SIMONE MONTEIRO E SILVA

On The Physical Refining of Edible Oils for Obtaining High Quality Products

Investigação sobre o Refino Físico de Óleos Vegetais para Obtenção de Produtos de Alta Qualidade

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University of Campinas
Faculty of Food Engineering

Ghent University
Faculty of Bioscience Engineering

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On The Physical Refining of Edible Oils for Obtaining High Quality Products

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"How strangely do we diminish a thing as soon as we try to express it in words."

(Maurice Maeterlinck)

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"The mind loves the unknown. It loves images whose meaning is unknown, since the meaning of the mind itself is unknown."

"A mente ama o desconhecido. Ela ama imagens cujo significado é desconhecido. A mente por si própria é desconhecida."

(René Magritte, Belgian artist, artista belga)

SUMMARY

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ABSTRACT

Vegetable oils are important compounds of the human diet and they should be refined before consumption. Consumers demand for healthier products as well as stiff environmental legislation are forcing refining industries towards changes and improvement of processes. In this context, this thesis has as main objective to investigate/improve the physical refining of vegetable oils, emphasizing the bleaching step. As first step, a HPLC methodology for simultaneous quantification of carotenes and tocols was developed and validated, and lately, it was used by our research groups. Then, bleaching step of palm oil, nowadays the most consumed oil in the world, was studied under different aspects: (1) determining kinetics, equilibrium and thermodynamic parameters of adsorptive removal of carotenes and phosphorus onto acid activated bleaching earth; (2) influence of different procedures on final color of palm oil; (3) influence of bleaching earth kind on final color of palm oil. These studies were important for a better understanding of bleaching process of palm oil, and some conclusions were obtained: adsorptive removal of carotenes and phosphorus onto acid activated bleaching earth occurs by chemisorption and it is endothermic; new procedures in the bleaching step can improve final color of palm oil when using the same amount of bleaching earth and deodorization time; a hypothesis was proposed to explain how the kind of bleaching earth can interfere in the final color of palm oil. Further studies are still necessary to optimize bleaching step and the new procedures suggested. Later, physical deacidification was studied by computer simulation and experimental data from literature. It was compared two mathematical approaches: differential and flash distillations. This last one presented better results regarding acidity and neutral oil loss profiles. In this approach, it was considered the heat transfer equations. In this way, this thesis presents an advance in refining process towards high quality products and less consumption of inputs.

RESUMO

Os óleos vegetais são importantes componentes da dieta humana e devem ser refinados antes do consumo. A demanda dos consumidores por produtos mais saudáveis, assim como regulamentações ambientais cada vez mais rígidas têm forçado os processadores de óleos vegetais a buscarem mudanças e aperfeiçoamento dos processos. Neste contexto, este trabalho de tese tem como objetivo investigar e aperfeiçoar o refino físico de óleos vegetais, com ênfase na etapa de branqueamento. Numa primeira etapa, uma metodologia para quantificar simultaneamente carotenos e tocoferóis foi desenvolvida e validada, e posteriormente, utilizada pelo grupo de pesquisa. Então, a etapa de branqueamento de óleo de palma, atualmente o óleo mais consumido mundialmente, foi estudada sob diferentes aspectos: (1) determinação da cinética, equilíbrio e parâmetros termodinâmicos do processo de adsorção de carotenos e fósforo em terra clarificante acidamente ativada; (2) influência de diferentes procedimentos na cor do óleo de palma refinado; (3) influência do tipo de terra clarificante na cor do óleo de palma refinado. Estes estudos foram importantes para um melhor entendimento do processo de branqueamento de óleo de palma, e algumas conclusões foram obtidas: a adsorção de carotenos e fósforos pela terra clarificante acidamente ativada ocorre por via química, e é um processo endotérmico; a utilização de novos procedimentos na etapa de branqueamento do óleo de palma pode melhorar a coloração obtida ao final do processo; o refino em duas etapas utilizando duas desodorizações em condições moderadas de temperatura apresentou melhor coloração final quando comparado ao processo utilizando a mesma quantidade de terra clarificante e tempo de desodorização; uma hipótese foi sugerida para explicar como o tipo de terra clarificante (neutra ou acidamente ativada) pode interferir na coloração final do óleo de palma. Ainda são necessários mais estudos para otimizar a etapa de branqueamento e os novos procedimentos sugeridos. Por último, a etapa de desacidificação por via física foi estudada através de simulação computacional e dados experimentais disponíveis na literatura. Foram comparadas duas abordagens matemáticas: a diferencial e a flash. Esta última apresentou resultados mais realísticos quanto aos perfis de acidez e perda de óleo neutro. Foi ainda considerada na abordagem flash equações de transferência de calor. Dessa forma, pode-se concluir que este trabalho de tese apresentou avanços nos processos de refino físico de óleos vegetais para obtenção de produtos de maior qualidade final e menor consumo de insumos.

Chapter 1. Introduction

Vegetable oils are important compounds of the human diet, providing energy, essential fatty acids and liposoluble vitamins. Additionally, they are responsible for the flavor and texture of foods as well as promote satiety. They are predominantly formed by triesters of glycerol and fatty acids, known as triacylglycerides, and continue to show, at lower levels, other constituents such as free fatty acids, partial acylglycerides, sterols, tocopherols, hydrocarbons, pigments, vitamins, heavy metals, glycolipids, fragments of proteins, resins, and mucilages. Some of these compounds affect the quality of the oil and should be removed during the refining process, which can be chemical (adding sodium hydroxide) or physical (steam stripping). The refining process is of vital importance for the quality of the final product (odor, flavor, color), its function (fatty acids composition, vitamins and antioxidants) and cost (neutral oil loss).

Chemical refining consists of removing free fatty acids by adding alkali and separating the soap by centrifugation (sludge). Differently, physical refining removes free fatty acids by steam stripping. Choosing the best process depends on the individual characteristics of each oil as well as economic and environmental issues. Low-quality oils should preferably be refined by the chemical process, since adding alkali is more efficient in removing undesirable compounds. For high-quality oils, physical refining should be chosen as it provides higher yield, use less chemical reagents and water, causing a lower environmental impact.

Bleaching is the first step of the physical refining process whereby phospholipids, pigments, contaminants, soaps and peroxidation products are removed from the oil. It is the most expensive step in edible oils refining due to the large amounts of bleaching earth spent. Moreover, stiff environmental laws are forcing refining industries to minimize the amount of solid waste produced, as they are difficult to treat.

After bleaching, the oil is ready for deacidification which involves extreme processing conditions, i.e. high temperatures and low pressure, as well as steam

injection. High temperature can lead to the formation of undesirable compounds, for instance, *cis-trans* isomerization of unsaturated fatty acids. The deacidification step can increase the *trans* fatty acid content from 0.3 % (crude oil) to 5 % (refined oil). This reaction must be avoided as high *trans* diets are related to the development of cancer, heart diseases and stunts in child growth.

Such extreme conditions also promote removal/degradation of desirable compounds, such as tocopherol, tocotrienols and carotenes (provitamin A). The main factors affecting the loss of tocopherols are those that directly influence its volatility, i.e., the temperature of deodorization, temperature and pressure and the amount of steam injected. In the case of carotenes, thermal degradation is the most important. Therefore, it is notable that despite the refining processes already being consolidated in the edible oil industry, there is room for improvement towards environment-friendly practices and healthier products. In fact, the various perceptions of what is desirable in oil-based products can incite changes in the technology used. Thus, the refining process parameters (temperature, pressure, amount of carrier agent) should be adjusted to maintain the original quality of vegetable oils in their crude state.

In this context, this thesis investigated experimentally or by simulation the improvement of processes involved in edible oils refining, including bleaching and deodorization steps. A new methodology for simultaneous quantification of carotenoids and tocopherols was developed and validated, and subsequently, it was applied by our research group to control the loss/degradation of minor compounds during refining. The bleaching step was studied using crude palm oil - the highest consumed oil in the world, currently. The deacidification by physical refining was investigated by computer simulation using experimental data present in scientific literature. This thesis was conducted under a joint supervision of Dr. Antonio José de Almeida Meirelles, Professor in the Faculty of Food Engineering of the University of Campinas and Prof. Christian Stevens, Professor in the Faculty of Bioscience Engineering of the University of Ghent according to the agreement signed by both Universities. Moreover, this thesis counted with the collaboration of

Dr. Wim De Greyt who kindly made the facilities of the R&D Center of Desmet Ballestra Company available for the experiments on adsorption and bleaching of vegetable oils.

The present thesis is organized in the following chapters:

Chapter 1 (Introduction) presents a brief description of the problems in the edible oil industries and the main objectives of this thesis work.

Chapter 2 (Literature Review) contextualizes this thesis in relation to other works already published on the same topics studied.

Chapter 3 presents the scientific article titled "*Validation of a method for simultaneous quantification of β-Carotene and Tocopherols in vegetable oils by HPLC*", published in *Food Chemistry*. This work validates a new HPLC methodology used to simultaneously quantify three classes of compounds: β-carotene, tocopherols and tocotrienols. The compounds were separated on a normal phase column (Lichrospher, Merck), using a mobile phase gradient consisting of hexane and isopropanol and a flow gradient ranging from 1 to 2 mL/min^{-1}. A diode array detector set at 292 nm (tocols) and 455 nm (β-carotene) and a fluorescence detector set at 290 nm excitation and 330 nm emission were used. A linear response was achieved over the concentration range 2.5 - 37.5 mg/L^{-1} for the tocols and over 0.05-10 $mg \cdot L^{-1}$ for the β-carotene. The method has been applied to the quantification of these compounds in Amazon oils. This methodology was used to study the thermal degradation of these compounds in oil subjected to similar temperatures as those usually employed in steam stripping ("Thermal degradation Kinetics of Carotenoids in Palm Oil", *Journal of American Oil Chemists' Society*, DOI: 10.1007/s11746-012-2156-1).

Chapter 4 presents the article, "*Adsorption of Carotenes and Phosphorus from Palm Oil by Acid Activated Bleaching Earth: Equilibrium, kinetics and thermodynamics*", submitted to *Journal of Food Engineering*. In this study, the adsorption of carotenes and phosphorus from crude palm oil by acid activated bleaching earth was investigated under bleaching conditions, i.e. high temperature and low pressure. Kinetic models and isotherms were adjusted to experimental

data. Furthermore, thermodynamic parameters of adsorption as Gibbs free energy, enthalpy and entropy were calculated, demonstrating that the process is spontaneous, endothermic and entropy driven. It could be observed that increasing the bleaching temperature leads to an increase of the adsorption efficience. However, there are other factors which should be considered when establishing optimal bleaching temperature to obtain a light, fully-refined, palm oil.

As a result, **Chapter 5** presents the article, "*Influence of Refining Practices on Palm Oil Color*", to be submitted to the *European Journal of Lipid Science and Technology*. This article studied the effects of different refining procedures on refined palm oil, especially in regards to color. The effects of storing, addition of a maturation step with citric acid, addition of an extra-dry step, multi-stage bleaching and a new approach using two refining procedures (two bleaching and two mild deodorizations) were studied. This new approach proved to be promising with an improvement in the final color of the palm oil compared to that obtained using the same amount of bleaching earth and deodorization time, but refined by the traditional approaches. More studies about process parameters and the kind of bleaching earth are still necessary in order to optimize two-step refining.

Chapter 6 presents the article "*Effect of Type of Bleaching Earth on Final Color of Refined Palm Oil*", to be submitted to *Food Chemistry*. In this paper, the effect of the kind of bleaching earth on the final color of palm oil was studied. An inverse correlation was found between p-anisidine value after bleaching and residual color after deodorization using acid activated bleaching earth, but not with the neutral earth. Moreover, heat bleaching was more efficient in oils refined with acid activated earth. Those results indicate that oxidation products are important to predict the final color of refined palm oil. In addition, a hypothesis was suggested to explain how the type of bleaching earth can define β-carotene oxidation pathway.

After completing the bleaching steps, edible oil should be deodorized to remove free fatty acids. Computer simulation emerges as an alternative for the better understanding of the deodorization process. **Chapter 7** discusses the article, *"Comparison between Differential and Flash Distillation for Simulating the*

Deodorization Process of Vegetable Oils" (originally in Portuguese), published and orally presented at the *XVIII Brazilian Congress on Chemical Engineering, COBEQ*. This work was important for understanding deodorization process by two different approaches. It was observed that the results obtained by differential distillation equation presented a linear trend for free fatty acid removal. However, experimental results have shown fatty acids removal presents an exponential behavior, like the one obtained by flash distillation equations. It was concluded that flash distillation provides more realistic results and it was chosen for further studies on computational simulation of deacidification of vegetable oils.

Chapter 8 presents the article "*Simulation of Batch Steam Deacidification of Coconut Oil*", published in the *Proceedings of 11th International Congress on Engineering and Food, ICEF*. In this work, the flash distillation approach was compared with experimental data of the deacidification of coconut oil presented in the literature. This approach presented good results in relation to the final oil acidity. Vaporization rate was not an input, and varied with time, according to the energy balance. The oil acidity showed an exponential decrease and the temperature presented small variation along the stripping period. These results are more realistic than those found in the literature on simulation of batch steam deacidification which considers the vaporization rate constant with time and so a linear acidity decrease. Simulation works presented in Chapter 8 and 9 of this thesis are necessary basis for better understanding and studying of vegetable oil deacidification under computer simulation. Such works should be improved in order to consider degradation/removal of desirable compounds and undesirable reactions. The necessary data for this study were simultaneously determined by the research group through hydrolysis studies which determined the fatty acids and thermal degradation of carotenoids in the deodorization process conditions, i.e. low temperature and high pressure.

Chapter 9 (Conclusions) presents a summary of the results obtained in this thesis.

1.1 Objectives

1.1.1 Overall Objective

- To investigate and improve the physical refining of edible oils, with emphasis on the bleaching step.

1.1.2 Specific Objectives

- To develop and validate an analytical method for the simultaneous quantification of carotenoids and tocols;
- To study kinetics, equilibrium and thermodynamic parameters of adsorption of carotenoids and phosphorus from palm oil onto bleaching earth;
- To evaluate the effect of different bleaching procedures on the quality of refined palm oil;
- To evaluate the effect of the type of bleaching earth on the color of fully refined palm oil;
- To improve and validate simulation of batch deacidification of vegetable oils, using flash distillation equations.

Chapter 2. Literature Review

2.1 Vegetable oils

Vegetable oils are important ingredients in numerous industrial products, such as margarine, salad oils, mayonnaise, bread as well as home made products (O'BRIEN, 1998). Vegetable oils are popular due to their ability to provide lubricity, texture, and flavor to products. Furthermore, it provides an effective means of transferring heat by immersion for frying (STANTON, 1996).

Fats and oils present compounds which can be divided into two major groups: glycerides and non-glycerides. Glycerides are the most important part of fats and oils, consisting of glyceryl esters of fatty acids (triacylglycerides, diacylglycerides and monoacylglycerides). Edible vegetable oils are composed mainly by triacylglycerols (more than 95 %) (MORETTO; FETT, 1998). All triacylglycerides have the same glycerin unit, so it is the fatty acids that contribute for their different properties. Usually, vegetable oils are liquid at room temperature, due to the presence of unsaturated fatty acids (WATKINS et al., 1996). The non-glyceridic parts are formed by minor compounds such as phosphatydes, vitamins and hydrocarbons (MORETTO; FETT, 1998).

In fact, physicochemical properties of vegetable oils are functions of the composition of their glyceridic part and depend on the fatty acids composition and their position in triacilglycerol molecule, i.e. if they are esterified to carbon 1, 2 or 3 of the glycerol molecule. Fatty acids differ due to chain length, number and position of double bonds. Meanwhile, triacylglycerols differ in the type of fatty acid and the position in which they were esterified. Variations in these characteristics are responsible for the chemical and physical differences shown by edible fats and oils (O'BRIEN, 1998).

Therefore, vegetable oils are classified according to the composition in terms of major fatty acids, as is shown in Table 2.1. The main three fatty acids are

palmitic, oleic and linoleic, and sometimes accompanied by stearic acid and by linolenic acid (GUNSTONE, 2005).

Table 2.1. Vegetable oils by fatty acid type (GUNSTONE, 2005)

Fatty acids	Vegetable oils
Lauric	Coconut and palm kernel
Palmitic	Palm oil and cottonseed
Oleic/Linoleic	Sunflower, sesame, cottonseed, canola, soybean
High oleic	Olive, sunflower, canola, soybean
Linoleic	Flax seed, canola, soybean

Currently, demand for oils rich in unsaturated fatty acids has increased, instead of saturated fats. Even though it is convenient to classify oils by their fatty acid composition, this is not the only index of their nutritional value or of their oxidative stability. Attention must also be given to the minor components in the crude oil and to what remains after refining (GUNSTONE, 2005).

Among desirable compounds with nutraceutical value, essential fatty acids and liposoluble vitamins, such as A, D, E and K, have remarkable importance (KITTS, 1996). Inevitably, the extraction step promotes the formation and/or removes undesirable compounds such as free fatty acids, hydrocarbons, heavy metals, glycolipids, fragments of proteins, resins, and mucilages from the oilseed. The refining steps are designed to remove these compounds, with the least possible damage to triacylglycerides and loss of nutritional compounds (DE GREYT; KELLENS, 2005).

2.1.1 Fatty acid composition

In this topic some features of triacylglycerols is discussed based on their fatty acid composition, but it should be kept in mind that in this case the fatty acids are those linked to the glycerol by ester bonds, not the free fatty acids.

Fatty acids are chain aliphatic carboxylic acids, formed basically by a non-polar chain with a hydrophilic polar group in the end. The characteristics of this chain are responsible for different properties of fatty acids and their derivatives.

Saturated fatty acids have a straight hydrocarbon chain. One *trans* double bond can change the chain shape slightly, whilst a *cis* double bond introduces a pronounced bend in the chain (Figure 2.1), modifying the physical properties of the fatty acid (SCRIMGEOUR, 2005).

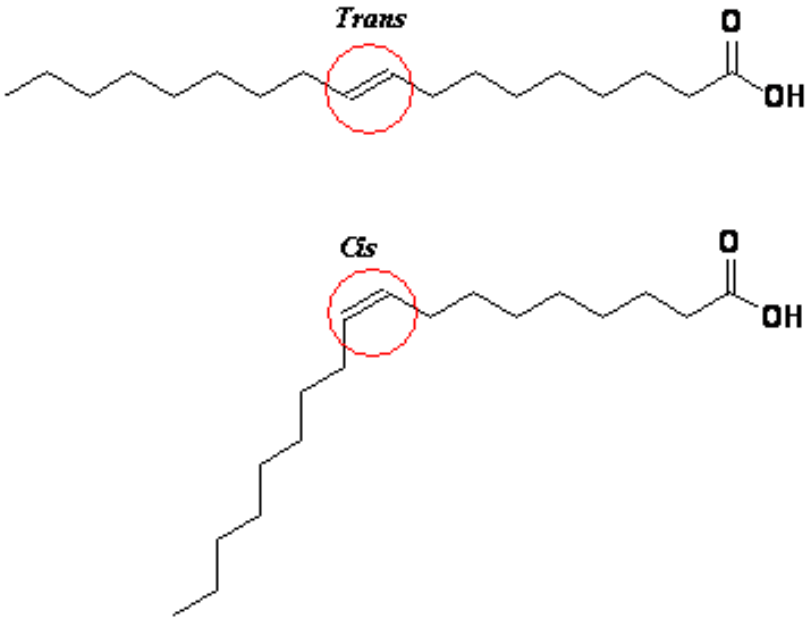

Figure 2.1. *Trans* and *cis* fatty acids chemical structure

Trans fatty acids can be mono or poly unsaturated, and contain one or more double bonds in this configuration. Their shape is similar to that of a saturated fatty acid, thus, their melting point is much higher than a *cis* isomer (DE GREYT; KELLENS, 2005). Several studies demonstrate that high *trans* diets are related to development of cancer and heart diseases (KITTS, 1996).

The most abundant fatty acids have from 4 to 22 carbons, the most common of those having 16 and 18 carbons: the saturated, palmitic (C16:0) and stearic (C18:0) acids; and unsaturated, oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids (SCRIMGEOUR, 2005).

In vegetable oils, most fatty acids are esterified into a glycerol molecule (triglyceride). Large amounts of non-esterified fatty acids (free fatty acids) indicate that the lipid was damaged permanently (CHRISTIE, 2003).

2.1.2 Minor Components

2.1.2.1 Free Fatty Acids (FFA)

Free fatty acids (FFA) virtually do not exist *in vivo*, however, they can be released by enzymatic action after the tissue death or harvest, when enzymatic deactivation does not occur (ARAÚJO, 2004). Fatty acids containing 14 - 22 carbons are sensorially inactive, but those containing 4 - 10 provide typical off-flavor in foods or act as precursors of other compounds which have active flavor. Moreover, the presence of free fatty acids lead to a fast oxidation (FRANKEL, 2005) and reduce the smoke point of oil (ARAÚJO, 2004).

Because they are more volatile than triacylglycerides and partial acylglycerides (mono-and di-acylglycerides), it is possible to remove the free fatty acids by physical separation using high temperatures (up to 260 °C) and low absolute pressure (up to 5 mbar).

2.1.2.2 Carotenes

Carotenes are pigments which are synthetized by vegetables, being precursors of vitamin A. They can be yellow, red or purple. They are basically tetraterpens, synthesized from eight isoprens. Carotenes are stable in their natural environment, but thermolabile if extracted or heated (NAWARR, 1996). Carotenes are minor components in several vegetable oils, including palm oil (GUNSTONE, 2005) and buriti oil (ALBUQUERQUE et al., 2005; FRANÇA et al., 1999; MARIATH et al., 1989; SILVA et al., 2009). During refining steps, enormous amounts of carotenes are lost due to adsorption (TAYLOR, 2005) and thermal degradation during deacidification step (SAMPAIO et al., 2012). According to Mayamol et al. (2007) the amount of β-carotene destroyed in the refining process would be enough to meet the world's Vitamin A requirements.

Some alternatives to traditional processing have been developed to retain or recovery carotenes lost during refining (GUNSTONE, 2005).

Vitamin A is essentially half of a β-carotene (Figure 2.2), with a water molecule added in its lateral chain. Therefore, β-carotene is a powerful pro-vitamin A, with 100 % activity (RODRIGUEZ-AMAYA, 1996). A study has shown that the ingestion of 50 000 IU of vitamin A by a newborn can reduce mortality by 15 % or more in developing countries (KLEMM et al., 2008).

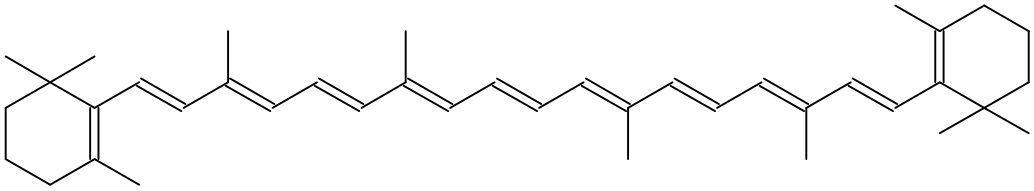

Figure 2.2. β-carotene chemical structure

2.1.2.3 Tocopherols and Tocotrienols

The tocopherols are found in the unsaponifiable part of vegetable oils. They consist of α-, β-, δ- and γ-tocopherols, with varied antioxidant activity (KITTS, 1996). The most important antioxidants are those with a phenolic structure. The composition is specific for each vegetable oil, and sometimes can be used for identification. Besides antioxidant activity, tocopherols also exhibit Vitamin E activity, especially the α-tocopherol (DE GREYT; KELLENS, 2005).

Tocotrienols are known to have potent properties that protect the nervous system, decrease serum cholesterol and aid cancer prevention. However, such properties are not usually attributed to tocopherols (SEN et al., 2004).

Figure 2.3 represents the structure of both tocopherols and tocotrienols.

Studies show that the volatilization and thermal degradation of tocopherols during both deodorization and deacidification steps are responsible for about two thirds of the total losses of these compounds (GOGOLEWSKI et al., 2000).

The losses during the refining process as a whole may reach values of up to 25 % of total tocopherols and up to 70 % of the γ-tocopherols present in the oil of sunflower seed (GOGOLEWSKI et al., 2000), and up to 25 % for canola oil (ALPASLAN et al., 2001). The main factors affecting the loss of tocopherols are

those that directly influence its volatility, i.e. the deodorization temperature, the vacuum intensity and the amount of steam injected (DE GREYT; KELLENS, 2005).

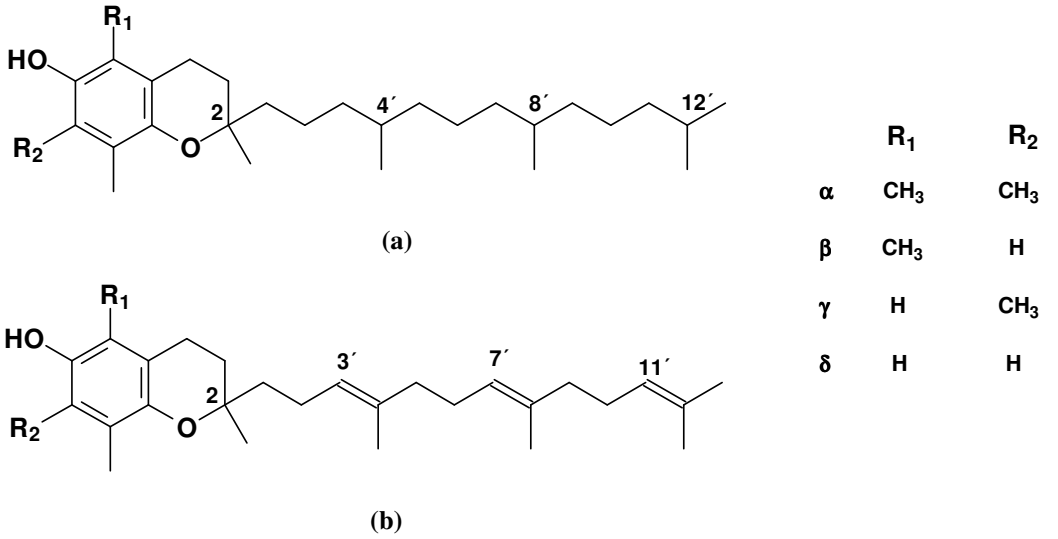

Figure 2.3. Chemical Structure of Tocopherols (a) and Tocotrienols (b).

2.1.3 Palm Oil

In the last years, palm oil has occupied a prominent position in the world production of fats and oils. According to the Food and Agriculture Organization of the United Nations, the production of palm oil in Brazil was 265 thousand tons in 2009, being only the 10th producer in the world (FAO, 2011). However, Brazil is the country with the highest potential to produce this oil due to its 75 million hectares available for cultivating palm trees (UNEP, 2011).

Palm oil is obtained from the mesocarp fruit of the *Elaeis guineensis* palm. The fruit consists of a seed (endosperm) which lies inside a shell and is covered with a fleshy mesocarp. The mesocarp is comprised of approximately 49 % oil. The palm kernel oil is obtained from the seed, or nut (Figure 2.4) (LAI, 2005).

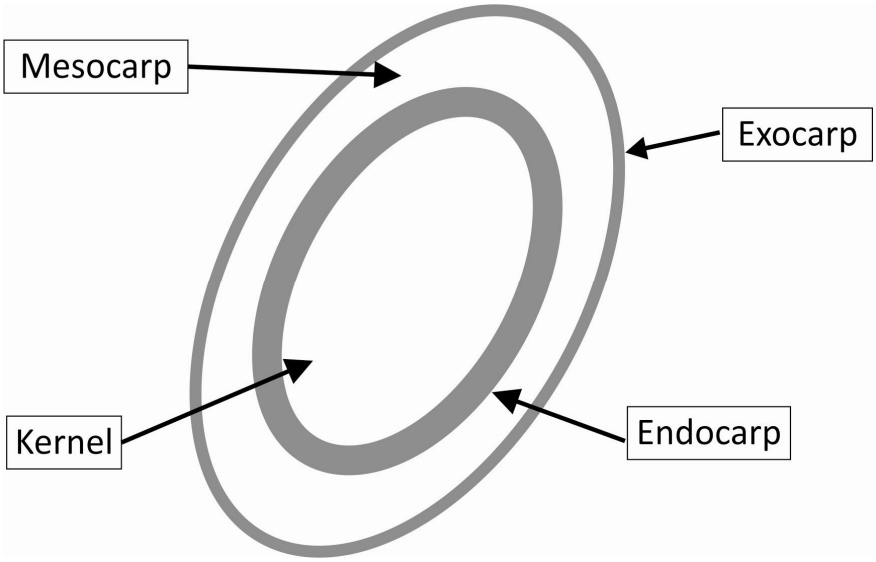

Figure 2.4. The palm fruit (POKU, 2002)

Palm oil contains 42 - 47 % palmitic acid (C16: 0) and 37 – 41 % oleic acid (C18: 1), while palm kernel oil is comprised of approximately 50 % lauric acid, thus being more saturated than palm oil (BASIRON, 2005; LAI, 2005).

The minor compounds of palm oil such as tocopherols, tocotrienols, carotenoids, phosphatides, sterols, triterpene alcohols and aliphatic constitute less than 1 % of the oil. Some of these compounds such as tocopherols, tocotrienols and carotenoids are nutritionally benefic (LAI, 2005). Apart from the nutritional aspect, these compounds also enhance the oxidative stability of the oil (QUIJANO, 1999).

According to Lai (2005), palm oil contains 350 - 450 mg/kg vitamin E in the form of tocopherol (30 %) and tocotrienol (70 %). It is the only edible oil that can be consumed in sufficient quantities in order to provide tocotrienols. According to Gibon et al. (2007), the tocopherol and tocotrienol content in palm oil can range from 600 to 1000 ppm, with the tocopherol:tocotrienol ratio approximately equal to 20 %.

As it is an oil derived from fruit, palm oil is produced by cooking, pressing and clarifying. The quality of the crude oil will affect the performance and efficiency

of the refining stage as well as the quality of the product after the end of its processing (GIBON et al., 2007).

Palm oil may contain up to 700 mg/kg of carotenoids, according to Lai (2005). According to Gibon et al. (2007), the carotenoid content in palm oil may vary from 500 to 2000 mg/kg. The carotenoids from palm oil are constituted mainly by α- and β-isomers (approximately 90 % of the total). Most carotenoids are destroyed during the refining steps, producing a light-colored oil when refined (GIBON et al., 2007).

Furthermore, palm oil may contain phospholipids in highly varied amounts (values are reported between 5 and 30 mg/kg) (GEE, 2007). Palm oils with low phosphatide content, high initial acidity and high carotene content should preferably be refined by means of the physical refining.

2.2 Processing Vegetable Oils

Crude oils and fats extracted from seeds and pulp oil may contain high levels of impurities and extremely unpleasant odors. Only some oils can be consumed without going through purification steps to remove gums, free fatty acids, metals and other impurities. These steps can also remove color, odor, and change the crystallization trend, making it adaptable for the means of its use (O'BRIEN, 2008).

Both the extraction and processing of vegetable oils involve a series of steps in which chemical and physical changes occur in the crude material. Choosing the processing technique depends on a number of factors, such as the quality and quantity of the raw material processed daily (O'BRIEN, 2008). The role of each of these steps will be briefly discussed.

2.2.1 Extraction

Vegetable oils and/or fats can be extracted from the oil seeds or fruit pulp by mechanical extraction or solvent extraction. In mechanical extraction, the oleaginous material undergoes a process that uses high temperature and high

pressure, forcing the oil out of the cells. This oil is usually of higher quality than those extracted with solvents, since mechanical extraction does not remove certain compounds such as phospholipids, which are considered harmful during the other processing stages. In fact, some vegetable oils extracted by pressing, like olive oil and evening primrose oil, require no additional processing, and can be directly used for consumption. The solid portion remaining from the pressing stage can still continue to a solvent extraction step (usually hexane) in a combined process which increases the product yield, or may even be destined for animal feed production (ANDERSON, 2005).

In solvent extraction, the oil from the oleaginous material is leached with a solvent, usually hexane. Elevated temperatures reduce the oil viscosity and increase diffusion; however, in the case of using hexane as solvent the temperatures should be limited up to 50 °C because of its high volatility (vapor pressure). The oil and the solvent are distilled until the oil is completely solvent-free, and the solvent can be recovered for next extraction (O'BRIEN, 1998).

Although the hexane solvent is currently considered the most efficient, there is concern about its flammability and environmental impacts. Because of this, other solvents that perform well and show improved safety are being studied; such as ethanol, for example (ANDERSON, 2005).

In the case of vegetable oils that are rich in phospholipids (soybean, corn and sunflower oils, for example), the next step is degumming, which must be performed prior to refining to avoid a dark color in the final product. In this process, crude oil is degummed by adding water or phosphoric acid to separate hydratable and non-hydratable phospholipids by precipitation (SOUZA, 2004).

2.2.2 Physical Refining

After extraction, vegetable oils proceed to the refining steps, which are either chemical or physical (Figure 2.5). Chemical refining consists of removing free fatty acids by adding alkali and separating the soap by centrifugation (sludge) (O'BRIEN, 1998). Physical refining removes free fatty acids and other compounds

by stripping (O'BRIEN, 1998). Choosing the best process depends on the individual characteristics of each oil. The palm, palm kernel and coconut oils, which have low levels of phospholipids, are almost always physically refined. The oil from seeds such as canola, sunflower and corn, can be refined both ways, and the choice depends on economic and environmental issues, such as managing soap and water waste generated by chemical refining. To obtain a good quality oil via physical refining, the phosphorus content should be below 5 mg/kg before the process (ANDERSON, 2005). According to O'Brien (1998), the phosphorus content should be below 10 mg/kg.

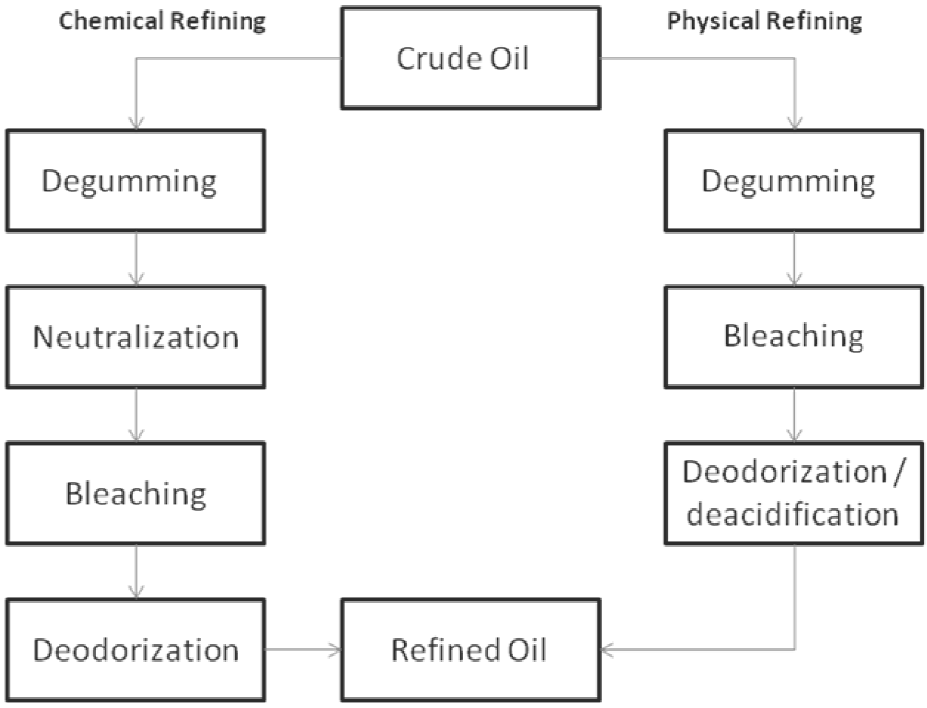

Figure 2.5. General overview of physical and chemical refining lines (DE GREYT; KELLENS, 2000)

The physical refining method of vegetable oils is an alternative to the traditional method which uses caustic substances to remove free fatty acids. The traditional process of refining oils with high phospholipid content should be avoided because it generates large amounts of soap and large losses of neutral oil.

Physical refining removes free fatty acids and other compounds by stripping with the aid of a carrier gas, high temperatures and very low pressure (close to the absolute vacuum). However, it is still necessary to pre-treat oil by degumming and bleaching, which aims to remove impurities that cause a change in color, and other quality losses when heating the oil (O'BRIEN, 2008). This work aims to study and improve the physical refining of vegetable oils, especially palm oils. Details of the sequence of steps used in physical refining are discussed as follows.

2.2.3 Degumming

The main objective of the degumming step is to remove phospholipids, which are oil-soluble substances present in most vegetable sources. Phosphatides are formed by one or two fatty acid chains and a phosphate ester (Figure 2.6). Many of these phosphatides become oil-insoluble when hydrated, forming *gums*, the term used in the industry to refer to such substances (XU; DIOSADY, 2004).

The phospholipid content in the oil varies according to the species and oilseed and sometimes even varies among seeds of the same species. Table 2.2 shows typical values of the amount of phospholipids present in different vegetable oils (XU; DIOSADY, 2004).

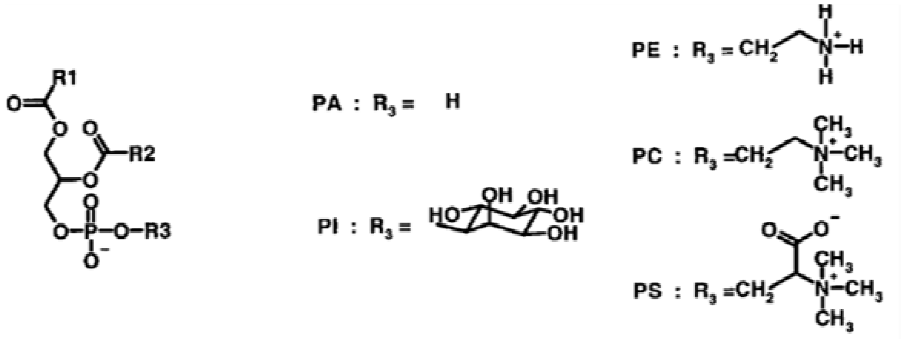

Figure 2.6. Structure of common phospholipids: PA, phosphatidic acid; PI, phosphatidyl inositol; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; PS phosphatidyl serine OS (WÜRTZ CHRISTENSEN; PEARCE, 2004)

Phospholipids should be removed, since they can generate a lot of problems, such as the formation of flocculent particles due to absorption of water

present in the air, as well as emulsion formation during chemical refining, causing a great loss in neutral oil. Some phospholipids have a pro-oxidant action, which contributes to an occurrence of off-flavor and the formation of unwanted color during physical deacidification.

Table 2.2. Phospholipid content in vegetable oils (XU; DIOSADY, 2004)

Oil	Phospholipids content (%)
Soybean	Up to 3.2 %
Canola	0.1
Cottonseed	1.5 – 1.8
Corn	0.04 (pressed); 0.5 (solvent)
Sunflower	Up to 1.5
Flaxseed	0.3

Phospholipids can be classified into hydratable and non-hydratable (NHP - non-hydratable phospholipids) according to their affinity for water. When phosphatides are complexed with metal ions such as Ca^{+2} and Mg^{+2}, its hydratability is greatly reduced. The NHP content is also influenced by the quality of oilseed, affected by the growth and storage conditions. Phosphatil choline (PC) is the phospholipid with the highest hydration capacity, while phosphatidic acid (PA) has the smallest. PA is largely produced as a phospholipid degradation product with phospholipase D, endogenous enzyme of oilseeds. This enzyme is activated by high temperatures and humidity in the places where the oilseed is stored. If phospholipase D is activated, the NHP content will increase, hindering the degumming process (WÜRTZ CHRISTENSEN; PEARCE, 2004).

The two main techniques used for degumming in the industry today are the degumming with water or acid. When degumming with water, hydratable phospholipids are removed. A quantity of water ranging from 50 % to 100 % of the mass content of gums present is added to the oil. The water content must be enough so that all the hydratable phosphatides are hydrated and precipitate; yet it should not be in excess so as to avoid formation of three phases during the

process. This technique was developed for soybean oil and cannot be applied to oils from different oil sources (XU; DIOSADY, 2004) and generates a large oil loss (approximately 35 % of the lecithin stream generated during refining is oil) (DIJKSTRA, 2010).

When degumming with acid, concentrated phosphoric acid (0.02 % to 1 % by weight of oil) is added to hot oil (70 – 90 °C). The gums are removed by centrifugation and the oil proceeds to chemical refining, when it is necessary to add a sufficient amount of caustic substances in order to neutralize the phosphoric acid and residual free fatty acids. This technique has a few disadvantages: the high corrosiveness of phosphoric acid, which requires use of very expensive materials for constructing the equipment; high consumption of NaOH to neutralize the excess acid, and produces very dark lecithin (mixture obtained from the degumming oil soybean). Dark lecithin has no commercial value (XU; DIOSADY, 2004).

2.2.4 Bleaching

The function of bleaching is to prepare the oil for subsequent refining steps, and in the case of physical refining, preparing oil for physical deacidification. This step removes impurities such as pigments, soaps, gums, pro-oxidant metals and the products of decomposition of peroxides which are either dissolved or in colloidal suspension (O'BRIEN, 2008).

Bleaching is an adsorptive process involving the mass transfer of the adsorbate (solute) from solution onto the surface of the adsorbent. When the thermodynamic equilibrium between the solution and the adsorbent is reached, the mass transfer ceases. Equilibrium is governed by the temperature and pH of the system and the properties of the adsorbent and adsorbate. There are several mathematical models that describe the equilibrium (Langmuir, Brunauer-Emmett-Teller, and Freundlich) (NEUMAN; TURGUT DUNFORD, 2004). The vegetable oil industry uses mainly three types of adsorbents in the bleaching process: clay, activated carbon and silica (DIJKSTRA; SEGERS, 2007).

Bleaching generally occurs under vacuum pressure to reduce the quantity of oxidizing agents and moisture, and under elevated temperatures to decrease the oil's viscosity. The process takes 15 to 30 minutes under a temperature of 80 to 120 °C. Although the use of high temperatures improves the kinetics of adsorption, it can also lead to unwanted reactions (NEUMAN; TURGUT DUNFORD, 2004).

This step is the more expensive in oil refining due to large amounts of bleached land used. Moreover, strong environmental regulations are forcing refining industries to minimize the amount of solid waste generated, as it is very difficult to treat (GIBON et al., 2007). Therefore, one objective of this study will be to optimize the bleaching step of vegetable oils.

2.2.5 Physical deacidification

The deacidification, either physical or via deodorization, is the final step in refining of edible oils. During stripping, a carrier gas is mixed with the oil, facilitating the mass transfer of the impurities to the gas phase, which is withdrawn continuously so as to avoid the volatile impurities from condensing on the liquid (BALCHEN et al., 1999).

The amount of stripping reducing agent necessary is also an important parameter and that cost is greatly influenced by the size of deodorizer and the vacuum system. Almost all commercial applications use water vapor as a stripping agent due to its ability to condense under mild conditions, reducing the cost of the vacuum system (BALCHEN et al., 1999).

The optimum parameters for this refining step (temperature, retention time, operating pressure and amount of carrier gas) must be defined according to the initial oil, with the specifications of the final product, the limitations of the equipment, and the need to minimize costs. Fatty acids should be removed to quantities that range from 0.03 to 0.05 %, in accordance with standard industry practices (DE GREYT; KELLENS, 2005). Brazilian legislation and Codex Alimentarius specify a maximum of 0.3 % of acidity (expressed as oleic acid) (ANVISA, 2005; CODEX ALIMENTARIUS, 1981).

Deacidification by physical processes is performed with the same type of equipment used for deodorization. Therefore, this study will use the trade name "deodorizer" to refer to the type of equipment being investigated in this work. Since free fatty acids are relatively less volatile, removing them also efficiently removes other more volatile compounds such as odors (DE GREYT; KELLENS, 2005). Therefore, it can be said that the oil that was physically deacidified was also deodorized.

Deodorization or deacidification by physical processes can occur in different forms: continuous, semi-continuous or batch. The batch process is suitable for small-scale production, its major advantages being simple construction and low cost. However, the operation cost is high, has low capacity and it requires a relatively long process time. Although deacidification by physical processes in a batch way is less used in the industry, it does include the main phenomena that occur in the continuous equipment, in addition it is simpler to investigate in a lab scale. For this reason, it has been an object of study in several research works (DECAP et al., 2004; MANUELA PRIETO et al., 2008; PETRAUSKAITE et al., 2000; SAMPAIO et al., 2011).

In the batch process, the crude oil is slowly heated under vacuum until the deodorization temperature is reached when the carrier gas starts to be injected. When the final product specifications are achieved, the oil is cooled under vacuum and stocked (ANDERSON, 2005).

However, the use of high temperatures is also related to the loss of oil quality. In fact, besides increasing the loss of neutral oil, high temperatures favor the hydrolysis of triacylglycerols and the isomerization of unsaturated fatty compounds to their *trans* forms.

Neutral Oil Loss (NOL)

In addition to volatile components such as free fatty acids, oxidation products, tocopherols and sterols, the distillate originating from deodorizers may

also have a portion of tri-, di-and mono-acylglycerides (neutral oil) (VERLEYEN et al., 2001).

The presence of neutral oil in the distillate mainly occurs by mechanical entrainment of oil drops by the vapor phase (DE GREYT; KELLENS, 2005) and, to a lesser extent, by the volatilization of lower molecular weight acylglycerides. In general, the loss of neutral oil is relevant to the processes carried out at high temperatures, high vacuum and high amounts of stripping agent. In most oils (such as soybean and palm), the loss of neutral oil is caused mainly by mechanical drag. In this case, installation of baffles significantly reduces losses. However, in the case of lauric oils, some loss occurs due to evaporation of short chain mono- and di-acylglycerides. This loss is inherent to the process conditions and is not affected by the design of the deodorizer (DE GREYT; KELLENS, 2005).

Hydrolysis

Hydrolysis is the reaction that occurs when oils and fats mix with water. Moisture causes triacylglycerols (TAG) to decompose into free fatty acids (FFA), monoacylglycerides (MAG) and diacylglycerides (DAG) what increases neutral oil losses during refining. This is essentially a reverse reaction of the fatty molecule synthesis and requires high temperatures (higher than 100 °C) and usually a long time (several hours) (Equation 2.1). Hydrolysis occurs in part due to the improper handling and storage of seeds, such as high humidity, high temperature and mechanical damage (LIST et al., 2005).

$$\begin{aligned} \mathrm{TAG} + \mathrm{H_2O} &\underset{heating}{\Longleftrightarrow} \mathrm{DAG} + \mathrm{RCOOH} \\ \mathrm{DAG} + \mathrm{H_2O} &\underset{heating}{\Longleftrightarrow} \mathrm{MAG} + \mathrm{RCOOH} \\ \mathrm{MAG} + \mathrm{H_2O} &\underset{heating}{\Longleftrightarrow} \mathrm{GLYCEROL} + \mathrm{RCOOH} \end{aligned} \tag{2.1}$$

Hence, during vegetable oil deodorization, this is an important reaction as it involves the use of very high temperatures and steam injection. According to De

Greyt and Kellens (2005), the acidity of the final deodorized oil is usually not less than 0.005% due to hydrolysis.

Sarkadi (1959) studied the hydrolysis of peanut oil at 180 °C, 400 mmHg and 4.5 hours, concluding that up to 1.1 % of acidity can be generated. It was also observed that the rate of hydrolysis is directly proportional to pressure.

Cis-trans Isomerization

High temperatures together with the residence time of the vegetable oil are the factors that affect the isomerization reaction of unsaturated fatty acids. Generally, the formation of *trans* fatty acids is insignificant at temperatures below 220 °C. However, it becomes significant at temperatures between 220 °C and 240 °C, and grows exponentially at temperatures above 240 °C (DE GREYT; KELLENS, 2005).

According to Schwarz (2000a; 2000b) the initial *trans* fatty acid content of 0.1 and 0.3 % in the crude oil may reach up to 5 % in refined oils, formed exclusively during the deodorizing or physical deacidification stages

2.3 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is the technique recommended by the American Oil Chemists' Society (AOCS) for identification and quantification of tocopherols and tocotrienols. Several studies are also found using HPLC for identifying classes of carotenes (KANDLAKUNTA et al., 2008; MARINOVA; RIBAROVA, 2007; MELENDEZ-MARTINEZ et al., 2007; VALLS et al., 2007).

Chromatography is based on a property called molecular polarity. It is very sensitive, and the adequate separation of the components of a mixture depends on choosing the correct mobile phase of the chromatographic column (stationary phase) and detection system (MCMASTER, 1994).

The mobile phase solvent is pumped through the chromatography column to elute the sample. When the composition of the mobile phase is constant, it is called

isocratic elution. In the case of a binary mobile phase, gradient elution can be used to increase the resolution of peaks. In this case, the composition of the mobile phase changes during the chromatographic run, modifying the separation obtained in the column (MCMASTER, 1994). This type of elution consists of using a "weak" solvent at the beginning of the run and to increase proportionally the “strong” solvent during the separation. This elution can increase the resolution and separation of compounds with a low affinity column, just as it can decrease the retention time of compounds with high affinity (ROBARDS et al., 1994).

After separating the components of the mixture, it is important that a detection system is capable of transforming the components, and only the components, in an electronic signal proportional to its concentration (ARAÚJO, 2004). Therefore, the detector used in chromatographic analysis must be sensitive and specific to the components analyzed.

Among the most frequently used detectors for tocopherols, the fluorescence detector is considered by the official method (AOCS, 1998) to be one of the most selective used in liquid chromatography. This selectivity is due to the fact that few molecules possess enough fluorescence to enable fluorescence detection, as well as the high degree of adjustability for each type of molecular spectra due to specific excitation and emission, thereby improving both the identification and the quantification of the examined compounds (YEUNG, 1986). Because, tocopherols are naturally fluorescent substances, this type of detector is extremely specific to analyze these compounds.

An even more versatile type of detector is the diode array detector (DAD) that can detect substances having absorption in a wide wavelength range varying from 190 nm to 800 nm, and is very useful for analysis of β-carotene which has maximum absorption at 455 nm (HUBER; GEORGE, 1993).

Recently, the appeal of foods rich in nutritionally beneficial compounds makes it necessary that such compounds are analytically determined (ASENSIO-RAMOS et al., 2009). Also, there is a tendency to seek analytical methods that can quantify different compounds simultaneously in order to save time and reactants.

Prates et al. (2006) propose a few methods in which it is possible to simultaneously quantify β-carotene, tocopherols and cholesterol in meat by HPCL as well as suggest the method of Tasioula-Daisy and Okogeri (2001) for the simultaneous determination of tocopherols and phenols in olive oil.

This work completed the development and validated a method that allows the simultaneous analysis of tocopherols and tocotrienols with β-carotene, using a gradient elution. A preview of this method was published in the *"Characterization of Oil Extracted from Buriti Fruit (Mauritia flexuosa) Grown in the Brazilian Amazon Region"*, in the Journal of the American Oil Chemists' Society (SILVA et al., 2009). However, this study was not conducted to validate the analytical method. It should be recognized that for a consistent interpretation of analytical results, it is essential to assess the reliability of the method, measured by the accuracy and precision (DIAS et al., 2008).

2.4 Adsorption

Adsorption is the phenomenon that selectively segregates atoms or molecules from a fluid onto the exposed surface of a solid (KENT, 2008). It is used to separate mixtures whose components present similar physical properties (volatility, solubility, etc.) or whose concentration is very low. Furthermore, adsorption can perform several separations which are impossible or impractical by conventional techniques, such as distillation, absorption (gas – liquid), and even membrane-based systems (KENT, 2008). The temperatures of adsorption are usually much lower, so the energetic costs are reduced when compared to other conventional methods. For this reason, adsorption is one of the most used alternatives to eliminate contaminants in gas and liquid streams, the drying of air and organic liquids, and the purification of biochemicals (COCERO; CALVO, 2008). In the food industry, it is mainly used to remove colors and other impurities. For instance, adsorption occurs in the bleaching of vegetable oils to remove compounds such as phospholipids, colorants, soaps, and contaminants; lipid

peroxidation products are also removed to obtain the desirable characteristics of edible oils (ZSCHAU, 2001).

2.4.1 Adsorption phenomena

Adsorption is the preferential partitioning of substances from a gaseous or liquid phase onto the surface of a solid substrate. The solid phase has active sites that take up specific compounds from the fluid phase and is called adsorbent. The fluid phase, a liquid or a gas, has a compound to be taken up, which is called the adsorbate (KENT, 2008).

Adsorbent and adsorbate can interact through chemisorption or physisorption. During physisorption, or physical adsorption, the interaction between the adsorbent and the adsorbate is through physical attraction, i.e. Van der Waals and eletrostatic forces. Thus, the magnitude of the interaction is affected by the size, polarity, polarizability, and quadrupolarity of sorbate atoms or molecules, as well as the electric field strength and the local field gradient of the solid surface (KENT, 2008). On the other hand, chemisorption, or chemical adsorption, involves the formation of chemical bonds and/or an electron transfer between the adsorbent and the adsorbate.

One way to distinguish between physisorption and chemisorption is by the effects of heat and temperature. For example, physisorption is always exothermic, i.e., $\Delta H_{ads} < 0$. Furthermore, the adsorbed state is more ordered than the fluid state, so $\Delta S < 0$. To be thermodynamically feasible, $\Delta G_{ads} < 0$, so $\Delta H_{ads} < T\Delta S$. In contrast, endothermic adsorption is possible for dissociative chemisorption even if the entropy of the adsorbate decreases, because the entropy of the adsorbent may increase to more than offset (KENT, 2008).

Adsorption separation is based on three distinct mechanisms: steric, equilibrium, and kinetic. In the steric separation mechanism, the porous solid has pores with dimensions that permit small molecules to enter while excluding large molecules from entering. The equilibrium mechanism is based on the solid having different abilities to accommodate different species, that is, the stronger adsorbing

species is preferentially removed by the solid. The kinetic mechanism is based on the different rates of diffusion of different species into the porous structure of the solid phase. Thus, by controlling the exposure time, the faster diffusing species is preferentially removed by the solid (DO, 1998).

2.4.2 Adsorbents

Adsorbent selection is an important step and it can determine whether or not a process will succeed or fail (DO, 1998). To be a good adsorbent, the solid needs to have good adsorptive capacity and selectivity.

Adsorption capacity refers to the amount of adsorbate taken up by the adsorbent per unit of mass (or volume), and it is probably the most important characteristic of an adsorbent. Adsorption capacity depends on the fluid-phase concentration, the temperature, as well as other conditions (especially the initial condition of the adsorbent). It has vital importance to capital cost, as it will define the amount of adsorbent, for instance bleaching earth required by a process (KENT, 2008).

Selectivity, on the other hand, can be defined as the ratio of "what is adsorbed" to "what remains in the fluid phase" at equilibrium (KENT, 2008). It is defined by the chemical nature of the material and will determine the components to be adsorbed (COCERO; CALVO, 2008). In the vegetable oil industry, the most common adsorbents are neutral and acid activated bleaching clays. Besides those adsorbents, activated carbon and silica gel have been proposed (ZSCHAU, 2001).

Naturally activated clays present some bleaching activity due to a number of layered silicates such as bentonites, hectorite and sepiolite. Differently, acid activated clays, are obtained by activating bentonite using elevated temperatures and some mineral acids (sulphuric or hydrochloric acids). The essential difference between both natural and acid activated bleaching clays is that in aqueous suspension, natural bleaching clays is slightly acid or neutral, whereas acid activated bleaching earth is highly acidic. This treatment greatly increases the specific surface from 40 – 60 m^2/g to about 300 m^2/g. Moreover, acid activation

ascribes other characteristics to the adsorbent, so that it can act as acidic catalyst, solid acid, cation exchanger and filter aid (ZSCHAU, 2001).

2.5 Computer Simulation

Computer simulation has become an important tool for the study and optimization of complex processes, involving a large number of variables, and is widely used in the development of industrial processes and equipment. In this research group, computer simulated steps by physical deacidification and deodorization of vegetable oils have been as widely studied in batch equipment (CERIANI; MEIRELLES, 2004b; CERIANI; MEIRELLES, 2007) as in continuous equipment (CERIANI; MEIRELLES, 2004c; CERIANI; MEIRELLES, 2007). The isomerization of fatty acids to the *trans* conformation (CERIANI; MEIRELLES, 2007) and its content after deodorization were also studied (CERIANI et al., 2008). In this work, computer simulation was used to study the deacidification step using experimental results found in the literature in terms of the final acidity and neutral oil loss.

To model the physical deacidification process in a batch process, Ceriani e Meirelles (2004b) used the vapor pressure equations and thermodynamic approach proposed by Ceriani e Meirelles (2004a) to predict the vapor-liquid equilibrium (VLE) of fatty compounds involved. Akterian (2009) used the vapor pressure equations proposed by Ceriani e Meirelles (2004c) to measure the temperature of sunflower oil with high oleic acid content during batch deodorization.

2.5.1 Differencial Distillation

In a study performed by Ceriani and Meirelles (2004b), physical deacidification was treated as a differential distillation, in which the tank is fed with oil and then heated. The superheated steam was then bubbled continuously into the oil, acting as a depletion agent carrying the most volatile fatty compounds in the vapor phase, which was then condensed and collected in a container. The oil

composition in the tank and the deodorizer distillate varied with time. It is important to note that this is a dynamic process that can not be modeled in steady state. The authors treated the process as a sequence of numerous, successive evaporation steps in equilibrium (CERIANI; MEIRELLES, 2004b).

Ceriani e Meirelles (2004b) developed three alternative approaches to modeling the process and compared their results with experimental data from Petrauskaitè et al. (2000) for coconut oil. The first and simplest approach, called Model 1, does not rely on steam injection. Model 2 employed steam injection, but steam was considered as an inert gas. In this case, it is assumed to be completely immiscible in the oil phase. Model 3 considered that small amounts of water originating from the injected steam dissolved in the oil, increasing the volatility of fatty acids and decreasing the boiling point of the mixture. In fact, the third model was more close to the experimental data from Petrauskaitè et al. (2000) and has been detailed in the equations that follows.

The ELV model is described in equations 2.2 and 2.3 (CERIANI; MEIRELLES, 2004b).

$$\kappa_i = \frac{y_i}{x_i} = \frac{\gamma_i \cdot f_i^0}{P \cdot \phi_i} \tag{2.2}$$

$$f_i^0 = P_i^{vp} \cdot \phi_i^{sat} \cdot exp\left(\frac{V_i^L \cdot \left(P - P_i^{vp}\right)}{R \cdot T}\right) \tag{2.3}$$

where κ_i is the coefficient of distribution of compound i between liquid and vapor phases, f_i^0 is the fugacity in the reference state; x_i and y_i represent the mole fraction of the component *i* in the liquid and vapor stages, respectively; *P* is the total pressure; *R* is the universal gas constant; *T* is the absolute temperature of the system P_i^{vp} and ϕ_i^{sat} , respectively, the vapor pressure and the fugacity of pure component *i*; γ_i the activity coefficient; ϕ_i^{st} the fugacity coefficient; V_i^L is the molar volume of component *i* in the liquid state. The exponential term of Equation 2.3 is

the Poynting factor. The vapor pressure of fatty compounds was estimated according to the method proposed by Ceriani and Meirelles (2004a).

The total and component mass balances around the batch still are presented by Equations 2.4 and 2.5.

$$\frac{dL}{dt} = -\upsilon \tag{2.4}$$

$$\frac{d(L \cdot x_i)}{dt} = -\upsilon \cdot y_i \tag{2.5}$$

where L is the number of moles of the liquid in the tank; $\upsilon$the molar vaporization rate in moles / time; x_i and y_i are molar fractions of the component i in the liquid and vapor phases, respectively.

The total and component mass balances and components for the distillate container are presented in Equations 2.6 e 2.7:

$$\frac{dD}{dt} = \upsilon \tag{2.6}$$

$$\frac{d(D_i)}{dt} = \upsilon \cdot y_i \tag{2.7}$$

where D is the total number of moles in the distillate; and D_i the number of moles of component i in the distillate container.

The work of Taham (2009) and Silva (2009) used this differential approach to simulate experiments in batch deacidification of fatty systems models and Buriti oil, respectively, utilizing equipment jointly developed by the Laboratory of Extraction, Applied Thermodynamics and Equilibrium (Extrae) and the company MARCONI. The results demonstrate that the simulation was an efficient way to describe the final oil acidity, however, when the removal of acidity was analyzed over time, the differences became significant. The curve of decreasing acidity obtained with the differential approach was linear while the experimental curves

obtained by Taham (2009) and Silva (2009) showed an exponential decay of acidity.

2.5.2 Flash Distillation

A batch deacidification process can also be analyzed according to the *flash* approach. The process is performed in batches in which steam is continuously fed into a specified quantity of oil, a single equilibrium stage and without the use of reflux, according to the operation of a ordinary industrial equipment.

The approach used in the present work corresponds to an adaptation of the prior work of Ceriani and Meirelles (2006) for crossflow deodorizers. In the present case each stage of a crossflow equipment is assumed to be a different and small time interval along the total residence time of the amount of oil in the unique stage of the batch deodorizer.

The work of Ceriani and Meirelles (2006) described the ongoing process in a crossflow continuous deodorizer using equations of mass and enthalpy balances and equilibrium relationships for each stage, as described in Equations 2.8 to 2.10.

$$F_{1(n,i)} = l_{n,i} - l_{n-1,i} + V_n \cdot e_n \cdot \frac{l_{n,i}}{L_n} - l_{n+1,i} - f_{n,i} - F_{n,i} = 0 \tag{2.8}$$

$$F_{2(n)} = h_n + H_n + e_n \cdot V_n \cdot \frac{h_n}{L_n} - h_{n+1} - h_{f,n} - H_{f,n} = 0 \tag{2.9}$$

$$F_{3(n,i)} = \eta_{n,i} \cdot \kappa_{n,i} \cdot V_n \cdot \frac{l_{n,i}}{L_n} - \upsilon_{n,i} + \left(1 - \eta_{n,i}\right) \cdot V_n \cdot \frac{F_{n,i}}{\sum_i F_{n,i}} = 0 \tag{2.10}$$

where n is the stage number, the subscript i is the component, H is the enthalpy of the vapor phase (J/hour), h is the enthalpy of the liquid phase (J/h), h_f is the enthalpy of the feed (J/hour), V total flow of vapor (mol/hour), υ is the component vapor flow, L is the total flow of liquid, l is the flux of the component i in the liquid phase (mol/hour), f is the feed stream in the liquid phase, F is the feed stream in the vapor phase, e is the entrainment term, η is the Murphree plate efficiency.

In this thesis, this approach will be adapted to describe the process of physical batch deacidification. In this case, each stage of the column is understood as a small time interval along the total residence time of the oil in the batch deodorizer.

2.5.3 Triacylglycerols composition

The probable fatty acid composition needed for a data entry program can be estimated by the statistical method developed by Antoniosi et al. (1995), which has been successfully used in several studies from the research group. This requires the weight composition of the vegetable oils in fatty acids, which should be determined by gas chromatography and the trisaturated fat content, which is available in studies for certain oils. This method is based on the theory of casual 1,3-random-2-random distribution and, wherein the fatty acids are randomly distributed, statistically, among the three positions of the glycerol molecule (ANTONIOSI et al., 1995).

The composition of monoacylglycerols (MAG) and diacylglycerols (DAG) can be obtained stoichiometrically, since each tag may be broken at one 1.3 - and 1.2 - DAG and each DAG, in turn, can be divided into MAGs. The concentration of each class of acylglycerides can be obtained in the research of some oils, and also determined by size exclusion chromatography (AOCS, 1998).

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Chapter 3. "VALIDATION OF A METHOD FOR SIMULTANEOUS QUANTIFICATION OF β-CAROTENE AND TOCOPHEROLS IN VEGETABLE OILS BY HPLC"

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Abstract

A normal-phase HPLC method for analysis of carotenes, tocopherols and tocotrienols has been developed and validated. In this work we present a modification to the official AOCS method for analysis of tocols which allowed simultaneous quantification of the three groups of compounds, including carotenes. Analytes were separated using a gradient mobile phase (hexane and isopropanol) and with a gradient flow rate (1 to 2 $mL \cdot min^{-1}$). The column effluent was monitored by Photo Diode Array detector (PDA) set at 292 nm (tocols) and 455 nm (β-carotene) and by fluorescence detector set at an excitation wavelength of 290 nm and 330 nm emission. Inter- and intra-run accuracies and precision of the analytical method were better than ± 15%. The lower limit of quantification was 5.0 $mg \cdot L^{-1}$ for the tocols and 0.1 $mg \cdot L^{-1}$ for carotenes. The method has been applied for the quantification of these compounds in Amazon oils.

3.1 Introduction

Vegetable oils are important compounds of human nourishment, providing energy, essential fatty acids and fat-soluble vitamins. Among these vitamins, provitamin A and vitamin E are highlighted. Tocopherols are natural antioxidants that also present Vitamin E activity, especially the α-tocopherols (De Greyt, & Kellens, 2005) which are frequently found in serum (Krčmová et al., 2009). Tocotrienols possess powerful neuroprotective, anti-cancer and cholesterol lowering properties that are often not exhibited by tocopherols (Sen, Khana, & Roy, 2005). During deodorization, it was observed that tocopherol losses exceeded 30%, two thirds of which resulted from their distillation (Gogolewsky, Nogala-Kalucka, & Szeliga, 2000). Both analytes, tocopherols and tocotrienols, present a

maximum UV absorption between 280 and 300 nm with minimum absorption between 250 and 260 nm. Tocopherols and tocotrienols have also intense native fluorescence when excited at 210 or 290 to 292 nm. Excitation of the chroman ring at these wavelengths produces a maximal emission at 320 nm or slightly higher wavelengths. Fluorescence detection provides sensitivity, specificity, and cleaner chromatograms compared to UV detection. Fluorescence detection is essential for the successful assay of vitamin E in complex food matrices. UV detection can be used for concentrated supplements or fortification premixes (Eitenmiller, Landen, 1999). In this work we study the uses of both Fluorescence and PDA (based on UV-vis spectrophotometry) detector to provide flexibility to this methodology, so it can be applied in laboratories that have only one of these detectors.

Many methods for determining tocopherol composition in oils have been published using normal phase or reversed-phase HPLC (RP-HPLC). Rodrigues, Darnet, & Silva (2010) quantified tocopherols in several Amazon fruits using reversed-phase HPLC according to the methodology of Brubacher, Müller-Mulot, & Southgate (1986). This method only quantifies tocopherols in saponified samples and cannot distinguish between β- and γ- fractions. Costa, Ballus, Teixeira-Filho, & Godoy (2010) quantified tocopherols in some Brazilian fruits according to the official AOCS Ce 8-89 method (1998), with the mobile phase modified by Sadler, Davis, & Dezman (1990). The mobile phase composition consisted in a mixture of 67:27:6 (v/v) methanol:tetrahydrofuran:water. This method could not quantify β-tocopherol and all tocotrienol homologues.

Carotenes are pigments synthesized by plants from eight isoprene units. Vitamin A makes up essentially half of the β-carotene molecule, with a water molecule added to its side chain (Rodriguez-Amaya, 1996). These molecules are thermolabile if extracted and heated (Nawar, 1996). They are found in high concentration in red oils, like crude palm oil (Gunstone, 2005) and Buriti oil (Silva, Sampaio, Taham, Rocco, Ceriani, Meirelles, & 2009; Albuquerque et al., 2005; França, Reber, Meireles, Machado, & Brunner, 1999; Mariath, Lima, & Santos, 1989). The amount of carotenes destroyed daily by the high temperatures

employed during the refining process of these oils is sufficient to meet the vitamin A requirement of the world population (Mayamol, Balachandran, Samuel, Sundaresan, & Arumughan, 2007). The total carotene quantification in oils may be done by UV-vis spectrophotometry, as suggested by PORIM (1990).

Recently, the potential occurrence of nutraceutical components in food has increased the presence of products on the market claiming to contain these substances, requiring analytical methods (Asensio-Ramos, Hernández-Borges, Rocco, & Fanali, 2009). There is a tendency to search analytical methods that can simultaneously quantify different components, saving reagents and time. Some recent examples are the method of Prates, Quaresma, Bessa, Fontes, & Alfaia (2006), in which a simultaneous quantification of β-carotene, cholesterol and tocopherols using HPLC in meat is presented, and the method of Tasioula-Margari, & Okogeri (2001) to determine simultaneously tocopherols and phenols in olive oils.

More recently, our research group presented a detailed characterization of Buriti oil, including tocopherols, tocotrienols and total carotenes in its composition (Silva et al., 2009). In this work, a new HPLC methodology for simultaneous quantification of these analytes was developed. However, no validation was included in this previous work. It is recognized that for a consistent interpretation of the results of an analytical method it is essential to evaluate the inherent confidence, which is calculated by the quantification of its accuracy, i.e., trueness and precision (Dias, Camões, & Oliveira, 2008). This was, in fact, one of the goals of the present article: to validate the HPLC method previously developed by our research group to quantify simultaneously total carotenes, tocopherols and tocotrienols. Furthermore, the method was used to quantify the presence of compounds in some Amazon oils.

3.2 Material and Methods

3.2.1 Chemical and reagents

All solvents and reagents used in this study were of HPLC grade. The mobile phase used in the HPLC system was vacuum-filtered through a 0.45 μm filter (USA). Hexane was purchased from Mallinckrodt (USA) and isopropanol from Tedia (Brazil). α-, β-, δ- and γ-tocopherol standards were purchased from Calbiochem (USA) and the β-carotene standard from Fluka (Germany).

3.2.2 Chromatography

Chromatographic analyses were carried out using a Shimadzu HPLC, series LC-20AT (Japan), equipped with a quaternary pump, an autosampler (SIL-20A), a degasser, and a SPD-M20A spectrophotometric detector (Photo Diode Array detector - PDA), which was set at 292 nm and 455 nm, and a RF-10AXL fluorescence detector, which was set at 290 nm for excitation and 330 nm for emission. Chromatographic separation of the compounds was achieved at 30 ºC, using a normal-phase Lichrospher column (Merck, 250 × 4.6 mm i.d.; 5 μm particle size) with a guard column (10 × 4.6 mm) purchased from Merck (Germany). The concentration gradient used was as follows: 0 – 7 min 99.5 % hexane and 0.5 % isopropanol; 7 – 9 min linear gradient of 0.5 – 1 % isopropanol; 9 – 20 min 99.0 % hexane and 1.0 % isopropanol; 20 – 25 min reconditioning of the column with 0.5 % isopropanol isocratic for 10 min. The flow gradient was: 0 - 4 min 1.0 $mL \cdot min^{-1}$, 4 - 7 min linear gradient of 1 - 1.5 $mL \cdot min^{-1}$, 7 - 9 min 1.0 $mL \cdot min^{-1}$, 9 - 15 min linear gradient of 1.5 - 2.0 $mL \cdot min^{-1}$, 15 - 17 min linear gradient of 2.0 - 1.0 $mL \cdot min^{-1}$, 17 - 35 min 1.0 $mL \cdot min^{-1}$.

The total chromatographic run time was 35 min, being the time required for the analysis of tocopherols. Although the analyses of carotenes and tocopherols were carried out simultaneously, calibration curves were performed separately due to the ease of preparing the standards separately. For the calibration curve of

β-carotene, a 5 minute run was used with a mobile phase composed of 99.5 % hexane and 0.5 % isopropanol, and a flow rate of 1.0 mL min^{-1}.

System control, data acquisition and processing were performed with an Intel-Celeron D PC, operated with Microsoft Windows XP Professional version 2002 and LC Solutions® version 2002 chromatography software with the system suitability option installed. Calibration curves were calculated by linear regression analysis of the peak area versus the concentration of the nominal standard for each compound. The goodness-of-fit of various calibration models were evaluated by visual inspection, the correlation coefficient and by intra- and inter-run accuracy and precision values.

3.2.3 Preparation of stock solutions, calibration standards and quality control samples

Stock solutions of α-, β-, δ- and γ-tocopherol were prepared by dissolving about 50 mg of each tocopherol fraction in 25 mL of hexane. Note that these stock solutions have the four tocopherol fractions in the same concentration. Serial dilution (37.50, 25.00, 17.50, 10.00, 5.00 and 2.50 $mg \cdot L^{-1}$) of a 2 $mg \cdot mL^{-1}$ tocopherol solution was carried out. Tocotrienols were quantified based on the area of tocopherol homologues. In the same way, stock solutions of β-carotene were prepared by dissolving 5 mg in 25 mL of hexane. Serial dilution (10.00, 5.00, 2.50, 1.00, 0.50, 0.25, 0.10 and 0.05 $mg \cdot L^{-1}$) of the 0.2 $mg \cdot mL^{-1}$ β-carotene solution was then performed. Total carotenes were quantified based on the area of β-carotene. These calibration standards were freshly prepared in triplicate for each analytical run.

Triplicates of quality control samples were prepared in hexane using the concentrations of 5.00 (LOQ), 15.00 and 35.00 $mg \cdot L^{-1}$ for the tocopherol system and in concentrations of 0.10, 0.35 and 9.00 $mg \cdot L^{-1}$ for β-carotene, as described above for the calibration standards. These quality control samples were used to investigate intra- and inter-run variations.

3.2.4 Validation Procedures

A chromatographic validation run included a set of calibration samples assayed in triplicate and quality control samples at three levels in triplicate, which was carried out on six separate occasions. The validation method was performed in accordance with the previously reported procedures (USDHHS, 2001; Shah et al., 2000; Marin, Franchini, & Rocco, 2007).

Calibration curves in the range of 2.5 to 37.5 mg·L^{-1} for each tocopherol in hexane and in the range of 0.05 to 10.00 mg·L^{-1} for β-carotene were plotted based on the peak-areas of each compound (axis *y*) against the respective nominal concentrations (axis *x*). All calibration curves were required to have a correlation coefficient of at least 0.9800.

The intra- and inter-run accuracy and precision of the assays were assessed by the average relative percentage deviation (DEV %) from the nominal concentrations and the coefficient of variance (CV %) values, respectively, based on reported guidelines (USDHHS, 2001; Shah et al., 2000; Marin et al., 2007). Precision (CV %) and accuracy (DEV %) were calculated from Equations 3.1 and 3.2:

$$\mathrm{CV}\ (\%) = \left(\frac{\mathrm{SD}}{\text{Average calculated concentration}}\right) \times 100 \tag{3.1}$$

$$\mathrm{DEV}\ (\%) = \left(1 - \frac{\text{Average calculated concentration}}{\text{Nominal concentration}}\right) \times 100 \tag{3.2}$$

where SD stands for standard deviation.

Intra-run precision and accuracy measurements were performed on the same day using tocopherol concentrations (n=3) of 5.00, 15.00 and 35.00 mg·L^{-1} in hexane and β-carotene concentrations (n=3) of 0.10, 0.350 and 9.000 mg·L^{-1}. Inter-run precision and accuracy of the analytical method were determined simultaneously from the results of the calibration curve and quality control samples run on six days. Each set of quality control samples containing tocopherols or β-carotene was evaluated from recently obtained calibration curves.

3.2.4.1 Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification (LOQ) was determined by considering the signal-to-noise ratio larger than 10 and the lowest concentration at which precision expressed by the coefficient of variance is lower than 20 %, and accuracy expressed by relative difference of the measured and true values is also lower than 20 %. The Limit of detection (LOD) was defined as the lowest concentration to be detected, taking into consideration a signal-to-baseline noise ratio larger than 3 (USDHHS, 2001; Shah et al., 2000; Marin et al., 2007).

3.2.4.2 Stability

According to the FDA Guidance (USDHHS, 2001), solvent evaporation stability during storage in the autosampler for a 24 hour period was established at five concentrations of 37.50, 25.00, 17.50, 10.00 and 5.00 $mg \cdot L^{-1}$ in triplicate and was tested only for tocopherols. Considering that solvent evaporation would affect the concentrations of tocopherols and carotenes in the same proportion, no specific stability test was required for carotenes.

3.2.5 Application of the method

3.2.5.1 Amazon Oils Tocopherols and Tocotrienols Quantification

Three Amazon oils were selected: Buriti (*Mauritia flexuosa*), Patawa (*Oenocarpus bataua*) and Tucuma (*Astrocaryum aculeatum*). Samples were dissolved in hexane and aliquots of 20 µL were injected in the HPLC system.

The following fruits pulps were purchased at local markets in the Amazon Region: Buriti pulp was acquired in Abaetuba (Pará, Brazil), and Patawa and Tucuma pulps in Belém (Pará, Brazil), during harvest time. Thirty fruits of each species were gathered in three different places which were separated by a distance of at least two kilometers from each other, adding up to 90 fruits from each species. The Bligh and Dyer (1959) method was used to extract oils from the dried pulps. The total lipid fraction was extracted by exhaustive maceration with chloroform and methanol, followed by filtration of solids and separation of the

solvent/fat layer. Dried samples (10% moisture) were used to facilitate extraction with organic solvents.

All data are presented as mean values ± SD and the mean values were analyzed by one-way ANOVA and Tukey-HSD at $p<0.05$ with SAS.

3.3 Results and Discussion

3.3.1 Chromatography

Reproducible separation of β-carotene was obtained in the same silica normal-phase column used for tocopherol analysis. Retention time of β-carotene was 1.9 min, showing that this compound has lower affinity with the column. Peaks were sharp, symmetrical and all homologues were efficiently separated (Figure 3.1). Tocopherols were analyzed using both PDA and fluorescence detectors. Retention times for tocopherols using the fluorescence detector were respectively, 7.6, 16.6, 19.9 and 29.1 min for the α-, β-, γ- and δ-tocopherol homologues. For the PDA detector, retention times were 7.2, 16.4, 19.3 and 28.5 min, respectively, for the α-, β-, γ- and δ-tocopherol homologues. Note that retention times for PDA were lower than for fluorescence. This difference is due to the system configuration: the samples pass through the PDA detector and then the fluorescence detector. It is also important to highlight that retention times can vary slightly on different days and analysis. In all chromatograms, we can note an interfering peak which does not disturb the analyses as its retention time is different of all other analyzed compounds. Furthermore, this specific peak is not symmetrical and not well resolved. These data confirm the efficiency of the specified flow and composition gradients of the mobile phase to separate carotenes and tocopherols. Previous studies performed by Rodrigues et al. (2010) and Costa et al. (2010) were not able to quantify tocotrienols, nor distinguish β- and γ-tocopherols.

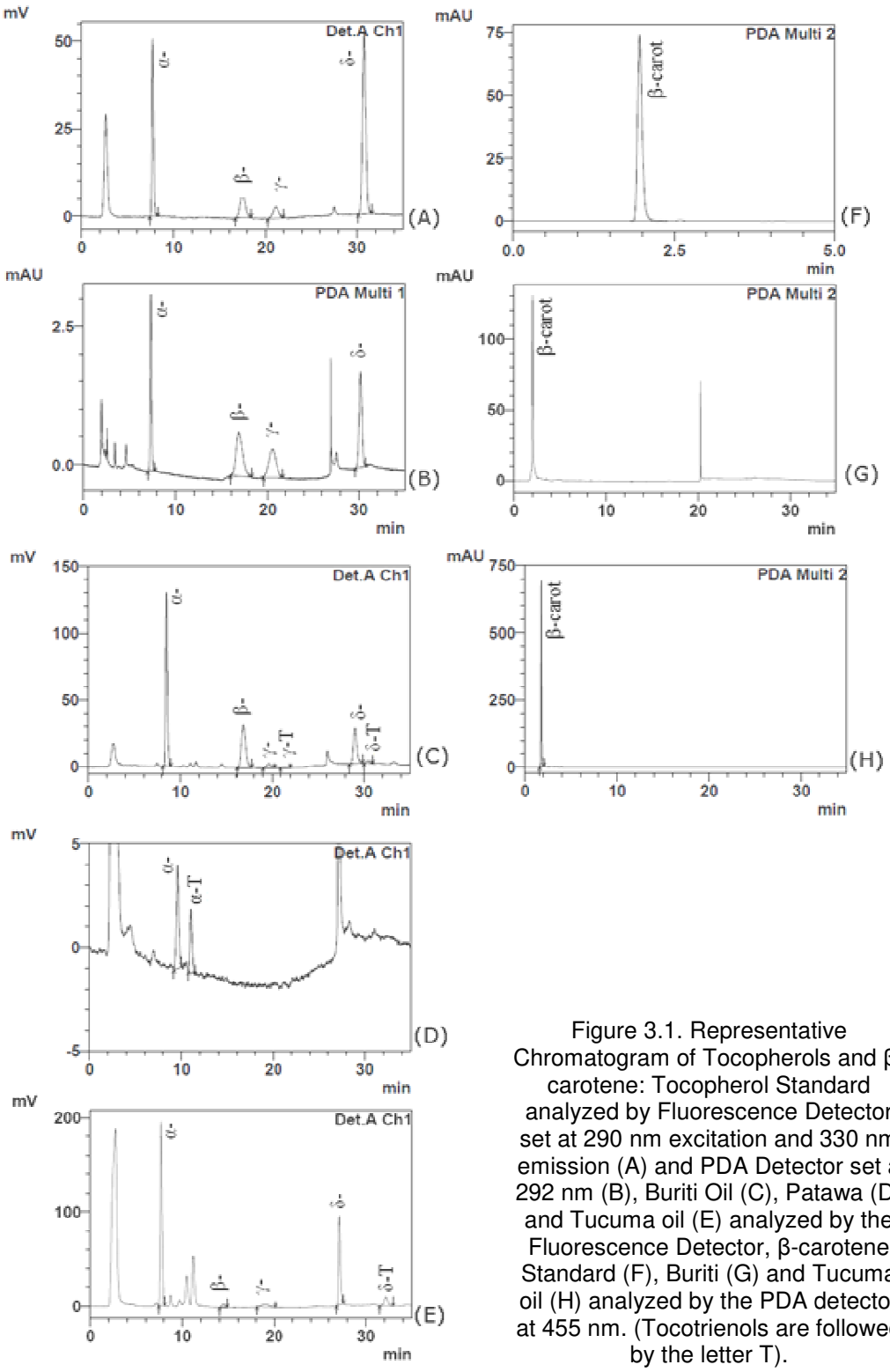

Figure 3.1. Representative Chromatogram of Tocopherols and β-carotene: Tocopherol Standard analyzed by Fluorescence Detector set at 290 nm excitation and 330 nm emission (A) and PDA Detector set a 292 nm (B), Buriti Oil (C), Patawa (D) and Tucuma oil (E) analyzed by the Fluorescence Detector, β-carotene Standard (F), Buriti (G) and Tucuma oil (H) analyzed by the PDA detector at 455 nm. (Tocotrienols are followed by the letter T).

3.3.2 Validation of the method

3.3.2.1 Linearity, accuracy, precision and sensitivity assays

Samples with standard concentrations of α-, β-, γ- and δ-tocopherols in hexane ranging from 2.50 to 37.50 $mg \cdot L^{-1}$ and samples of β-carotene in hexane ranging from 0.05 to 10.00 $mg \cdot L^{-1}$ were used to construct the calibration curves. Results for the six different sequences of tocopherols standard samples performed in triplicate on different days using the fluorescence detector are shown in Table 3.1. The relationship between tocopherol concentrations and the peak areas was described by the linear regression equations, and in all equations *x* is the tocopherol homologue concentration in $mg \cdot L^{-1}$ and *y* is the chromatogram peak area divided by $1x10^5$. All R^2 obtained were higher than 0.9810. At the upper limit of quantification (i.e. 37.50 $mg \cdot L^{-1}$) the percentage deviation and the inter-run variability values were less than 4.10 %, an appropriate value according to the literature (USDHHS, 2001; Shah et al., 2000; Marin et al., 2007). For all the other tocopherol concentrations, excluding the LOQ (2.5 $mg \cdot L^{-1}$), the percent deviation and the inter run variability values were less than 13.30 %.

Data of the same six sequences of tocopherol standard samples run in triplicate on different days, obtained using the PDA detector set at 292 nm, are also shown in Table 3.1. The relationship between each tocopherol concentration and peak area (divided by $1x10^5$) was described by linear regressions in the same way as for the fluorescence detector. All R^2 values obtained were higher than 0.9970. At the upper limit of quantification (i.e. 37.50 $mg \cdot L^{-1}$) the percent deviation and the inter-run variability values were less than 4.60 %. For all the other concentrations of tocopherols, excluding the LOQ (2.5 $mg \cdot L^{-1}$), the percent deviation and the inter run variability values were also less than 13.50 %.

Table 3.1. Inter-run variation – accuracy, precision and linearity of standard curve samples of tocopherols from six separate assays.[a]

	Nominal Conc. (mg/L)	***ALFA***			***BETA***			***GAMA***			**DELTA**		
		Calculated Conc. (mg/L)	**CV[b] (%)**	**DEV[c] (%)**	**Calculated Conc. (mg/L)**	**CV (%)**	**DEV (%)**	**Calculated Conc. (mg/L)**	**CV (%)**	**DEV (%)**	**Calculated Conc. (mg/L)**	**CV (%)**	**DEV (%)**
1[d]	**2.50 (LOD)**	3.43±0.21	6.22	-37.06	3.76±1.07	28.32	-50.43	4.36±1.00	23.01	-74.46	3.04±0.79	26.17	-21.44
	5.00 (LOQ)	5.23±0.12	2.32	-4.66	5.29±0.58	10.96	-5.72	5.63±0.36	6.48	-12.52	5.05±0.67	13.23	-0.81
	10.00	9.52±0.23	2.42	4.79	9.18±0.65	7.08	8.18	9.07±0.54	5.94	9.25	9.38±0.76	8.08	6.24
	17.50	16.81±0.22	1.28	3.96	16.64±0.99	5.92	4.89	15.88±0.90	5.68	9.24	16.62±0.82	4.92	5.02
	25.00	24.49±0.29	1.19	2.04	24.33±1.00	4.09	2.70	23.84±0.77	3.21	4.62	24.17±0.99	4.08	3.32
	37.50	37.73±0.61	1.62	-0.60	38.36±1.56	4.06	-2.30	38.73±0.74	1.92	-3.28	37.26±1.40	3.75	0.63
	R^2	0.9975±0.0016	0.16	0.25	0.9929±0.0031	0.31	0.71	0.9819±0.0073	0.74	1.81	0.9979±0.0011	0.11	0.21
2[e]	**2.50 (LOD)**	3.12±0.22	7.18	-25.29	2.71±0.79	29.13	-8.34	2.81±0.56	20.02	-12.21	2.67±0.23	8.74	-6.91
	5.00 (LOQ)	5.23±0.10	1.90	-4.55	4.97±0.67	13.46	0.69	5.28±0.21	4.03	-5.54	5.02±0.21	4.24	-0.48
	10.00	9.72±0.14	1.43	2.80	9.72±0.76	7.80	2.82	9.82±0.41	4.19	1.81	9.77±0.17	1.75	2.29
	17.50	19.87±0.15	0.92	3.59	17.50±0.96	5.51	0.00	17.09±0.31	1.84	2.33	17.18±0.27	1.57	1.82
	25.00	24.63±0.30	1.22	1.48	24.91±0.95	3.81	0.35	24.65±0.22	0.91	1.40	24.96±0.28	1.13	0.17
	37.50	37.70±0.48	1.26	-0.54	38.25±1.74	4.55	-2.00	97.78±0.46	1.22	-0.75	38.13±0.39	1.02	-1.69
	R2	0.9987±0.0008	0.08	0.13	0.9974±0.0038	0.38	0.26	0.9988±0.0011	0.11	0.12	0.9993±0.0004	0.04	0.07

[a] A linear curve was fitted to the data for response of tocopherol versus theoretical concentration as described in the Experimental section. The calculated concentration was derived from reading the response for the standard sample against calibration curve. Each entry corresponds to the average value of 6 assay analyses.

[b] CV (coefficient of variation, precision) = Calculation according to Equation 3.1.

[c] Accuracy (DEV %) = the deviation of the calculated concentration from the nominal value. Calculation according to Equation 3.2.

[d] Fluorescence detector data; [e] PDA data

Table 3.2. Inter-run variation - accuracy, precision and linearity of standard curve samples of β-carotene from six separate assays[a] and intra- and inter-run precision and accuracy for β-carotene in quality control samples.[b]

	β-CAROTENE				**QUALITY CONTROL**			
Nominal Concentration (mg/L)	**Calculated Concentration (mg/L)**	**CV (%)**	**DEV (%)**		**Nominal Concentration (mg/L)**	**Calculated Concentration (mg/L)**	**CV (%)**	**DEV (%)**
0.05 (LOD)	0.05±0.02	33.74	-6.28	***Inter-run (n=24)***	0.100 (LOQ)	0.09±0.01	9.35	12.97
0.10 (LOQ)	0.10±0.02	18.40	-3.32		0.350	0.31±0.02	7.28	12.04
0.25	0.28±0.03	9.87	-10.15		9.000	8.80±0.98	11.16	2.24
0.50	0.56±0.05	9.26	-11.09					
1.00	1.01±0.06	6.40	-0.80					
2.50	2.33±0.16	6.68	6.82	***Intra-run (n=3)***	0.100 (LOQ)	0.09±0.00	1.42	5.70
5.00	4.59±0.41	9.03	8.27		0.350	0.31±0.03	10.59	10.14
10.00	10.19±0.16	1.59	-1.91		9.000	7.95±0.27	3.45	11.71
R^2	0.9942±0.0091	0.92	0.58					

[a] A linear curve was fitted to the data for response of β-carotene versus theoretical concentration as described in the Experimental section. The calculated concentration was derived from reading the response for the standard sample against calibration curve. Each entry corresponds to the average value of six assay analyses.

[b] The data are shown as averages, SD (standard deviation), accuracy (percent deviation, DEV%) and CV (coefficient of variation, precision). Accuracy and precision calculations were carried out using Equations 3.1 and 3.2, respectively.

Data of the six different sequences of β-carotene standard samples performed in triplicate on different days, using the PDA detector set at 455 nm, are show in Table 3.2. R^2 value was higher than 0.9940. At the upper limit of quantification (i.e. 10.00 mg·L^{-1}) the percent deviation and the inter-run variability values were less than 2.00 %. For all the other concentrations of β-carotene, excluding the LOQ (0.10 mg·L^{-1}), the percent deviation and the inter run variability values were less than 11.10 %.

Reproducibility of the method was evaluated by analyzing replicates of tocopherol quality control samples at concentrations of 5.00 (LOQ), 15.00 and 35.00 mg L^{-1}, using both fluorescence and PDA detectors. The intra- and inter-run average results are reported in Table 3.3. Accuracy and precision of the assays are demonstrated by DEV values ≤ 14.92 and C.V. values ≤ 13.64%, respectively (Table 3.3). Reproducibility of the method was also evaluated by analyzing replicates of β-carotene quality control samples of 0.10 (LOQ), 0.35 and 9.00 mg L^{-1}, using the PDA detector. The intra- and inter-run average results are reported in Table 3.2. Accuracy and precision of the assays are demonstrated by DEV values ≤ 12.97 % and by C.V. values ≤ 11.16 %, respectively.

3.3.2.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) was determined as the sample whose signal-to-noise ratio (S/N) was slightly greater than 3 and corresponded to 2.50 mg·L^{-1} of each tocopherol. For tocopherols, the lower limit of quantification (LOQ), estimated at 5.00 mg·L^{-1} of each tocopherol, displayed a S/N ratio equal to 10. Furthermore, accuracy values (DEV %) were found ranging within ± 15.00% of the nominal concentration values (Table 3.1). The intra- and inter-run variabilities (quality controls) were demonstrated by CV ≤ 14.70% (Table 3.3). Note that tocopherols and tocotrienols can be quantified in very small amounts due to their natural fluorescence.

Table 3.3. Intra- and inter-run precision and accuracy for tocopherols in quality control samples.[a]

		Nominal Conc. (mg/L)	*ALFA*			*BETA*			*GAMA*			*DELTA*		
			Calculated Conc (mg/L)	*CV (%)*	*DEV (%)*	*Calculated Conc (mg/L)*	*CV (%)*	*DEV (%)*	*Calculated Conc (mg/L)*	*CV (%)*	*DEV (%)*	*Calculated Conc (mg/L)*	*CV (%)*	*DEV (%)*
1[b]	*Inter-run*	5.000 (LOQ)	5.23±0.28	5.25	-4.72	*5.67±0.77*	*13.64*	*-13.47*	*4.97±0.67*	*13.53*	*0.53*	*4.26±0.17*	*3.97*	*14.70*
	(n= 24)	15.000	14.01±0.77	5.51	6.59	*14.27±1.46*	*10.24*	*4.84*	*15.78±1.33*	*8.44*	*-5.20*	*13.02±1.06*	*8.14*	*13.20*
		35.000	32.56±1.51	4.65	6.98	*34.59±2.37*	*6.84*	*1.18*	*35.24±2.17*	*6.16*	*-0.68*	*31.27±1.82*	*5.81*	*10.66*
	Intra-run	5.000 (LOQ)	5.47±0.52	9.53	-9.35	*5.72±0.41*	*7.17*	*-14.37*	*4.95±0.29*	*5.90*	*0.92*	*4.306±0.18*	*4.11*	*14.00*
	(n=3)	15.000	14.64±1.80	12.31	2.39	*13.36±0.18*	*1.38*	*10.96*	*15.31±0.34*	*2.19*	*-2.08*	*14.25±1.75*	*12.28*	*4.98*
		35.000	33.49±3.14	9.38	4.31	*33.38±0.97*	*2.30*	*4.62*	*32.55±2.38*	*7.31*	*7.01*	*33.22±2.04*	*6.14*	*5.07*
2[c]	*Inter-run*	5.000 (LOQ)	4.66±0.45	9.57	6.7898	*4.86±0.41*	*8.34*	*2.70*	*5.26±0.54*	*10.33*	*-5.22*	*5.07±0.34*	*6.76*	*-1.34*
	(n= 24)	15.000	12.82±0.81	6.33	14.55	*13.19±1.50*	*11.35*	*12.04*	*16.48±1.34*	*8.10*	*-9.87*	*12.76±0.95*	*7.43*	*14.92*
		35.000	31.27±1.64	5.24	10.67	*37.40±3.78*	*10.12*	*-6.86*	*34.29±3.87*	*11.29*	*2.01*	*29.92±1.77*	*5.90*	*14.50*
	Intra-run	5.000 (LOQ)	5.01±0.30	6.04	-0.29	*5.67±0.16*	*2.86*	*-13.36*	*5.32±0.28*	*5.30*	*-6.31*	*4.82±0.41*	*8.46*	*3.69*
	(n=3)	15.000	12.99±0.22	1.73	13.36	*13.36±0.18*	*1.38*	*10.96*	*13.36±0.18*	*1.38*	*10.96*	*12.75±0.66*	*5.15*	*14.96*
		35.000	31.56±0.56	1.77	9.89	*33.38±0.97*	*2.30*	*4.62*	*33.38±0.97*	*2.90*	*4.62*	*30.78±1.62*	*5.25*	*12.04*

[a] The data are shown as averages, SD (standard deviation), accuracy (percent deviation, DEV%) and CV (coefficient of variation, precision). Accuracy and precision calculations were carried out using Equations 3.1 and 3.2, respectively.

[b] Fluorescence detector data; [c] PDA detector data

The lower limit of quantification (LOQ) of β-carotene, estimated as 0.10 $mg \cdot L^{-1}$, showed accuracy values (DEV%) lower than 3.32 % and precision values lower than 18.40 %. The intra- and inter-run variabilities (quality controls) were demonstrated by CV ≤ 11.16 % (Table 3.2).

3.3.2.3 Stability

Stability of samples was tested only for solvent evaporation. Even after 24 hours in the autosampler, the precision and the accuracy of the analysis indicated satisfactory values (CV and DEV lower than 15.0 %) (Table 3.4). Autosampler stability testing showed that tocopherols can remain 24 h without solvent evaporation, allowing the solubilisation of a large number of oil samples for each analytical run and use of the autosampler for injection. Considering that no solvent evaporation was detected, the concentration of carotenes was not affected by storage in the autosampler.

3.3.3 Application of the HPLC method

3.3.3.1 Quantification of Tocopherols, Tocotrienols and β-carotene in Amazon Oils

Applicability of this method was tested by quantifying tocopherols, tocotrienols and total carotenes in three Amazon oils: Buriti, Patawa and Tucuma oils. Table 3.5 presents the results for the tocopherol, tocotrienol and carotenes analyses and Figure 3.1 shows the chromatograms. Buriti oil presented all tocopherols, detected by both PDA and fluorescence means. β-tocopherol was encountered in the highest concentration (759 $mg \cdot L^{-1}$ and 711 $mg \cdot L^{-1}$, by PDA and Fluorescence, respectively), followed by γ-tocopherol (319 $mg \cdot L^{-1}$ and 310 $mg \cdot L^{-1}$), α-tocopherol (306 $mg \cdot L^{-1}$ and 299 $mg \cdot L^{-1}$) and δ-tocopherol (87 $mg \cdot L^{-1}$ and 89 $mg \cdot L^{-1}$). Buriti oil also presented tocotrienols. γ-Tocotrienol was detected by Fluorescence, however in concentrations below the LOQ, and was not detected by PDA. δ-Tocotrienol was encountered in the concentration of 20 $mg \cdot L^{-1}$ and 26 $mg \cdot L^{-1}$. Total tocol content was 1488 $mg \cdot L^{-1}$ and 1435 $mg \cdot L^{-1}$, by PDA and fluorescence, respectively.

Table 3.4. Stability evaluation (average ± SD) of tocopherols in hexane (n=3).[a]

		Nominal Concentration (mg/L)				
		5.00 (LOQ)	**10.00**	**17.50**	**25.00**	**37.50**
ALFA	Fresh Samples	5.03 ± 0.03	9.76 ± 0.07	17.00 ± 0.06	24.61 ± 0.14	37.99 ± 0.12
	Autosampler (24 h)	5.32 ± 0.04	9.34 ± 0.20	16.84 ± 0.08	24.41 ± 0.07	38.21 ± 0.18
	Average	5.18 ± 0.16	9.55 ± 0.27	16.92 ± 0.11	24.51 ± 0.15	38.10 ± 0.18
	CV(%)	3.13	2.82	0.63	0.61	0.48
	DEV(%)	-3.58	4.50	3.30	1.98	-1.60
BETA	Fresh Samples	5.32 ± 0.33	9.03 ± 0.04	16.22 ± 0.41	24.40 ± 0.76	38.44 ± 2.36
	Autosampler (24 h)	5.62 ± 0.26	8.58 ± 0.29	16.15 ± 0.03	23.88 ± 0.82	38.74 ± 0.72
	Average	5.47 ± 0.32	8.80 ± 0.31	16.18 ± 0.26	24.14 ± 0.76	38.59 ± 1.57
	CV(%)	5.78	3.52	1.62	3.16	4.07
	DEV(%)	-9.36	11.97	7.53	3.43	-2.90
GAMA	Fresh Samples	5.60 ± 0.02	8.83 ± 0.23	15.35 ± 0.54	24.00 ± 0.05	38.85 ± 0.63
	Autosampler (24 h)	5.58 ± 0.20	8.63 ± 0.17	15.92 ± 1.40	23.77 ± 0.09	38.84 ± 0.11
	Average	5.59 ± 0.13	8.73 ± 0.21	15.64 ± 0.99	23.89 ± 0.14	38.84 ± 0.40
	CV(%)	2.29	2.42	6.38	0.59	1.04
	DEV(%)	-11.81	12.73	10.65	4.45	-3.58
DELTA	Fresh Samples	5.17 ± 0.06	9.71 ± 0.07	17.07 ± 0.16	24.50 ± 0.05	38.02 ± 0.29
	Autosampler (24 h)	5.35 ± 0.11	9.53 ± 0.06	16.81 ± 0.18	24.19 ± 0.06	38.32 ± 0.09
	Average	5.26 ± 0.13	9.62 ± 0.12	16.94 ± 0.21	24.34 ± 0.18	38.17 ± 0.25
	CV(%)	2.38	1.20	1.24	0.72	0.65
	DEV(%)	-5.12	3.83	3.22	2.63	-1.79

[a] The data are shown as averages, SD (standard deviation), accuracy (percent deviation, DEV%) and CV (coefficient of variation, precision). Accuracy and precision calculations were carried out using Equations 3.1 and 3.2, respectively.

The concentration of carotenes was 1576 mg·L^{-1}. These results are similar to those found in studies performed by Silva et al. (2009) where Buriti oil was analyzed, presenting 1517 mg·L^{-1} of total tocols in which β-tocopherol was the most important homologue. However, it was followed by α-tocopherol and γ-tocopherol respectively, probably due to the different post-harvest treatments of the oil (Silva et al., 2009).

Patawa oil contained only α-homologues in both analyses: 38 mg·L^{-1} and 41 mg·L^{-1}, by PDA and fluorescence, respectively of α-tocopherol and 35 mg·L^{-1} and 33 mg·L^{-1} of α-tocotrienol (Table 3.5). The α-tocopherol content obtained was similar to that found by Rodrigues et al. (2010), however they did not analyze tocotrienols. The same authors also found β- + γ-tocopherols (7.8 mg·L^{-1}) and δ-tocopherol (7.7 mg·L^{-1}) in very low concentrations that were not detected during the analyses of Patawa oil done in this work (Table 3.5). The total tocol content was 73 mg·L^{-1}, by both PDA and fluorescence, respectively. Carotenes were not detected in Patawa oil. In Patawa chromatogram (Figure 1-D) the interfering peak (retention time approximately 26 minutes) is higher than peaks of the tocols. Comparing it with Buriti chromatogram it can be noted that the interfering compound has a different retention time compared to all other analyzed compounds, so it do not disturb the analysis.

The Tucuma oil contained all tocopherol homologues in both analyses. The most important was α-tocopherol (241 mg·L^{-1} and 234 mg·L^{-1}, by PDA and fluorescence, respectively), followed by γ-tocopherol (68 mg·L^{-1} and 65 mg·L^{-1}), β-tocopherol (37 mg·L^{-1} and 40 mg·L^{-1}) and δ-tocopherol (19 mg·L^{-1} and 22 mg·L^{-1}). The oil also presented δ-tocotrienol (25 mg·L^{-1}). Rodrigues et al. (2010) found only α-tocopherol and β- + δ-tocopherols. The total tocol content was 399 mg·L^{-1} and 385 mg·L^{-1}, by PDA and fluorescence, respectively. The carotenes content was 1934 mg·L^{-1}, a result in accordance with those found by Rodriguez-Amaya (1996). Note that tucuma oil contains more carotenes than Buriti oil (Silva et al., 2009).

Table 3.5. Tocopherols, Tocotrienols and total carotenes concentration ($mg \cdot L^{-1}$) in three Amazon Oils

Measured Concentration (mg/L)	Buriti		Patawa		Tucumã	
	FLUORO	PDA	FLUORO	PDA	FLUORO	PDA
α-tocopherol	306 ± 7	299 ± 11	38 ± 1	41 ± 7	241 ± 4	234 ± 33
β-tocopherol	759 ± 17	711 ± 31	ND	ND	37 ± 5	40 ± 0
γ-tocopherol	319 ± 17	310 ± 27	ND	ND	68 ± 1	65 ± 4
δ-tocopherol	87 ± 1	89 ± 3	ND	ND	19 ± 0	22 ± 3
α-tocotrienol	ND[a]	ND	35 ± 1	33 ± 7	ND	ND
γ-tocotrienol	LLOQ[b]	ND	ND	ND	ND	ND
δ-tocotrienol	20 ± 1	26 ± 9	ND	ND	25 ± 0	25 ± 1
TOTAL TOCOLS	1488 ± 23	1435 ± 54	73 ± 1	73 ± 26	390 ± 3	385 ± 6
Total Carotenes	1576 ± 30		ND		1934 ± 131	

[a] Not detected

[b] Detected, but in a concentration lower than LOQ

Mean concentration values obtained by PDA and fluorescence were compared using the Tukey test. There was no significant difference between mean values of each tocopherol/tocotrienol (0.95 confidence level) measured by both detectors. From that, it can be concluded that there is no interfering compound in the samples that is detected with tocols and both detectors can be used to quantify tocopherols and tocotrienols in these oils. Although, it can be noted in Table 3.5 that the fluorescence detector has in general lower values of CV%, so its use may be preferred.

The analytical procedure used by Rodrigues et al. (2010) required several sample preparation steps, including saponification. Besides being time consuming, the sequence of several preparation steps may increase the uncertainty of the results. Despite the fact that the samples used in this work and those analysed by Rodrigues et al. (2010) were obtained at different times and/or places, and also submitted to different post-harvesting processing, both works show similar results in regards to the content of tocopherols. As emphasized by Sampaio, Ceriani, Silva, Taham, & Meirelles (2010), crop seasonality and fruit ripeness are responsible for fluctuations in the composition of vegetable oils.

3.4 Concluding remarks

We have developed a simple, sensitive and reproducible HPLC method for simultaneously quantification of tocols and total carotenes. The lower limit of quantification (LOQ) was 5.0 $mg \cdot L^{-1}$ for the tocols and 0.1 $mg \cdot L^{-1}$ for carotenes, whereas the lower limit of detection (LOD) was 2.50 $mg \cdot L^{-1}$ for tocols and 0.05 $mg \cdot L^{-1}$ for carotenes. There was no significant solvent evaporation during samples storage in the autosampler, allowing the solubilisation of a large number of oil samples for each analytical run. The methodology was applied for the quantification of the mentioned compounds in Amazon oils, concluding that both PDA and fluorescence detection can be used to quantify tocopherols and tocotrienols in these vegetable oils.

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Chapter 4. "ADSORPTION OF CAROTENES AND PHOSPHORUS FROM PALM OIL ONTO ACID ACTIVATED BLEACHING EARTH: EQUILIBRIUM, KINETICS AND THERMODYNAMICS"

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Abstract

In this work, the adsorption of carotenes and phosphorus from Crude Palm Oil onto acid activated bleaching earth was investigated under bleaching conditions, i.e. high temperature (90, 105 and 115 °C) and low pressure (less than 50 mbar). Bleaching earth was added to palm oil in a range of 0.5-3.0 wt%. Results suggest that adsorption of β-carotene increases with temperature, while phosphorus adsorption was not affected. Both the pseudo-first-order and the pseudo-second-order kinetic model describe efficiently the β-carotene experimental data. Intra-particle diffusion is involved in β-carotene adsorption mechanism, although it is not the sole rate limiting step in the adsorption onto acid activated bleaching earth. Phosphorus adsorption was too fast, resulting in a lack of kinetic data. The equilibrium data were described better by Langmuir and Freundlich models, for β-carotene and phosphorus, respectively. A multi-component Freundlich type isotherm was tested. Its competition coefficients were too low, and it assumed the same form as the monocomponent Freundlich. A thermodynamic study demonstrated that β-carotene and phosphorus adsorption is spontaneous, endothermic and an entropy-driven process. Isosteric heat values suggest that the interactions between adsorbate and adsorbent are heterogeneous.

4.1 Introduction

Adsorption is a complex chemical process employed in the refining of vegetable oils during which oil impurities are removed by adsorbent materials after alkali or before physical refining (Proctor and Brooks, 2005). During bleaching, compounds such as phospholipids, colorants, soaps, contaminants and lipid peroxidation products are removed to obtain desirable characteristics in edible oils

(Zschau, 2001). Adsorptive bleaching is mostly affected by temperature and humidity, but structure and type of bleaching earth also plays a role (Gibon et al., 2007). Activated bleaching earth is the most common adsorbent used in edible oil bleaching. It adsorbs preferentially those components which are cationic or polar in nature (Zschau, 2001). Furthermore, adsorptive processes can also be used in vegetable oil industry for the removal of free fatty acids (Cren et al., 2009; Cren and Meirelles, 2005, 2012).

Crude Palm Oil (CPO) is purified by physical refining, meaning that the free fatty acids (FFA) are removed in the last refining step at high temperature (240 - 260 °C) and low pressure (less than 5 mbar), avoiding excessive loss of neutral oil during alkali neutralization (chemical refining) (Rossi et al., 2001; Sampaio et al., 2011).

To avoid color fixation during the deodorization step, bleaching needs to reduce phosphorus and iron to sufficiently low levels and minimize oxidation products (Gibon et al., 2007). In the case of carotenes, full reduction by bleaching is not necessary, as they can be thermally decomposed during the subsequent deacidification (so-called heat bleaching) (Gibon et al., 2007). In fact, in some refining processes, only 20 % of the carotenoid-related color is removed during bleaching. Remaining carotenoids are then destroyed during heat bleaching: after 20 min at 240 °C, more than 98 % of the total carotenoids are destroyed (Maclellan, 1983).

Usually, work on bleaching is dealing with color/pigments removal by physical adsorption on the bleaching earth surface. Some authors have suggested that pigments and phospholipids removal is performed through chemisorption and that bleaching earth acidity is related with its adsorption capacity for pigments (Taylor, 2005). β-carotene adsorption was studied for different types of oil such as soya (Ma and Lin, 2004), palm oil (Low et al., 1998), rapeseed oil (Sabah et al., 2007), maize and sunflower (Christidis and Kosiari, 2003). None of these studies looked at the adsorption under bleaching conditions, i.e. high temperature, low pressure and without solvent addition.

Besides pigments removal, the bleaching process is responsible for removing trace amounts of metals, adsorption of phosphorus, soaps and for decomposing primary oxidation products (Taylor, 2005).

There is a lack of scientific knowledge on phosphorus adsorption onto bleaching clays. Gutfinger and Letan (1978) studied phospholipids removal from soybean oil onto various adsorbents and proposed the Freundlich model to describe the equilibrium isotherm. However, they did not determine thermodynamics parameters. Brown and Snyder (1985) studied the removal of phospholipids from miscellas by adsorption onto silica.

Although adsorption is used on industrial scale for bleaching of edible oils, the understanding of its thermodynamic basis is still very restricted. One of the reasons for this lack of understanding is that the proposed adsorption is multicomponent, involving at least carotenes, phospholipids and, eventually, metals removal. Furthermore, the use of model systems able to correctly mimetize the studied vegetable oil is difficult due to the unavailability of pure compounds and/or the difficult solubilization of the compounds in solvent-free systems. For these reasons, the use of model systems can not properly represent all events that occur in the real system.

In this context, the objective of this work was to evaluate the adsorption of carotenes and phosphorus from Crude Palm Oil (CPO) onto acid activated bleaching earth under industrially applied conditions, i.e. high temperature, low pressure and without solvent. Acid activated bleaching earth was chosen as it is the most common adsorptive agent used industrially (Taylor, 2005). Through batch adsorption experiments, the kinetics (pseudo-first-order and pseudo-second order) and mechanism (intra-particle diffusion) of removal were evaluated. The effect of temperature on the adsorption process was also studied. Moreover, thermodynamic parameters as Gibbs free energy, enthalpy and entropy were calculated. Those results are important to better understand and to improve palm oil refining.

4.2 Material and Methods

4.2.1 Adsorbent

Tonsil OPT 210 FF (Süd Chemie, Germany), a commercial bleaching earth, was used for the adsorption experiments. It is a highly acid activated bleaching earth manufactured by acid activation of calcium bentonite with a surface area (B.E.T.) of 200 m^2/g. Particle size was characterized by a sieve analysis of the dry powder, presenting the following average values: 60 % >25 µm, 40 % >45 µm, 29 % >63 µm, 17 % >100 µm and 5 % >150 µm.(Süd-Chemie). Table 4.1 presents the bleaching earth physical chemical characterization.

Table 4.1 Physical Chemical Characterization of Tonsil OPT 210 FF (Süd-Chemie)

Apparent bulk density g/l	550
Free moisture (2 h, 110 °C) %	~ 10
Loss on ignition (predried, 2 h, 1000 °C) %	8.0
pH (10% suspension, filtered)	2.2 – 4.8
Acidity mg KOH/g	4.5
Chloride content mg Cl/g	0.5
Surface area (B.E.T.) m^2/g	200
Micropore volume	
0 - 80 nm ml/g	0.29
0 - 25 nm ml/g	0.25
0 - 14 nm ml/g	0.23

4.2.2 Oil Characterization

Crude palm oil was obtained from a local processor in Belgium and it was characterized in terms of free fatty acids (FFA) (4.6 wt%, expressed as palmitic acid) and phosphorus content (19.1 ± 0.02 mg/kg). Carotenes, which are susceptible to degradation due to oxidation, were determined before each experimental set. The initial carotenes concentration was 454 ± 5.5 mg/kg, but this initial value decreased along the storage time to 399 ± 3.3 mg/kg.

4.2.3 Batch Adsorption

Batch adsorption experiments were carried out in a rotary evaporator at a constant speed, reproducing the bleaching process of palm oil. In each test, 0.400 kg of crude palm oil was placed into 500 mL flasks and the following procedure was performed: heating the crude palm oil to 85 °C (Rotavapor, BUCHI, B-480, Switzerland); adding 0.09 % citric acid, as 30 % aqueous solution (Sigma-Aldrich ACS reagent, >99 %, Germany); high shear mixing at 16000 rpm during 1 minute (mixer, IKA-WERKE Digital, EURO-ST D, Germany); adding adsorbent (bleaching earth); 15 minutes of maturation at 85 °C and atmospheric pressure; applying vacuum (50 mbar) and maintaining the bleaching for 30 minutes at the selected temperature (Rotavap, BUCHI, R-124, Switzerland); removing the bleaching earth by filtration over a Buchner funnel and a paper filter (pore size 11 µm, Whatman). In this way, the bleaching earth contact time with palm oil sums 45 minutes. For adsorption kinetics, time zero was considered immediately after the bleaching earth (BE) addition, before the 15min maturation step. Sampaio et al. (2012) found that carotene loss during dry pretreatment with silica gel under 85 °C was lesser than 5 %. Moreover, a blank run was made to evaluate the thermal degradation during this procedure. The experiment was performed in triplicate according to the procedure previously described and using 105 °C of bleaching temperature. The initial and final concentrations of carotenes were measured. It was obtained a loss in carotenes of 6.3 ± 1.3 wt%. Based on the small amounts of carotenes lost by thermal degradation, it is assumed that almost all carotene removal is due to the adsorption process.

The kinetics of adsorption was determined at 105 °C and using 3.0 wt% of bleaching earth. To determine this value, preliminary experiments were performed using bleaching earth in a range of 0.5 to 3.0 wt%. The bleached oil was deodorized afterwards, and Lovibond color was analyzed. The experiment using 3.0 wt% of acid activated bleaching earth resulted in a fully refined oil with a light color (3.4 R) as specified by PORAM (Palm Oils Refiners Association of Malaysia)

for processed palm oil (Taylor, 2005). To obtain the adsorption isotherms, different concentrations of bleaching earth were added (ranging from 0.5 and 3.0 wt%) and agitated at 90, 105 and 115 °C. The adsorbate concentrations in solid phase q_t (mg/kg) at time t, and at equilibrium, q_e (mg/kg), were obtained by mass balance, according to Equations 4.1 and 4.2:

$$q_t = \frac{W_{oil} \cdot (C_0 - C_t)}{W_{BE}} \quad (4.1)$$

$$q_e = \frac{W_{oil} \cdot (C_o - C_e)}{W_{BE}} \quad (4.2)$$

Where W_{oil} is the weight of crude palm oil treated in kg, W_{BE} is the weight of bleaching earth in kg, C_0 is the initial concentration of adsorbate (carotenes or phosphorus) in (mg/kg), C_t and C_e are the liquid phase concentration of adsorbate at time t (min) and at equilibrium, respectively.

4.2.4 Analytical Measurements

Palm oil has a high carotenoid content, β-carotene being the most abundant (Gee, 2007). Total carotene content, expressed as β-carotene, was determined measuring the absorbance at 446 nm of samples homogenized and diluted in iso-octane (Spectrophotometer UV-240, Shimadzu Graphicord, Japan). In the same way, phosphorus in palm oil mostly occurs as inorganic phosphates (Goh et al., 1984a) and about 10 - 30 % occurs as phosphatides (Gibon et al., 2007). In this work, phosphorus content was expressed as total phosphorus, measured using an Inductively Coupled Plasma (ICP) (Thermo Scientific, iCAP 6000 series, USA) method according to the AOCS Official Method Ca 20–99 (AOCS, 1998).

4.2.5 Modeling

Adsorption models were fitted to experimental data using nonlinear regression analysis performed using Origin™ 8 and Levenberg-Marquardt

interactive method (OriginLab, 2007). The parameters and coefficients of determination (R^2) were obtained at 95 % confidence interval.

4.3 Results

4.3.1 Adsorption mechanism and kinetics

There are several mathematical models proposed to describe the kinetics of adsorption. They can be classified in two types: adsorption reaction models and adsorption diffusion models. Adsorption diffusion models are based on three consecutive steps, being (1) diffusion across the liquid film surrounding the adsorbent particle; (2) diffusion in the liquid contained in the pores and/or along pore walls, so called intra-particle diffusion; and (3) adsorption and desorption between adsorbate and active sites. The latter is a very rapid step in a physical process and can be negligible for kinetic studies (Qiu et al., 2009). Therefore, film liquid diffusion and/or intra-particle diffusion are always the limiting steps for the adsorption process and adsorption diffusion models are mostly constructed to describe one or both of those phenomena. Adsorption reaction models are based on the whole process of adsorption, without considering these steps mentioned above. In this work, we studied two reaction models and one diffusion model.

4.3.1.1 Pseudo-first-order rate equation

The pseudo-first-order equation can be expressed in a non-linear form (Equation 4.3) (Ho and McKay, 1998; Yousef et al., 2011).

$$q_t = q_e\left(1 - exp(-k_1 t)\right) \tag{4.3}$$

Where q_t and q_e are sorption capacities at time t and at equilibrium e, respectively, i.e. the amount of adsorbate per unit of adsorbent (mg/kg); k_1 is the pseudo-first-order constant t^{-1}. This model basically differs from first-order model of Lagergren as it is based on adsorption capacity instead of solution concentration.

Mostly, this model is suitable to describe the initial 20 - 30 minutes of the adsorption process (Ho and McKay, 1998).

4.3.1.2 Pseudo-second-order rate equation

The pseudo-second-order equation can be expressed in a non-linear equation (Equation 4.4) (Ho and McKay, 1998; Yousef et al., 2011).

$$q_t = \frac{q_e^2 k_2 t}{1 + q_e k_2 t} \tag{4.4}$$

where k_2 is the pseudo-second-order constant t^{-1}. The pseudo-second-order equation is based on the sorption capacity of the solid phase and it is in agreement with chemisorption being the rate controlling step (Ho and McKay, 1998). In the same way, Ho's second order-equation has been a so-called pseudo-second-order rate equation to distinguish from the one based on the solution concentration.

4.3.1.3 Intra-particle diffusion

The Weber-Morris intra-particle diffusion model is expressed as (Equation 4.5);

$$q_t = k_{id} t^{0.5} + C_i \tag{4.5}$$

where k_{id} (mg/kg min$^{0.5}$) is the intra-particle diffusion rate constant and C_i (mg/kg) is associated to the boundary layer thickness. If intra-particle diffusion is the limiting step of adsorption process, the plot q_t against $t^{0.5}$ provides a straight line. According to the Weber-Morris model, it is essential that the plot goes through the origin if intra-particle diffusion is the only rate-limiting step (Alkan et al., 2007).

The concentration plot of adsorbed carotene and phosphorus versus contact time is shown in Figure 4.1. For carotenes, the coefficient of determination were high for both pseudo-first-order and pseudo-second-order models (R^2 = 0.9992 and 0.9948, respectively, Table 4.2). The T-test shows a high accuracy in parameter

prediction for both kinetic models. The q_e values calculated by the pseudo-first-order model (14.25 × 10^3 mg/kg) and the pseudo-second-order model (q_e = 14.10 × 10^3 mg/kg) presented a good agreement with experimental data (14.40 × 10^3 mg/kg). These data suggests that both kinetic models are suitable to describe carotene adsorption data. The curve-fitting plot of intra-particle model presented high R^2, ($q_t = 478.82 \cdot t^{0.5} + 11243$, $R^2 = 0.9837$), for carotenes, but it did not pass through the origin (Figure 4.2). This result indicates that, even though intra-particle diffusion is involved in the adsorption process, it is not the sole rate limiting step (Alkan et al., 2007). Liquid film resistance may be also involved in the process. Phosphorus adsorption was faster than carotene adsorption and it reached equilibrium in less than 25 minutes of contact time. For this reason, a lack of concentration data occurred to calculate the kinetic parameters. In fact, bleaching earth adsorbs preferentially cationic and polar molecules, such as phospholipids (Zschau and Grp, 2001).

Table 4.2 Fitting kinetics and mechanism parameters of adsorption of carotene onto acid activated bleaching earth according to pseudo-first-order, pseudo-second-order and intra-particle diffusion at 105 °C (Initial concentration: carotenes 454 ± 5.5 mg/kg and phosphorus 19.1 ± 0.02 mg/kg)

		Carotenes	
	q_{exp} × 10^{-3} (mg/kg)	14.40	
			t-value
Pseudo-First-Order	q_e× 10^{-3} (mg/kg)	14.25±0.06	113
	K_1 (min^{-1})	0.16±0.01	20
	R^2	0.9992	
	F-test	11187	
	P-value	<0.0001	
Pseudo-Second-Order	q_e × 10^{-3} (mg/kg)	14.10±0.27	205
	K_2 × 10^3 (kg/mg min^{-1})	0.08±0.01	17
	R^2	0.9948	
	F-test	4587	
	P-value	<0.0001	
Intra-particle	K_{id} × 10^{-3} (mg/kg $min^{0.5}$)	478.82±4.40	47
Diffusion	C_i	11243±23	11
	R^2	0.9837	
	F-test	120	
	P-value	0.0083	

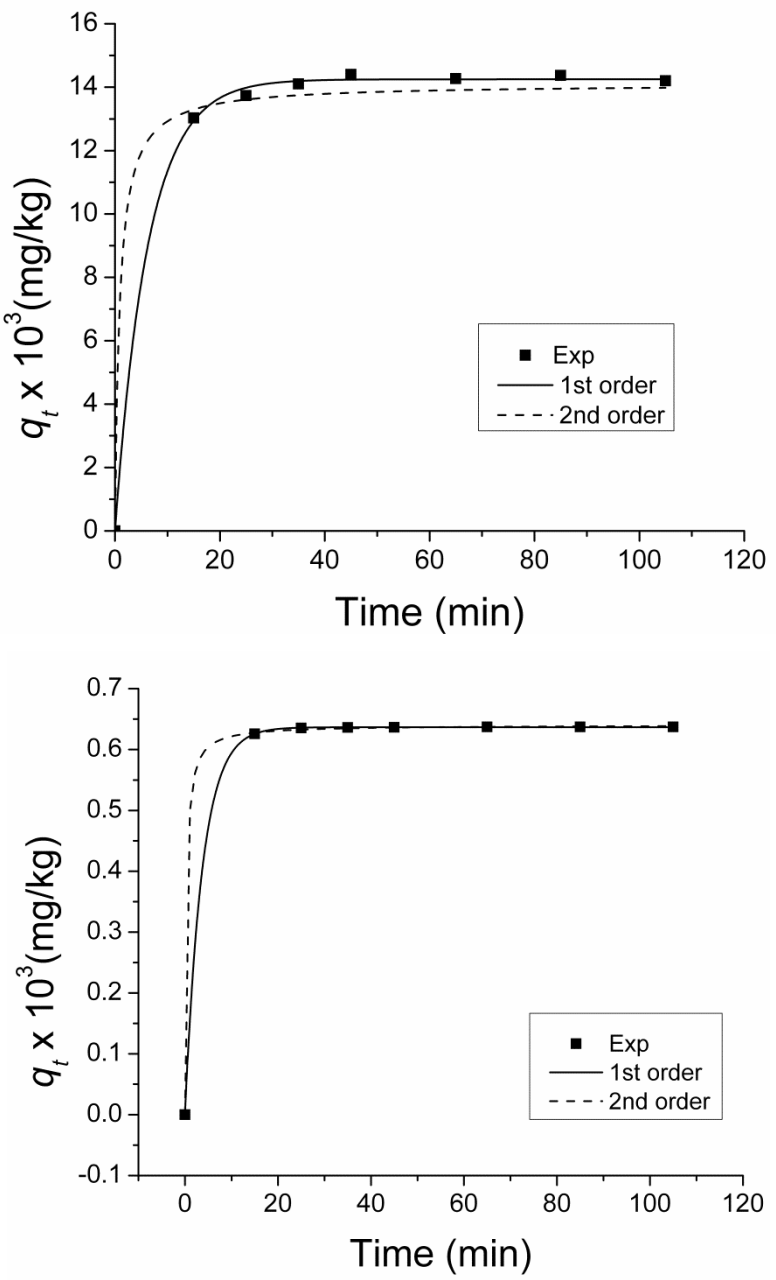


Figure 4.1. Adsorption kinetics of Carotenes (A) and phosphorus (B) onto acid activated bleaching earth at 105 °C and using 3.0 wt% of bleaching earth. (Initial concentration: carotenes 454 ± 5.5 mg/kg and phosphorus 19.1 ± 0.02 mg/kg)

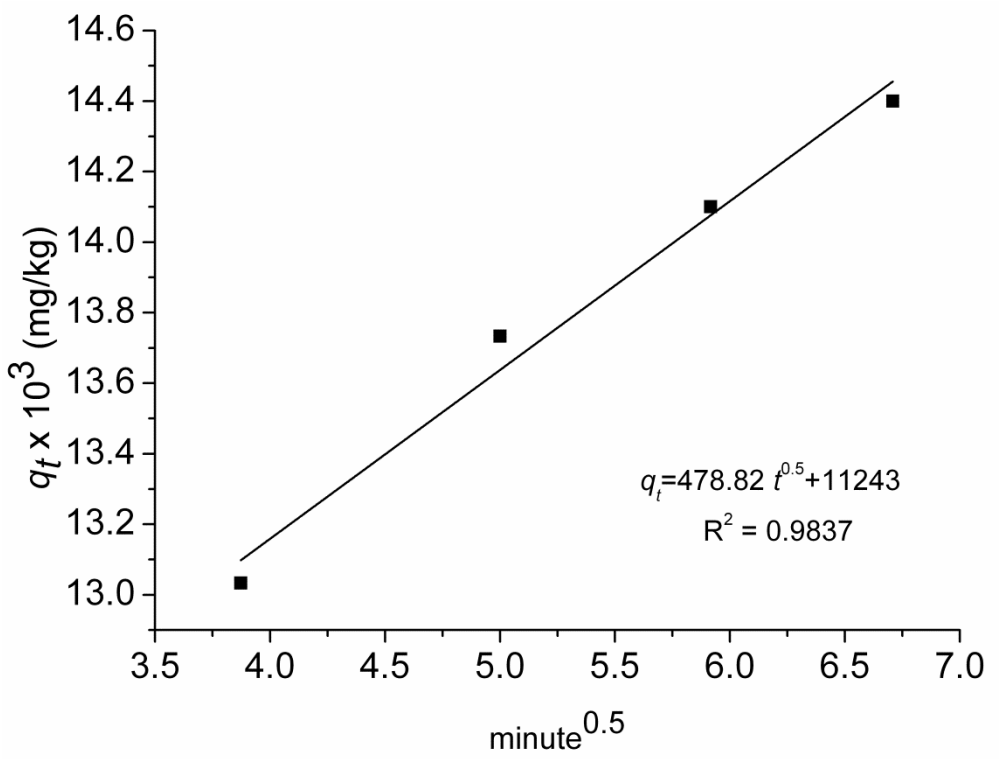


Figure 4.2. Intra-particle diffusion plots for the adsorption of β-carotenes onto acid activated bleaching earth at 105 °C and using 3.0 wt% of bleaching earth

4.3.2 Isotherm studies

Adsorption equilibria information is important to understand, to design and to implement the process. The temperature affects adsorption capacity of adsorbents. Thus, isotherms can provide the relation between the amount of a solute adsorbed at a constant temperature and its concentration in the equilibrium solution (Yousef et al., 2011). In this work, four adsorption isotherms were applied to evaluate adsorption of carotenes and phosphorus onto bleaching earth: Langmuir, Freundlich, Temkin and Multi-component Freundlich.

4.3.2.1 Langmuir

The Langmuir model was the first model presenting a coherent theory of adsorption. It assumes that the adsorbent surface is homogenous, adsorption is localized in specific sites, which can adsorb only one adsorbate molecule (Do, 1998). It is generally expressed as (Equation 4.6):

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \quad (4.6)$$

where q_e (mg/kg) is the amount of adsorbate per unit mass of adsorbent, C_e (mg/kg) is the equilibrium concentration of adsorbate in solution, q_{max} (mg/kg) and K_L (mg/kg)$^{-1}$ are Langmuir constants related to the adsorption capacity and rate of adsorption, respectively.

4.3.2.2 Freundlich

The Freundlich model is empirical (Do, 1998), assuming a heterogeneous surface energy, i.e. stronger binding sites are occupied first and the binding strength decreases with an increasing degree of site occupation (Yousef et al., 2011). It is described as in Equation 4.7:

$$q_e = K_F C_e^n \quad (4.7)$$

where K_F [(mg/kg)·(mg/kg)$^{-n}$] is defined as the adsorption capacity of the adsorbent. The *n* values range from zero to one, reflecting the adsorption intensity or surface heterogeneity.

4.3.2.3 Temkin

The Temkin model is also an empirical one and does not assume a saturation limit. It takes into account indirect interactions between adsorbate and adsorbent and is expressed as follow (Equation 4.8 and 4.9):

$$q_e = \frac{TR}{b} ln(K_T C_e) \quad (4.8)$$

$$B = \frac{TR}{b} \quad (4.9)$$

where R is the ideal gas constant (8.134 J/mol K), T is the absolute temperature, K_T (mg/kg)$^{-1}$ is the equilibrium binding constant corresponding to the maximum binding energy and B (mg/kg) is related to the heat of adsorption (Do, 1998; Yousef et al., 2011).

4.3.2.4 Multi component isotherms

A Freundlich based isotherm was chosen to study multi component adsorption of carotenes and phosphorus onto acid activated bleaching earth, as Freundlich model correlated well both monocomponent experimental data. It is written in the form (Equation 4.10) (Sheindorf et al., 1982):

$$q_i = K_i C_i \left(\sum_{j=1}^{k} a_{ij} C_j \right)^{n-1} \tag{4.10}$$

where a_{ij} (kg/mg) describes the inhibition to the adsorption of component i by component j, and can be determined from thermodynamic data, or more likely, from experimental data of bicomponent systems. The equation is based on the assumption that each component individually obeys the Freundlich isotherm and that for each component in a multicomponent adsorption there is an exponential distribution of adsorption energy sites (Sheindorf et al., 1982).

Table 4.3 presents the carotenes and phosphorus removal from crude palm oil by acid activated bleaching earth. For both adsorbents, there is a greater removal when using more bleaching earth, as expected. Concerning to the temperature influence, Table 4.3 shows that for carotenes there is a clear trend of improving removal with higher temperatures. In case of phosphorus adsorption, the influence of temperature on the percentage of removal seems to be negligible, with practically the same removal values in the entire range of temperatures investigated. In the conditions tested, it was possible to reach a removal up to 99 % of both compounds.

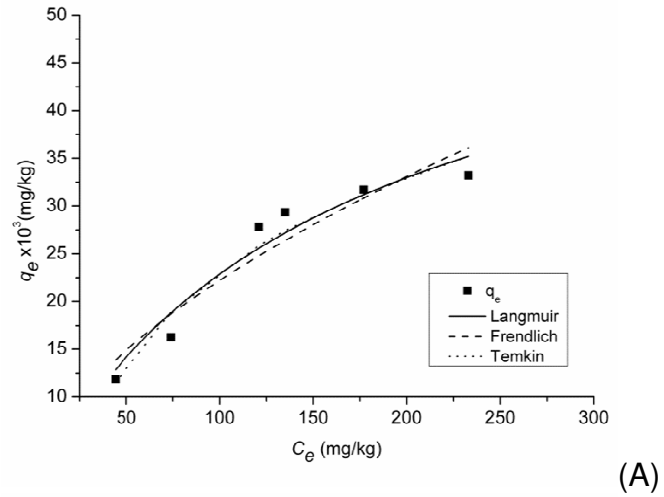
Table 4.3. Removal of carotenes and phosphorus from Crude Palm oil onto acid activated bleaching earth at 90, 105 and 115 °C

	90 °C			105 °C			115 °C		
BE (%)	C_e mg/kg	$q_e \times 10^{-3}$ mg/kg	% *removal*	C_e mg/kg	$q_e \times 10^{-3}$ mg/kg	% *removal*	C_e mg/kg	$q_e \times 10^{-3}$ mg/kg	% *removal*
Carotene									
0	399			454			399		
0.5	233	33	42	258	42	43	96	43	76
0.7	177	32	56	178	41	61	52	39	87
0.9	135	29	66	128	38	72	47	35	88
1.0	121	28	70	93	37	80	21	25	95
2.0	74	16	81	40	21	91	15	19	96
3.0	44	12	89	16	15	96	10	13	97
Phosphorus									
0	19.1	3.3		19.1			19.1		
0.5	2.9	2.4	85	2.7	3.3	86	3.4	3.2	82
0.7	2.3	1.9	88	2.3	2.4	88	2.5	2.4	87
0.9	1.8	1.8	91	1.8	1.9	91	1.9	1.9	90
1.0	1.3	1.2	93	1.3	1.8	93	1.5	1.8	92
2.0	0.4	0.6	98	0.4	0.9	98	0.3	0.9	98
3.0	0.3	0.6	98	0.2	0.6	99	0.1	0.6	99

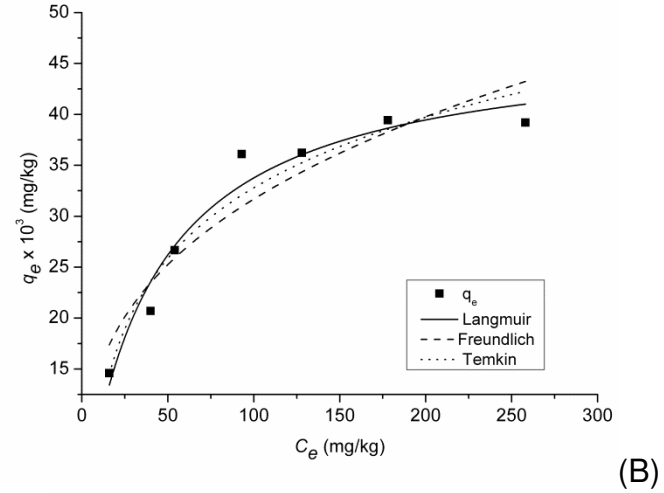
Langmuir, Freundlich and Temkin parameters were determined from non-linear fitting for both carotenes and phosphorus and results are presented in Table 4.4. For carotenes adsorption at 90 °C, the Temkin model presented the highest R^2 (0.9524). At 105 °C and 115 °C, the Langmuir model described the experimental data in a better way (R^2 = 0.9691 and R^2 = 0.9917, respectively). Our results are in accordance to previous works found in the literature. Low et al. (1998) studied the decolorization of CPO using acid activated spent bleaching clay, presenting Langmuir a better adjustment to experimental data, but Freundlich still presenting a good description. The same results were observed by Ahmad et al. (2009) in the adsorption of β-carotene from a n-hexane solution onto a silica-based adsorbent. On the other hand, in the study perfomed by Boki et al. (1992), Freundlich isotherm presented a better agreement with pigments adsorption. However, it should be highlighted that, this study used different kinds of oil (rapessed, soybean, wheatgerm, safflower, corn, cottonseed and sunflower) in which β-carotene is not the most important pigment. The calculated isotherms are plotted in Figure 4.3.

Table 4.4 Isotherms constants for carotenes and phosphorus adsorption onto Tonsil OPT 210 FF at 90, 105 and 115 °C.

		Carotenes						**Phosphorus**					
Model	Parameters	**90 °C**	t-	**105 °C**	t-	**115 °C**	t-	**90 °C**	t-	**105 °C**	t-	**115 °C**	t-
Langmuir	$q_{max} \times 10^{-3}$ (mg/kg)	59.21±11.15	5.3	50.53±2.73	18.5	57.05±2.99	19.3	5.23±1.39	3.8	5.18±1.70	3.1	3.93±1.05	3.7
	K_L $(mg/kg)^{-1}$	0.01 ± 0.00	2.8	0.03±0.00	5.7	0.04±0.01	7.4	0.43±0.14	3.1	0.45±0.17	2.7	0.70±0.28	2.5
	R^2	0.9453		0.9691		0.9917		0.9267		0.9019		0.8532	
	F-test	388		878		1428		179		132		92	
	P-value (95 %)	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.0002	
Freundlich	$K_F \times 10^3$ $[(mg/kg)\cdot(mg/kg)^{-n}]$	1.57±0.86	1.8	6.84±1.86	3.7	5.98±1.49	4.0	1.53±0.10	15.3	1.57±0.12	13.5	1.59±0.10	15.1
	n	0.58±0.11	5.3	0.34±0.06	6.1	0.45±0.06	7.1	0.64±0.08	8.2	0.62±0.10	6.7	0.48±0.07	7.0
	R^2	0.9048		0.9106		0.9260		0.9533		0.9390		0.9469	
	F-test	222		312		272		283		213		258	
	P-value (95 %)	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Temkin	K_T $(mg/kg)^{-1}$	0.051±0.011	8.9	0.24±0.08	9.7	0.28±0.04	7.7	6.01±1.65	3.6	8.88±3.73	2.38	25.25±16.92	1.5
	$B \times 10^{-3}$(mg/kg)	14.23±1.59	4.8	10.77±1.10	3.1	13.72±0.79	17.3	0.97±0.13	7.2	0.83±0.15	5.48	0.57±0.12	4.9
	R^2	0.9524		0.9523		0.9830		0.9121		0.8571		0.8297	
	F-test	446		577		1252				89		79	
	P-value (95 %)	<0.0001		<0.0001		<0.0001				0.0002		0.0003	
Freundlich	$Ki \times 10^3$ $[(mg/kg)\cdot(mg/kg)^{-n}]$	1.57±0.70	2.2	6.90±1.47	4.7	5.98±1.22	4.9	1.56±1.30	1.2	1.57±1.04	1.2	1.53±1.61	0.9
Type	a_{ij} $(mg/kg)^{-1}$	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Multi	n_i	0.57±0.09	6.5	0.34±0.04	7.8	0.45±0.05	8.7	0.61±1.00	0.6	0.62±1.04	0.6	0.53±1.04	0.5
	R^2	0.9607		0.9738		0.9735		0.9607		0.9738		0.9735	



(A)



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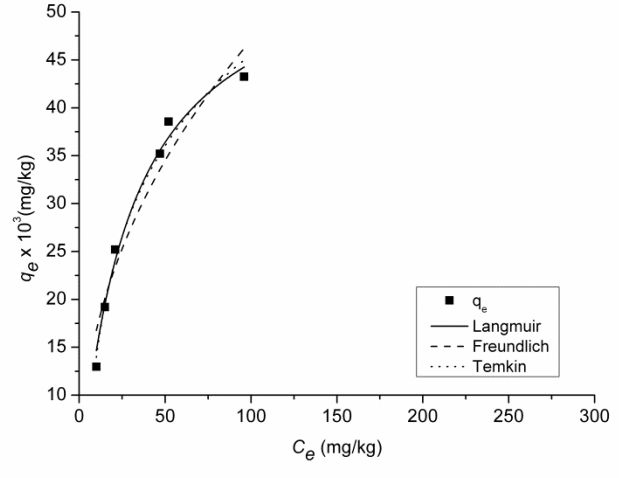


(C)

Figure 4.3. Adsorption isotherm plots for carotenes onto acid activated bleaching earth at (A) 90 °C, (B) 105 °C and (C) 115 °C.

It is interesting to note that better agreements between experimental and theoretical data were observed at higher temperatures, according to the higher R^2 values. The T-test shows a low accuracy in parameter estimation by Langmuir model at 90 °C. For all other models and temperatures, T-test shows a high accuracy, i.e. t-values > than standard deviation. The lowest q_{max}, i.e. maximum adsorption capacity and the complete coverage of adsorbent surface by adsorbate, was obtained at 105 °C. The highest value was obtained at 90 °C, however, as previously discussed, Langmuir model did not present a good accuracy at this temperature.

The Langmuir parameter K_L presented a positive correlation with temperature, i.e. increasing the temperature the affinity between adsorbent and adsorbate also increases.

Phosphorus adsorption was described more accurately by the Freundlich model, presenting R^2 higher for all tested temperatures (R^2 = 0.9533, 0.9390 and 0.9469 for 90 °C, 105 °C and 115 °C, respectively, Table 4.4). The T-test shows a high accuracy in parameter estimation of all models tested for phosphorus. K_F values, a measure of adsorption capacity, increases with temperature for phosphorus adsorption. Gutfinger and Letan (1978) observed that the Freundlich isotherm described the adsorption of phospholipids from soybean oil onto silica gel, which is an empirical model that takes into account different energy of interactions between adsorbent and adsorbate. The same result was observed by Brown and Snyder (1985). In both studies, *n* values were lower than 1, as in our work. Considering that Langmuir model was the best to describe the carotenes adsorption, it can be inferred that heterogeneity is due to the adsorbate. In fact, phosphorus occurs in vegetable oils as phospholipids, a lipid class which can have different properties depending on its organic chain.

Calculated isotherms for phosphorus are plotted in Figure 4.4. The fit accuracy of Freundlich models can be observed for the entire concentration range. The Temkin model presented the highest deviations from experimental data at lower concentration, which is confirmed by low R^2 values.

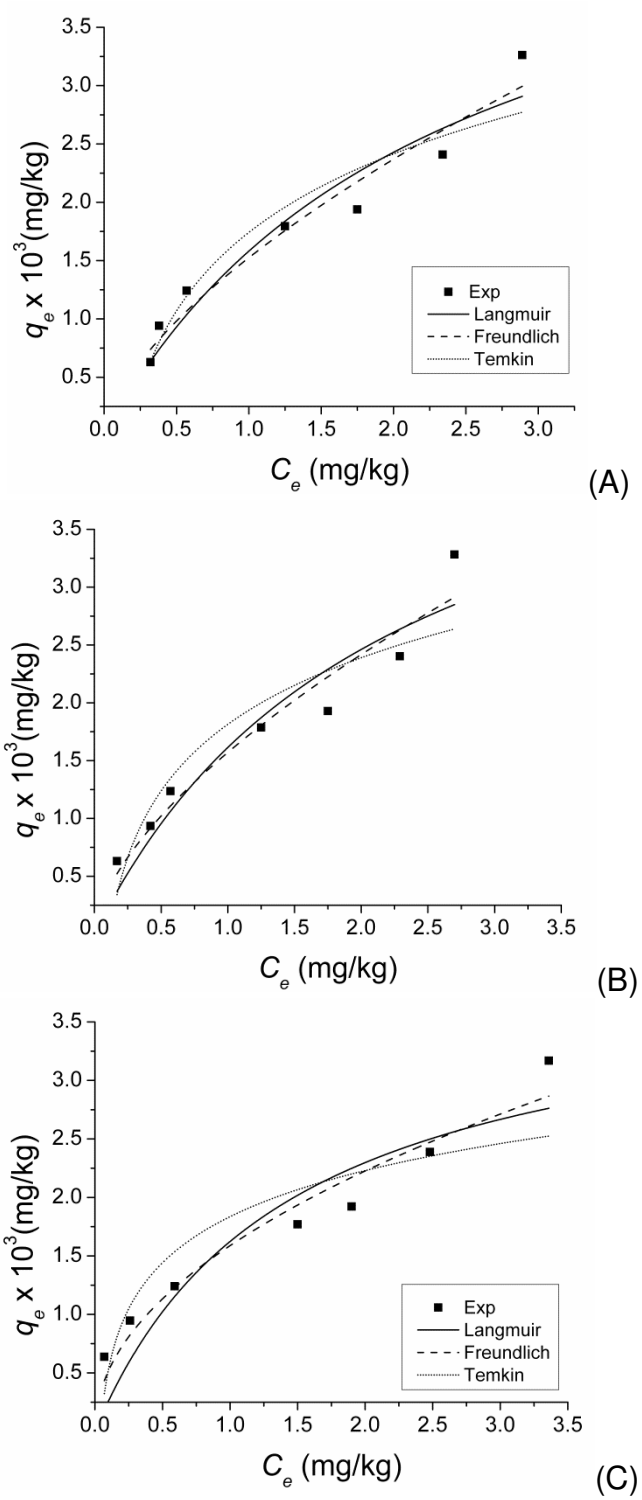


Figure 4.4. Adsorption isotherm plots for phosphorus onto acid activated bleaching earth at (A) 90 °C, (B) 105 °C and (C) 115 °C.

Parameters for multi component Freundlich type isotherm are shown in Table 4.4. The competition coefficients a_{ij} presented low values, lower than 0.0001 kg/mg, so that the multi component Freundlich type isotherm assumes the same form as the monocomponent Freundlich model. It is interesting to highlight that the coefficients K_i and n_i obtained for the mono and multi component Freundlich equations are quite similar, differing, in the worst case, in the magnitude of the second decimal. For that reason, it can be concluded that under the tested conditions, i.e. bleaching conditions and initial adsorbates' concentrations, the competitive effects of carotenes on phosphorus adsorption and vice-versa are not important.

4.3.2.5 Estimation of thermodynamic parameters

Thermodynamic parameters can provide useful information to design an adsorption process. For instance, they indicate if the process is spontaneous or not and if it is exo- or endothermic. Thermodynamic parameters are calculated using the variation of equilibrium constants K_0 (or the solute coefficient of distribution between the solid and liquid phases at equilibrium) which changes with temperature (Khan and Singh, 1987; Zuim et al., 2011) (Equation 4.11).

$$K_0 = \lim_{q_e \to 0} \frac{q_e}{C_e} \tag{4.11}$$

The standard Free energy (ΔG^0_{ads}) was calculated by Equations 4.12 and 4.13 (Calvet, 1989; Khan and Singh, 1987; Zuim et al., 2011). The standard Enthalpy (ΔH^0_{ads}) and standard entropy (ΔS^0_{ads}) were calculated by Van't Hoff equation (Equation 4.13).

$$\Delta G^0_{ads} = -RTln(K_0) \tag{4.12}$$

$$ln(K_0) = -\frac{\Delta G^0_{ads}}{RT} = \frac{\Delta S^0_{ads}}{R} - \frac{\Delta H^0_{ads}}{RT} \quad (4.13)$$

where *R* (8.134 J/mol K) is the universal gas constant, and *T* (K) is the absolute bulk temperature. The thermodynamic parameters are presented in Table 4.5. Both carotenes and phosphorus presented negative values for Gibbs Free Energy, indicating that the adsorption process of the studied compounds onto Tonsil OPT 210 FF is feasible and spontaneous (Srivastava et al., 2006). The magnitude of the Gibbs free energy increases with temperature, showing that adsorption is more spontaneous at higher temperatures. The phosphorus adsorption presented higher absolute values for Gibbs free energy, suggesting that this process is more spontaneous than β-carotene adsorption, which is in accordance with the kinetic results found in this work (section 4.4.3.1).

Table 4.5 Equilibrium constants, standard Gibbs free energy, enthalpy and entropy of carotenes and phosphorus adsorption from Crude Palm Oil onto Tonsil OPT 210 FF at 90 °C, 105 °C and 115 °C

	Carotenes				Phosphorus			
°C	K_0	ΔG^0_{ads} (kJ/mol)	ΔH^0_{ads} (kJ/mol)	ΔS^0_{ads} (kJ/mol K)	K_0	ΔG^0_{ads} (kJ/mol)	ΔH^0_{ads} (kJ/mol)	ΔS^0_{ads} (kJ/mol K)
90	333	-17.15	126.65	0.40	2731	-23.37	46.17	0.19
105	1944	-23.29			3516	-25.11		
115	5210	-27.02			7947	-28.35		

On the other hand, ΔH^0_{ads} was positive for both compounds at all temperatures, indicating an endothermic process, i.e. an increase in temperature improves adsorption. It is also indicative for a chemical adsorption process, as physisorption is always exothermic (Erbil, 2006). Those results are in agreement with Sarier and Guler (1988) stating that β-carotenes are chemisorbed onto acid activated bleaching earth. Ahmad et al. (2009) also found that β-carotene adsorption from hexane solutions onto silica gel was an endothermic process.

ΔS^0_{ads} was positive indicating that the randomness at solid/liquid interface increased during the adsorption process (Srivastava et al., 2006; Zuim et al., 2011), i.e. the molecules are in a less ordered state than in solution, as a result of redistribution of energy between the adsorbate and the adsorbent (Ahmad et al., 2009). Positive values for entropy during β-carotene adsorption onto silica gel (Ahmad et al., 2009) and sepiolite (Sabah et al., 2007) were also observed in previous studies.

The positive values of ΔH^0_{ads} and ΔS^0_{ads} indicate that both carotene and phosphorus adsorption onto acid activated bleaching earth is entropy-driven rather than energy driven. The reason of this gain in entropy may be related to a release of compounds and other species originally presented in the adsorbent surface (Bouhamed et al., 2007; de Reese and Plank, 2011). For instance, in the present case it is possible that water molecules originally present in the adsorbent due to its natural humidity be released as a consequence of the adsorption process. Moreover, knowing the relative portion of enthalpic and entropic contribution allows the adsorption optimization by modifying the process conditions, for instance, the adsorption temperature.

4.3.2.6 Isosteric Heat

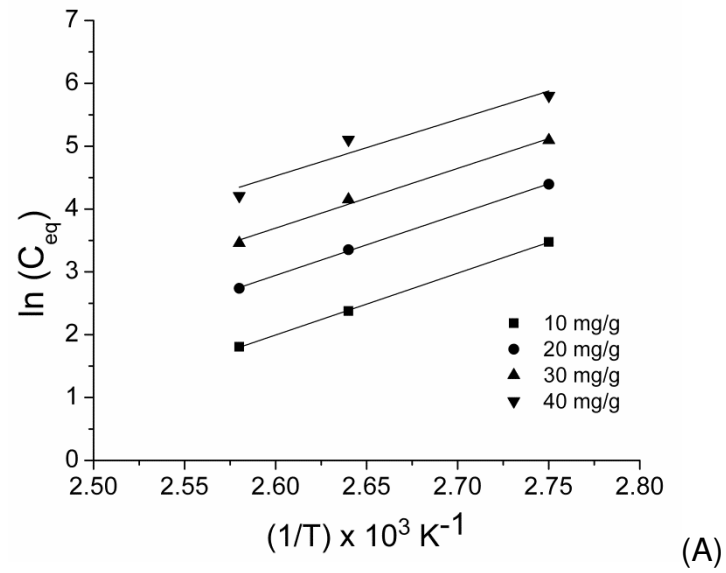
The heat of adsorption depends on the adsorbent fraction covered. The heat of adsorption at a constant surface area, denominated isosteric heat of adsorption, can be calculated by the following equations (Equations 4.12 and 4.13) (Do, 1998; Erto et al., 2010):

$$\frac{d(lnC_e)}{dt} = -\frac{\Delta H_{st,a}}{RT^2} \tag{4.12}$$

$$\Delta H_{st,a} = R\frac{d(lnC_e)}{d(1/T)}\bigg|_{q_e} \tag{4.13}$$

Carotenes and phosphorus equilibrium concentrations at a constant amount of adsorbed compounds were determined through Langmuir isotherms. The isosteric heat of adsorption was obtained from the slope of the plot of ln(C_e) versus

(1/*T*) for different amounts of adsorbed adsorbates (Figure 4.5). The $\Delta H_{st,a}$ vary with surface loading for both carotenes and phosphorus (Figure 4.6), indicating that interaction between Tonsil OPT 210 FF and the studied compounds are energetically heterogeneous. In fact, when the adsorbent surface has different energetic sites, molecules will adsorb preferentially at sites with the highest energy of adsorption and progressively go to lower energetic ones, resulting in a decrease of the heat of adsorption with the loading (Do, 1998). This can be due to differences in adsorbent surface or difference in adsorbed molecules. In fact, crude palm oil has different carotenoids, mostly α- and β-, (Gee, 2007) and phospholipids (Goh et al., 1984b), which in this work are all expressed as β-carotenes and phosphorus, respectively. Positive values of $\Delta H_{st,a}$ confirm that the adsorption is endothermic (Do, 1998; Srivastava et al., 2006).



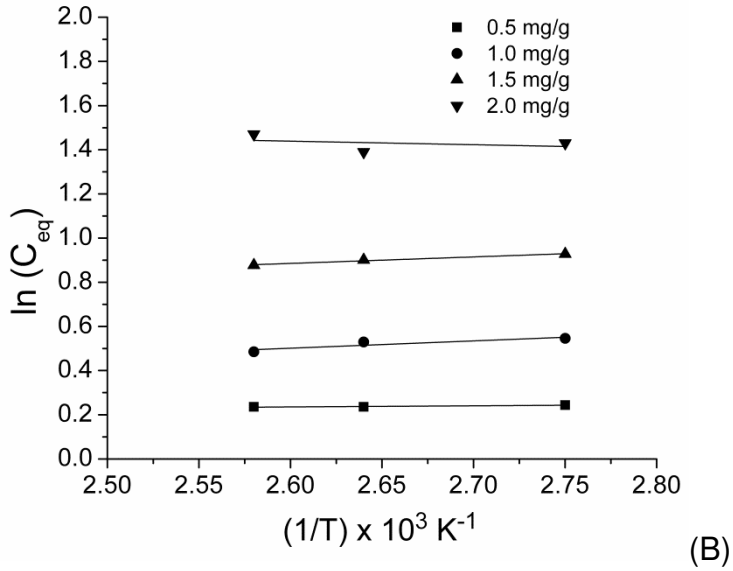


Figure 4.5. Adsorption isosters used to determine the isoteric heat of carotenes (A) and phosphorus (B)

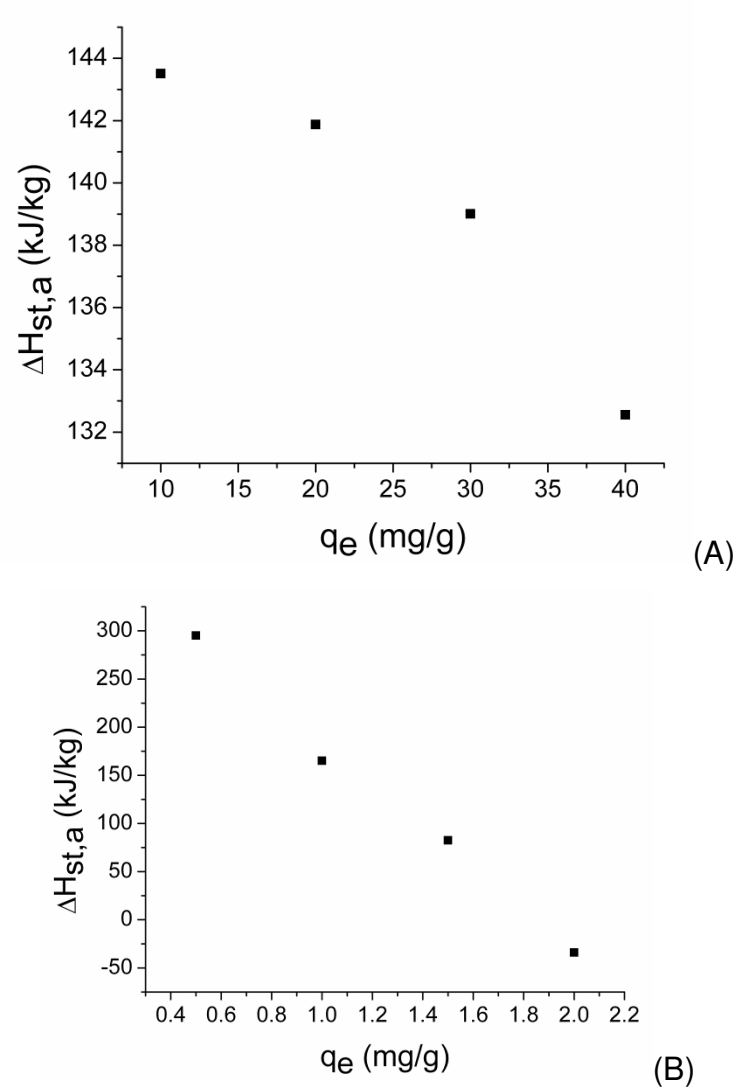


Figure 4.6. $\Delta H_{st,a}$ as a function of the amount adsorbed of carotenes (A) and phosphorus (B)

4.4 Conclusion

Results presented in this work suggest that adsorption of carotenes and phosphorus onto acid activated bleaching earth increases with temperature. It was possible to reach a removal up to 99 % of both compounds at tested conditions. The pseudo-second-order kinetic model describes the β-carotene experimental data accurately. Intra-particle diffusion is involved in β-carotene adsorption process, although it is not the sole rate limiting step in the adsorption onto Tonsil

OPT 210 FF. The equilibrium data are described more accurately by Langmuir and Freundlich models, for β-carotene and phosphorus, respectively. A thermodynamic study demonstrated that β-carotene and phosphorus adsorption is spontaneous, endothermic and an entropy-driven process. Isosteric heat values suggest that interaction between adsorbate and adsorbent are heterogeneous. High temperatures are better for adsorptive removal of carotenes and phosphorus. However, industrially it should be taken into account other factors, as unwanted side reactions, to establish the optimum bleaching temperature.

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Chapter 5. "INFLUENCE OF REFINING PRACTICES ON PALM OIL COLOR"

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Abstract

In this work the effect of different bleaching procedures on the final color of palm oil final color, i.e. after bleaching and deodorization, was tested using both neutral and acid activated bleaching earth. Firstly, the effect was evaluated on the final color of storage between bleaching and deodorization. Deodorization procedures were performed immediately after and after 3 and 7 days of storage time. The best color was obtained after storing the oil for 3 days under a nitrogen at deep-freezing (-18 °C). Secondly, the effect was evaluated on final color after a maturation step with citric acid before bleaching earth addition and an extra-drying step. The maturation step was advantageous in case of the process using neutral bleaching earth, whereas a reference procedure gave the best result with an acid activated bleaching earth. Lastly, a new refining approach in two-steps was suggested, leading to a color improvement in comparison to the reference procedure using the same amount of bleaching earth and deodorization residence time. The two-step refining has to be further optimized concerning the process parameters and the kind of adsorbent used.

Nomenclature:

ABE: Acid Activated Bleaching Earth

BE: Bleaching Earth

FFA: Free Fatty Acid

NBE: Neutral Bleaching Earth

P1, P2, P3: Procedure 1, 2 and 3, respectively

p-AnV: p-anisidine value

PV: Peroxide Value

$T_{addition}$: temperature of the oil before bleaching earth addition

T_{BE}: temperature of the oil during adsorption process

W_{CA}: amount of added citric acid, expressed in %

W_{water}: amount of added water, expressed in %

5.1 Introduction

Prior to human consumption, crude palm oil (CPO) has to be refined to remove minor compounds as phospholipids, free fatty acids, oxidation products and other undesirable compounds. Phospholipids, for instance, should be removed for several reasons: they have emulsifying properties, which can increase neutral oil losses; they are usually connected to metals that are the main cause of oxidative stability reduction; and further, they can cause color inversion [3]. Especially in oils submitted to physical refining, phospholipids must be removed to avoid color fixation that can occur due to the high temperatures employed during the deodorization step. In the case of palm oil, physical refining is usually preferred [4], including a degumming pretreatment, a bleaching step and a high-temperature, low pressure step [8].

For palm oil, dry degumming is usually chosen [4]. It is integrated into the bleaching operation and involves introduction of acid, usually phosphoric acid, with the combination of either a brief retention time, high temperature and high shear retention, or a longer retention time, lower temperature, and a less vigorous agitation system. The acid and the precipitated phosphatides are removed in the subsequent bleaching operation [1]. The phosphoric acid dosage is a critical point: under- or over-dosage can promote darkening during deodorization. Also, it is possible that some phosphorus containing compounds originating from the acid is retained by the oil [3]. An alternative is citric acid addition, but for economical reasons phosphoric acid is mainly used by Malaysian refiners [4].

After a dry-pretreatment, palm oil is bleached at 95 – 110 °C and <50 mbar. Refining cannot be done without bleaching even for oils with a very low phospholipid content, except in raw material of excellent quality and with low

content of oxidation products [10]. Bleaching can be performed in batch or continuous units. The batch procedure has as advantages simplicity of operation and flexibility [3], and is usually conducted in multi-purpose units in which neutralization and bleaching are conduced, as well as the pretreatment for physical refining [10]. Continuous and semi-continuous processes are used to treat large quantities of the same type of oil and they have as advantages reduced energy consumption and simple process control [10]. More recently, a multi-step process has been developed in order to reduce bleaching earth consumption, and it can be co-current or counter-current. In the co-current process bleaching earth is added in two consecutive steps, with a filtration after each addition. The counter-current procedure re-uses bleaching earth from the first one in the second step, resulting in saving up to 15 % [4]. All those processes should be optimized in each industrial plant according to the quality of the oil to be processed and to the empirical experience of engineering companies and processors.

Bleaching is by far the most expensive process in edible oil refining [4], due to the relative high cost of bleaching clays as well as neutral oil losses in the earth and the disposal costs of bleaching adsorbents [3]. The bleaching step is the most delicate step of the refining procedure. In general, it has been insufficiently exposed to proper engineering and it is open to developments mainly in the fundamentals of the process. In that way, it is clear that an optimization of the process can reduce significantly the costs of refining. Some studies have been reported in the literature to optimize bleaching. Langmaack and Eggers [5] studied the optimization of bleaching of rapeseed oil by improving mass transfer between oil and adsorbent. Skevin et al. [9] studied the effect of bleaching parameters, i.e. temperature, duration and clay content on oxidative stability, bleaching efficiency and concentration of bioactive compounds. Rossi et al. [8] studied the effect of bleaching and physical refining on palm oil color and its content of carotenoids and tocopherols. It is interesting to note that those studies do not evaluate different or new procedures during refining.

In this context, the objective of this work was to evaluate the effect of different procedures on the quality of fully refined (RBD) palm oil, especially the color. The study was divided in several steps to cover different aspects of palm oil refining. Firstly, the effect of storage time between the bleaching and deodorization steps, especially its effect on the final oil color obtained after deodorization. This procedure was important for establishing a appropriate lab methodology for deodorizing all dry pre-treated oil samples. Secondly, the effect of a maturation step with citric acid before adding bleaching earth was tested. Here, the effect of bleaching temperature was evaluated. Finally, multi-step procedures were tested. All trials were performed using an acid activated bleaching earth (here, so-called ABE) and a neutral bleaching earth (here, so-called, NBE).

5.2 Material and Methods

5.2.1 Reagents

5.2.1.1 Palm oil

Crude palm oil was obtained from a local processor in Belgium (Fuji Oil, Ghent, Belgium). Crude, bleached and full refined palm oil were characterized in terms of free fatty acids (FFA), color, carotenes and elements content, p-Anisidine (p-AnV) and Peroxide (PV) Values. The deterioration of the bleachability index (DOBI) was determined for crude palm oil.

5.2.1.2 Bleaching Earth

Batch trials were performed using two types of bleaching earth: non-activated bleaching earth (Pure Flo B80, provided by Oil-Dri) and acid activated bleaching earth (Tonsil Opt. 210 FF, provided by Süd-Chemie AG).

5.2.2 Analytical

5.2.2.1 Color:

Sample color was determined according to AOCS Official Method Cc 13e-92, using a Lovibond Tintometer Color Scale at 70 °C, ensuring that color was determined in a completely melted sample. Analyses were carried out using 5" ¼ (133.4 mm) glass cells. In the cases of crude palm oil samples, color was determined using a 1" (25.4 mm) glass cell [2].

5.2.2.2 Free Fatty Acids (FFA)

Free Fatty Acids content (FFA) was determined according to AOCS Official Method Ca 5-40, by titration. Results were expressed as percentage of palmitic acid (C16:0, MW = 256 g/mol) [2].

5.2.2.3 Deterioration of the bleachability index (DOBI)

The deterioration of the bleachability index (DOBI) was measured using a UV-vis spectrophotometer. It is a numerical value of the ratio of the spectrophotometric absorbance at 446 nm (non-oxidized carotenes maximum) to the absorbance at 268 nm (oxidized carotenes maximum) [4].

About 0.1 mg of completely molten and homogenized oil is diluted in 25 mL of iso-octane. Absorbances are measured at 268 nm and 446 nm. The DOBI value is obtained by Equation 5.1.

$$\mathrm{DOBI} = \frac{\text{absorbance at } 446 \text{ nm}}{\text{absorbance at } 268 \text{ nm}} \qquad \text{Eq. 5.1}$$

The higher the DOBI value, the lower the amount of oxidized carotenes, the easier the palm oil will be heat bleached, as color due oxidized carotenes is difficult to remove. DOBI values between 2.5 and 4.0 indicate average to good crude oil quality. Values below 2.0 indicate a poor quality crude palm oil which is difficult to bleach [4].

5.2.2.4 Carotene Content

The carotene content, expressed as β-carotene, was determined measuring the absorbances at 446 nm of homogenized samples and diluted in iso-octane, according to the PORIM official method [7].

5.2.2.5 p-Anisidine and Peroxide values

The peroxide (PV) and p-anisidine (p-AnV) values were determined according to the AOCS Official Method Cd 8-53 and Cd 18-90, respectively [2].

5.2.2.6 Elements Content

The Phosphorus (P) and Iron (Fe) content was measured using an Inductively Coupled Plasma (ICP) method, according to the AOCS official method Ca 20-99, using kerosene as solvent [2].

5.2.3 Refining Procedure

Batch adsorption experiments were carried out in a rotary evaporator at a constant speed, reproducing the bleaching process of palm oil. In each test, crude palm oil was placed into a flask and a reference procedure, established according to usual dry pre-treatment practices, was performed. This reference procedure, here so-called *P1*, is described as follows: heating the crude palm oil; adding citric acid; high shear mixing (16000 rpm during 1 minute); adding adsorbent (bleaching earth); 15 minutes of maturation at 85 °C and atmospheric pressure; applying vacuum (50 mbar) and maintaining the bleaching for 30 minutes at the selected temperature; removing the bleaching earth by filtration over a Buchner funnel and a paper filter (pore size 11 µm, Whatman). The bleaching parameters varied in each trial.

After bleaching, the oil was submitted to a deodorization step since some color effects can only be observed after heat bleaching. The deodorization was always carried out one day after the bleaching to avoid differences due to storage effects. The deodorization was performed in a lab-scale equipment described by

Petrauskaité et al. [6]. Deodorization parameters were fixed and set as 260 °C of deodorization temperature, 3.0 mbar, 1.5 % sparge steam injection and 60 minutes of residence time.

Some changes in *P1* (Reference Procedure) were implemented to evaluate the final color, i.e. the color after bleaching and deodorization step. The first change consisted in the addition of a maturation step before addition of the bleaching earth, and the procedure *P2* becomes as follows: heating the crude palm oil; adding citric acid; high shear mixing; 15 minutes of maturation at 85 °C and atmospheric pressure; adding adsorbent; applying vacuum (50 mbar) and maintaining the bleaching for 30 minutes at the selected temperature; removing the bleaching earth by filtration over a Buchner funnel and a paper filter.

The second change consisted in the addition of an extra drying step after maturation at atmospheric pressure before the addition of the bleaching earth. Note that this change was done based in *P2*. The hypothesis is that drying after acid maturation would lead the phospholipids to precipitate, which should facilitate/improve the subsequent adsorption by the bleaching earth. The procedure *P3* becomes as follows: heating the crude palm oil; adding citric acid; high shear mixing; 15 minutes of maturation at 85 °C and atmospheric pressure; extra drying step: 15 minutes under vacuum and at 105-110 °C; adding adsorbent; applying vacuum (50 mbar) and maintaining the bleaching for 30 minutes at the selected temperature; removing the bleaching earth by filtration over a Buchner funnel and a paper filter.

5.3 Results

5.3.1 Characterization of Crude Palm Oil

Crude palm oil was characterized regarding FFA, DOBI, OSI, color, carotene and elements content (mg/kg) (Table 5.1). The DOBI values are slightly higher than 2.0, indicating that final color is difficult to predict after complete

refining of the oil [4]. The phosphorus and iron content is on average, i.e. content between 10 and 20 mg/kg for phosphorus and between 5 and 10 mg/kg for iron.

Table 5.1. Characterization of Crude Palm Oil

FFA (%, as palmitic acid)	4.61
DOBI	2.06
Carotenes (mg/kg)	467
OSI (97.8 °C)	37.50
Elements content (mg/kg)	
P	19.1±0.2
Fe	7.4±0.9
Color (1 in)	
Red (R)	28.1

5.3.2 Effect of storage of bleached oil on the heat bleaching during deodorization

The effect of storage time on the final color was evaluated to determine if bleached oils can be stored before deodorization. Even though this practice is not commonly used in the edible oils industry, it has fundamental importance in lab-scale trials, as sometimes it is not possible to deodorize oils immediately after bleaching.

Firstly, the color deviation after deodorization was evaluated to determine the color difference expected due to experimental variation on this step. For this purpose, three batches of 900 g of CPO were bleached according to the reference procedure using ABE. Each batch was divided in two equal parts and they were immediately deodorized at 250, 260 and 270 °C, respectively. Average color values were 4.4, 4.5 and 5.5, for 250, 260 and 270 °C, respectively. The amplitude was equal or lower than 0.10 R in each tested temperature. From those results, it can be concluded that temperatures of 250 and 260 °C do not cause significant differences in the final color, and thus, temperatures oscillations in the range of 250 – 260 °C during deodorization process will not affect the final color. Therefore, differences higher than 0.2 R in palm oil final color obtained from the same bleached oil are considered significant. This value can be considered as the

estimated uncertainty associated mainly with the deodorization step, since the same batch of bleached oil was deodorized at each temperature.

Secondly, a storage effect was evaluated in duplicate: 900 g of CPO was bleached and deodorized according to *P1* in two separated tests. Each batch of 900 g was divided in 3 parts: the first one was immediately deodorized; the second and the third were stored under deep-freezing (-18 °C) and under a nitrogen atmosphere. The results are presented in Table 5.2. In both tests performed, the lowest color was obtained after 3 days of storage time. A long storage time resulted in the highest value of R color. It is clear in this way, that in order to evaluate and compare color differences in fully refined palm oils, the period between the bleaching process and deodorization must be the same for the different samples. It is worth to mention that for the results obtained in case of 3 and 7 days of storage time, the differences in color observed in tests 1 and 2 were higher than the prior value of 0.2. In the present case the uncertainty was high because it also reflects the errors in the bleaching step, since tests 1 and 2 involve two bleaching experiments performed independently. It seems also that the value of this combined uncertainty increases with storage time.

Table 5.2. Effect of storage period on color of final color of refined palm oil (Bleaching parameters *P1*: $T_{addition}$: 85 °C; T_{BE}: 105 °C; W_{CA}: 0.09 %; W_w: 0.21 %)

	Test 1		*Test 2*	
	pAn	*Color (R)*	*pAn*	*Color (R)*
Non-stored	8.8	4.5	10.2	4.5
3 days	8.8	3.4	10.2	4.1
7 days	2.4	5.1	3.2	4.6

5.3.3 Effects of a maturation time at atmospheric pressure and of extra dry step

The effects of a maturation time at atmospheric pressure and of an extra dry step were evaluated in CPO refining using 0.5 % ABE and NBE (Table 5.3). All the trials were efficient to reduce the free fatty acids (FFA) content to satisfactory

levels after bleaching and deodorization. When ABE was used, P1 presented the best reduction of phosphorus, followed by *P2* and *P3* (Table 5.3). Note that only P1 reached the desired value of 3 mg/kg. No trial was capable to reduce the iron content to a satisfactory level (bellow 0.2 mg/kg) and it is not possible to observe a significant difference in terms of this element reduction.

Table 5.3. Effects of maturation time at atmospheric pressure (*P2*) and of an extra dry step (*P3*) on palm oil refining after bleaching using 0.5 % BE and Deodorization. (Bleaching parameters: $T_{addition}$: 85 °C; T_{BE}: 105 °C; W_{CA}: 0.09 %; W_{water}: 0.21 %)

	ABE			NBE		
	P1	***P2***	***P3***	***P1***	***P2***	***P3***
After Bleaching						
FFA (%)	4.8	4.8	4.8	4.8	4.9	4.8
Elements content (mg/kg)						
P	2.7±0.1	3.3±0.1	3.9±0.1	3.1±0.1	3.4±0.0	3.9±0.0
Fe	0.7±0.1	0.6±0.0	0.6±0.0	0.5±0.0	0.5±0.0	0.5±0.0
Carotenes (mg/kg)	258	274	274	280	296	274
Color (5 ¼ in) *Red (R)*	68.0	70.0	70.0	68.0	68.0	70.0
After deodorization						
FFA (%)	0.04	0.03	0.03	0.07	0.06	0.06
Color (5 ¼ in) *Red (R)*	8.2	8.9	8.5	9.1	8.1	7.7

Still using ABE, *P1* presented the lowest carotene content after bleaching (258 mg/kg) and also the lighter oil after bleaching (68.0 R) and deodorization (8.2 R). *P2* and *P3* presented the same carotene content after bleaching (274 mg/kg) and the same red color (70.0 R). It was not possible to quantify the carotene content after deodorization due the absence of a peak at 450 nm in RBD oil spectrum [8].

Despite of the bigger phosphorus content, *P3* presented a lighter color after the deodorization than *P2* (8.5 R and 8.9 R, respectively). So, in this step, it was not possible to observe any relation between P and Fe content and the color after deodorization process neither to reach the desired color, i.e. bellow 3.0 R.

When using NBE, *P1* gave a better phosphorus removal, followed by *P2* and *P3*, respectively. Concerning the carotenes, *P3* presented the best reduction. There was no significant difference between the procedures related to the iron

reduction. As for the refining using ABE, no correlation between P, Fe or carotenes content and final color was observed.

There is an indication that, when using 0.5 % of bleaching earth, non-activated bleaching earth was less efficient in removing carotenes. Note that, despite the higher carotenes content, the red color after deodorization was lighter when using non-activated bleaching earth. *P1* seems to be the most efficient procedure for decolorization with ABE and *P3* with NBE.

5.3.4 Effects of bleaching temperature

Three bleaching temperatures (T_{BE} = 85, 105 and 115 °C) were evaluated using ABE (Table 5.4) and *P1*. The carotenes and phosphorus removals are more efficient at higher temperatures. The best color after deodorization was obtained when bleaching was performed at 105 °C. In fact, bleaching with acid activated BE involves catalytic reactions next to an adsorption process and there is an optimum temperature to increase the adsorption process and avoid unwanted side reactions. Note that, all bleached oil achieved the recommended phosphorus and iron content (lower than 3.0 mg/kg and 0.2 mg/kg, respectively) to avoid color fixation during heat bleaching. Thus, the oil bleached at the highest temperature (115 °C) had the darkest final color, probably due to unwanted side reactions.

Table 5.4. Effects of bleaching temperature (T_{BE}) on palm oil refining after Bleaching (*P1)* using 3.0 % ABE and Deodorization. (Bleaching parameters: $T_{addition}$: 85 °C; W_{CA}: 0.09 %; W_{water}: 0.21 %)

	ABE		
	85	***105***	***115***
After Bleaching			
FFA (%)	4.8	4.8	4.9
Elements content (mg/kg)			
P	0.3 ± 0.0	0.22 ± 0.0	0.14 ± 0.0
Fe	0.2 ± 0	0.2 ± 0.0	0.3 ± 0.0
Carotenes (mg/kg)	44	16	10
After deodorization			
FFA (%)	0.03	0.03	0.04
Color (5 ¼ in) *Red (R)*	4.3	3.4	4.8

5.3.5 Two-stage bleaching

Efforts to reduce the amount of BE necessary for the bleaching process are leading to new developments and optimization of this process and of the so-called multi-step bleaching processes. In this work, two steps bleaching had been tested. Both steps were performed according to *P2*, but without adding citric acid in the second one. Both kinds of BE were tested in four experiments: (1) addition of ABE in the first bleaching followed by NBE in the second one; (2) addition of NBE followed by ABE; (3) adding two times ABE; and (4) adding two times NBE. The amount of BE in each step was always 1.5 %. Color results are shown in Figure 5.1.

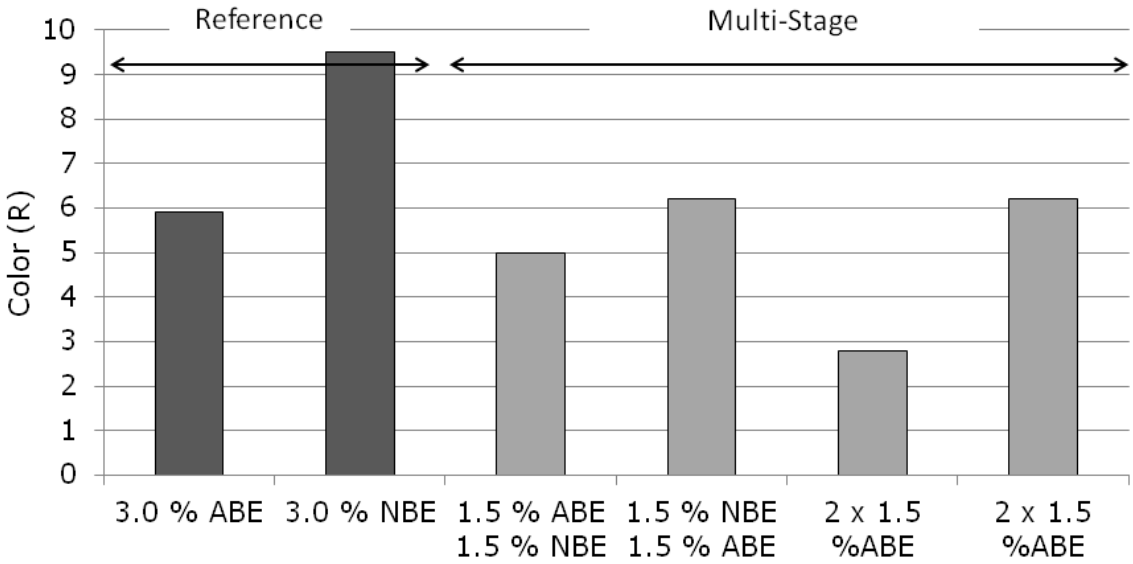

Figure 5.1. Color value of oils fully refined by two-stage bleaching and reference procedure. Reference procedure: (bleaching parameters: $T_{addition}$: 85 °C; T_{BE}: 105 °C; W_{CA}: 0.09 %; W_{water}: 0.21 %; deodorization: 260 °C, 3 mbar, 1.5 % sparge steam injection, 60 minutes)

All two-stage bleaching procedures tested resulted in a color improvement. The best result was obtained in the test 2 x ABE, followed by ABE + NBE. There was no difference in color between the processes NBE + ABE and 2 x NBE. This suggests that the contact between the CPO and the NBE lead the formation of

compounds that cannot be removed by adsorption and generate the color fixation. Indeed, previous studies by our research group, suggest that the type of BE used defines the oxidation pathways of β-carotene: in non-polar environments β-carotene oxidizes through radical addition, meanwhile in polar ones radical cations are formed.

5.3.6 Two-step refining

The last step of this work was to evaluate the effect of color in reprocessing fully refined palm oil. CPO was fully refined two times, i.e. a fully refined palm oil was used as feedstock in subsequent refining trials. As a prospective test, procedures were done according to the reference methodology: (1) bleaching *P2*; (2) reference deodorization (260 °C, 3.0 mbar, 1.5 % sparge steam injection, 60 minutes); (3) bleaching *P2*; (4) reference deodorization.

The first refining procedure was responsible for a great color reduction using both kinds of BE (Figure 5.2). The second bleaching promoted color reduction to a level that could not be reduced anymore by heat bleaching. Regarding p-AnV, values present different behavior depending on the kind of BE. ABE leads to a constant reduction through all refining steps, being most significant in its first part. On the other hand, bleaching with NBE does not reduce p-AnV and the secondary oxidation products are removed exclusively during the deodorization step, probably by volatilization.

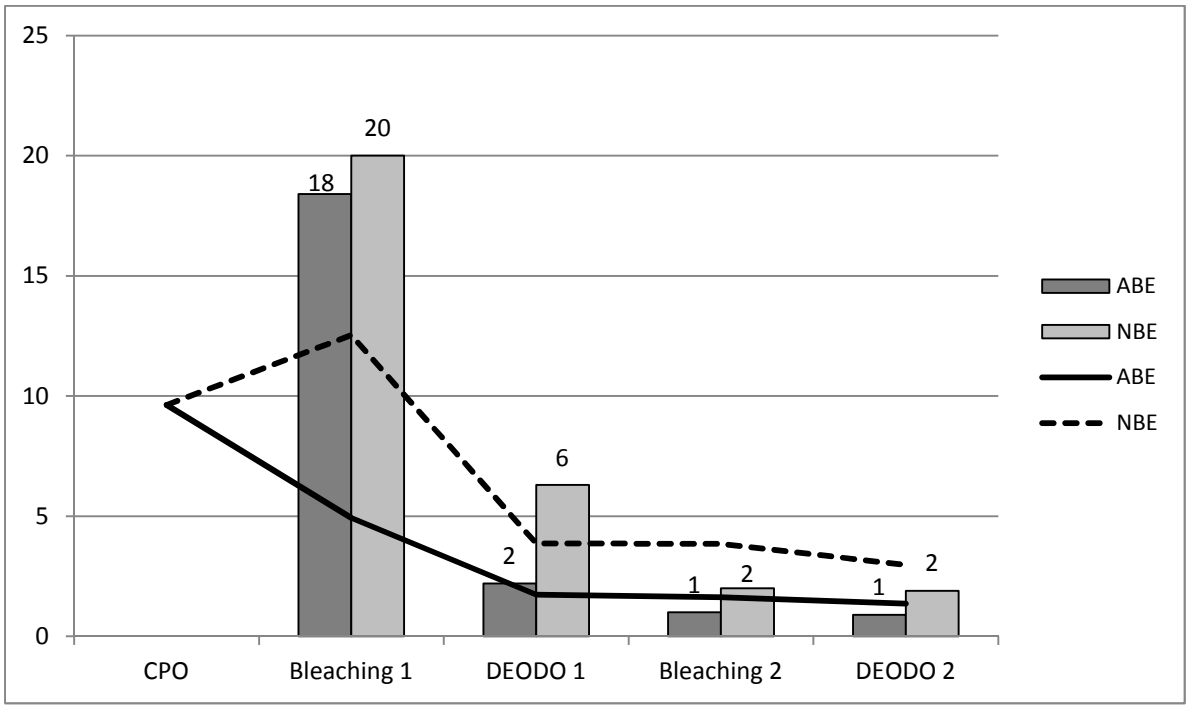

Figure 5.2. Color (bars) and p-Anisidine (lines) value of two times refined palm oil with two deodorization steps (Bleaching Parameters: $T_{addition}$: 85 °C; T_{BE}: 105 °C; W_{CA}: 0.09 %; W_{water}: 0.21 %; Deodorization: 260 °C, 3mbar, 1.5 % sparge steam injection, 60 minutes)

After those results, mild deodorization was tested. The hypothesis here is, that the first deodorization should reduce the FFA content just enough to avoid adsorption competition among adsorbates. Then, the second one should finish the FFA reduction to the desired levels. The procedure is as follows: (1) bleaching *P2* with 1.5 % BE; (2) mild deodorization (220 °C, 3 mbar, 3.75 % sparge steam injection, 15 minutes); (3) bleaching *P2* with 1.5 % BE; (4) final deodorization (260 °C, 3 mbar, 1.5 % sparge steam injection, 45 minutes). Both kind of BE were used in four tests: (1) addition of ABE followed by NBE; (2) addition of NBE followed by ABE; (3) adding two times ABE; and (4) adding two times NBE.

The Lovibond color after each step is plotted on Figure 5.3. All double refining tests lead to an improvement in final color of the refined palm oil when compared with reference procedures. The best result was obtained using two times ABE (3.0 R). Note that BE added in both bleaching steps together amounts up to 3.0 % and both deodorization steps together takes 60 minutes of residence time,

same as the reference procedures. Thus, it was possible to obtain in lab-scale experiments a lighter palm oil using the same amount of BE and with approximately the same energy consumption.

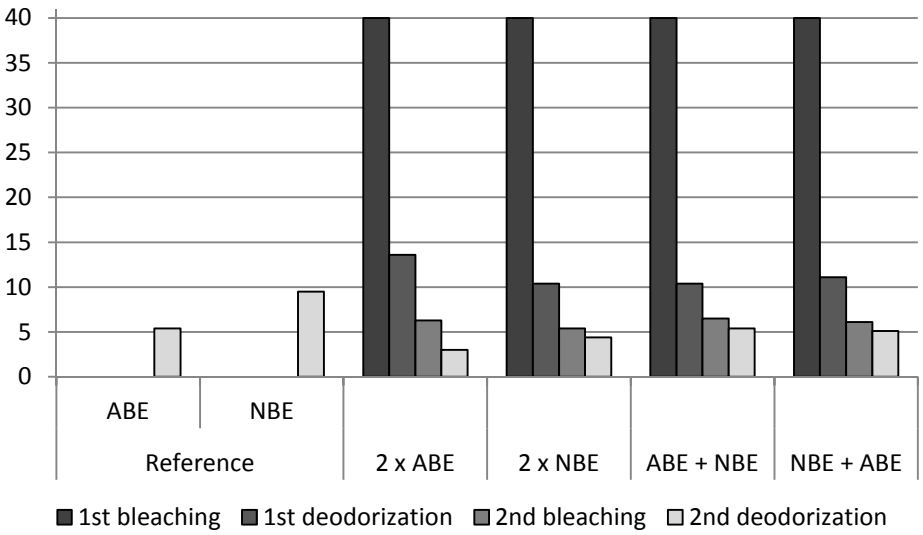

Figure 5.3. Color of two times refined palm oil (Bleaching Parameters: $T_{addition}$: 85 °C; T_{BE}: 105 °C; W_{CA}: 0.09 %; W_w: 0.21 %; Mild Deodorization: 220 °C, 3 mbar, 3.75 % sparge steam injection, 15 minutes; Final Deodorization: 260 °C, 3mbar, 1.5 % sparge steam injection, 45 minutes)

5.4 Conclusion

Bleaching is a delicate and important step of edible oil processing, involving high BE consumption. However, this step was not sufficiently studied from the point of view of process development. Results obtained in this work show that different bleaching procedures can improve the palm oil final color, and that this procedures should be optimized according to the kind of BE used. Furthermore, a new processing approach was suggested, consisting of a two-step refining, i.e. two mild-deodorization steps, instead of one extreme, as normally used in industrial practice. The new approach may present a higher initial investment, however it may be paid back by lower BE consumption. The optimization of this approach and the utilization of different adsorbents, as silica and activated carbon, should be performed in the future.

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Chapter 6. "EFFECT OF TYPE OF BLEACHING EARTH ON FINAL COLOR OF REFINED PALM OIL"

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Abstract

Despite the fact that some studies indicate chemical changes are probably responsible for the color fixation of palm oil, this mechanism is not clear. The objective of this work was to study the effect of the type of bleaching earth on the refined palm oil color. Two types of bleaching earth were tested, one neutral (NBE) and one acid-activated (ABE), being Pure Flo B80 and Tonsil Optimum 210 FF, respectively, in a range of 0.5 - 3.0 % (w/w). An inverse correlation between p-anisidine value after bleaching and residual color after deodorization was found with acid activated bleaching earth, but not with the neutral earth. Moreover, heat bleaching was more efficient in oils refined with acid activated earth. Those results indicate that oxidation products are important to predict the final color of refined palm oil. The type of bleaching earth (acid activated or neutral) can define possible oxidations pathways, so that a better explanation for, palm oil final color was obtained.

6.1 Introduction

Crude palm oil is produced from palm fruits and contains about 1 % of minor components, such as phospholipids, metals, phytosterols, carotenes, tocopherols and tocotrienols (Gee, 2007). It has to be refined to achieve desirable characteristics, such as a light color, bland taste and a good oxidative stability (Gibon , De Greyt, & Kellens, 2007; Sampaio, Ceriani, Silva, Taham, & Meirelles, 2011). Physical refining is generally preferred to avoid a too high neutral oil loss (Rossi, Gianazza, Alamprese, & Stanga, 2001), and usually consists of a bleaching and a deodorization/deacidification step (Gibon , De Greyt, & Kellens, 2007). Both steps are important for the removal of coloring components. Part of the color pigments are removed during bleaching by adsorption on a suitable bleaching

earth. The remaining color components are then thermally degraded during deodorization at high temperature.

In the case of palm oil refining, bleaching is integrated in a so-called dry pretreatment step which aims at simultaneous bleaching and element removal with minimal formation of oxidation products (Gibon , De Greyt, & Kellens, 2007). Complete removal of carotenes during bleaching is not possible and also not necessary since these components are not heat stabile and can be degraded during high temperature deodorization (Gibon , De Greyt, & Kellens, 2007). In fact, some refineries only remove 20% of the color of the carotenoid content of the crude palm oil at the bleaching step, and after 20 min at 240 °C more than 98 % of the total carotenoids has been destroyed (Maclellan, 1983). Part of the color pigments are physically adsorbed by bleaching earth. Other components are chemically bound to bleaching clay via covalent or ionic bonds (Gibon , De Greyt, & Kellens, 2007; Taylor, 2005). Moreover, bleaching earth acidity is related with pigment adsorption capacity (Taylor, 2005). Acid activated bleaching earth work simultaneously as a solid, acidic catalyst, adsorptive agent, cation exchanger and filter aid (Zschau & Grp, 2001), whereas neutral bleaching earth acts basically only as a adsorptive material (Taylor, 2005). In fact, there is no consensus on the adsorption mechanisms of β-carotene onto bleaching earth. Boki, Kubo et al. (1992) found that pigment adsorption onto clay minerals occurs through physisorption. Sabah, Cinar et al. (2007) suggested β-carotene adsorption onto sepiolite is also physical. On the other hand, according to Khoo, Morsingh et al. (1979), β-carotene adsorption onto different commercial bleaching earths is not purely physical. Later, other works found that β-carotene adsorption onto acid activated bleaching earth (Sarier e Guler, 1988) and onto silica gel (Ahmad, Chan et al., 2009) was controlled by chemisorption.

The crude palm oil dark orange color is due to the high carotenoid content (500 - 700 mg/kg) (Gee, 2007), nonetheless it is not completely understood which compounds are responsible for its final color, i.e. the color after the deodorization step (Fraser & Frankl, 1981; Gibon , De Greyt, & Kellens, 2007). It is well accepted

that there is no correlation between the β-carotene content after bleaching and the palm oil final color (A. Dijkstra & Segers, 2007; Fraser & Frankl, 1981; Maclellan, 1983). Some studies suggest that in the finished oil, the color is mostly due to high molecular weight (HMW) compounds derived from oxidation reactions, especially in the case of carotenoids (Fraser & Frankl, 1981). Also, aggressive bleaching conditions, as excessive temperatures or time process or both, lead to darker final colors (Maclellan, 1983).

Sarier and Güler (1988) observed that, besides adsorption of carotenes during bleaching, acid activated clay can also catalyze chemical changes in the carotene molecule probably leading to oxidized forms. Conjugated double bonds are responsible for carotenes color (Kamal-Eldin, 2003; Zollinger, 1991) and antioxidant activity (Kamal-Eldin, 2003), so the bleaching process consists basically in the interruption of this system either by cleavage or by radical addition to one of the double bonds (Krinsky & Yeum, 2003). There are at least three mechanisms for these reactions, being (1) radical addition, (2) electron transfer to the radical and (3) allylic hydrogen abstraction. Which mechanism will take place depends on the reaction conditions. For instance, increasing the oxygen concentration, the formation of secondary peroxyl radicals becomes important, resulting in the loss of antioxidant behavior. With sufficiently high oxygen partial pressures (above 150 mmHg) (Burton & Ingold, 1984), β-carotene reactions have a pro-oxidant effect since they could generate more radicals than they consume (Krinsky & Yeum, 2003). Electron transfer reactions have been reported either in formation of a carotenoid cation CAR^+, anion CAR^- or in the formation of an alkyl radical (Krinsky & Yeum, 2003), stable because of its resonance structure (Kamal-Eldin, 2003). Finally, allylic hydrogen abstraction by peroxyl radical occurs at an oxygen pressure of less than 760 mmHg, producing a carotene radical. Those radicals undergo addition of oxygen producing dicarbonyls (Kamal-Eldin, 2003). It is clear, that allylic hydrogen abstraction does not occurs in bleaching conditions due to its moderate temperature and pressures, being always lower than 50 mbar.

Carotenoids oxidation during bleaching can occur through radical addition or electron transfer.

6.2 Material and Methods

6.2.1 Oil characterization

Standard quality parameters, including free fatty acids (FFA) content, color, carotene content, deterioration of bleachability index (DOBI) and element content, were analyzed for crude, bleached and full refined palm oil samples.

6.2.2 Color

Sample color was determined according to AOCS Official Method Cc 13e-92, using a Lovibond Tintometer Color Scale at 70 °C, ensuring that color was determined in a completely melted sample. Analyses were carried out using 5” ¼ (133.4 mm) glass cells. In the cases of crude palm oil samples, color was darker than scale in a 5” ¼ glass cell and a 1” (25.4 mm) was used (AOCS, 1998).

6.2.3 Free Fatty Acids (FFA)

Free Fatty Acids content (FFA) was determined according to AOCS Official Method Ca 5-40, by titration. Results were expressed as percentage of palmitic acid (C16:0, MW = 256 g/mol) (AOCS, 1998).

6.2.4 Deterioration of the bleachability index (DOBI)

The deterioration of bleachability index (DOBI) was measured using a UV-vis spectrophotometer. This index corresponds to the ratio of the spectrophotometric absorbance at 446 nm (non-oxidized carotenes maximum) to the absorbance at 268 nm (oxidized carotenes maximum).

About 0.1 mg of completely molten and homogenized oil is diluted in 25 mL of iso-octane. Absorbencies are measured at 268 nm and 446 nm. The DOBI value is obtained by Equation 5.1.

$$\text{DOBI} = \frac{\text{absorbance at } 446 \text{ nm}}{\text{absorbance at } 268 \text{ nm}} \quad (5.1)$$

The higher the DOBI value, the lower the amount of oxidized carotenes, the easier it will be to heat bleaching, as color due to oxidized carotenes is difficult to remove. DOBI values between 2.5 and 4.0 indicate average to good crude oil quality. Values below 2.0 indicate a poor quality crude palm oil which is difficult to bleach.

6.2.5 Carotenes Content

Carotenes content, expressed as β-carotene, was determined measuring the absorbances at 446 nm of samples homogenized and diluted in iso-octane, according to PORIM (1990).

6.2.6 p-Anisidine and Peroxide values

Peroxide (PV) and p-anisidine (p-AnV) values were determined according to AOCS Official Method Cd 8-53 and Cd 18-90, respectively (AOCS, 1998).

6.2.7 Elements Content

The content of Phosphorus (P) and Iron (Fe) were measured using an Inductively Coupled Plasma (ICP) method, according to AOCS official method Ca 20-99, using kerosene as solvent (AOCS, 1998).

6.2.8 Refining Procedure

Bleaching trials were performed according to the following steps: heating the crude palm oil to 85 ºC; adding of citric acid (0.3 % of a 30 % solution); high shear mixing (16000 rpm during 1 minute); addition of Bleaching Earth; 15 minutes of maturation time at 85 ºC and atmospheric pressure; applying vacuum (50 mbar) and maintaining bleaching for 30 minutes at 105-110 °C; removing bleaching earth by filtration over a Buchner filter (pore size 11 µm, Whatman).

Experiments were performed using two kinds of bleaching earth: non-activated bleaching earth (Pure Flo B80, purchased from Oil-Dri, here so-called NBE) and acid activated bleaching earth (Tonsil OPT. 210 FF, purchased from Süd-Chemie AG, here so-called ABE).

After bleaching, the oil was submitted to a deodorization step since some color effects can just be observed after heat bleaching. To avoid differences due to storage effects, the deodorization was always carried out one day after the bleaching. The deodorization was performed in a lab-scale equipment described by Petrauskaite, De Greyt, & Kellens, 2000. Deodorization parameters were fixed to all experiments and set as 260 °C of deodorization temperature, 3.0 mbar, 1.5 % steam and 60 minutes of residence time.

6.3 Results

Crude palm oil (obtained from a local processor) was characterized regarding FFA, DOBI, color, carotenes and elements content (mg/kg). The DOBI value was 2.06 ± 0.01, indicating that the final color is difficult to predict after complete refining of the oil (Gibon , De Greyt, & Kellens, 2007). The initial content (mg/kg) of phosphorus, iron and carotenes was 19.1 ± 0.2, 7.4 ± 0.9 and 467 ± 2, respectively. The crude palm oil presented a initial FFA of 4.61 %, expressed in palmitic acid, and 37.8 hour OSI.

A bleaching procedure was tested using both kinds of bleaching earth at three different concentrations: 0.5, 1.5 and 3.0 % (Table 6.1). Phosphorus and iron contents lower than 3.0 and 0.3 mg/kg, respectively, were achieved by both bleaching earths when using 3.0 %. ABE leads to a better reduction of phosphorus especially when small amounts of bleaching earth are used. However, increasing the amount, this effect becomes less important, and the phosphorus content obtained using each bleaching earth is quite similar. The opposite behavior was observed for iron adsorption.

Concerning the carotenes analysis, it can be noted that for the lower concentration level tested, ABE resulted in a higher adsorption of carotene than

NBE. Using larger quantities, NBE gives higher adsorption. It was not possible to observe a relation between the carotene content after bleaching and the color after deodorization (Table 6.1), as suggested by Taylor (2005).

Table 6.1. Palm Oil Color, β-carotenes and elements content (mg/kg) after Bleaching and deodorization process

	ABE			NBE		
	0.5	***1.0***	***3.0***	***0.5***	***1.0***	***3.0***
After Bleaching						
Elements content (mg/kg)						
P	2.7±0.1	0.5±0.1	0.2±0.1	3.1±0.1	0.6±0.1	0.2±0.0
Fe	0.7±0.1	0.2±0.0	0.2±0.0	0.5±0.0	0.1±0.0	0.2±0.0
Carotenes (mg/kg) [b]	258	83	10	280	87	ND[a]
Color (5 ¼ in) *Red (R)*	68.0	42.0	15.8	68.0	39	12.5
After deodorization						
Color (5 ¼ in) *Red (R)*	8.2	7.3	3.4	9.1	8.4	6.1

ND[a]: Not detected, [b]: Carotenes were not detected in any sample after deodorization

Regarding the color, it can be noted that after bleaching using 0.5 % lead to 68.0 R for both BE. When a higher amount of BE was used, differences in color could be observed; NBE leads to oil about 3.0 R lighter then ABE after bleaching. After deodorization the oil bleached with ABE presents a lighter color, even though it was darker after bleaching. It is interesting to highlight that the carotene content and Lovibond color obtained after bleaching with ABE were higher than that obtained with NBE, however, it gave instead a lighter color after deodorization.

The oxidative state of the oils after bleaching using ABE and NBE are presented in Figure 6.1. For both bleaching earths, peroxides value decrease with BE concentration. Note that, amounts slightly higher than 1.0 % ABE reduces the PV to zero, while for NBE about 2.0 % is necessary. Small amounts of BE increases p-AnV. In the case of ABE, p-AnV increases and then starts to decrease until the initial value. In the case of NBE, p-AnV shows an increasing trend up to an approximately constant value. It is interesting to note that maximum p-AnV values coincide with the point the peroxides value reaches zero for the first time, suggesting that the peroxides are being converted into secondary oxidation products.

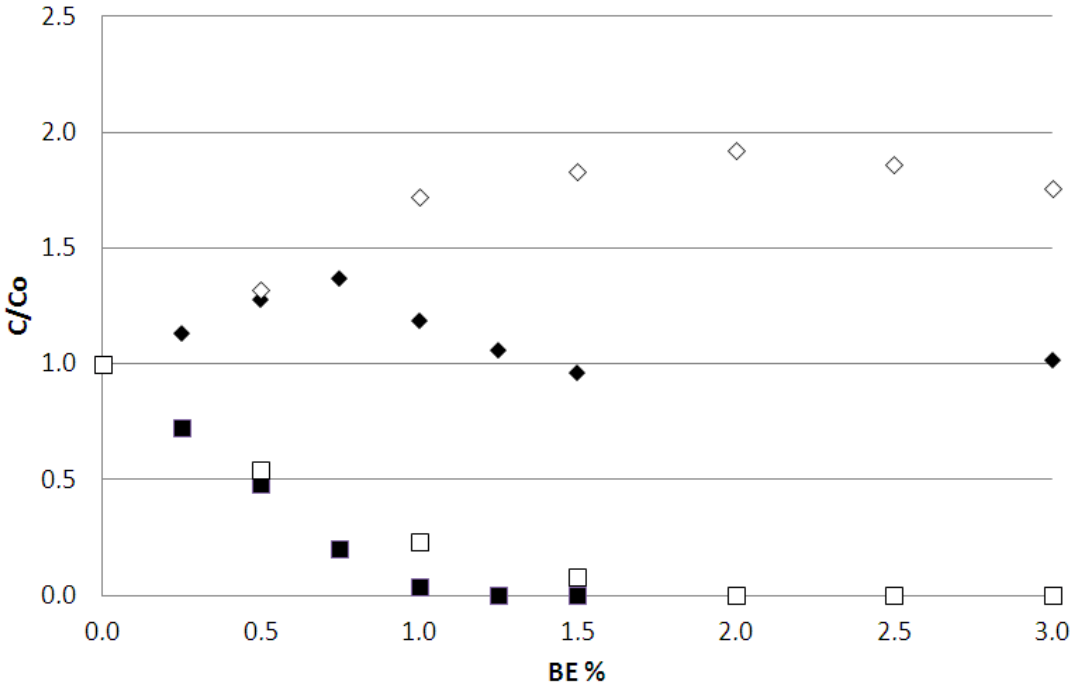

Figure 6.1. Oxidative State of Bleached Oils: Peroxide value, (■ ABE and □ NBE) and p-Anisidine value (♦ ABE and ◊ NBE)

Different p-AnV values were obtained by adjusting the bleaching earth amount and by repeating the bleaching procedure in fully refined oils. A correlation between p-AnV after bleaching and palm oil color after deodorization was observed when ABE was used (Figure 6.2). The same correlation was not observed for NBE.

As a second part of this work, the effect of the amount of citric acid added during bleaching was evaluated. Citric acid can act in two ways during oil degumming: firstly, displacing phosphatidic acid (PA) in its salts; secondly, forming a chelating agent capable to form a stronger bond with alkaline earth than PA does (A. J. Dijkstra, 2010). Here, we assume that adding more citric acid, more elements species can be chelated, reducing their concentration in the oil. Moreover, citric acid reduces the pH of the solution and competes with compounds to be adsorbed. The effect of citric acid amount (added as 30 % solution) was tested using 2.0 % of bleaching earth (Table 6.2). Note that this represents an increase in citric acid and water addition.

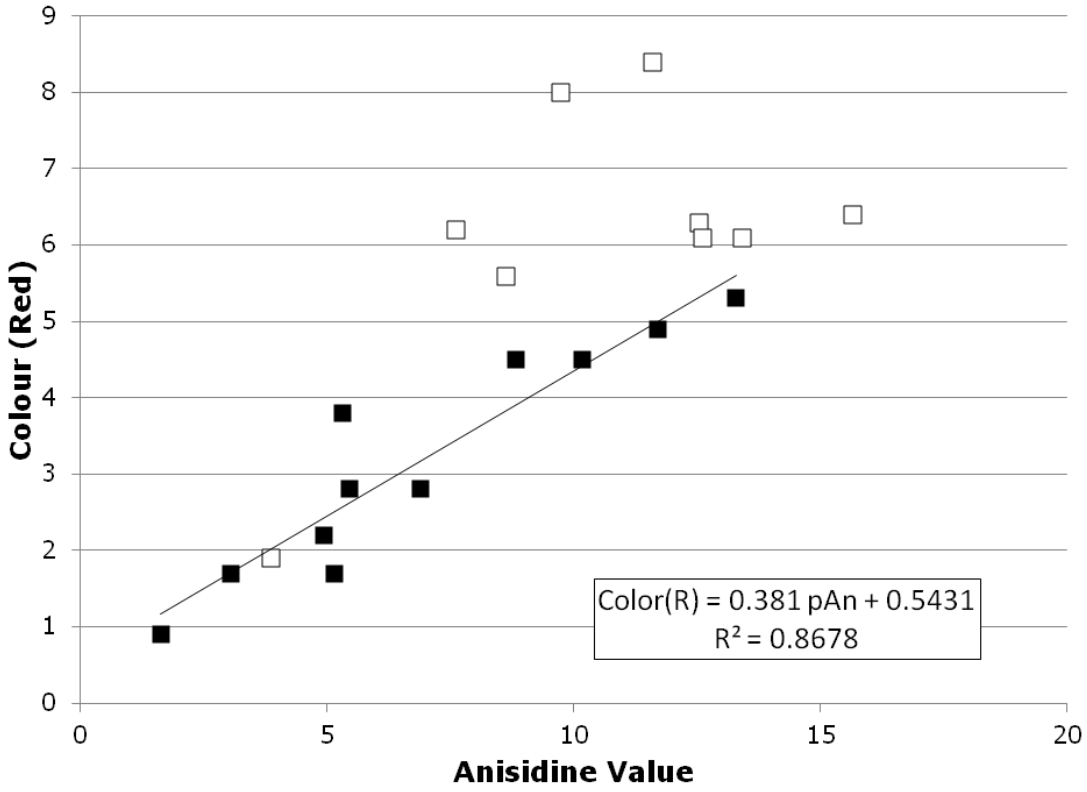

Figure 6.2. p-Anisidine value vs Colour (■ ABE and □ NBE)

Table 6.2. Effect of citric acid and water amount in bleaching using 2.0 % of bleaching earth

	ABE		NBE	
	0.09 % CA 0.21 % water	***0.27 % CA 0.63 % water***	***0.09 % CA 0.21 % water***	***0.27 % CA 0.63 % water***
After Bleaching				
FFA (%)	5.0	5.0	4.7	4.8
Elements content (mg/kg)				
P	0.4±0.1	0.5±0.1	0.6±0.0	0.8±0.1
Fe	0.2±0.0	0.2±0.0	0.3±0.0	0.3±0.0
Carotenes (mg/kg) [a]	40	21	13	19
Color (5 ¼ in) *Red (R)*	29.0	25.0	19.2	22.0
After deodorization				
Color (5 ¼ in) *Red (R)*	6.1	7.3	8.9	8.2

[a]: Carotenes were not detected in any sample after deodorization

Oils showed a higher FFA after bleaching with ABE than those bleached with NBE. It can be due to the inherent acidity of ABE or due to a catalytic effect forming FFA. Phosphorus adsorption decreases with the amount of added citric acid for both bleaching earths, but the decrease is more significant with NBE. There is no difference between final iron content using the same bleaching earth.

Concerning carotenes content, increasing the citric acid concentration resulted in different behaviors for each bleaching earth. In the case of ABE, adding more citric acid caused that more carotenes were adsorbed. On the other hand, when NBE was used as bleaching earth, the increasing amount of citric acid resulted in a lower adsorption of carotenes. It was not possible to observe any relation between the color after bleaching and after deodorization, as suggested by Fraser and Frankl (1981). In the case of ABE, the use of more citric acid leads to a lower color after bleaching but a higher color after deodorization. In the other hand, when NBE was used, more citric acid leads to a higher color after bleaching and a lower color after deodorization. From those results, it can be inferred that chelating power is not the factor affecting the palm oil final color. A possible explanation is that the pH interferes in β-carotenes oxidation pathways, and this will be further discussed in the following section.

6.4 Discussion

In this work, no correlation was found between the β-carotene concentration of crude oil and the palm oil final color, as it has been suggested by Fraser (1981). On the other hand, a high correlation was found between p-AnV after bleaching with ABE and the palm oil color after deodorization/heat bleaching. The same correlation was not observed when the bleaching was done with NBE. It is also interesting to highlight that heat bleaching was more efficient after bleaching with ABE, even though NBE was capable of removing more efficiently the color during bleaching. Oxidation of compounds such as β-carotenes may be an explanation for those results as described hereafter.

Tonsil OPT 210 FF is an acid activated bleaching earth manufacturated by acid activation of calcium bentonite. It has acidic and catalytic activity, leading most importantly hydroperoxide decomposition, forming sub-products such as aldehydes, ketones and conjugated polyenes (Zschau & Grp, 2001). Pure Flo B 80 is a neutral bleaching earth with no catalytic activity. These properties can be confirmed through oxidative state data (Figure 6.1). Note, for instance, less amount of ABE is needed to reach zero peroxides compared to NBE. Furthermore, the first point presenting a maximum p-AnV value (secondary oxidation products) is correlated to the minimum PV value. Note that ABE decreases p-AnV until a constant value and NBE keeps it constant at the maximum. Indeed, as suggested by Sarier and Güler (1988), β-carotene which remains in solution with acid activated bleaching earth is rapidly oxidized later on, even more than those submitted to oxygen for 48 hours. Consequently, it may be said that acid activated bleaching earth initiates the oxidation of unadsorbed β-carotene.

Carotenoids can react with radical species in three different ways, including (1) radical addition, (2) electron transfer to the radical or (3) allylic hydrogen abstraction. Burton and Ingold (1984) suggested that β-carotene reacts with peroxyl groups by addition, rather than by hydrogen abstraction. Liebler and McClure (1996) identified radical adducts formed during β-carotene oxidation, which then combines with a second radical to form an addition product. The formation of alkyl- and alkoxyl-containing addition products indicates that both may add directly to β-carotene. In contrast, peroxyl radical addition yields an unstable intermediate radical adduct that collapses to an epoxide and releases an alkoxyl radical.

The polarity of the medium determines the pathways that β-carotene oxidation undergoes: in nonpolar solvents, only addition radicals are formed, meanwhile in polar ones carotenoid radical cations are formed (El-Agamey & McGarvey, 2003). For both solvents, the first product is an addition radical formed between the acylperoxyl radical and the carotenoid (Eq.6.2, Figure 6.3) (El-Agamey & McGarvey, 2002). In fact, the Gibbs free energy presents similar

negative values for both system polarities, thus, the exergonicity of reactions depends mostly on the nature of the free radical, being •OH radical the most exergonic and peroxyl radicals the least one (Martinez, Vargas, & Galano, 2010). Those radicals have no reactivity toward oxygen, even at high oxygen pressures as 760 mmHg (Eq. 6.3) (El-Agamey & McGarvey, 2003).

$$BC + \bullet ROO \rightarrow ROOBC\bullet \quad (6.2)$$

$$ROOBC\bullet + O_2 \rightarrow \times \quad (6.3)$$

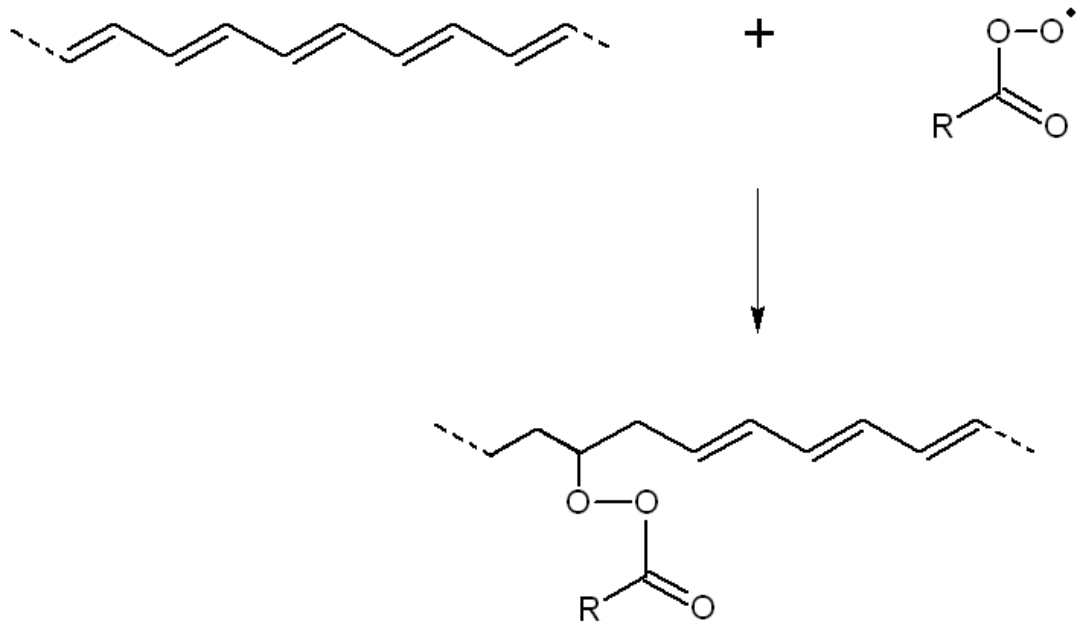

Figure 6.3. First Addition

Subsequent steps of β-carotene oxidation follow different pathways for polar and non-polar solvents (Figure 6.4). In non-polar solvents, the radicals decompose in epoxides and cyclic ethers, therefore, with an acyloxyl radical elimination (Eq. 6.4) (El-Agamey & McGarvey, 2003), leading to volatiles products such as apocarotenals (Krinsky & Yeum, 2003). Note that this reaction releases a radical,

thus, showing no net consumption of radical, being an autoxidation reaction (Liebler, 1993).

$$ROOBC\bullet + ROO\bullet \rightarrow \text{“nonradical product”} \qquad (5.4)$$

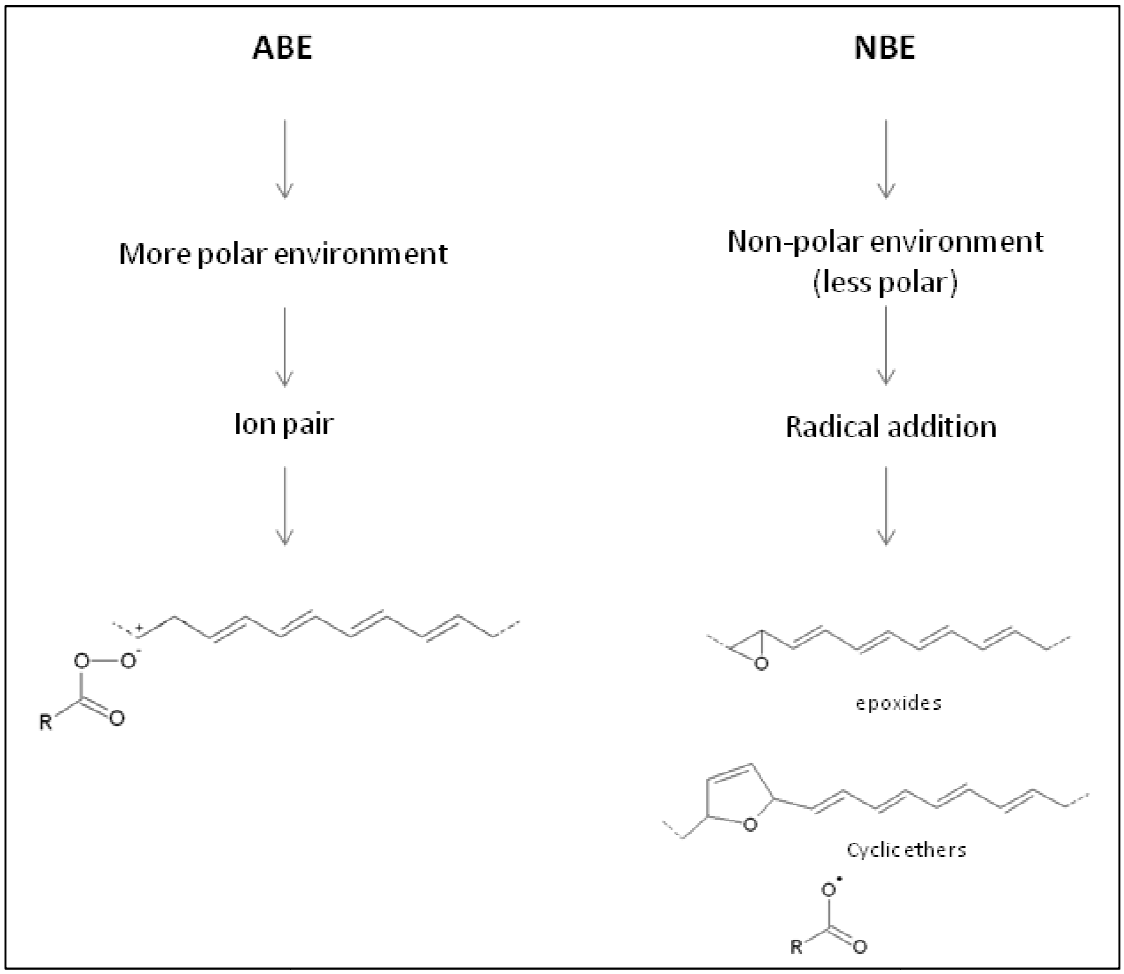

Figure 6.4. Second Addition

Nonetheless, in polar solvents, an ion-pair composed by a peroxyl anion (ROO-) and a cation (CAR+) is formed, which absorbs near-infrared radiation (El-Agamey & McGarvey, 2002) and after, it is transformed to carotenoid radical cation. Even though vegetable oil is nonpolar, BE negative sites may make a polar

layer close to its surface, supporting reactions associated with polar environments. Negative charged sites of ABE can readily adsorb those cations. Indeed, cationic compounds are preferentially adsorbed by acidic centers of bleaching earth, as suggested by Zschau (2001), due to a chemisorption mechanism instead of physisorption (Kent, 2008).

During the deodorization step, β-carotene degradation progress follows different mechanisms. Heat excited carotenoids undergo *cis-trans* isomerization or react with oxygen to form diradicals (Kamal-Eldin, 2003). β-carotene can undergo autoxidation much easier than compounds containing fewer conjugated double bonds, as β-apo-8´-carotenal and retinal, due to *trans-cis* thermal isomerization. During twisting of the β-carotene backbone unpaired spin density will develop in each half of the molecule, reaching a maximum in the perpendicular transition state and forming cyclic peroxides which may self-initiate autoxidation reactions (Mordi, Walton, Burton, Hughes, Ingold, Lindsay, et al., 1993). The main stable products to thermal degradation are higher molecular weight components, epoxy-β-carotenes, apo-carotenals and apo-carotenones, other low molecular weight di- and tri-oxygenated compounds, probably including carboxylic and/or peracids, and carbon dioxide (Mordi, et al., 1993) (Figure 6.5). Marty and Berset (1990) suggested β-carotene degrades by progressive shortening of the polyene chain by two carbon atoms at a time. So, oxidative break of a double bond in the β-carotene molecule leads to the formation of two carbonyl fragments of which one may be colorless.

The effect of citric acid addition on the palm oil final color reinforces this hypothesis. The citric acid can induce an increasing competition among compounds to be adsorbed and reducing the solution pH. The increase of competition among compounds to be adsorbed has a negative effect, as it reduces the adsorption of P and Fe. This phenomenon is observed in processes using both BE, and the concentrations of those elements after processing with more citric acid are higher. The reduction of pH can change the β-carotene pathways, as explained previously. In the case of process using NBE, the use of more citric acid leads to a more polar environment and makes the formation of cations more favorable. The

process using ABE shows an already sufficient low pH, so that even with less citric acid addition those reactions were promoted.

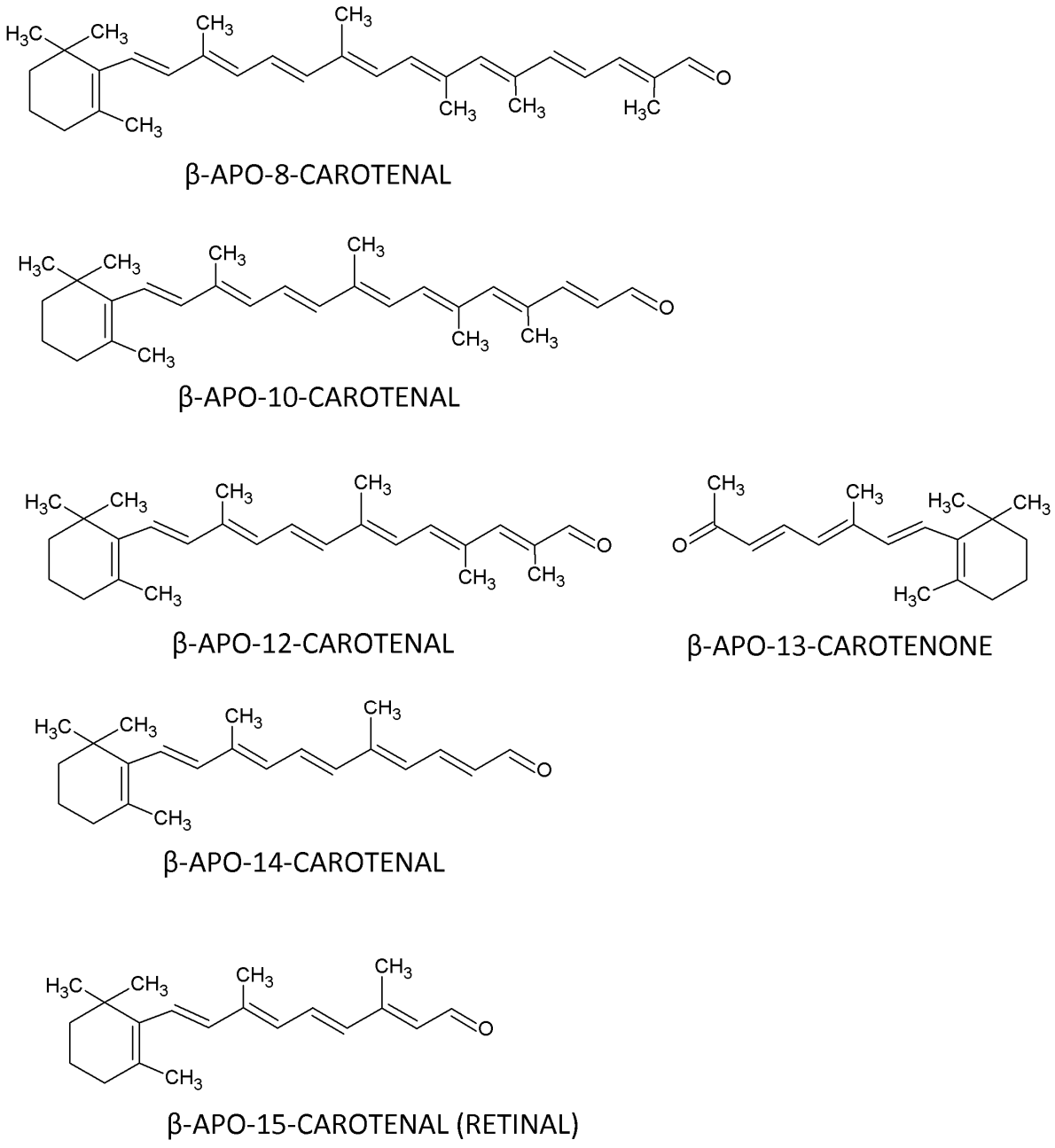

Figure 6.5. Products formed during β-carotene oxidation through radical addition

In this way, the positive effect of pH is more pronounced with NBE, whilst the negative effect of competition with ABE. Processing with NBE tends to have the same behavior as processing with ABE when more citric acid is added: lower carotenes adsorption, darker color after bleaching and lighter color after deodorization.

Although the results presented in this work are not conclusive, they are important to improve the understanding of palm oil refining and some concluding remarks can be drawn so far: The type of bleaching earth can define oxidation pathways in two different manners. At the first moment, ABE decomposes peroxides in alkoxyl and alkyl radicals, which can react faster with β-carotene. Later, ABE makes the environment more polar, supporting radical cation formation which absorbs near infra-red. Furthermore, acid activation enhances adsorptive power due to an increasing surface area (Taylor, 2005).

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Chapter 7. "COMPARING DIFFERENTIAL AND FLASH DISTILLATIONS IN THE SIMULATION OF VEGETABLE OIL DEACIDIFICATION"

"COMPARAÇÃO ENTRE A DESTILAÇÃO DIFERENCIAL E FLASH PARA A SIMULAÇÃO DA DESACIDIFICAÇÃO DE ÓLEOS VEGETAIS"

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Computer simulation, physical refining, flash distillation

Summary

In batch physical refining of vegetable oils, the batch of oil is continuously injected with steam under high vacuum pressure. This process has been studied and treated as differential distillation process, showing good results for predicting the acidity at the end of the process. However, when analyzed over time, the differences regarding the experimental data become more significant. Flash distillation already considers that a batch of oil achieves single-stage equilibirum without the use of reflux, when the oil batch achieves steam distillation under high vacuum pressure. This study aims to compare the two approaches in computer simulation programs. It was observed that differential distillation has a linear acidity reduction while in flash distillation the acidity reduces exponentially.

7.1 Introduction

The deacidification by physical means (deodorization) is the final stage of the refining process of edible oils. A stripping operation, whose objective is to reduce undesirable volatile components, may also be considered. Here, a carrier agent is mixed into the oil, facilitating the mass transfer of volatile impurities into the vapor phase, which are then removed in order to avoid the impurities from condensing onto the liquid (BALCHEN et al., 1999). This process may also result in neutral oil loss in the distillate, mainly in part by the vapor entrainment of oil drops (DE GREYT e KELLENS, 2005) and, on a lesser scale by the volatization of lower molecular weigh acylglycerides.

The optimal parameters for the refining step (temperature, retention time, operating pressure and quantity of carrier gas) must be defined according to the crude oil, with the specifications of the final product, the limitations of the equipment and the need to minimize costs (DE GREYT and KELLENS, 2005). Therefore, simulating physical deacidification is promising for improving the quality

of the refined oil as it defines the process parameters in accordance to the raw material and the final product specifications.

Ceriani and Meirelles (2004) already studied this process considering it as a process of differential distillation, wherein the still is fed with oil and then heated. The superheated steam is bubbled continuously into the oil, acting as a depletion agent by carrying the most volatile fatty compounds to the vapor phase, which are then condensed and collected in a flask. The oil compositions within the reactor and the distillate vary with time. The authors regard the process as a sequence of numerous, successive evaporation steps in equilibrium (CERIANI and MEIRELLES, 2004). This approach proved to be acceptable for predicting acidity at the end of the experiment, however, when acidity removal is analyzed over time; the differences become significant (SILVA, 2009; TAHAM; 2009). However, for the batch deacidification process can be analyzed according to the *flash* approach, steam must be continuously fed into oil until it reaches a single equilibrium stage, without the use of reflux, specific to the operation of a ordinary industrial equipment.

Thus, the objective of this study is to compare the differential and flash distillation approaches for simulating deacidification process.

7.2 Methodology

7.2.1 Vegetable Oil Composition

Simulations were performed for an oil constituted mainly of oleic, palmitic and linoleic acid (about 75 %, 15 % and 5 %, respectively) with 3.1 % of acidity, expressed as oleic acid modeled according to Buriti oil composition as determined by Silva et al. (2009). Thus, following the methodology developed by Antoniosi (1995) it was possible to determine the composition of triacylglycerols (TAG). Only the 4 main TAGs and 12 fatty acids were considered, as shown in Table 7.1.

Table 7.1. Oil composition studied.

Component	Molar Fraction	Mass Fraction
POP	6.4291×10^{-2}	0.07539
OOP	3.0089×10^{-1}	0.36385
OOO	3.5428×10^{-1}	0.44140
OOLi	7.1419×10^{-2}	0.08878
C12:0	3.5036×10^{-5}	0.00001
C14:0	8.1953×10^{-5}	0.00002
C16:0	1.5309×10^{-2}	0.00513
C16:1	2.9426×10^{-4}	0.00010
C18:0	1.4556×10^{-3}	0.00054
C18:1	6.1463×10^{-2}	0.02269
C18:2	4.1209×10^{-3}	0.00151
C18:3	8.7384×10^{-4}	0.00032
C20:0	8.9826×10^{-5}	0.00004
C20:1	3.9931×10^{-4}	0.00016
C22:0	6.1820×10^{-5}	0.00002
C24:0	5.7116×10^{-5}	0.00003

7.2.2 Modeling

For the computer simulations in this study, the vapor pressure equations and the thermodynamic approach proposed by Ceriani and Meirelles (2004) were used to describe the vapor-liquid equilibrium (VLE) of fatty compounds (Equations 7.1 and 7.2).

$$k_i = \frac{y_i}{x_i} = \frac{\gamma_i \cdot f_i}{P \cdot \phi_i} \tag{7.1}$$

$$f_i^0 = P_i^{vp} \cdot \phi_i^{sat} \cdot exp\left(\frac{V_i^L \cdot \left(P - P_i^{vp}\right)}{R \cdot T}\right) \quad (7.2)$$

where k_i is the distribution coefficient of component *i* between the liquid and vapor phases, the f_i is the fugacity in the reference state, x_i and y_i represent the mole fraction of component *i* in the liquid and vapor phases, respectively; *P* is the total pressure, *R* is the gas constant, *T* is the absolute system temperature, P_i^{vp} and ϕ_i^{sat} respectively, the vapor pressure and fugacity coefficient of pure component *i*[1], γ_i is the activity coefficient, ϕ_i the fugacity coefficient of component *i*, V_i^L the molar volume of component *i* in the liquid state. The exponential term in Equation 7.2 is the Poynting factor. Vaporization efficiencies were considered equal to unity. The equations were compiled using the Matlab software.

7.2.2.1 Differential distillation:

The equations used to differential distillation are presented below. The mass balances for all components and the still are given by Equations 7.3 and 7.4.

$$\frac{dL}{dt} = -\dot{v} \quad (7.3)$$

$$\frac{dl_i}{dt} = -\dot{v}_i \quad (7.4)$$

where *L* is the oil moles in the still; and l_i the mole charge of component *i*, $\dot{v}_i$ is the rate of total vaporization in moles/min.

The balances for the total mass and for the distillate components are given by Equations 7.5 and 7.6.

[1] Ceriani and Meirelles (2004) considered the non-ideality in both vapour and liquid phases. The activity coefficients were calculated according to the predictive UNIFAC model, modified by Fornari et al. (1994). The fugacity coefficients were calculated by the Virial equation. The Poyting factor was considered equal to 1.

$$\frac{dD}{dt} = \dot{v} \tag{7.5}$$

$$\frac{d(d_i)}{dt} = \dot{v}_i \tag{7.6}$$

where D is the total number of moles of the distillate; and d_i the number of moles of component i in the distillate balloon.

Ceriani and Meirelles (2004b) developed three alternative approaches to process modeling and compared their results with experimental data from Petrauskaitè et al. (2000) for coconut oil. The first and simplest approach, called Model 1, does not rely on steam injection. Model 2 employed steam injection, but as an inert gas. In this case, the steam is considered to be completely immiscible in the oil phase. Model 3 studied small amounts of water originating from the injected steam dissolved in the oil, increasing the volatility of fatty acids and decreasing the boiling point of the mixture. In fact, the third model was closest to the experimental data from Petrauskaitè et al. (2000) and for this reason was chosen to be addressed in this study. Thus, the concentration of water in liquid oil at a given pressure and temperature has been determined by Equation 7.7.

$$f = P - \sum_{i=1}^{n+1} \left[\gamma_i \cdot x_i \cdot P_i^{vp} \cdot \frac{\phi_i^{sat}}{\phi_i} \right] \cdot \left[exp\left(\frac{V_i^L \cdot \left(P - P_i^{vp}\right)}{R \cdot T} \right) \right] \tag{7.7}$$

7.2.2.2 Flash distillation:

The following present the equations used to model flash distillation. The balances of mass and enthalpy for each component are shown in Equations 7.8 and 7.9. Equation 7.10 presents the equilibrium used.

$$F_{1(t,i)} = l_{t,i} - l_{t-1,i} + \dot{v}_{t,i} - \dot{s}_{t,i} = 0 \tag{7.8}$$

$$F_{2(t)} = h_t - h_{t-1} + H_{t,v} - H_{t,s} = 0 \tag{7.9}$$

$$F_{3(t,i)} = \dot{v}_t \cdot k_{t,i} \cdot \frac{l_{t,i}}{L_t} - \dot{v}_{t,i} = 0 \tag{7.10}$$

Where $l_{t,i}$ and $l_{t-1,i}$ represents the charge mol of component *i* in the oil at points t and *t-1*, respectively, $\dot{v}_{t,i}$ corresponds to the vapor phase formed in the flash in moles/min and $\dot{s}_{t,i}$ represents the vapor injection of component *i* in moles/min. Note that in this case, the flow rate of injected steam per minute is equal to 1 when compound *i* is water and for all other compounds in the system $\dot{s}_{t,i}$ is equal zero.

In each interval of time between *t-1* and t, Equations 7.8 and 7.10 generate (2NC +1) system of relations, where NC is the number of system components. The function discrepancies represented by $F_{j(t)}$ were resolved by the Newton-Raphson method, generating the values $l_{t,i}$, $\dot{v}_{t,i}$ and *T* (CERIANI and MEIRELLES, 2006).

Thus, one can achieve variations in the composition of the oil batch and the distillate over time by means of equations 7.11 and 7.12 below:

$$x_{t,i} = \frac{l_{t,i}}{\sum_{i=1}^{NC} l_{t,i}} \tag{7.11}$$

$$y_{t,i} = \frac{\dot{v}_{t,i}}{\sum_{i=1}^{NC} \dot{v}_{t,i}} \tag{7.12}$$

Note that in the case of differential distillation, the amount of water dissolved in the oil corresponds to the solubility of the compound under the specified conditions of temperature and pressure; and that through this balance of components; the water exerts its influence on the equilibrium phases of the system. In flash distillation, equilibrium is also affected by the ratio between the

amount of oil and the amount of steam injected in a small time interval. In fact the latter simulation strategy represents more correctly what happens in the batch distillation of vegetable oils, which corresponds to an evaporation batch with steam injection.

The main objective of this paper is to compare these two simulation strategies for future in order to evaluate them in comparison with experimental data in the future.

7.3 Results and Discussion

The following are the results obtained for the flash and differential distillation simulations performed under similar conditions as used in the industry (temperature: 230 to 250 °C, pressure: 5 to 10 mmHg and steam injection: 0.5 to 2.5 %, the latter represents the total mass of steam as a percentage of the initial oil cargo and this vapor mass should be distributed over the 60 minutes of stripping used in the process).

Figures 7.1A and 7.1B show the profiles for acidity and neutral oil loss (NOL) obtained from the two simulations. Note that the acidity obtained with the two approaches is approximately equal at the end of 60 minutes, however, the profiles obtained over time are different (Fig. 7.1A).

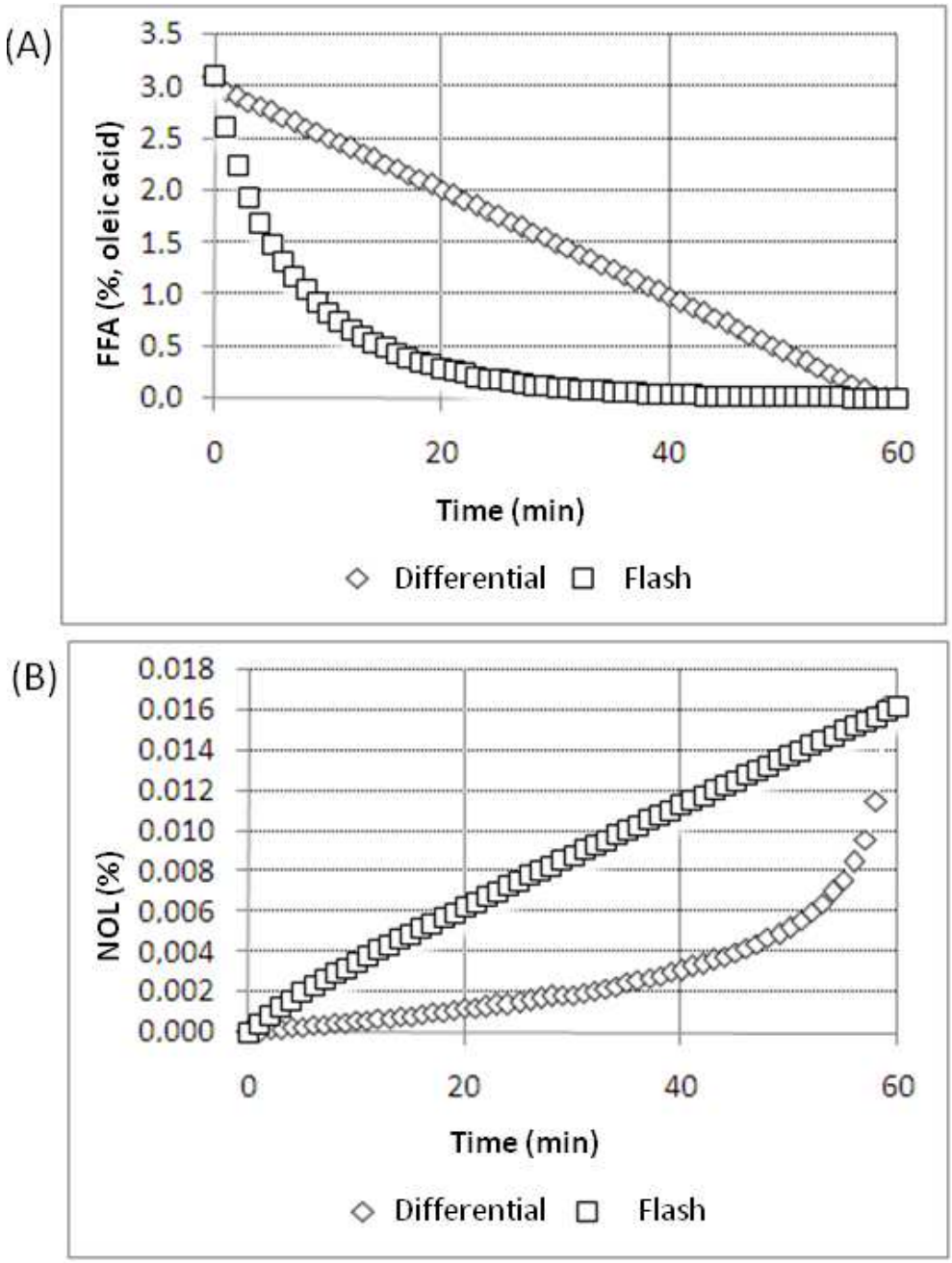

Figure 7.1. Comparison of FFA (A) and NOL (B) obtained from the differential and flash approaches and (250 °C, 2.5 mmHg and 5% steam).

The differential approach generates a linear reduction in acidity. This occurs because in this approach, the vaporization rate as well as process temperature is fixed over time. In this case, there is the variation of the amount of water dissolved

in the oil, and thus the concentrations of the other components are calculated. In the flash approach, temperature, together with the compositions of the vapor and liquid phases, is one of the responses of the program and varies with time. Thus, one can observe that the vaporization rate is not constant and the acidity reduces exponentially. In fact, experiments have proved that in the physical refining of vegetable oils, the acidity reduces exponentially (SILVA, 2009; TAHAM, 2009).

In the case of NOL (Figure 7.1B), the tendencies reverse with an exponential increase in differential distillation and a linear increase in flash distillation. It is also interesting to note that the opposite happens with acidity: the values obtained with NOL flash distillation are higher during the entire process time. Therefore, the amount of water vaporized at each instant is approximately equal in the two approaches.

It is believed that flash distillation represents a more adequate deacidification of vegetable oils in batches by simultaneously keeping the transient character of the vaporization of fatty compounds present in the oil batch, and the continuous injection of sparge steam, taking into account both the effect of water on system phase equilibrium of the system and its influence on mass balances and enthalpy of the process. Therefore, the effects of temperature, pressure and steam flow rate will be discussed below based on this approach.

Figure 7.2A shows the temperature profile obtained with the flash approach. Note that the temperature remains very close to that determined for means of the process, however with a slight tendency to decrease. Model 3 proposed by [15], which regards that the temperature is constant throughout the stripping process, despite being a good approximation, it is not the best way to describe the process.

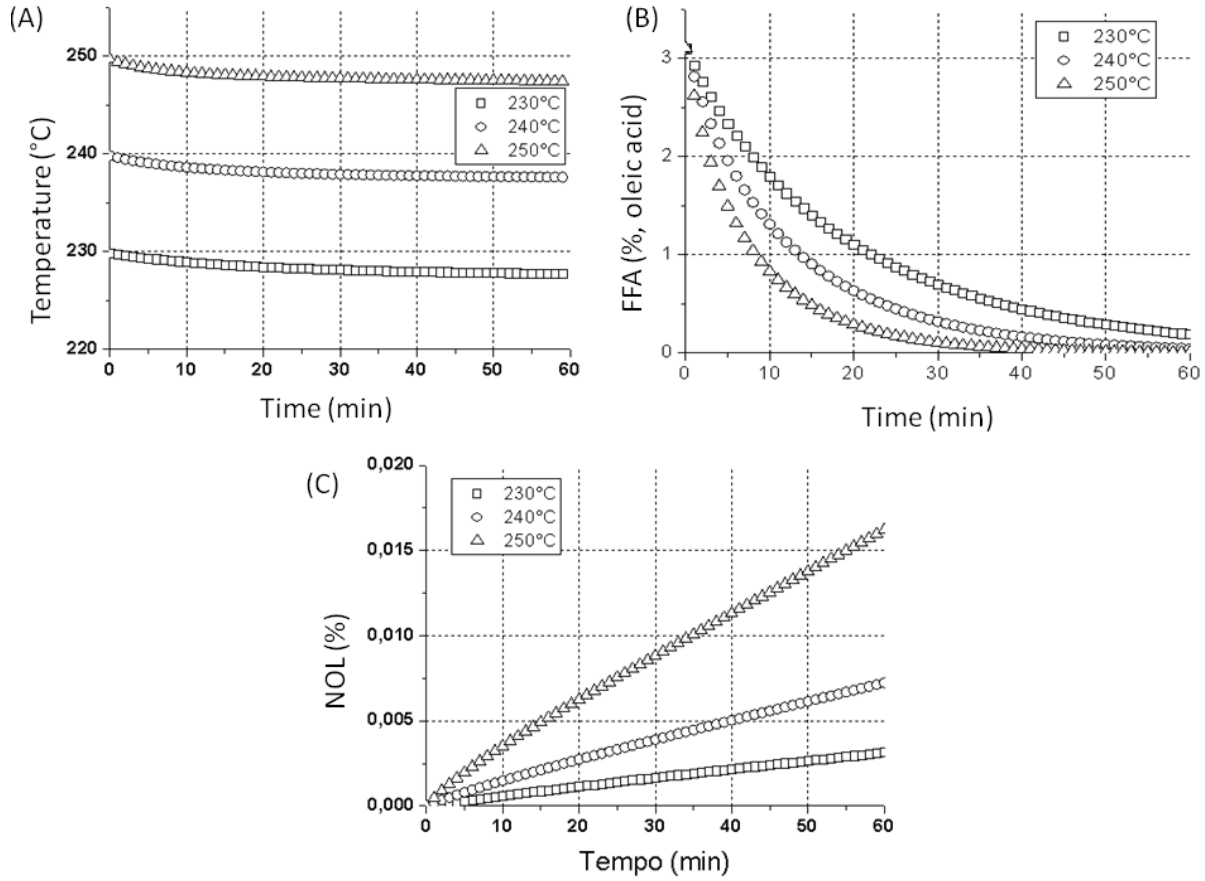

Figure 7.2. Temperature profiles (A), FFA (B) and NOL (C) obtained from the flash approach 2.5 to 5 mmHg and steam.

Figures 7.2B and 7.2C show the profiles of NOL and acidity observed at 5 mmHg and with an steam injection of 2.5% vapor, with varied process temperatures (230, 240 and 250 °C). Under such experimental conditions, the increased temperature causes a slight decrease in the acidity of the final oil. Note that the differences in final acidity obtained with the highest and lowest temperature did not exceed 0.2 %. NOL is more sensitive to temperature, increasing up to four times the heat.

Figure 7.3A shows the temperature profiles obtained by setting the initial temperature of the oil batch at 250 °C and the pressure at 5 mmHg, with the steam flow variation rate (0.5, 1.5 and 2.5 %). Note that the greater the flow of steam used, the greater the decrease in temperature with time. This occurs because the

supply of heat to the system is disregarded. Thus, the greater the flow of steam, the greater the rate of vaporization, and therefore the greater the amount of energy removed from the oil to guarantee the volatilization of the compounds.

Figures 7.3B and 7.3C show the acidity and NOL profiles obtained at 250 °C and 5 mmHg, with variation of steam flow rate (0.5, 1.5 and 2.5%). Interestingly, the acidity values obtained after 60 minutes are very close when compared with the steam injections of 1.5 and 2.5 %. However, the FFA reduction was significantly lower when using 0.5 % steam. In the case of NOL, significant differences occurred when comparing all steam flows.

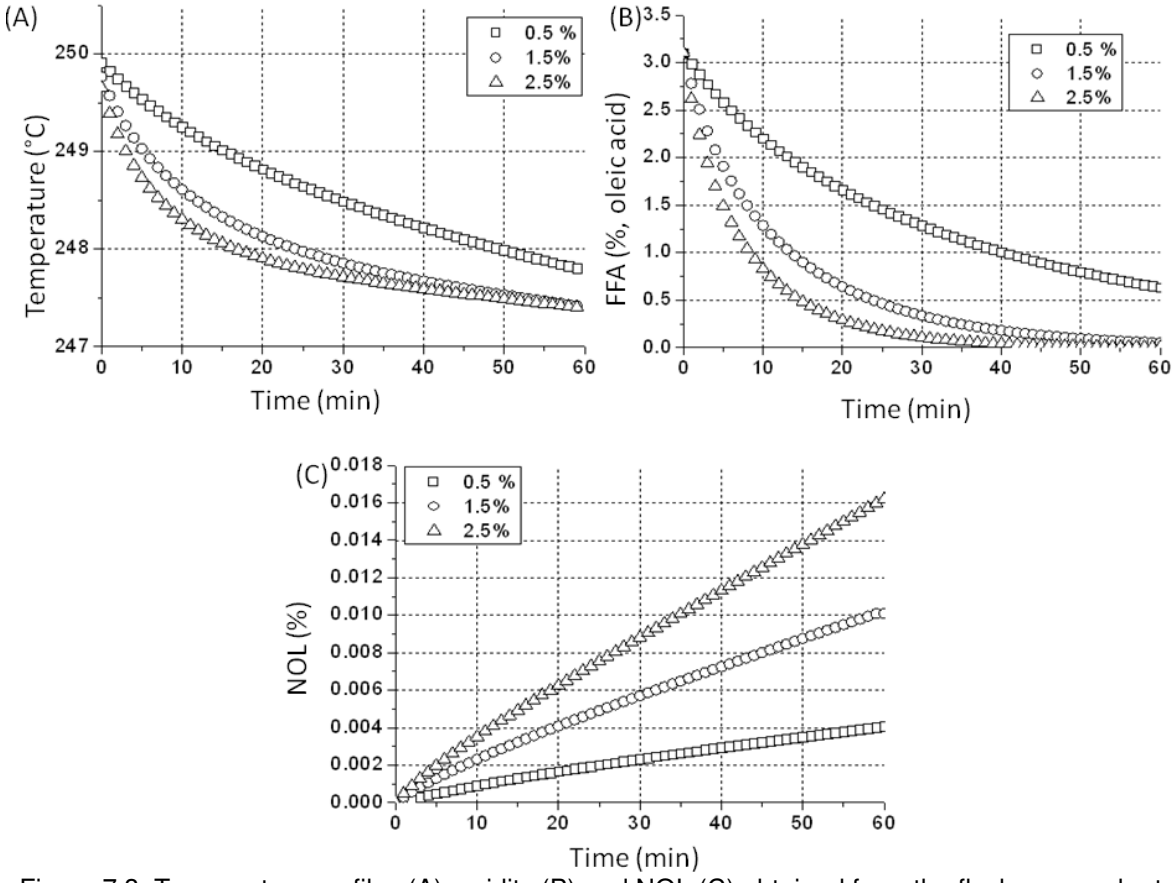

Figure 7.3. Temperature profiles (A), acidity (B) and NOL (C) obtained from the flash approach at 250 °C and 5 mmHg.

Figure 7.4A shows temperature profiles obtained by setting the process temperature to 250 °C and the steam flow rate at 2.5 %, and varying the pressure (5, 7, and 10 mmHg). One may note that the lower the pressure, the greater the decrease in temperature. As in the case of increased steam flow rate, the decrease in pressure increases the rate of vaporization of the compounds and thus a greater amount of energy must be removed from the oil by reducing its temperature.

The NOL is more sensitive to variations in system pressure, increasing up to two times compared to those with the highest and lowest pressures.

The results presented in Figures 7.2 to 7.4 show a lower sensitivity of the final acidity of the oil, compared to the neutral oil loss with changes in temperature, pressure and flow rate of steam injected directly. Such findings may provide a better choice of operating conditions that ensure that acidity of the final product lies within the amount required by law (0.3 %) as well as low neutral oil loss.

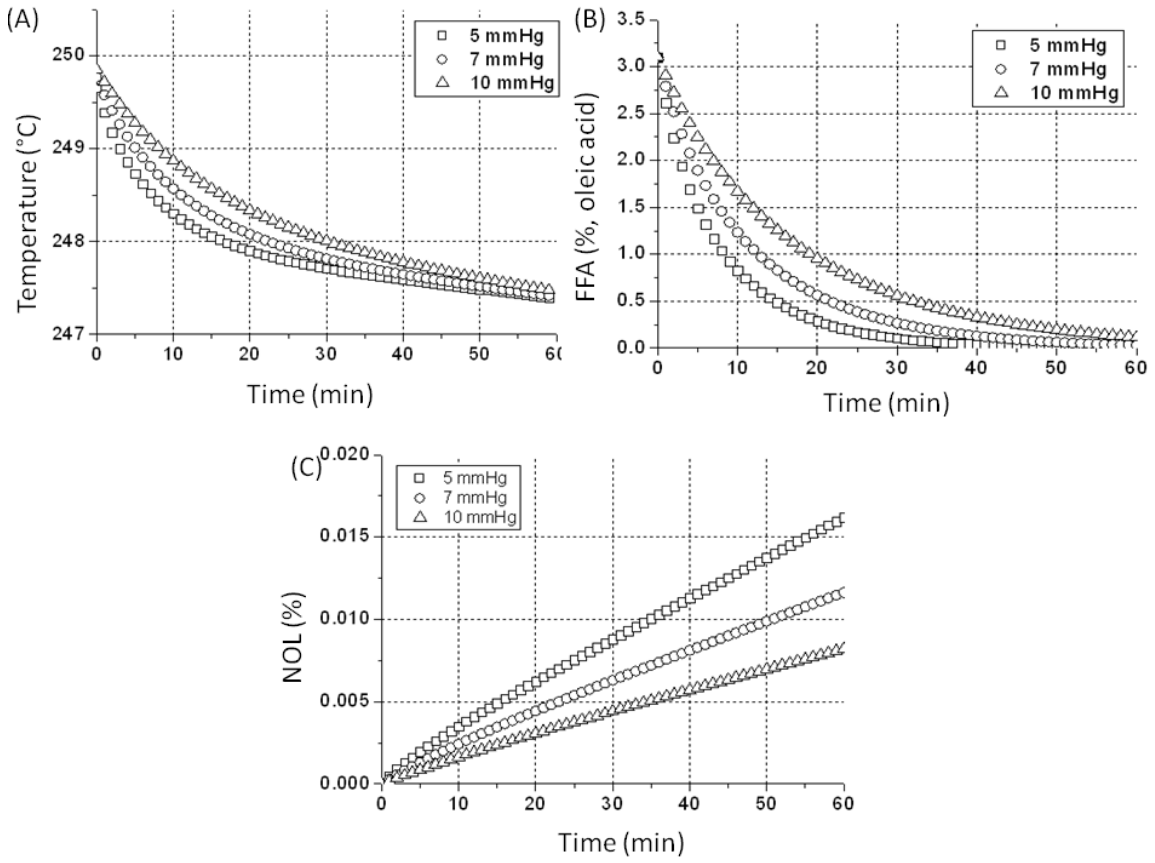

Figure 7.4. Temperature profiles (A), FFA (B) and NOL (C) obtained from the flash approach at 250 °C and 2.5% steam.

Figure 7.5 shows a comparison of the values of NOL obtained at 250 °C and 5 mmHg, with a range of steam flow rates. Interestingly, the values obtained with differential distillation are equal in the three flow rates tested, and therefore, its graph lines are overlapped, making it difficult to view the three curves. This result had been anticipated because in the differential approach the injected water is regarded only in the liquid phase, and its mole fraction corresponds to its solubility in oil at a given pressure and temperature. Note that in the three situations tested, the temperature and pressure are equal and therefore have the same water solubility in oil. The remaining water in the vapor phase does not interfere with NOLs. In the case of the flash approach, the greater the flow of steam, the higher the NOL- a result that better describes the actual process.

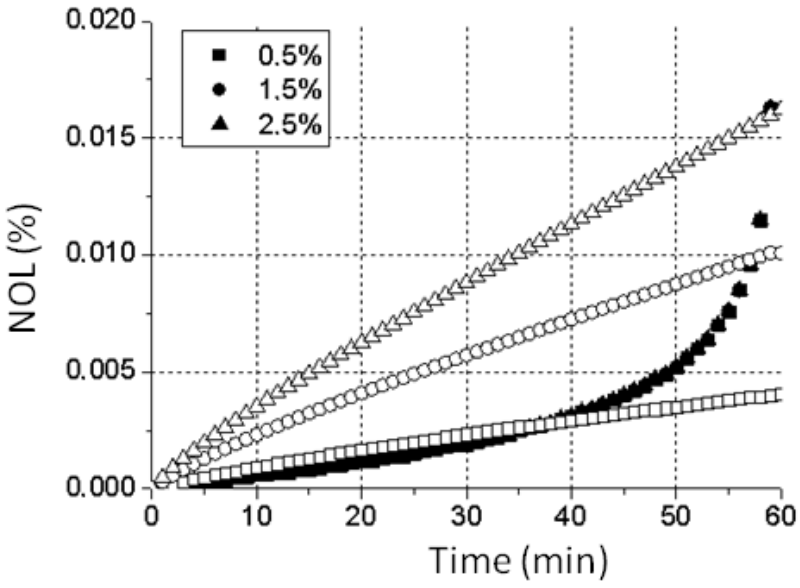

Figure 7.5. NOL profiles obtained at 250 °C and 5 mmHg with the flash approach (open symbols) and differential (filled symbols) with different steam flow rates.

7.4 Conclusion

The flash approach represented a better example of the actual process when compared to differential distillation simulation. Therefore, this approach will be further studied in future experiments where the heat supply system, and hydrolysis reactions of *cis-trans* isomerization occurring in the oil due to high temperatures and mechanical drag of the liquid phase will be considered. Thus, future simulation data may be compared to data obtained experimentally.

This study indicates that NOL is more sensitive to variations in process than acidity. This fact enables the study of optimal parameters, which generate the desired final acidity (as established by law), with less neutral oil loss and the lowest cost process to produce steam and vacuum.

Nomenclature

d_i - number of moles for component *i* in the distillate container.

D - Total number of moles of the distillate

VLE - Vapor -Liquid Equilibrium

f_i - fugacity of component *i*

f_i^0 - fugacity of component *i* in the reference state

h_t - enthalpy of the compounds in the liquid phase at time*t*

$H_{t,v}$ - enthalpy of fatty compounds in the vapor phase at time *t*

$H_{t,s}$ - enthalpy of live steam at time *t*

k_i - distribution coefficient of component *i* between the liquid and vapor phases

L - number of moles of liquid in the tank

l_i - quantity of component *i* in the liquid phase (mole)

P - total pressure

NOL – Neutral Oil Loss

P_i^{vp} - vapor pressure of pure component *i*

R - gas constant

$\dot{s}_{t,i}$ - steam injection of compound *i* in moles / min

T - absolute system temperature;

x_i - molar fraction of component *i* in the liquid state

y_i- molar fraction of component *i* in the vapor state

$\dot{v}$ - molar vaporization rate in moles / time

$\dot{v}_{t,i}$ - vaporization rate of component *i* in moles / min

V_i^L - molar volume of component *i* in the liquid state

TAG – triacylglycerol

γ_i - activity coefficient

ϕ_i - fugacity coefficient

ϕ_i^{sat} - fugacity of pure component *i*

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Chapter 8. "SIMULATION OF BATCH STEAM DEACIDIFICATION OF COCONUT OIL"

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Keywords:

flash distillation; edible oils; lauric acid; neutral oil loss

Abstract

A new approach that considers mass and enthalpy balances, equilibrium relationships and heat transfer was tested to simulate a steam deacidification process of coconut oil. These equations had already been used successfully to describe a continuous crosscurrent cascade process in a trayed column. In this work, this approach was adapted to a steam deacidification process in which oil is supplied only at the beginning of the process (batch) while steam is continuously injected into the still, improving the mass transfer of compounds into vapour phase, which is also removed continuously. The equations were partitioned by Naphtali-Sandholm method and then solved by the Newton-Raphson method using Matlab software. Group contribution methods were selected to calculate all the physical properties needed in the equilibrium relationships and energy balances. This approach presented good results in relation to the oil final acidity. The oil acidity profile showed an exponential decrease and the temperature presented small variation along the stripping period, which is in accordance with experimental results. In this way, the suggested procedure can be considered as a valuable tool to deeply investigate common processes of the oil and fat industry.

8.1 Introduction

The steam deacidification is the final step in edible oils processing. It can be considered as a stripping process with as main objective reduction of undesirable volatile compounds [1]. Steam deacidification can occur in different ways: continuous, semi-continuous and batch. The latter is suitable for small scale processes and has as advantages the low cost and easy construction [2]. Although a batch process is not commonly used on industrial scale, it includes the most important phenomena that occur during the continuous process. For that reason, the batch processing was studied in several works reported in literature [3-5].

In the batch process, oil is supplied only at the process beginning while steam is continuously injected into the oil, improving the mass transfer of compounds into the vapour phase, which is also removed continuously. During this process, undesirable removal also occurs, like the loss of triacylglycerols (TAG), diacylglicerols (DAG) and monoacylglycerols (MAG) [2]. Therefore, the process parameters (temperature, retention time, pressure and amount of stripping agent) have to be optimized to ensure final product specification and to avoid neutral oil losses and high costs. In this context, the computational simulation emerges as a tool that allows the process analyses and optimisation without doing experiments leading to additional costs.

The batch process was already studied using the computational simulation as a differential distillation [5]: a tank (still) is charged with feed and then heated, vapour flows overhead, is condensed, and is collected in a receiver. In this study, oil and distillate composition varies with time. The authors considered the process as a sequence of numerous and successive vaporisations [5]. This approach was satisfactory to estimate the final oil acidity but presents significant differences when this response is analysed throughout the process [6].

Continuous steam deacidification was also studied by computational simulation presenting good results [7]. Both counter current and cross flow were modelled using mass and enthalpy balance equations and vapour-liquid equilibria (VLE) relations. These equations were adapted in our work to describe a batch steam deacidification.

Thus, the goal of this work was to simulate the batch physical refining of coconut oil, using a realistic approach that considers mass and enthalpy balances, equilibrium relations and heat transfer.

8.2 Materials & Methods

Ceriani & Meirelles described the crossflow steam deacidification process by mass and enthalpy balances and VLE relations [7] for each stage. In this work, the same equations are used to describe the batch steam deacidification as a flash

distillation, however time is used instead of stages. The batch process is divided in t times that are considered as stages of Ceriani & Meirelles column, receiving a certain amount of steam that is removed in the same stage/time (Figure 8.1).

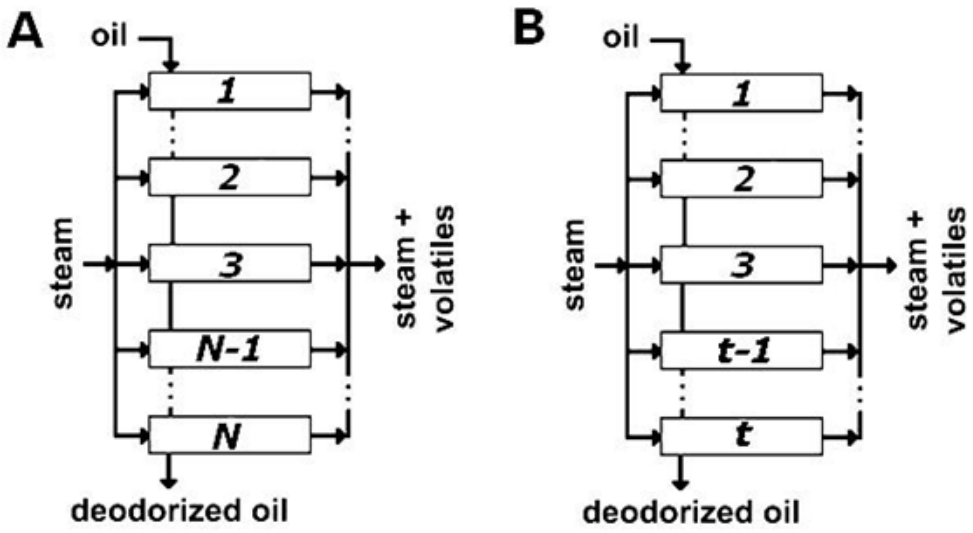

Figure 8.1. Steam deacidification design: (A) Ceriani & Meirelles Crossflow and (B) Batch

To simulate deacidification experiments, vapour pressure equations and thermodynamic approach proposed by Ceriani & Meirelles [8] was used to describe Vapour-Liquid Equilibria (VLE) of fatty compounds (Equations 8.1 and 8.2).

$$k_i = \frac{y_i}{x_i} = \frac{\gamma_i \cdot f_i}{P \cdot \phi_i} \tag{8.1}$$

$$f_i^0 = P_i^{vp} \cdot \phi_i^{sat} \cdot exp\left(\frac{V_i^L \cdot \left(P - P_i^{vp}\right)}{R \cdot T}\right) \tag{8.2}$$

where k_i is the distribution coefficient of *i* in both liquid and vapour phases; f_i^0 is the fugacity of component *i* in reference; x_i and y_i are molar fractions of component *i* in both liquid and vapour phases, respectively; P is the total pressure; R is the gas constant; T is the absolute system temperature; P_i^{vp} and ϕ_i^{sat} are respectively,

vapour pressure and fugacity coefficient of the pure component *i*; γ_i is the activity coefficient[2] of component *i*; ϕ_i is the fugacity coefficient of component *i*; V_i^L is the liquid molar volume of component *i*. The exponential term in Equation 8.2 is called the Poynting factor (POY).

Mass and enthalpy balances (Equations 8.3 and 8.4) and equilibria relations (Equation 5.5) were adapted from Ceriani & Meirelles [7] to this work.

$$F_{1(t,i)} = l_{t,i} - l_{t-1,i} + \dot{v}_{t,i} - \dot{s}_{t,i} = 0 \tag{8.3}$$

$$F_{2(t)} = h_t - h_{t-1} + H_{t,v} - H_{t,s} - UA = 0 \tag{8.4}$$

$$F_{3(t,i)} = \dot{v}_t \cdot k_{t,i} \cdot \frac{l_{t,i}}{L_t} - \dot{v}_{t,i} = 0 \tag{8.5}$$

where $l_{t,i}$ and $l_{t-1,i}$ is total moles of liquid in the still at instant *t* and *t-1*, respectively; $\dot{v}_{t,i}$ is the vapour phase formed during the flash in mols/min and $\dot{s}_{t,i}$ is the vapour injection of component *i* in mols/min. Note that, $\dot{s}_{t,i}$ is equal to the injected steam flow per minute when *i* is water and for all the other components, $\dot{s}_{t,i}$is equal to zero. h_t is liquid phase enthalpy in time *t*; $H_{t,v}$ is vapour enthalpy of fatty components in time *t*; $H_{t,s}$ is vapour phase enthalpy of steam in time *t*. *UA* is the overall heat transfer coefficient times heat exchange surface area in J/K·min (estimated according to literature [9]). $k_{t,i}$ is distribution coefficient of component *i* in time *t*.

This approach generates for each time interval *NC* mass balances, 1 enthalpy balance and *NC* equilibrium relations where *NC* is equal to the number of

[2] Ceriani and Meirelles (2004) considered the non-ideality in both vapour and liquid phases. The activity coefficients were calculated according to the predictive UNIFAC model, modified by Fornari et al. (1994) [15]. The fugacity coefficients were calculated by the Virial equation. The Poyting factor was considered equal to 1.

components that constitute the oil. All the equations were partitioned using the Naphtali and Sandholm [10] calculation method of multicomponent separation. The equations are related only to times t and t-1, so, the Jacobian matrix formed is very sparsed and it was solved using the Newton-Raphson method giving as responses $l_{t,i}$, $\dot{v}_{t,i}$ and T. Variations in composition in liquid and vapour phase can be obtained throughout the time using Equations 8.6 and 8.7.

$$x_{t,i} = \frac{l_{t,i}}{\sum_{i=1}^{NC} l_{t,i}} \tag{8.6}$$

$$y_{t,i} = \frac{\dot{v}_{t,i}}{\sum_{i=1}^{\mathrm{NC}} \dot{v}_{t,i}} \tag{8.7}$$

The vaporisation efficiency was considered equal to one. All equations were compiled using Matlab software. Heat capacities and vapour pressure of fatty compounds were predicted using group contribution method. Necessary input data are retention time, pressure and temperature of the process, percentage of steam and oil composition.

This work used the coconut oil deacidification data obtained by Petrauskaitè et al. [3] to run the simulations. The authors have done the experiments in a batch scale equipment containing 250 g of bleached coconut oil. The experiments were conducted for 60 minutes under temperatures and pressures between 190 °C and 230 °C and 1.6 and 3.0 mbar, respectively. Steam percentage varied from 0.6 % to 1.2 %. Since Petrauskaitè et al. [3] have not described the coconut oil composition in terms of partial acylglycerols, this work performed computational simulations considering a coconut oil with the composition used by Ceriani & Meirelles [5] to simulate these experiments. The composition that presented the best results in the differential distillation was choosen: 0.89 % of diacylglycerols (DAG) and 0.27 % of monoacylglycerols (MAG).

8.3 Results & Discussion

This work simulated the experiments of coconut oil deacidification done by Petrauskaitè et al. [3], using an flash distillation in which VLE is affected by the system pressure, the quantity of oil in the still and the quantity of steam injected in a short period of time. In a first step, simulations were done without considering the heat transfer between the equipment and the oil, i.e. *UA* equals zero. Then, the same simulations were done considering that *UA* equals 29.50 J/K·min[3] (according to the literature [9]).

Table 8.1 presents the results for the final acidity, expressed in lauric acid percentage. Calculated final acidity presents satisfactory values for all process conditions. Note that, all experiments have as intermediate response to those obtained in computational simulation. Considering *UA* equals zero tends to overestimate acidity responses. On the other hand, considering a *UA* equals 29.50 J/K·min tends to slightly underestimate the responses. In this last case, absolute deviations were lower. Thus, it can be noted that is important to add a heat transfer term in the enthalpy balance to obtain more realistic results. As expected, our results were more approximated than that found by Ceriani & Meirelles [5].

Table 8.1. Comparison of Refined final acidity by Petrauskaitè et al. (2000) and this work.*

	Process conditions			FFA oil out (%, lauric)		
Exp	Temp (℃)	Steam (%)	Pressure (mbar)	Petrauskaitè et al.	*UA* = 0 J/K·min	*UA* = 29.5 J/K·min
1	190	0.6	1.6	0.235	0.403	0.232
2	210	0.8	1.6	0.070	0.135	0.058
3	230	0.7	1.6	0.019	0.045	0.011
4	230	0.6	2.3	0.033	0.091	0.034
5	230	0.6	3.0	0.035	0.126	0.005
6	230	1.2	3.0	0.017	0.058	0.016

*Experiments numbers correspond exactly to those reported by Petrauskaitè et al. (2000)

[3] This value was experimentally determined in a previous work, as described in Appendix 2.

Figure 8.2 shows acidity profiles for Experiment 6 (230 °C, 3 mbar and 1.2 % steam) considering both *UA* values. It can be noted that acidity presents a non-linear behaviour: free fatty acids removal is larger in the first minutes and then there is only a slightly decrease in acidity. This behaviour is due to different vaporisation rates calculated throughout the process in our approach. Ceriani & Meirelles [5] obtained a linear decrease of acidity once in their approach the vaporisation rate is constant. In fact, experimental acidity profiles shows that acidity removal is not constant with time [13][14]. It can also be noted that acidity values were lower for larger *UA* during all the process.

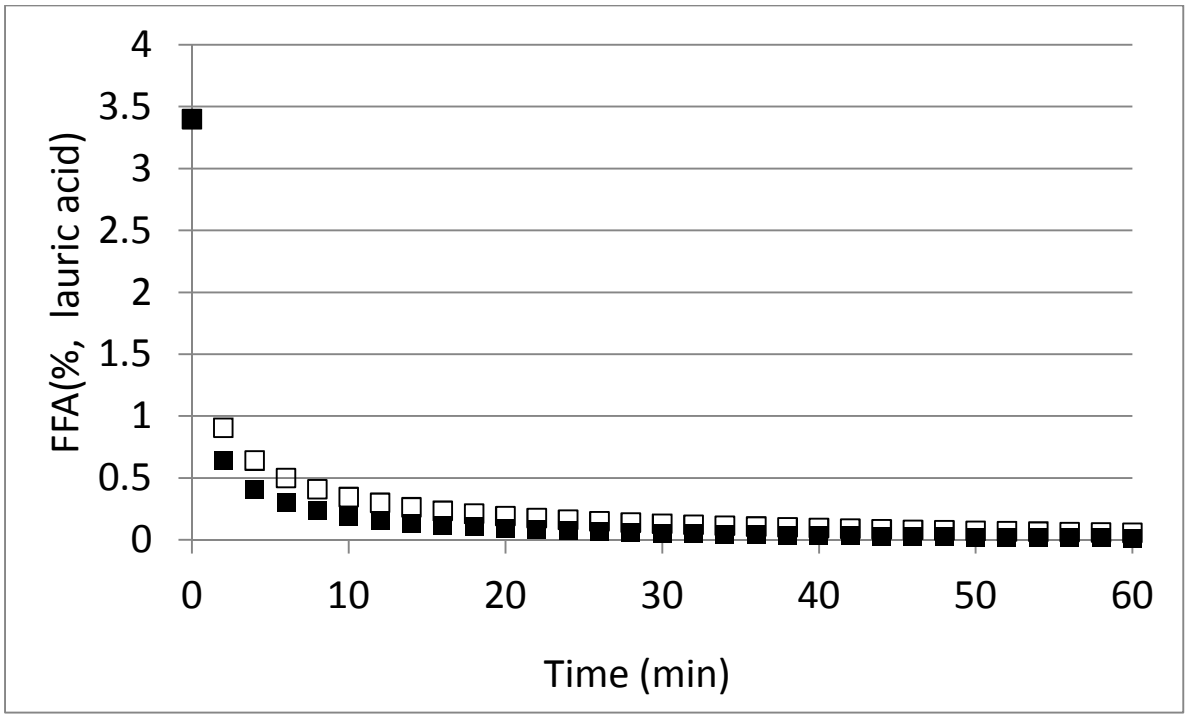

Figure 8.2. Simulated acidity profile for Exp 6: UA equals 0 (empty square) and UA equals 29.5 J/K·min (full square)

The flash distillation approach can predict satisfactorily the neutral oil loss (NOL) results with low absolute deviation values (Table 8.2). If the heat transfer is considered, NOL tends to approximate to the experimental values. The simulated values are lower than the experimental for both *UA* cases. One probable

explanation is the entrainment of partial acylglycerols. During the experiments, small quantities of neutral oil can be mechanically carried by the steam used improve fatty acids transfer to the vapour phase. This is not predicted in our equation.

Table 8.2. Comparison of Neutral Oil Loss by Petrauskaitè et al.(2000) and this work.

	Process conditions			Neutral Oil Loss (%)		
Exp	Temp (℃)	Steam (%)	Pressure (mbar)	Petrauskaitè et al.	*UA* = 0 J/K·min	*UA* = 29.5 J/K·min
1	190	0.6	1.6	0.28	0.12	0.18
2	210	0.8	1.6	0.57	0.26	0.41
3	230	0.7	1.6	1.28	0.47	0.85
4	230	0.6	2.3	1.21	0.35	0.60
5	230	0.6	3.0	0.89	0.30	0.55
6	230	1.2	3.0	0.93	0.43	0.77

Figure 8.3 presents the NOL profile for Experiment 6 considering both *UA* values. NOL increases with time: in the first minutes a considerable loss occurs and then it tends to increase linearly. It can be seen that a higher UA value provides a higher loss. It is in agreement with the acidity results: when the heat transfer is considered, the system achieves higher temperatures, and thus, it has more energy to volatilise fatty compounds, increasing the acidity removal and the oil loss. It was not found in the literature NOL profiles to compare with the simulations carried out in this work.

The last response analysed by this simulations was the temperature profile. Figure 8.4 presents profiles for Experiments 5 and 6 considering both *UA* values. It can be noted that when *UA* value is equal to zero, the oil temperature decreases due to the energy reduction of the system, i.e. the energy to volatilise the fatty compounds is removed from the oil resulting in a temperature reduction. When *UA* value is different from zero, so that, the necessary energy to volatilise the fatty compounds is obtained from the heat source, the temperature maintain quite constant. It is important to highlight that both experiments were done at 230 °C, and so, the temperature at time zero is equal to 230 °C for all profiles.

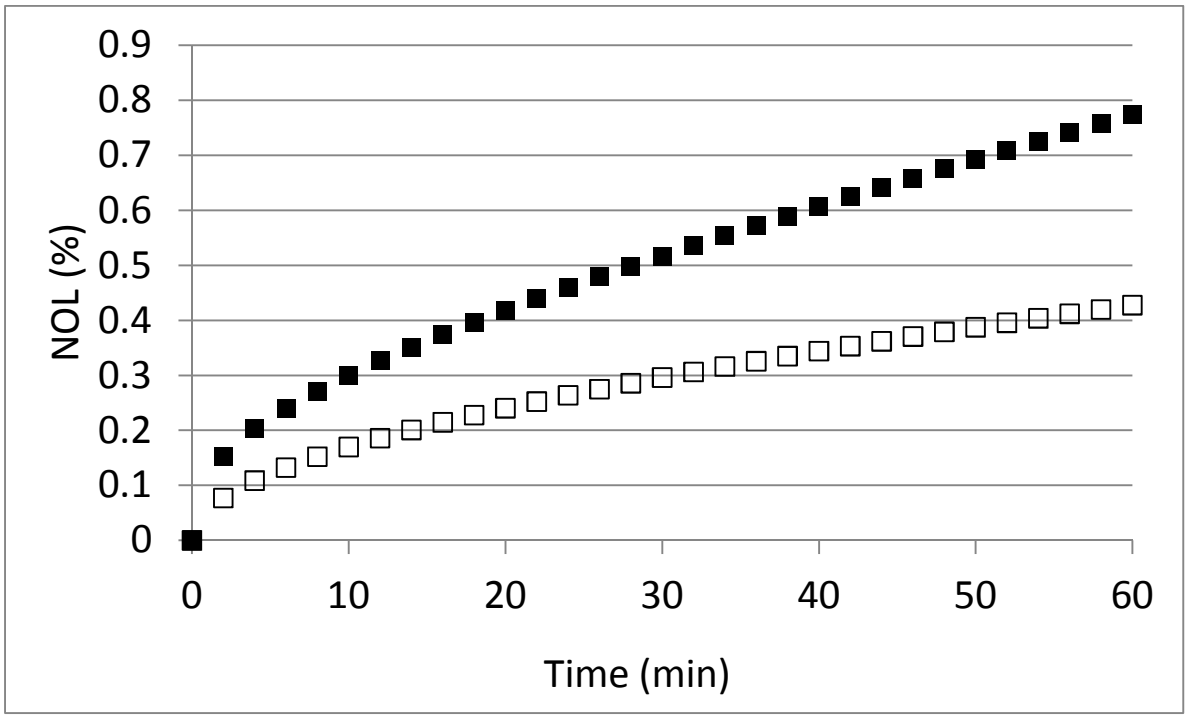

Figure 8.3. Simulated Neutral Oil Loss for Exp 6: *UA* equals 0 (empty square) and *UA* equals 29.5 J/K·min (full square)

It is also interesting to note in the temperature profiles considering the heat transfer that the Experiment 5 profile tends to slightly increase while the Experiment 6 profile tends to slightly decrease. As these experiments were performed using the same temperature and pressure; the difference is only the steam percentage (0.6 % and 1.2 %, for Experiments 5 and 6, respectively). The use of higher amounts of steam entails more fatty compounds. As the same *UA* was used in all experiments, it can be noted that in Experiment 5, the heat given to the system is larger than the necessary to volatilize the compounds with 0.6 % steam and lower than the necessary to volatilize the compounds with 1.2 % steam. In fact, it was observed in experimental works that the system temperature tends to decrease during stripping if not enough heat is given to the process [13][14].

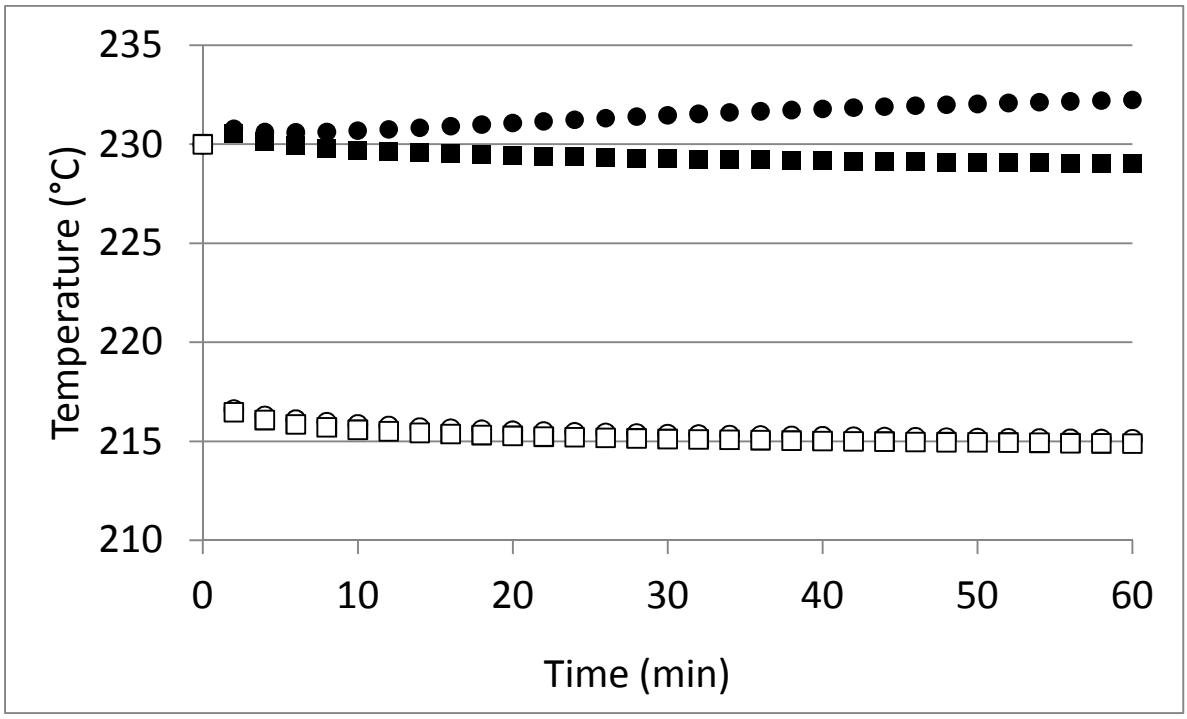

Figure 8.4. Simulated Temperature Profiles for Exp 5 (circles) and Exp 6 (squares): *UA* equals 0 (empty symbols) and *UA* equals 29.5 J/K·min (full symbols)

8.4 Conclusion

In this work, a batch steam deacidification was simulated using a new approach. It presented satisfactory results to describe the final acidity and the neutral oil loss. This approach was also efficient to describe the process parameters profile. In future work, *cis-trans* isomerisation and hydrolysis reactions and mechanical entrainment may be included. Thus, this computational simulation is a valuable tool to deeply investigate common processes of the vegetable oil industry.

Nomenclature

VLE - Vapor -Liquid Equilibrium

f_i - fugacity of component *i*

f_i^0 - fugacity of component *i* in the reference state

h_t - enthalpy of the compounds in the liquid phase at time *t*

$H_{t,v}$ - enthalpy of fatty compounds in the vapor phase at time *t*

$H_{t,s}$ - enthalpy of live steam at time *t*

k_i - distribution coefficient of component *i* between the liquid and vapor phases

$l_{t,i}$ - quantity of component *i* in the liquid phase (mole) at instant *t*

L - number of moles of liquid in the tank

P - total pressure

POY – Poynting Factor

NOL – Neutral Oil Loss

P_i^{vp} - vapor pressure of pure component *i*

R - gas constant

$\dot{s}_{t,i}$ - steam injection of compound *i* in moles / min

T - absolute system temperature;

x_i- molar fraction of component *i* in the liquid phase

y_i- molar fraction of component *i* in the vapor phase

$\dot{v}$ - molar vaporization rate in moles / time

$\dot{v}_{t,i}$ - vaporization rate of component *i* in moles / min

V_i^L - molar volume of component *i* in the liquid state

TAG – triacylglycerol

γ_i - activity coefficient of component *i*

ϕ_i - fugacity coefficient of component *i*

ϕ_i^{sat} - fugacity of pure component *i*

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Chapter 9. Conclusion

This thesis provides a better understanding on physical refining of vegetable oils as well as some advances towards high quality products and process improvement by consuming fewer inputs. The bleaching process was deeply studied concerning adsorption phenomena and the effect of different procedures and kinds of bleaching earth on the final color. The deacidification process was investigated by computer simulation, providing the basis for a better understanding of vegetable oils deacidification.

Chapter 3 presented the development and validation of a HPLC methodology for simultaneous quantification of carotenes and tocols. This method using normal phase is simple, sensitive and reproducible. The lower limit of quantification (LOQ) and the lower limit of detection (LOD) were established. The methodology was successfully applied for quantification of those compounds in Amazon oils, concluding that both photo diode array (PDA) and fluorescence detectors can be used to quantify tocopherols and tocotrienols in vegetable oils. Later, it was used by our research group for determining thermal degradation of carotenes (Thermal Degradation Kinetics of Carotenes in Palm Oil, Journal of American Oils Chemists' Society, DOI: 10.1007/s11746-012-2156-1) and liquid-liquid equilibrium of fatty systems (Experimental data for liquid–liquid equilibrium of fatty systems with emphasis on the distribution of tocopherols and tocotrienols, Fluid Phase Equilibria, DOI: 10.1016/j.fluid.2012.09.033).

Chapter 4 presents a study on the adsorption phenomena of carotenes and phosphorus during bleaching processes. Two kinetic models were tested for carotenes, being the pseudo-second-order model which is better to describe carotene adsorption. Phosphorus adsorption was too fast, resulting in a lack of data for analyzing its kinetics. The equilibrium data are described more accurately by Langmuir and Freundlich models, for β-carotenes and phosphorus, respectively. A thermodynamic study demonstrated that β-carotenes and phosphorus adsorption is spontaneous, endothermic and an entropy-driven process. High temperatures

should be preferred to increase adsorption of carotenes and phosphorus. However, other factors as undesirable side reactions should be taken into account when defining the best bleaching temperature to obtain a light color oil.

Chapter 5 presents the effect of new bleaching procedures, showing that this step can still be optimized concerning final color and the kind of bleaching earth. A two step refining, i.e. a procedure based on two mild deodorization steps was suggested and presented an improvement in color although the same total amount of bleaching earth and total deodorization residence time were used. In case of keeping the same quality of the final product, i.e. the same color obtained in the traditional approach, the new method would make it possible to decrease the amount of bleaching earth or the deodorization time. The optimization of this approach and the utilization of different adsorbents, as silica and activated carbon, should be investigated as future work.

Chapter 6 presented work on the effect of the kind of bleaching earth on the palm oil final color. An inverse correlation between p-anisidine values after bleaching and the final color after deodorization was observed. Moreover, a hypothesis to explain how the kind of bleaching earth (neutral or acid activated) can interfere on the palm oil final color was presented and discussed; this hypothesis suggested that the reduction of pH can change β-carotene oxidative pathways and the kind of products formed along the refining steps.

Chapter 7 compared two different mathematical approaches to simulate the steam stripping (physical refining) of vegetable oils: differential and flash distillations. The flash approach represented a more reliable simulation procedure when compared to differential distillation. Differential distillation presented a linear trend for free fatty acids removal. However, experimental results have shown that fatty acids removal has an exponential behavior, like the one obtained by flash distillation equations. Furthermore, this study indicated that neutral oil loss is more sensitive to variations in process than acidity. It was concluded that flash distillation equations provide more realistic results and this procedure was chosen for further studies on computer simulation of vegetable oils.

Chapter 8 adapted successfully an algorithm used to describe a continuous crosscurrent deodorizer for simulating a batch deodorization versus time. The algorithm took into account the continuous feed of sparge steam as well as the heat transfer to the oil in batch, features that are able to properly reproduce the operation of batch deodorizers. This approach, the so-called flash distillation procedure, presented satisfactory results concerning the final oil acidity. The oil acidity profile showed an exponential decrease and the temperature presented a small variation along the stripping period, which is in accordance to the experimental results. In this way, the suggested procedure can be considered as a valuable tool to deeply investigate common processes of the oil and fat industry.

9.1 Suggestions for further works

- To study the adsorption process (kinetics, equilibrium and thermodynamics parameters) of carotenes and phosphorus removal by neutral bleaching earth;
- To study the competitive effect of different components (carotenes, phosphorus and other elements, free fatty acids) on adsorption;
- To analyze by HPLC, mass spectrophotometry or other advanced techniques, the oxidation products formed during bleaching with different types of bleaching earth;
- To simulate deacidification processes considering reactions, such as hydrolysis of triacylglycerols and thermal degradation of carotenes.

Appendix 1

Table A1 - Experimental Data from β-carotene adsorption at 105 °C onto Tonsil OPT 210 FF

Time (min)	***C (mg/kg)***
0	454±5.5
15	63±0.0
25	42±0.6
35	31±0.0
45	22±14.33
65	25±1.0
85	23±0.6
105	28±0.0

Table A2- Experimental Data from phosphorus adsorption at 105 °C onto Tonsil OPT 210 FF

Time (min)	***C (mg/kg)***
0	19.11±0.02
15	0.34±0.09
25	0.05±0.00
35	0.03±0.04
45	0.02±0.00
65	ND*
85	ND
105	ND

**Not Detected*

Table A3- Experimental Data from β-carotene isotherm adsorption at 90 °C, 105 °C and 115 °C onto Tonsil OPT 210 FF

	90 °C		105 °C		115 °C	
BE (%)	C_e (mg/kg)	q_e (mg/g)	C_e (mg/kg)	q_e (mg/g)	C_e (mg/kg)	q_e (mg/g)
0	399		467		399	
0.5	233	33	258	42	96	43
0.7	177	32	178	41	52	39
0.9	135	29	128	38	47	35
1.0	121	28	93	37	21	25
1.5			54	28	15	19
2.0	74	16	40	21	10	13
3.0	44	12	16	15	96	43

Table A4- Experimental Data from phosphorus isotherm adsorption at 90 °C, 105 °C and 115 °C onto Tonsil OPT 210 FF

BE (%)	C_e (mg/kg)	q_e (mg/g)	C_e (mg/kg)	q_e (mg/g)	C_e (mg/kg)	q_e (mg/g)
0	19.1		19.1		19.1	
0.5	2.9	3.3	2.7	3.3	3.4	3.2
0.7	2.3	2.4	2.3	2.4	2.5	2.4
0.9	1.8	1.9	1.8	1.9	1.9	1.9
1.0	1.3	1.8	1.3	1.8	1.5	1.8
1.5	0.6	1.2	0.6	1.2	0.6	1.2
2.0	0.4	0.9	0.4	0.9	0.3	0.9
3.0	0.3	0.6	0.2	0.6	0.1	0.6

Table A5 – Linear adjusts for K_0 calculation - β-carotenes adsorption onto Tonsil OPT 210 FF

Temperature (°C)	Equation	K_o
90	$ln\left(\frac{q_e}{C_e}\right) = -10343q_e + 5.8074$	333
105	$ln\left(\frac{q_e}{C_e}\right) = -27661q_e + 7.5725$	1944
115	$ln\left(\frac{q_e}{C_e}\right) = -30805q_e + 8.5583$	5210

Table A6 – Linear adjusts for K_0 calculation - phosphorus adsorption onto Tonsil OPT 210 FF

Temperature (°C)	Equation	K_o
90	$ln\left(\frac{q_e}{C_e}\right) = -10282q_e + 7.9123$	2731
105	$ln\left(\frac{q_e}{C_e}\right) = -13242q_e + 8.1650$	3516
115	$ln\left(\frac{q_e}{C_e}\right) = -26120q_e + 8.9805$	7947

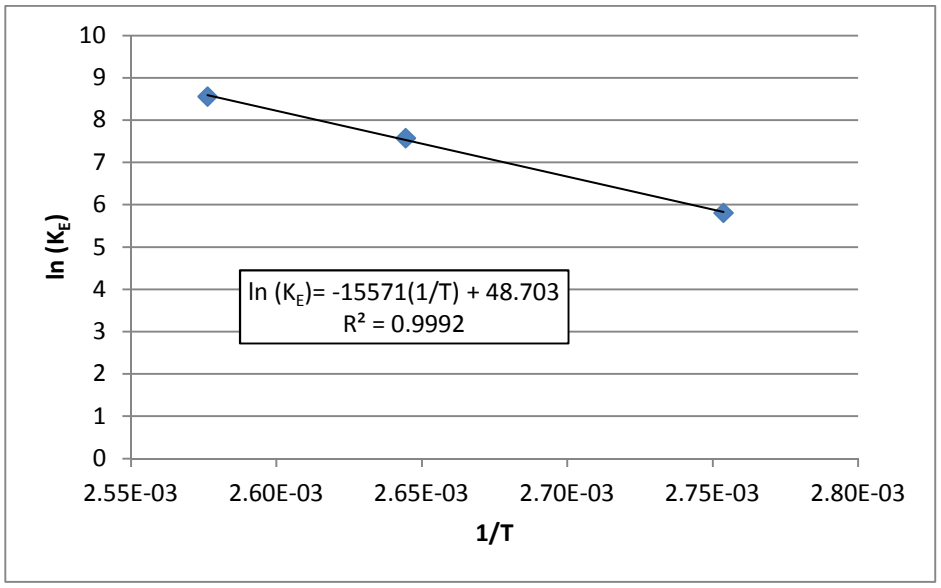

Figure A1 – Van't Hoff graph for β-carotene adsorption onto Tonsil OPT 210 FF

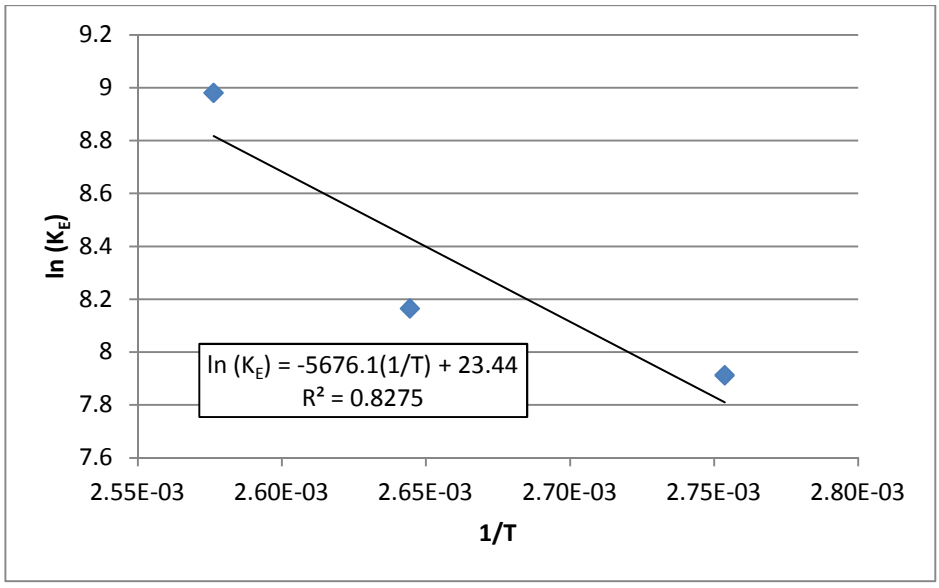

Figure A2 – Van't Hoff graph for phosphorus adsorption onto Tonsil OPT 210 FF

Appendix 2

UA was experimentally determined in a lab-scale deodorizer, the same described by Sampaio et al. (2011). This work was performed as part of the undergraduate research of Pedro Menchik, conducted under the supervision of Dr. Roberta Ceriani. This work was published in the Proceedings of the *8th Latin American Symposium of Food Science, 2009* (**Calculation of the Overall Heat Transfer Coefficient for a Lab-Scale Vegetable Oils Deodorizer** – *in Portuguese***,** Pedro Menchik, Simone Monteiro e Silva, Thiago Taham, Antonio J. A. Meirelles and Roberta Ceriani).

The determination was done in duplicate, using 900 g of fully refined soybean oil (Cargill). It was considered a typical fatty acid composition for soybean oil: 10.82 % palmitic, 4.89 % stearic, 25.21 % oleic, 51.61 % linoleic and 7.47 % linolenic. The amount of sparge steam injection and the heat source temperature were 5.0 % and 290 °C, respectively. The temperatures of heat source and of the oil were measured every two minutes.

The heat capacity was considered as a function of temperature and of composition in fatty acids. It was calculated according to the Equation proposed by Wang and Briggs (2002):

$$C_p = 1.8146 + 0.0021\,T \tag{A2.1}$$

where C_p is the heat capacity (J/g·°C) and T is the oil temperature (°C).

Then, the overall heat transfer coefficient was determined by the following equations, assuming a constant heat source temperature and a well mixed liquid phase:

$$UA = mc_p \frac{\left(T_{jacket} - T_{oil,2}\right)}{\left(T_{jacket} - T_{oil,1}\right)} \tag{A2.2}$$

$$T_{ad} = \frac{\left(T_{jacket} - T_{oil,2}\right)}{\left(T_{jacket} - T_{oil,1}\right)} \tag{A2.3}$$

where *UA* is the overall heat transfer coefficient (J/K), *m* is the oil mass (g), T_{jacket} is the temperature of the heating source, $T_{oil,1}$ and $T_{oil,2}$ are the temperature of the oil at time 1 and 2, respectively and T_{ad} is the adimensional temperature.

The *UA* was determined only for the step in which the heat source temperature was constant, i.e. T_{jacket} equal to 290 °C. It was not considered in the calculation the heating-up step. The Figure A3 presents the plot of the adimensional temperature against time. A linear correlation was obtained (R^2 = 0.95). From the slope of linear equation, it was calculated *UA* (29.5 J/min). This results is in accordance to the values reported for overall heat transfer coefficient between steam and vegetable oils (PERRY; D.W, 1984).

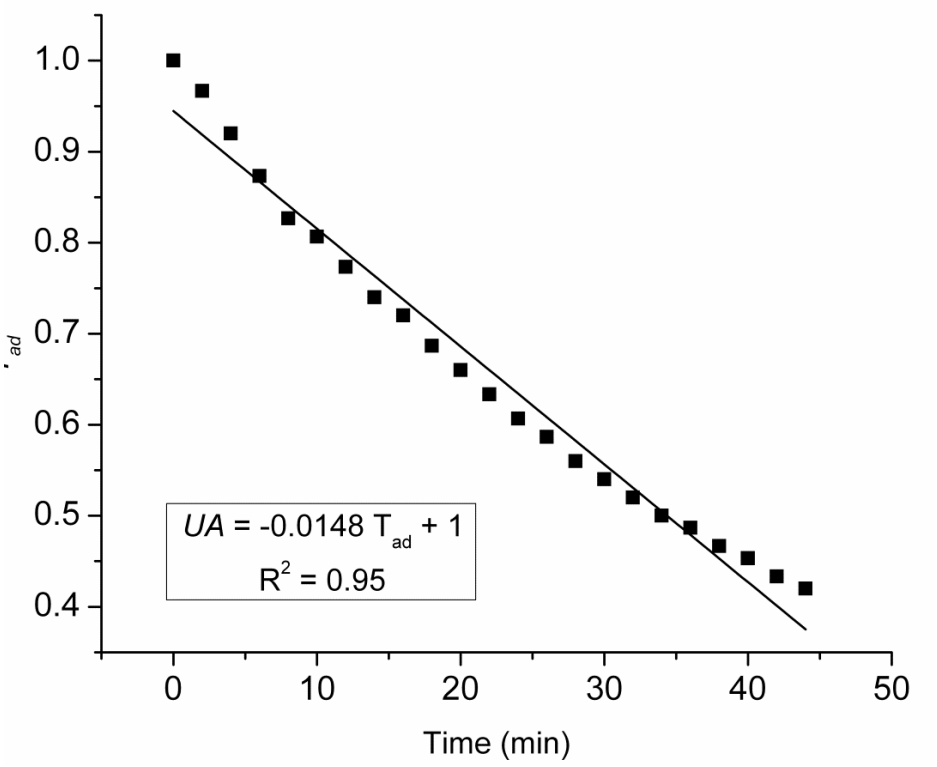

Figure A3 - Adimensional temperature versus time

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