|---|---|---|
| C10:0 | $0.05^{b} \pm 0.00$ | $0.04^{b} \pm 0.00$ | $0.01^{a} \pm 0.00$ | $0.01^{a} \pm 0.00$ | $0.01^{a} \pm 0.00$ | $0.01^{a} \pm 0.00$ |
| C12:0 | $0.06^{b} \pm 0.01$ | $0.05^{b} \pm 0.00$ | $0.02^{a} \pm 0.00$ | $0.02^{a} \pm 0.00$ | $0.02^{a} \pm 0.00$ | $0.01^{a} \pm 0.00$ |
| C14:0 | $1.86^{c} \pm 0.14$ | $1.62^{c} \pm 0.03$ | $0.61^{b} \pm 0.02$ | $0.43^{ab} \pm 0.03$ | $0.33^{ab} \pm 0.03$ | $0.24^{a} \pm 0.01$ |
| C16:0 | $23.23^{d} \pm 0.22$ | $22.13^{d} \pm 0.29$ | $11.42^{c} \pm 0.14$ | $9.21^{b} \pm 0.15$ | $8.36^{ab} \pm 0.26$ | $7.40^{a} \pm 0.05$ |
| C17:0 | $1.20^{c} \pm 0.07$ | $1.08^{c} \pm 0.01$ | $0.47^{b} \pm 0.01$ | $0.36^{ab} \pm 0.02$ | $0.30^{ab} \pm 0.02$ | $0.22^{a} \pm 0.00$ |
| C18:0 | $22.54^{b} \pm 4.05$ | $26.14^{b} \pm 0.66$ | $12.50^{a} \pm 0.18$ | $9.48^{a} \pm 0.04$ | $7.78^{a} \pm 0.28$ | $6.47^{a} \pm 0.19$ |
| Σ saturated | $48.94^{c} \pm 3.61$ | $51.06^{c} \pm 0.32$ | $25.04^{b} \pm 0.36$ | $19.51^{ab} \pm 0.24$ | $16.79^{ab} \pm 0.03$ | $14.35^{a} \pm 0.12$ |
| C14:1 | $0.32^{b} \pm 0.03$ | $0.30^{b} \pm 0.01$ | $0.11^{a} \pm 0.00$ | $0.07^{a} \pm 0.00$ | $0.05^{a} \pm 0.00$ | $0.04^{a} \pm 0.00$ |
| C16:1 | $2.52^{b} \pm 0.22$ | $2.33^{b} \pm 0.05$ | $0.07^{a} \pm 0.00$ | $0.56^{a} \pm 0.01$ | $0.48^{a} \pm 0.05$ | $0.34^{a} \pm 0.014$ |
| C18:1 n9c | $36.32^{c} \pm 4.25$ | $32.87^{bc} \pm 0.49$ | $24.49^{ab} \pm 0.10$ | $22.24^{a} \pm 0.10$ | $21.55^{a} \pm 0.60$ | $20.83^{a} \pm 0.19$ |
| C24:1 | $0.01 \pm 0.00$ | $0.02 \pm 0.00$ | $0.02 \pm 0.00$ | $0.02 \pm 0.00$ | $0.02 \pm 0.00$ | $0.01 \pm 0.00$ |
| Σ mono unsaturated | $39.17^{b} \pm 4.50$ | $35.53^{b} \pm 0.56$ | $24.69^{a} \pm 0.09$ | $22.88^{a} \pm 0.12$ | $22.11^{a} \pm 0.66$ | $21.22^{a} \pm 0.20$ |
| C18:2 n6c | $7.88^{a} \pm 0.39$ | $9.17^{b} \pm 0.19$ | $12.43^{c} \pm 0.02$ | $13.13^{cd} \pm 0.12$ | $13.56^{d} \pm 0.09$ | $14.04^{d} \pm 0.04$ |
| C18:3 n6 | $0.10^{a} \pm 0.00$ | $0.10^{a} \pm 0.00$ | $0.17^{b} \pm 0.00$ | $0.18^{b} \pm 0.00$ | $0.20^{bc} \pm 0.00$ | 0.22c ± 0.01 |
| C18:3 n3 | $1.03^{a} \pm 0.34$ | $0.84^{a} \pm 0.02$ | $36.47^{b} \pm 0.51$ | $43.52^{c} \pm 0.21$ | $46.69^{d} \pm 0.65$ | $49.65^{e} \pm 0.01$ |
| C20:3 n6 | $0.52^{c} \pm 0.03$ | $0.60^{c} \pm 0.01$ | $0.23^{b} \pm 0.01$ | $0.15^{ab} \pm 0.00$ | $0.13^{a} \pm 0.01$ | $0.11^{a} \pm 0.01$ |
| C20:3 n3 | $0.02^{a} \pm 0.00$ | $0.03^{a} \pm 0.00$ | $0.06^{b} \pm 0.00$ | $0.07^{b} \pm 0.00$ | $0.07^{b} \pm 0.00$ | $0.07^{b} \pm 0.00$ |
| C20:4 n6 | $1.88^{c} \pm 0.10$ | $2.17^{d} \pm 0.04$ | $0.73^{b} \pm 0.03$ | $0.45^{a} \pm 0.02$ | $0.37^{a} \pm 0.02$ | $0.26^{a} \pm 0.01$ |
| C20:5 n3 | $0.36^{c} \pm 0.01$ | $0.39^{c} \pm 0.01$ | $0.14^{b} \pm 0.00$ | $0.09^{a} \pm 0.00$ | $0.08^{a} \pm 0.00$ | $0.06^{a} \pm 0.00$ |
| C22:6 n3 | $0.10^{c} \pm 0.00$ | $0.12^{d} \pm 0.00$ | $0.04^{b} \pm 0.00$ | $0.02^{a} \pm 0.00$ | $0.02^{a} \pm 0.00$ | $0.01^{a} \pm 0.00$ |
| Σ Poli unsaturated | $11.89^{a} \pm 0.89$ | $13.41^{a} \pm 0.23$ | $50.27^{b} \pm 0.45$ | $57.61^{c} \pm 0.36$ | $61.11^{d} \pm 0.69$ | $64.43^{e} \pm 0.08$ |
| Σ unsaturated | $51.06^{a} \pm 3.61$ | $48.94^{a} \pm 0.32$ | $74.96^{b} \pm 0.36$ | $80.49^{bc} \pm 0.24$ | $83.21^{bc} \pm 0.03$ | $85.65^{c} \pm 0.12$ |
| PUFA/SFA | $0.24^{a} \pm 0.00$ | $0.26^{a} \pm 0.00$ | $2.01^{b} \pm 0.04$ | $2.95^{c} \pm 0.05$ | $3.64^{d} \pm 0.05$ | $4.49^{e} \pm 0.03$ |
| w-6/w-3 | $7.18^{b} \pm 1.36$ | $8.75^{b} \pm 0.27$ | $0.37^{a} \pm 0.01$ | $0.32^{a} \pm 0.00$ | $0.30^{a} \pm 0.00$ | $0.29^{a} \pm 0.00$ |
| Cholesterol | $73.03^{c} \pm 1.97$ | $43.59^{b} \pm 0.55$ | $39.95^{b} \pm 0.80$ | $39.92^{b} \pm 1.59$ | $28.59^{a} \pm 0.49$ | $26.89^{a} \pm 1.59$ |

Control=0% FS+0% TP; T1=0% FS+20% TP; T2=5% FS+15% TP; T3=10% FS+10% TP; T4=15% FS+5% TP; and T5=20% FS+0% TP

[1]Percentage of total fatty acids

[2]mg/100g product

FS = flaxseed flour, TP = tomato paste

Average of three determinations ± SE. Different letters within the same row, indicate significant differences ($P < 0.05$)

## Artículo 4

# Use of avocado and tomato paste as ingredients to improve nutritional quality of pork frankfurter

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**Summary**

The objective of this research was to study the effect of avocado (A) and tomato paste (T) addition on the physicochemical, nutritional and sensory quality of pork frankfurters. Treatments were: 1) Control; (2) A10=10%; (3) A20=20%; (4) T10=10% T; (5) T20=20% T; and (6) A10+T10. Addition of T increased *a** and *b** values. Frankfurters with T and with a combination of T and A had the best acceptance by the sensory panel. Addition of A increased the proportion of monounsaturated fatty acids in the finished product. Antioxidant activity increased significantly ($P < 0.05$) with addition of tomato, much higher than that observed by adding avocado. Addition of tomato paste and avocado pulp in the formulation of pork frankfurters can be a good strategy to improve their nutritional quality and antioxidant properties.

**Keywords:** pork frankfurters, functional foods, *Persea americana*, *Lycopersicum esculentum*

## Introduction

One negative aspect that has come to affect the marketing and consumption of meat and meat products is its possible association as causal agent of cardiovascular disease (CVD) and certain types of cancer, due to its contents of saturated fat, cholesterol and salt (Cross et al., 2007b; WCR, 2007). This issue presents a challenge for the food technologist and at the same time an opportunity for the meat industry to innovate and develop new food alternatives that tend to maintain consumer's health. One of these opportunities to achieve the innovation and transformation of meat is in the field of functional foods (Bhat and Bhat, 2011; Jiménez-Comenero, 2007). Strategies for the development of functional meat products are diverse, one of them being the reformulation of meat products (Jiménez-Colmenero et al., 2010a).

Several studies have shown the feasibility of adding different ingredients in the formulation of meat products such as dietary fiber (Garcıa et al., 2002; Kerr et al., 2005), natural antioxidants (Alves et al., 2012; Doménech-Asensi et al., 2013b) or polyunsaturated fatty acids (Rodríguez-Carpena et al., 2011; Sing et al., 2011).

Tomato and avocado are fruits that when consumed as part of a daily diet can provide several health benefits. Tomato is an excellent source of vitamins, minerals, and phytochemical compounds like lycopene (Erge and Karadeniz, 2011; Navarro-González et al., 2011). It has been demonstrated that consumption of foods rich in lycopene, as tomato and its by-products, improve health due to its following properties: anti-inflammatory and anticoagulant (Yaping et al., 2003), anticarcinogenic (Guttenplan et al., 2001; Wertz et al., 2004) and antioxidant (Borguini and Ferraz Da Silva Torres, 2009). Avocado is a food rich in unsaturated fatty acids (Dreher and Davenport, 2013) tocopherols, phytosterols, phenolic compounds, and procyanidins (Wang et al., 2010); and it has been demonstrated that its consumption diminishes cancer incidence (Ding et al., 2007; Ding et al., 2009).

To take advantage of the biological properties of tomato and avocado they could be used as ingredients in meat products of major consumption as pork frankfurters. However, it is necessary to know the correct amount to which they should be added, without compromising the quality of the new meat product. Therefore, the objective of the present study was to evaluate the physicochemical, sensory and nutritional quality of pork frankfurters, added with tomato and avocado.

## Materials and Methods

### *Experimental design*

Quality characteristics of pork frankfurters as affected by avocado pulp (A) and tomato paste (T) addition were studied. Both ingredients were incorporated into the formulation according to the following treatments: Control (without addition of A and T), treatment with 10% of A (A10), treatment with 20% of A (A20), treatment with 10% of T (T10), with 20% of T (T20) and treatment with 10% to 10% of T (A10+T10). Product quality was evaluated by determination of pH, color, proximate analysis, sensory analysis, fatty acids profile, antioxidant, and total phenols and flavonoids. One-way analysis was performed on quality measurements with Number Cruncher Statistical Systems 2007 (NCSS, Kaysville, UT) software (Hintze, 2007). Significant differences were estimated at a probability level in the type I error of 0.05. When significant differences between treatments were found Tukey multiple range test was performed.

*Ingredient acquisition and meat preparation*

For the preparation of pork frankfurter, 3 kg lots were used for each treatment. Meat (73% moisture, 6% fat), avocado (77% moisture, 15% fat), tomato paste (80% moisture) and spices, were obtained from the local market. Pork meat was cut into 5 x 5 cm and was transferred to a cutter (Kilia Fleischereimaschinenfabrik., Kiel, Germany), where particle size was reduced to a fine paste. Subsequently salts, condiments, and the rest of the ingredients for the emulsion were added. The entire process was carried out in the shortest time possible (no more than 5 min), not exceeding a temperature of 10 °C.

Once the emulsion was obtained, the meat batter was stuffed (RISCO RS 2050, NJ, USA) in collagen casings (2 x 10 cm). Product was cooked in a smoked house oven (EnviroPak CVU350E, OR, USA) to an internal temperature of 71.1 °C. After heat treatment, product was subjected to a cold water bath (5 min) and subsequently refrigerated at 2 °C.

*Proximate composition, color and pH*

Moisture, ash, protein and fat content were determined according to AOAC methods (AOAC, 2011). Moisture (g water/100 g sample) was determined by drying a 5 g sample at 100 °C to constant weight. Ashing was performed at 550 °C for 4 h (g ash/100 g sample). Protein (g protein/100 g sample) was analyzed according to the micro-Kjeldahl method. Factor 6.25 was used for conversion of nitrogen to crude protein. Fat (g fat/ 100 g sample) was calculated by weight loss after an extraction with petroleum ether in a goldfish apparatus. Surface color was measured with a Minolta colorimeter using the D65 illuminant and 10°

standard observer (Chroma meter CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) recording *L**, *a** and *b** values. *L** indicates lightness; *a** redness; and *b** yellowness. For pH measurement, 5 g of ground patty sample was weighed into 100 mL beakers and 45 mL of distilled water was added and the mixture homogenized. A portable pH meter (Hanna, Model HI 98140, Woonsocket, RI, USA) equipped with a puncture type combination pH electrode was used, and the reading was taken once stabilized.

*Sensory evaluation*

Sensory evaluation was performed by a trained panel of 8 members (ISO-8586-1, 1993b) in a controlled environment (21 ± 1 °C, 55 ± 5% relative humidity). Frankfurters were cut into slices of 2 x 2.5 cm, using 6 pieces per treatment for each attribute. Each sample was randomly coded with three digits. Panelists were instructed to clean their palate between samples using water. Color, taste, firmness, juiciness and overall acceptability were evaluated using a 9-point hedonic scale; 1 = dislike extremely, and 9 = extremely like.

*Lipid profile*

Composition of methyl esters were analyzed by gas chromatography using a Hewlett Packard 6890, equipped with a flame ionization detector (FID) and auto-sampler. An analytical capillary column, Supelco SPTM-2560 (Supelco Bellefonte, PA) (100 m x 0.25 mm x 0, 20 µm), was used. Injection port temperature was maintained at 250 °C and detector at 300 °C. Chromatograms were recorded and stored using the ChemStation software version A.10.01. Identification of fatty acids was made according to retention time and elution pattern of commercial standards (SupelcoTM-37 component FAME Mix, Supelco Bellefonte, PA). Quantification was carried out by measuring the area under the curve, expressed as fatty acid percentage of total fatty acids. Likewise, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and the ratio of PUFA/SFA and n-6/n-3 were calculated.

*Total phenols and flavonoids*

Total phenols were determined using spectrophotometry described by Singleton and Rossi (1965) with some modifications. Absorbance was read at 765 nm on a FLUOstar Omega (BMG Labtech Inc., Durham, NC, USA) microplate reader. Results were reported as mg of Gallic Acid Equivalent /100 g fresh weight (mg GAE/100 g FW). Total flavonoids content were determined according to the method described by Zhishen et al. (1999) with some modifications. Absorbance was read at 415 nm on a FLUOstar Omega (BMG Labtech Inc., Durham, NC, USA) microplate reader. Results were expressed as mg of Quercetin Equivalent/100 g fresh weight (mg QE/100 g FW).

*Antioxidant capacity*

The capacity of the extracts to inactivate radical 2, 2-diphenyl-1-picryl-hidracil (DPPH) was calculated according to the method by Brand-Williams et al. (1995) with some modifications. Radical reduction was determined at 518 nm on a FLUOstar Omega (BMG Labtech Inc., Durham, NC, USA) microplate reader. The activity was expressed as micromoles of Trolox Equivalent /100 g fresh weight (µM TE/100 g FW). Trolox Equivalents Antioxidant Capacity (TEAC) was determined according to the methodology by Brand-Williams et al. (1995). The ability of extracts to reduce the radical ABTS• was monitored for 5 min at 762 nm on a FLUOstar Omega (BMG Labtech Inc., Durham, NC, USA) microplate reader. TEAC final value was calculated using a regression equation between the percentage of inhibition of the radical and the concentration of Trolox. Results were reported as micromoles Trolox Equivalent /100 g fresh weight (µM TE/100 g FW). The determination of the Oxygen Radical Absorption Capacity (ORAC) measures the effect of the antioxidant compounds in a sample on the reduction of fluorescein induced by peroxyl radicals AAPH generator (2, 2' - azobis (2-amidinopropane) dihydrochloride) (Ou et al., 2001). The decrease of the fluorescence was measured at an excitation wavelength of 485 nm and emission at 520 nm in a FLUOstar Omega (BMG Labtech Inc., Durham, NC, USA) microplate reader. Results were expressed as micromoles of Trolox Equivalent /100 g fresh weight (µM TE/100 g FW).

## Results and Discussion

*Proximate composition, color and pH*

Table 1 shows proximate composition of frankfurters. Moisture content ranged between 66.49 (A10) and 70.08 % (T20), while that of fat ranged from 5.34 to 7.33%, being higher in treatment A20 and lower in the control. The contents of protein and ash in all treatments were approximately 19.0% and 2.0%, respectively. Protein content is high if compared with many of the products of this type available in the market (US Department of Agriculture, 2013; PROFECO, 2010), an attribute that may be attractive to the consumer at the time of purchase.

On the other hand, it is observed that addition of avocado increased lipid content of the final product (A10 and A20 treatments). This increase is caused by the high lipid content (15.4%) in the avocado pulp (greater proportion of monounsaturated fatty acids).

In general, from the balance of nutrients stand point, proximate composition of the product developed in this research is better with respect to the frankfurters sold in Mexico. PROFECO (2010), in a quality study on 62 brands of frankfurters, reported contents of moisture, fat, and protein in the range of 66.8 to 68.3%, 4.6 to 12.0% and 6.0 to 11.9%, respectively. Besides, all of them contained starch as extender in the range between 4.9 and 15.0% with no health benefit, on the contrary adding only empty calories.

Table 2 shows the results of color ($L$*, $a$* and $b$*) and pH of control frankfurters and those added with A and T. Avocado addition to frankfurters did not affect ($P > 0.05$) luminosity (L). However, $a$* value decreased ($P < 0.05$), while $b$* value increased ($P < 0.05$). Avocado has a typical green color due to chlorophyll, the main pigment present in the pulp (Wang et al., 2010), keeping this color during the development of products (A10 and A20 treatments).

Rodríguez-Carpena et al. (2012) studied the effect of avocado oil addition on the quality properties of pork burgers, reporting a decrease of $a$* value, an increase in $b$* and without changes in the $L$*. This same behavior was reported by Rodríguez-Carpena et al. (2011) when aqueous extract of avocado rind was added to pork burgers. On the other hand, the addition of tomato paste in the formulation of sausages decreased $L$* value but increased $a$* and $b$*. Lycopene is a red pigment that imparts the characteristic color to the tomato (Borguini and Ferraz Da Silva Torres, 2009) and its addition in the formulation of meat products can change the color towards reddish-orange tones (Garcia et al., 2009), what explains the increase in $a$* and $b$* values observed in the present study. Calvo et al. (2008) developed a fermented sausage enriched with lycopene, and as in our study reported an increase in the values of $a$* and $b$*.

Avocado and tomato paste addition affected pH of product. Avocado slightly increased pH values, while tomato paste decreased them ($P < 0.05$). The acidic nature of tomato paste (pH = 4.2) caused this pH reduction. Similar results were reported by Candogan (2002) in beef burgers made with 10 and 15% of tomato paste. Garcia et al. (2009) and Deda et al. (2007) also reported a decrease in pH by adding tomato paste in different meat products.

*Sensory evaluation*

Sensory evaluation results are presented in Table 3. It is observed that flavor of the treatments with avocado (A10 and A20) was assessed with a lower score than the rest of the treatments. Juiciness was equal among the evaluated treatments ($P > 0.05$). Firmness, color and overall acceptance of treatments with 20% of tomato paste and the combination of avocado and 10% tomato paste had the highest evaluation by the panelists. On the other hand, the color is favored when tomato paste is added in the formulation of sausages, noting otherwise when adding avocado. Color is a very important quality parameter in meat products and one that most influences consumer purchase decision (Troy and Kerry, 2010). It is also a parameter that is easily altered by the proportion of non-meat ingredients in the formulation (Whyte, 2006), as observed in our study.

Deda et al. (2007) reported that acceptance of sausages by panelists increased as the addition of tomato paste increased in formulation, having an optimal at a 12% level.

*Lipid profile*

Lipid profile is presented in Table 4. Predominant saturated fatty acids (SFA) were Palmitic (C16:0) and stearic (C18:0); amongst the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were oleic (C18:1n-9) and linoleic (C18:2n-6), respectively. The lipid profile of the control treatment was similar to that reported in pork sausages by Valencia et al. (2008) and by Delgado-Pando et al. (2010). There are significant differences ($P < 0.05$) in the type and quantity of lipids between the control treatment and those with the addition of avocado. The largest quantities of SFA occur in control treatments and in those which only contain tomato, while the MUFA were higher in treatments with avocado, mainly due to the high content of oleic acid present in the avocado (Ozdemir and Topuz, 2004). SFA decreased by 8% and MUFA increased in the same amount when 20% of avocado was added in the formulation of the product. PUFA/SFA ratio was in the range of 0.35 to 0.46 for all treatments, and as it was expected was greater for the treatment with 20% of avocado. With respect to the n-6/n-3 ratio, it was similar among treatments ranging from 11.24 to 12.80. These results are similar to those obtained by Delgado-Pando et al. (2010) for low fat pork sausages, who reported a ratio of PUFA/SFA and n-6/n-3 of 0.27 and 9.20, respectively.

*Phenolic compounds and antioxidant capacity*

Figure 1 shows the effect of tomato and avocado addition to frankfurters on phenols and flavonoids content. It can be observed that 10% of avocado or 10% of tomato addition had no effect on phenols content ($P > 0.05$), but there was an increase ($P < 0.05$) at 20% addition either avocado or tomato or in the combination treatment. On the other hand, flavonoids contents only increased in the 20% avocado treatment.

As for the antioxidant capacity, shown in Figure 2 and measured by the methods of DPPH, TEAC and ORAC, addition of 10% or 20% of avocado or 10% tomato had no significant effect ($P > 0.05$). However, the addition of 20% tomato or 10% tomato and 10% avocado increased ($P < 0.05$) antioxidant capacity, regardless of method used. Apparently the contribution of antioxidant compounds contained in tomato is greater than those in avocado. The addition of 10% avocado and 10% tomato had an additive effect on the antioxidant capacity. However, the 20% tomato treatment had the highest antioxidant capacity. The main antioxidant compound in tomato is lycopene (Erge and Karadeniz, 2011) and apparently its contribution to the antioxidant capacity was greater than those antioxidant compounds provided by the avocado.

## Conclusion

Tomato and avocado addition affected nutritional and sensory qualities of pork frankfurters in different ways. Tomatoes did not modify fatty acid profile, but increased antioxidant capacity and overall acceptance. On the other hand, avocado increased oleic acid (C18:1) and decreased stearic acid (C18:0) contents. However antioxidant capacity was not modified and sensory attributes decreased. Nevertheless the combination of both, tomato and avocado, showed better fatty acid profile and antioxidant capacity compared to control and exceeded sensory characteristics to all treatments. For these reasons, the use of tomato and avocado as ingredients in pork frankfurters could be a good alternative to consumers looking for healthy food choices. Further studies to determine the stability of the product during refrigeration storage and research to show beneficial effects on health are in progress.

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Table 1. Proximate analysis of frankfurters added with avocado and tomato paste.

| Sample | Moisture | Lipid | Protein | Ash |
|---|---|---|---|---|
| Control | $69.99^{c}$ | $5.34^{a}$ | $20.85^{c}$ | $2.27^{ab}$ |
| A10 | $68.49^{abc}$ | $6.01^{a}$ | $19.53^{b}$ | $2.35^{ab}$ |
| A20 | $66.49^{a}$ | $7.33^{c}$ | $19.15^{ab}$ | $2.56^{b}$ |
| T10 | $69.65^{bc}$ | $6.14^{ab}$ | $18.91^{ab}$ | $2.16^{ab}$ |
| T20 | $70.08^{c}$ | $6.04^{ab}$ | $18.33^{a}$ | $1.96^{a}$ |
| A10 + T10 | $67.09^{ab}$ | $7.01^{bc}$ | $19.39^{ab}$ | $2.01^{a}$ |
| SEM | 0.54 | 0.19 | 0.19 | 0.07 |

A10 = 10% avocado, A20 = 20% avocado, T10 = 10% tomato paste,
T20 = 20% tomato paste, A10 + T10 = 10% avocado + 10% tomato paste
Average of three determinations. Different letters within the same column, indicate significant differences ($P < 0.05$)
SEM = Standard error of the mean

Table 2. Effect of avocado and tomato paste addition on color and pH of pork frankfurters.

| Sample | *L** | *a** | *b** | pH |
|---|---|---|---|---|
| Control | 73.08$^{c}$ | 8.24$^{c}$ | 12.09$^{a}$ | 6.21$^{c}$ |
| A10 | 73.06$^{c}$ | 3.37$^{b}$ | 18.96$^{b}$ | 6.26$^{cd}$ |
| A20 | 73.68$^{c}$ | 1.39$^{a}$ | 20.92$^{b}$ | 6.27$^{d}$ |
| T10 | 64.10$^{b}$ | 19.28$^{e}$ | 31.14$^{c}$ | 6.03$^{b}$ |
| T20 | 58.30$^{a}$ | 25.49$^{f}$ | 43.24$^{e}$ | 5.81$^{a}$ |
| A10 + T10 | 63.09$^{b}$ | 17.75$^{d}$ | 36.,09$^{d}$ | 6.20$^{c}$ |
| SEM | 0.44 | 0.26 | 0.61 | 0.015 |

A10 = 10% avocado, A20 = 20% avocado, T10 = 10% tomato paste,
T20 = 20% tomato paste, A10 + T10 = 10% avocado + 10% tomato paste
Average of three determinations. Different letters within the same column, indicate significant differences (P < 0.05)
SEM = Standard error of the mean

Table 3. Sensory analysis of pork frankfurters added with avocado and tomato paste.

| Sample | Flavor | Firmness | Juiciness | Color | Overall Acceptance |
|---|---|---|---|---|---|
| Control | $6.37^{ab}$ | $5.00^{a}$ | $5.75^{a}$ | $7.00^{ac}$ | $5.87^{ab}$ |
| A10 | $4.87^{a}$ | $5.50^{ab}$ | $5.12^{a}$ | $5.62^{ab}$ | $5.12^{a}$ |
| A20 | $5.75^{ab}$ | $5.62^{ab}$ | $5.50^{a}$ | $5.12^{b}$ | $5.75^{ab}$ |
| T10 | $6.75^{b}$ | $6.00^{ab}$ | $6.00^{a}$ | $7.37^{c}$ | $6.75^{b}$ |
| T20 | $6.50^{ab}$ | $6.00^{ab}$ | $5.87^{a}$ | $7.00^{ac}$ | $6.62^{b}$ |
| A10-T10 | $7.19^{b}$ | $6.34^{b}$ | $6.00^{a}$ | $6.62^{abc}$ | $7.08^{b}$ |
| SEM | 0.42 | 0.25 | 0.22 | 0.39 | 0.33 |

A10 = 10% avocado, A20 = 20% avocado, T10 = 10% tomato paste,
T20 = 20% tomato paste, A10 + T10 = 10% avocado + 10% tomato paste
Average of three determinations. Different letters within the same column, indicate significant differences ($P < 0.05$)
SEM = Standard error of the mean

Table 4. Fatty acid profile of pork frankfurters added with avocado and tomato paste.

| | Control | A10 | A20 | T10 | T20 | A10 + T10 |
|---|---|---|---|---|---|---|
| Saturated | | | | | | |
| C10:0 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 |
| C12:0 | 0.05 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.00 |
| C14:0 | $1.24^{c}$ ± 0.12 | $0.87^{ab}$ ± 0.01 | $0.78^{a}$ ± 0.05 | $1.09^{bc}$ ± 0.02 | $1.11^{bc}$ ± 0.00 | $0.84^{ab}$ ± 0.01 |
| C16:0 | $24.14^{b}$ ± 0.70 | $22.044^{a}$ ± 0.00 | $22.61^{ab}$ ± 0.40 | $23.60^{ab}$ ± 0.04 | $23.58^{ab}$ ± 0.01 | $23.07^{ab}$ ± 0.22 |
| C17:0 | $0.36^{c}$ ± 0.02 | $0.26^{abc}$ ± 0.00 | $0.20^{a}$ ± 0.04 | $0.33^{c}$ ± 0.01 | $0.31^{bc}$ ± 0.01 | $0.21^{ab}$ ± 0.01 |
| C18:0 | $14.47^{c}$ ± 0.06 | $11.42^{b}$ ± 0.03 | $8.88^{a}$ ± 0.03 | $14.47^{c}$ ± 0.18 | $13.89^{c}$ ± 0.02 | $11.03^{b}$ ± 0.16 |
| C20:0 | $0.20^{b}$ ± 0.10 | $0.20^{b}$ ± 0.10 | $0.16^{a}$ ± 0.00 | $0.19^{b}$ ± 0.10 | $0.17^{ab}$ ± 0.09 | $0.17^{ab}$ ± 0.09 |
| Σ saturated | $40.25^{c}$± 0.99 | $34.88^{ab}$ ± 0.14 | $32.71^{a}$ ± 0.52 | $39.79^{c}$ ± 0.23 | $39.18^{c}1$ ± 0.11 | $35.39^{b}$ ± 0.31 |
| mono insaturated | | | | | | |
| C14:1 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.02 ± 0.01 | 0.02 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 |
| C16:1 | $2.12^{ab}$ ± 0.01 | $3.27^{c}$ ± 0.02 | $4.60^{d}$ ± 0.01 | $2.05^{a}$ ± 0.03 | $2.22^{b}$ ± 0.02 | $3.33^{c}$ ± 0.02 |
| C18:1 n9c | $41.88^{a}$ ± 1.42 | $46.83^{c}$ ± 0.02 | $46.94^{c}$ ± 0.74 | $43.15^{abc}$ ± 0.03 | $42.82^{ab}$ ± 0.08 | $46.23^{bc}$ ± 0.53 |
| C20:1 | $0.85^{b}$ ± 0.01 | $0.74^{ab}$ ± 0.00 | $0.59^{a}$ ± 0.00 | $0.84^{b}$ ± 0.06 | $0.75^{ab}$ ± 0.04 | $0.71^{ab}$ ± 0.01 |
| C22:1 n9 | $0.13^{b}$ ± 0.00 | $0.10^{ab}$ ± 0.00 | $0.08^{a}$ ± 0.01 | $0.10^{ab}$ ± 0.00 | $0.12^{ab}$ ± 0.00 | $0.09^{ab}$ ± 0.00 |
| Σ mono insaturated | $44.69^{a}$ ± 1.41 | $50.75^{b}$ ± 0.04 | $52.07^{b}$ ± 0.72 | $45.95^{a}$ ± 0.01 | $45.72^{a}$ ± 0.02 | $50.21^{b}$ ± 0.50 |
| Poli insaturated | | | | | | |
| C18:2 n6c | 12.80 ± 0.40 | 12.44 ± 0.00 | 13.23 ± 0.35 | 12.35 ± 0.14 | 13.03 ± 0.1 | 12.52 ± 0.06 |
| C18:3 n6 | 0.13 ± 0.03 | 0.09 ± 0.00 | 0.11 ± 0.04 | 0.11 ± 0.01 | 0.11 ± 0.00 | 0.09 ± 0.00 |
| C18:3 n3 | 0.79 ± 0.03 | 0.77 ± 0.00 | 0.81 ± 0.00 | 0.75 ± 0.02 | 0.75 ± 0.04 | 0.82 ± 0.00 |
| C20:3 n6 | $0.13^{d}$ ± 0.00 | $0.12^{c}$ ± 0.00 | $0.09^{a}$ ± 0.00 | $0.12^{c}$ ± 0.00 | $0.13^{d}$ ± 0.00 | $0.10^{b}$ ± 0.00 |
| C20:3 n3 | $0.11^{a}$ ± 0.00 | $0.09^{c}$ ± 0.00 | $0.07^{a}$ ± 0.00 | $0.11^{d}$ ± 0.00 | $0.10^{cd}$ ± 0.00 | $0.08^{b}$ ± 0.00 |
| C20:4 n6 | $0.52^{d}$ ± 0.01 | $0.45^{c}$ ± 0.00 | $0.35^{a}$ ± 0.01 | $0.43^{c}$ ± 0.01 | $0.52^{d}$ ± 0.00 | $0.40^{b}$ ± 0.00 |
| C20:5 n3 | 0.08 ± 0.01 | 0.06 ± 0.00 | 0.05 ± 0.00 | 0.06 ± 0.01 | 0.08 ± 0.00 | 0.05 ± 0.00 |
| C22:6 n3 | 0.16 ± 0.02 | 0.11 ± 0.00 | 0.32 ± 0.17 | 0.14 ± 0.00 | 0.14 ± 0.00 | 0.15 ± 0.05 |
| Σ Poli insaturated | 14.74 ± 0.53 | 14.14 ± 0.01 | 15.05 ± 0.20 | 14.08 ± 0.13 | 14.89 ± 0.04 | 14.21 ± 0.10 |
| Σ insaturated | $59.74^{a}$ ± 0.89 | $65.11^{bc}$ ± 0.04 | $67.28^{c}$ ± 0.52 | $60.21^{a}$ ± 0.14 | $60.82^{a}$ ± 0.02 | $64.60^{b}$ ± 0.40 |
| AGP/AGS | $0.37^{ab}$ ± 0.00 | $0.40^{c}$ ±0.00 | $0.46^{d}$ ± 0.00 | $0.35^{a}$ ± 0.00 | $0.38^{b}$ ± 0.00 | $0.40^{c}$ ± 0.00 |
| n-6/n-3 | 11.93 ± 0.27 | 12.65 ± 0.01 | 11.24 ± 1.85 | 12.25 ± 0.36 | 12.80 ± 0.45 | 11.86 ± 0.50 |

A10 = 10% avocado, A20 = 20% avocado, T10 = 10% tomato paste, T20 = 20% tomato paste, A10 + T10 = 10% avocado + 10% tomato paste
Average of three determinations ± SE. Different letters within the same column, indicate significant differences (P< 0.05)

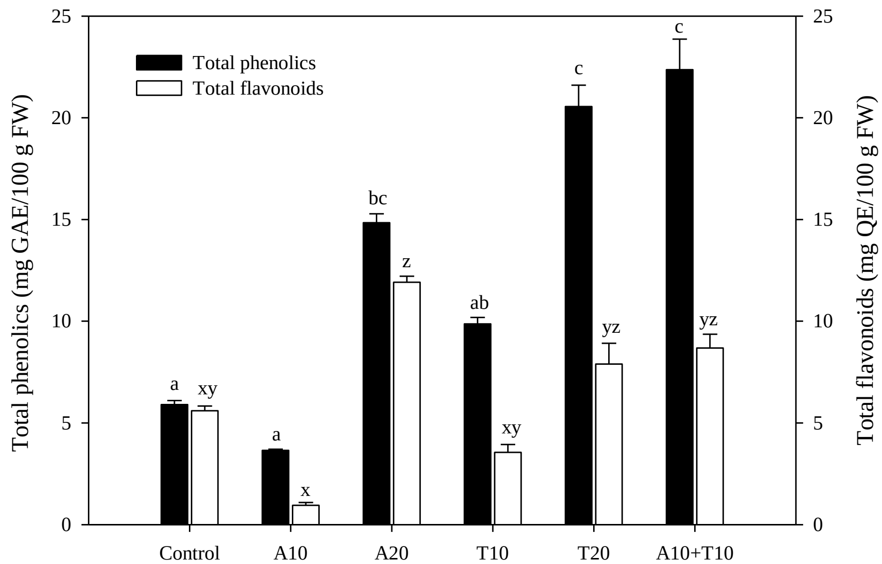


Figure 1. Tomato and avocado effect on phenolics and flavonoids contents in pork frankfurters. A10= 10% Avocado; A20= 20% avocado; T10=10% tomato; T20=20% tomato; A10+T10= 10% avocado+10% tomato.
Different letters on columns (a-c), (x-z), show significative differences ($P < 0.05$).
Each value is the average of 6 replicates ± SE.

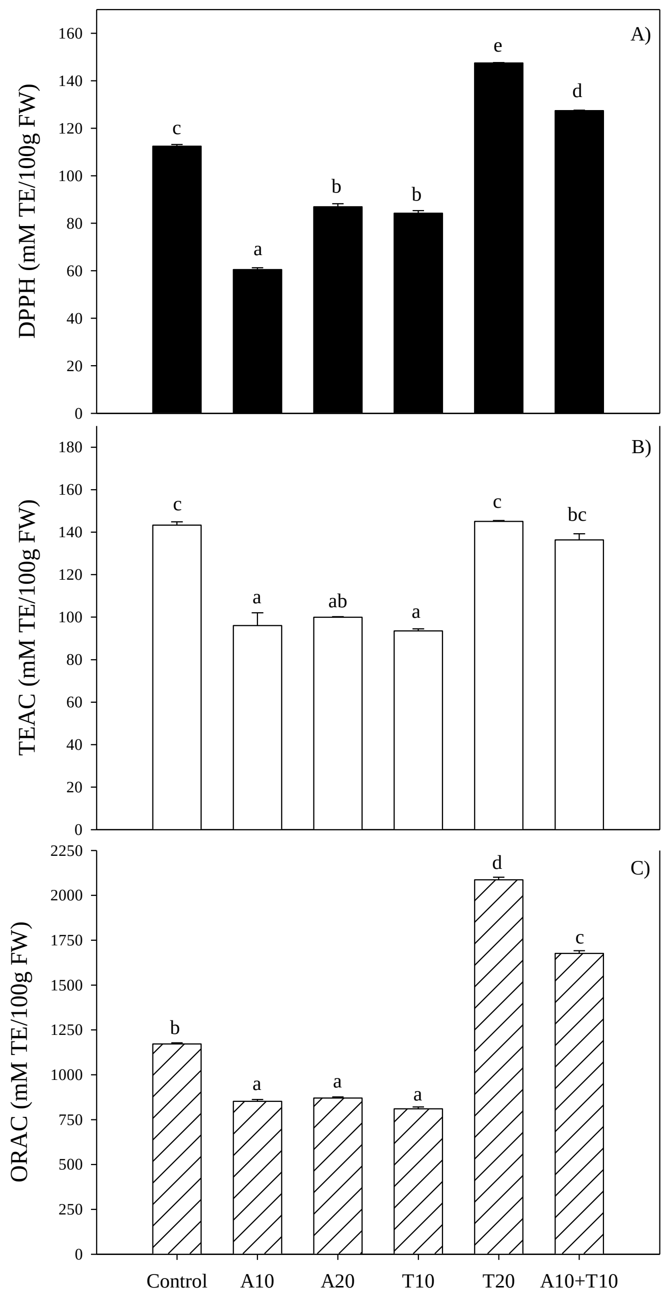


Figure 2.- Tomato and avocado effect on (a) DPPH, (b) ORAC and (c) TEAC of pork frankfurters extracts.
A10= 10% Avocado; A20= 20% avocado; T10=10% tomato; T20=20% tomato; A10+T10= 10% avocado+10% tomato.
Different letters on columns show significative differences (P < 0.05).
Each value is the average of 6 replicates ± SE.

# Capítulo 4

## CONCLUSIONES GENERALES

La calidad fisicoquímica y sensorial de hamburguesas de res adicionadas con harina de linaza y pasta de tomate puede estimarse utilizando los modelos de predicción desarrollados en el presente estudio. De acuerdo a dichos modelos, la formulación óptima que predice las mejores características de calidad en hamburguesas de res, incluye 3% de harina de linaza y 10.8% de pasta de tomate. Sin embargo una combinación de 10% de harina de linaza y 10% de pasta de tomate en la formulación de hamburguesas de res muestra un mejor perfil nutricional sin comprometer la calidad sensorial del producto. Desde el punto de vista de inocuidad, los modelos de predicción generados estiman una reducción de 3.62 log UFC/g de *Escherichia coli* O157:H7 cuando se tiene una formulación de 10% HL+10% PT, respecto a la formulación óptima en la que se tiene una reducción de 2.74 log UFC/g. Al simular condiciones de almacenamiento en abuso de temperatura (15°C durante 5 d), y una contaminación inicial de 2.5 log UFC/g, el modelo estima un crecimiento de *Escherichia coli* O157:H7 de 2.91 log UFC/g en la formulación de 10% HL+10% PT, mientras que en la formulación óptima la predicción fue de 3.63 log UFC/g.

Existe un gran potencial para utilizar la harina de linaza y pasta de tomate como ingredientes en el desarrollo de hamburguesas de res con una connotación más saludable, más seguros y de calidad aceptable. Estudios posteriores son necesarios para evidenciar los efectos benéficos que dichos productos tienen en los organismos vivos.