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"Efecto del Extracto de Romero *(Rosmarinus officinalis) y* el Proceso de Fritura sobre la Calidad y Vida útil de Aceite y Productos Pre-fritos"

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La presentación de la tesis doctoral titulada "Efecto del extracto de romero (Rosmarinus officinalis) y el proceso de fritura sobre la calidad y vida útil de aceite y productos pre-fritos" realizada por Dña. María del Rocío Teruel Gutiérrez, bajo mi inmediata dirección y supervisión, y que presenta para la obtención del grado de Doctor por la Universidad de Murcia.

En Murcia a 23 de marzo de 2015

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"A veces sentimos que lo que hacemos es tan solo una gota en el mar, pero el mar seria menos si le faltara una gota" ***Maria Tersesa de Calcuta.***

RESUMEN

Los cambios socio-culturales que se ha producido en las últimas décadas han afectado fuertemente a los hábitos de consumo alimentario, y por ende, a la preparación y consumición de alimentos. El consumidor actual demanda alimentos de conveniencia, fáciles de preparar, que le permitan gestionar su falta de tiempo libre. Entre este tipo de alimentos destacan los productos fritos debido a sus fácil y rápido modo de preparación, su económico precio y sus deseables características organolépticas.

La fritura es esencialmente un proceso de deshidratación, en el cual el alimento es introducido en un medio graso a elevadas temperaturas (140-180 ºC). El proceso engloba una rápida transferencia de calor y masa entre el alimento y el medio de fritura, resultando en una sucesión de cambios físico-químicos tales como la gelatinización del almidón, la desnaturalización de las proteínas y las reacciones de Maillard. Dichos cambios son los responsables del aroma, color, textura y características organolépticas propios de este tipo de productos. Sin embargo, las condiciones de altas temperaturas a las que tiene lugar el proceso, unidas a la presencia de oxígeno y agua provocan el desarrollo de reacciones de descomposición (hidrolisis, oxidación, isomerización y polimerización). Los compuestos generados en dichas reacciones de descomposición provocan el decremento de la palatabilidad y las características nutricionales de los productos y aceites de fritura.

Otro punto crítico a tener en cuenta en el consumo de productos fritos es su alto contenido graso. Dado que actualmente existe una grave epidemia de obesidad que afecta tanto a los países desarrollados como a los países en vías de desarrollo, donde los alimentos ricos en azucares simples y grasas resultan extremadamente económicos. Si bien existen múltiples estudios que han correlacionado el consumo de productos fritos

con el incremento de riesgos en la salud, no existe ninguna señal que sugiera que el consumo de este tipo de productos disminuirá en un futuro. Por el contrario, la popularidad de este método se evidencia en el gran incremento del consumo producido en los últimos años. No obstante, este hecho converge con otras tendencias de la sociedad. Los consumidores no solo demandan productos de conveniencia, sino que a su vez solicitan productos saludables y sostenibles. Por todos estos motivos, la presente tesis tiene como objetivo principal mejorar la calidad y estabilidad del medio y los productos de fritura. Con este propósito han sido estudiadas diferentes estrategias y tecnologías emergentes como la aplicación de antioxidantes naturales extraídos del romero (*Rosmarinus officinalis*), la fritura bajo condiciones de vacío y la fritura por medio de aire caliente.

La aplicación de antioxidantes es una práctica común en la industria de la fritura. En esta línea, la aplicación de antioxidantes sintéticos como el hidroxianisol butilado (BHA) y el Butil hidroxitolueno (BHT) ha sido frecuentemente usada para mejorar la vida útil del aceite de fritura. Sin embargo, estudios recientes han asociado este tipo de compuestos con fenómenos de carcinogénesis y mutagénesis. Lo que ha provocado una nueva tendencia de consumo que solicita "productos naturales".

Entre las múltiples fuentes de antioxidantes naturales destaca el romero (Rosmarinus officinalis L.), un arbusto aromático propio de la región mediterránea cuya aplicación como conservante cárnico ha sido recientemente autorizada por la Unión Europea bajo la Directiva 95/2/EC y al cual le han asignado el numero E-392 (Directivas de la Union Europea 2010/67/EU y 2010/69/EU). Los extractos de romero poseen un gran número de compuestos que actúan como fuente de actividad antioxidantes, mayoritariamente

diterpenos fenólicos como el ácido carnosico, carnosol, rosmanol, epirosmanol, isorosmanol, metil carnosato, y también, ácidos fenólicos como por ejemplo el ácido rosmarínico. Los compuestos con mayor actividad son el carnosol y ácido carnósico. La actividad antioxidante de los compuestos fenólicos del romero es el resultado de su capacidad de eliminar radicales libres debido a su estructura química. En el presente estudio, tres extractos comerciales de romero (*Rosmarinus officinalis*) obtenidos con diferentes procedimientos (combinaciones de solvente-formato) fueron caracterizados. Posteriormente se evaluó el efecto de los mismos en nuggets de pollo congelados y en la vida útil del aceite de fritura.

Los resultados mostraron que tanto el formato como el solvente afectaron sobre el contenido de ácido carnosico y carnosol extraído, así como, en la concentración de compuestos fenólicos. Observándose una relación directa entre la actividad antioxidante y la concentración de compuestos fenólicos. Concluyendo, que el extracto de acetona en polvo mostro el mayor potencial antioxidante seguido del extracto liquido extraído con acetona y extracto liquido extraído con metanol. Para evaluar el efecto antioxidante de los extractos se realizaron dos ensayos. El primer ensayo evaluó la aplicación de los tres extractos sobre nuggets de pollo pre-fritos a lo largo del proceso de almacenamiento en congelación. Con la finalidad de prevenir las reacciones de oxidación lipídica, que se considera la principal forma de deterioro en productos cárnicos durante el almacenamiento y puede continuar su desarrollo durante el almacenamiento en congelado. La dosis utilizada fue la dosis máxima propuesta por la Directiva de la Unión Europea 2010/69/EU, la cual determina la cantidad límite de 150 ppm (mg/kg de grasa) de ácido carnosico y carnosol en productos cárnicos. Los análisis físico-químicos y sensoriales fueron realizados en las muestras a los 0, 3, 6, y 9 meses de

almacenamiento en congelación. Los resultados indicaron que los extractos no afectaron a las características físico-químicos (color, pH) y de calidad sensorial del producto, lo cual demuestra que la aplicación de este tipo de extractos puede ser una alternativa en la industria de los productos pre-frito. El efecto sobre la oxidación lipídica no pudo observarse a lo largo del periodo de nueve meses de almacenamiento, probablemente debido a que el proceso de congelación evita el deterioro del producto. Cabe destacar, que en el último punto de control (9 meses) los nuggets tratados con extracto de romero mostraron una leve tendencia protectora. Para confirmar este efecto antioxidante sería recomendable realizar un estudio de almacenamiento más largo.

Un segundo ensayo se diseñó con el objetivo de valorar la capacidad protectora del extracto de romero durante la fritura por inversión en aceite de girasol. La oxidación lipídica es una reacción en cadena promovida por los radicales y es una de las causas más importantes del deterioro en los aceites vegetales. Los hidroperóxidos generados en la oxidación, debido a las altas temperaturas alcanzadas durante el proceso de fritura, se descomponen con facilidad formando aldehídos, cetonas, ácidos, ésteres, alcoholes e hidrocarburos de cadena corta. Estos subproductos disminuyen la palatabilidad y calidad nutricional de los aceites, y además, provocan una pérdida importante de aceptabilidad en los productos fritos. En el presente ensayo se aplicó una dosis de 50 mg de ácido carnosico y carnosol por kg de aceite de girasol, coincidiendo con lo legislado por la EFSA para aceites destinados a fritura. El proceso de fritura se llevó a cabo a 180 ºC a lo largo de un total de cuatro ciclos de fritura. Realizándose los siguientes análisis: estabilidad oxidativa, compuestos polares totales, viscosidad, color, contenido de ácido carnosico y carnosol sobre el aceite de fritura y análisis sensorial sobre el producto frito. Los resultados indicaron que el aceite enriquecido con romero

mostraba una mejora de la estabilidad oxidativa y una disminución de la generación de compuestos polares, sin modificar las características organolépticas del producto frito. Sin embargo, la adición de extracto de romero al máximo legal permitido no mostro efecto sobre los valores de viscosidad y color, probablemente debido a la degradación de los diterpenos durante la fritura a altas temperaturas (180 ºC). Concluyendo que el enriquecimiento del aceite con compuestos activos de romero, al máximo legal permitido, no fue suficiente para obtener la protección total del aceite.

La fritura a vacío ofrece algunas ventajas como la disminución de la temperatura o la disminución de la concentración de oxígeno durante el proceso de fritura. Esta tecnología se caracteriza por llevar a cabo el proceso de fritura a presión sub-atmosférica, preferiblemente por debajo de 50 Torr (6,65 KPa), donde tanto el punto de ebullición del agua como el del aceite disminuyen. Han sido múltiples las ventajas atribuidas a este proceso, entre las que se encuentran: (1) disminución del contenido de aceite absorbido en el producto frito; (2) la mejora de la conservación del color y sabor propio debido a las bajas temperaturas y a la ausencia de oxígeno durante el proceso; (3) el incremento de la vida útil del aceite de fritura; (4) la disminución del contenido de acrilamida, o (5) la conservación de nutrientes. Razones por las cuales la fritura a vacío fue valorada como alternativa para en el procesado y la obtención de nuggets de pollo más saludables, determinando también las condiciones óptimas-fritura al vacío. El estudio considero tres niveles temperaturas (130, 140 y 150 ºC) del aceite durante el proceso de fritura a vacío. En cada una de ellas se tomó muestra a los 2, 4, 6 y 8 minutos de fritura. Para evaluar el efecto del proceso de fritura a vacío, las características de composición, color, textura y sensoriales de los producto fueron analizadas y se compararon con nuggets de pollo cocinados en condiciones atmosféricas

a una temperatura 160 ºC (tradicional). Las temperaturas evaluadas en el proceso a vacío fueron suficientes para el desarrollo de las reacciones de Maillard, aunque la evolución de estas fue más lenta que la sucedida en condiciones atmosférica, esto es debido a que pardeamiento no enzimático es altamente dependiente de la temperatura. La disminución del punto de ebullición en las muestras fritas en condiciones de vacío produjo un descenso inicial de la humedad más rápido, lo conllevo una caída adicional de la luminosidad. El tratamiento a vacío no produjo nuggets de pollo con un menor contenido graso, probablemente debido a la uniforme costra que recubre el producto y que se forma durante el proceso de pre-fritura, la cual es el principal obstáculo en la absorción de aceite para ambos procesos (vacío y atmosférico). El proceso de fritura a vacío en las condiciones estudiadas produjo productos con una composición y características sensoriales similares a las obtenidas por el procedimiento tradicional. Los consumidores no mostraron preferencia alguna para el atributo "aceptación general" ni "oleosidad" para los productos preparados por ambos métodos, aunque determinaron que los productos obtenidos a vacío eran más crujientes. Por tanto, la fritura a vacío puede ser considerada como una alternativa en la producción de productos empanados. Considerando interesante para futuras investigaciones incluir otro tipo de parámetros a evaluar, tales como el contenido de micronutrientes y productos del deterioro en el aceite y el producto, que pueden verse mejorados a consecuencia de la disminución de las temperaturas de cocinado caracteristica en condiciones de vacío.

En último lugar, se estudió la tecnología de aire caliente como posible alternativa en la producción de productos saludables con características sensoriales deseables (textura, color, aroma y sabor). La fritura con aire caliente tiene como objetivo producir "alimentos fritos" por medio de aire caliente y una pequeña cantidad de aceite en lugar de sumergirlo en aceite caliente, tal y como se hace de forma tradicional. El producto se

deshidrata durante el proceso y gradualmente comienza a aparecer la corteza típica de los productos fritos. La cantidad de aceite usado es significativamente menor a la utilizada en la fritura por inversión en aceite, y como resultado, los productos poseen un muy bajo contenido en grasa. Actualmente, una gran variedad de freidoras de aire están disponibles en el mercado, pero no existen publicaciones científicas que aborden el efecto de esta tecnología sobre las características de calidad del producto. Para evaluar esta tecnología emergente, se planteó un último ensayo, en el cual se usó como materia prima patatas y se valoró el proceso a los largo de 30 minutos, para un mejor entendimiento a su vez se comparó con el proceso de fritura tradicional (inversión en aceite caliente). La toma de muestras para ambos tipos de fritura se realizó cada 3 minutos y las muestras eran sometidas a análisis físico-químicos. Aunque los análisis se realizados sobre el producto en una escala de tiempo prolongada (30 min), el producto final se definió conforme a los criterios de calidad fijados por la industria, que estipulan que el contenido de humedad del producto final debe de poseer un contenido de entre 38% y 45 % de su peso. En el producto final también se llevaron a cabo los análisis de color, la textura, composición, y además se realizaron análisis de microestructura, propiedades calorimétricas y sensoriales. Este estudio determinó que la evolución de la temperatura, el contenido de humedad, y el color fueron significativamente más lentos. Como consecuencia, son requeridos tiempos más largo de cocción en el caso de freír con aire caliente. La evaluación del producto final de ambos tratamientos confirmo que para contenidos de humedad similares, los productos cocinados con tecnología de aire caliente mostraron un contenido de aceite significativamente menor y características de color similares. Las diferencias encontradas a nivel visual, de textura y características sensoriales entre ambos productos se deben a los diferentes grados de gelatinización del almidón. Los análisis de SEM y DSC determinaron que las muestras tratadas con aire

caliente mostraban un menor grado de gelatinización que las muestras cocinadas por inmersión en aceite caliente. El flujo de transferencia de calor fue diferente dependiendo del tratamiento, estimando que el flujo de calor fue 3,7 veces mayor en el tratamiento de fritura tradicional. La tasa de transferencia de calor tuvo un efecto directo sobre la gelatinización del almidón y el proceso de evaporación. En las muestras de aire la evaporación se produjo lentamente dando lugar a la formación de una corteza más delgada, homogénea y sin irregularidades, lo que proporciona una sensación en boca diferente. Las observaciones visuales también mostraron diferencias entre tratamientos, las muestras tratadas con aire caliente mostraron mayor expansión de la corteza y una distribución porosa del núcleo más regular. Durante el enfriamiento, se produjo la contracción de la corteza en las muestras tratadas con aire, fenómeno que no tuvo lugar en las muestras cocinadas en aceite. En lo que se refiere al núcleo, ambos productos mostraron un aspecto gelatinizado, aunque el grado de gelatinización fue mayor en los productos fritos por inversión en aceite. En general, el proceso de fritura mediante aire caliente permite obtener productos con menor contenido de graso, sin embargo las características sensoriales de los mismos diferentes de las obtenidas en la fritura tradicional. Los resultados de este estudio permitirán a la industria avanzar en el desarrollo y perfeccionamiento de la tecnología de fritura por medio de aire caliente y así obtener productos similares a los obtenidos por medio del proceso de inversión en aceite, por lo que en un futuro este proceso podrá considerarse una alternativa.

SUMMARY

Societal changes produced in recent decades are having a dramatic influence on food preparation and consumption. The search of free time by consumers has driven the demand of convenience foods, easy-to-prepare products. Among them emphasize the fried products due to the fact that besides their easy and fast cooked, these are cheap products with desirable sensory characteristics.

Frying is essentially a dehydration process that involves rapid heat and mass transfer in food immersed in hot oil (140–180 ºC), which leads to a succession of physical and chemical changes in the product such as starch gelatinisation, protein denaturation and Maillard reactions. These changes produced the unique flavors, colors and textures characteristics of fried foods. But at the same time the exposition at high heat in presence of water and air which produce decomposition processes (hydrolysis, oxidation, isomerization and polymerization) that give rise to the formation products that decrease of palatability, nutritional quality, and could be toxic of edible oils and fried products. Other critical point is the fat uptake due to the epidemic obesity that currently prevalent in developed and even in developing regions, where meals high in fat and sugar are the cheapest. Regardless of the many studies correlating fried product consumption with rising of health risks, there is no sign to suggest that we will give up eating fried products. On the contrary, their popularity of method is evidenced by a great increase in fried food consumption in the recent years. However, this fact converge with other society trends. Consumer's demand convenient products with additional attributes, such as foods that are healthy, ethical, and comforting.Due to all these reasons this thesis is focused on the improvement of quality and stability of frying medium and fried products. For this purpose has been evaluated use of different emerging technologies and strategies such as the application of natural antioxidants

from rosemary (*Rosmarinus officinalis*), frying under vacuum conditions, and fried by hot air.

The application of antioxidants is a common practice in the frying industry. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been commonly used to improve frying oil shelf life. However, recent studies have associated these compound with carcinogenesis or mutagenesis phenomena. This have resulted in a consumer trend towards "natural products". Among the natural antioxidant sources is found rosemary (Rosmarinus officinalis L.), a woody aromatic herb that is native to the Mediterranean countries. This herb has recently been authorized by the European Union under Directive 95/2/EC and assigned E-392 as its E number (European Union Directives 2010/67/EU and 2010/69/EU) for use in meat product preservation. Rosemary extract contains a large number of compounds that act as a major source of natural antioxidants, mainly phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methyl carnosate, phenolic acids, such as rosmarinic acid. The most active components are carnosol and carnosic acid. The antioxidant activity of these phenolic compounds of rosemary is the result of their ability to scavenge free radicals due to their chemical structure.In this study, three commercial rosemary extracts (*Rosmarinus officinalis*) obtained in different ways (format-solvent combinations) were characterized. Then was evaluated the effect of these extract on frozen chicken nuggets and on frying oil shelf life.

The results showed that format and solvent used in rosemary extracts influenced the carnosic acid and carnosol concentration and the amount of phenolic compounds obtained. Therefore, a direct relation between the antioxidant activity and concentration

of phenolic compounds was observed. Concluding the powder acetone had the higher antioxidant potential followed by liquid methanol and liquid acetone.To evaluate the antioxidant effect of these rosemary extracts trials were made. The first one evaluated the three extracts application on pre-fried chicken nuggets along frozen storage. In order to prevent lipid oxidation reactions, which are considered the major deterioration form in stored muscle foods and this may still occur during frozen storage. The dose used was the maximum dose proposed by the European Union Directive 2010/69/EU for the carnosic and carnosol compounds [150 ppm (mg/kg fat basic)] in meat products. Physical-chemical and sensory measurement were carried out in the samples after 0, 3, 6 and 9 month of frozen storage. The extracts had no effect on the physical-chemical characteristics (color, pH) and sensory quality of the product that pointed out to their potential use as alternatives in the production of pre-fried products. The effect on lipid oxidation was not observed until the ninth month of storage, probably because the freezing processavoid the deterioration of the product. At 9 months a slight tendency antioxidant was observed in the nuggets with rosemary extracts, but possibly a longer storage would be required to confirm this subject.

A second trial was focused in the study of application the of rosemary extract on sunflower oil to preserved it of lipid oxidation during deepfat frying. Lipid oxidation is a catalytic process involving a free radical chain reaction mechanism, is an important cause of quality loss in vegetable oils, since it involves the generation of hydroperoxides. At high temperature, such as those reached during the frying process, hydroperoxides are readily decomposed to alkoxy radicals to form aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons. These products decreased palatability and nutritional quality of edible oils and promote an important loss of

acceptability on the fried products. In the assay, the maximum dose established by EFSA for rosemary extract in frying oils (50 mg of carnosic acid and carnosol/kg oil) was applied. Frying process was carried out at 180 ºC along a total of four frying cycles. Oxidative stability, total polar compounds, viscosity, color, carnosol and carnosic acid content oil and sensory analysis of fried product were evaluated. The results showed that rosemary extract improved the oxidative stability and diminished the generation of polar compounds in sunflower oil, without modifying the organoleptic characteristics of the fried product. However, rosemary extract addition at the legal maximum had no effect on the viscosity and color values, probably due to the degradation of diterpenes during the cycles at high temperature (180 ºC). Consequently, the application of the legal maximum of the active compounds cannot be deemed sufficient to provide complete protection to the oil.

Vacuum cooking offers some advantages such the decreased of temperature or the limited the oxygen content during cooking process that make it an interesting alternative in the fried industry. This technology is defined as frying carried out below atmospheric pressure, preferably below 50 Torr (6.65 kPa) when the boiling points of both the oil and the water contained in the foods are lowered, several advantages has been attributed to this technology such as: (1) can reduced oil content in the fried product; (2) the preservation of natural color and flavors due to the low temperature and the absence of oxygen during the process; (3) fewer adverse effects on oil quality; (4) a decreased acrylamide content, and (5) the preservation of nutritional compounds.For this reason, vacuum frying was evaluated as an alternative process to make chicken nuggets healthier and determinate the optimum vacuum-frying conditions. Three levels of oil temperature for vacuum-frying (130, 140, and 150 ºC) were considered in this study.

For each temperatures were analyzed 2, 4, 6 and 8 min of frying. To evaluate the effect of vacuum-frying on the products characteristics the compositional, color, textural and sensory properties of vacuum fried chicken nuggets were evaluated and compared with the properties of deep fat fried nuggets.The temperatures evaluated in this study for vacuum frying were sufficient for the evolution of Maillard reactions though the evolution was slower than in atmospheric frying because non-enzymatic browning is highly temperature-dependent. The decreased boiling point of samples fried under vacuum produced an initial faster rate of moisture loss and thus an additional fall in luminosity. The vacuum frying treatment did not produce nuggets with a lower fat content than atmospheric conditions, the formation of an uniform coating on the surface of chicken meat during the dipping and pre-frying phase being the main hurdle for the mass transfer of oil during both types of frying.The process of frying under vacuum in the conditions studied provided products with compositional and sensory attributes similar to those of chicken nuggets fried in atmospheric conditions. Consumers did not show any significant preferences concerning “overall acceptance” and “oiliness” for products prepared by either of these methods, but determined as more crunchy the nuggets cooked under vacuum conditions. Therefore vacuum frying can be considered a worthwhile alternative for making fried batter products. However, it would be recommended to future researches include other parameters such as micronutrients and deterioration product formed in the oil and in the product itself as a consequence of high temperatures of traditional frying and that could be reduced by decreasing of temperature that allows the vacuum-frying process.

Finally, hot air technology was studied as a possible alternative to produce healthier products without compromising on the desirable appearance, texture, flavour and taste

attributes. Hot air frying, which aims to produce a “fried product” with hot air and a small oil amount around material instead of immersing it in hot oil. The product gets dehydrated in the process and the typical crust gradually appears on the product. The amount of oil used is significantly lower than in deep oil frying giving, as a result, very low fat products. Currently, a variety of air fryers are available in the market, but there is not scientific publication about the effect of hot air frying in product quality characteristic.To evaluate this emergent technology, the hot air frying was studied on potatoes along of 30 minutes and was compared with deep-oil frying process. Samples were removed from the frying equipment at 3 min intervals in both process, for up to a maximum of 30 minutes, and subjected to physico-chemical analysis. Although the above analyses were carried out over an extended time scale, the final product was defined in accordance with the quality control criteria set by frying industry, which stipulates that the moisture content of the ideal product must be in the range between 38% and 45% on a wet weight basis. On the final product were also carried out analysis of color, texture, microstructure, calorimetric properties and sensory characteristics.This study shows that the evolution of temperature, moisture content, and color were significantly slower in the case of air frying than deep fat frying. As a consequence, longer cooking times are required in the case of air frying.The evaluation of final product confirmed that for similar moisture content, air fried products have a significantly lower oil content and similar color. However, the two types of frying also resulted in products having significantly different texture and sensory characteristics. The differences found in visual, texture and sensorial characteristics of the two products are due to the different degrees of starch gelatinization. SEM and DSC analyses determined that air fried samples had a lower degree of gelatinization than deep fat fried samples. The heat flux was different in each treatment, in the case of deep-oil frying is

estimated that the heat flux is 3.7 times greater than in the case of air frying. The rate of heat transfer had a direct effect on starch gelatinization and evaporation process. A slowly evaporates was produced in air samples causing the surface crust to be thinner, homogeneous and without irregularities, which gives a perceptible difference in mouth feel. The visual observations of the crust also showed that air-fried samples expanded to a greater extent and contained regular pore distribution in core region in contrast to deep fat fried samples. Furthermore, during cooling too, the air-fried samples showed crust shrinkage, which was not observed in deep fat fried product. As far as the core is concerned, both products showed gelatinized appearance, although the extent of gelatinization was higher in the deep fat fried product.Overall, air frying process permits the manufacture of lower fat content products, though these products have different sensory characteristics. The results of this study will enable industry to find ways and means of converging product characteristics obtained by the two processes, so that the processes can be viewed as being truly alternatives.

ÍNDICE

1. INTRODUCTION

1.1. PRE-COOKED FROZEN FOOD

Broad changes in recent decades, including the increasing number of women in the workforce, changes in consumer purchasing patterns, busy lifestyles and the availability of a wide range of frozen products in different categories have been led factors tacking to the growth of the frozen food market and have it turned into one of the most dynamic and largest sectors of the food industry (Albert, 2011). Precooked frozen meals are products resulting from an uncompleted cooked, packaged subsequently and subjected to a process of preservation by cold. These are an alternative to fresh, canned and cooked foods because frozen processed food is easier to prepare than fresh food and can be stored in freezers for extended shel-life, because low temperatures (-9.5 ºC) prevent the growth of microorganisms, which helps to slow down the process of decomposition and keep the food for longer time period. Also, these foods maintain the same nutritional values as fresh food, which makes them more preferred than fresh food. For these reasons, it is widely considered to be one of next generation of convenient ready foods.

Over the past year the sector reached 629,309 thousand Euros in sales in our country, Spain. In addition, the projected outlook for the frozen food sector is promising, with significant opportunities for new development in emerging markets. The global frozen prepared food industry is already experiencing strong progression and is expected to reach sales of 142 billion Euros by 2015 (Houston Chronicle, 2014).

Significant growth is likely to come from emerging markets in Asia, Latin America and Eastern Europe, rising incomes, changing lifestyles and interest in ethnic and international cuisine will continue to drive the success of the category. A good example

is China, the fastest increasing market for frozen foods in Asia. As the pace of life has increased, sales of frozen dumplings have experienced double digit yearly growth, reaching over 152 million Euros in 2011. Crucially, the basic raw materials used in the production of frozen food include chicken, potatoes, fish, pizza, beans, (Houston Chronicle, 2014).

Among frozen pre-cooked products highlights are fried products due to the fact that their cooked is convenient, fast, relatively inexpensive and have a desired sensory characteristics. Regardless of the many studies correlating fried product consumption with rising of health risks, there is no sign to suggest that we will give up eating fried products. On the contrary, their popularity of method is evidenced by a great increase in fried food consumption in the recent years, of which more than 20 million tons of world annual oil production is extensively utilized for frying (Gertz, 2004; Dana and Saguy, 2006; Tarmizi and Ismail, 2008; Tarmizi et al., 2013; Sayon-Orea et al., 2013).

1.2. THE FRYING OPERATION

Deep fat frying is defined as the process of cooking foods by immersing them in edible oil at a temperature above the boiling point of water, usually 150–200ºC. Oil is used as the heat transfer medium to dehydrate the food, acts as "lubricant" between the frying vat and food, and makes the fried product more palatable and desirable (Tarmizi and Ismail, 2008; Andrés-Bello et al., 2011; Dueik and Bouchon, 2011; Andrés-Bello, 2012).

It is one of the most olden methods of food preparation; Egyptian wall paintings show dough being the fried in oil, indicating that Europe and North Africa were using this

method in food preparation well before the new Era (Stier, 2004). Currently it is a well-established method of food preparation and is extensively employed in domestic as well as industrial practice due to its ability to create unique sensory properties, including texture, flavour and appearance.

During frying, a series of complex processes occur between the food being fried and the adjacent oil (Ziaiifar et al., 2008; Kalogianni and Papastergiadis, 2014). On the one hand, the food conducts chemical and physical transformation due to the high operating temperature range of 140–180 employed, such us dehydration, cooking, starch gelatinisation, protein denaturation and Maillard reactions (Ziaiifar et al., 2008). Whereas, on the other hand, the frying oil becomes a part of the fried product (Chiou et al., 2012), and so will become part of our diet. The oil is also a source of fat-soluble vitamins (A, D, E and K), phytosterols, polyphenols, triterpenic acids, carotenoids and provides essential fatty acids such as oleic, linoleic and linolenic acids, which are necessary for human metabolism and essential for normal growth and development and may be an important factor in the prevention and treatment of certain diseases (Giese, 1996, Simopoulos, 1999; Chiou et al., 2012). However, the benefits of oil, can be canceled when oil is exposed to heat, water and air, which generate a series of changes that result in its decomposition due to hydrolysis, oxidation, isomerization and polymerization processes (Choe and Min, 2007; Lalas, 2007; Karoui et al., 2011; Chiou et al., 2012). The formation of these products entails in the decrease of palatability, nutritional quality, and an increase in toxicity of edible oils and fried products (Boskou, 2003; Choe and Min, 2007; Lalas, 2007; Chiou et al., 2012).

1.2.1. Transference heat and mass

Phenomena occurring during deep fat frying are extraordinarily complex as it involves heat and mass transference reactions simultaneously. During the immersion in hot oil, the heat is transferred by convection from the oil to the external layer of the food and, as a consequence crust is developed with their characteristic color and flavor. While mass transference is characterized by water vapor output the food due to increase of temperature and oil enters into pores provided (Andrés-Bello, 2012).

According to Farkas et al. (1996 a, b), deep-frying period is divided into four stages: initial-heating, surface boiling, falling rate, and bubble end point. These are described as follow:

- Initial-heating is described as the initial immersion of a raw material into hot oil and is characterized by heating of food until boiling water temperature. Heat is transferred by natural convection from the oil to the food and via conduction through the food. The heat is required for raising the temperature and there is absence of water vaporization.

- The stage two is observed a few seconds after product immersion into hot oil. Heat is mainly use to vaporize water and the result is the outflow of steam bubbles from the product surface. Bubbling promotes mixed convective heat transfer by stirring the oil; this brings on an increasing of surface heat transfer. The initial superficial vaporization creates a porous dried overheated region and is generically called “crust”, where is generated main organoleptic characteristics of fried food.

- In the falling rate stage, the crust region continues growing and heating inside the core region is established at the boiling water temperature. The amount of moisture removed in this period may be relatively small, but the required time may be long due to decreased heat transfer rate associated to crust growing. During this period, transformations such as starch gelatinization and protein denaturation are developed in core region and improve digestibility of fried food.

- Bubble end point is characterized by the absence of bubbles from food to the oil, this occurs by the decrease of water inside the product. It is unusual that products are kept until this stage due to develops of hard texture, overcooking and oiliness taste on products.

1.2.2. Chemistry of frying oil

Exposure to excessive heat in the presence of water and oxygen produces changes in frying oil properties (Ziaiifa et al., 2008; Zhang et al., 2012a; Aladedunye et al., 2013). The physic-chemical changes taking place in oil during frying may be broken into three reactions types: hydrolysis, oxidation and polymerization (Figure 1).

The evaluation of oil quality is important not only because it is absorbed by the food and becomes part of the product, but also for its great influence on the full costs of the process.

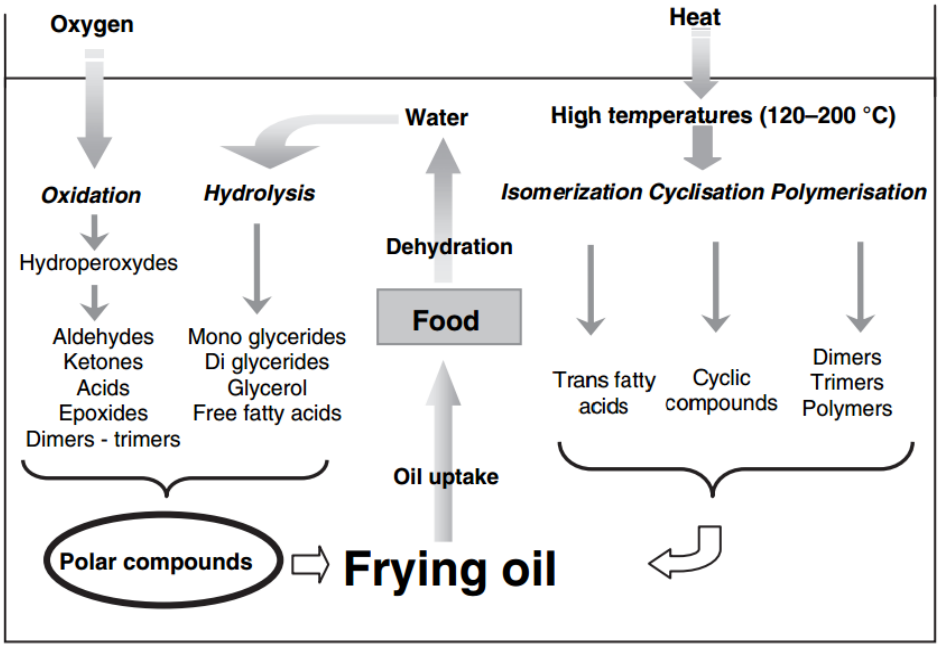


Figure 1. Outline of the oil degradation during frying and the consequences on the product quality (Ziaiifar et al., 2008).

1.2.2.1. Hydrolysis

Hydrolysis is one of the degradation reactions that occurs along the course of frying, where the water leached from food reacts with oil. Water breaks, a weak nucleophile, the ester linkages of triacylglycerol (TAG) and separates into diacylglycerol (DAG), monoacylglycerol (MAG), glycerol and free fatty acid (FFA) (Figure 2) (Choe and Min, 2007; Dueik and Bouchon, 2011; Zhang et al., 2012a).

Moreover, through partial hydroperoxides decomposition of oil at high temperatures is observed a breakdown of these compounds in the presence of moisture and air (Bensmira et al., 2007).

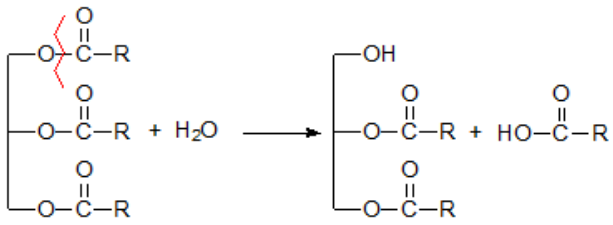


Figure 2. Formation of fatty acids and diacylglycerols (Dobarganes, 2009).

The free fatty acid content change with frying process time, therefore can be considered as indicator of the degree of hydrolysis. This is one and total polar compound levels are the most important measures in oil deterioration (Choe and Min, 2007). As result, FFA is widely used by food industry to determine the quality of used frying oil (Maskan and Bagci, 2003; Tarmizi and Siew, 2008). Respect to the discard point, there are several different approaches. Tseng et al (1996) suggests that frying oil should be discarded at levels around 1%, while others like Gupta (2005) determined that levels to be below 0.5%. Some countries have regulatory guidelines which provide the maximum level of FFA permitted in frying oil. For example, the United States Department of Agriculture provides that FFA content should be lower than 2%. The Japan limit is 1.25% and the limits of European countries are in a range from 1.0 at 2.5% (Sebastian et al., 2014). Some authors (Ismail, 2005; Tarmizi and Ismail, 2007; Aladedunye and Przybylski, 2013) related the discard point with the type of food cooked, for frying breaded and battered chicken product usually are considered a limit value around 2.0-2.5% in fast food chains.

Other method to evaluate the degree of oil hydrolysis is the determination of oil smoke point. This technique is usually used by cooks because is possible quantification by visual observation and is highly correlated with FFA content (Sebastian et al. 2014). The smoke point can be described as the lowest temperature in which the oil start to

release smoke. This temperature are inversely proportional to the content of low molecular constituents (FFA, DAG, MAG ...) produced in hydrolysis process (Tarmizi and Ismail, 2008).

There are numerous determining factors in hydrolysis oil degradation, such as water content, oil structural properties or frying conditions. The water content is one of the most important parameters, this is because it acts as reactant in the hydrolysis y the amount affect directly to the reaction. For this reason, the water content of food products used along frying is a limiting factor for oil shelf life (Dana et al., 2003; Choe and Min, 2007). Moreover, the chemical structure of oil also affect the hydrolysis degree. Choe and Min (2007) explained that water has easier access to short and unsaturated fatty acids than long and saturated fatty acids, since these are more water soluble and therefore water can access easily to break the chains. Regarding frying conditions it is well-demonstrated that hydrolysis and therefore FFA increase with time and temperature, as long as, there are not replacement of frying oil with fresh oil during the process. In the case of frequent replacement (as continuous frying) is produced a trend change, FFA level increase in initial stage and then remains constant (Ismail, 2001, 2005; Choe and Min 2007).

1.2.2.2. Oxidation

Oxidation is the most important reaction leading to oil deterioration and is defined as interaction between oxygen with unsaturated fatty acids. This decomposition reaction has raised a major issue from the quality point of view, by affecting the aroma, flavour, colour and nutrient content, as well as other health related attributes in the fried product (Dana and Saguy, 2001).

Oxidation of unsaturated fatty acids occurs via a free radical chain reaction, which are divided in three sub-processes denominated initiation, propagation and termination (Figure 3) (Shahidi and Zhong, 2005). Initiation stage involves the subtraction of hydrogen of an unsaturated fatty acids, in the presence of initiators such as heat, trace metals and light, and is formed an alkyl radical (Lawson, 1995; Dobarganes, 2009).

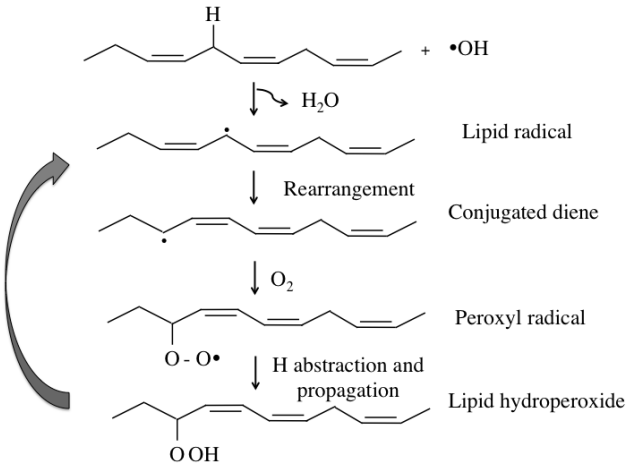


Figure 3. Simplified oxidation scheme (Jairam et al., 2012).

Afterward, the alkyl radicals reacts with oxygen to result peroxyl radicals, which in turn reacts with new triacylglycerol molecules giving rise to the formation of hydroperoxides and new alkyl groups . This period is generally referred to as propagation stage and their products are called as primary products of oxidation (Shahidi and Zhong, 2005; Tarmizi and Siew, 2008; Dobarganes, 2009; Casarotti and Jorge, 2012; Zhang et al., 2012a). In the termination stage, these hydroperoxides are decompose into other forms more stable (aldehydes, ketones, alcohols, hydrocarbons and acids), which are termed secondary products of oxidation (Choe and Min, 2007; Lalas, 2007).

Numerous analytical methods are used for measuring lipid oxidation, primary oxidation is routinely expressed in terms of peroxide value (PV) or the diene conjugation value. However, both are considered as unstable to measure oil oxidation due to peroxides are highly susceptible to decomposition in secondary oxidation products (Matthäus et al., 2009; Debnath et al., 2012). For this reason, there are another kinds of analyzes, as Thiobarbituric acid test or p -Anisidine Value, have been developed to evaluated these secondary products, which are more resistance than peroxides. The total oxidation values (Totox) is a measure of the primary (peroxides) and secondary oxidation (aldehydes), offers overall estimation of the progressive oxidative deterioration of the oil (Shahidi and Zhong, 2005). Gas chromatographic analysis measures volatile compounds that are directly related to the flavor of fried food. Also provides information of oil deterioration, but the interpretation of this data should be made carefully because there are fluctuations in the formation and degradation of these compounds during frying (Romano et al., 2012). Other types of analyses are the accelerated stability tests that are usually employed to determine the oil shelf-life (Farhooshet al., 2013). The most of the accelerated tests are designed to speed up the oxidation process by exposing oil samples to elevated temperatures in the presence of excess amounts of air or oxygen (Farhoosh et al., 2013).

1.2.2.3. Polymerisation

Polymerisation is an advanced stage of secondary oxidation, this means that constituents such as carbonyls, alcohols and fatty acids react further with oxygen and produce higher molecular weight compounds (Zhang et al., 2012a). Also the exposition of the oil to excessive heat can result in thermal alteration even in the absence of

oxygen. In these reactions, the fatty acids are cleaved by heat and associated with each other to form polymeric compounds (Gupta, 2005; Zhang et al., 2012a).

Regarding to polymerization mechanism involves complicated reactions and are still needed to be investigated. Temperature and frying time are strongly associated with the polymerized triglyceride formation and oxygen accelerates this reactions. However, the fatty acid structures do not appear to have slight effect, the saturated fatty acids are more stable than unsaturated but at temperatures above 150ºC these also are decomposed (Dana and Saguy, 2001).

The limiting value of polymer compounds in used oil is between 10 to 16%. Though, some European countries apply more restrictive standards, and discard the oil when this value reaches 10% (Tarmizi and Ismail, 2007). In other way, countries like the Netherlands and South Africa have authorized a higher limit of polymer compounds (16%) for used oil (Berger, 2005). Polymeric compounds cause diverse changes in oil, such as an increase of viscosity, tendency of foaming during frying, increase oil uptake and imparting bitter taste to the fried product (Tseng et al., 1996; Samah and Fyka, 2002; Tarmizi and Ismail, 2007; Choe and Min, 2007). Polymers produce a brown resin like residue that is visible along surface of the fryer (Choe and Min, 2007).

The content of total polar compounds is an indicator of the degree of oil deterioration along frying (Warner and Gupta, 2003). The fractions of polar compounds include free fatty acids, hydroperoxides, aldehydes, acids, alcohols, ketones, and epoxides, among others, and are a global measure of all oxidation and hydrolysis (Dobarganes et al., 2003; Sebastian et al., 2014). Moreover, there are many simple instruments designed

for this purpose available on the market (Sebastian et al., 2014). About 25 to 27 per cent of polar compounds are the limiting values in frying oil. Some countries, like Belgium, Chile, France, Italy, Spain and South Africa, only permit a maximum of 25% of polar compounds while others like Austria and Germany permit a 27% (Berger, 2005). Oil that contains polar compounds of more than 25% is unfit for human consumption (Rossell, 1997).

Table 1. Typical oil breakdown during frying.

Reaction	Description	Causative agent	Main Constituents
Hydrolysis	Interaction between oil, steam and water, which hydrolyze triglycerides.	Moisture	MAG, DAG , glycerols, FFA
Oxidation	Interaction between oil and oxygen at high temperature that promotes the formation of primary and secondary oxidation compounds.	Air	Hydroperoxides, carbonyl constituents (ketones, aldehydes), acids, epoxides, hydrocarbons, alcohol, FFA.
Polymerisation	Wide variety of chemical reaction, such us, oxidation of the secondary oxidation components at high temperature, and alteration of oil molecules or fatty acids by heat, which leads to the formation of large molecules.	Temperature	Polymerized triglyceride, oxidated triglyceride, dimers, cyclic fatty acids monomers

Source: Tarmizi and Ismail (2007), Dobarganes (2009).

As a conclusion, the reactions of hydrolysis, oxidation and polymerisation are interrelated to each other. As these examples illustrate, free fatty acids is developed mainly by hydrolysis but can also by oxidation and cleavage of double bonds. In the same way, fatty acids can be oxidized and/or undergo thermal breakdown (Dana and Saguy, 2001). In Table 1 are tabulated a summary of typical oil breakdown during frying oil.

1.2.3. Operating conditions

Quality of food is affected by several parameters, such as, operating conditions, oil composition and quality, type of fryer, texture, size and shape of the food, among others. Therefore, these factors must be optimized to ensure that the food is properly cooked (Tarmizi and Ismail, 2008).

One of those parameters is temperature, which directly influences the extent of the process. The effect of frying at high temperatures has been heavily researched. Some authors support that the amount of oil uptake is lowered, since the time needed to decrease the moisture content is shorter as a result of rapid heat transfer, and the crust formation acts as barrier for oil penetration (Dobarganes et al., 2000; Dana and Saguy, 2006). The fast evaporation at high temperature also produces a rapid pressure build up that prevents oil from entering the food structure (Yamsaengsung and Moreira, 2002). Many other studies (Garayo and Moreira, 2002; Mariscal and Bouchon, 2008; Kita et al., 2008; Tsuzuki et al., 2010) support the opposite, that the product oil content is higher when frying temperature increases. It is explained because higher thermal driving force leads to a rapid release of overheated steam, which in turn opens up capillary channels, defects and cracks inside the cellular structure and thus facilitates oil absorption.

The lower temperatures along frying process cause a slower heat transfer, therefore, longer frying times are required to obtain similar residual water content. Consequently, the water vapor released is lessened and the crust developing is delayed, allowing more oil to be drawn into the food structure (Blumenthal, 1991; Pedreschi and Moyano, 2005; Moyano and Pedreschi, 2006). Even if the crust is formed, it is likely to exhibit low

level of firmness that could allow oil to penetrate easily into the food structure (Ziaiifar et al., 2008). Kita et al. (2005) reported an inverse correlation between temperature and oil content, a reduction of 3% oil content was demonstrated for every increase in frying temperature of 20 ºC in potato chips. Bouchon et al. (2001) observed that frying temperature had no effect on the product oil content when frying time was relatively short, this probably because the crust was not fully developed during this time. Others studies even showed that the effect has minimal or no influence on the amount of oil taken into the product at 140 to 190ºC (Gamble and Rice, 1987; Moreira et al., 1997). McDonough et al. (1993) stated a correlation between oil uptake and water evaporation, because the oil diffused into products through small channels formed as water evaporated from the product. On the contrary, Kalogianni and Smith (2013) observed that oil uptake is independent of water removal and the thickness of the formed crust in their work on the effect of frying variables on French fry properties. The water content of French fries was affected by potato-to-oil ratio, distribution of potatoes in the fryer, potato variety and specific gravity, whereas the oil content was only affected by the potato variety.Tsuzuki et al. (2010) explained that this phenomenon depends on the frying oil and frying time, others studies at higher frying temperature increased or had no effect on the oil content of fried product.

Time plays significant role in quantifying the amount of oil taken into the product and on the tendency of oil deterioration. It is generally accepted that the oil content in a product increases with frying time (Maneerote et al., 2009). Rossell (2001) stated that mainly of food products have an optimum cooking time and temperature. An excessive frying time may produce a higher oil content in the final product, as the oil adhering to the surface of the product is drawn into its pore structure. An insufficient frying time

may be produce a soggy texture in products due to the moisture has not been totally released (Maneerote et al., 2009).

The selection of the type of the frying oil also affects the fried product quality. Several authors emphasized that the product oil content can be reduced when oil that demonstrates higher resistance against oxidation and lower degree of degradation is used for frying (Romero et al., 2000; Ngadi et al., 2007). Moreover, reduce the tendency of oil deterioration which in turn lowers the operation cost and oil utilization (Mehta and Swinburn, 2001).Kita and Lisińska (2005) and Kita et al. (2005) observed that when the oils used to fry contain higher unsaturated fatty acid level, the final oil content was increased in products. Ngadi et al. (2007) reported that increase in oil hydrogenation degree gave chicken nuggets lesser oil content. Ziaiifar et al. (2008) argued that the use of saturated oil produces higher oil content in products since the oil tends to solidify and remain on the surface once the foods are removed from the oil. Further, unsaturated oil which is less viscous has more ability to drain out from the product surface. In other hand, Krokida et al. (2000) found that the degree of hydrogenation of frying oil did not affect the amount of oil taken into French fries.Tarmizi and Ismail (2008) studied the effect of frying temperatures greater than 200 ºC on oil breakdown and observed premature foaming and increase in oil viscosity. Increase in oil viscosity enhances the wetting properties of oil and therefore increases the contact between food and oil, and this could led to a higher oil content in the product. Tseng et al. (1996) reported that frying tortilla chips using unsaturated oil increased the rate of degradation, which resulted in higher surface oil; this finding seems to be similar when saturated oil is used for frying.

The frying oil is often used several times before being renewed. It is important take into account this fact in order to ensure the nutritional and economic sustainability of the process, because the micronutrients are lost during the baths. Other way to increase quality of frying is adding fresh oil into the fryer to replace the oil taken up and compensate for other losses occurring during frying. Frequent replenishment of fresh oil improves the life-span and quality of frying oil (Romero et al., 1998; Choe and Min, 2007). In contrast, the replenishment with used oil can cause foaming and rapid breakdown of the origin oil (Tarmizi and Ismail, 2008). Most of the continuous fryers are equipped with an automated system to compensate fresh oil regularly. Romero et al. (1998) and Shimizu et al. (2004) compared the frying stability with and without oil replenishment during frying. They concluded that frequent addition of fresh oil throughout frying process minimized both oxidative and hydrolytic changes and hence prolonged its life-span.

1.2.4. Changes in product during frying and storage

The physical and chemical changes development during the frying process and storage directly affect to the product final quality through development of color, flavor, taste, and texture (Moreira et al., 1999; Bordin et al., 2013). The main changes in the composition of foods during the frying process are summarizes in Table 2.

Table 2. Physical and chemical changes in food during the frying process.

Component	Changes during frying	Changes during frozen storage
Fat	Increased concentration and change in composition	Small alteration of the composition and oxidation
Water	Significant loss	Moderate loss
Reducing sugars	Maillard reaction	
Starch	Gelatinization	Retraction
Proteins	Alteration of the composition	Small alteration of the composition and oxidation
Amino acids	Formation of heterocyclic flavoring substances	
Flavoring substances	Formed by oxidative and Maillard reactions	Small modification formed by oxidative
	Interaction with frying oil	
Vitamins	Moderate loss	Small loss
Minerals	Small loss	Small loss
Antioxidants	Moderate loss	Small loss

Source: Miller et al. (1980); Bárcenas and Rossell (2006); Bordin (2013).

1.2.4.1. Texture

Texture is an attribute of uppermost importance for fried product preference and it is a critical parameter for fried quality (Ross and Scalon, 2004; Albert, 2011). The development of texture inside the food during the frying process is a result of the combination of changes in proteins, fats and carbohydrate polymers similar to those that occur during boiling or baking (Bordin et al., 2013). The structure of a protein is the result of many interactions, as attraction and repulsion forces. Changing the

temperature, pH, surface tension, presence of salt, agents that disrupt the intra and intermolecular interactions, change the natural structural conditions of the protein and break it in amino acids chains. These amino acids can form spherical aggregates which interact and originate the gel network (Bordin et al., 2013). In the external part of product is developed an external oily and crispy crust as a major structural transformation occurring due to high temperature (160–190°C) and oil infiltration (Pedreschi et al., 2001). This superficial crust is quickly formed, acting as a barrier to evaporation, decreasing the loss of water and keeping it inside the food.

According to Ruiz de Huidobro (2005), texture is a sensory parameter that can be perceive, describe and quantify only by human being. It is a broad concept which includes a serie of sensorial stimuli, such as, tactile sensation, taste sensation, mechanical stimulation, and even, olfactory and visual stimuli. The market for fried products has motivated the developing of instrumental characterizations of texture properties through definitions of the mechanical attributes.To evaluate the texture instrumentally is used a texturometer, a device that allows measured the resistance both to shearing and compression, besides ithas demonstrated a good correlation with sensory analyses. Many authors have used this technique in their studies, for example, Thomas et al. (2007,2014) andDas et al. (2008) used a double compression cycle test, called texture profile analysis, to analyze textural properties on nuggets.Dogans et al. (2005) also used a compression test to evaluate the fracturability parameter, defined force applicate at the first fracture peak. Other indicator used inthe measurement of texture is the force maxima, which is defined like a maximum force necessary to break a product (Salvador et al., 2005; Kita el al., 2007).

The kinetics of texture evolution during frying process has been evaluated through texturometer. Products showed two phases, a first where whole tissue softens and other one where progressively crust is developed and hardened (Gołubowska et al., 2005; Pedreschi and Moyano, 2005; Moyano et al., 2007).Accordantly, Lima and Singh (2001) realized that texture parameter, puncture force and flexural strength of the crust increased with frying time and temperature, which is associated with the decreasing of moisture. Moyano et al. (2007) observed the same trend in a specific shear force and toughness.In agreement, Moreira et al. (1995), showed that the crispness of tortilla chips increased as frying time increased. Pedreschi et al. (2001) determinated that higher frying temperature accelerated hardening of the crust, resulting in harder crusts.

Frozen storage keeps almost intact the food quality because low temperatures. However produces phenomena that damage the food structure. Miller et al. (1980) observed a reduction of quality in frozen storage product, since less juiciness and harder in samples was descried. This damage is result of the ice crystals formated during the freezing process and the recrystallization phenomenon, which involves changes in the number, size and shape of the crystals during the frozen storage. The crystal growth occurs at constant temperature and it is accelerated by the temperature fluctuations, resulting in a decrease in the number of crystals and an increase in their size (Bárcenas and Rossell, 2006).

1.2.4.2. Color

Color is other major component of quality in fried products, as it could determine their subsequent sale on the market.The appeareance and color of the food surface is the first quality parameter evaluated by consumers and is critical in the acceptance of the

product. Color measurement, as a tool for quality evaluation, is interesting due to its nondestructive nature (Miller et al., 1980; Nourian and Ramaswamy, 2003). It has been measured usually in units L∗a∗b∗ using either a colorimeter or specific data acquisition and image processing systems (Pedreschi et al.,2005). L∗, a∗, b∗ is an international standard for color measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976. L∗ is the luminance or lightness component, which ranges from 0 to 100, and parameters a∗ (from green to red) and b∗ (from blue to yellow) are the two chromatic components, which range from −120 to 120. In the L∗a∗b∗ space, the color perception is uniform which means that the Euclidean distance between two colors corresponds approximately to the color difference perceived by the human eye (Pedreschi et al., 2005). In particular, there are specific values of lightness, which have been established in the food fried industry (Krokida et al., 2001).

During frying, heat and mass transfer phenomena take place, causing physicochemical changes which affect the color of the fried products and oil. Process variables such as oil temperature, oil type and sample dimensions are expected to affect the color of the fried products (Krokida et al., 2001). The fried products, like potatoes, increase the redness values accompanied by a simultaneous decrease in yellowness (b*) and lighness (L*) values.

Changes in the food surface are resulted by the effects of caramelization and Maillard reaction (both responsible for the development of gold to brown), as well as evaporation of surface water and oil uptake. Maillard (non-enzymatic browning) is considered the main reaction in the browning of food. This reaction involves different groups of amino acids, peptides, proteins, and carbonyl groups or other aldehydes, and ketones of sugars.

The development of the color is limited by the reduction of sugars as glucose, fructoseand sucrose. Several intermediate products, called Amadori products or pre-melanoidins, are rapidly polymerized at frying temperatures, forming dark-colored molecules (melanoidins). Above temperatures of 150 °C browning is faster. Among all the compounds produced by Maillard reaction, there is an increasing in toxic compounds, like acrylamide (Bordin, 2013). Pedreschi et al. (2005) found a linear correlation between acrylamide content and their color, represented by the redness component.Moreover, excessive browning during frying produces an undesirable color and unacceptable bitter taste (Krokida et al., 2001; Nourian and Ramaswamy, 2003). Besides Maillard and caramelization, frying oil can also take part in the non-enzymatic browning process by reaction of lipid oxidation products with amines, amino acids and proteins. By reducing glucose oxidase, Jiang and Ooraikul (1989) observed less non-enzymatic browning in fried potatoes. Shallenberger et al. (1959) observed the same effect reducing sugar and sucrose. The oil degradation may affect the color of fried product and oil. The characteristics of color and flavor of fried products are also developed by a combination of reactions and compounds absorbed by the frying oil. Paul and Mittal (1996) and Aladedunye and Przybylski (2009) examined how the degradation of oil during frying of canola affected the color of the fried product.

Regard freezing, it may also affect color of a product due to difference in light diffraction of ice in water. However, this change in color is reversible when product is defrosted. There are other changes that are permanent, resulting in active enzymes and oxidative processes, or also denatures pigments and associated proteins. For example, the red pigment in meat, myoglobin, gets oxidized either during freezing or prolonged frozen storage if the packing is unadequated (persintence of oxygen, film damaged ...).

1.2.4.3. Lipid oxidation

Lipid oxidation represents a key barrier in the development of food products, because oxidation products produces changes in foods that include loss of flavor, development of off-flavors, loss of color, nutrient value and functionality, severely compromising the quality of some foods and limits the shelf-life of others (Karakaya et al., 2011).

As explained previously, unsaturated fatty acids are extremely susceptible to the oxidation and can undergo great changes during handling the raw material, processing into a product or during storage. In frying process, the changes occur in initial fat of the food and in which acquires during the frying process. As result of fatty acids oxidation, monohydroperoxides are formed, which eventually break down into mainly volatile products. This group includes aldehydes, ketones, alcohols, acids, hydrocarbons, furanones, and lactones. Due to the low odor threshold of the majority of these compounds, the presence of volatile degradation products even at low concentration impairs the sensory properties of products and development of off flavors that persist even after the cooking of the products (W´sowicz et al., 2004).

Pre-fried frozen food are especially affected because are subjected to cooked high temperatures and subsequently stored. During the frozen storage of meat and vegetables products, volatile compounds derivate of oxidation are accumulated in their tissues. The rate of lipid oxidation in frozen products are dependent on the unsaturation degree of fatty, storage temperature, presence and concentration of pro-oxidant (oxygen)and presence or not of antioxidants.

Various methods have been developed to determine the extent of lipid oxidation. An iodometric method is often used to estimate the hydroperoxide level of lipids, and a colorimetric method using thioharhituric acid (TBA) has been employed extensively to measure the amount of a secondary decomposition production food. This is based on the measurement of the absorbance of TBA-malonaldehyde complex at 532–535 nm. Malonaldehyde is a three carbon dialdehyde being one of the intermediates formed in the oxidation of lipids. Likewise sensory profile of odour and taste attributes often has been used to describe sensory quality of stored food. A standardized descriptive language to evaluate the off-flavor has been developed.

1.3. STRATEGIES TO IMPROVE THE FRYING PROCESS AND PRODUCTS

1.3.1. Antioxidant strategies

Antioxidants have been widely used in food industry for oxidation control and to maintain the quality. The term antioxidant is refered to any substance that when it is present at low concentration compared with an oxidizable substrate, significantly delays or prevents oxidation of that substrate by inhibiting the initiation or propagation of oxidizing chain reactions (Al-Mamarya et al., 2002). In general, the antioxidant can be divided in two basic categories, natural and synthetic. The most synthetic antioxidantused to preserve food are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) (Zheng and Wan, 2001). However, the increased understanding over the safety of synthetic food additives and their potential effect associated with carcinogenesis or

mutagenesis phenomena have result in a trend towards "natural products" (Moure et al., 2001; Lalas and Dourtoglou, 2003).

1.3.1.1. Natural antioxidant

Researchers have been studied the availability, chemical structures and effectiveness of natural sources as potential antioxidants to prevent deterioration in food products. Vegetables, fruits, spices, and medicinal herbs have been described as a wide source bioactive substances that possess a variety of antioxidant, antimicrobials, antibacterial, anti-mutagen, anti-inflammatory, and antibiotics effects, due to their composition rich in carotenoids, flavonoids, anthocyanins and phenolic compounds (McCarthya et al., 2001; Moure et al., 2001; Shan et al., 2005). In particular, their antioxidant activity is closely associated with their phenolic compounds composition. These molecules are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens and are characterized by a structure formed several hydroxyl groups on aromatic rings (Manach et al., 2004; Babsundram et al., 2006). The following Table 3 and table 4 shows the top sources of antioxidant plant phenols.

Phenolic compounds can be categorized into different groups according to the number of phenol rings that they contain and the structural elements that join these rings: Phenolic acids, flavonoids, stilbenes, and lignans (Figure 4). The flavonoids are characterized by having a common structure consisting of 2 aromatic rings bound together by 3 carbon atoms that form an oxygenated heterocycle, which are further divided into 6 subclasses as a function of the type of heterocyclic involved: flavonols, flavones, isoflavones, flavonoids, anthocyanins, and flavanols. Furthermore

polyphenols can be associated with other compounds, such us, carbohydrates and organic acids (Manach et al., 2004).

Natural antioxidants in extract form have been studied in a wide variety of food substrates to prove their effectiveness. In particular, many studies had evaluated the potential of these natural compounds in meat products during frozen storage. Mohamed and Mansour (2012) concluded that addition of essential oils of marjoram and rosemary (200 ppm) reduce oxidation and improve sensory characteristics of beef patties with mechanically deboned poultry meat (200 g/Kg) during frozen storage for three months.The potential of the grape seed extract was examined by Kulkarni et al. (2011) in precooked beef sausages (28% fat) stored four months at -18 ºC. The concentrations used were 100, 300 and 500 ppm. In regard sensory evaluation, the samples with grape extract retained their fresh odor and flavor longer than control and obtained lower rancid odor and flavor scores.Likewise, TBARS of samples treated with grape remained during the four months. Selani et al. (2011) evaluated two extracts obtained from different grape varieties (*Isabel* and *Niagara*) on raw and cooked chicken during 9 months of storage (-18 ºC). Extracts showed be as effective as BHT or sodium erythorbate in the delay of lipid oxidation. Green tea and grape seed extracts were used as preservatives in raw beef patties by Díaz et al. (2005) and Bañón et al. (2007). The application of the extracts by themselves does not delay the colour deterioration of raw beef patties. However, when these extracts were used with a low SO2 concentration a protective effect was observed on the major causes of raw meat deterioration: microbial spoilage, redness loss and lipid oxidation. Also, these extacts were applied in cooked pork meatballs during refrigerated storage at aerobiosis conditions. The samples containing green tea and grape seed extracts showed lower levels of TBARS, major

volatile compounds, microbiological counts and cholesterol oxidation products. Although, these extracts provided an atypical brown color to the samples (Price et al. 2013). Other authors like Rojas and Brewer (2008) reported the reduction of lipid oxidation in vacuum-packaged cooked beef and pork samples stored at -18 ºC during 4 months by usin oregano as antioxidant source. This reduction only was significant in beef samples.

El-Alim et al. (1999) evaluated the effect of diferent spices (marjoram, wild majoram, caraway, clove, peppermint, nutmeg, curry, cinnamon, basil, sage, thyme, and ginger) on the stability of fresh chicken minced meat, and the effect of ethanolic extract of spices sage, basil, thyme and ginger on fresh and cooked pork patties during refrigerated and frozen storage. The lipid oxidation was effectively inhibited in the chicken meat after 180 days at -18 ºC treated with several spices except curry and cinnamon, diminishing the TBARS (between 32% and 83%). Marjoram, wild marjoram and caraway were the most effective dry spices. Also, ethanolic extracts of spices had a very strong antioxidative effect in pork products. Modi et al. (2006) study the quality changes in chicken curry during frozen storage, where TBARS values were maintained constant for 6 months (-18 ºC) associated to spices addition.

Table 3. Antioxidant sources (Fruits and Vegetables).

	Antioxidants	**References**
Fruits		
Berries	Flavanols hydroxycinammic acids, hydroxybenzoicacids, anthocyanins	Hakkinen et al. (1998), Belitz and Grosch (1999),Wang and Lin (2000), Yanishlieva-Maslarova and Heinonen (2001), and Manach et al. (2004)
Cherries	Hydroxycinnamicacids, anthocyanins Belitz and Grosch (1999), Yanishlieva-Maslarova et al.,	Belitz and Grosch (1999), Yanishlieva-Maslarova et al.,(2001), and Manach et al. (2004)
Blackgrapes	Anthocyanins, flavonols	Belitz and Grosch (1999), Yanishlieva-Maslarova et al.(2001), and Manach et al. (2004)
Citrus fruits	Flavanones, flavonols, phenolic acids	Yanishlieva-Maslarova et al. (2001), Beecher (2003), and Manach et al. (2004)
Plums, prunes, apples, pears,kiwi	Hydroxycinnamic acids, catechins	Belitz and Grosch (1999), Yanishlieva-Maslarova et al.(2001), and Manach et al. (2004)
Vegetables		
Aubergin	Anthocyanins, hydroxycinnamic acids	Manach et al. (2004)
Chicory, artichoke	Hydroxycinnamic acids	Manach et al. (2004)
Parsley	Flavones	Manach et al. (2004), and Beecher (2003)
Rhubarb Anthocyanins Manach et al. (2004)	Anthocyanins	Manach et al. (2004)
Sweet potato	Flavonols, flavones,	Chu et al. (2000)
Yellow onion, curly, kale, leek	Flavonols	Manach et al. (2004)
Parsley	Flavones	Manach et al. (2004)
Beans	Flavanols	Manach et al. (2004)
Spinach	Flavonoids, p-coumaric acid	Bergman et al. (2001)
Flours, oats, wheat, rice	Caffeic and ferulic acids	Yanishlieva-Maslarova et al. (2001), and Manach et al.(2004)

Source: Dimitrios (2006).

Table 4. Antioxidant sources (Teas and Herbs and spices).

	Antioxidants	**References**
Teas		
Black, green	Flava-3-ols, flavonols	Manach et al. (2004), and Beecher (2003)
Alcoholic drinks		
Red wine	Flavan-3-ols, flavonols, anthocyanins	Manach et al. (2004), and Beecher (2003)
Cider	Hydroxycinnamic acids	Manach et al. (2004)
Other drinks		
Orange juice	Flavanols	Manach et al. (2004)
Coffee	Hydroxycinnamic acids	Manach et al. (2004),Sanchez-Gonzales et al. (2005)
Chocolate	Flavanols	Beecher (2003), and Manach et al. (2004)
Herbs and spices		
Rosemary	**Carnosic acid, carnosol,**	Yanishlieva-Maslarova et al. (2001)
	Rosmarinic acid rosmano	lbanez et al. (2003)
Sage	Carnosol, Carnosic acid, lateolin, rosmanul,rosmarinic acid	Yanishlieva-Maslarova et al. (2001)
	Rosmarinic acid	Zheng and Wang (2001)
Oregano	Rosmarinic acid, phenolic acids, flavonoids	Yanishlieva-Maslarova et al. (2001), Exarchou et al. (2002), and Belhattab et al. (2004)
Thyme	Thymol, carvacrol	Yanishlieva-Manach et al. (2004))
	Flavonoids, lubeolin	Zheng et al. (2001)
Summer savory	Rosmarinic, carnosol, carvacrol, flavonoids	Yanishlieva-Maslarova et al. (2001)
Ginger	Gingerd and related companids	Yanishlieva-Maslarova et al. (2001),Moure et al. (2001)

Source: Dimitrios (2006).

Ganhão et al. (2010) studied the effects of wild Mediterranean fruits (arbutus-berries, common hawthorns, dog roses and elm-leaf blackberries). Most fruit extracts reduced the formation of protein carbonyls and inhibited color and texture deterioration during chill storage. Though, the application of blackberries and hawthorns extracts had a negative effect in color and texture characteristics, respectively.

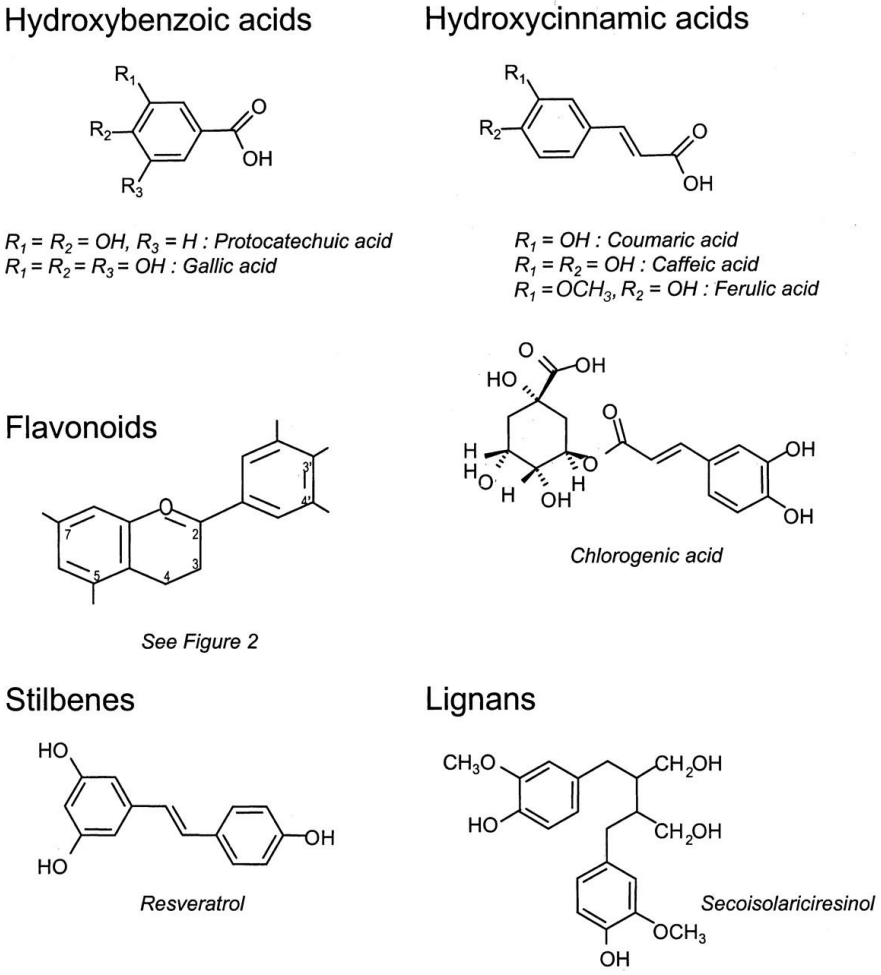


Figure 4. Chemical polyphenols structures (Manach et al., 2004).

Nuñez de Gonzalez et al. (2008) and Yildiz-Turp and Sedaroglu (2010) proved the antioxidant effectiveness to incorporate plum extract into meat product on frozen storage. First authors assessed raw and precooked pork sausages with no antioxidant, dried plum puree (3%,6%), dried plum and apple puree (3%,6%), and butylated hydroxytoluene and butylated hydroxyanisole (0.02%) stored at 4 ºC for 28 days and at -20 ºC for 90 days. The incorporation of both levels of dried plum puree retarded the lipid oxidation at refrigeration and frozen conditions, plum was equally as effective as synthetic antioxidant and even greater for doses 6% in frozen conditions. Yildiz- Turp and Sedaroglu (2010) evaluated the effects of three amounts of plum puree (5%, 10% or 15%) on low fat beef patties stored 45 days at -18 ºC. The incorporation of extract exhibited a protective effect for all doses; the results showed TBARS values lower than control at the end of the storage period. The increase of plum concentration affect to pH, moisture, color, juiciness and texture scores.

Rodríguez-Caperna et al. (2011b) showed the effectiveness of avocado extracts (Hass and Fuerte varieties) in meat products. Both extracts were used in raw porcine patties during chilled storage, the patties treated with avocado extracts had lower amounts of TBARS, a minor formation of protein carbonyls and obtained lower color deterioration.

Banerjee et al. (2012) examined the antioxidant capacity of broccoli power extract in goat meat nuggets. Three different concentrations (1, 1.5, and 2%) were used and were compared with a control without antioxidant and other with butylated hydroxyl toluene (100 ppm). The thiobarbituric acid reactive substances values of goat nuggets with broccoli powder extracts, in general, were lower than control throughout the storage. The effect was associated with increasing doses since the implementation at 2% showed

significant differences with regard control nuggets and had a similar protection to the oxidation that nuggets with 100 ppm BHT.

Other interesting food substrate to evaluate the effectiveness of natural antioxidant is fried oil due to intense thermoxidation phenomenon produced during cooking process. For this reason many studies have focused in addition of antioxidants into frying medium. Aladedunye and Matthäus (2014) stated that polyphenolic fractions from rowanberry (*Sorbus aucuparia*) and crabapple (*Malus baccata*) offered a wide potential as antioxidants for rapeseed oils both during frying and storage. The results showed that oxidative degradation was lower in oils enriched with these fruit extracts, and the protection was better than the protection offered by the synthetic antioxidant BHT.

Du and Li (2008) researched antioxidant effect of cassia essential oil on deep-fried beef during the frying process. They evaluated frying time (1.5 min, 2.5 min, and 3 min), frying temperature (130, 150, and 190ºC), oil types (rapeseed oil, soybean oil, palm oil, peanut oil, and sunflower seed oil) and concentration of essential oil (0, 0.004%, 0.008%, 0.012%, 0.016%, 0.02%, 0.024%, 0.028%, and 0.032%) to determinate the optimum conditions. Results showed that cassia oil improve oxidation stability during frying process. Furthermore, the authors conclude that optimum frying conditions to use cassia oil as antioxidant are 30 µL cassia oil/250 mL palm oil, 1.5 min at 150 C.

Chirinos et al. (2011) characterized the phenolic compounds of Inca muña (*Clinopodium bolivianum*) leaves and evaluated their application to improve the oxidative stability of soybean oil during frying. They stated that Inca muña extract

prevents the oxidation of unrefined soybean oil during frying at 180 ºC, at concentration of 600 ppm the extract showed the highest efficacy.

Nor et al. (2008) evaluated the antioxidative properties of *Pandanus amaryllifolius* leaf extracts (0.1%, 0.2%, 0.3%, and 0.4%) in refined, bleached and deodorized palm olein, using accelerated oxidation and deep frying studies at 180 °C. The Pandanus extracts retard the oil oxidation, including the dose at 0.1%, and may be as effectively as BHT.

Jaswir et al. (2000) proved antioxidant capacity and synergistic of rosemary, sage, and citric acid on palm olein during deep frying of chips.

1.3.1.2. Mechanism and factors

The antioxidant effect of phenolic compounds is mainly due to their redox properties and is the result of various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity. They are also known to play an important role in stabilizing lipid peroxidation and to inhibit various types of oxidizing enzymes (Shan et al., 2005). For convenience, these have been divided in primary and secondary antioxidant:

The primary antioxidants are the most effective ones. They are able to interrupt the oxidative chain reactions by inactivating the free radicals in initiation and propagation steps. This kind of antioxidant proceeds operates via hydrogen atom transfer or electron donation to the free radical and to convert it into stable non-radical products or more

stable radicals (Brewer, 2008; Baek, 2012). Chain- breaking mechanisms are represented as following:

$$L^* + AH \rightarrow LH + A.$$

$$LO^* + AH \rightarrow LOH + A.$$

$$LOO^* + AH \rightarrow LOOH + A.$$

Figure 5. Chain-breaking electron donors.

Free radicals produced in initiation (lipid radical: L*) or propagation (alkoxyl: LO* or peroxyl: LOO* radicals) phases, reacting with antioxidant (AH) by donating an electron/hydrogen atom or accepting an impaired electron from radical and this fact allows obtained a stable molecule (Baek, 2012).

Secondary antioxidants, also called, preventive antioxidants retard the rate of oxidation. These type of antioxidant rather that convert free radicals to non-radicals products, are generally aimed to inhibit the factors initiating oxidation. For example metal quelators (citric acid), quenchers of single oxygen (tocopherols, carotenoids), enzymatic antioxidant and hydroperoxide decomposers (α-tocopherol) (Baek, 2012).

In the case of phenolic compounds, their chemical structure is a key determinant of their radical scavenging and metal chelating activity. For example, in the case of phenolic acid the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group determined their antioxidant activity. A large degree of hydroxylation

increase the activity, this fact provides high antioxidant activity of trihydroxylated gallic acid. However, substitution of hydroxyl groups by methoxyl groups at the 3- and 5-position (syringic acid) reduces the activity. Hydroxycinnamic acids exhibit higher antioxidant activity compared to the corresponding hydroxybenzoic acids (Balasundrama et al., 2006).

1.3.1.3. Antioxidant extraction

Bioactive compounds from plant materials can be extracted by various liquid-solid extraction techniques, such as Soxhlet extraction, maceration, supercritical extraction, ultrasound assisted extraction, and pressure assisted extraction. Most of these techniques are based on the extracting power of different solvents in use and the application of heat and mixing (Azmir et al., 2013). The selection of the extraction process and optimization of the extraction conditions (solvents, temperatures, pressures and times) directly affects to yield of compounds (Wang et al., 2013). Usually, the substrate is cleaned, dried and ground into a powder before the solvent extraction (Shah et al., 2014). Extraction efficiency of any conventional method mainly depends on the choice of solvents; the polarity of the targeted compound is the most important factor for solvent choice. Molecular affinity between solvent and solute, mass transfer, use of co-solvent, environmental safety, human toxicity and financial feasibility should also consider in selection of solvent for bioactive compound extraction (Azmir et al., 2013). The solvents usually used are ethanol, methanol, acetone, dimethyl sulfoxide and water, either separately or in combination (Shan et al., 2014). In particular, methanol and acetone provide a high antioxidant yield due to their hydrogen-bonding ability which is crucial for the extraction of phenolic diterpenes responsible for antioxidant properties in

many plant materials, such as rosemary leaves (Erkan et al., 2008; Trabelsi et al., 2010; Rodrguez-rojo et al., 2012; Wang et al., 2013).

Garrido et al. (2011) used two different methods to extracts the natural antioxidant of red grape pomace. The first extract was obtained through a triple extraction with methanol on grinded dry skin. The entire volume of three extractions was mixed and evaporated in rotary system. The second extract was subject a pre-treatment consisting in applying fast change in pressure to improve tissues permeability to the extraction solvent. The new extraction procedure permitted to improve the yield and quality of extract.Han and Rhee (2005) evaluated antioxidant properties of selected oriental herbs (white peony, red peony, sappanwood, moutan peony, rehmania and angelica) in raw and cooked meat. The extracts were prepared through triple ethanoic (95%) extraction at 40 ºC during 3 h and the filtered and them combined filtrate was evaporated to dryness under vacuum. They concluded that alcohol extracts from red peony root, white peony root, sappanwood tree heartwood and Moutan peony root-bark are promising sources of natural antioxidants for meat products.Zhao et al. (2006) worked with four kinds of solvent extracts to obtain the phenolic compounds of three Chinese barley varieties. The acetone extract contained the highest amount of phenolic compounds followed by 80% ethanol, 80% methanol and water extract.Wang et al. (2013) prepared four types of extracts from *Malus baccata L.* using different solvent mixture. The solvents used were distilled water, 80% ethanol, 80% acetone, and 80% ethyl acetate. The process consisted in mixing the powder sample with the corresponding solvent, and apply ultrasonic wave for 10 min and then 1 hour at thermostat-controlled water bath (45 ºC), after the extraction was concentrated under reduced pressure at 50 °C. They tasted that recovery of phenolic, flavonoid and anthocyanins from the sample matrix

was dependent on solvent used. The major yield was obtained in samples extracted with 80% acetone, ethanol, ethyl acetate and distilled water, respectively.

Rodríguez-Rojo et al. (2012) studied using different extraction processes (conventional, microwave assisted and ultrasound assisted) and plant pretreatments (deoiled and milled, deoiled and fresh plant) to obtain rosemary antioxidants. Moreover, two solvent were used: ethanol and water. Their results indicated that without any pretreatment, the use of ethanol improved the concentration of target compounds obtained. The extraction was improved using any of the assisted techniques; amoung them the microwave was the best .In the same way, the pre-treatment step overall increases the antioxidant activity.

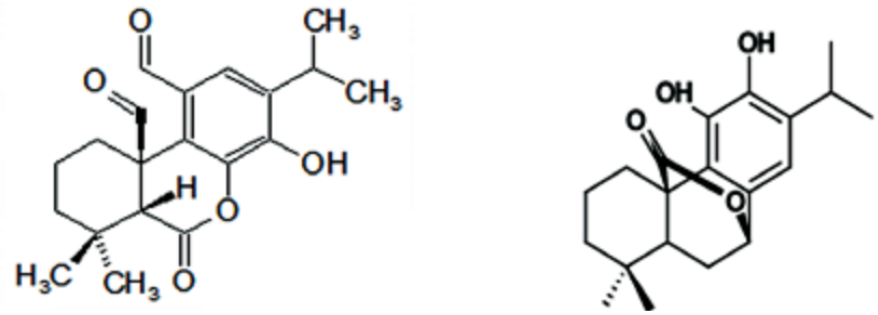
Extraction efficiency depends on the solvent with polarity, pH, temperature, extraction time and composition. However, the most important parameters, under same time and temperature conditions, are solvent and samples compositions of sample (Shah et al., 2014).

1.3.1.4. Rosemary

Rosemary (*Rosmarinus officinalis*) is an aromatic Mediterranean plant belongs to the mint family Lamiaceae. The name derives from the Latin for "dew" (ros) and "sea" (marinus), or "dew of the sea". Rosemary has been extensively used in traditional meals and several industrial food applications. The use of rosemary extracts in foodstuffs has been recently authorized by the Directive 95/2/EC which included rosemary extract under the E-392 number (European Union Directives 2010/67/EU and 2010/69/EU),

which confirms the importance of this ingredient as a natural preservative in foods and beverages.

Rosemary active compounds have been well documented and a large number of compounds with antioxidants properties have been isolated, mainly phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methyl carnosate and other phenolic acids, such as caffeic acid, rosmarinic acid, caffeoyl derivatives (Shan, 2005). Of these, the antioxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol (Figure 6) (Nogala-Kalucka et al., 2005). They are found mainly in rosemary leaves chloroplasts, subcellular organelles with their own double membrane (Rodríguez-Rojo et al., 2012).



Carnosic acid Carnosol

Figure 6. Chemical structures of carnosic acid and carnosol (Pubchem compounds).

Antioxidant activity of these compound is mainly associated with their phenolic region. As was explained before, the usual mechanisms in phenolic compounds are the hydrogen donating and chain breaking. When carnosic acid donates a hydrogen to radical spices, the rearrangement of their structure occurs to fill the empty electron

shell. The most common rearrangement is the donations of their second hydrogen which form an O-quinone and then may be formed carnosol. Moreover, carnosic acid is converted carnosol in presence of air in alcoholic solutions, this converts carnosol in one of the most important compounds in rosemary extracts. Carnosic acid and carnosol are degraded into other phenolic diterpenes with antioxidant capacity, this explains that antioxidant effects remain along the time after the total degradation of carnosic acid and carnosol (Santos-Gomes et al., 2002).

Several authors have evaluated the effect of rosemary extract on different food matrixes. The effectiveness along the frying process was evaluated by Lalas and Dourtoglou (2003), who applied rosemary extract in 400 mg/kg (this amount of extract is equivalent to 60 mg of carnosic acid and carnosol/kg oil) in soybeans oil and showed a lower oxidation in samples with rosemary extract. Furthermore a study of antioxidant activity of rosemary extract in doses between 1000-3000 mg/kg in soybean showed a protective effect on oxidation at high temperature (Ramalho and Jorge, 2008; Casarotti and Jorge, 2012). This finding is in line with the work done by Réblová et al. (1999) that using silicone oil and rosemary extract observed that polar and polymer compounds as well as decomposition products from polyunsaturated triacylglycerols in canola oil and palm olein-canola oil mixture decreased during intermittent frying for five consecutive days. Urbančič et al. (2013) studied the effects of rosemary extract on the stabilization of sunflower oil and the reduction of acrylamide formation in potato during deep fat frying. They shows that the oil treated with rosemary extract was more effectiveness for deteriorating that tocopherol and synthetic antioxidants (butylated hydroxyanisole, and tertiary butylhydroquinone) used as controls. Che-Man and Jaswir (2000) also observed a retarded of oil deterioration during frying when oleoresin rosemary extract (0.4%) and

sage extract (0.4%) were added into to refined, bleached and deodorized (RBD) palm. Upadhyay and Mishra (2015) also found a direct association between rosemary antioxidants and the oxidative stability of sunflower oil blended at low (60 ºC) and high (100-130 ºC) temperatures. They applied different combinations of oleoresin rosemary and ascorbyl palmitate ranging from 200 to 1500 ppm and 100 to 300 ppm,

Jaswir et al. researchers investigated the synergistic effects of natural antioxidants from rosemary, sage and citric acid on oxidative stability of palm olein and flaxseed oil (Jaswir et al., 2000, 2004). They proved that addition of these antioxidants were able to hinder thermal oxidation of oil during frying. Rosemary activity as color protector of caroteinoides and delayed lipid oxidation were displayed in oils and meat derivatives (Madsen et al., 1996).

The effectiveness of rosemary as an inhibitor of lipid oxidation also has been studied along storage of meat products. Naveena et al. (2013) evaluated the effect of carnosic acid in cooked chicken patties during refrigerated storage and observed a reduction of lipid oxidation. In agreement, Georgantelis et al. (2007) investigated the effect of rosemary extract and chitosan added individually or in combination on lipid oxidation and color stability on beef burgers stored in frozen condition for 180 days. The combination of chitosan and rosemary that retarding lipid oxidation and improve color retention of beef burgers, moreover individual use of rosemary improved the resistance to oxidation. Sebraneket al. (2005) evaluated antioxidant effectiveness of rosemary at concentrations of 1500 and 2500 ppm in frozen and precooked-frozen pork sausage, and from 500 to 3000 ppm in refrigerated, fresh pork sausage. The application at 2500 ppm was as effective as the maximum permitted concentrations of BHA/BHT (synthetic

antioxidant) in refrigerated, fresh pork sausage and in cooked-frozen sausage, and was superior to BHA/BHT in raw-frozen pork sausage patties. Therefore they determined that rosemary extract clearly provides an alternative to synthetic antioxidants for extending the shelf life of processed meats such as pork sausage and appears to be particularly effective for raw-frozen pork sausage products. Thongtan et al. (2005) studied the effect of refined rosemary extract on pre-cooked frozen beef patties stored for 12 weeks. Samples with rosemary extract showed lower PV and TBA values; furthermore, samples with refined rosemary extract reported a significantly higher liking score for flavor.

A number of studies have demonstrated the antioxidant properties of rosemary extracts. Rosemary compounds are effective at inhibiting lipid oxidation in different matrix and their application does not affect overall sensory attributes of the final products. More researchs should be conducted to expand knowledge about this type of extracts and their active compounds. Futher researchs as regarding the activity of the maximum European doses permitted in food focusing on the effects of extraction method and format of rosemary extract should be carried out.

1.3.2. Vacuum frying process

The excessive fat content and the deterioration have been associated to multiples adverse effects on health. By this fact in the recent decades the fried industry has research other way to obtain products with similar organoleptic characteristic and healthy quality. Vacuum frying is one of the potential frying techniques to improve healthy quality of frying products. The vacuum frying consists to cook food below the atmospheric pressure in a closed system, which leads to decrease the boiling point of

water in food. Hence, it is possible frying at low temperature (Shyu and Hwang, 2001; Andrés-Bello et al., 2011; Mir-Bel et al., 2012). For this purpose the product is placed inside the frying basket once the oil reaches the target temperature. The lid is then closed, and the chamber is depressurized. Subsequently, the basket is immersed in the oil bath, where it remains for the required time. Afterwards it is lifted out and the vessel is pressurized using a pressure release valve. Previous studies applied this technology and found several advantages such as, reduced oil content, preservation of color, flavors and nutritional compound, the increase of oil shelf-life and decreased of production of toxic compounds (Granda et al., 2005; Shyu and Hwang, 2001; Da Silva and Moreira, 2008; Dueik et al., 2010). Mariscal and Bouchon (2008) compared atmospheric and vacuum frying of apple slices and suggested that can be used to improve apple slices quality. Three treatment were evaluated (atmospheric, vacuum and pre-drying vacuum) using different equivalent thermal driving forces (ΔT = 40, 50, 60 °C). Vacuum technology application overall reduced oil content. In particular, the pre-dried vacuum treatment with a driving force of ΔT = 60 °C reduce the oil content to less than 50%. Furthermore, a protector effect was described in color at vacuum conditions. Tarmizi et al. (2013) also observed a reduction of oil content but applying vacuum during cooling, in order to study a possible combination of moderate vacuum frying followed by post-frying high vacuum application during the oil drainage stage. Continuous release of water vapor prevent the surface oil penetrate into the product structure and released it from the surface of the product. Their data showed that the lowering pressure after frying to a value well below the frying pressure reduced the oil taken up around 48%. Shelf-life increase of oil by using vacuum frying was confirmed by Crosa et al. (2014) in a study based on the vacuum and traditional frying on oil degradation, in two different refined sunflower oils (sunflower oil with high oleic acid content and

sunflower oil with synthetic antioxidant), during 10 consecutive days (4h per day). Conclusion indicated that vacuum frying achieves a significant decrease in the rate of deterioration reactions in oil compared with the traditional process. Granda and Moreira (2005) confirmed that vacuum frying is an alternative to reduce acrylamide formation. Their results shown that potatoes fried under vacuum (10 Torr) have a 94% decrease in acrylamide content if are compared to those fried under atmospheric conditions. Effects of vacuum frying on nutritional quality were evaluated by Perez-Tinoco et al. (2008) in pineapple chips. Who that concluded vacuum frying process produces healthy snacks and partially preserves the color, antioxidant capacity and nutritional compounds.

Many authors have shown that vacuum frying may be used as alternative frying technology and provides a wide variety of advantages. However, most these of these studies have been evaluated the effect of this technique on fruit and vegetables. Further investigations are needed to evaluate the effects of vacuum frying in meat product characteristics.

1.3.2.1. Water transfer

During vacuum frying process, as in the traditional frying process, mass loss is mainly due to two concurrent flows: water loss and oil uptake (Andrés et al., 2014). The moisture loss exhibit a typical drying profile, similar to the profile described at traditional fried products (Yagua and Moreira, 2011; Andrés-Bello, 2012).

The kinetic of evaporation was associated to three regimens. The initial regimen, also called initial warm-period, is corresponds with the immersion of product in oil at temperatures above the water boiling point. The material reaches the evaporation

temperature and use mainly heat to vaporize water. Water starts to evaporate and is followed by a flow of steam bubbles in the oil. This initial vaporization is related to the surface vaporization of water because core temperature remains below of saturation temperature (Achir et al., 2009; Yagua and Moreira, 2011; Andrés-Bello, 2012). In vacuum frying, this initial heat-up period is very short (1-5 seconds) water starts evaporating as soon as the raw products is in contact with oil medium and therefore it is difficult to quantify. This is due to the boiling point of water is lower at vacuum pressure, therefore, products only need warm up a few degrees to reach the boiling point. For example, Garayo and Moreira (2002) worked at pressure of 3.115 kPa where the boiling water point is around 25 ºC, which practically rise the room temperature.

The second drying regime corresponds with a flux of constant evaporation, product core temperature is close to saturation temperature (Yagua and Moreira, 2011). The drying rate is limited by two factors, the heat transfer from the drying medium to the product and the liquid water transport from core to surface. The internal liquid water can be transported inside core region due to pressure gradients. This stage continues as long as the food surface remains wetted with water (Andrés-Bello et al., 2011). Andrés-Bello (2012) described a constant phase when study vacuum frying in sea bream fried. Conversely, Garayo amd Moreira (2002) and Liu-Ping et al. (2005) did not observe constant rate period due to the higher evaporate rate in vegetables chips fried in vacuum conditions, potato and carrot, respectively. The initial surface vaporization and a subsequent depth one occurred in the regimes described, creates a porous dried region that is denominated crust. The extent of crust formation is determines by the amount of water remove, that is definitive in oil transfer phenomenon (Andrés-Bello et al., 2011).

Then product moisture level decreases very low, the liquid water transport from core to crust is not possible and decreased the flux of vaporization. Therefore the drying rate decreases and this regimen is called falling rate period, also called third regimen.

In relation to the effect of frying conditions, such as pressure, time and temperature, several authors have reported significant differences between both processes. However, there are still controversy about the role of vacuum conditions in water transfer. In one hand, some authors (Shyu and Hwang,2001; Garayo and Moreira, 2002; Fan et al., 2005; Liu-Ping et al., 2005; Shyu et al., 2005; Mir-Bel et al., 2012) stated that drying rate increase with decrease pressure and increase temperature and time. This is due to increase of vacuum reduced the water boiling point, as result water begins to vaporize faster than in traditional frying.

In other hand, Mariscal and Bouchon (2008) concluded that initial vigorous escape of water is similar in vacuum and atmospheric conditions, but for longer frying time, a lower water loss for products cooked at vacuum conditions was observed. These authors explained that these variations are associated with microstructural changes that occur during the initial depressurization step and which affect to water loss in following evaporation steps. This difference in moisture loss rate can be accentuated by vapor accumulation in the fryer head space resulting of drying, that do slower the water transfer. Effect is accentuated at higher thermal driving forces.

1.3.2.2. Fat transfer

The other flow that occurs during frying is the oil absorption. Oil uptake is a complex phenomenon and depends of a wide variety of factors, like, initial product structure, the

various interchanges between the product and the heating medium, the variation of product and oil properties, and the chemical reactions including interaction between food constituents and oxidized lipids and hydrolysis of frying fats due to food moisture. Garayo and Moreira (2002) divided the process into three periods: the frying, the pressurization, and the cooling period. At the beginning of the frying period, as explained in the previous section, the water starts to evaporate. In vacuum conditions, the water in the products evaporates quicker and it less vigorous due to decrease of water boiling temperature. The capillary pressure between oil and gas is negligible, as result any oil is absorbed at this stage.

The second period in vacuum frying is the pressurization from vacuum to atmospheric conditions. This stage begins when the products are removed from the frying oil and are kept in the vessel closed without altering the vacuum and temperature conditions. Then vessel is vented and pressure increase. Garayo and Moreira (2002) assert that when vacuum is released, the pressure in the pores of products increases fast to atmospheric level and a suction effects is produced. The gas diffuses much faster into the pores at low pressures and obstructs the penetrating from surface oil to structure. On the contrary, other authors (Tan and Mittal, 2006; Troncoso et al., 2009; Tarmizi and Niranjan, 2010) argue that the rapid increase in pressure to atmospheric values, forces the surface oil to penetrate into the product. This controversy leads to the conclusion that pressurization process plays an important role in the oil absorption mechanism. This could be increase or decrease oil absorption depending on the amount of surface oil and free water present in the product (Garayo and Moreira, 2002). Given the repercussions of surface oil in the final product oil absorption, these authors consider that it is necessary a de-oiling process to remove surface oil under vacuum (before of

vented process and after the product is fried). The application of their de-oiling mechanism (centrifuging system) before the pressurization step showed a reduction of the total oil content between 80–90%. In respect to the effect of pressurization, Mir-Bel et al. (2009) found that the volume of oil absorbed by the product is inversely proportional to the pressurization speed. Low speeds of vacuum break increase oil content around 70% more than when it is broken abruptly. Same authors also observed that this increase follow a linear relation.

Finally, the third period starts when the products are removed from the fryer and it is known as the cooling period, when part of the adhered oil continues to penetrate the pore spaces, which depends proportionately from oil adhered in surface. The work concluded that a draining time of 30 s reduces the oil content, but longer draining times have practically no influence on oil absorption, and also asserted that the effect of the draining time on the oil uptake is less than that of the vacuum break speed.

Regarding the effect of pressure and temperature on the oil uptake, Garayo and Moreira (2002) and Yagua and Moreira (2011) state that higher oil temperature produces the increase of the total oil up take in potato chips. In agrement, Mir-Bel (2010) determined that higher temperature produced further degradation of the product surface and oil, which united to increased in the water evaporation rate resulting in an increase of the oil absorption. In contrast, Tan and Mittal (2006) (donut) and Andrés-Bello (2012) (seabream) concluded that oil content is greater at higher vacuum level and lower oil temperature. Yagua and Moreira (2011) observed that, although the vacuum pressure affects the absortion fluxes speed in oil and water, at the end of processthe same oil

content was obtained as if different pressure levels were used.Concluding that the vaccum pressure did not affect to the final oil content.

The influence of frying time was studied by Shyu and Hwang (2001), Garayo and Moreira (2002), and Granda and Moreira (2005), Yagua and Moreira (2011), who reported that absorption oil mainly occurred in the first frying seconds, and then the total oil content remain constant in time.

1.3.2.3. Heat transfer

The main aim of deep fat frying is transfer heat to product, for which purpose is used a heat reservoir created by large volume of hot oil (Achir et al., 2009). At beginning of the frying process, food is submerged into the oil. Heat is transferred via natural convection between the oil and the food surface, the product is warmed up to the boiling point of the water. The continue heat transfer cause the evaporation of surface water of the food and subsequent bubbling. In vacuum frying, the boiling point is lower than in atmospheric condition since heat required is lower. The turbulence changes the heat transfer from natural convection to forced convection, which increases the rate of heat transfer. While internal temperature of food is slowly increased to boiling point by conduction from the food surface. The transfer speed inside of food depends on the thermal conductivity coefficient. At the end of frying process, the products practically do not have water which results in the decrease of evaporation and bubbling, modifying the heat transfer coefficient again.

The heat transfer during frying process depends of a large number of factors, such as, differences in temperatures between the foods, oil type, product characteristic, flying

time, frying temperature, or bubble flow (flow direction, velocity, bubble frequency and magnitude of oil agitation) (Farinu and Baik, 2007). Therefore, the modification of pressure conditions also must be affected to heat transfer coefficient along cooked process.

Heat transfer coefficient during super-atmospheric pressure frying was studied by Erdogdu and Dejmek (2010), who compared convective heat transfer coefficient during frying at atmospheric and high pressure (2 bar) in potato. Authors conclude that as higher pressure frying was reacheda higher heat transfer coefficient values, were described almost double, but temperature effect was not evaluated. Pandey and Moreira (2011) and Yagua and Moreira (2011) studied convective heat coefficient at vacuum frying and determined that convective heat transfer coefficient changed considerably as frying progressed and temperature fluctuated during process. These authors did not compare heat transfer coefficients obtained with atmospheric conditions. The effects of low reduce pressure also was investigated by Mir-Bel et al. (2012), who analyzed the influence of vacuum conditions (19.5-25.9 kPa) and temperature (100, 120 and 140 ºC) on the convective heat transfer coefficient and developed a parameter called the "bubbling efficiency" to quantifying the influence of parameter (Mir-Bel et al., 2012).

1.3.3. Air frying process

Air frying is an emerging technology developed by the industry as an answer to the new trends of consumers. Currently, society demands healthy food based on a fast and easy cooking process. In the market there are a wide variety of cooking devices (oven, microwave or deep fryer) but that does not meet the consumer needs. Conventional ovens obtain products with good organoleptic characteristics but long cooking times.To

obtain fast cook process have been used microwave oven and deep frying machine. Microwave use has difficult to get the browning of food and although fryers develop desirable characteristics on product, they have other limitations. One such inherent disadvantage is the high level of fats of fried products. The air frying machine provides a last cooked by developing browning and flavoring of the cooked product. Moreover, air frying machine can controlthe oil content in the products, through use of the high-speed hot air circulation to cook. Air flow is distributed rapidly around the food product at similar temperature that in frying process, and produces a uniform cooking. High-speed hot air is possible due to chamber shape that works by the achievement of air velocities much higher than those obtained in traditional oven. Oil application could be done before or during the process in order to provide the taste, texture and appearance typical of fried products. The product is dehydrated and the typical crust of fried product appears. The amount of oil used is significantly low and as a result products obtained are less oily as was corroborated by Andrés et al. (2012). These machines are on the market as alternative to produce products with low oil content but with the same organoleptic characteristics that traditional fried products.

1.3.3.1. Water transfer

The evolution of moisture loss during air frying was determinate by Andrés et al. (2012), whoe observed that air moisture curve is similar to a frying classical profile with three regions: increasing rate region, constant rate region, and falling rate region (described previously in this doctoral thesis). At the beginning of the process, in the increasing rate region, the product is heating until reached the required temperature to evaporate their water composition. After obtained the boiling temperature starts the constant region, when heat is used for the evaporation and hence the product

temperature remains constant. The drying continues until the moisture level in the product is so low that decrease the rate of drying. However, compared with deep oil frying, the three regions are expanded in time and slope of tangents to the drying curves are lower in air frying. Andrés et al. (2012) explained that these differences in speed drying due to the heat transfer (value of Biot number) are much slower when the fluid phase is air than when it is oil. These authors also studied the effect of pretreatment, freezing and blanching, in water loss during air frying process which was not affected.

1.3.3.2. Fat transfer

Fat transfer is a complex phenomenon resulting from interactions between oil and products, which depends of numerous physical and chemical factors (Ziaiifar et al., 2008). Many authors (Moreira et al., 1997; Pedreschi et al., 2008) have assumed that both, as oil uptake is a superficial process but also cooling period are critical. During deep fat frying, the vapor flow move out from food to oil and obstructs the incorporation oil. Therefore, the largest proportion of oil which ends up into the food is sucked into the porous crust region after the fried product is removed from the oil bath. The water outlet produces voids in the crust that determine oil input during cooling. Pedreschi et al. (2008) tasted that surface structure plays an important role in the phenomenon and the greatest part of oil is retained in form of drops in the crust of the fried piece after it is cooled. Moreira et al. (1997) determined in their study in tortilla chips, that the around 20% of the final oil content is absorbed during frying and that around 64% is absorbed during cooling, leaving only 36% the total oil at the tortilla surface.Bouchon et al. (2003) divides the products oil in three different fractions: structural oil which represents the oil absorbed during frying, penetrated surface oil which represents the oil suctioned into the food during cooling after removal from the

fryer, and surface oil which is the oil that remains on the surface and does not penetrate into microstructure. Andrés et al. (2012) when evaluated the kinetics of oil transfer in deep fat and air frying, observed that both processes described a similar trend, however, the amount absorbed was very different. During the initial frying time, the total oil content of the product increases considerably, and then it remains almost constant when increase the frying time in agreement with Bouchon and Pyle (2005). Oil content was much lower in the case of air frying, probably these differences are associated with the type of external fluid surrounding to heat the product which limits fats transfer. In terms of mass transfer mechanism, this is the main difference between the two types of frying.

1.3.3.3. Heat transfer

Heat is transferred from the fluid phase, in this case is hot air, to the product surface. The main characteristic of this technology is high speed of hot air. That is possible because this kinds of machine has a shape and distribution that works with the air flow and rather than against flow. In other words, no offers resistance to the smooth flow of air.Unlike conventional oven devices, the blower is placed in the top center of the cooking chamber. The air then travels downward until bottom, which have a special design to facilitate air circulate, this allows that heat circulates fast around a metal mesh cooking basket. The air cools as it contacts the food, but simultaneously accelerates on the center of the chamber. This change in velocity allows more effectively exchanging the heat and compensates for the dropping temperature (Erickson, 1989).

Compared with conventional oven, heat transfer occurs faster with air fryer due to the air velocity (Erickson 1989). However, Andrés et al. (2012) compared heat transfer in air and deep fat frying process, and observed that air process had lower energy

efficiency, which is explained because the individual heat transfer coefficient, since is lower in air frying than in deep oil frying and therefore heat transfer is much lower.

2. OBJETIVOS

2.1. OBJETIVO PRINCIPAL

El objetivo principal de esta Tesis Doctoral se centra en la mejora de la calidad y estabilidad del medio de fritura y de los productos fritos, mediante el empleo de diferentes tecnologías emergentes tales como la fritura en condiciones de vacío, la fritura por medio de aire caliente y la aplicación de antioxidantes naturales procedentes del romero (Rosmarinus officinalis).

2.2. OBJETIVOS ESPECIFICOS

Los objetivos específicos hacen referencia a cada una de las tecnologías estudiadas y se desarrollan en cuatro capítulos cuya finalidad es:

1. Caracterizar diferentes extractos comerciales de romero (Rosmarinus officinalis) obtenidos mediante diferentes procedimientos y evaluar el efecto de los mismos en nuggets de pollo pre fritos, durante su almacenamiento en congelación.

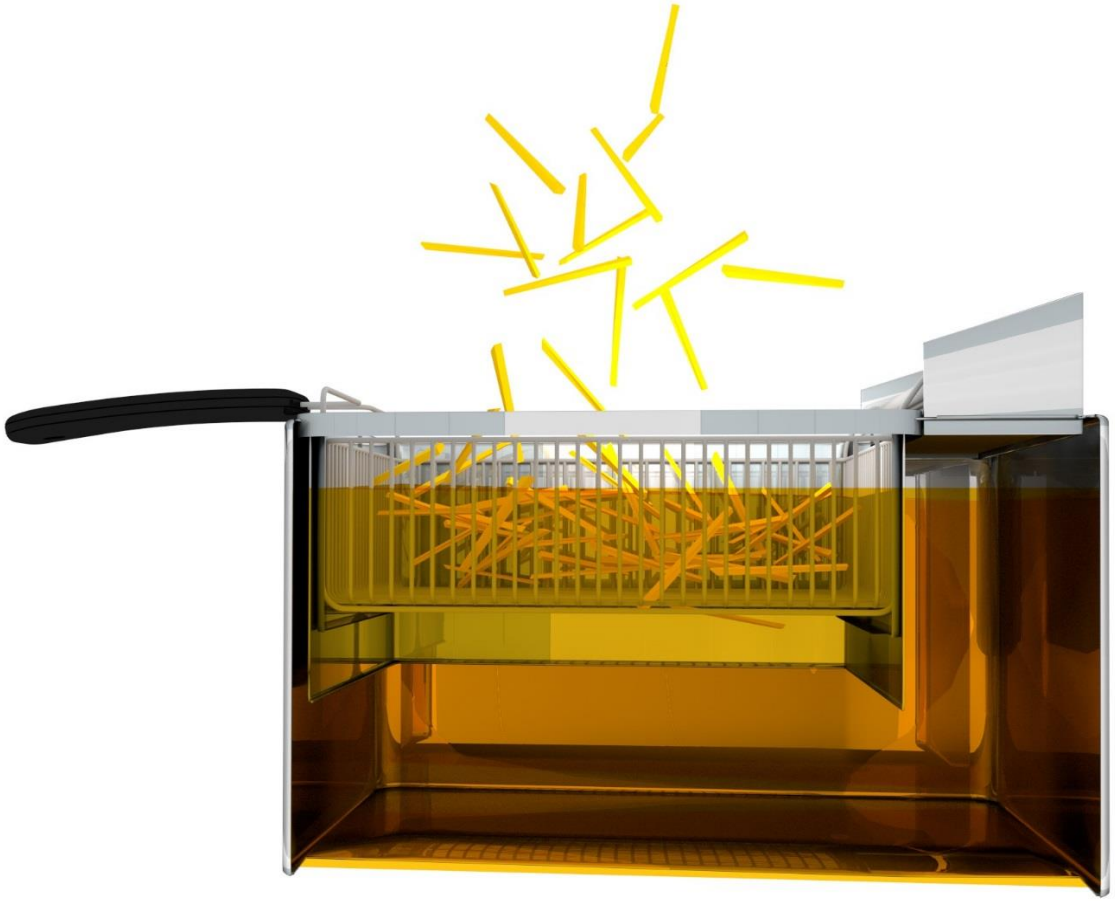
2. Estudiar la aplicación de un extracto comercial de romero en aceite de girasol a lo largo del proceso de fritura de cara a evaluar su efecto sobre la calidad del aceite y sobre los productos cocinados en el mismo.

3. Estudiar el proceso de fritura a vacío en nuggets de pollo pre fritos para evaluar aspectos relacionados con su calidad y analizar las condiciones óptimas de procesado del producto.

4. Estudiar el proceso de fritura mediante aire caliente en patatas tipo "French fries" para evaluar aspectos relacionados con su calidad, analizar las condiciones optimas de

procesado del producto y valorar si puede ser considerado una alternativa al producto tradicional.

3. MATERIAL Y MÉTODOS



En la presente tesis doctoral se evaluó el efecto de diferentes métodos de fritura y del extracto de romero sobre la calidad y estabilidad del aceite y de los productos cárnicos "nuggets" prefritos. El trabajo se ha estructurado en 4 ensayos. A continuación se describe la metodología seguida para cada uno de ellos.

3.1. EFECTO DE DIFERENTES EXTRACTOS DE ROMERO (*Rosmarinus officinalis*) EN LA CALIDAD DE NUGGETS DE POLLO CONGELADOS.

3.1.1. MATERIAS PRIMAS Y PREPARACIÓN DE MUESTRAS

3.1.1.1. Elaboración de nuggets de pollo

Para la realización del presente ensayo, se procedió a la elaboración de un producto cárnico pre-frito, nuggets de pollo, en la Planta Piloto de Tecnología de los Alimentos de la Universidad de Murcia. Con la finalidad de lograr un producto lo más similar al producto comercial de referencia, se realizaron ensayos preliminares hasta obtener una formulación óptima de la masa cárnica, así como del rebozado.

La elaboración de la masa cárnica incluyó: pechuga de pollo (60%) (SADA, Valencia, España), copos de puré de patatas (15%) (McCain alimentarie S.A.S, Harnes, Francia), hielo (23%), sal (1%) (Salinas del Odiel S.L., Huelva, España) y albúmina de huevo (1%) (Huevos Guillén S.L, Valencia, España). La carne y el hielo se picaron en una picadora de disco (Robot coupe, Borgoña, Francia) durante 30 segundos. Posteriormente, y en la misma picadora, se procedió a la incorporación del resto de condimentos y mezclado durante 30 segundos. A continuación se formaron los nuggets en una formadora manual adquiriendo unas dimensiones de 5×3×1 cm y un peso

aproximado de 25 g y se congelaron a -18 ºC. Seguidamente, los nuggets se rebozaron por inmersión durante 30 segundos, en una mezcla de harina de trigo (93.57%) (Harinera del Mar siglo XXI S.L., Castellón, España), sal (1.17%) (Salinas del Odiel S.L., Huelva, España), bicarbonato (0.24%) (Jesus Navarro S.A., Alicante, España), levadura (2.34%) (Mondelez Internacional, Madrid, España) y goma xantana (1.17%) (Solegraells, Barcelona, España).

Los antioxidantes se adicionaron al empanado, obteniéndose finalmente 5 tipos de productos distintos en función del antioxidante empleado:

-**Control** (Control): Sin antioxidantes.

-**Líquido Tocoferol** (Liquid-tocopherol): 300 ppm.

- **Polvo-acetona** (Powder-acetone): 600 ppm.

- **Líquido-acetona** (Liquid-acetone): 300 ppm.

- **Líquido-metanol** (Liquid-methanol): 900 ppm.

Las dosis elegidas para los diferentes extractos fueron seleccionadas de cara a asegurar que se alcanzaba la dosis máxima de 150 ppm (carnósico+carnosol) recogida en la Directiva Europea 2010/69.

Para la pre-fritura del producto se empleó aceite de girasol (Hacendado, S.A, España) y una freidora comercial con control automático de temperatura (marca Taurus S.L. Lérida, España). Los nuggets se pre-frieron a una temperatura de 165 ºC durante 30 s, tras lo cual, los nuggets se envasaron en bolsas de polietileno y se congelaron (−18 °C) durante 9 meses. Un total de 480 unidades fueron manufacturadas, 200 fueron utilizados para los análisis físico químicos (5 nuggets x 5 lotes x 4 tiempos) y 280 para el análisis

sensorial (7 nuggets x 5 lotes x 4 tiempos) x 2 réplicas. Los análisis de los nuggets durante el periodo de almacenamiento se realizaron en los meses: 0, 3, 6 y 9.

3.1.1.2. Extractos de romero

Para este ensayo se utilizaron 3 extractos de romero facilitados por la empresa Ingrenat S.L. (Cartagena, España).

-Polvo-acetona: extracto de romero en polvo obtenido usando acetona como disolvente.

-Líquido-metanol: extracto de romero en fase oleosa obtenido usando metanol como disolvente.

-Líquido-acetona: extracto de romero en fase oleosa obtenido usando acetona como disolvente.

La empresa certifica que los extractos son solubles en grasas y aceites hasta una concentración máxima de 1000 ppm. A nivel microbiológico, los tres extractos mostraron un recuento total inferior a 1000 ufc/g, un contenido de mohos y levaduras inferior a 100 ufc/g, ausencia de Salmonella en 25g y ausencia de E.Coli en 1g.

3.1.2. DETERMINACIONES ANALITICAS

3.1.2.1. Análisis en los extractos

Determinación de ácido carnósico y carnosol

El carnosol y el ácido carnósico fueron identificados y cuantificados en las muestras de extracto mediante cromatografía líquida de alta resolución (HPLC), de acuerdo con lo descrito por Okamura y col. (1994). Para su determinación, los extractos fueron

disueltos en acetona (1:10, p/v) y para eliminar aquellas sustancias insolubles se sometieron a agitación por medio de ultrasonidos y filtración con un filtro de nylon de 0,45 micras. La medida fue llevada a cabo con un cromatógrafo Agilent 1200 (Agilent Technologies, Inc., Estados Unidos) equipado con un detector de red de diodos (diode array), bomba cuaternaria y un auto-muestreador automático. Se empleó una columna analítica Hichrom Hi- RPB 18, 0,46 × 250 mm, con un diámetro de tamaño de partícula 5 µm. La columna se mantuvo a temperatura ambiente y se empleo una fase móvil compuesta por acetonitrilo (A) y ácido acético al 1% en agua purificada (B) con el siguiente gradiente: 0-10 min 30 % A, 70 % B; 10 a 22,5 min 65 % A, 35 % B; 22,5-27,6 min 100 % A, 0 % de B; 27,6 min 30 % A, 70 % B, deteniéndose a los 30 min. La velocidad de flujo fue constante a 1,2 ml/min. La cuantificación se realizó en base a soluciones patrón de carnosol y ácido carnósico.

Actividad antioxidante

Para la medida de la actividad antioxidante de los extractos de romero in vitro se emplearon los ensayos: FRAP, ABTS y Polifenoles totales.

El **método FRAP** se realizó siguiendo lo descrito por Benzie y Strain (1996). Este método evaluó la capacidad antioxidante de las muestra de acuerdo con su capacidad para reducir el hierro férrico (Fe^{+3}-TPTZ) presente en el complejo 2,4,6-tri(2-piridil)-s-triazina (TPTZ) hasta su forma ferrosa (Fe^{+2}-TPTZ), color azul, que presenta un máximo de absorción a 593 nm.

$$\text{Fe (III)} - \text{TPTZ} + 1e^- \longrightarrow \text{Fe (II)} - \text{TPTZ}$$

(incoloro) (azul)

Para la obtención del complejo fueron necesarios los siguientes reactivos: tampón acetato (300 mM) (Panreac Quimica SAU, Barcelona, España), pH 3,6, una solución de $FeCl_3\ _6H_2O$ (20 mM) (Panreac Quimica SAU, Barcelona, España) y una solución de TPTZ (2, 4, 6-tripyridyl-s-triazine)(10 mM) (TCI Europe N.V., Zwijndrecht, Bélgica) en HCl (40 mM) (Panreac Quimica SAU, Barcelona, España), los cuales se mezclaron en la proporción 10:1:1. Posteriormente, 1 ml de la solución FRAP, 1 ml de agua destilada y una alícuota de 25 µL de muestra, se mezclaron, incubando durante 4 min a 37 ºC y se determinó la absorbancia a una longitud de onda de 593 nm. Los resultados se expresaron como mM FeSO4. La recta de calibrado se realizó a partir de una solución estándar de $FeSO_4$.

El **método ABTS +** se realizó siguiendo lo descrito por Rodríguez-Caperna y col. (2011a). Este método se fundamenta en la cuantificación de la decoloración del radical ABTS•+, que es reducido en presencia de antioxidantes donadores de hidrógeno. El radical catiónico ABTS•+ es un cromóforo que absorbe luz a una longitud de onda de 734 nm. Para elaborarlo se mezcló una solución de ABTS (7 mM) (Sigma-Aldrich, Missouri, Estados Unidos) con la solución de persulfato potásico (2,45 mM) (Sigma-Aldrich, Missouri, Estados Unidos), después de la incubación a temperatura ambiente en la oscuridad 12 h. Posteriormente, la solución de ABTS se diluyó con etanol hasta obtener una absorbancia de 0,700 (±0,04 a 734 nm). Las muestras de los diferentes tipos de extractos de romero se diluyeron (1:6000) y una parte alícuota de 10 µl se mezcló con 1000 µl de solución de ABTS. Esta mezcla se dejó en reposo, a temperatura ambiente y condiciones de oscuridad, durante 4 min e inmediatamente se midió la absorbancia a 734 nm que se comparó con una curva estándar de Trolox (Sigma-Aldrich, Missouri, Estados Unidos), expresando los resultados como equivalentes de mM Trolox.

Los **polifenoles** presentes en los extractos fueron cuantificados colorimétricamente mediante el Índice de Folin Ciocalteu (Rodríguez-Carpena y col. 2011a). Para lo cual se tomo una alícuota de 200 µL de cada extracto (1:2500) y se le adicionó 1000 µL de reactivo de Folin-Ciocalteu (1:10) (Panreac Química S.A., Barcelona, España) y 800 µL de disolución de sodio carbonatado al 7.5% (p/v). Los compuestos fenólicos se oxidaron por el reactivo de Folin-Cicalteu (Panreac Química S.A., Barcelona, Spain), lo que resultó en una coloración azul, la cual fue medida en un espectrofotómetro modelo UNICAM UV/Vis Spectrometer (Spectronic Unicam, Texas, Estados Unidos) a una longitud de onda de 765 nm tras 20 minutos de incubación. El contenido de compuestos fenólicos se calculó en base a una curva de calibrado realizada con ácido gálico, y los resultados se expresaron como equivalentes de ácido gálico (mg/100 g materia fresca).

3.1.2.2. Análisis en el producto (nuggets de pollo)

pH

La determinación de pH se realizó según la norma ISO (1999) sobre el producto cárnico pre-frito descongelado. Para ello se pesaron 5 g de muestra picada, se añadieron 5 ml de agua destilada y con ayuda de una varilla de vidrio se llevo a cabo su completa homogeneización durante 5 min. Posteriormente, se dejó reposar la muestra durante otros 5 min antes de proceder a la medida del pH con un pHmetro (Crison GLP 21, Crison Instruments, Barcelona, España) con electro combinado Crison, Cat nº 52-22 (Ingold Electrodes, Inc. Wilmington, Estados Unidos). Los análisis fueron realizados por triplicado.

Color

La medida de color de las muestras se realizó por reflexión en superficie sobre el producto cárnico pre-frito descongelado, utilizando un colorímetro portátil Minolta Chroma Meter II Reflectance, CR-200/08 (Minolta Limited, Milton Keynes, Reino Unido) a partir del espectro de reflexión de las muestras, utilizando el observador estándar de 10º y el iluminante D65. Los análisis fueron realizados por triplicado.

Sustancias reactivas con el ácido 2-tiobarbitúrico (TBARS)

La valoración del grado de oxidación lipídica se realizó mediante el método del TBARS descrito por Tarladgis y col. (1960). Para ello 10 g de muestra fueron homogenizados con 1 ml de butil hidroxi anisol (BHA, Acros Organics, New Jersey, USA) y 49 ml de agua destilada. El volumen total se traspasó a un matraz esférico de 1000 ml de capacidad, al cual se añadieron 2.5 ml de disolución de HCl (4N). La destilación se llevó a cabo en un matraz esférico en contacto con una manta calefactora a 130 ºC y se conectó la columna de refrigerante. Posteriormente 50 ml de destilado fueron filtrados y depositados en un tubo con tapón de rosca, cubierto con papel de aluminio para evitar el contacto con la luz. Para realizar la reacción con TBA se debe utilizar la misma proporción de destilado y de reactivo de TBA, por lo que una alícuota de 5 ml del extracto se introdujo en un tubo de vidrio pirex con tapón de rosca, añadiéndoles 5 ml de la disolución de ácido 2-tiobarbitúrico (0,8%) (TBA) (Acros Organics Geel, Bélgica). Para la preparación de la solución blanco, se utilizaron 5 ml de agua destilada y 5 ml de TBA (0.8%). Una vez cerrados, las muestras y el blanco se introdujeron en un baño de agua en ebullición durante 35 min. Transcurrido el período de incubación, la muestra fue rápidamente enfriada en un baño a temperatura ambiente (22±2 ºC) durante 5 min y se midió la absorbancia a 532 nm. La cuantificación de TBARS se realizó

siguiendo el protocolo de Tardlagis y col. (1960), obteniendo el resultado en mg de malondialdehido (MDA)/kg de muestra. Los análisis fueron realizados por triplicado.

Análisis sensorial

La evaluación sensorial de los productos, a diferentes tiempos y tratamientos, se realizó mediante un análisis sensorial descriptivo cuantitativo (QDA) por un panel de cata entrenado. La selección y entrenamiento del panel se realizó de acuerdo con la ISO (2012). El panel estuvo formado por 9 jueces (6 mujeres y 3 hombres) y fue sometido a una etapa de entrenamiento específico llevado a cabo en 7 sesiones teórico-prácticas de 1 hora de duración cada una, orientadas hacia el aprendizaje de los atributos. Las tres primeras sesiones se dedicaron a la evaluación y discusión de las características sensoriales propias del producto. Las cuatro siguientes a la generación y selección de los descriptores y cuantificación de los mismos. Los atributos seleccionados fueron: Textura (crujiente, jugosidad, firmeza, cohesividad), olor (intensidad de olor, olor a rancio), color (del empanado, de la masa), intensidad de sabor y sabor rancio.

Las catas se realizaron en una sala estandarizada (ISO, 2007) equipada con una mesa redonda para las sesiones de entrenamiento, 7 cabinas individuales y un equipo de aire acondicionado.

Las muestras de nuggets de pollo pre-fritas y congeladas se cocinaron en una freidora comercial (Taurus S.L., Lérida, España) provista de aceite de girasol a 165 ºC (Sovena S.A., Sevilla, España) durante 5 minutos. Una vez cocinadas se obtenían piezas de dimensiones uniformes (2x1,5cm) (4 piezas por nugget), se envolvían en papel de aluminio y se codificaban con 3 dígitos. Las muestras se entregaron instantáneamente a los panelistas. Entre las muestras, los panelistas dispusieron de agua mineral y biscotes.

La evaluación de los atributos se realizó con una escala no estructurada de 10 cm. Los panelistas realizaron los análisis de cada muestra por cuadruplicado. Las muestras fueron numeradas con 3 dígitos y fueron asignadas a los miembros del panel siguiendo un modelo aleatorio.

3.2. ESTABILIDAD DEL ACEITE DE GIRASOL CON EXTRACTO DE ROMERO (*Rosmarinus officinalis*) DURANTE EL PROCESO DE FRITURA.

3.2.1. MATERIAS PRIMAS Y PREPARACIÓN DE LAS MUESTRAS

Para la realización de este estudio se utilizaron patatas tipo French-Fries congeladas (Agristo NV, Harelbeke-Hulste, Bélgica), adquiridas en un comercio local y conservadas en congelación (-18 ºC) hasta su uso.

Para la fritura se empleó aceite de girasol (Sovena S.A., Sevilla, España) el cual fue almacenado en refrigeración y en oscuridad hasta el inicio del experimento.

Los extractos de romero utilizados fueron proporcionados por la empresa Ingrenat S.L. (Cartagena, España). Para su obtención se utilizaron hojas de romero (*Rosmarinus officinalis*) y se llevó a cabo una extracción sólido líquido utilizando como disolvente metanol. El proceso de extracción fue optimizado por la empresa para mejorar la pureza y la calidad del extracto, y se encuentra actualmente bajo proceso de patente.

3.2.2. PROCEDIMIENTO DE FRITURA

La fritura se llevó a cabo con aceite de girasol estableciéndose dos lotes de trabajo:

-Control (SO): aceite de girasol.

-Aceite de girasol con extracto de romero (SOR): Aceite de girasol con 500 ppm de extracto de romero (liquido-metanol).

De entre los 3 extractos mencionados anteriormente en el apartado 3.1.1.2., se eligió el extracto de romero líquido obtenido con metanol para este trabajo, ya que cumple, además de la normativa prevista en materia de aditivos para la Unión europea, también podría ser comercializado en otros países como Estados Unidos y Japón.

La fritura se realizó en una freidora eléctrica (Taurus, España), siguiendo el protocolo descrito por Lalas y Dourtoglou (2003). El proceso de fritura se realizó a 180°C, para lo cual la freidora se conectó 20 minutos antes de comenzar el ensayo y se mantuvo un ratio de producto en aceite de 1.2:20 (masa/volumen). Se realizaron 4 ciclos de fritura conformados cada uno de ellos por 10 lotes. En cada lote se emplearon 150± 5g de patatas. Para cada ciclo se procedió del siguiente modo: 150 g de patatas fueron fritos durante 5 minutos, finalizados los cuales el cestillo fue retirado del aceite y se procedió a un escurrido de 1 minuto. Para asegurar que la temperatura durante la fritura fuera constante, se esperó 10 minutos entre lotes y se utilizó una sonda IKA Labortechnik ETS-D4 fuzzy (IKA®-WERKE GMBH & CO.KG, Staufen, Alemania). Tras cada ciclo de fritura, se procedió a la toma de muestra. Treinta gramos de aceite fueron retirados y almacenados en botellas opacas, con una atmosfera de nitrógeno y almacenados en congelación para su posterior análisis. El volumen de aceite no fue repuesto durante el proceso de fritura (4 ciclos).

3.2.3. DETERMINACIONES ANALITICAS

3.2.3.1. Análisis en los extractos

Ácido carnósico y carnosol

El ácido carnósico y carnosol fueron identificados y cuantificados en las muestras de extracto mediante cromatografía líquida de alta resolución (HPLC) como se describió anteriormente en el apartado 3.1.2.1.

3.2.3.2. Análisis en el aceite

Compuestos polares

La determinación de compuestos polares en el aceite se llevó a cabo mediante una sonda Testo 265 (Testo, Barcelona, España), realizándose una medida en cada intervalo de fritura.

Viscosidad

La viscosidad del aceite fue monitorizada usando un viscosímetro tipo copa Ford (ASTM D 1200).

Índice de estabilidad oxidativa

La medida de la estabilidad oxidativa del aceite se determinó usando el Equipo Rancimat modelo 743 (Herisau, Suiza). Este equipo permite la medida del grado en el que un aceite se oxida en condiciones pre-establecidas de oxígeno y temperatura, mediante la evaluación de los subproductos generados en la oxidación. El procedimiento analítico seguido fue el descrito por Martínez-Tomé y col. (2001). De cada aceite se pesaron 3 g que se introdujeron en el equipo programado para mantener la muestra a temperatura de 110 ºC y con un flujo de oxígeno constante. En este método la estabilidad oxidativa se define como el tiempo (en horas) necesario para que la

reacción de oxidación alcance el punto de inflexión en la representación gráfica de la conductividad vs tiempo.

Color

La medida de color se realizó con un colorímetro portátil Minolta Chroma Meter II Reflectance, CR-200/08 (Minolta Limited, Milton Keynes, Reino Unido), utilizando el observador estándar de 10º y el iluminante D65.

Para realizar la medida se tomaron 100 ml de aceite que fueron introducidos en un tubo de vidrio a partir del cual se procedió a realizar la medición por triplicado.

3.2.3.3. Análisis en el producto (patatas)

Análisis sensorial

La evaluación sensorial del producto obtenido, patatas fritas, en cada ciclo se llevó a cabo por un panel entrenado formado por 9 jueces, utilizando una prueba descriptiva cuantitativa (QDA). La selección y entrenamiento del panel se realizó de acuerdo con la ISO (2012). El panel fue sometido a una etapa de entrenamiento específico llevado a cabo en 7 sesiones teórico-prácticas de 1 hora de duración cada una, orientadas hacia el aprendizaje de los atributos. Las tres primeras sesiones se dedicaron a la evaluación y discusión de las características sensoriales propias del producto. Las cuatro siguientes a la generación y selección de los descriptores y cuantificación de los mismos: intensidad de color, intensidad de olor, sabor, persistencia, aceitoso, crujiente, calidad global.

Al igual que en el primer ensayo, las catas se realizaron en una sala estandarizada según la norma ISO (2007) equipada con una mesa para las sesiones de entrenamiento, 7 cabinas individuales y un equipo de aire acondicionado.

Las muestras se presentaron a los panelistas inmediatamente después de ser cocinadas. Entre muestras, los panelistas dispusieron de agua mineral y biscotes. La cuantificación de los atributos se realizó con una escala no estructurada de 10 cm. Los panelistas realizaron los análisis de cada muestra por cuadruplicado. Las muestras fueron numeradas con 3 dígitos y fueron asignadas a los miembros del panel siguiendo un modelo aleatorio.

3.3. USO DE FRITURA A VACÍO EN EL PROCESADO DE NUGGETS

3.3.1. MATERIAS PRIMAS Y PREPARACIÓN DE MUESTRAS

3.3.1.1. Elaboración de nuggets de pollo

La elaboración de nuggets se llevó a cabo según lo descrito en el apartado 3.1.1.1. Para la realización de este experimento se fabricaron cuatro lotes de nuggets de pollo. Un total de 542 unidades fueron producidas, 200 destinadas al análisis físico-químico y 342 al análisis sensorial.

3.3.2. PROCEDIMIENTO DE FRITURA

Se realizaron dos procesos de fritura, fritura a vacío Gastrovac® y fritura a presión atmosférica.

3.3.2.1. Fritura a vacío

Para la fritura en condiciones de vacío a diferentes tiempos y temperaturas se utilizó el equipo de cocción a vacío Gastrovac®. El esquema del equipo y los diferentes componentes del mismo se muestran a continuación.

Figura 7. Esquema del equipo de fritura (Gastrovac).

1. Olla de acero inoxidable. Su vaso está conectado a la fuente de calor mediante una sonda de temperatura.

2. Tapa. Realizada en metacrilato resistente a las condiciones de trabajo que permite la visualización del proceso.

3. Selector de temperatura. Permite establecer la temperatura del líquido de fritura.

4. Manómetro para el registro de la presión de vacío en el interior y una válvula de salida que permite el mantenimiento del vacío, para lo que está conectada a través de un tubo de conexión de vacío a la bomba de vacío.

5. Bomba de vacío. Provoca la disminución de la presión atmosférica hasta alcanzar el equilibrio correspondiente a la presión de evaporación de líquido introducido en el vaso de la olla a la temperatura seleccionada.

6. Sistema de calefacción o fuente de calor, conectado al selector de temperatura.

7. Tubo de conexión de vacío de material adecuado, goma, para soportar las condiciones de trabajo especiales en las que se encuentra el mismo.

El proceso de fritura de las muestras se realizó según el protocolo desarrollado por Andrés-Bello (2010). En primer lugar se llenó el vaso de la olla con dos litros de aceite de girasol y se introdujeron las muestras en un cestillo metálico que se mantiene suspendido de la tapa mediante una ballesta. A continuación se cerró herméticamente con dicha tapa, que conecta la bomba de vacío mediante un tubo de material resistente a las condiciones de trabajo a una válvula que permite el mantenimiento del vacío constante en el interior del equipo. Mediante el selector de temperatura se establecieron las diferentes temperaturas del aceite y, utilizando la bomba de vacío, se disminuyó la presión atmosférica hasta alcanzar el equilibrio correspondiente a la presión de evaporación del líquido a la temperatura seleccionada. Transcurrido el tiempo estimado para cada ensayo, se apagó la bomba de vacío y antes del restablecimiento de la presión atmosférica, se elevó el producto sobre el aceite y se centrifugó manualmente para disminuir el fenómeno de impregnación. El aceite de girasol empleado fue filtrado tras cada una de los ensayos.

3.3.2.2. Fritura a presión atmosférica

La fritura a presión atmosférica se llevó a cabo en una freidora redonda de agua Movilfrit F5 (Movilfrit S.A., Barcelona, España) con cestillo, filtro especial para residuos y sin cubeta desmontable. Este equipo está fabricado con exterior de metal, disponiendo de un depósito especial que permite usar agua y aceite, evitando que se mezclen sabores y tiene una capacidad de 5 l de aceite y 1 l de agua.

3.3.3. DETERMINACIONES ANALÍTICAS

Evolución de la temperatura

Para determinar la evolución de la temperatura en el centro del producto se empleó una sonda de punción Testo 925 (Testo GmbH y Co., Lenzkirch, Germany). La temperatura se midió después de cada tratamiento térmico para evaluar las variaciones de temperatura en el centro de cada nugget.

Humedad

La determinación de la humedad de las muestras se realizó siguiendo lo descrito en la norma ISO (1997). Este análisis se efectúa por método gravimétrico a través de la pérdida de masa por desecación. Siguiendo el protocolo descrito por Varela y col. (2008) el análisis de la humedad se realizó en la masa cárnica y rebozado por separado. Previamente al análisis, se procedió al secado de placas Petri de vidrio en una estufa Vaciotem modelo P-selecta (Selecta S.A., Barcelona, España) a 105 ºC durante 24 h, dejándose enfriar, antes de pesar la muestra, en un desecador hasta temperatura ambiente.

Para determinar la humedad se pesaron 5.0 ± 0.5 g de la muestra homogeneizada sobre las placas y se introdujeron en una estufa ST 6120 (Heraeus, Barcelona, España) a 105 ºC hasta peso constante. El porcentaje de humedad expresado en g de agua por 100 g de muestra, se calculó mediante la siguiente ecuación:

$$\%Humedad = \frac{(m1 - m0) - (m2 - m0)}{(m1 - m0)} * 100$$

m0 = masa del pesa sustancias (g).

m1 = masa del pesa sustancias más la muestra antes de la desecación (g).

m2 = masa del pesa sustancias más la muestra después de la desecación (g).

Contenido en grasa

La determinación del contenido en grasa total en materia seca se realizó gravimétricamente mediante una extracción tipo Soxhlet con éter de petróleo (ISO,1973). El equipo utilizado para la extracción de grasa fue Soxtec System 2055 Tecator (FOSS, Hillerød, Dinamarca). Para la determinación de la cantidad de grasa en las muestras, se pesaron 5.0 ± 0.5 g de las mismas, previamente deshidratadas en estufa a vacío (Heraeus, Barcelona, España) hasta peso constante.

Las muestras se introdujeron en cartuchos de celulosa y se colocaron en el equipo de extracción. Se adicionaron 90 ml de éter de petróleo como solvente orgánico (Panreac Química, S.A., Castellar del Vallés, España). La extracción se realiza en dos etapas y concluye con una tercera de recuperación del solvente. Tras la extracción es necesaria la evaporación de los restos de éter, para lo cual los botes de extracción se introdujeron en estufa a 105 ºC durante 30 min. Posteriormente, se enfriaron hasta temperatura ambiente en un desecador, calculándose el porcentaje de grasa total en materia seca con la siguiente expresión:

$$Xg = \frac{(Pbg - Pbs)}{Pm}$$

Xg = cantidad de grasa de la muestra (g grasa/ g muestra seca).

Pbg = peso del bote + grasa (g).

Pbs = peso del bote antes de extracción (g).

Pm= peso de la muestra (g).

Color

La medida de color de las muestras se realizó por reflexión en superficie del producto cocinado, con un colorímetro Minolta modelo CM3600d (Minolta Co. Ltd, Tokio, Japón), según lo descrito en los apartados 3.1.2.2. y 3.2.3.2. Además de las coordenadas L*, a* y b*, para monitorizar los cambios inducidos durante el proceso de cocinado se calcularon los parámetro Chroma* y Hue*.

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

$$H^* = arctg(b^*/a^*)$$

Textura

La textura de los nuggets de pollo se evaluó instrumentalmente con un texturómetro TA-XT2 Texture Analyser (Texture Technologies Corp., Nueva York, Estados Unidos) equipado con una célula de carga de 50 Kg. Se realizó el análisis del perfil de textura (TPA) de las muestras con una sonda cilíndrica de 7,5 cm de diámetro. El ensayo se realizó mediante la aplicación de dos ciclos consecutivos a una velocidad constante de 0,5 mm/segundo hasta alcanzar el 50% de la altura inicial de la muestra. Los parámetros analizados fueron: dureza, elasticidad, adhesividad, cohesividad, gomosidad y masticabilidad y se calcularon según lo descrito por Bourne (1978). La Figura 8 muestra una curva tipo de TPA y las definiciones de cada uno de los parámetros de textura analizados. Para determinar el perfil de textura, se realizaron 3 réplicas por muestra a una temperatura de sala de 20 ºC.

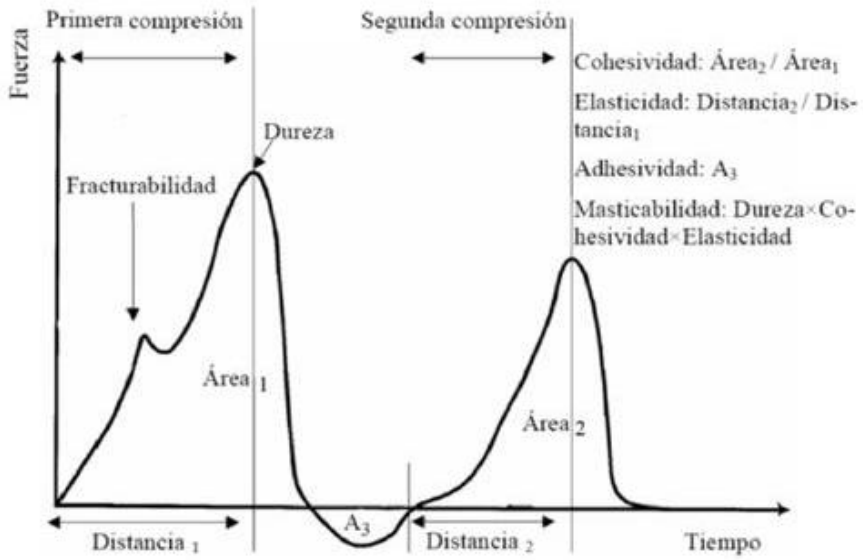


Figura 8. Curva tipo para el Análisis del Perfil de Textura (TPA) (Bourne y col., 1978; Breene, 1975).

Análisis sensorial

La preparación de las muestras se llevó a cabo tal y como se describe anteriormente (3.1.2.2.). Una vez cocinadas las muestras se cortaban en piezas rectangulares de aproximadamente 2 x 1,5 cm, se codificaban con un número de tres dígitos y se presentaban de inmediato a los panelistas. Los análisis sensoriales se realizaron en cabinas individuales de acuerdo a la norma ISO (2007).

Se realizaron dos análisis sensoriales sobre el producto, "Just about right" y prueba pareada, los cuales se describen a continuación.

El objeto del primer análisis fue evaluar cómo afecta el tiempo de cocinado en la aceptabilidad y preferencia del consumidor de nuggets de pollo. Para lo cual se evaluaron los atributos color, textura y jugosidad de la masa, así como la aceptación general. El análisis sensorial se llevó a cabo con un panel de 60 catadores, no expertos y no entrenados. Cada catador evaluó tres muestras en la misma sesión, que correspondían a tres tiempos de cocción de fritura tradicional a 165 ºC (4 min, 6 min y 8 min). Los

atributos color, textura y jugosidad de la masa se evaluaron con una escala de punto ideal de 5 puntos, cuyo extremo izquierdo (1) corresponde a "poco", el centro (3) a "ideal" y el extremo derecho (5) a "mucho". Las escalas de punto ideal, también denominadas JAR (Just about right), miden las desviaciones de un atributo del producto relativo al ideal teórico de un nivel de respuesta. Estas escalas tienen un punto medio anclado en "justo lo ideal" o "ideal", y los extremos representan los niveles de intensidad de los atributos que son más alto y más bajo que el ideal. Siguiendo las consideraciones de Gacula y col. (2007), las puntuaciones individuales 1, 2, 3, 4 o 5 se transformaron a -2, -1, 0, 1, o 2, respectivamente. Las puntuaciones extremas -2, -1, 1, 2 fueron divididas en dos grupos de datos: un grupo que representa a aquellos consumidores que sentían que las muestras no cumplían con el nivel esperado para el atributo (por debajo de lo ideal, -2 y -1 puntos) y otro para aquellos consumidores que sentían que el producto superó el nivel esperado (por encima del JAR, 1 y 2 puntos). Por tanto, para cada muestra, la media de los valores por debajo del punto ideal o cero, corresponde a los valores de la desviación negativos (insuficientes del atributo) y la media de los valores por encima del punto ideal correspondía al valor de desviación positiva (excesivo del atributo). Se consideró que las muestras analizadas estaban dentro de la puntuación ideal, cuando más del 60% de los consumidores las determinaron como ideales y sus desviaciones negativas y positivas no superaban los 0,25 puntos (Arcia y col. 2010). Por otra parte se evaluó la aceptación general del producto mediante el uso de una escala estructurada de 9 puntos. Para complementar la información se preguntó a los catadores cuál fue el atributo decisivo en su elección.

El segundo análisis sensorial consistió en una prueba pareada (ISO, 2005) con objeto de verificar si existen diferencias de percepción entre las muestras procesadas a presión atmosférica y las procesadas a presión sub-atmosférica. Este análisis sensorial se llevó a

cabo con un panel de 81 catadores, no expertos y no entrenados que evaluaron los atributos sensación grasa, jugosidad, crujiente, color y aceptación general en una escala de 10 puntos.

3.4. ESTUDIO COMPARATIVO DE LAS CARACTERÍSTICAS DE PATATAS PRODUCIDAS MEDIANTE FRITURA EN PROFUNDIDAD Y FRITURA CON AIRE.

3.4.1. MATERIAS PRIMAS Y PREPARACIÓN DE MUESTRAS

Patatas

Para la realización de este ensayo se escogieron patatas de la variedad tipo "Maris Piper" envasadas en bolsa de polietileno, adquiridas en un comercio local (Morrisons, Reino Unido) y almacenadas en refrigeración 12 horas antes de su uso. Las patatas se lavaron con agua potable para eliminar residuos y se eliminó su piel mediante pelado manual. Posteriormente, se cortaron para obtener tiras de patatas homogéneas de dimensiones 9 x 9 x 30 mm. El producto fue lavado durante 1 minuto con el objetivo de eliminar el almidón residual de la superficie y secado con papel de filtro.

Aceite

El aceite utilizado fue aceite refinado de girasol siguiendo lo descrito en anteriores ensayos. El aceite fue obtenido en un comercio local (Morrisons, Reino Unido) y almacenado en condiciones de oscuridad y refrigeración hasta su uso.

3.4.2. PROCEDIMIENTO DE FRITURA

La metodología se llevó a cabo según lo descrito por Andrés y col. (2010). En ambos tipos de fritura los equipos fueron previamente conectados (30 minutos) hasta obtener una temperatura del medio de transmisión del calor de 180 ºC, controlada mediante una sonda de temperatura localizada en la parte interna del equipo. Una vez alcanzada la temperatura, 100 g del producto se introdujeron en las cestillas de ambos equipos. En la freidora por inmersión se mantuvo un ratio de producto en aceite de 1:20 y para los experimentos de fritura en aire caliente previo al cocinado se añadieron 0,45 g de aceite por 100 g de patatas. Se evaluaron los tiempos de cocinado en intervalos de 3 minutos hasta un tiempo final de 30 minutos.

3.4.2.1. Fritura por inmersión en aceite

Para la fritura por inmersión en aceite se utilizó una freidora Morphyrichards modelo 45470 (Morphyrichards, South Yorkshire, Reino Unido). Este equipo está fabricado con exterior de metal y contiene un cestillo metálico, cubeta desmontable, termostato e indicador de temperatura. La capacidad es de 3,2 litros de aceite y una potencia nominal de 2000 W.

3.4.2.2. Fritura con aire caliente

El proceso de fritura con aire se realizó con el equipo Philips Viva Collection Airfryer modelo AH-9000 con tecnología Rapid Air (Philips, Ámsterdam, Países Bajos). La tecnología Rapid Air utiliza aire caliente combinado con circulación de aire a alta velocidad, por lo cual el producto recibe calor de forma uniforme. El equipo está fabricado con materiales plásticos y contiene cestillo metálico, temporizador, termostato

e indicador de temperatura. La capacidad de producto es de 800 g y tiene una potencia nominal de 1300 W.

3.4.3. DETERMINACIONES ANALITICAS

3.4.3.1. Análisis realizados durante el proceso de fritura

Humedad

La determinación del contenido de humedad de las patatas tras los diferentes tratamientos se realizó siguiendo lo descrito en el apartado 3.3.2.2. con modificaciones (Ahmad Tarmizi y Niranjan 2010) al tratarse de un producto vegetal con mayor contenido en agua en su composición.

Contenido en grasa

La medida del contenido graso se realizó gravimétricamente mediante una extracción con éter de petróleo (ver apartado 3.3.3.). En este caso el equipo utilizado para la extracción de grasa consistió en un Soxhlet Quickfit (BDH, Reino Unido), evaporador rotacional modelo RE 111 (Büchi Labortechnik AG, Suiza) y una estufa Weiss-Gallenkamp (Reino Unido). El protocolo seguido fue estandarizado por Ahmad Tarmizi y Niranjan (2010) siguiendo lo descrito por AOAC.

Se pesaron 5.0 ± 0.5 g de las muestra deshidratadas y homogenizadas. Las muestras se dispusieron en el interior de cartuchos de celulosa (Fisher Scientific) y se dispusieron en el equipo de extracción. Un matraz de fondo redondo previamente secado y pesado de 250 ml (Quickfit-BDH, Poole, Reino Unido) se llenó con 150 ml de éter de petróleo (Fisher Scientific) y se conectó al sistema extractor-calefactor. El proceso de extracción se mantuvo 4 horas. Una vez finalizada la etapa de extracción, el disolvente se eliminó

mediante el uso de una evaporación rotativa bajo vacío (380 a 510 mm de Hg) a 50 ºC. El matraz que contenía la grasa extraída se secó hasta peso constante a 105 ºC. El cálculo del contenido de grasa se realizó según lo descrito en el apartado 3.3.3. (g de aceite por 100 g de materia seca desgrasada).

Color

La medida de color de las muestras se realizó por reflexión en superficie del producto, con un colorímetro HunterLab modelo ColorQUEST CT-1100 (Hunter Associates Laboratory, Inc., Estados unidos), siguiendo lo descrito anteriormente.

Textura

La textura de las patatas se determinó instrumentalmente mediante un analizador de textura Brookfield CT3 Texture (Estados Unidos) equipado con una célula de carga de 25 Kg. Para la recopilación y el análisis de los datos utilizo el software Texture Pro CT. El análisis se realizó mediante la aplicación de un ciclo de compresión con una sonda cilíndrica de extremo plano de 2 mm de diámetro. La velocidad de la prueba fue de 1,6 mm/s. Se realizaron dos análisis sobre la tira de patata (9 x 9 x 30 mm), el primero comprimió el producto hasta 2 mm y la segunda prueba lo comprimió hasta los 6 mm. De esta forma se pudo evaluar tanto la región de la corteza como la del núcleo del producto. El parámetro textura obtenido fue dureza o fuerza máxima (mJ).

3.4.3.2. Análisis realizados en el producto final

Análisis de la microestructura. Cryo-SEM

El análisis estructural de las muestras se realizó mediante la técnica Cryo-SEM (microscopía electrónica de barrido). Se utilizó un equipo FEI Quanta FEG 600 y una cámara externa de criocongelación Quorum PP2000T del servicio de microscopía de la Universidad de Reading (Reino Unido). Se cortaron piezas de 4 mm de largo, 4 de ancho y 2 mm de alto del producto cocinado. Las muestras se situaron en un portamuestras y se congelaron por inmersión en nitrógeno líquido (-183 ºC). Después se pasaron a la criocámara del microscopio donde se sublimaron y se observaron en la cámara fría por microscopia electrónica de barrido (SEM).

Calorimetría diferencial de barrido

La temperaturas y entalpías de gelatinización de las muestras se determinarón usando un calorímetro diferencial de barrido Perkin Elmer DSC 200 (Waltham, Massachusetts, Estados Unidos). Las muestras no necesitaron tratamiento previo, se extrajeron del interior del núcleo del producto, se pesaron y dispusieron en un porta-muestras de aluminio (TA Instruments, New Castle, Estados Unidos). Las muestras se mantuvieron 1 minuto a 20 ºC para equilibrar la temperatura, posteriormente se calentaron en un intervalo de temperaturas de 20 a 200 ºC a una velocidad de calentamiento de 10 ºC/min. Para finalizar el ciclo se enfriaron las muestras hasta la temperatura inicial, 20 ºC, a una velocidad de 200 ºC/min. Como referencia se utilizó un porta-muestras de aluminio vacío (Steeneken y Woortman 2009).

Análisis Sensorial

El análisis descriptivo cuantitativo (QDA) del producto se llevó a cabo por un panel entrenado formado por 9 jueces siguiendo lo establecido en el apartado de análisis sensorial del apartado 3.1.2.2. Se llevaron a cabo tres sesiones de entrenamiento de una duración 2 horas, en las cuales se determinaron los descriptores a estudio (Apariencia: color marrón, hinchado, sequedad, uniformidad de cocinado, sensación grasa.; Olor: patata al horno, patata cocida, frito, aceite viejo; Sensación en boca: suavidad de la piel exterior, dureza de la piel exterior, crujiente de la piel exterior, sequedad, aceitoso, huecos, humedad del interior de la patata, correoso, denso, cantidad de patata en el interior, harinoso; Sabor: dulce, acido; Flavor: aceitosa, patata al horno, patata cocida, arenosa: Retrogusto: Amargo, metálica, ácida, película aceitosa, dedos grasientos).

Perfil de temperatura

La medida de la temperatura en el interior del producto y del equipo fue registrada utilizando una sonda HI9880 (HANNA Instruments, Leighton Buzzard, Reino Unido).

La medida se realizó desde que el producto se introdujo en el equipo con una frecuencia de 30 segundos.

Análisis estadístico

El análisis estadístico de cada una de las experiencias se ha realizado empleando el programa SPSS 15.0 (SPSS Ibérica S.L.U., Madrid, España). Se ha aplicado el análisis descriptivo, tanto intragrupal como intergrupal, en los diferentes datos obtenidos. Para valorar la significación de las diferencias descritas se ha utilizado el análisis de varianza (ANOVA) y el análisis de contrastes post-hoc test de Tukey.

4. RESULTS AND DISCUSSION

4.1. EFFECT OF DIFFERENT FORMAT-SOLVENT ROSEMARY EXTRACTS (Rosmarinus officinalis) ON FROZEN CHICKEN NUGGETS QUALITY

4.1.1. INTRODUCTION

Chicken-based foodstuffs are becoming increasingly popular mainly as "ready-to-eat" products, such as frozen chicken nuggets, because of the reduced preparation time, their good nutritional quality as a protein source and the low cost and longer shelf-life in frozen conditions (Magdelaine et al., 2008). The high polyunsaturated fatty acid profile of chicken meat, while nutritionally interesting, makes the product very susceptible to oxidative reactions, which may be intensified by deep-frying, the usual preparation way of this product. Moreover, these lipid oxidation reactions, which are considered the major deterioration form in stored muscle foods, may still occur during frozen storage (Soyer et al., 2010). Such changes could affect the physical-chemicals parameters and sensory attributes (odor, color, and flavor) of the product, in addition to diminish the shelf-life (Selani et al., 2011).

Synthetic antioxidants have been successfully used to prevent lipid oxidation in chicken meat. However, increasing concerns over the safety of synthetic food additives have resulted in a trend towards "*natural products*". As a result, the industry faces a challenge to find effective antioxidants from natural sources to prevent deterioration in meat and meat products during processing and storage (Brannan, 2009). Among natural antioxidant sources, rosemary (*Rosmarinus officinalis L.*), a woody aromatic herb that is native to the Mediterranean countries, has recently been authorized by the European Union under Directive 95/2/EC and assigned E-392 as its E number (European Union Directives 2010/67/EU and 2010/69/EU) for use in meat product preservation. The addition of rosemary extract to poultry products has been shown to be effective in retarding lipid oxidation, and previous studies in chicken sausages (Liu et al.,2009) and

patties (Naveena et al., 2013) have pointed to the protective effect of rosemary extract (500-1500 ppm) and leaves (22.5-130 ppm) in inhibiting lipid oxidation.

Rosemary antioxidant activity is related to components such as phenolic diterpenes, carnosol (CAS No 5957-80-2) and carnosic acid (CAS No 3650-09-7) (Rodríguez-Rojo et al., 2012). The antioxidant capacity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms and chelate metal cations (Shan et al., 2005). Previous studies (Azmir et al., 2013; Wang et al., 2013) have reported that the yield of bioactive compounds can be changed or modified by using different extraction procedures, solvents, temperatures, pressures and times. In an earlier paper (Garrido et al., 2011) extraction systems to obtain red grape pomace extracts were studied, and the extraction process was seen to have a clear effect on the extract composition (antioxidant activity, total polyphenols and total anthocyanins) and on the inhibition of lipid oxidation in pork burgers.

Therefore, the aims of this study were (1) to characterize three different commercial rosemary extracts (*Rosmarinus officinalis)* obtained in different ways (format-solvent combinations) and (2) to evaluate the effect of these rosemary extracts on the physical-chemical and sensory quality of frozen chicken nuggets during 9 months of storage.

4.1.2. MATERIAL Y METHODS

4.1.2.1. Characterization of rosemary extracts

The rosemary extracts used in this study were elaborated by Natural Ingredients S.L. (Ingrenat S.L., Cartagena, Spain). The extracts were obtained from rosemary leaves by "*Liquid-Solid Extraction*" with methanol or acetone as principal extract and solvents.

Both solvents are usually used for phenolic diterpene extraction due to their hydrogen-bonding ability that provides a high antioxidant yield (Erkan et al., 2008**).** Both extraction processes (with acetone or methanol) were optimized by the company to improve the purity and deodorization of the extract, and are currently under patent. Two format types were considered: liquid and powders. Finally, the company obtained three types of extracts:

– Powder-acetone: powdered rosemary extract obtained using acetone as solvent.

– Liquid -methanol: liquid rosemary oil extract obtained using methanol as solvent.

– Liquid- acetone: liquid rosemary oil extract obtained using acetone as solvent.

Concentration of Carnosic Acid and Carnosol

Carnosol and carnosic acid were identified and quantified in the extract samples using high performance liquid chromatography (HPLC), as described by Okamuraet al. (1994). Extract samples were dissolved in acetone (1:10, w/v), and insoluble substances were removed by ultrasonic stirring and filtration through a 0.45 µm nylon mesh. The analysis was performed with an Agilent 1200 series HPLC instrument (Agilent Technologies, Waldbroon, Germany) equipped with an autosampler. The column was a HiChrom Hi-RPB 18 type with 0.46 × 250 mm with a 5 µm particle size diameter (Hichrom Ltd, Reading, United Kingdom). The mobile phase consisted ofacetonitrile HPLC (A) and purified water containing 1% acetic acid HPLC (B) applying the following gradient: 0-10 min 30% A, 70% B; 10-22.5 min 65% A, 35% B; 22.5-27.6 min 100% A, 0% B; 27.6 min 30% A, 70% B; stop 30 min. The flow rate was constant at 1.2 ml/min. The detector was equipped with a diode array detector (DAD) operating at 284 nm based on the standard solutions of carnosol and carnosic acid.

Antioxidant capacity

Total Phenolic Content: The total phenolic content (TPC) was determined by the Folin-Ciocalteu colorimetric technique (Rodríguez-Carpena et al., 2011a).

FRAP Assay: The total antioxidant capacity of the different extracts was determined using FRAP assay by the method of Benzie and Strain (1996) with some modifications. The method is based on the reduction of a ferric 2, 4, 6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) by antioxidants to the ferrous form (Fe^{2+}-TPTZ). The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl3·6H2O solution. All three solutions were mixed together in the ratio 10:1:1. The FRAP assay was performed by warming 1 mL of dH2O to 37 °C before adding 25 µL of sample and 1 mL of reagent and incubating at 37 °C for 4 min. Absorbance at 593 nm was determined relative to a reagent blank also incubated at 37 °C. The total antioxidant capacity of samples was determined against a standard of known FRAP value, ferrous sulphate.

ABTS Assay: Radical cation scavenging capacity was measured for the extract against ABTS+ generated as described by Rodríguez-Caperna et al. (2011a). The 2, 20 – azinobis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation (ABTS) solution was generated by the reaction of 7 mmol of ABTS and 2.45 mmol of potassium persulfate (in equal quantities) after incubation at room temperature in darkness for 12 h. The ABTS solution was then diluted with phosphate buffered saline (pH 7.4) to obtain an absorbance of 0.700 (0.04 at 734 nm). The samples of different types of rosemary extracts were diluted 1:6000. An aliquot of 10 µL of each diluted extract was added to 1000 µL of ABTS solution and mixed thoroughly. The reaction mixture was allowed to

stand at room temperature in the dark for 4 min, and the absorbance at 734 nm was immediately recorded. The absorbance of the reaction was compared to the Trolox standard curve previously described, and the results were expressed as mmoles of Trolox equivalents per gram of fresh matter.

4.1.2.2. Chicken nuggets

Sample formulation, preparation and storage conditions

The nuggets were experimentally manufactured following commercial practices for pre-fried products. For this purpose deboned skinless chicken breasts (60%) were minced with ice (23%) in a chopper for 30 seconds. The usual additives for commercial nuggets, 15% potato flakes (McCain alimentarie S.A.S., Harnes, France); 1% salt (Salinas del Odiel S.L, Huelva, España) and 1% albumin (Huevos Guillén S.L., Valencia, España) were used. All components were thoroughly mixed to provide a uniform blend, and the chicken nugget samples were prepared in characteristic shapes of 5×3×1 cm, each weighing 25 g, and frozen at −18 °C. The pieces were dipped in the prepared batter (wheat flour 93.57 %, salt 1.17% bicarbonate 0.24%, yeast 2.34% and xanthan gum 1.17%) for 15 seconds. Chicken nuggets were distributed into five different batches according to the following formulas: A control batch without any extract (1) and a batch with tocopherol extract (2) were used to check the rosemary extract effect. Tocopherol was selected because it is a commonly antioxidant used in food matrixes (McCarthy et al., 2001). The doses of the three different rosemary extracts were selected to ensure 150 ppm of carnosic and carnosol expressed as fat basic in products according to European Union Directive 2010/69/EU on food additives. Given the differences in the composition and antioxidant capacity of the three of extracts, the following doses were used doses to reach 150 ppm carnosic acid and

carnosol: 600 ppm powder-acetone (3), 900 ppm liquid-methanol (4) and 300 ppm liquid-acetone and liquid-tocopherol (5). All the nugget batches were pre fried using a household fryer (Taurus S.L., Lérida, Spain) for 30 seconds at 165 ºC in sunflower oil (Sovena España S.A., Sevilla, Spain). The pre-fried nuggets were then packaged in polyethylene bags and stored at −18 °C for 9 months. Physical-chemical (lipid oxidation, color and pH) and sensory analysis were carried out in the samples after 0, 3, 6 and 9 month. Physical-chemical (Lipid oxidation, color and pH) and sensory analyze were carried out in the samples after 0, 3, 6 and 9 month. A total of 480 chicken nuggets were used, 200 for the physical-chemical analysis (5 nuggets x batch x 4 time) by duplicated and 280 for the sensorial analysis (7 nuggets x 5 batch x 4 time) by duplicated.

Lipid oxidation

Thiobarbituric acid reactive substances (TBARS) were measured according to the method of Tarladgis et al. (1960). The analysis was repeated by triplicate.

Color coordinates (L*, a*, b*)

Color was measured using a Minolta CR400 colorimeter standardized using a white calibration plate (Minolta Camera Co., Osaka, Japan) (8-mm-diameter aperture, d/0 illumination system, D65 illuminant and a 2º standard observer angle) by triplicate. Lightness (L*), green-red chromacity (a*) and blue-yellow chromacity (b*) were measured according to the CIELab system.

pH

The pH of the nugget samples was measured by triplicate using Crison GLP21 equipment (Crison Instruments S.A., Barcelona, Spain) (ISO 1999).

Sensory analyses: Qualitative Descriptive Analysis (QDA)

For the sensory analysis, all evaluations were conducted in individual booths which contained the instructions for the evaluation procedure. The tasting room for sensory evaluation was air-conditioned and free of disturbing factors. The pre-fried nuggets samples were fried in a household deep fat fryer (Taurus S.L., Lérida, Spain) at 165 ºC for 5 minutes, until reaching an internal temperature of 72 ºC, as measured by a portable T200 thermometer (Digitron Instrumentation Ltd., Hertford, United Kingdom). Rectangular pieces of approximately 2 x 1.5 cm were obtained and immediately presented to the panelists.

The panelists were trained according to (ISO, 2012). Seven training sessions were carried out: in the three first, descriptors of chicken nuggets were studied and the following four sessions were concerned with identifying, selecting and quantifying attributes to evaluate the nuggets. In training and panel performance sessions, samples were coded with random three digit numbers and were presented individually to the panelists (Macfie et al., 1989). Mineral water was provided for mouth rinsing between samples. Sensory analysis was carried out using an unstructured scale of 10 cm. The textural parameters analyzed were crispness, juiciness, firmness, and cohesiveness. For the color, odor and taste characteristics the following attributes were considered: odor intensity, rancid odor, crust color, mass color, taste intensity, rancid taste. The sensory test was repeated by triplicate.

4.1.2.3. Statistical analysis

Data were analyzed with the statistical package SPSS 15.0 (Statistical Package for the Social Science for Window) (IBM, Armonk, New York, USA). The effect of the different type of extract on chicken nuggets quality was analyzed using ANOVA. When the differences among batches were significant ($p < 0.05$), Tukey's test at a significance level

4.1.3. RESULT AND DISCUSSION

4.1.3.1. Characterization of rosemary extracts

Composition of rosemary extract and antioxidant capacity

Table 5 shows the composition (total phenolics content, carnosic acid, carnosol, and essential oil content) of the rosemary extracts (powder acetone, liquid acetone, and liquid rosemary). Phenolic compounds constitute the main type of secondary metabolite with antioxidant activity in plant and herbs (Shan et al., 2005). In rosemary extracts, carnosic acid and its derivatives, carnosol, rosmadial, rosmanol, rosmanol isomers and methyl carnosate are the main compounds involved in such activity (Naveena et al., 2013). The results showed that the phenolic contents varied considerably (from 18.62 to 23.23 g GAE/100g extract) as a function of the solvent and the format type, a finding similar to that found by Dorman et al. (2003) for de-odourised aqueous rosemary extract. Shan et al. (2005) studied the total phenolic constituents of 26 spice extracts and found the value of 5.07 g GAE/100gr for the methanolic extract of rosemary, which is lower than the results the present study. In contrast, Han and Rhee (2005) found higher values (30.4 g GAE/100g) for the total phenolic content in rosemary dry powder extract

than those found in the current research, probably because their extract was not subjected to deodorizing and bleaching processes. Previously Sundram and Gapor (1994) observed that refined, bleached and deodorized process in oil, olein and stearin palm showed a partial loss (around 25%) of one of its mainly antioxidant, vitamin E.The phenolic content was higher in the powder acetone extract (23.23 g GAE/100g) than in the two liquid extracts. It seems that the dissolution process carried out to obtain the liquid extracts decreases the phenolic compound content. As regards the liquid extracts, the results revealed that liquid methanol obtained higher values than liquid acetone. Acetone and methanol have been extensively used as solvents for the extraction of phenolic compounds due to these compounds are distributed in low-to mid-polar extract (Zhao et al., 2006; Trabelsi et al., 2010; Wang et al., 2013). However, the yield in the liquid extraction process mainly depends on the solvents chosen, probably due to the polarities involved and therefore to the capacity to solubilize chemical constituents of the samples (Azmir et al., 2013). In disagreement, Wang et al. (2013) studied the effect of different polarity solvents on the extraction of phenolic compounds of various extracts of *Malus baccata* L. and obtained a major yield in samples extracted with 80% acetone, ethanol, ethyl acetate and distilled water, respectively. Zhao et al., (2006) also found that the acetone extract contained the highest amount of phenolic compounds followed by ethanol, methanol and water extract in barley (*Hordeum vulgare* L.). The results of the present study could be explained by the extraction of other chemical constituents in rosemary leaves such as essential oils, which would be included in the final extract. Rosemary essential oil includes compounds such as p-cimeno, linalool, gamma-terpineno, timol, beta-pineno, alfa-pineno, and eucalyptol, monoterpene hydrocarbons with a low molecular weight, high vapor pressure and low polarity (Da Cruz Francisco and Sivik, 2002), which have more

affinity for the acetone solvent, that interfering in phenolic compounds extraction. This is evident in the essential oil values obtained: liquid acetone extract is approximately 4 times more concentrated than liquid methanol extract (197.27 vs. 943 mg/kg extract). The results obtained for carnosic acid and carnosol concentration are in accordance those obtained for the measurement of total phenolics.

Table 5. Composition (Total phenolics, Carnosic acid, Carnosol, Essencial oil) and antioxidant capacities (FRAP, ABTS) of rosemary extracts.

	Type of extract		
	Powder acetone	**Liquid acetone**	**Liquid methanol**
Composition [A, B]			
Total phenolics (mg GAE/ 100 g extract)	23.23±0.39 [c]	18.62±0.55 [a]	20.41±0.20 [b]
Carnosic acid (g/Kg)	179.16±0.14 [c]	46.53±0.35 [a]	67.44±0.74 [b]
Carnosol (g/Kg)	28.88±0.76 [c]	5.84±0.17 [a]	15.57±0.06 [b]
Essencial oil (mg/Kg)	1052.00±39.68 [c]	943.00±50.21 [b]	197.27±8.00 [a]
Antioxidant activity [A, B]			
FRAP (mM/ g)	2063.82±7.00 [c]	660.31±4.04 [a]	1186.54±3.91 [b]
$ABTS^+$ (mM Trolox / g)	1043.47±12.85 [c]	247.30±2.06 [a]	811.66±17.12 [b]

[A] All date are expressed as mean value ± standard deviation (n=3). [B] Means with different letters ([a-b]) within the same column are significantly different ($p < 0.05$).

The powder rosemary extract was the most concentrated of active compounds and antioxidant capacity measured by FRAP and ABTS analysis. Some authors like Erkan et al. (2008) found that rosemary extract was a more powerful antioxidant than blackseed essential oil due to its higher phenolic content. Wang et al. (2013) studied the effect of solvent in *Malus baccata* L. extracts and described a higher antioxidant activity

in the extract containing the higher concentration of phenolic compounds. Amarowicz et al. (2004) observed a direct relation between the antioxidant activity and reducing power of certain plant extracts, which have been shown to exert an antioxidant action by breaking the free radical chain through the donation of hydrogen atom. The antioxidant activity mechanism of rosemary extracts is similar to that of other polyphenol and flavonoids. The presence of a catechol group in the aromatic ring (C_{11}-C_{12}) of the rosemary phenolic diterpene skeleton is probably the most important structural element in the antioxidant activity of these compounds (Shan et al., 2005). Therefore, the extracts containing more phenolic compounds, in particular carnosic acid and carnosol, prevent the formation of reactive radical species and produce a higher antioxidant effect. As can be viewed in the Table 5 where it is reported that the extract with more phenolic compounds is powder acetone extract and has more antioxidant activity, followed by liquid methanol extract and liquid acetone extract after this one.

4.1.3.2. Quality evaluation of chicken nuggets

Lipid oxidation

Figure 9 shows the TBARS (mg malondialdehyde/kg sample) values of the different chicken nugget batches stored at -18 ºC. The values varied between 4.07 and 5.88 mg malondialdehyde/kg during the 9 months storage period. These results agree with those obtained by Wang et al. (1976) in a study that evaluated frozen commercial fried chicken products, in which TBARS values ranged between 2.1 to 9.2 mg malondialdehyde/kg sample. The control group (no antioxidant) showed significantly higher ($p < 0.05$) lipid oxidation values associated with the storage time at 9 months. In the same way, Modi et al. (2004) reported increases in TBARS values during frozen storage (6 months) of chicken nuggets. However, the batches formulated with

antioxidant (powder-acetone, liquid-methanol, liquid-acetone, and liquid-tocopherol) maintained constant TBARS values ($p > 0.05$). A similar trend was observed by Modi et al. (2006) in chicken curry during 6 months of frozen storage, behavior that they associated with the effect of both the low temperatures and the antioxidant capacity of the spices added.

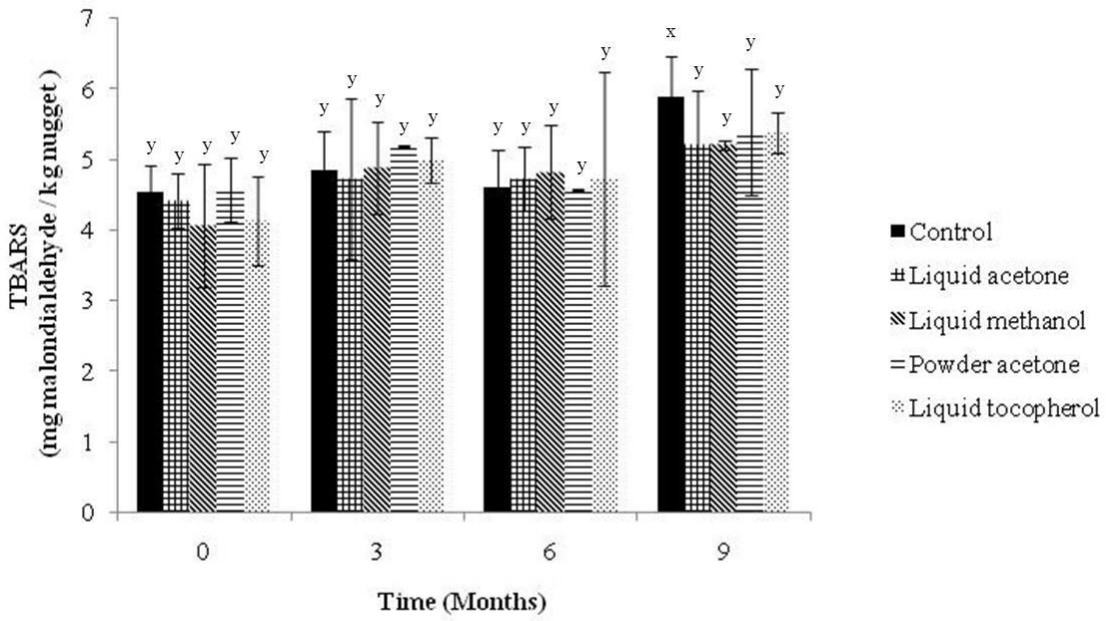


Figure 9. Thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde/Kg) in different chicken nuggets batters formulated samples for each frozen storage times (0, 3, 6, 9 months). Different letters (y-z) indicate significant differences among treatments ($p < 0.05$).

The differences between the treatment groups were not statistically significant, probably because freezing made it difficult to detect a clear deterioration of the product. The effectiveness of rosemary as an inhibitor of lipid oxidation in meat products has been documented so, some authors (Naveena et al., 2013) evaluated the effect of carnosic acid extracted from dried rosemary leaves in cooked chicken patties during refrigerated storage and observed a reduction of TBARS from 37 to 87% compared with the control group. Others (Hwang et al., 2013) showed an increase in the shelf life of raw and deep

fried chicken nuggets containing other natural antioxidants (*Artemisia princeps Pamp* and ascorbic acid) whose polyphenolic constituents inhibited lipid oxidation.

pH and color

The pH and color measurements of nuggets during the 9 months of frozen storage period are presented in Table 6. The initial pH values were similar to those found in previous studies in refrigerated chicken nuggets (6.35) (Kumar et al., 2011). Neither tocopherol nor rosemary extract significantly affected pH of the chicken nuggets ($p > 0.05$). Modi et al. (2006) also did not find significant differences in pH during a storage period of 6 months in curry chicken, while Naveena et al. (2013) found no effect of rosemary addition or storage on pH in buffalo and chicken patties. Selani et al. (2011) and Mohamed and Mansour (2012) neither showed changes due to the addition natural extracts or frozen storage in cooked chicken meat and beef patties, respectively.As respect the color coordinates, no significant effect ($p > 0.05$) of storage time was evident in any of the nugget groups. Selani et al. (2011) observed similar results in cooked chicken meat stored frozen in similar time (9 months). Machado-Velasco andVélez-Ruiz (2008) studied the physical properties of different types of Mexican foodstuffs, including cereal-based foods similar to nugget crusts, during frozen storage, finding no alterations after two months storage. In contrast, others like Giannou et al. (2005) and Kindt et al. (2008) observed an increase in crust color during the frozen storage of a mass-fried-bakery product (dough) and pasta, which was attributed to the formation of white ice spots on the crust surface. However, even after 9 months of frozen storage, the samples remained fairly acceptable from a commercial point of view.

Table 6. Color CIELab *(L*. a* and b**) and pH values in different chicken nuggets batters formulated samples for each frozen storage time (0, 3, 6, 9 months).

Color CIELab and pH values				
Treatment	**Storage time (months)** [A, B,]			
	0	**3**	**6**	**9**
L*(lightness)				
Control	67.69±1.85	70.58±1.97	70.05±1.72	68.60±1.79
Liquid acetone	68.37±3.95	70.46±1.28	70.83±2.16	69.79±1.00
Liquid methanol	67.35±1.92	69.61±1.32	69.88±0.52	68.90±1.96
Powder acetone	68.95±1.88	69.60±0.95	70.08±0.60	69.62±1.98
Liquid tocopherol	68.23±0.89	69.77±0.95	69.98±1.85	69.16±1.29
a* (redness)				
Control	-0.59±0.34^{b}	-0.64±0.41^{b}	-1.20±0.18	-0.93±0.19^{a}
Liquid acetone	-0.34±0.43^{b}	-0.61±0.27^{b}	-0.66±0.55	-0.40±0.31^{b}
Liquid methanol	-1.23±0.22^{a}	-1.16±0.23^{a}	-1.27±0.44	-0.86±0.30^{ab}
Powder acetone	-0.89±0.25^{ab}	-0.92±0.34^{ab}	-0.98±0.26	-0.55±0.11^{ab}
Liquid tocopherol	-0.86±0.31^{ab}	-1.03±0.40^{a}	-0.84±0.41	-0.59±0.12^{ab}
b* (yelowness)				
Control	12.23±6.88	14.23±2.11	13.46±1.81	12.29±2.97
Liquid acetone	13.89±1.96	14.03±1.90	14.46±2.81	12.65±1.40
Liquid methanol	13.71±0.85	13.47±1.57	14.70±1.02	13.49±1.82
Powder acetone	14.36±1.10	12.94±1.35	13.55±0.42	13.45±1.27
Liquid tocopherol	14.11±3.01	12.67±1.51	12.38±0.67	12.95±0.58
Ph				
Control	6.30±0.03	6.32±0.07	6.36±0.17	6.28±0.13
Liquid acetone	6.32±0.08	6.34±0.04	6.34±0.05	6.33±0.12
Liquid methanol	6.33±0.18	6.35±0.07	6.39±0.01	6.31±0.03
Powder acetone	6.43±0.13	6.42±0.09	6.49±0.03	6.40±0.07
Liquid tocopherol	6.39±0.08	6.38±0.07	6.41±0.04	6.41±0.03

[A] All date are expressed as mean value ± standard deviation (n=3). [B] Means with different letters ($^{a-b}$) within the same column are significantly different ($p < 0.05$).

As regards the impact of treatment, there were no significant differences ($p > 0.05$) between treatments and the control samples in L* and b* values. However, a significant effect ($p < 0.05$) was observed with regard to a* values, although this trend did not follow a consistent evolution, probably due to the small differences resulting from the "homemade" production process more than to the effect of extracts themselves. Accordingly, this result realized that the color of the extracts has not interfered with the characteristic batter color and therefore the doses used can be commercially applied in such products. Other natural antioxidants have been seen to adversely affect color in chicken and chicken products; for example, the application of wine-making residues in precooked and cooked chicken meat (Ganhão et al., 2011; Selani et al., 2011).

Sensory evaluation

Tables 7 and 8 show the sensory analysis results for the five treatment groups (control, powder-acetone, liquid-methanol, liquid-acetone, and liquid-tocopherol) after frozen storage for 0, 3, 6, and 9 months.

The texture attributes (crispness, juiciness, firmness, and cohesiveness) (Table 7) showed no clear treatment-related trends. In agreement, Mohamed and Mansour (2012) did not detect significant changes in juiciness and firmness scores in beef patties with added antioxidant extracts (rosemary and majoran). Significant differences ($p < 0.05$) were found in crispness and juiciness due to frozen storage, possibly associated with the ice crystals produced by the frozen process that may damage the tissues and result in drip losses during thawing (Totosaus and Kerry, 2012).The use of natural extracts (rosemary and tocopherol) did not affect mass or crust color, odor intensity or taste intensity (Table 8).

Table 7. Texture attributes of different chicken nuggets batters formulated in different frozen storage times (0, 3, 6, 9 months).

Sensory Evaluation of texture attributes*				
Treatment	**Storage time (months)** [A, B, C]			
	0	**3**	**6**	**9**
Crispness				
Control	6.40±1.45^{z}	6.27±1.64^{z}	5.07±0.94^{y}	4.67±1.21^{ab,y}
Liquid acetone	6.00±1.41^{z}	6.18±1.72^{z}	5.40±1.60^{yz}	4.60±1.37^{a,y}
Liquid methanol	6.43±1.61	6.34±1.75	5.93±1.34	5.62±1.23^{b}
Powder acetone	6.29±1.44	6.14±1.71	4.88±1.37	5.42±1.02^{ab}
Liquid tocopherol	6.84±1.25^{z}	5.88±1.29^{y}	5.67±1.10^{y}	5.20±1.22^{ab,y}
Juiciness				
Control	6.52±1.00^{z}	5.82±1.20^{yz}	5.10±0.86^{xy}	4.99±0.73^{x}
Liquid acetone	6.35±1.19^{z}	6.16±1.27^{z}	5.53±1.22^{yz}	5.10±0.83^{y}
Liquid metanol	6.31±1.30^{z}	6.13±1.47^{yz}	5.42±0.96^{y}	5.45±0.82^{y}
Powder acetone	6.41±1.12^{z}	6.20±1.28^{z}	4.99±1.48^{yz}	5.33±0.78^{y}
Liquid tocopherol	6.69±1.08^{z}	6.17±1.44^{yz}	5.64±0.98^{xy}	5.26±0.88^{x}
Firmness				
Control	4.00±1.70	4.68±0.98	4.08±1.20	4.00±0.94
Liquid acetone	4.33±1.28	3.97±1.15	3.90±1.01	3.83±1.08
Liquid methanol	3.70±1.08	4.23±1.43	3.96±1.19	3.75±1.65
Powder acetone	3.67±0.93	3.93±0.82	3.60±.98	3.63±1.33
Liquid tocopherol	3.72±1.47	3.89±1.17	3.99±1.15	3.63±1.12
Cohesiveness				
Control	3.84±1.56	4.53±1.14	4.86±1.42^{b}	4.00±1.20
Liquid acetone	4.53±1.46	4.06±1.45	4.56±0.90^{ab}	3.88±1.11
Liquid metanol	4.42±1.58	4.03±1.33	3.87±1.23^{a}	4.18±1.33
Powder acetone	3.93±1.28	4.35±1.08	3.87±1.06^{a}	3.97±1.46
Liquid tocopherol	4.03±1.24	3.73±1.70	3.68±1.47^{a}	3.87±1.44

[A] All date are expressed as mean value ± standard deviation (n=3). [B] Means with different letters (a-b) within the same column are significantly different ($p < 0.05$). [C] Means with different letters (x-z) within the same line are significantly different ($p < 0.05$).

Table 8. Sensory attributes of different formulationsat0, 3, 6, 9 months offrozen storage.

Sensory Evaluation of taste and odor attributes				
Treatment	**Storage time (months)** [A, B, C]			
	0	**3**	**6**	**9**
Odor intensity				
Control	9.96±0.19^{z}	9.38±0.70^{xy}	9.00±0.63^{x}	9.45±0.61^{y}
Liquid acetone	9.92±0.41^{z}	9.14±0.87^{yz}	9.29±0.64^{yz}	9.04±2.00^{y}
Liquid metanol	9.86±0.45^{z}	9.44±0.62^{y}	9.28±0.54^{y}	9.43±0.59^{y}
Powder acetone	9.92±0.28^{z}	9.62±0.50^{yz}	9.00±0.73^{x}	9.30±0.53^{xy}
Liquid tocopherol	9.96±0.14^{z}	9.34±0.83^{y}	9.35±0.65^{y}	9.50±0.5y
Rancid odor				
Control	0.00±0.00	0.00±0.00	0.06±0.25	0.05±0.22
Liquid acetone	0.01±0.04	0.10±0.31	0.00±0.00	0.16±0.47
Liquid methanol	0.00±0.00	0.00±0.00	0.00±0.00	0.04±2.09
Powder acetone	0.00±0.00	0.00±0.00	0.03±0.14	0.03±0.11
Liquid tocopherol	0.00±0.00	0.03±0.18	0.00±0.00	0.00±0.00
Crust color				
Control	9.85±0.60^{b,z}	9.54±0.64^{z}	8.94±0.68^{a,y}	9.60±0.59^{z}
Liquid acetone	9.33±1.17^{ab}	9.21±1.01	9.23±0.67^{ab}	9.52±0.77
Liquid methanol	9.57±0.92^{ab}	9.00±1.67	9.48±0.59^{b}	9.74±0.45
Powder acetone	9.69±0.63^{ab,z}	9.65±0.43^{yz}	9.28±0.61^{ab,y}	9.51±0.52^{yz}
Liquid tocopherol	8.96±1.58^{a}	9.03±0.78	9.48±0.60^{b}	8.88±1.95
Mass color				
Control	9.78±0.64^{z}	9.50±0.51^{b,yz}	9.06±0.57^{y}	9.65±0.59^{z}
Liquid acetone	9.79±0.66^{z}	8.76±2.41^{a,y}	9.32±0.75^{yz}	9.52±0.77^{yz}
Liquid metanol	9.43±1.62	9.38±0.61^{b}	9.40±0.58	9.74±0.45
Powder acetone	9.73±0.81^{z}	9.54±0.52^{b,yz}	9.18±0.63^{z}	9.51±0.52^{yz}
Liquid tocopherol	9.85±0.36	9.31±0.78^{ab}	9.09±0.80	9.62±0.57
Taste intensity				
Control	9.89±0.32^{z}	9.42±0.75^{yz}	8.69±0.94^{y}	8.65±2.13^{y}
Liquid acetone	9.88±0.45^{z}	8.41±2.51^{y}	9.17±0.70^{yz}	9.24±0.78^{yz}
Liquid methanol	9.89±0.42^{z}	9.38±0.71^{y}	9.24±0.60^{y}	9.52±0.67^{yz}
Powder acetone	9.74±0.50^{z}	9.41±0.65^{yz}	8.78±0.87^{yz}	8.42±2.50^{y}
Liquid tocopherol	9.96±0.20^{z}	9.13±0.87^{xy}	8.52±1.93^{x}	8.88±1.95^{yz}
Rancid taste				
Control	0.00±0.00	0.00±0.00	0.13±0.34	0.15±0.49
Liquid acetone	0.00±0.00	0.14±0.35	0.06±0.25	0.48±1.82
Liquid methanol	0.00±0.00	0.03±0.18	0.36±1.80	0.17±0.38
Powder acetone	0.00±0.02	0.02±0.12	0.50±1.78	0.13±0.41
Liquid tocopherol	0.00±0.00	0.03±0.18	0.13±0.34	0.42±1.77

[A] All date are expressed as mean value ± standard deviation (n=3). [B] Means with different letters (a-b) within the same column are significantly different ($p < 0.05$). [C] Means with different letters (x-z) within the same line are significantly different ($p < 0.05$).

The results of the sensory evaluation are similar to those described with the instrumental evaluation (Table 6), in which color did not vary between treatments or between sampling times (0, 3, 6, and 9 months). Naveena et al. (2013) described lower color and higher off-odor scores in raw and cooked ground buffalo meat patties and chicken patties containing unrefined rosemary extract (22.5-130 ppm), when was used refined oleoresins resulted in just detectable off-odor and did not affect (p >0.05) either color or overall acceptability scores. Liu et al. (2009) also reported that use of rosemary at higher concentrations than 1500 ppm resulted in lower color scores and higher off-odor scores than control values in fresh chicken sausages during refrigerated storage. Selani et al. (2011) observed that the addition with natural antioxidants in this case of industry residue extract led to a significantly lower color score ($p < 0.05$) than the control which remark the importance of bleaching and deodorizing of extracts in addition to the doses added in products.

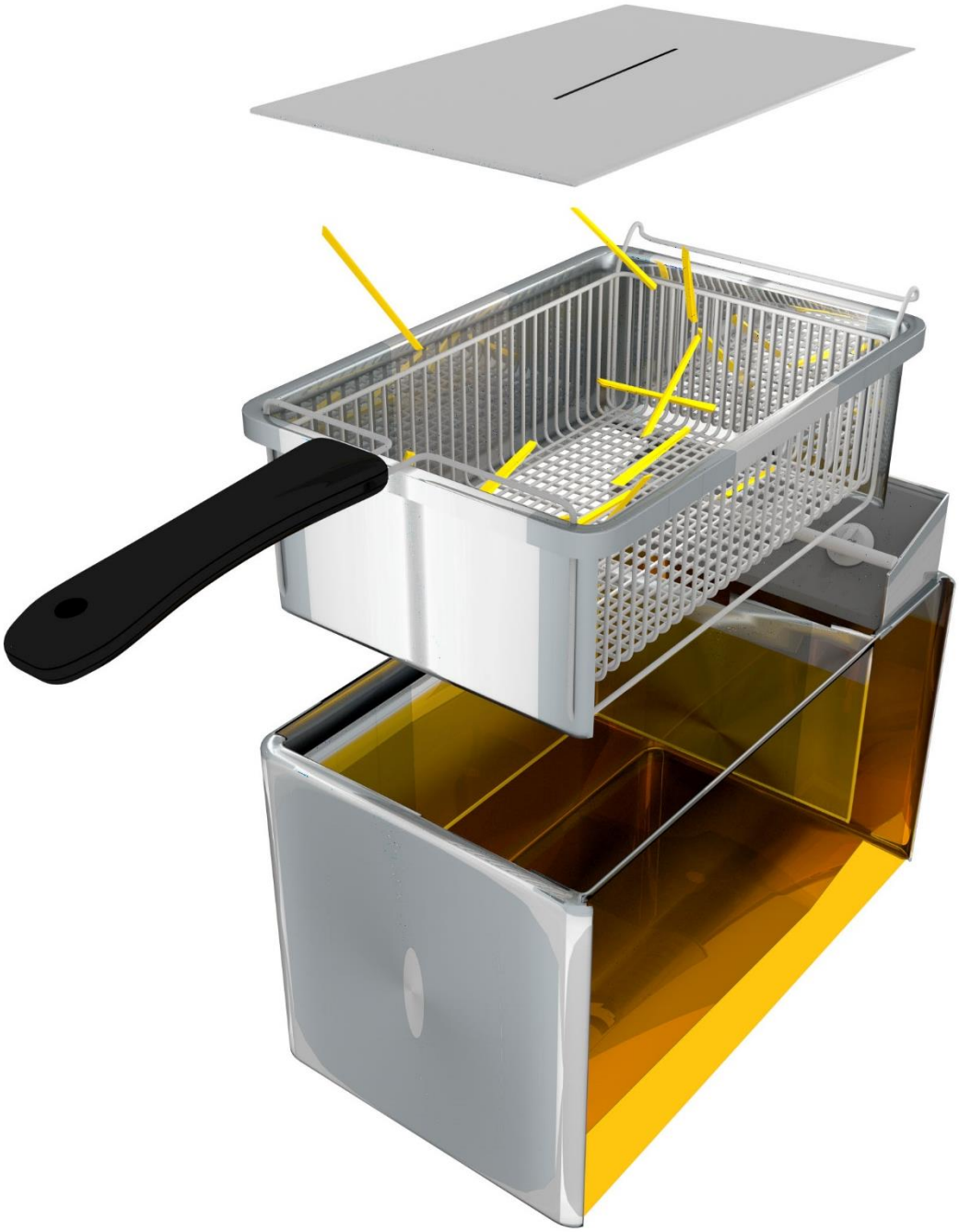
Regarding effect of frozen storage (9 months), the attributes crust color and mass color were unaffected. A similar result was found Seleni et al. (2011) in cooked chicken meat with respect frozen storage (9 months). In general, the odor and taste intensity scores gradually decreased ($p < 0.05$) during the 9 months of frozen storage compared with initial values (Table 4), although scores were still high (8.90-9.41). A decrease in sensory scores with time has been reported by other authors in frozen chicken curry (Modi et al., 2006) and refrigerated chicken nuggets (Modi et al.,2004). According to Mohamed and Mansour (2012), the addition of antioxidants (rosemary and majoran) to beef patties did not significantly affect flavor scores during frozen storage, although the same authors found significant differences ($p < 0.05$) when samples were prepared with mechanically deboned poultry meat (200 g/kg) due to the reduction in the rate of lipid oxidation by antioxidants during storage.

4.1.4. CONCLUSION

The format and solvent types used in the present study influenced the amount of phenolic compounds in the rosemary extracts obtained and therefore in their antioxidant capacity. After characterization of the different extracts, it can be concluded that the powder acetone had the higher antioxidant potential followed by liquid methanol and liquid acetone.

The addition of these rosemary extracts to chicken nuggets had no effect on the physical-chemical characteristics (color, pH) and sensory quality of the product that pointed out to their potential use as alternatives in the production of pre-fried products. After 9 months of storage, a slight tendency as regarding the effectiveness of these natural extracts as antioxidant compounds was observed, but possibly a longer storage would be required to confirm this subject.

4.2. SUNFLOWER OIL STABILITY ADDED WITH ROSEMARY EXTRACT (E-392) IN FRYING PROCESS.



4.2.1. INTRODUCTION

Deep fat frying is one of the most popular cooking methods, both at home and at industrial scale, because it improves food sensory characteristics by increasing palatability (Che-Man and Jaswir, 2000; Saguy and Dana, 2003). During the frying process, the oil is exposed to heat, water and air, which produce a series of physical and chemical changes that result in its decomposition due to hydrolysis, oxidation, isomerization and polymerization processes (Choe and Min, 2007; Lalas, 2007). Among these, lipid oxidation, a catalytic process involving a free radical chain reaction mechanism, is an important cause of quality loss in vegetable oils, since it involves the generation of hydroperoxides (Choe and Min, 2007; Casarotti and Jorge, 2012). These primary oxidation products are relatively stable at room temperature, but, at high temperature such as those reached during the frying process (180 ºC), they are readily decomposed to alkoxy radicals to form aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons (Boskou, 2003; Choe and Min, 2007; Lalas, 2007). These secondary products results in the decreased palatability and nutritional quality of edible oils and therefore in an important loss of acceptability on the fried products, moreover these compounds can be toxic (Jaswir et al., 2000; Boskou, 2003; Choe and Min, 2007; Lalas, 2007).

Antioxidants have been applied to retard oxidative deterioration during storage and frying operations and to prolong the shelf-life of both the oil and fried food. Although synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used in commercial cooking oil, consumers tend to prefer natural additives since synthetic ones are associated with carcinogenesis or mutagenesis phenomena (Lalas and Dourtoglou, 2003).

Plant extracts have been extensively reviewed as potential sources of antioxidants, since they contain carotenoids, flavonoids, anthocyanins and phenolic compounds that function as oxygen scavengers in both primary and secondary oxidation processes. Grapes, green tea, berries, tomatoes and rosemary extracts have been particularly praised in this respect (Ahn et al., 2008). Rosemary extract contains a large number of compounds that act as a major source of natural antioxidants, mainly phenolic diterpenes like carnosic acid, carnosol, rosmanol, rosmadial, epirosmanol, isorosmanol, methyl carnosate and other phenolic acids such as rosmarinic acid (Herrero et al., 2005). Among these, the most active components are carnosol and carnosic acid. The antioxidant activity of phenolic compounds of rosemary is the result of their ability to scavenge free radicals due to their chemical structure. In this respect, the presence of catechol group in the aromatic ring (C11-C12) of the rosemary phenolic diterpene skeleton is probably the most important structural element in the antioxidant activity of these compounds (Shan et al.,2005).

Previous studies evaluated the rosemary antioxidant activity during the degradation of different vegetable oils. In refined palm oil, doses of rosemary oleoresin between 200-4000 mg/kg were effective in inhibiting oil oxidation (Che-Man and Jaswir, 2000; Jaswir et al., 2000). Lalas and Dourtoglou (2003) observed lower oxidation in vegetables oil samples when rosemary extract at 400 mg/kg was used. Furthermore a study of the antioxidant activity of rosemary extract in doses between 1000-3000 mg/kg in soybean confirmed its protective effect against oxidation at high temperature (Casarotti and Jorge, 2012; Ramalho and Jorge, 2008).

The Directive 95/2/EC authorized the use of rosemary extracts, E-392, in foodstuffs (European Union Directives 2010/67/EU and 2010/69/EU). This fact permits various types of production process using solvent extraction and super- critical carbon dioxide

extraction. The EFSA establishes that maximum of 50 ppm (expressed as the sum of carnosol and carnosic acid) as safety threshold for frying oils.

The aim of this work was: (1) to evaluate the effect of adding a rosemary extract below the maximum permitted for active compounds (50 ppm for the sum of carnosol and carnosic acid) on the quality of sunflower oil during deep fat frying, taking into account oxidative stability, total polar compounds, viscosity, color, carnosol and carnosic acid (2) and to determine the effects on the sensory characteristics of a fried standard product.

4.2.2. MATERIALS AND METHODS

4.2.2.1. Materials

Refined sunflower oil and frozen pre-fried French fries (Hacendado, España S.A., Sevilla, Spain) were purchased from a local market. The French fried potatoes were used as a standard product to determine the sensory characteristics of oil (Boskou, 2003). The rosemary extract used in this study was obtained by methanol extraction by Ingrenat S.A. (Murcia, Spain) from rosemary leaves using the liquid-solid extraction technique involves removing solid soluble components. The carnosic acid and carnosol composition of the extract, as declared by Ingrenat S.A, were 85.4 g of carnosic acid/kg extract and 17.1 g of carnosol/kg extract. Microbiological counts of the extract were: Total count < 1000 cfu/g, Molds and Yeasts < 100cfu/g; absence Salmonella spp. in 25 g and E.coli in 1g. All the chemicals and solvents used in extraction process were of maximal quality.

Two different treatments were used: Sunflower oil with no rosemary extract added (SO) and sunflower oil with 500 ppm of rosemary extract (SOR). To obtain a final concentration of 50 ppm carnosol and carnosic acid in the oil (European Union directives 2010/69/EU) the extract was added at 500 ppm.

4.2.2.2. Frying process

Frying process was done as described Lalas and Dourtoglou (2003). The potatoes were fried at 180 ºC in deep fryers (Taurus, Lleida, Spain), using a potato/oil ratio of 0.06 g/mL. The pre-heating time until the frying temperature (180 ºC), was reached at 20 minutes and then a batch of about 150 g of potatoes was put in to the oil, fried for 5 minutes and drained for one minute. Ten successive batches were fried to complete each cycle. The temperature was monitored with an IKA Labortechnik ETS-D4 fuzzy (Staufen, Germany) digital thermometer.

Between the different cycles, the deep fryers were cooled to room temperature until a new cycle was begun. Thirty grams of oil sample were removed from each fryer and stored at 0°C in a brown bottle under a nitrogen atmosphere. The volume of oil was not replenished during the frying process. A total of four cycles were carried out for each treatment (SO and SOR).

4.2.2.3. Analyses of frying oil

The following analyses were carried out to evaluate the quality of the different oils:

Oxidative stability

The oxidative stability of the oil was measured as described by Martínez-Tomé et al. (2001) using Rancimat equipment, model 743 (Herisau, Switzerland), with a flow of air

of 20 L/h at 110 °C. The time that elapses until these secondary reaction products appear is known as the induction time or induction period (IP), which is a characteristic of the resistance of the sample to oxidation.

Longer IPs suggests stronger antioxidant activity of rosemary extract. The relative activity of the antioxidants is expressed by the protection factor (PF), which is calculated as a ratio between the IP of oil with antioxidants added and the IP of the control. A protection factor greater than 1 indicates inhibition of lipid oxidation.

Total polar compounds

Polar compounds were measured (as a percentage based on the dielectric constant measurement) using a Testo 265 polarimeter (Barcelona, Spain) as described by Sánchez-Gimeno et al. (2008).

Viscosity

Oil viscosity was determined using a Ford Viscosity Cup calibrated using an official calibration standard of 120 centipoises (ASTM D 1200 Standard Test Method for Viscosity by Ford Viscosity Cup).

Color

The instrumental color coordinates, lightness (L*), redness (a*, red-green) and yellowness (b*, yellow-blue) of the sunflower oil samples, were assessed with a Minolta Chroma Meter CR-400 (Osaka, Japan) and the results were expressed in accordance with the CIELab system (CIE, 1986) with reference to illuminant D65 and a visual angle of 10°.

Carnosol and carnosic acid

Carnosol and carnosic acid were identified and quantified in the SOR samples by HPLC analysis, according to the procedure described by Okamura et al. (1994). The samples were kept in brown bottles under freezing conditions at −18 °C in N2 atmosphere until analysis. For this purpose, the oil samples were dissolved in acetone (1:10, w/v) and the insoluble substances were removed by ultrasonic stirring and filtered with a 0.45 µm nylon filter. The analysis was performed with an Agilent 1200 series HPLC instrument equipped with an autosampler. The column used was a HiChrom Hi-RPB 18 (0.46 × 250 mm) with a 5 µm particle size diameter. The mobile phase consisted of acetonitrile (A) and distilled water containing 1% acetic acid (B), applying the following gradient: 0-10 min, 30% A, 70% B; 10-22.5 min, 65% A, 35% B; 22.5-27.6 min, 100% A, 0% B; 27.6 min, 30% A, 70% B and stop 30 min. The flow rate was constant at 1.2 ml/min. Chromatographic analysis was performed on an Agilent 1200 Series (Agilent, Germany) ultra-fast HPLC system equipped with a binary pump, micro degasser, an auto plate-sampler and a thermostatically controlled column apartment and quantification at was carried out at 284 nm based on the standard solutions of carnosol and carnosic acid from Sigma–Aldrich Co. (St. Louis, MO).

Sensory evaluation of French fries

For the sensory analysis, all evaluations were conducted in individual booths, which contained the instructions for the evaluation procedure. The tasting room for sensory evaluation was air-conditioned and free of disturbing factors. The panelists were trained according to ISO (2012). Eight training sessions were carried out: in the three first, descriptors of the product were studied and the following four sessions were concerned with identifying, selecting and quantifying the attributes to be evaluated. The

descriptors considered in this study were: color intensity, odor intensity, persistence, oiliness, crispiness and overall quality. In the training and tasting sessions, the samples were coded with random three digit numbers. Mineral water was provided for mouth rinsing between samples. Sensorial analyses were carried out using an unstructured scale of 10 cm.

4.2.2.4. Statistical Analysis

Data were analyzed with the statistical package SPSS 15.0 (Statistical Package for the Social Sciences). All data were analyzed following an analysis of variance (ANOVA) and, when the differences between the groups were statistically significant ($p < 0.05$), Tukey's test at $p < 0.05$ was applied to evaluate the differences between pairs of groups.

4.2.3. RESULTS AND DISCUSSION

4.2.3.1. Analyses of frying oil

Oxidative stability

Both SO and SOR were evaluated by the Rancimat method. Table 9 show the induction period for each treatment and time. Both oils showed a significant decrease ($p < 0.05$) in oxidative stability as the total frying time increased.

Between the fresh oil and the first frying cycle there were was a pronounced reduction in stability (oxidative). Although a tendency to decrease with frying time was observed for both oils, this reduction was less drastic from the first cycle, in agreement with Ramalho and Jorge (2008) who also observed a similar result in soybean oil at 180 ºC. The decrease in stability is related to the oxidation reactions associated with high

temperatures in the presence of oxygen during frying (Saguy and Dana, 2003; Farhoosh and Tavassoli-Kafrani, 2011; Zhang et al., 2012a).

Table 9. Induction Period of oil samples (SO: sunflower oil, SOR: sunflower oil with 500 ppm of rosemary extract) in different frying cycles.

Number of batches	**Type of oil[a]**	**IP (hour)**	***Stabilization factor F***
Fresh oil	SO	3.65 [b] ±0.30^{AZ}	
	SOR	4.14±0.01^{BZ}	1.13
10	SO	0.92 ±0.14^{AY}	
	SOR	1.72±0.03^{BY}	1.87
20	SO	1.10±0.08^{AY}	
	SOR	1.80±0.14^{BY}	1.63
30	SO	1.09±0.01^{AY}	
	SOR	1.64±0.01^{BY}	1.50
40	SO	0.98±0.03^{AY}	
	SOR	1.38±0.07^{BX}	1.42

[a]SO: sunflower oil; SOR: sunflower oil with 500 ppm of rosemary extract.[b]Data are given as mean ± standard deviation (number of replication=3). Mean values in each treatment with different letters are significantly different (Tukey's significant differences test, *p < 0.05). A, B: indicate statistically differences among treatments; X, Y, Z: indicate differences among number of batches.

As regards the treatment significant difference ($p < 0.05$) in the oxidative stability values were observed between both treatments for each cycle. The induction time of SOR was longer than for SO, suggesting its greater stability as a result of the addition of rosemary extract. Furthemore, the Rancimat method provided information about the protective effect against oxidation expressed as the protection factor (PF). The result obtained for the SOR protection factor were above the unit in all cases and also indicated a lower oxidative deterioration in the supplemented oil (Table 9).

Other authors have observed similar results in vegetable oil with rosemary extract added i.e. Lalas and Dourtoglou (2003) observed an increase in the oxidative stability of oil with 400 ppm of rosemary extract (60 ppm of carnosic acid and carnosol) during potato frying. Ramalho and Jorge (2008) and Casarotti and Jorge (2012) also observed improved oxidative stability with rosemary extract at 1000 mg/kg and 3000 mg/kg (82.03 GAE/g of extract), respectively, in soybean oil subjected to heating. Others like Nogala-Kalucka et al. (2005) in rapeseed oil triacylglycerols (500 ppm rosemary extract) and Merrill et al. (2008) in high-oleic vegetable oils (1000 ppm rosemary extract) used the Rancimat test and observed protective effect of rosemary on oil oxidative process.

The protective effect of rosemary extract is related to its phenolic constituents. The primarily phenolic diterpenes in rosemary extract are carnosic acid and carnosol (Nogala-Kalucka et al., 2005, Zhang et al., 2012b). However, these compounds were completely degraded after the third cycle of frying although their action lasted until the end of the study. This is because the deterioration of these compounds gives rise to other phenolic diterpene compounds that also act as antioxidants (rosmarinic acid, rosmanol, epirosmanol, methyl carnosate, isorosmanol, 7-methylepirosmanol, rosmaridiphenol and rosmariquinone acid) (Naveena et al., 2013), although with a lower activity than carnosic acid and carnosol.

Polar compounds

The heating process produces different chemical reactions which, in turn, generate polar compounds, which may be of low (volatile) or high (non-volatile) molecular weight and are related with oil deterioration. The measurement of polar compounds considered the non-volatile compounds, including triglyceride monomers, oxidized and dimerized

triglycerides, polymers of triglycerides, diglycerides and free fatty acids (Gharachorloo, et al. 2010; Lalas, 2007).

The initial polar compounds values of both oils were unusually high, probably due to storage conditions prior to purchase. Several factors, such as light, temperature and type of containers can affect the quality stability of oil products. The oil in the present study was purchased in a supermarket where was stored in transparent plastic containers. Naz et al. (2005) determined that this type of container is not appropriate for storage, since it offers little protective action agents light and oxygen so that the oil deteriorates rapidly. Another cause of these higher values may be the method used; Dobarganes (2007) showed that polar compound values were overestimated when Testo 265 was used. One of the explanations for such behavior is the moisture contained in the frying oil, since it increases the dielectric constant, and, as a result total polar compounds values increase (Osawa et al., 2012).

Polar compounds of the sunflower oil increased ($p < 0.05$) during the heating process as a result of oxidative alteration at high temperature (Figure 10), as described by other authors (Farhoosh and Tavassoli-Kafrani, 2011; Guillén and Uriarte, 2012; Urbančič et al., 2013).

As regards the treatment, there were significant differences ($p < 0.05$) for each cycle between SO and SOR. The results suggest that rosemary extract delayed the formation of polar compounds throughout the frying process. In Spain, a threshold value of 25% has been established as safety limit in frying oil (BOE, 1989). The sunflower oil (SO) reached 25% PC after 36 cycles, while the sunflower oil supplemented with rosemary extract (SOR) had not reached this value when the study finished after 40 batches. Other researchers also found that rosemary extract delayed the formation of polar compounds

following heating in soybean oil (Casarotti and Jorge, 2012), in rapeseed oil (Réblová et al., 1999) and in sunflower oil (Urbančič et al., 2013).

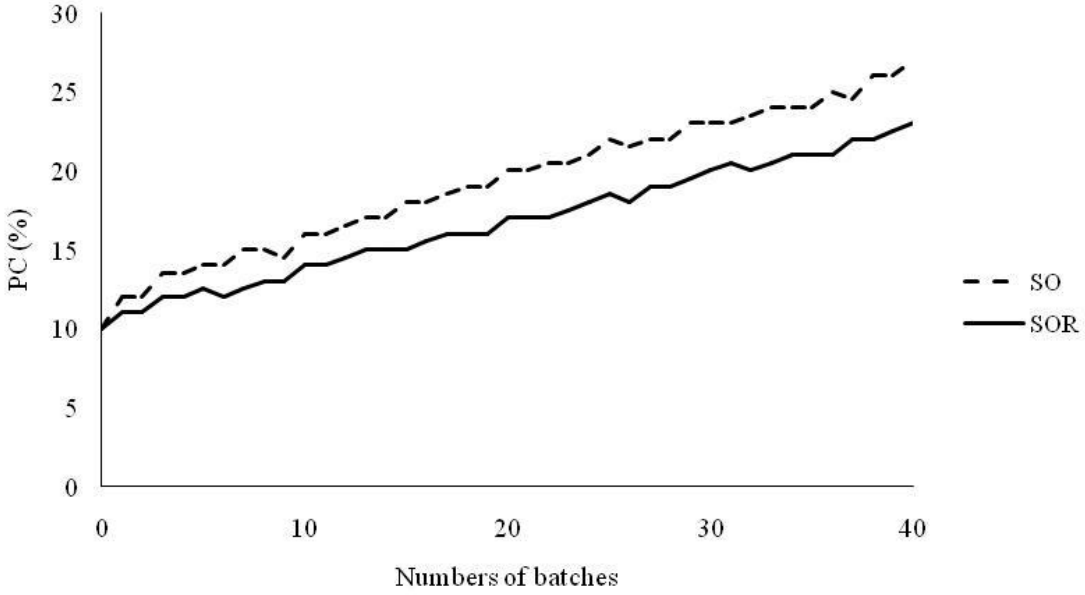


Figure 10. Evolution of polar compounds (P.C) content in oil samples (SO: sunflower oil, SOR: sunflower oil with 500 ppm of rosemary extract) in different frying times.

This diminution in polar compound formation observed in the enriched oil would be due to the capacity of rosemary compounds to react with the radicals of initiating and propagating steps, involve in a retardation of oxidation and thus in an extension of the shelf-life of the oil (Lalas, 2007; Angelo and Jorge, 2008; Guillén and Uriarte, 2012; Urbančič et al., 2013).

Viscosity and color coordinates (CIELab)

Table 10 shows the viscosity and the color coordinate values of the sunflower oil samples. Viscosity increased significantly ($p < 0.05$) with the number of batches, in agreement with other authors who studied the effect of frying with different types of oils (Sánchez-Gimeno et al., 2008; Serjouie et al., 2010). However, no significant

differences were observed between SO and SOR at this respect. In contrast, Che-Man and Jaswir (2000) found that the increasing in viscosity was minor ($p < 0.05$) in palm olein during deep frying when rosemary extract was added at 0.4 %. Other researchers (Jaswir et al., 2000; Ghos et al., 2012) also observed a lower viscosity in oils supplemented with antioxidants than in the control oils. This increase in viscosity is due to polymer formation, which is determined by two main factors, the oxygen content and the increased temperature, both of which promote oxidation processes and thermal polymerization. Moreover, two different types of polymer are formed, depending on the presence or not of oxygen. Polar polymers are formed in the oxygen presence and non-polar polymers are produced without oxygen (Zhang et al., 2012a). The presence of a catechol group in the aromatic ring (C11-C12) on the rosemary phenolic diterpene skeleton had a significant effect on delaying oxidative degradation, which is reflected in the reduced content of polar compounds observed during frying (Figure 10). However the lower polar compound content of SOR than SO was not enough to produce a decrease in the viscosity of the oil with rosemary extract.

The color of oils has been widely used as an index to determine it quality. The characteristic color of sunflower oil is mainly due to carotenoid pigments (yellow pigment) and a small quantity of chlorophyll (green pigment) from unripe seed and plant impurities (Kaynak et al., 2004). When oils are heated (180 ºC) the above mentioned chemical changes, including oxidation or polymerization, occur a rapid change in oil color from light yellow to orange brown (Maskan, 2003). Table 10 shows the CIELab coordinates values for SOR and SO samples. The parameter L*(luminosity) decreased significantly ($p < 0.05$) for both treatments as the frying time increased.

This decrease is associated with a darkening of the samples, this effect has been previously described by other researchers (Lalas, 2007; Sanchez-Gimeno et al., 2008),

who associated these variations with polymer formation and non-polar compounds solubilized in the oil. Maskan (2003) explained that the darkening (decrease in L* value) may be caused by the Maillard products resulting for interactions of food with frying oil.

The a* value was also affected by the frying process, increasing with the numbers of frying cycles. The value was almost constant during the first 3 frying cycle and then increased sharply in both, SOR and SO. This indicates that frying process modified the initial color of the oil ($a^* < 0$) to a redder color ($a^* > 0$). In general the redness of oil is not acceptable from an oil quality point of view because it is related with combined oxidized fatty acid and pyrolytic condensation products (Blumenthal, 1991). Similar results were described by Maskan (2003) who observed that the values of a* were constant until 3 cycles, after which a significant increase occurred due to a reduction in the oil coloring pigment chlorophyll.

Table 10. Quality changes during frying in samples of sunflower oil (SO) and sunflower oil with 500 ppm of rosemary extract (SOR).

Number of batches	Type of oil[a]	Viscosity	*L**	*a**	*b**
1	SO	$20.47^{b}\pm0.36^{X}$	31.91 ± 0.05^{AX}	-0.37 ± 0.03^{BX}	0.41 ± 0.03^{AX}
	SOR	20.21 ± 0.29^{X}	33.27 ± 0.02^{BY}	-0.57 ± 0.01^{AW}	1.31 ± 0.01^{BX}
10	SO	20.95 ± 0.35^{XY}	32.86 ± 0.01^{BY}	-1.00 ± 0.03^{V}	2.72 ± 0.00^{AY}
	SOR	21.23 ± 0.80^{XY}	32.84 ± 0.01^{AW}	-0.96 ± 0.05^{V}	3.17 ± 0.00^{BY}
20	SO	21.74 ± 0.71^{XY}	33.21 ± 0.01^{BZ}	-0.54 ± 0.03^{W}	3.27 ± 0.02^{BZ}
	SOR	21.99 ± 0.35^{Y}	33.05 ± 0.04^{AX}	-0.59 ± 0.05^{W}	3.20 ± 0.03^{AZ}
30	SO	22.49 ± 0.35^{Y}	30.03 ± 0.00^{BW}	0.91 ± 0.01^{BY}	-1.48 ± 0.01^{BW}
	SOR	22.24 ± 0.71^{Y}	29.97 ± 0.01^{AV}	0.77 ± 0.01^{AX}	-1.61 ± 0.00^{AV}
40	SO	24.98 ± 0.34^{Z}	29.54 ± 0.00^{AV}	1.25 ± 0.01^{BZ}	-1.84 ± 0.01^{BV}
	SOR	25.47 ± 0.34^{Z}	29.96 ± 0.01^{BV}	1.03 ± 0.01^{AY}	-1.50 ± 0.01^{AW}

[a]SO: sunflower oil; SOR: sunflower oil with 500 ppm of rosemary extract.[b]Data are given as mean ± standard deviation (number of replication=3). Mean values in each treatment with different letters are significantly different (Tukey's significant differences test, $*p < 0.05$). A, B: indicate statistically differences among treatments; V, X, Y, Z: indicate differences among number of batches.

A higher b* in oil is due to the natural carotenoids and xanthophylls present in sunflower oil, which are desirable for this oil (Maskan, 2003). During the first cycles a slight increase in b* value was observed, after then a stronger decrease was observed ($p < 0.05$). This loss of the typical yellow color of fresh sunflower oil is usually promoted by a reduction in natural carotenoids and xanthophylls, resulting from oxidation or decomposition during frying, the presence of combined peroxides and aldehydes and also by fine particulates dispersed in the oil (Lalas, 2007).

In general, color degradation was associated with the frying time and increase strongly from the second cycle on wards for both treatments. As regards the effect of treatment, significant differences were detected although there was no clearly defined tendency. In agreement with Jaswir et al. (2000), in terms of color (redness and yellowness), no significant differences were found between samples with or without antioxidants during deep fat frying, although both samples were modified by frying time.

Identificacion of Carnosol and Carnosic Acid

The main compounds with antioxidative activity in rosemary extract are carnosic acid and carnosol (diterpenes) (Nogala-Kalucka et al., 2005; Zhang et al., 2012b). Quantitative analysis of carnosic acid and carnosol in the SOR samples using high performance liquid chromatography (Table 11) showed that carnosic acid and carnosol content in SOR decreased with frying time (0, 1, 2, 3 and 4 frying cycles). The initial values were 11 mg/kg of carnosic acid and 34 mg/kg of carnosol, but, after the first cycle carnosic acid had been totally degraded, while carnosol had fallen to 10 mg/kg. The total degradation of carnosol was evident in the third cycle.

Table 11. Concentration of carnosic acid and carnosol during frying in samples of sunflower oil with 500 ppm of rosemary extract (SOR).

Number of batches	Carnosic acid	Carnosol	Antioxidant capacity
1	11±0.06 mg/kg	34±0.17 mg/kg	45±0.25 mg/kg
10	N.d.[a]	10±0.09 mg/kg	10±0.04 mg/kg
20	N.d.	10±0.03 mg/kg	10±0.12 mg/kg
30	N.d.	N.d.	N.d.
40	N.d.	N.d.	N.d.

[a]N.d: not detected.

The presence of carnosol longer that carnosic acid are due to the higher initial values of this molecule and the conversion process of carnosic acid to carnosol (Nogala-Kalucka et al., 2005). These observations concerning to the degradation of carnosic acid and carnosol with frying, agree with Zhang et al. (2012b) which showed that temperature directly affects the degradation of the major phenolic diterpenes of rosemary (carnosic acid and carnosol). In a study of the radical scavenging activity and oxidative stability of virgin olive oil enriched with carnosic acid Zunin et al. (2010) observed a protective effect of rosemary extract against lipid oxidation at 60 ºC but at 180 ºC the addition of carnosic acid did not inhibit oil oxidation due to its rapid degradation at this temperature.

4.2.3.2. Sensory evaluation of French fries.

The influence of rosemary extract on the organoleptic quality of French fries was also examined. Table 12 shows the sensory evaluation scores for samples fried in oil with and without antioxidants after 1, 10, 20, 30 and 40 batches.

In both treatments (SO and SOR) the sensory score of French fries were high throughout the frying process. In general, the attributes evaluated did not show

significantly difference ($p < 0.05$) between the different frying cycles. In fact the French fries were equally acceptable whether or not the oil contained antioxidant until the last frying cycle. Lalas and Dourtoglou (2003) also observed no sensory changes in French fries for similar treatments (400 ppm of rosemary extract in soybean oil).

Only in the first frying samples from the SOR group shows significantly lower scores for color intensity and taste ($p < 0.05$) compared with the SO samples, although the differences may be considered unimportant since the scores were always high (more than 9 on a 10 point scale). A similar trend was described by Réblová et al. (1999) in a study where some spicy notes were detected from the rosemary included in the oil, although this disappeared after few batches. The slight differences in sensory attributes detected by panelists in the first frying batches may have been related with the presence of small quantities of the characteristic compounds of rosemary such as, oxygenated monoterpenes (linalool, verbenone, isobornyl acetate) (Martinello and Pramparo, 2005), which could be transferred to the fried product. However, when these compounds are subjected to the high temperatures (180 ºC) would be degraded, which explains why these changes were observed only in the first frying.

Generally, no significantly changes between treatments were observed during the frying process, indicating that the organoleptic characteristics of French fried were not modified even after four frying cycles. This means that adding rosemary extract at 500 ppm to the frying oil as natural antioxidant does not alter the sensory quality.

Table 12. Sensory Evaluation of French fries fried in sunflower oil (SO) and sunflower oil with 500 ppm of rosemary extract (SOR).

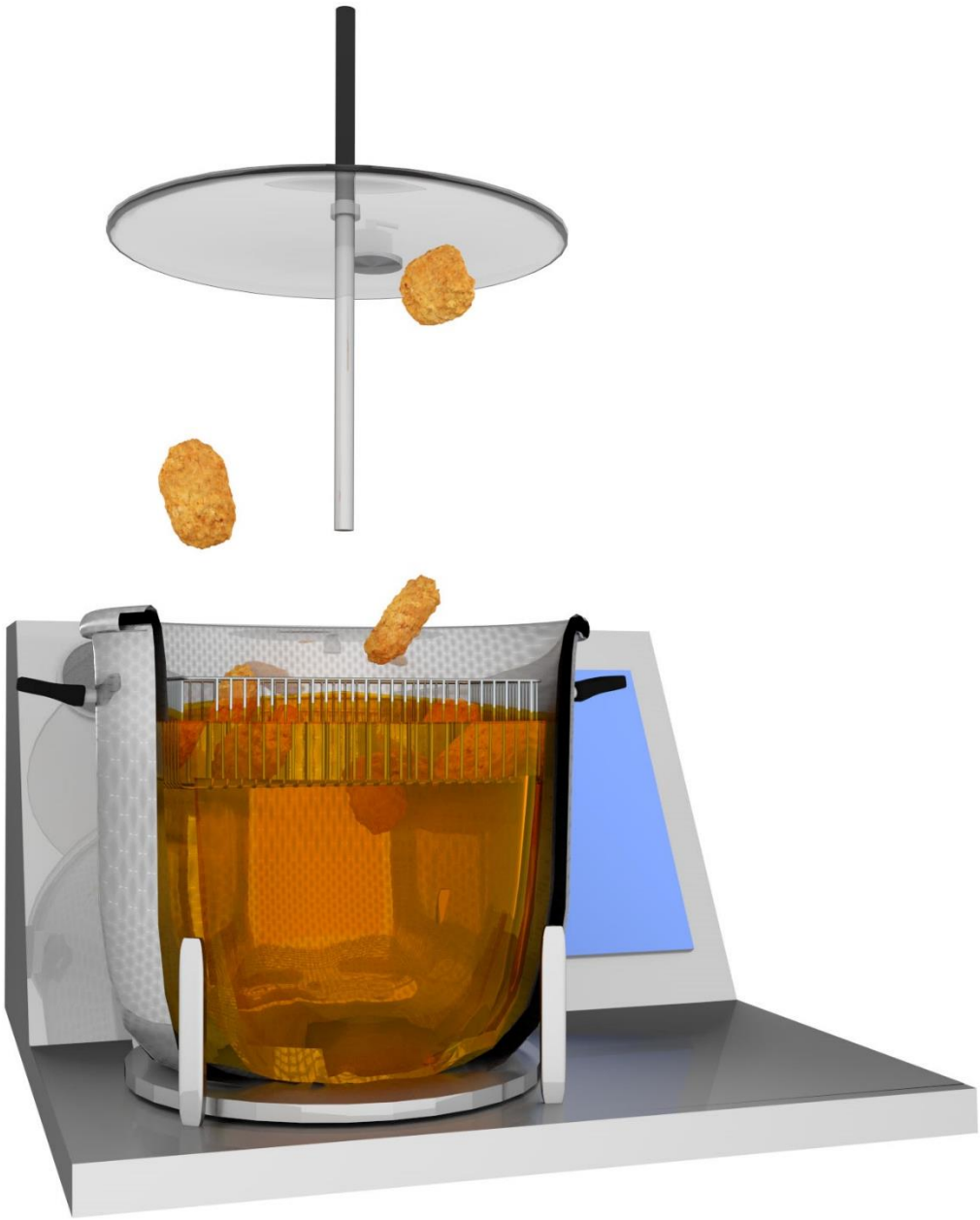
Number of batches	Type of oil[a]	Colour intensity	Odour intensity	Persistence	Taste	Oiliness	Overall quality
1	SO	10.00[b]±0.00[B]	10.00±0.00[Z]	10.00±0.00	10.00±0.00[B]	1.09±0.50	9.90±0.30
	SOR	9.38±0.02[A]	9.89±0.33	9.48±1.07	9.33±0.07[A]	1.40±0.92	9.64±0.47
10	SO	9.21±0.79	9.50±1.27[YZ]	8.95±0.92	9.19±1.59	1.75±0.80	9.18±1.00
	SOR	9.44±1.38	8.70±0.64	9.49±1.48	8.88±1.46	1.45±0.53	8.90±1.16
20	SO	9.43±0.74	9.10±0.89[Y]	9.06±1.06	9.05±1.32	1.28±0.55	8.86±1.19
	SOR	9.39±0.60	9.40±0.64	8.98±1.00	8.93±1.07	1.36±1.36	8.65±1.18
30	SO	9.70±0.40	9.80±0.29[YZ]	9.77±0.44	9.67±0.46	1.63±1.46	9.47±0.56
	SOR	9.64±0.40	9.47±0.83	9.57±0.77	9.49±0.68	1.54±1.47	9.47±0.67
40	SO	9.51±0.51	9.52±0.57[YZ]	9.03±1.21	9.10±1.31	1.35±0.33	9.29±0.93
	SOR	9.43±0.41	9.06±1.20	8.83±1.45	8.86±1.00	1.30±0.30	9.14±1.07

[a]SO: sunflower oil; SOR: sunflower oil with 500 ppm of rosemary extract.[b]Data are given as mean ± standard deviation (numbers of replication=3).Mean values in each treatment with different letters are significantly different (Tukey's significant differences test, *$p < 0.05$). A, B: indicate statistically differences among treatments; Y, Z: indicate differences among number of frying.

4.2.4. CONCLUSION

In the present research we applied the maximum doses of rosemary extract established by EFSA for frying oils (50 mg of carnosic acid and carnosol/kg oil). The results showed that rosemary extract improved the oxidative stability and diminished the generation of polar compounds in sunflower oil, without modifying the organoleptic characteristics of the fried product. However, rosemary extract addition at the legally maximum had no effect on the viscosity and color values, probably due to the degradation of diterpenes (carnosol and carnosic acid) during the cycles at high temperature (180 ºC). Consequently, the application of the legal maximum of the active compounds cannot be deemed sufficient to provide complete protection to the oil. In conclusion, a higher dose of this rosemary extract would be necessary in order to be considered as alternative line to protect frying sunflower oil.

4.3. USE OF VACUUM-FRYING IN CHICKEN NUGGETS PROCESSING



4.3.1. INTRODUCTION

Deep fat frying is one of the oldest and most popular food preparation techniques at both domestic and industrial venues (Granda and Moreira, 2005; Choe and Min, 2007; Mir-Bel et al., 2009). Deep fat frying can be defined as a thermal food processing method in which food in immersed in oil at a temperature of 150-200 ºC, which is well above the boiling temperature of water (Mir- Belet al., 2012; Sravan et al., 2013). The heat and simultaneous mass transfer of oil and air promote a number of chemical changes, such as water loss, oil uptake, crust formation, starch gelatinization, aromatation and color change via Maillard reactions, hydrolysis or oxidation, and oil polymerization (Mir-Bel et al., 2012). The results are products with unique and distinctive qualities of flavor, color, appearance, taste, aroma and texture (Saguy and Dana, 2003). This process is widely used to cook French fries, potato chips, extruded snacks, donuts, fish sticks and fried chicken products such as the nuggets studied in this article (Moreira et al., 1999).

Due to its simplicity and versatility and the organoleptic qualities of the resulting products, frying is widely accepted as a food preparation technique which has led to an important increase in the consumption of fried food worldwide (Dana and Saguy, 2006). However, multiple studies have recognized that an excessive consumption of fat has a significant adverse effect on health, leading to an increased incidence of coronary heart disease (CHD), cancer, diabetes or hypertension (Krokida et al., 2001). In addition, during frying, potentially toxic compounds can be formed as a consequence of deterioration, among them acrylamide (Granda and Moreira, 2005). Nevertheless, despite intensified consumer awareness of the relationships among food, nutrition and health, frying remains one of the principal cooking methods (Saguy and Dana, 2003).

For this reason, numerous studies have been published on frying technology in recent years with the aim of providing nutritional and safe products to satisfy the expectations of meat consumers.

Among possible methods, vacuum-frying is defined as frying carried out below atmospheric pressure, preferably below 50 Torr (6.65 kPa) when the boiling points of both the oil and the water contained in the foods are lowered (Andrés-Bello et al., 2011). Several advantages has been attributed to this technology such as: (1) reduced oil content in the fried product; (2) the preservation of natural color and flavors due to the low temperature and the absence of oxygen during the process; (3) fewer adverse effects on oil quality (Shyu et al. 1998); (4) a decreased acrylamide content (Granda et al., 2004), and (5) the preservation of nutritional compounds (Da Silva and Moreira, 2008; Shyu and Hwang, 2001; Dueik et al.,2010).

As regards the absorption of fat, there is considerable controversy about the role that the pressurization process plays in the oil absorption mechanism. While several studies have shown that less oil is absorbed during the vacuum-frying process (Garayo and Moreira, 2002; Mariscal and Bouchon, 2008; Moreira et al.,2009), others, including Troncoso and Pedreschi (2009), have found that oil absorption at the surface increases during vacuum-frying, due to higher heat and mass transfer rates and the existence of a pressurization step. Moreover,Garayo and Moreira(2002) indicated that oil absorption may increase or decrease depending on the amount of surface oil and free water present in the product, and suggested that vacuum-frying may be an alternative to traditional frying to obtain healthier fried foods.

Despite the many studies evaluating the effect of this technique on fruit and vegetables (Shyu and Hwang, 2001; Garayo and Moreira, 2002; Granda et al., 2004; Fan et al.,

2005; Shyu et al., 2005; Moyano and Pedreschi, 2006; Da Silva and Moreira, 2008; Mariscal and Bouchon, 2008; Mir-Bel et al., 2009; Troncoso and Pedreschi, 2009; Troncosoet al., 2009; Mir-Bel et al., 2012), no reports are available on the effect of vacuum-frying on the physical-chemical and organoleptic characteristics of meat products.

The objective of the present study was (1) to evaluate the effect of vacuum-frying on the characteristics (oil content, moisture, color, texture and sensory quality) of chicken nuggets and (2) to analyze the optimum vacuum-frying conditions to obtain a lower fat content product.

4.3.2. MATERIAL AND METHODS

4.3.2.1. Sample formulation, preparation and storage conditions

The nuggets used for this study were manufactured in the same way as commercial pre-fried products. For this purpose deboned skinless chicken breasts (60%) were first chopped in a mincer (Kenwood Model A920 PK001) with ice (23%) for 30 s. They were then mixed with the usual ingredients and seasonings used for commercial nuggets (15% potato flakes, 1% salt and 1% albumin). All the components were thoroughly mixed to provide a uniform blend. The chicken nugget samples were formed into characteristic shapes (5×3×1 cm), each weighing 25-g and were chilled to below −18 °C. The frozen pieces were dipped in the prepared batter for 15 s (wheat flour 93.57 %, salt 1.17% bicarbonate 0.24%, yeast 2.34% and xanthan gum 1.17 %). These battered nuggets were pre-fried using a traditional fryer (Movilfrit, Barcelona, Spain) for 30 seconds in sunflower oil at 165 ºC before packing in polyethylene bags and storing at −

40 °C until use. A total of 542 chicken nuggets were used, 200 for the physical-chemical analysis (50 per batch) and 342 for the sensorial analysis.

4.3.2.2. Vacuum frying

For the frying tests Gastrovac equipment (International Cooking concepts, Barcelona, Spain) was used. The equipment was designed, and patented at the Polytechnic University of Valencia (Martínez-Monzó et al., 2004). The electrical appliance consists of a pressure cooker vessel with an inner basket, a membrane vacuum pump (Model 24207, Selecta S.L., Barcelona, Spain) to provide vacuum to the vessel and a heating system controlled by a temperature probe.

Three levels of oil temperature for vacuum-frying (130, 140, and 150 ºC) were considered in this study. For each temperatures investigated the frying times were 2, 4, 6 and 8 min, times that were selected on the basis of previous experiments. The vacuum vessel was set to the target temperature and allowed to operate for 30 min before frying started. Two liters of Sunflower oil (Hacendado, España S.A., Sevilla, Spain) were used in all the experiments and the oil was filtered through a sieve (filter pore size, 1 mm) after each batch and changed for each temperature. The nuggets/oil ratio of around 0.05 w/w was maintained by replenishing with fresh oil. The temperature in the centre of the nugget was controlled before and after the frying process with a Testo 925 probe (Testo GmbH y Co., Lenzkirch, Germany).

Three nuggets were fried each time. Once the oil temperature had reached the target value, the nuggets (previously weighed) were placed into the basket, the lid was closed and the vessel was evacuated. The frying operation consisted of an initial depressurization step with the product outside the oil (1 min), immersion of the product

once the vacuum had reached the target value at ± 1 ºC, the frying period, and the time taken atmospheric pressure (1 min).

Then, the lid of the vessel was opened, the nuggets were removed from the basket and the temperature of the product was measured. The nuggets were then allowed to cool at room temperature (25 ºC), dried with paper towels to remove the excess surface oil and kept in desiccators until analysis (Da Silva and Moreira, 2008).

4.3.2.3. Atmospheric frying

For the atmospheric frying experiments, a commercial deep fat fryer was used (Movilfrit, Barcelona, Spain). To compare the effects of atmospheric frying and vacuum-frying on the quality and frying rate of nuggets, an oil temperature of 165 ºC was used since this is within the range of temperatures normally used for frying (between 150 and 180 °C) (Choe and Min, 2007).

Frying times were 2, 4, 6 and 8 min. The atmospheric fryer was set to the required frying temperature and left for 30 min to ensure that the oil temperature was constant before introducing the nuggets. The nuggets were then allowed to cool at room temperature (25 ºC), dried with paper towels to remove the excess surface oil and kept in desiccators until analysis.

4.3.2.4. Proximate composition

Moisture content: The initial moisture content of pre-fried nuggets and the same nuggets after frying was measured for each time and temperature analyzed. The moisture content of the meat and breaded cover was determined (Varela et al., 2008) in

three homogeneous 5-g samples of each, which were dried until a stable weight (48 h at 110 ºC) (ISO, 1997).

Fat content: The total fat content of three dried samples of meat mass and breaded cover (5 g) was extracted with petroleum ether (BP 40-60 ºC) for four hours in a Soxtec System 2055 Tecator extracting unit (FOSS, Hillerød, Denmark) and gravimetrically determined (ISO, 1973).

4.3.2.5. Characteristics of the fried product

Color was measured using a Minolta CM3600d colorimeter (Minolta Co. Ltd, Tokyo, Japan) (Illuminant D 65, 10 º viewing angle). Following the CIELab system, Lightness (L*), green-red (a*), blue-yellow (b*), chroma (C*) and hue (H*) were measured in triplicate samples. The colorimeter was standardized using a white calibration plate.

Textural analysis was performed using a TA-XT2 Texture Analyser (Texture Technologies Corp., Scarsdale, NY, USA). The samples were placed on the base plate of the analyser with a cylindrical aluminum probe (7.5 cm in diameter) using a 50-kg load cell. The crosshead speed was 0.5 mm/s, with a rest period of 5 s between cycles, and the deformation was 50% of the original length. Six textural parameters were determined from each curve: hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness (Bourne 1978). Analysis determinations were performed for each frying treatment in triplicate

Sensorial analyses were conducted in laboratory conditions in individual booths located in the tasting room, which was air-conditioned and free of disturbing factors (ISO, 2007). The pre-fried nugget samples were fried as described above. Rectangular pieces of approximately 2 x 1.5 cm were cut, codified with a three-digit number and

immediately presented to the panelists. Two types of sensory test (Just about right (JAR) and paired test) were performed. The aim of the first analysis was to assess how the traditional frying time influences the acceptability and preferences of consumers for chicken nuggets. The overall acceptance and the level of consumer preference for the attributes (covering color, covering texture and mass juiciness) were measured on a scale of 9 points (Gacula et al., 2007). Chicken nuggets prepared by traditional frying (165 ºC) for three different cooking times (4, 6 and 8 min) were served. The samples were presented consecutively to each consumer (n=60). Consumers were required to rate the adequacy of color, crispness and juiciness of the fried nuggets on a 5-point just about right (JAR) scale (1 = too soft, 3 = just about right; 5 = too hard). The deviation below and above point 3 on the scale (JAR) was estimated according to Gacula et al. (2007). Individual scores 1, 2, 3, 4 or 5 were transformed to -2, -1, 0, +1, or +2, respectively. The extreme scores -2, -1, +1, +2 were divided into two groups of data from this calculation: one for those consumers who felt that the samples did not meet the expected level for the attribute (below JAR, -2 and -1 points) and another for those consumers who felt the product exceeded the expected level (above the JAR, +1 and +2 points). For each sample, the mean of the values below JAR point 0 corresponded to the negative deviation values (too little of the attribute) and the mean of values above JAR point corresponded to the positive deviation value (too much of the attribute). Moreover, the last question in the test was: "Which attribute is more important for your choice of preferred sample?" and proposing "crispness, juiciness, oiliness, color, overall acceptance or other" as possible answers.

The second analysis was a paired test (ISO, 2005) to verify whether there were perceptive differences between the traditional fried and vacuum-fried samples with similar physical-chemical characteristics (n=81) considering crispness, juiciness,

oiliness, color and overall acceptance. To analyze the data obtained with the paired test, the significance of any difference in preferences and sensory properties was established (α=0.05).

4.3.2.6. Statistical analysis

The effect of temperature and vacuum pressure on the drying curve, the oil content, weight loss, color, texture and sensorial qualities of chicken nuggets was evaluated using a factorial design with four levels for temperature and four levels for frying time. The statistical analysis of the data was conducted using statistical package SPSS 15.0 (Statistical Package for the Social Science for Window). Statistical significance was expressed at the $p < 0.05$ level.

4.3.3. RESULTS AND DISCUSSION

4.3.3.1. Characteristics of the fried product

Temperature profile

Figure 11 shows the temperature at the center of the chicken nuggets after the atmospheric pressure and vacuum treatments. The products fried at atmospheric pressure showed an internal temperature increase that was almost linear with time up to the boiling point of water (100 °C). This then flattened out as a result of the phase change of water at its boiling point (Wang, 2005). In a study of transport mechanisms during the deep fat frying of chicken nuggets at atmospheric pressure (175 and 190 ºC), Sravan et al. (2013) recorded a steady rise in temperature for the first 2 min of frying, after which it became constant. Their results indicated that a negative pressure exists in

the nugget core during most of the frying process except for the first few seconds, which can be attributed to the oil uptake that occurs throughout the frying process.

The three vacuum curves show similar temperature profiles. In the vacuum treatments the water boiling temperature is a function of oil temperature, chamber temperature and the characteristics of the product (Andrés-Bello et al., 2010). In all cases, the boiling point in lower than 100 ºC the vacuum treatments was close to 80 ºC. The heating slope is steeper for vacuum treatments than for atmospheric frying (Figure 11), and the boiling point was reached in 2 to 4 min.

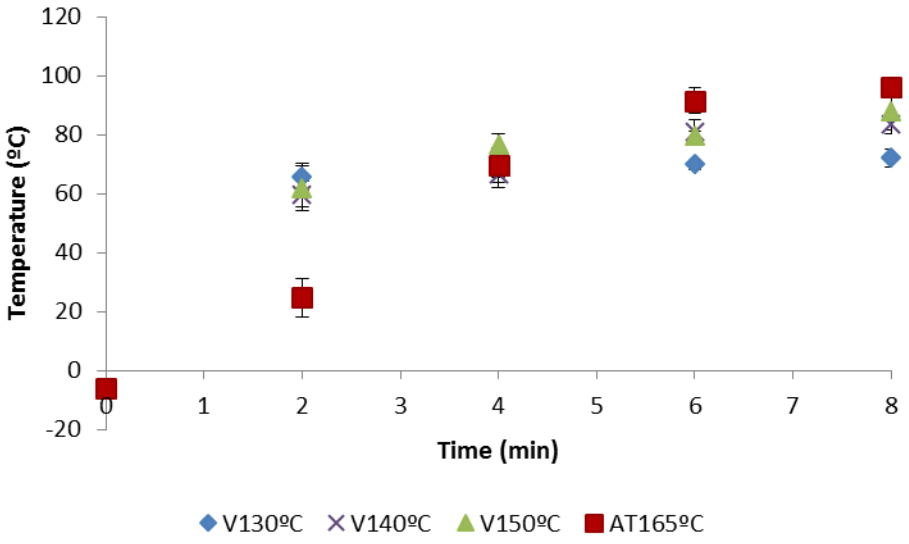


Figure 11. Temperature at the center of chicken nuggets after atmospheric pressure frying (AT) and under conditions of continuous vacuum frying (V).

The maximum temperatures reached inside the chicken nuggets at 8 min were, obtained in products processed by traditional frying at 165 °C, followed by the vacuum treatments in order of decreasing of temperature, which agrees with the boiling points of water for each of the working pressures.

Moisture content

Figure 12 shows the development of moisture for the breaded cover and meat mass at the chicken nugget frying times in atmospheric (165 ºC) and vacuum conditions (130, 140 and 150 ºC). The moisture content of raw samples was 0.84±0.02 (g water/g dry solid) for the breaded cover and 2.13±0.05 (g water/g dry solid) for the meat mass. After frying, the moisture content of breaded cover was above 0.44±0.02 (g water/g dry solid) at 165 °C, 0.27±0.01(g water/g dry solid) at 150 °C, 0.20±0.01 (g water/g dry solid) at 140 °C and 0.26±0.03 (g water/g dry solid) at 130 °C after 8 min of treatment. In the meat mass, the equivalent values were 2.02±0.09 (g water/g dry solid) at 165 °C, 1.83±0.09 (g water/g dry solid) at 150 °C and 1.5±0.2 (g water/g dry solid) at 140 °C, 1.8±0.1 (g water/g dry solid) at 130 °C after 8 min of treatment. The moisture content of the samples decreased significantly ($p < 0.05$) during frying. The higher rate of moisture loss was observed during the first 2 min of frying in the breaded cover of samples fried in vacuum conditions due to the high moisture evaporation rates from the product. In vacuum-frying, the free water of the breaded covering is rapidly vaporized, a phenomenon that may be responsible for the difference in the moisture content between vacuum and atmospheric fried samples.

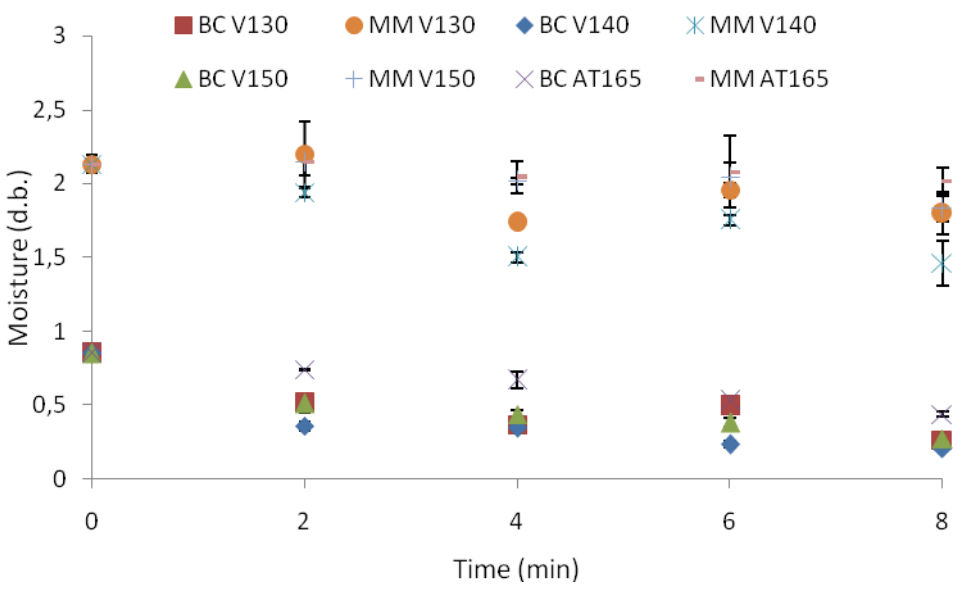


Figure 12. Moisture loss (d.b) of meat mass (MM) and breaded cover (BC) of chicken nuggets frying for different treatments.

This effect may also result from the changes in the sample temperature profiles shown in Figure 11. Oil temperature had a negative effect on the moisture content of fried nuggets (meat mass and breaded covering) for all the treatments. When moisture data were fitted to an empirical model as a function of the square root of time (Gamble et al.,1987), the breaded covering data fitted ($r > 0.94$) this model properly and reflected the faster water loss in vacuum-fried samples than in atmospheric fried samples. For meat mass, the correlation coefficients obtained for the fitted data were lower than 0.8. The thickness of the crust surface increase in during drying, producing a decreasing the heat and of vapor transfer rate from the meat mass, which may explain why the moisture content in the meat mass showed no significant difference with respect to time or treatment.

Fat content

Table 13 shows the fat uptake by the breaded covering and meat mass of control and vacuum-fried nuggets. No significant differences due to the time or treatments were detected, suggesting that there was no significant oil uptake after the pre-frying process.

Oil absorption is a complex mechanism, which is still not clearly understood under vacuum conditions (Garayo and Moreira, 2002). There are many factors that make this a complex phenomenon, such as the initial product structure, the various interchanges between the product and the heating medium, product variation and oil properties, chemical reactions, food moisture content, the cooling phase, the frying time, the temperature, the drainage time or pressurization time (Vitrac, 2000)..

Table 13. Oil content (d.b) of chicken nuggets after atmospheric pressure frying (AT) and under conditions of continuous vacuum frying (V).

	Temperature	**Time (minutes)**				
	(ºC)	**0**	**2**	**4**	**6**	**8**
Breaded cover (g oil/g dry solid)	V130	0.04±0.01	0.07±0.02	0.06±0.01^{b}	0.03±0.01^{a}	0.11±0.04
	V140	0.04±0.01	0.03±0.01	0.04±0.01^{a}	0.05±0.02^{ab}	0.07±0.01
	V150	0.04±0.01^{y}	0.07±0.00^{yz}	0.05±0.00^{ab,yz}	0.09±0.02^{b,z}	0.06±0.02^{yz}
	AT165	0.04±0.01	0.05±0.02	0.04±0.01^{a}	0.03±0.01^{a}	0.07±0.00
Meat mass (g oil/g dry solid)	V130	0.14±0.03	0.19±0.00^{b}	0.14±0.04	0.09±0.04^{a}	0.22±0.05
	V140	0.14±0.03^{yz}	0.11±0.00^{a,yz}	0.07±0.02^{y}	0.14±0.02^{ab,yz}	0.19±0.02^{z}
	V150	0.14±0.03^{yz}	0.12±0.00^{ab,y}	0.15±0.01^{yz}	0.21±0.03^{bc,z}	0.09±0.03^{y}
	AT165	0.14±0.03	0.18±0.03^{ab}	0.16±0.06	0.28±0.02^{c}	0.19±0.09
Total nuggets (g oil/g dry solid)	V130	0.09±0.00	0.13±0.00^{b}	0.11±0.02^{b}	0.06±0.02^{a}	0.16±0.05
	V140	0.09±0.00^{y}	0.08±0.00^{a,xy}	0.05±0.01^{a,x}	0.10±0.00^{ab,y}	0.13±0.01^{z}
	V150	0.09±0.00^{y}	0.10±0.00^{ab,y}	0.10±0.01^{ab,y}	0.15±0.03^{b,z}	0.08±0.00^{y}
	AT165	0.09±0.00	0.12±0.02^{b}	0.11±0.03^{ab}	0.16±0.01^{b}	0.10±0.04

^{a,b,c}: Effect of treatment (p b 0.05); ^{x,y,z,w}: Effect of frying time (p b 0.05)

Several studies (Moreira et al., 1999; Saguy and Dana, 2003; Dana and Saguy, 2006; Mir-Bel et al., 2009) demonstrated that most of the oil does not penetrate the product during frying but during the cooling period, when the product is removed from the fryer and the product starts to cool, leading to water vapor condensation and a subsequent decrease in internal pressure.

Oil adhering to the food surface is sucked in due to the consequent 'vacuum effect'. Therefore, oil uptake is a surface phenomenon, involving equilibrium between adhesion and drainage as the food is removed from the oil bath (Ufheil and Escher, 1996; Moreira et al., 1997; Moreira and Barrufet, 1998). The pressurization step plays an important role in reducing oil absorption during vacuum-frying. It can increase or decrease oil absorption in the product depending on the amount of surface oil and free water present in the product (Garayo and Moreira, 2002).

Previous studies of vacuum-frying were made using vegetables with no significant initial fat content, which facilitates the observation of the absorption behaviour. Da Silva and Moreira (2008) determined significantly lower oil absorption by vacuum-fried sweet potato chips, and Mariscal and Bouchon (2008) and Dueik et al. (2010) observed significantly lower oil content in apple and carrot chips, respectively. In contrast Tan and Mittal (2006) and Troncoso et al. (2009) found a significant increase in oil content in potato chips and grater oil uptake in donuts fried at similar temperatures. Andrés-Bello et al. (2010) studied the effect of vacuum-frying in a more complex product, seabream (*Sparus Aurata*) fillets, and found a lower fat content in fillets cooked under vacuum conditions. Mariscal and Bouchon (2008) concluded that permeability was of great importance because oil absorption is essentially a surface-related phenomenon resulting from the competition between drainage and suction into the porous crust once the food is removed from the oil bath and begins to cool (Bouchon et al., 2003;Andrés-

Bello et al., 2010). Therefore, a crust with a lower permeability would reduce the uptake.

The batter and breading treatments used in fried foodstuffs, such as chicken nuggets, modify the permeability of the crust (Ngadi et al., 2009). Pinthus et al. (1995), Fiszman and Salvador (2003) and García et al. (2004) demonstrated that different coatings had different degrees of effectiveness in controlling mass transfer in the frying process. Consequently, the absence of differences in the results obtained for chicken nuggets may indicate that the formation of a uniform coating on the surface of chicken meat during the pre-frying phase is the main hurdle for the mass transfer of oil during frying of this type of food product.

Color

The evolution of color is considered one of the most important quality characteristics of fried products, Table 14 shows the evolution of this parameter as a function of time and treatment. The initial values (0 min) of the CIELab parameters correspond to pre-fried nuggets before treatment, and are therefore the same for all groups. L* is a critical parameter in the frying industry, and is usually used as a quality control factor (Mariscal and Bouchon, 2008). In this study, L* decreased significantly ($p < 0.05$) with time as the samples got darker. The L* values for the pre-fried nuggets (66.87±3.95) decreased to 39.87-53.08 (after frying for 8 min.) depending on the treatment. The decrease in luminosity (L*) during deep fat frying is a typical change that has been reported for many fried products, including chicken nuggets, tofu, pork meat and donuts (Baik and Mittal,2003; Sosa-Morales et al.,2006; Ngadi et al., 2007). Significant differences ($p < 0.05$) between the vacuum treatments were observed for darkening, because non-enzymatic browning reactions are temperature-dependant. As regards differences

between vacuum and atmospheric treatments, in general L* decreased more quickly with the vacuum. Özkan et al. (2003) identified a positive correlation between light and moisture in foods, which means that lower water content leads to decreased brightness. In the present study, it was found that surface losses of moisture were significantly higher and faster in vacuum-fried products, pointing to an additional loss of moisture in these groups.

Previous studies (Da Silva and Moreira, 2008; Mariscal and Bouchon, 2008; Troncoso et al., 2009) of L* coordinates in vegetables pointed to an absence of color degradation in vacuum-fried products and a darkening of traditionally fried products. This may be due to the importance of PPO activity in vegetables, which produce the oxidation of phenols, resulting in enzymatic browning in a reaction that is oxygen dependent and therefore limited in vacuum treatments, at least for this type of product (Snoeck et al., 2011). The absence of air during frying may inhibit enzymatic browning, although caramelization and Maillard kinetic reactions continue if temperatures are higher than 100 ºC. Our results indicate that non-enzymatic reactions and water losses had a predominant effect on luminosity in chicken nuggets.

The redness (a*) values increased significantly ($p < 0.05$) with frying time and temperature (Table 14). Baik and Mittal (2003), Pedreschi, et al. (2005) and Ngadi et al. (2007) also reported that redness increased gradually with traditional frying time, finding that the higher the frying temperature, the darker the resulting potato slices. This suggests that the Maillard reaction was limited by temperature and not by the pressure conditions. Analysis of variance showed that both frying time and temperature had a significant effect ($p < 0.05$) on yellowness (b*) in fried chicken nuggets. A similar phenomenon was observed by Ngadi et al. (2007) in nuggets.

Chroma (C*) and Hue (H*) index were used to describe color saturation and tone of the chicken nuggets. The Chroma index increased with frying time and the Hue index decreased. Moreover, Chroma and Hue analysis indicated significant differences between treatments, the greatest saturation values and the lowest tone being recorded for the traditional treatment.

In conclusion, vacuum-frying decreases the boiling points of the oil and the food water content, so food is totally cooked at lower temperatures. The lower temperature than that used in atmospheric frying, together with the absence of air during frying, may reduce enzymatic browning reactions. The temperatures used in the present study were sufficiently high to promote non-enzymatic browning reactions, although the rate of this reaction depends on the temperature. However, changes in the L* parameter were more intense in the vacuum treatments because water losses are intensified in the vacuum-frying process.

Table 14. CIE L*, a*, and b* values of chicken nuggets after atmospheric pressure frying (AT) and under conditions of vacuum frying (V).

		Time (min)				
	Temperatura (°C)	**0**	**2**	**4**	**6**	**8**
L*	V130	66.87 ± 3.95^{z}	58.41 ± 3.36^{yz}	58.07 ± 4.71^{ab,y}	60.16 ± 3.13^{c,yz}	53.08 ± 2.30^{b,y}
	V140	66.87 ± 3.96^{z}	54.53 ± 6.63^{y}	54.59 ± 3.03^{ab,y}	52.11 ± 2.02^{b,xy}	44.10 ± 2.33^{ab,x}
	V150	66.87 ± 3.97^{z}	52.08 ± 8.05^{y}	50.30 ± 3.95^{a,xy}	41.27 ± 0.32^{a,xy}	39.87 ± 4.62^{a,x}
	AT165	66.87 ± 3.98^{z}	63.43 ± 1.35^{yz}	60.42 ± 0.69^{b,yz}	59.11 ± 1.82^{c,y}	46.39 ± 4.15^{ab,x}
a*	V130	− 1.93 ± 0.10^{y}	− 1.69 ± 0.93^{y}	− 1.65 ± 0.38^{a,y}	− 1.28 ± 0.49^{a,y}	0.54 ± 1.30^{a,z}
	V140	− 1.93 ± 0.11^{x}	− 0.98 ± 0.25^{xy}	− 1.12 ± 0.45^{a,xy}	0.84 ± 1.70^{ab,yz}	1.66 ± 1.58^{a,z}
	V150	− 1.93 ± 0.12^{y}	1.13 ± 2.340^{yz}	2.26 ± 1.40^{b,yz}	3.04 ± 0.46^{bc,z}	4.77 ± 3.70^{ab,z}
	AT165	− 1.93 ± 0.13^{x}	− 1.72 ± 0.65^{x}	− 0.80 ± 0.24^{a,x}	4.36 ± 1.57^{c,y}	7.97 ± 1.76^{b,z}
b*	V130	12.25 ± 2.82^{y}	18.91 ± 3.01^{yz}	17.98 ± 4.38^{ab,yz}	18.32 ± 2.73^{a,yz}	22.73 ± 1.51^{a,z}
	V140	12.25 ± 2.83	16.16 ± 3.56	15.91 ± 0.96^{a}	19.64 ± 5.49^{a}	20.01 ± 2.20^{a}
	V150	12.25 ± 2.84^{y}	20.67 ± 5.15^{z}	23.16 ± 1.26^{b,z}	23.30 ± 1.19^{ab,z}	25.22 ± 0.91^{b,z}
	AT165	12.25 ± 2.85^{x}	16.72 ± 6.72^{x}	17.67 ± 3.26^{ab,xy}	29.85 ± 3.31^{b,z}	26.82 ± 0.91^{b,yz}
C*	V130	12.41 ± 2.80^{y}	19.01 ± 2.95^{yz}	18.06 ± 4.35^{ab,yz}	18.37 ± 2.69^{a,yz}	22.75 ± 1.49^{a,z}
	V140	12.41 ± 2.81	16.19 ± 3.57	15.96 ± 0.94^{a}	19.69 ± 5.58^{a}	20.12 ± 2.29^{a}
	V150	12.41 ± 2.82^{y}	20.79 ± 5.20^{z}	23.30 ± 1.13^{b,z}	23.50 ± 1.2^{ab,z}	25.87 ± 0.91^{b,z}
	AT165	12.41 ± 2.83^{x}	16.84 ± 6.67^{y}	17.69 ± 3.26^{ab,y}	30.19 ± 3.46^{b,z}	28.02 ± 0.65^{b,z}
H*	V130	99.34 ± 2.21^{z}	95.32 ± 3.43^{z}	95.56 ± 1.87^{b,z}	94.16 ± 2.15^{c,yz}	87.37 ± 0.77^{b,y}
	V140	99.34 ± 2.22^{z}	93.45 ± 0.52^{yz}	94.08 ± 1.74^{b,yx}	88.38 ± 4.01^{bc,xy}	85.58 ± 4.29^{ab,x}
	V150	99.34 ± 2.23^{z}	87.93 ± 6.53^{yz}	84.30 ± 3.71^{a,y}	82.58 ± 0.73^{ab,y}	79.34 ± 8.29^{ab,y}
	AT165	99.34 ± 2.24^{z}	96.75 ± 3.18^{yz}	92.66 ± 0.83^{b,y}	81.82 ± 2.30^{a,x}	73.45 ± 3.82^{a,w}

Lightness (L*), Green-red (a*), Blue-yellow (b*), Hue (H*) and Chroma (C*).^{a,b,c} Effect of treatment and^{x,y,z,w}: Effect of frying time ($p < 0.05$).

Textural analysis

Texture Profile Analysis (TPA) was used to measure the characteristic texture of nuggets in this study (Roudaut et al.,2002).Figure 13 shows the effect of frying method on the texture characteristics of chicken nuggets at different temperatures of both atmospheric and vacuum-frying. Neither the time nor frying method produced significant differences in the texture profile ($p > 0.05$) of the products.

This agrees with the results of other authors (Da Silva and Moreira, 2008; Moreira et al., 2008; Dueik et al., 2010), who observed no significant differences in the texture of vegetables prepared by atmospheric and vacuum-frying processes. In contrast, Shyu et al. (2001) indicated that vacuum-frying temperature and time greatly affect the texture quality of fried apple chips. In addition, Troncoso et al. (2009) found that textural parameters (maximum breaking force, hardness, and crispness) of chips were significantly decreased by vacuum-frying. It should be noted that all such studies have focused on the preparation of vegetable foods, which have different physical-chemical characteristics from meat products.

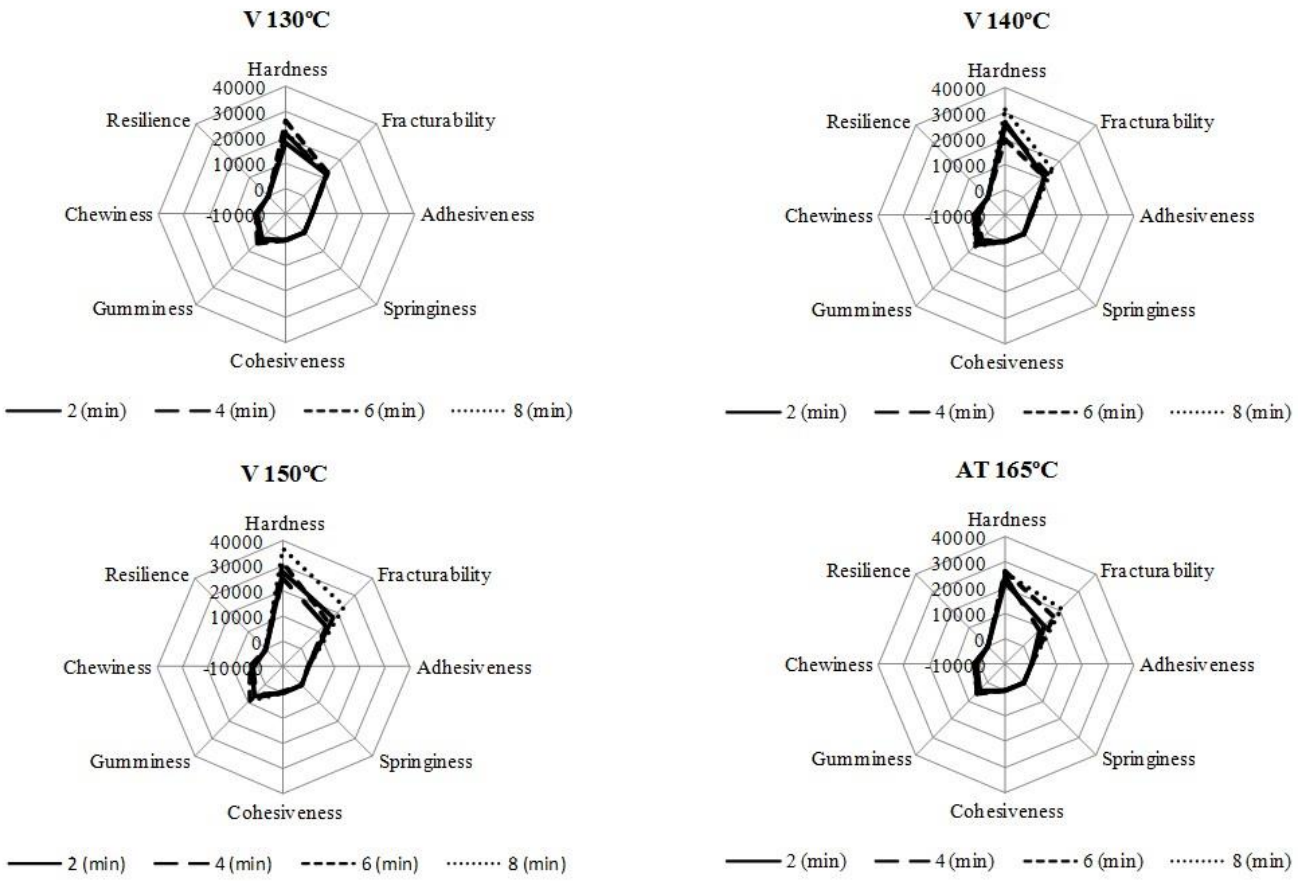


Figure 13. Texture profile of chicken nuggets after atmospheric pressure frying (AT) and under conditions of continuous vacuum frying (V). Hardness (N), fracturability (N), adhesiveness (Ns), springiness (mm). cohesiveness, gumminess (N), chewiness (N mm) and shear force (kg/cm^3).

Ngadi et al. (2007) showed that breaded products, particularly the batter itself, changed their physical and chemical properties during frying, implying a modification of their textural properties. Among these changes can be included moisture loss, protein denaturation and starch gelatinization (Loewe, 1993). Protein denaturation and starch gelatinization reactions occur at 60-80°C, and, given that the temperatures used in this study were higher, the reactions would have occurred already, which explain the absence of differences between treatments since such processes would occur early in both treatment; however, hardness showed a trend to increase (Figure11) with frying time, which could be due to the moisture loss that occurs in the nugget crust during the frying process. Also, it should be noted that the highest hardness values were observed in the 140 ºC and 150°C vacuum-frying treatments, which produced the greatest losses in moisture. Higher values of maximum load indicate an increasing crispy texture (Ngadi et al., 2007; Hirte et al., 2012).

The mechanical, morphological and compositional differences between the layers of the sandwich-like structure of nuggets make it difficult to assess the texture of this kind of crusted food piece (Albert et al.,2011), since the greater thickness of the meat mass could induce very slight moisture changes in the crust. However, the profile curves obtained for vacuum-frying and traditional-frying differed (data not shown), the curves for vacuum-frying presenting a profile with many force peaks and drops associated with fracture events, describe by Albert et al. (2011) as typical of the behavior of crisp products. In contrast, the corresponding curve for the traditionally fried product did not present drops in force and no fracture events occurred during the test, indicating that the product was not crisp. This trend is consistent with the results observed in the sensory analysis, in which consumers thought that vacuum treatment led to greater crispness than the traditional method.

4.3.3.2. Sensory evaluation

The aim of the first sensory analysis was to evaluate the best frying time for chicken nuggets under atmospheric conditions and to determine how much each sample varied from or approached the desirable level for this product. The averages of overall acceptance were judged by a nine-point hedonic liking scale. All samples tested positively, with the samples cooked for 6 min scoring highest (6.98), closely followed by samples cooked for 8 min (6.95) while the lowest score was obtained by samples fried for 4 min (5.85). Crispness, juiciness and color, in order of importance, were determinant attributes in the consumer preference. In relation to the JAR scale, we study considered a JAR score of over 60% as correct and a value above 0.25 and below − 0.25 as "too much" and "too little", respectively (Arcia et al.,2010).

The percentage of consumers that considered the 6 min samples as just about right was higher in the case of juiciness (65%) and texture (60%). As regards the color attribute, the sample cooked for 8 min had the highest score, with 63% compared with the 6 min samples score of 58%. The 8 min samples showed a "too much" deviation outside the defined range. For all these reasons, 6 min was selected as reference time for the traditional (atmospheric) frying of chicken nuggets.

The second analysis was a paired test (ISO, 2005) to assess whether there were perceived differences between traditional fried and vacuum-fried samples, using 150 ºC for 6 min for the traditionally and 150 ºC for 6 min for the vacuum-frying samples, since there combinations of time and temperature were seen to provide the product with similar physical and chemical characteristics. Figure 14 shows the sensory test results for this comparison. Consumers did not show any significant preferences concerning "overall acceptance" and "oiliness" for the products prepared by these two methods.

Neither was there any significant preference for juiciness, which may be associated with the moisture content of the meat mass (which did not show any significant differences either).

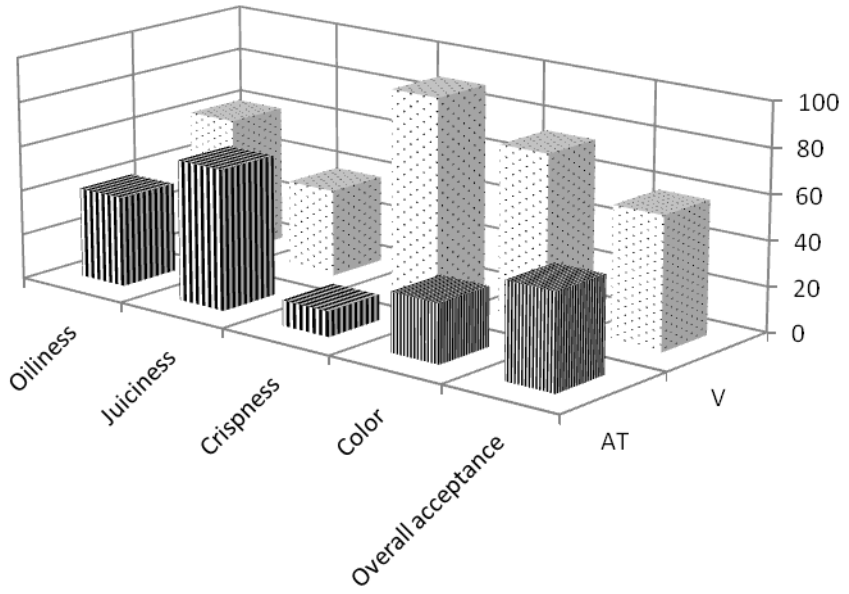


Figure 14. Sensory evaluation (%) of chicken nuggets frying at different pressure and temperature.

A significant number of panelists ($p < 0.05$) preferred the color of the chicken nuggets cooked under vacuum. The instrumental color recording showed significant differences in the L* parameter, the average value of the vacuum samples being 39.87 compared with the 59.11 of the atmospheric samples. Therefore, consumers preferred the darker samples. Moreover significant differences in crispiness (determined as more crunchy nuggets) were observed for the samples cooked under vacuum. This trend is consistent with that described for texture, which showed that, although the texture parameters were

similar, the vacuum-frying curves presented a highly jagged profile with many force peaks and drops associated with a crispy texture. However, these differences were not decisive in the election made by consumer.

4.3.4. CONCLUSIONS

The process of frying under vacuum in the conditions studied provided products with compositional and sensorial attributes similar to those of chicken nuggets fried in atmospheric conditions. The temperatures (130, 140, and 150 ºC) evaluated in this study for vacuum-frying were sufficient for the evolution of Maillard reactions though the evolution was slower than in atmospheric frying (165 ºC) because non-enzymatic browning is highly temperature-dependent. The decreased boiling point of samples fried under vacuum produced an initial faster rate of moisture loss and thus an additional fall in luminosity. Consumers did not show any significant preferences concerning "overall acceptance" and "oiliness" for products prepared by either of these methods, but determined as more crunchy the nuggets cooked under vacuum conditions. The vacuum-frying treatment did not produce nuggets with a lower fat content than atmospheric conditions, the formation of a uniform coating on the surface of chicken meat during the dipping and pre-frying phase being the main hurdle for the mass transfer of oil during both types of frying. Therefore vacuum-frying can be considered a worthwhile alternative for making fried batter products. However, it would be recommended to future researches include other parameters such as micronutrients and deterioration product formed in the oil and in the product itself as a consequence of high temperatures of traditional frying and that could be reduced by decreasing of temperature that allows the vacuum-frying process.

4.4. A COMPARATIVE STUDY OF THE CHARACTERISTICS OF FRENCH FRIES PRODUCED BY DEEP FAT FRYING AND AIR FRYING.

4.4.1. INTRODUCTION

Frying is essentially a dehydration process involving rapid heat and mass transfer in food immersed in hot oil, which leads to a succession of physical and chemical changes in the product (Tarmizi and Ismail, 2008; Andrés-Bello et al., 2011; Dueik and Bouchon, 2011). Frying is extensively employed in domestic as well as industrial practice, due to its ability to create unique sensory properties, including texture, flavour and appearance, which make the food more palatable and desirable (Dana and Saguy, 2006). Furthermore, its operational simplicity in the context of commercial practice, convenience, and economic viability, has resulted in extensive sales of a large variety of fried products (Mehta and Swinburn, 2001). Despite, the many studies correlating fried product consumption with increased health risks (Krokida et al., 2001; Mariscal and Bouchon, 2008), and increasing consumer awareness of this relationship (Mariscal and Bouchon, 2008), there is no sign to suggest that we will give up eating fried products (Dana and Saguy, 2006; Tarmizi and Ismail, 2008; Sayon-Orea et al., 2013). These issues have prompted the fried product industry to search for ways and means to produce healthier products without compromising on the desirable appearance, texture, flavour and taste attributes (Garayo and Moreira, 2002; Fan et al., 2005; Da Silva and Moreira, 2008; Mariscal and Bouchon, 2008; Andrés-Bello et al., 2011; Andrés et al., 2013).

One such process is hot air frying, which aims to produce a "fried product" by sparging, essentially, hot air around the material instead of immersing it in hot oil. A variety of proprietary air fryer designs are currently available in the market, which create the frying effect by bringing direct contact between a fine mist of oil droplets in hot air and the product, inside a chamber. Most designs provide for extremely high heat transfer

rates uniformly between air and the product being fried. Some achieve this simply with a built-in air blower, while others also couple high convective rates with radiative heat transfer. A number of manufacturers also claim that the shape of the chamber in which air and product are being contacted is profiled in such a way that air velocities are significantly higher than in typical ovens (Erickson, 1989). Moreover, the air is also distributed more uniformly through the product, which minimizes variations in product quality. A schematic of a typical air fryer is shown in Fig. 1. The product gets dehydrated in the process and a crust, typically associated with frying, gradually appears on the product. Oil application could be done before or during the process to lightly coat the food product, in order to provide the taste, texture and appearance typical of fried products. The amount of oil used is significantly lower than in deep oil frying giving, as a result, very low fat products (Andrés et al., 2013). To date, there is only a scientific publication about hot air frying. Andrés et al. (2013) analyzed the kinetics of mass transfer and volume changes in hot air frying and deep-oil frying at the same temperature (180ºC) and concluded that both are affected by medium type. Heat transfer was slower when the fluid phase is air than when it is oil, due to lower heat transfer coefficient of air. Moreover, they also observed that product mass losses in air frying were higher than in deep frying, because the water lost during air frying was not offset by any significant oil uptake. Unfortunately, this paper makes little or no reference to the quality and sensory parameters of the product, and this is a major knowledge gap. In the present work, we have aimed to draw a comparison between: 1) process parameters of air frying and hot air frying - such as moisture content time profile, product temperature versus time profile and product oil content versus time profile, and 2) product characteristics yielded by the two frying methods, which include starch gelatinization profile, microstructure using SEM as well as sensory characteristics. This

detailed comparison has been drawn by holding the same frying medium temperature in both cases, i.e. 180ºC. Further, the product characteristics mentioned above, including sensory analysis, have been compared after fixing the final product moisture content at a value that consumers normally consume (91.7 ±6.03 g water/ 100g defatted dry matter), which also helps us to evaluate whether air frying can produce a true alternative to traditional frying.

4.4.2. MATERIALS AND METHODS

4.4.2.1. Raw materials

Maris Piper potatoes packaged in polyethylene bags and sunflower oil were purchased from a local supermarket (Morrisons, Reading, UK), and stored in a refrigerator at 4 ºC.

Analytical grade petroleum ether (boiling point of 40 to 60 ºC) and cellulose extraction thimbles (single thickness, 22 mm x 80 mm), used in the determination of oil content were purchased from Fisher Scientific UK Ltd (Loughboroug, UK).

4.4.2.2. Frying equipment used

Commercial deep oil frying (model: 45470, Morphyrichards) with a nominal power: 2,000 W) and hot air frying equipment (model: AH-9000 Viva Collection Airfryer HD9220/40, Philips) with a nominal power: 1,300 W. Figure.

4.4.2.3. Sample preparation

The samples were prepared following the methodology described by Tarmizi and Niranjan (2010). Potatoes ranging in moisture content between 445.37 ± 107.77 g water/100 g dry matter were selected for this study. The potatoes were taken out from

the fridge in which they were stored at least 12 h before being used in experiments, then washed, peeled and manually cut into strips (9 x 9 x 30 mm). The strips were soaked in running water for 1 min to eliminate occluded starch and blotted using tissue paper.

4.4.2.4. Frying protocol

The frying methodology, described by Andrés et al.(2013), was used in this study. In the case of deep fat frying, about 100 g of potato strips were immersed in 2 L of oil to give a product to oil ratio of 1:20 (w/v) which was deemed by Andrés et al.(2013) to be sufficient to avoid major changes occurring in terms of product-to-oil ratio, oil composition and temperature. In the case of hot air frying experiments, 0.45 g of oil per 100 g of potatoes strips, was added into the air chamber.

The potato strips were only introduced into the oil in the case of deep fat frying or into the hot air frying chamber in the case of air frying, after an operating temperature of 180°C was reached, the temperature being confirmed by thermocouples located at the bottom of both frying equipment. Samples were removed from the frying equipment at 3 min intervals, for up to a maximum of 30 mins, and subjected to physico-chemical analysis.

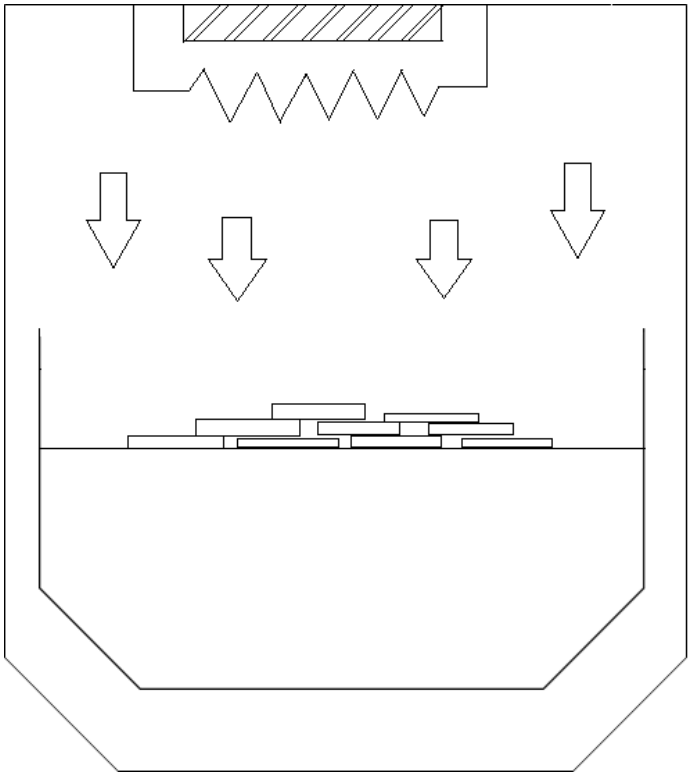


Figure 15. Schematic representation of air fryer: (a) fan, (b) electrical resistance heater, (c) hot air and (d) samples. It may be noted that there are a variety of proprietary hardware designs available each claiming heat and mass transport advantages as well as improved product quality, for instance, see Erickson (1989).

4.4.2.5. Analyses of French fries during the Frying Processes

Proximate composition

Samples were analysed according to American Oil Chemists' Society official methods, also described by Tarmizi and Niranjan (2010).

Moisture content: The moisture content was determined by taking three homogenized samples of 10 g collected at each processing time, and drying these for 48 hours at 105 ºC in the convection oven (Weiss-Gallenkamp, Loughborough, U.K.) to obtain a constant weight.

Oil content determination: The total fat content of three dried samples (5 g) collected at a given processing time was measured. The dried samples were ground using a mortar and transferred to a single-thickness cellulose extraction thimble (Fisher Scientific UK Ltd, Loughboroug, UK). A dried and weighed 250-mL round-bottom flask (Quickfit-BDH, Poole, U.K.) was filled with 150 mL of petroleum ether (Fisher Scientific UK Ltd, Loughboroug, UK), and oil was extracted gravimetrically using a Soxhlet extraction system (Quickfit-BDH) for 4 h. The solvent was then removed by rotary evaporation (Rotavapor RE 111, Büchi Labortechnik AG, Flawil, Switzerland) under vacuum of 380 to 510 mmHg at 50 ºC. The flask containing oil was dried to constant weight at 105 ºC using the same convection oven described above (Weiss-Gallenkamp, Loughborough, U.K.). The oil content was expressed as g oil/100 g defatted dry matter.

Color

The color of the potato French fries was measured using a reflectance colorimeter (HunterLab CT-1100 ColorQUEST, Reston, VA). According to the CIE LAB system, Lightness (L*), green-red chromacity (a*), and blue-yellow chromacity (b*) were measured. The illuminant used was D 65 and the colorimeter was standardized using a cylindrical light trap (black), followed by standard white and grey calibration plates. All measurements were undertaken in triplicate.

Texture

Texture measurements were made with Brookfield CT3 Texture fitted with 25kg load cell. Data collection and analysis was accomplished by using electronic Texture Pro CT software. A single cycle puncture test was performed using a cylindrical flat-end punch

(2mm diameter probe) by fixing the test speed at 4.6 mm/s; the punch was allowed to travel into the samples for: 2mm (covering the crust region) and 6mm (which covered the core). Six samples were measured and punctured at 2 random positions for each processing time.

4.4.2.6. Analyses of the final product (i.e. ready to consume)

Although the above analyses were carried out over an extended time scale, which was much longer than what will be used in practice, the final product was defined in accordance with the quality control criteria set by frying industry, which stipulates that the moisture content of the ideal product must be in the range between 38% and 45% on a wet weight basis (Matthäus et al., 2004). The moisture and oil contents, color and texture of the final product were determined as above. In addition, SEM, DSC and sensory analyses were also carried out on the final product.

Scanning electron microscopy (SEM)

Sections taken from the core and crust regions of the product were freeze-dried and their fractured surface was examined and photographed using a scanning electron microscope (FEI Quanta FEG 600 with a Quorum PP2000T Cryo Stage, Eindhoven, Netherlands) at different magnifications, and representative images were chosen.

Differential scanning calorimetry (DSC)

The method of Steeneken and Woortman (2009) was used. Heating scans were performed on core samples of French fries by employing a Perkin Elmer DSC 200, by heating from 20 to 210 ºC at 10 ºC/min followed by cooling to 20 ºC at 200 ºC/min.

Sensory analysis

For the sensory analysis, all evaluations were conducted in individual booths which contained the instructions for the evaluation procedure. The tasting room for sensory evaluation was air-conditioned and free of disturbing factors. Samples were fried in a commercial deep fat fryer (model: 45470, Morphyrichards) at 180 ºC for 9 minutes and in a commercial air fryer (AH-9000 Viva Collection Airfryer HD9220/40, Philips) at 180 ºC for 21 minutes. Samples were obtained, and immediately after, were presented to the panelists.

The panelists were trained according to ISO (2012). The training program consisted of three sessions aiming to develop sensory descriptors and ensure competent usage of these by the panel. For each sample the panelists registered the perceived intensities of each of the attributes. These attributes were individually recorded using an unstructured scale of 100 mm, and the data sets checked by ANOVA. Mineral water and bread were provided for mouth rinsing between samples.

4.4.2.7. Statistical analysis

The statistical analysis of the data was conducted using statistical package SPSS 15.0 (Statistical Package for the Social Science for Windows). Statistical significance was expressed at the p b 0.05 level.

4.4.3. RESULTS AND DISCUSSION

4.4.3.1. Analyses of French fries during the Frying Processes

Temperature profile

The temperature of French fries, measured at a point, more or less, near the centre, under different frying conditions (deep fat frying and air frying) is presented in Figure 16. The deep fat fried samples behaved in a manner similar to the one described in earlier work (Budžaki and Seruga, 2005; Farinu and Baik, 2008; Mir-Bel et al., 2012). The initial temperature increased, almost linearly with time, until it reached the boiling point of water (~100 ºC). The temperature then increased gradually for a period of time, before increasing more sharply. The air fried samples also showed the same initial trend, i.e. temperature increasing linearly up to the boiling point of water, but at a significantly slower rate than deep fat frying. The oil fried sample took 1.5 minutes to reach the boiling point of water, whereas the air fried sample took nearly 5.5 minutes.

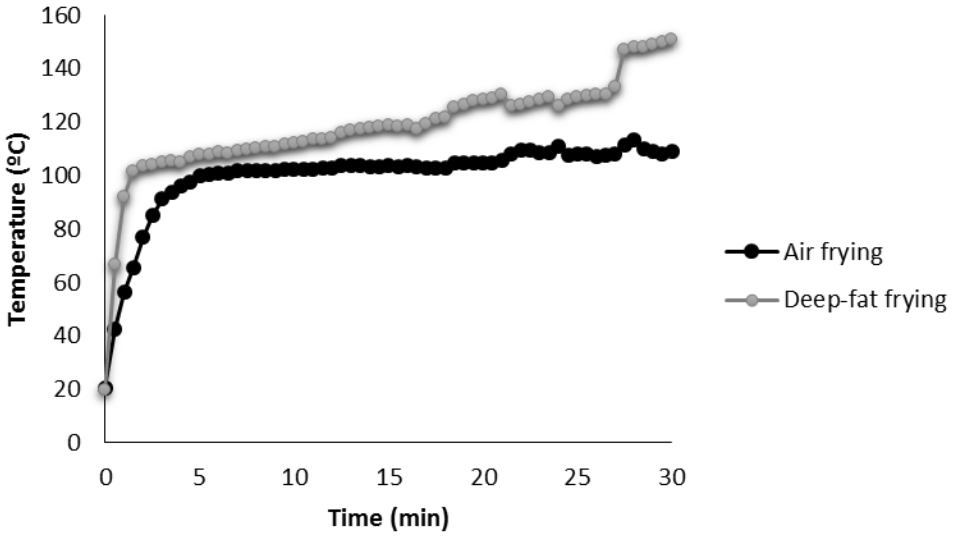


Figure 16. Evolution of temperature inside French fries in both types processes at 180 ºC, deep fat and air frying.

A second difference between oil frying and air frying is that the temperature, in the case of the latter process, remains, more or less, constant at the boiling point of water till the end of the process, and the gradual, but significant, increase in temperature above 100 ºC observed in the case of deep oil frying is not evident. Based on the times taken for the product centre to reach the boiling point of water, it can be estimated that the heat flux in the case of oil frying is 3.7 times greater than in the case of air frying, which seems to provide enough energy in the form of latent heat as well as sensible heat. The post boiling heat transfer is accompanied by physicochemical changes occurring such as: gelation of starches, increase in the thickness of superficial crust and reduction in the rate of steam release from the product (Mir-Bel et al., 2012).

Moisture and oil content

Frying process normally implies a series of complex mass transfer processes between the food and fluid phase giving, as a result, two counter current-fluxes: a water/steam flow from the food to the hot oil and an oil inlet into the food (Ziaiifar et al., 2008; Krotida et al., 2000; Andrés et al.,2013; Kalogianni and Popastergiadis, 2014), although such simplistic explanations have been questioned (Bouchon and Pyle, 2005).

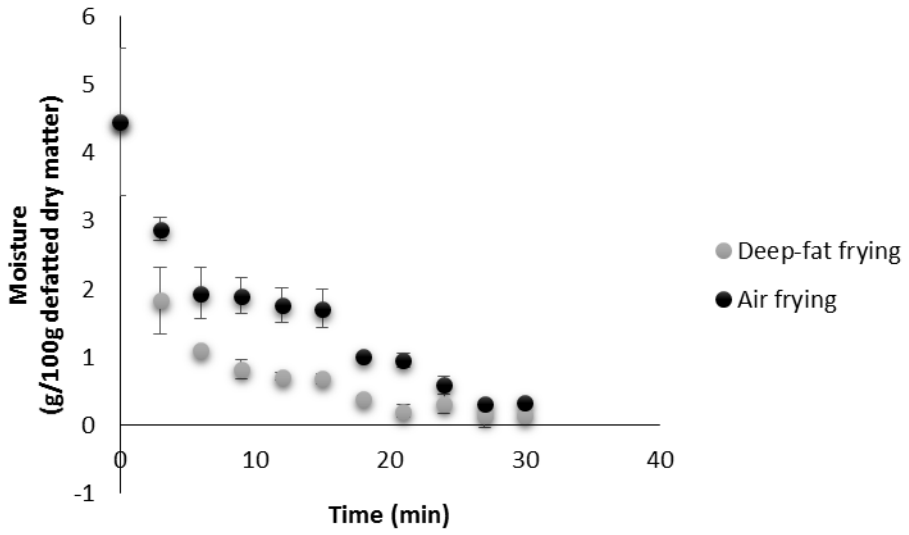


Figure 17. Evolution of moisture values of French fries in both types processes at 180 ºC, deep fat and air frying.

The variation of moisture content (expressed as g/100g defatted dry matter) with time for different frying conditions is shown in Figure 17. As expected, the moisture decreases with frying time ($p < 0.05$) for both deep fat as well as air frying. The mechanism of water loss during frying has been interpreted previously as a dehydration process (Mir-Bel et al., 2012; Bingol et al., 2014). It is clear from Figure 17 that the moisture content decreases more rapidly in deep fat frying than air frying ($p < 0.05$). These results are consistent with higher heat flux observed in the case of deep fat frying and are also in agreement with Andrés et al. (2013) who compared moisture loss kinetics between the two frying methods.

Figure 18 shows fat content variation with time in of the two frying process. The values varied between 0.37-1.12 g/100g defatted dry matter for samples processed by air frying, and between 5.63-13.77 g/100g defatted dry matter for deep fat fried samples. The differences between the oil contents may be attributed to the ”frying medium”

surrounding the products: hot oil in the case of deep fat frying, and a mist of oil droplet in air in the case of deep fat frying.

This observation is also in agreement with the findings of Andrés et al. (2013) who showed that the main difference between the two types of frying is the final fat content and these differences are due to the type of frying medium employed. In the case of deep fat frying, it is known that the oil absorption (64-90% of the total oil absorbed) predominantly occurs at the end of frying, due to the condensation of water vapor inside product caused by the fall in temperature below the boiling point of water, which creates a suction pressure gradient between the surface and the inner structure of the product (Mellema, 2003; Saguy and Dana, 2003; Dana and Saguy, 2006; Ziaiifar et al., 2008; Mir-Bel et al., 2009; Tarmizi and Niranjan, 2010).

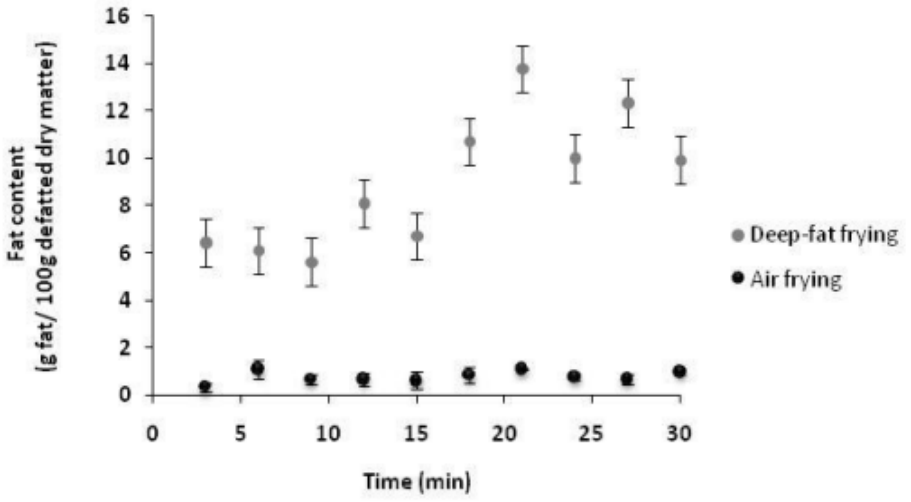


Figure 18. Evolution of fat values of French fries in both types processes at 180 ºC, deep fat and air frying.

Deep fat frying is undertaken in oil (20 g of oil per gram of potatoes), whereas air-frying samples are mixed with a small oil amount before "frying" (0.003 g of oil by

gram of potatoes). This implies that, in the case of the latter process, a limited amount of oil is in contact with the sample surface and therefore oil absorption is limited.

Color

The color of the fried potatoes is one of the most significant quality factors determining acceptance (Krokida et al., 2001). Instrumental color coordinates (CIELab) for both types of French fries are shown in Figure 19. As expected, L* decreased with frying time in the two processes whereas a* and b* increased ($p < 0.05$). This is consistent with the potatoes turning darker and more red-yellow as described by Nourian and Ramaswamy (2003) and Romani et al. (2009a, b).

The characteristic color of French fried potatoes essentially result from the Maillard reaction (non-enzymatic browning) involving reducing sugars and amino acids (Nourian and Ramaswamy, 2003; Pathare et al., 2013).

It is also clear from Figures 17 that a* and b* drop initially, attain a minimum value,and then increase progressively before leveling off around the same values for both types of products. A closer analysis of the figures also shows that the minimum values of a* and b* are attained much more rapidly in the case of deep fat frying ($p < 0.05$) The rapid evolution of colour is consistent with the higher rates of temperature rise observed in the case of deep fat frying (Figure 16). Baik and Mittal (2003), Pedreschi et al. (2005) and Ngadi et al. (2007) and Pathare et al. (2013) reported that the non-enzymatic browning reactions are highly temperature dependant. Thus, air frying process can potentially achieve the characteristic color of deep fat fried French fries but requires significantly longer processing time.

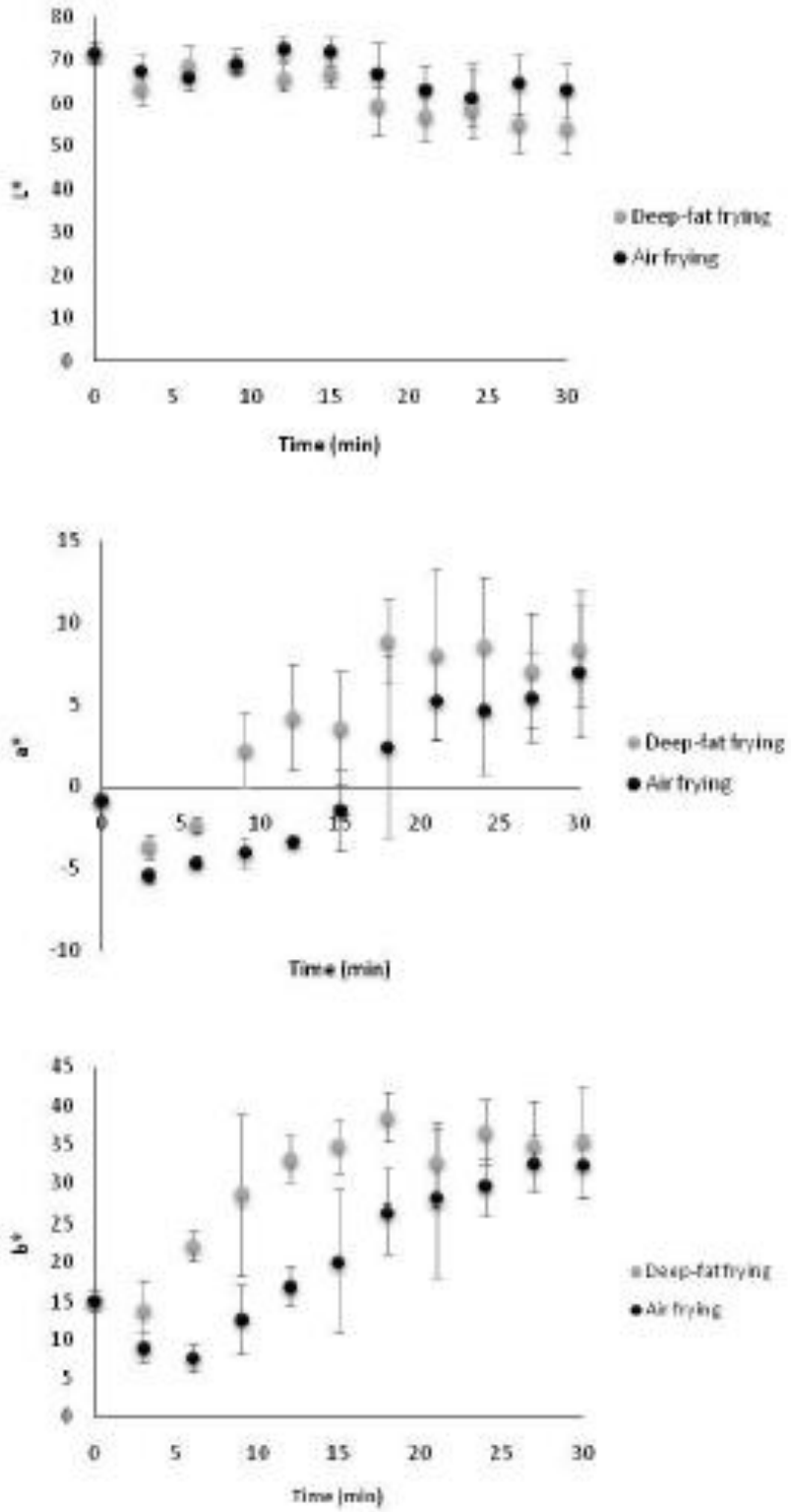


Figure 19. Evolution of CIELab values of French fries in both types processes at 180 ºC, deep fat and air frying.

Texture

The kinetics of textural changes occurring in the two types of products was studied using a compression test. Table 15 shows hardness work (mJ) for the probe to penetrate the surface (2 mm) and core (6mm) of samples.

Moyano et al. (2007) and Pedreschi and Moyano (2005) observed that heating of potato tissue causes drastic physical, chemical, and structural changes, which could be divided into two stages: the tissue softening during the first few minutes of frying followed by crust formation and subsequent hardening. The same trend was observed in the present study for deep fat, as well as, air fried products. Table 15 shows the hardness work to decrease initially. The evaluation of texture parameter (hardness work) at 2 mm and 6 mm allowed studying the crust development and the modifications in product core, respectively. The initial stage of frying resembles a cooking process when a part of the starch gelatinizes and the lamellar media solubilizes at temperatures of around 60 to 70 ºC (Moyano et al., 2007). The softening phase of the tissue, at the surface as well as core, was much faster in deep fat frying ($p < 0.05$) which required only 3 minutes (105 ºC) to be completely softened, compared to 6 minutes (100 ºC) required for air fried samples.

The second stage is characterized by the development of a porous dried region and an overheated region which is generically called "crust". This region is result of a vaporization front located close to the heat exchange surface which progressively moves towards the product center with the frying time. Miranda and Aguilera (2006) showed that the exposure of potato products to temperatures above 100 ºC, such as the temperatures encountered during frying, causes starch granules and cells located on the surface to become dehydrated and form an external crust, which makes the product

crispy. Both processes showed increase in hardness work values for the crust and core regions with time ($p < 0.05$).

With regard to the effect of frying methods, in general no differences were observed between the two frying methods for crust region at different frying times. However in the case of the core region, the air fried samples showed higher hardness work values ($p< 0.05$) than the deep fat fried samples. These differences in core texture may be due to a smaller degree of gelatinization occurring in air fried samples, associated with the prevalence of lower temperatures inside the product.

As evident in Table 15, with time, the evaporation continues until the products are completely dry, in both processes, and the hardness work converge to more or less identical values at very long process times. In practice, however, it is necessary to note that this final stage is never reached since the products are removed much earlier at process end-points defined by consumer acceptability of the product.

The quality parameters of the final product, withdrawn at this end point, i.e. the products which are meant to be consumed, are discussed below. In terms of texture data shown in Table 15, it is clear that both products have different texture characteristics in both the regions: crust and core. Air fried samples (21minutes) had hardness work values about 1.38 and 7.29 mJ for crust and core respectively, while that deep fat fried samples (9 minutes) were about 4.23 and 11.49 mJ ($p< 0.05$; $p< 0.001$).

Table 15. Compression test results (Hardness Work, mJ) of products air fried and deep fat fried at 180 ºC, as a function of processing time.

	Processing time (minutes)										
	0	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**
2mm											
Deep fat	16.84±0.55^{c}	1.26±0.52^{a,y}	1.28±0.92^{a}	1.38±1.26^{a}	2.26±1.41^{a}	2.49±1.19^{a}	1.84±1.77^{a}	5.41±3.56^{a}	3.10±2.53^{a}	10.32±6.95^{b}	14.93±6.11^{b,c}
Air	16.84±0.55^{d}	10.18±3.80^{bc,z}	2.03±0.71^{a}	1.61±1.03^{a}	2.06±0.99^{a}	1.44±0.52^{a}	1.83±1.78^{a}	2.84±1.40^{a}	3.49±1.86^{a}	8.84±8.06^{b}	5.52±7.98^{c}
6mm											
Deep fat	75.74±8.23^{d}	4.51±2.32^{a,y}	4.44±1.95^{a,y}	4.23±1.32^{a,y}	3.84±2.30^{a,y}	4.76±1.77^{a,y}	7.48±3.93^{a}	5.51±3.61^{a,y}	5,71±2.05^{a}	30.92±22.56^{b}	50.83±26.76^{c}
Air	75.74±8.23^{c}	29.24±11.95^{b,z}	7.46±1.70$^{a.z}$	7.29±3.20^{a,z}	8.27±3.67^{a,z}	8.95±3.47^{a,z}	8.07±4.09^{a}	11.49±3.04^{a,z}	10.59±5.85^{a}	27.73±16.19^{b}	29.72±16.46^{b}

Represent averages of three independent repeat ± standard deviations. $^{a, b, c, d}$: indicate statistically significant differences ($p < 0.05$) among frying time; $^{x, y}$: indicate statistically significant differences ($p < 0.05$) among treatments.

4.4.3.2. Analyses of the final product deemed to be fit for consumption

Quality control criteria of frying industry stipulate that the moisture content of the final product must be in the range between 38% and 45% on a wet weight basis (Gökmen et al., 2006; Romani et al., 2008). To meet this criterion the samples used in this study were processed for 9 minutes in the case of deep fat frying and for 21 minutes in the case of air frying, both at 180 ºC. SEM, DSCand sensory analyses were undertaken to compare the two products.

SEM and DSC analyses

Figure 20 show the microstructure of the raw and fried potato chips. Figure 20 (a-b) shows the cross section of raw potato chips. The core of the chips contain non-deformed flesh cells with starch granules, while the outer surface reveals mechanical damage of cells caused by the cutting process; these results are similar to the ones described by Lisińska and Golubowska (2005).When we compare the raw potato tissue consisting of cells appearing pentagonal/hexagonal in shape (Figure 20 a-b) with the tissue resulting after "frying" (Figure 20 c-h), irreversible changes can be seen and two particularly clear areas appear: crust and core. Aguilera et al. (2001) and Pedreschi and Aguilera (2002) postulated that cells in the crust of fried potato tended to change their shape while shrinking, and their walls became wrinkled and convoluted around dehydrated gelatinised starch; there was however, little or no rupture evident. The crust of air fired samples (Figure 20 f and h) showed higher empty spaces and smaller cells than deep fat fried samples, because the temperatures and rates of water evaporation were different in the two process; moreover, any empty spaces formed during deep fat frying would be filled with oil. On the other hand, in both products, starch swelling mainly occurred in the core region, which is a result of grain hydration and gelatinisation to form an

amylase and amilopectin reticulum which completely fills the cellular lumen (García-Segovia et al., 2008), although this process occurred to a greater degree in deep fat fried samples (Figure 20 d) than air fried sample (Figure 20 g).

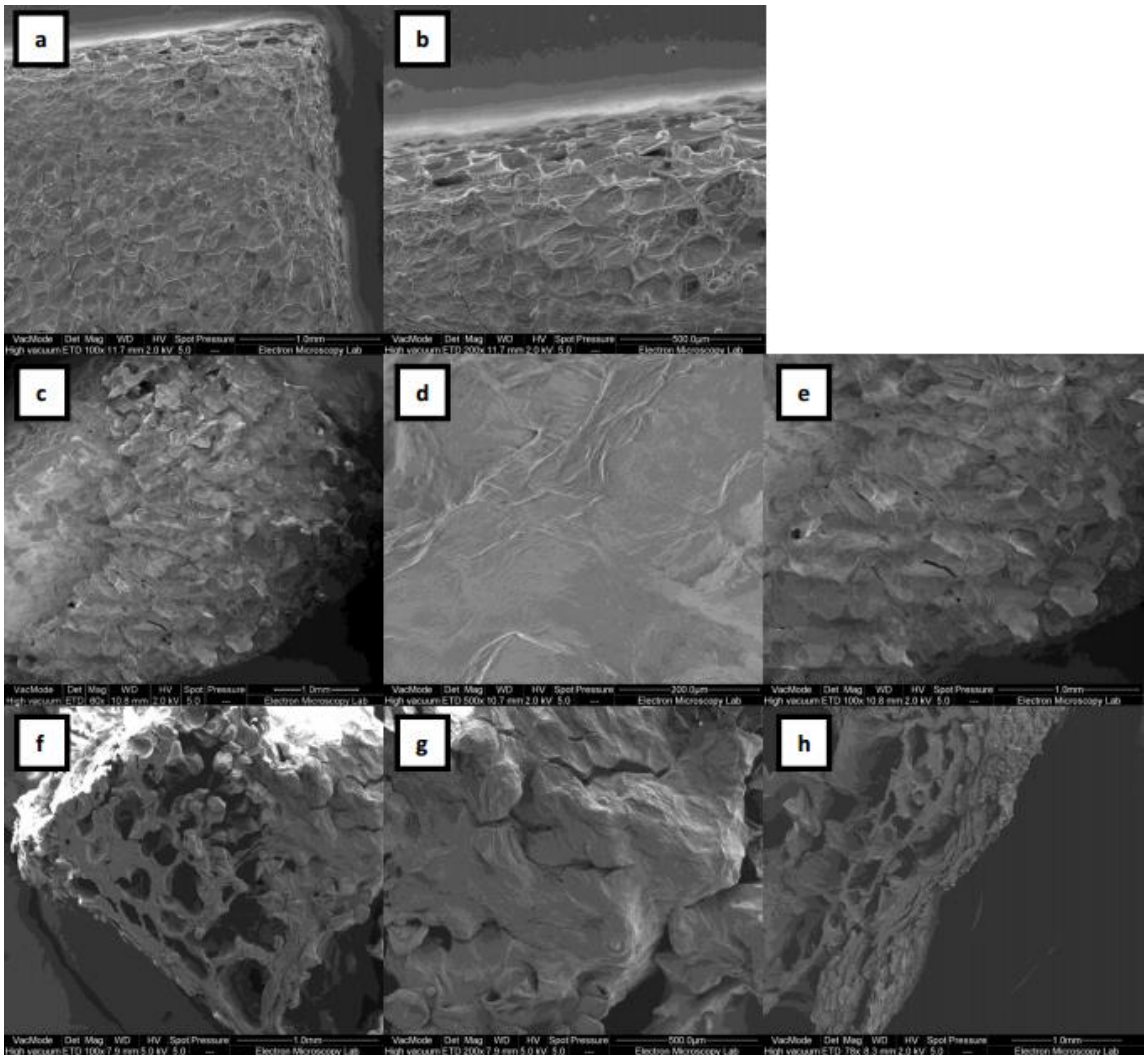


Figure 20. Scanning electron microscope of French fries raw, deep fat frying (9 minutes) and air frying (21 minutes): (a-b) raw samples, (c-e) deep fat fried samples, and (f-h) air fried samples.

Similar results were noted for the DSC analyses given in Figure 21. Both process showed higher gelatinization temperature and weaker endotherms than raw samples, which indicates the modification of starch structure due to gelatinization process

(Garzón, 2006; Liu et al., 2009). Furthermore, deep fat fried samples have a lower value of the enthalpy of gelatinization (ΔH) than air fried samples. According to Bello (2009) lower values of enthalpy indicates a higher proportion of gelatinized starch. Thus, a key difference between air fired and deep fat fried products is the higher extent of gelatinization occurring in the latter.

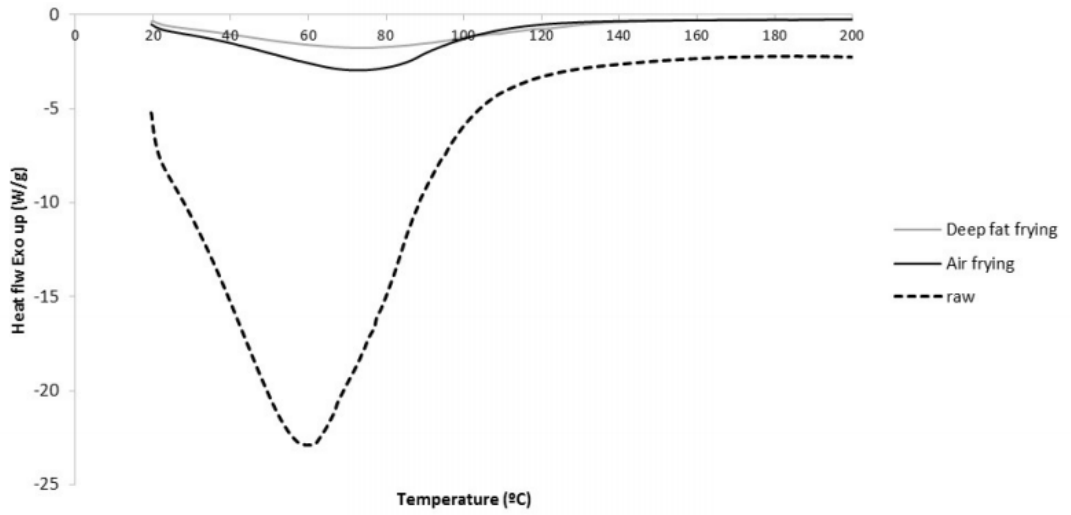


Figure 21. Gelatinization endotherms of French fries: raw, deep fat fried (9 minutes) and air fried samples (21 minutes).

Sensory analyses

A panel evaluated appearance, odor, mouthfeel, taste, flavor and after effects of products obtained by both types processes, based on 31 descriptors (Table 16). There were statistically significant differences found for 22 of the 31 attributes ($p < 0.05$) used, which indicates major difference in the perceived product characteristics. It may be noted that the air fired product was processed for 21 minutes, whereas the deep fat fried

product was processed for 9 minutes. Under these conditions, both products had average moisture content about 45%.

In terms of appearance, the extent of brownness and evenness of cooking were not significantly different between air fried and deep fat fried samples, which is also in agreement with instrumental color measurement. However, air fried samples stood out in terms of appearing puffed and dry, when compared with deep fat fried samples which also highlighted oiliness attributes ($p < 0.05$); the SEM images shown in Figure 20 are consistent with these sensory observations. With regard to odor, the deep fat fried product gave a fried smell and flavor, while the air fried samples give what was described as "jacket potato smell" ($p < 0.05$). In the same way, the after effects attributes only show differences in terms of the deep fat fried product giving an oily mouth coating and greasy fingers. The skin mouth feel was smoother and it felt tough in the case of air fried samples ($p < 0.05$) which is also consistent with the texture test that showed higher values of hardness work for air fried samples than deep fat fried samples. However, the crispness was similar ($p > 0.05$). In traditional deep fat frying, oil migrates to intracellular spaces formed by cell wall shrinkage and water evaporation (Costa Rui et al., 2001), resulting in a more oily mouth feel ($p < 0.001$).

On the other hand, in air fried samples, these spaces remain void and gave a desiccated mouth feel. The floury mouthfeel and earthy flavor were significantly higher in deep fat fried samples. The mealiness sensation in potatoes is associated with a greater volume of the gelatinized starch filled up in their cells (Bordoloi et al., 2012). These observations are also supported by DSC and SEM measurement (Figure 20 and 21).

Table 16. Quantitative descriptive analysis of French fries in both types processes: Deep fat (9 minutes) and Air (21 minutes) frying.

		Deep fat frying	Air frying	
Appearance	Brown	10.85±6.33 [a]	8.33±7.83 [a]	ns
	Puffed	42.45±15.84 [a]	1.00±3.16 [b]	***
	Dryness	62.95±15.21 [a]	29.08±17.74 [b]	***
	Evenness of Cook	48.93±16.83 [a]	54.45±15.15 [a]	ns
	Oil release to fingers	0.50±0.99 [b]	41.55±16.38 [a]	*******
Odor	Jacket Potato	43.10±12.66 [a]	1.08±3.40 [b]	***
	Boiled Potato	5.33±7.70 [b]	18.83±5.72 [a]	**
	Fried Odour	2.55±8.06 [b]	40.63±11.84 [a]	*******
	Old Fat	2.00±6.32 [a]	1.38±3.36 [a]	ns
Mouthfeel	Smoothness of Outer Skin	55.73±18.40 [a]	31.80±18.39 [b]	**
	Toughness of Outer Skin	48.40±16.70 [a]	22.73±11.00 [b]	***
	Crispness of Outer Skin	39.58±23.68 [a]	36.55±14.11 [a]	ns
	Dessicated	58.70±14.31 [a]	20.75±16.92 [b]	*******
	Oily mouthfeel	1.80±4.65 [b]	26.83±11.09 [a]	***
	Hollow Gap 1/2	1.05±0.16 [b]	2.00±0.00 [a]	***
	Moistness of Core Potato	15.93±8.53 [b]	28.88±11.65 [a]	*
	Chewy	42.30±14.42 [a]	21.58±13.23 [b]	***
	Dense	22.98±12.28 [a]	31.63±14.52 [a]	ns
	Amount of potato inside	24.20±13.72 [b]	54.60±20.04 [a]	***
	Floury	9.15±8.14 [b]	34.05±19.44 [a]	**
Taste	Sweet	11.68±11.04 [b]	19.33±6.60 [a]	*
	Acidic	4.60±7.04 [a]	3.75±5.58 [a]	ns
Flavour	Oily Flavour	2.10±5.59 [b]	26.38±8.38 [a]	***
	Jacket Potato Flavour	40.55±19.07 [a]	0.63±1.98 [b]	***
	Boiled Potato	6.80±10.52 [b]	21.28±7.65 [a]	*****
	Earthy	7.35±8.69 [a]	0.60±1.90 [b]	*
After Effects	Bitter	9.05±8.12 [a]	3.70±4.11 [a]	ns
	Metallic	0.25±0.79 [a]	0.00±0.00 [a]	ns
	Acidic	3.78±7.67 [a]	2.60±3.51 [a]	ns
	Oily film coating mouth	1.20±3.71 [b]	17.73±7.17 [a]	***
	Greasy Fingers	0.53±1.11 [b]	33.88±16.53 [a]	***

Represent averages of three independent repeat ± standard deviations. [a, b]: indicate statistically significant differences among treatments.

In general, the QDA results indicate that sensory characteristics of the products obtained from the two processes are significantly different, and the key differences will be summarized below.

4.4.3.3. Key appearance differences between air fried and deep fat fried products

The external appearance of the samples is shown in Figure 22. The color of air fried and deep fat fried products may not be significantly different, however, the visual presence of fat in deep fat fried product is amply evident. Another major difference between samples fried in air and oil is the structure of the products formed. Visual observations indicate that deep fat fried samples have a surface crust structure which is dry, crisp and thick. This is the result of the high temperatures being reached rapidly at the product surface which causes intense local water evaporation that impedes gelatinization of the starch in the region. In the case of air fried product, the water evaporates much more slowly causing the surface crust to be thinner, homogeneous and without irregularities, which gives a perceptible difference in mouth feel. The visual observations of the crust also showed that air-fried samples expanded to a greater extent and contained regular pore distribution in core region in contrast to deep fat fried samples. During cooling too, the air-fried samples showed crust shrinkage, which was not observed in the deep fat fried product. Higher crust shrinkage during cooling is indeed a feature of air fried product, which does not seem to happen to the same extent in the case of deep fat fried product. This is most probably because crust cooling of air fried product occurs with concomitant steam condensation that leaves voids in the crust causing it to collapse. In contrast, the presence of oil in the crust of deep fried products minimizes crust collapse.

As far as the core is concerned, both products showed gelatinized appearance, although the extent of gelatinization was higher in the deep fat fried product.

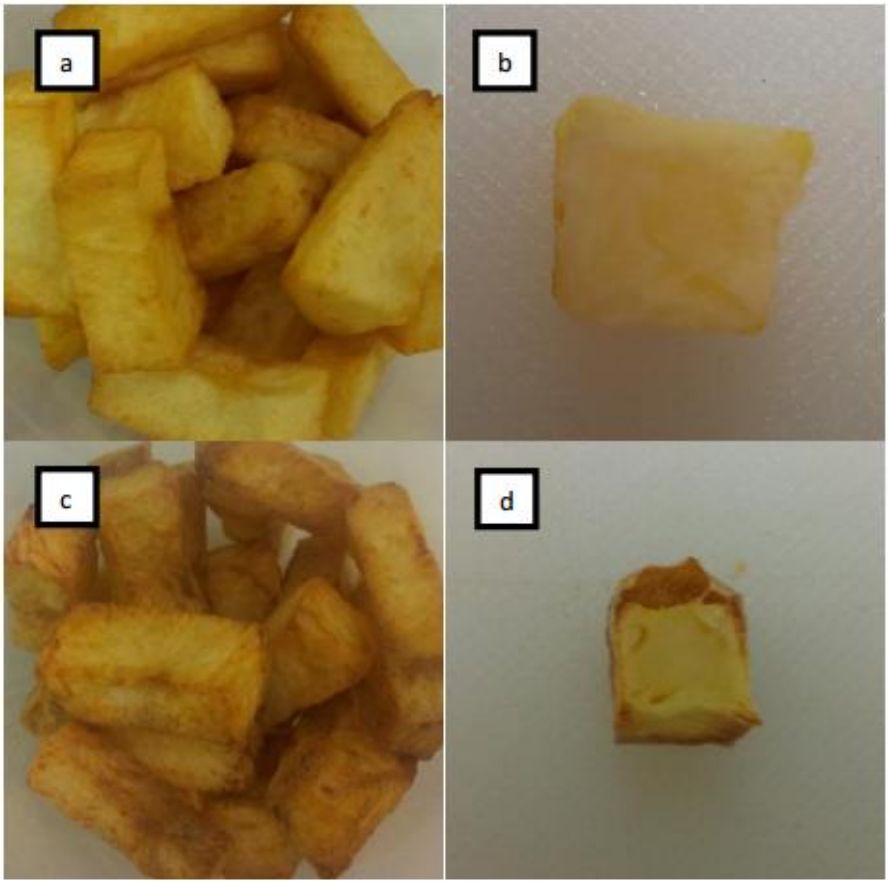


Figure 22. Picture of French fries samples: deep fat samples for 9 minutes (a-b) and air for 21 minutes (c-d).

4.4.4. CONCLUSION

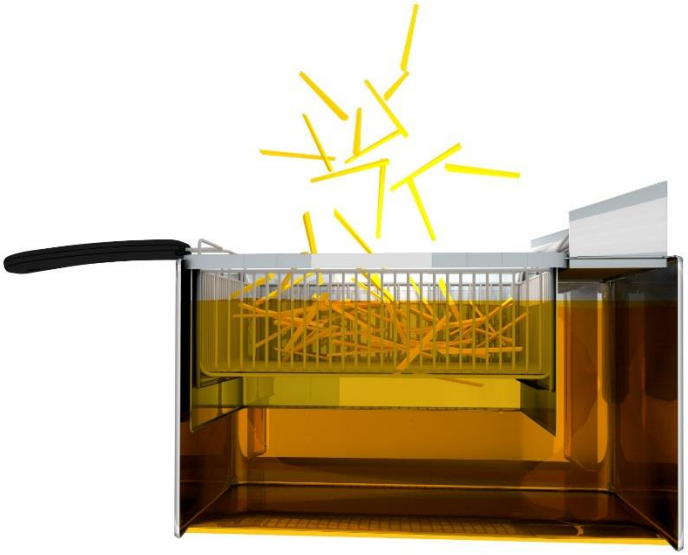
The present study shows that the oil content of French fries having similar moisture content and color was significantly lower when the product is air fried: the values were 5.63 g oil/100 g defatted dry matter for deep-oil frying and 1.12 g oil/100 g defatted dry matter for air frying. On the other hand, the evolution of temperature, moisture content,

and color were significantly slower in the case of air frying than deep fat frying. As a consequence, longer cooking times are required in the case of air frying.

The final product evaluation by SEM and DSC analyses showed that air fried samples had a lower degree of gelatinization than deep fat fried samples, which may explain the differences found between texture and sensorial characteristics of the two products.

Overall, air frying process permits the manufacture of lower fat content products, though these products have different sensory characteristics.

5. CONCLUSIONS



1. The characterization of three rosemary extract (*Rosmarinus officinalis*) obtained through different liquid-solid extraction process, has allowed to demonstrate the effect of solvent and format on phenolic compounds and antioxidant capacity of rosemary extracts. The highest antioxidant potential is for powder acetone extract followed by liquid methanol and liquid acetone.

2. The extracts containing more phenolic compounds, in particular carnosic acid and carnosol, prevent better the formation of reactive radical species and produce a higher antioxidant effect.

3. The addition of rosemary extracts (powder-acetone, liquid-methanol and liquid-acetone) in chicken nuggets in maximum doses established did not alter on the physical-chemical characteristics and sensory quality of the product, so rosemary can be used as alternative in pre-fried production. In addition, the extracts showed a trend to oxidative protective along frozen storage.

4. The addition of rosemary extract in frying oil at the maximum dose established improved the oxidative stability and diminished the generation of polar compounds in sunflower oil without modifying the fried product organoleptic characteristics. The extract has not protective effect on the viscosity and color.

5. The main compounds with antioxidative activity of rosemary extract, carnosic acid and carnosol, are degraded along frying time.

6. The process of frying under vacuum provided products with similar compositional and sensorial attributes to those of chicken nuggets fried in atmospheric conditions. In addition, consumers determined as more crunchy the nuggets cooked under vacuum conditions.

7. Temperature evolution in vacuum treatment products is initially faster than atmospheric treatment producing an initial faster moisture loss and an additional fall in luminosity. After the first minutes the temperature for all treatment became constant around boiling point, about 80 ºC in nuggets fried under vacuum and 100 ºC in nuggets fried at atmospheric pressure.

8. The application of vacuum at 130, 140, and 150 ºC allows the development of Maillard reactions though the evolution is slower than in atmospheric frying (165 ºC) because non-enzymatic browning is highly temperature-dependent.

9. The vacuum-frying treatment not produces nuggets with a lower fat content than atmospheric conditions.

10. In air frying technology the rate of heat transferred is lower than in deep fat frying due different fluid thermal conductivities, resulting in a slower evolution of temperature, moisture loss, texture and color.

11. The application of air frying technology reduces significantly oil content in French fries.

12. The sensorial analysis (QDA), SEM and DSC indicates that structure of French fries is influenced by type of process. The differences in the temperature and moisture kinetics result in different starch gelatinization degree and distribution.

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