Instituto Tecnológico y de Estudios Superiores de Monterrey

Campus Monterrey

School of Engineering and Sciences

CHARACTERIZATION OF SAUSAGES ADDED WITH A FUNCTIONAL CARROT POWDER INGREDIENT RICH IN PRO-VITAMIN A CAROTENOIDS AND FORTIFIED WITH CHLOROGENIC ACID

A thesis presented by

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Submitted to the

School of Engineering and Sciences

in partial fulfillment of the requirements for the degree of

Master of Science

In Biotechnology

Monterrey Nuevo León, May 27th, 2017

Instituto Tecnológico y de Estudios Superiores de Monterrey

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Declaration of Authorship

I, Melissa Alvarado Ramírez declare that this thesis titled, 'Characterization of sausages added with a functional carrot powder ingredient rich in pro-vitamin A carotenoids and fortified with chlorogenic acid" and the work presented in it are my own. I confirm that:

- This work was done wholly or mainly while in candidature for a Master of Science degree at this University.
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.
- Where I have consulted the published work of others, this is always clearly attributed.
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.
- I have acknowledged all main sources of help.
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

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Melissa Alvarado Ramírez

Monterrey Nuevo León, May 27th, 2017

Dedication

I dedicate this thesis to my family, specially to my mother Irma Ramírez and my father Francisco Alvarado. Without your advices, scowls, and coffee cups in the middle of the night I could not have done it. Thanks for all your unconditional support, patience, and encouragement. You were my main motivation for pushing through this work.

I also dedicate this thesis to my friends. Thank you, Erika Ortega because without you I could not have gone through all this process; you are and will be one of my dearest friends.

To my dearest lifelong friends, Mónica Leyva and Fernanda Llanas. Even when you were not here, I felt your support all the time. Thank you because you always believed in me. Your friendship through all these years have helped me to become the person I am today.

Acknowledgements

I would like to show my deepest gratitude to my advisor, Daniel Alberto Jacobo Velázquez, who taught me and guided me to become a better person and scientist. I appreciated all the times you showed me my strengths and weaknesses. You always took care of me and I will never forget your support, patience, and wisdom.

Thanks to Dr. Jorge Alejandro Benavides Lozano for accepting to be a committee member, as well as, M.Sc. Joaquín Tirado Escobosa who believed in this project and kindly accepted to be part of the committee.

I would like to deeply thank Sigma Alimentos for their technical assistance and facilities, specially to Carlos Sada and Ulises who helped in the manufacturing process.

I would also like to thank Dr. Perla Ramos, for her assistance in carotenoid analysis. As well as, Héctor Velázquez, and Erika Leal, who facilitated the equipment in order to accomplished my experiments. Also thanks to Lily Ramos for her teachings in HPLC analyses.

Specially thanks to Erika Ortega for her commitment in this project as well as her advices through my Master studies. I really appreciated every single moment we laugh, cried, sang, danced, and made science in the laboratory.

Thanks to my old friends who supported me and put up with my mood in times of crisis: Javier Villela, Carlos Martínez, Gaby Sánchez, Jorge Montalvo, César Ortiz, Mónica Leyva, and Fernanda Llanas.

Thanks to all my new friends I made during this process: Miguel Aguilar, Melissa Moreira, Alejandro Maeda, Alejandro Robles, Mariel Torres, Calef Sánchez, Alejandro González, Alonso Ornelas, Aidee Sánchez, Gilberto Rodríguez, Leticia Barraza, Jacob Bañuelos, Jesús Santana, Héctor Castañeda, Adán Mendoza, Carlos Moreno, Caro García and José García.

Thanks to Dr. Marco Rito Palomares and the Bioprocess and Synthetic Biology Research Group for their financial support in this project.

Thanks to CONACYT for the support scholarship (#710330), and to Tecnológico de Monterrey for the academic scholarship.

Thanks again to my mentor, family, friends, and acquaintances for their support and good moments.

Abstract

CHARACTERIZATION OF SAUSAGES ADDED WITH A FUNCTIONAL CARROT POWDER INGREDIENT RICH IN PRO-VITAMIN A CAROTENOIDS AND FORTIFIED WITH CHLOROGENIC ACID

By

Melissa Alvarado Ramírez

Metabolic syndrome has become a worldwide health issue that results in the increased risk of chronic degenerative diseases such as diabetes, cancer, and cardiovascular diseases. These metabolic conditions could be prevented if the intake of nutraceuticals is increased. The main source of these compounds in the diet are fruits and vegetables, however the consumption of such foods in the Mexican population is low, and thus it is relevant to design strategies that allow the incorporation of nutraceutical compounds in high-consumed foods in the population.

The transformation of fruits and vegetables into powders, for later incorporation in food formulations as ingredients could be a strategy to increase the consumption of nutraceuticals. However, the heat treatments to which vegetables are subjected during drying to obtain powders induce a significant loss of these compounds. Also, there are additional losses during processing when the powder is added to a food formulation. In the past years, postharvest abiotic stresses such as wounding, modified atmospheres, and UV-radiation, have been studied as an effective tool to improve the accumulation of bioactive compounds in horticultural crops.

In this context, it has been reported that wounding stress induces the accumulation of phenolic compounds, specially, chlorogenic acid (CHA) in carrots. Thus, the transformation of stressed carrots into a powder (carrot powder, CP) and its further incorporation into highly consumed foods, would be an effective strategy to overcome thermal losses of nutraceuticals observed during processing and to increase the consumption of antioxidants in the Mexican population.

In the present study, a carrot powder with high concentration of phenolic compounds (functional carrot powder, FCP) was produced and added as an ingredient to sausage formulations. To obtain FCP, carrots were shredded, stored for 48 h at 15 °C, dried at 60 °C and grounded by milling. The nutraceutical content of phenolic compounds, carotenoids, water absorption index (WAI), and oil absorption index (OAI) were evaluated and compared to regular carrot powder (control carrot powder, CCP), which was obtained by drying carrots immediately

after shredding (without storing the samples). Both CPs showed similar levels of α-carotene, β-carotene, and retinol equivalents (RE); whereas FCP showed 612.4% and 798.4% higher levels of total phenolics and CHA, respectively, as compared with CCP. Likewise, FCP showed higher values of WAI and OAI.

Preliminary studies were performed to determine the optimum FCP concentration in sausages. Based on the results, formulation with 4% w/w FCP was chosen as the optimum concentration for a complete characterization of the product.

A proximate analysis and dietary fiber content was determined in sausages added with 4% FCP and 4% CCP. Moreover, pH and purge values were evaluated as well as color, texture properties, and nutraceutical content (carotenoids and phenolic compounds) for 42 d of storage at 4 °C. Finally, a sensory acceptability test was performed. It was observed an increment of 72% in total dietary fiber by the addition of 4% CP, and a significant decrease of moisture in CCP formulation. Likewise, FCP addition decreased the pH of sausage, while CCP showed higher purge loss than the control and FCP formulation during storage. Color as well as texture parameters were also affected by CP addition. In general, phytochemical content showed stability during storage of sausages added with CP. Compared to CCP sausages, FCP formulation showed 377.7% higher concentration of total phenolic compounds. No significate differences in carotenoids were observed between CP formulations, while a portion of 62.5 g of 4% CP sausage contributes to 32.5% of daily retinol equivalent intake in Mexican population. Finally, a consumer's acceptability test showed adequate acceptability of CCP and FCP formulations by consumers.

Results demonstrated that FCP addition in a sausage formulation resulted on a product accepted by consumers, while providing nutraceutical compounds such as fiber, phenolic compounds and carotenoids, which could greatly aid on the prevention of chronic and degenerative diseases.

Resumen

CARACTERIZACIÓN DE SALCHICHAS ADICIONADAS CON HARINA DE ZANAHORIA FUNCIONAL RICA EN CAROTENOIDES PRO-VITAMINA A Y FORTIFICADA CON ÁCIDO CLOROGÉNICO

Por

Melissa Alvarado Ramírez

El síndrome metabólico se ha convertido en un problema de salud mundial que resulta en un mayor riesgo de enfermedades crónicas y degenerativas tales como diabetes, cáncer y enfermedades cardiovasculares. Dichas enfermedades se pueden prevenir si se incrementara la ingesta de compuestos nutracéuticos. La principal fuente de estos compuestos en la dieta son las frutas y hortalizas, sin embargo, el consumo de estos alimentos en la población mexicana es bajo, por lo que es relevante diseñar estrategias que permitan la incorporación de compuestos nutracéuticos en alimentos de alto consumo en la población. La transformación de frutas y verduras en harinas, para su posterior incorporación como ingredientes en formulaciones alimenticias, podría ser una estrategia para aumentar el consumo de compuestos nutracéuticos.

Sin embargo, los tratamientos térmicos a los que se someten las hortalizas durante el secado y la molienda para la obtención de harinas inducen una pérdida significativa de estos compuestos. Además, cuando la harina de vegetales se añade a las formulaciones de alimentos, hay pérdidas adicionales durante el procesamiento debido a tratamientos térmicos. En los últimos años, se han estudiado a los estreses abióticos poscosecha tales como el estrés de corte, las atmósferas modificadas y la radiación UV, como herramientas eficaces para mejorar la acumulación de compuestos bioactivos en los cultivos hortofrutícolas.

En este contexto, se ha reportado que el estrés de corte, induce la acumulación de compuestos fenólicos, especialmente ácido clorogénico. Por lo tanto, la transformación de zanahorias estresadas en harinas (harina de zanahoria, CP) y su posterior incorporación en alimentos de alto consumo sería una estrategia eficaz para superar las perdidas térmicas de nutracéuticos observadas durante el procesamiento y a su vez, incrementar el consumo de antioxidantes en la población mexicana.

En el presente estudio, se produjo una harina de zanahoria con alta concentración de compuestos fenólicos (harina de zanahoria funcional, FCP) y se añadió como ingrediente a formulaciones de salchicha. Para obtener FCP, las zanahorias se rallaron, se almacenaron durante 48 h a 15 °C, se secaron a 60 °C y se molieron. Posteriormente, se determinó el contenido de compuestos fenólicos, carotenoides, el índice de absorción de agua (WAI) y el índice de absorción de aceite (OAI) en el FCP y se compararon los valores con los obtenidos en harina de zanahoria regular (CCP) producida con zanahoria secada inmediatamente después de rallarlas (sin almacenarlas). Ambas harinas de zanahoria mostraron niveles similares de α-

caroteno, β-caroteno y equivalentes de retinol (RE), mientras que FCP reportó incrementos de 612.4% y 798.4% en compuestos fenólicos totales y CHA, respectivamente, en comparación con CCP. Del mismo modo, FCP mostró valores mayores de WAI y OAI comparado con CCP.

Se realizaron estudios preliminares para determinar la concentración óptima de FCP en salchichas. En un estudio inicial, las formulaciones, añadidas con 4, 5, 6, 8 y 12% p/p de FCP fueron evaluadas por su contenido nutracéutico, aceptabilidad sensorial y propiedades fisicoquímicas. Basándose en los resultados del estudio preliminar, se seleccionó la formulación con FCP al 4% p/p como la concentración óptima para una caracterización completa del producto.

Se realizó un análisis proximal y de contenido de fibra dietética en salchichas con FCP y CCP al 4%. Adicionalmente, se evaluaron los valores de pH y de purga, así como el color, las propiedades de textura y el contenido nutracéutico (carotenoides y compuestos fenólicos) durante 42 días de almacenamiento a 4 °C. Finalmente, se realizó un test de aceptabilidad sensoria con consumidores. Se observó un incremento del 72% en la fibra dietética total en las salchichas adicionadas con CP. Además, se observó una disminución significativa en el contenido de humedad en la formulación de CCP comparada con FCP. Asimismo, la adición de CP disminuyó el pH, mientras que CCP mostró mayores pérdidas de purga que el control y FCP durante el almacenamiento. El color, así como los parámetros de textura también se vieron afectados por la adición de CP. En general, el contenido de fitoquímicos mostró estabilidad durante el período de almacenamiento de las salchichas con CP. A diferencia de las salchichas con CCP, la formulación FCP mostró una concentración de 377% mayor de compuestos fenólicos totales. Asimismo, no se observaron diferencias significativas en el contenido de carotenoides (α-caroteno y β-caroteno) entre las formulaciones con CP. También se reportó que una porción de 62.5 g de salchicha al 4% de CP contribuyen con el 32.5% de la ingesta diaria recomendada de equivalente de retinol en la población mexicana. Finalmente, una prueba de aceptabilidad sensorial reportó valores adecuados para las formulaciones de CCP y FCP.

Los resultados demostraron que la adición de FCP en salchichas resultó en un producto altamente aceptado por los consumidores además de proporcionar compuestos nutracéuticos tales como fibra, compuestos fenólicos y carotenoides que podrían en gran medida contribuir a una mayor ingesta de vitamina A y a prevención enfermedades crónicas y degenerativas en la población mexicana.

List of Figures

List of Tables

CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

According to recent statistical data, the main cause of death in Mexico are diseases derived from obesity such as diabetes, cardiovascular diseases, and hypertension (INEGI, 2016). In most cases, these diseases could be prevented if the consumption of chemopreventive compounds increase in our population. The main source of these compounds in the diet are fruits and vegetables, however the consumption of such foods in the Mexican population is low (Rivera et al., 2002), and thus it is relevant to design strategies that allow the incorporation of nutraceutical compounds in high-consumed foods.

A functional food is defined as a food that improves health by providing chemical compounds (nutraceuticals) that help on the prevention of chronic diseases (Biswas et al., 2011). In this context, nutraceutical compounds such as carotenoids, phenolics, tocopherols, dietary fiber, among others, which are naturally present in fruits and vegetables, are being added to food formulations to produce functional foods (O'Shea, Arendt, & Gallagher, 2012). Meat is an integral component of the human diet as it is an important source of protein, essential fats, vitamins and minerals. In developed countries, meat is a significant portion of a normal diet, contributing 15% of energy, 40% of protein and 20% of fat daily (Daniel, Cross, Koebnick, & Sinha, 2011). Hence, the addition of nutraceutical compounds such as phenolic compounds in meat products has taken interest in the last decade (Mehta et al., 2015).

The transformation of fruits and vegetables into powders, for later incorporation in food formulations as ingredients could be a strategy to increase the consumption of nutraceuticals in the population. However, the heat treatments to which vegetables are subjected during drying and grinding to obtain powders induce a significant loss of these compounds. Also, when the powder is added to food formulation, there are additional losses during processing due to thermal treatments (Chantaro et al., 2008). Therefore, scientific community has now focused its attention on the development of technologies to increase the levels of these compounds in fruits and vegetables (Rommens et al., 2008). The controlled application of postharvest abiotic stresses (wounding stress, UV radiation, phytohormones, ultrasound treatments, etc.) has been proposed as a simple and efficient technology to increase the nutraceutical content of horticultural crops (Cisneros-Zevallos, 2003; Jacobo-Velázquez & Cisneros-Zevallos, 2012).

Among plants, carrot is considered a nutraceutical crop for its content of carotenoids, fiber, and phenolic compounds. In the last decade, it has been demonstrated that carrots respond to different postharvest abiotic stresses producing phenolic compounds, and from the different abiotic stresses evaluated wounding stress is the most effective for this purpose (Jacobo-Velázquez et al., 2011, 2015; Becerra-Moreno, 2015). The main phenolic compound produced in carrots as a response to wounding stress is the chlorogenic acid (CHA) (Jacobo-Velázquez et al., 2012). Health promoting properties of CHA have been extensively studied. Reports have showed its protective effect against different chronic and degenerative diseases, mainly to those related with the metabolic syndrome (Santana-Gálvez et al., 2017).

In the present study, a FCP was produced by applying wounding stress to carrot tissue prior to dehydration and milling and compared to a regular carrot powder (CCP). The effects of FCP addition on the physicochemical, sensory and nutraceutical characteristics of sausages was evaluated. Likewise, a shelf-life study was performed to evaluate the physicochemical and nutraceutical stability of the formulated product.

1.2 Hypothesis

The hypothesis of the present study was that the incorporation of FCP ingredient into a sausage formulation would result on a product accepted by consumers and with high levels of nutraceutical compounds (provitamin A carotenoids, and chlorogenic acid) that are stable during its shelf-life.

1.3 General Objective

Evaluate the effect of FCP addition, on the sensory acceptability, phytochemical content and shelf-life stability of sausage formulations.

1.4 Specific Objectives

1) Compare the phytochemical content and functional properties of CCP and FCP.
2) Determine the optimum concentration of FCP ingredient that results on a sausage formulation highly acceptable by consumers and with high content of nutraceutical compounds.

3) Evaluate and compare the consumer's acceptability, chemical composition, phytochemical content and shelf-life stability of sausage added with 4% (w/w) FCP or CCP.

1.5 Thesis structure

This thesis is composed of 5 chapters. Chapter 1 presents a general overview of the principal topics that were covered in the thesis. Chapter 2 comprises a literature review of the main causes of death in Mexico, follow by the benefits of nutraceutical compounds intake. Then, the dietary patterns in Mexican population is addressed. Subsequently, the addition of carrot powder in food formulations and the application of wounding stress to enhance phenolic content of carrots were reviewed. Finally, the state of the art regarding the addition of different ingredients in sausages to improve their nutraceutical and organoleptic characteristics is presented. Chapter 3 shows the materials and methods applied in the experimental stage of the investigation. Chapter 4 presents results and discussion regarding the characterization of sausages added with FCP. Finally, Chapter 5 presents the main conclusions of the investigation and recommendations for future studies.

CHAPTER 2

BACKGROUND INFORMATION

2.1 Prevention of chronic and degenerative diseases by nutraceutical compounds

A change in lifestyle, increasing physical activity and improving dietary habits, are the most important steps in term of prevention and cost/effectiveness in disease such as diabetes, CVD and obesity (Flores et al., 2010). Currently, there has been a trend towards lifestyle modification and use of natural products. This derives from publications and reports that have provided scientific evidence to support hypotheses that, for instance, phytochemicals in foods and in isolated form provide health benefits to the consumer (Dilldard & German, 2000). In addition, consumers are seeking complementary or alternative beneficial products besides expensive, high-tech disease-treatments (Das et al., 2011).

The term “nutraceuticals” was coined from “nutrition” and “pharmaceutical” by Stephen De Felice, founder and chairman of the Foundation for Innovation in Medicine in 1989 (Das *et al*., 2011; Dillard & German, 2000). Nutraceutical can be defined as a food (or a part of food) that provides medical or health benefits, including the prevention and/or treatment of diseases. From this point of view, phytochemicals present in these foods have a wide range of therapeutic effects against diseases such as diabetes, CVD, cold, arthritis, cancer, hypertension, dyslipidemia, depression, metabolic syndrome, among others (Cicero & Colletti, 2015; Bjørklund & Chirumbolo, 2016; Dahiya, 2013; Das et al., 2011; Dillard & German, 2000).

Nutraceuticals can be classified based on food sources or chemical nature. Some of the most important are: dietary fiber, probiotics, polyunsaturated fatty acids, antioxidant vitamins, and phenolic compounds (Dahiya, 2013). They are found naturally in fruits and vegetables, but can also be obtained in optimum concentrations through dietary supplements (Cencic and Chingwaru, 2010). In some countries, such as the United States and Germany, people are already aware of the health benefits that nutraceutical compounds provided and thus, more people are consuming them. That is why the global market for supplements of nutraceuticals is growing fast at $ 140 billions and an annual growth of 14.7% is estimated (Wadhwa, Charles & Pandit, 2010).

On the other hand, food industry has taken interest in this area due to the increasing demand and the desire of people for an improved quality of life. New products are being develop in which, nutraceutical compounds are being added as functional ingredients. Functional food

products have been defined as providing an added health benefit over and above the food product's traditional nutritional value (Frewer, Scholderer, & Lambert, 2003; Heasman & Mellentin, 2001).

Functional foods may be classified into (1) conventional food containing naturally occurring bioactive substance; (2) foods that have been modified, by enrichment or other means, with bioactive substances; and (3) synthesized food ingredients, such as some specialized carbohydrates intended to have probiotic effects (Henry, 2010).

The main consumer driving force for purchasing functional foods is the growing desire to use foods either to help prevent chronic illnesses such as cardiovascular disease, diabetes, hypertension and obesity, or to optimize health, for example by increasing energy, boosting the immune system, generation of wellbeing (Regmi & Gehlhar, 2005; Sadler, 2005). This need has led to one of the fastest growing food sectors, with a compound annual growth rate of 8.6% in the 10 years to 2012 (Euromonitor, 2010b). The emergence of a new market segment called 'Health and Wellness' reached a global value of US$625 billion in 2012. Of this market, the functional foods part alone was valued at US$168 billion in a global market that is 2.5 times the size that of vitamins and dietary supplements (Euromonitor, 2010a).

Nevertheless, the consumption of such compounds, alone or added into food products in Mexico is low because of a preference for a western diet (high in sugar and protein, low in vegetables and fruits), which contributes to higher rates of obesity (Dávila-Torres, González-Izquierdo & Barrera-Cruz, 2015). In Mexico, the rate of obesity in the adult population, based on actual measures of height and weight was 32.4% in 2012 (compared to a rate of 24.2% in 2000), which represents the second highest rate of the OECD countries, after the United States (35.3% in 2012).

Additionally, there is evidence of the association between Mexican dietary patterns with higher risk of CVDs, and metabolic syndrome, hence cases of chronic degenerative diseases are rising (Flores et al., 2010; Córdoba-Villalobos, 2008). Chronic degenerative diseases are a public health issue in Mexico since lack of prevention strategies, have led into high expenditures in the health sector (OECD, 2014). In addition, there are a lot of reported cases, which contributes to general mortality (INEGI, 2016). Thus, it is important to aboard recent trends of food consumption in Mexican population.

2.2 Mexican dietary patterns in meat consumption

Changes in food consumption in Mexico such as a high intake of fats and refined sugars as well as a reduction of fruits and vegetables intake have been reported since last decade (Rivera *et al.*, 2002). Unfortunately, several studies have associated Mexican dietary patterns with metabolic syndrome (Denova-Gutierrez et al., 2010; Flores et al., 2010; Acosta-Cázares & Escobedo-de la Peña, 2010). During a short time, there has been a notable increase and preference in Mexican population for a western diet, which is composed of high intake of refined sugars from soft drinks, increment of protein from red meat, and an important reduction of fruits and vegetables (Denova-Gutiérrez et al., 2010). These changes are now contributing to the growing number of obese people and nutrition-deficiencies issues in Mexico (OECD, 2014). For instance, Rivera (2012) have reported that 30% of Mexican children younger than five years have an energy deficit and low consumption of zinc, iron, vitamin B12, folates, vitamin A among other micronutrients. While in adults, according to the Mexican National Health and Nutrition Survey of 2012 (ENSANUT-2012), the incidence in diabetes, hypercholesterolemia and hypertension increased compared with previous reports from 2006 (Ramírez-Silva et al., 2011).

ENSANUT-2006 showed that, the typical Mexican diet has increased consumption of proteins from sources like meat. In terms of protein, 69.5% of the food intake was contributed by five products: maize, milk, wheat, beans and beef, which covered 72.5% of the protein consumption recommended. Likewise, the energy intake by product came mainly from the consumption of cereals (46.6%), sugar (15.4%), vegetable oils (8.7%) and meats (6.9%). Regarding meats products, they occupied the fourth place within the total caloric energy supply and the second in the energy supply from proteins and fats. The contribution to the total calories of the different types of meats was: pork 3.1%, poultry 1.9% and beef 1.7% (Martínez & Villezca, 2005).

Meat is an integral component of the human diet. It is a great source of protein, essential fats, vitamins and soluble minerals. In developed countries, meat constitutes a significant portion of a normal diet, contributing 15% of energy, 40% of protein and 20% of fat daily (Daniel et al., 2011). Likewise, a shift towards a diet pattern characterized by high meat consumption seems to supplant the consumption of cereals and other foods of plant origin, so the trend indicates an increase in meat intake in developing countries (Lippi et al., 2015).

Regarding this aspect, the latest Food and Agriculture Organization (FAO) statistics showed that the current world meat intake is 311.8 million tons/year and production will double by 2050, especially in developing countries. As well as a steady demand for processed meat

(FAO, 2015). Furthermore, in 2013, Mexico occupied the 8th position worldwide in processed food production. For instance, the production value of livestock and cold meats sector increased from $ 27 billion in 2007 to $ 41 billion in 2014, with one of the greatest dynamism in the sector, growing on average 5.05%. Specifically, cold meat products had an annual growth rate of 30.2%, in which the highest participation was generated by the meat and poultry preserves with 45.0%, followed by cold meats and sausages with 35.0%, and thirdly red meat hams with 20.0% (Actinver, 2015). This national tendency implies that cold meats sector will still growth as FAO (2015) predicted in a global scale.

This means that the impact of fresh and processed meat on human health is expected to grow exponentially in the coming decades. In Mexico, meat consumption patterns in the Northeast indicate a predilection for red meat. Among some dishes that stand out in preference are those that are made from roast beef. However, cold meat such as sausages are also commonly eating by Mexicans and its market is growing as previously mentioned (Taddei, et al., 2012).

Unfortunately, meat do not provide chemopreventive compounds in the diet. Hence new strategies for the incorporation of nutraceuticals compounds are needed. The transformation of fruits and vegetables into powders, for later incorporation in food formulations highly consumed such as sausages, could be a strategy to increase the consumption of nutraceuticals in the population. Among different fruits and vegetables, carrot has been considered a nutraceutical crop because it provides basic nutrition and bioavailable phytochemicals (Arscott & Tanumihardjo, 2010), making it a good candidate for its transformation and incorporation in to meat products. Thus, next section will discuss the nutraceutical properties of carrot.

2.3 Carrot (*Daucus carota*) as a source of nutraceutical compounds

Carrot (*Daucus carota*), among vegetables, is considered an important horticultural crop due its nutritional value, source of natural antioxidants and high concentration of provitamin-A compounds. Its production has increased steadily and it is considered a primary vegetable in many countries. In Mexico, carrot production was of 331 thousand tons in 2014 and its consumption is still increasing (FAOSTAT, 2014). A study with 21,072 eight-year-old and nine-year-old Mexican children suggest that carrot is the most preferable eaten vegetable in the country, followed by lettuce and tomato (Secretaría de Salud, 2010).

From a human diet perspective, carrots do not supply significant amounts of calories. However, carrot provides high amounts of nutraceutical compounds such as dietary fiber and pro-vitamin A carotenoids (Arscott & Tanumihardjo, 2010). Raw carrots are composed of

approximately 88% water, 1% protein, 7% carbohydrates, 0.2% fat, and 3% fiber. In addition, carrot is a good source of minerals such as Ca, P, Fe, and Mg (Sharma, *et al.* 2012). Also, carrot provides vitamins like thiamin, riboflavin, and niacin in appreciable quantities when compared with other consumed vegetables (Arscott & Tanumihardjo, 2010).

In the last decade, carrot has been extensively studied by our research group, since it responds to different postharvest abiotic stresses by producing secondary metabolites such as phenolic compounds (Jacobo-Velázquez et al., 2011; Jacobo-Velázquez & Cisneros Zevallos, 2012; Becerra-Moreno et al., 2015). It has been reported that wounding is the most effective abiotic stress to increase the content of phenolic compounds in this crop (Jacobo-Velázquez et al., 2011, 2015; Becerra-Moreno, 2015). The procedure to induce the accumulation of phenolic compounds in carrots consist on shredding the tissue followed by storage for 48 h at 15-20 °C (Jacobo-Velázquez et al., 2011, 2015). This procedure induces increase of 300% and 3,000% in the content of total phenolic compounds and chlorogenic acid (CHA), respectively. Additionally, it has been observed that the concentration of other nutraceutical compounds present in carrot such as dietary insoluble fiber increases, while β-carotene are not affected by the application of such treatments (Santana-Gálvez et al., 2016).

Considered as a functional food and due to its high acceptance by consumers, the interest to incorporate carrot in different presentations (raw, cooked, pomace, powder) in food formulations has increased. This thesis is focused on the incorporation of carrot powder rich in nutraceuticals in sausage formulations to add phenolic compounds, carotenoids, and fiber. Therefore, the health benefits of these compounds are briefly described in the following sections.

2.3.1 Phenolic Compounds (PC)

Phenolics are ubiquitous plant components that are primarily derived from phenylalanine via the phenylpropanoid metabolism (Dixon and Paiva, 1995). In plants, they are involved in defense against ultraviolet radiation or physiological damage by pathogens (Denny & Buttriss, 2007). Phenolics can be classified in phenolic acids, flavonoid, stilbenes, and lignans.

In carrot, PC are present throughout the roots but are highly concentrated in the periderm tissue, like the peel (Mercier et al., 1994) and are mostly phenolic acids specifically, hydroxycinnamic acids and derivatives (Arscott & Tanumihardjo, 2010). The main hydroxycinnamic acid present in carrot is the 5-*O*-caffeoylquinic acid (5-*O*-CQA) also known as chlorogenic acid (82%) followed by dicaffeoylquinic acids (diCQAs, 17%), ferulic acid (FA, 1.2%), and in less amount isocoumarin (Heredia & Cisneros-Zevallos, 2009).

2.3.1.1 Chlorogenic acid (CHA)

CHA is one of the principal polyphenolics in human diet since it is widely spread among fruit and vegetables (Santana-Gálvez et al., 2017). It is the most representative of hydroxycinnamic acids and it is the main phenolic acid in carrots (42.2-61.8%) (Sharma *et al.,* 2012). Chemically, CHA is the esterification of caffeic acid, with (-)-quinic acid (Arscott & Tanumihardjo, 2010). Moreover, studies have found that chlorogenic acid can be absorbed and hydrolyzed by intestinal microbiota into various aromatic acid metabolites (Gonthier, et al., 2003). Bioactivity of chlorogenic acid relies in its antioxidant activity (Sato et al., 2010) and its many health promoting properties have been extensively studied.

For instance, *in vitro* and *in vivo* studies have supported the ability of chlorogenic acid to scavenge reactive oxygen species (Sato et al., 2010). Anti-inflammatory effects have also been reported by suppression of pro-inflammatory cytokines (IL-8, IL-6, IL-1β, and TNF-α) (Liang & Kitts, 2015). In addition, anticarcinogenic and antimutagenic properties have also been attributed to CHA (Jiang, et al., 2000; Noratto et al., 2009; Belkaid et al., 2006). Likewise, positive effects in obesity, dyslipidemia and diabetes have been associated with chlorogenic acid consumption (Meng et al., 2013; Thom, 2007). With these reports, it has been suggested that chlorogenic acid could prevent and treat chronic and degenerative diseases, mainly those assocated with the metabolic syndrome (Santana-Gálvez et al., 2017).

2.3.2 Carotenoids

Carotenoids are a group of more than 600 lipophilic pigments such as red, orange, and yellow found in the photosynthetic tissues of plants, algae, and microorganisms (Sharma *et al.,* 2012). Humans nor animals cannot synthesize them thus, they serve as the source for all animal carotenoids. Carotenoids are tetraterpenes with a conjugated system of double bonds with delocalized π-electrons (Boon *et al.,* 2010). In general, carotenoids in foods are classified into carotenes and xanthophylls. Carotenes include lycopene, α-carotene, and β-carotene; and xantophylls include lutein, zeaxanthin, violaxanthin, and neoxanthin. Those that predominate and are often quantified in human serum include lutein, zeaxanthin, β-cryptoxanthin, lycopene, α- and β-carotene (Arscott & Tanumihardjo, 2010).

In plants, carotenoids serve as accessory pigments in photosynthesis and photoprotection. This is due to the polyene structure of carotenoids, which allows the molecules to absorb light and to quench, or inactivate, singlet oxygen and free radicals. However, carotenoids are important micronutrients for human health (Castermiller and West 1998). **Table 2.1** shows α- and β-carotene content of selected fruit and vegetables consumed by Mexican population.

Table 2.1 Alfa and beta carotene content in fuits and vegetables commonly consumed by the Mexican population.

Food[a]	Values[b]	
	α-carotene [ppm]	β-carotene [ppm]
Broccoli	1	779
Carrots	4649	8836
Cilantro	72	3440
Corn[c]	33	30
Mango	17	445
Onion	6	391
Orange	16	51
Papaya	--	276
Red pepper	59	2379
Potatoe	---	6940
Tomato[d]	--	410
Spinach	--	5597

[a] References of raw vegetable concentrations excepted noted otherwise

[b] Data according to USDA Carotenoid Data Base for U.S. Food 1998.

[c] Canned

[d] Canned sauce

Carotenoids have biological functions, such as the regulation of gene expression or effect on cell functions like inhibition of monocyte adhesion and platelet activation. These biological effects have been attributed to the antioxidant property of carotenoids through deactivation of free radicals and singlet oxygen quenching (Arscott & Tanumihardjo, 2010). They have been linked with enhancement of immune system and decreased risk of degenerative diseases such as cancer, cardiovascular disease, age related macular degeneration and cataract formation. Numerous animal experiments have indicated that carotenoids inhibit carcinogenesis in mice and rats and may have anticarcinogenic effects in human (Silva et al., 2008; Fergunson et al., 2004)

Specifically, β-carotene have attracted considerable attention because of its possible protective effect against some types of cancer since β-carotene functions as a free radical-trapping agent and singlet oxygen quencher (Palafox-Carlos et al., 2010). β-carotene has the highest provitamin A activity, where one molecule of β-carotene can yield two retinol (vitamin A) molecules in the human body.

Vitamin A deficiency (VAD) is a major public health problem. VAD affects 163 million children and the highest prevalence of VAD is in parts of Africa (48%) and Southeast Asia (44%) (UN-SCN 2010). Vitamin A, helps to the maintenance of normal eye health, epithelial function, embryonic development, and immune system function (Arscott & Tanumihardjo, 2010). Cardiovascular protection has also been attributed to β-carotene. For instance, D'Odorico et al. (2000) have shown that the presence of α- and β-carotene in blood has a protective effect against atherosclerosis. Likewise, Nocolle et al. (2003) has demonstrated that high carotenoid diets are associated with a reduced risk of heart disease. In **Table 2.2** are shown some clinical studies in relation with carotenoids intake and different diseases.

In carrots, the main carotenoids present are β-carotene, α-carotene, and lutein (Sánchez et al, 2014). The total carotenoids content in the edible portion of carrot roots range from 6,000 to 54,800 µg/100 g. The predominant carotenoids are the provitamin A carotenes, that is, α- and β-carotene, accounting for 13% to 40% and 45% to 80% of the carotenoids in orange carrots, respectively (Simon, 1989). Total root carotenoid content can vary significantly between cultivars (Simon & Wolff 1987; Nicolle et al., 2004; Grassmann et al., 2007) and is the major source of variation in reported carrot carotenoid concentrations. Additionally, the growing season, soil, maturity, and genetic factors also influence carotenoid content of carrots.

In addition to α- and β-carotene, orange carrots have detectable levels of lutein. Lutein has no provitamin A activity, but is localized in the macular region of the eye in humans and may have importance in eye health and protection from age-related macular degeneration (Tanumihardjo & Yang, 2005).

2.3.3 Dietary Fiber (DF)

Dietary fiber is a substance of vegetable origin composed of carbohydrates or derivatives, except lignin, which resist the hydrolysis catalyzed by human digestive enzymes and arrive intact to the colon where it can be hydrolyzed and fermented by the microbiome (Elleuch et al., 2011). The most accepted classification for dietary fiber is to differentiate dietary components according to their solubility. Thus, fiber is classified into two categories: insoluble or partially fermentable fiber (cellulose, hemicellulose, lignin) and soluble or fermentable fiber (pectin, gums and mucilages) (Matos & Chambilla, 2010). Lineback (1999) has reported that carrot cell wall is composed of pectin, cellulose, lignin, and hemi-cellulose. Nawirska and Kwasniewska (2005) have reported the composition of dietary fiber constituents in the fresh carrot on dry weight basis as pectin (7.41%), hemi-cellulose (9.14%), cellulose (80.94%) and lignin (2.48%).

Table 2.2 Clinical studies demonstrating the health benefits of carotenoids.

Carotenoid source	Disease	Subject	Study details	Experimental findings	References
Common diet	SCD	1031 Finnish men aged 45-65 of the KIHD cohort	Follow-up of 15.9 years. Serum concentrations of carotenoids were analyzed among other biomarkers	Serum β-carotene concentrations may increase the risk of SCD in middle-aged Finnish men (p=0.044). Furthermore, low serum b-carotene concentrations may be related to the risk of CVD and total mortality.	Karppi et al., 2012
9-cis β-carotene-rich powder of the alga *Dunaliella bardawil* in combination with fibrates	Hypercolesterolemia	First trial: 20 fibrate-treated men with plasma HDL-chloresterol levels below 40mg/dl Second trial: 22 fibrate-treated patients	Open-labeled first trial. Capsules provided 60mg β-carotene/ day for 6 weeks. Double blind placebo controlled second trial for 6 weeks. Control capsules had β-carotene-deficient alga.	Plasma HDL-cholesterol increased by 24.5 and 12.7% in the first and second trials, respectively (p=0.002 and 0.012)	Shaish et al., 2006
Common diet	Atherosclerosis	392 men and women aged 45-65 years	Follow up period of 5 years. Carotid and femoral artery atherosclerosis was assessed by high-resolution duplex ultrasound.	α- and β-carotene plasma levels were inversely associated with the prevalence of atherosclerosis in the carotid and femoral arteries (p=0.004)	D'Odorico et al., 2000

SCD: Sudden cardiac death; KIHD: Kuopio Ischemic Heart Disease Risk Factor

Table 2.2 (Continue) Clinical studies demonstrating the health benefits of carotenoids.

Carotenoid source	Disease	Subject	Study details	Experimental findings	References
B- carotene source: Dunaliella salina	Erythema formation	36 healthy adults of skin type II between 22 and 55 years old (12 men, 24 women)	Placebo controlled parallel study. Comparison between intake of 24 mg/d β-carotene from algal source vs a mix of three carotenoids 24 mg/d (b-carotene, lutein, and lycopene; 8mg/d each) and a control group with a placebo for 12 weeks	Long-term supplementation for 12 weeks with 24 mg/d of a carotenoid mix supplying similar amounts of β-carotene, lutein, and lycopene ameliorates UV-induced erythema in humans; the effect was comparable to daily treatment with 24 mg of B-carotene alone.	Heinrich et al., 2003
Common diet	NPC	198 histologically confirmed NPC cases of Caucasian ethnicity of 18-76 years old	Hospital-based case control study between 1992 and 2008. Nutrient supplementation was not assessed.	Study findings suggest a protective effect of carotenoids against NPC in a low-risk population, adding further support to a possible beneficial role of a diet rich in fruits and vegetables in cancers of the head and neck.	Polesel et al., 2012
Common diet	Breast cancer	36,664 Swedish women	Population-based cohort study. Follow up of 9.4 years	Dietary α-carotene and β-carotene are inversely associated with the risk of breast cancer among smokers and among women who do not use dietary supplements.	Larsson, Bergkvist and Wolk (2010)

SCD: Sudden cardiac death; KIHD: Kuopio Ischemic Heart Disease Risk Factor; NPC: nasopharyngeal carcinoma

There is considerable epidemiological evidence suggesting that a diet high in fiber reduces the risk of cardiovascular disease, type II diabetes and cancer. For instance, Ning et al. (2014) examined the data generated during five years of 11,113 participants with no history of CVD between the ages of 20 and 79, and concluded that the higher the dietary fiber intake, the lower the risk of CVD. Likewise, Xu et al. (2014) reported an inverse relationship between DF intake and C-reactive protein concentration. Regarding diabetes, Yao et al. (2014) evaluated the data generated in observational studies from 1974 to 2013 with a total of 488,293 participants in which they concluded that the intake of total DF, insoluble DF, fruit fiber and cereal reduces the risk of type II diabetes. The mechanism of action seems to be related to the reduction of the glycemic index of foods. Finally, the mechanism of action of DF on cancer prevention may be associated with increased fecal matter and accelerated intestinal transit, thus decreasing the time of exposure of large intestinal epithelial cells to carcinogenic agents. Furthermore, fermentable DF induces the synthesis of short-chain fatty acids (SCFAs), also referred to as volatile fatty acids (VFAs), such as butyrate that promotes cell differentiation and apoptosis of mutant colonocytes (Ben et al., 2015).

2.4 Carrot as a functional food ingredient

Inverse associations between the consumption of β-carotene and risk of cancer and cardiovascular diseases reported in section 2.3 as well as, total dietary fiber, and phytochemical content (pro-vitamin A carotenoids and phenolic compounds) in carrots, have attracted the attention of scientific community to generate new approaches in order to take advantage of such crop. The idea to transform carrot into a functional food ingredient has been around since a carotenoid-based antioxidant was developed by Bombardelli and Morazzoni (1996) for functional purposes. Since then, several authors have added carrot in different presentations such as fresh/ raw, pomace, and juice into different food products in order to reach the recommended intake values of pro-vitamin A carotenoids, as an additive for its antioxidant properties, or as a functional ingredient for its phytochemical content (Bhosale et al., 2010; Bombardelli & Morazzoni, 1996).

For instance, Stoll et al. (2003) enzymatically extracted total carotene content from carrot pomace and incorporated it to prepare a functional drink. Similarly, Shanmugam & Ashokkumar (2015), generated a carrot juice emulsions beverage with flaxseed oils. Likewise, Mestry et al. (2011) developed a fermented mixed juice of carrot and watermelon. On the other hand, a few attempts have been carried out for utilizing carrot pomace in foods such as bread (Kumar & Kumar, 2012; Kohajdová et al., 2012), cookies and biscuits (Turksoy & Özkaya, 2011; Lumari & Grewal,

2007), chicken nuggets (Bhosale et al., 2010) and pasta (Day et al., 2009). All these previous studies have reported the detection of β-carotene content and the increase of dietary fiber in foods added with carrot pomace.

However, the addition of carrot in to food products, can modify sensory and organoleptic properties of these products, therefore there are new technologies and strategies for its incorporation to minimize sensory changes. Technologies like spray drying and microencapsulation (Maestry, Mujumdar & Thorat, 2011), milling processing (Ferreira et al., 2015), emulsions (Shanmugam & Ashokkumar, 2015), convection drying (Turksoy & Özkaya, 2011), and freeze drying (Pandey et al., 2013) have been recently studied. Particularly, the addition of carrot powder in different concentrations (0.5% to 30%) have showed positive results in the development of new products. Unfortunately, some of these processes required high temperatures and the effect of heating can cause degradation of some bioactive compounds (Chantaro et al., 2008). As a consequence of the latter, the scientific community has now focused its attention on the development of technologies that increase the levels of these compounds in fruits and vegetables (Rommens et al., 2008). Next section will aboard strategies to increase nutraceutical compounds in carrots.

2.4.1 Postharvest abiotic stresses as a strategy to increase nutraceutical compounds

Plant secondary metabolites are often referred to as compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense. In higher plants a wide variety of secondary metabolites are synthesized from primary metabolites (Ramakrishna & Ravishankar, 2011). They are needed in plant defense against herbivores and pathogens. Often, they may confer protection against environmental stresses. Due to their biological activity, secondary metabolites are used commercially as insecticides, fungicides, pharmaceuticals, fragrances, flavorings, medicinal drugs, and industrial materials (Cisneros-Zevallos, 2003).

Besides industrial application, the enormous positive impact of consumptions of nutraceutical compounds on human health, has interested the scientific community in the development of strategies to increase their content in fruits and vegetables. The traditional way of doing this is through genetic engineering. However, this strategy is technically complex and the extensive cultivation of transgenic plants is very limited and questioned because they could generate an ecological imbalance. As an alternative to genetic engineering, it has been proven that the application of post-harvest abiotic stresses in fruits and vegetables (i.e. wounding stress, modified atmospheres, exposure to UV light, exogenous phytohormones, etc.) induces

accumulation of nutraceuticals in a practical and effective way (Jacobo-Velázquez et al., 2015; Jacobo-Velázquez & Cisneros-Zevallos, 2009; Cisneros-Zevallos, 2003).

Wounding stress has been proven to be one of the most effective postharvest abiotic stresses for the activation of the phenylpronoid metabolic pathway in plants (Jacobo-Veláquez et al., 2012). Phenolic compounds in wounded plants are produced in part as a mechanism to support the biosynthesis of lignin, which acts as a barrier to prevent water loss (Becerra-Moreno et al., 2015). Fruits and vegetables as living tissues, show physiological response and changes involved at the transcriptome and cellular levels. This stress response seems to be the result of the activation of the phenylpropanoid metabolism together with those metabolic pathways involved in the supplementation of carbons skeletons needed for phenolics biosynthesis such as the glycolysis, the oxidative pentose phosphate pathway (OPPP), and the shikimate pathway, but global response is still not well understood. Recently, it was suggested that ATP may trigger wound-response in plants, since it is liberated to the tissue matrix. This would cause the initiation of a signal transduction network leading to the activation or *de novo* synthesis of transcription factors that regulate gene expression involved in the secondary metabolism of a plant (Cisneros-Zevallos *et al*, 2014). Data also suggests that upon the application of wounding stress, reactive oxygen species play the major role on the activation of the primary and secondary metabolism as well as on the accumulation of secondary metabolites (Jacobo-Vélazquez et al., 2015).

Carrot has been used as a model system to study the effect of wounding stress on the phenylpropanoid metabolism. Phenolic compounds that accumulate in this vegetable due to wounding stress are mainly hydroxycinnamic acids, being chlorogenic acid the hydroxycinnamic acid that accumulates the most (Jacobo-Velázquez & Cisneros-Zevallos, 2012). This distinctive characteristic in carrots can be used as a new approach to increase the content of phenolic compounds. With this technology, the degradation of nutraceutical compounds by processes related to the transformation of carrots into a powder will be overcome, by still producing a carrot powder with considerable amounts of phytochemicals. The technologies to generate a carrot powder rich in nutraceuticals compounds was previously characterized by our research group (Santana-Galvéz et al., 2016), and its addition to highly-consumed food products such as sausages, make this approach feasible to improve consumption and distribution of nutraceutical compounds among the Mexican population.

2.5 Sausages enriched with functional ingredients

Meat has a great potential for delivering important nutrients like quality proteins and some essential fat-soluble vitamins and minerals with high bioavailability as compared to other food products (Chan, 2004; Biesalski, 2005). Further processing of meat had led to the generation of many compounds beneficial to human health (Saiga *et al.,* 2003) and understanding the relationship between nutrition and health has resulted in the development of the concept of functional foods even in meat products (Bhat & Bhat 2011).

Among processed meat products, sausages are widely consumed by Mexican children and adults due to the simplicity and versatility usage in different dishes. As previously discuss in section 2.2, the demand for these products is increasing globally. Hence, in an effort to develop new products with greater nutritional values, and to raise the consumption of nutraceuticals among the populations, recent investigations are being focus on the addition of vegetable or fruit sources for nutritional enhancement in sausages.

Table 2.3 shows some of the nutritional and nutraceutical enhancements that have been made in sausages. Unfortunately, many of the studies do not focus in the enhancement of nutraceutical content and only fewer researchers have identified, quantified and characterized nutraceuticals compounds such as phenolics or carotenoids in sausages (Calvo, García & Selgas, 2008; Fernández-López, et al., 2006; Ribas-Agustí et al., 2013). For instance, Fernández-López, *et al.,* (2006), added orange fiber from juice industry by-products and evaluated polyphenol composition. The most concentrated phenolic compound was herperidin with a concentration of 0.062 mg/g in a formulation of 20 g/kg of orange fiber in dry-cured sausage. While, Ribas-Agustí *et al.,* (2013) added cocoa extracts and grape seeds extracts to dry fermented sausages in which they found flavan-3-ols, procyanidins, glycosides of quercetin and galloylated derivatives. However, the concentrations of those nutraceuticals compounds added were still very low to provide a nutraceutical benefit.

Most of the studies have focused on the addition of fiber from vegetable sources from a technical point of view more than a nutritional view. It is known that fibers act through their solubility, viscosity, gel forming ability, water-binding capacity, oil adsorption capacity, and fermentability (Biswas et al., 2010). All the previous characteristics could help to improve physicochemical parameters such as texture, cooking loss and purge loss (Mehta et al., 2013). However, even with the technical benefits provide by the addition of fiber, sensory acceptability is still a restriction in the concentration levels of these ingredients. As it can be appreciated, most studies done with sausages used concentrations lower than 3.0% (w/w).

Table 2.3. Quality attributes of sausages added with different nutrients or nutraceuticals.

Type of sausage	Nutrient/ nutraceutical incorporated	Concentration of ingredient	Experimental findings	References
Pork cooked sausage	Carrot fiber and potato starch	Fiber: 0.5%, and 1.5% (w/w) Starch: 2.0% and 3.8% (w/w)	Salt could be reduced from pork sausages by the addition of these ingredients. No negative effects on WBC, color, and texture were found Sensory assessment resulted in sausages containing potato starch with better sensory quality than fiber-rich sausages	Grossi et al., 2012
Bologna sausage	Citrus Fiber	From 0.5% to 2% (w/w)	Fiber content was 217% higher in 2% citrus fiber. Delayed in oxidation process and a decreased residual nitrite levels was found. Sausages were harder, chewy and less springy. Sausage color were more red than conventional bologna sausage. However, sausages were not sensory accepted.	Fernández-Ginés et al., 2003
Raw and cooked pork sausages	Lutein/ sesamol, ellagic acid/ olive leaf extract	200/ 250/ 300/ 200 µg/ g meat	Ingredients reduced lipid oxidation, except lutein. Their incorporation did not have detrimental effect on pH, cooking loss, tenderness, juiciness, texture or flavor.	Hayes et al., 2010
Dry fermented sausages	Cocoa and grape seed extracts	0.5% (w/w)	Overall stability of the phenolic compound found (epicatechin, catechin, gallic acid, galloylated flavan-3-ols) was not affected. Sensory acceptability was also not affected.	Ribas-Agustí et al., 2013
Bologna sausages	Orange dietary fiber (ODF) and oregano essential oil (OEO)	ODF: 1.0% (w/w) OED: 0.02% (w/w)	Addition of ODF and OEO together showed lower lipid oxidation values and the lowest aerobic and lactic acid bacteria counts. Sensory evaluation values were similar for both samples.	Viuda-Martos et al., 2010

Abbreviations: WBC= water binding capacity.

Table 2.3 (Continued) Quality attributes of sausages added with different nutrients or nutraceuticals.

Type of sausage	Nutrient/ nutraceutical incorporated	Concentration of ingredient	Experimental findings	References
Pork sausages	Carrot fiber (78% insoluble, 14% soluble)	2.0% (w/w)	Carrot dietary fiber increased emulsion strength resulting in firm sausages. Color changes increase L* values and decrease a*. Sensory evaluation was acceptable.	Grossi, et al., 2011
Low-fat pork sausages	Tomato peel powder	10% (w/w)	Tomato peel reduce hardness in sausages as well as springiness. During storage, PUFAS were stable. L* and a* values increased with the addition of tomato peel powder.	Wang et al., 2016
Low-fat fermented pork sausage	Soy Fiber	1.0% (w/w) and 2.0% (w/w)	Volatile compounds from lipid oxidation were reduce. However, 2.0% sausage was retracted from the consumer study, since it caused texture depreciation. Fat was reduced by approximately 40%.	Bastianello et al., 2012
Low-fat pork Lyon-style and	Inulin and citrus fiber	1.0% to 5.6% (w/w)	Addition of inulin and citrus fiber led to sensory characteristics similar to the full-fat reference. Color, intensity, spiciness and firmness increased. Consumer test revealed acceptable values for low-fat and fiber enriched sausage	Tomaschunas, et al., 2013
Dry Fermented sausages	Vegetables powders: celery, celery juice, parsnip and leek	17.5 kg/ 100kg	Addition of vegetables powders showed no negative effect on the fermentation and ripening process. Color or celery powder was the most stable. Celery juice powder, show lower levels of hardness	Eisinaite, et al., 2016

Abbreviations: PUFAS= polyunsaturated fatty acids

Table 2.3. (Continued) Quality attributes of sausages added with different nutrients or nutraceuticals.

Type of sausage	Nutrient/ nutraceutical incorporated	Concentration of ingredient	Experimental findings	References
Cured sausages	Caffeic acid + carnosic acid and quercetin + rutin	0.05g/ 100g meat and 0.08 g/ 100g meat, respectively	All the phenolic compound mixtures were able to maintain oxidative stability in sausages. No sensory differences between samples was found. Samples containing caffeic and carnosic acid presented harder textures.	Capitani et al., 2012
Frankfurters sausages	Chia flour	10.0% (w/w)	Increased in TDF (98% insoluble) and ash content (K, Mg, Ca, Mn). Fat and energy were reduced a 26%. Reduce purge loss in all samples. Color was affected as well as texture.	Pintado, et al., 2015
Low-fat pork sausages	Tomato powder	0.8%, 1.2% and 1.5% (w/w)	Lightness (L*) increased as the concentration of tomato increased, as well as WHC. As for pH, values were lower than the control. Cohesiveness and springiness parameters were also lower. Lycopene content was not measure.	Kim et al., 2011
Low-fat beef sausages with pork back fat	Pineapple dietary fibers (PDF)	1.0% (w/w)	Different content of moisture, protein, fat, ashes and TDF was found in samples with PDF. Cooking loss and an increase of purge loss were also found	Henning, Tshalibe, & Hoffman, 2016
Semi-dried sausages	Kimchi powder	1.0% and 3.0% (w/w)	pH decreased as the concentration of kimchi increased. Color was affected (L* decrease, a* and b* increased). Overall sensory properties were improved. Softness and tenderness was also increased.	Lee et al., 2009

Abbreviations: TDF= total dietary fiber; WBC= water binding capacity.

Table 2.3. (Continued) Quality attributes of sausages added with different nutrients or nutraceuticals.

Type of sausage	Nutrient/ nutraceutical incorporated	Concentration of ingredient	Experimental findings	References
Dry fermented sausages	Tomato peel	0.6%, 0.9% and 1.2% (w/w)	Approximately 0.26-0.58 mg of lycopene / 100g of sausages remained at the end of 21 days. Sensory and textural properties and overall acceptability of all formulations were good	Calvo, García & Selgas, 2008
Cooked sausages inoculated with LAB	Cactus pear flour	3% (w/w)	Cactus flour increased moisture in sausages. In inoculated sausages, a harder structure but less cohesive and resilient was reported. Rancidity was also decrease during storage	Díaz-Vela, Totosaus & Pérez-Chabela, (2015)
Chicken sausages	Corn bran, dried Apple pomace and dried tomato pomace	Each at 3.0%, 6.0% and 9.0%	Organoleptic acceptability of 3% were the closest to control. Moisture content decreased significantly. Ash content increased while emulsion stability and cooking yield was significantly higher in 6.0% and 9.0% formulations	Yadav et al., 2016
Reduced-fat turkey frankfurters	Modified corn starch	2.4% to 6.6%	Low levels of starch showed more yellow, firmer and no cohesive as those with higher levels. For optimal sensory and physical attributes, it was suggested to add 2.4% of modified starch	Beggs, Bowers & Brown, 1997
Dry cured sausages	Orange fiber	5.0, 10.0, 15.0 and 20 g/kg	Decrement in the radio of lipid oxidation was showed in orange fiber rich sausages. Hesperidin, a phenolic compound from orange was found in concentrations of .062 mg/g in samples of greater concentration of orange fiber	Fernández-López, et al., 2006

Abbreviations: LAB= lactic acid bacteria

For instance, Tomaschunas *et al.* (2013), analyzed consumer's acceptance of reduce fat pork Lyon-style and liver sausages containing inulin (0.2% to 2%) and citrus fiber (0.2% to 3%) in combination. Results revealed high acceptability in formulations with fibers amounts of 1%. Moreover, Calvo, García & Selgas (2008), described the development of a dry fermented sausage with addition of dry tomato peel. The overall acceptability of all formulations (0.6%, 0.9%, and 1.2%) were satisfactory. However, Eim *et al.* (2008), studied four formulations of dry fermented sausage (*sobrassada*) containing carrot dietary fiber in 3.0%, 6.0%, 9.0%, and 12.0%, in which formulation of 9.0% and 12.0% were not sensory acceptable. These results denoted that a low concentration of vegetable fibers is generally preferred in meat products.

2.5.1 Meat quality attributes

Addition of new ingredients even in low concentrations, can cause changes in quality attributes. Alterations in sensory quality properties such as color and texture, influences consumer acceptance of meat products, and consequently, these attributes are the most reported and studied during the development of a new functional meat product (Biswas et al., 2011). The three sensory properties that consumers judge are appearance, texture and flavor. The consumer must need first to be entire satisfied with the sensory properties of the product, before other quality dimension become relevant. Once appearance have been satisfied, flavor becomes a critical determinant of quality. Therefore, the characterization of these attributes as well as their acceptability is important in the success of functional meat products.

Specifically, color is known to play an important role in the acceptability of meat. The surface color of meat depends on the quantity of myoglobin and hemoglobin present, on its chemical state and on the chemical and physical conditions of other components in the meat. Meat showing a bright red color is assumed to be fresh, while oxidation of heme-iron to form metmyoglobin produces the brown color which consumers associated with spoilage (Mastromatteo, et al., 2011).

Also, textural parameters of food are important aspects of consumer acceptance. It is a group of properties resulting from natural structure of the food elements, their mutual arrangement and interactions. Meat texture is determined by cohesion and the strength between the myofibrillar, conjunctive and cytoskeletal structures, which is influenced by biological and technological variables such, age and species (Cañeque &, Sañudo, 2005). Meat texture is highly correlated with water absorption and swelling indexes of proteins (WBC), which affects hardness and tenderness, a sensory attribute (Cierach & Majewska, 1997).

Many instrumental methods have been developed for food textural property determination (Biswas et al., 2011). Nowadays, the most commonly used instrumental method is probably, the compression method of texture profile analysis (TPA). This method mimics the conditions to which the material is subjected throughout the mastication process (Bourne, 1978). **Table 2.4** shows, the parameter measure by TPA and its relationship with sensory characteristics. Among texture attributes, hardness is the most important factor to the consumer, as it decides the commercial value of a meat.

Table 2.4 Parameters measure by Texture Profile Analysis and relationship with sensory characteristics.

Parameter	Sensory Definition	Instrumental Definition	Units
Hardness	Force required to compress a sample between molars	Maximum force required to compress the sample during first cycle	Kg, g, Newton
Springiness	Measures how much of the original structure is broken by the initial compression	The distance of the detected height during the second compression divided by the original compression distance.	mm
Cohesiveness	Force required to break intern matrix bonds of a piece of food	How well the product withstands a second deformation relative to its resistance under the first deformation. (A2/A1, where A1 is the total energy required for the first compression and A2 is the total energy required for the second compression)	Adimensional
Chewiness	Energy required to break up a solid food until it is ready to be swallow	The work required to masticate the sample for swallowing (Hardness x Cohesiveness x Springiness)	g*mm

Source: Bourne, 1978

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and plant material

Acetone (HPLC grade), ethanol, hydrochloric acid (HCl) and ortophosphoric acid were purchased from Desarrollo de Especialidades Químicas (San Nicolás de los Garza, NL., México). Butylated hydroxytoluene (BHT), *tert*-butyl methyl ether (*t*-BME, HPLC grade), methanol (HPLC grade), butylated hydroxytoluene (BHT), β-carotene and chlorogenic acid (CHA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Chlorine (Cloralex, 6% sodium hypochlorite), and fresh carrots (*Daucus carota*) were purchased from a local supermarket (HEB, Monterrey, México).

3.2 Functional carrot powder (FCP) production

FCP was produced in a pilot plant as previously described by Santana-Gálvez et al. (2016). Fresh carrots with no visible signs of fungi or significant damage were selected and washed with tap water, disinfected with a chlorine solution (200 ppm, pH 6.5-7.0) for 5 min and dried with paper towel. Thereafter, both ends of carrots were cut with a knife and disposal. The remaining was shredded using a food processor (Waring Commercial, WFP11, Torrington, CT, USA). Afterwards, shredded carrots were placed in 19-L plastic containers (3 kg per container) with 4 holes (0.8 cm diameter) on the lids to prevent CO_2 accumulation. Then, the containers with shredded carrots were placed in a Symphony incubator (VWR, Radnor, PA, USA) for 48 h at 15 °C. After storage, shredded carrots were spread in vented trays and placed in a food dehydrator (Edel Ingenieros, MMWD, Monterrey, NL, México) at 60 °C for 30 h to reach a final moisture content of 10%. To obtain FCP, dehydrated carrots were grounded in a knife mill (Wiley Mill, Swedesboro, NJ), passed through a 2 mm sieve, then a 1 mm sieve, and finally a No. 40 sieve. Two types of carrot powders (CPs) were obtained: a control carrot powder (CCP) produced from carrots that were shredded but not stored, and a functional carrot powder (FCP) obtained from shredded carrots stored for 48 h at 15 °C to allow the accumulation of phenolic compounds.

3.3 Water absorption index (WAI) and oil absorption index (OAI) determination of carrot powders (CPs)

The water absorption index (WAI) of carrot powders was determined based on the method described by Serna-Saldívar (2012) with slight modifications. Samples of 0.5 g were placed on 15 mL centrifuge tubes and 7.5 mL of distilled water were added to them. Samples were vortexed for 30 s and incubated at room temperature (21 °C) for 30 min with manual stirring every 5 min. The tubes were centrifuged at 10,000 x *g* and 21 °C for 10 min. The supernatant was discarded and the pellet was weighed. WAI was calculated as grams of wet pellet per gram of dry sample.

The oil absorption index (OAI) of carrot powders was determined following the procedure described by Rodríguez-Miranda et al. (2011) with slight modifications. Nine grams of corn oil (Mazola®) were added to 1 g of carrot powder in 50 mL centrifuge tubes. Samples were vortexed for 30 s and incubated at room temperature (21 °C) for 30 min with manual stirring every 5 min. The tubes were centrifuged at 5,000 x *g* and 21 °C for 10 min. The supernatant was discarded and the pellet was weighed. OAI was expressed as grams of oily pellet per gram of dry sample.

3.4 Preparation of sausages added with 4% (w/w) carrot powder (CP)

Additional experiments were done to determine the optimum concentration of FCP that minimized the effect on the physicochemical properties and consumer's acceptability of sausages (See Supplementary Data, Appendix A). Optimum FCP concentration was determined to be 4% w/w. Sausage preparation was performed in the pilot plant of Sigma Alimentos (Apodaca, N.L., México) using the formulations shown in **Table 3.1.**

To obtain sausages, the ingredients were mixed properly in a cutter for 5 min at high speed and then the meat batter was stuffed into a 26 mm cellulose casings. Samples were hand linked and heat processed at 80 °C for 25 min, and then at 85 °C until inner temperature of the product was 78 °C. Samples were cooled at 8 °C and separated from their casings by hand. Finally, sausages were vacuum packed (packs of 6 sausages of ~62.5 g each) and stored at 4 °C. A shelf-life study was performed, where sausages were stored for 42 days at 4 °C. Color, texture, pH, purge, and phytochemical content (phenolic compounds and carotenoids) in sausages were evaluated at the beginning of the shelf-life study and every 7 d during 42 d. Likewise, a proximate analysis, dietary fiber determinations and a sensory acceptability test were performed in the samples before storage.

Table 3.1. Sausage formulations containing 4% (w/w) of CCP or FCP.

Ingredients	Percentage in each formulation (w/w)		
	Control[a]	4% CCP	4% FCP
Turkey meat	68.700	68.700	68.700
Water	21.700	21.200	21.200
NaCl	1.867	1.867	1.867
SFM	0.350	0.350	0.350
Sodium nitrite	0.016	0.016	0.016
Sodium erythorbate	0.050	0.050	0.050
Sodium phosphate	0.410	0.410	0.410
Carmine (E120)	0.007	0.007	0.007
Soy protein concentrate	3.000	3.000	3.000
Modified starch	1.500	---	---
Corn starch	2.000	---	---
CCP	---	4.000	---
FCP	---	---	4.000
Soy fiber	0.400	0.400	0.400

[a] Control= 0% of CP. Abbreviations: CP = carrot powder; CCP = control carrot powder; FCP = functional carrot powder; SFM = standard flavor mixture containing species, NaCl, starch, flavor and hydrolyzed vegetable protein.

3.5 Proximate and dietary fiber (DF) analyses

Proximate and dietary fiber (DF) analyses of sausages were performed with the following methods: moisture content (AACC 44.15.02), total fat (Soxhlet method), total protein (Kjeldahl method), ash (dry ashing method), total carbohydrates (by difference), total dietary fiber (TDF) (AOAC 985.29), insoluble dietary fiber (IDF) (AOAC 991.42) and soluble dietary fiber (SDF) (AOAC 993.19). Total calories (kcal) were calculated as 4 calories per g of carbohydrates, 9 calories per g of fat, and 4 calories per g of protein.

3.6 pH, purge, color, and texture profile analyses

The pH values were measured using a H30PCO sympHony pH-meter (VWR, United States) at room temperature on homogenates of sample with distilled water (ratio of 1:10 w/v). Purge values were calculated as the weight difference of the sausage package before and after cleaning the exudates with paper towels, divided by the initial weight of the packages, and multiplied by 100. Colorimetric analysis [CIE-LAB, lightness, L*; redness, a* and yellowness, b*, Chroma (C*) and Hue Angle (H°)] was performed on the external surface of sausages using a

Chroma Meter CR-400 (Konica Minolta Business Technologies, Tokyo, Japan). Textural properties were analysed by texture profile analysis (TPA) performed in a TA-XT.plus Texture Analyser (Texture Technologies Corp., Scarsdale, NY, USA). Sausages sections of 20 mm high were axially compressed twice to 70% of their original height. The cross-head speed was 5 mm/s. The following parameters were determined: hardness (g), the maximum force required to compress the sample; springiness (mm), the ability of the sample to recover its original form after the deforming force was removed; cohesiveness, the extent to which the sample could be deformed prior to rupture (A2/A1, where A1 is the total energy required for the first compression and A2 is the total energy required for the second compression); chewiness (g*mm), the work required to masticate the sample for swallowing.

3.7 Phytochemical analyses

3.7.1 Phenolic compounds

Phenolic quantification of carrot powder and sausages were determined with the procedure described by Santana-Gálvez et al. (2016). Samples (0.5 g of carrot powder or 5.0 g of sausage) were homogenized in 20 mL of methanol using a tissuemizer (Advance homogenizing system, VWR). Thereafter, the homogenates were centrifuged (12,000 *xg*, 4 °C, 15 min) and the supernatants were recovered as methanol extracts, which were filtered through a nylon syringe filter (0.45 µm, VWR), prior to injection.

3.7.1.1 Identification and quantification of phenolic compounds

The identification and quantification of phenolic compounds in methanol extracts were performed by high performance liquid chromatography system coupled to diode array detection (HPLC-DAD) (Agilent Technologies, 1260 Infinity, Santa Clara, CA, USA). Methanol extracts (10 µl) were injected into the system. Compounds were separated on a 250 × 4.6 mm, 5 µm particle size, C18 reverse phase column (Luna, Phenomenex, Torrance, CA, USA). Vial chamber and column temperatures were 4 and 25 °C, respectively. Mobile phases consisted of water (phase A) and methanol:water (60:40 v/v, phase B), both adjusted to pH 2.4 with orthophosphoric acid. Gradient solvent system used was 0/100, 3/70, 8/50, 35/30, 40/20, 45/0, 50/0, and 60/100 (min/% phase A) at a constant flow rate of 0.8 mL per min. Chromatographic data were processed with OpenLAB CDS ChemStation software (Agilent Technologies). Chlorogenic acid was detected at 320 nm and identified by comparing its retention times and absorption spectra as compared with an authentic chemical standard. To obtain the concentration of CHA in mg per kg of sausages, a

standard curve (5-250 ppm) of the compound was prepared. Similarly, the concentration of total phenolics was determined as the sum of all individual phenolic compounds identified in the methanol extracts by comparing their retention time and spectra characteristics with previous reports (Becerra-Moreno et al., 2012, 2015; Santana-Gálvez et al., 2016).

3.7.2 Carotenoids

Carotenoid profiles of carrot powder and sausages were determined as indicated by Santana-Gálvez et al. (2016) with some modifications. Extractions were done under dark conditions and at room temperature. Samples (0.1 g of carrot powder or 1.0 g of sausage) were homogenized 4 consecutive times with 20 mL of acetone added with 0.1% (w/v) BHT. Homogenates were filtered under vacuum through Whatman No. 1 filter paper. Filtrates were collected and transferred to a 100-mL volumetric flask. Volume was completed to 100 mL using the acetone-BHT solution. Prior to injection to the chromatographic system, acetone extracts were filtered with a polytetrafluoroethylene syringe filter (0.45 µm, Millipore, Billerica, MA, USA) and placed in amber glass vials with nitrogen in the headspaces to prevent oxidation of carotenoids.

3.7.2.1 Identification and quantification of carotenoids

The identification and quantification of carotenoids was performed in the same chromatographic system utilized for phenolic analyses. Acetone extracts (25 µL) were injected to the HPLC-DAD system, and carotenoids were separated on a C30 reverse phase column (4.6 × 150 mm, 3 µm particle size) (YMC, Wilmington, NC, USA), coupled to a corresponding C30 guard cartridge. Vial chamber and column temperatures were 4 °C and 25 °C, respectively. The mobile phase consisted of 50% methanol, 45% *t*-BME, and 5% water. The system was isocratic, where total elution time was 30 min at a flow rate of 0.5 mL per min. Carotenoids were detected at 450 nm, and identified by comparing their retention times and absorption spectra with reference standards and a previous report (Santana-Gálvez et al., 2016). β-Carotene was quantified using a calibration curve (5-250 ppm) of a reference standard, while the rest of carotenoids (α-carotene and lutein) were quantified as β-carotene equivalents. Retinol equivalents (RE) were calculated as 1 µg of retinol= 6 µg β-carotene and 12 µg α-carotene.

3.8 Sensory acceptability test of sausages added with 4% carrot powder (CP)

Sensory evaluation tests were performed to compare the acceptability of sausages added with 4% CCP or FCP and a control (0% CP). To ensure microbiological stability and safety of

sausages prior to sensory evaluations, sausage CPC and FPC as well as the control were assayed for microbiological growth (See Supplementary Data, Appendix B). The consumer panel was composed of 98 students and workers from Tecnológico de Monterrey – Campus Monterrey that consumed sausages at least once a week. The mean age of the consumers was 23 years old. Sausage portions of 2.0 cm height of each sample were placed in plastic containers. Each formulation was assigned a different random 3-digit number. Water and crackers (with neutral flavor) were available for consumers to clean the palate between samples. Consumers were asked to evaluate appearance, flavor, texture, and overall acceptability of sausages using the following 9-point hedonic scale:1- “Dislike extremely”, 2 - “Dislike very much”, 3 - “Dislike moderately”, 4 - “Dislike slightly”, 5 - “Neither like nor dislike”, 6 - “Like slightly” 7 - “Like moderately”, 8 - “Like very much”, and 9 - “Like extremely”. The samples were presented at random to the consumers. Consumers that evaluated the overall acceptability of the control sample with a 6 or higher value were considered to truly like sausages; therefore, only those surveys were considered for data analysis.

3.9 Statistical analyses

Results were expressed as mean values ± standard error of the mean. Each experiment was done at least with 3 replicates unless otherwise indicated. Data were analyzed with full factorial ANOVA to evaluate significant effects and interactions, followed by LSD test to determine significant differences among groups ($p < 0.05$), using JMP software version 9.0 (SAS Institute Inc., Cary, NC, USA).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Phytochemical characterization and functional properties of carrot powder (CP)

4.1.1 Phytochemical content

Two types of CPs were obtained: a control carrot powder (CCP) and a functional carrot powder (FCP). CCP was obtained from carrots that were shredded and immediately dehydrated; whereas FCP was obtained from shredded carrots that were stored for 48 h at 15 ºC prior to dehydration to allow the accumulation of phenolic compounds as a response to wounding stress. The carotenoid and phenolic content were analyzed in CCP and FCP (**Figure 4.1**). Both CPs showed similar levels of α-carotene, β-carotene, and RE, indicating that the application of wounding stress conditions did not affect the carotenoid content of carrot (**Figure 4.1A**). On the other hand, FCP showed 612.4% and 798.4% higher levels of total phenolics and CHA, respectively, as compared with CCP (**Figure 4.1B**). Previously, Santana-Gálvez, et al. (2016) followed the same procedure to obtain CP from carrot treated with wounding stress. The authors also reported higher content of chlorogenic acid in CP obtained from carrots treated with wounding stress (2530 mg per kg) compared with regular CP (547 mg per kg), and no significant difference in the carotenoid content between both CPs was detected. It is well known that wounding stress induces the accumulation phenolic compounds in carrots (Jacobo-Velázquez et al., 2011; Surjadinata and Cisneros-Zevallos, 2012; Becerra-Moreno et al., 2015; Jacobo-Velázquez et al., 2015). In the specific case of CHA, it is accumulated in wounded tissue because it is required as substrate for the biosynthesis of lignin, which prevents water loss in wounded carrots (Becerra-Moreno et al., 2015).

4.1.2 Hydration and oil-binding properties

The WAI and OAI from CCP and FCP are presented on **Table 4.1**. As observed, the production of carrot powder from carrot treated with wounding stress prior to its dehydration (FCP) resulted on a carrot powder with higher WAI and OAI values. The higher values of WAI in FCP could be attributed to the biosynthesis of lignin content that occurs during storage of wounded carrots (Becerra-Moreno et al., 2015). Lignin, is an insoluble fiber that prevents water loss in wounded-crops. Generally, WAI of vegetable fibers is related with the insoluble fiber fraction having a greater ability than soluble fiber to trap water within the cell matrix rather than due to its

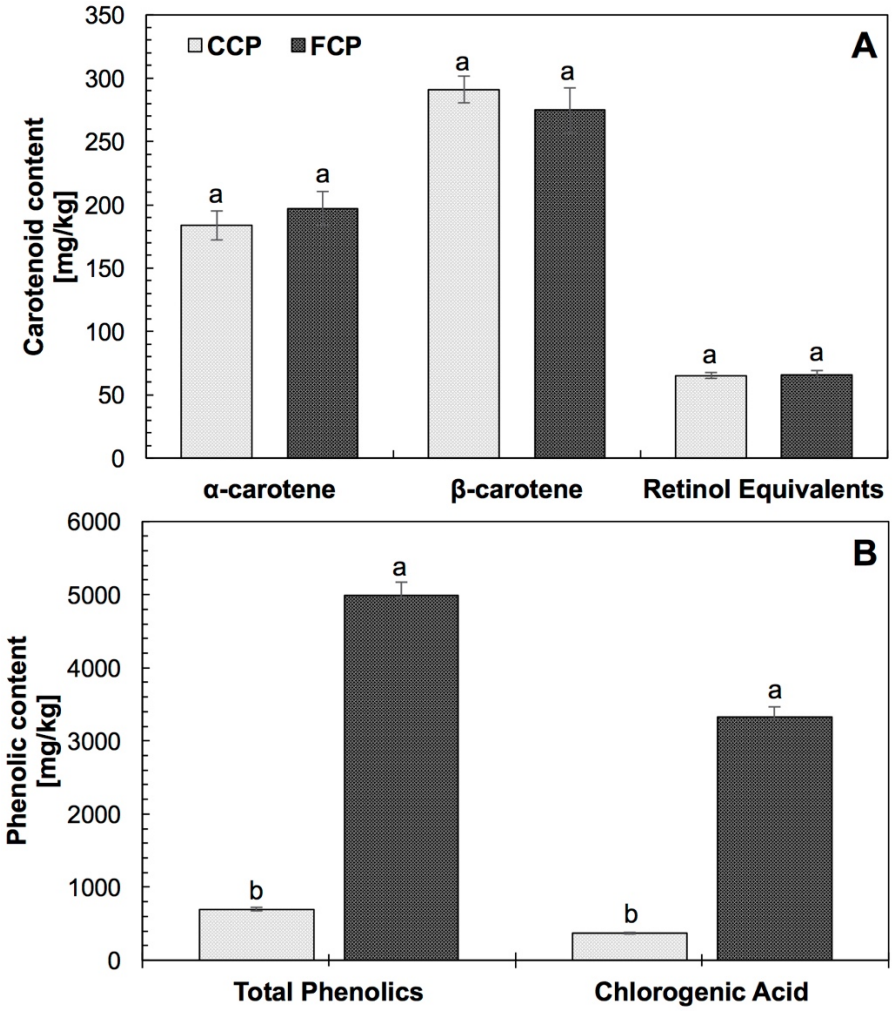


Figure 4.1 Carotenoid (A) and phenolic (B) content in CCP and FCP**.** Values represent the mean of three replicates with their standard error bars. Bars with different letters indicate statistically difference by the LSD test ($p<0.05$). CCP= control carrot powder; FCP= functional carrot powder.

Table 4.1 Water absorption index (WAI) and oil absorption index (OAI) of CCP and FCP.

Parameters[b]	Samples[a]	
	CCP	FCP
WAI	5.28 ± 0.07 b	6.65 ± 0.04 a
OAI	3.09 ± 0.01 b	3.21 ± 0.01 a

[a] Different letters in the same row indicate significant differences by the LSD test ($p<0.05$).
[b] Values represent the mean of three replicates with their standard error.
CCP= control carrot powder; FCP= functional carrot powder; WAI= water absorption index; OAI= oil absorption index.

ability to bind water (Robertson & Eastwood, 1980). In addition, hydroxycinnamic acids with polar nature, such as chlorogenic acid, can also stablish bonds to water molecules, which could be in part responsible for the higher water absorption index detected in FCP.

Likewise, high OAI in vegetable fibers is associated with the surface particle size of the insoluble fiber. It has been observed that insoluble fiber has greater capacity to absorb components of oily nature than soluble fiber, by trapping them on the surface (Matos & Chambilla, 2010). Thus the higher lignin content in FCP could also explain the higher values of OAI when compared with CCP.

4.2. Chemical composition of sausages added with 4% CCP or FCP

Chemical analyses of sausages added with either 4% CCP or 4% FCP is presented in **Table 4.2**. The addition of CP increased the content of total (TDF), insoluble (IDF) and soluble dietary fiber (SDF) by 72.7%, 54.6% and 269.2%, respectively, as compared with the control; where no significant difference was detected between CCP and FCP formulation.

The higher levels of dietary fiber detected in sausages added with CPs was mainly due to the addition of insoluble fiber (cellulose, lignin, and hemi-cellulose) present in carrot (Nawirska & Kwasniewska, 2005; Lineback 1999). The addition of CCP induced a significant decrease (from 67.88 to 67.24%) in the moisture content of sausages as compared to the control and FCP formulation, which was expected since FCP ingredient possessed higher water absorption index (WAI) than the CCP ingredient (**Table 4.1**). Additionally, higher moisture content detected in FCP formulation could be attributed to the addition of higher levels of CHA present in the FCP ingredient (**Figure 4.1B**). Similar observations have been reported for sausage formulations added with phenolic acids such as caffeic acid and carnosic acid (Capitani *et al.,* 2012; Hayes *et al.,* 2011). The mechanism by which phenolic compounds increases water retention of processed meat formulations has not been elucidated. However, a proposed mechanism is related with the inhibition of protein oxidation by phenolic antioxidants, since protein oxidation is a key factor affecting water holding capacity of processed meats (Huff-Lonergan & Lonergan, 2005).

4.3. pH and purge values of sausages as affected by 4% (w/w) CCP or FCP addition and storage

The effects of CPs addition and storage time on the pH and purge values of sausages are shown on **Table 4.3**. As observed, CCP addition did not affect the pH of sausage before storage

Table 4.2. Proximate analyses (%) and energy content of sausages added with 4% (w/w) CCP or FCP.

Determinations[a]	Treatment[b]		
	Control[c]	4% CCP	4% FCP
Calories (kcal/ 62.5 g)	101.39 ± 1.52 a	99.48 ± 0.57 a	97.02 ± 1.62 a
Moisture	67.88 ± 0.02 a	67.24 ± 0.12 b	67.75 ± 0.12 a
Carbohydrates	6.28 ± 0.49 a	6.05 ± 0.45 a	6.80 ± 1.02 a
Fat	10.50 ± 0.48 a	10.75 ± 0.37 a	10.16 ± 0.57 a
Proteins	12.18 ± 0.02 a	12.22 ± 0.07 a	11.79 ± 0.37 a
Ash	3.16 ± 0.03 c	3.75 ± 0.02 a	3.50 ± 0.03 b
TDF	1.54 ± 0.01 b	2.66 ± 0.09 a	2.65 ± 0.03 a
IDF	1.41 ± 0.10 b	2.18 ± 0.14 a	2.13 ± 0.05 a
SDF	0.13 ± 0.09 b	0.48 ± 0.05 a	0.52 ± 0.02 a

Significance[d]	FCP	CPC	FCP x CPC
Calories	NS	NS	NS
Moisture	*	*	*
Carbohydrates	NS	NS	NS
Fat	NS	NS	NS
Proteins	NS	NS	NS
Ash	**	***	**
TDF	NS	***	NS
IDF	NS	**	NS
SDF	NS	**	NS

[a] Value represent mean of 2 replicates in FW basis. [b] Values with different letters within the same row indicate significantly difference by the LSD test ($p<0.05$). [c] Control= 0% of CP. [d] Asterisks indicate significant difference by ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: NS = non-significant; CP = carrot powder; CCP = control carrot powder; FCP = functional carrot powder; TDF = total dietary fiber; IDF = insoluble dietary fiber; SDF = soluble dietary fiber; CPC = carrot powder concentration.

(day 0 samples); however, FCP addition decreased the pH from 6.67 to 6.57. The decrease in pH value in FCP formulation could be attributed to the lower pH of FCP (pH = 6.0) as compared to CCP (pH = 6.3) ingredient. This lower pH value in the FCP ingredient could be attributed to the CHA produced in carrots due to wounding stress prior to its dehydration and milling process, since it has been stated that although CHA is a week acid, it is sufficiently acidic to lower the pH of food formulations (Gülçin, 2006). Regarding storage time, the pH value of sausages showed a significant decrease from 28 to 42 days (**Table 4.3**), where no significant difference was observed between formulations. This decrease in pH values suggest that lactic acid was produced by lactic acid bacteria (LAB), which growth is a common deterioration mechanism during storage of processed meat products (Hugo & Hugo, 2015; Capillas et al., 2014; Eim, et al., 2008).

Table 4.3. Effect of 4% (w/w) CCP or FCP addition and storage (42 d at 4° C) on pH and purge values of sausages.

Samples	Storage time (d)	Parameters[a]	
		pH[b]	Purge[c]
Control[d]	0	6.67 ± 0.03 bcd	ND
	7	6.70 ± 0.01 abc	0.65 ± 0.01 bcd
	14	6.73 ± 0.03 ab	0.33 ± 0.01 d
	21	6.67 ± 0.03 bcd	0.49 ± 0.16 cd
	28	6.77 ± 0.03 a	0.64 ± 0.01 bcd
	35	6.60 ± 0.06 de	0.65 ± 0.01 bcd
	42	6.63 ± 0.03 bcd	0.48 ± 0.1 6cd
4% CCP	0	6.60 ± 0.06 de	ND
	7	6.63 ± 0.03 cde	0.99 ± 0.33 abc
	14	6.70 ± 0.01 abc	1.15 ± 0.17 ab
	21	6.63 ± 0.03 cde	1.16 ± 0.16 ab
	28	6.70 ± 0.01 abc	1.22 ± 0.40 a
	35	6.57 ± 0.07 e	1.00 ± 0.20 abc
	42	6.60 ± 0.01 de	1.21 ± 0.21 a
4% FCP	0	6.57 ± 0.03 e	ND
	7	6.57 ± 0.03 e	0.50 ± 0.16 cd
	14	6.63 ± 0.07 cde	0.49 ± 0.15 cd
	21	6.70 ± 0.01 abc	0.66 ± 0.01 bcd
	28	6.70 ± 0.01 abc	0.66 ± 0.01 bcd
	35	6.57 ± 0.03 e	0.44 ± 0.11 d
	42	6.60 ± 0.01 de	0.33 ± 0.01 d
Significance[e]			
FCP		NS	***
CPC		***	***
S		***	NS
FCP x CPC		NS	***
FCP x S		NS	NS
CPC x S		NS	NS
FCP x CPC x S		NS	NS

[a] Values with different letters within the same column indicate significantly difference by the LSD test ($p<0.05$).

[b] Values represent the mean of three replicates.

[c] Values represent the mean of two replicates in control sample and three replicates in 4% CPs formulations. Purge was not evaluated in day 0 samples.

[d] Control= 0% of CP

[e] Asterisks indicate significant difference by ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Abbreviations: CP = carrot powder; ND = no determined; NS = non-significant; CCP = control carrot powder; FCP = functional carrot powder; CPC = carrot powder concentration; S = storage time.

Purge was also analyzed during storage of sausage formulations **(Table 4.3)**. At 7 days of storage, non-significant difference was observed between the purge values of the three formulations. However, from 14 to 42 days, FCP addition did not affect the purge values of sausages, whereas the CCP formulation showed significant higher purge values as compared with the control. As previously described, for the differences in WAI between FCP and CCP ingredients as well as the higher CHA content added in the FCP formulation, FCP sausages retained their moisture with no significate differences with the control. Water retention in meat can be affected by proteolysis and protein oxidation, thus higher CHA content could minimize those effects (Huff-Lonergan & Lonergan, 2005). Lower purge loss in FCP are also supported by results discussed in section 4.1 in which, FCP ingredient had a higher WAI. By this means, purge loss from FCP formulation could be minimized. Thus, WAI and CHA content in FCP ingredient are the main contributors to improve retention of water of FCP sausage formulation as compared to CCP.

4.4. Color (CIE LAB) and texture properties of sausages as affected by 4% (w/w) CCP or FCP addition and storage

The effect of CPs addition and storage time on the instrumental color values of sausages are shown in **Table 4.4**. In general, the addition of CPs in sausage changed the color from soft-pink to dark-orange, mainly due to the carotenoid pigments present in the ingredient. These changes were reflected on all the color parameters evaluated. When comparing the color of CCP and FCP formulations, except for the b* values, the evaluated parameters showed significant difference. Lightness (L*) and redness (a*) parameters showed lower values in FCP as compared to CCP formulation. During CP production, difference in color between FCP batch and CCP batch are easily perceived after dehydration. FCP usually has a gold orange-brownish color, while CCP presents a bright-orange color, which could explain differences between L* and a* values of CCP and FCP sausage formulations.

During storage, L* values of sausages were not affected **(Table 4.4)**. Likewise, comparing 0 to 42 day samples, the a* values increased in the control and CCP samples, as well as C*. Likewise, the Hº value decreased only in the CCP. Color remained unaltered in the FCP formulation during storage time. These results are in agreement with previous reports were only slight changes in instrumental color values have been reported for meat products (frankfurters sausages, bologna sausages, and cooked pork sausages) stored under refrigeration (Pintado *et al.,* 2015; Hayes *et al.,* 2011; Fernández-Ginés *et al.,* 2003). On the other hand, addition of antioxidant has shown positive effect on color stability and elimination

Table 4.4 Effect of 4% (w/w) CCP or FCP addition and storage time (42 d at 4 ºC) on color CIE LAB values of sausages.

Samples[a]	Storage time (d)	Parameters[b]				
		L*	a*	b*	C*	H°
Control[c]	0	57.17 ± 0.33 ab	17.80 ± 0.56 bc	9.50 ± 0.26 ij	20.18 ± 0.57 j	28.11 ± 0.62 ij
	7	57.72 ± 0.10 a	17.84 ± 0.25 bc	9.54 ± 0.31 ij	20.23 ± 0.34 j	28.14 ± 0.58 ij
	14	57.49 ± 0.29 a	17.68 ± 0.18 cd	10.34 ± 0.08 h	20.48 ± 0.19 ij	30.32 ± 0.08 h
	21	57.59 ± 0.35 a	18.33 ± 0.09 ab	10.01 ± 0.10 hij	20.88 ± 0.09 ij	28.64 ± 0.25 i
	28	57.65 ± 0.70 a	17.96 ± 0.05 bc	9.39 ± 0.21 j	20.27 ± 0.08 j	27.59 ± 0.56 j
	35	57.78 ± 0.18 a	18.30 ± 0.29 ab	10.03 ± 0.04 hi	20.87 ± 0.24 ij	28.73 ± 0.49 i
	42	57.24 ± 0.19 ab	18.67 ± 0.19 a	9.91 ± 0.17 hij	21.14 ± 0.15 i	27.95 ± 0.57 ij
4% CCP	0	56.13 ± 0.52 c	16.18 ± 0.28 e	22.53 ± 0.59 ef	27.74 ± 0.60 efg	54.31 ± 0.52 de
	7	55.90 ± 0.27 c	17.12 ± 0.12 d	23.49 ± 0.34 abcd	29.07 ± 0.33 abc	53.92 ± 0.32 def
	14	55.68 ± 0.42 c	17.78 ± 0.36 bcd	23.93 ± 0.21 a	29.81 ± 0.34 a	53.40 ± 0.46 ef
	21	56.23 ± 0.46 c	17.73 ± 0.71 bcd	22.60 ± 0.84 cdef	28.74 ± 0.89 bcde	51.88 ± 1.32 g
	28	55.88 ± 0.14 c	17.88 ± 0.21 bcd	23.50 ± 0.32 abc	29.53 ± 0.16 ab	52.73 ± 0.67 fg
	35	56.25 ± 0.20 c	17.35 ± 0.47 cd	23.40 ± 0.12 abcde	29.14 ± 0.31 ab	53.47 ± 0.73 ef
	42	56.52 ± 0.32 bc	17.89 ± 0.37 bcd	22.60 ± 0.20 def	28.82 ± 0.38 abcd	51.64 ± 0.34 g
4% FCP	0	52.58 ± 0.58 e	14.60 ± 0.26 g	22.52 ± 0.37 ef	26.84 ± 0.45 gh	57.05 ± 0.12 ab
	7	53.04 ± 0.57 de	15.25 ± 0.08 fg	23.41 ± 0.48 abcde	27.95 ± 0.38 def	56.90 ± 0.62 ab
	14	52.52 ± 0.18 e	15.10 ± 0.08 fg	23.68 ± 0.25 ab	28.09 ± 0.23 cdef	57.47 ± 0.23 a
	21	53.85 ± 0.62 d	15.32 ± 0.20 fg	21.29 ± 0.64 g	26.23 ± 0.63 h	54.24 ± 0.53 de
	28	53.06 ± 0.33 de	15.15 ± 0.10 fg	22.83 ± 0.42 bcdef	27.40 ± 0.38 fg	56.43 ± 0.40 abc
	35	52.91 ± 0.33 de	15.48 ± 0.14 ef	22.36 ± 0.36 f	27.19 ± 0.37 fgh	55.31 ± 0.27 cd
	42	53.18 ± 0.25 de	15.33 ± 0.20 fg	22.53 ± 0.40 ef	27.26 ± 0.33 fg	55.76 ± 0.63 bc
Significance[d]						
FCP		***	***	NS	***	***
CPC		***	***	***	***	***
S		NS	***	***	*	***
FCP x CPC		***	***	NS	***	***
FCP x S		NS	NS	NS	NS	NS
CPC x S		NS	NS	**	**	***
FCP x CPC x S		NS	NS	NS	NS	NS

[a] All values are mean ± standard error of three replicates. [b] Values with different letters within the same column indicate significantly difference by the LSD test ($p<0.05$). [c] Control = 0% of CP. [d] Asterisks indicate significant difference by ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: NS = non-significant; CP = carrot powder; CCP = control-carrot powder; FCP = functional carrot powder; CPC = carrot powder concentration; S= storage time.

of color variations in different parts of sausages (Kim & Choi, 2014; Kim et al., 2011). Hence, phenolic compounds added from FCP could be a possible explanation for the higher stability of color in FCP sausages compare with the control and CCP formulations.

The results obtained agree with the data from the preliminary study performed to determine the optimum concentration of FCP in sausage formulations (**See Supplementary Data, Appendix A, Table S1-S2**). In those experiments, the effect of FCP addition at 4%, 8% and 12% (w/w) on the color of sausage was evaluated. Results showed a decrement in L* and a*, while an increment in b* as FCP concentration increased. Also, color parameters in 4% FCP formulation were stable during storage time (**Table S2**).

A texture profile analysis (TPA) was also performed to evaluate changes in texture parameters in sausages due to CP addition and storage time (**Table 4.5**). Although the addition of CP affected all the texture parameters evaluated (hardness, springiness, cohesiveness, and chewiness), FCP formulation showed closest values to the control as compared with CCP formulation.

In general, the changes in texture observed in sausage formulations, could be attributed to the replacement of starch and modified starch by CPs (**Table 3.1**). It is well known that starches have a positive effect on texture and rheological properties of processed meat formulations. For instance, starches improve texture by increasing water holding capacity (WHC). Likewise, starches reduce water loss during cooking and purge during storage (Li & Yeh, 2003). Furthermore, the addition of insoluble fiber to sausage formulations has been related with decreased values of cohesiveness as herein reported (Kim *et al.,* 2011; García *et al.,* 2006).

The close texture values of FCP to the control than CCP formulation could be attributed to the higher content of CHA and lignin present in the FCP ingredient. It has been reported that insoluble dietary fibers such as lignin improves the texture due to their water and fat-binding properties, which results in harder meat products (Mehta *et al.,* 2015; Biswas *et al.,* 2011; Viuda-Martos *et al.,* 2010; Pintado *et al.,* 2016; Ruiz-Capillas *et al.,* 2014; Todd & Cunningham, 1988). Likewise, as earlier discussed phenolic compounds such as CHA prevents oxidative degradation of muscle proteins, which can also explain the higher hardness value observed in FCP formulation as compared with CCP formulation (Ganhãoa, Morcuende & Mario Estévez, 2010). These results, agree with other report, in which the addition of caffeic acid and carnosic acid increased hardness in sausages (Capitani et al., 2012).

Storage time also affected the value of all texture parameters evaluated. No clear pattern was shown for chewiness and springiness, but hardness parameter in all formulations increased during storage time, while cohesiveness slightly decreased (**Table 4.5**). At the end of the shelf-

Table 4.5 Texture parameter values as affected by 4% (w/w) CCP or FCP addition and storage time (42 d at 4 °C).

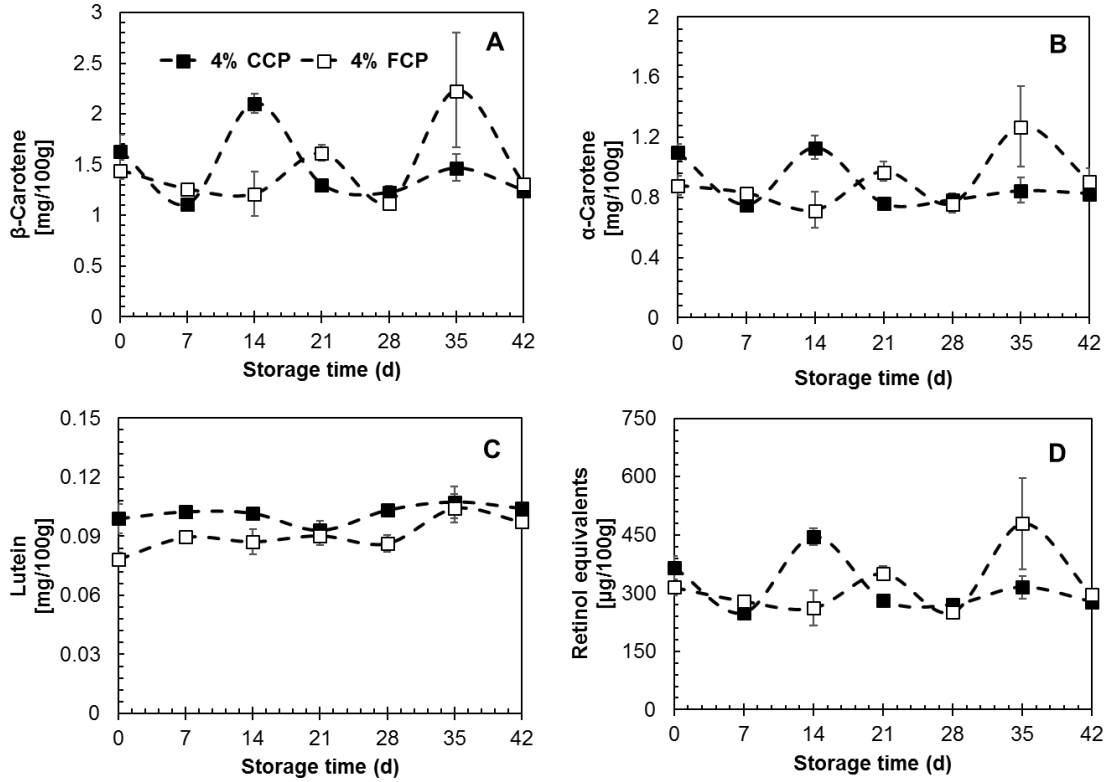
Samples[a]	Storage time (d)	Parameters[b]			
		Hardness (g)	Springiness (mm)	Cohesiveness	Chewiness (g*mm)
Control[c]	0	6526.18 ± 209.11 gh	0.85 ± 0.02 a	0.38 ± 0.01 ab	2079.02 ± 91.01 abc
	7	6774.13 ± 70.86 efg	0.82 ± 0.02 abc	0.39 ± 0.01 a	2161.85 ± 59.85 ab
	14	6958.08 ± 139.02 de	0.79 ± 0.01 bcde	0.36 ± 0.01 bc	1987.08 ± 82.63 abcd
	21	7014.45 ± 135.20 de	0.78 ± 0.01 bcde	0.36 ± 0.01 bc	1972.66 ± 46.53 bc
	28	6748.85 ± 99.13 efg	0.83 ± 0.01 ab	0.35 ± 0.01 cd	1976.01 ± 52.41 bcd
	35	7222.76 ± 108.51 cd	0.75 ± 0.01 defg	0.32 ± 0.01 fgh	1756.01 ± 67.01 ef
	42	7782.02 ± 137.55 a	0.80 ± 0.01 abcd	0.35 ± 0.01 cd	2167.13 ± 81.33 a
4% CCP	0	5295.11 ± 92.34 j	0.76 ± 0.01 cdefg	0.31 ± 0.01 hi	1225.14 ± 44.05 i
	7	6503.05 ± 84.53 gh	0.71 ± 0.03 fgh	0.34 ± 0.01 de	1588.28 ± 74.19 fg
	14	5795.28 ± 124.40 i	0.70 ± 0.03 gh	0.32 ± 0.01 fgh	1294.20 ± 116.14 hi
	21	6872.57 ± 120.52 ef	0.75 ± 0.05 defg	0.30 ± 0.01 ij	1551.24 ± 117.69 g
	28	5919.11 ± 114.44 i	0.77 ± 0.03 cdef	0.31 ± 0.01 hi	1420.81 ± 99.23 gh
	35	6473.70 ± 142.57 gh	0.63 ± 0.02 i	0.28 ± 0.01 j	1150.38 ± 40.77 i
	42	6699.03 ± 119.90 efg	0.69 ± 0.02 h	0.31 ± 0.01 ghi	1423.23 ± 55.36f gh
4% FCP	0	6257.96 ± 104.52 h	0.75 ± 0.01 defg	0.32 ± 0.01 fg	1529.44 ± 42.10 g
	7	7252.46 ± 110.29 cd	0.73 ± 0.01 efgh	0.34 ± 0.01 c	1823.26 ± 65.43 de
	14	6732.02 ± 116.03 efg	0.73 ± 0.03 efgh	0.32 ± 0.01 fgh	1585.19 ± 109.29 fg
	21	7419.46 ± 118.04 bc	0.75 ± 0.01 defg	0.33 ± 0.01 ef	1829.79 ± 52.55 de
	28	6616.40 ± 98.23 fg	0.76 ± 0.02 cdefg	0.32 ± 0.01 fgh	1601.68 ± 53.39 fg
	35	7649.82 ± 93.34 ab	0.69 ± 0.04 h	0.30 ± 0.01 ij	1575.21 ± 77.64 fg
	42	7590.87 ± 78.44 ab	0.73 ± 0.01 efgh	0.31 ± 0.01 fghi	1743.71 ± 44.68 ef
Significance[d]					
FCP		***	NS	*	***
CPC		***	***	***	***
S		***	***	***	***
FCP x CPC		***	NS	*	***
FCP x S		NS	NS	NS	NS
CPC x S		***	NS	***	***
FCP x CPC x S		NS	NS	NS	NS

[a] Values represent the mean of 20 replicates ± standard error of the mean. [b] Values with different letters within the same column indicate significantly difference by the LSD test ($p<0.05$). [c] Control= 0% of CP. [d] Asterisks indicate significant difference by ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Abbreviations: NS = non-significant; CP = carrot powder; CCP = control carrot powder; FCP = functional carrot powder; CPC = carrot powder concentration; S = storage time.

life study FCP and control formulations showed similar hardness values, whereas lower values were detected in CCP formulation. Increases in hardness during storage of sausage is associated with water loss. These results have been reported before by several authors in shelf-life studies (Feng et al., 2017; Pintado et al., 2016; Ruiz-Capillas et al., 2014, Capitani et al., 2012; Fernandez-Ginés, 2003). At the end of the shelf-life study, CCP sausages showed greater increment in hardness compared with FCP and control. CCP sausages increased 26% in hardness compared to 0-day CCP samples. These results were expected since CCP formulation experienced higher purge loss.

4.5 Carotenoid and phenolic content of sausages as affected by 4% (w/w) CCP or FCP addition and storage

The effects of CP addition and storage time on the carotenoid content of sausages is shown in **Figure 4.2**. As expected, carotenoids were not detected in control samples. Likewise, addition of CP resulted on sausages with high levels of β-carotene **(Figure 4.2A)**, α-carotene **(Figure 4.2B)**, and lutein **(Figure 4.2C)**. No significant differences were detected in β-carotene and α-carotene content between CCP and FCP formulation. However, FCP formulation showed slight lower (10.86%) lutein content as compared with CCP. Interestingly, when comparing the theoretical amount of α-carotene and β-carotene per 100 g of sausage, using the reference values obtained for the CPs ingredient **(Figure 4.1)**, higher amounts of α-carotene and β-carotene were detected in CCP and FCP formulations. Theoretical values of α-carotene, β-carotene and lutein for CCP formulation were, 0.73, 1.16, and 0.63 mg/100 g, respectively; whereas for FCP formulation theoretical values of α-carotene, β-carotene and lutein were 0.78, 1.09, and 0.67 mg/100 g, respectively. However, as can be observed in **Figure 4.2** in both cases the content of α-carotene, and β-carotene were higher. For instance, in the FCP formulation, the experimental values for α-carotene, and β-carotene 11%, and 31% respectively, higher, as compared with theoretical values. These results agree with results obtained in the preliminary study about the optimization of the sausage formulation to be tested in the present study (**See Supplementary Data, Appendix A, Table S4**). According to Seybold *et al.* (2004), depending on the variety of the vegetable, carotenoids can be associated with different proteins, indicating that the cellular matrix of the vegetable may influence the release or retention of carotenoids. Protein–carotenoid complexation has been shown to exert an inhibitory effect on carotenoid extractability. Thermal treatments such as cooking denature the proteins, softening the cell walls and releasing carotenoids from these complexes (Carisa, Da Cunha & Vera, 2014). Hence, it is likely that the



Significance	β-carotene	α-carotene	Lutein	Retinol equivalents
FCP	NS	NS	***	NS
CPC	***	***	***	***
S	**	*	*	**
FCP x CPC	NS	NS	***	NS
FCP x S	**	**	NS	**
CPC x S	**	**	*	**
FCP x CPC x S	**	**	NS	**

Figure 4.2 β-carotene (A), α-carotene (B), lutein (C), and retinol equivalents (D) content before and during storage (42 d at 4°C) of sausages added with 4% (w/w) CCP or FCP. Values represent the mean of 3 replicates with their standard error bars. Asterisks indicate significant difference by ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001. Abbreviations: NS = non-significant, CCP = control carrot powder, FCP = functional carrot powder, CPC = carrot powder concentration, S= storage time.

higher levels of carotenoids detected as compared with the theoretical calculations, could be attributed to higher extractability of carotenoids due to heat treatment applied during sausage manufacturing.

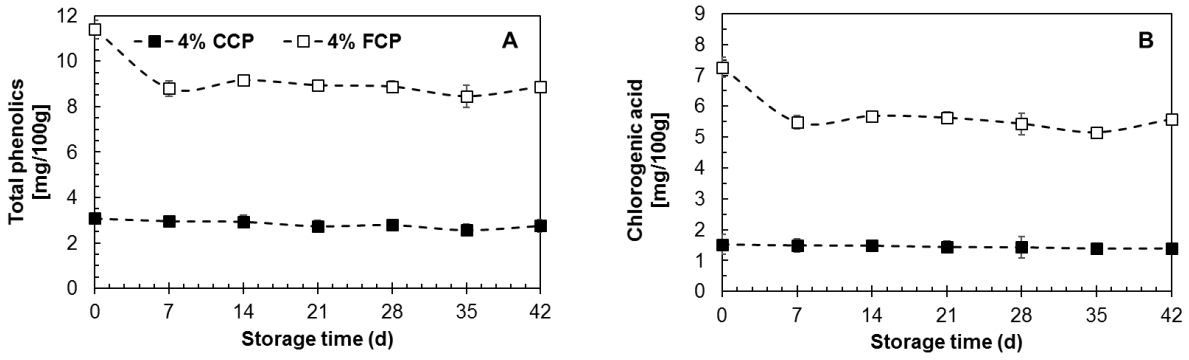
During storage, the content of carotenoids remained relatively stable and at the end of the shelf-life study only slight decrements were detected in β- and α-carotene, whereas lutein content remained unaltered. In this context, at 42 days of storage CCP formulation showed 23.55% and 24.87% lower levels of β-carotene and α-carotene, respectively, as compared with day 0; whereas FCP showed 9.11% and 3.44% lower levels of β- and α-carotene, respectively, as compared to time 0 days samples. However, at the end of the shelf-life, no significant difference was detected between the carotenoid content of CCP and FCP.

Several mechanisms have been proposed for the oxidation and degradation of carotenoids in food systems by the presence of free radicals. Once oxidation has been initiated by one of several oxidizing agents, carotenoids further react with themselves or other chemical species within the environment to form different degradation products (Boon *et al.,* 2010).

Results presented herein, showed that sausages, were an adequate food matrix to prevent carotenoid degradation, since β-carotene, α-carotene, and lutein were stable during the shelf-life. A possible reason to explain these results, is the presence of antioxidant phenolic compounds in CPs. Studies with the addition of antioxidants such as BHT, resulted in a reduced rate of oxidation product formation (Boon, *et al.,* 2010). Hence presence of CHA could have help maintain the stability of carotenoids during storage period in both CP formulations by protective them from free radicals. Several studies have confirmed that CHA could act as an antioxidant additive. For instance, Laguerre et al. (2009), as well as, Sasaki et al. (2010), studied separately the antioxidant capacity of chlorogenic acid against oxidation of emulsified lipids. Both experiments reported evidence of inhibition of lipid oxidation by chlorogenic acid.

Since vitamin A deficiency is considered a public health issue in developing countries, interest in incorporating pro-vitamin A carotenoids into functional foods has risen in the last decade (UN-SCN 2010). It is recommended a daily consumption of 568 µg of retinol equivalents by the Mexican population (SEGOB, 2010). At the beginning of the shelf-life of CCP and FCP formulations, one portion of sausages (62.5 g) contributed with 40.16% and 34.62%, respectively of the recommended vitamin A daily intake. Likewise, at the end of the 42-d shelf-life study, retinol equivalents values per portion of sausage remained high providing 30.57% and 32.48%, respectively of daily vitamin A intake recommendations.

The total phenolic and chlorogenic acid content before and during storage of CCP and FCP sausage formulations are shown in **Figure 4.3**. As expected, phenolic compounds were not



Significance	Total phenolics	Chlorogenic acid
FCP	***	***
CPC	***	***
S	***	***
FCP x CPC	***	***
FCP x S	NS	***
CPC x S	***	***
FCP x CPC x S	NS	***

Figure 4.3 Total phenolic (A) and chlorogenic acid (B) content before and during storage (42 d at 4 ºC) of sausages added with 4% (w/w) of CCP or FCP. Values represent the mean of 3 replicates with their standard error bars. Asterisks indicate significant difference by ANOVA. *p < 0.05, **p < 0.01, ***p < 0.001. Abbreviations: NS = non-significant, CCP = control carrot powder, FCP = functional carrot powder, CPC = carrot powder concentration, S= storage time

detected in the control. Likewise, FCP sausage formulations showed 270% and 377% higher total phenolic and CHA content, respectively, as compared with CCP formulation. When comparing the theoretical values expected of total phenolic and CHA based on the levels of chlorogenic acid in CPs **(Figure 4.1)**, the content quantified in FCP and CPC formulations were lower, indicating that phenolic compounds are partially degraded during sausage manufacturing. Theoretical values of total phenolic and CHA per 100 g of FCP sausage formulations were 19.95 mg and 13.32 mg, respectively; whereas values detected were 11.40 mg and 7.26 mg, respectively. These results are in agreement with the result observed in the preliminary study, where degradation of CHA was detected during sausage manufacturing. Lower levels of CHA detected after sausage manufacturing could be attributed to decarboxylation and oxidative reactions induced by thermal processing (Napolitano & d'Ischia, 2002).

During storage time, the content of total phenolic and CHA in the FCP formulation decreased from 11.40 and 7.26 to 8.87 and 5.57 mg/100g, respectively at day 7, whereas for the further storage period the levels remained constant. On the other hand, the content of phenolic in the CPC formulation remained constant during storage. At the end of the 42-day shelf-life study, the total phenolic and CHA in the FCP formulation were 23% and 22% lower as compared to time 0 days samples. It is likely that CHA present in sausage formulations is acting as antioxidant preventing the degradation of other bioactive compounds, thus degradation of the compound was observed during storage (Nardini et al., 2002). Chlorogenic acid content per portion of FCP and CCP formation were 3.48 and 0.86 mg per portion, respectively.

4.6 Sensory acceptability test of sausages added with 4% (w/w) CCP or FCP

A sensory evaluation test was performed to compare the acceptability between the sausage formulations (**Table 4.6**). To ensure microbiological stability and safety of sausages prior to sensory evaluations, sausage CPC and FPC as well as the control were assayed for microbiological growth (See Supplementary Data, Appendix B). The addition of CPC and FCP decreased the acceptability of all the parameters evaluated (appearance, flavor, texture, overall acceptability, and no significant difference were detected between the CPC and FCP values) **(Table 4.6)**.

Table 4.6. Sensory acceptability values of sausages added 4% (w/w) CCP or FCP.

Parameters	Samples[a]		
	Control[b]	4% CCP	4% FCP
Appearance	7.84 ± 0.12 a	6.08 ± 0.20 b	5.91 ± 0.19 b
Flavor	7.52 ± 0.12 a	6.42 ± 0.20 b	6.53 ± 0.18 b
Texture	7.61 ± 0.13 a	6.10 ± 0.20 b	6.37 ± 0.18 b
Overall acceptability	7.66 ± 0.11 a	6.44 ± 0.17 b	6.62 ± 0.16 b

[a] Different letters in the same row indicate significant differences by the LSD test ($p < 0.05$).
[b] Control= 0% of carrot powder.
Abbreviations: CCP = control carrot powder; FCP = functional carrot powder.

Although the formulations containing carrot powder showed lower acceptability as compared with the control, most values were above the acceptable range (between 6 and 7 i.e. “Like slightly” and “Like moderately”). In preliminary study performed to determine the optimum CP concentration in sausage formulations, the acceptability of 4% FCP sausage formulation was evaluated in two separate sensory acceptability test (see Supplementary Data, Appendix A,

Tables S5-S6). In the first test, the acceptability of sausage formulations added with 0%, 4% and 8% FCP were compared, where no significant difference was detected between the overall acceptability of 0% and 4% FCP formulations, and the acceptability of 8% FCP showed significant lower values as compared with 0% FCP. Similar results were obtained when the acceptability of 0%, 4%, 5% and 6% FCP formulations were evaluated; no significant difference was detected between the overall acceptability of 0% and 4% FCP formulations, whereas FCP addition at higher concentration significantly decreased the acceptability of sausage formulations (See Supplementary Data, Appendix A, Table S5 and S6). However, when the acceptability of 4% CCP, 4% FCP and the control (0 % CP) formulations were compared, lower acceptability values for all parameters evaluated were obtained in the 4% FCP formulation compared with the preliminary test. This could be due to the degree of hedonic disconfirmation that a consumer end-up experiencing (Piqueras-Fiszman & Spence, 2015). The latter refers to the difference between the expected and actual liking of the product when tasted. Uncertainty and familiarity are two factors that have a significant effect in product expectations. The more familiar consumers are with a given product the more certain their expectations are likely to be (Ludden, Schifferstein & Hekkert, 2009). Since the organoleptic properties of the different formulations of FCP tested were significant different among them as the concentration of FCP increased, being 4% FCP the one with the closest values to the control, it was expected to have higher acceptability values. However, when only 4% concentration was tested, it was easier for the consumers to defined what formulation was the most conventional to them and thus, affecting acceptability parameters of 4% FCP and CCP.

Comments provided by the consumers in the survey were analyzed in search for reasons that could explain the collected data. Even though, appearance value was in the acceptable range, it was the most criticized parameter, since most of the comments were related to the color of both samples. They showed displeased in the color, but also mentioned, that 4% CCP and FCP sausages tasted much better than what they looked, which is supported by the higher flavor acceptability values obtained. Color (CIE LAB) analysis was also correlated with appearance values. While, no significant differences were obtained between CP formulation, FCP showed slight lower appearance score. Since CIE values from CCP were closer to the control values, it is suggested that consumers felt more comfortable with the visual appearance of this sample.

Comments from consumers also criticized the texture of sausages added with CPs. No significant difference was found between CP formulations in the texture acceptability, and both values were above the acceptable range. Data from the TPA could explained such results. Since

hardness values were higher in FCP formulation, consumers preferred FCP texture rather than CCP.

4.7 Potential health benefits in sausage added with 4% functional carrot powder (FCP)

The development of a 4% FCP sausage rich in nutraceutical compounds such as dietary fiber (DF), carotenoids, and phenolic compounds could benefit health in several ways. For instance, the consciousness among consumers for inclusion of DF in a daily diet is increasing. For adults, the recommended intakes of DF are 28-36 g/day, 70-80% of which must be insoluble fiber (Mehta *et al.,* 2015). For Mexican population, the recommended reference value is 30 g/day (SEGOB, 2010). A portion (one sausage of 62.5 g) of 4% FCP sausage formulation provided ~1.66 g DF, so the intake of 2 sausages will provide 11% of recommended daily intake of DF.

Furthermore, considering an average moisture content of 90% for fresh carrots, one portion of sausage (62.5 g) would provide 25 g of fresh carrot. In addition, Mexican standards declare that 1 portion of fresh shredded carrots is equivalent to half cup (55 g or 1 small carrot) (Secretaría de Salud, 2010) and according to the U.S. Dietary Guidelines Advisory Committee (2015), the recommended daily vegetables intake (RDVI) is 2.5 cups, where ~0.80 cups per day of red and orange vegetables are recommended. Therefore, one sausage (62.5 g) would provide 0.45 Mexican portions of fresh carrot, 18% of RDVI and 56% of the daily intake of red and orange vegetables.

In addition, dietary provitamin A carotenoids are a major source of vitamin A requirements. Vitamin A is an essential vitamin for the promotion of general growth, maintenance of visual function, regulation of differentiation of epithelial tissues, and embryonic development (Tang, 2010). Vitamin A deficiency is common among children in developing countries, and a world-wide public health issue. Results presented here, showed that a portion of 4% FCP sausage, contributed to 32.5% of daily retinol equivalent intake, according to the nutritional reference values for Mexican population (568 µg of retinol equivalents).

Health-promoting properties of chlorogenic acid includes prevention and treatment of diseases related with the metabolic syndrome, such as obesity, dyslipidemia, diabetes and hypertension. An intake of 30 mg per day of chlorogenic acid have showed positive effects in human health (Santana-Gálvez et al., 2017). A portion of 4% FCP sausage formulation could provide 5.54 mg of chlorogenic acid, representing 18.5% of the daily intake that provides health benefits.

CHAPTER 5

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The use of postharvest treatment such as wounding stress in order to increase phenolic compounds content in carrot as well as, its subsequent dehydration and milling to produce a carrot powder ingredient rich in nutraceuticals (FCP) is an attainable option to enhance the intake of these compounds in Mexican population by the incorporation of this ingredient in different food products. CP production yield for both CCP and FCP is approximately 1 kg per 12 kg of shredded carrot fresh weight. However, due to the granulometry of the CPs, only CP with particle size <0.425 mm (45% of total yield) was used in the production of CP sausages. Further experiments should be oriented to optimize the FCP production process to increase the total yield.

Based on the general results achieved in this study, the production and the addition of FCP in sausages resulted to be a factual approach in the development of a novel nutraceutical ingredient and the production of a new nutraceutical-fortified product, respectively. The addition of FCP, proved to be a successful strategy in the increment of nutraceutical content in meat products specifically, sausages, even when they were thermally treated during production. Due to its dietary fiber, carotenoids content, and phenolic compounds, sausages added with FCP could become a product that helps to prevent chronic diseases by improving consumption of nutraceutical compounds in Mexican population.

Furthermore, from this study, several scientific data were generated respect to the effect on physicochemical parameters, nutraceutical content and sensory acceptability of FCP in sausages. The proximate analysis and fiber content showed a suitable increment of total dietary fiber. Likewise, addition of FCP slight decreased pH values. The analysis of pH during shelf-life is very important since is related with lactic acid bacterial (LAB) growth. While purge loss values were similar between FCP and control. In general, during a shelf-life study it is observed viscosity due to the acid production by LAB. Such viscosity that occurs out of the products is more apparently in wet surfaces, typical environment of sausage packs. For this point of view, FCP addition in sausages could increased water retention and avoid the production of viscosity which, is an unpleasant aspect of meat products that has become a rejection parameter for consumers; therefore, a negative factor that affect shelf-life of sausages. This represents a suitable choice since water holding capacity is a very important property in food industry.

In addition, color of meat, which is an important quality attribute that influences consumer acceptance, was altered by the addition of CP making them orange. While this change, made the

consumers' opinion divided, it could be generally accepted once consumers know that sausages are orange due to the pro-vitamin A carotenoids. Similarly, texture is a key quality attribute used in the fresh and processed food industry to assess product acceptability. Sausages herein produced showed texture parameters such as hardness, higher with the addition of FCP compared to the CCP formula.

More important, 4% FCP sausages formulation successfully demonstrated higher levels of phenolic compounds, specially chlorogenic acid. Also, good stability of these compounds was achieved during the shelf-life study. Moreover, the addition FCP in sausages incorporated pro-vitamin A carotenoids. Since Vitamin A deficiency represents a public health issue, the addition of these compounds in highly-consumed products could help to fight this problem. However, more than for their nutritional and functional application, β-carotene and phenolic compounds have been added in meat formulations as natural additives in order to avoid oxidation of lipids and proteins. Hence, it would be interesting to test these sausages from a nutritional point of view in clinical studies.

As for sensory acceptability of sausages added with FCP, addition of 4% w/w FCP resulted to be the most acceptable value among the consumers. On the other hand, concentrations of 12% w/w resulted in technical issues and a product with organoleptic properties remarkable different from a conventional sausage.

It is important to emphasized that during this study sausages were not cooked or heated again. So, the degradation analysis of nutraceutical compounds once sausages are cooked twice, remains for future work. Finally, the addition of FCP in other products such as beverages and baked products are opportunity areas that are not study yet. The generated scientific knowledge will contribute to the production and development of food products with the potential to prevent chronic diseases in the Mexican population.

Appendix A

Determination of optimum functional carrot powder (FCP) concentration in sausage formulations

A.1 Materials and Methods

A.1.1 Determination of the optimum concentration of functional carrot powder (FCP) in sausage formulation

To determine the optimum concentration of FCP in sausage formulation, sensory acceptability tests of sausages with different levels of FCP were performed as described in section A.1.1.1. In the first set of experiments, three different levels of FCP (4%, 8%, and 12%) and a control sample (0% FCP) were produced. A batch of 8 kg for each formulation were elaborated with the formulations shown in **Table S1**. In addition to sensory acceptability, chlorogenic acid and carotenoid contents were analyzed in the different sausage formulations. Likewise, the instrumental texture and color were determined every 7 d during 21 d of storage. However, 12% FCP formulation was not considered for the sensory acceptability test, since the organoleptic characteristics were extremely different when compared with the control (i.e. high sweet flavor, odor and taste).

According with the results obtained, a new experiment where FCP was added at 4%, 5%, and 6% was performed **(Table S1)**. For these treatments, the sensory acceptability was determined as described in section A.1.1.1. Based on the results, the 4% formulation was chosen as the final formulation to perform a complete characterization of the product.

A.1.1.1 Sensory acceptability test of sausages added with different concentrations of functional carrot powder (FCP)

Sensory evaluation tests were performed to determine the optimum concentration of CP in sausage formulations. Before sensory evaluations, the samples were testes for their microbial safety following the procedure described in Appendix B. The consumer panel was composed of 212 students and workers from Tecnológico de Monterrey – Campus Monterrey that consumed sausages at least once a week. The mean age of the consumers was 23 years old. Three sensory evaluation tests were performed. In the first test, 97 consumers evaluated the acceptability of sausages added with 0, 4 and 8% of FCP; whereas in the second test 115 consumers evaluated

the acceptability of sausages added with 0, 4, 5, and 6% of FCP. Based on the results from the first and second tests, the formulation of sausages added with 4%.

Table S1. Formulations of sausages tested to determine optimum FCP concentration.

Ingredients	Percentage in each formulation (w/w)					
	Control[a]	FCP 4%	FCP 5%	FCP 6%	FCP 8%	FCP 12%
Turkey meat	68.700	68.700	68.700	68.700	68.700	58.700
Water	21.700	21.200	20.600	19.600	17.600	24.600
NaCl	1.867	1.867	1.867	1.867	1.867	1.867
SFM	0.350	0.350	0.350	0.350	0.350	0.350
Sodium nitrite	0.016	0.016	0.016	0.016	0.016	0.016
Sodium erythorbate	0.050	0.050	0.050	0.050	0.050	0.050
Sodium phosphate	0.410	0.410	0.410	0.410	0.410	0.410
Carmine (E120)	0.007	0.007	0.007	0.007	0.007	0.007
Soy protein concentrate	3.000	3.000	3.000	3.000	3.000	3.000
Modified starch	1.500	---	---	---	---	---
Corn starch	2.000	---	---	---	---	---
FCP	---	4.000	5.000	6.000	8.000	12.000
Soy fiber	0.400	0.400	---	---	---	---

[a] Control = 0% of FCP.
Abbreviations: FCP = functional carrot powder; SFM = standard flavor mixture containing species, NaCl, starch, flavor and hydrolyzed vegetable protein.

Sausage portions of 2.0 cm height of each sample were placed in plastic containers. Each formulation was assigned a different random 3-digit number. Water and crackers (with neutral flavor) were available for consumers to clean the palate between samples. Consumers were asked to evaluate appearance, flavor, texture, and overall acceptability of sausages using a 9-point hedonic scale, where 1 was “Dislike extremely”, 2 – “Dislike very much”, 3 – “Dislike moderately”, 4 – “Dislike slightly”, 5 – “Neither like nor dislike”, 6 – “Like slightly” 7 – “Like moderately”, 8 – “Like very much”, and 9 – “Like extremely”. The samples were presented at random to the consumers. Consumers that evaluated the overall acceptability of the control sample with a 6 or higher value were considered to truly like sausages; therefore, only those surveys were considered for the data analysis.

A.1.2 Results and Discussion

To determine optimal CP concentration in sausage formulations a preliminary study was performed. The study consisted on evaluating two separate set of formulations containing different concentrations of FCP. In the first set of formulations, FCP was added at 0, 4, 8; and

12% whereas in the second set of formulations FCP was added at 0, 4, 5 and 6% (**Table S1).** In the first set of formulations, the content of carotenoids and phenolics was determined before storage of samples, whereas the color and texture were evaluated during 21 d of storage at 4 ºC. Furthermore, a sensory acceptability test of the 0, 4, and 8% formulations was performed before storage of sausages formulations. In the second set of experiments, the 0, 4, 5 and 6% formulations were only tested for sensory acceptability.

FCP addition at 4%, replaced corn starch and modified starch in sausage formulations; whereas FCP addition at 5%, 6%, and 12% also replaced soy fiber addition, and water percentage was modified. Furthermore, in 12% FCP formations, turkey meat content was reduced by 10% and water percentage was increased, since the meat batter too dense to mix properly in the cutter.

A.1.2.1 Effect of functional carrot powder (FCP) addition at 0, 4, 8, and 12% (w/w) on the color (CIE) and texture properties of sausages

The instrumental CIE color values of sausages added with 0, 4, 8 and 12% of FCP were evaluated before and during 21 d of storage a 4 ºC (**Table S2**). The addition of FCP induced a significant change the color parameters evaluated. Lightness (L*) values decreased, while, b* (yellowness), C*, and H° values increased as FCP concentration increased. These results were expected since FCP contains carotenoid compounds. Their characteristic yellow, orange, and red colors are due to the presence of several conjugated double bonds in a polyene chain that functions as a chromophore (Rodriguez-Concepcion & Stange, 2013) and consequently addition of these compounds in food formulations increase b* values and decrease lightness. Similar colorimetry results were reported by Eim *et al*. (2008) in the production of sobrassada sausages added with carrot dietary fiber, where L* values decreased, and b* values increased as DF was increased. Authors, also attributed these results, to the addition of carotenoids in the formulation. Storage time significantly affected the redness (a*), chroma (C*) and hue (H°) values of sausages. However, these effects were observed in the 12% FCP formulation, which showed higher a*, C* and H° values at 21 d of storage. It is suggested that color in high CP concentration (<12% w/w) formulas is not stable through time, however a longer shelf-life study with formulations with high concentration of CP are necessary to support this hypothesis.

Table S2. Color (CIE) analyses during storage (21 days at 4 °C) of sausages added with FCP (4, 8, and 12% w/w).

Samples[a]	Storage time (d)	Parameters[b]				
		L*	a*	b*	C*	H°
Control[c]	0	57.03 ± 0.14 a	18.73 ± 0.10 a	9.86 ± 0.05 e	21.17 ± 0.13 d	27.76 ± 0.10 e
	7	57.20 ± 0.05 a	17.80 ± 0.64 b	9.69 ± 0.43 e	20.27 ± 0.76 d	28.55 ± 0.22 e
	14	57.27 ± 0.35 a	18.74 ± 0.36 a	10.19 ± 0.23 e	21.33 ± 0.42 d	28.53 ± 0.09 e
	21	56.60 ± 0.25 a	19.39 ± 0.18 a	10.42 ± 0.18 e	22.01 ± 0.24 d	28.26 ± 0.22 e
4% FCP	0	55.71 ± 0.27 b	16.72 ± 0.17 de	17.39 ± 0.62 d	24.13 ± 0.68 c	46.07 ± 0.97 cd
	7	54.57 ± 0.16 c	16.37 ± 0.24 e	18.11 ± 0.18 d	24.41 ± 0.29 c	47.89 ± 0.16 c
	14	55.24 ± 0.10 bc	17.24 ± 0.11b cd	17.66 ± 0.14 d	24.68 ± 0.12 c	45.69 ± 0.30 d
	21	55.78 ± 0.36 b	17.75 ± 0.13 b	18.13 ± 0.22 d	25.37 ± 0.21 c	45.60 ± 0.30 d
8% FCP	0	53.15 ± 0.25 d	16.08 ± 0.09 e	23.09 ± 1.13 bc	28.15 ± 1.15 b	55.03 ± 1.48 ab
	7	53.18 ± 0.20 d	16.15 ± 0.08 e	23.51 ± 0.18 bc	28.53 ± 0.11 b	55.51 ± 0.34 a
	14	53.10 ± 0.44 d	16.68 ± 0.16 de	22.31 ± 0.39 c	27.86 ± 0.35 b	53.21 ± 0.49 b
	21	52.87 ± 1.07 d	16.68 ± 0.18 cde	23.33 ± 1.21 bc	27.86 ± 1.49 b	54.67 ± 1.60 ab
12% FCP	0	50.47 ± 0.38 e	16.43 ± 0.20 e	24.58 ± 1.32 ab	29.59 ± 1.41 b	56.10 ± 1.58 a
	7	50.76 ± 0.06 e	16.24 ± 0.09 e	24.42 ± 0.37 ab	29.33 ± 0.34 b	56.37 ± 0.34 a
	14	50.19 ± 0.11 e	16.73 ± 0.05 de	23.61 ± 0.41 bc	28.94 ± 0.36 b	54.67 ± 0.42 ab
	21	50.22 ± 0.47 e	17.45 ± 0.27 bc	26.37 ± 0.60 a	31.62 ± 0.60 a	56.50 ± 0.49 a
Significance[d]						
CPC		***	***	***	***	***
S		NS	***	NS	*	*
CPC x S		NS	NS	NS	NS	NS

[a] All values are mean ± standard error of three replicates.

[b] Different letters in the same column indicate significant differences by the LSD test ($p < 0.05$).

[c] Control= 0% FCP.

[d] Asterisks indicate significant difference by ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Abbreviations: NS = non-significant; CCP = control carrot powder; FCP = functional carrot powder; CPC = carrot powder concentration; S= storage time.

In addition to color measurements, the texture of sausages added with 0, 4, 8, and 12% of FCP was also evaluated during storage **(Table S3)**. FCP concentration as well as storage time showed a significant effect ($p < 0.05$) in texture parameters evaluated (hardness, springiness, cohesiveness and chewiness). Likewise, the interaction between the two variables evaluated (FCP concentration and storage time) showed a significant effect on hardness, springiness and cohesiveness of the sausages. In general, hardness of sausages increased, while springiness values decreased as the concentration of FCP increased. Formulation of 4% FCP had the closest values to control results, whereas 8% and 12% FCP formulations had not significant differences between cohesiveness and chewiness parameters. FCP contains cellulose and lignin (Sharma et al., 2012) which are insoluble fibers (Boulos, Greenfield & Wills, 2000) that slightly dry and harder meat products (Pintado *et al.,* 2016; Ruiz-Capillas *et al.,* 2015; Todd & Cunningham, 1988). Likewise, it has been reported that the increase in hardness associated with dietary fiber addition in cooked meat sausages is related with the inclusion of fiber particles in the protein matrix, which would presumably reinforce binding during cooking (Ruiz-Capillas, 2014; Viuda-Martos et al., 2010). Similar results have been reported by other authors, where the addition of orange fiber (1% w/w) increase hardness and chewiness in nitrite-free hot dog sausages (Ruiz-Capillas *et al.,* 2015); whereas the addition of chia flour (10% w/w) increased the hardness in frankfurters sausages (Pintado *et al.,* 2016).

Table S3. Texture analyses during storage (21 days at 4°C) of sausages added of sausages added with FCP (4, 8, and 12% w/w).

Samples[a]	Storage time (d)	Parameters[b]			
		Hardness (g)	Springiness (mm)	Cohesiveness	Chewiness (g*mm)
Control[c]	0	5416.81 ± 103.68 ghi	0.82 ± 0.01 a	0.32 ± 0.01 ab	1427.38 ± 69.29 ab
	7	5368.57 ± 88.25 hi	0.81 ± 0.01 a	0.29 ± 0.01 def	1264.22 ± 59.89 cd
	14	5577.63 ± 147.70 gh	0.82 ± 0.01 a	0.32 ± 0.01 b	1461.67 ± 51.62 a
	21	5632.92 ± 129.18 gh	0.81 ± 0.01 a	0.28 ± 0.01 def	1317.48 ± 71.38 bc
4% FCP	0	5270.82 ± 76.63 ij	0.71 ± 0.01 bc	0.33 ± 0.01 a	1240.74 ± 52.96 cd
	7	5575.25 ± 106.32 gh	0.68 ± 0.02 c	0.31 ± 0.01 bc	1198.75 ± 55.19 cd
	14	5640.19 ± 84.22 fgh	0.74 ± 0.01 b	0.32 ± 0.01 b	1330.17 ± 47.68 abc
	21	5907.55 ± 97.99 ef	0.69 ± 0.01 c	0.30 ± 0.01 cd	1255.12 ± 56.96 cd
8% FCP	0	5022.18 ± 70.15 j	0.63 ± 0.02 d	0.29 ± 0.01 de	933.95 ± 38.27 f
	7	5662.10 ± 52.09 efg	0.60 ± 0.01 de	0.28 ± 0.01 def	966.06 ± 25.45 f
	14	5927.80 ± 78.60 de	0.61 ± 0.02 d	0.29 ± 0.01 def	1039.89 ± 46.97 ef
	21	6197.06 ± 105.25 cd	0.55 ± 0.02 fg	0.27 ± 0.01 f	935.39 ± 35.90 f
12% FCP	0	6210.35 ± 56.37 c	0.53 ± 0.01 gh	0.29 ± 0.01 de	951.79 ± 31.17 f
	7	7097.82 ± 86.81 a	0.46 ± 0.01 i	0.28 ± 0.01 ef	920.57 ± 23.26 f
	14	7229.11 ± 78.71 a	0.50 ± 0.01 hi	0.29 ± 0.01 de	1054.03 ± 36.26 ef
	21	6748.77 ± 147.91 b	0.57 ± 0.01 ef	0.30 ± 0.01 cd	1157.78 ± 46.67 de
Significance[d]					
CPC		***	**	***	***
S		***	***	***	***
CPC x S		***	***	***	NS

[a] All values are mean ± standard error of 20 replicates.

[b] Different letters in the same column indicate significant differences by the LSD test ($p < 0.05$).

[c] Control= 0% FCP.

[d] Asterisks indicate significant difference by ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Abbreviations: NS = non-significant; FCP = functional carrot powder; CPC = carrot powder concentration; S= storage time.

A.1.2.2 Effect of functional carrot powder (FCP) addition at 0, 4, 8, and 12% (w/w) on the carotenoid and chlorogenic acid content of sausages added with 0, 4, 8, and 12% (w/w).

Chlorogenic acid, carotenoid and retinol equivalents content were determined in sausage formulations added with 4, 8, and 12% FCP, in order to determine their stability to thermal treatments during sausages manufacturing (**Table S4**). The content of chlorogenic acid in the FCP ingredient used to produce these formulations was 4563.92 mg/kg; whereas the α-carotene, β-carotene, lutein and retinol equivalents (RE) content were 179.01 mg/kg, 308.16 mg/kg, 55.3 and 66.27 mg/kg, respectively. As expected, neither chlorogenic acid nor carotenoids were detected in sausage with 0% FCP.

The content of CHA in the 4%, 8% and 12% FCP formulation were 11.67, 31.98 and 48.70 mg/100 g, respectively (**Table S4**); whereas, the theoretical values calculated of CHA (based on CHA content in FCP ingredient) were 18.25, 36.51, and 54.76 mg/100 g, respectively. These results indicate that there is a slight degradation of CHA during sausage manufacturing, which is mainly attributed to decarboxylation and oxidative reactions induced during thermal processing (Jiang & Peterson, 2010).

Regarding the carotenoid content, the theoretical values calculated for α-carotene/β-carotene/lutein/RE were 0.71/1.23/0.57/260, 1.43/2.46/0.59/530, 2.14/3.69/0.61/790 mg/100 g, for 4%, 8% and 12%, respectively. As shown in **Table S4**, the content detected in sausage formulations were slightly higher than the theoretical values.

Table S4. Carotenoids and chlorogenic acid content in sausages added with 4, 8, and 12% FCP.

Samples[a]	Carotenoids[b] (mg/100g)			Retinol Equivalents (µg/100g)	Chlorogenic[c] acid (mg/100g)
	α-Carotene	β-Carotene	Lutein		
Control[d]	ND	ND	ND	ND	ND
4% FCP	1.07 ± 0.05 c	1.58 ± 0.10 c	0.57 ± 0.01 c	351.93 ± 20.62 c	11.67 ± 1.06 c
8% FCP	1.50 ± 0.01 b	2.43 ± 0.01 b	0.60 ± 0.01 b	530.16 ± 2.02 b	31.99 ± 1.03 b
12% FCP	2.05 ± 0.03 a	3.45 ± 0.07 a	0.62 ± 0.01 a	746.07 ± 13.44 a	48.71 ± 3.08 a

[a] All values are mean ± standard error of three replicates.
[b] Values with different letter within columns indicate statistically significant difference by the LSD test ($p < 0.05$). Units are in β-carotene equivalents.
[c] Units are in chlorogenic acid equivalents.
[d] Control= 0% of FCP.
Abbreviations: FCP = functional carrot powder; ND= no detected.

This could be attribute to the heat treatments applied to formulations during sausage manufacturing, as well as to the fat content in formulations, since they have been associated with an increased extractability and bioavailability of carotenoids in foods (Boon et al., 2010; Stahl & Sies, 1996). According with the retinol equivalents calculated in each formulation, one portion (62.5 g, 1 sausage) of the 4%, 8%, and 12% FCP formulations provided ~39%, 72% and 101%, respectively, of the dietary reference value of retinol equivalents (568 µg per day) for the Mexican population (SEGOB, 2010).

A.1.2.3 Sensory acceptability test of sausages added different concentrations (0, 4, 5, 6 and 8% w/w) of functional carrot powder (FCP)

Sensory evaluation tests to determine optimum FCP concentration in sausage formulations were performed in two different set of experiments. Formulation with 12% FCP was discarded for the sensory evaluation, since its organoleptic characteristics were extremely different as compared with 0% FCP sausage. The first set of sensory evaluations included 0, 4, and 8% FCP formulations (**Table S5**). In general, the 8% FCP formulation showed lower acceptability values in all parameters evaluated (appearance, flavor, texture, overall acceptability). On the other hand, for the 4% FCP formulation, appearance values slightly decreased as compared with the control. However, the overall acceptability, which is the most important parameter in the sensory acceptability test, shown no significant difference between 4% FCP formulation and then control. Comments provided by the consumers in the survey were analyzed in search for reasons that could explain the collected data. For both formulations (4% and 8% FCP), appearance was the most commented parameter, since some people referred to the color as “darker, orange-brownish, strange, and unusual.” Results from instrumental color analysis (**Table S2**) correlates with the comments obtained from consumers, since the darker and orange-brownish color is associated with decrement in the L (lightness) value and with increases in the b* values. Color of meat is an important quality attribute that influences consumer acceptance of meat and meat products. Interestingly, for some consumers, the changed in color of 4% FCP formulation was positive since it was related with a better-quality meat.

Likewise, texture is a key quality attribute in the fresh and processed food industry. Results from texture analyses (**Table S3**) were correlated with the data obtained from the sensory acceptability test **(Table S5)**. For instance, participants agreed in the increment of hardness as the concentration of FCP increased. In general, there were negative comments related with texture from 8% FCP formulation, since it was perceived as harder, fibrous and lumpy. This is due to the addition of carrot fiber, especially insoluble fraction as previously described.

Table S5. Sensory acceptability values of sausages added with 4% and 8% (w/w) of functional carrot powder (FCP).

Parameters	Samples[a]		
	Control[b]	4% FCP	8% FCP
Appearance	7.72 ± 0.12 a	6.66 ± 0.15 b	5.68 ± 0.19 c
Flavor	7.15 ± 0.15 a	6.77 ± 0.16 a	5.58 ± 0.20 b
Texture	7.24 ± 0.15 a	6.84 ± 0.17 a	6.06 ± 0.18 b
Overall Acceptability	7.23 ± 0.15 a	6.85 ± 0.15 a	5.66 ± 0.18 b

[a] Different letters in the same row indicate significant differences by the LSD test ($p < 0.05$).
[b] Control= 0% of functional carrot powder.

Since 8% FCP acceptability values were between 5 and 6, i.e. “Neither like nor dislike” and “Like slightly”, it was decided to discard this formulation, and a second sensory acceptability test was performed to determine if FCP added at levels between 4 and 8% were acceptable by consumers. Results from the second sensory acceptability test are shown in **Table S6**. In general, as the concentration of FCP in the formulations was increased, a slight decrease in the acceptability values of appearance and texture of the sausages was observed. However, all values were above the acceptable range (between 6 and 7 i.e. “Like slightly” and “Like moderately”). For flavor parameter, there were no significant differences between 4% and the control. This result is similar to the values obtained in the first acceptability test **(Table S5)**. However, the 5% and 6% FCP formulation showed lower acceptability values.

Table S6. Sensory acceptability values of sausages added with different concentrations of functional carrot powder (FCP).

Parameters	Samples[a]			
	Control[b]	4% FCP	5% FCP	6% FCP
Appearance	7.51 ± 0.15 a	6.73 ± 0.14 b	6.23 ± 0.18 c	6.19 ± 0.17 c
Flavor	7.02 ± 0.15 a	6.71 ± 0.16 a	6.09 ± 0.20 b	5.84 ± 0.21 b
Texture	7.37 ± 0.15 a	6.91 ± 0.14 b	6.51 ± 0.18 c	6.46 ± 0.17 c
Overall Acceptability	7.24 ± 0.14 a	6.89 ± 0.13 a	6.29 ± 0.18 b	6.10 ± 0.19 b

[a] Different letters in the same row indicate significant differences by the LSD test ($p<0.05$).
[b] Control= 0% of functional carrot powder.

Regarding the overall acceptability, addition of FCP at 4% did not affect the value, whereas 5% and 6% FCP formulations showed a decrease. Nevertheless, all the FCP formulations were maintained in the acceptance range (between 6 and 7 i.e. “Like slightly” and “Like moderately”).

According with the analyses of the sensory evaluation tests, 4% FCP was the most adequate formulation since it did not show significant differences as compared with the control. Likewise, in textural profile analyses 4% FCP formulation showed the results closest to the 0% FCP (control sample). For phytochemical analyses, results indicated that 4% FCP still contributes with high levels of nutraceuticals (mention hear the values). Thus, evaluating together all parameters the 4% FCP formulation was selected to further characterize its physicochemical characteristics and shelf-life stability.

Appendix B

Methodology of the microbiological analyses of sausages

To ensure microbiological safety of sausages prior to sensory evaluations, sausages added with 4% of CPC and FCP as well as the control were assayed for total mesophilic bacteria and lactic acid bacteria count. For microbial determinations, sausage samples (10 g) were put into sample bag (Whirl-Pak, Nasco, USA), diluted with sterile peptone water (0.1% w/v) (BD Bioxon, México) and homogenized for 2 min in a stomacher (IUL Instruments, Spain). Additional decimal dilutions in peptone water were prepared for plate count microbiological analyses. All analyses were performed in aseptic conditions inside a laminar flow cabinet. Triplicate counts were performed for all dilutions.

For lactic acid bacteria determinations, Sterile Difco TM Lactobacilli Mann, Rogosa Sharpe agar (MRS; Difco Laboratories, Dickinson and company, France) was prepared according to manufacturing specifications. Aliquots (0.1 mL) of serial decimal dilution of sausage samples were count by plate method and incubated at 37° C for 48 h. For total mesophilic bacterial determinations, general purpose Difco TM Tryptic soy agar (TSA; Difco Laboratories, Dickinson and company, France) were prepared according to manufacturing specifications and sterilized at 121°C for 15 min. The decimal dilutions of sausage sample were mixes and aliquots (0.1 mL) for the standard spread-plate method. Plates were incubated at 37°C for 24 h.

Results indicated that sausages were safe for human consumption, since no bacterial counts were detected in the different formulations before storage.

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This document was typed in using Microsoft Word by Melissa Alvarado Ramírez