UNIVERSIDAD DE COSTA RICA

SISTEMA DE ESTUDIOS DE POSGRADO

IMPACT OF VACUUM FRYING ON A CAROTENOID-RICH FRUIT: FROM THE MODELING OF DEGRADATION KINETICS DURING PROCESSING AND STORAGE TO THE *IN VIVO* EVALUATION OF THE HEALTH EFFECT IN RATS

Tesis sometida a la consideración de la Comisión del Programa de Doctorado en Ciencias para optar al grado y título de Doctorado en Ciencias

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Ciudad Universitaria Rodrigo Facio, Costa Rica

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Dedication

I dedicate this thesis to my family.

To all the people who believed in me, thank you!

In memoriam, Carolina Rojas Garbanzo.

How many roads must a man walk down, before you call him a man?
Bob Dylan

Acknowledgments

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Resumen

El objetivo de esta investigación fue diseñar un *snack* alternativo a partir de papaya (*Carica papaya* L.) que contenga carotenoides (β-caroteno, BC; β-criptoxantina, BCX; y licopeno, LYC) y ácidos grasos esenciales (ω-6 y ω-3) aplicando la tecnología de fritura al vacío. Las principales preguntas de investigación fueron: *¿Cuáles son los principales factores involucrados en la degradación de los carotenoides durante la fritura al vacío y el posterior almacenamiento de los chips de papaya?* y *¿Los cambios en la microestructura de los chips de papaya fritos al vacío y el tipo de aceite usado como medio para freír influyen en la absorción y bioconversión de carotenoides?*

Para alcanzar este objetivo, primero se determinó el estado de madurez más adecuado para procesar la papaya y obtener chips fritos con propiedades fisicoquímicas y sensoriales adecuadas. Posteriormente, se modelaron los parámetros de fritura al vacío y las condiciones de almacenamiento para optimizar el contenido de carotenoides de los chips de papaya para ofrecer a los potenciales consumidores. Además, se monitorearon los cambios fisicoquímicos de los chips de papaya durante la fritura al vacío y se evaluó el efecto de la composición lipídica del medio de fritura (aceite de palma: un aceite saturado versus aceite de soya: un aceite insaturado) sobre la reactividad de los carotenoides. Finalmente, se evaluó el efecto del consumo de chips de papaya sobre la absorción y bioconversión de carotenoides en un modelo animal (ratas Wistar). Asimismo, se determinó el perfil lipídico (triglicéridos, colesterol total, HDL y LDL) en el plasma de ratas después del consumo de chips de papaya fritos al vacío. Además, se utilizaron herramientas de microscopía para explicar la relación entre la microestructura del alimento procesado y la absorción de carotenoides.

Como principales resultados, se estableció primero que el estado de madurez 4 (41-55% de color amarillo de la cáscara) era el más adecuado para procesar la papaya y así obtener chips de papaya de calidad. En segundo lugar, se encontró que durante la fritura al vacío de papaya solo se produjo la degradación de BCX (alrededor de un 40-60% de pérdidas), mientras que hubo un aumento de la extractabilidad del BC total (1,3 - 2,7 veces de su concentración inicial) y del LYC total (1,9 - 2,8 veces de su concentración inicial). En tercer lugar, la incorporación de nitrógeno durante el empacado y el uso de un aceite insaturado (aceite de soya) como medio de fritura conservaron de mejor forma el contenido de carotenoides en los chips de papaya durante el almacenamiento. Finalmente, se determinó que el consumo de chips de papaya no alteró el perfil lipídico en el plasma de ratas Wistar. Asimismo, se demostró que la presencia del aceite de soya (rico en ácidos linoleico y α-linolénico) en los chips de papaya fritos al vacío favoreció la formación de una emulsión fina en las mezclas administradas a las ratas. Este fenómeno, sumado a la forma globular disuelta en lípidos de los carotenoides provitamina A (BC y BCX), tuvo un efecto positivo significativo sobre la bioconversión de BCX y BC en el hígado de las ratas.

En conclusión, el presente estudio demostró que la fritura al vacío es una tecnología adecuada para producir un *snack* frito saludable con carotenoides provitamina A (145±21 - 249±44 µg RAE/100 g de chips) y licopeno (13±2 - 34±5 mg/100 g de chips). El incremento de la absorción de carotenoides y de la extractabilidad de estos en los chips de papaya podría explicarse por una mayor liberación de estos compuestos de los tejidos de las células de papaya debido a la alteración de la estructura de la matriz durante la fritura al vacío, y por un aumento de su solubilidad debido a la absorción del aceite de fritura en los chips de papaya. Los chips de papaya fritos al vacío podrían ser parte de una dieta diversa para la población con deficiencia de carotenoides.

Abstract

The goal of this research was to design an alternative snack from papaya fruit (*Carica papaya* L.) that contains carotenoids (β-carotene, BC; β-cryptoxanthin, BCX; and lycopene, LYC) and essential fatty acids (ω-6 and ω-3) applying vacuum frying technology. Our research questions were: *What are the main factors involved in the degradation of carotenoids during vacuum frying and subsequent storage of papaya chips?* and *do the microstructure changes of the vacuum-fried papaya chips and the type of oil used as frying medium influence the absorption and bioconversion of carotenoids?*

To accomplish this goal first, we determined the most suitable ripening stage for processing papaya fruit to obtain fried chips with appropriate physicochemical and sensory properties. Next, we modelized the vacuum frying parameters and storage conditions to optimize the carotenoid content in papaya chips delivered to potential consumers. In addition, physicochemical changes in papaya chips were monitored during vacuum frying, and the effect of lipid composition of frying medium (palm: oil saturated oil versus soy oil: unsaturated oil) on carotenoid reactivity was assessed. Finally, we evaluated the effect of papaya chips consumption on carotenoid absorption and bioconversion in an animal model (Wistar rats). Measuring of the lipid profile (triglycerides, total cholesterol, HDL, and LDL) in rat plasma after the consumption of vacuum-fried papaya chips was assessed. Also, microscopy tools were used to explain the relationship between processed food microstructure and carotenoid absorption.

As the main results, we got first that the ripening stage 4 (41-55 % of skin yellowing) was the most suitable ripening stage for processing papaya fruit to obtain quality papaya chips. Second, we found that during vacuum frying of papaya only occurred the degradation of BCX (around 40-60 % losses), while an extractability increase of total BC (1.3-2.7-fold of its initial concentration) and total LYC (1.9-2.8-fold of its initial concentration) took place. Third, the incorporation of nitrogen during packaging and the use of unsaturated oil (soy oil) as frying medium best preserved the carotenoid content in papaya chips during storage. Finally, we found that the consumption of papaya chips did not alter the lipid profile in plasma of Wistar rats. Likewise, we showed that the presence of soy oil (rich in linoleic and α-linolenic acids) in vacuum-fried papaya chips favored the formation of a fine emulsion in diet mixtures given to rats. This phenomenon added to the globular lipid-dissolved form of provitamin A carotenoids (BC and BCX) had a significant positive effect on the bioconversion of BCX and BC in the liver of rats.

In conclusion, our study demonstrated that vacuum frying is a suitable technology to produce a healthy fried snack with provitamin A carotenoids (145±21 - 249±44 µg RAE/100 g chip) and lycopene (13±2 - 34±5 mg /100 g chip). The increase of carotenoid absorption and extractability of carotenoids from papaya chips could be explained by a higher release of these compounds from papaya cell tissues due to the matrix structure disruption during vacuum frying and by the increase of their solubility enhanced by frying oil absorption in the chips. Vacuum-fried papaya chips could be part of a diversified diet for the population with carotenoid deficiencies.

Résumé

L'objectif de cette recherche était d'obtenir un *snack* alternatif à base de papaye (*Carica papaya* L.) enrichi en caroténoïdes (β-carotène, BC; β-cryptoxanthine, BCX; et lycopène, LYC) et en acides gras essentiels (ω-6 et ω-3) en utilisant la technologie de friture sous vide. Nos principales questions de recherche pour cette étude sont: *¿Quels sont les principaux facteurs impliqués dans la dégradation des caroténoïdes lors de la friture sous vide et du stockage des chips de papaye?* et *¿Dans quelle mesure le procédé de friture sous vide et l'huile utilisée modifient-ils la microstructure des chips de papaye et par conséquent son influence sur l'absorption et la bioconversion des caroténoides?*

Pour atteindre cet objectif, nous avons préalablement déterminé l'état de maturité le plus adapté afin d'obtenir des chips de papaye aux propriétés physico-chimiques et sensorielles appréciables. Nous avons ensuite modélisé les paramètres de friture sous vide et les conditions de stockage afin d'optimiser la teneur en caroténoïdes des chips de papaye en vue de consommateurs potentiels. De plus, les changements physico-chimiques des chips de papaye pendant la friture sous vide ont été évalué par rapport à la réactivité des caroténoïdes en tenant compte de la composition en acides gras de l'huile utilisée (soja: huile dite insaturée et palme: huile dite saturée). Enfin, nous avons étudié l'effet de la consommation de chips de papaye sur l'absorption et la bioconversion des caroténoïdes *in vivo* chez le rat. Le profil lipidique (triglycérides, cholestérol total, HDL et LDL) a été mesuré dans le plasma des rats après consommation des chips de papaye. De plus, des outils de microscopie ont été utilisés pour expliquer la relation entre la microstructure de l'aliment transformé et l'absorption des caroténoïdes.

Les principaux résultats indiquent que l'état de maturité 4 (41 à 55% du jaunissement de la peau) était l'état le plus approprié pour traiter les fruits afin d'obtenir des chips de papaye de qualité. Nous avons également constaté une dégradation de la BCX (environ 40 à 60% de pertes) ainsi qu'une augmentation de l'extractibilité du BC total (1,3 à 2,7 fois sa concentration initiale) et du LYC total (1,9 à 2,8 fois sa concentration initiale) lors du traitement de friture sous vide. Enfin, le traitement à l'azote lors du stockage des chips associé à l'utilisation d'une huile non saturée (huile de soja) ont le mieux préservé la teneur en caroténoïdes des chips de papaye. Nous avons finalement montré que la consommation de chips de papaye ne modifiait pas le profil lipidique plasmatique chez le rat. De même, nous avons montré que l'huile de soja (riche en acides linoléique et α-linolénique) présente dans les chips de papaye favorisait la formation d'une émulsion fine dans les mixtures diététiques administrées aux rats. Ce phénomène, ajouté à la forme globulaire lipidique des caroténoïdes de pro-vitamine A (BC et BCX) dans les chromoplastes de la papaye a eu un effet positif significatif sur la bioconversion du BCX et du BC dans le foie des rats.

En conclusion, notre étude a démontré que la friture sous vide est une technologie adaptée pour produire un *snack* diététique enrichi en caroténoïdes pro-vitaminique A (145±21 - 249±44 µg RAE/100 g de chips), et en lycopène (13±2 - 34±5 mg/100 g de chips). L'augmentation de l'absorption des caroténoïdes *in vivo* ainsi que de l'extractabilité des caroténoïdes des chips de papaye pourraient s'expliquer par une plus grande libération de ces composés lié au traitement de friture sous vide qui modifierait la structure de la matrice. De plus, l'augmentation de la solubilité des caroténoïdes a été améliorée par l'absorption d'huile de friture dans les chips de papaye. De ce fait, les chips de papaye frites sous vide pourraient faire partie d'un régime alimentaire diversifié pour des populations présentant des carences en vitamine A.

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List of abbreviations

all-*E*, *Z*	carotenoid isomer forms
ANOVA	analysis of variance
a_w	water activity (dimensionless)
BC, BC_o	β-carotene, and initial β-carotene content ($mg \cdot kg^{-1}$, FW or NF-DW)
BCX, BCX_o	β-cryptoxanthin, and initial β-cryptoxanthin content ($mg \cdot kg^{-1}$, FW or NF-DW)
BI, BI_o	browning index, and initial browning index (dimensionless)
b_{aw}, b_{BI}, b_{suc}	time to reach the sigmoid's midpoint for a_w, browning index and sucrose (min)
bw	body weight of animal (g)
C	constant related to the monolayer heat of sorption (dimensionless)
C*	chroma parameter (color saturation) (dimensionless)
CCD	central composite design
CHO	total cholesterol ($mg \cdot dL^{-1}$)
CTs	carotenoids
D_{90}	size distribution of lipid droplets
DW	dry weight
E_a	activation energy ($J \cdot mol^{-1}$)
Exp	experimental data
FFA	free fatty acids (nmol/mL)
FDP+P	freeze-dried papaya mixed with palm oil
FDP+S	freeze-dried papaya mixed with soy oil
FW	fresh weight
GAB	Guggenheim-Anderson-de Boer model
h°	hue angle parameter (dimensionless)
HDL	high-density lipoprotein cholesterol ($mg \cdot dL^{-1}$)
HPLC-DAD	high performance liquid chromatographic with diode array detector
k	reaction rate constant for carotenoids ($kg \cdot mg^{-1} \cdot day^{-1}$)
K	factor related to the heat of sorption of the multilayer (dimensionless)
k_{aw}, k_{BI}, k_{suc}	rate constant of a_w, browning index, and sucrose (min^{-1})
k_{BCX}	β-cryptoxanthin degradation rate constant ($kg \cdot mg^{-1} \cdot min^{-1}$)
k_M	rate of water loss (min^{-1})
k_O	rate of oil uptake (min^{-1})
k_{ref}	rate constant at the reference temperature ($kg \cdot mg^{-1} \cdot min^{-1}$ or $kg \cdot mg^{-1} \cdot day^{-1}$)
L*, a*, b*	CIELab color space parameters (dimensionless)
L_{aw}, L_{BI},	curve's maximum of a_w, and browning index (dimensionless)
LC-ESI-MS	liquid chromatography - electrospray ionization - mass spectrometry
LDL	low-density lipoprotein cholesterol ($mg \cdot dL^{-1}$)
L_{suc}	curve's maximum of sucrose ($g \cdot 100\ g^{-1}$, NF-DW)
LYC, LYC_o	lycopene, and initial lycopene content ($mg \cdot kg^{-1}$, FW or NF-DW)
m	mass (g or kg)
M, M_o	moisture content, and initial moisture content ($g \cdot g^{-1}$, DW)

M_g	GAB monolayer moisture content ($g \cdot g^{-1}$, DW)
M_w	moisture content ($g \cdot g^{-1}$, DW)
n	number of repetitions
NF-DW	non-fat dry weight
O, O_e	oil content, and oil content at infinite time ($g \cdot g^{-1}$, DW)
p	probability
PC-P	papaya chips with palm oil
PC-S	papaya chips with soy oil
PCs	papaya chips
PETm/PE	polyethylene terephthalate metallized/polyethylene
r	correlation coefficient
R	gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$)
R^2	coefficient of determination
R^2-adj	adjusted R^2
RAE	retinol activity equivalent
RDI	reference daily intake
RS	ripening stage
RSM	response surface methodology
S-Max	chips with saturated oil and maximum lipid content
S-Min	chips with saturated oil and minimun lipid content
t	time (min or days)
$t_{1/2}$	theoretical half-life (days)
T	temperature (°C or K)
T_{ref}	reference temperature (°C or K)
TRIG	triglycerides ($mg \cdot dL^{-1}$)
TSS	total soluble solids (°Brix)
UnS-Min	chips with unsaturated oil and minimum lipid content
ΔE	color difference (dimensionless)
ω-3	omega-3 fatty acid (alpha linolenic acid)
ω-6	omega-6 fatty acid (linoleic acid)

The results of this work have been published in the form of articles:

Soto, M., Brenes, M., Jiménez, N., Cortés, C., Umaña, G., & Pérez, A.M. (2021). Selection of optimal ripening stage of papaya fruit (*Carica papaya* L.) and vacuum frying conditions for chips making. *CyTA-Journal of Food*, *19*(1), 273-286.

Soto, M., Pérez, A.M., Servent, A., Vaillant, F., & Achir, N. (2021). Monitoring and modelling of physicochemical properties of papaya chips during vacuum frying to control their sensory attributes and nutritional value. *Journal of Food Engineering*, *299*, 110514.

Soto, M., Dhuique-Mayer, C., Servent, A., Jiménez, N., Vaillant, F., & Achir, N. (2020). A kinetic study of carotenoid degradation during storage of papaya chips obtained by vacuum frying with saturated and unsaturated oils. *Food Research International*, *128*, 108737.

Soto, M., Servent, A., Portet, K., Conéjéro, G., Vaillant, F., Poucheret, P., & Dhuique-Mayer, C. (2021). Carotenoid absorption in rats fed with vacuum-fried papaya chips depends on processed food microstructure associated with saturated and unsaturated oils. *Food Research International*, *142*, 110223.

The results were also the subject of oral communications and a poster:

Oral communications

Soto, M., Pérez, A.M., Brenes, M., Achir, N., & Vaillant, F. (2018). Fritura al vacío: una tecnología alternativa para la producción de *snacks* de frutas y vegetales. *5th International Congress of Agroindustrial Engineering*. October 22-27, 2018. San Carlos, Costa Rica.

Soto, M., Dhuique-Mayer, C., Servent, A., Jiménez, N., Vaillant, F., & Achir, N. (2019). Degradation kinetics of carotenoids during storage of papaya chips obtained by vacuum frying with saturated and unsaturated oils. *XII Iberoamerican Congress of Food Engineering – CIBIA 2019*. July 1-4. Faro, Algarve, Portugal.

Soto, M., Dhuique-Mayer, C., Servent, A., Jiménez, N., Vaillant, F., & Achir, N. (2019). Reactividad de carotenoides durante el almacenamiento de chips de papaya obtenidos mediante fritura al vacío con aceite saturado e insaturado. *VI International Congress of Food Science and Technology - CITA 2019*. September 17-19. San José, Costa Rica.

Dhuique-Mayer, C., **Soto, M.**, Conéjéro, G., Portet, K., Servent, A., & Poucheret, P. (2022). Carotenoid absorption in rats fed with vacuum-fried papaya chips depends on processed food microstructure associated with saturated and unsaturated oils. *3rd International Conference on Food Bioactives & Health*. June 22-25. Parma, Italy.

Poster

Soto, M., Dhuique-Mayer, C., Servent, A., Jiménez, N., Vaillant, F., & Achir, N. (2019). Impact of vacuum frying on carotenoid-rich fruit: carotenoid degradation during storage of papaya chips obtained with saturated and unsaturated oils. *Journée de la filière APAB 2019 – École Doctorale GAIA*. June 13. Montpellier, France.

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Chapter I

Introduction

Chapter I. Introduction

1. Snack foods

For decades, consumers (mainly in low- and middle-income countries) have preferred diets based on energy-dense and nutrient-poor foods. For example, consumers in Latin America and the Caribbean are attracted by diets based on foods that are composed mainly or solely of sugars and saturated lipids but slight or no content in vitamins, minerals, protein, fiber, or essential fatty acids (Poti *et* al., 2014). Part of the foods in these diets are snacks. A snack is a small portion of food, usually consumed between meals, and normally designed to be easy to eat and temporarily satisfy hunger (Njike *et al.*, 2016). The snacks represent a big market in food industry. In fact, the consumption of snacks is continuously growing, and according to Euromonitor International (2015), the snack consumption worldwide was projected to increase in 30% from 2015 to 2019.

The traditional snacks that we know are made basically of starchy foods such as potato chips, tortillas chips, pretzels, and extruded products made from cereals, among others (Da Silva & Moreira, 2008) as shown in Figure 1.1. And they usually have a high content of saturated lipids (if they are fried foods) also they contain salt and refined carbohydrates and present artificial ingredients to improve their flavor and color.

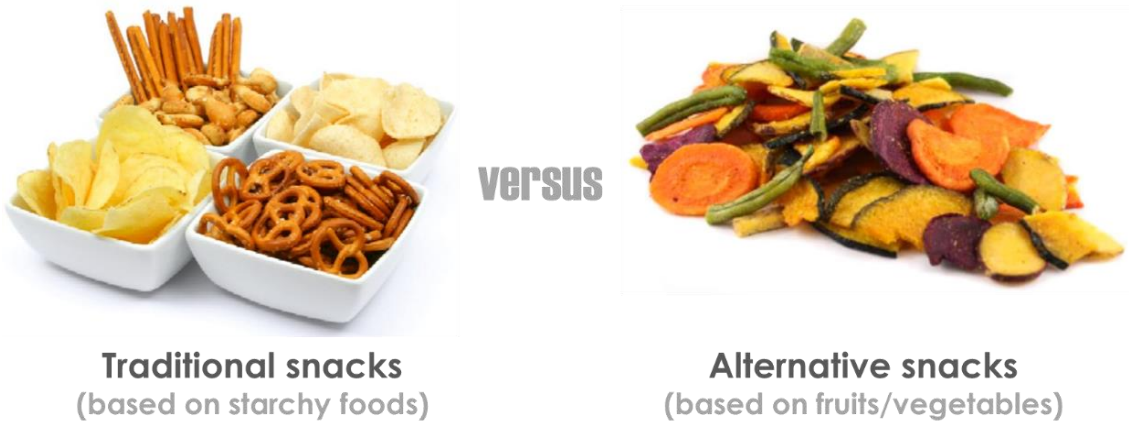

Figure 1. 1. Comparison of traditional snacks and alternative snacks.

Contrary, there are alternative snacks based on fruits and vegetables which are healthier than traditional snacks (Figure 1.1). Fruits and vegetables contain a wide variety of phytochemicals, such as polyphenols, carotenoids, tocopherols, tocotrienols, phytosterols, glucosinolates, among others (Abuajah *et al.*, 2015). In this regard, it has been shown that a diet rich in fruits prevents the decrease of the antioxidant capacity in the blood due to the high content of antioxidants that these foods present (Jensen *et al.*, 2008). Numerous epidemiological studies promote the consumption of fruits and vegetables to prevent non-communicable diseases (Angelino *et al.*,2019; Gan *et al.*, 2015).

2. Vacuum frying technology

The consumer trends towards healthier foods (including snacks) require alternative strategies to the consumption of deep-fat fried products rich in simple sugars, saturated lipids, and salt, *e.g.*, potato chips, French fries, doughnuts, extruded snacks, cheese sticks, among others (Da Silva & Moreira, 2008). Manufacturing of foods based on fruits and vegetables with quality nutritional properties represents one of these strategies. In this context, vacuum frying is an alternative technology to produce fruit and vegetable-based snacks bearing the desired sensory quality and better preserving their nutrients than the traditional fried snacks (Figure 1.2) (Da Silva & Moreira, 2008; Dueik & Bouchon, 2011b).

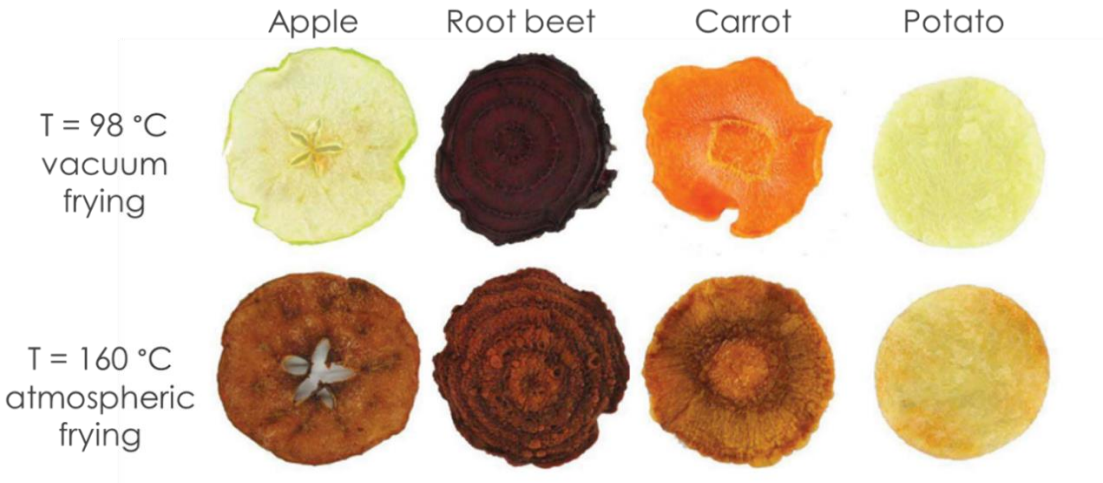

Figure 1. 2. Images of vacuum (top) and atmospheric (botton) fried apple, red beet, carrot, and potato slices, using a thermal driving force of 60 °C. Source: Dueik & Bouchon (2011a).

In addition, vacuum frying allows the use of healthier unsaturated vegetable oils (soybean, corn, sunflower, rice bran, among others) due to the low operation temperatures (frying medium) and the absence of oxygen during the process (compared to atmospheric frying), thus minimizing oil deterioration (Andrés-Bello *et al.*, 2011). Da Silva & Moreira (2008) showed that vacuum frying retained 1.2 and 2-fold more carotenoids than atmospheric frying in mango and sweet potato chips, respectively.

3. Carotenoids

Among the phytochemicals that are presented in fruits and vegetables, the carotenoids (organic pigments with colorations ranging from yellow to red) are well known to be natural antioxidants with beneficial health effects. These compounds are from the group of isoprene present in plants and animals. According to their chemical structure, these compounds are classified into two main groups: carotenes such as β-carotene and lycopene, which contain only a parent hydrocarbon chain, and xanthophylls such as β-cryptoxanthin, which present an oxygen functional group (Ribeiro *et al.*, 2018). Some carotenoids with a β-ring and an 11-carbon polyene chain in their chemical structure (Figure 1.3), such as β-carotene and β-cryptoxanthin, represent an important source of vitamin A (Woollard, 2012). These carotenoids can be converted through enzymes into retinol (vitamin A) and other related retinoids (retinol esters) in the organism (Figure 1.3), playing a vital role in growth, visual cycle, and gene regulation (Kulczynski *et al.*, 2017). Vitamin A deficiency remains a public health problem in developing countries (West *et al.*, 2002). According to WHO (2020), 250 million preschool children are vitamin A deficient (retinol <0.7 μmol/L serum).

On the other hand, carotenoids that do not have provitamin A activity have been implicated in the protection against certain forms of cancer, cardiovascular diseases, and macular degeneration due to the great antioxidant capacity of these compounds (Müller *et al.*, 2016). For instance, several epidemiological studies have concluded that diets rich in lycopene foods are associated with a reduced

risk of diseases including cancer (especially prostate cancer) and cardiovascular disease (Willcox *et al.*, 2003; Rao & Agarwal, 2000; Müller *et al.*, 2016).

However, these compounds must be bioaccessible and bioavailable in order to be transferred from the food matrix to the different tissues of the human body (Rodriguez-Amaya, 2010; Cardoso *et al.*, 2015). The term bioavailability refers to the amount of a compound that is absorbed into the body and enters the systemic circulation (Fernández-García *et al.*, 2009). On the other hand, bioaccessibility is defined as the fraction or proportion of the compound ingested and released from the food matrix to the gastrointestinal system, thus being available for intestinal absorption (Rodriguez-Amaya, 2010).

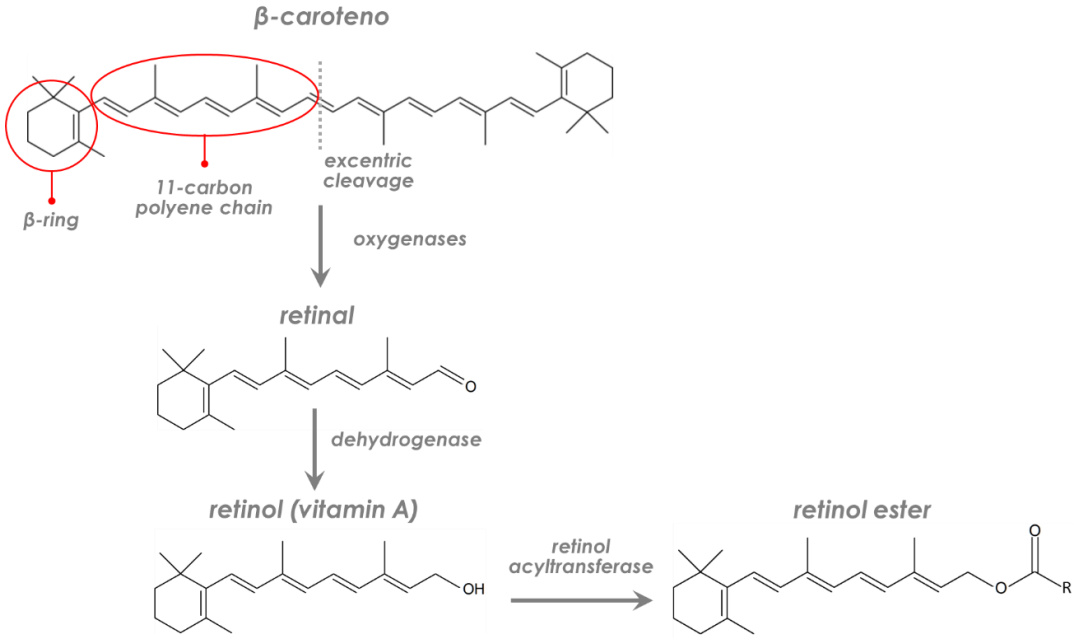

Figure 1. 3. Conversion of β-carotene to retinol (vitamin A).

4. Metabolism of carotenoids

Figure 1.4 shows carotenoid absorption from the fruit matrix until accumulation or bioconversion (into other metabolites) in human tissues. Initially, carotenoids are deposited in the food matrix in different structures: within photosynthetic plastid membranes (example, chloroplasts) and non-photosynthetic plastids (chromoplasts) (Wieslaw, 2010), as shown in Figure 1.4. Carotenoids are partially

released during food preparation, processing, and mastication (Kotake-Nara & Nagao, 2012).

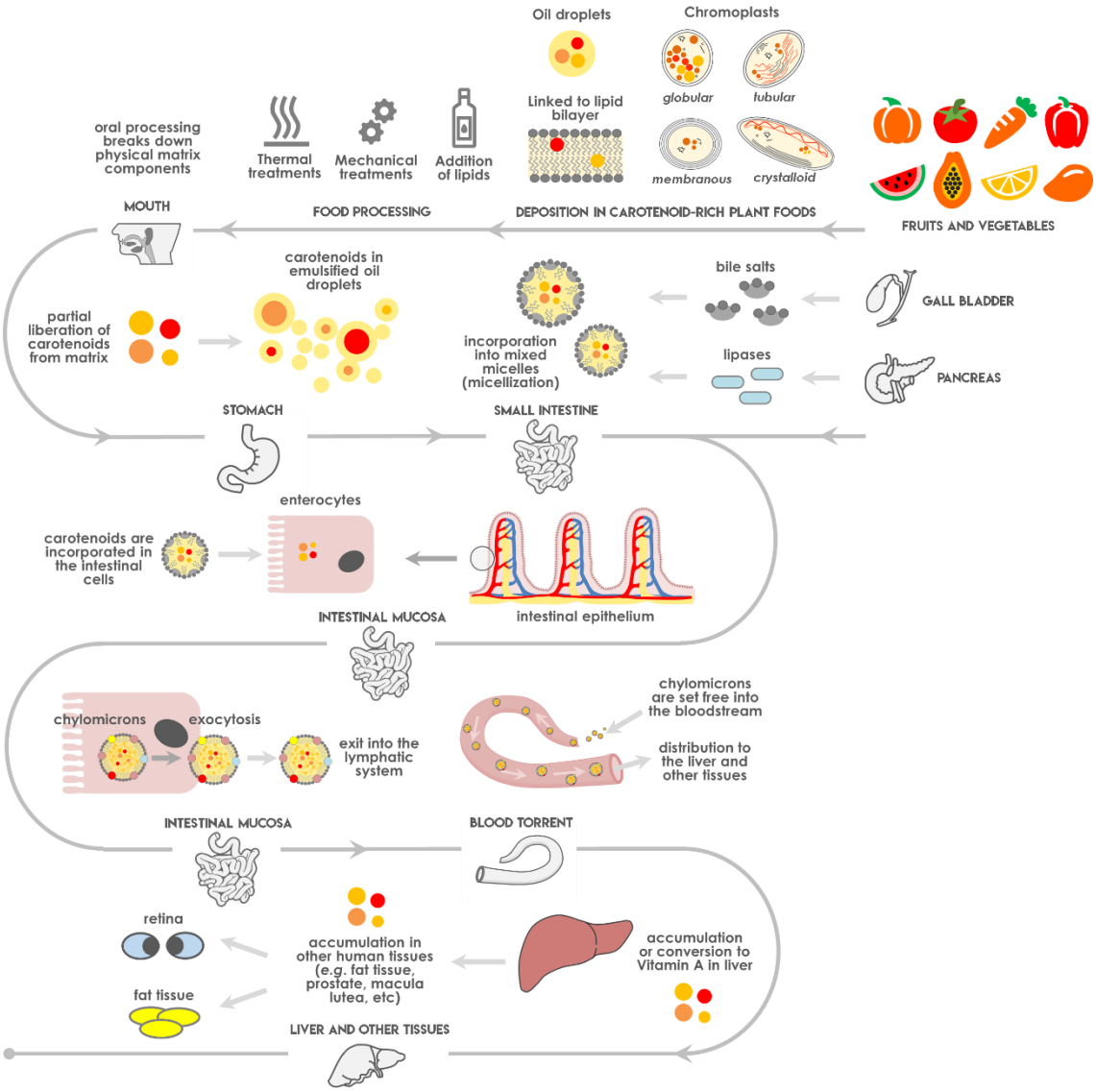

Figure 1. 4. Carotenoid absorption process diagram, from food matrix to human body. Source: prepared by the author.

Once in the stomach, carotenoids are incorporated into lipid emulsion droplets derived from dietary co-consumed lipids. In the small intestine, secreted bile salts (by the gall bladder) and lipases (by the pancreas) help to break these droplets and finally transfer the carotenoids to the mixed micelles (micellization). Micelles are molecular aggregates able to transport hydrophobic compounds, acting as emulsifiers and encapsulating carotenoids for their transport through the small

intestine and later incorporation into the intestinal epithelium. Micelles are constituted by bile salts, cholesterol, fatty acids, mono acyl glycerides, and phospholipids (Deming *et al.*, 2012).

The micelles containing carotenoids then come into contact with the intestinal epithelial cells (enterocytes), leading to the absorption of these compounds (Yonekura & Nagao, 2007). After the incorporation of carotenoids into the cytosol/plasma membrane of enterocytes, the conversion of provitamin A carotenoids to vitamin A could take place (bioconversion). Then, the enterocyte's Golgi apparatus packs the compounds in chylomicrons to be transported into the lymph and blood torrent (Almiger *et al.*, 2012; Parker, 1996). Chylomicrons reach a size of 75 to 1200 nm and are characterized by their apoprotein B-48 content, which is essential to the assembly of these particles (Yonekura & Nagao, 2007). Once in the blood torrent, they can be distributed to the liver, where the synthesis and accumulation of vitamin A take place as well. In the liver, the carotenoids are released and incorporated into lipoprotein particles that are subsequently secreted into the blood torrent delivering lipidic compounds (including carotenoids) to other tissues (Canene-Adams & Erdman, 2009; Deming *et al.*, 2002) (Figure 1.4).

As shown in Figure 1.4, carotenoids need to be released from the food matrix to be absorbed by the human body. The absorption of carotenoids is affected by several factors, including their molecular structure, physicochemical properties, food matrix composition, processing and storage conditions, the addition of lipids, human factors, among others (Deming *et al.*, 2002). Carotenoid polarity influences the absorption. For instance, the polar compounds can be incorporated more easily into micelles than apolar pigments, like β-carotene (Yeum & Russell, 2002). The food matrix and subcellular localization of carotenoids is another main factor. Free carotenoids or those inside chromoplasts are easier to extract than those within lipid bilayers or associated with proteins or polysaccharides (During, 2007), as shown in Figure 1.4. One of the most important factors to influence micelle formation is the presence of dietary fat because the lipids are required for incorporation into micelles. Also, the presence of lipids stimulates the release of bile to facilitate micelle formation (Parker,

1996). Increasing the dietary fat increases the extent of micelle formation and bioavailability, but only until an optimal threshold is reached (Yonekura and Nagao 2007). The carotenoid content incorporated into the micelles is also affected by the carotenoid polarity and the composition and saturation degree of the fatty acids constituting the micelles (Lemmens *et al.*, 2014).

Food processing, including mechanical treatments (*e.g.*, peeling, cutting, slicing, grinding, extraction) and thermal treatments (*e.g.*, blanching, pasteurization, sterilization, frying), improves the bioavailability because of the cell food matrix disruption (Figure 1.5). During processing, a portion of carotenoids is degraded, but the amount released from the matrix is higher (Boon *et al.*, 2010), as shown in Figure 1.5.

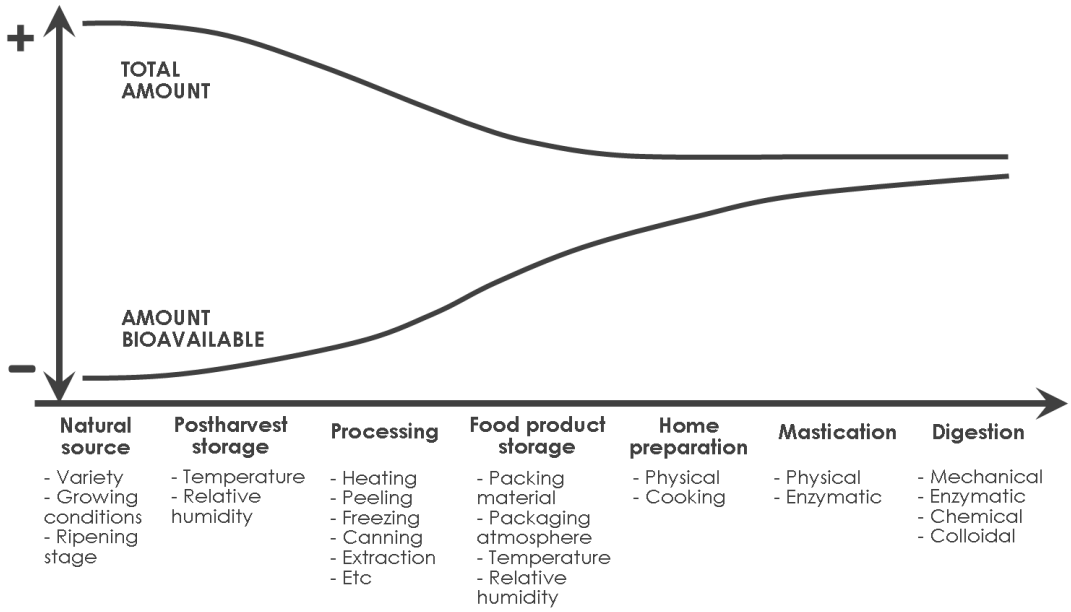

Figure 1. 5. Example of a food in which the total amount of a nutrient decreases with time, but the amount of bioavailable compounds increases due to suppression of food matrix effects. Source: adapted from Parada & Aguilera (2007).

5. Papaya fruit

Papaya fruit (*Carica papaya* L.) is a valuable source of pro-vitamin A carotenoids, such as β-carotene and β-cryptoxanthin. Besides, red-fleshed papaya genotypes contain higher amounts of lycopene than other fruits and

vegetables such as mango, pumpkin, carrot, and grapefruit (USDA, 2020), as shown in Table 1.1. Besides its nutritional value, papaya fruit is of commercial importance in the developing countries where this crop is grown (FAO, 2020; Saran *et al.*, 2016). During the past two decades, high growth rates for papaya production were reported worldwide. On average, total papaya world production increased from 5.49 million tons in 1994 to 13.02 million tons in 2017 (FAO, 2020).

Table 1.1. Content of carotenoids in different fruits and vegetables.

Fruit/vegetable	Carotenoids (µg/100 g fresh weight)		
	Lycopene	β-carotene	β-cryptoxanthin
Pink guava	5204	129	-
Watermelon	4532	303	78
Red tomato	4088	103	-
Papaya	1828	274	589
Pink grapefruit	1419	686	6
Red pepper	484	1570	77
Pumpkin	-	3100	-
Mango	2	640	-
Carrot	1	8285	-

Source: USDA (2020).

Papaya is sold primarily as a fresh product; however, it is a highly perishable fruit, and about 30 % of the production is compromised due to postharvest losses (Albertini *et al.*, 2016). Commercially ripe fruits find possible applications in the preparation of syrups, dried products, yogurt, jam, jellies, nectars, and sweets, among other foods (Annegowda & Bhat, 2016). An alternative to adding value to papaya fruit is the application of vacuum frying to obtain a novel and healthy snack, vacuum-fried papaya chips.

6. Impact of food processing on carotenoids

During processing and storage of dried products, the carotenoids are prone to isomerization and oxidation as a result of exposure to high temperature, oxygen, light, or pro-oxidant molecules (Achir *et al.*, 2016; Achir *et al.*, 2010; Caris-Veyrat

et al., 2003; Colle *et al.*, 2010b; Xiao *et al.*, 2018). The loss of carotenoids during storage may reach 90 % due to storage conditions (Bechoff *et al.*, 2010). Therefore, there is a problem in choosing a process that preserves the carotenoids and matches the optimal storage conditions that allow their maximum retention during the food shelf-life.

There is no information about the behavior of vacuum-fried papaya chips. Moreover, literature about kinetic parameters of carotenoids in this kind of product during processing and storage is scarce. The manufacturing of papaya chips implies some challenges because papaya fruit is a complex matrix with high moisture content, rich in sugars and carotenoids with varied chemical structures (xanthophyll, β-cryptoxanthin; carotenes, β-carotene, and lycopene). During frying, this product undergoes various physicochemical and microstructural changes, added to the oil uptake, that can affect the stability and reactivity of carotenoids present in the papaya fruit, as well as their absorption after ingestion.

7. Snack rich in carotenoids and essential fatty acids

In this context, the goal of this research was to design an alternative snack from papaya fruit rich in carotenoids and essential fatty acids (ω-6 and ω-3) applying vacuum frying technology. Our main research questions were:

- *What are the main factors involved in the degradation of carotenoids during vacuum frying and subsequent storage of papaya chips?*

- *Do the microstructure changes of the vacuum-fried papaya chips and the type of oil used as frying medium influence the absorption and bioconversion of carotenoids?*

To answer these questions, the carotenoid degradation kinetics, during vacuum frying and storage of papaya chips, were followed, applying different experimental and modeling methods. Furthermore, microscopy techniques were performed in order to relate the effect of processed food microstructure and the absorption/bioconversion of carotenoids in rats fed with papaya chips. Thus, this

document was organized around four scientific articles, each one corresponding to each specific objective or stage carried out to achieve the goal of the study, as shown in Figure 1.6.

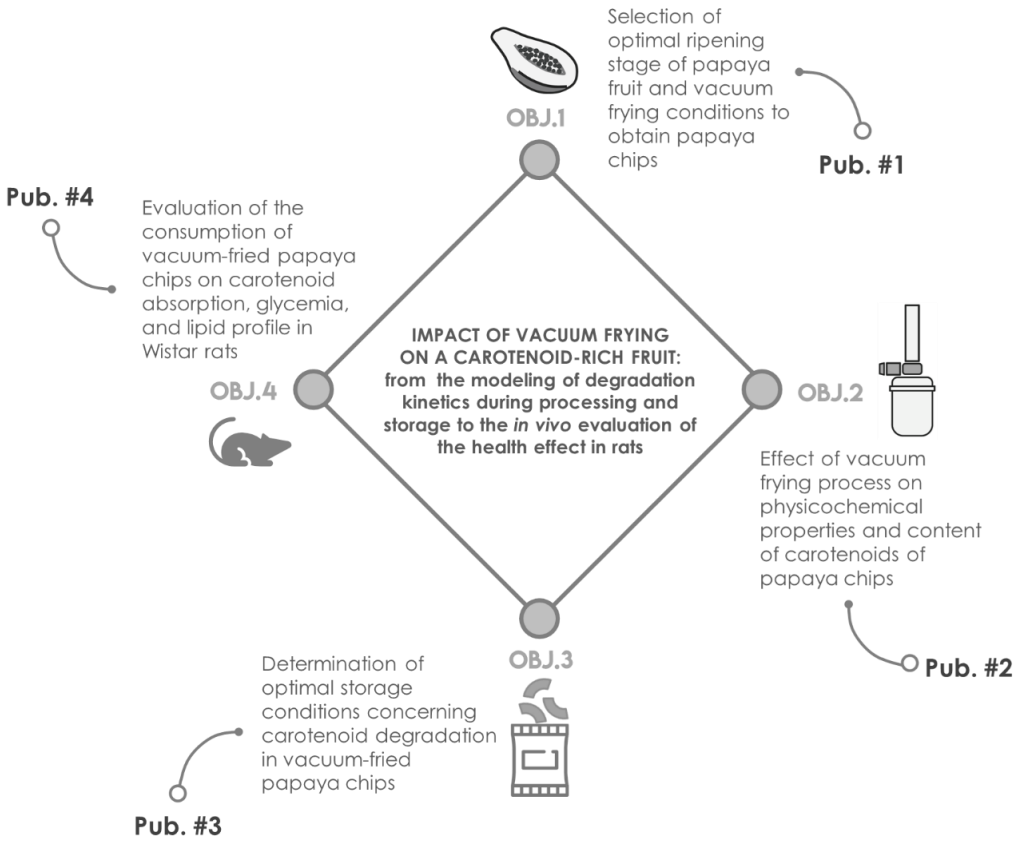

Figure 1. 6. Scheme of specific objectives (OBJ.) or stages of this study and the distribution of publications (Pub.).

This study first determined the most suitable ripening stage for processing papaya fruit to obtain fried chips with appropriate physicochemical and sensory properties (Chapter III, Publication #1). Next, it was performed the modeling of carotenoid degradation and physicochemical changes of papaya chips during vacuum frying to predict the sensory attributes and nutritional value of the final fried product (Chapter IV, Publication #2). In addition, determination of storage conditions was conducted in order to optimize the carotenoid content in papaya chips delivered to potential consumers considering the lipid composition of the oil (saturated oil and unsaturated oil) and the presence of oxygen and nitrogen inside the packages (Chapter V, Publication #3). Finally, the evaluation of papaya chips consumption on the absorption and bioconversion of carotenoids in an

animal model (Wistar rats) was evaluated. Measuring of the lipid profile in rat plasma after digestion of vacuum-fried papaya chips was assessed as well. Also, microscopy tools were used to explain the relationship between processed food microstructure and carotenoid absorption (Chapter VI, Publication #4).

Chapter II

Materials and methods

Chapter II. Materials and methods

The evaluation of the impact of vacuum frying on papaya fruit to obtain a healthy snack was carried out with papaya fruits from Costa Rica and Brazil. The experiments were carried out in two countries: Costa Rica (experiments from Objectives 1 and 2) and France (experiments from Objectives 3 and 4). This chapter recapitulates the protocols for preparing the different samples used to accomplish the different experiments of this study and presents the details of the main physicochemical analysis methods and the use of the results to characterize and model the reaction kinetics.

1. Materials

1.1. Chemicals

The following chemicals were used: SigmaUltra standards for glucose, fructose, and sucrose from Sigma-Aldrich (St. Louis, MO, USA); β-carotene, β-cryptoxanthin and lycopene standards from Sigma-Aldrich (St. Louis, MO, USA). Methanol, methyl tert-butyl ether (MTBE), hexane, formic acid, and chloroform were obtained from Merck (Darmstadt, Germany). Other analytical grade chemicals and HPLC grade solvents were purchased from JT Baker Inc. (Phillipsburg, NJ, USA).

1.2. Fruits and oils

Table 2.1 describes the different materials used for the experiments with specific information about their origin (brand, supplier, country). Red-fleshed papaya fruits (*Carica papaya* L.) from two varieties were used for the different experiments, as shown in Table 2.1. Two varieties were used due to the availability of the fruit in each country where the experiments were carried out. In Costa Rica, we worked with Pococí variety, and in France, with Formosa variety. Papaya fruits were selected for color, elongated shape, and absence of physical or microbiological damage. Fruit skin color was assessed as a percentage of yellowing on the whole fruit (0–100 %), using a ripening scale with seven

categories or stages developed at *Laboratorio de Tecnología Poscosecha* (CIA, Universidad de Costa Rica).

Table 2.1. Information about materials used for vacuum frying experiments.

Objective	Experiment	Papaya fruits			Frying oils		
		Ripening stage*	Variety/ Country	Supplier	Type	Brand	Supplier
Obj. 1	Selection of optimal ripening stage of papaya fruit	RS3, RS4, RS5	Pococí/ Costa Rica	OroFruits (Orotina, Alajuela, Costa Rica)	Palm olein	D´orofrit	Grupo NUMAR, (San José, Costa Rica)
	Optimization of vacuum frying conditions to obtain papaya chips	RS4					
Obj. 2	Effect of vacuum frying on physicochemical properties and carotenoid content of papaya chips	RS4			Soy oil	Clover®	Grupo NUMAR, (San José, Costa Rica)
Obj. 3	Optimal storage conditions concerning carotenoid degradation in vacuum-fried papaya chips	RS4	Formosa/ Brazil	TerreAzur (Montpellier, France)	Soy oil	-	Huileries Cauvin, (Nimes, France)
Obj. 4	Effect of papaya chips consumption in an animal model (Wistar rats)	RS4			Hydroge-nated palm oil	Risso®	Vandemoortele, (Gent, Belgium)

*Ripening stages: RS3, 26-40% skin yellowing; RS4, 41-55% skin yellowing; RS5, 56-70 % skin yellowing.

For each experiment, a single batch of the fruit was acquired according to the ripening stage. The physicochemical composition of papaya fruits from Pococí and Formosa varieties is presented in Table 2.2. The two varieties selected had a similar proximal composition and carotenoid profile.

Table 2.2. Physicochemical composition of papaya fruits from Pococí and Formosa varieties at ripening stage 4 (RS4, 41-55 % skin yellowing).

Parameter	Fresh papaya at RS4)	
	Pococí	**Formosa**
Moisture (g·100 g^{-1} FW)	88.04 ± 0.68	86.96 ± 0.69
Lipids (g·100 g^{-1} FW)	ND (<0.10)	ND (<0.10)
Protein (g·100 g^{-1} FW)	0.58 ± 0.03	0.66 ± 0.07
Ash (g·100 g^{-1} FW)	0.46 ± 0.03	0.64 ± 0.05
Dietary fiber (g·100 g^{-1} FW)	1.75 ± 0.09	2.13 ± 0.23
Sugars (g·100 g^{-1} FW):		
Glucose	4.07 ± 0.38	4.30 ± 0.10
Fructose	3.99 ± 0.33	4.23 ± 0.12
Sucrose	ND (<0.25)	ND (<0.25)
Organic acids (g·100 g^{-1} FW):		
Citric acid	0.101 ± 0.002	0.031 ± 0.002
Succinic acid	0.039 ± 0.002	0.050 ± 0.003
Malic acid	ND (<0.01)	0.044 ± 0.004
Carotenoids (mg·kg^{-1} FW):		
Total β-cryptoxanthin	9.43 ± 1.46	4.20 ± 0.46
Total β-carotene	3.00 ± 0.43	3.70 ± 0.84
Total lycopene	33.24 ± 4.40	13.63 ± 1.90

Values are expressed as the mean ± standard deviation (n=3). Total β-cryptoxanthin, sum of free and ester forms; Total β-carotene sum of all-*E*- and *Z*-forms; Total lycopene, sum of all-*E*- and *Z*-forms.

2. Vacuum frying system

The papaya chips were elaborated using two vacuum frying systems from the same manufacturing company (Auriol, Marmande, France), having the same dimensions and operation parameters (see Appendix A1). Thus, it was possible to replicate experiments in both countries. Figure 2.1 shows the sketch of the two vacuum frying systems used. The system consists of a stainless-steel vessel

(capacity of 80 L), electric heat resistors for heating the oil, a lid with a rotary axis coupled to a piston in which a stainless-steel basket is placed, a temperature transducer, a filter, a heat exchanger to condense the water vapor generated during the process, a condensate vessel, and a liquid ring vacuum pump. The frying process consisted of heating the oil (55 L or ~50 kg) to the target temperature, loading the papaya products (slices or discs) into the basket, closing the lid, and depressurizing the vessel. Once the pressure reached 25 kPa, the basket was immersed into the oil for the predefined time to obtain the chips. When the frying step was completed, the basket was raised, and the centrifuging system was applied to control the oil uptake in the samples. Finally, the vessel was pressurized, and the papaya chips were removed from the fryer and cooled to ambient temperature before packaging.

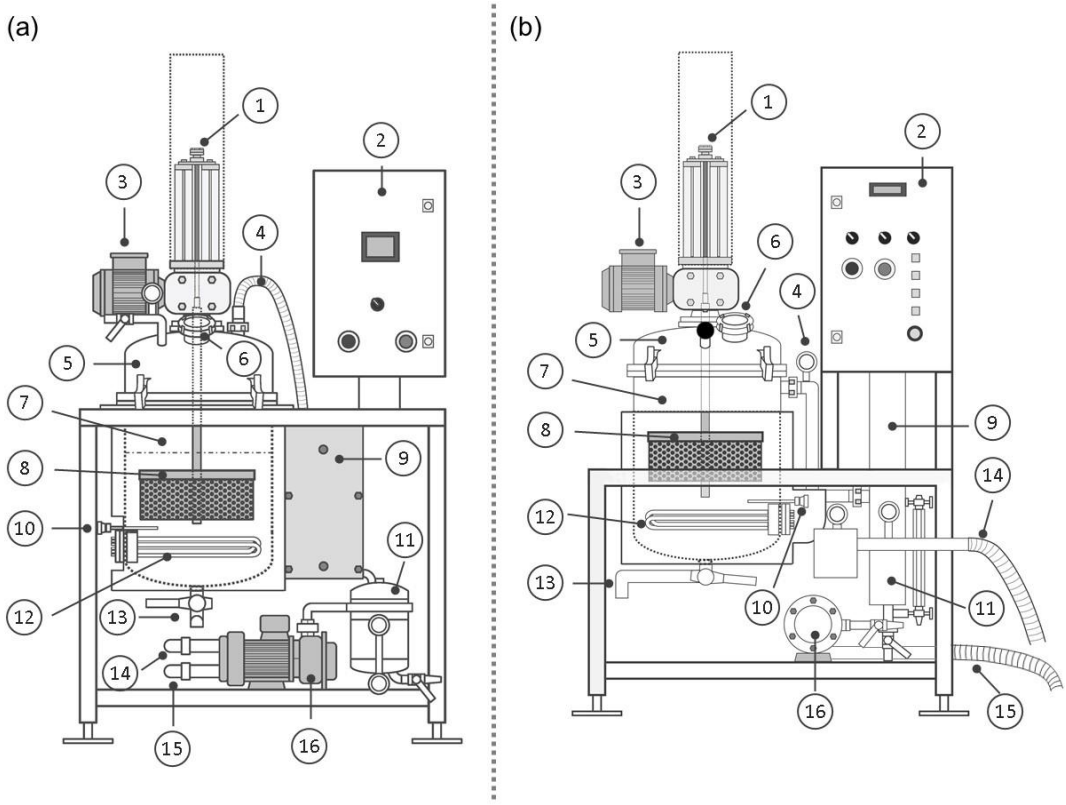

Figure 2.1. Sketch of the vacuum frying system used for obtaining the papaya chips: (a) vacuum fryer from Costa Rica; (b) vacuum fryer from France. System components: 1. Rotary axis of basket with piston, 2. Control panel, 3. Engine of rotary shaft, 4. Air and steam outlet to keep vacuum, 5. Lid, 6. Glass visor, 7. Frying vessel, 8. Basket, 9. Heat exchanger, 10. Temperature transducer, 11. Condensate vessel, 12. Electric heating resistors, 13. Oil outlet valve, 14. Water outlet, 15. Water inlet, 16. Liquid ring pump.

3. Protocols of experiments

3.1. Selection of optimal ripening stage of papaya fruit (Objective 1)

3.1.1. Ripening stages of papaya

A ripening scale with seven categories or stages, developed at *Laboratorio de Tecnología Poscosecha* (CIA, Universidad de Costa Rica), was used to select the fruits (see Appendix A2). This scale is based on fruit skin color as a percentage of yellowing on the whole fruit (0–100 %): RS1 (0-15 % yellowing), RS2 (16-25 % yellowing), RS3 (26-40 % yellowing), RS4 (41-55 % yellowing), RS5 (56-70 % yellowing), RS6 (71-80 % yellowing), and RS7 (81-100 % yellowing). The ripening stages used in the study were RS3, RS4, and RS5, as shown in Figure 2.2. Preharvest RS2 papaya fruits were harvested manually and selected for color, elongated shape, and absence of physical or microbiological damage. Then, the fruits were stored at 25 °C for ripeness until obtaining the skin color corresponding to the three ripening stages (RS3, RS4, and RS5). These ripening stages were selected because they presented the most promising sensory and physicochemical features for papaya chips making.

3.1.2. Vacuum frying

Papaya fruits at the three ripening stages were washed and peeled manually. Each fruit was cut vertically into four pieces and the seeds were removed. The pre-cut pieces were then cut into 4-mm thick slices (dimensions of approximately 3 cm x 3 cm x 6 cm) using a slicing machine (FP-100 Hobart, CA, USA) before vacuum frying. The papaya chips were elaborated using the vacuum frying system shown in Figure 2.1a.

Chips were obtained under fixed conditions (25 kPa, 120 °C, 12 min) using the central point of the central composite design described in section 3.2. After frying, the samples were centrifuged (under vacuum) at fixed conditions (300 rpm, 16.6 x g, for 2 min) in order to remove excess oil. The physicochemical properties measured in the papaya chips were moisture, aw, lipid content, color variation, and browning index. Also, the sensory acceptance of papaya chips was evaluated. Yields for each step in the process to obtain the papaya chips were determined for

each ripening stage. The final goal was to select the ripening stage in papaya fruits that resulted in chips with the lowest a_w, moisture and lipid content, color variation and browning index, and the highest sensory acceptance and yield. Three frying trials or repetitions were conducted for each ripening stage.

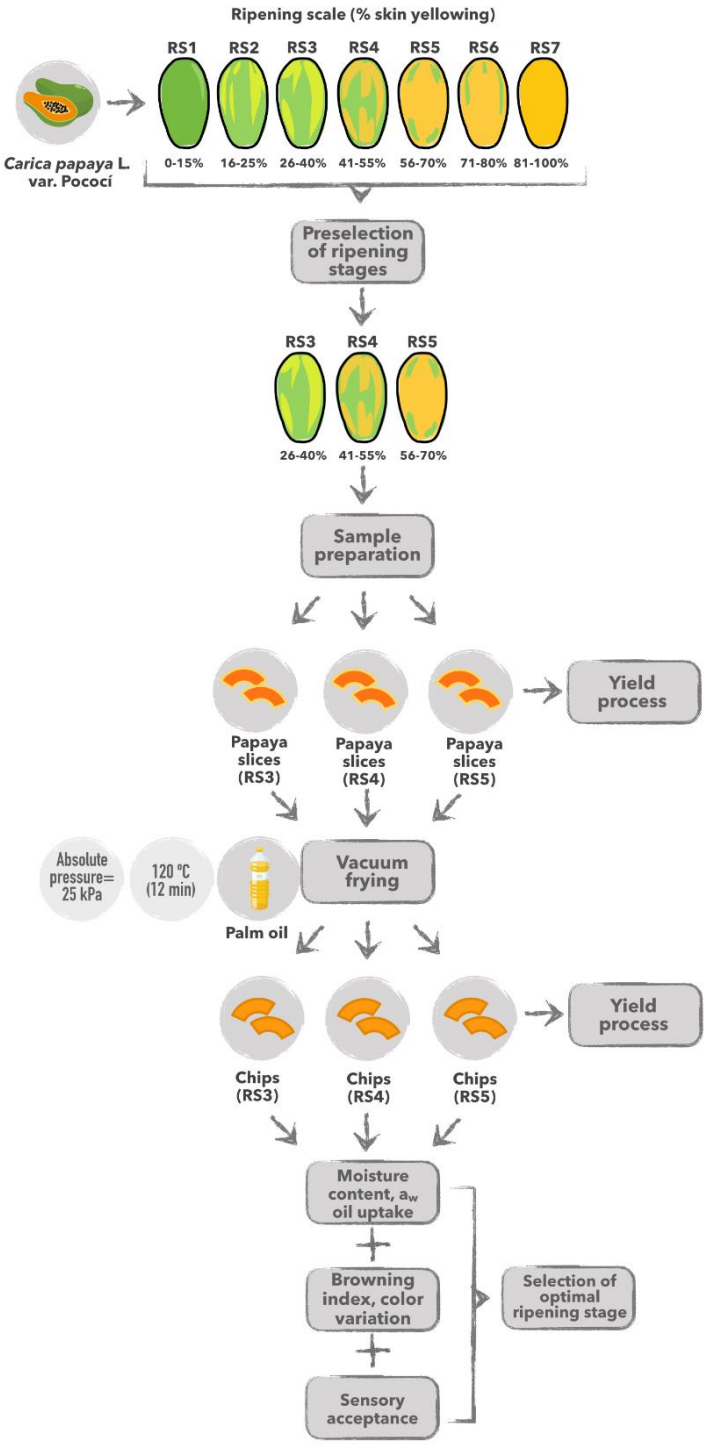

Figure 2.2. Process flow diagram to select the most promising ripening stage in papaya fruits for vacuum-fried chips making.

3.2. Optimization of vacuum frying conditions to obtain papaya chips (Objective 1)

Considering the physicochemical properties, sensory acceptance, and overall process yield, the RS4 ripeness stage (41-55 % skin yellowing) was chosen for processing to obtain the vacuum-fried papaya chips. Then, response surface methodology (RSM) was applied to optimize the vacuum frying conditions (Figure 2.3). The papaya chips were obtained using the vacuum frying system shown in Figure 2.1a. A central composite design (CCD), with two independent variables, was used: temperature / X1 (100-140 °C) and time / X2 (9-15 min), which resulted in an experimental design of 11 points, including three replicates from the central point. Regression analysis was performed on data for dependent variables affected by the vacuum frying conditions (moisture content, a_w, color variation, browning index, and lipid content).

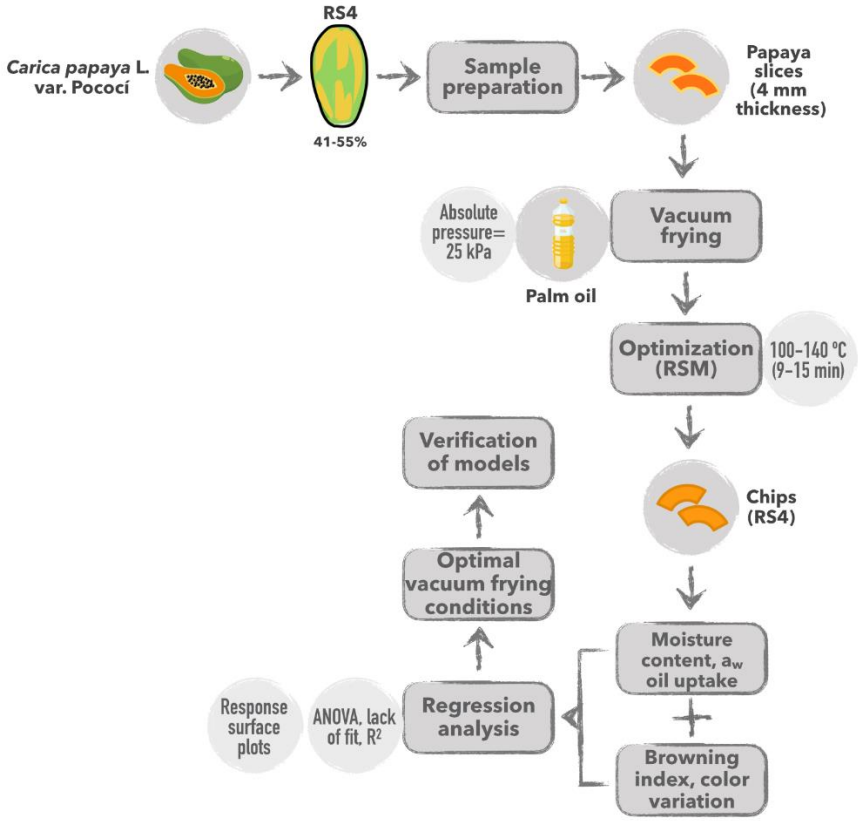

Figure 2.3. Process flow diagram to optimize vacuum frying conditions to obtain papaya chips.

3.3. Effect of vacuum frying on physicochemical properties and content of carotenoids of papaya chips (Objective 2)

Papaya fruits (RS4) were selected and then washed and peeled manually. Each fruit was vertically cut into four pieces, and the seeds were removed. The pre-cut pieces were sliced, firstly into 4-mm thick pieces using a food processor (FP-100 Hobart, CA, USA) and then into discs (diameter 30.0 ± 0.2 mm) using a circle-shaped cookie cutter as shown in Figure 2.4 (also see Appendix A3).

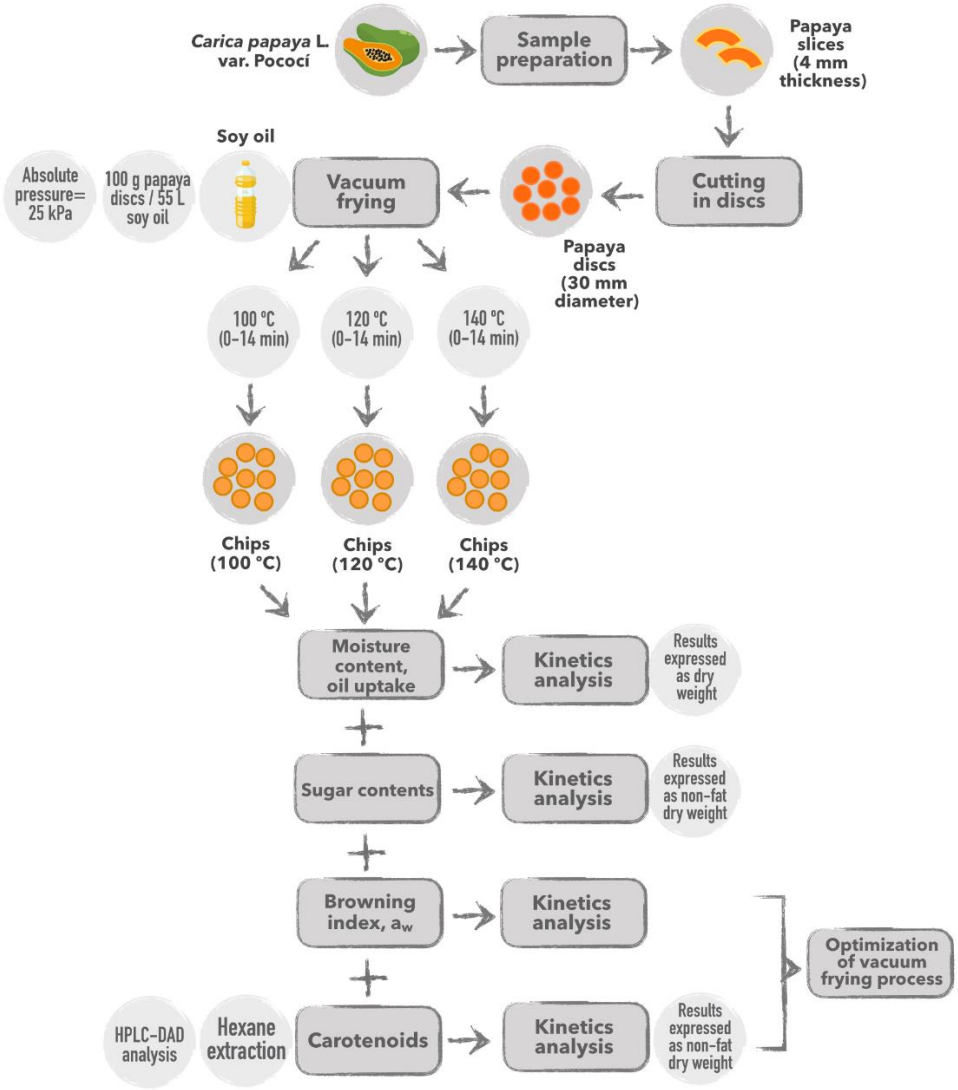

Figure 2.4. Process flow diagram to evaluate the effect of vacuum frying on physicochemical properties and carotenoid degradation in papaya chips.

The papaya chips were obtained using a vacuum frying system shown in Figure 2.1a. Papaya discs were fried at 25 kPa at three different oil temperatures (100,

120, and 140 °C) for seven frying times (0, 2, 4, 6, 8, 10, and 14 min) to obtain the papaya chips. For each frying experiment, a product/oil ratio of 1/500 (100 g papaya discs/50 kg oil) was performed to avoid a decrease in the oil temperature and pressure during the vacuum frying trials (to maintain isothermal conditions) (see Appendix A4 and A5). Once the papaya discs were fried, the samples were centrifuged (under vacuum) at fixed conditions (300 rpm, 16.6 x g, for 2 min) in order to remove excess oil. Three frying trials or repetitions were conducted for each frying condition (the combination of temperature/time). After vacuum frying, samples were packaged in metallized PET/PE bags (Multivac, France) and stored at -80 °C before analyses.

Different physicochemical properties were measured in the papaya chips: moisture, a_w, oil content, sugar content, color parameters (L*, a*, b*), and carotenoid content. Also, the browning index was measured in aqueous extracts obtained from papaya chips. Finally, kinetics analyses were performed to optimize process conditions to produce quality papaya chips with adequate sensory attributes (color and texture) and high nutritional value (carotenoid content).

3.4. Kinetic study of carotenoid degradation during storage of papaya chips (Objective 3)

3.4.1. Vacuum frying

Papaya fruits were selected and then washed and peeled manually. Each fruit was vertically cut into four pieces, and then the seeds were removed. The pre-cut pieces were cut into 4-mm thick slices (dimensions of approximately 3 cm x 3 cm x 6 cm) using a slicing machine before vacuum frying. The papaya chips were obtained using the vacuum frying system shown in Figure 2.1b.

Frying was performed under fixed conditions (25 kPa, 120 °C, 13 min) to achieve a final moisture content of 2 %. After frying, the samples were centrifuged in order to control the oil content absorbed by the samples. Papaya chips with palm oil were centrifuged at two-speed values, 530 rpm or 51.8 x g (maximum) and 100 rpm or 2.1 x g (minimum), to reach 24 % and 29 % lipid content, respectively. For

papaya chips with soy oil, a centrifugation speed of 100 rpm was applied to obtain 24 % lipids in the final product (Figure 2.5).

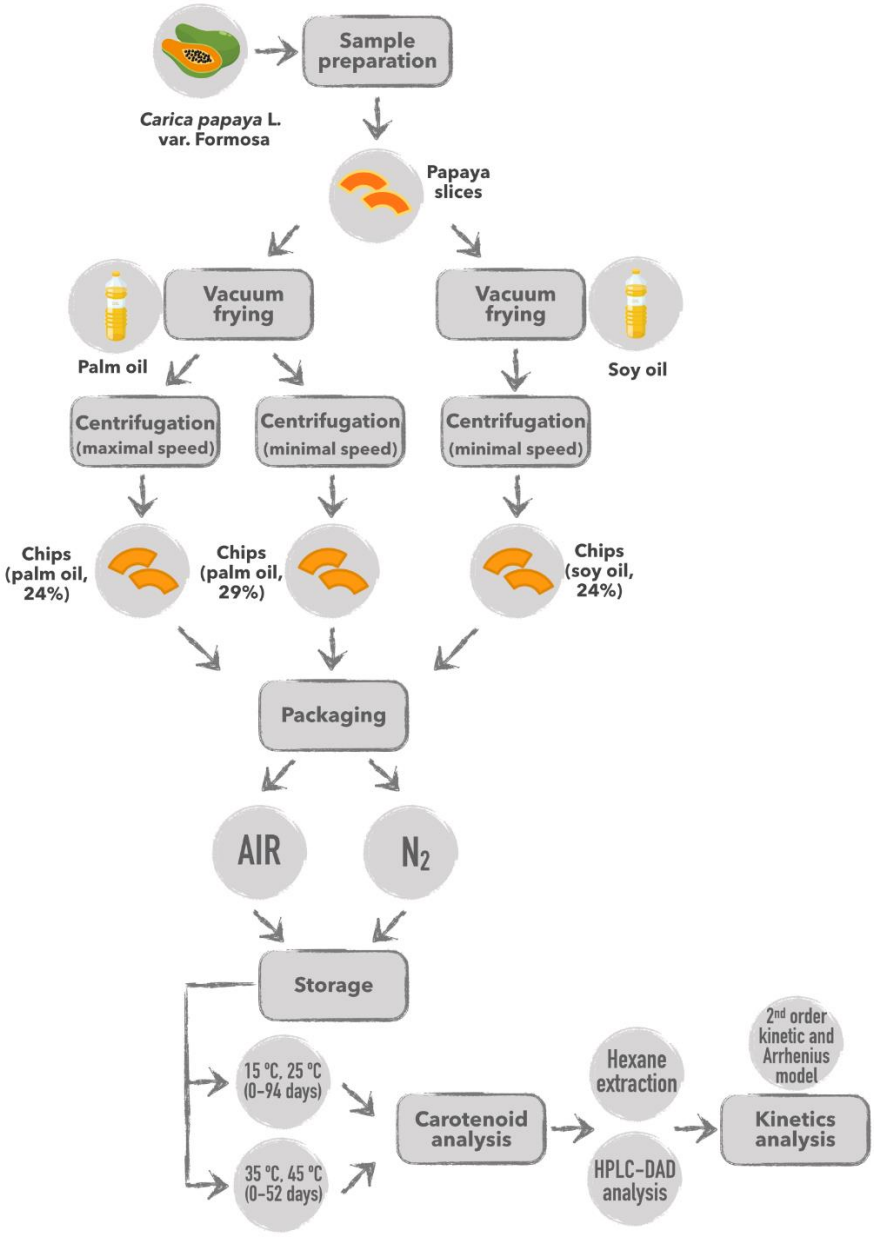

Figure 2.5. Process flow diagram to evaluate the carotenoid degradation during storage of vacuum-fried papaya chips.

3.4.2. Packaging and storage conditions

Papaya chips obtained after vacuum frying were packaged in polyethylene terephthalate metallized/polyethylene (PETm/PE, 12/70 µm) bags (see the technical data in Appendix A6) under compressed air or N_2 conditions using a

sealing machine (C200, Multivac, Germany). Approximately 5 g of papaya chips were weighed and placed in each bag and a gas injection pressure of 0.5 bar was applied into the bags (air or N_2) and then sealed. Samples were stored at 15 °C and 25 °C for 94 days (with sampling times at 15, 25, 45, 60, 75 and 94 days), and at 35 °C and 45 °C for 52 days (with sampling times at 3, 8, 15, 25, 35 and 52 days) as shown in Figure 2.5 (also see Appendix A7). For each frying treatment, packaging atmosphere, and storage temperature, three bags with papaya chips (repetitions) were analyzed at each sampling time. A total of 432 bags were stored for the study. Papaya chips obtained at the initial day (*t=0*) were also analyzed.

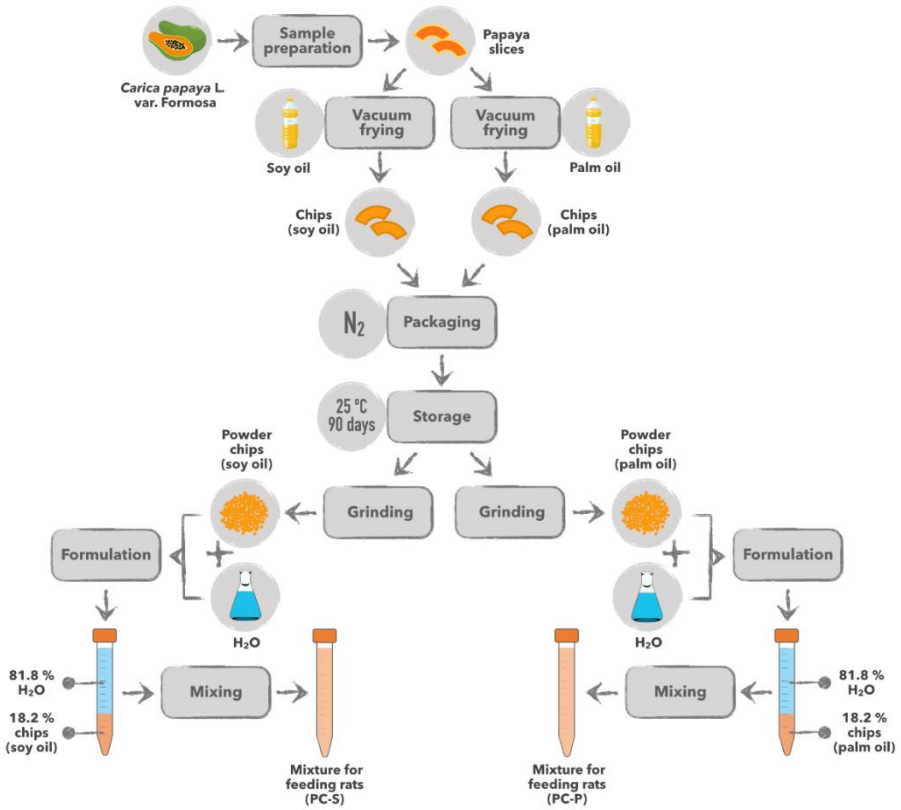

Figure 2.6. Flow process diagram to obtain the mixtures from papaya chips for feeding rats. PC-S, papaya chips with soy oil; PC-P, papaya chips with palm oil.

3.5. Effect of papaya chips consumption in an animal model (Wistar rats) (Objective 4)

3.5.1. Formulation of papaya mixtures

Four mixtures based on papaya products were elaborated for feeding the rats during experimentation. Papaya chips first were obtained after vacuum frying

(120 °C, 13 min, 25 kPa) either with soy oil (~26 % oil in chips) or palm oil (~24 % oil in chips), and then they were packaged in PET-metallized/PE bags under nitrogen conditions and stored for 90 days at 25 °C as previously described in section 3.4. After the storage period, the chips were ground (18 %, w/w) and mixed with 82 % (w/w) of distilled water to obtain the diet mixture with the adequate viscosity for feeding the rats via oral administration (Papaya chips - soy oil, PC-S, and Papaya chips - palm oil, PC-P) as shown in Figure 2.6.

Table 2. 3. Program steps for freeze dryer as function of pressure, temperature, and time of process.

Step	Pressure (mbar)	Temperature (°C)	Time (min)	Observations
Freezing step				
F-01	-	-10	1	F-01: maximum speed to cool down. F-02: Holding time for -10 °C. F-06: sublimator is cooling with maximum power for the set time. Reached temperature is not significant. F-07: all product sensors must reach this setpoint.
F-02	-	-10	60	
F-03	-	-45	1	
F-04	-	-45	60	
F-05	-	0	0	
F-06	-	0	60	
F-07	-	-45	-	
Main drying step				
D-01	1,0	-25	1	D-05: at this step the freeze dryer uses 120 min to go from 10 °C to 20 °C.
D-02	1,0	-25	300	
D-03	1,0	-10	120	
D-04	1,0	10	120	
D-05	1,0	20	120	
D-06	0,5	25	120	
D-07	0,5	25	300	
D-08	0,5	35	300	
D-09	0,5	35	60	
D-10	0	0	0	
Past drying step				
P-01	0,1	-	5	P-01: this step is to check if the product is dry. The freeze dryer closes the flap between chamber and condenser for 5 min and checks the pressure increase of the chamber (<0,1 mbar).
P-02	-	20	1	
P-03	-	20	300	
P-04	-	0	0	

On the other hand, papaya fruit was cut in small pieces (3 cm x 3 cm) and then freeze-dried (Usifroid SMH 15, Élancourt, France) for 72 h. For freeze drying of

papaya, it was performed different steps in order to obtain a product with the adequate final moisture. These steps were programmed in the freeze-dryer as shown in Table 2.3.

To obtain the mixtures from freeze-dried papaya, the papaya pieces were ground (14 %, w/w), and then mixed with 81-82 % (w/w) of distilled water and 4-5 % (w/w) of the different oils (soy or palm oils) to obtain the other diet mixtures (Freeze-dried papaya + soy oil, FDP+S, and Freeze-dried papaya + palm oil, FDP+P) with the adequate texture as well (Figure 2.7). Papaya was freeze-dried in order to obtain a product with similar content of carotenoids (especially of lycopene, the main carotenoid in papaya) with respect to papaya chips but without the application of a thermal treatment that occurs in vacuum frying. In addition, it was added oil to freeze-dried papaya to obtain a product with similar lipid content compared to papaya chips.

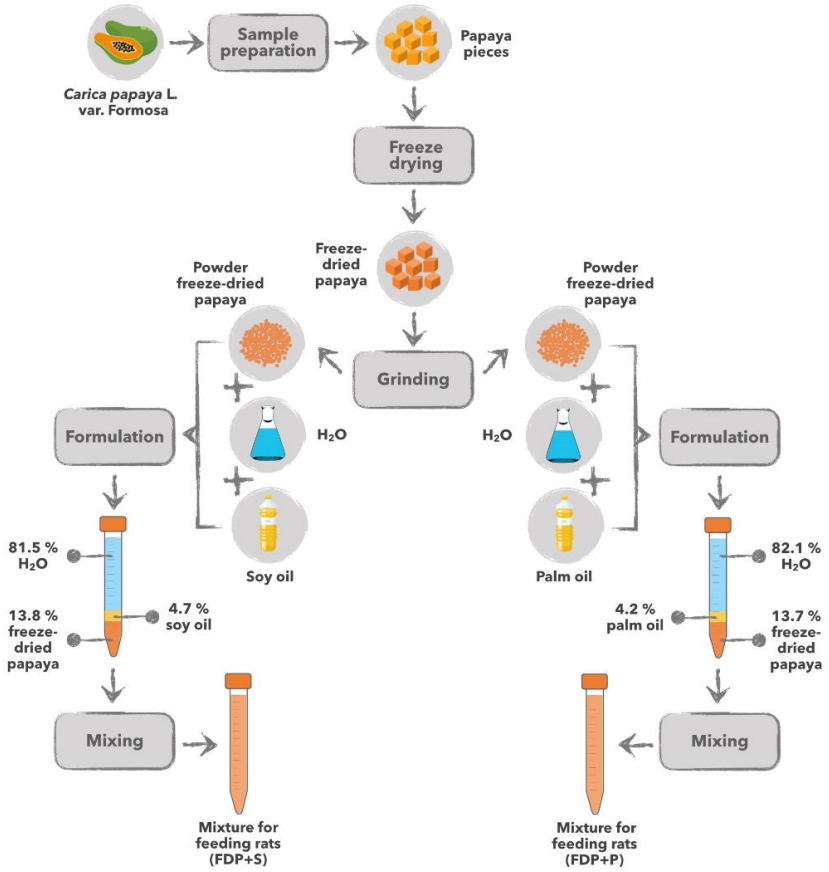

Figure 2.7. Flow process diagram to obtain the mixtures from freeze-dried papaya for feeding rats. FDP+S, freeze-dried papaya mixed with soy oil; FDP+P, freeze-dried papaya mixed with palm oil.

The formulation of different mixtures was made considering the lycopene concentration (major carotenoid) and the lipid content (Table 1) leading to products with a density of ~1.1 g/mL. Concentrations of lycopene, total carotenoids, and lipids in the diet mixtures expressed per body weight (bw) of rats were 0.29 mg/kg bw/day, 0.35 mg/kg bw/day and 0.28 g/kg bw/day, respectively. The different mixtures were prepared one week before the diet period and stored at -20 °C (Figure 2.8). The physicochemical composition of the processed papaya products for obtaining the mixtures used in the animal experimentation is shown in Table 2.4.

Table 2.4. Composition of the different papaya products processed for obtaining the mixtures used in the animal experimentation.

Component	PC-S	PC-P	FDP
Moisture (g/100 g FW)	2.35 ± 0.54	1.22 ± 0.10	7.12 ± 0.43
Lipids (g/100 g FW)	25.95 ± 3.71	24.01 ± 3.12	0.75 ± 0.01
Sugars (g/100 g FW):			
Sucrose	34.92 ± 1.99	32.76 ± 1.08	0.86 ± 0.23
Glucose	5.55 ± 0.33	6.55 ± 0.55	34.15 ± 0.90
Fructose	5.58 ± 0.30	5.78 ± 0.60	32.46 ± 0.80
Carotenoids (mg/kg FW):			
β-cryptoxanthin	10.55 ± 0.05	11.29 ± 0.63	30.96 ± 3.55
β-carotene	12.68 ± 0.44	16.62 ± 1.23	31.00 ± 1.35
Lycopene	180.32 ± 3.82	176.25 ± 2.08	248.49 ± 30.93
Total carotenoids	203.55 ± 3.47	204.17 ± 1.59	310.44 ± 32.82
Vitamin A content (μg RAE/100 g)	130.68 ± 3.34	160.21 ± 7.65	369.42 ± 21.52

PC-S, papaya chips with soy oil; PC-P, papaya chips with palm oil; FDP, freeze-dried papaya. Values are expressed as the means ± standard deviation, SD (n=3). FW, fresh weight. Vitamin A content is expressed as Retinol Activity Equivalent (RAE). RAE estimate was calculated for a bioconversion ratio (carotenoid:retinol) of 12:1 for all-E-β-carotene, and 24:1 for all-E-β-cryptoxanthin and Z-β-carotene (US IOM, 2000).

3.5.2. Animal experimentation

A total of 38 healthy male Wistar rats (258 ± 11 g body weight), seven weeks old, were obtained from Janvier Labs (Saint Berthevin), France. Animals were handled in compliance with European Union rules and according to the guidelines of the National Institute of Health and the Committee for Animal Care

at the University of Montpellier (France). Experimental procedures were carried out following the conditions for the maintenance and use of laboratory animals (12 h light/12 h dark and 25 ± 1 °C). During all the experimental period, standard commercial diet A04 (SAFE, Scientific Animal Food, and Engineering; Augy, France) (see the technical data in Appendix A8) and tap water *ad libitum* were provided to rats. During the adaptation period (4 days), rats were only fed with the standard diet without any carotenoid source (carotenoid deficient diet), as shown in Figure 2.8.

After the adaptation period, the animals were randomly divided into five experimental groups. One group of animals (n=6) received only the standard A04 diet (Control). The other 32 rats were orally fed with 1 mL of the different mixtures for seven days two times per day (morning, 8-9 am and afternoon, 3-4 pm) in addition to the standard A04 diet. Four groups (n=8/group) were fed with PC-S, PC-P, FDP+S, and FDP+P (Figure 2.8). For each treatment or diet, the different mixtures were prepared one week before the diet period. The mixtures were stored in 14 tubes of 15 mL (2 per day of diet period) at -20 °C. Before the oral feed, the mixtures were thawed at 37 °C in a water bath for 20 min (Figure 2.8). The minimum number of rats is 6 to be statistically accurate for the analysis. It was selected 8 rats for the groups fed with papaya mixtures and 6 for the control to limit the impact of the animals in accordance with the recommendations of ethics in animal experimentation.

At the end of the experimental period, rats were anesthetized (20 µL pentobarbital/100 g body weight), and blood samples were collected by cardiac puncture before euthanasia. For each blood sample, an aliquot (500 µL) was taken for glycemia determination. Then, tubes with heparin containing the blood samples were centrifuged at 1500 rpm (Jouan BR41 Multifunction, Thermo Electron Corporation, France) for 20 min at 15 °C to collect plasma. Liver samples were collected and weighed after being washed with ice-cold saline solution (0.9 % NaCl, w/v). Then, they were packaged in plastic bags and immediately frozen in liquid nitrogen. All the samples were stored at -80 °C before analysis (Figure 2.8).

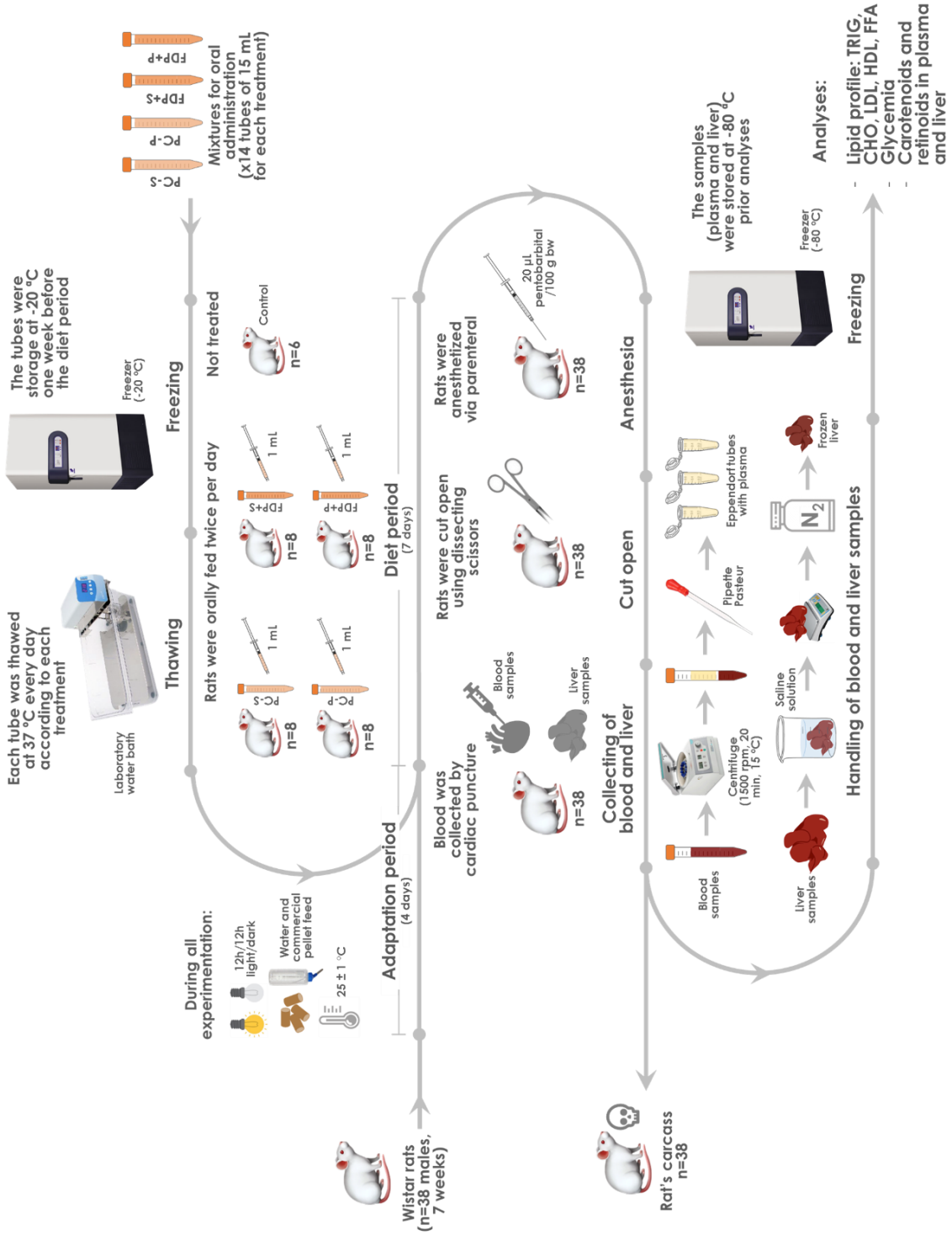

Figure 2.8. Scheme of animal experimentation. TRIG, triglycerides; CHO, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FFA, free fatty acids.

4. Methods of analyses

As follow are presented the methods used for the determination of the main physicochemical parameters, sensory analysis, as well as the analysis of carotenoids, lipid profile (TRIG, CHO, HDL, and LDL), and free fatty acids in rat plasma.

4.1. Physicochemical analysis

4.1.1 Moisture content

It was determined according to the AOAC 920.151 method (AOAC, 2015).

4.1.2 Lipid content

It was determined by the method described method of Carpenter et al. (1993).

4.1.3 Protein content

It was determined according to the AOAC 920.152 method (AOAC, 2015). A nitrogen conversion factor of 6.25 was used to calculate protein content.

4.1.4 Ash content

It was determined according to the AOAC 940.26 method (AOAC, 2015).

4.1.5 Fatty acid profile

The fatty acid profile was determined using the AOAC 996.06 standard method (AOAC, 2015) and the AOCS Ce 1e-91 method (AOCS, 2012).

4.1.6 Sugar content

Sugars (sucrose, glucose, and fructose) were determined by the Shimadzu HPLC system (Shimadzu Manufacturing, Inc., Canby, Oregón, USA) equipped with a RID-10A refractive index detector, a DGU-20A5 degasser, a SIL-20AHT autosampler, a CTO-20A column oven and a LC-20AT binary gradient pump. Sugars were separated using a Zorbax carbohydrate column (250 mm x 4.6 mm i.d., 5 μm) (Agilent, CA, USA) with a guard column. The mobile phase was acetonitrile/H_2O (80/20). The operation temperature was set at 30 ^oC. The flow

rate was set at 1mL/min and the injection volume was 10 µL. Isocratic conditions were programmed with a run time of 15 min. Quantification was performed after obtaining linear calibration curves of glucose, fructose, and sucrose. In order to keep the same basis among samples, the results were expressed as non-fat dry weight.

4.1.7 Hardness in fresh papaya

Skin and pulp firmness, expressed in Newtons (N), were determined in papaya fruits (n=25) with a penetrometer (Chatillon, NY, USA) using a tooth-shaped tip as shown in Figure 2.9. All measurements were performed at three points in the equatorial zone of each papaya.

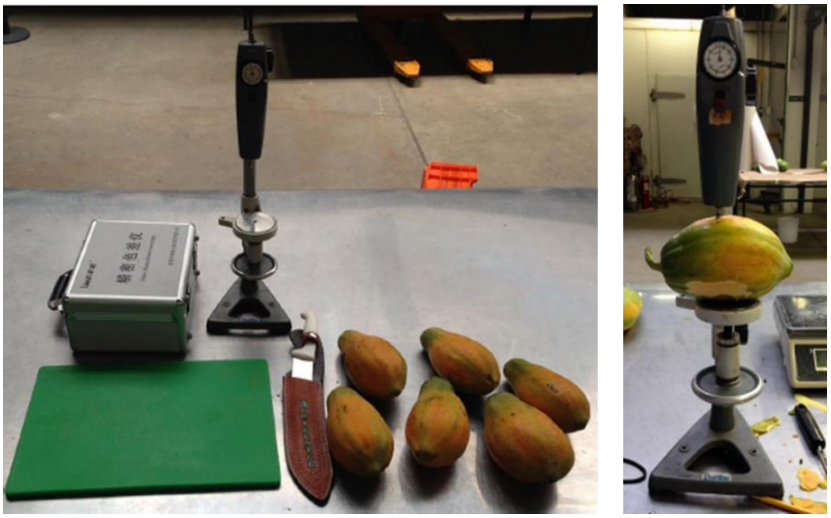

Figure 2. 9. Penetrometer used for the measuring of skin and pulp firmness in papaya fruits.

4.1.8 Color parameters

Color was measured in fresh papaya pulp on the whole fruit with a portable digital colorimeter WR-10 (Iwave, China), and in papaya chips with a color measurement spectrophotometer (model ColorFlex, HunterLab,Virginia, USA). For color determinations in both pulp fruit and papaya chips, color parameters were expressed in CIELab units L*, a* and b* using illuminant D65 and a 10◦ observer angle.

4.1.9 Browning index in aqueous extracts from papaya chips

Aqueous extracts were obtained from the vacuum-fried papaya chips as shown in Figure 2.10. These samples were previously defatted and dehydrated, to express the results on the same basis. Briefly, the samples were ground and then degreased with petroleum ether (b.p. 40-60 °C) using an E-812 fat extractor unit (Büchi, Fawil, Switzerland) To avoid an undesirable change in color, the defatted samples were dehydrated at 25 °C in desiccators with a saturated solution of $CaCl_2$ (a_w at equilibrium of 0.280). It was considered that samples reached the equilibrium point when they showed a constant weight during three consecutive readings.

Then the defatted and dehydrated samples were weighed (300 mg) into 40 mL centrifuge tubes. Then 5 mL of distilled H_2O was added and mixed using an IKA® Ultra-Turrax® (Merck KgaA, Darmstadt, Germany) for 5 min. The tubes were incubated for 30 min in a water bath at 70 ° C. After incubation, the tubes were cooled in an ice bath and centrifuged at 1400 x g (Allegra 21 centrifuge, Beckman Coulter, Switzerland) for 10 min at 15 °C. The supernatant consisting of the water-soluble compounds were filtered through a membrane (0.45 µm) before color analysis. Color of the extracts was measured using the same protocol as previously mentioned in section 4.1.8. Then, the browning index (BI) was calculated using the L *, a * and b * values according to Equation 1.

$$BI = 100 \times \left[\frac{\left(\frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \right) - 0.31}{0.17} \right] \qquad [1]$$

where L^*, $a^*$and b^* represented the values of aqueous extracts from papaya chips after vacuum frying. The BI at each frying time was represented with respect to its initial value (BI_0).

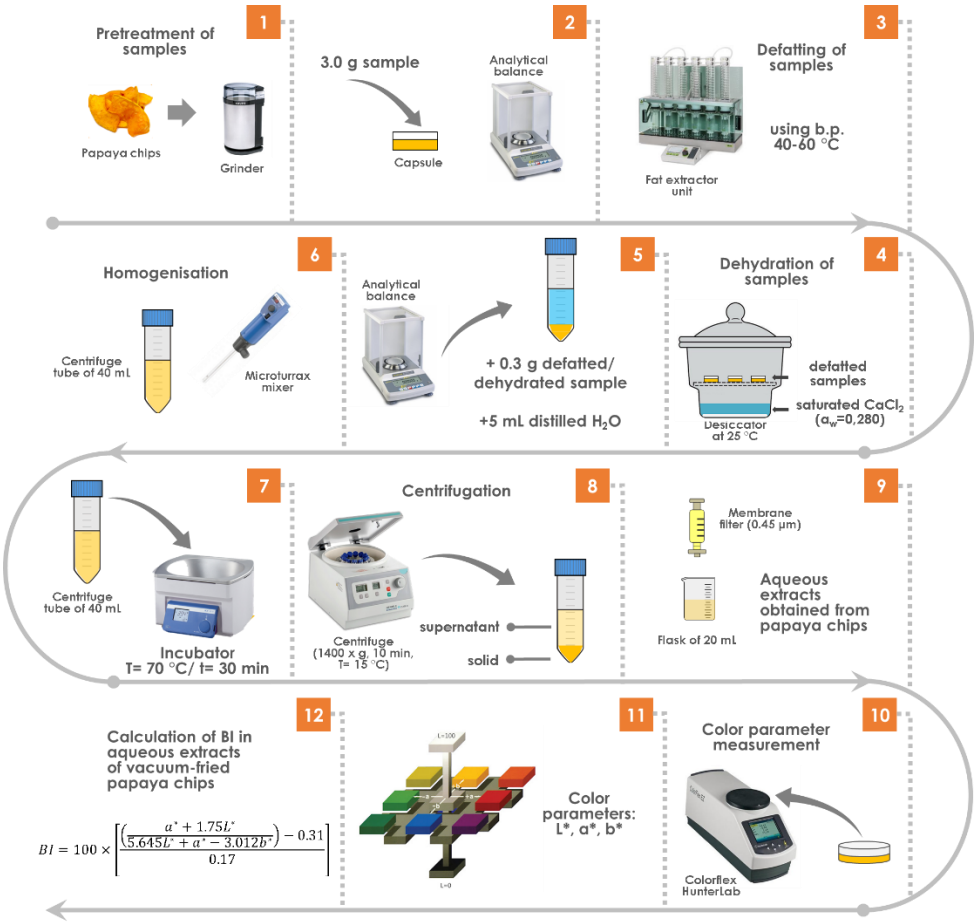

Figure 2. 10. Scheme of the protocol for the determination of browning index in aqueous extracts obtained from papaya chips.

4.1.10 Water activity and moisture sorption isotherm

Water activity of the samples were determined at 25 °C using a water activity meter (Aqualab, model CX-2, Decagon Devices, Inc., Pullman, WA). To determine the moisture sorption isotherm, the papaya chips were blended and approximately 5 g of sample were placed in weighing capsules. Samples were transferred to vacuum desiccators containing saturated solutions of different salts to generate the desired water activity in the papaya chips at equilibrium. The desiccators were kept in ovens at 25 °C. The weighing capsules containing the samples were hermetically sealed before transfer to the desiccators containing the saturated solutions. The saturated salt solutions and the a_w at equilibrium were: LiCl (0.113), $CaCl_2$ (0.280), K_2CO_3 (0.436), $NaNO_2$ (0.640), NaCl (0.756), MgCl, (0.326), KNO_3 (0.946). Samples (5 replicates for each a_w at equilibrium) were

considered to have reached the equilibrium point when the weight remained constant for three consecutive determinations. The equilibrium moisture content of the sample was determined using the AOAC method 920.151 (AOAC, 2015). A test tube containing thymol was placed inside the jars of higher water activity to prevent mold growth during storage.

4.2. Sensory acceptance

In order to evaluate the sensory acceptance of papaya chips prepared from fruits at different ripening stages, a sensory test was applied to regular consumers of snacks and fruits. The participants (n=100) were between 18 and 60 years of age (40 men and 60 women). A hybrid hedonic scale was used with verbal affective anchors in the middle and extreme regions of the scale (0=disliked extremely, 5= neither liked nor disliked; 10= liked extremely). This scale was used because it is more consumer-friendly than other unstructured scales (Villanueva *et al.*, 2005) as shown in Figure 2.11. Papaya chips were prepared and packaged in PET-m/PE bags one week before the sensory test. Samples were removed from the bags 15 min prior to evaluation and 3-4 chips were placed on white plates coded with random 3-digit numbers and maintained at room temperature (25 °C). Samples were randomly presented, and the participants were asked to drink water between samples.

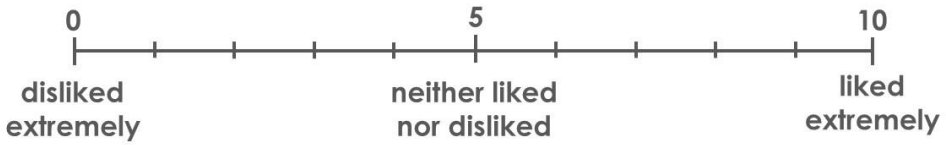

Figure 2. 11. A hybrid hedonic scale used to measure the sensory acceptance of vacuum-fried papaya chips.

4.3. Carotenoid determination

4.3.1. Carotenoid extraction from papaya products: traditional method without a saponification step

Extraction procedures and conditions for analysis were described previously by Schweiggert *et al.* (2012). Sample preparation was carried out under dim light. Samples were ground and weighed (3 g for papaya fruit and 2 g for papaya chips)

in a 50 mL Pyrex flask and 0.1 g of $NaHCO_3$ was added. The papaya chip samples were rehydrated with 4 mL of distilled water and homogenized. Carotenoids were extracted in 15 mL of a mixture (1:1:1, v/v/v) of methanol, ethyl acetate and light petroleum containing 0.1 g/L of BHT and 0.1 g/L of BHA (LP/BHT/BHA) using an IKA® Ultra-Turrax® (Merck KGaA, Darmstadt, Germany). The suspension was filtered through a Büchner funnel with Whatman qualitative filter paper No.1, and the recovered solids were re-extracted 2–3times until a colorless filtration residue was obtained (Figure 2.12).

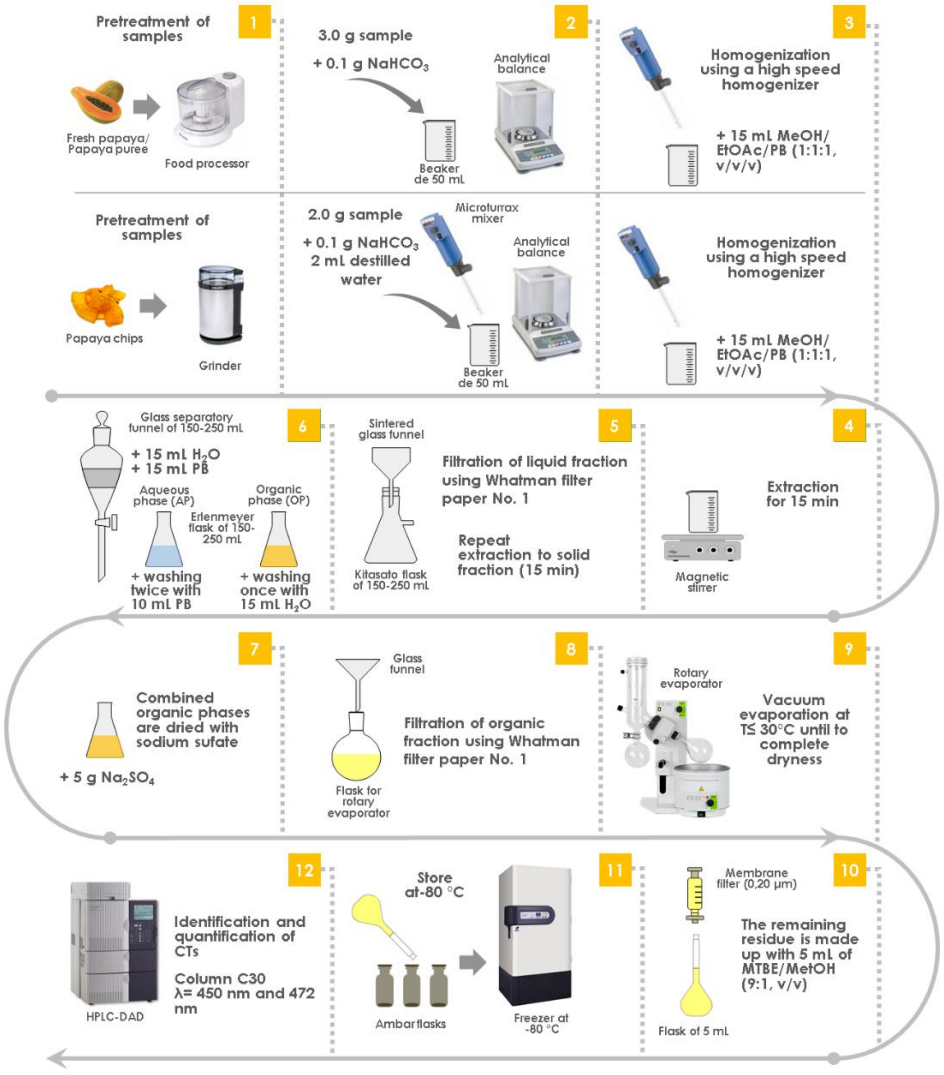

Figure 2. 12. Scheme of carotenoid extraction from papaya products applying a traditional method without a saponification step.

Filtrates were transferred to a separatory funnel with 15 mL H_2O and 15 mL LP/BHT/BHA, and the upper fraction was washed with 15 mL H_2O and

recovered. For quantitative carotenoid extraction, the lower aqueous fraction was re-extracted 2–3 times with 10 mL LP/BHT/BHA. The combined upper fractions were dried with 5 g Na_2SO_4 and evaporated to complete dryness under reduced pressure at ≤25 °C using a parallel evaporation system (Büchi Multivapor™ P-6, Flawil, Switzerland). The remaining residue was mixed with 5 mL of MTBE/methanol (9:1 v/v), membrane filtered (0.45 µm) and stored at -80 °C in amber glass vials until HPLC analysis (Figure 2.12).

4.3.2. Carotenoid extraction from papaya products: fast method with a saponification step

Extraction procedures and conditions for analysis were described previously by Dhuique-Mayer *et al.* (2016). Briefly, samples were ground and then weighed (0.5 g for papaya fruit, 0.15 g for freeze-dried papaya, 0.2 g for papaya chips, and 0.7 g for papaya mixtures used for feeding rats) in 20 mL tubes. The papaya chip samples were rehydrated with 2 mL of distilled water and homogenized. Then, 2 mL of an ethanol solution containing 1 % pyrogallol was added. The mixture was homogenized using a Vortex mixer and incubated for 2 min in the dark in a water bath at 70 °C.

Then, after cooling the samples saponification was performed for 30 min in a water bath at 70 °C by adding 1.5 mL of saturated KOH (12 N). After incubation, the tubes were cooled in an ice bath and 2 ml of distilled water and 5 mL of hexane were added. Then, after mixing and decantation, the organic phase was recovered, and the aqueous phase was extracted two more times with 5 mL of hexane. The organic phases were combined and evaporated to dryness using a vacuum evaporation system (GeneVac EZ-2, SP Scientific, UK). Finally, the residue was re-dissolved in 500 µL of methyl tert-butyl ether (MTBE)/methanol (80/20) and placed in an amber vial prior to HPLC analysis. A scheme of the followed extraction protocol is shown in Figure 2.13.

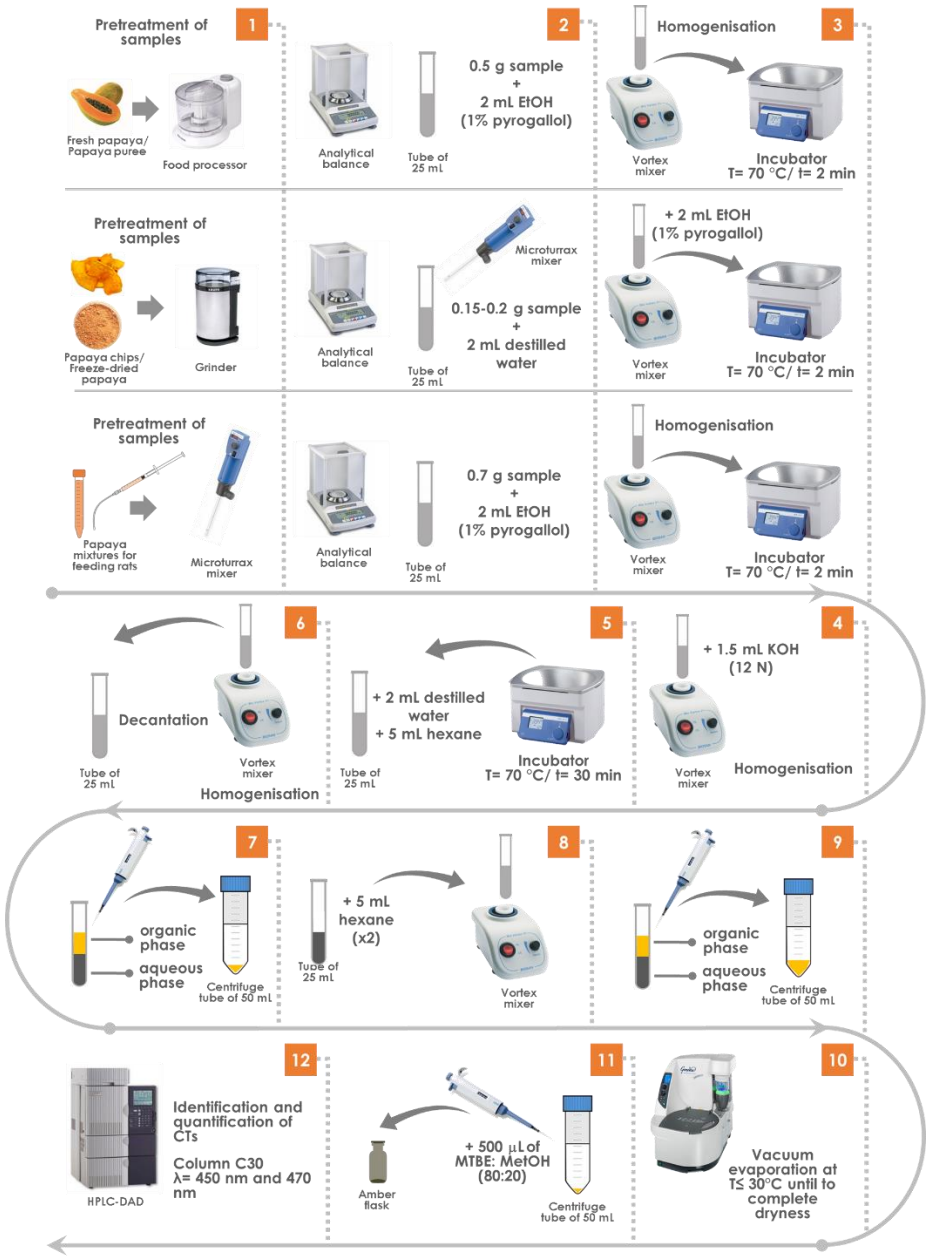

Figure 2. 13. Scheme of carotenoid extraction from papaya products applying a fast method with a saponification step.

4.3.3. Carotenoid and retinoids extraction from rat plasma

The carotenoids were extracted according to the method previously described by Poulaert *et al.* (2014) with some modifications. The plasma previously obtained (700 µL) was put into a tube of 8 mL, then 500 µL of ethanol 96 % (v/v) containing canthaxanthin (2 mg/mL) as internal standard and 2 mL of hexane were added. The mixture was homogenized (using a Vortex) during 60 s and then

centrifuged (Allegra 21 Centrifuge, Beckman Coulter, Switzerland) at 1400 x g for 5 min at 25 °C.

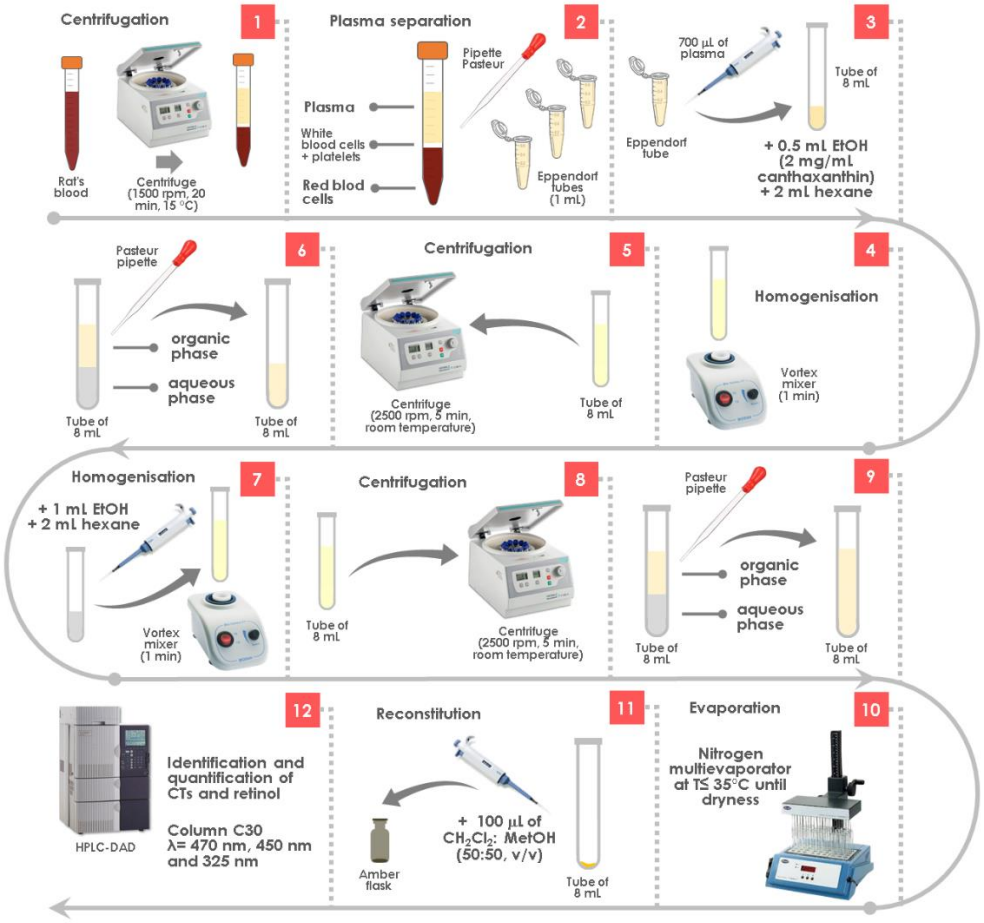

Figure 2. 14. Scheme of carotenoid and retinoids extraction from rat plasma.

The organic phase was collected, then the aqueous phase was reextracted with 1 mL of ethanol and 2 mL of hexane. The organic phases were pooled and evaporated under nitrogen at 30 °C until dryness. The dried extract was dissolved with 100 µL of CH_2Cl_2/Methanol (50:50, v/v) prior HPLC analysis. A scheme of the followed extraction protocol is shown in Figure 2.14.

4.3.4. Carotenoid and retinoids extraction from rat liver

The frozen liver was cut into small pieces on an ice-cold table. Then, 1 g of liver was mixed with 1 mL phosphate-buffered saline (PSB) and homogenized (using a 15 mL Potter tissue grinder) at 600-1000 rpm during 4 min. This step was done

twice. Then the homogenates were mixed and homogenized using an Ultra-turrax homogenizer (IKA T10 Basic, Germany) for 60 s. For carotenoid extraction (CE) and retinyl palmitate extraction (RPE), 900 µL and 500 µL of liver homogenates, respectively, were extracted using 500 µL of ethanol 96 % (v/v) containing canthaxanthin (2 mg/mL) as internal standard and 2.5 mL or 2 mL of hexane for CE and RPE, respectively. The mixture was homogenized again using a Vortex for 60 s and then centrifuge (Allegra 21 Centrifuge, Beckman Coulter, Switzerland) at 1600 x g for 5 min, at 25 °C. The organic phase was collected, and the aqueous phase was reextracted with 1 mL of ethanol and 2 mL of hexane. The organic phases were pooled and evaporated under nitrogen at 30 °C until dryness. The dried extract was dissolved with 100 µL and 850 µL of CH_2Cl_2/Methanol (1 g/20 mL) for CE and RPE analysis, respectively. A scheme of the followed extraction protocol is shown in Figure 2.15.

4.3.5. HPLC analysis in papaya products (coupled to traditional extraction method without a saponification step)

Carotenoid were identified using a reverse-phase HPLC-DAD Shimadzu system (Shimadzu Manufacturing, Inc., Canby, Oregon, USA) equipped with an SPD-M20A diode array detector, a DGU-20A5 degasser, a SIL-20AHT autosampler, a CTO-20A column oven and a LC-20AT binary gradient pump. Carotenoids were separated using a C30 column (150 x 4.6 mm i.d., 3 µm) (YMC America Inc., PA, USA) with a guard column. The mobile phase was methanol as eluent A and MTBE as eluent B. Operation temperature was set at 30 °C. The flow rate was set at 0.6 mL/min and the injection volume was 10 µL. A solvent gradient was programmed as follows: 0-5 min, isocratic 80 % A (initial conditions); 5–7 min, 73 % A; 7-15 min, 62.5 % A; 15-20 min, isocratic 62.5 % A; 20-30 min, 45 % A; 30-35 min, 10 % A; 35-40 min, isocratic 10 % A; 40-45 min, 80 % A with a return to the initial conditions for rebalancing. All-E-β-cryptoxanthin (free form and ester forms), all-E-β-carotene and its isomers were detected at 450 nm, and all-E-lycopene and their isomers were detected at 470 nm (Dhuique-Mayer *et al.*, 2007). Z-β-carotene and Z-lycopene contents were expressed as the sum of all Z-β-carotene and all Z-lycopene isomers, respectively. Isomers were identified by relative retention times, *i.e.*, elution order and combined spectral data, based on

previously published data obtained with the same mobile phase (MTBE/methanol) and detection wavelength range (Achir *et al.*, 2010; Achir *et al.*, 2015; Chanforan *et al.*, 2012; Martins *et al.*, 2016; Schweiggert *et al.*, 2012; Schweiggert *et al.*, 2011b). Analyses were done in triplicate.

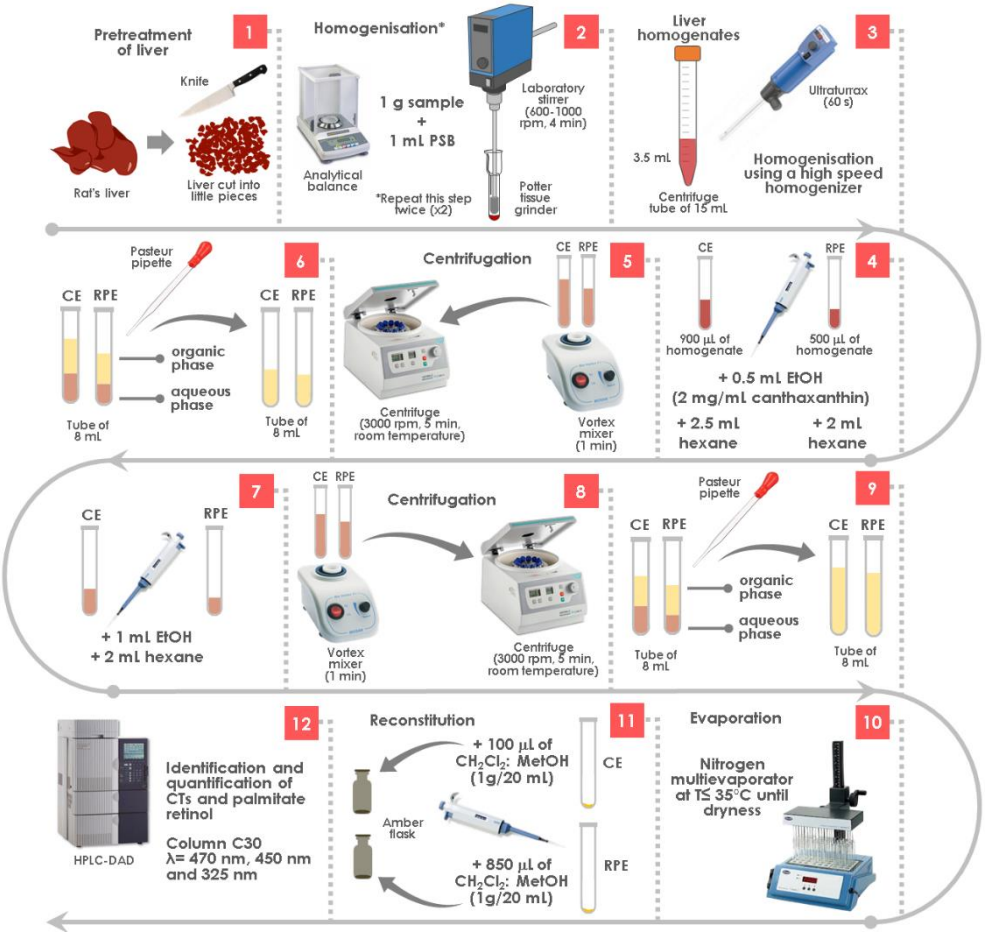

Figure 2. 15. Scheme of carotenoid and retinoids extraction from rat liver.

4.3.6. HPLC analysis in papaya products (coupled to fast method with a saponification step)

Carotenoid identification was performed on a reverse-phase HPLC-DAD Agilent 1100 system (Massy, France) with a diode array detector according to Dhuique-Mayer *et al.* (2007). Carotenoids were separated using a C30 column (250 x 4.6 mm i.d., 5 µm) (YMC EUROP GmbH, Germany) with a guard column, and the mobile phase was H2O as eluent A, methanol as eluent B, MTBE as eluent C. Operation temperature was set at 25 ºC. The flow rate was set at 1mL/min and

the injection volume was 20 µL. A solvent gradient was programmed as follows: 0-2 min, isocratic 40 % A – 60 % B (initial conditions); 2–5 min, 20 % A – 80 % B; 5-10 min, 4 % A – 81 % B – 15 % C; 10-60 min, 4 % A – 11 % B – 85 % C; 60-70 min, isocratic 4 % A – 11 % B – 85 % C; 70-71 min, 100 % B; 71-72 min, with a return to the initial conditions for rebalancing. All-E-β-cryptoxanthin, all-E-β-carotene and its isomer were detected at 450 nm, and all-E-lycopene and their isomers were detected at 470 nm (Dhuique-Mayer *et al.*, 2007). Z-lycopene content was expressed as the sum of all Z-lycopene forms. Isomers were identified by relative retention times, *i.e.*, elution order and combined spectral data (see Appendix A9). Analyses were done in triplicate.

4.3.7. HPLC analysis in plasma and liver samples

Carotenoid and retinoids were separated on the same C30 column as previously described in section 4.1.6. The mobile phase was H_2O as eluent A, methanol as eluent B, and MTBE as eluent C. Temperature was set at 25 °C. The flow rate was set at 1mL/min and the injection volume was 60 µL. A solvent gradient was programmed as follows: 2 % A – 96 % B – 2 % C (initial conditions); 0-27 min, 2 % A – 18 % B – 80 % C; 27-35 min, 4 % A – 11 % B – 85 % C and back to the initial conditions for re-equilibration. Chromatograms were generated at 325 and 470 nm to identify retinol, retinyl esters and lycopene (see Appendix A10). Carotenoid and retinoids were identified by comparing their retention time and spectra with the respective standards. Quantification was achieved by establishing calibration curves with all-E-lycopene, retinol and retinyl palmitate standards. Analyses were done in duplicate.

4.4. Lipid profile in plasma

4.4.1 Triglycerides

The measurement of the concentration of triglycerides (TRIG) in plasma of rats was performed using a Biolabo enzyme kit (Biolabo SAS, Maizy, France). The reaction scheme is shown in Figure 2.16.

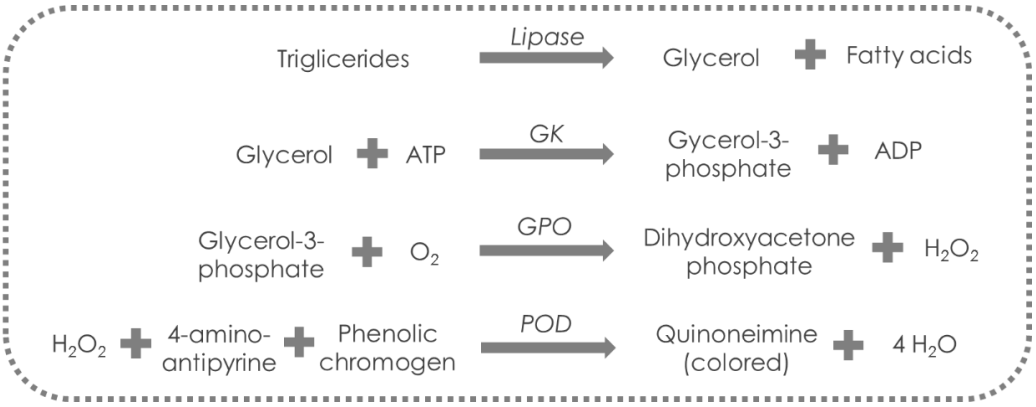

Figure 2. 16. Reaction scheme to measure the concentration of triglycerides. ATP, adenosine triphosphate; ADP, adenosine diphosphate; GK, glycerol kinase; GPO, glycerol 3 phosphate oxidase; POD, peroxidase.

- *Reagents*

The enzymatic method to measure TRIG utilizes the following reagents shown in Table 2.5.

Table 2.5. Reagents utilized in enzymatic method to determine concentration of triglycerides.

R1 (Buffer)	R2 (Enzymes)	R3 (Standard)
piperazine-N,N′-bis(2-ethanesulfonic acid): 100 mmol/L Magnesium chloride: 9.8 mmol/L Chrolo-4-phenol: 3.5 mmol/L Preservative	Lipase: ≥1000 IU/L Peroxidase (POD): ≥1700 IU/L Glycerol 3 phosphate oxidase (GPO): ≥3000 IU/L Glycerol kinase (GK): ≥660 IU/L 4-amino-antipyrine (PAP): 0.5 mmol/L Adenosine triphosphate (ATP): 1.3 mmol/L	Glycerol (equivalent to trioleine or triglycerides: 200 mg/dL (2.28 mmol/L)

- *Protocol*

The reagent R2 was dissolved in the R1 flask to form R4. The R3 was the standard with a glycerol concentration (Cn) of 200 mg/dL or 2.28 mmol/L. The samples were thawed at room temperature during 10-20 min. The test tubes (2 mL tube Eppendorf) were identified according to the type of sample: Blank, Standard and

Assay (rat plasma) (see Appendix A11.a). Then, 1 mL of R4 was added in each test tube. For the Blank tubes 40 µL of distilled water was added. For the Standard tubes 40 µL of R3 was added. Finally, for the Assay tubes 40 µL of rat plasma was added. Then, after mixing (using a Vortex), the tubes were incubated for 5 min in a water bath at 37 °C. Samples were transferred to a 96-well microplate (300-350 µL/well) and the absorbance (A) was read against the Blank at 500 nm using a Spark spectrophotometry-fluorescence device (TECAN, Männedorf, Switzerland). The absorbance of the colored complex (quinoneimine, pink color) was proportional to the amount of TRIG in the sample (see Appendix A12.a). The reaction was linear up to at least 700 mg/dL (7.9 mmol/L). The content of TRIG was determined according to Equation 2 and the results were expressed as mg/dL.

$$TRIG = \frac{A_{Assay} - A_{Blank}}{A_{Standard} - A_{Blank}} \times Cn_{Standard} \qquad [2]$$

4.4.2 Total cholesterol

The measurement of total cholesterol (CHO) in plasma of rats was performed using a Biolabo enzyme kit (Biolabo SAS, Maizy, France). The reaction scheme is shown in Figure 2.17.

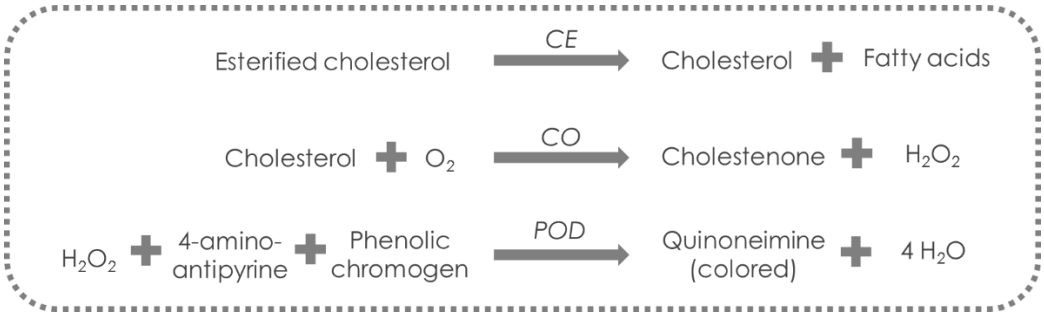

Figure 2. 17. Reaction scheme to measure the concentration of total cholesterol. CE, cholesterol esterase; CO, cholesterol oxidase; POD, peroxidase.

- *Reagents*

The enzymatic method to measure CHO utilizes the following reagents shown in Table 2.6.

Table 2.6. Reagents utilized in enzymatic method to determine concentration of total cholesterol.

R1 (Buffer)	R2 (Enzymes)	R3 (Standard)
Phosphate buffer: 100 mmol/L	Cholesterol oxidase (CO): ≥100 IU/L	Cholesterol: 200 mg/dL (5.17 mmol/L)
Sodium cholate: 2.3 mmol/L	Cholesterol esterase (CE): ≥170 IU/L	
Chrolo-4-phenol: 5.0 mmol/L	Peroxidase (POD): ≥1200 IU/L	
Triton x 100: 1.5 mmol/L	4-amino-antipyrine (PAP): 0.25 mmol/L	
Preservative	PEG 6000: 167 µmol/L	

- *Protocol*

The reagent R2 was dissolved in the R1 flask to form R4. The R3 was the standard with a cholesterol concentration (Cn) of 200 mg/dL or 5.17 mmol/L. The samples were thawed at room temperature during 10-20 min. The test tubes (2 mL tube Eppendorf) were identified according to the type of sample: Blank, Standard and Assay (rat plasma) (see Appendix A11.b). Then, 1 mL of R4 was added in each test tube. For the Blank tubes 40 µL of distilled water was added. For the Standard tubes 40 µL of R3 was added. Finally, for the Assay tubes 40 µL of rat plasma was added. Then, after mixing (using a Vortex), the tubes were incubated for 5 min in a water bath at 37 °C. Samples were transferred to a 96-well microplate (300-350 µL/well) and the absorbance (A) was read against the Blank at 500 nm using a Spark spectrophotometry-fluorescence device (TECAN, Männedorf, Switzerland). The absorbance of the colored complex (quinoneimine, pink color) was proportional to the amount of CHO in the sample (see Appendix A12.b). The reaction was linear up to at least 500 mg/dL (12.9 mmol/L). The content of CHO was determined according to Equation 3 and the results were expressed as mg/dL.

$$CHO = \frac{A_{Assay} - A_{Blank}}{A_{Standard} - A_{Blank}} \times Cn_{Standard} \qquad [3]$$

4.4.3. LDL cholesterol

The quantitative determination of LDL cholesterol in plasma was performed using a Biolabo enzyme kit (Biolabo SAS, Maizy, France). This determination occurs in two phases:

- 1rst phase: during this phase only non-LDL lipoproteins (HDL, VLDL and chylomicrons) are solubilized by detergent 1 releasing free cholesterol. Then, the cholesterol generated is subjected to the action of cholesterol oxidase and cholesterol esterase producing a colorless compound.
- 2nd phase: during this phase, the detergent 2 solubilizes LDL cholesterol. The chromogenic pair develops a color reaction that is proportional to the concentration of LDL cholesterol. The absorbance (A) is measured at 546 nm.

- *Reagents*

The enzymatic method to measure LDL utilizes the following reagents shown in Table 2.7.

Table 2.7. Reagents utilized in enzymatic method to determine concentration of LDL cholesterol.

R1 (Enzymes)	R2 (Selective detergent)	R3 (Calibrator)
Cholesterol oxidase	Buffer (morpholino-ethanesulfonic acid)	Bottle R3.1: lyophilizate
Cholesterol esterase	pH 6.3	Bottle R3.2: diluent
Detergent 1	Detergent 2	* Reconstitute the lyophilizate (bottle R3.1)
Preservative	DSBmT (N, N-bis (4-sulphobutyl) -m-toludine-disodium	with an aliquot of 2 mL of R3.2 (diluent)
Buffer (morpholino-ethanesulfonic acid) pH 6.3	Preservative	
Ascorbic acid oxidase		
4-amino-antipyrine (4-AAP)		
Peroxidase		

- *Protocol*

The samples were thawed at room temperature during 10-20 min. The protocol was realized using a 96-well microplate. The different wells (maximum volume of 400 µL) were identified according to the type of sample: Blank, Calibrator and Assay (rat plasma) (see Appendix A11.c). The R1 (aliquot of 180 µL was added into all wells (96-well microplate). Then, 10 µL of R3 (Cn= 77.4 mg/dL or 2.00 mmol/L) was added in the Calibrator well. For the Assay wells 10 µL of rat plasma was added. Then, the microplate was mixed vigorously and let stand for 10 min at room temperature. After this time, the absorbance (A1) was read against the Blank (well with R1) at 546 nm using a Spark spectrophotometry-fluorescence device (TECAN, Männedorf, Switzerland). Then, 60 µL of R2 was added into all wells. The microplate with samples, after mixing, was incubated for 5 min at 37 °C. Finally, the absorbance (A2) was read against the Blank (well with R1+R2) at 546 nm using the same spectrophotometry-fluorescence device (see Appendix A12.c). The reaction was linear from 7 to 900 mg/dL (0.18 to 23 mmol/L). The content of LDL was determined according to Equations 4-6 and the results were expressed as mg/dL.

For Calibrator and Assay samples:

$$\Delta A_{Calibrator} = (A2_{Calibrator} - A2_{Blank}) - (0.75 \times (A1_{Calibrator} - A1_{Blank})) \qquad [4]$$

$$\Delta A_{Assay} = \left(A2_{Assay} - A2_{Blank}\right) - \left(0.75 \times (A1_{Assay} - A1_{Blank})\right) \qquad [5]$$

And then:

$$LDL = \frac{\Delta A_{Assay}}{\Delta A_{Calibrator}} \times Cn_{Calibrator} \qquad [6]$$

4.4.4. HDL cholesterol

The quantitative determination of HDL cholesterol in plasma was performed using a Biolabo enzyme kit (Biolabo SAS, Maizy, France). This determination occurs in two phases:

- 1rst phase: LDL particles, VLDL and chylomicrons generate free cholesterol, which through an enzymatic reaction, produce hydrogen peroxide. The generated peroxide is consumed by a peroxidase reaction with DSBmT yielding a colorless product.
- 2nd phase: during this phase, specific detergent solubilizes HDL cholesterol. In conjunction with cholesterol oxidase and cholesterol esterase action, peroxidase and 4-amino-antipyrine develop a colored reaction. The absorbance of the colored complex formed is proportional to the concentration of HDL cholesterol, read at 600 nm.

- *Reagents*

The enzymatic method to measure HDL utilizes the following reagents shown in Table 2.8.

Table 2.8. Reagents utilized in enzymatic method to determine concentration of HDL cholesterol.

R1 (Accelerator)	R2 (Selective detergent)	R3 (Calibrator)
Good's buffer	Good's buffer	Bottle R3.1: lyophilizate
Cholesterol oxidase	Cholesterol esterase	Bottle R3.2: diluent
Peroxidase	4-amino-antipyrine (4-AAP)	* Reconstitute the lyophilizate (bottle R3.1) with an aliquot of 2 mL of R3.2 (diluent)
DSBmT (N, N-bis (4-sulphobutyl) -m-toludine-disodium	Detergent	
	Stabiliser	
Accelerator	Ascorbic acid oxidase	
Preservative	Preservative	

- *Protocol*

The samples were thawed at room temperature during 10-20 min. The protocol was realized using a 96-well microplate. The different wells (maximum volume of 400 µL) were identified according to the type of sample: Blank, Calibrator and Assay (rat plasma) (see Appendix A11.d). The R1 (aliquot of 180 µL was added into all wells (96-well microplate). Then, 10 µL of R3 (Cn= 107.0 mg/dL or

2.77 mmol/L) was added in the Calibrator well. For the Assay wells 10 uL of rat plasma was added. Then, the microplate was mixed vigorously and let stand for 5 min at 37 °C. After this time, the absorbance (A1) was read against the Blank (well with R1) at 600 nm using a Spark spectrophotometry-fluorescence device (TECAN, Männedorf, Switzerland). Then, 60 µL of R2 was added into all wells. The microplate with samples, after mixing, was incubated for 5 min at 37 °C. Finally, the absorbance (A2) was read against the Blank (well with R1+R2) at 600 nm using the same spectrophotometry-fluorescence device (see Appendix A12.d). The reaction was linear from 2.5 to 200 mg/dL (0.065 to 5.17 mmol/L). The content of HDL was determined according to Equations 7-9 and the results were expressed as mg/dL.

For Calibrator and Assay samples:

$$\Delta A_{Calibrator} = (A2_{Calibrator} - A2_{Blank}) - (0.75 \times (A1_{Calibrator} - A1_{Blank})) \qquad [7]$$

$$\Delta A_{Assay} = \left(A2_{Assay} - A2_{Blank}\right) - \left(0.75 \times (A1_{Assay} - A1_{Blank})\right) \qquad [8]$$

And then:

$$HDL = \frac{\Delta A_{Assay}}{\Delta A_{Calibrator}} \times Cn_{Calibrator} \qquad [9]$$

4.5. Free fatty acids in plasma

Free fatty acid (FFA) quantification with Abcam enzyme kit (Allscience, Miami, USA) provides a sensitive method for detecting the long-chain free fatty acid in various biological samples, such as serum, plasma and other body fluids, food, or growth media. In this assay, fatty acids are converted to their CoA derivatives, which are subsequently oxidized with the concomitant generation of color.

4.5.1. Reagent preparation

For this determination different reagents were used: fatty acid assay buffer (FAAB), fatty acid probe (FAP), acyl-CoA synthetase reagent (ACS), enzyme mix (EM), Enhancer (E), and palmitic acid standard (PAS). The reagents were prepared as is indicated in Table 2. 9.

Table 2.9. Reagents utilized in enzymatic method to determine concentration of free fatty acids.

Reagent	Reagent preparation
FAAB	1. This reagent is ready to use as supplied. 2. Equilibrate to room temperature before use. 3. Store at -20 ºC.
FAP	1. This reagent is ready to use as supplied. 2. Warm by placing in a 37 ºC bath for 1 -5 min to thaw the DMSO solution before use. 3. Keep on ice during the assay. 4. Store aliquots at -20 ºC protected from light and moisture.
ACS	1. Reconstitute the ACS with 220 µL FAAB. 2. Keep on ice during the assay. 3. Store aliquots at -20 ºC protected from light and moisture.
EM	1. Reconstitute the EM with 220 µL FAAB. 2. Keep on ice during the assay. 3. Store aliquots at -20 ºC protected from light and moisture.
E	1. This reagent is ready to use as supplied. 2. Equilibrate to room temperature before use. 3. Store at -20 ºC.
PAS	1. Place PAS in a hot water bath (80-100 ºC) for 1 min or until PAS looks cloudy. 2. Vortex for 30 s until PAS becomes clear. 3. Repeat the heat and vortex one more time. 4. The PAS is in solution and ready to use.

4.5.2. Protocol

- *Standard preparation*

The palmitic acid standard (PAS) with a concentration of 1 mmol/µL, was prepared fresh for every use. From PAS was prepared a standard curve dilution as described in Table 2.10 using a 96-well microplate. The standard curve was made for duplicate.

Table 2.10. Standard preparation utilized in enzymatic method to determine concentration of free fatty acids.

No. Standard	Volume PAS (µL)	Volume FAAB (µL)	Final volume of standard in well (µL)	Palmitic acid Cn in well (nmol/well)
1	0	150	50	0
2	3	147	50	1
3	6	144	50	2
4	12	138	50	4
5	18	132	50	6

PAS, palmitic acid standard; FAAB, fatty acid assay buffer; Cn, concentration.

- *Reaction mix preparation*

The reaction mix (colorimetric assay) was prepared fresh for every use. The mix was prepared with reagents described in Table 2.11. It was prepared with a master mix according to the number of assays (samples and standards).

Table 2.11. Calculation to prepare the reaction mix utilized in enzymatic method to determine concentration of free fatty acids.

Reagent	Volume (µL)*
Fatty acid assay buffer (FAAB)	44
Fatty acid probe (FAP)	2
Enzyme mix (EM)	2
Enhancer (E)	2

*For the master mix prepare: X µL of reagent x (number of assays + 1).

- *Determination of free fatty acids*

The samples were thawed at room temperature during 10-20 min. The protocol was realized using a 96-well microplate. The different wells (maximum volume of 400 µL) were identified according to the type of sample: Standard dilutions (Table 2.9) and Sample (rat plasma) (see Appendix A11.e). Aliquots of 50 µL of samples and standards dilutions were placed in the different wells of microplate (for duplicate). Then, 2 µL of ACS reagent was added into all standard and sample wells. Then, the microplate was mixed vigorously and incubated for 30 min at 37 °C. After this time, 50 µL of reaction mix (Table 2.10) was added to each well. Then, the microplate with samples was incubated for 30 min at 37 °C protected from light. Finally, the absorbance was read against the Blank (Standard No.1) at 570 nm using a Spark spectrophotometry-fluorescence device (TECAN, Männedorf, Switzerland). The content of free fatty acids (FFA) was calculated according to Equation 10.

$$FFA = \left(\frac{F_a}{S_v}\right) \times D \qquad [10]$$

where F_a represents the amount of fatty acid in the sample well calculated from standard curve (nmol), S_v the amount of sample volume added in sample wells (mL), and D the sample dilution factor. The results were expressed as nmol/mL.

5. Expressions and kinetic analysis

5.1. Carotenoid and sugar contents expressed as non-fat dry weight

The concentration of carotenoids and sugars presented in papaya chips, obtained by vacuum frying at the different conditions (oil temperatures and frying times), were expressed as non-fat dry weight (NF-DW) to maintain the same basis. This was necessary for evaluating the real effect of vacuum frying on the carotenoid and sugar contents of papaya chips. During frying, a counterflow of water vapor and oil occurs between the food matrix and the frying medium. Thus, papaya chips obtained at different frying times had varied contents of moisture and lipids. Data expressed as NF-DW basis were used to realize the kinetic study and generate the respective models (Chapter IV). Also, this allowed us to compare carotenoid content between fresh papaya (before vacuum frying) and papaya chips (after vacuum frying) (Chapters III and V). First, for all papaya chip samples, it was determined the moisture content using AOAC standard method 920.151 (AOAC, 2015) and the lipid content, following the method described by Carpenter *et al.* (1993). Then, after determinations of carotenoid and sugar contents (in fresh weight), it was performed the following calculations:

$$C_t^{NF-DW} = \frac{C_t^{FW}}{1-M_t-O_t} \qquad [11]$$

$$S_t^{NF-DW} = \frac{S_t^{FW}}{1-M_t-O_t} \times 100 \qquad [12]$$

where $C_t^{NF\text{-}DW}$ represents the carotenoid content (β-carotene, β-cryptoxanthin, and lycopene) at time t (expressed as mg·kg^{-1} NF-DW) and $S_t^{NF\text{-}DW}$ the sugar content (glucose, fructose, and sucrose) at time t (expressed as g·100 g^{-1} NF-DW); C_t^{FW} represents the carotenoid content at time t (expressed as mg·kg^{-1} fresh weight, FW) and S_t^{FW} the sugar content at time t (expressed as g·g^{-1} FW); M_t is the

moisture content at time t (kg·kg^{-1} FW for carotenoids or g·g^{-1} FW for sugars); O_t is the lipid content at time t (kg·kg^{-1} FW for carotenoids or g·g^{-1} FW for sugars); t is the frying time (min).

5.2. Kinetics analysis

Different kinetic models were considered for describing the behavior of the variables evaluated (contents of carotenoids, sucrose content, moisture content, lipid uptake, a_w, and browning index) during vacuum frying as a function of frying time at different oil temperatures. Also, the modeling of carotenoid degradation during the storage of papaya chips packaged at two different atmospheres (air and nitrogen) at four store temperatures was assessed.

Kinetic parameters of physicochemical properties were identified from experimental data measured in papaya chips by triplicate. The uncertainty of the parameters was calculated by non-linear error propagation using SigmaPlot software (Systat Software Inc., CA, USA). Equations and kinetic parameters from models are described in Table 2.12.

In the case of carotenoids, the rate constants were assumed to vary with the temperature according to the Arrhenius equation described by Equation 13.

$$k = k_{ref} exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \quad [13]$$

where k_{ref} is the rate constant (in kg·mg^{-1}·min^{-1} for Obj.2; in kg·mg^{-1}·day^{-1} for Obj.3) at the reference temperature T_{ref} chosen in the middle of the studied temperature range (393 K for Obj.2; 303 K for Obj.3), with E_a, T, and R respectively denoting the activation energy (J·mol^{-1}), medium temperature (K), and gas constant (8.314 J·mol^{-1}·K^{-1}). Kinetic constants and activation energies were identified by nonlinear least-squares regression with SigmaPlot software (Systat Software Inc., CA, USA). The model's parameters were identified from experimental data measured in triplicate. The uncertainty of the parameters was calculated by non-linear error propagation using SigmaPlot software (Systat Software Inc., CA, USA).

Table 2.12. Equations and kinetic parameters from models generated to describe the behavior of different variables during vacuum frying and storage of papaya chips.

Objective (Chapter)	Experiment	Model	Independent variables	Dependet variables	Equation	Kinetic parameters
Obj. 2 (Chapter IV)	Effect of vacuum frying on physico-chemical properties and content of carotenoids of papaya chips	Exponential	• T= 100, 120, 140 °C • t= 0, 2, 4, 6, 8, 10, 14 min	Moisture content, *M* ($g \cdot g^{-1}$ DW)	$M = M_0 * exp^{(-k_M * t)}$ [14]	• M_o: initial moisture content (t=o) • k_M: specific rate of water loss
		First-order		Oil content, *O* ($g \cdot g^{-1}$ DW)	$O = O_e * (1 - exp^{(-k_O * t)})$ [15]	• O_e: oil content at infinite time (t=∞) • k_O: specific rate of oil uptake
		Second-order		β-cryptoxanthin, *BCX* ($mg \cdot kg^{-1}$ NF-DW)	$BCX = \left(\frac{1}{1/BCX_0 + k_{BCX} * t}\right)$ [16]	• BCX_o: initial concentration (t=o) • k_{BCX}: reaction rate constant
		3-parameter logistic function		• Water activity, a_w (dimensionless) • Sucrose content, *Suc* ($g \cdot 100\ g^{-1}$ NF-DW) • Browning index, BI/BI_o (dimensionless)	$X = \frac{L_X}{1 + e^{-k_X * (t - b_X)}}$ [17] *X is the variable (a_w, *Suc* or BI/BI_o)	• L_X: curve's maximum of the variable • b_X: time to reach the sigmoid's midpoint • k_X: constant rate
Obj. 3 (Chapter V)	Optimal storage conditions concerning carotenoid degradation in vacuum-fried papaya chips	Second-order	• T= 15, 25, 35, 45 °C • At 15 and 25 °C, t= 0, 15, 25, 45, 60,75, 94 days • At 35 and 45 °C, t= 0, 3, 8, 15, 25, 35, 52 days	• β-carotene, *BC* ($mg \cdot kg^{-1}$ NF-DW) • β-cryptoxanthin, *BCX* ($mg \cdot kg^{-1}$ NF-DW) • Lycopene, *LYC* ($mg \cdot kg^{-1}$ NF-DW)	$CT = \left(\frac{1}{1/CT_0 + k_{CT} * t}\right)$ [18] *CT is the variable (*BC*, *BCX* or *LYC*)	• CT_o: initial concentration (t=o) • k_{CT}: reaction rate constant

DW, dry weight; NF-DW, non-fat dry weight.

Chapter III

Selection of optimal ripening stage of papaya fruit and vacuum frying conditions to obtain papaya chips

Chapter III. Selection of optimal ripening stage of papaya fruit and vacuum frying conditions to obtain papaya chips

Publication #1

Selection of optimal ripening stage of papaya fruit (*Carica papaya* L.) and vacuum frying conditions for chips making

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Abstract

The aim of this study was to compare the physicochemical characteristics of papaya fruits from Costa Rica (*Carica papaya* L. cv Pococí) and vacuum-fried chips obtained from these fruits at three different postharvest ripening stages (RS3, RS4, and RS5) and select the optimal stage for vacuum frying. In addition, response surface methodology was used to optimize vacuum frying conditions (temperature and time) to obtain papaya chips with desirable characteristics. RS4 was found the most adequate ripening stage for processing papaya fruits to obtain vacuum-fried chips with suitable physicochemical characteristics and good sensory acceptance. The optimal vacuum frying conditions at 25 kPa were: 120 °C and 13.1 min. Vacuum frying caused 40 and 60 % degradation of β-carotene and β-cryptoxanthin, respectively, and a 1.5-fold increase in the extractability of lycopene. The total carotenoid content of the vacuum-fried papaya chips was 381 mg/kg chips fresh weight.

Keywords

Optimization; Physicochemical properties; β-carotene; β-cryptoxanthin; Lycopene; Response surface methodology.

1. Introduction

Papaya (*Carica papaya* L.) is a fruit native to America (possibly southern Mexico and neighboring Central America) belonging to the family *Caricaceae* (Saran *et al.,* 2016). Papaya fruits are widely produced and consumed in almost all tropical and subtropical regions, the main producers being India, Brazil and Mexico (FAO, 2020). According to FAO statistics, about 13.02 million tons of papaya were produced in 2017 on 440,629 ha in different countries (FAO, 2020). In addition to its economic value, papaya is a valuable source of bioactive compounds, in particular provitamin A carotenoids, such as β-carotene (BC) and

β-cryptoxanthin (BCX), and lycopene (LYC) (in red-fleshed papaya fruits) (USDA, 2020). Several epidemiological studies have concluded that diets rich in LYC foods are associated with a reduced risk of diseases including cancer (especially prostate cancer) and cardiovascular disease (Willcox *et al.*, 2003; Rao & Agarwal, 2000; Müller *et al.*, 2016). Papaya cultivars from Costa Rica and specially Pococí cultivar have been reported to contain high amounts of BC, BCX and LYC (Schweiggert *et al.*, 2012).

Fruit quality is influenced greatly by fruit maturity at harvest. Changes in fruit skin color has been used as criterion during harvest to judge ripening. For instance, papaya fruit should be harvested when skin color changes from dark green to light green or when some skin yellowing develops (Saran *et al.*, 2016). In many fruits, the physicochemical composition and micronutrient content vary with ripening. In papaya, total soluble solids, pulp firmness, color values, pigment contents, and carotenoid profiles change significantly during ripening (Schweiggert *et al.*, 2011b).

Papaya is sold primarily as a fresh product; however, it is a highly perishable fruit and about 30 % of the production is compromised due to postharvest losses (Albertini *et al.*, 2016). Commercially ripe fruits find possible applications in the preparation of syrups, dried products, yogurt, jam, jellies, nectars and sweets, among other foods (Annegowda & Bhat, 2016). Vacuum frying is an alternative technology for production of fruit snacks with higher sensory and nutritional quality than traditional fried snacks. This process is performed at pressures below atmospheric levels, which decreases the boiling points of the frying oil and the water in the product. The absence of air during the process inhibits lipid and pigment oxidation and preserves the natural color and nutrients of fried fruits (Andrés-Bello *et al.*, 2011; Da Silva & Moreira, 2008; Dueik & Bouchon, 2011).

The application of vacuum frying for the development of papaya chips constitutes a promising option for processing this fruit, thus generating a novel product (that meets consumer expectations) and creating added value to this crop. There are a few studies about vacuum frying of papaya fruit. For instance, Wexler *et al.* (2016) optimized the vacuum frying conditions for unripe papaya (green skin), a food matrix that does not present carotenoids and has lower moisture and sugar

content than ripe papaya. Likewise, other authors have studied the effect of pretreatments of ripe papaya such as partial drying and freezing (Pandey *et al.*, 2020) and the effect of frying temperature, time, and thickness of papaya slices (Pandey & Chauhan, 2019) on physicochemical and sensory properties of vacuum-fried papaya chips. However, in these last studies only one ripening stage of papaya was evaluated.

Under this context, the present study compared the physicochemical characteristics and carotenoid profile of fresh Pococí papaya and vacuum-fried papaya chips obtained from these fruits at three different postharvest ripening stages (based on their skin yellowing). In addition, the sensory acceptance of papaya chips, and the process yield were considered. Looking into above mentioned gaps this study was conducted to find out optimum ripening stage and frying conditions for making papaya chips.

2. Materials and methods

2.1. Materials

Red-fleshed papaya fruits (*Carica papaya* L.) from hermaphrodite plants of the commercial Costa Rican Pococí hybrid were acquired from OroFruits (Orotina, Alajuela, Costa Rica: 9°54'45.8"N 84°30'21.7"W) in August 2017 at three different postharvest ripening stages. Commercial palm olein frying oil D'orofrit (Grupo NUMAR, Costa Rica) was used.

2.2. Chemicals

The following chemicals were used: SigmaUltra standards for glucose, fructose, and sucrose from Sigma-Aldrich (St. Louis, MO, USA); β-carotene, β-cryptoxanthin and lycopene standards from Sigma-Aldrich (St. Louis, MO, USA). Other analytical grade chemicals and HPLC grade solvents were purchased from JT Baker Inc. (Phillipsburg, NJ, USA).

2.3. Characterization of papaya fruits at three different postharvest ripening stages

Papaya fruits at three postharvest ripening stages were evaluated for physicochemical characteristics and suitability for use in a vacuum frying process to obtain papaya chips. Fruit skin color was used as criterion to determine different ripening stages. This was assessed as a percentage of yellowing on the whole fruit (0–100 %), using a ripening scale with seven categories or stages developed at *Laboratorio de Tecnología Poscosecha* (CIA, Universidad de Costa Rica): RS1 (0-15 % yellowing), RS2 (16-25 % yellowing), RS3 (26-40 % yellowing), RS4 (41-55 % yellowing), RS5 (56-70 % yellowing), RS6 (71-80 % yellowing), and RS7 (81-100 % yellowing). The ripening stages used in the study, RS3, RS4 and RS5, are shown in Figure 3.1a and Figure 3.1b. Preharvest RS2 papaya fruits (n=75) were harvested manually and selected for color, elongated shape, and absence of physical or microbiological damage. Then, the fruits were stored at 25 °C for ripening until obtaining the skin color corresponding to the three ripening stages (RS3, RS4 and RS5), 25 fruits for each stage.

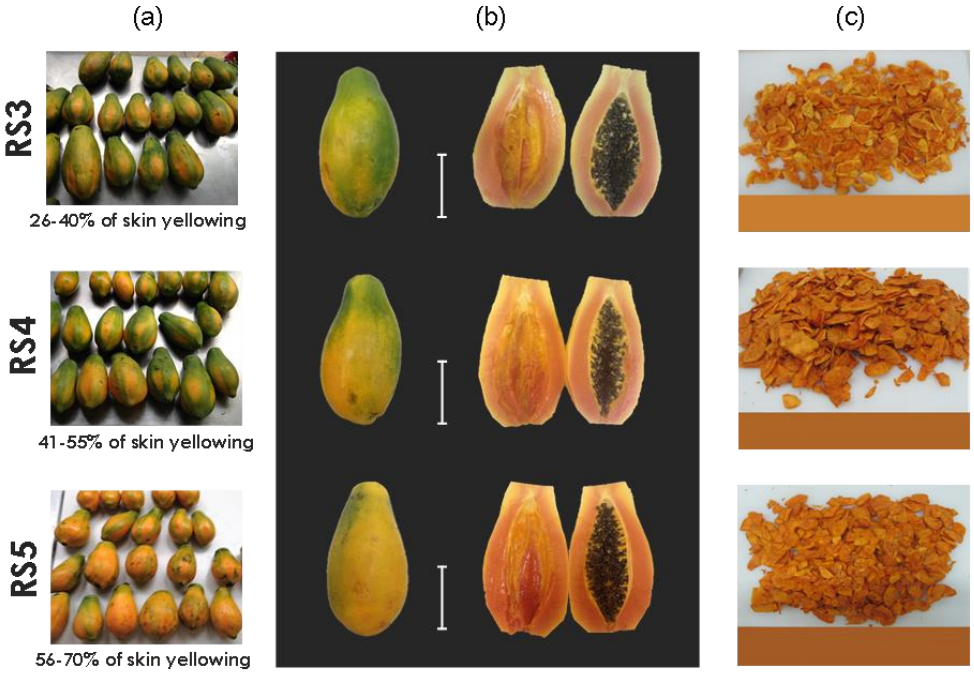

Figure 3.1. Papaya fruits (*Carica papaya* L., cv Pococí) at three ripening stages based on the percentage of skin yellowing: a) batches of papaya fruits, b) entire and halved fruits, c) papaya chips obtained by vacuum frying. White bars mark 10 cm.

In order to characterize and standardize the three ripening stages, the following physicochemical properties were evaluated: skin and pulp firmness, pulp color (parameters L*, C* and h°), total soluble solids, proximal composition (moisture, protein, lipids, ash, total fiber), sugars, organic acids, and carotenoid content.

2.4. Effect of ripening stage on properties of vacuum-fried papaya chips

2.4.1. Sample preparation

Papaya fruits at the three postharvest ripening stages were washed and peeled manually. Each fruit was cut vertically into 4 pieces and the seeds were removed. The pre-cut pieces were then cut into 4-mm thick slices (dimensions of approximately 3 cm x 3 cm x 6 cm) using a slicing machine (FP-100 Hobart, CA, USA) prior to vacuum frying.

2.4.2. Vacuum frying

The papaya chips were obtained using a vacuum frying system (Auriol, Marmande, France) as shown in Figure 3.2. The system consisted of a stainless-steel vessel (capacity of 80 L), electric heat resistors for heating the oil, a lid with a rotary axis coupled to a piston where a stainless-steel basket was placed, a temperature transducer, a filter, a heat exchanger to condense the water vapor generated during the process, a condensate vessel and a liquid ring vacuum pump. The frying process consisted of heating the oil (55 L) to the target temperature, loading the papaya slices (about 1 kg) into the basket, closing the lid, and depressurizing the vessel. Once the pressure reached 25 kPa, the basket was immersed into the oil for the predefined time to obtain the chips. When the frying step was completed, the basket was raised, and the centrifuging system was applied at 300 rpm (16.6 x g) for 3 min to control the oil absorption by the samples. Finally, the vessel was pressurized, and the papaya chips were removed from the fryer and cooled to ambient temperature prior to packaging (Figure 3.1c).

2.4.3. Study design

In order to select the most promising ripening stage in papaya fruits for vacuum-fried chips, a random unrestricted design with a single factor (postharvest ripening stage: RS3, RS4 and RS5) was applied. Chips were obtained by vacuum frying under fixed conditions (25 kPa, 120 °C, 12 min) using the central point of central composite design described below. The physicochemical properties measured in the papaya chips were moisture, a_w, lipid content, color variation and browning index. Sensory acceptance of the chips was also evaluated. Yields for each step in the process to obtain the papaya chips were determined for each ripening stage. Data for physicochemical properties, sensory panel acceptance, and yields were statistically analyzed with an analysis of variance (ANOVA) (p<0.05). Significantly different means were further compared using Tukey's test. The final goal was to select the ripening stage in papaya fruits that resulted in chips with the lowest a_w, moisture and lipid content, color variation and browning index, and the highest sensory acceptance and yield.

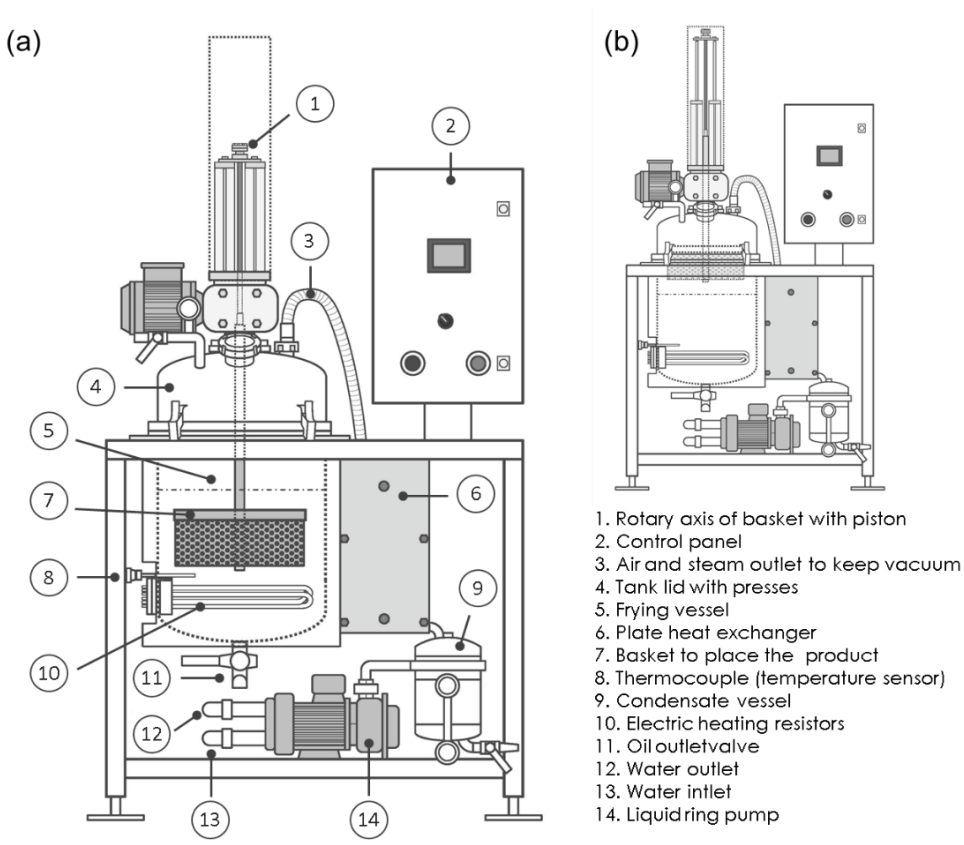

Figure 3.2. Sketch of the vacuum frying system used for obtaining papaya chips: a) basket in "bottom" position, b) basket in "top" position.

2.5. Optimization of vacuum frying conditions

Response surface methodology (RSM) was used to optimize the vacuum frying process for papaya at the most promising ripening stage. A central composite design (CCD) with two independent variables was used: temperature / X_1 (100-140 °C) and time / X_2 (9-15 min). The range of values for the selected variables was determined in preliminary RSM experiments. The two independent variables were coded at five levels (-1.414, -1.0, 0, +1, +1.414), which resulted in an experimental design of 11 experimental points including three replicates of the central point. Regression analysis was performed on data for dependent variables affected by the vacuum frying conditions (moisture content, a_w, color variation, browning index, and lipid content) using the second order polynomial model shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} {X_i}^2 + \sum \sum_{j<i} \beta_{ij} X_i X_j \qquad [1]$$

where Y represents the response variable; β_o, β_i, β_{ii} and β_{ij} are regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively; and X_i and X_j are independent variables. ANOVA, regression analysis, and plotting of response surfaces were performed using Statistica 7.0 (Statsoft Inc., USA). Models and regression coefficients with p-values below 0.05 were considered significant. The adequacy of the models was verified in extra experimental runs (three replicates) at optimal conditions (120 °C, 13.1 min) selected from contour surfaces for the different response variables. The model was considered adequate if confidence intervals for experimental data and predicted values overlapped.

2.6. Characterization of vacuum-fried papaya chips

The impact of the vacuum frying process was evaluated using papaya chips obtained from papaya fruits at the most promising ripening stage and processed at optimized vacuum frying conditions. The following physicochemical properties of the chips were evaluated and compared to raw material (fresh papaya): color

(parameters L*, C*, h°, color difference (ΔE) and browning index (BI)), proximal composition (moisture, protein, lipids, ash, dietary fiber), sugars, a_w, and carotenoid content. The sorption isotherm of papaya chips at 25 °C was also determined.

2.7. Physicochemical analyses

Moisture, ash, protein, and total dietary fiber contents were determined by standard AOAC methods 920.151, 940.26, 920.152, and 985.29, respectively (AOAC, 2015). A nitrogen conversion factor of 6.25 was used to calculate protein content. Lipid content was determined by the method described by Carpenter *et al.* (1993). Sugars (sucrose, glucose, and fructose) were determined by HPLC using a Shimadzu LC-6A (Kyoto, Japan) equipped with an EconosphereTM NH2 column (Alltech Associates, Deerfield, IL, USA) and a Shimadzu RID-6A refractive index detector (Kyoto, Japan), using conditions described previously (Gancel *et al.*, 2011).

Total soluble solids (TSS, expressed as °Brix) were measured on a refractometer with a digital thermometer (model NAT-1T, Atago Co., Ltd., Tokyo, Japan) and automatic temperature compensation. Water activities of the samples at 25 °C were determined in triplicate using a water activity meter (Aqualab, model CX-2, Decagon Devices, Inc., Pullman, WA).

Color was measured in fresh papaya pulp on the whole fruit with a portable digital colorimeter WR-10 (Iwave, China), and in papaya chips with a color measurement spectrophotometer (model ColorFlex, HunterLab, Virginia, USA). For color determinations in both pulp fruit and papaya chips, color parameters were expressed in CIELab units L*, a* and b* using illuminant D65 and a 10◦ observer angle. Chroma (C*), and hue angle (h°) were calculated from a* and b* values as follows:

$$C^* = \sqrt{(a^{*2} + b^{*2})} \qquad [2]$$

$$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right) \qquad [3]$$

The color difference (ΔE) was calculated using positions in the three-dimensional (3-D) L*a*b* color space for papaya before (fresh papaya) and after (papaya chips) vacuum frying as shown in Equation 4.

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \qquad [4]$$

where L_o^*, $a_o^*$and b_o^* represented the values from fresh papaya, and L^*, a^* and b^* represented the values from papaya chips, after vacuum frying. The browning index (BI) was calculated as follows (Pathare *et al.*, 2013):

$$BI = 100 \times \left[\frac{\left(\frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}\right) - 0.31}{0.17}\right] \qquad [5]$$

where L^*, $a^*$and b^* represented the values from papaya chips, after vacuum frying. Skin and pulp firmness, expressed in Newtons (N), were determined in papaya fruits (n=25) with a penetrometer (Chatillon, NY, USA) using a tooth-shaped tip. All measurements were performed at three points in the equatorial zone of each papaya. All physicochemical analyses were done in triplicate except for determination of pulp color parameters, TSS, and skin and pulp firmness in fresh papaya (n=25).

2.8. Carotenoid analyses

Extraction procedures and conditions for analysis were described previously by Schweiggert *et al.* (2012). Sample preparation was carried out under dim light. Samples were ground and weighed (3 g for papaya fruit and 2 g for papaya chips) in a 50 mL Pyrex flask and 0.1 g of $NaHCO_3$ was added. Carotenoids were extracted in 15 mL of a mixture (1:1:1, v/v/v) of methanol, ethyl acetate and light petroleum containing 0.1 g/L of BHT and 0.1 g/L of BHA (LP/BHT/BHA) using an IKA® Ultra-Turrax® (Merck KGaA, Darmstadt, Germany). The suspension was filtered through a Buchner funnel with Whatman qualitative filter paper No.1, and the recovered solids were re-extracted 2–3 times until a colorless filtration residue was obtained. Filtrates were transferred to a separatory funnel with 15 mL H_2O and 15 mL LP/BHT/BHA, and the upper fraction was washed

with 15 mL H_2O and recovered. For quantitative carotenoid extraction, the lower aqueous fraction was re-extracted 2–3 times with 10 mL LP/BHT/BHA. The combined upper fractions were dried with 5 g Na_2SO_4 and evaporated to complete dryness under reduced pressure at ≤25 °C using a parallel evaporation system (Büchi Multivapor™ P-6, Flawil, Switzerland). The remaining residue was mixed with 5 mL of MTBE/methanol (9:1 v/v), membrane filtered (0.45 μm) and stored at -80 °C in amber glass vials until HPLC analysis.

Carotenoid were identified using a reverse-phase HPLC-DAD Shimadzu system (Shimadzu Manufacturing, Inc., Canby, Oregon, USA) equipped with a SPD-M20A diode array detector, a DGU-20A5 degasser, a SIL-20AHT autosampler, a CTO-20A column oven and a LC-20AT binary gradient pump. Carotenoids were separated using a C30 column (150 x 4.6 mm i.d., 3 μm) (YMC America Inc., PA, USA) with a guard column. The mobile phase was methanol as eluent A and MTBE as eluent B. Operation temperature was set at 30 °C. The flow rate was set at 0.6 mL/min and the injection volume was 10 μL. A solvent gradient was programmed as follows: 0-5 min, isocratic 80 % A (initial conditions); 5–7 min, 73 % A; 7-15 min, 62.5 % A; 15-20 min, isocratic 62.5 % A; 20-30 min, 45 % A; 30-35 min, 10 % A; 35-40 min, isocratic 10 % A; 40-45 min, 80 % A with a return to the initial conditions for rebalancing. All-E-β-cryptoxanthin (free form and ester forms), all-E-β-carotene and its isomers were detected at 450 nm, and all-E-lycopene and their isomers were detected at 470 nm (Dhuique-Mayer *et al.*, 2007). Z-β-carotene and Z-lycopene contents were expressed as the sum of all Z-β-carotene and all Z-lycopene isomers, respectively. Isomers were identified by relative retention times, i.e. elution order and combined spectral data, based on previously published data obtained with the same mobile phase (MTBE/methanol) and detection wavelength range (Achir *et al.*, 2010; Achir *et al.*, 2015; Chanforan *et al.*, 2012; Martins *et al.*, 2016; Schweiggert *et al.*, 2012; Schweiggert *et al.*, 2011b). Analyses were done in triplicate and results were expressed in mg/kg.

2.9. Sensory acceptance

In order to evaluate the sensory acceptance of papaya chips prepared from fruits at different ripening stages, a sensory test was applied to regular consumers of

snacks and fruits. The participants (n=100) were between 18 and 60 years of age (40 men and 60 women). A hybrid hedonic scale was used with verbal affective anchors in the middle and extreme regions of the scale (0=disliked extremely, 5= neither liked nor disliked; 10= liked extremely). This scale was used because it is more consumer-friendly than other unstructured scales (Villanueva *et al.*, 2005). Papaya chips were prepared and packaged in PET-m/PE bags one week before the sensory test. Samples were removed from the bags 15 min prior to evaluation and 3-4 chips were placed on white plates coded with random 3-digit numbers and maintained at room temperature (25 °C). Samples were randomly presented, and the participants were asked to drink water between samples.

2.10. Process yield

The yield for the process to obtain papaya slices prior to vacuum frying was determined by measuring the mass of the whole fresh papayas (m_{papaya}) and the mass of the papaya slices (m_{slices}) after the peeling, deseeding and slicing (Equation 6). The frying yield was calculated using the mass of papaya slices prior to frying and the mass of fried chips (m_{chips}) (Equation 7). Finally, the overall process yield was determined as shown in Equation 8.

$$\text{Yield}_{\text{peeling+deseeding+slicing}}(\%) = \frac{\text{m}_{slices}}{m_{papaya}} \times 100 \qquad [6]$$

$$\text{Yield}_{\text{frying}}(\%) = \frac{\text{m}_{chips}}{m_{slices}} \times 100 \qquad [7]$$

$$\text{Yield}_{\text{overall}}(\%) = \frac{\text{m}_{chips}}{m_{papaya}} \times 100 \qquad [8]$$

2.11. Water sorption isotherm

Papaya chips were blended and approximately 5 g of sample were placed in weighing capsules. Samples were transferred to vacuum desiccators containing saturated solutions of different salts to generate the desired water activity in the papaya chips at equilibrium. The desiccators were kept in ovens at 25 °C. The

weighing capsules containing the samples were hermetically sealed before transfer to the desiccators containing the saturated solutions. The saturated salt solutions and the a_w at equilibrium were: LiCl (0.113), $CaCl_2$ (0.280), K_2CO_3 (0.436), $NaNO_2$ (0.640), NaCl (0.756), MgCl, (0.326), KNO_3 (0.946). Samples (5 replicates for each a_w at equilibrium) were considered to have reached the equilibrium point when the weight remained constant for three consecutive determinations. The equilibrium moisture content of the sample was determined using the AOAC method 920.151 (AOAC, 2015). A test tube containing thymol was placed inside the jars of higher water activity to prevent mold growth during storage. The Guggenheim-Anderson-de Boer (GAB) model shown in Equation 9 was applied to predict the sorption isotherm of papaya chips.

$$\mathrm{M_w} = \frac{\mathrm{M_g C K a_w}}{[(1 - \mathrm{K a_w})(1 - \mathrm{K a_w} + \mathrm{C K a_w})]} \qquad [9]$$

where M_w is the moisture content (g water/g dry solids); M_g is the GAB monolayer moisture content (g water/g dry solids); C is a constant related to the monolayer heat of sorption and K is a factor related to the heat of sorption of the multilayer.

2.12. Statistical analyses

The physicochemical characteristics of papaya fruits at three ripening stages were analyzed by one-way ANOVA and the *post hoc* Tukey-HSD test to detect significant differences (p<0.05). Data obtained from the sensory panel, color evaluations and yield data were also analyzed by one-way ANOVA and *post hoc* Tukey-HSD (p<0.05). The influence of vacuum frying on carotenoid content was evaluated using the Student's t-test to detect significant differences (p<0.05) between fresh papaya and papaya chips. Data were presented as mean ± standard deviation of replicates. Statistical analyses were performed using JMP 8.0 (SAS Institute, Inc., NC, USA).

3. Results and discussion

3.1. Characterization of papaya fruits at three different postharvest ripening stages

Papaya fruits at ripening stages RS3, RS4 and RS5 are shown in Figures 3.1a and 3.1b. These ripening stages were selected after preliminary analyses (data not shown), because they presented the most promising sensory and physicochemical features for production of papaya chips. The proximal composition (moisture, lipids, protein, ash, dietary fiber) of fresh papaya fruits at the three ripening stages is shown in Table 3.1. The results from proximal composition were similar to those reported for red-fleshed papaya fruit (USDA, 2020). Lipid content in Pococí papaya at the three ripening stages was below the detection limit of 0.10 g/100 g fresh weight (FW).

Table 3.1 also shows organic acid content, total soluble solids (TSS), and sugar contents of papaya fruits. Glucose and fructose were the sugars present in fresh Pococí papaya, whereas sucrose was not detected (limit detection of 0.25 g/100 g FW). TTS content (expressed as °Brix), and organic acids presented in Pococí papaya, citric and succinic acids, were consistent with values reported by USDA (2020) but were slightly higher than those reported for other papaya cultivars from Costa Rica and Mexico (Sancho *et al.* 2011; Schweiggert *et al.*, 2011b).

Color parameters in papaya pulp were similar to those reported previously for Pococí papaya (Schweiggert *et al.*, 2011b; Schweiggert *et al.*, 2012). Skin firmness values were higher than those reported by Schweiggert *et al.* (2011b) for Pococí papaya (14-16 N for fruits at ripening stages from RS3 to RS5). Pulp firmness in Pococí papaya was comparable to that reported by Barragán-Iglesias *et al.* (2018) for Maradol papayas at ripening stages similar to those in our study.

Table 3.1 also shows the carotenoid content of papaya fruit pulp. Ten carotenoids were identified in fresh papaya (Figure 3.3a and Table 3.2) based on standard retention times and combined spectral data (Chanforan *et al.*, 2012; Martins *et al.*, 2016; Schweiggert *et al.*, 2012; Soto *et al.,* 2020). β-carotene was presented as all-E-form (BC) and as a Z-isomer tentatively identified as 13Z-β-carotene

(peak 2, Figure 3.2 and Table 3.2). All-E-β-cryptoxanthin (BCX) was detected as free form and the corresponding caprate, laurate and myristate esters, peaks 5, 6 and 7, respectively (Figure 3.3 and Table 3.2).

Table 3.1. Physicochemical properties and carotenoid contents of papaya Pococí (*Carica papaya* L.) at three ripening stages.

Parameter	Fresh papaya - Ripening stage		
	RS3	**RS4**	**RS5**
Moisture (g/100 g FW)†	88.52 ± 0.82[a]	88.04 ± 0.68[a]	87.55 ± 0.91[a]
Lipids (g/100 g FW)†	ND	ND	ND
Protein (g/100 g FW)†	0.57 ± 0.02[a]	0.58 ± 0.03[a]	0.50 ± 0.06[a]
Ash (g/100 g FW)†	0.47 ± 0.01[a]	0.46 ± 0.03[a]	0.47 ± 0.04[a]
Dietary fiber (g/100 g FW)†	1.79 ± 0.18[a]	1.75 ± 0.09[a]	1.66 ± 0.12[a]
Glucose (g/100 g FW)†	3.60 ± 0.01[a]	4.07 ± 0.38[a]	4.13 ± 0.25[a]
Fructose (g/100 g FW)†	3.52 ± 0.08[a]	3.99 ± 0.33[a]	4.06 ± 0.25[a]
Sucrose (g/100 g FW)†	ND	ND	ND
Citric acid (g/100 g FW)†	0.095 ± 0.007[b]	0.101 ± 0.002[ab]	0.110 ± 0.001[a]
Succinic acid (g/100 g FW)†	0.045 ± 0.007[a]	0.039 ± 0.002[a]	0.035 ± 0.007[a]
TSS (°Brix)‡	12.17 ± 1.30[a]	12.07 ± 0.65[a]	11.75 ± 1.21[a]
Skin firmness (N)‡	33.07 ± 7.76[a]	32.71 ± 6.81[a]	24.45 ± 8.07[b]
Pulp firmness (N)‡	16.98 ± 6.18[a]	11.89 ± 5.41[b]	7.79 ± 2.92[c]
Pulp color‡:			
L*	51.16 ± 2.39[a]	47.80 ± 3.21[b]	46.23 ± 3.02[b]
C*	36.71 ± 2.22[b]	37.5 ± 2.25[ab]	38.78 ± 2.07[a]
h°	55.82 ± 1.76[a]	54.90 ± 2.24[a]	55.19 ± 1.26[a]
Carotenoids (mg/kg FW)†:			
all-E-β-cryptoxanthin	1.19 ± 0.31[a]	1.86 ± 0.24[a]	2.93 ± 0.81[a]
all-E-β-cryptoxanthin-caprate	1.37 ± 2.22[b]	2.17 ± 0.39[ab]	3.03 ± 0.08[a]
all-E-β-cryptoxanthin-laurate	3.92 ± 0.59[b]	6.03 ± 1.13[ab]	7.98 ± 0.17[a]
all-E-β-cryptoxanthin-myristate	1.35 ± 0.25[b]	1.84 ± 0.10[ab]	2.29 ± 0.08[a]
all-E-β-carotene	0.95 ± 0.01[c]	1.94 ± 0.17[b]	3.38 ± 0.11[a]
all-E-lycopene	23.50 ± 2.12[b]	31.43 ± 4.11[ab]	34.98 ± 0.85[a]
Z-lycopene	1.71 ± 0.36[a]	1.81 ± 0.29[a]	2.87 ± 1.24[a]
Z-β-carotene	0.38 ± 0.17[a]	1.06 ± 0.26[a]	1.58 ± 0.53[a]
Total β-cryptoxanthin	6.17 ± 0.49[b]	9.43 ± 1.46[ab]	12.92 ± 0.57[a]
Total β-carotene	1.33 ± 0.17[c]	3.00 ± 0.43[b]	4.96 ± 0.42[a]
Total lycopene	25.22 ± 2.48[a]	33.24 ± 4.40[a]	37.85 ± 2.09[a]

†Values are expressed as the mean ± standard deviation (n=3). ‡Values are expressed as the mean ± standard deviation (n=25). FW, fresh weight. TSS, total soluble solids. ND, not detectable (lipids < 0.10 g/100 g; sucrose < 0.25 g/100 g). Means in the same row with the same lower-case letters are not significantly different from each other (Tukey's test, $p < 0.05$).

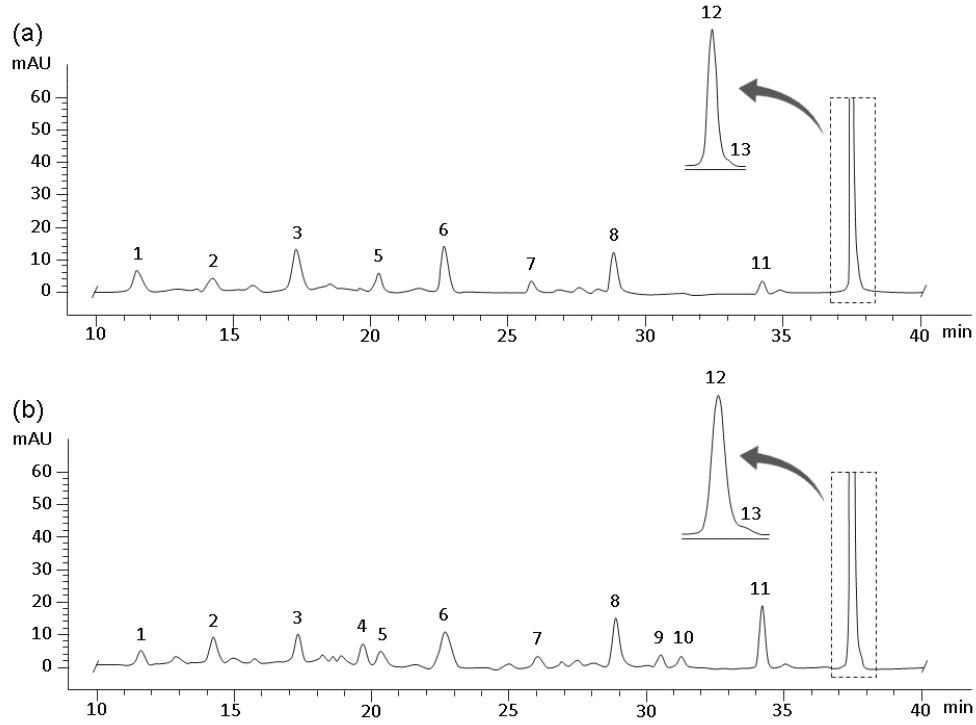

Figure 3.3. HPLC separation of analyzed carotenoids monitored at 450 nm from: a) fresh papaya, b) papaya chips. For peak assignment, see Table 3.2.

All-E-lycopene (LYC) was identified and three of its Z-isomers were detected and identified, namely 13Z-, 9Z- and 5Z-lycopene based on the III/II ratio, *cis* peak intensity, and absorption wavelength (peaks 8, 11 and 13, Figure 3.3 and Table 3.2). The total carotenoid content of papaya fruits was 32.72-55.73 mg/kg FW; these results were similar to those reported for other varieties of papaya fruits from Costa Rica (Schweiggert *et al.*, 2012), but higher than values reported for Maradol papaya from Mexico (Sancho *et al.*, 2011), papaya from Sri Lanka (Chandrika *et al.*, 2003) and Formosa papaya from Brazil (Soto *et al.*, 2020). LYC was the main carotenoid in papaya fruits, representing 62.8-77.1 % of total carotenoids, followed by BCX (free and ester forms) which, represented 18.9-23.2 %. Laurate ester was the BCX form in the highest amounts. BC was found in lower amounts than the other all-E-carotenoids (2.9-6.1 %). The relative proportion of Z-LYC (sum of Z-isomers) and Z-BC in papaya fruits was 4.0-5.2 % and 1.2-2.8 %, respectively.

Table 3.2. UV-Vis spectra of analyzed carotenoids in fresh papaya and papaya chips.

No.	Retention time (min)	Carotenoid	Sample	HPLC-DAD UV-Vis spectrum (nm)	III/II (%)	A_B/A_{II} (%)
1	10.5	*all-E*-β-cryptoxanthin	FP, PC	(430), **452,** 476	18	-
2	13.5	*13Z*-β-carotene	FP, PC	344, (422), **448**, 466	9	44
3	17.2	*all-E*-β-carotene	FP, PC	(428), **450**, 476	13	-
4	19.7	*9Z*-β-carotene	PC	336, (420), **446**, 470	9	31
5	20.4	β-cryptoxanthin caprate	FP, PC	(430), **452**, 476	17	-
6	22.7	β-cryptoxanthin laurate	FP, PC	(430), **452**,476	18	-
7	25.7	β-cryptoxanthin myristate	FP, PC	(430), **452**, 476	19	-
8	28.8	*13Z*-lycopene	FP, PC	362, 440, **466**, 494	47	66
9	30.8	*5Z*-13'Z-lycopene	PC	360, 434, **458**, 486	43	26
10	31.5	*DiZ*-lycopene	PC	362, 434, **458**, 486	28	10
11	34.2	*9Z*-lycopene	FP, PC	362, 442, **466**, 496	72	20
12	37.5	*all-E*-lycopene	FP, PC	446, **472**, 502	74	-
13	37.7	*5Z*-lycopene	FP, PC	446, **472**, 502	74	-

Values in bold represent the main maximum. Values in brackets mark point of inflection. III/II, ratio of the height of the longest-wavelength absorption peak (III) and that of the middle absorption peak (II), taking the minimum between the two peaks as baseline, multiplied by 100. A_B/A_{II}, ratio of the height of *cis*-peak (A_B) and that of the middle main absorption peak (A_{II}), multiplied by 100. FP, fresh papaya; PC, papaya chips.

There were no significant differences (p<0.05) in proximal composition, TSS or succinic acid content among papaya fruits from different ripening stages. In contrast, skin and pulp firmness, L* and C* pulp color parameters and carotenoid content were significantly different (p<0.05) in papaya fruits at the different ripening stages evaluated. Skin firmness was significantly lower at RS5 than at RS4 and RS3, while pulp firmness decreased at each successive ripeness stage. Fruit softening during ripening results from degradation of cell wall components through hydrolysis of pectin and hemicellulose caused by increased activity of polygalacturonase, β-galacatosidase and pectinesterase (Manrique & Lajolo, 2004; Fabi *et al.*, 2009). Table 3.1 shows a decrease of L* (lightness) and an increase of C* (color saturation) parameters with increasing ripeness. These differences were significant (p<0.05) between RS3 and RS5. Color differences (ΔE) were less obvious between consecutive ripening stages RS3-RS4($\Delta E_{RS3\ to}$

$_{RS4}$=3.5) and RS4-RS5($\Delta E_{RS4\ to\ RS5}$=2.1) than between RS3 and RS5 ($\Delta E_{RS3\ to\ RS5}$=5.4). According to Obón *et al.* (2009), ΔE values greater than 5 represent evident color differences that can be observed visually. Color changes in pulp during ripening are expected and result from changes in carotenoid accumulation.

Analysis of carotenoid content showed that all BCX ester forms, BC and LYC increased significantly ($p<0.05$) in papaya fruits during the RS3-RS5 ripening stage. Z-isomer forms were not significantly different. Total BC (sum of all-E- and Z-forms) showed the greatest increase and was 3.7-fold higher in RS5 than at RS3. Total BCX and total LYC (sum of all-E- and Z-forms) contents were 2.1 and 1.5-fold higher, respectively. All-E-lycopene and β-cryptoxanthin laurate were the most abundant pigments in papaya pulp at the studied ripening stages. These results are consistent with those previously reported for Pococí papaya (Schweiggert *et al.*, 2011b). Skin/pulp firmness, pulp color parameters and carotenoid content were the parameters that differed among the Pococí papaya fruits at the different ripening stages of our study.

3.2. Effect of ripening stage on properties of vacuum-fried papaya chips

Papaya fruits at three ripening stages were vacuum fried to obtain papaya chips. Physicochemical properties (moisture, a_w, lipids, color parameters) and sensory acceptance of papaya chips were analyzed. Yields were calculated to evaluate the feasibility of processing each ripening stage (Table 3.3). There were no significant differences ($p>0.05$) in a_w, moisture or lipid contents among papaya chips at different ripening stages produced under the vacuum frying conditions described in section 2.4.2 (120 °C, 12 min, 25 kPa). However, color parameters in papaya chips showed significant differences ($p<0.05$). this was expected because of the color differences in the fresh papaya pulp at different ripening stages. The color parameters L*, C*, and h° were significantly higher ($p<0.05$) for RS3 chips than for RS5 (Table 3.3). Likewise, ΔE (color difference relative to fresh papaya) was significantly higher ($p<0.05$) for RS3 papaya chips than for RS5. The BI did not differ significantly among papaya chips obtained from different ripening stages. Color variation in papaya chips was most influence by L* (lightness). Papaya

chips at RS3 were lighter in color than chips obtained from RS4 and RS5 fruits (Figure 3.1c). Color differences between RS3 and RS5 papaya chips could be explained by the differences in the carotenoid content of the fresh papaya fruits before vacuum frying. Carotenoid content was higher in fresh papaya fruits at RS5 (55.74±3.08 mg/kg FW) than at RS3 (32.72±2.81 mg/kg FW).

Table 3.3. Physicochemical properties and sensory acceptance of vacuum-fried papaya chips, and yield process according to each ripening stage of papaya Pococí (*Carica papaya* L.).

Parameter	Papaya chips - Ripening stage		
	RS3	**RS4**	**RS5**
Moisture (g/100 g FW)	1.48 ± 0.42^{a}	1.41 ± 0.24^{a}	1.18 ± 0.16^{a}
Lipids (g/100 g FW)	31.86 ± 1.79^{a}	36.62 ± 3.90^{a}	30.77 ± 1.23^{a}
a_w	0.186 ± 0.016^{a}	0.187 ± 0.017^{a}	0.195 ± 0.001^{a}
Color parameters:			
L*	54.09 ± 4.60^{a}	47.13 ± 2.58^{ab}	45.13 ± 1.59^{b}
C*	54.37 ± 2.23^{a}	49.93 ± 1.87^{ab}	48.80 ± 1.24^{b}
h°	63.37 ± 2.11^{a}	60.84 ± 0.40^{ab}	59.97 ± 1.30^{b}
ΔE	19.72 ± 3.71^{a}	13.94 ± 3.71^{ab}	11.10 ± 1.42^{b}
BI	200.16 ± 11.11^{a}	212.90 ± 11.11^{a}	216.97 ± 0.38^{a}
Overall sensory acceptance^{+}	5.9 ± 0.5^{b}	6.5 ± 0.4^{a}	6.6 ± 0.4^{a}
Process yields (%):			
Peeling+deseeding+slicing	79.47 ± 1.20^{a}	75.77 ± 1.53^{a}	68.42 ± 4.67^{b}
Frying	17.23 ± 0.57^{b}	19.36 ± 1.15^{a}	14.64 ± 0.29^{c}
Overall	13.69 ± 0.45^{a}	14.67 ± 0.87^{a}	10.02 ± 0.20^{b}

Values are expressed as the mean ± standard deviation (n=3, $^{+}$n=100). ΔE, color difference; BI, browning index. FW, fresh weight. Means in the same row with the same lower-case letters are not significantly different from each other (Tukey's test, $p < 0.05$).

The overall sensory acceptance of papaya chips by regular consumers of snacks and fruits ranged from 5.9-6.6 (Table 3.3). A score of 5 indicated that the evaluator neither liked nor disliked the product. Sensory acceptance was lower for RS3 chips than for RS4 and RS5 ($p<0.05$). The purpose of this evaluation was to determine whether consumers liked the papaya chips and whether they preferred a specific ripening stage. Further sensory evaluations should be conducted to determine consumer preferences regrading appearance, aroma, taste, flavor, mouthfeel, and texture.

Overall process yields and yields for the operations for obtaining papaya slices (peeling+deseeding+slicing) and papaya chips (vacuum frying) are shown for each ripening stage in Table 3.3. Overall yields were lowest ($p<0.05$) for RS5 papaya fruit. Overall yields for RS3 and RS4 fruit were not significantly different ($p>0.05$). Skin and pulp firmness of papaya fruits were lowest at RS5. Fruit softening at this ripeness stage caused losses during the peeling and slicing steps; further losses occurred during vacuum frying due to the RS5 papaya slices sticking to the basket. Considering the physicochemical properties, sensory acceptance and overall process yield, the RS4 ripeness stage (41-55 % skin yellowing) was selected for processing to obtain vacuum-fried papaya chips.

3.3. Optimization of vacuum frying conditions

3.3.1. Influence of vacuum frying conditions on physicochemical properties of papaya chips

Response surface methodology (RSM) was used to optimize vacuum frying conditions to obtain papaya chips from fruits at RS4. Temperature (X_1) and time (X_2) of the frying process were evaluated using a central composite design (CCD). Results of the RSM experiments on moisture content, lipid content, water activity, color change (ΔE), and browning index (BI) of vacuum-fried papaya chips are shown in Table 3.4. Moisture ranged from 0.45 to 2.25 g/100 g FW, a_w ranged from 0.177 to 0.270, lipid content ranged from 20.57 to 39.43 g/100 dry weight (DW), ΔE from 11.45 to 31.92 and BI ranged from 162.97 to 227.35 (Table 3.4). All of the models were significant ($p<0.05$) with R^2 and R^2-adj values higher than 0.87 and 0.81, respectively, for all models except lipid content ($R^2=0.74$, R^2-adj= 0.63) (Table 3.5). Lack of fit was non-significant for all of the models ($p>0.05$).

Table 3.4. Two factor-five level central composite design and experimental data for the responses.

Standard order	Run order	X_1	X_2	Y_1, moist.	Y_2, a_w	Y_3, lipids	Y_4, ΔE	Y_5, BI
1	8	105.86 (-1)	9.88 (-1)	1.69	0.270	22.58	30.61	227.35
2	3	105.86 (-1)	14.12 (+1)	1.19	0.219	28.94	24.83	195.24
3	2	134.14 (+1)	9.88 (-1)	0.92	0.210	25.92	17.88	162.97
4	11	134.14 (+1)	14.12 (+1)	0.50	0.186	30.20	14.83	167.19
5	7	100.00 (-1.414)	12.00 (0)	2.25	0.262	20.57	31.92	212.06
6	10	140.00 (+1.414)	12.00 (0)	0.45	0.228	33.48	21.08	167.36
7	6	120.00 (0)	9.00 (-1.414)	1.13	0.259	28.01	26.36	164.41
8	4	120.00 (0)	15.00 (+1.414)	0.49	0.201	39.43	11.45	180.03
9	9	120.00 (0)	12.00 (0)	1.15	0.177	29.40	17.90	164.56
10	5	120.00 (0)	12.00 (0)	1.21	0.180	30.06	15.13	171.25
11	1	120.00 (0)	12.00 (0)	1.39	0.198	27.85	17.07	171.56

X_1, temperature (°C); X_2, time (min); Y_1 moisture content (g/100 g fresh weight); Y_2, lipid content (g/100 g dry weight); Y_3, water activity (a_w); Y_4, color difference (ΔE); Y_5, browning index (BI).

As shown in Table 3.5, the moisture content of papaya chips was significantly ($p<0.05$) affected by frying temperature and frying time. The contour plot (Figure 3.4a) for moisture revealed that the moisture content of papaya chips decreased with increasing temperature and increased slightly with shorter frying times at all temperatures. As seen in Table 3.5, a_w was significantly affected ($p<0.05$) by frying temperature and frying time (both linear and quadratic effects). The lowest values for a_w were reached at high temperatures and long vacuum frying times (Figure 3.4b). This was expected since frying is a dehydration/cooking process; higher temperatures and longer times cause more evaporation in the sample and result in lower food product moisture content and a_w. These results are consistent with those reported for other food products obtained by vacuum frying: carrot (Fan *et al.*, 2005), pineapple (Pérez-Tinoco *et al.*, 2008), gold kiwi (Diamante *et al.*, 2011), apple (Bravo *et al.*, 2011), apricot (Diamante *et al.*, 2012) and green papaya (Wexler *et al.*, 2016).

Table 3.5. Regression coefficients and analysis of the model for response variables.

Regression coefficients	Predicted values				
	Moisture	**a_w**	**Lipids**	**ΔE**	**BI**
β_0	1.313***	0.185**	30.462***	17.581**	172.281****
β_1	-0.501**	-0.018*	2.857*	-4.758*	-19.456**
β_2	-0.228*	-0.020*	3.349*	-3.739*	-
β_{11}	-	0.026*	-2.331*	4.458*	11.113*
β_{22}	-0.246*	0.018*	-	-	-
β_{12}	-	-	-	-	9.083*
R^2	0.943	0.892	0.739	0.917	0.869
R^2-adj	0.918	0.819	0.628	0.882	0.813
p value of model	<0.0001	0.005	0.019	<0.0001	0.002
p value of lack of fit	0.237	0.388	0.094	0.249	0.121
MS of pure error	0.030	0.000	12.782	6.667	118.142
MS of lack of fit	0.009	0.000	1.288	2.027	15.656

ΔE, color difference; BI, browning index. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Figure 3.4c shows that lipid content of papaya chips (expressed as dry weight) increased with increasing temperatures and longer frying times. The final oil content of fried products can be affected by product shape, temperature and frying time, moisture content, porosity, pore-size distribution, and pre- and post-treatments (Moreira, 2012). Oil absorption results from competition between drainage and suction into the porous crust once the food is removed from the frying medium and cooling begins (when the temperature decreases and the gas pressure inside the pores is lower than outside) (Moreira, 2012). Transport phenomena during food processing are greatly influenced by product microstructure. Dueik *et al.* (2012) found a good correlation between porosity and final oil content in vacuum-fried products; oil absorption was highest in products with the highest accumulative pore volume. Mass transfer phenomenon (water loss and oil uptake) not only depends on the process conditions (oil temperature, frying time, and pressure) but also on other factors related to the food product, such as its size, shape, and thickness (Moreira, 2012).

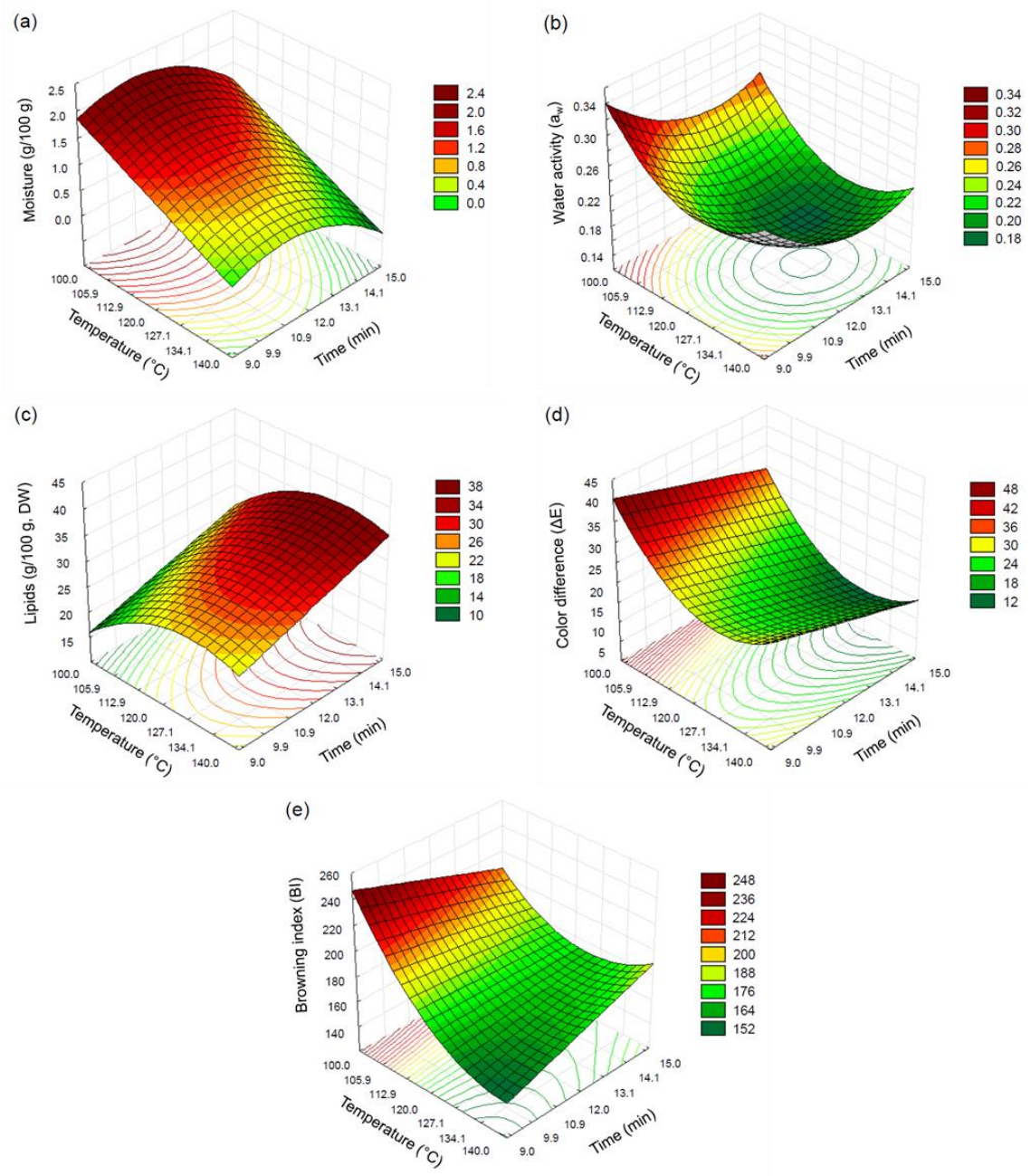

Figure 3.4. Response surface plots for moisture (a), a_w (b), lipids, dry weight (c), color difference (d) and browning index (e) as function of temperature and time of vacuum frying.

Figure 3.4d shows the surface plot for color difference (ΔE) of papaya chips as affected by frying temperature (linear and quadratic effects) and frying time (linear effect). The color difference of vacuum-fried papaya chips decreased with increasing frying time and temperature (Figure 3.4d). These results are similar to

those reported for vacuum-fried apricot slices (Diamante *et al.*, 2011). Changes in papaya chip color with respect to fresh papaya were expected due to carotenoid degradation and non-enzymatic browning reactions (*e.g.*, Maillard reaction, caramelization reaction) that depend on the content of reducing sugars and amino acids or proteins on the surface, as well as the temperature and frying time (Andrés-Bello *et al.*, 2011). Figure 3.4e shows the surface plot for the browning index (BI) of papaya chips as affected by frying temperature (linear and quadratic effects) (Table 5). BI in papaya chips increased with decreasing temperature and was highest at low temperature and short frying times (temperature-time interaction effect) as shown in Figure 3.4e. Similar results were observed by Ihns *et al.* (2011) for apricot slices dried at different temperatures. Reducing sugars could be related to Maillard or caramelization reactions producing colored compounds. However, further studies are needed to identify the specific compounds involved in browning and elucidate the mechanisms involved in sugar reactions during frying of papaya.

3.3.2. Optimal vacuum frying conditions and model verification

RSM showed that the moisture content, a_w, and color difference in papaya chips decreased at high temperatures (120-140 °C) and long frying times (12-15 min). The browning index was lower at short frying times (9-13.1 min) at high temperatures (120-140 °C), while lipid content was lower at short frying times (9-13.1 min) at low temperatures (100-120 °C). For chips, color and texture (crispiness) are quality factors that must be considered. Loss of crispiness during storage of dried chips is related to an increase in moisture content; therefore, moisture content and water activity in these products must be low enough to ensure adequate shelf life (Wexler *et al.*, 2016). In addition, low lipid content is desirable in fried products as current consumer trends require food products with better nutritional profiles.

Table 3.6 shows the verification of models using the optimum conditions (120 °C and 13.1 min) selected after the RSM analysis. The confidence intervals (α=5 %) overlapped for experimental and predicted values for moisture, a_w, lipid content, ΔE and BI. This indicates that the regression models can predict response

variables for any combination of frying temperature and frying time in the experimental ranges tested in this study.

Table 3.6. Predicted and experimental values of responses under optimum vacuum frying conditions.

Optimum point[a]	Responses				
	Moisture (g/100 g)	a_w	Lipids (g/100 g, DW)	ΔE	BI
Experimental value[b]	1.90 ± 0.55	0.170 ± 0.025	29.82 ± 1.83	15.43 ± 2.66	171.89 ± 8.73
Predicted value[c]	1.14 ± 0.28	0.180 ± 0.043	32.14 ± 2.91	15.71 ± 3.66	172.28 ± 7.15

ΔE, color difference; BI, browning index. [a]Optimum point: temperature, 120 °C and time, 13.1 min. [b]Results are the means ± confidence interval at 95% confidence level for n = 3. [c]Results are the means ± confidence interval at 95% confidence level. DW, dry weight.

3.4. Characterization of vacuum-fried papaya chips

Table 3.7 shows the proximal composition (moisture, lipids, protein, ash, and dietary fiber), total sugar content, a_w, color parameters (L*, C*, h°, ΔE, and BI) and carotenoid contents of fresh papaya at ripening stage RS4 and papaya chips obtained from RS4 Pococí papaya by vacuum frying at optimized conditions. The values for moisture content (<2 g/100 g) and aw (<0.2) of the papaya chips were typical for dried snack products. These results are similar to those obtain for vacuum-fried papaya chips made with Coorg honey dew variety (Pandey & Chauhan, 2019; Pandey *et al.*, 2020) and Formosa variety (Soto *et al.*, 2020).

Figure 3.5 shows the moisture sorption isotherm for papaya chips at 25 °C, which is the average daytime storage temperature in tropical conditions. The sorption isotherm shown in Figure 3.5 corresponds to the type III behavior found in sugar-rich foods (*e.g.,* dried fruit products) due to the solubility of sugars in water (Al-Muhtaseb *et al.,* 2002). The GAB model adequately fit the experimental data (R^2= 0.999, R^2-adj= 0.998) to describe the adsorption behavior of vacuum-fried papaya chips at 25 °C. The GAB model better described the moisture content in papaya chips than the regression model obtained by RSM. Predicted values for moisture content in papaya chips (a_w= 0.170± 0.025, moisture= 1.90 ± 0.55 FW)

were 1.62±0.28 g/100 g FW (GAB model) and 1.14±0.28 g/100 g FW (RSM-regression model).

Table 3.7. Physicochemical properties and carotenoid contents of papaya Pococí (*Carica papaya* L.) at RS4 and papaya chips obtained at optimum vacuum frying conditions.

Parameter	Fresh papaya (RS4)	Papaya chips
Moisture (g/100 g FW)	88.04 ± 0.68	1.90 ± 0.48
Lipids (g/100 g FW)	ND	29.25 ± 1.53
Protein (g/100 g FW)	0.58 ± 0.03	3.34 ± 0.62
Ash (g/100 g FW)	0.46 ± 0.03	3.23 ± 0.48
Dietary fiber (g/100 g FW)	1.75 ± 0.09	14.10 ± 0.09
Total sugars (g/100 g FW)	8.06 ± 0.71	42.87 ± 1.55
a_w	0.987 ± 0.001	0.170 ± 0.025
Color parameters:		
L*	47.84 ± 6.71†	50.81 ± 2.92
C*	37.52 ± 6.01†	45.81 ± 1.18
h°	54.93 ± 4.07†	67.44 ± 1.42
ΔE	-	15.43 ± 2.35
BI	-	171.89 ± 7.71
Carotenoids (mg/kg FW):		
all-E-β-cryptoxanthin	1.86 ± 0.24	4.32 ± 0.55
all-E-β-cryptoxanthin-caprate	2.17 ± 0.39	5.55 ± 1.00
all-E-β-cryptoxanthin-laurate	6.03 ± 1.13	19.60 ± 3.66
all-E-β-cryptoxanthin-myristate	1.84 ± 0.10	3.20 ± 0.18
all-E-β-carotene	1.94 ± 0.17	6.75 ± 0.60
all-E-lycopene	31.43 ± 4.11	264.44 ± 34.60
Z-lycopene	1.81 ± 0.29	77.97 ± 12.40
Z-β-carotene	1.06 ± 0.26	8.92 ± 1.67
Total β-cryptoxanthin	9.43 ± 1.46	22.98 ± 3.57
Total β-carotene	3.00 ± 0.43	15.67 ± 2.26
Total lycopene	33.24 ± 4.40	342.41 ± 47.00

Values are expressed as the mean ± standard deviation (n=3, †n=25). ΔE, color difference; BI, browning index. FW, fresh weight.

The GAB model is widely used because this 3-parameter equation provides estimates of the monolayer moisture content (M_g), which is defined as the water necessary to cover the entire food surface by absorption to hydrophilic and polar

groups. From a practical point of view, the M_g value is the moisture content for maximum product stability (Caballero-Cerón *et al.*, 2015). In papaya chips, this value was 0.0929 g water/g dry weight (DW). In dried foods with a high sugar content such as papaya chips (~43 g sugars/100 g chips FW), values lower than the monolayer moisture content are recommended to improve food stability (Welti-Chanes *et al.*, 2007). The final moisture content of the papaya chips (0.0194 g water/g DW) was lower than the M_g given by the model. Dried products with aw values between 0.1 and 0.3 are microbiologically stable and have a long shelf-life as long as there is no change in moisture content during storage (Labuza & Altunakar, 2007).

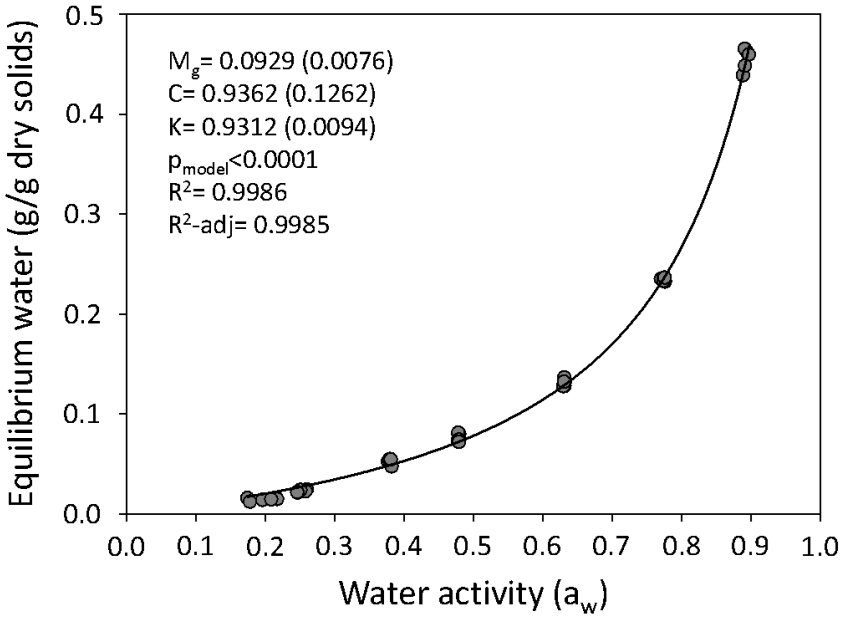

Figure 3.5. Experimental data for vacuum-fried papaya chips and predicted sorption isotherm using GAB equation at 25 °C. M_g, C and K are the parameters obtained from GAB equation (see section 2.10). The standard error for these parameters is shown in parentheses (n=5).

The lipid content was ~29 g/100 g FW (~30 g/100 g dry weight, DW). The oil content of papaya chips obtained in this study was higher than that reported by Pandey *et al.* (2020). These authors obtained an oil content that ranged from 19.61 ± 0.08 to 25.45 ± 0.14 g/100 DW. In order to reduce the oil content in the chips, it is necessary to allow an adequate oil drainage (using a centrifuge) when

the product is removed from the fryer under vacuum (before pressurization of the vessel). For instance, by applying a higher speed of centrifugation to the fried product the more oil on the surface product could be removed (Moreira, 2012). In addition, partial drying before vacuum frying and an increase in thickness of slices reduce oil uptake in papaya chips (Pandey & Chauhan, 2019; Pandey *et al.*, 2020).

Protein, ash, and dietary fiber contents in papaya chips were 7, 6 and 8-fold higher than in fresh papaya. The increase in these parameters was expected and is a consequence of dehydration during vacuum frying. The sugar content was less than 10 % of the fresh weight of fresh papayas and almost 43 % of the fresh weight of papaya chips. Similar results were obtained previously by Soto *et al.* (2020) for vacuum-fried papaya chips obtained from Formosa papaya at the same ripening stage (RS4).

Table 3.7 shows that the color parameters for papaya chips and fresh papaya pulp were similar. The color difference (ΔE) in papaya chips with respect to fresh papaya was 15.43. ΔE values greater than 5 represent an evident color difference for human visual observation (Obón *et al.*, 2009). Diamante *et al.* (2011) suggested that a ΔE of 20 or less was acceptable for vacuum-fried gold kiwi, but this value depends on each food product. The browning index (BI) in papaya chips was ~172. BI is defined as brown color purity and is one of the most common indicators of browning in food products containing sugars (Pathare *et al.*, 2013). In general, vacuum-fried fruit snacks retain more of their natural color due to lesser oxidation and lower frying temperatures (Andrés-Bello *et al.*, 2011) as shown in Figure 3.1c.

Thirteen carotenoids were identified in papaya chips (Figure 3.3b and Table 3.2). Three additional carotenoids not found in fresh papaya were present in papaya chips due to the formation of Z- β-carotene isomer (peak 4, tentatively identified as 9Z-β-carotene) and Z-lycopene isomers (peaks 9 and 10, tentatively identified as 5Z-13'Z- and DiZ-lycopene, respectively) shown in Figure 3.3b. The 9Z-β-carotene is usually formed under severe process conditions such as thermal treatments above 100 °C, while 13Z-β-carotene is predominant at lower temperatures (Pénicaud *et al.*, 2011). Mono-Z-lycopene isomers (such as 5Z-,9Z-

, and 13Z-lycopene) can be present in fresh fruits, whereas di-Z-lycopene isomers are more likely to be in processed food products (Chanforan *et al.*, 2012). Fresh papaya and papaya chips obtained from fruits at RS4 contained 45.67±2.50 mg/kg FW and 381.06±41.17 mg/kg FW of total carotenoids, respectively. The carotenoid content in papaya chips was comparable to other similar products such as non-fat orange sweet potato chips (Bechoff *et al.*, 2010), but was 2, 2.4 and 26-fold higher than that of fried orange sweet potato chips (Da Silva & Moreira, 2008), fried papaya chips obtained from Formosa papaya fruits (Soto *et al.*, 2020), and fried mango chips (Da Silva & Moreira, 2008), respectively. These results confirm that papaya chips are a good source of carotenoids, especially lycopene.

The carotenoids in papaya chips identified by HPLC-DAD were, in descending order by content, all-E-lycopene (LYC), Z-lycopene (Z-LYC), all-E-β-cryptoxanthin (BCX, free and ester forms, primarily laurate ester), Z-β-carotene (Z-BC) and all-E-β-carotene (BC). LYC was the main carotenoid in both fresh papaya and chips, representing 68.8 and 69.4 % of total carotenoids, respectively. The relative proportion of BCX and BC decreased after vacuum frying, from 20.7 % to 6.0 % (BCX) and 4.2 % to 1.8 % (BC). The relative proportion of Z-LYC increased from 4.0 % (fresh papaya) to 20.5 % (chips). There was no change in Z-BC (2.3 % in both fresh papaya and chips).

Figure 3.6 compares the carotenoid content expressed as non-fat dry weight of fresh papaya and papaya chips. There was a significant effect of vacuum frying on BCX, BC and Z-LYC content, according to Student's test ($p<0.05$). Under the optimized conditions (T= 120 °C, t=13.1 min, P= 25 kPa), BCX and BC were degraded by 60 % and 40 %, respectively, whereas LYC content was 1.5-fold higher after vacuum frying. LYC has poor solubility and accumulates in the papaya matrix as crystals associated with plastid membranes. Oil absorption in chips increases the dissolution of lycopene crystalline structures and enhances its extractability. The Z-forms, Z-BC and Z-LYC increased 1.4 and 7.4-fold, respectively. This agrees with previous reports that thermal processes favor isomerization of carotenoids from all-E to Z-forms, especially at temperatures

higher than 100 °C when these compounds are dissolved in lipids (Achir *et al.*, 2011; Ayari *et al.*, 2015; Ahmed *et al.*, 2002).

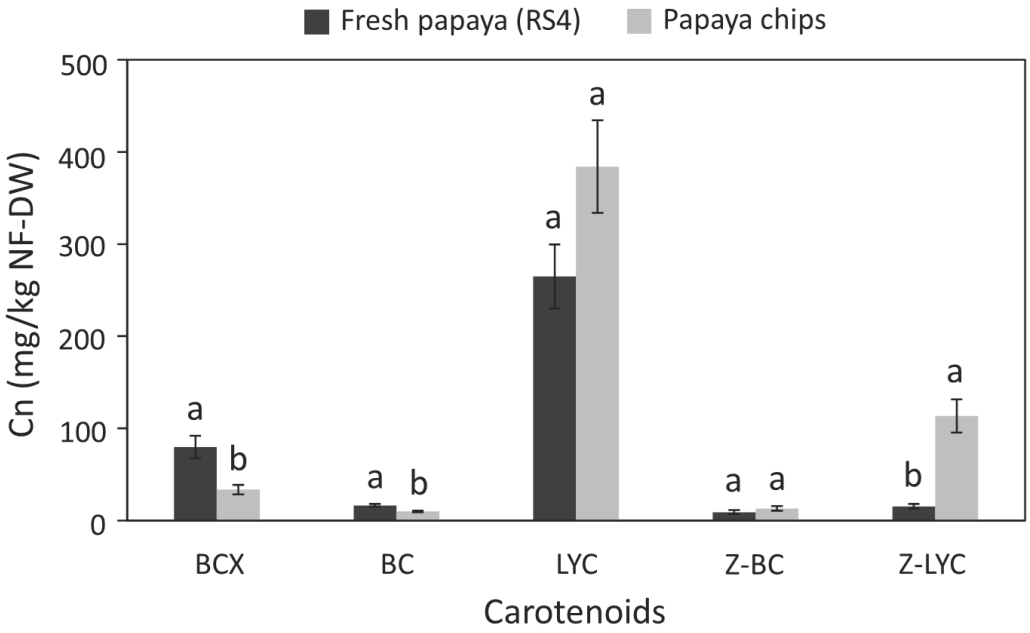

Figure 3.6. Carotenoid concentration in fresh papaya and papaya chips. Bars show the mean ± standard deviation (n=3). For each carotenoid, means with the same letter are not significantly different (Student's t-test, $p < 0.05$). RS4, ripening stage 4; NF-DW, non-fat dry weight. BCX, all-E-β-cryptoxanthin, sum of free and esters forms; BC, all-E-β-carotene; LYC, all-E-lycopene; Z-BC, Z-β-carotene; Z-LYC, Z-lycopene.

4. Conclusions

Differences in physicochemical composition were found among Costa Rican papaya fruits (*Carica papaya* L., cv Pococí) at three ripening stages (RS3, RS4, and RS5), such as skin and pulp firmness, color parameters, and carotenoid content. Pococí papaya fruits at ripening stage RS4 were the most suitable for vacuum frying to obtain chips with acceptable physicochemical and sensory characteristics. At an operating pressure of 25 kPa, the optimum oil temperature was 120 °C and the optimum frying time was 13.1 min. These frying conditions allowed us to obtain quality vacuum-fried papaya chips. Concerning the carotenoid content, at optimal frying conditions, degradation of 40 % and 60 % for BC and BCX occurred, respectively, and there was a 1.5-fold increase in LYC

extractability. These results showed that papaya chips are a good source of carotenoids, especially LYC, and could be an alternative source for vitamin A, as BC and BCX provide retinol activity. Further research is needed to understand the physical and chemical phenomena involved in carotenoid reactivity during vacuum frying of papaya fruits.

Chapter IV

Effect of vacuum frying process on physicochemical properties and content of carotenoids of papaya chips

Chapter IV. Effect of vacuum frying process on physicochemical properties and content of carotenoids of papaya chips

Publication #2

Monitoring and modelling of physicochemical properties of papaya chips during vacuum frying to control their sensory attributes and nutritional value

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Abstract

Vacuum frying is an alternative technology to obtain fruit snacks with higher sensory and nutritional quality compared to traditional fried snacks. Vacuum frying of a carotenoid-rich fruit (red-fleshed papaya) was performed at 25 kPa, using soybean oil at 100, 120, and 140 °C from 0 to 14 min. The study aimed to monitor and model physicochemical changes that chips undergo during vacuum frying, which are related to their sensory and nutritional attributes. Moisture content, a_w, oil uptake, sugar content, browning index (BI) and carotenoid contents were monitored. Water loss and oil uptake followed first-order kinetics while the decrease in a_w followed a logistic trend. Glucose and fructose followed the same degradation pattern while BI and sucrose content increased as a function of frying time and oil temperature. β-cryptoxanthin (BCX) loss followed second-order kinetics and retention was 60 and 40% after 14 min at 120 and 140 °C, respectively. Contents of lycopene and β-carotene were increased suggesting an improvement in availability of these compounds to extraction. Optimal vacuum frying conditions for processing papaya fruit comprise a combination of temperatures (107-120 °C) and frying times (9-14 min) to produce quality papaya chips with a_w ranging from 0.1 to 0.3, low color degradation and BCX loss ≤30 %.

Keywords

Kinetics; Browning index; Sugars; β-cryptoxanthin; β-carotene; Lycopene

1. Introduction

The consumer trends towards healthier foods require alternative strategies to the consumption of traditional fried snacks, which are rich in simple sugars, saturated lipids, and salt (Da Silva & Moreira, 2008). The manufacturing of fried chips based on fruits having good nutritional and sensory properties represents

such an alternative. Vacuum frying is a unit operation in which the food is under pressure below atmospheric level. Thus, decreasing the boiling point of the water in the product and consequently the frying medium. The lower temperatures (from 90 to 140 °C) compared to atmospheric frying, and the absence of oxygen during the process minimize some undesirable chemical reactions, including the degradation of natural pigments and Maillard and caramelization reactions, thus preserving the natural color and nutrients in fried foods (Dueik & Bouchon, 2011b). In addition, this technology allows the use of unsaturated vegetable oils as frying medium due to the process conditions that reduce oil deterioration (Andrés-Bello *et al.*, 2011). For these reasons, vacuum frying is currently used for making healthy and attractive snacks from fruits (Andrés-Bello *et al.*, 2011; Da Silva & Moreira, 2008; Dueik & Bouchon, 2011).

Several factors influence the quality attributes of vacuum-fried fruits such as vacuum frying equipment, properties of the raw fruit (composition of fruit matrix), pre- and post-treatments, and processing conditions. Oil temperature and frying time influence color, texture, nutrients, and content of oil and sugars of fried fruits (Ayustaningwarno *et al.*, 2020a; Dueik *et al.*, 2010; Mariscal & Bouchon, 2008; Troncoso & Pedreschi, 2009).

Different physicochemical changes occur in foods during frying affecting their sensory and nutritional attributes. During frying, the food product loses water rapidly and gains oil, specially at the food's surface (Fan *et al.*, 2010). Moisture content in dehydrated foods (those with a_w in the range of 0.1 to 0.4) is of upmost importance to obtain a product with adequate mechanical properties, such as crispness. Likewise, during frying, some compounds (*e.g.*, sugars, vitamins, and pigments) undergo reactions in the fried food that may significantly impair the product quality (Moreira, 2012). For instance, the color variation of fruit fried products is due to pigment degradation (*e.g.*, carotenoids) or the presence of brown compounds formed from Maillard and caramelization reactions (*e.g.*, hydroxymethylfurfural, HMF) (Dueik & Bouchon, 2011b). Degradation of sugars is involved in Maillard and caramelization reactions causing the formation of volatile molecules and browning in foods processed at high temperatures (Martins *et al.*, 2000). The degree of browning is considered one of the most

important sensory parameters in the definition of the quality of fried foods (Pathare *et al.*, 2013).

In this study, papaya fruit (*Carica papaya* L.) was selected as a model fruit because it is a valuable source of lycopene and pro-vitamin A carotenoids (β-carotene and β-cryptoxanthin) (Schwiggert *et al.*, 2011b; USDA, 2020). Carotenoids are well known to be natural antioxidants with beneficial health effects such as provitamin A activity; in addition, they enhance the immune system functions and lower the risk of developing chronic diseases (*e.g.*, macular degeneration, type 2 diabetes, and cardiovascular diseases) (Kopec & Failla, 2018; Kulczynski *et al.*, 2017). Besides its nutritional value, papaya fruit is consumed worldwide, representing a world production of about 13 million tons in 2017 (FAO, 2020). Thus, vacuum-fried papaya chips could represent a novel and healthy fried food compared to traditional snacks due to their high carotenoid content (Soto *et al.*, 2021a).

In this context, this study first proposes to monitor and model different physicochemical properties (water content, a_w, oil uptake, sugar content, carotenoid degradation, and browning index) of papaya chips during vacuum frying. The selected physicochemical properties are related to the sensory attributes and nutritional value of the final fried product, such as color, texture, and provitamin A content. Finally, the study proposes to define the best conditions of the vacuum frying process to optimize the quality of papaya chips taking into account the most relevant physicochemical characteristics for this type of product. The information generated from this study will be of great interest for manufacturers of fried products who try to obtain attractive and healthy snacks.

2. Materials and methods

2.1. Materials

Red-fleshed papaya fruits (*Carica papaya* L.) from hermaphrodite plants of the commercial Costa Rican hybrid Pococí were acquired from OroFruits (Orotina,

Alajuela, Costa Rica) in October 2019 at ripening stage 4 (41-55 % of skin yellowing). Commercial soybean frying oil Clover® (Grupo NUMAR, Costa Rica) was used as frying medium.

2.2. Chemicals

The following chemicals were used: SigmaUltra standards for glucose, fructose, and sucrose from Sigma-Aldrich (St. Louis, MO, USA); β-carotene, β-cryptoxanthin and lycopene standards from Sigma-Aldrich (St. Louis, MO, USA). Other analytical grade chemicals and HPLC grade solvents were purchased from JT Baker Inc. (Phillipsburg, NJ, USA).

2.3. Sample preparation

Papaya fruits were selected and then washed and peeled manually. Each fruit was vertically cut into 4 pieces and the seeds were removed. The pre-cut pieces were sliced, firstly into 4-mm thick pieces using a food processor (FP-100 Hobart, CA, USA) and then into discs (diameter 30.0 ± 0.2 mm) using a circle-shaped cookie cutter.

2.4. Vacuum frying

The papaya chips were obtained using a vacuum frying system (Auriol, Marmande, France) as previously described by Soto *et al.* (2021a). Briefly, this system consists of a stainless-steel vessel (capacity of 80 L), with a lid equipped with a rotary axis coupled to a piston, a stainless-steel basket, electric heat resistors to heat the oil, a temperature transducer, a filter, a heat exchanger to condense the water vapor generated during the process, a condensate vessel and a liquid ring vacuum pump. The frying vessel was filled with 55 L (~50 kg) of soy oil which was heated to the target temperature. Once the temperature was reached, the papaya discs (100 ± 1 g) were placed into the basket, then the lid was closed, and the vessel depressurized. When the pressure in the vessel achieved the vacuum (25 kPa), the basket was immersed into the oil for the set time to obtain the chips. Once the papaya discs were fried, the basket was raised, and the centrifuging system was applied at 300 rpm (16.6 x g) for 2 min in order to remove excess oil. Finally, the vessel was pressurized, and the papaya chips were

removed from the fryer and left to cool down at ambient temperature prior to packaging.

2.5. Study design

Papaya discs were fried at three different oil temperatures (100, 120 and 140 °C) for seven frying times (0, 2, 4, 6, 8, 10 and 14 min) to obtain the papaya chips. For each frying experiment a product/oil ratio of 1/500 was performed to avoid a decrease in the oil temperature during the vacuum frying trials (to maintain the frying medium at a constant temperature) (appendix A4) and to keep the vacuum pressure stable during process (appendix A5). Three frying trials or repetitions were conducted for each frying condition (combination of temperature/time). After each trial extra oil was added to the vessel to keep the same product/oil ratio. The soybean oil was filtered after every three trials and was replaced after 12 trials to keep the oil fresh. After vacuum frying, samples were packaged in metallized PET/PE bags (Multivac, France) and stored at -80 °C prior to analyses. Different physicochemical properties were measured in the papaya chips: moisture, a_w, oil content, sugar content, color parameters (L*, a*, b*) and carotenoid content. In addition, the browning index was measured in aqueous extracts obtained from papaya chips.

2.6. Physicochemical analyses

2.6.1. Moisture content, oil content, a_w, protein content and pH

Moisture was determined using AOAC standard method 920.151 (AOAC, 2015). Lipid content was determined by the method described by Carpenter *et al.* (1993). Water activity of the samples were determined at 25 °C using a water activity meter (Aqualab, model CX-2, Decagon Devices, Inc., Pullman, WA).

In addition, protein content and pH value were determined in fresh papaya. Protein content was measured by standard AOAC method 920.152 (AOAC, 2015) using a nitrogen conversion factor of 6.25. To measure pH, a digital pH-meter (Metrohm 827 pH lab meter, Metrohm Ion Analysis, Switzerland) was used.

2.6.2. Sugar content

Briefly, samples were ground and then defatted with petroleum ether (b.p. 40-60 °C) using a fat extraction unit E-812 (Büchi, Fawil, Switzerland). This was done to avoid any interference of lipids. Defatted samples were weighed (300-1000 mg) in 40 mL centrifuge tubes. Then 5 mL of distilled H_2O was added and mixed using an IKA® Ultra-Turrax® (Merck KgaA, Darmstadt, Germany) for 5 min. The mixture was incubated for 30 min in a water bath at 70 °C. After incubation, the tubes were cooled in an ice bath and centrifuged at 1400 x g (Allegra 21 Centrifuge, Beckman Coulter, Switzerland) for 10 min at 15 °C. The supernatant was membrane filtered (0.45 μm) prior to sugar analysis.

Sugars (sucrose, glucose, and fructose) were determined by the Shimadzu HPLC system (Shimadzu Manufacturing, Inc., Canby, Oregón, USA) equipped with a RID-10A refractive index detector, a DGU-20A5 degasser, a SIL-20AHT autosampler, a CTO-20A column oven and a LC-20AT binary gradient pump. Sugars were separated using a Zorbax carbohydrate column (250 mm x 4.6 mm i.d., 5 μm) (Agilent, CA, USA) with a guard column. The mobile phase was acetonitrile/H_2O (80/20). The operation temperature was set at 30 °C. The flow rate was set at 1mL/min and the injection volume was 10 μL. Isocratic conditions were programmed with a run time of 15 min. Quantification was performed after obtaining linear calibration curves of glucose, fructose, and sucrose. In order to keep the same basis among samples, the results were expressed as non-fat dry weight.

2.6.3. Color analysis in papaya chips

Color was measured in the papaya chips with a spectrophotometer (ColorFlex, HunterLab, Virginia, USA). Color parameters were expressed in CIELab units L^*, a^* and b^* using illuminant D65 and a 10◦ observer angle.

2.6.4. Color analysis in aqueous extracts from papaya chips

Aqueous extracts were obtained from papaya chips that had been previously defatted and dehydrated, to express results on the same basis. Briefly, samples were ground and then defatted with petroleum ether (b.p. 40-60 °C) using a fat extractor unit E-812 (Büchi, Fawil, Switzerland). To avoid undesirable color

change, defatted samples were then dehydrated at 25 °C in desiccators containing saturated solution of $CaCl_2$ (a_w at equilibrium of 0.280). It was considered that samples reached the equilibrium point when they showed a constant weight during three consecutive readings. Then defatted and dehydrated samples were weighed (300 mg) in 40 mL centrifuge tubes. Then 5 mL of distilled H_2O was added and mixed using an IKA® Ultra-Turrax® (Merck KgaA, Darmstadt, Germany) for 5 min. The tubes were incubated for 30 min in a water bath at 70 °C. After incubation, the tubes were cooled in an ice bath and centrifuged at 1400 x g (Allegra 21 Centrifuge, Beckman Coulter, Switzerland) during 10 min at 15 °C. The supernatant constituted by the hydrosoluble compounds was membrane filtered (0.45 µm) prior to color analysis. Color of the extracts was measured using the same protocol as previously mentioned with the papaya chips. Then, the browning index (*BI*) was calculated using L^*, a^* and b^* values as follow:

$$BI = 100 \times \left[\frac{\left(\frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \right) - 0.31}{0.17} \right] \qquad [1]$$

where L^*, $a^*$and b^* represented the values of aqueous extracts from papaya chips after vacuum frying. *BI* represents the purity of brown color and is considered to be an important parameter associated with browning in food products containing sugar (Pathare *et al.*, 2013). *BI* at each frying time was represented with respect to its initial value (BI_o).

2.6.5. Carotenoid content

Procedures and conditions for carotenoid extraction of papaya chips were described previously by Soto et al. (2020). Briefly, samples were weighed (200-500 mg) in 20 mL tubes. Then, 2 mL of an ethanol solution containing 1 % pyrogallol was added. The mixture was homogenized using a Vortex mixer and incubated for 2 min in the dark in a water bath at 70 °C. Then after cooling, saponification of the samples was performed for 30 min in a water bath at 70 °C by adding 1.5 mL of saturated KOH (12 N). After incubation, the tubes were cooled in an ice bath and 2 mL of distilled water and 5 mL of hexane were added. Then, after mixing and decantation, the organic phase was recovered, and the

aqueous phase was extracted two more times with 5 mL of hexane. The organic phases were mixed and evaporated under nitrogen at 30 °C until dryness. Finally, the residue was re-dissolved in 500 µL of methyl tert-butyl ether (MTBE)/methanol (80/20) and placed in an amber vial prior to HPLC analysis.

Carotenoid identification was performed by HPLC using an Agilent 1100HPLC-DAD system (Massy, France). Carotenoids were separated using a C30 column (150 mm x 4.6 mm i.d., 3 µm) (YMC EUROP GmbH, Germany) with a guard column, and the mobile phase was methanol as eluent A and MTBE as eluent B. Operation temperature was set at 30 °C. The flow rate was set at 0.6 mL/min and the injection volume was 10 µL. The gradient program was described by Soto *et al.* (2021a). All-E-β-cryptoxanthin, all-E-β-carotene and their isomers were detected at 450 nm, and all-E-lycopene and their isomers were detected at 470 nm (Soto *et al.*, 2020). The contents of Z-β-carotene and Z-lycopene were expressed as the sum of all their Z-isomers, respectively. In order to maintain the same basis among samples, the results were expressed on a non-fat dry weight basis.

2.6.6. Vitamin A activity

Vitamin A activity was expressed as Retinol Activity Equivalent (RAE). RAE estimate was calculated for a bioconversion ratio (carotenoid:retinol) of 12:1 for all-E-β-carotene, and 24:1 for all-E-β-cryptoxanthin and Z-β-carotene (US IOM, 2000).

2.7. Kinetic modelling

2.7.1. Moisture content

An exponential model was chosen to describe the moisture content in papaya chips during the frying process as shown in Eq. 2 (Krokida *et al.*, 2000; Manjunatha *et al.*, 2014; Ayustaningwarno *et al.*, 2020b). It is based on the following assumptions: 1) the oil temperature is constant during frying, 2) initial water content ($t=0$) in papaya chips is uniform, 3) the moisture content at an infinite process time ($t=\infty$) is negligible.

$$M = M_0 * exp^{(-k_M * t)} \quad [2]$$

where M is the moisture content at time t (g·g^{-1} dry weight, DW); M_o is the initial moisture content (g·g^{-1} dry weight, DW); k_M represents the specific rate of water loss for this model (min^{-1}); t is the frying time (min).

2.7.2. Oil content

A first-order kinetic model was chosen to describe the moisture content in papaya chips during the frying process as shown in Eq. 3 (Krokida *et al.*, 2000). This model assumes that the oil temperature is constant during frying. The initial oil content (t=0) in papaya chips was negligible.

$$O = O_e * (1 - exp^{(-k_O * t)}) \quad [3]$$

where O is the oil content at time t (g·g^{-1} DW); O_e is the oil content at infinite time (t=∞); (g·g^{-1} DW); k_O represents the specific rate of oil uptake for this model (min^{-1}); t is the frying time (min).

2.7.3. Water activity (a_w), sucrose content, and browning index (BI)

A logistic model of three parameters was used to describe a_w, and sucrose content in papaya chips and *BI* in aqueous extracts from papaya chips during vacuum frying as shown in Eq. 4 (Nambi *et al.*, 2016; Vaikousi *et al.*, 2008).

$$X = \frac{L_X}{1+e^{-k_X*(t-b_X)}} \quad [4]$$

where X is the variable measured at time t: a_w (dimensionless), sucrose content (expressed as g·100 g^{-1} non-fat dry weight, NF-DW), and *BI* (represented in the dimensionless form BI/BI_o); L_X is the curve's maximum of the variable (dimensionless for a_w and BI/BI_o; for sucrose in g·100 g^{-1} NF-DW); b_X represents the time to reach the sigmoid's midpoint (min); k_X represents the constant rate (min^{-1}); t is the frying time (min).

2.7.4. β-cryptoxanthin

A second-order kinetic model was used to describe the degradation of β-cryptoxanthin (*BCX*) during vacuum frying as described by Eq. 5 (Soto *et al.*, 2020). *BCX* degradation was presented in the dimensionless form BCX/BCX_o.

$$BCX = \frac{1}{((1/BCX_0)+k_{BCX}*t)} \quad [5]$$

where *BCX* represents the carotenoid concentration at time t and BCX_o is the initial concentration ($t=0$) (mg·kg^{-1} non-fat dry weight); k_{BCX} is the reaction rate constant (kg·mg^{-1}·min^{-1}); t is the frying time (min). The rate constants were assumed to vary with the temperature according to the Arrhenius equation described by Eq. 6. Isothermal conditions within the product (papaya chips) were assumed to simplify the calculation of the kinetic parameters.

$$k = k_{ref} exp\left(\frac{-E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right) \quad [6]$$

where k_{ref} is the rate constant (kg·mg^{-1}·min^{-1}) at the reference temperature T_{ref} chosen in the middle of the studied temperature range (393 K), with E_a, T, and R respectively denoting the activation energy (J·mol^{-1}), medium temperature (K), and gas constant (8.314 J·mol^{-1}·K^{-1}).

2.8. Parameter identification

The estimation of kinetic parameters was performed using a non-linear regression analysis method which is based on the minimization of the residual sum of squares (RSS) between the prediction and experimental data set for each model (Eq. 7).

$$RSS = \sum_{i=1}^{n}(y_i - \hat{y}_i)^2 \quad [7]$$

where y_i is the experimental value of the dependent variable (moisture content, oil content, a_w, sucrose content, browning index, β-cryptoxanthin content); $\hat{y}_i$ is the calculated value from the model; n_i is the number of experimental points. The

coefficient of determination R^2 was calculated from the ratio of the explained variance to the total variance (TSS), with the explained variance being TSS minus RSS:

$$R^2 = \frac{\sum_{i=1}^{n}(y_i-\bar{y})^2-RSS}{\sum_{i=1}^{n}(y_i-\bar{y})^2} \quad [8]$$

where $\bar{y}$ is the mean value of the dependent variable (moisture content, oil content, a_w, sucrose content, browning index, β-cryptoxanthin content), and n is the number of experimental points. Kinetic parameters for different physicochemical properties in papaya chips were identified using SigmaPlot software (Systat Software Inc., CA, USA). The parameter uncertainly was calculated as the standard error of regression.

2.9. Statistical analyses

The different frying conditions were performed in three repetitions. Data were presented as mean ± standard deviation of replicates. Kinetic parameters for different physicochemical properties of papaya chips were expressed as mean with its respective standard error. The variation of lycopene and β-carotene isomers in papaya chips during vacuum frying was analyzed by one-way ANOVA and post hoc Tukey-HSD test to detect significant differences ($p<0.05$). ANOVA was performed using Statistica 7.0 (Statsoft Inc., USA). Correlation analyses from experimental data of sugar contents, a_w and *BI* of papaya chips were done with SigmaPlot software (Systat Software Inc., CA, USA).

3. Results and discussion

3.1. Physicochemical properties of fresh papaya

Some characteristics were determined in fresh Pococí papaya at ripening stage of 4 (RS4: 41-55 % of skin yellowing) selected to produce papaya chips. Moisture content (88.99 ± 0.67 g·100 g^{-1} fresh weight (FW)) and a_w value (0.989 ± 0.005) were in agreement with previously published data for papaya Pococí (Soto *et al.*,

2021a). The protein content was 0.58 ± 0.03 g·100 g^{-1} FW. Concerning the lipid content, it was considered negligible as the detection limit is 0.10 g·100 g^{-1} FW. These results were similar to those reported for red-fleshed papaya fruit (USDA, 2020). Total sugar content (sum of glucose and fructose) was 6.51 ± 0.44 g·100 g^{-1} FW. Sucrose was not detected in the papaya fruit (detection limit of 0.25 g·100 g^{-1} FW) as previously reported in literature (Soto *et al.*, 2020; Soto *et al.*, 2021a). The pH value of Pococí papaya was 5.43 ± 0.41 being a fruit with low acidity compared to other tropical fruits. The fruit contained 24.70 ± 5.34 mg·kg^{-1} FW of total carotenoids. This carotenoid content is in agreement with that reported for red-fleshed papaya fruit (Schweiggert *et al.*, 2011b; Soto *et al.*, 2020; USDA, 2020).

3.2. Moisture content, oil uptake, and a_w

Results of experimental data for the moisture content (dry weight) of papaya chips during frying at different oil temperatures are shown in Figure 4.1a. The treatments were performed at constant temperature (100, 120 or 140 °C) with minimal variation of oil temperature during the frying process. Figure 4.1a shows that the moisture content in papaya chips decreased significantly during frying. Water started evaporating as soon as the raw material was in contact with the oil. Initially, the rate of water loss was high, leading to an initial rapid fall of moisture content (85-98 % of water loss, Figure4.1a) during the first minutes of frying (0-4 min) due mainly to the loss of surface moisture, and then it slowed down as the product reached equilibrium.

As frying temperature increased the moisture content for the same frying time decreased. After 10 min of vacuum frying at 100 and 120 °C the moisture content in papaya chips reached equilibrium values of 0.024 and 0.020 g·g^{-1} DW, respectively. At 140 °C the equilibrium moisture value was of 0.005 g·g^{-1} DW and was reached after 8 min of frying. The moisture content model fitted the experimental data accurately (Figure 4.1a). It was shown that the rate constant of moisture loss (k_M), presented in Table 4.1, increased with temperature as previously described by several authors during the frying process (Troncoso & Pedreschi, 2009; Krokida *et al.*, 2000; Manjunatha *et al.*, 2014; Ayustaningwarno *et al.*, 2020b).

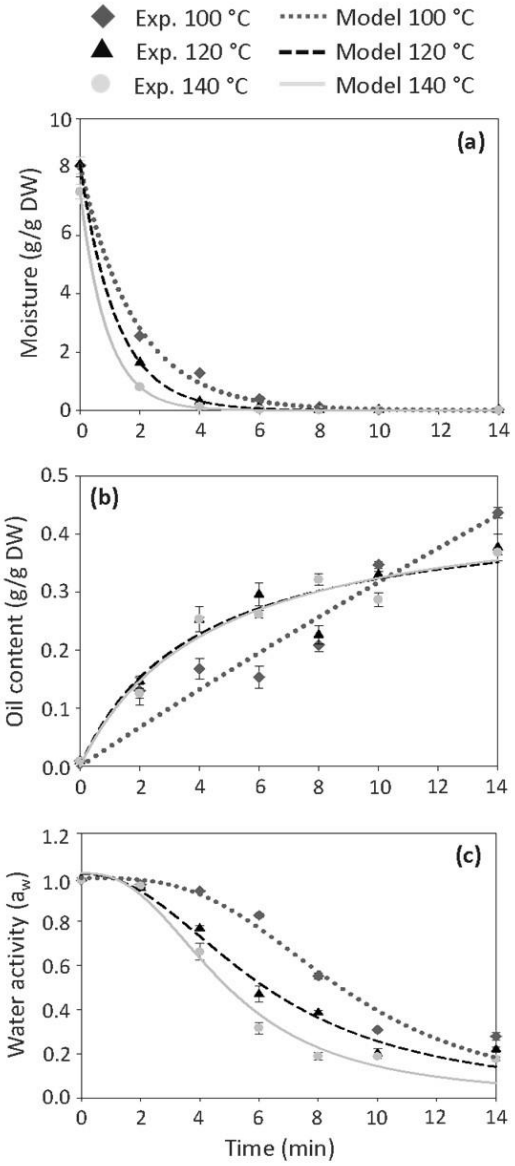

Figure 4.1. Effect of vacuum frying on (a) moisture content, (b) oil content and (c) a_w of papaya chips at different oil temperatures. Values are expressed as the mean ± standard deviation (n=3). Exp: experimental data, Model: predicted data by different models, DW: dry weight.

Figure 4.1b shows the increase of oil uptake (dry weight) versus frying time applying different oil temperatures. Generally, the longer the product remained in the frying vessel, the more the oil was absorbed. There were no significant differences ($p>0.05$) for the rate constant of oil uptake (k_O) between curves at 120 and 140 °C (Table 4.1). The general pattern was an initial rapid increase of oil uptake (0-8 min) followed by a gradually decreasing rate, with a final increase for

the longest frying time (14 min). At 100 °C the model had a linear behavior with time. In general, at low temperatures and for short times, the proposed model had a linear behavior with time, whereas at higher temperatures (≥120 °C) and for longer times, oil content reached an equilibrium value as shown in Figure 4.1b.

Table 4.1. Kinetic parameters from models generated for moisture content, oil content, a_w, sucrose, browning index and β-cryptoxanthin.

Parameter	T (°C)	p-value of model	R^2	Kinetic parameters
Moisture content (M)	100	<0.0001	0.997	k_M= 0.5474 (0.0253)
	120	<0.0001	0.999	k_M= 0.8145 (0.0086)
	140	<0.0001	0.999	k_M= 1.1132 (0.0115)
Oil content (O)	100	0.0007	0.915	O_e= 2.6259 (8.5157); k_O= 0.0129 (0.0447)
	120	0.0010	0.903	O_e= 0.3518 (0.0424); k_O= 0.2594 (0.0901)
	140	0.0001	0.961	O_e= 0.3567 (0.0285); k_O= 0.2470 (0.0551)
Water activity (a_w)	100	0.0042	0.9355	L_{aw}= 1.0968 (0.1491); b_{aw}= 8.4770 (1.2244); k_{aw}= -0.3214 (0.1111)
	120	0.0020	0.9551	L_{aw}= 1.3988 (0.4917); b_{aw}= 4.2074 (3.2026); k_{aw}= -0.2549 (0.0930)
	140	0.0037	0.9394	L_{aw}= 1.2307 (0.3446); b_{aw}= 4.2260 (1.8508); k_{aw}= -0.4012 (0.1621)
Sucrose (suc)	100	<0.0001	1.0000	L_{suc} = 16.6508 (0.0463); b_{suc} = 9.31601 (0.0100); k_{suc} = 1.5685 (0.0160)
	120	0.0214	0.8536	L_{suc} = 34.4856 (6.7104); b_{suc} = 5.4333 (1.3868); k_{suc} = 0.5479 (0.3291)
	140	0.0028	0.9470	L_{suc} = 37.0830 (2.5857); b_{suc} = 3.5910 (0.4192); k_{suc} = 1.8472 (1.4312)
Browning index (BI)	100	0.0038	0.939	L_{BI}= 5.0128 (5.8417); b_{BI} = 14.5270 (20.9550); k_{BI}= 0.1064 (0.0655)
	120	0.0025	0.950	L_{BI}= 5.8191(1.4827); b_{BI} = 7.1559 (2.8715); k_{BI}= 0.2124 (0.0721)
	140	<0.0001	0.997	L_{BI} = 17.2378 (0.2696); b_{BI} = 3.9264 (0.0925); k_{BI}= 1.1519 (0.1210)
β-crypto-xanthin (BCX)	100	0.6043	0.037	k_{BCX}= 0
	120	<0.0001	0.910	k_{BCX}= 5.00 x10^{-4} (4.27 x10^{-5})
	140	<0.0001	0.940	k_{BCX}= 9.00 x10^{-4} (8.04 x10^{-5})

Standard error is expressed in brackets (n=3). Units of kinetic parameters: k_M, k_O, k_{aw}, k_{suc}, k_{BI}, and k_{BCX} are expressed in min^{-1}; O_e is expressed in g·g^{-1} DW; L_{aw}, and L_{BI} are expressed in dimensionless form, and L_{suc} is expressed in g·100 g^{-1} NF-DW; b_{aw}, b_{suc},and b_{BI} are expressed in min.

During frying at low temperatures, oil absorption took place not only during the cooling period but also to some extent during the frying period; this can be explained by the fact that low temperatures and thus weak water flows lead to the formation of a crust with a structure more favorable to oil absorption during the frying period (Pedreschi & Moyano, 2005; Ziaiifar *et al.*, 2010). This can be observed in Figure 4.1b, after 10 min at 100 °C the oil uptake in papaya chips was higher compared to 120 and 140 °C.

Moisture loss is directly related to oil absorption because oil enters the voids replacing the water that has evaporated during the frying, which happens especially during cooling (Ziaiifar *et al.*, 2010). Oil uptake increased as the moisture content diminished. In this study, an equilibrium value was observed in moisture content (~2 g·100 g^{-1} FW) when the papaya chips reached oil contents above 25 g oil·100 g^{-1} FW.

Figure 4.1c shows the change of a_w values in papaya chips during vacuum frying. It was observed that sigmoidal behavior described a_w values in papaya chips during the frying process. A decreasing logistic function (Eq. 4) fitted the a_w data accurately. The models for a_w at 120 and 140 °C presented a similar behavior. L_{aw} and b_{aw} parameters of curves at 120 and 140 °C were significantly different ($p<0.05$) from those obtained at 100 °C (Table 4.1).

Figure 4.1c also shows that a_w values of papaya chips decreased during frying according to three distinct stages: settling period, constant rate period and falling rate period. The settling period occurs during the first minutes of process (0-2 min at 120 and 140 °C, and 0-4 min at 100 °C), with a_w values close to one. Then, there is a constant rate period in which the unbound water is removed. This period represents the condition of equilibrium temperature at the product surface and ends when the surface of the solid is no longer wet and represents the critical moisture content or critical a_w value (Barbosa-Cánovas & Juliano, 2007). Finally, the last stage comprises the period in which the surface of the food product is dried, and the water is removed from the center to the surface as vapor (Barbosa-Cánovas & Juliano, 2007). At this point, the drying rate approaches the equilibrium moisture content as is shown in Figure 4.1a.

Water loss and oil uptake phenomena depend not only on the process conditions (oil temperature, frying time, and pressure) but on physical factors related to the food, such as its size, shape, and thickness (Moreira, 2012). The thickness significantly affects the contents of moisture and oil of food products during frying. Water loss and oil uptake are higher at smaller sample thickness (keeping the same oil temperature and frying time) (Krokida *et al.*, 2000). The selection of the thickness of fruit/vegetable slices to obtain the chips depends on the ripening stage and composition of the raw material (moisture, proteins, polysaccharides, sugars) and the physical changes in the fried product (crispness, shrinkage, puffing). For instance, the ripening stage and the chemical composition of fruits are factors that influence the skin/pulp firmness of these food matrices, affecting the process conditions during the peeling, cutting, and slicing steps (Soto *et al.*, 2021a). A greater firmness in the fruit allows obtaining a lesser thickness in the product during the slicing operation. In this study was selected a thickness of 4 mm in the papaya discs according to pilot experiments. This value is similar to that applied for other fruits to obtain vacuum-fried chips: 4 mm for mango (Ayustaningwarno *et al.*, 2020a); 3.5-4.5 mm for banana (Yamsaengsung *et al.*, 2011); 5 mm for kiwi (Diamante *et al.*, 2011), and apple (Mariscal & Bouchon, 2008). For vegetables, with a higher starch content and lower moisture and sugar content than fruits, the thickness is usually less than in fruits. For instance, 1.5 mm for potato (Crosa *et al.*, 2014), blue potato, and sweet potato (Da Silva, & Moreira, 2008); 2 mm for carrot (Dueik *et al.*, 2010).

3.3. Sugar reactions

Figure 4.2 shows the variation of different sugars present in papaya chips during vacuum frying at different oil temperatures. The sugars present in fresh papaya discs (t=0) were glucose and fructose, representing 53 and 47 % of total sugars, respectively. Figure 4.2a and Figure 4.2b show that concentrations of glucose and fructose of papaya chips varied as function of frying time and oil temperature. Curves of glucose and fructose followed the same degradation pattern. At 100 °C, it was observed that degradation of both sugars started after 10 min of frying. At 120 and 140 °C, these sugars decreased significantly after 4 min of frying. Also, it was observed that the highest retentions of glucose and fructose occurred at

100 °C. For instance, after 14 min of vacuum frying the average retention of glucose and fructose at 100 °C was 62 %. At 120 and 140 °C after 14 min the average retention of these two sugars was 39 and 27 %, respectively.

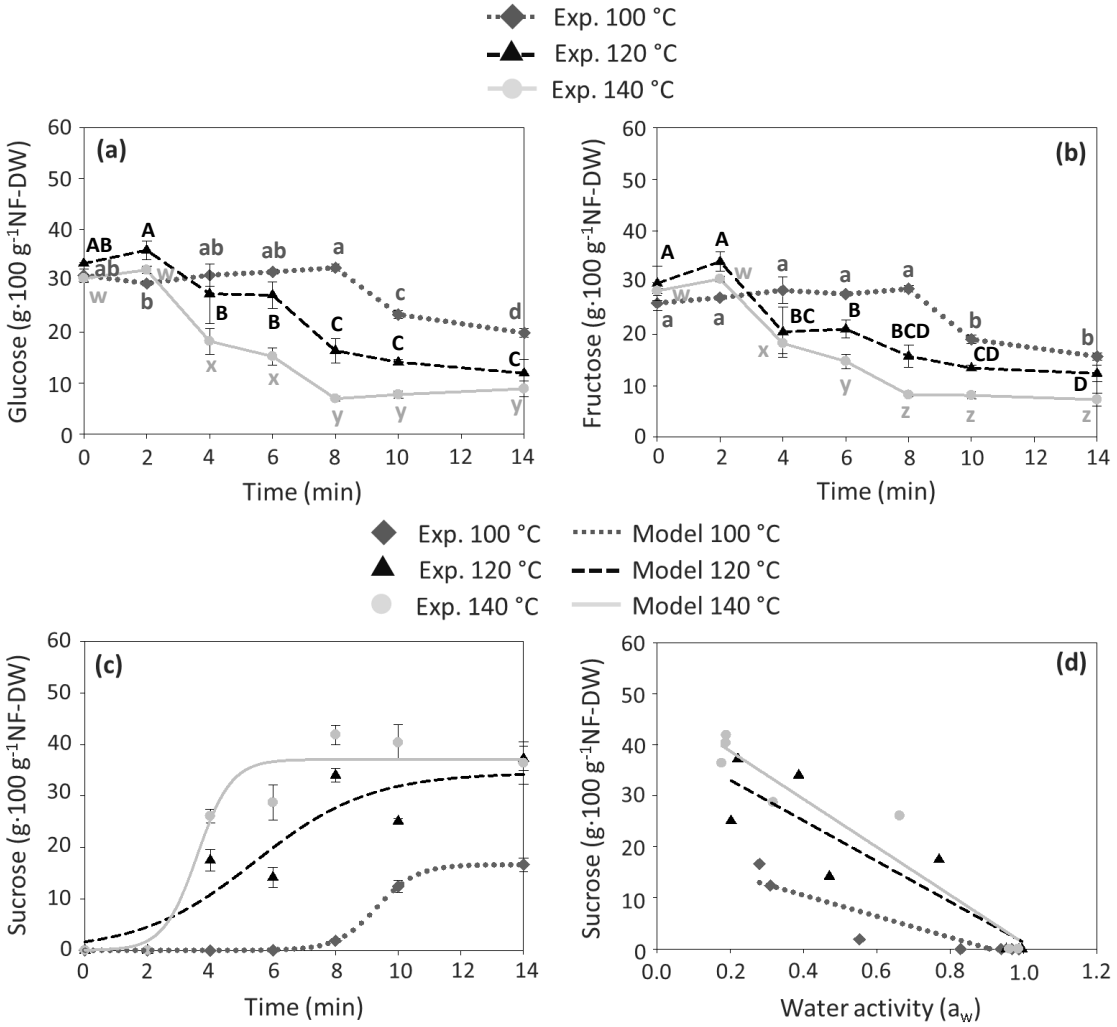

Figure 4.2. Variation of concentrations of glucose, fructose, and sucrose (expressed as non-fat dry weight, NF-DW) in papaya chips during vacuum frying at different oil temperatures: (a) glucose, (b) fructose, (c) sucrose, values are expressed as the mean ± standard deviation (n=3), and (d) sucrose as a function of a_w values. Exp: experimental data, Model: predicted data by different models. For glucose (a) and fructose (b) curves, mean values with the same letters (*a-d* for 100 °C, *A-D* for 120 °C, and *w-z* for 140 °C) are not significantly different from each other (Tukey's test, $p < 0.05$).

On the other hand, during vacuum frying the formation of sucrose occurred. Experimental data for sugar content fitted well with a 3-parameters logistic

function (Equation 4). Sucrose formation in papaya chips also followed sigmoidal behavior during the frying process as shown in Figure 4.2c. Experimental data showed that initial formation of sucrose varied according to the oil temperature, increasing at 120 and 140 °C (Figure 2.c). Moreover, Figure 4.2c shows that the lowest sucrose formation was reached at 100 °C. In fact, the time to reach the sigmoid's midpoint (b_{suc}) was significantly longer ($p<0.05$) at 100 °C than at 120 and 140 °C (Table 4.1). Likewise, the lowest curve's maximum of sucrose formation (L_{suc}) was observed at 100 °C (~17 g·100 g^{-1} NF-DW) while L_{suc} value at 120 and 140 °C was ~2-fold higher than at 100 °C (Table 4.1, Figure 4.2c).

Sucrose content in fresh papaya Pococí is negligible (Soto et al., 2021a). Thus, its formation during vacuum frying of papaya could be explained by two simultaneous phenomena: i) condensation reaction between glucose and fructose, or ii) thermal degradation of cell wall and middle lamella polysaccharides (*e.g.*, fructooligosaccharides) that could lead to the release of sucrose during frying of papaya. There is no information in the reviewed literature showing a similar reaction as seen in this study. Nevertheless, sugar condensation reaction is frequently used in chemical industry for producing low-caloric polysaccharides (*e.g.*, polydextrose). The formation of disaccharides by condensation reaction is carried out under temperatures from 100 to 300 °C, preferably at temperatures close or higher to melting point of saccharides serving as the substrate (Shah *et al.*, 2004). In addition, vacuum conditions are required in order to minimize decomposition and discoloration of sugars (Shah *et al.*, 2004).

Specific factors related to the frying process such as temperature and rate of water loss as well as intrinsic characteristics of papaya (such as proximal composition, sugar concentration, organic acids, acidity) are involved in sucrose formation. It seemed that high temperatures during processing and low a_w values in food matrix favor this reaction. For instance, a negative linear correlation was observed between sucrose content and a_w. Correlation coefficients (R values) were -0.911, -0.887 and -0.964 for the curves at 100, 120 and 140 °C, respectively, as shown in Figure 4.2d. As a_w values decreased, sucrose content increased. Furthermore, glucose and fructose could undergo not only condensation reaction

to produce sucrose but also Maillard or caramelization reactions to generate colored compounds affecting the browning color of papaya chips.

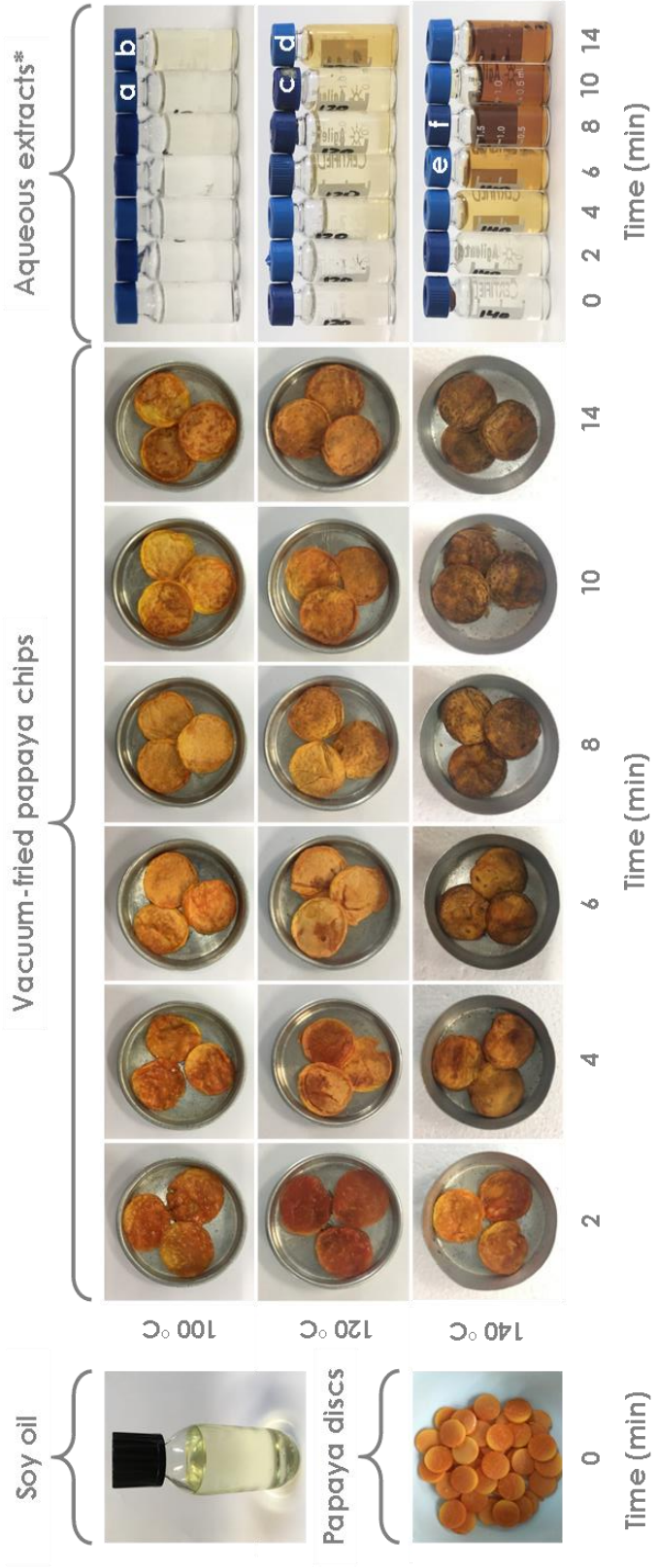

Figure 4.3. Papaya discs, soy oil, and papaya chips and their aqueous extracts obtained during vacuum frying at different oil temperatures (100-140 °C). *Letters indicate browning index of some aqueous extracts with respect to its initial value (BIo, t=0 min): (a) 2*BIo, (b) 3*BIo, (c) 4*BIo, (d) 5*BIo, (e) 15*BIo, and (f) 18*BIo.

3.4. Color parameters

Color is one of the most important quality parameters in fruit and vegetable food products. The average L*, a* and b* color values of fresh papaya discs were 34.9 ± 2.5, 25.6 ± 2.2 and 40.0 ± 2.0, respectively. A low color variation was observed in the papaya chips fried at 100 °C and 120 °C, but at 140 °C the color degradation was higher and more evident with the longest frying times (Figure 4.3). For instance, after 14 min at 140 °C, L*, a* and b* parameters significantly decreased ($p<0.05$) to 29.2 ± 1.2, 16.3 ± 0.9 and 22.1 ± 1.8, respectively. On the other hand, at 100 °C and after 14 min of process, color parameters significantly increased ($p<0.05$) and L*, a* and b* values in papaya chips were 47.0 ± 1.3, 35.6 ± 0.4 and 52.2 ± 1.6, respectively. At 120 °C there was only a significant difference ($p<0.05$) for b* parameter; after 14 min of frying, papaya chips had a b* value of 28.1 ± 0.5.

There was not a clear trend for color change in papaya chips during vacuum frying and the L*, a*, b* parameters could not be modelized during the process (data not shown). Papaya fruit is a complex matrix and the color variations in vacuum-fried papaya chips could be the result of carotenoid degradation (loss of red-orange color) and formation of molecules from Maillard or caramelization reactions (development of browning), and to a lesser extent the adsorbed oil in the chips during the frying process.

Nevertheless, the measurement of browning index (BI) in aqueous extracts obtained from papaya chips allowed the browning reaction that alters the color in the chips to be evaluated more accurately. Figure 4.3 clearly shows that the browning color in aqueous extracts increased with increasing frying time and oil temperature. The variation of dimensionless value of browning index (BI/BI_0) in aqueous extracts of papaya chips at different temperatures is shown in Figure 4.4a. Experimental data for BI/BI_0 were fitted well with a 3-parameters logistic function (Eq. 4). Figure 4.4a shows that BI of aqueous extracts was sensitive to temperature change. For instance, the constant rate of browning formation (k_{BI}) increased by ~2-fold when the frying temperature was increased from 100 to 120 °C but increased by ~5.4-fold when temperature was increased from 120 to 140 °C (Table 1). Contrary to L*a*b* parameters of papaya chips BI, obtained by L*a*b* measurements on aqueous extracts, was much more sensitive

to the processing conditions and therefore more relevant for monitoring color variation.

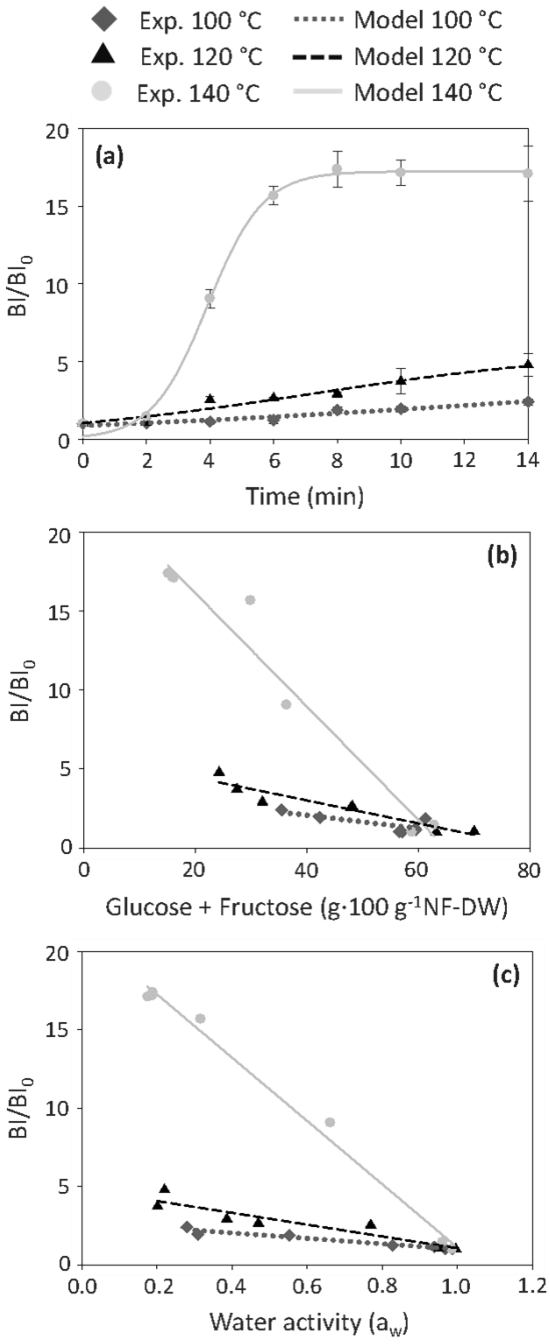

Figure 4.4. Variation of dimensionless value of browning index (BI/BI_0) in aqueous extracts of papaya chips at different oil temperatures: (a) BI/BI_0 as a function of frying time (values are expressed as the mean ± standard deviation, n=3), (b) BI/BI_0 as a function of reducing sugars (glucose + fructose), and (c) BI/BI_0 as a function of a_w values. Exp: experimental data, Model: predicted data by different models, NF-DW: non-fat dry weight.

Browning in papaya chips could be more related to caramelization than Maillard reaction due to intrinsic characteristics of papaya fruit. Papaya is a low acidity fruit (pH= 5.43) with a reducing sugar content of 6.51 g·100^{-1} FW (59.19 g·100 g^{-1} DW), specifically glucose and fructose, and a low protein content (~0.6 g·100 g^{-1} FW). The Maillard reaction takes place where reducing sugars react with amino acids and proteins during heating (Purlis, 2010; Martins *et al.*, 2000). Caramelization requires temperatures >120 °C and starts with an enolization reaction of sugars, in particular reducing sugars and sucrose (Kroh, 1994). Thus, BI is related to sugar degradation, specifically to reducing sugars (glucose and fructose). Figure 4.4b showed the linear correlation between BI and concentration of reducing sugars at different oil temperatures. R values were -0.760, -0.949 and -0.979 for the curves at 100, 120 and 140 °C, as reducing sugars decreased, browning index increased.

Moreover, browning reactions mainly depend on temperature and a_w since this variable represents the availability of water for chemical reactions in food. Low a_w and high temperatures favor the formation of color during thermal treatment (Purlis, 2010). For instance, the production of colored compounds such as HMF always needs at least one dehydration step during the Maillard and caramelization reactions (Kroh, 1994).

In vacuum-fried papaya chips a negative linear correlation was observed between a_w and BI/BI_0 as shown in Figure 4.4c with a higher slope at 140 °C. R values were -0.972, -0.929 and -0.996 for the curves at 100, 120 and 140 °C, respectively. As oil temperature increased and a_w decreased in papaya chips during frying, browning development increased being faster at temperatures >120 °C.

3.5. Carotenoid reactivity

In terms of carotenoid reactivity, a clear difference was observed between carotenes and xanthophyll during vacuum frying of papaya fruit. Carotenes such as β-carotene (BC) and lycopene (LYC), contain only a parent hydrocarbon chain while xanthophylls such as β-cryptoxanthin (BCX), contain an oxygen functional group (Ribeiro *et al.*, 2018).

Table 4.2. Variation of dimensionless concentration of total lycopene and total β-carotene and their isomers in papaya chips during vacuum frying at different oil temperatures.

T (°C)	Time (min) 0	2	4	6	8	10	14
all-E-BC/Total BC$_o$							
100	0.91 ± 0.19^{c,A}	2.93 ± 0.56^{a,A}	2.76 ± 0.13^{a,A}	2.05 ± 0.07^{b,A}	1.75 ± 0.02^{b,A}	1.93 ± 0.15^{b,A}	1.68 ± 0.10^{b,A}
120	0.95 ± 0.16^{bc,A}	1.98 ± 0.21^{a,B}	1.22 ± 0.05^{b,B}	1.22 ± 0.04^{b,B}	0.93 ± 0.08^{bc,B}	0.78 ± 0.10^{c,B}	0.74 ± 0.13^{c,B}
140	0.92 ± 0.28^{bc,A}	1.41 ± 0.03^{a,B}	1.00 ± 0.15^{b,B}	0.94 ± 0.09^{bc,C}	0.65 ± 0.09^{bc,C}	0.57 ± 0.03^{c,B}	0.62 ± 0.10^{bc,B}
Z-BC/Total BC$_o$							
100	0.093 ± 0.003^{d,A}	0.62 ± 0.09^{bc,A}	0.59 ± 0.07^{c,A}	0.82 ± 0.07^{b,A}	0.81 ± 0.04^{b,A}	0.81 ± 0.12^{bc,A}	1.05 ± 0.09^{a,A}
120	0.053 ± 0.001^{d,C}	0.45 ± 0.01^{c,A}	0.68 ± 0.15^{ab,A}	0.68 ± 0.09^{ab,AB}	0.74 ± 0.05^{a,A}	0.49 ± 0.01^{bc,B}	0.57 ± 0.12^{abc,B}
140	0.085 ± 0.003^{d,B}	0.44 ± 0.13^{c,A}	0.51 ± 0.11^{bc,A}	0.53 ± 0.07^{bc,B}	0.51 ± 0.08^{bc,B}	0.83 ± 0.09^{a,A}	0.70 ± 0.09^{ab,B}
Total BC/Total BC$_o$							
100	1.00 ± 0.18^{c,A}	3.55 ± 0.64^{a,A}	3.35 ± 0.20^{ab,A}	2.86 ± 0.04^{ab,A}	2.56 ± 0.04^{b,A}	2.74 ± 0.27^{b,A}	2.74 ± 0.19^{b,A}
120	1.00 ± 0.16^{d,A}	2.42 ± 0.20^{a,B}	1.91 ± 0.19^{b,B}	1.90 ± 0.13^{b,B}	1.67 ± 0.13^{bc,B}	1.26 ± 0.11^{cd,B}	1.31 ± 0.25^{cd,B}
140	1.00 ± 0.28^{b,A}	1.85 ± 0.15^{a,B}	1.50 ± 0.25^{ab,B}	1.46 ± 0.16^{ab,C}	1.15 ± 0.15^{b,C}	1.40 ± 0.11^{ab,B}	1.32 ± 0.15^{b,B}
all-E-LYC/Total LYC$_o$							
100	0.83 ± 0.12^{d,A}	1.72 ± 0.25^{c,A}	2.14 ± 0.12^{b,A}	2.12 ± 0.09^{b,A}	2.38 ± 0.07^{ab,A}	2.54 ± 0.04^{a,A}	2.20 ± 0.15^{ab,A}
120	0.84 ± 0.10^{d,A}	1.98 ± 0.06^{b,A}	2.49 ± 0.21^{a,A}	2.07 ± 0.13^{ab,A}	1.88 ± 0.26^{bc,B}	1.83 ± 0.18^{bc,B}	1.53 ± 0.07^{c,B}
140	0.83 ± 0.07^{c,A}	1.66 ± 0.08^{b,A}	2.07 ± 0.27^{a,A}	1.51 ± 0.02^{b,B}	1.06 ± 0.04^{c,C}	0.99 ± 0.05^{c,C}	0.87 ± 0.10^{c,C}
Z-LYC/Total LYC$_o$							
100	0.17 ± 0.01^{e,A}	0.32 ± 0.00^{d,A}	0.41 ± 0.03^{bc,B}	0.34 ± 0.01^{cd,C}	0.39 ±0.02^{bcd,C}	0.46 ± 0.04^{b,B}	0.56 ± 0.00^{a,C}
120	0.16 ± 0.02^{d,A}	0.30 ± 0.02^{c,A}	0.53 ± 0.02^{b,B}	0.59 ± 0.03^{b,B}	0.58 ± 0.05^{b,B}	0.57 ± 0.01^{b,B}	0.70 ± 0.03^{a,B}
140	0.17 ± 0.01^{c,A}	0.38 ± 0.07^{c,A}	0.89 ± 0.12^{b,A}	1.17 ± 0.03^{a,A}	1.12 ± 0.05^{a,A}	1.01 ± 0.12^{ab,A}	1.03 ± 0.06^{ab,A}
Total LYC/Total LYC$_o$							
100	1.00 ± 0.13^{d,A}	2.04 ± 0.26^{c,A}	2.56 ± 0.08^{b,A}	2.47 ± 0.10^{b,A}	2.78 ± 0.08^{ab,A}	3.00 ± 0.05^{a,A}	2.76 ± 0.16^{ab,A}
120	1.00 ± 0.12^{c,A}	2.27 ± 0.08^{b,A}	3.02 ± 0.23^{a,A}	2.66 ± 0.15^{ab,A}	2.45 ±0.31^{b,AB}	2.41 ± 0.17^{b,B}	2.23 ± 0.08^{b,B}
140	1.00 ± 0.07^{d,A}	2.04 ± 0.15^{c,A}	2.95 ± 0.39^{a,A}	2.68 ± 0.03^{ab,A}	2.19 ± 0.09^{cb,B}	2.00 ± 0.16^{c,C}	1.90 ± 0.10^{c,C}

Values are expressed as the mean ± standard deviation (n=3). For each carotenoid, mean values in the same row with the same lower-case letters are not significantly different from each other; and mean values in the same column with the same upper-case letters are not significantly different from each other (Tukey's test, $p<0.05$). Carotenoids: all-E-BC, all-E-β-carotene; Z-BC, Z-β-carotene; Total BC, total β-carotene (sum of all-E-BC + Z-BC); all-E-LYC, all-E-lycopene; Z-LYC, Z-lycopene; Total LYC, total lycopene (sum of all-E-LYC + Z-LYC).

The variation of dimensionless concentrations of total BC and total LYC and their respective isomers (expressed in non-fat dry weight) are represented in Table 4.2. In general, at first there was an increase in BC and LYC attributed to an extractability phenomenon in the first minutes of frying, followed by a decrease phase because of degradation during the rest of the process including isomerization (all-E- to Z-forms).

For all-E-BC (the main β-carotene isomer) the highest content ($p<0.05$) was reached at 2 min of vacuum frying for all tested temperatures. In the case of Z-BC, there was an increase in the formation of these isomers throughout the frying process, reaching the highest concentrations after 8-14 min (Table 4.2). The Z-BC isomers found in papaya chips were 9Z- and 13Z-β-carotene (Soto *et al.*, 2020; Soto *et al.*, 2021a). It was observed that above 100 °C the total BC degradation generally increased with oil temperature during the period studied. The highest retentions ($p<0.05$) of total BC and all-E-BC in papaya chips were obtained at 100 °C. In the case of all-E-LYC, there was a constant increase of extractability during frying at 100 °C. For longest frying times all-E-LYC was better preserved at 100 °C than at 120 and 140 °C (Table 4.2). The Z-LYC content increased during vacuum frying, with the highest concentration after 14 min at 100 and 120 °C and after 6 min at 140 °C. In general, isomerization of lycopene increased at 140°C compared to 100 or 120 °C. The main Z-LYC isomers found in papaya chips were 5Z-, 9Z- and 13Z-lycopene. In addition, at 120 and 140 °C there was a formation of two di-Z-lycopene forms but in a lower concentration compared to the mono-Z-lycopene isomers.

There are few reports about carotenoid reactivity in foods during atmospheric frying and even less during vacuum frying. Degradation of β-carotene during deep-fat frying (under atmospheric conditions) varied according to the fruit or vegetable matrix. For instance, retentions of 80-98 % was found in plantain cylinders (Rojas-Gonzalez *et al.*, 2006); 72-86 % in orange-fleshed sweet potato chips (Vimala *et al.*, 2006); 28-32% in carrot crisps (Dueik *et al.*, 2010); 32 % in mango chips (Nunes & Moreira, 2009). In the case of the impact of frying on lycopene degradation the information is scarce. The phenomenon of the extractability of β-carotene and especially of lycopene increasing during vacuum

frying of papaya could be explained by two factors: i) a greater release of these carotenoids from papaya cell tissues (*e.g.*, chromoplasts) due to the matrix structure disruption during frying (100-140 °C), and ii) an increase of their solubility enhanced by frying oil absorption in papaya chips. These findings are in agreement with those reported for several studies that described an increase of β-carotene and lycopene extractability during thermal processing of certain vegetables such as tomato, sweet potato and bell pepper (Colle *et al.*, 2010b; Dewanto *et al.*, 2002; Re *et al.*, 2002; Kidmose *et al.*, 2006).

On the other hand, BCX presented a different behavior and was the least stable carotenoid compared to the others. There was an effect of frying time on dimensionless concentrations of BCX, expressed as non-fat dry weight at 120 and 140 °C (Figure 4.5). For instance at 120 and 140°C, after 14 min of process there were losses of 40 and 60%, respectively. At 100 °C there was no BCX degradation. Notably, BCX decreased more rapidly at higher temperatures (120–140 °C). The mathematical model that best described the trend of BCX degradation in papaya chips was the second-order model as described previously during storage (Soto *et al.*, 2020).

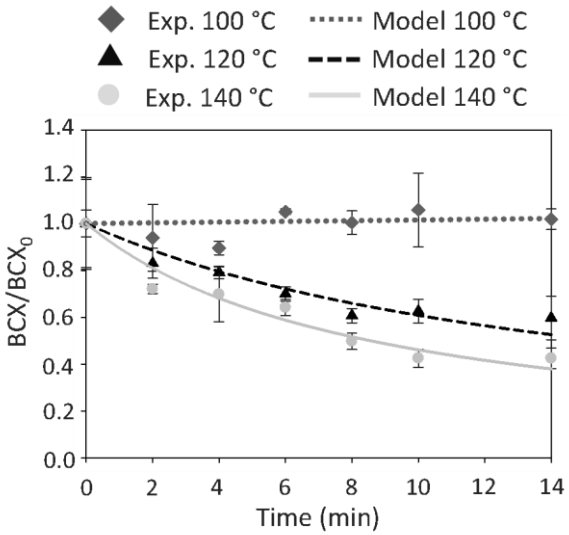

Figure 4.5. Variation of dimensionless concentration of β-cryptoxanthin (BCX/BCX_0) in papaya chips during vacuum frying at different oil temperatures (expressed as non-fat dry weight). Values are expressed as the mean ± standard deviation (n=3). Exp: experimental data, Model: predicted data by model.

The resulting rate constants are presented in Table 1. The constant rate of degradation (k_{BCX}) increased with temperature (Table 1). BCX followed the Arrhenius temperature-dependency pattern (R^2 = 0.85). The rate constant of reference (k_{ref}) at the reference temperature of 120 °C was 4.0×10^{-4} kg·mg^{-1}·min^{-1} and the activation energy (E_a) was 52 kJ·mol^{-1}.The E_a value of BCX in the papaya chips was in accordance with that previously reported by Hadjal *et al.* (2013) in thermally treated blood orange juice (62 kJ·mol^{-1}). Aparicio-Ruiz *et al.* (2011) also found similar activation energies in virgin olive oil enriched with BCX during a heat treatment, 63-69 kJ·mol^{-1}.

These results show that orange color degradation in papaya chips, measuring by L*a*b* parameters, is related to BCX degradation and not to BC or LYC loss. Chemical differences between xanthophyll and carotenes could affect their reactivity during vacuum frying. In the case of BCX, there was no effect of vacuum frying on its extractability as with carotenes. BC and LYC have poor solubility compared to BCX. For instance, lycopene has the lowest solubility and accumulates in the papaya matrix as crystals associated with the plastid membranes (Schweiggert *et al.*, 2011b). Thus, the oil uptake in the chips allows the crystalline structures of lycopene to dissolve increasing its extractability during analyses. For total BC, the maximum extractability was reached when the oil content was ~0.13 g oil·g^{-1} chip DW. The maximum extractability of total LYC occurred when the chips had ~0.26 g oil·g^{-1} chip DW (at 120 and 140 °C) and ~0.33 g oil·g^{-1} chip DW (at 100 °C). Conformation, concentration, and lipid solubility of carotenoids in the papaya chips seemed to have a key effect on reactivity of carotenoids during vacuum frying.

3.6. Optimization of vacuum frying process of papaya

In our study we focused on the influence of oil temperature and frying time on the physicochemical properties that are related to sensory attributes and nutritional value of vacuum-fried papaya chips as summarized in Figure 4.6. Desirability of a_w, color and nutritional value in papaya chips was determined according to literature considering the most relevant physicochemical characteristics for this kind of products.

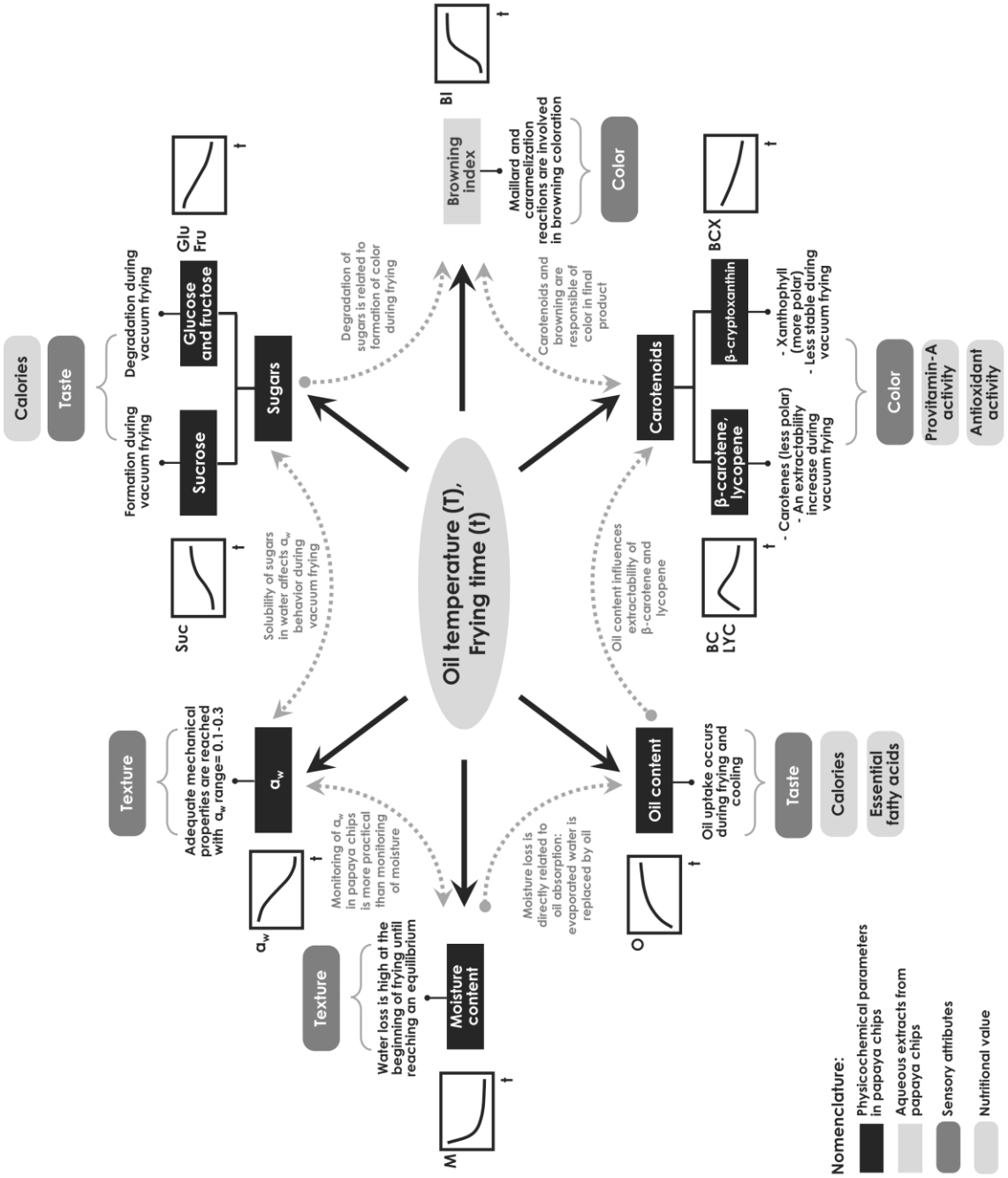

Figure 4.6. Diagram of influence of oil temperature and frying time on physicochemical properties related to sensory attributes and nutritional value of vacuum-fried papaya chips.

3.6.1. Desirability of a_w, color and nutritional value

- a_w

Control of a_w in dried food products with high hygroscopicity such as papaya chips (due to low moisture content and high sugar content), is critical to ensure

microbiological quality and to maintain a suitable texture during storage. Physical changes such as loss of crispness occur when the dried products gain water above the critical a_w (Welti-Chanes *et al.*, 2007). It was observed that adequate crispness is obtained when a_w values in papaya chips ranged from 0.1 to 0.3 (moisture content ≤2 g·100 g^{-1} chip FW) (Soto *et al.*, 2021a). Konopacka *et al.* (2002) found a critical a_w value of 0.2 for fat-free apple chips (moisture content 3.5 g·100 g^{-1} chips); they described that increasing a_w in these chips above 0.3 resulted in complete loss of crispness. Critical a_w values for fruit chips are usually lower than those for chips based primarily on starch-protein mixtures (a_w 0.35-0.50) (Konopacka *et al.*, 2002).

- *Color*

The reaction impacting the color in papaya chips was browning (Maillard or caramelization) which affects quality. Therefore, it is desired that the BI be as low as possible. There is no information in the reviewed literature about decoupling the pigment degradation from browning during fruit or vegetable processing and even less about BI determination in aqueous extracts from a solid food matrix as control parameter. Food color acceptance by consumers depends on the food product. Some authors suggested that a color difference between raw and vacuum-fried fruits (ΔE) of 20 or less is acceptable; for instance, this was found for gold kiwifruit (Diamante *et al.*, 2011) and apple (Mariscal & Bouchon, 2008). In our case when aqueous extracts from papaya chips obtained BI/BI_0 greater than 5, the papaya chips presented ΔE >20 (data not shown). For this reason, it was determined that a BI/BI_0 of 5 (or 5^*BI_0, Figure 4.3) should be considered as a criterion for a maximum limit in the chips.

- *Nutritional value*

Our results showed that papaya chips are a good source of carotenoids, especially lycopene (70-77 % of total carotenoids), and could be an alternative for vitamin A consumption as they provide retinol activity thanks to β-carotene and β-cryptoxanthin. For instance, after 10 min of vacuum frying the papaya chips presented a nutritional value of 91-128 μg Retinol Activity Equivalent (RAE) (portion of 40 g chips), which corresponds to 10-14 % of the reference daily intake

(RDI) (900 μg for adults and children ≥4 years) (FDA, 2020). It could be claimed that food products with 10 % or more of the RDI for vitamin A (RAE) as "good source of vitamin A" (FDA, 2020). In papaya chips, the BCX represented ~70 % of total provitamin A activity expressed as RAE (see section 2.6.6). Therefore, the retention of BCX during vacuum frying is of importance for having provitamin A activity in papaya chips. In our study it was calculated that BCX losses lower than 30 % ensured 10 % or more of the RDI for vitamin A (RAE) in the papaya chips.

3.6.2. Optimization of vacuum frying conditions

Prediction of a_w, BI and BCX loss in papaya chips using the developed kinetic models is shown in Figure 4.7. This figure shows that values of a_w between 0.1 and 0.3 (grey zone) are reached with frying times that range from 7 to 14 min. When the chips have a_w values ≤ 0.3 an equilibrium is reached, thus a_w is slightly affected by temperature. Contrary to final a_w, BCX and BI were more affected by oil temperature. BCX degradation and color variation increased with increasing oil temperature and frying time (Figure 4.7). Low BCX loss and BI (under 20 % and $3*BI_o$, respectively) are not of interest because a_w values in papaya chips are close or above to 0.3 and would generate a product that is neither stable nor crispy. On the contrary, obtaining papaya chips with a_w values lower than 0.1 implies not only longer frying times but also higher BCX degradation and browning.

Figure 4.7 shows a triangular zone with stripes embedded in the a_w stable zone in which the loss of BCX is lower than 30 %, thus ensuring ≥10% RDI of vitamin A (RAE) in the vacuum-fried papaya chips. This region comprises a combination of temperatures from 107 to 120 °C and frying times from 9 to14 min that produce quality papaya chips with a_w values from 0.1 to 0.3 and low color degradation (BI $<4*BI_o$). These frying conditions could be useful for processing papaya or other carotenoid-rich fruits keeping the same vacuum pressure and isothermal conditions. However, these conditions could change depending on the type of vacuum frying machine, type of oil, size, thickness, and shape of fruit slices, ratio product/oil, and ripening stage of fruit, among other factors.

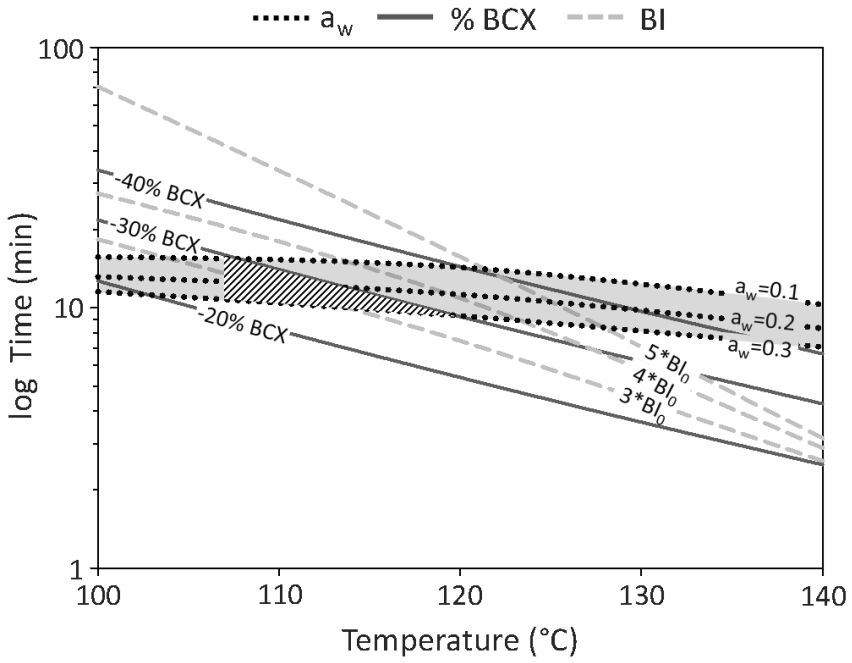

Figure 4.7. Prediction curves of water activity (a_w), browning index of aqueous extracts with respect to its initial value (BI_0), and β-cryptoxanthin loss (% BCX) with oil temperature and with frying time in papaya chips vacuum-fried at 100-140 °C.

3.7 Other sensory and nutritional attributes

Other factors, such as oil type, oil content, and sugar content, may be analyzed for producing vacuum-fried fruit chips. The sugars and oil not only provide calories in fruit chips but also contribute to their final taste. Therefore, it is important to control the quality and content of these compounds. The type of oil affects the nutritional value of the final fried product. For instance, unsaturated vegetable oils are a source of essential fatty acids such as linoleic and alpha-linolenic acids, which are susceptible to oxidation during atmospheric frying (Márquez-Ruíz *et al.*, 2010). However, one of the benefits of vacuum frying is preserving the oil quality because of the low temperatures employed and the minimal exposure to oxygen (Andrés-Bello *et al.*, 2011; Belkova *et al.*, 2018). For instance, the fatty acid profile of the soybean oil used as frying medium remained similar during vacuum frying of papaya fruit, after 12 trials at 120 °C (Appendix A13). Nevertheless, it is necessary to verify the quality of frying medium by analyzing other parameters such as total polar compounds, oxidized fatty acids,

and polymer triglycerides, which are the most reliable methods for monitoring oils during frying process (Gertz, 2000).

In the current study, the soybean oil used was mainly composed of polyunsaturated fatty acids such as linoleic acid (ω-6, 49.3 % of total lipids), oleic acid (25.8 % of total lipids), and alpha-linolenic acid (ω-3, 6.3 % of total lipids). Therefore, it is relevant to find the right balance between the caloric intake provided by the oil and the contribution of essential fatty acids in the final product. In order to reduce the oil content in the chips, it is necessary to allow adequate oil drainage (using a centrifuge) when the product is removed from the fryer under vacuum (before pressurization of the vessel). For instance, by applying a higher centrifugation speed to the fried product, higher oil content is removed from the product surface (Moreira *et al.*, 2009).

In the case of sugars, they provide the sweetness of the fried product. In addition, Maillard and caramelization reactions, which are involved in browning, depend on sugars. During vacuum frying, the sugars are concentrated, which is intrinsic to the process and inevitable. But it is relevant to determine the process conditions that generate the least degradation of sugars, thus preserving the color and flavor of the final product. Because our study aimed to study the changes of the main physicochemical properties in chips, further sensory evaluation with consumers should be conducted to validate our results.

4. Conclusions

Physicochemical parameters measured in a carotenoid-rich fruit such as moisture, a_w, oil uptake, sucrose content, and browning index (BI) could be modeled as function of frying time at different oil temperatures. During vacuum frying of papaya fruit, the degradation of reducing sugars (glucose and fructose) was observed whereas the formation of sucrose occurred. Reducing sugars could be related to a condensation reaction producing sucrose and to Maillard or caramelization reactions producing colored compounds. BI in aqueous extracts obtained from papaya chips facilitated the characterization of the kinetics of

Maillard or caramelization reactions that alter color in the chips. However, further studies in papaya and other carotenoid-rich fruits are needed to identify the specific compounds involved in browning and elucidate the mechanisms involved in sugar reactions during frying. There was a clear difference in reactivity between xanthophylls (BCX) and carotenes (BC and LYC). BCX was the less stable carotenoid while BC and LYC contents were influenced by the extraction efficiency from the matrix. Moisture content (measured by a_w) plays a key role for most of the important quality parameters, either directly, *e.g.*, oil uptake and texture, or indirectly for chemical reactions, *e.g.*, browning. Prediction of a_w, BI (in aqueous extracts), and BCX loss can be used to optimize process conditions to produce quality fried chips from papaya fruit. The results obtained allowed the monitoring and modelling of physicochemical parameters related to sensory and nutritional attributes of great relevance for this type of product. This research brings new information about the reactivity of carotenoids and sugars during the frying of fruits. From a practical point of view, this information will be useful for snack producers who want to make fried products based on fruits rich in carotenoids with high moisture and sugar content.

Chapter V

Determination of optimal storage conditions regarding carotenoid degradation in vacuum-fried papaya chips

Chapter V. Determination of optimal storage conditions regarding carotenoid degradation in vacuum-fried papaya chips

Publication #3

A kinetic study of carotenoid degradation during storage of papaya chips obtained by vacuum frying with saturated and unsaturated oils

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Abstract

The aim of the study was to evaluate the degradation kinetics of carotenoids (CTs) in vacuum-fried papaya (*Carica papaya* L.) chips (PCs) during storage at four temperatures (15, 25, 35 and 45 ºC) for 52 and 94 days for the two highest and lowest temperatures, respectively. Three treatments were applied to obtain the chips: chips with soy oil (24 % lipids) and chips with palm oil (24 % and 29 % lipids). All the chips were packaged under air or nitrogen conditions. The CTs analyzed by HPLC-DAD were per order of content all-E-lycopene (LYC), Z-lycopene (Z-LYC), all-E-β-carotene (BC), all-E-β-cryptoxanthin (BCX) and Z-β-carotene (Z-BC). The all-E-forms represented 80 % of carotenoids in PCs. No significant carotenoid degradation was observed in the PCs packaged under nitrogen conditions during storage. For chips stored under air conditions, a second-order kinetic model best fitted the experimental data. Rate constants for LYC degradation were the lowest, while BCX and BC presented similar rate constants 4-23-fold higher depending on lipid composition. All Z-isomers degraded faster than all-E-forms, but Z-BC degraded only 2-4-fold faster than Z-LYC. All CTs followed Arrhenius temperature-dependency pattern and LYC showed the lowest activation energies (5-21 kJ/mol). A higher lipid content in the chips with palm oil enhanced the carotenoid retention in PCs. Moreover, a greater retention ($p<0.05$) of CTs was observed in PCs with soy oil. The use of soy oil instead of palm oil increased the theoretical half-life (at 25 ºC) by 2.2, 1.3 and 5.9-fold for BCX, BC and LYC, respectively. Packaging under nitrogen conditions and lipid composition may be considered to optimize the shelf life and carotenoid retention in PCs during storage.

Keywords

Carotenoids; Papaya chips; Kinetics; Vacuum frying; Soy oil; Palm oil; Oxygen; Storage; Temperature.

1. Introduction

Fruits and vegetables are a source of bioactive compounds such as vitamins, polyphenols and carotenoids (CTs). The beneficial effects of CTs on human health have been reviewed and evaluated in several studies. For instance, CTs such as β-carotene (BC) and β-cryptoxanthin (BCX) represent an important nutritional source of vitamin A in the human diet which is necessary for vision, growth, cell differentiation, and other physiological processes (Fernández-García *et al.*, 2012). Other CTs without provitamin A activity such as lycopene (LYC) have health-promoting properties. For example, the consumption of lycopene-rich foods has been reported to be inversely associated with the incidence of cardiovascular disease and cancer (Müller *et al.*, 2016; Rao & Agarwal, 2000).

The papaya fruit (*Carica papaya* L.) is a valuable source of micronutrients, in particular pro-vitamin A carotenoids, BC and BCX, and red-fleshed papaya genotypes contain higher amounts of LYC compared to other fruits and vegetables such as mango, pumpkin, carrot and grapefruit (USDA, 2019). Besides its nutritional value, the papaya fruit represents a commercial importance in the developing countries where this crop is grown. During the past two decades, high growth rates for papaya production were reported worldwide. On average, total papaya world production increased from 5.49 million tons in 1994 to 13.02 million tons in 2017 (FAO, 2020).

Papaya is traded as both a fresh and processed product. During processing and storage, the concentration of nutrients and their biological activity may be changed. It is well known that food processing can cause many effects, not all of which result in a loss of quality and health properties. Because of this it is important to understand how food processing and storage influence the nutritional properties of food products.

Frying is a process widely used in the food industry that involves a process of simultaneous heat and mass transfers. During this operation, the food is cooked, dried, and formulated because of the oil uptake in the product. Nowadays, consumers are more interested in healthy products with good sensory and

nutritional properties. Following this trend, vacuum frying is an alternative technology to atmospheric frying as it improves the sensory and nutritional quality of fried foods because of better control of oil quality and content in the fried products. In addition, during vacuum frying, the product is heated at a lower temperature and lower oxygen pressure thus inhibiting lipid and pigment oxidation and therefore, preserving the natural color and nutrients of fried fruits (Andrés-Bello *et al.*, 2011; Da Silva & Moreira, 2008; Dueik & Bouchon, 2011). Da Silva and Moreira (2008) showed that vacuum frying retained 1.2 and 2-fold more CTs than atmospheric frying in mango and sweet potato chips, respectively.

However, after frying, the chips are usually stored at ambient temperature for several weeks or months. The loss of CTs during storage may reach 90 % as a function of storage conditions (Bechoff *et al.*, 2010). Therefore, there is an issue when choosing a process that preserves CTs to match it with optimal storage conditions that enable their maximum retention during the shelf-life of chips.

During processing and storage of dried products, CTs are prone to isomerization and oxidation as a result of exposure to high temperature, oxygen, light or pro-oxidant molecules (Achir *et al.*, 2016; Achir *et al.*, 2010; Caris-Veyrat *et al.*, 2003; Colle *et al.*, 2010b; Xiao *et al.*, 2018). Isomerization of all-E-carotenoids to Z-forms and cleavage occur due to heating during thermal treatments, while oxidation is a slower reaction that may occur at ambient temperature during storage in the presence of oxygen, metals, enzymes, unsaturated lipids, and other pro-oxidant compounds (Pénicaud *et al.*, 2011). Kinetic studies of carotenoid degradation during storage have been conducted in carotenoid degradation of non-fat dehydrated products (low a_w) (Bechoff *et al.*, 2010; Song *et al.*, 2018). It has been shown that storage of dehydrated products under nitrogen conditions enhanced the retention of carotenoids. For instance, Bechoff *et al.* (2010) found a β-carotene loss of 16 and 83 % in sweet potato chips stored under N_2 and air, respectively, at 40 °C after 21 days. Also, storage of dehydrated pumpkins under N_2 packaging retained more β-carotene and induced a low rate of carotenoid isomerization (especially at 4 °C) in comparison with storage under air conditions (Song *et al.*, 2018). Moreover, the presence or addition of lipids has demonstrated an increase in extractability and bioaccessibility of carotenoids in different food

matrices. Oil can then play a role as carrier of carotenoids during processing, storage as well as during digestion (Colle *et al.*, 2012; Dhuique-Mayer *et al.*, 2016, 2018; Lemmens *et al.*, 2014; Maity *et al.*, 2014).

There is no information about behavior of vacuum-fried papaya chips. What is more, there is no literature about kinetic parameters of carotenoids in this kind of product during storage. In the present study, β-carotene, β-cryptoxanthin and lycopene degradation kinetics were investigated in vacuum-fried papaya chips during storage influenced by temperature (range of 15-45 ºC), considering the lipid composition of the chips and the presence of oxygen inside the packages. This study gives insight into carotenoid degradation trends as a function of their chemical structure and gives an indication of how to optimize frying oil choice and storage conditions especially under different scenarios to maximize carotenoid retention.

2. Materials and methods

2.1. Materials

Red-fleshed papaya fruits (*Carica papaya* L. var. Formosa from Brazil) from a single batch were acquired from TerreAzur (Montpellier, France) at ripening stage 4 (41-55 % of skin yellowing). Papaya skin color was assessed as a percentage of skin yellowing on the whole fruit (0–100%).

Commercial frying oils, soy oil (Huileries Cauvin, Nimes, France) and hydrogenated palm oil Risso® (Vandemoortele, Gent, Belgium) were used. These oils were chosen because they are widely used for frying purposes and they present a very different fatty acid profile, a saturated oil (palm) and an unsaturated oil (soy) as is shown in Table 5.1. In addition, these oils present a lower vitamin E content in contrast to other vegetable oils (Table 5.1) to avoid any interaction with CTs.

2.2. Sample preparation

Papaya fruits were selected and then washed and peeled manually. Each fruit was vertically cut into 4 pieces and then the seeds were removed. The pre-cut pieces were cut into 4-mm thick slices (dimensions of approximately 3 cm x 3 cm x 6 cm) using a slicing machine prior to vacuum frying.

2.3. Vacuum frying

The papaya chips (PCs) were obtained using a vacuum frying system (Auriol, Marmande, France) as is shown in Figure 5.1. This system consists of a stainless-steel vessel (capacity of 80 L), electric heat resistors for heating the oil, a lid with a rotary axis coupled to a piston where is placed a stainless-steel basket (radius of 16.5 cm), a temperature transducer, a filter, a heat exchanger to condense the water vapor generated during the process, a condensate vessel and a liquid ring vacuum pump. The frying process consists of heating the oil (55 L) to the target temperature (120 ^{o}C). Then loading the papaya slices (about 1 kg) into the basket, closing the lid, and depressurizing the vessel. Once the pressure reached 25 kPa, the basket was immersed into the oil (palm or soy) for 13 min to achieve a final moisture content of 2 %. When the chips were fried, the basket was raised, and the centrifuging system was applied for 3 min in order to control the oil content absorbed by the samples. Two speed values were applied during centrifugation of papaya chips with palm oil, 530 rpm or 51.8 x g (maximal speed) and 100 rpm or 2.1 x g (minimal speed), to reach 24 % and 29 % lipid content, respectively. For papaya chips with soy oil a centrifugation speed of 100 rpm was applied to obtain 24 % lipids in the chips. Finally, the vessel was pressurized, and the papaya chips were removed from the fryer and cooled down at ambient temperature prior to packaging and storage.

Three treatments were evaluated: 1) S-Min, chips with saturated oil and a minimum lipid content; 2) S-Max, chips with saturated oil and a maximum lipid content; 3) UnS-Min, chips with unsaturated oil and a minimum lipid content. Five frying trials or repetitions were carried out for each frying treatment. After each trial extra oil was added to the vessel to keep the volume of 55 L. Then, the chips from each trial (repetition) were combined manually to obtain a better

homogenization of samples prior to packaging and storage. New oil was used for each treatment (2 batches of palm oil was used for S-Min and S-Max treatments, and 1batch of soy oil was used for UnS-Min treatment).

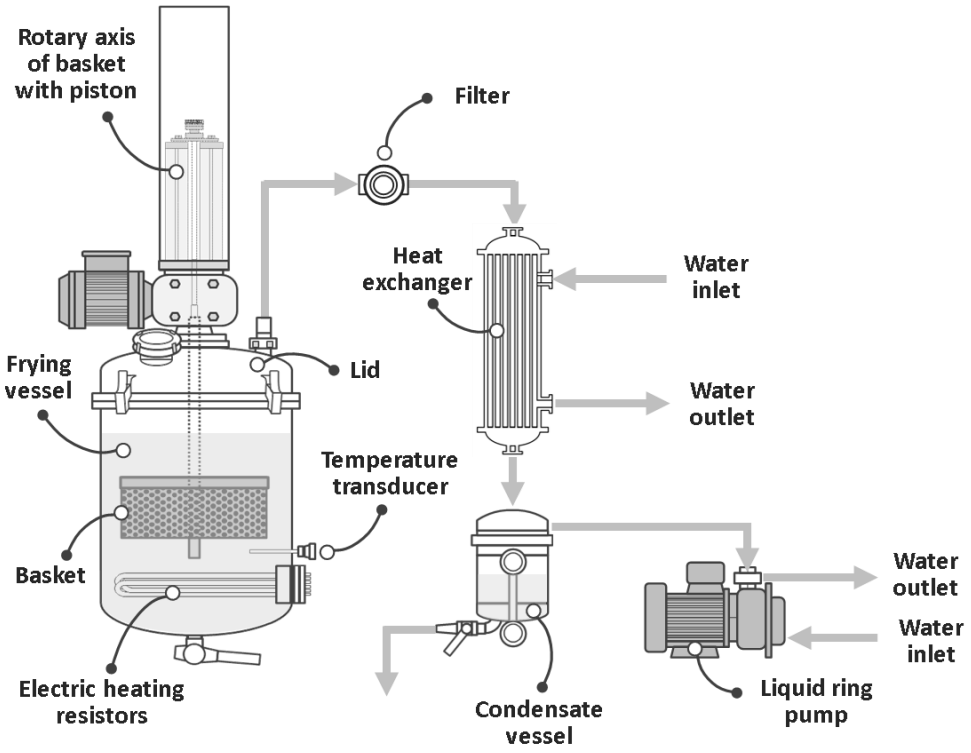

Figure 5.1. Sketch of the vacuum frying system used for obtaining the papaya chips.

2.4. Packaging and storage conditions

Papaya chips obtained by the different treatments were packaged in polyethylene terephthalate metallized/polyethylene (PETm/PE, 12/70 μm) bags (Multivac, France) under compressed air or N_2 conditions using a sealing machine (C200, Multivac, Germany). These bags have a water vapor transmission rate of 1 g/m²·day and an oxygen transmission rate of 1 cm³/m², representing a good barrier against moisture and oxygen, avoiding oxidation reactions in the product. Approximately 5 g of papaya chips were weighed and placed in each package and a gas injection pressure of 0.5 bar was applied into the bags (air or N_2) and then sealed. Samples were stored at 15 °C and 25 °C for 94 days (with sampling times

at 15, 25, 45, 60, 75 and 94 days) and at 35 °C and 45 °C for 52 days (with sampling times at 3, 8, 15, 25, 35 and 52 days). For each frying treatment, packaging atmosphere and storage temperature, three packages of chips (repetitions) were analyzed at each sampling time. A total of 432 packages were stored for the study.

2.5. Kinetic modelling

Kinetic experimental data were presented in the dimensionless form *C/Co* at different storage time intervals *t*, where *C* is the carotenoid concentration and *Co* is the initial amount of the different carotenoids in the chips (*t=0*). Carotenoid degradation can be described by Equation 1.

$$\frac{dC}{dt} = -kC^n \quad [1]$$

where *C* represents the carotenoid concentration (mg/kg fresh weight), *t* the storage time (day), *k* the reaction rate constant (kg/mg·day), and *n* the apparent reaction order.

The apparent order 0, 1 and 2 were tested. To discriminate the appropriate kinetic model, goodness of fit for each kinetic mathematical model (order 0, 1 and 2) was evaluated by R^2 as follow:

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} \quad [2]$$

with SS_{res} being the residual sum of square ($SS_{res} = \sum_i^n (y_i - \hat{y}_i{}^2)$) measuring the discrepancy between the experimental data y_i and the simulated ones $\hat{y}_i$. A small SS_{res} indicates a suitable goodness of fit of the model to the data. $SS_{tot} = \sum_i^n (y_i - \bar{y}^2)$ is the total variance between the experimental data y_i and the mean of the data set $\bar{y}^2$.

The rate constants were assumed to vary with the temperature according to the Arrhenius equation described by Equation 3.

$$k = k_{ref} exp\left(\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \quad [3]$$

where k_{ref} is the rate constant (kg/mg·day) at the reference temperature T_{ref} chosen in the middle of the studied temperature range (303 K), with E_a, T, and R respectively denoting the activation energy (J/mol), medium temperature (K), and gas constant (8.314 J/mol·K). Kinetic constants and activation energies were identified by nonlinear least-squares regression with SigmaPlot software (Systat Software Inc., CA, USA). The model's parameters were identified from experimental data measured in triplicate. The uncertainty of the parameters was calculated by non-linear error propagation using SigmaPlot software (Systat Software Inc., CA, USA).

2.6. Physicochemical analyses

Moisture was determined using AOAC standard method 920.151 (AOAC, 2015). Lipid content was determined by the method described by Carpenter *et al.* (1993). Sugars (sucrose, glucose, and fructose) were determined by HPLC using a Shimadzu LC-6A (Kyoto, Japan) equipped with an EconosphereTM NH2 column (Alltech Associates, Deerfield, IL, USA) and a Shimadzu RID-6A refractive index detector (Kyoto, Japan), using conditions described previously (Gancel, Feneuil, Acosta, Pérez, & Vaillant, 2011). The fatty acid profile was determined using AOAC standard method 996.06 (AOAC, 2015) and AOCS method Ce 1e-91 (AOCS, 2012). Water activities of the samples at 25 °C were determined in triplicate using a water activity meter (Aqualab, model CX-2, Decagon Devices, Inc., Pullman, WA). Vitamins A and E were determined by the method described previously (Lee *et al.*, 2011) with certain modifications. Identification of both vitamins was performed on a reverse-phase HPLC-DAD Shimadzu Prominence (Kyoto, Japan) equipped with a Zorbax Eclipse XDB-C8 column (150 x 4.6 mm i.d, 5 µm) (Agilent, CA, USA) with a guard column, and the mobile phase was a mixture of methanol and water (95:5, v/v). Operation temperature was set at 50 °C. The flow rate was set at 1 mL/min and the injection volume was 10 µL. For vitamin A, retinol-acetate and retinol-palmitate were detected and quantified at 325 nm using their standard curves. For vitamin E, α-tocopherol, δ-tocopherol

and γ-tocopherol were detected and quantified at 295 nm using their standard curves.

2.7. Carotenoid analyses

Extraction procedures and conditions for analysis were described previously by Dhuique-Mayer *et al.* (2016). Briefly, samples were ground and then weighed (500 mg for papaya fruit and 200 mg for papaya chips) in 20 mL tubes. The papaya chip samples were rehydrated with 2 mL of distilled water and homogenized. Then, 2 mL of an ethanol solution containing 1 % pyrogallol was added. The mixture was homogenized using a Vortex mixer and incubated for 2 min in the dark in a water bath at 70 °C. Then, after cooling the samples saponification was performed for 30 min in a water bath at 70 °C by adding 1.5 mL of saturated KOH (12 N). After incubation, the tubes were cooled in an ice bath and 2 ml of distilled water and 5 mL of hexane were added. Then, after mixing and decantation, the organic phase was recovered, and the aqueous phase was extracted two more times with 5 mL of hexane. The organic phases were combined and evaporated to dryness using a vacuum evaporation system (GeneVac EZ-2, SP Scientific, UK). Finally, the residue was re-dissolved in 500 µL of methyl tert-butyl ether (MTBE)/methanol (80/20) and placed in an amber vial prior to HPLC analysis.

Carotenoid identification was performed on a reverse-phase HPLC-DAD Agilent 1100 system (Massy, France) with a diode array detector according to Dhuique-Mayer *et al.* (2007). Carotenoids were separated using a C30 column (250 x 4.6 mm i.d., 5 µm) (YMC EUROP GmbH, Germany) with a guard column, and the mobile phase was H_2O as eluent A, methanol as eluent B, MTBE as eluent C. Operation temperature was set at 25 °C. The flow rate was set at 1mL/min and the injection volume was 20 µL. A solvent gradient was programmed as follows: 0-2 min, isocratic 40 % A – 60 % B (initial conditions); 2–5 min, 20 % A – 80 % B; 5-10 min, 4 % A – 81 % B – 15 % C; 10-60 min, 4 % A – 11 % B – 85 % C; 60-70 min, isocratic 4 % A – 11 % B – 85 % C; 70-71 min, 100 % B; 71-72 min, with a return to the initial conditions for rebalancing. All-E-β-cryptoxanthin, all-E-β-carotene and its isomer were detected at 450 nm, and all-E-lycopene and their isomers were detected at 470 nm (Dhuique-Mayer *et al.*, 2007). Z-lycopene

content was expressed as the sum of all Z-lycopene isomers. Isomers were identified according to their relative retention times, *i.e.*, elution order and the combined use of their spectral data. The identifications were based on previously published data obtained with the same mobile phase (water/methanol/MTBE) and the same detection wavelength range (Achir *et al.*, 2015; Achir *et al.*, 2010; Chanforan *et al.*, 2012; Martins *et al.*, 2016; Schweiggert *et al.*, 2012).

2.8. Statistical analyses

The influence of vacuum frying was evaluated using the Student's t-test to detect significant differences ($p<0.05$) between treatment values. In order to evaluate the effect of lipid content and type of oil on carotenoid degradation in papaya chips packaged under air conditions during storage a comparison of Arrhenius parameters was carried out using the Student's t-test ($p<0.05$). Two-way ANOVA was used to analyze the effects of storage temperature and packaging atmosphere (effects were considered significant when $p<0.05$). If significantly different, means were further compared using Tukey's test. Statistical analyses were performed with JMP 14 software (SAS Institute Inc., USA).

3. Results and discussion

3.1. Physicochemical properties of fresh papaya and papaya chips

Table 5.1 shows moisture, lipids, sugar contents, a_w and carotenoid contents in fresh papaya and papaya chips obtained after vacuum frying. The means of the different physicochemical properties of the chips obtained from the three frying treatments were reported, except for the lipid content. The papaya chips presented a low moisture content (~2 g/100 g) and a_w (~0.2), which are typical values for this kind of food product. The lipid content was ~24 g/100 g fresh weight (FW) for S-Min and UnS-Min treatments, and ~29 g/100 g FW from S-Max treatment as expected (*section 3.4*). Sugar content in fresh papaya was less than 10 % of its composition (fresh weight). Whereas, in papaya chips almost 44 % of the product (fresh weight) was made up of sugars.

Table 5.1. Physicochemical properties of the oils (palm and soy oils) and the papaya products (fresh papaya and papaya chips obtained by vacuum frying at *t=0*).

Frying oils		
Compound	**Palm oil**	**Soy oil**
Lipids (g/100 g FW)*	99.97 ± 0.02	99.99 ± 0.01
Vitamin A (mg/100 g FW)*	ND	ND
Vitamin E (mg/100 g FW)*	228.10 ± 23.77	265.24 ± 0.83
Fatty acid profile (% relative):		
Saturated	54.47	15.45
Monounsaturated	36.18	19.08
Polyunsaturated (ALA, ω-3)	0.22	9.13
Polyunsaturated (LA, ω-6)	9.15	56.34
Papaya products		
Compound	**Fresh papaya**	**Papaya chips**
Moisture (g/100 g FW)**	86.96 ± 0.40	1.90 ± 0.12
Water activity, a_w**	0.988 ± 0.003	0.226 ± 0.013
Lipids (g/100 g FW)**	ND	23.90 ± 1.38[a] 29.25 ± 1.43[b] 24.10 ± 0.96[c]
Total sugars (g/100 g FW)**	8.51 ± 0.24	44.33 ± 0.75
Carotenoids (mg/kg FW)***:		
all-E-β-cryptoxanthin	4.20 ± 0.46	9.56 ± 1.57
all-E-β-carotene	3.30 ± 0.70	11.11 ± 2.29
all-E-lycopene	13.07 ± 1.73	105.06 ± 14.86
Z-β-carotene	0.40 ± 0.06	7.46 ± 1.43
Z-lycopene	0.56 ± 0.09	23.26 ± 3.49
Total carotenoids	21.53 ± 2.16	156.45 ± 20.98
Carotenoids (mg/kg NF-DW)***:		
all-E-β-cryptoxanthin	32.42 ± 2.84	13.42 ± 2.29
all-E-β-carotene	25.37 ± 4.84	15.38 ± 3.22
all-E-lycopene	101.01 ± 13.15	145.97 ± 25.66
Z-β-carotene	3.09 ± 0.41	10.32 ± 2.00
Z-lycopene	4.34 ± 0.62	32.22 ± 5.10
Total carotenoids	166.25 ± 14.24	217.14 ± 35.0

*Values are expressed as the mean ± standard deviation (n=2). **Values are expressed as the mean ± standard deviation (n=3). ***Values are expressed in fresh weight as the mean ± standard deviation (n=9). FW, fresh weight; NF-DW, non-fat dry weight; ND, not detectable (vitamin A < 0.18 mg/100 g; lipids < 0.10 g/100 g). ALA, alpha linolenic acid; LA, linoleic acid. Lipid content in papaya chips: [a]for S-Min treatment, [b]for S-Max treatment and [c]for UnS-Min treatment.

Eight carotenoids were identified in papaya chips using HPLC-DAD (Figure 5.2, Table 5.2). Three E-carotenoids, all-E-β-cryptoxanthin (BCX), all-E-β-carotene

(BC), and all-E-lycopene (LYC) were identified according to their standard retention times and the combined use of their spectral data (Chanforan *et al.*, 2012; Martins *et al.*, 2016; Schweiggert *et al.*, 2012). One Z-isomer for β-carotene (Z-BC) was tentatively identified, namely 13Z-β-carotene (Achir et al., 2015; Achir *et al.*, 2010) (Table 5.2). Finally, four Z-lycopene isomers (Z-LYC) were detected, these were tentatively identified, namely 13Z-, 5Z-13'Z-, 9Z- and 5Z-lycopene according to their III/II ratio, their *cis* peak intensity, and maximal absorption wavelength (Chanforan *et al.*, 2012; Schweiggert *et al.*, 2012) (Table 5.2). Fresh papaya fruit presented only one LYC isomer (5Z) as is shown in Figure 5.2a. Otherwise, three more Z-LYC isomers were detected in papaya chip samples (Figure 5.2b) as a consequence of heating during vacuum frying.

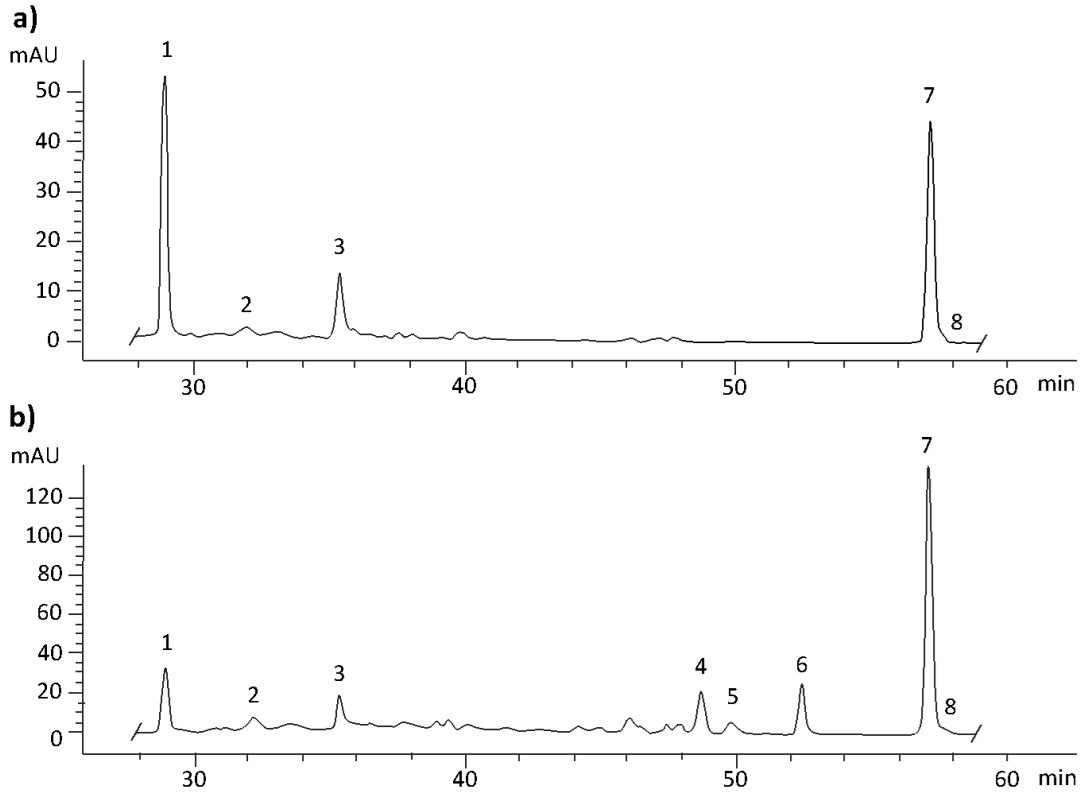

Figure 5.2. HPLC separation of analyzed carotenoids monitored at λ=450 nm from: a) fresh papaya, b) papaya chips. For peak assignment see Table 5.2.

Table 5.1 also shows the carotenoid contents in fresh papaya and papaya chips. Fresh papaya samples contained 21.53 ± 2.16 mg/kg fresh weight (FW) of total carotenoids. These results are similar to those reported for red-fleshed papaya

fruit (Gayosso-García Sancho *et al.*, 2011; Schweiggert *et al.*, 2011a; Schweiggert *et al.*, 2011b; USDA, 2020). Total carotenoid content in papaya chips was 156.45 ± 20.98 mg/kg FW. There is no previous information about carotenoid content in papaya chips, but this content is comparable to other similar products such as non-fat orange sweet potato chips (Bechoff *et al.*, 2010) and fried orange sweet potato chips (Da Silva & Moreira, 2008), but it is 11 and 17-fold higher in comparison to fried carrot chips (Dueik & Bouchon, 2011), and fried mango chips (Da Silva & Moreira, 2008), respectively. These results showed that papaya chips are a good source of carotenoids, especially LYC, and could be an alternative for vitamin A consumption as they provide retinol activity thanks to BC and BCX.

Table 5.2. UV-Vis spectra of analyzed carotenoids in fresh papaya and papaya chips.

No.	Retention time (min)	Carotenoid	HPLC-DAD UV-Vis spectrum (nm)	III/II (%)	A_B/A_{II} (%)
1	28.9	*all-E*-β-cryptoxanthin	(424), **450**, 478	19	-
2	31.9	*13Z*-β-carotene	342, (418), **438**, 464	6	35
3	35.4	*all-E*-β-carotene	(422), **452**, 476	10	-
4	47.5	*13Z*-lycopene	362, 440, **466**, 496	49	62
5	48.8	*5Z-13'Z*-lycopene	360, 432, **458**, 488	46	30
6	51.9	*9Z*-lycopene	362, 440, **466**, 496	63	18
7	57.3	*all-E*-lycopene	446, **472**, 502	68	-
8	57.8	*5Z*-lycopene	446, **472**, 502	69	-

Values in bold represent the main maximum. Values in brackets mark point of inflection. III/II, ratio of the height of the longest-wavelength absorption peak (III) and that of the middle absorption peak (II), taking the minimum between the two peaks as baseline, multiplied by 100. A_B/A_{II}, ratio of the height of *cis*-peak (A_B) and that of the middle main absorption peak (A_{II}), multipled by 100.

LYC in both fresh papaya and chip samples was the main carotenoid, representing 61 and 67 % of total carotenoids, respectively. Moreover, BC and BCX decreased their relative proportion after vacuum frying, from 15 % (BC) and 20 % (BCX) in fresh papaya to 7 % (BC) and 6 % (BCX) in the chips. The relative proportion of Z-BC increased from 2 % (fresh papaya) to 5 % (chips), and from 3 % (fresh papaya) to 15 % (chips) for Z-LYC.

Indeed, by comparing the amount of CTs expressed as non-fat dry weight between fresh papaya and papaya chips (Table 1), there was a significant effect of vacuum frying on all carotenoid contents according to Student's t-test ($p<0.05$). Under the applied conditions (T= 120 °C, P= 0.25 bar, t= 13 min), 60 % and 40 % of BCX and BC degradation occurred, respectively, whereas LYC showed an increase of 45 % after vacuum frying. LYC has poor solubility and accumulates in the papaya matrix as crystals associated with the plastid membranes (Schweiggert *et al.*, 2011a). Thus, oil uptake in the chips allows the crystalline structures of lycopene to dissolve which increases its extractability. For the Z-forms, there was an increase in Z-BC and Z-LYC of 2.3 and 6.4-fold, respectively. This is in agreement with previously reported literature, which indicates that food processing favors the conversion of CTs from all-E to Z-isomers at temperatures higher than 100 °C especially when these compounds are dissolved in lipids (Achir *et al.*, 2015, Achir *et al.*, 2011; Ahmed *et al.*, 2002; Ayari *et al.*, 2015; Kourouma *et al.*, 2019).

3.2. Effect of packaging atmosphere (air versus nitrogen) and storage temperature on carotenoid degradation in papaya chips

The oxygen present in packaging had a drastic effect on total carotenoid content. Indeed, for all chips stored under nitrogen, a slight or no significant degradation was observed whatever the temperature, the type of oil or the lipid content. Figure 5.3 compares the means of carotenoid retention of chips packaged under both air and N_2 conditions after 1 month of storage at 15 and 45 °C (chips obtained from the 3 treatments). Results from the two-way ANOVA showed that the interactions between the two factors (storage temperature and packaging atmosphere) were significant ($p<0.05$) only for BCX retention. Total BC was affected by both packaging atmosphere and storage temperature ($p<0.05$). In the case of LYC, only the packaging atmosphere was significant ($p<0.05$).

Stored samples packaged under compressed air (21 % oxygen) exhibited a higher degradation of BCX (35-62 %), total BC (47-69 %) and total LYC (53-63 %). With no oxygen present during the storage conditions (bags flushed with N_2), only a small decrease was observed in most CTs. This phenomenon was observed throughout the storage study for all the test temperatures. Minimal or no

degradation of CTs was achieved regardless of lipid composition (type of oil and lipid content) in papaya chips (data not shown). Papaya chips packaged under N_2 and stored at 15 and 45 ºC did not present significantly different BCX and total LYC concentrations ($p>0.05$). In the case of the chips packaged under air conditions, the storage temperature altered only BCX and total BC retention ($p<0.05$); after 1 month of storage, an increase of 30 ºC caused a degradation of 42 % and 41% of BCX and total BC, respectively.

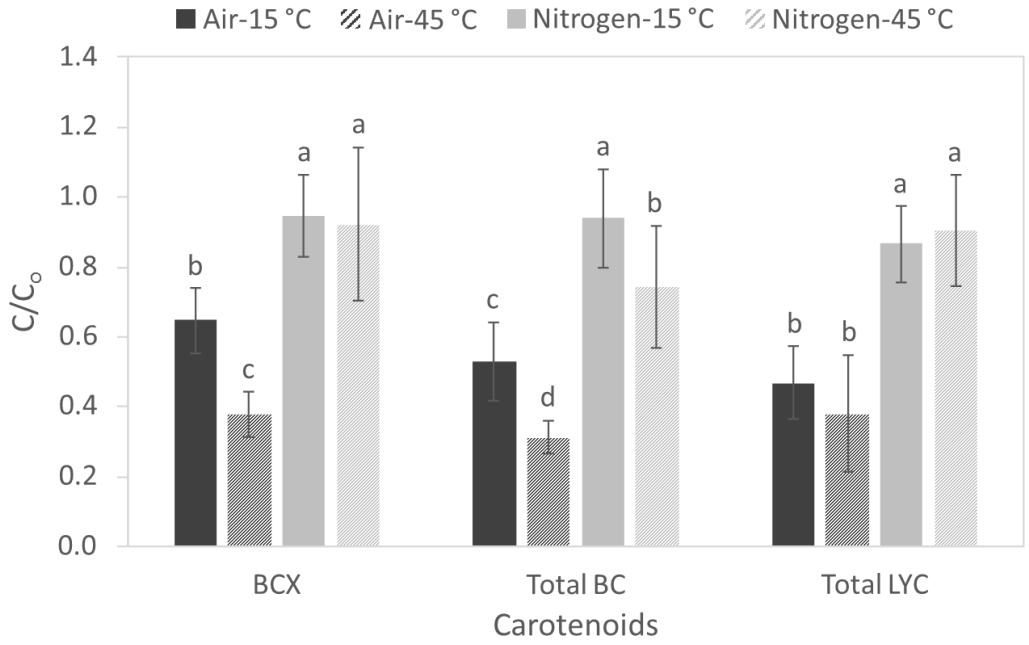

Figure 5.3. Variation of dimensionless concentrations (C/C_0) of different carotenoids in papaya chips packaged under air or nitrogen conditions after 1 month of storage at 15 and 45 ºC**.** Bars are expressed as the means ± standard deviation (n=9). For each carotenoid means with the same letter are not significantly different (Student's t-test, $p<0.05$). BCX, all-E-β-cryptoxanthin; Total BC, all-E-β-carotene + Z-β-carotene; Total LYC, all-E-lycopene + Z-lycopene.

Figure 5.4 shows the clear positive effect of packaging papaya chips under N_2 conditions at 45 ºC (maximum temperature evaluated in this study). These results showed how oxygen in package head space has a severe impact on carotenoid degradation. Oxygen diffuses in the fried product and generates carotenoid oxidation during storage, having an even greater effect than during the vacuum-frying process. This reveals that packaging under low oxygen

conditions may be applied to preserve the maximal carotenoid content in this kind of food product during storage.

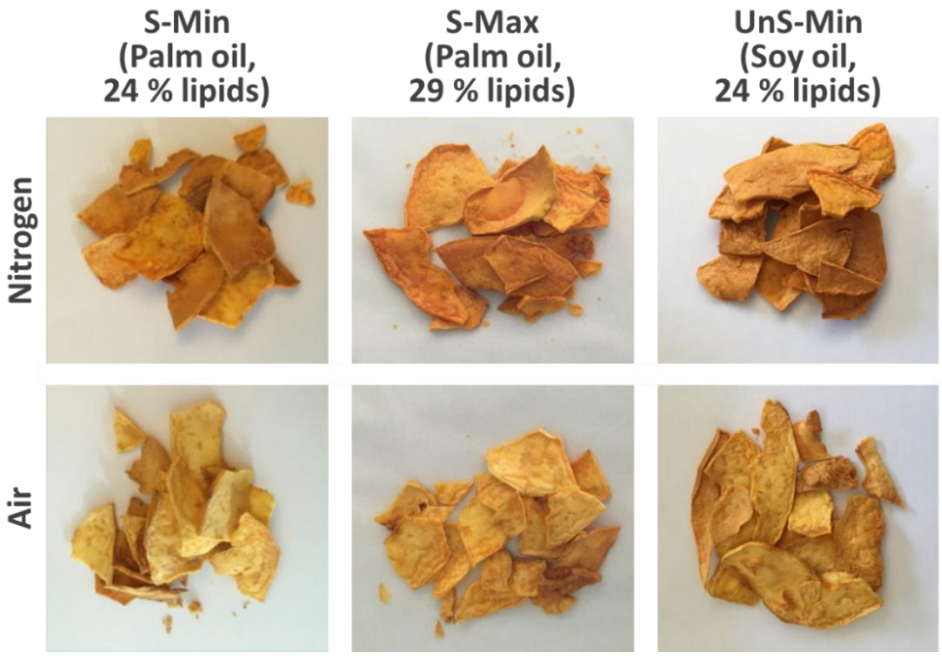

Figure 5.4. Papaya chips packaged under air or nitrogen conditions after 1 month of storage at 45 °C.

3.3. Carotenoid degradation kinetics in papaya chips packaged with air during storage

In the case of papaya chips packaged with air, a degradation of different CTs was observed during the storage time, regardless of the treatment as is shown in Figure 5.5. The dimensionless concentrations of all CTs decreased with storage time at all the test temperatures. Notably, CTs decreased more rapidly at higher temperatures (35-45 °C). Kinetic models were set up based on the analysis of experimental dimensionless concentrations over the storage time for each carotenoid. Second-order kinetics appeared to best fit the experimental data compare to first or zero-order reactions. Indeed, calculations made for BCX, BC and LYC revealed that second order showed the highest R^2 (Equation 2) for 32 data set over 36. Therefore, the mathematical model that describe best the trend of carotenoid degradation in papaya chips is the second-order model. This is in

accordance with the result of different papers that deals with carotenoid degradation during thermal degradation (Achir *et al.*, 2011; Hadjal *et al.*, 2013).

When considering an elementary reaction, the second order model is associated to a bimolecular reaction. In our case, this is probable since degradation was proven to be linked with oxidation and the co-reactant may be a reactive species derived from oxygen. However, it is not possible to go further into the reaction mechanism since this order is only an apparent one and do not represent precisely the complex reaction mechanism that involve isomerization reaction and various oxidation pathways as depicted in a paper about β-carotene degradation in dried orange-fleshed sweet potato during storage (Achir *et al.*, 2014).

Carotenoid degradation is a complex reaction in food that is supposed to change as a function of the matrix and its storage conditions (Penicaud *et al.*, 2011). In this case, carotenoid degradation in the chips occurs due to oxidative reactions because of the presence of oxygen inside the bags. The resulting rate constants are presented in Table 5.3. All rate constants increased with temperature. Globally, the all-E-carotenoids were more stable than the Z-forms. The LYC rate constants were the lowest (S-Min, from 0.46 to 0.95 x 10^{-3} kg/mg·day, S-Max, from 0.25 to 0.31 x 10^{-3} kg/mg·day, UnS-Min, from 0.11 to 0.22 x 10^{-3} kg/mg·day) which indicates that LYC was the most stable carotenoid in papaya chips during storage, regardless of the treatment. BCX and BC presented similar degradation rate constants for S-Max and UnS-Min treatments. Nevertheless, in chips obtained by S-Min, rate constants for BCX (from 1.84 to 11.63 x 10^{-3} kg/mg·day) were higher than those for BC (from 1.21 to 7.40 x 10^{-3} kg/mg·day).

No isomers (Z-BC and Z-LYC) were produced during storage, certainly because of the low temperature. Isomerization is a reaction assumed to be sensitive to temperature that does not occur at temperatures lower than 100 ºC (Colle *et al.*, 2010a). Among the Z-carotenoids present after frying, Z-BC showed the highest degradation rate constants for all treatments (S-Min, from 4.39 to 24.68 x 10^{-3} kg/mg·day, S-Max, from 2.65 to 16.25 x 10^{-3} kg/mg·day; UnS-Min, from 3.47 to 17.37 x 10^{-3} kg/mg·day).

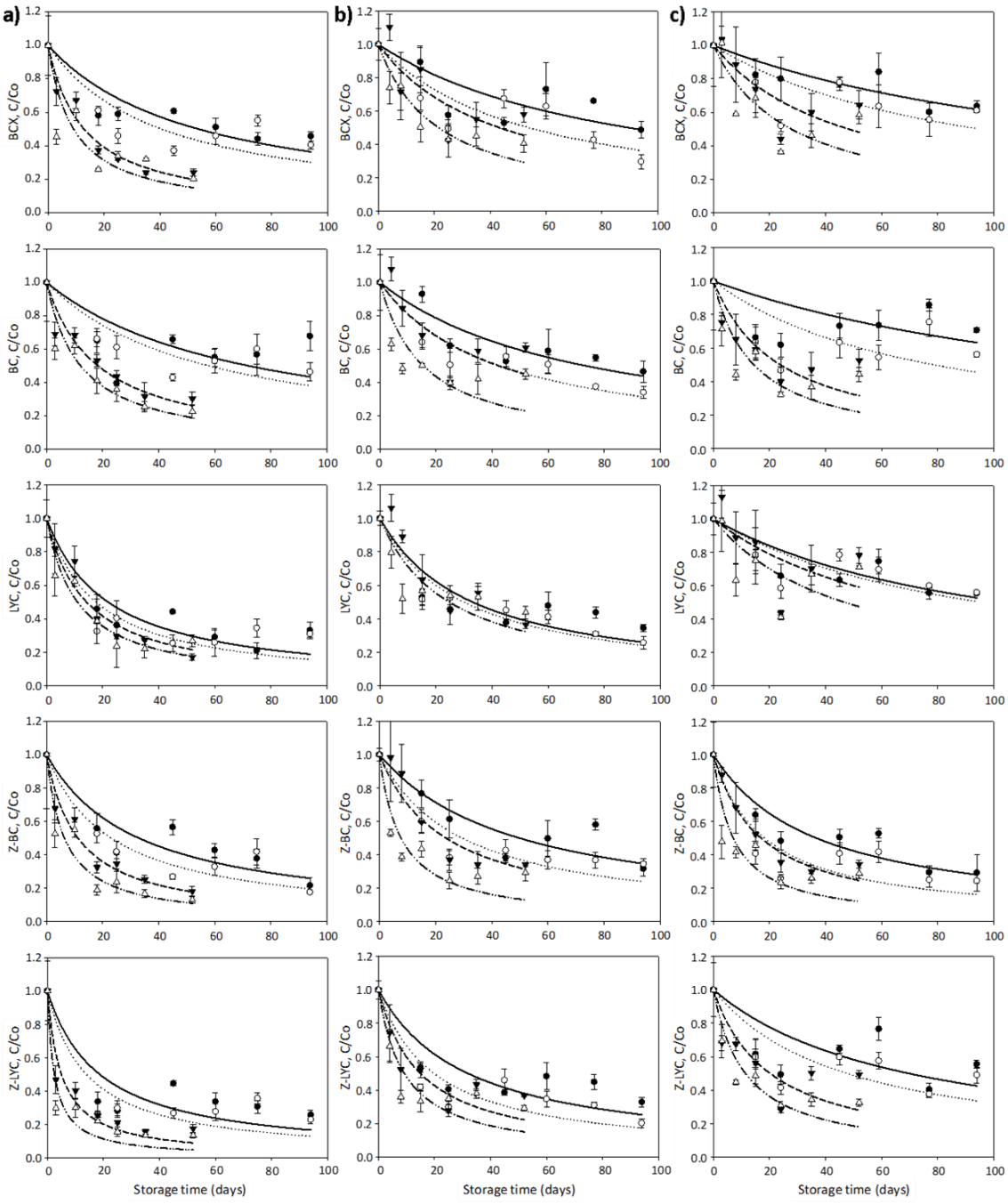

Figure 5.5. Variation of dimensionless concentrations (C/C_o) of different carotenoids in papaya chips packaged under air conditions during the storage at four temperatures (● 15 ºC, ○ 25 ºC, ▼ 35 ºC, △ 45 ºC): a) S-Min (palm oil, 24 % lipids), b) S-Max (palm oil, 29 % lipids), c) UnS-Min (soy oil, 24 % lipids). Lines represent modeled data (— 15 ºC, ···· 25 ºC, – – 35 ºC, —·· 45 ºC). BCX, all-E-β-cryptoxanthin; BC, all-E-β-carotene; LYC, all-E-lycopene; Z-BC, Z-β-carotene; Z-LYC, Z-lycopene. Values are expressed as the means ± standard deviation at each storage time (n=3).

Table 5.3. Second-order rate constants and theoretical half-life of different carotenoids in papaya chips packaged under air conditions during the storage at four temperatures.

Treatments*	k x 10^{-3} (kg/mg·day)				$t_{1/2}$ (days)			
	15 °C	25 °C	35 °C	45 °C	15 °C	25 °C	35 °C	45 °C
BCX, all-E-β-cryptoxanthin								
S-Min	1.84 (0.21)	2.44 (0.34)	7.70 (0.86)	11.63 (2.35)	54	41	13	8
S-Max	1.12 (0.17)	1.86 (0.25)	2.26 (0.43)	4.68 (0.57)	90	54	45	21
UnS-Min	0.77 (0.13)	1.28 (0.24)	2.43 (0.51)	3.99 (0.82)	150	91	48	29
BC, all-E-β-carotene								
S-Min	1.21 (0.25)	1.51 (0.21)	4.77 (0.55)	7.40 (1.09)	72	58	18	12
S-Max	1.20 (0.14)	2.05 (0.22)	2.08 (0.37)	5.36 (0.89)	74	43	42	16
UnS-Min	0.61 (0.16)	1.26 (0.29)	3.86 (0.72)	6.08 (1.18)	156	75	24	16
LYC, all-E-lycopene								
S-Min	0.46 (0.05)	0.57 (0.09)	0.70 (0.07)	0.95 (0.13)	22	18	14	11
S-Max	0.25 (0.03)	0.27 (0.02)	0.25 (0.03)	0.31 (0.04)	33	30	32	26
UnS-Min	0.11 (0.01)	0.10 (0.01)	0.14 (0.04)	0.22 (0.05)	94	104	75	49
Z-BC, Z-β-carotene								
S-Min	4.39 (0.68)	6.29 (0.96)	12.93 (1.65)	24.68 (4.91)	33	23	11	6
S-Max	2.65 (0.31)	4.48 (0.47)	5.75 (0.87)	16.25 (2.20)	50	29	23	8
UnS-Min	3.47 (0.42)	7.10 (1.27)	7.49 (0.84)	17.37 (3.39)	37	18	17	7
Z-LYC, Z-lycopene								
S-Min	2.14 (0.35)	2.88 (0.50)	7.95 (1.08)	16.94 (3.51)	19	14	5	2
S-Max	1.30 (0.17)	2.09 (0.29)	2.66 (0.36)	4.17 (0.61)	32	20	16	10
UnS-Min	0.72 (0.14)	1.06 (0.18)	2.37 (0.44)	4.01 (0.55)	67	45	20	12

*Treatments: S-Min, saturated oil and minimum lipid content (palm oil with 24 % lipids); S-Max, saturated oil and maximum lipid content (palm oil with 29 % lipids); UnS-Min, unsaturated oil and minimum lipid content (soy oil with 24 % lipids). Standard error is expressed in brackets (n=3).

Figure 5.6 shows the rate constants of reference (k_{ref}) and the activation energies (E_a) for the different carotenoids in PCs. All CTs followed Arrhenius temperature-dependency pattern (most models with $R^2>0.95$). The rate constants of reference (k_{ref}) varied from 1.80 to 4.80 x 10^{-3} kg/mg·day for BCX, from 2.20 to 3.00 x 10^{-3} kg/mg·day for BC, from 0.10 to 0.70 x 10^{-3} kg/mg·day for LYC, from 5.10 to 9.50

x 10^{-3} kg/mg·day for Z-BC, and from 1.70 to 5.20 x 10^{-3} kg/mg·day for Z-LYC. Approximately, the BCX and BC were degraded 7-18 and 4-23-fold faster than LYC, respectively. However, when comparing the Z-isomers, the k_{ref} of Z-BC was 2-4-fold superior to the values obtained for Z-LYC. This indicates that isomerization has a more dramatic effect on lycopene degradation, which is the main carotenoid in papaya.

E_a were 40-48 kJ/mol for BCX, 45-52 kJ/mol for BC, 5-21 kJ/mol for LYC, 43-60 kJ/mol for Z-BC, and 29-63 kJ/mol for Z-LYC. Lycopene was thus less sensitive to an increase in temperature than the other carotenoids in its all-E-conformation. However, when isomerized, Z-LYC tends to have a similar sensitivity to temperature than Z-BC. The E_a values of BC and LYC in the papaya chips were in accordance with those previously reported by Achir *et al.* (2015) in thermally treated grapefruit juice (45 ± 10 kJ/mol for BC, and 26 ± 10 kJ/mol for LYC); Colle *et al.* (2012) also found similar activation energies in a tomato emulsion during a heat treatment, 45 and 28 kJ/mol for BC and LYC, respectively.

Because this study did not analyze the degradation products especially from oxidation reactions, it is not possible to deepen the reaction mechanism, but some authors have studied and proposed some mechanisms. In the case of β-carotene, the oxygen can attack this molecule either on the β-ring or on the chain. This radical attack followed by homolytic internal substitution gives the epoxides, which appear as initial products. The stable final products, the apocarotenones and apocarotenals, could be formed from the epoxides (Mohamed *et al.*, 2001; Pénicaud *et al.*, 2011). If the attack of the oxygen molecule is done on the chain of β-carotene, in chain epoxides are formed, especially on *cis* isomers, due to the higher availability of the π-electrons in these *cis* forms (Pénicaud *et al.*, 2011). Oxidation of lycopene occurs on both the isolated double bonds and the terminal double bonds of the conjugated double bond system, followed by rearrangements, resulting in many lycopene epoxides (Rodriguez & Rodriguez-Amaya, 2009). Kobori *et al.* (2014) found that interaction of oxygen with the lycopene molecule is manifested by a high abundance of the compounds 6-methyl-5-hepten-2-one, geranial, 2,3-epoxy-geranial and rose oxide. For β-cryptoxanthin, information

about oxidation mechanisms is scarce. For instance, in a model system two oxidation products derived from this carotenoid affected by non-iodine-catalyzed illumination were identified, 5,6-epoxy-β-β-carotene-3-one and β-β-carotene-3-one (Li *et al.*, 2015).

3.4. Effect of lipid content (24 % versus 29 %) and type of oil (soy versus palm) on carotenoid degradation in papaya chips packaged with air during storage

According to data shown in Table 5.3, degradation was faster in the S-Min treatment (chips with palm oil and 24 % lipid content) whatever the carotenoid and its configuration (all-E and Z- forms). In S-Max samples (chips with palm oil and 29 % lipid content) the degradation rate constants of Z-BC were the lowest. Moreover, it was found that LYC, Z-LYC and BCX were more stable in the chips obtained with the UnS-Min treatment (chips with soy oil and 24 % lipid content). Table 5.3 also shows the theoretical half-life ($t_{1/2}$) of each individual carotenoid that was calculated as representative of the second-order kinetic reaction according to the Equation 4.

$$t_{1/2} = \frac{1}{kC_o} \quad [4]$$

The t½ is dependent on initial carotenoid concentration. For the same reactivity, the highest C_0 the lowest t½. For each carotenoid as temperature increased, t½ values decreased in a consistent manner with faster reactions accompanied by higher rate constants (Table 5.3). The increase in storage temperature from 15 to 45 ºC caused a reduction of the t½ of 4.2-6.3, 4.5-10.0 and 1.3-2.1-fold for BCX, BC and LYC, respectively. The t½ values for LYC (the main carotenoid) at 25 ºC (typical shelf storage temperature for this kind of product) were 18, 30 and 104 days for S-Min, S-Max and UnS-Min treatments, respectively. Moreover, their isomers (Z-LYC) presented t½ values of 14, 20 and 45 days for S-Min, S-Max and UnS-Min treatments, respectively. The lower t½ of lycopene in S-Min demonstrates that this carotenoid is more sensitive to degradation when papaya chips are obtained under S-Min conditions (palm oil and 24 % lipid content) in comparison to the other treatments, S-Max and UnS-Min.

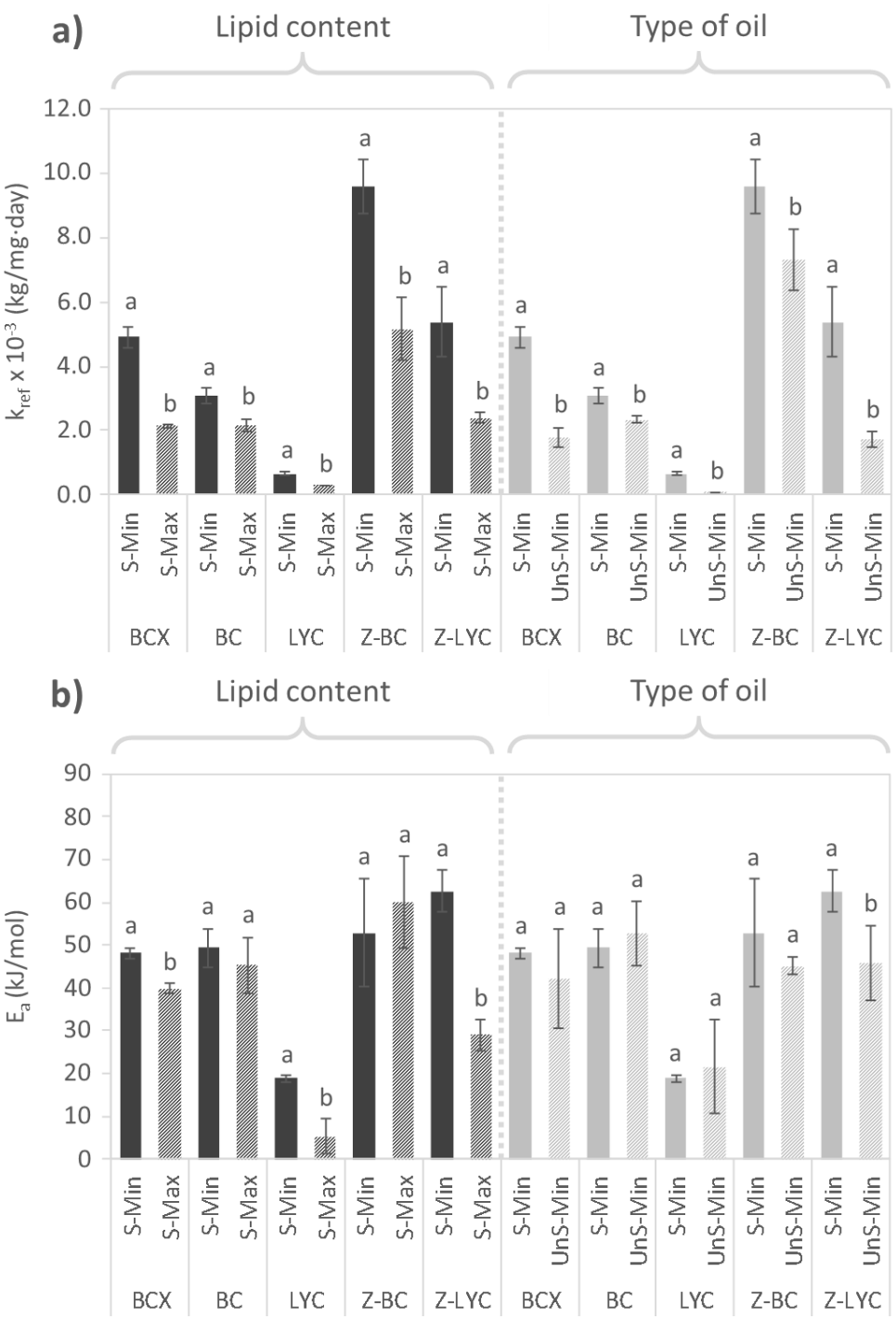

Figure 5.6. Arrhenius parameters (at a reference temperature of 30 °C) of carotenoids in papaya chips packaged under air conditions during the storage at different temperatures (15-45 °C): a) k_{ref}, b) E_a. Bars are expressed as the means ± standard deviation (n=3). For each carotenoid means with the same letter are not significantly different (Student's t-test, $p<0.05$). BCX, all-E-β-cryptoxanthin; BC, all-E-β-carotene; LYC, all-E-lycopene; Z-BC, Z-β-carotene; Z-LYC, Z-lycopene. S-Min, palm oil with 24 % lipids; S-Max, palm oil with 29 % lipids; UnS-Min, soy oil with 24 % lipids.

A comparison of Arrhenius parameters of different carotenoids in the papaya chips packaged with air was carried out in order to analyze the effect of lipid content (S-Min vs S-Max) and type of oil (S-Min vs UnS-Min) as shown in Figure 5.6. When evaluating the effect of palm oil content in the papaya chips on carotenoid degradation, it was found that the k_{ref} values for the different CTs in S-Min (24 % lipids) were significantly higher ($p<0.05$) compared to those obtained in S-Max (29 % lipids). The k_{ref} values were ~1.4-fold higher for BC, and ~2-fold higher for BCX, LYC, Z-BC, and Z-LYC (Figure 5.6a). This means that CTs are less stable during storage when papaya chips have a lower lipid content. A hypothesis to explain this behavior is that the presence of a higher lipid content in the chips could cause a protective effect by replacing the voids that are filled with air, thus reducing the oxidation of CTs. Figure 5.6b shows that BCX, LYC and Z-LYC in the chips with higher lipid content (S-Max) had significantly lower activation energies ($p<0.05$). When increasing the lipid content, the values of E_a decreased from 48 to 40 kJ·mol^{-1}, from 19 to 5 kJ·mol^{-1} and from 63 to 29 kJ·mol^{-1} for BCX, LYC and Z-LYC, respectively.

Analyzing the effect of the type of oil (palm vs soy) in papaya chips with 24 % lipids, it was observed that the k_{ref} values of all CTs were significantly higher ($p<0.05$) in S-Min compared to those in UnS-Min. The k_{ref} values were ~1.3-fold higher for BC and Z-BC, ~3-fold higher for BCX and Z-LYC, and ~7-fold higher for LYC (Figure 5.6a). The palm oil used in this study presented 54.45 % and 45.55 % of saturated and unsaturated fatty acids (UFA), respectively; while UFA represented 84.55 % of soy oil (mostly linoleic acid, ω-6) (Table 5.1). The greater retention of CTs in papaya chips with soy oil could be explained by several hypotheses: 1) competition for oxygen is generated between the double bonds of UFA present in soy oil and carotenoids, however, once these UFAs are oxidized, this oil could turn pro-oxidant, in this case further studies would be necessary to evaluate the lipid stability in PCs during long term storage; 2) the lower viscosity of soy oil during cooling compared to palm oil could favor deeper oil absorption inside the pores that are generated in the fried product, replacing the voids that are filled with air and thus reducing carotenoid oxidation. This alternative to saturated oil could be nutritionally interesting provided that the PCs are stored

for a limited period and the soy oil is of good quality. Therefore, matrix lipid composition (lipid content and type of oil) affected kinetic parameters of CTs and demonstrated a protective role on CTs, especially for lycopene. In addition, more research shall be required to understand how differences in food matrix (type of oil, lipid content) can affect kinetic parameters of CTs.

3.5. Effect of packaging atmosphere (air versus nitrogen) and type of oil (soy versus palm) on the nutritional features of papaya chips during storage

The relationship between storage time and experimental data of vitamin A activity, total lycopene content and theoretical content of polyunsaturated fatty acids for papaya chips packaged with air and nitrogen are described in Figure 5.7, depending on the type of oil. According to experimental data, PCs with an initial nutritional value of 163 μg Retinol Activity Equivalent (RAE) (portion of 100 g) packaged under air conditions and stored for 2 months at an ambient temperature of 25 ºC, which is the average storage temperature in the daytime under tropical temperatures, retained about 52 and 48 % of this value for chips with soy oil (UnS-Min) and palm oil (S-Min), respectively. On the other hand, total lycopene content (sum of all-E and Z-isomers) in PCs packaged with air (initial content of 12.8 mg/100 g chips, fresh weight) retained about 60 and 26 % of the initial total lycopene content after 2 months at 25 ºC, for UnS-Min and S-Min, respectively. Figure 5.7 also shows vitamin A activity and total lycopene for chips packaged with nitrogen based on experimental data. Provitamin A and lycopene were stable in chips packaged under N_2 conditions after 2 months of storage, as no degradation was observed.

Arrhenius model predictions of vitamin A and total lycopene in PCs after 6 months of storage (typical shelf-life of this kind of product) are also shown in Figure 5.7. If PCs packaged with air were stored for 6 months at 25 ºC, only 18 and 12 % of initial RAE would be retained for UnS-Min and S-Min, respectively. About 31 and 7 % of total lycopene would be retained if the chips were stored for 6 months at 25 ºC. In chips packaged with nitrogen there would be no carotenoid degradation after 6 months by assuming a k_{ref} value of zero. Provitamin A and lycopene were more stable in the presence of soy oil in chips packaged with air.

This is remarkably interesting from a nutritional point of view, since the chips with soy oil would have a better nutritional composition providing essential fatty acids.

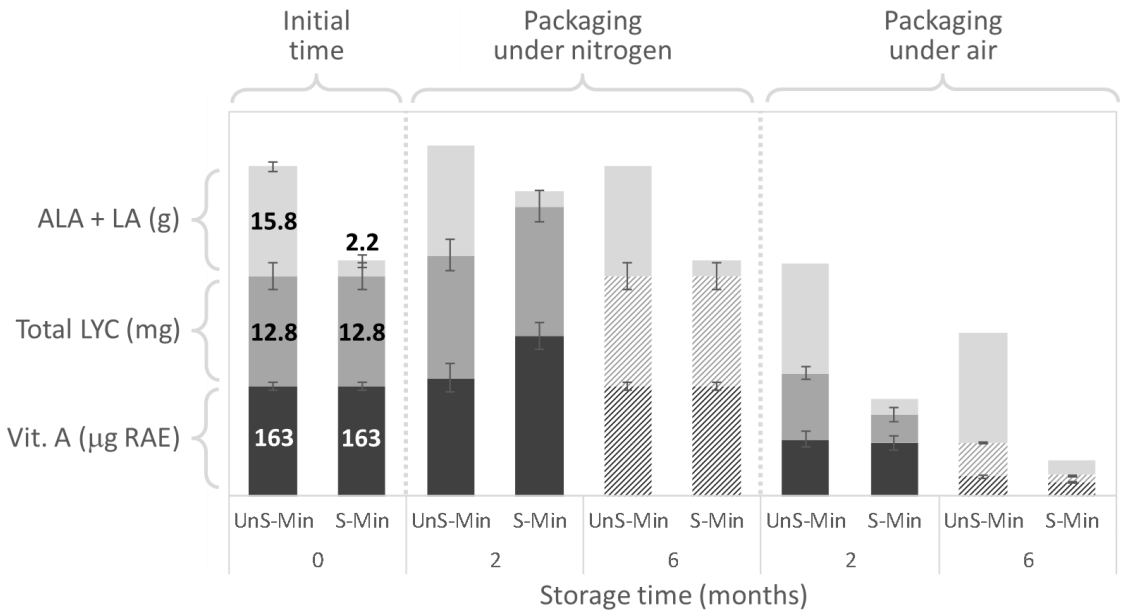

Figure 5.7. Vitamin A activity, total lycopene content and theoretical content of polyunsaturated fatty acids in 100 g portion of papaya chips packaged under air or nitrogen conditions during the storage at 25 ºC. Solid bars: data based on experimental results. Stripe bars: data based on Arrhenius model predictions. Bars are expressed as the means ± standard deviation (n=3). Vit A, Vitamin A activity: Retinol Activity Equivalent (RAE) estimate was calculated for a bioconversion of 12:1. RAE= [(all-E-β-carotene/12) + (minor compounds/24)] x unit (g). Minor compounds are Z-β-carotene and all-E-β-cryptoxanthin. Total LYC, Total lycopene content: it was calculated as the sum of all-E and Z-isomers in papaya chips, fresh weight. ALA + LA, alpha linolenic acid (ω-3) + linoleic acid (ω-6). S-Min, palm oil with 24 % lipids; UnS-Min, soy oil with 24 % lipids.

The absence of air during vacuum frying may inhibit lipid oxidation in the product (Andrés-Bello *et al.*, 2011). Lipid stability in fried products depends on the quality of the frying oil and process and storage conditions; for instance, Lee and Pangloli (2013) showed a lipid stability in potato chips fried in an unsaturated oil (mid-oleic sunflower oil) stored during 6 weeks at ambient temperature (22 °C). Assuming that there is a slight or minimal degradation of polyunsaturated fatty acids during a limited storage time, the chips with soy oil

would exhibit a higher content of alpha linolenic and linoleic acids (total sum of 15.8 g/100 g chips, fresh weight) in comparison to chips with palm oil (2.2 g/100 g chips, fresh weight). Further studies are needed to verify the oxidation stability of fatty acids in vacuum-fried chips during storage. Globally, packaging under nitrogen conditions and the use of unsaturated oil may be considered for optimal carotenoid retention and for improving the nutritional properties of chips.

4. Conclusions

The present study evaluated the reactivity of different CTs present in vacuum-fried papaya chips during storage, taking into account the lipid composition of matrix (type of oil and lipid content). Conservation of CTs in this kind of product during storage is of commercial and nutritional importance. Our results showed that there was a positive effect of packaging papaya chips with N_2 on carotenoid retention (under the tested conditions of temperature and time) allowing a minimal degradation of CTs. Degradation of carotenoids in the case of a packaging with air followed a second order kinetic model. Retention of all-E-carotenoids was higher than that of Z-carotenoids, and lycopene (the main carotenoid in papaya chips) was the most stable in comparison to the other CTs. The Arrhenius model correctly described the carotenoid degradation in papaya chips during storage at 15-45 °C. Vacuum frying allows the use of both saturated and unsaturated oils (due to the absence of oxygen and lower temperatures during the process) maintaining a high quality of the frying medium that favors a higher preservation of CTs during storage. Packaging under nitrogen conditions and the use of an unsaturated oil may be considered for optimal shelf life and carotenoid retention of these food products during storage. The data generated in this study brings new scientific knowledge about how important storage conditions and matrix composition are on carotenoid degradation.

In a more cognitive perspective, this study shows that the degradation kinetic is driven by oxygen. Therefore, further research is needed to understand the

physical and chemical phenomena involved in carotenoid degradation during the storage of carotenoid-rich fried products. Regarding the uncertainty of analysis and the difficulty to have access to different degradation products in real matrices, a kinetic approach should be probably conducted in both a model medium and the real matrix.

Chapter VI

Evaluation of the consumption of vacuum-fried papaya chips on carotenoid absorption, glycemia, and lipid profile in Wistar rats

Chapter VI. Evaluation of the consumption of vacuum-fried papaya chips on carotenoid absorption, glycemia, and lipid profile in Wistar rats

Publication #4

Carotenoid absorption in rats fed with vacuum-fried papaya chips depends on processed food microstructure associated with saturated and unsaturated oils

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Abstract

Many studies indicate that food matrix microstructure and type of dietary oil or fat play a key role in carotenoid absorption. Therefore, this work was designed to highlight the relationship between processed food microstructure and carotenoid absorption. This study aimed to evaluate the consumption of a carotenoid-rich fruit snack on lipid profile, glycemia, and especially on carotenoid absorption/bioconversion in Wistar rats. Animals were orally fed with mixtures based on vacuum-fried papaya chips with either soy oil (PC-S) or palm oil (PC-P) for 7 days, receiving 0.29 mg lycopene/kg/day and 0.35 mg total carotenoids/kg/day. Lycopene and retinoids were analyzed in plasma and liver of rats by HPLC-DAD. Results showed that the consumption of mixtures based on papaya chips did not affect the lipid profile or glycemia in rat plasma, regardless of the type of oil. Wide-field and confocal microscopy analyses of food matrix helped to understand why lycopene accumulation in the liver was higher ($p<0.05$) in rats fed with PC-P (0.442 μg/g liver) than in those which were fed with PC-S (0.291 μg/g liver). A better dissolution of crystalloid lycopene was found in PC-P. Conversely, a higher bioconversion of provitamin A carotenoids was observed for soy products. The effect of the type of oil was underlined by epifluorescence microscopy of papaya mixtures showing homogeneous and small lipid droplets for soy products. These results showed that PC-S could be recommended as a healthy snack, being a source of provitamin A carotenoids and bioavailable lycopene in a diversified diet.

Keywords

Food microstructure; Lycopene; Carotenoid bioconversion; Lipid profile; Vacuum frying; Wistar rats.

1. Introduction

Over the last few decades, a dramatic rise of obesity and metabolic syndrome has been occurred in Latin America and the Caribbean, leading to a higher incidence of non-communicable diseases (*e.g.*, cardiovascular diseases and diabetes) which are the major causes of death (Popkin & Reardon 2018; Mattei *et al.*, 2015). This is attributed to nutrition transition (an increase of unhealthy dietary habits), negative changes in lifestyle, and migration from rural to urban areas (Cuevas *et al.*, 2011). For instance, during the year 2016 in Latin America and the Caribbean, 58.8 % and 60.0 % of adult men and women, respectively, were overweight or obese (OPS/OMS, 2019). Besides, during the same year, in this region, the prevalence of high blood pressure and high blood glucose/diabetes for men and women was 23.8 and 8.8%, and 18.0 and 9.6%, respectively (OPS/OMS, 2019). Among numerous factors, the high availability of energy-dense and nutrient-poor snack foods in low- and middle-income countries represent a problem (Pries *et al.*, 2019). Consumers in Latin America and the Caribbean are attracted by diets based on this kind of foods that are composed mainly or solely of sugars and saturated lipids but slight or no content in vitamins, minerals, protein, fiber, or essential fatty acids (Poti *et al.*, 2014).

The consumer trends towards healthier foods require alternative strategies to the consumption of deep-fat fried products rich in simple sugars, saturated lipids and salt, *e.g.*, potato chips, French fries, doughnuts, extruded snacks, cheese sticks, among others (Da Silva & Moreira, 2008). Numerous epidemiological studies promote the consumption of fruits and vegetables for the prevention of the non-communicable diseases (Angelino *et al.*, 2019; Gan *et al.*, 2015). Consequently, the intake of fruits and vegetables must be increased in diets because they represent a source of bioactive health-promoting compounds such as vitamins, polyphenols, carotenoids, and fiber.

Among phytochemicals, the carotenoids, such as lycopene, are natural antioxidants with beneficial health effects. These compounds enhance the functions of the immune system and lower the development of chronic diseases such as macular degeneration, type 2 diabetes, obesity, cardiovascular diseases,

and a certain type of cancers (Cheng *et al.*, 2017; Kopec & Failla, 2018). However, the most relevant function of carotenoids is the provitamin A activity. Some carotenoids such as β-carotene and β-cryptoxanthin can be converted into retinol (vitamin A) and other related retinoids in the organism, playing a key role in growth, visual cycle, and gene regulation (Kulczynski *et al.*, 2017).

The application of technological processes is of utmost importance to obtain novel and healthy products from fruits and vegetables. In this context, vacuum frying is an alternative technology to produce fruit and vegetable-based snacks bearing the desired sensory quality and better preserving their nutrients compared to the traditional fried snacks (Da Silva & Moreira, 2008; Dueik & Bouchon, 2011). In addition, vacuum frying allows the use of healthier unsaturated vegetable oils due to the low operation temperatures and the absence of oxygen during the process (compared to atmospheric frying), thus minimizing oil deterioration (Da Silva & Moreira, 2008). For instance, vacuum-fried papaya chips are a good source of lycopene and provitamin A carotenoids such as β-cryptoxanthin and β-carotene (Soto *et al.*, 2021b). These chips may be an alternative in Latin America and the Caribbean to control vitamin A deficiency which remains a public health problem in countries of this region (Cediel *et al.*, 2015).

However, the bioaccessibility and bioavailability of carotenoids in fruit and vegetable-based foods are influenced by various factors. For instance, food matrix microstructure and the presence and type of oil have a great impact (Lemmens *et al.,* 2014; Schweiggert & Carle, 2017; Xavier & Mercadante, 2019). Carotenoids are enclosed in cell organelle structures (*e.g.*, chromoplasts in fruits). Therefore, disruption of cell food matrix during processing (thermal and mechanical treatments) may increase carotenoid bioavailability (Van Buggenhout *et al.*, 2010). Otherwise, the presence of lipids plays a role in the transfer and diffusion of carotenoids during processing, as well as during digestion, since carotenoids need to be released from the food matrix and incorporated into lipid emulsion droplets and finally transferred to mixed micelles (Xavier & Mercadante, 2019). Location and deposition forms of carotenoids in raw fruit/vegetable tissues have been widely studied concerning to their bioavailability (Schweiggert & Carle,

2017). In contrast, few studies have investigated the impact of both factors, processed food microstructure and oil presence, on carotenoid absorption and bioconversion.

From a nutritional point of view, this study was embedded in the general approach of making a carotenoid-rich snack using papaya in order to offer a healthier product than traditional fried snacks. Papaya fruit was chosen for several reasons: 1) it is a fruit widely spread and consumed in America Latina and the Caribbean (FAO, 2020; Saran *et al.*, 2016), 2) it presents a varied profile of carotenoids, xanthophyll (β-cryptoxanthin) and carotenes (β-carotene and lycopene) (Soto *et al.*, 2021b), and 3) carotenoids are more bioavailable from papaya than from other plant foods such as tomato and carrot (Schweiggert *et al.*, 2014). The objective of the present study was to highlight the relationship between processed food microstructure and carotenoid absorption/bioconversion in rats fed with a mixture based on vacuum-fried papaya chips obtained with saturated or unsaturated oils. To better understand the effect of processing, freeze-dried papaya mixtures with the addition of oils were administered to rats to compare with mixtures from papaya chips. Lipid profile (triglycerides, total cholesterol, HDL, and LDL) and glycemia were analyzed in plasma, while retinoids and lycopene were measured in the liver and plasma of rats. Wide-field and confocal microscopy tools were used to explain the results concerning the processed food microstructure.

2. Materials and methods

2.1. Materials

Red-fleshed papaya fruits (*Carica papaya* L. var. Formosa from Brazil) from a single batch were acquired from TerreAzur (Montpellier, France) at ripening stage 4 (41-55 % of skin yellowing). Commercial frying oils, soy oil (Huileries Cauvin, Nimes, France) and hydrogenated palm oil Risso® (Vandemoortele, Gent, Belgium) were used as described previously by Soto *et al.* (2020).

2.2. Obtention of papaya chips and mixtures for animals

Papaya chips were obtained after vacuum frying (120 °C, 13 min, 25 kPa) either with soy oil (~26 % oil in chips) or palm oil (~24 % oil in chips) and then were packaged in metallized PET/PE bags under nitrogen conditions and stored for 90 days at 25 °C as previously described by Soto *et al.* (2020). After the storage period the chips were ground (18 %, w/w) and mixed with 82 % (w/w) of distilled water to obtain the diet mixtures with the adequate viscosity to feed the rats by oral administration (Papaya chips - soy oil, PC-S, and Papaya chips - palm oil, PC-P).

On the other hand, papaya fruit slices were freeze-dried (Usifroid SMH 15, Élancourt, France) for 72 h. Freeze-dried papaya was ground (14 %, w/w) and mixed with 81 % (w/w) of distilled water and 5 % (w/w) of the different oils (soy or palm oils) to obtain the other diet mixtures (Freeze-dried papaya + soy oil, FDP+S, and Freeze-dried papaya + palm oil, FDP+P) with the adequate texture as well. The formulation of different mixtures was made considering the lycopene concentration (major carotenoid) and the lipid content (Table 6.1) leading to products with a density of ~1.1 g/mL. Contents of lycopene, total carotenoids and lipids in the diet mixtures expressed per body weight (bw) of rats were 0.29 mg/kg bw/day, 0.35 mg/kg bw/day and 0.28 g/kg bw/day, respectively. The different mixtures were prepared one week before the diet period and stored at -20 °C. Prior to oral administration to the rats, the mixtures were thawed in a water bath at 37 °C.

2.3. Animals

Male Wistar rats (n=38), 7 weeks old, were obtained from Janvier Labs (S^{t} Berthevin, France). Animals were handled in compliance with European Union animal care regulations rules and the guidelines of the National Institute of Health and the Committee for Animal Care at the University of Montpellier (France). Animals were housed two by two in plexiglass cages (enriched with toys) at 25 ± 1 °C, subjected to 12 h light/dark cycle and fed with standard diet A04 (SAFE, Scientific Animal Food and Engineering; Augy, France) and water *ad libitum*. The retinol content in the standard diet was 7500 UI/kg. During the

adaptation period (4 days), rats were only fed with standard diet (A04) without any carotenoid source.

2.4. Study design

The animals were randomly divided into 5 groups for the diet period (7 days) as shown in Figure 6.1. One group of animals (n=6) received only the regular A04 diet (Control). The other four groups (n=8) were orally fed with 1 mL of the different diet mixtures for 7 days two times per day (morning, 8-9 am and afternoon, 3-4 pm) in addition to regular A04 diet. Two groups were fed with PC-S or PC-P, and the other two groups treated with FDP+S or FDP+P (Figure 6.1). At the end of the experimental period the animals were fasted overnight, then they were anesthetized (20 µL pentobarbital/100 g bw) and blood samples were collected by cardiac puncture before euthanasia. For each blood sample an aliquot (500 µL) was taken for glycemia determination. Then, tubes with heparin containing the blood samples were centrifuged (Jouan BR41 Multifunction, Thermo Electron Corporation, France) at 1500 rpm for 20 min at 15 °C to collect the plasma. Liver samples were collected and weighed after being washed with ice-cold saline solution (0.9 % NaCl, w/v), and then immediately snap frozen in liquid nitrogen. All the samples were stored at -80 °C for further analyses.

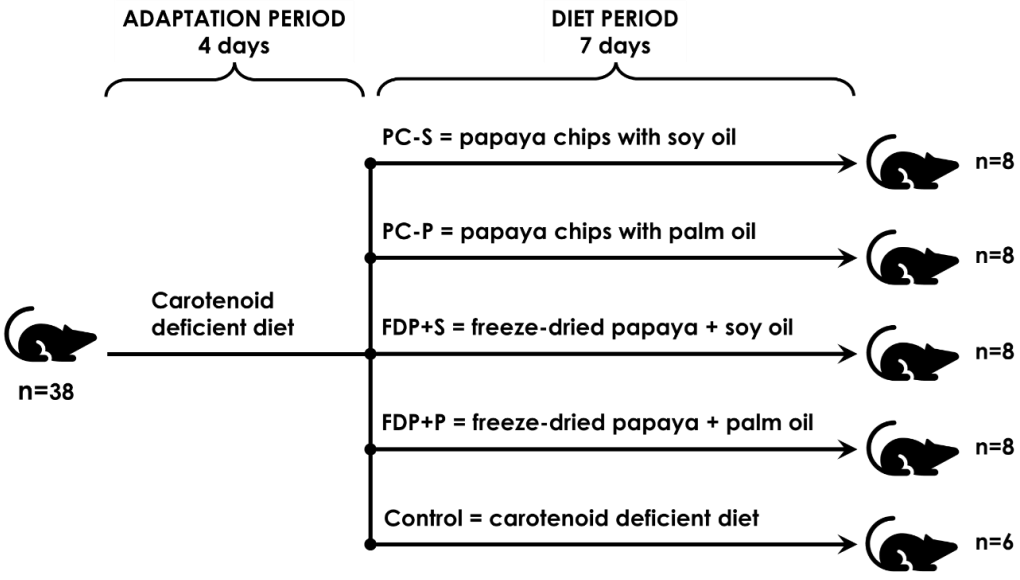

Figure 6.1. Study design to evaluate the effect of the consumption of different papaya mixtures on carotenoid absorption and lipid profile in plasma and liver of Wistar rats.

2.5. Growth performance

Animals from different groups were weighted to determine their weight gain after adaptation and diet periods. Weights were recorded at the initial day of experimentation (day 0), at the end of the adaptation period (day 4), and at the end of the diet period (day 7) using a top-pan electronic balance.

2.6. Chemical analyses of papaya mixtures

Moisture and protein contents were determined by standard AOAC methods 920.151 and 920.152, respectively (AOAC, 2015). Lipid content was determined by the method described by Carpenter *et al.* (1993). Sugars (sucrose, glucose, and fructose) were determined using UPLC-1290 System Infinity II (Agilent, CA, USA) equipped with a refractive index detector. Sugars were separated using a Shodex SH1011 column (300 x 8 mm i.d., 6 μm) (Showa Denko K.K., Tokyo, Japan) with a guard column, and the mobile phase was H_2O with H_2SO_4 (0.01%). The operation temperature was set at 30 ºC. The flow rate was set at 0.7 mL/min and the injection volume was 10 μL. Isocratic condition was programmed with a run time of 20 min. Quantification was performed after obtaining linear calibration curves of glucose, fructose, and sucrose. The fatty acid profile was determined using AOAC standard method 996.06 (AOAC, 2015) and AOCS method Ce 1e-91 (AOCS, 2012).

2.7. Carotenoid analyses

2.7.1. Carotenoid extraction from papaya mixtures

Extraction procedures and conditions for analysis were described previously by Gies *et al.* (2019), and di Corcia *et al.* (2020) with some modifications (Soto *et al.*, 2020). Briefly, samples were weighed (700 mg) in 20 mL tubes. Then, 2 mL of an ethanol solution containing 1 % pyrogallol was added. The mixture was homogenized using a Vortex mixer and incubated for 2 min in the dark in a water bath at 70 °C. Then, after cooling saponification of samples was performed for 30 min in a water bath at 70 °C by adding 1.5 mL of saturated KOH (12 N). After incubation, the tubes were cooled in an ice bath and 2 ml of distilled water and 5 mL of hexane were added. Then, after mixing and decantation, the aqueous phase was extracted twice with 5 mL of hexane. The organic phases were pooled and

evaporated under nitrogen at 30 °C until dryness. Finally, the residue was dissolved in 500 µL of methyl tert-butyl ether (MTBE)/methanol (80/20) and placed in an amber vial prior to HPLC analysis.

2.7.2. Carotenoid and retinoids extraction from plasma

The carotenoids were extracted according to the method previously described by Poulaert *et al.* (2014) with some modifications. The plasma previously obtained (700 µL) was put into a tube of 8 mL, then 500 µL of ethanol 96 % (v/v) containing canthaxanthin (2 mg/mL) as internal standard and 2 mL of hexane were added. The mixture was homogenized (using a Vortex) for 60 s and then centrifuged (Allegra 21 Centrifuge, Beckman Coulter, Switzerland) at 1400 x g for 5 min at 25 °C. The organic phase was collected, then the aqueous phase was reextracted with 1 mL of ethanol and 2 mL of hexane. The organic phases were pooled and evaporated under nitrogen at 30 °C until dryness. The dried extract was dissolved in 100 µL of CH_2Cl_2/Methanol (50:50, v/v) prior HPLC analysis.

2.7.3. Carotenoid and retinoids extraction from liver

The frozen liver was cut into small pieces on an ice-cold table. Then, 1 g of liver was mixed with 1 mL phosphate-buffered saline (PSB) and homogenized (using a 15 mL Potter tissue grinder) at 600-1000 rpm for 4 min. This step was done twice. Then the homogenates were pooled and homogenized using an Ultra-turrax homogenizer (IKA T10 Basic, Germany) for 60 s. For carotenoids and retinyl palmitate extraction, 900 µL and 500 µL of liver homogenates, respectively, were extracted using 500 µL of ethanol 96 % (v/v) containing canthaxanthin (2 mg/mL) as internal standard and 2.5 mL or 2 mL of hexane for carotenoids and retinyl palmitate, respectively. The mixture was homogenized again using a Vortex for 60 s and then centrifuged (Allegra 21 Centrifuge, Beckman Coulter, Switzerland) at 1600 x g for 5 min, at 25 °C. The organic phase was collected, and the aqueous phase was reextracted with 1 mL of ethanol and 2 mL of hexane. The organic phases were pooled and evaporated under nitrogen at 30 °C until dryness. The dried extract was dissolved in 100 µL and 850 µL of CH_2Cl_2/Methanol (1 g/20 mL) for carotenoid and retinyl palmitate analysis, respectively.

2.7.4. HPLC analysis in papaya mixtures

Carotenoid identification was performed by HPLC using a HPLC-DAD Agilent 1100 system (Massy, France). Carotenoids were separated using a C30 column (250 x 4.6 mm i.d., 5 μm) (YMC EUROP GmbH, Germany) with a guard column, and the mobile phase was H_2O as eluent A, methanol as eluent B, MTBE as eluent C. Operation temperature was set at 25 ºC. The flow rate was set at 1mL/min and the injection volume was 20 μL. The gradient program was described by Soto *et al.* (2020). β-cryptoxanthin and β-carotene were detected at 450 nm, and lycopene was detected at 470 nm. In papaya mixtures the β-carotene and lycopene contents were expressed as the sum of their all-E- and Z-isomers. For carotenoid HPLC analysis the limit of detection (LOD) was 0.0040 μg, and the limit of quantification (LOQ) was 0.0150 μg.

2.7.5. HPLC analysis in plasma and liver samples

Carotenoid and retinoids were separated with the same C30 column as previously described in section 2.6.4. The mobile phase was H_2O as eluent A, methanol as eluent B, and MTBE as eluent C. Temperature was set at 25 ºC. The flow rate was set at 1mL/min and the injection volume was 60 μL. A solvent gradient was programmed as follows: 2 % A – 96 % B – 2 % C (initial conditions); 0-27 min, 2 % A – 18 % B – 80 % C; 27-35 min, 4 % A – 11 % B – 85 % C and back to the initial conditions for re-equilibration. Chromatograms were generated at 325 nm to identify retinol and retinyl esters, and at 470 nm for lycopene identification. Carotenoid and retinoids were identified by comparing their retention time and spectra with the respective standards. Quantification was achieved by establishing calibration curves with all-E-lycopene, retinol and retinyl palmitate, being the determination coefficients 0.994, 0.997 and 0.998, respectively.

2.8. Glycemia determination

Determination of glucose was performed in rat samples using the Accu-Chek Performa blood glucose meter (Roche, Basel, Switzerland).

2.9. Lipid profile and free fatty acids in plasma

Lipid profile analyses were performed in plasma: triglycerides (TRIG), total cholesterol (CHO); low-density lipoprotein (LDL) and high-density lipoprotein (HDL) using the Biolabo enzyme kits (Biolabo SAS, Maizy, France). Free fatty acids content (FFA) was determined using the Abcam enzyme kit (Allscience, Miami, USA). These analyses work under colorimetric reactions, for which a Spark 96-plate micro spectrophotometry-fluorescence device was used (TECAN, Männedorf, Switzerland).

2.10. Microscopy analyses

Mixtures from vacuum-fried papaya chips and freeze-dried papaya were directly observed with a wide-field microscope Eclipse Ni-E (Nikon Instruments Inc., NY, USA). The pictures were obtained with the 20X Plan-APO 0.75 NA objective under transmitted light or differential interference contrast (DIC). Size distribution of lipid droplets, expressed as D_{90}, was calculated by using ImageJ v1.8.0 software. D_{90} means that 90 % of spherical particles have a diameter less than the specified value (μm). The lipids were visualized in fluorescence with the lipophilic stain Nile Red (3.14 μM) with a blue excitation filter (B2A: 450-490 nm, long-pass emission 505 nm).

The same mixtures and the papaya chips were observed with a confocal microscope Zeiss 880 (Zeiss, Jena, Germany) with a 488 Argon laser with an objective 20X Plan APO 1.0 NA to visualize the autofluorescence of carotenoids (lycopene and β-carotene/β-cryptoxanthin). Spectral analysis was carried out using the advanced Linear Unmixing function (LSM 880 software, Zeiss) which separates mixed signals pixel by pixel using the entire emission spectrum of each defined autofluorescent compound in the sample. This function was applied with the advanced iterative option and one residual channel. After spectral imaging acquisitions on samples, this Linear Unmixing function allowed visualization with coded colors of the fluorescence of each standard (lycopene, β-carotene, and β-cryptoxanthin) based on their reference spectra (Talamond *et al.*, 2015). The spectral detector of this microscope was used to obtain the emission spectra of

lycopene and β-carotene/β-cryptoxanthin and visualize specifically these molecules in the samples between 500 and 690 nm.

2.11. Statistical analyses

Results obtained from plasma and liver samples were analyzed by one-way analysis of variance (ANOVA) after examining for homogeneity of variances by Barlett's test. P-value <0.05 was considered significant. If significantly different, means were further compared using Fisher's test ($p<0.05$). Composition of the different papaya mixtures was also compared by one-way ANOVA and post hoc Tukey-HSD ($p<0.05$). Statistical analyses were performed using the XLSTAT Software version 2020 (Addinsoft Inc, USA).

3. Results and discussion

3.1. Carotenoid consumption and growth performance

Table 6.1 shows the composition of the different papaya mixtures (PC-S, PC-P, FDP+S, FDP+P) that were made to feed the rats by oral administration. There were no significant differences ($p>0.05$) in lycopene, total carotenoid, and lipid contents among the different papaya mixtures. During the diet period rats were orally fed with 1 mL of the different papaya mixtures (PC-S, PC-P, FDP+S, FDP+P) two times per day, which means 2 mL papaya mixture/day. Therefore, the animals fed with mixtures consumed per day ~96 μg of lycopene, ~113 μg of total carotenoids, and ~90 mg of lipids (either from soy or palm oils).

Mixtures from papaya chips (PC-S and PC-P) presented around 2-fold lower ($p<0.05$) contents of β-cryptoxanthin, β-carotene, and vitamin A (expressed as Retinol Activity Equivalent, RAE) than those in mixtures from freeze-dried papaya (FDP+S and FDP+P). This was explained by thermal degradation of these carotenoids during the vacuum frying process (Soto *et al.*, 2020).

Daily lycopene intake in this study was similar to that applied in previous studies realized in rats by Jain *et al.* (1999) (142 μg/day) and in gerbils by Mills *et al.* (2007) (60 μg/day). In most studies with rats (in which the absorption,

bioconversion, or the health effects of carotenoids are analyzed), the authors usually apply much higher doses of carotenoids in diets to feed animals. For instance, some studies reported a daily lycopene intake that ranged from 720 to 10.000 μg (Takayama *et al.*, 2013; Shalaby & El Shaer, 2019; Yilmaz *et al.*, 2018; Xu *et al.*, 2019).

Table 6.1. Composition of the different papaya mixtures used for feeding the Wistar rats.

Component	Papaya mixtures			
	PC-S	**PC-P**	**FDP+S**	**FDP+P**
Moisture (mg/mL)	906.38 ± 0.73^{b}	910.71 ± 0.94^{a}	903.97 ± 0.74^{c}	909.66 ± 0.95^{a}
Lipids (mg/mL)	44.67 ± 3.65^{a}	43.93 ± 1.03^{a}	45.01 ± 2.42^{a}	46.00 ± 1.81^{a}
Protein (mg/mL)	8.10 ± 0.96^{a}	8.19 ± 1.44^{a}	4.90 ± 0.12^{b}	4.66 ± 0.10^{b}
Carotenoids (μg/mL):				
β-cryptoxanthin	3.01 ± 0.33^{b}	2.50 ± 0.13^{b}	5.67 ± 0.53^{a}	5.38 ± 0.50^{a}
β-carotene	2.73 ± 0.27^{b}	2.38 ± 0.11^{b}	6.28 ± 0.42^{a}	5.89 ± 0.43^{a}
Lycopene	49.50 ± 5.28^{a}	46.44 ± 5.12^{a}	48.50 ± 5.45^{a}	47.30 ± 4.47^{a}
Total carotenoids	55.24 ± 5.41^{a}	51.31 ± 5.15^{a}	60.45 ± 5.77^{a}	58.57 ± 5.31^{a}
Total carotenoids (μg/mg FM)	1.24 ± 0.12^{a}	1.17 ± 0.12^{a}	1.34 ± 0.13^{a}	1.27 ± 0.12^{a}
Vitamin A content (μg RAE/mL)	0.32 ± 0.03^{b}	0.27 ± 0.02^{b}	0.70 ± 0.02^{a}	0.65 ± 0.05^{a}
Sugars (mg/mL):				
Sucrose	71.24 ± 4.25^{a}	69.72 ± 2.95^{a}	1.79 ± 0.06^{b}	1.89 ± 0.12^{b}
Glucose	14.55 ± 0.89^{b}	15.55 ± 1.09^{b}	49.07 ± 0.42^{a}	50.98 ± 0.83^{a}
Fructose	13.85 ± 0.74^{b}	14.52 ± 0.90^{b}	47.84 ± 0.56^{a}	49.79 ± 0.83^{a}
Fatty acid profile (mg/mL):				
Saturated	7.45 ± 0.29^{c}	21.04 ± 0.30^{b}	7.06 ± 0.10^{c}	22.80 ± 0.10^{a}
Monounsaturated	12.95 ± 0.22^{b}	17.20 ± 0.36^{a}	12.39 ± 0.55^{b}	18.14 ± 0.32^{a}
Polyunsaturated	23.44 ± 0.43^{b}	4.86 ± 0.21^{c}	25.01 ± 0.45^{a}	5.04 ± 0.26^{c}

PC-S, papaya chips with soy oil; PC-S and PC-P, vacuum-fried papaya chips obtained with soy and palm oil, respectively; FDP+S and FDP-P, freeze-dried papaya mixed with soy and palm oil, respectively. FM, fat matter. Values are expressed as the means ± standard deviation, SD (n=3). Means in the same row with the same letter are not significantly different (Tukey's test, $p<0.05$). Vitamin A content is expressed as Retinol Activity Equivalent (RAE). RAE estimate was calculated for a bioconversion ratio (carotenoid:retinol) of 12:1 for all-E-β-carotene, and 24:1 for all-E-β-cryptoxanthin and Z-β-carotene (US IOM, 2000).

Rats gained weight during the experimental period, and the final body weight ranged from 326.6 g to 346.6 g (Table 6.2). However, after the diet period, there

were no significant differences ($p>0.05$) in weight gain between rats consuming papaya mixtures (PC-S, PC-P, FDP+S, FDP+P) and the Control group (Table 6.2).

Table 6.2. Body weight and weight gain of Wistar rats fed with papaya mixtures and Control group at different periods of the study.

Weight	Control	Papaya mixtures			
		PC-S	PC-P	FDP+S	FDP+P
Body weight (g):					
Initial	254.8 ± 4.5	252.6 ± 2.5	267.8 ± 2.9	259.1 ± 4.1	253.0 ± 3.8
After adaptation period (4 days)	300.9 ± 4.0	290.7 ± 3.9	306.1 ± 2.0	297.1 ± 5.1	288.5 ± 4.3
After diet period (7 days)	346.6 ± 5.0	326.6 ± 6.2	343.9 ± 5.6	337.3 ± 7.7	331.5 ± 5.3
**Weight gain (g):*					
During adaptation period (4 days)	46.2 ± 2.2^{a}	38.1 ± 3.9^{b}	38.3 ± 2.2^{ab}	38.0 ± 2.8^{b}	35.5 ± 0.8^{b}
During diet period (7 days)	45.7 ± 1.4^{a}	35.9 ± 3.4^{a}	37.9 ± 4.7^{a}	40.1 ± 3.7^{a}	42.9 ± 3.4^{a}

Control, carotenoid deficient diet; PC-S and PC-P, vacuum-fried papaya chips obtained with soy and palm oil, respectively; FDP+S and FDP-P, freeze-dried papaya mixed with soy and palm oil, respectively. Values are expressed as the means ± standard error of the mean, SEM (n=6 for Control, n=8 for the other groups). *For weight gain, means in the same row with the same letter are not significantly different (Fisher's t-test, $p<0.05$).

3.2. Glycemia

Figure 6.2 shows the results of glycemia measured in rats from the Control group and rats fed with the different papaya mixtures (PC-S, PC-P, FDP+S, FDP+P). The glycemia of rats fed with mixtures from papaya chips (185.75 ± 34.56 mg/dL for PC-S, and 178.13 ± 30.71 mg/dL for PC-P) was not significantly different from the Control group (160.67 ± 20.70 mg/dL). Unexpectedly, the glycemia increased in rats consuming freeze-dried papaya mixtures (223.00 ± 48.09 mg/dL for FDP+S, and 198.50 ± 36.98 mg/dL for FDP+P). These values were significantly higher ($p<0.05$) than those in the Control group. These differences in glycemia could be attributable to the higher contents of glucose and fructose present in freeze-dried papaya mixtures (FDP+S, FDP+P) (~98 % of total sugars) compared to the mixtures made with papaya chips (PC-S, PC-P) (~30 % of total sugars)

(Table 6.1). During vacuum frying of papaya, the degradation of glucose and fructose was observed, whereas the formation of sucrose occurred (Soto *et al.*, 2021b). According to these authors, factors related to the frying process (high temperature and fast rate of water loss) and intrinsic characteristics of papaya (proximal composition, sugar concentration, organic acids, acidity) are involved in sucrose formation.

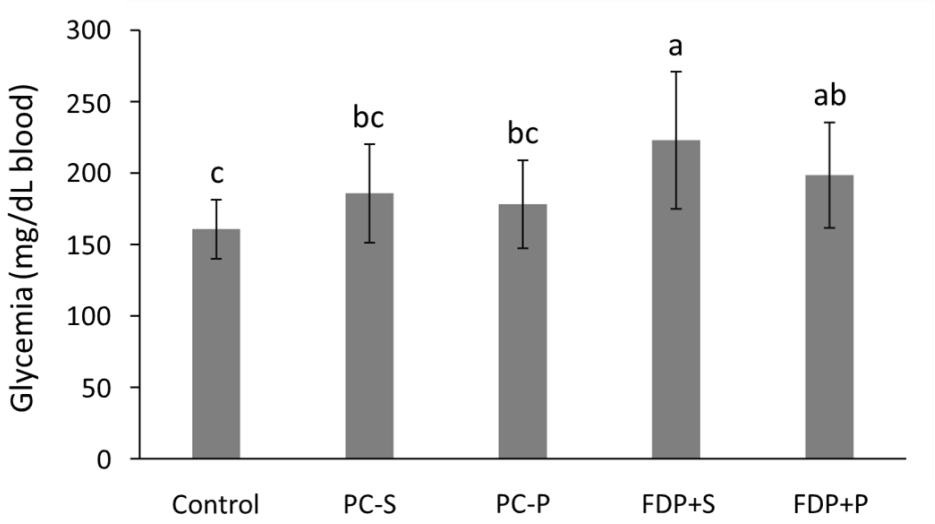

Figure 6.2. Glycemia of the Control group and rats fed with papaya mixtures: vacuum-fried papaya chips with either soy oil (PC-S) or palm oil (PC-P), and freeze-dried papaya mixed with either soy oil (FDP+S) or palm oil (FDP+P). Bars are expressed as the means ± standard error of the mean, SEM (n=6 for Control, n=8 for the other groups). Means with the same letter are not significantly different (Fisher's t-test, $p<0.05$).

3.3. Lipid profile and free fatty acids in plasma

Table 6.3 shows the lipid profile and content of free fatty acids (FFA) in plasma of rats fed with the papaya mixtures (PC-S, PC-P, FDP+S, FDP+P) and the Control group. No significant differences ($p>0.05$) were found in cholesterol determinations (total, CHO; high-density lipoprotein, HDL; and low-density lipoprotein, LDL) among rats fed with papaya chip mixtures (PC-S, PC-P) and rats from Control group. Similarly, there were no significant differences ($p>0.05$) for CHO, HDL, and LDL among the Control group and rats fed with freeze-dried papaya mixtures (FDP+S, FDP+P) (Table 6.3).

Moreover, the level of triglycerides (TRIG) in plasma was similar among rats of the Control group and rats fed with papaya chips mixtures (PC-S, PC-P) and those fed with FDP+S mixture. Rats consuming the FDP+P mixture presented the highest TRIG level ($p<0.05$). These results demonstrated that consumption of mixtures based on papaya chips (PC-S, PC-P) did not increase CHO, HDL, LDL, or TRIG in rat plasma, regardless of the type of oil.

Table 6.3. Lipid profile and free fatty acids in plasma from Wistar rats fed with papaya mixtures and the Control group.

Papaya mixtures	CHO (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TRIG (mg/dL)	FFA (nmol/mL)
Control	90.05 ± 1.7^{ab}	20.80 ± 3.10^{a}	14.35 ± 1.32^{a}	70.69 ± 6.06^{b}	27.70 ± 9.41^{b}
PC-S	92.05 ± 4.9^{a}	27.06 ± 2.90^{a}	15.77 ± 1.05^{a}	59.00 ± 3.25^{b}	64.93 ± 14.7^{a}
PC-P	78.82 ± 2.78^{b}	19.91 ± 3.17^{a}	13.80 ± 1.48^{a}	65.37 ± 4.15^{b}	58.61 ± 7.34^{a}
FDP+S	85.52 ± 2.99^{ab}	22.69 ± 1.88^{a}	14.93 ± 1.21^{a}	67.59 ± 5.12^{b}	43.14 ± 2.52^{ab}
FDP+P	93.43 ± 3.91^{a}	21.47 ± 1.26^{a}	16.06 ± 1.64^{a}	92.95 ± 5^{a}	40.37 ± 5.26^{ab}

Control, carotenoid deficient diet; PC-S and PC-P, vacuum-fried papaya chips obtained with soy and palm oil, respectively; FDP+S and FDP-P, freeze-dried papaya mixed with soy and palm oil, respectively. CHO, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; FFA, free fatty acids; TRIG, triglycerides. Values are expressed as the means ± standard error of the mean, SEM (n=6 for Control, n=8 for the other groups). Means in the same column with the same letter are not significantly different (Fisher's t-test, $p<0.01$).

Only FFA increased significantly ($p<0.05$) in plasma of rats consuming papaya chips mixtures (PC-S, PC-P) compared to the Control group. The heat treatment (120 °C) applied during vacuum frying of papaya could induce some hydrolysis of triglycerides from frying medium (soy and palm oils) (Chung *et al.*, 2004). Thus, the oil absorbed in papaya chips (PC-S, PC-P) during the process could alter the levels of FFA in the plasma of rats. On the other hand, the mixtures based on freeze-dried papaya (FDP+S, FDP+P) were made with fresh oils without any thermal degradation.

3.4. Lycopene absorption

Lycopene was detected in the liver of rats fed with the different mixtures based on freeze-dried papaya (FDP+S, FDP+P) and papaya chips (PC-S, PC-P) (Figure 6.3). Significant differences ($p<0.05$) were observed between groups of rats fed with FDP+S and PC-S. Lycopene accumulation in the liver was higher in rats fed with FDP+S (0.522 μg/g liver) than in those which were fed with PC-S (0.291 μg/g liver).

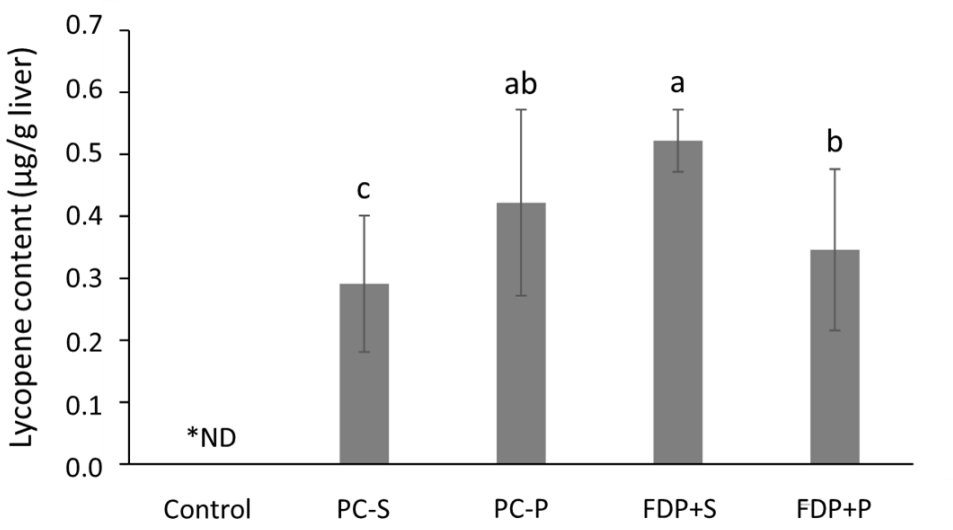

Figure 6.3. Lycopene content in liver of the Control group and rats fed with papaya mixtures: vacuum-fried papaya chips obtained with either soy oil (PC-S) or palm oil (PC-P), and freeze-dried papaya mixed with either soy oil (FDP+S) or palm oil (FDP+P). Bars are expressed as the means ± standard error of the mean, SEM (n=8). Means with the same letter are not significantly different (Fisher's t-test, $p<0.05$). *ND, not detected.

To support these results, wide-field microcopy of the mixtures was performed and revealed that the FDP+S mixture exhibited a fine emulsion with numerous droplets (the D_{90} statistical diameter was 18 μm) probably due to the soy oil-based formulation (Figure 6.4a). In opposite the oil droplet size of the PC-S mixture was higher (the D_{90} statistical diameter was 46 μm) and crystalloid remnants of lycopene were identified (Figure 6.4b, see arrows). A better dissolution of crystalline lycopene in the soy oil fine emulsion was hypothesized.

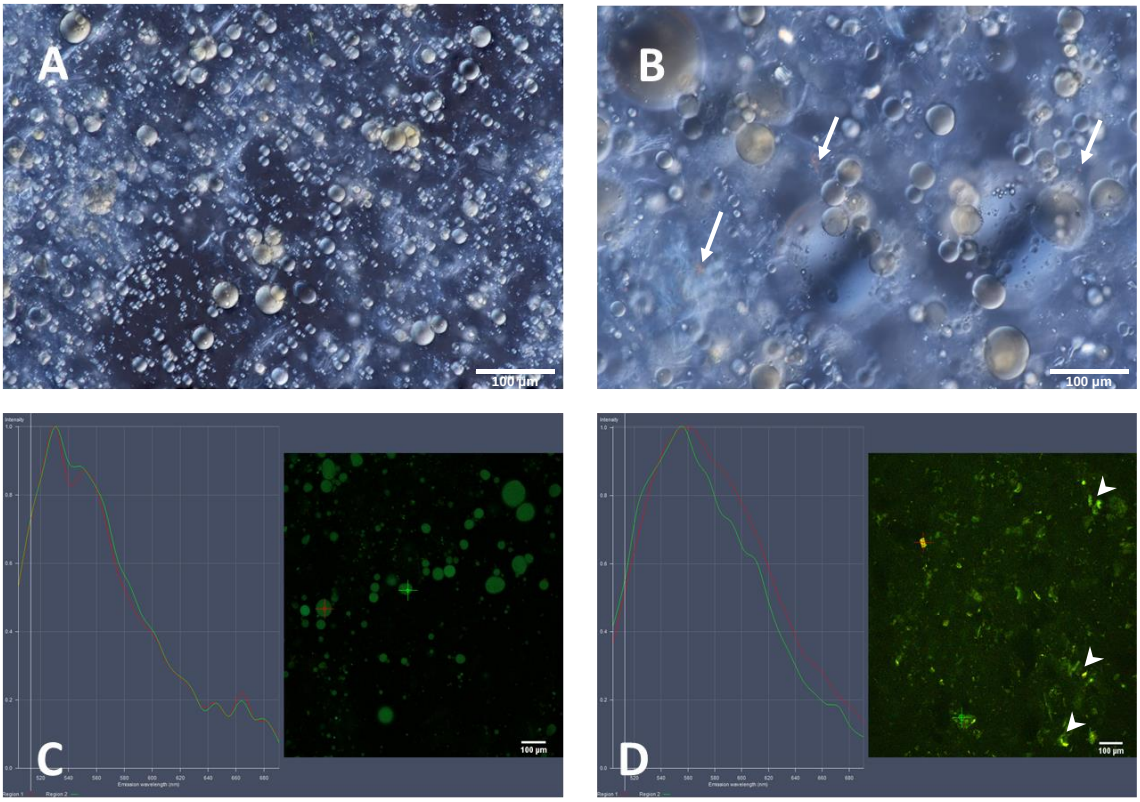

Figure 6.4. Wide-field and confocal microscopy of papaya soy mixtures for feeding rats. (A) Wide-field microscopy (DIC 20 X objective) of freeze-dried papaya soy mixture (FDP+S). (B) Wide-field microscopy (DIC 20 X objective) of vacuum-fried papaya chips soy mixture (PC-S). (C) Spectral imaging by confocal microscopy of FDP+S mixture (red line, lycopene; green line, β-carotene/β-cryptoxanthin). (D) Spectral imaging by confocal microscopy of PC-S mixture (red line, lycopene). *Arrows and arrowheads* mark crystalloid remnants of lycopene. The scale bars represent a length of 100 µm.

To confirm the identification of carotenoids, the spectral imaging of confocal microscopy allowed to identify these compounds with fluorescence spectra, as well as to locate the carotenoids in papaya mixtures. In the FDP+S mixture, the two types of carotenoids β-carotene/β-cryptoxanthin (provitamin A carotenoids) and lycopene seemed to dissolve in the core of lipid droplets of emulsion (Figure 6.4c). Inversely, only fluorescence emission spectra of lycopene were found in the PC-S mixture within solid aggregates (Figure 6.4d, see arrowheads).

According to Schweiggert *et al.* (2012), and Schweiggert and Carle (2017), lycopene from papaya occurs in solid crystalline deposition form whereas β-

carotene/β-cryptoxanthin are liquid-crystalline or lipid-dissolved in the globular-tubular substructure of chromoplast. The morphology of chromoplast and deposition form strongly influence carotenoid bioavailability. Also, the storage of lycopene crystalloid form was associated with a lower bioaccessibility and absorption (Schweiggert *et al.,* 2014). The addition of soy oil and water to freeze-dried papaya (FDP+S) generated a fine emulsion favoring the dissolution of small crystalline lycopene, thereby increasing its absorption during digestion (Salvia-Trujillo *et al.*, 2017). Indeed, before carotenoid absorption, the lipid digestion rate was higher for a fine emulsion with small droplets contributing to micelle formation, which represents the absorbable form for enterocytes (Salvia-Trujillo *et al.*, 2019). For PC-S, the formed emulsion was coarse with larger oil droplets and crystalloid remnants of lycopene (Figure 6.4b).

On the other hand, significant differences ($p<0.05$) in lycopene absorption were observed between rats fed with PC-S mixture (0.291 μg/g liver) and those with PC-P mixture (0.422 μg/g liver). As shown in Figure 5, crystalloid lycopene form remained in PC-S (Figure 6.5a). This figure shows specific red color aggregates (lycopene) within the food matrix. On the other hand, lycopene seemed to be better dissolved in the matrix of PC-P. In fact, the red color represents a diffuse veil within the food matrix (Figure 6.5b).

Lycopene transfer to oil during vacuum-frying of papaya fruit could have been better with palm oil than with soy oil. The fatty acid composition of oils (chain length and degree of unsaturation) influences the incorporation of lycopene into micelles (Lemmens *et al.*, 2014). Lycopene bioaccessibility or absorption was reported higher with olive oil and could be explained by the presence of C18:1 (Clarke *et al.*, 2000; Colle *et al.*, 2012; Nagao *et al.*, 2013). In addition, fatty acids with a medium-chain length such as C16:0 increased lycopene bioaccessibility compared to those with long-chain length. This phenomenon was particularly significant when up to 5 % of lipids were added. Medium-chain fatty acids are hydrolyzed to a greater extent than those with long-chain length. Complete digestion of medium-chain fatty acids led to better lycopene solubilization (Colle *et al.*, 2012). This can also explain why upon the addition of sunflower oil up to 5%, lycopene bioaccessibility from papaya remained unaffected (Schweiggert

et al., 2012). In the current study, the lipid content in papaya mixtures was ~4.5 % (w/v).

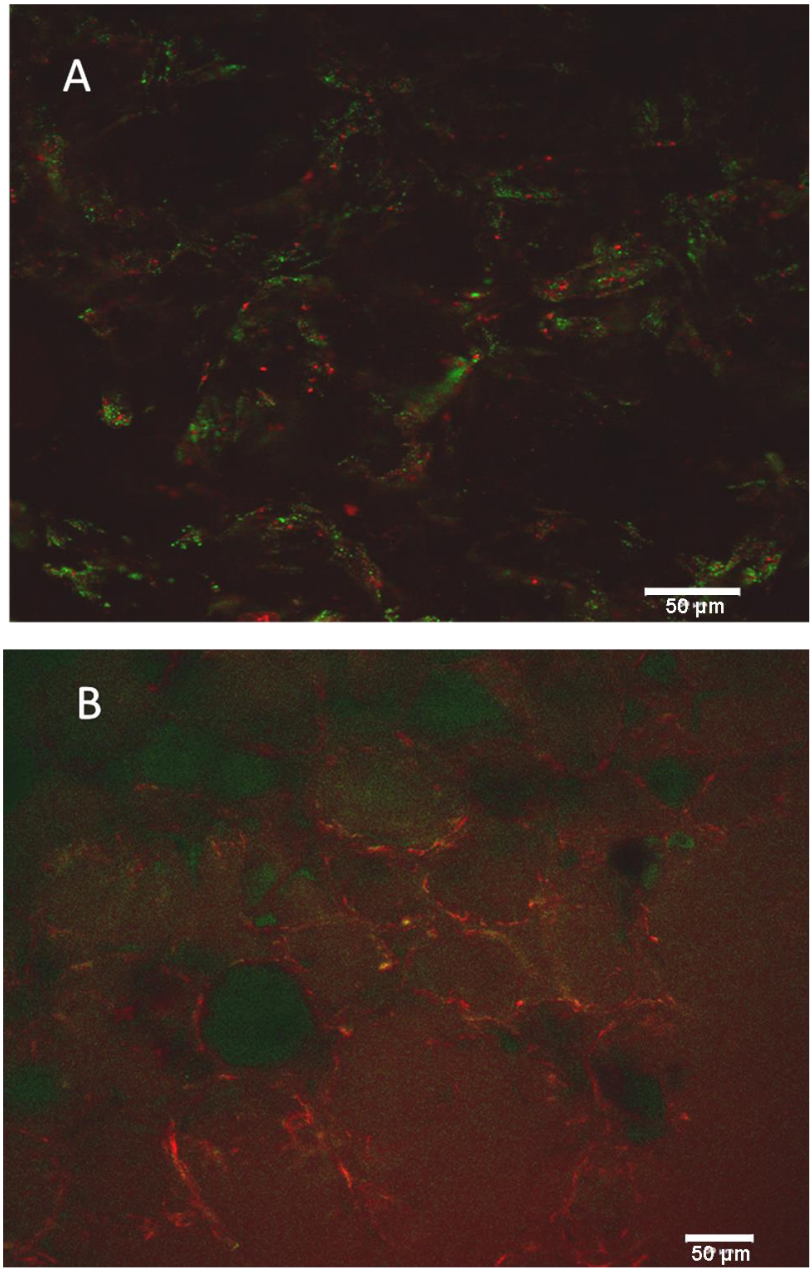

Figure 6.5. Spectral imaging by confocal microscopy (20X objective) of (A) Vacuum-fried papaya chips with soy oil, (B) Vacuum-fried papaya chips with palm oil. Lycopene is represented by red color and β-carotene/β-cryptoxanthin by green color. The scale bars represent a length of 50 µm.

Palm oil contained 39 % of C18:1 and 41 % of C16:0, whereas soy oil only contained 26 % and 11 %, respectively. Therefore, the fact that palm oil has a

distinct fatty acid composition, added to the effect of processing, could explain the difference for lycopene absorption in rats consuming the papaya chips but processed with a different oil. Furthermore, saturated coconut oil (rich in medium-chain triglycerides) enhanced lycopene tissue accumulation to a greater degree than safflower oil (rich in long-chain triglycerides) in Mongolian gerbils fed with tomato powder (Conlon *et al.*, 2012). Borel *et al.* (1996) pointed out that the solubility of carotenes in bulk triglycerides increased with decreasing of fatty acid chain length.

3.5. Provitamin A carotenoid bioconversion in plasma and liver

The bioconversion of pro-vitamin A carotenoids was demonstrated by an increase of retinol in plasma and retinyl palmitate content in the liver of rats consuming the papaya mixtures (PC-S, PC-P, FDP+S, FDP+P) comparatively with those of the Control group fed with a regular diet (non-supplemented with carotenoids). Retinol content in plasma was significantly higher ($p<0.05$) in rats fed with the papaya mixtures (PC-S, 3.05 ± 0.20 μmol/L; PC-P, 2.80 ± 0.31 μmol/L; FDP+S, 3.11 ± 0.38 μmol/L; FDP+P, 2.87 ± 0.14 μmol/L) than rats of Control group (2.38 ± 0.25 μmol/L). However, no significant differences ($p>0.05$) were found in retinol content among the groups of rats fed with the different papaya mixtures (PC-S, PC-P, FDP+S, FDP+P).

Figure 6.6 shows that contents of retinyl palmitate in rats following administration of mixtures with palm oil (PC-P, FDP+P) were lower than in those which were fed with soy oil mixtures (PC-S, FDP+S). The better bioefficacy observed after ingestion of soy products could be explained by the different behavior of emulsions formed in mixtures administrated to the rats. Wide-field epifluorescence microscopy with help of lipid coloration showed emulsions with small and round-shape oil droplets for the soy products (Figure 6.7a, and Figure 6.7c) in opposition to emulsions with larger and undefined-shape oil droplets for the mixtures with palm oil (Figure 6.7b, and Figure 6.7d).

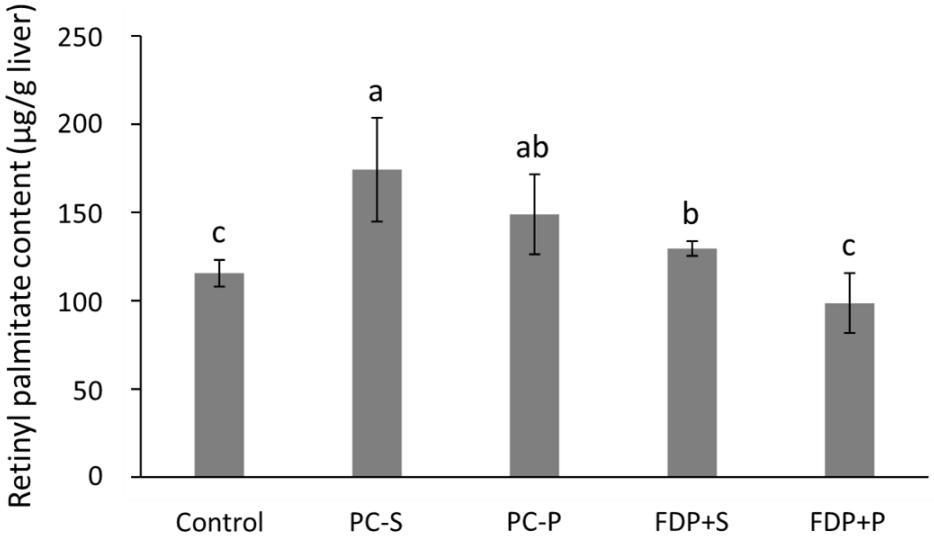

Figure 6.6. Retinyl palmitate content in the liver of the Control group and rats fed with papaya mixtures: vacuum-fried papaya chips obtained with either soy oil (PC-S) or palm oil (PC-P), and freeze-dried papaya mixed with either soy oil (FDP+S) or palm oil (FDP+P). Bars are expressed as the means ± standard error of the mean, SEM (n=6 for Control, n=8 for the other groups). Means with the same letter are not significantly different (Fisher's t-test, $p<0.05$).

Carotenoids require their liberation from the food matrix and their solubilization into oil droplets to be then transferred to bile salts mixed micelles. According to Salvia-Trujillo *et al.* (2013), the droplet size of oil in water emulsions generated during the first step of digestion was one of the most important factors in carotenoid absorption due to the hydrolysis of triglycerides. Digestion of triglycerides and carotenoid bioaccessibility were higher for small lipid droplets than for bigger ones (Salvia-Trujillo *et al*, 2013). In addition, the lipid digestion of long-chain fatty acids such as acid oleic and linoleic acids induced the assembly and basolateral secretion of chylomicrons for their transfer to the lymph (McClements *et al.*, 2015). Failla *et al.* (2014) showed that unsaturated fatty acids from soy oil promoted the uptake and secretion of β-carotene by Caco-2 cells.

It was also observed a processing effect between vacuum-fried papaya chips mixtures (PC-S, PC-P) and freeze-dried papaya mixtures (FDP+S, FDP+P). The bioconversion of provitamin A carotenoids was generally better when vacuum-fried chips products were ingested (average of retinyl palmitate was 161 µg/g liver

for papaya chips mixtures versus 114µg/g liver for freeze-dried papaya mixtures). This is relevant because the vitamin A content (RAE) in mixtures based on papaya chips was around 2-fold lower than in freeze-dried papaya mixtures (Table 6.1).

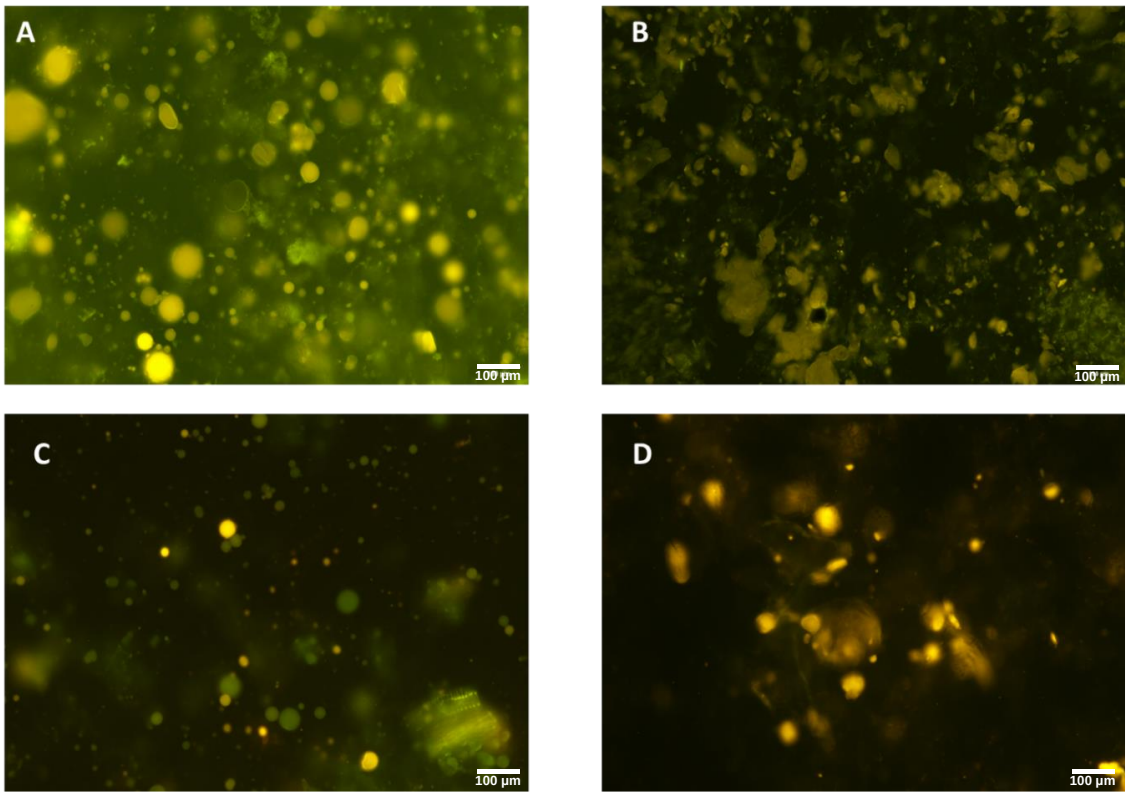

Figure 6.7. Wide-field epifluorescence microscopy (20X objective-Nile Red coloration fluorescent stain for lipids) of papaya mixtures for feeding rats. (A) Vacuum-fried papaya chips soy mixture (PC-S). (B) Vacuum-fried papaya chips palm mixture (PC-P). (C) Freeze-dried papaya soy mixture (FDP+S). (D) Freeze-dried papaya palm mixture (FDP+P). The scale bars represent a length of 100 µm.

This result underlined that the vacuum frying process favored carotenoid bioconversion. The incorporation of oil into the papaya matrix during vacuum frying (120 °C, 13 min, 25 kPa) could play a major role in the absorption of provitamin A carotenoids compared to freeze drying with subsequent oil addition. It was reported that heat treatments increased carotenoid bioavailability especially when lipids were absorbed into the food matrix during deep-fat frying or vacuum frying (Lemmens *et al.*, 2011; Berni *et al.*, 2015; Tumuhimbise *et al.*, 2009). Mutsokoti *et al.* (2016) showed that high

temperatures (>100°C) and short time process (10 min) were sufficient to observe a maximal carotenoid transfer to the oil. These conditions were similar to those used during the vacuum frying of papaya. The increase of carotenoid solubility due to vacuum frying of papaya fruit resulted in a higher bioconversion of provitamin A carotenoids and accumulation of lycopene in the liver of rats.

4. Conclusions

Our study showed that a carotenoid-rich healthy snack obtained by vacuum frying did not increase glycemia, cholesterol, or triglycerides in rat plasma regardless of the type of oil. Moreover, the consumption of mixtures based on vacuum-fried papaya chips favored the absorption of lycopene and provitamin A carotenoids in rats. It was shown that carotenoid absorption depends on the type of oil and processing as well as carotenoid deposition in food microstructure. The crystalloid form of lycopene in papaya was better dissolved in palm oil during vacuum frying and was better absorbed in rats. This phenomenon could be explained by the presence of C18:1 and medium-chain fatty acids such as C16:0 in palm oil. Inversely, the consumption of mixtures based on vacuum-fried chips with soy oil increased the bioconversion of provitamin A carotenoids in the liver of rats. The lipid-dissolved form of provitamin A carotenoids associated with the presence of unsaturated soy oil (rich in linoleic and α-linolenic acids) in vacuum-fried papaya chips favored the formation of a fine emulsion in diet mixture. This facilitated the formation of micelles and consequently a better carotenoid absorption in rats. Finally, these results showed that vacuum-fried papaya chips obtained with soy oil represent an interesting source of provitamin A carotenoids and bioavailable lycopene in a diversified diet. In other words, for the contributions of provitamin A carotenoids, a portion of 25 g of vacuum-fried papaya chips presented a nutritional value of 75 µg Retinol Activity Equivalent (RAE), which corresponds to 10 % of the recommended daily intake (RDI) (800 µg for adults). It could be claimed that food products with 10 % or more of the RDI for vitamin A (RAE) as “good source of vitamin A” (FDA, 2020).

Vacuum-fried papaya chips could be an alternative to offer consumers a healthier product than traditional snacks. For instance, 25-30 g could be a portion for papaya chips (this corresponds to an individual portion for classic commercial potato chips). According to Soto *et al.* (2020), 25 g of papaya chips contains 6.4 g of lipids (which corresponds to 9.8% of the daily intake recommended for lipids) and 3.9 mg of carotenoids. Daily intake recommended for carotenoids does not exist; however, 4.8 mg/day is needed to meet the requirement of 800 µg of vitamin A for an adult (Toti *et al.*, 2018).

Chapter VII

General discussion and complementary results

Chapter VII. General discussion and complementary results

The goal of this research was to design a carotenoid-rich snack from papaya fruit containing essential fatty acids (ω-6 and ω-3), applying vacuum frying to offer a healthier product than traditional fried snacks. The development of this type of snack could represent an alternative strategy to the consumption of traditional deep-fat fried products rich in simple sugars, saturated lipids, and salt. Vacuum-fried papaya chips could be manufactured and consumed in regions such as Latin America and the Caribbean, where this fruit is an important crop. In this region, there is a high prevalence of non-communicable diseases (*e.g.*, cardiovascular diseases and diabetes) and vitamin A deficiency in children (Stevens *et al.*, 2015).

Papaya fruit was chosen as the food matrix for this study because of various reasons: 1) it is a fruit widely spread and consumed in Latin America and the Caribbean (FAO, 2020; Saran *et al.*, 2016); 2) this fruit presents a varied profile of carotenoids, xanthophyll (β-cryptoxanthin) and carotenes (β-carotene and lycopene) (Schweiggert *et al.*, 2012); and 3) the carotenoids are more bioavailable from papaya than from other plant foods such as tomato and carrot due to the deposition form of these compounds in the papaya chromoplasts (globular and tubular substructures) (Schweiggert *et al.*, 2014).

Vacuum frying is performed at low temperatures (from 90 to 140 °C) compared to atmospheric frying (150-180 °C). The low temperatures coupled to the absence of oxygen during the process minimize some undesirable chemical reactions, including pigment degradation and Maillard and caramelization reactions (due to the presence of sugars). Therefore, vacuum frying represents a suitable technology for the processing of fruits and vegetables such as papaya, which present high contents of moisture, sugars, pigments, and nutrients. From a nutritional point of view, vacuum frying of papaya fruit allows the preservation of carotenoids. In addition, the oil uptake in the chips is of utmost importance due to the role of lipids in the transfer and diffusion of carotenoids during processing as well as during digestion (Xavier & Mercadante, 2019). The application of vacuum frying for the development of papaya chips constitutes a

promising food processing alternative to generate a novel product (that meets consumer expectations), adding value to this crop, reducing the losses of raw material (about 30 % of papaya production is compromised due to postharvest losses) (Albertini *et al.*, 2016), and improving the current situation of papaya producers (Figure 7.1).

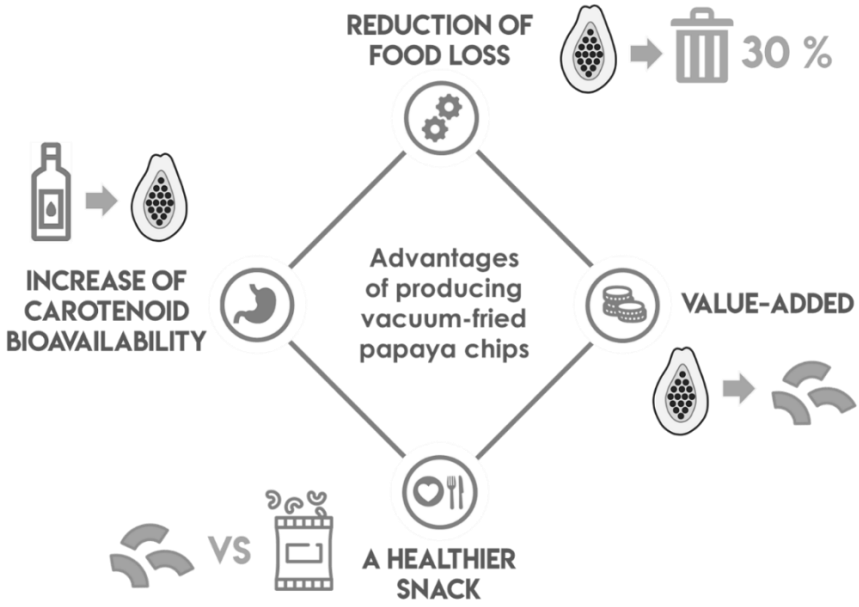

Figure 7.1. Advantages of the application of vacuum frying for papaya chips making.

For making this healthy snack from papaya fruit, it was necessary to follow different stages to obtain not only a product with a high content of carotenoids but with bioavailable carotenoids. Four objectives were proposed around which the thesis project revolved to study the behavior of the carotenoids in raw papaya fruit according to the ripening stage (objective 1), and during the processing (objective 2) and storage (objective 3) of vacuum-fried papaya chips, and finally the absorption and bioconversion of carotenoids after papaya chips consumption (objective 4). The results obtained from each stage allowed us to propose the best conditions for making a snack with adequate sensory attributes (*e.g.*, color, texture, sensory acceptance) and high nutritional value (high carotenoid content and the presence of essential fatty acids, ω-3 and ω-6) as shown summarized in Table 7.1. In addition, it was demonstrated that the selected conditions allowed the elaboration of a food product with bioavailable carotenoids (Table 7.1).

Table 7.1. Behavior of carotenoids in papaya fruit and vacuum-fried papaya chips, and main results obtained according to the different stages of the thesis project.

STAGE	OBJECTIVE 1 RAW MATERIAL	OBJECTIVE 2 VACUUM FRYING	OBJECTIVE 3 STORAGE	OBJECTIVE 4 ABSORPTION/ BIOCONVERSION
WHAT WAS STUDIED?	• Three different ripening stages of papaya fruit: - RS3 (26-40% skin yellowing) - RS4 (41-55% skin yellowing) - RS5 (56-70% skin yellowing) • Different vacuum frying conditions to obtain papaya chips	• Physicochemical changes (moisture, a_w, oil uptake, sugars, color, and carotenoids) of papaya chips during vacuum frying (T=100-140 °C, t=0-14 min)	• Degradation of carotenoids in vacuum-fried papaya chips during storage (T=15-45 °C, t=0-94 days) packaged under air or N_2 conditions • Three treatments: chips with soy oil (24 % lipids) and chips with palm oil (24 % and 29 % lipids)	• Five groups of rats fed with different papaya mixtures for 7 days: - Carotenoid deficient diet (Control) - Vacuum-fried papaya chips with either soy oil (PC-S) or palm oil (PC-P) - Freeze-dried papaya mixed with either soy oil (FDP+S) or palm oil (FDP+P)
WHAT WAS THE OBJECTIVE?	• To select the optimal ripening stage of papaya fruit for papaya chips making • To optimize vacuum frying conditions to obtain papaya chips with desirable characteristics	• To monitor and model the main physicochemical properties of papaya chips during vacuum frying to control their sensory attributes and nutritional value	• To determine the optimal storage conditions regarding the carotenoid degradation in vacuum-fried papaya chips	• To evaluate the effect of consumption of vacuum –fried papaya chips on carotenoid absorption, glycemia, and lipid profile in Wistar rats
WHAT HAPPENS TO CAROTENOIDS? **PROVITAMIN A CAROTENOIDS: β-CAROTENE, β-CRYPTOXANTHIN** ↑	• *Fresh papaya at three ripening stages:* - BC: RS5 presented the highest content and RS3 the lowest content - BCX: RS4 and RS5 presented higher content than RS3 • *Vacuum frying of papaya RS4:* At optimal frying conditions there was a degradation of 40% and 60% of BC and BCX, respectively	• There was a BC increase attributed to an extractability phenomenon in the first minutes of frying followed by a decrease phase because of degradation (including isomerization) • BCX was the least stable carotenoid compared to BC and LYC. BCX degradation followed a second-order model	• Degradation of BCX and BC in papaya chips, packaged under air, followed a second-order model • BCX and BC presented similar rate constants (higher than LYC) • No isomerization was observed in BC (all-E- to Z-forms) during storage • Degradation of BCX and BC was lower in papaya chips with soy oil	• A higher bioconversion of provitamin A carotenoids was observed for soy products (PC-S and FDP+S) due to the emulsions with small oil droplets • The bioconversion of provitamin A carotenoids was generally better when vacuum-fried chips products were ingested (PC-S and PC-P)
LYCOPENE ↓	• *Fresh papaya at three ripening stages:* RS3 presented the lowest LYC content • *Vacuum frying of papaya RS4:* At optimal frying conditions, there was a 1.5-fold increase in the extractability of LYC	• There was an increase of LYC extractability during vacuum frying • Isomerization of LYC was observed, especially at 140 °C • The maximum extractability of total LYC occurred when the chips had ~0.26 g oil·g^{-1} chip DW (at 120 and 140 °C) and ~0.33 g oil·g^{-1} chip DW (at 100 °C)	• LYC was the most stable carotenoid compared to BCX and BC • Rate constants for LYC degradation were the lowest • Z-LYC degraded 2-4-fold slower than Z-BC • Degradation of LYC was lower in papaya chips with soy oil	• A better dissolution of crystalloid LYC was found in PC-P compared to PC-S • A fine emulsion favored the dissolution of small crystalline LYC, thereby increased its absorption during digestion
MAIN RESULTS	• The **RS4** was selected as the **most suitable ripening stage** for processing **to obtain vacuum-fried papaya chips** • Based on physicochemical properties of papaya chips, the **optimal vacuum frying conditions**, for the experiments in objectives 3 and 4, at **25 kPa** were: **120 °C and 13.1 min**	• During vacuum frying of papaya, the **degradation of reducing sugars** (glucose and fructose) **was observed**, whereas the **formation of sucrose occurred** • **Prediction of a_w, browning index, and BCX loss** can be used to **optimize process conditions** to produce quality papaya chips	• **Packaging under nitrogen** conditions caused **no degradation of carotenoids** in vacuum-fried papaya chips during storage (3 months) • **Provitamin A carotenoids and lycopene** were **more stable** in the **presence of soy oil** in chips packaged with air, **especially at** low storage temperature **(15-25 °C)**	• Consumption of **vacuum-fried papaya chips did not alter glycemia, cholesterol, or triglyceride**s in rat plasma • Consumption of **vacuum-fried papaya chips favored the absorption** of lycopene (especially with palm oil) and **provitamin A carotenoids** (especially with soy oil) in rats

BC, all-E-β-carotene; BCX, all-E-β-cryptoxanthin; LYC, lycopene; Z-BC, Z-β-carotene; Z-LYC, Z-lycopene. DW, dry weight. PC-S, papaya chips with soy oil; PC-P, papaya chips with palm oil; FDP+S, freeze-dried papaya mixed with soy oil; FDP+P, freeze-dried papaya mixed with palm oil.

7.1. Selection of optimal ripening stage of papaya fruit

The physicochemical composition and micronutrient content vary with ripening fruit, such as happens in papaya. Therefore, it was necessary to select the most promising ripening stage in papaya fruits for vacuum-fried chips making. That means a ripening stage to obtain a product with suitable physicochemical properties (moisture, a_w, lipids, color parameters) and good sensory acceptance. Also, a ripening stage that would allow papaya processing with the highest possible yield. The objective was to select an optimal ripening stage to standardize the frying process for the rest of the experiments, thus avoiding variations in the results due to a matrix effect.

In this way, from a seven-stages scale (from RS1 to RS7), previously developed at *Laboratorio de Tecnología Poscosecha* (CIA, Universidad de Costa Rica), three ripening stages (RS3, RS4, and RS5) were preselected. Fruits at low ripeness (RS1 and RS2) were not considered because they presented less sweetness, color (low content of carotenoids), and aroma. On the other hand, fruits at high ripeness (RS6 and RS7) were unsuitable for processing due to the lack of pulp firmness, causing low yields during peeling and slicing.

The chemical composition of fruits at the three ripening stages analyzed (RS3, RS4, and RS5) was similar in many components, such as moisture, protein, ash, lipids, dietary fiber, sugars, total soluble solids, and organic acids. There were no significant differences ($p>0.05$) for these parameters. However, it was found that the skin and pulp firmness, color parameters, and carotenoid content differed significantly ($p<0.05$). In general, it was found that in the ripening range evaluated with increasing ripeness, pulp and skin firmness, and the L* parameter decreased, but the C* parameter and the carotenoid content increased as shown in Figure 7.2.

Papaya fruits at RS3 and RS4 showed a higher skin and pulp firmness ($p <0.05$) than those at RS5. Regarding the color parameters, RS3 had the highest L* value (luminosity) and the lowest C * value (color saturation), which is linked to a lower content of total carotenoids compared to RS4 and RS5. Between RS4 and RS5,

there were no significant differences for the color parameters and carotenoid content ($p>0.05$).

Regarding the papaya chips obtained after vacuum frying, in general, it was observed that as the ripening stage increased, the sensory acceptance increased, but the process yields decreased (Figure 7.2). According to the results, RS4 and RS5 presented a greater sensory acceptance than RS3. The sensory test was performed by a panel of 100 consumers of snacks and fruits. On the other hand, the lowest process yield (to obtain the papaya slices and the papaya chips) was reached by processing fruits at RS5. The highest frying yield was obtained processing the fruits at RS4.

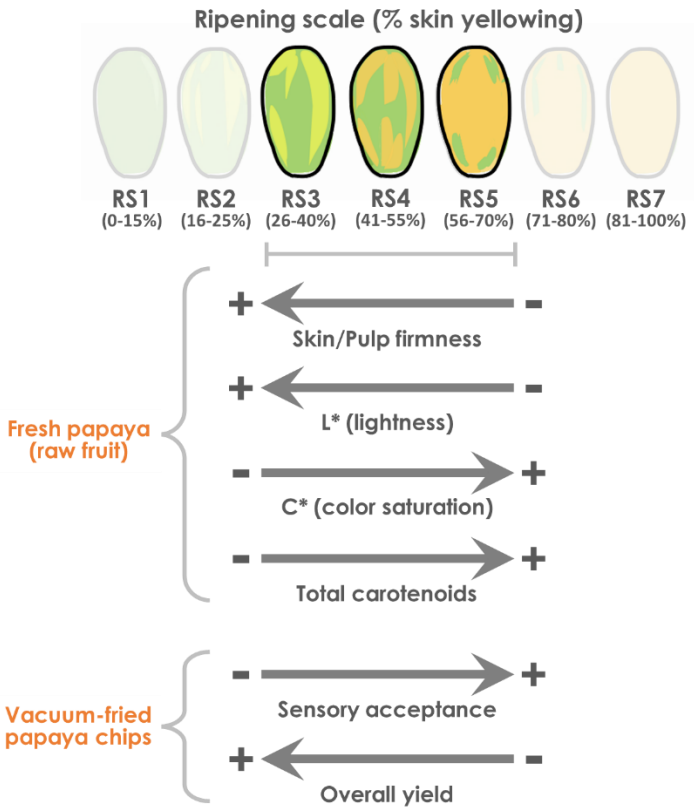

Figure 7.2. Main changes found in fresh papaya and vacuum-fried papaya chips according to the three ripening stages (RS) evaluated (+ means that parameter increased with increasing or decreasing ripeness; - means that parameter decreased with increasing or decreasing ripeness).

Taking into account the parameters that are shown in Figure 7.2, it was found that the ripening stage 4 (RS4, 41-55 % skin yellowing) was the most suitable for

the production of vacuum fried papaya chips. This stage allowed the production of papaya chips with adequate physicochemical properties and sensory acceptance. In addition, from a technical point of view, papaya fruits at RS4 generated an overall process yield higher than the others. Once the optimal ripening stage was selected, the response surface methodology (RSM) was performed to optimize the vacuum frying conditions for papaya chips making, using a product/oil ratio of 1/50. The independent variables were frying temperature (100-140 °C) and frying time (9-15 min). The vacuum pressure was set at 25 kPa since it was the minimum reached by the equipment.

The significance, goodness of fit, and contour surfaces of the models (second-order polynomial model) generated for the different physicochemical parameters were analyzed. The optimal frying conditions were selected, having papaya chips with the lowest moisture content and a_w values to ensure their adequate shelf life during storage (Wexler *et al.*, 2016). In addition, the product presented a low lipid content (better nutritional profile), low color variation (taken as a reference to fresh fruit), and a low browning index. These latest color parameters are related to carotenoid degradation and non-enzymatic browning reactions (*e.g.*, Maillard and caramelization reactions) (Andrés-Bello *et al.*, 2011).

At an operating pressure of 25 kPa, the optimum oil temperature was 120 °C and the optimum frying time was 13.1 min, keeping a product/oil ratio of 1/50. These frying conditions allowed us to obtain quality vacuum-fried papaya chips. Concerning the carotenoid content, at optimal frying conditions, degradation of 40% and 60% for BC and BCX occurred, respectively, and there was a 1.5-fold increase in the extractability of LYC. The frying conditions were established for making the papaya chips for the rest of the experiments of this study, in Objectives 3 and 4.

7.2. Effect of vacuum frying on physicochemical properties and content of carotenoids of papaya chips

During frying, various physicochemical changes take place in the food products, affecting their sensory properties and nutritional value. These changes occur due to the mass transfer phenomena between the frying medium and the product

(water loss and oil absorption in the food). Also, chemical reactions in both the food (degradation of pigments, loss of vitamins, degradation of sugars, among others) and the frying medium (oxidation, hydrolysis, and polymerization reactions) are responsible for these changes.

In this objective, it was studied the effect of different vacuum frying conditions (oil temperature, 100-140 °C, and frying time, 0-14 min) on some physicochemical properties related to sensory attributes and nutritional value of vacuum-fried papaya chips, such as moisture, oil absorption, water activity, sugar content, browning index, and carotenoids. Then, different kinetic models were evaluated to describe the changes observed for each physicochemical property in the papaya chips. Finally, some of the generated kinetic models (a_w, browning index, and loss of BCX) were selected to optimize the vacuum frying conditions considering the impact of these parameters on the texture, color, and nutritional value (provitamin A activity) of papaya chips. The desirability of a_w, color, and nutritional value in papaya chips was determined according to the literature. It was considered the most relevant physicochemical characteristics for this kind of products.

Figure 4.6 (Chapter IV) summarizes very well the influence of oil temperature and frying time on physicochemical properties and how these affect the sensory attributes and nutritional value of vacuum-fried papaya chips. Regarding the loss of moisture, a_w, and oil absorption (parameters related to the mass transfer phenomenon), the observed behavior coincides with that reported by the literature in frying processes (Krokida *et al.*, 2000; Troncoso & Pedreschi, 2009; Ziaiifar *et al.*, 2010; Manjunatha *et al.*, 2014; Ayustaningwarno *et al.*, 2020b).

Regarding the color, there was not a clear trend for color variation in papaya chips during vacuum frying and the L *, a *, b * parameters could not be modeled during the process. Papaya fruit is a complex matrix and color variations in vacuum-fried papaya chips could be the result of carotenoid degradation (loss of red-orange color) and formation of molecules from Maillard or caramelization reactions (development of browning), and to a lesser extent the adsorbed oil in the chips during the frying process. In this way, it was necessary to decouple the color

measurement in papaya chips since the reactions that affect the color variation are diverse.

Determination of browning index (BI) in aqueous extracts obtained from papaya chips allowed us to describe best the reaction that alters the color in the chips. Experimental data for browning (expressed as the dimensionless form BI/BI_0) were fitted well with a 3-parameter logistic function as shown in Chapter IV (section 2.7.3). Browning is related to Maillard or caramelization reactions (that involved sugar degradation). These reactions generate colored compounds (*e.g.*, hydroxymethylfurfural that can form melanoidins) affecting the color of papaya chips.

The most relevant results generated in this objective were about the reactivity of carotenoids and sugars during frying of papaya fruit. These compounds have a direct influence not only on the color variation of the final fried product but on the nutritional value of the snack. Regarding carotenoid reactivity, it was shown that carotenoids in papaya fruit have a different reactivity behavior during vacuum frying influenced by their chemical structure, as summarized in Figure 7.3.

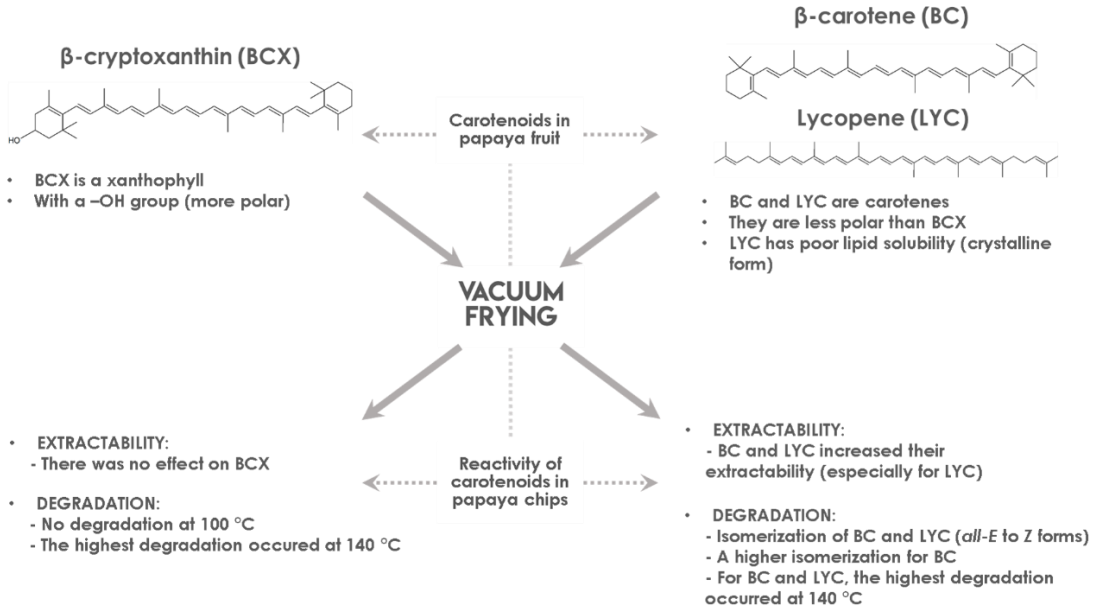

Figure 7.3. Reactivity of different carotenoids analyzed in vacuum-fried papaya chips.

There was a clear difference between xanthophylls (BCX) and carotenes (BC and LYC). In the case of BCX, it was the least stable carotenoid compared to the others. At 100 °C, there was no BCX degradation, but it decreased more rapidly at higher temperatures (120–140 °C) during the process, following a second-order reaction model. In addition, there was no effect of vacuum frying on its extractability, as happened with BC and LYC.

On the other hand, BC and LYC were influenced by vacuum frying conditions on their extractability. In general, at first, there was an increase of BC and LYC attributed to an extractability phenomenon in the first minutes of frying followed by a decrease phase because of degradation during the rest of the process, including isomerization (all-E- to Z-forms). Increasing of extractability of β-carotene and especially of lycopene during vacuum frying of papaya could be explained by two factors: i) a higher release of these carotenoids from papaya cell tissues (*e.g.*, chromoplasts) due to the matrix structure disruption during frying (100-140 °C), and ii) an increase of their solubility enhanced by frying oil uptake in papaya chips.

BCX has a higher lipid solubility compared to LYC and BC. LYC has the lowest solubility and accumulates in the papaya matrix as crystals associated with the plastid membranes (Schweiggert *et al.*, 2011b). Thus, the oil uptake in the chips allowed the crystalline structures of lycopene to dissolve, increasing its extractability during analyses. LYC needed a higher oil content in the chips to reach the maximum extractability during the process (0.26-0.33 g oil·g^{-1} chip DW) than BC (~0.13 g oil·g^{-1} chip DW). Conformation, concentration, and lipid solubility of carotenoids in the papaya chips seemed to have a key effect on the reactivity of these compounds during vacuum frying. The extractability increase of carotenoids could have a positive impact on their *in vivo* absorption during digestion.

For sugars, it was observed the degradation of glucose and fructose during vacuum frying of papaya fruit. Glucose and fructose are the main sugars detected in fresh papaya since sucrose content is negligible in this fruit (Figure 7.4a), representing 53 and 47 % of total sugars, respectively. Unexpectedly, the formation of sucrose occurred during vacuum frying. This phenomenon was

notorious at the highest temperatures (120 and 140 °C), as shown in Chapter IV (Figure 4.2). Forming of sucrose in a real food matrix was a new find because there is no information in the reviewed literature showing a similar reaction as seen in this study. Due to our skepticism about the nature of this compound, we originally named it as the "mysterious compound". Figure 7.4d shows the retention time of the sucrose standard, matching with the retention time observed for the "mysterious compound" found in papaya chips after vacuum frying (Figure 7.4b). To verify if this compound was sucrose, we applied two tests: i) enzymatic treatment with invertase, which catalyzes the hydrolysis of sucrose into fructose and glucose; and ii) mass spectrometry analysis of papaya chips and sucrose standard.

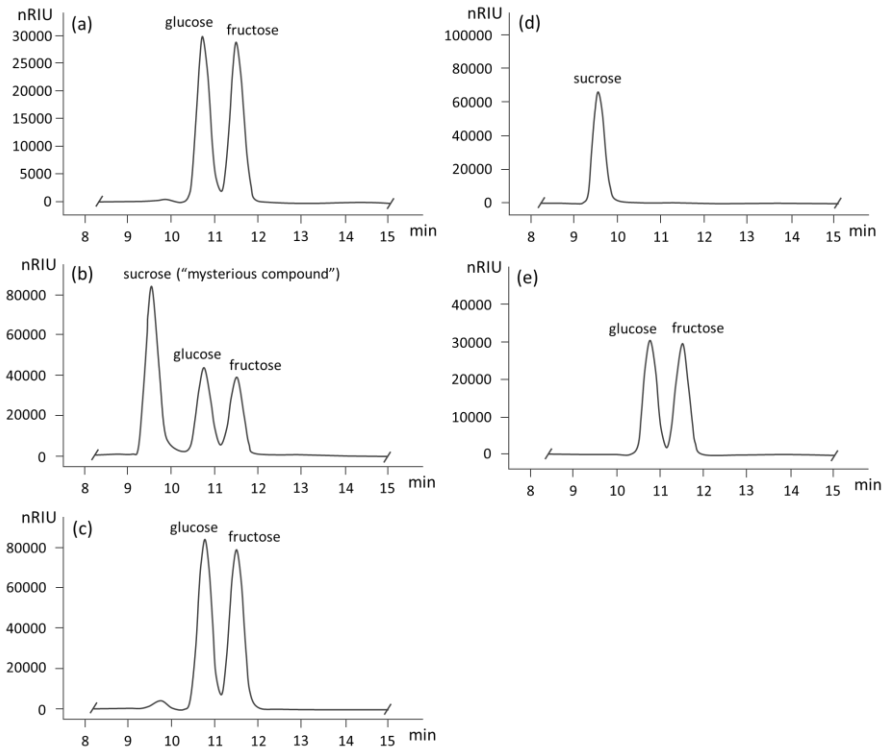

Figure 7.4. UPLC separation of analyzed sugars monitored with a refractive index (RI) detector from: (a) extract of fresh papaya (500 mg/mL distilled water), (b) extract of vacuum-fried papaya chips (50 mg/mL distilled water), (c) extract of vacuum-fried papaya chips after enzymatic treatment with invertase, (d) standard sucrose (13.6 mg/mL acetate buffer), (e) standard sucrose after enzymatic treatment with invertase.

Figure 7.4c shows that after performing enzymatic treatment on the papaya chip extract using invertase, an enzyme that acts specifically on sucrose, almost total reduction of the “mysterious compound” occurred while the contents of glucose and fructose increased. This result indicated that the “mysterious compound” was sucrose. The enzymatic treatment's efficacy was verified by applying the invertase on the standard sucrose (Figures 7.4d and 7.4e). However, despite this test, a mass spectrometric analysis was carried out to be 100 % sure about the identification of this compound (Figure 7.5).

Figure 7.5 shows the liquid chromatography - electrospray ionization - mass spectrometry (LC-ESI-MS) of vacuum-fried papaya chips and sucrose standard. For the papaya chips, three peaks (P_1, P_2, and P_3) were detected (Figure 7.5a). Figures 7.5c and 7.5d show identical MS and MS^2 spectra corresponding to sucrose $[M-H_2O]+H^+$. With the help of the sucrose standard, it was demonstrated that P_1 corresponded to sucrose (2-*O*-α-D-glucopyranosyl-D-fructose) and the other two peaks (P_2 and P_3) were isomers of sucrose (Figures 7.5a and 7.5b). Further studies should be conducted to identify these sucrose isomers since many of them have some interesting physicochemical properties and physiological effects. For instance, some isomers inhibit lipid accumulation in the human body, others have low glycemic index properties and prebiotic activity, and most of them are not cariogenic as sucrose (Tian *et al.*, 2019).

After applying these two tests, we were sure about the formation of sucrose in papaya chips during vacuum frying, probably due to a condensation reaction between glucose and fructose (decrease of these sugars during frying). However, further studies are needed to elucidate the mechanisms involved in sugar reactions during the frying of papaya fruit.

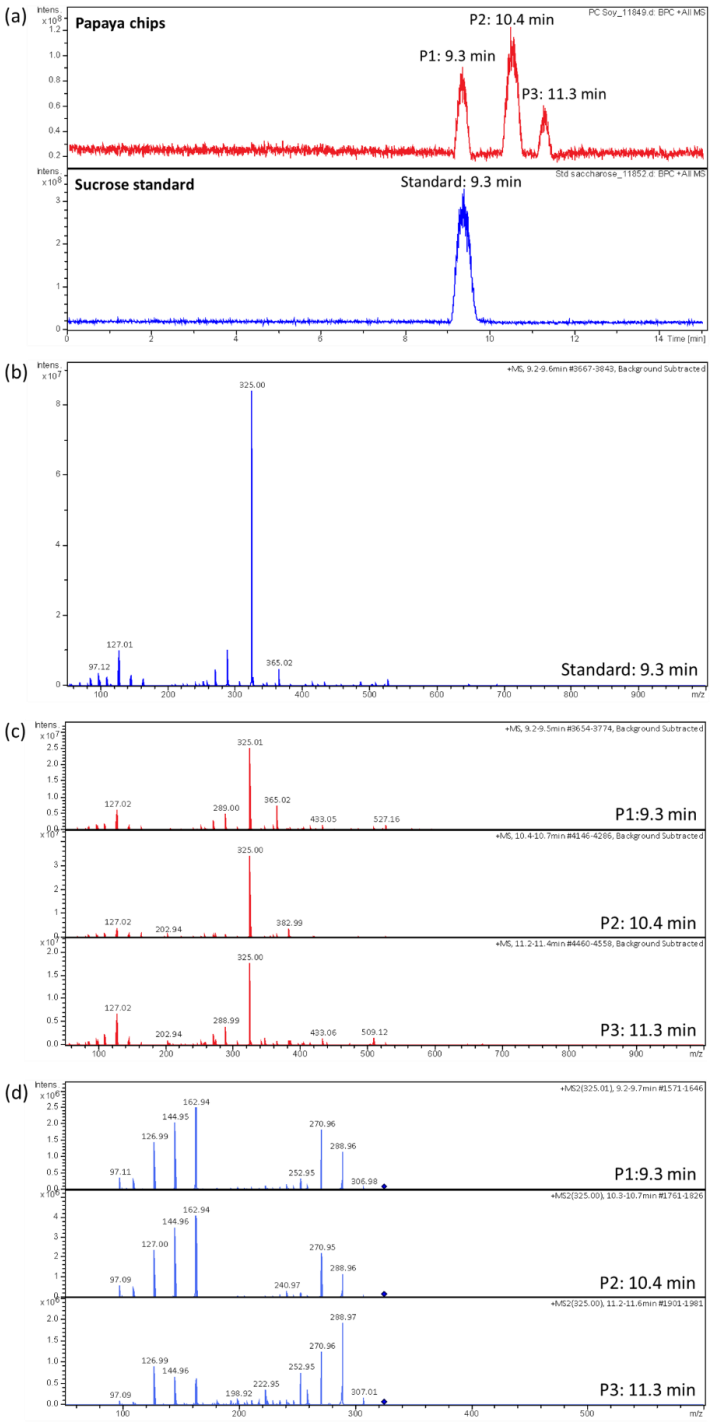

Figure 7.5. Positive ESI-MS^n spectra of extract of papaya chips (50 mg/mL distilled water) and sucrose standard (13.6 mg/mL distilled water): (a) LC-ESI-MS of papaya chips and sucrose standard; (b) spectrum MS of sucrose standard, (c) spectrum MS of extract of papaya chips, (d) spectrum MS^2 of ion *m/z* 325 (extract of papaya chips). Papaya chips presented sucrose (2-*O*-α-D-glucopyranosyl-D-fructose) (P_1) and two isomers of sucrose (P_2 and P_3).

Finally, the optimal process conditions (oil temperature and frying time) were determined, following the kinetic models generated for the variables a_w, BI, and loss of BCX. Control of a_w is critical to ensure microbiological quality and maintain a suitable texture during storage of dried products. According to Konopacka *et al.* (2002), adequate crispness is reached when a_w values in apple chips ranged from 0.1 to 0.3. Color variation was related to BI (measured in the aqueous extracts), possibly due to the degradation of sugars. Some authors suggested that a color difference between raw and vacuum-fried fruits (ΔE) of 20 or less is acceptable (Mariscal & Bouchon, 2008; Diamante *et al.*, 2011). For papaya chips, it was found that a ΔE <20 corresponds to BI/BI_0 of 5 (or $BI=5 * BI_0$). From a nutritional point of view, BCX losses lower than 30 % ensured 10 % or more of the recommended daily intake for vitamin A (RAE) in the papaya chips. Considering the desirability of these variables, optimal vacuum frying conditions comprise a region that is a combination of oil temperatures from 107 to 120 °C and frying times from 9 to 14 min, as shown in Figure 7.6.

Figure 7.6 compares the two approaches performed in the objectives 1 and 2 of this study to optimize the vacuum frying conditions (oil temperature and frying time). The optimal point obtained by RSM was congruent with the optimal region generated by the kinetic modeling analysis, even though the dependent variables or physicochemical parameters analyzed were different (except for a_w) and the conditions evaluated in objective 1 were performed at non-constant oil temperature, as shown in Figure 7.7. The frying trials for objective 1 were carried out with a product/oil ratio of 1/50 versus 1/500 in objective 2, which caused a drop in oil temperature during part of the frying process (Figure 7.7). Nevertheless, these frying conditions could be useful for processing papaya or other similar carotenoid-rich fruits keeping the same operating conditions. However, factors such as the type of vacuum fryer (equipment dimensions, heat system, vacuum system, etc.), type of oil (saturated, unsaturated), size, thickness, and shape of fruit slices, product/oil ratio, and ripening stage of fruit, among others, may be considered.

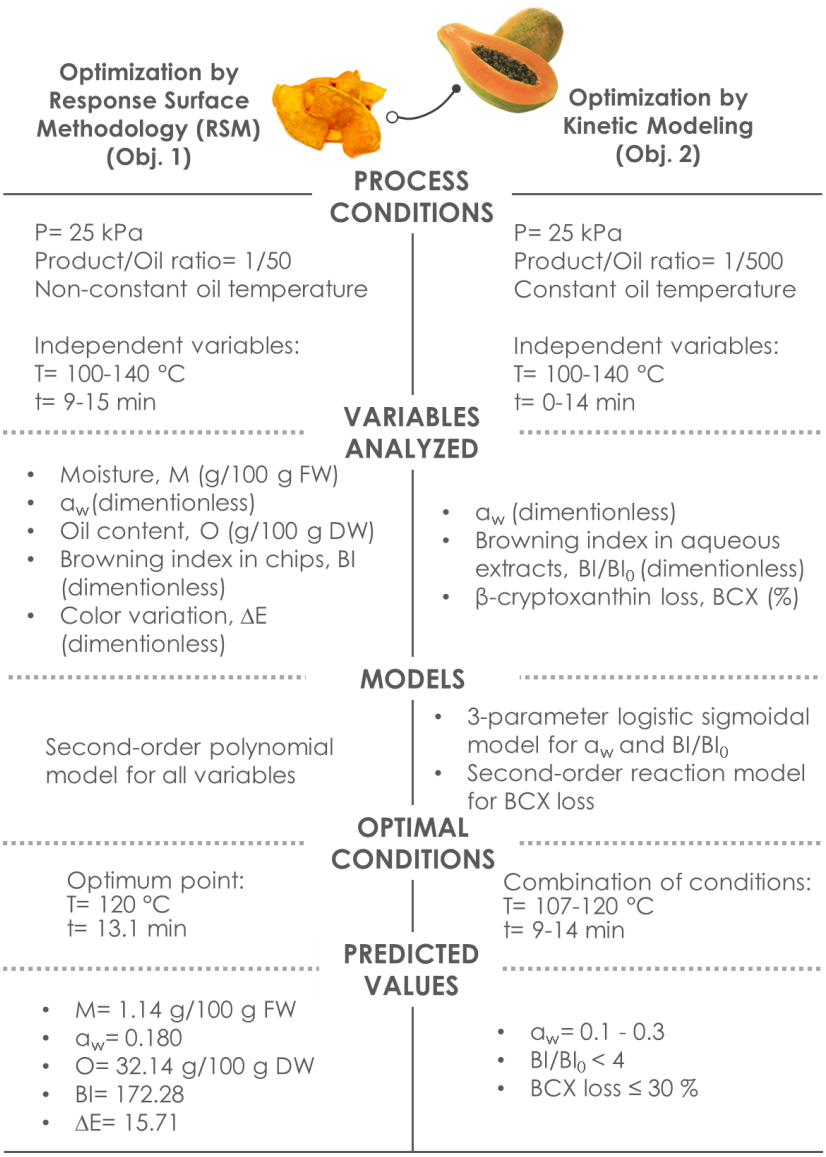

Figure 7.6. Comparison of different approaches performed to optimize vacuum frying conditions in objectives 1 and 2.

Other parameters such as the sugar and oil content may be analyzed for producing vacuum-fried fruit chips since they have a sensory and nutritional impact on the final product. The sugars and lipids not only provide calories in fruit chips but also contribute to their taste. In the literature, there is not much information about the quality limits for these parameters in this type of food. Thus, these parameters must be validated with sensory studies with consumers.

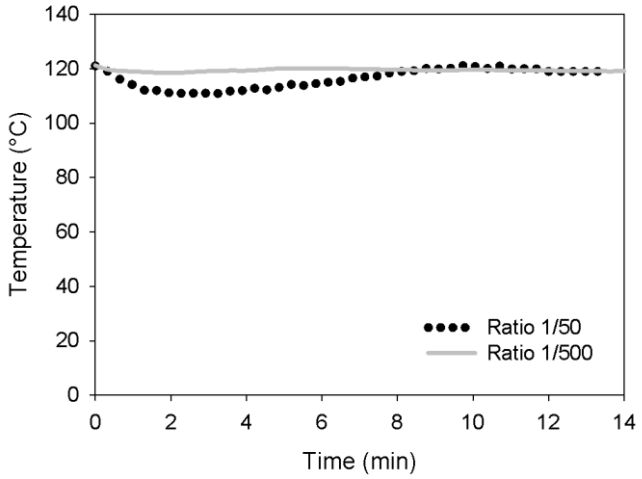

Figure 7.7. Temperature-time profile for soy oil used as frying medium (at two ratio product/oil) during vacuum frying of papaya chips at 120 °C. Ratio product oil: 1/50 (Objective 1) and 1/500 (Objective 2).

7.3. Determination of optimal storage conditions regarding carotenoid degradation in vacuum-fried papaya chips

It is useless to optimize the processing conditions to obtain a food product with good sensory and nutritional characteristics if subsequent storage conditions cause the product to lose its quality, reducing its shelf-life. For instance, in the case of papaya chips, loss of crispness, oxidation of pigments and lipids, and degradation of bioactive compounds such as carotenoids are factors that limit their quality. It should be noted that in products such as papaya chips, which are dehydrated foods with low a_w, hygroscopic, and rich in pigments and lipids, it is necessary to use a suitable packaging. This packaging must be a good barrier against moisture and oxygen, avoiding oxidation reactions and the loss of crispness in the product.

The degradation of carotenoids affects not only the color loss but also the loss of the nutritional value of papaya chips. During processing and storage of dried products, carotenoids are prone to isomerization and oxidation as a result of exposure to high temperature, oxygen, light, or pro-oxidant molecules (Caris-Veyrat *et al.*, 2003; Colle *et al.*, 2010b; Achir *et al.*, 2014; Xiao *et al.*, 2018). The carotenoids present in papaya chips provide provitamin A activity and

antioxidant activity to the product. Hence the importance of determining the storage conditions that allow minimizing the degradation of these compounds. In this objective, different parameters of the food were evaluated, such as type of oil (soy and palm oil) and oil content in the product (24 and 29 % lipids), as well as different conditions of packaging (presence of oxygen or nitrogen inside the packages) and storage (temperature and time). Papaya chips were stored at 15 °C and 25 °C for 94 days (with sampling times at 0, 15, 25, 45, 60, 75, and 94 days) and at 35 °C and 45 °C for 52 days (with sampling times at 0, 3, 8, 15, 25, 35, and 52 days). All papaya chips obtained by the different treatments were packaged in polyethylene terephthalate metallized/polyethylene (PETm/PE, 12/70 μm) bags.

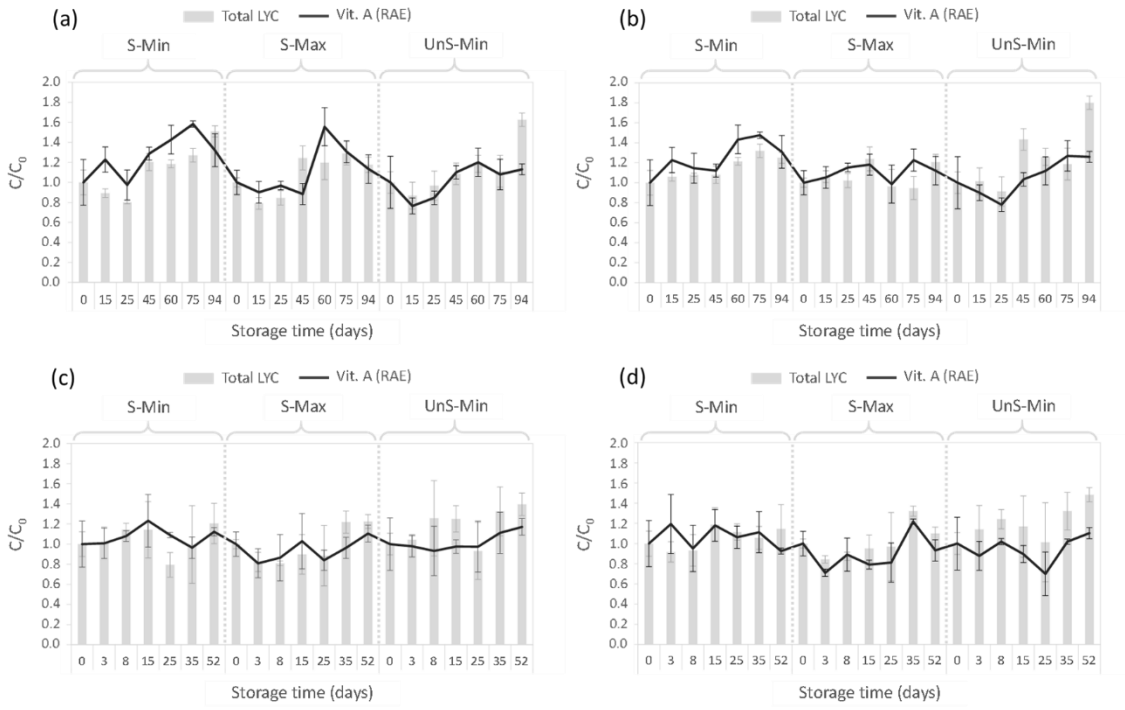

Figure 7.8. Variation of dimensionless concentrations (C/C_0) of vitamin A activity and total lycopene content in papaya chips packaged under nitrogen conditions during the storage at four temperatures: (a) 15 °C, (b) 25 °C, (c) 35 °C, (d) 45 °C. S-Min, palm oil, 24 % lipids; S-Max, palm oil, 29 % lipids; UnS-Min, soy oil, 24 % lipids. Vit. A, Vitamin A activity: Retinol Activity Equivalent (RAE). Total LYC, Total lycopene content: it was calculated as the sum of all-E and Z-isomers in papaya chips, fresh weight. Bars (for Total LYC) and lines (for Vit. A) are expressed as the means ± standard deviation (n=3).

As the most relevant results generated in this objective, it was observed a positive effect of nitrogen on carotenoid preservation during storage. No significant or minimum degradation of carotenoids was observed in papaya chips packaged under nitrogen conditions regardless of oil composition (the type of oil and oil content) and temperature, as shown in Figure 7.8. Modeling of lycopene content and vitamin A activity in papaya chips packaged under N_2 was not performed because there was no clear trend for these parameters. However, these results showed how oxygen is a key factor in carotenoid degradation, confirming that packaging under low oxygen conditions must be applied during storage to preserve the maximal carotenoid content in this type of food.

On the other hand, carotenoid degradation occurred in the chips stored under air conditions. A second-order kinetic model best described the experimental data for all carotenoids analyzed. In addition, all carotenoids followed Arrhenius temperature-dependency pattern. The rate constants (k) for LYC degradation were the lowest, while BCX and BC had similar rate constants from 4 to 23-fold higher than LYC depending on the lipid composition. The Z isomers degraded faster than all-E forms, and among the *cis* carotenoids, Z-BC showed higher rate constants than Z-LYC, as shown in Table 5.3 (Chapter V).

Regarding the effect of oil content (24 % versus 29 %), our results showed that higher lipid content in papaya chips (containing palm oil) improved carotenoid retention. To explain this phenomenon, it was hypothesized that the presence of more lipids in the chips could cause a protective effect by replacing the voids filled with air, thus reducing the oxidation of carotenoids. Moreover, it was observed that carotenoid retention was higher ($p<0.05$) in papaya chips with soy oil than in those with palm oil, having the same lipid content (24 % lipids). For instance, the use of soy oil instead of palm oil increased the theoretical half-life (at 25 °C) by 2.2, 1.3, and 5.9-fold for BCX, BC, and LYC, respectively.

Viscosity differences can be observed between palm and soy oils at ambient temperature. Whereas palm oil is solid (Figures 7.9a), soy oil is liquid (Figure 7.9d) because of the lower content of saturated fatty acids that present this oil than hydrogenated palm oil. At 120 °C, which was the operating frying temperature, both oils are liquids (Figures 7.9b and 7.9e), but the kinematic

viscosity of soy oil (5.68 $mm^2 \cdot s^{-1}$) remains lower than palm oil (5.85 $mm^2 \cdot s^{-1}$) (Esteban *et al.*, 2012). Thus, the observed behavior could be explained by the lower viscosity of soy oil during cooling than palm oil, favoring a deeper oil uptake inside the pores generated in the fried product (more structural oil than surface oil in the papaya chips). Thus, during the storage of the product, there are fewer spaces or voids to be filled with the air present in the bags reducing the carotenoid oxidation in the chips, as schematized in Figures 7.9c and 7.9f.

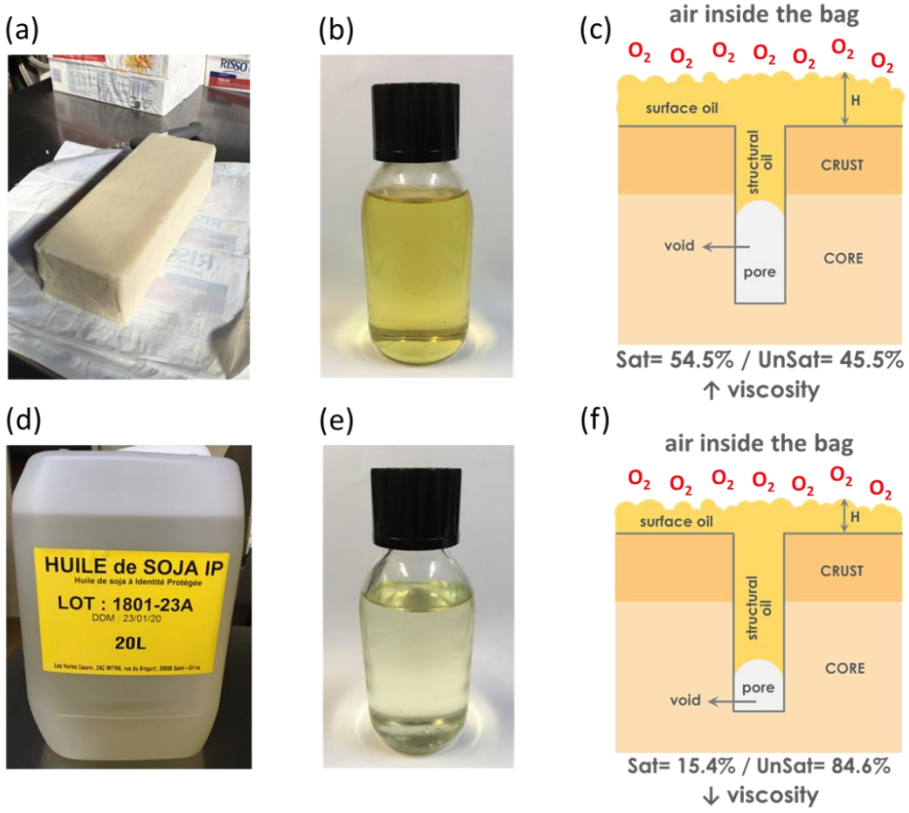

Figure 7. 9. Comparison of the two oils used as frying medium during vacuum frying to obtain papaya chips for the storage study: (a) hydrogenated palm oil at room temperature, (b) palm oil at 120 °C, (c) diagram of oil absorption in chips with palm oil, (d) soy oil at room temperature, (e) soy oil at 120 °C, (f) diagram of oil absorption in chips with soy oil. SFA, saturated fatty acids; UFA, unsaturated fatty acids.

Packaging under nitrogen conditions and lipid composition may be considered to optimize shelf-life and retention of carotenoids in papaya chips during storage. In a limited storage time and providing good quality of soy oil, this alternative to saturated oil could be nutritionally interesting since soy oil provides essential fatty acids such as linoleic acid (ω-6) and alpha-linolenic acid (ω-3).

7.4. Evaluation of consumption of vacuum-fried papaya chips on carotenoid absorption, glycemia, and lipid profile in Wistar rats

After studying the effect of the ripening stage of papaya fruit, and the effect of vacuum frying and storage conditions on carotenoid content and physicochemical properties of papaya chips, it was necessary to evaluate the health benefits of this snack. One of our hypotheses or assumptions was that vacuum frying would improve the *in vivo* absorption of carotenoids due to the microstructural changes and oil uptake that undergo the product, causing an extractability increase of carotenoids, as demonstrated in Objective 2, hence the importance of achieving this objective.

Despite a portion of carotenoids being degraded during processing, the amount released from the matrix is higher (Boon *et al.*, 2010). Moreover, lipids are essential in the transfer and diffusion of carotenoids during processing and digestion since these compounds need to be released from the matrix and then incorporated into the micelles for their absorption (Xavier & Mercadante, 2019). In addition, factors such as the chain length and saturation degree of the fatty acids affect the incorporation of carotenoids into the mixed micelles (Lemmens *et al.*, 2014).

In this way, it was of interest to evaluate the effect of food processing and the type of oil on the absorption/bioconversion of carotenoids using an animal model (Wistar rats). Mixtures based on vacuum fried papaya chips (processed at T= 120 °C for t= 13 min) were compared with those based on unheated papaya. Since fresh papaya has lower carotenoid content than papaya chips, the fruit had to be freeze-dried in order to obtain samples with similar carotenoid content. Regarding the effect of the type of oil, the oils evaluated previously in Objective 3 were used, soy oil (unsaturated) and palm oil (saturated). Thus, the vacuum-fried papaya chips were obtained with both soy and palm oils (PC-S and PC-P). Likewise, these oils were added to freeze-dried papaya for making the other mixtures (FDP+S and FDP+P), having all the samples the same lipid content, as described in section 3.5 (Chapter II). These oils were chosen because they are widely used for frying purposes, and they present a very different fatty acid

profile. In addition, these oils present a lower vitamin E content in contrast to other vegetable oils, thus avoiding any interaction with the carotenoids.

To exemplify the effect of processing on the microstructural changes of food, wide-field microscopy of papaya products was performed. Figure 7.10 shows how in fresh papaya fruit, the carotenoids are contained within the intact cells (Figure 7.10a, marked with arrows); however, after the fruit is processed either with freeze-drying (Figure 7.10b) or vacuum frying (Figures 7.10c and 7.10d), these cellular structures are disrupted allowing the release of carotenoids (marked with arrows). Regarding vacuum frying, the presence of oil allows a higher diffusion of the carotenoids into the matrix, as shown in Figures 7.10c and 7.10d.

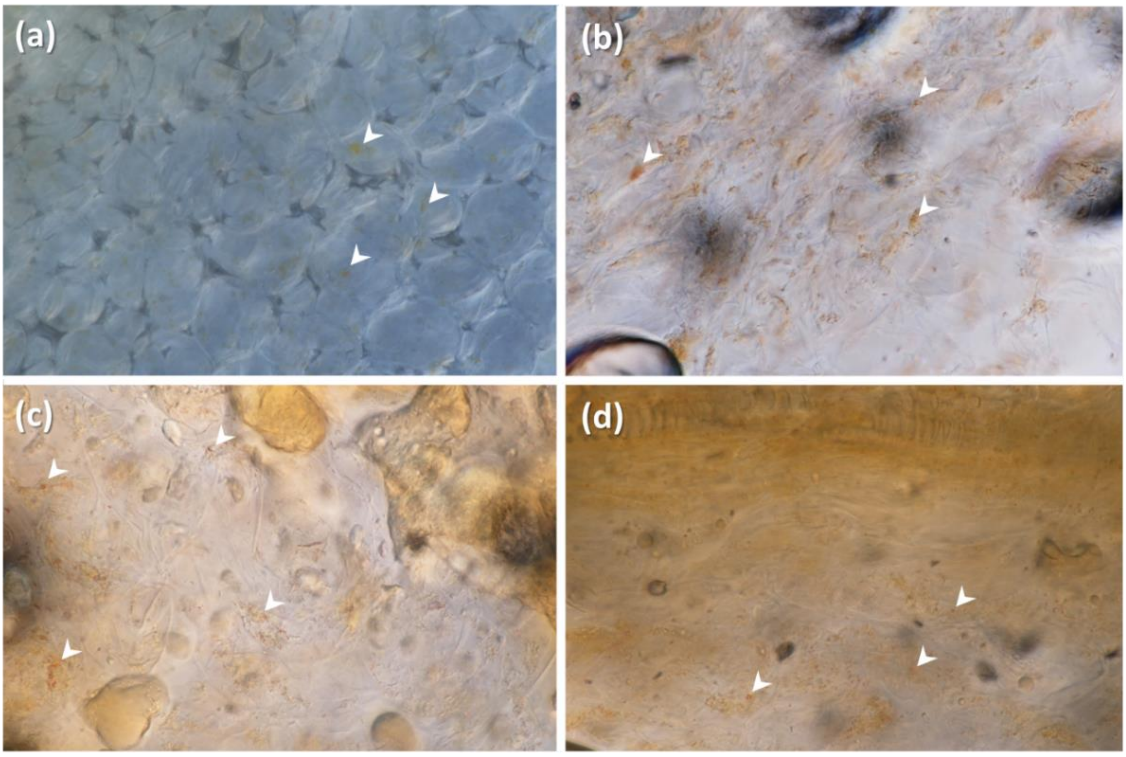

Figure 7. 10. Wide-field microscopy (DIC 40 X objective) of papaya products: (a) fresh papaya (var. Formosa), (b) freeze-dried papaya, (c) vacuum-fried papaya chips with soy oil soy, (d) vacuum-fried papaya chips with palm soy.

Because papaya chips are a complex food matrix with lipids and high sugar content, it was also necessary to evaluate if their consumption affected the glycemia, lipid profile (triglycerides, TRIG; total cholesterol, CHO; HDL

cholesterol, HDL; and LDL cholesterol, LDL), and weight gain of the rats. It is useless to develop a food product that is rich in bioavailable carotenoids but affects other metabolic parameters.

According to our results, the consumption of papaya chip mixtures did not alter the weight gain, lipid profile in the plasma, and the glycemia of rats, regardless of the type of oil. After the diet period, there were no significant differences ($p>0.05$) in weight gain, glycemia, and lipid profile (TRIG, CHO, HDL, and LDL) between rats consuming papaya chip mixtures (with soy and palm oils) and the Control group (animals with carotenoid deficient diet that received only the regular A04 diet). Only the content of free fatty acids (FFA) increased significantly in plasma of rats consuming papaya chips mixtures compared to the Control group. The heat treatment (120 °C) applied during vacuum frying of papaya could induce some hydrolysis of triglycerides from the frying medium (soy and palm oils). Thus, the oil absorbed in papaya chips during the process could alter the levels of FFA in the plasma of rats.

Regarding the absorption of carotenoids, the results showed that LYC accumulation in the liver was higher ($p<0.05$) in rats fed with PC-P mixture (0.422 μg/g liver) than in those which were fed with the PC-S mixture (0.291 μg/g liver). Wide-field and confocal microscopy analyses showed that a better dissolution of crystalloid LYC was found in PC-P, showing that LYC transfer to oil during vacuum-frying of papaya fruit could have been better with palm oil than with soy oil.

Conversely, a higher bioconversion of provitamin A carotenoids was observed for soy products (PC-S and FDP+S). The effect of oil type was highlighted by epifluorescence microscopy of papaya mixtures showing homogeneous and fine emulsions with smaller lipid droplets for soy products. Also, the effect of processing was observed comparing diets with vacuum-fried papaya chips mixtures (PC-S and PC-P) and those with freeze-dried papaya mixtures (FDP+S and FDP+P). Bioconversion of provitamin A carotenoids (BC and BCX) was better in rats consuming the vacuum-fried chips products. The average retinyl palmitate content was 161 μg/g liver for papaya chip mixtures versus 114 μg/g liver for freeze-dried papaya mixtures. This result is relevant since the vitamin A

content (RAE) of mixtures based on papaya chips was around 2-fold lower than in freeze-dried papaya mixtures.

Factors such as type of oil (fatty acid chain length), size of emulsion oil droplets, and frying process (heat treatment) could explain the results obtained, as shown in Figure 7.11. Added to these factors, the deposition and subcellular localization of carotenoids in the food matrix also influence the carotenoid absorption. In papaya fruit, LYC is deposited in crystalloid chromoplast, whereas BC and BCX in globular-tubular chromoplast (Schweiggert & Carle, 2017).

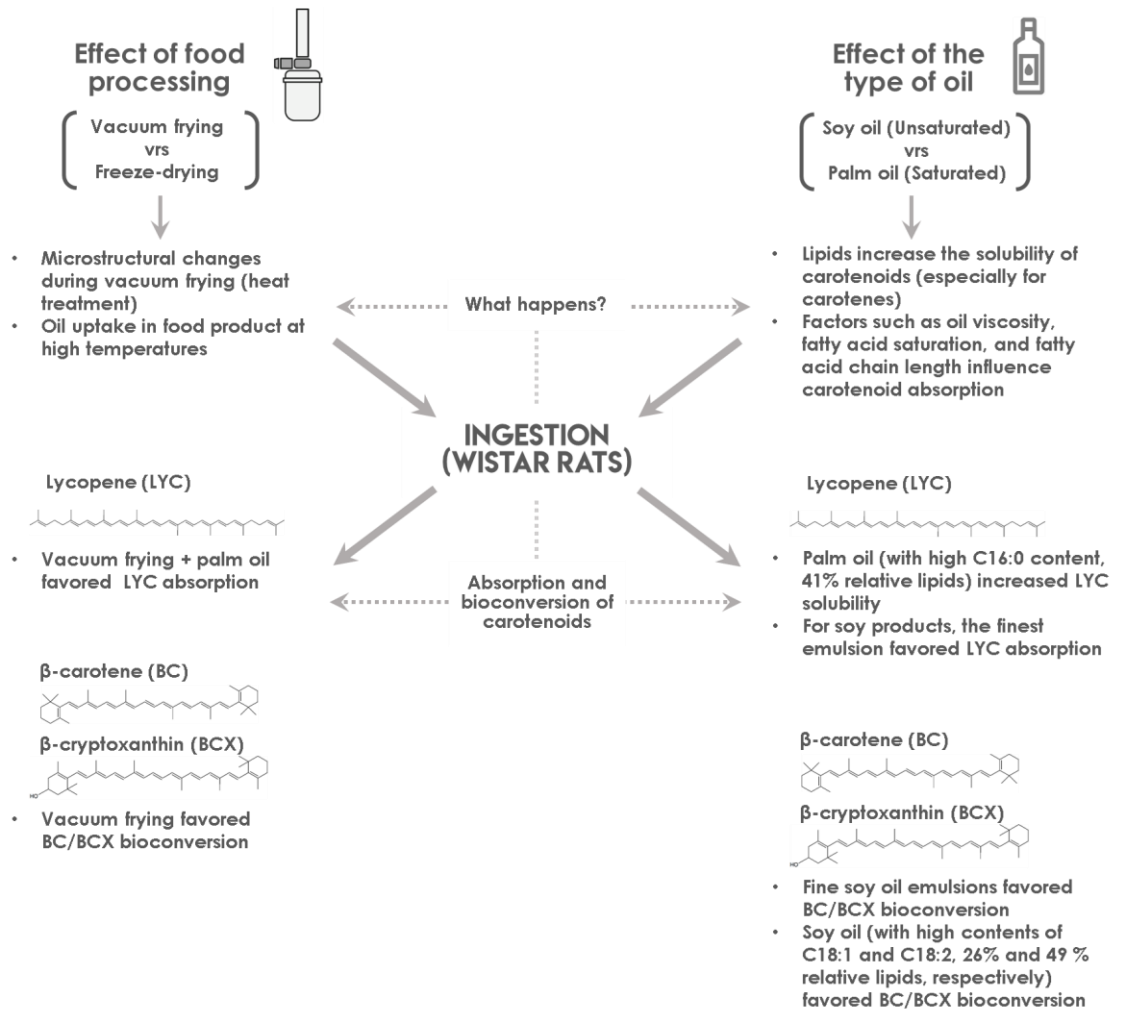

Figure 7. 11. Effect of food processing and the type of oil on the absorption and bioconversion of carotenoids using Wistar rats fed with mixtures based on vacuum-fried papaya chips.

Figure 7.11 shows that the vacuum frying process had a positive effect on the absorption of LYC in the presence of palm oil. Vacuum frying, which was performed at temperature of 120 °C, enhanced the incorporation of oil into the pores generated in the food matrix during frying, thus increasing diffusion and solubility of crystalline LYC. In addition, it has been demonstrated that fatty acids with a medium-chain length, such as palmitic acid (C16:0, major fatty acid in palm oil), increased lycopene bioaccessibility compared to those with long-chain length (Colle *et al.*, 2012).

These results showed that vacuum-fried papaya chips elaborated with soy oil (unsaturated oil) could be recommended as an alternative snack in a diversified diet, being a source not only of provitamin A carotenoids and bioavailable lycopene but essential fatty acids as well, as summarized in Figure 7.12. For instance, 25-30 g could be a portion for papaya chips (this corresponds to an individual portion for classic commercial potato chips). Regarding the provitamin A carotenoids, a portion of 25 g of vacuum-fried papaya chips presented a nutritional value of 75 μg Retinol Activity Equivalent (RAE) (Chapter IV, section 3.6), which corresponds to 10 % of the recommended daily intake (RDI) (800 μg for adults). It could be claimed that food products with 10 % or more of the RDI for vitamin A (RAE) as "good source of vitamin A" (FDA, 2020).

Also, according to results obtained in Chapter V (section 3.1), 25 g of papaya chips contain 6.4 g of total fat and 0.99 of saturated fat, which corresponds to 8.2 % and 5 % of the RDI for these compounds, respectively (FDA, 2020). Besides, this portion contains 3.9 mg of total carotenoids. The RDI for carotenoids does not exist; however, 4.8 mg/day is needed to meet the requirement of 800 μg of vitamin A for an adult (Toti *et al.*, 2018).

If we compare the papaya chips concerning traditional snacks (for example, potato chips), the snack based on papaya fruit has a lower content of total fat and saturated fat and a higher content of polyunsaturated fatty acids than potato chips. In addition, the papaya snack provides vitamin A and a higher content of dietary fiber.

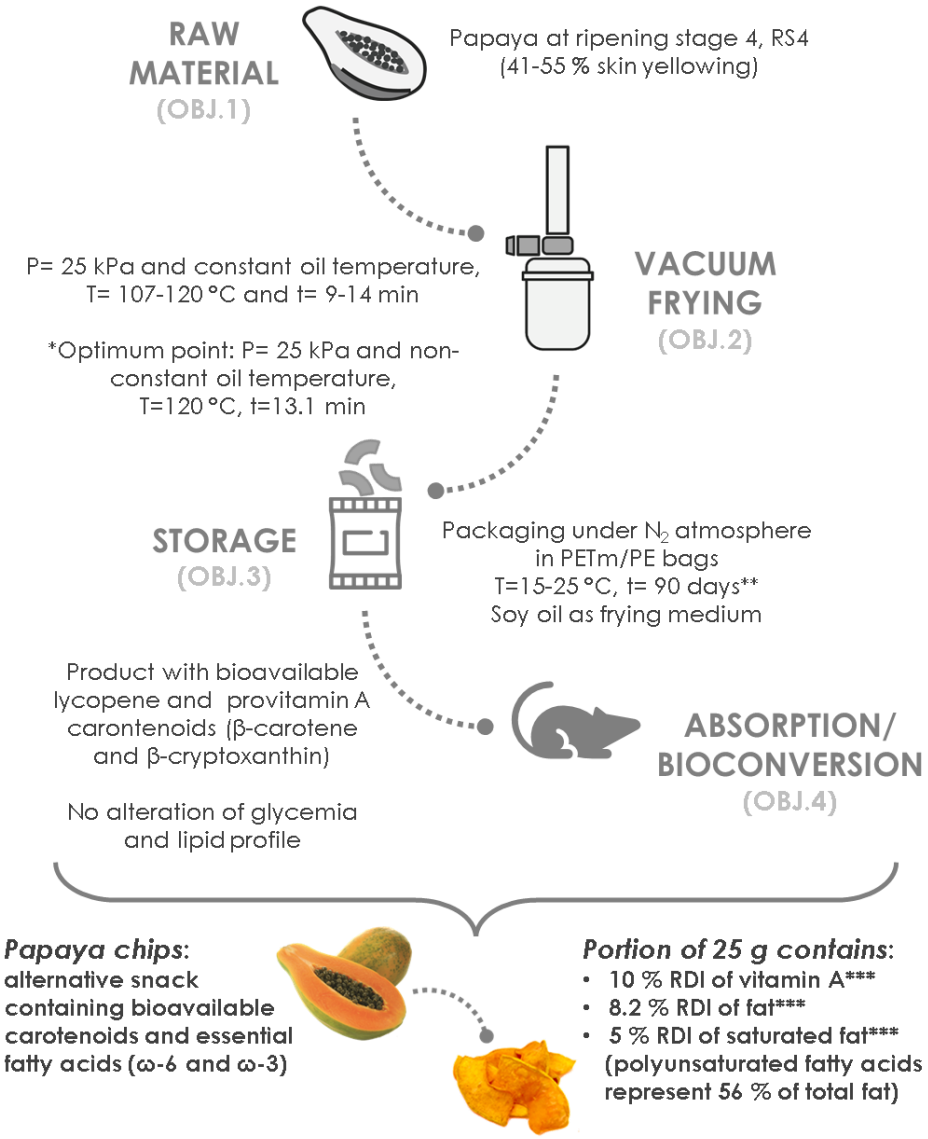

Figure 7. 12. Characteristics of raw material and recommended processing and storage conditions to obtain suitable vacuum fried papaya chips with physicochemical and nutritional quality. RDI, recommended daily intake. *Optimal conditions obtained by RSM, approach in Objective 1. **The storage study was limited to 90 days but this time could be longer. ***% RDI based on a 2000 kcal diet (FDA, 2020).

It is important to mention that the vacuum-fried papaya chips contains high levels of sugars and lipids, and it was observed in the animal experimentation that the level of free fatty acids (FFA) increased significantly in plasma of rats consuming papaya chips mixtures. According to Capurso and Capurso (2012) the excess of FFA induces or aggravates insulin resistance in liver and muscle through direct or indirect (from triglyceride deposits) generation of metabolites, altering

the insulin signaling pathway, which is key to the further development of type 2 diabetes. Thus, further studies should be conducted to verify the results obtained in our study considering other metabolic parameters related to insulin resistance and oxidative stress.

Figure 7.12 summarizes the characteristics of raw material and recommended processing and storage conditions, according to each stage or objective of this project to obtain suitable vacuum fried papaya chips with physicochemical and nutritional quality. These conditions can be used as a reference for scaling up for manufacturing at an industrial level. This information also will be useful for snack producers who want to make fried products based on fruits rich in carotenoids containing essential fatty acids.

Chapter VIII

Conclusion and perspectives

Chapter VIII. Conclusions and perspectives

Carotenoids are relevant compounds in foods not only because they provide color, but because they are bioactive compounds that provide many benefits for human health (provitamin A activity, antioxidant activity, enhance the functions of the immune system, and lower the development of chronic diseases, among others). Due to the lipophilic nature of carotenoids and the way they are deposited and localized in different plant tissues, these compounds often need to be released from the cellular substructures of the food matrix to be absorbed during digestion. Hence, food processing (thermal and mechanical treatments) contributes to increasing the extractability of carotenoids.

During food processing and storage, carotenoids are susceptible to degradation reactions (isomerization, oxidation, cleavage reactions) mainly due to several factors such as high temperatures, oxygen, light, the presence of metals, enzymes, and other pro-oxidant compounds. However, it has been shown that processing, even though it can generate carotenoid losses, also increases the bioaccessibility and bioavailability of these compounds. Due to this context, two research questions were posed at the beginning of the project:

- *What are the main factors involved in the degradation of carotenoids during vacuum frying and subsequent storage of papaya chips?*
- *Do the microstructure changes of vacuum-fried papaya chips and the type of oil used as frying medium influence the absorption and bioconversion of carotenoids?*

Effectively, after performing the four stages or objectives of the project, these questions were answered. Regarding the first question, it was found that the presence of oxygen during the processing and storage of foods rich in carotenoids such as papaya chips constitutes one of the main factors involved in the degradation of these compounds. The negative effect of oxygen on carotenoids increases in the presence of high temperatures (> 100 ° C) (synergistic effect). For this reason, vacuum frying for processing fruits such as papaya coupled to nitrogen atmosphere during the packaging of papaya chips (oxygen-free

packaging atmosphere) are essential factors to preserve carotenoids and improve their bioavailability, as demonstrated in this study. Although vacuum frying occurs at temperatures ranging from 100 to 140 °C, the absence of oxygen prevents or minimizes the degradation of carotenoids.

Regarding the second research question, it was observed that the heat treatment caused carotenoid degradation (including isomerization). However, the microstructural changes due to the disruption of the cellular substructures of the food matrix improved the release of carotenoids (mainly carotenes, β-carotene, and lycopene). This phenomenon added to the presence of lipids caused a higher extractability of carotenoids during frying, having a positive effect on their *in vivo* absorption, as demonstrated in the study with the animal model. Likewise, it is well known that the presence of lipids plays a role in the transfer and diffusion of carotenoids during processing as well as during digestion since these compounds need to be released from the food matrix and incorporated into lipid emulsion droplets and then transferred to mixed micelles (micellization) to be absorbable by the enterocyte.

Several factors such as oil viscosity, the degree of saturation, and the chain length of the fatty acids influenced the absorption of the carotenoids present in papaya chips. For example, the lower viscosity of soy oil compared to palm oil could have influenced the formation of finer emulsions containing smaller and round-shape oil droplets, improving the absorption of provitamin A carotenoids and lycopene. On the other hand, it was hypothesized, based on the information from literature, that fatty acids with a medium-chain length, such as palmitic acid (major fatty acid in palm oil), increased lycopene absorption after the consumption of papaya chips. Likewise, the presence of unsaturated long-chain fatty acids such as oleic acid and linoleic acid in soy oil promoted the absorption of provitamin A carotenoids such as β-carotene and β-cryptoxanthin.

In this context, vacuum frying is an adequate technology for making snacks from fruits and vegetables rich in carotenoids. As said before, this technology minimizes the degradation of carotenoids and improves their bioavailability since it combines three key factors that affect the reactivity of these compounds:

- Heat treatment at not so high temperatures of frying medium (compared to conventional frying, which occurs at 150-180 ° C) for short times.
- A partial or total absence of oxygen.
- Oil uptake in the food product.

The most valuable contribution of this thesis was the comprehensive approach that the project had since it analyzed what happened with the bioactive compound of interest (in this case, the carotenoids) during the different stages that the food undergoes. This project evaluated the effect that the characteristics of raw material and the processing and storage conditions have on the carotenoid reactivity in papaya chips affecting the *in vivo* absorption of carotenoids in the animal model.

In addition, other phenomena that the papaya fruit experienced during vacuum were observed, for instance, the reactions that sugars present in papaya undergo during frying: condensation reaction producing sucrose (the formation of three sucrose isomers was observed) and Maillard or caramelization reactions producing colored compounds (browning in papaya chips). In fact, it was possible to model the browning index (in aqueous extracts obtained from papaya chips) and facilitated the characterization of the kinetics of Maillard or caramelization reactions that alter color in the chips. From a nutritional point of view, the sugar reaction observed in papaya chips (decrease of glucose and fructose, and formation of sucrose isomers) was beneficial because it could reduce the glycemic index of the fried product compared to a non-heated papaya product (freeze-dried papaya). It was observed in the animal model study that the diet with mixtures based on papaya chips did not increase the glycemia of the rats. A phenomenon that did occur in the rats that consumed the mixtures based on freeze-dried papaya.

Because this work covered such broad topics in each objective, many perspectives arose to continue this research project. The most relevant are mentioned below:

- Although two papaya varieties were used (Pococí from Costa Rica and Formosa from Brazil), the results obtained with both varieties were

consistent with each other, as they presented a similar proximal composition and carotenoid profile. However, further studies may be performed to validate whether the phenomena observed in this study with the two varieties evaluated agree with other ones.

- We found that three isomers of sucrose were formed during vacuum frying of papaya chips, but further studies are needed to identify the mechanisms involved in sugar reactions during frying of papaya and other carotenoid-rich fruits with similar sugar composition.
- It would be of interest to evaluate if the changes in the sugar profile that papaya experiences during vacuum frying have any positive effect on health, for instance, inhibition of lipid accumulation in the human body, reduction of glycemia, prebiotic activity, among others.
- We observed that the lipid composition of papaya chips affected the stability of carotenoids during storage. We evaluated lipid contents of 24 % and 29 % in the final product. In further research, it would be of interest to evaluate the carotenoid reactivity and the oxidative stability of lipids in a product with a lower content of lipids, for example a fat content lower than 20% which is more according to the current trends that promote foods with low fat content. This research is needed to understand the physical and chemical phenomena involved in carotenoid degradation during the storage of carotenoid-rich fried products.
- We performed an exploratory animal study over a short period of time; however, it was sufficient to observe an effect on carotenoid absorption and bioconversion. Therefore, more studies should be conducted to verify the results obtained (acute versus short-term administration). In addition, it would be necessary to consider other metabolic parameters related to oxidative stress (such as superoxide dismutase activity, catalase activity, among others) and analyze other target tissues in which carotenoids can accumulate or store (for example, prostate, kidney, colon, spleen, among others).
- Finally, it is necessary to validate the results obtained from the animal study with human clinical trials. This snack has the potential to provide bioavailable lycopene and provitamin A carotenoids for child populations

in regions of Latin America, where deficiencies of this vitamin are common.

From a practical point of view, this study brought valuable information about the optimal conditions of vacuum frying for making a carotenoid-rich snack from papaya fruit containing essential fatty acids (ω-6 and ω-3). The information generated from this study will be of great interest for manufacturers of fruit/vegetable fried products who try to obtain attractive and healthy snacks.

Chapter IX

References

Chapter IX. References

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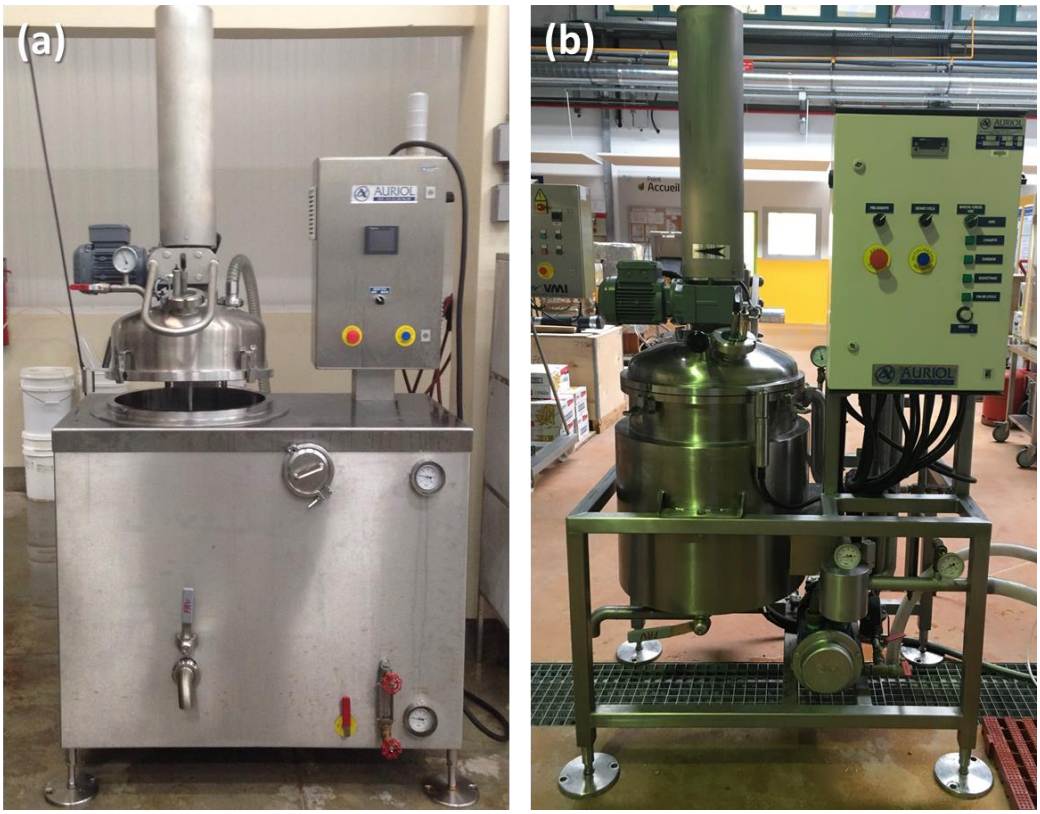
Chapter X

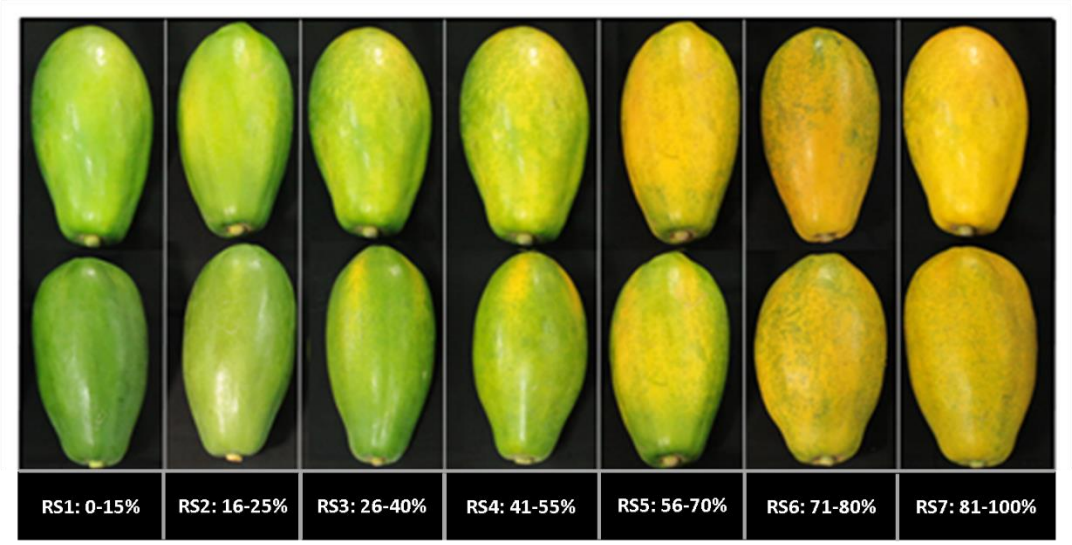
Appendix

Chapter X. Appendix

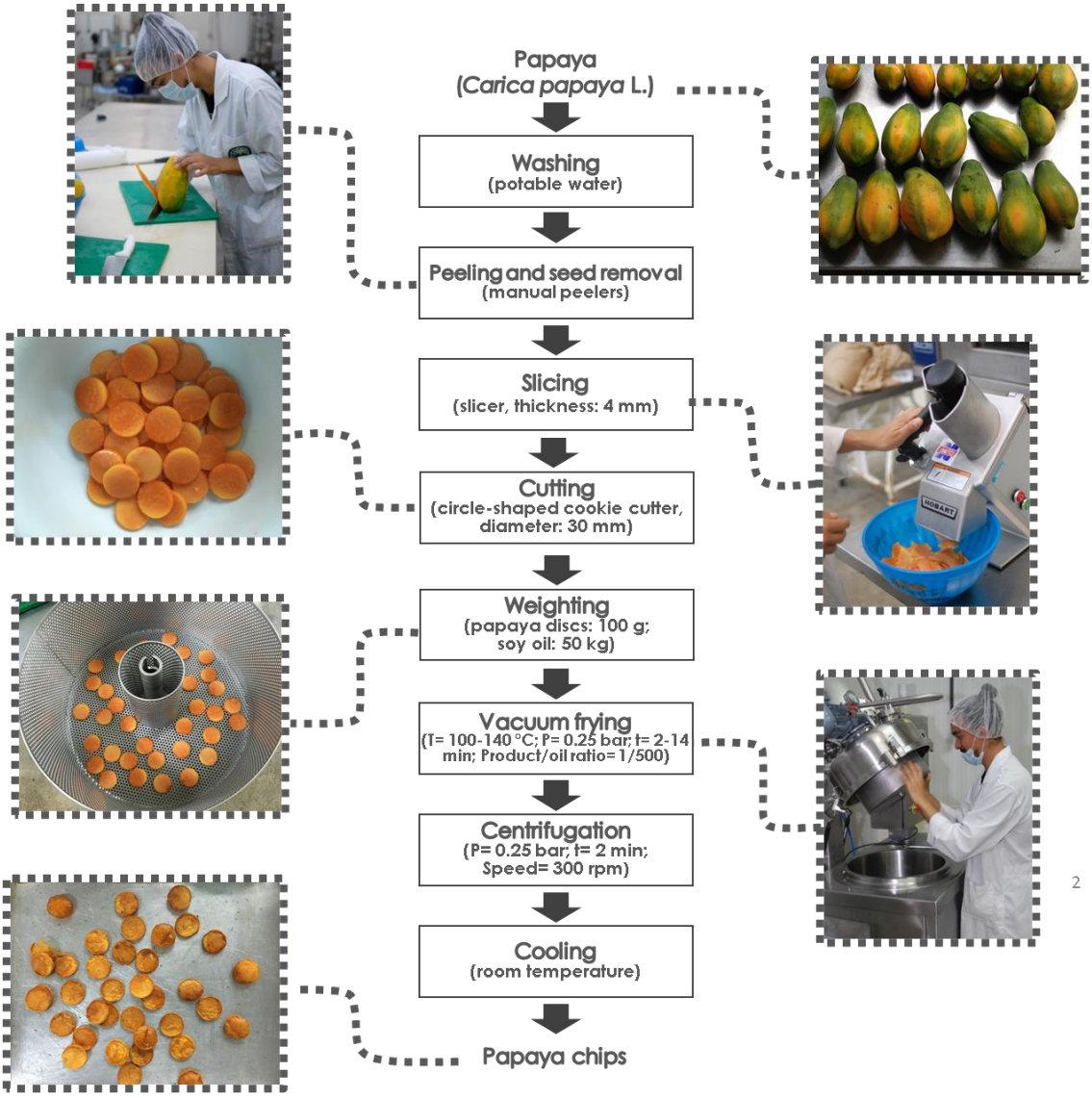
A1. Vacuum fryers used for obtaining the papaya chips: (a) vacuum fryer from Costa Rica; (b) vacuum fryer from France.



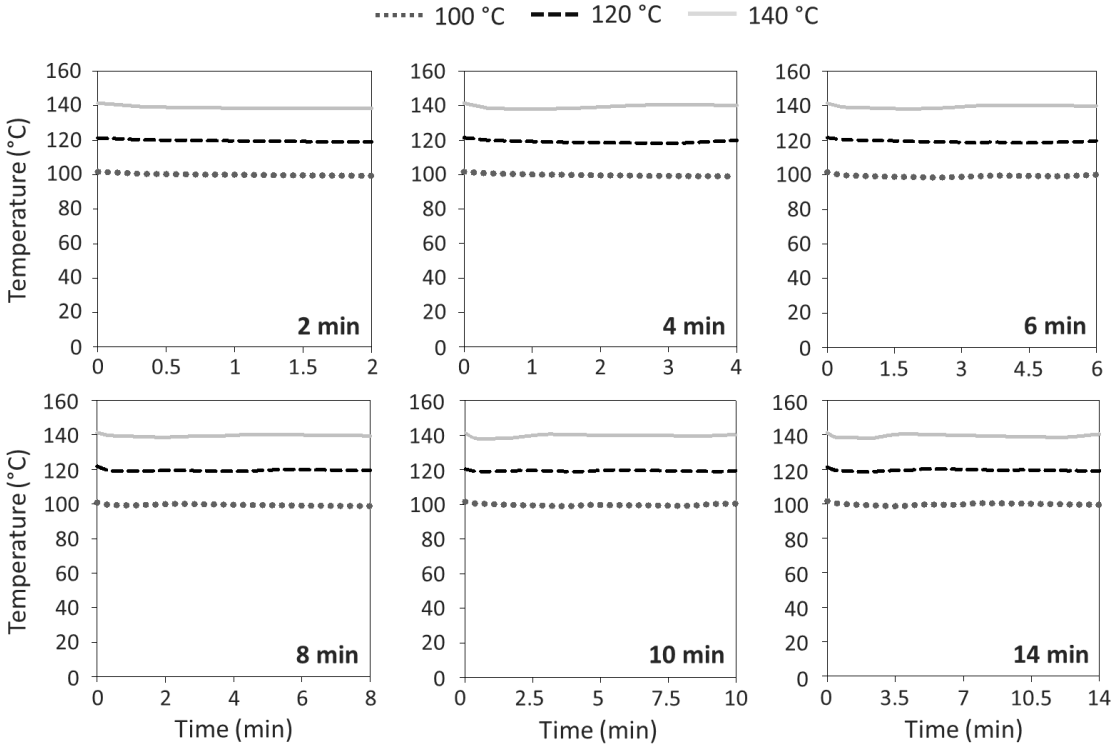
A2. Ripening scale with seven categories or stages according to skin yellowing (%) developed at Laboratorio de Tecnología Poscosecha (CIA, Universidad de Costa Rica).



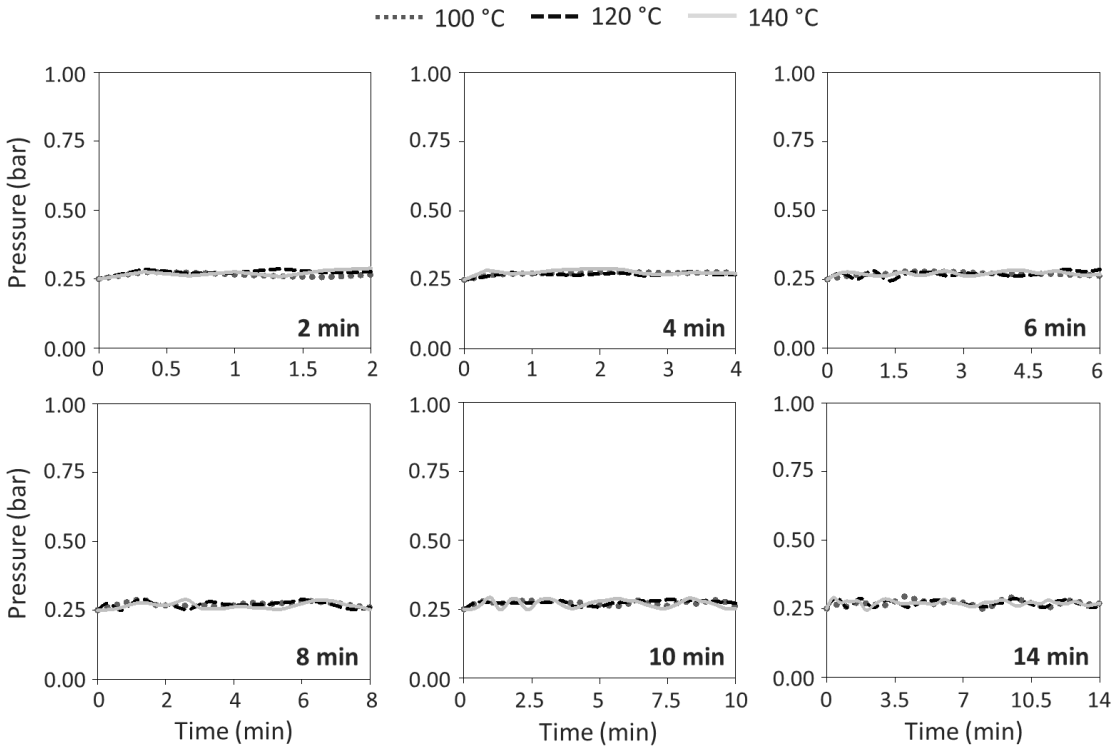
A3. Process flow to obtain vacuum-fried papaya chips at different oil temperatures and frying times.



A4. Time-temperature profiles for oil used as frying medium during vacuum frying (100-140 °C) of papaya chips.



A5. Time-pressure profiles for frying vessel during vacuum frying (100-140 °C) of papaya chips.



A6. Technical data of the metallized bags used for packaging of papaya chips.

Sachet sous vide métallisé PET/PE 12/70 µm

Caractéristiques :
Haute sécurité pour le produit et protection des arômes avec une excellente barrière au gaz et à la lumière.
Qualité certifiée de la matière première jusqu'au sachet fini
Résistance à la perforation élevée
Très bonne qualité de soudure.

Domaine d'utilisation :
Viande fraîche
Charcuterie
Poisson
Fromage non gazeux
Plats cuisinés
Non-Food

Certificats :
BRC pour la fabrication du film et des sachets.

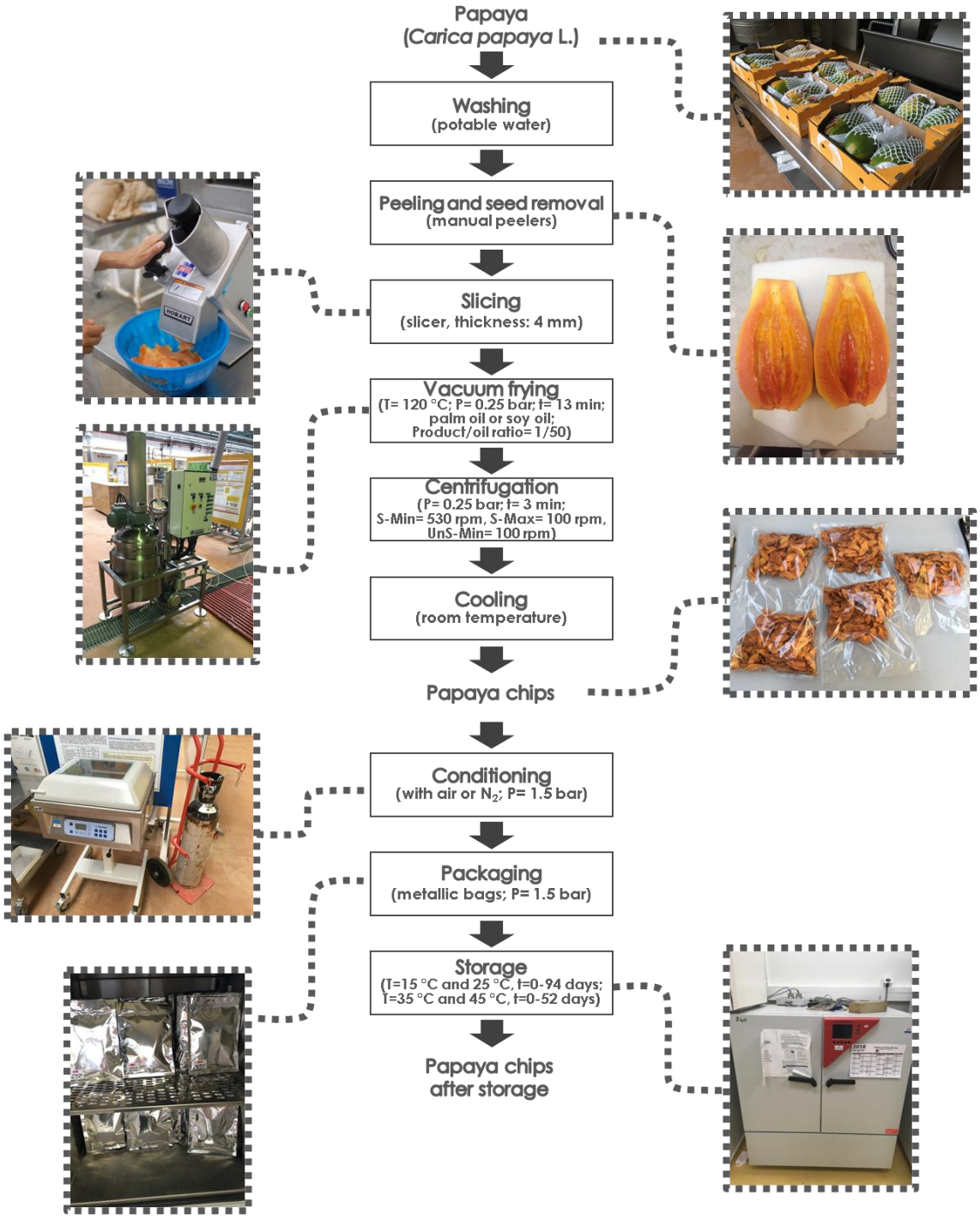
Plage d'utilisation :
-40°C jusqu'à +90°C

Conservation :
au frais et au sec
Durée de stockage de 1 an maximum entre 4 et 25 °C

Désignation	Unité	Valeur	Méthode
Composition du film	PA/PE		
Epaisseur	µm	12/70	DIN 53370 23°C / 50% r.F.
Perméabilité à			
Vapeur d'eau	g/m².d	1	DIN 53122 23°C / 85% r.F.
Oxygène	cm³/m²	1	DIN 53380 23°C / 75% r.F.
Gaz carbonique	cm³/m²	3	DIN 53380 23°C / 75% r.F.
Azote	cm³/m²	< 1	DIN 53380 23°C / 75% r.F.
Caractéristiques mécaniques			
Force déchirement longitudinale	N/15 mm	50	DIN 53455 23°C / 50% r.F.
Force déchirement transversale	N/15 mm	55	DIN 53455 23°C / 50% r.F.
Secteur de soudure	°C	115-155	
Résistance à la température	°C	-40/+90	

Les indications précédentes se basent sur l'état actuel de nos connaissances. La responsabilité de l'utilisateur pour vérifier l'usage de ce produit avec son application reste pleine et entière.

A7. Process flow to obtain vacuum-fried papaya chips packaged under air or nitrogen conditions and stored at different temperatures.



A8. Technical data sheet of standard commercial food A04 used to feed the rats during the animal experimentation.

SAFE
SCIENTIFIC ANIMAL FOOD & ENGINEERING
www.safe-diets.com

Aliment entretien Rats, Souris & Hamsters

A04

Composition Nutritive /kg

ACIDES AMINÉS		TOTAL *
Arginine	mg	9 000
Cystine	mg	2 500
Lysine	mg	7 200
Méthionine	mg	2 800
Tryptophane	mg	1 900
Glycine	mg	8 100

ACIDES GRAS		TOTAL *
Acide palmitique	mg	5 900
Acide palmitoléique	mg	150
Acide stéarique	mg	600
Acide oléique	mg	4 800
Acide linoléique	mg	15 000
Acide linolénique	mg	1 200

MINÉRAUX		TOTAL *
P	mg	5 500
Ca	mg	7 300
Na	mg	2 500
K	mg	6 000
Mg	mg	1 600
Mn	mg	70
Fe	mg	270
Cu	mg	16
Zn	mg	55
Cl	mg	4 000

VITAMINES		TOTAL *
Vitamine A	UI	7 500
Vitamine D3	UI	1 000
Vitamine B1	mg	7
Vitamine B2	mg	6,5
Vitamine B5	mg	16,5
Vitamine B6	mg	2,6
Vitamine B12	mg	0,02
Vitamine E	mg	30
Vitamine K3	mg	2,5
Niacine	mg	75
Ac.Folic	mg	0,5
Biotine	mg	0,04
Choline	mg	1 600

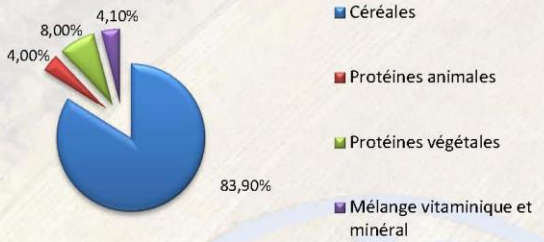
Technologie des Pellets

		Moyenne*
Diamètre	mm	16,43
Résistance à l'écrasement	kgf/cm²	22,7
Résistance à l'abrasion	%	97,3
Masse spécifique	g/l	645
Poids	g	5,319
Longueur	mm	22,64

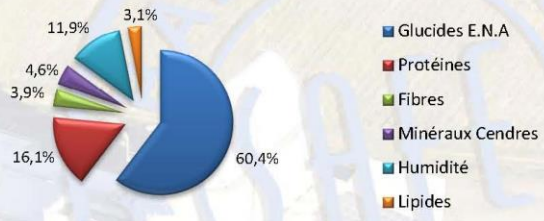
Composition

Orge, blé, maïs, tourteau d'extraction de soja, son de blé, protéine de poissons hydrolysée, phosphate bicalcique, prémélange de minéraux, carbonate de calcium, prémélange de vitamines.

Composition Centésimale en %



Composition Nutritive en %



Valeur Energétique**	Kcal/kg	Mj/kg	% Protéines	% Lipides	% Carbohydrates
Atwater	3 339	13,97	19,3	8,4	72,4
ME	3 145	13,17	-	-	-

** Information du calcul de l'Energie:
http://www.safe-diets.com/fre/produits/informations/energy/aliments-et-energie.html

Glucides E.N.A. dont		
- Amidon	(en %)	43,5
- Sucres totaux	(en %)	3,2

* Les valeurs sont données à titre d'information, il s'agit de moyennes calculées sur valeurs brutes du produit. Elles sont indicatives et n'ont pas de valeur contractuelle. Elles sont soumises à des variations liées aux conditions de production, stockage et de méthodes analytiques. Une analyse peut être réalisée sur demande.

Glucides E.N.A. : Extrait non azoté, valeur calculée.

Mise à jour le 22 février 2016

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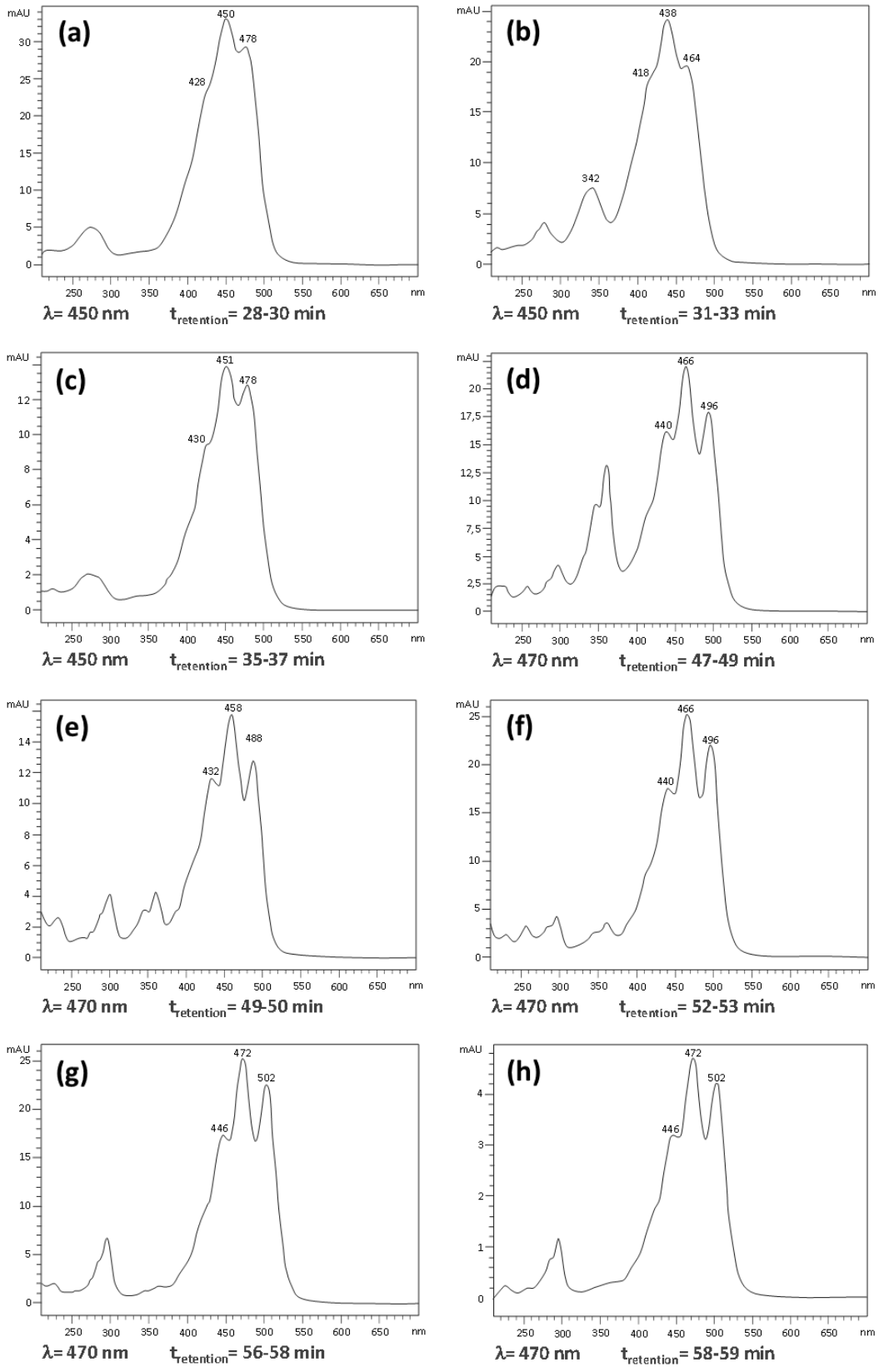
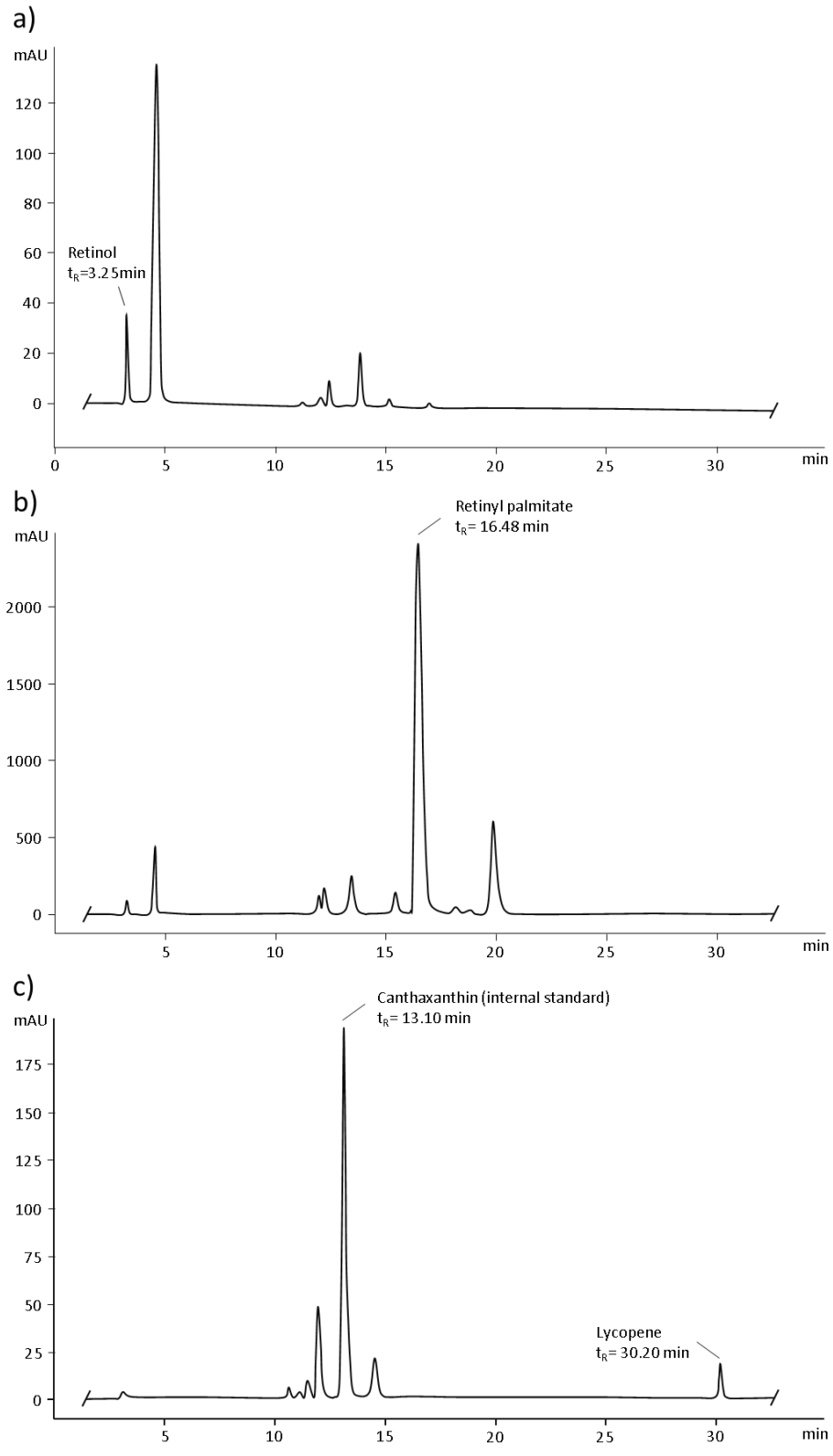
A9. UV-Visible spectra of analyzed carotenoids in fresh papaya and papaya chips: (a) *all-E*-β-cryptoxanthin, (b) *13Z*-β-carotene, (c) *all-E*-β-carotene, (d) *13Z*-lycopene, (e) *5Z-13'Z*-lycopene, (f) *9Z*-lycopene, (g) *all-E*-lycopene, (h) *5Z*-lycopene.

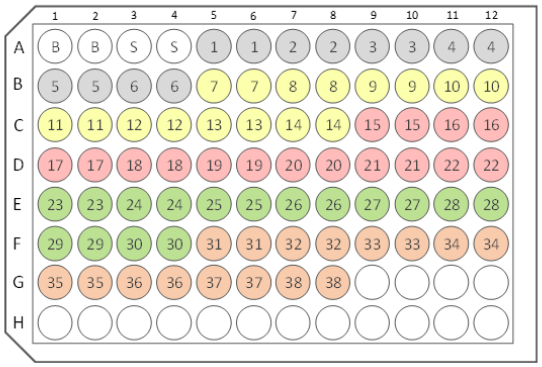


A10. HPLC-DAD separation of retinoids and carotenoids measured in plasma and liver samples: a) retinol in plasma of rats (λ=325 nm), b) retinyl palmitate in liver of rats (λ=325 nm), c) lycopene in liver of rats (λ=470 nm). t_R, retention time.

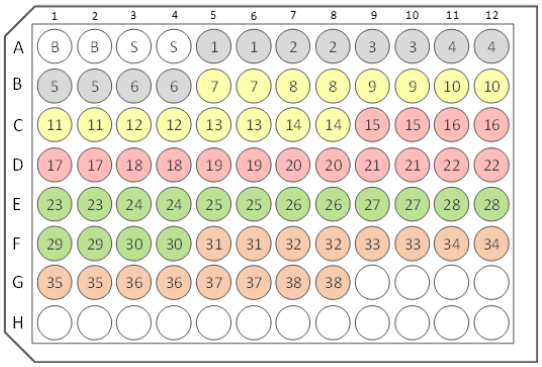


A11. Distribution of samples and standards in the 96-wells plates according to different analyses: (a) triglycerides, (b) total cholesterol, (c) low-density lipoprotein cholesterol, (d) high-density lipoprotein cholesterol, (e) free fatty acids.

(a)

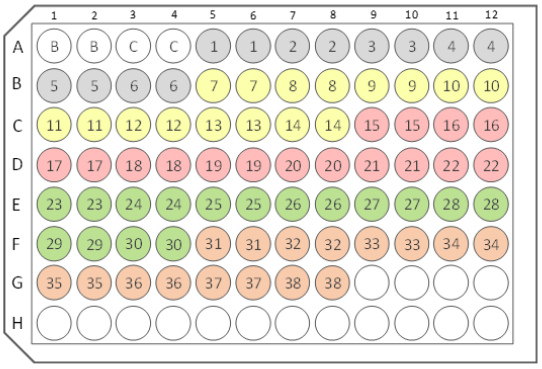


(b)

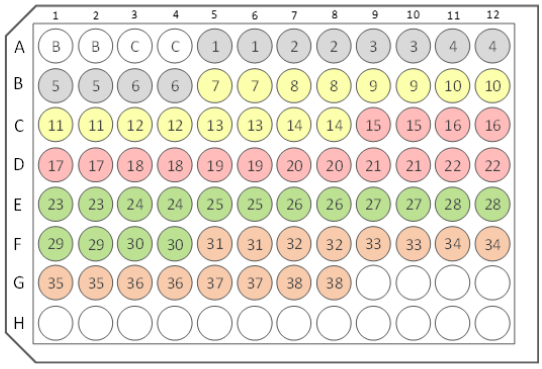


B= blanks; S= standards; 1-6= rat plasma (Control); 7-14= rat plasma (PC-S); 15-22= rat plasma (PC-P); 23-30= rat plasma (FDP+S); 31-38= rat plasma (FDP+P).

(c)

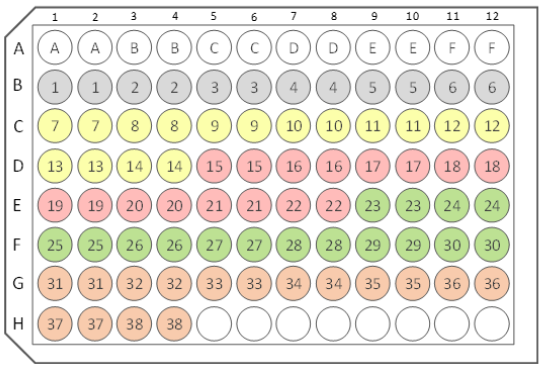


(d)



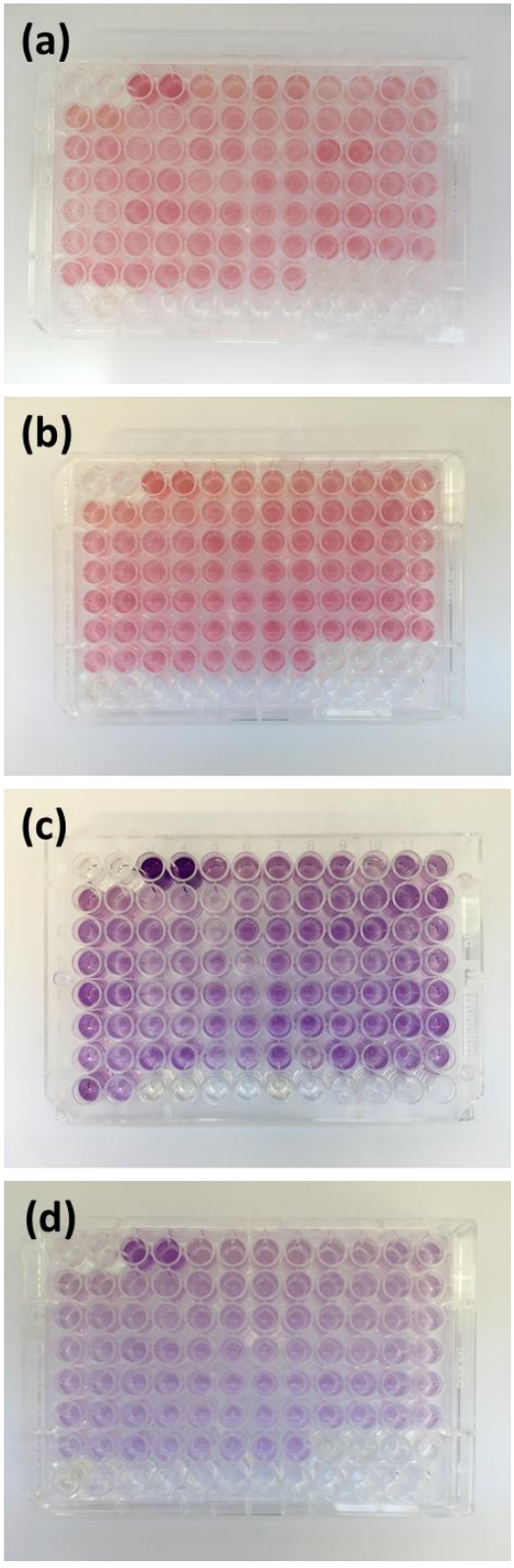
B= blanks; C= calibrators; 1-6= rat plasma (Control); 7-14= rat plasma (PC-S); 15-22= rat plasma (PC-P); 23-30= rat plasma (FDP+S); 31-38= rat plasma (FDP+P).

(e)



A-F= standards; 1-6= rat plasma (Control); 7-14= rat plasma (PC-S); 15-22= rat plasma (PC-P); 23-30= rat plasma (FDP+S); 31-38= samples (FDP+P).

A12. Pictures of different 96-wells plates according to different analyses: (a) triglycerides, (b) total cholesterol, (c) low-density lipoprotein cholesterol, (d) high-density lipoprotein cholesterol.



A13. Fatty acid profile of soybean oil used as frying medium during vacuum frying of papaya fruit.

Fatty acid profile (% relative)*	**Soybean oil****	
	Fresh oil	**After vacuum frying of papaya fruit (12 trials at 120 °C, and 0.25 bar)**
Palmitic acid, C16:0	10.5 ± 0.3[a]	10.9 ± 0.8[a]
Stearic acid, C18:0	4.3 ± 0.1[b]	4.9 ± 0.3[a]
Oleic acid, C18:1 (9*c*)	25.8 ± 1.0[a]	27.0 ± 0.4[a]
Elaidic acid, C18:1 (9*t*)	1.8 ± 0.2[a]	2.0 ± 0.1[a]
Linoleic acid, C18:2 (9*c*,12*c*)	49.3 ± 1.0[a]	46.6 ± 1.0[b]
Linolenic acid, C18:3 (6*c*,9*c*,12*c*)	6.3 ± 0.2[a]	5.9 ± 0.1[b]
Behenic acid, C22:0	0.86 ± 0.01[a]	0.88 ± 0.07[a]
Non identified	1.2 ± 0.1[b]	1.8 ± 0.1[a]

*Isomers: *c*, *cis*; *t*, *trans*. ** Values are expressed as the mean ± standard deviation (n=3). Mean values in the same row with the same letters are not significantly different from each other (Student's *t*-test, $p < 0.05$).