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43 **Abstract**

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

62

63 **Keywords**

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

66 **1. Introduction**

67 Over the last few decades, a dramatic rise of obesity and metabolic syndrome has been
68 occurred in Latin America and the Caribbean, leading to a higher incidence of non-
69 communicable diseases (e.g. cardiovascular diseases and diabetes) which are the major
70 causes of death (Popkin, & Reardon 2018; Mattei et al., 2015). This is attributed to
71 nutrition transition (increase of unhealthy dietary habits), negative changes in lifestyle, and
72 migration from rural to urban areas (Cuevas et al., 2011). For instance, during year 2016 in
73 Latin America and the Caribbean 58.8% and 60.1% of adult men and women, respectively,
74 were overweight or obese (OPS/OMS, 2019). Also, during the same year, in this region the
75 prevalence of high blood pressure and high blood glucose/diabetes for men and women was
76 23.8 and 8.9%, and 18.0 and 9.6%, respectively (OPS/OMS, 2019). Among numerous
77 factors, the high availability of energy-dense and nutrient-poor snack foods in low- and
78 middle- income countries represent a problem (Pries, Filteau, & Ferguson, 2019).
79 Consumers in Latin America and the Caribbean are attracted by diets based on this kind of
80 foods that are composed mainly or solely of sugars and saturated lipids but slight or no
81 content in vitamins, minerals, protein, fiber or essential fatty acids (Poti, Slining, & Popkin,
82 2014).

83 The consumer trends towards healthier foods requires alternative strategies to the
84 consumption of deep-fat fried products rich in simple sugars, saturated lipids and salt, e.g.
85 potato chips, French fries, doughnuts, extruded snacks, cheese sticks, among others (Da
86 Silva & Moreira, 2008). Numerous epidemiological studies promote the consumption of
87 fruits and vegetables for the prevention of the non-communicable diseases (Angelino et
88 al.,2019; Gan et al., 2015). Consequently, the intake of fruits and vegetables must be

89 increased in diets because they represent a source of bioactive health promoting compounds
90 such as vitamins, polyphenols, carotenoids, and fiber.
91 Among phytochemicals, the carotenoids, such as lycopene, are known to be natural
92 antioxidants with beneficial health effects. These compounds enhance the functions of
93 immune system and lower the development of chronic diseases such as macular
94 degeneration, type 2 diabetes, obesity, cardiovascular diseases, and certain type of cancers
95 (Cheng et al., 2017; Kopec & Failla, 2018). However, the most relevant function, is the
96 provitamin A activity. Some carotenoids such as β-carotene and β-cryptoxanthin have
97 provitamin A activity. This means they can be converted into retinol and other related
98 retinoids in organism, playing a key role on growth, visual cycle, and gene regulation
99 (Kulczynski, Gramza-Michalowska, Kobus-Cisowska, & Kmiecik, 2017).
100 The application of technological processes is of utmost importance to obtain novel and
101 healthy products from fruits and vegetables. In this context, vacuum frying is an alternative
102 technology to produce fruit and vegetable-based snacks bearing the desired sensory quality
103 and better preserving their nutrients compared to the traditional fried snacks (Da Silva &
104 Moreira, 2008; Dueik & Bouchon, 2011). In addition, vacuum frying allows the use of
105 healthier unsaturated vegetable oils due to the low operation temperatures and the absence
106 of oxygen during process (compared to atmospheric frying), thus minimizing oil
107 deterioration (Da Silva & Moreira, 2008). For instance, vacuum-fried papaya chips are a
108 good source of lycopene and provitamin A carotenoids such as β-cryptoxanthin and β-
109 carotene (Soto et al., 2020). These chips and may be an alternative in Latin America and
110 the Caribbean to control vitamin A deficiency which remains a public health problem in
111 countries of this region (Cediel et al., 2015). However, the bioaccessibility and
112 bioavailability of carotenoids in fruit and vegetable-based foods are influenced by various

113 factors. Especially, food matrix microstructure as well as the presence and type of oil have
114 a great impact (Lemmens, Colle, Van Buggenhout, Palmero, Van Loey, & Hendrickx,
115 2014; Schweiggert & Carle, 2017; Xavier & Mercadante, 2019). Carotenoids are enclosed
116 in cell organelle structures (e.g. chromoplasts in fruits). Thus, disruption of cell food matrix
117 during processing (thermal and mechanical treatments) may increase carotenoid
118 bioavailability (Van Buggenhout et al., 2010). Otherwise, the presence of lipids play a role
119 in the transfer and diffusion of carotenoids during processing as well as during digestion,
120 since carotenoids need to be released from food matrix and incorporated into lipid emulsion
121 droplets and finally transferred to mixed micelles (Xavier & Mercadante, 2019). Location
122 and deposition forms of carotenoids in raw fruit/vegetable tissues have been widely studied
123 in relation to their bioavailability (Schweiggert & Carle, 2017). In contrast, few studies
124 have investigated the impact of both processed food microstructure and oil presence on
125 carotenoid absorption and bioconversion.
126 From a nutritional point of view, this study was embedded in the general approach of
127 making a carotenoid-rich snack using papaya in order to offer a healthier product than
128 traditional fried snacks. Papaya fruit was chosen because: 1) it is a fruit widely spread and
129 consumed in America Latina and the Caribbean (FAO, 2019; Saran, Solanki, & Choudhary,
130 2016), 2) it presents a varied profile of carotenoids, xanthophyll (β-cryptoxanthin) and
131 carotenes (β-carotene and lycopene) (Soto et al., 2020) carotenoids are more bioavailable
132 from papaya than from other plant foods such as tomato and carrot (Schweiggert et al.,
133 2014). The objective of the present study was to highlight the relationship between
134 processed food microstructure and carotenoid absorption/bioconversion in rats fed with a
135 mixture based on vacuum-fried papaya chips obtained with saturated or unsaturated oils. To
136 better understand the effect of processing, freeze-dried papaya mixtures with addition of

oils were administred to rats comparatively to mixtures from papaya chips. Lipid profile (triglycerides, total cholesterol, HDL and LDL) as well as glycemia were analyzed in plasma while retinoids and lycopene were measured in liver and plasma of rats. Wide-field and confocal microscopy tools were used to explain the results in relation to 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. (2020b).

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 this 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 via 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) during 72 h. Freeze-dried papaya was ground (14%, w/w), then 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 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 regulation 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 wood 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 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 1). At the end of the experimental period, rats were being fasted overnight before 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.

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 adaptation period (day 4), and at the end of diet period (day 7) using a top-pan electronic balance.

2.6. Chemical analyses for 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

Procedures and conditions for extraction of different papaya mixtures were described previously by Soto et al. (2020b). 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

233 a tube of 8 mL, then 500 µL of ethanol 96 % (v/v) containing canthaxanthin (2 mg/mL) as
234 internal standard and 2 mL of hexane were added. The mixture was homogenized (using a
235 Vortex) for 60 s and then centrifuged (Allegra 21 Centrifuge, Beckman Coulter,
236 Switzerland) at 1400 x g for 5 min at 25 °C. The organic phase was collected, then the
237 aqueous phase was reextracted with 1 mL of ethanol and 2 mL of hexane. The organic
238 phases were pooled and evaporated under nitrogen at 30 °C until dryness. The dried extract
239 was dissolved in 100 µL of CH_2Cl_2/Methanol (50:50, v/v) prior HPLC analysis.
240
241 *2.7.3. Carotenoid and retinoids extraction from liver*
242 The frozen liver was cut into small pieces on an ice-cold table. Then, 1 g of liver was mixed
243 with 1 mL phosphate-buffered saline (PSB) and homogenized (using a 15 mL Potter tissue
244 grinder) at 600-1000 rpm for 4 min. This step was done twice. Then the homogenates were
245 pooled and homogenized using an Ultra-turrax homogenizer (IKA T10 Basic, Germany) for
246 60 s. For carotenoids and retinyl palmitate extraction, 900 µL and 500 µL of liver
247 homogenates, respectively, were extracted using 500 µL of ethanol 96 % (v/v) containing
248 canthaxanthin (2 mg/mL) as internal standard and 2.5 mL or 2 mL of hexane for
249 carotenoids and retinyl palmitate, respectively. The mixture was homogenized again using
250 a Vortex for 60 s and then centrifuged (Allegra 21 Centrifuge, Beckman Coulter,
251 Switzerland) at 1600 x g for 5 min, at 25 °C. The organic phase was collected, and the
252 aqueous phase was reextracted with 1 mL of ethanol and 2 mL of hexane. The organic
253 phases were pooled and evaporated under nitrogen at 30 °C until dryness. The dried extract
254 was dissolved in 100 µL and 850 µL of CH_2Cl_2/Methanol (1 g/20 mL) for carotenoid and
255 retinyl palmitate analysis, respectively.
256

257 *2.7.4. HPLC analysis in papaya mixtures*

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

266

267 *2.7.5. HPLC analysis in plasma and liver samples*

268 Carotenoid and retinoids were separated with the same C30 column as previously described in
269 section 2.6.4. The mobile phase was H_2O as eluent A, methanol as eluent B, and MTBE as eluent C.
270 Temperature was set at 25 ºC. The flow rate was set at 1mL/min and the injection volume was 60
271 µL. A solvent gradient was programmed as follows: 2% A-96% B-2% C (initial conditions); 0-27
272 min, 2% A-18% B-80% C; 27-35 min, 4% A-11% B- 85 C% and back to the initial conditions for
273 re-equilibration. Chromatograms were generated at 325 nm to identify retinol and retinyl esters, and
274 at 470 nm for lycopene identification. Carotenoid and retinoids were identified by comparing their
275 retention time and spectra with the respective standards. Quantification was achieved by
276 establishing calibration curves with all-E-lycopene, retinol and retinyl palmitate, being the
277 determination coefficients 0.994, 0.997 and 0.998, respectively. For carotenoid HPLC analysis
278 the limit of detection (LOD) was 0.0040 µg, and the limit of quantification (LOQ) was
279 0.0150 µg.

280

281

282 *2.8. Glycemia*

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

285

286 *2.9. Lipid profile and free fatty acids in plasma*

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

293

294 *2.10. Microscopy analyses*

295 Mixtures from vacuum-fried papaya chips and freeze-dried papaya were directly observed
296 with a wide-field microscope Eclipse Ni-E (Nikon Instruments Inc., NY, USA). The
297 pictures were obtained with the 20X Plan-APO 0.75 NA objective under transmitted light
298 or differential interference contrast (DIC). Size distribution of lipid droplets, expressed as
299 D_{90}, was calculated by using ImageJ v1.8.0 software. D_{90} means that 90% of spherical
300 particles have a diameter less than the specified value (µm). The lipids were visualized in
301 fluorescence with the lipophilic stain Nile Red (3.14 µM) with a blue excitation filter (B2A:
302 450-490 nm, long-pass emission 505 nm).
303 The same mixtures and the papaya chips were observed with a confocal microscope Zeiss
304 880 (Zeiss, Jena, Germany) with a 488 Argon laser with an objective 20X Plan APO 1.0

305 NA to visualize the autofluorescence of carotenoids (lycopene and β-carotene/β-
306 cryptoxanthin).
307 Spectral analysis was carried out using the advanced Linear Unmixing function (LSM 880
308 software, Zeiss) which separates mixed signals pixel by pixel using the entire emission
309 spectrum of each defined autofluorescent compound in the sample. This function was
310 applied with the advanced iterative option and one residual channel. After spectral imaging
311 acquisitions on samples, this Linear Unmixing function allowed visualization with coded
312 colors of the fluorescence of each standard (lycopene, β-carotene, and β-cryptoxanthin)
313 based on their reference spectra (Talamond, Verdeil, & Conéjéro 2015). The spectral
314 detector of this microscope was used to obtain the emission spectra of lycopene and β-
315 carotene/β-cryptoxanthin and visualize specifically these molecules in the samples between
316 500 and 690 nm.
317
318 *2.11. Statistical analyses*
319 Results obtained from plasma and liver samples were analyzed by one-way analysis of
320 variance (ANOVA) after examining for homogeneity of variances by Bartlett's test. P-
321 value <0.05 was considered significant. If significantly different, means were further
322 compared using Fisher's test ($p<0.05$). Composition of the different papaya mixtures was
323 also compared by one-way ANOVA and post hoc Tukey-HSD ($p<0.05$). Statistical
324 analyses were performed using the XLSTAT Software version 2020 (Addinsoft Inc, USA).
325
326 **3. Results and discussion**
327 **3.1 Carotenoid consumption and growth performance**

328 Table 1 shows the composition of the different papaya mixtures (PC-S, PC-P, FDP+S,
329 FDP+P) that were made to feed the rats by oral administration. There were no significant
330 differences ($p>0.05$) in lycopene, total carotenoid, and lipid contents among the different
331 papaya mixtures. During the diet period rats were orally fed with 1 mL of the different
332 papaya mixtures (PC-S, PC-P, FDP+S, FDP+P) two times per day, which means 2 mL
333 papaya mixture/day. Therefore, the animals fed with mixtures consumed per day ~96 µg of
334 lycopene, ~113 µg of total carotenoids, and ~90 mg of lipids (either from soy or palm oils).
335 Mixtures from papaya chips (PC-S and PC-P) presented around 2-fold lower ($p<0.05$)
336 contents of β-cryptoxanthin and β-carotene and thus of vitamin A (expressed as Retinol
337 Activity Equivalent, RAE) than those in mixtures from freeze-dried papaya (FDP+S and
338 FDP+P). This was explained by thermal degradation of these carotenoids during vacuum
339 frying process (Soto et al., 2020).
340 Daily lycopene intake in this study was similar to that applied in previously studies realized
341 in rats by Jain et al., (1999) (142 µg/day), and in gerbils by Mills et al., (2007) (60 µg/day).
342 In most studies with rats, in which are analyzed the absorption, bioconversion or the health
343 effects of carotenoids, the authors usually apply much higher doses of carotenoids, in diets,
344 to feed animals. For instance, some studies reported a daily lycopene intake that ranged
345 from 720 to 10.000 µg (Takayama et al., 2013; Shalaby, & Fouad, 2019; Yilmaz et al.,
346 2018; Xu et al., 2019).
347 Rats gained weight during the experimental period and the final body weight ranged from
348 326.6 g to 346.6 g (Table 2). However, after the diet period there were no significant
349 differences ($p>0.05$) in weight gain between rats consuming papaya mixtures (PC-S, PC-P,
350 FDP+S, FDP+P) and Control group (Table 2).
351

352 ### 3.2 Glycemia

353 Figure 2 shows the results of glycemia measured in rats from Control group and the rats fed
354 with the different papaya mixtures (PC-S, PC-P, FDP+S, FDP+P). The glycemia of rats fed
355 with mixtures from papaya chips (185.75 ± 34.56 mg/dL for PC-S, and 178.13 ± 30.71
356 mg/dL for PC-P) was not significantly different from Control group (160.67 ± 20.70
357 mg/dL). Unexpectedly, the glycemia increased in rats consuming freeze-dried papaya
358 mixtures (223.00 ± 48.09 mg/dL for FDP+S, and 198.50 ± 36.98 mg/dL for FDP+P). These
359 values were significantly higher ($p<0.05$) than those in Control group. These differences in
360 glycemia could be attributable to the higher contents of glucose and fructose present in
361 freeze-dried papaya mixtures (FDP+S, FDP+P) (~98% of total sugars) compared to the
362 mixtures made with papaya chips (PC-S, PC-P) (~30% of total sugars) (Table 1). During
363 vacuum frying of papaya, the degradation of glucose and fructose was observed whereas
364 the formation of sucrose occurred (Soto et al., 2020c). According to these authors, factors
365 related to frying process (high temperature and fast rate of water loss) and intrinsic
366 characteristics of papaya (proximal composition, sugar concentration, organic acids,
367 acidity) are involved in sucrose formation.

368

369 ### 3.3 Lipid profile and free fatty acids in plasma

370 Table 3 shows the lipid profile and content of free fatty acids (FFA) in plasma of rats fed
371 with the papaya mixtures (PC-S, PC-P, FDP+S, FDP+P) and the Control group. No
372 significant differences ($p>0.05$) were found in cholesterol determinations (total, CHO;
373 high-density lipoprotein, HDL; and low-density lipoprotein, LDL) among rats fed with
374 papaya chip mixtures (PC-S, PC-P) and rats from Control group. Similarly, there were no

375 significant differences ($p>0.05$) for CHO, HDL and LDL among Control group and rats fed
376 with freeze-dried papaya mixtures (FDP+S, FDP+P) (Table 3).
377 Moreover, the level of triglycerides (TRIG) in plasma was similar among rats of Control
378 group and rats fed with papaya chips mixtures (PC-S, PC-P) and those fed with FDP+S
379 mixture. Rats consuming FDP+P mixture presented the highest TRIG level ($p<0.05$). These
380 results demonstrated that consumption of mixtures based on papaya chips (PC-S, PC-P) did
381 not increase CHO, HDL, LDL or TRIG in rat's plasma, regardless the type of oil.
382 Only FFA increased significantly ($p<0.05$) in plasma of rats consuming papaya chips
383 mixtures (PC-S, PC-P) compared to the Control group. The heat treatment (120 °C) applied
384 during vacuum frying of papaya could induce some hydrolysis of triglycerides from frying
385 medium (soy and palm oils) (Chung, Lee, & Choe, 2004). Thus, the oil absorbed in papaya
386 chips (PC-S, PC-P) during process could alter the levels of FFA in plasma of rats. On the
387 other hand, the mixtures based on freeze-dried papaya (FDP+S, FDP+P) were made with
388 fresh oils without any thermal degradation.
389
390 **3.4 Lycopene absorption**
391 Lycopene was detected in liver of rats fed with the different mixtures based on freeze-dried
392 papaya (FDP+S, FDP+P) and papaya chips (PC-S, PC-P) (Figure 3). Significant differences
393 ($p<0.05$) were observed between groups of rats fed with FDP+S and PC-S. Lycopene
394 accumulation in the liver was higher in rats fed with FDP+S (0.522 µg/g liver) than in those
395 fed with PC-S (0.291 µg/g liver). To support these results, wide-field microcopy of the
396 mixtures was performed and revealed that FDP+S mixture exhibited a fine emulsion with
397 numerous droplets (the D_{90} statistical diameter was 18 µm) probably due to the soy oil-
398 based formulation (Figure 4.a). In opposite, the oil droplet size of PC-S mixture was higher

399 (the D_{90} statistical diameter was 46 µm) and crystalloid remnants of lycopene were
400 identified (Figure 4.b, see arrows). A better dissolution of crystalline lycopene in the soy oil
401 fine emulsion was hypothesized.
402 To confirm the identification of carotenoids, the spectral imaging of confocal microscopy
403 allowed to identify these compounds with fluorescence spectra as well as to locate the
404 carotenoids in papaya mixtures. In the FDP+S mixture, the two type of carotenoids β-
405 carotene/β-cryptoxanthin (provitamin A carotenoids) and lycopene seemed to dissolve in
406 the core of lipid droplets of emulsion (Figure 4.c). Inversely, only fluorescence emission
407 spectra of lycopene were found in PC-S mixture within solid aggregates (Figure 4.d, see
408 arrowheads).
409 According to Schweiggert et al. (2012) and Schweiggert & Carle (2017), lycopene from
410 papaya occurs in a solid crystalline deposition form whereas β-carotene/β-cryptoxanthin are
411 liquid-crystalline or lipid-dissolved in globular-tubular substructure of chromoplast. The
412 morphology of chromoplast and deposition form strongly influence carotenoid
413 bioavailability. Also, the storage of lycopene crystalloid form was associated with a lower
414 bioaccessibility and absorption (Schweiggert et al., 2014). The addition of soy oil and water
415 to freeze-dried papaya (FDP+S) generated a fine emulsion favorizing the dissolution of
416 small crystalline lycopene, thereby increasing its absorption during digestion (Salvia-
417 Trujillo et al., 2017). Indeed, before carotenoid absorption the lipid digestion rate was
418 higher for a fine emulsion with small droplets contributing to micelle formation, which
419 represents the absorbable form for enterocytes (Salvia-Trujillo et al., 2019). In the case of
420 PC-S, the formed emulsion was coarse with larger oil droplets and crystalloid remnants of
421 lycopene (Figure 4.b).

422 On the other hand, significant differences ($p<0.05$) in lycopene absorption were observed
423 between rats fed with PC-S mixture (0.291 µg/g liver) and those with PC-P mixture (0.422
424 µg/g liver). As shown in Figure 5, crystalloid lycopene form remained in PC-S (Figure 5.a).
425 This figure shows specific red color aggregates (lycopene) within the food matrix. Whereas
426 lycopene seemed to be better dissolved in the matrix of PC-P. In fact, the red color
427 represents a diffuse veil within the food matrix (Figure 5.b). Lycopene transfer to oil during
428 vacuum-frying of papaya fruit could have been better with palm oil than with soy oil. The
429 fatty acid composition of oils (chain length and degree of unsaturation) influences the
430 incorporation of lycopene into micelles (Lemmens et al., 2014). Lycopene bioaccessibility
431 or absorption was reported higher with olive oil and could be explained by the presence of
432 C18:1 (Clarke et al., 2000; Colle et al., 2012; Nagao et al., 2013). In addition, fatty acids
433 with medium-chain length such as C16:0 increased lycopene bioaccessibility compared to
434 long-chain length and this was particularly significant when up to 5% of lipids was added.
435 Medium-chain fatty acids are hydrolyzed to a higher extent than those with long-chain
436 length. A complete digestion of medium-chain fatty acids led to better lycopene
437 solubilization capacity (Colle et al., 2012). This can also explain why upon addition of
438 sunflower oil up to 5%, lycopene bioaccessibility from papaya remained unaffected
439 (Schweiggert et al., 2012).
440 Palm oil contained 39% of C18:1and 41% of C16:0 whereas soy oil only contained 26%
441 and 11%, respectively. Therefore, the fact that palm oil has a different fatty acid
442 composition, added to the effect of processing, could explain the difference for lycopene
443 absorption in rats consuming the papaya chips but processed with different oil.
444 Furthermore, saturated coconut oil (rich in medium-chain triglycerides) enhanced lycopene
445 tissue accumulation to a greater degree than safflower oil (rich in long-chain triglycerides)

446 in Mongolian gerbils fed with tomato powder (Conlon et al., 2012). Borel et al. (1996)
447 pointed out that the solubility of carotenes in bulk triglycerides increased with decreasing
448 of fatty acid chain length.
449
450 **3.5 Provitamin A carotenoid bioconversion in plasma and liver**
451 The bioconversion of pro-vitamin A carotenoids was demonstrated by an increase of retinol
452 in plasma and retinyl palmitate content in liver of rats consuming papaya mixtures (PC-S,
453 PC-P, FDP+S, FDP+P) comparatively with those of Control group fed with a regular diet
454 (non-supplemented with carotenoids). Retinol content in plasma was significantly higher
455 ($p<0.05$) in rats fed with the papaya mixtures (PC-S, 3.05 ± 0.20 µmol/L; PC-P, 2.80 ± 0.31
456 µmol/L; FDP+S, 3.11 ± 0.38 µmol/L; FDP+P, 2.87 ± 0.14 µmol/L) than rats of Control
457 group (2.38 ± 0.25 µmol/L). However, no significant differences ($p>0.05$) were found in
458 retinol content among the group of rats fed with the different papaya mixtures (PC-S, PC-P,
459 FDP+S, FDP+P).
460 Figure 6 shows that contents of retinyl palmitate following administration of mixtures with
461 palm oil (PC-P, FDP+P) were lower than those in soy oil mixtures (PC-S, FDP+S). The
462 better bioefficacy observed after ingestion of soy products could be explained by the
463 different behavior of emulsions formed in mixtures administrated to the rats. Wide-field
464 epifluorescence microscopy with help of lipid coloration showed emulsions with small and
465 round-shape oil droplets for the soy products (Figure 7.a, and Figure 7.c) in opposition to
466 emulsions with larger and undefined-shape oil droplets for the mixtures with palm oil
467 (Figure 7.b, and Figure 7.d).
468 Absorption of carotenoids requires their liberation from food matrix and their solubilization
469 into oil droplets to be then transferred to bile salts mixed micelles. According to Salvia-

Trujillo (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 because of the hydrolysis of triglycerides. Digestion of triglycerides and carotenoid bioaccessibility were higher for small lipid droplets than for bigger ones (Salvia-Trujillo, 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, Li, & Xiao, 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). In fact, 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 live 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 1). This result underlined that vacuum frying process favorized the carotenoid bioconversion. The incorporation of oil into the papaya matrix during vacuum frying (120 °C, 13 min, 25 kPa) could play a major role on 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, Namutebi, & Muyonga, 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 oil. These conditions were similar to those used during vacuum frying of papaya. The increase of carotenoid solubility due to

494 vacuum frying of papaya fruit resulted in a higher bioconversion of provitamin A
495 carotenoids and accumulation of lycopene in liver of rats.
496
497 **4. Conclusions**
498 Our study showed that a carotenoid-rich healthy snack obtained by vacuum frying did not
499 increase glycemia, cholesterol or triglycerides in rat plasma regardless the type of oil.
500 Moreover, the consumption of mixtures based on vacuum-fried papaya chips favorized the
501 absorption of lycopene and provitamin A carotenoids in rats. It was shown that carotenoid
502 absorption depends on the type of oil and processing as well as carotenoid deposition in
503 food microstructure. The crystalloid form of lycopene in papaya was better dissolved in
504 palm oil during vacuum frying and was better absorbed in rats. This could be explained by
505 the presence of C18:1 and medium-chain fatty acids such as C16:0 in palm oil. Inversely,
506 consumption of mixtures based on vacuum-fried chips with soy oil increased the
507 bioconversion of provitamin A carotenoids in liver of rats. The lipid-dissolved form of
508 provitamin A carotenoids associated with the presence of unsaturated soy oil (rich in
509 linoleic and α-linolenic acids) in vacuum-fried papaya chips favorized the formation of a
510 fine emulsion in diet mixture. This facilitated the formation of micelles and consequently a
511 better carotenoid absorption in rats. Finally, these results showed that vacuum-fried papaya
512 chips obtained with soy oil represent an interesting source of provitamin A carotenoids and
513 bioavailable lycopene in a diversified diet. . In other words for the contributions of pro-
514 vitamin A carotenoids, a portion of 25g of vacuum-fried papaya chips presented a
515 nutritional value of 75 µg Retinol Activity Equivalent (RAE) which corresponds to 10 % of
516 the recommended daily allowance (RDA) (800 µg for adults). It could be claimed that food
517 products with 10% or more of the RDA for vitamin A (RAE) as "good source of vitamin

518 A" (FDA, 2020). Vacuum-fried papaya chips could be an alternative to offer the consumers
519 a healthier product than traditional snacks. For intance, 25-30 g could be a portion for
520 papaya chips (this correspond to classic commercial potatoes chips for individual portion).
521 According to Soto et al. (2020) 25 g of papaya chips contains 6.4 g of lipids (which
522 corresponds to 9.8% of daily intake recommended for lipids) and 3.9 mg of carotenoids.
523 Daily intake recommended for carotenoids does not exist; however, 4.8 mg/day is needed
524 to meet the requirement of 800 µg of vitamin A for an adult (Toti et al ., 2018).
525
526
527 **Declaration of competing interest**
528 No conflict of interest involving any of the authors.
529
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535
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710

711

712 **Figure captions**

713 **Figure 1.** Study design for evaluating the effect of the consumption of different papaya
714 mixtures on carotenoid absorption and lipid profile in plasma and liver of Wistar rats.

715

716 **Figure 2.** Glycemia of Control group and rats fed with papaya mixtures: vacuum-fried
717 papaya chips with either soy oil (PC-S) or palm oil (PC-P), and freeze-dried papaya mixed
718 with either soy oil (FDP+S) or palm oil (FDP+P). Bars are expressed as the means ±
719 standard error of the mean, SEM (n=6 for Control, n=8 for the other groups). Means with
720 the same letter are not significantly different (Fisher's t-test, $p<0.05$).

721

722 **Figure 3.** Lycopene content in liver of Wistar rats fed with papaya mixtures: vacuum-fried
723 papaya chips obtained with either soy oil (PC-S) or palm oil (PC-P), and freeze-dried
724 papaya mixed with either soy oil (FDP+S) or palm oil (FDP+P). Bars are expressed as the
725 means ± standard error of the mean, SEM (n=8). Means with the same letter are not
726 significantly different (Fisher's t-test, $p<0.05$).

727

728 **Figure 4.** Wide-field and confocal microscopy of papaya soy mixtures for feeding rats. (A)
729 Wide-field microscopy (DIC 20 X objective) of freeze-dried papaya soy mixture (FDP+S).
730 (B) Wide-field microscopy (DIC 20 X objective) of vacuum-fried papaya chips soy mixture
731 (PC-S). (C) Spectral imaging by confocal microscopy of FDP+S mixture (red line,
732 lycopene; green line, β-carotene/β-cryptoxanthin). (D) Spectral imaging by confocal

733 microscopy of PC-S mixture (red line, lycopene). *Arrows and arrowheads* mark crystalloid
734 remnants of lycopene. The scale bars represent a length of 100 μm.
735
736 **Figure 5.** Spectral imaging by confocal microscopy (20X objective) of (A) Vacuum-fried
737 papaya chips with soy oil, (B) Vacuum-fried papaya chips with palm oil. Lycopene is
738 represented by red color and β-carotene/β-cryptoxanthin by green color. The scale bars
739 represent a length of 50 μm.
740
741 **Figure 6.** Retinyl palmitate content in liver of Control group and rats fed with papaya
742 mixtures: vacuum-fried papaya chips obtained with either soy oil (PC-S) or palm oil (PC-
743 P), and freeze-dried papaya mixed with either soy oil (FDP+S) or palm oil (FDP+P). Bars
744 are expressed as the means ± standard error of the mean, SEM (n=6 for Control, n=8 for the
745 other groups). Means with the same letter are not significantly different (Fisher's t-test,
746 $p<0.05$).
747
748 **Figure 7.** Wide-field epifluorescence microscopy (20X objective-Nile Red coloration
749 fluorescent stain for lipids) of papaya mixtures for feeding rats. (A) Vacuum-fried papaya
750 chips soy mixture (PC-S). (B) Vacuum-fried papaya chips palm mixture (PC-P). (C)
751 Freeze-dried papaya soy mixture (FDP+S). (D) Freeze-dried papaya palm mixture
752 (FDP+P). The scale bars represent a length of 100 μm.

Figure 1

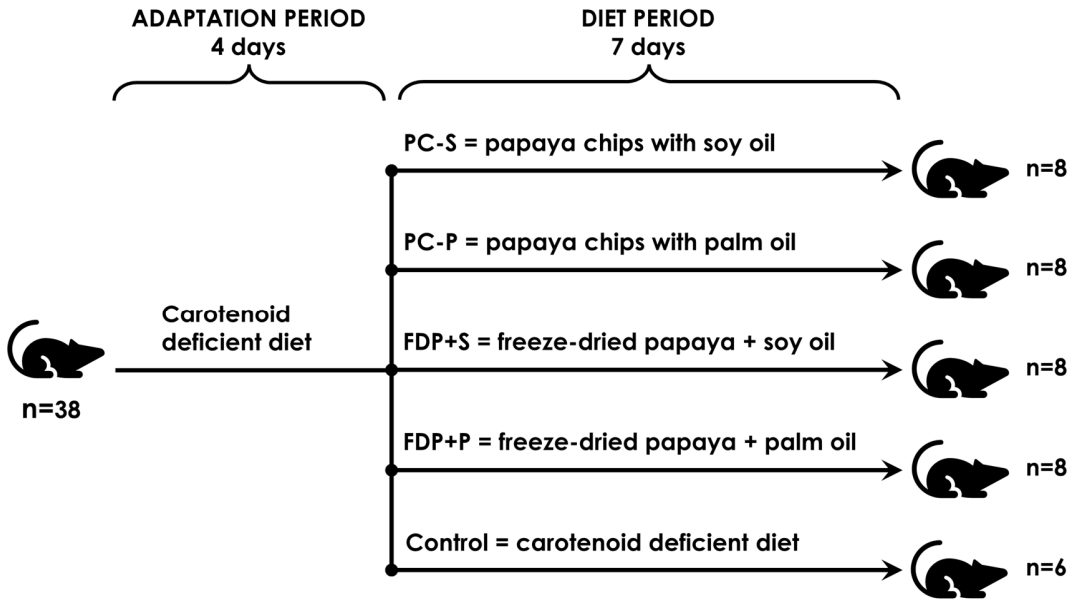
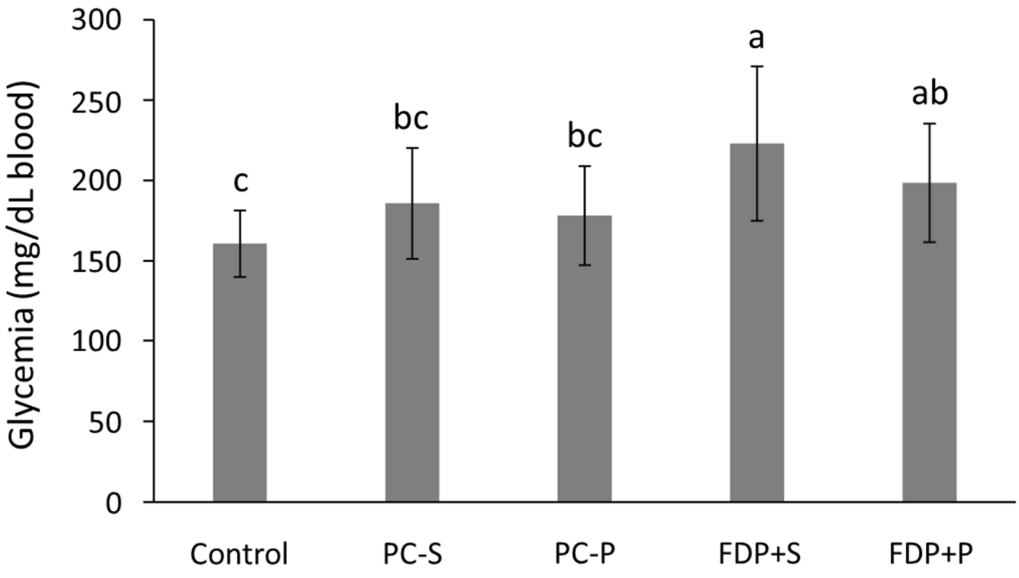

ADAPTATION PERIOD
4 days
DIET PERIOD
7 days
PC-S = papaya chips with soy oil
n=8
PC-P = papaya chips with palm oil
n=8
Carotenoid
deficient diet
FDP+S = freeze-dried papaya + soy oil
n=8
n=38
FDP+P = freeze-dried papaya + palm oil
n=8
Control = carotenoid deficient diet
n=6

Figure 2


Glycemia (mg/dL blood)
300
250
200
150
100
50
0
c
bc
bc
a
ab
Control
PC-S
PC-P
FDP+S
FDP+P

784 **Figure 3**

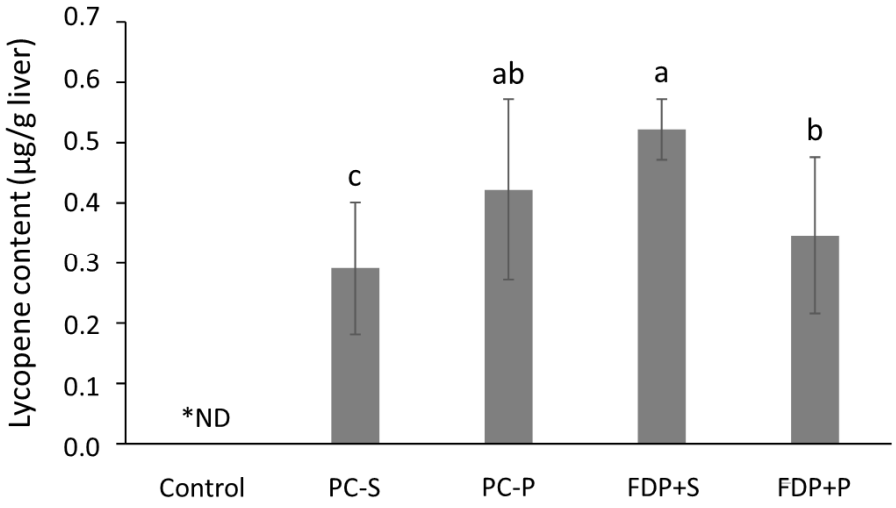


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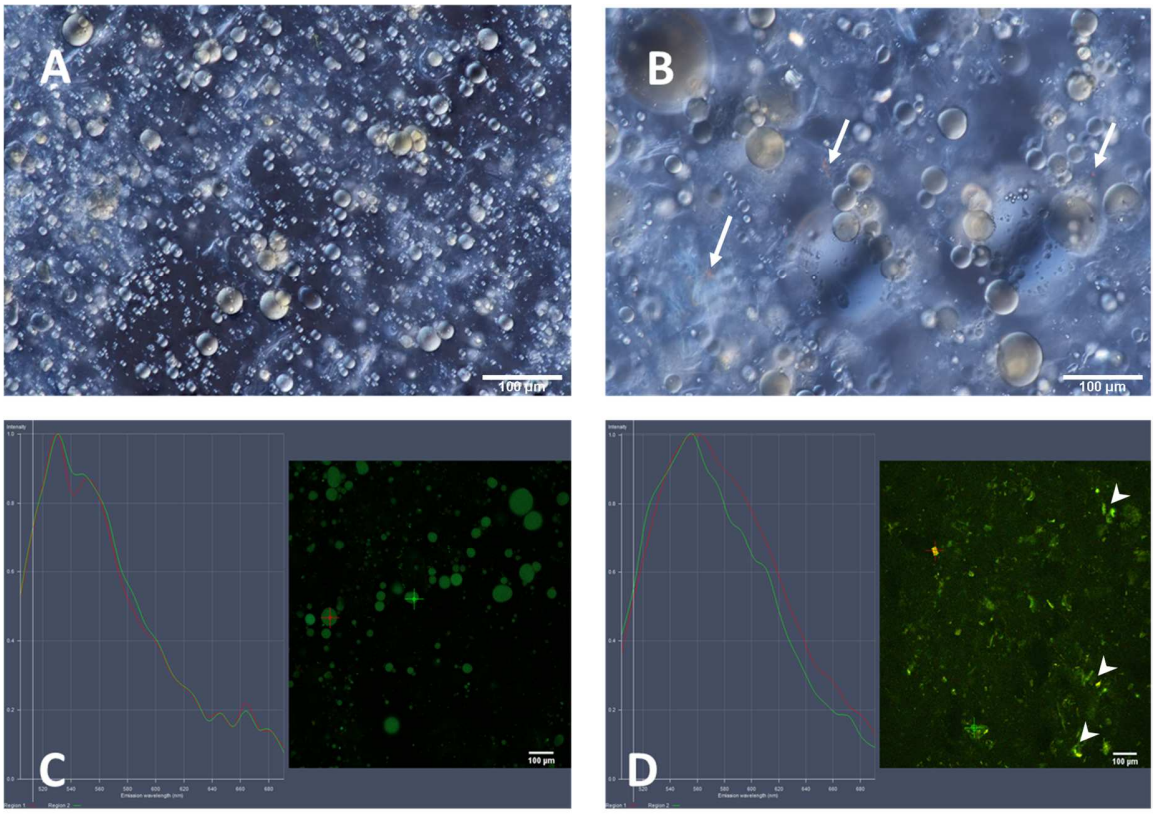
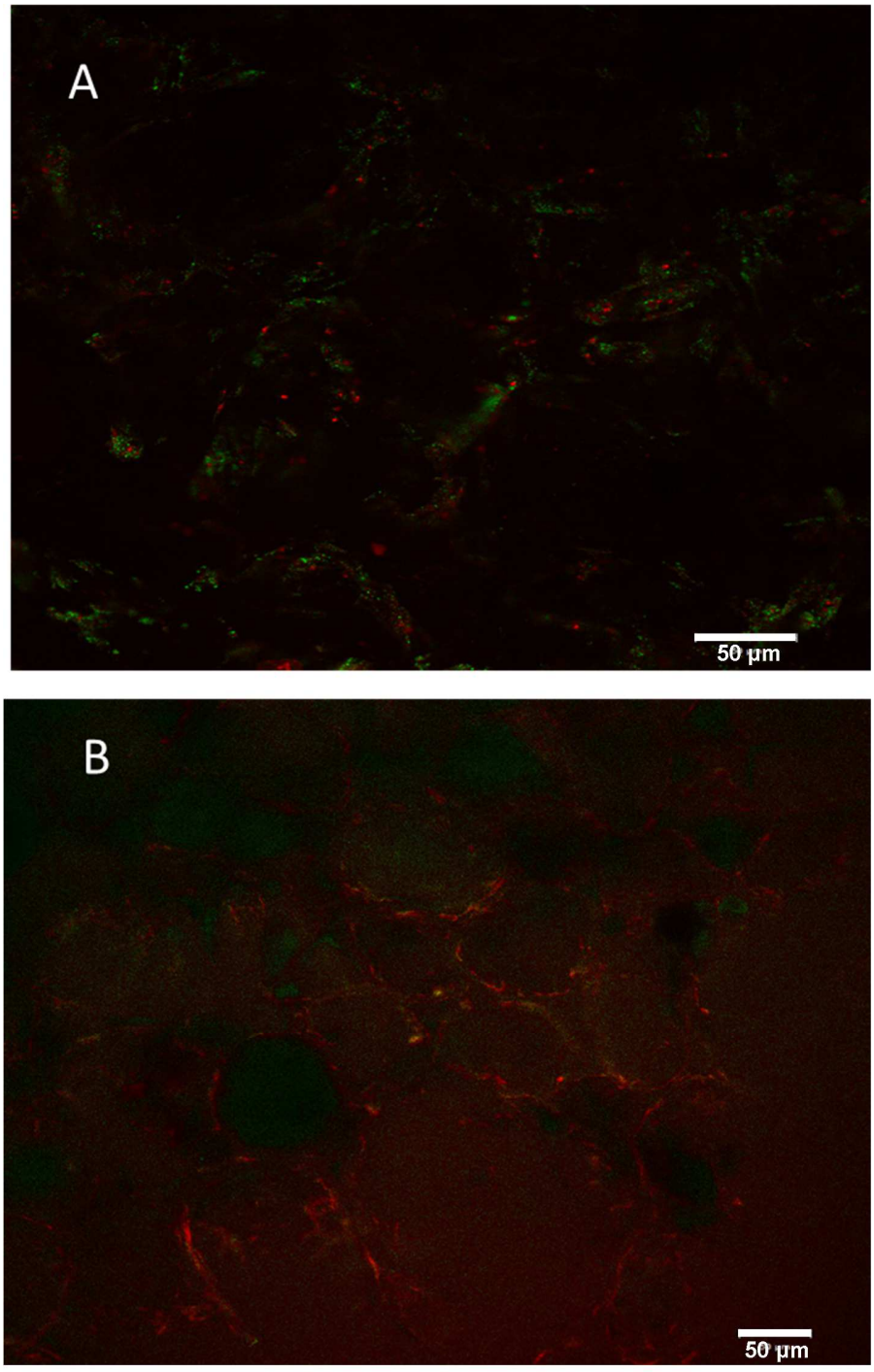
Figure 4



809

810 **Figure 5**

811



812

Figure 6

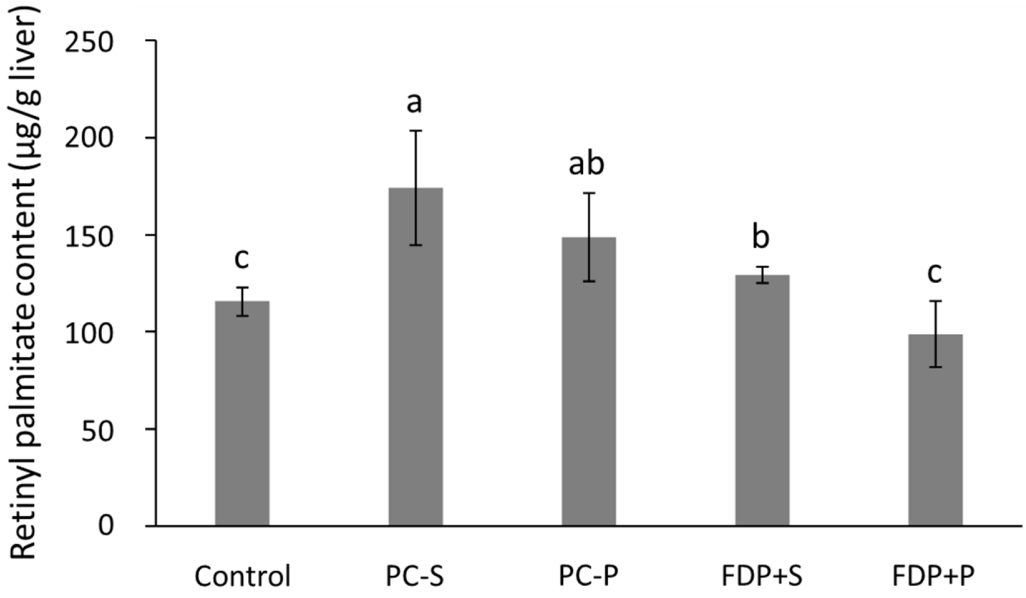
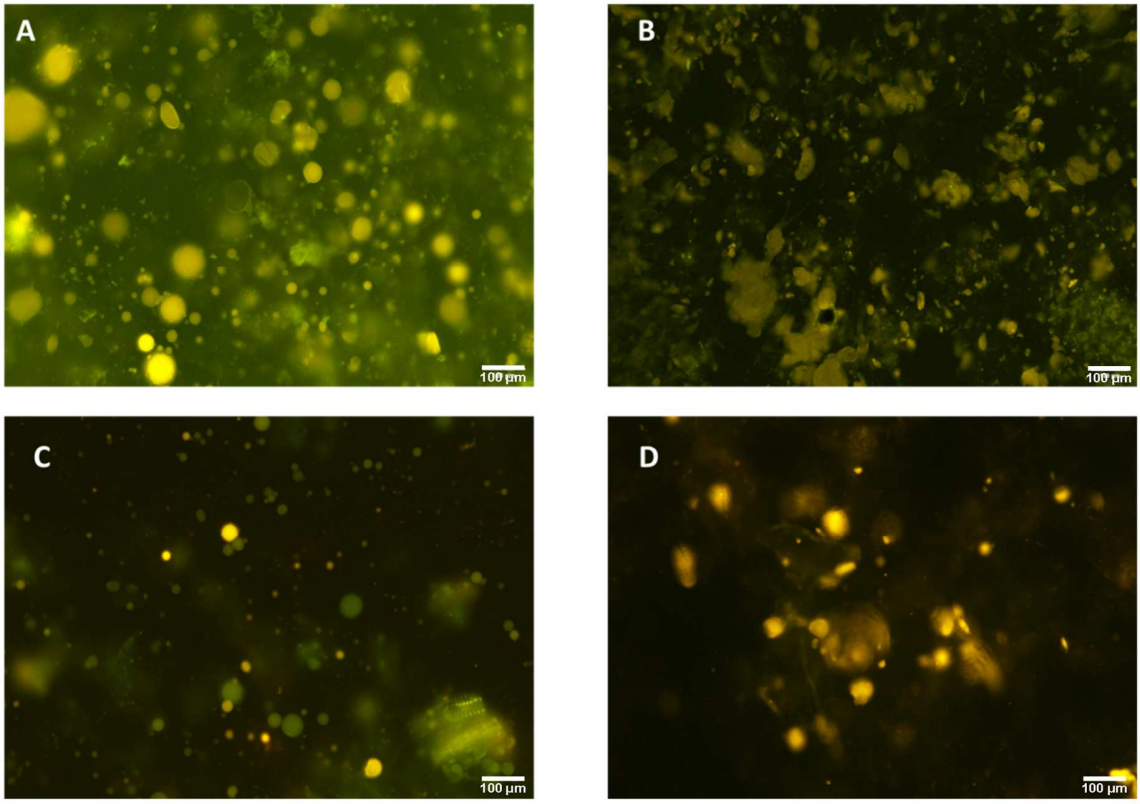

Retinyl palmitate content (µg/g liver)
250
200
150
100
50
0
a
ab
b
c
c
Control
PC-S
PC-P
FDP+S
FDP+P

Figure 7



841

842 **Table 1**. Composition of the different papaya mixtures used for feeding the Wistar rats.

843

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]

844

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

853

854 **Table 2**. Body weight and weight gain of Wistar rats fed with papaya mixtures and Control
855 group at different periods of the study.

856

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]

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

862 **Table 3**. Lipid profile and free fatty acids in plasma from Wistar rats fed with papaya
863 mixtures and Control group.
864

Papaya mixtures	CHO (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TRIG (mg/dL)	FFA (µmol/uL)
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}

865 Control, carotenoid deficient diet; PC-S and PC-P, vacuum-fried papaya chips obtained
866 with soy and palm oil, respectively; FDP+S and FDP-P, freeze-dried papaya mixed with
867 soy and palm oil, respectively. CHO, total cholesterol; LDL, low density lipoprotein; HDL,
868 high density lipoprotein; FFA, free fatty acids; TRIG, triglycerides. Values are expressed as
869 the means ± standard error of the mean, SEM (n=6 for Control, n=8 for the other groups).
870 Means in the same column with the same letter are not significantly different (Fisher's t-
871 test, $p<0.01$).
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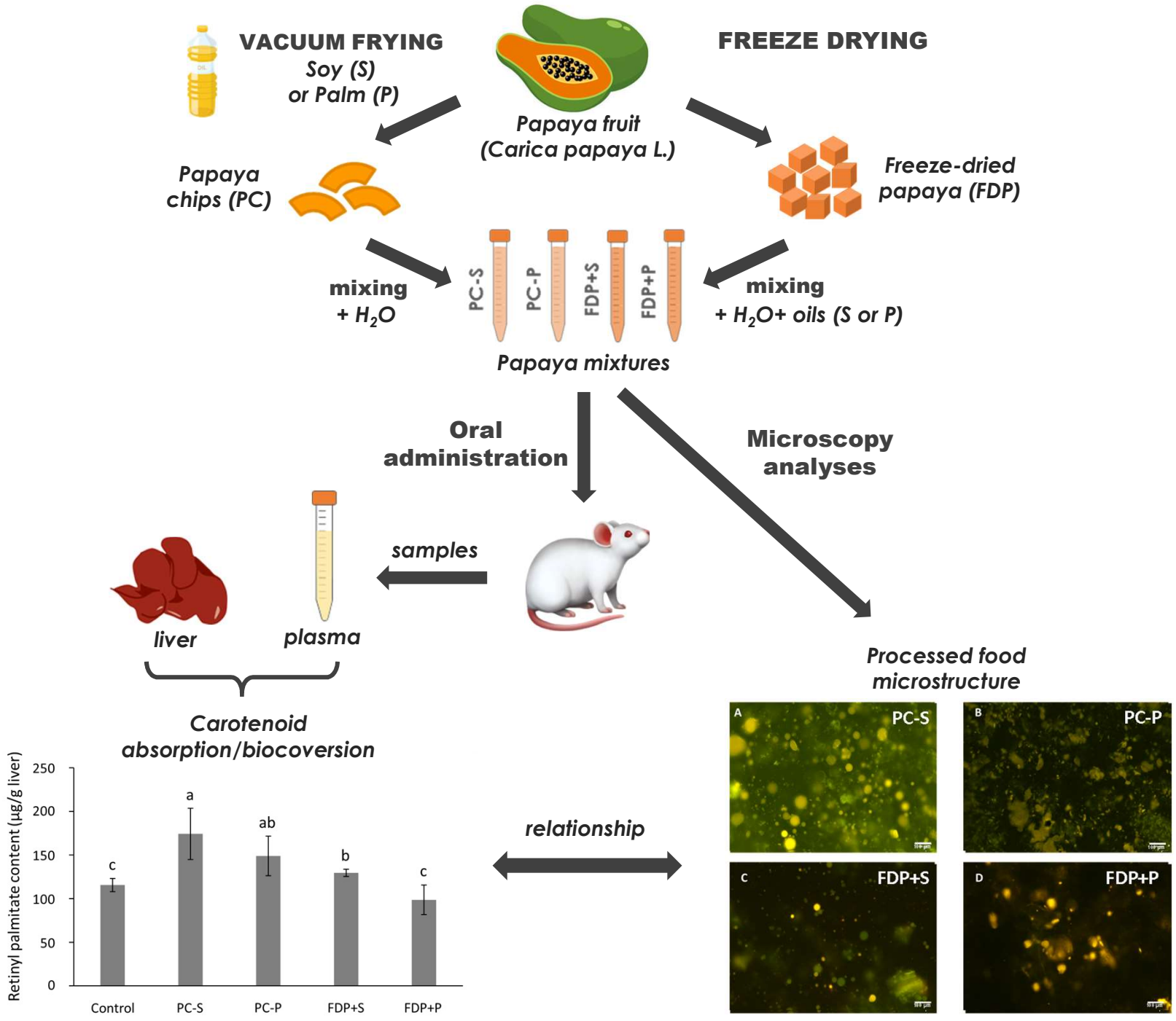
VACUUM FRYING
Soy (S)
or Palm (P)
FREEZE DRYING
Papaya fruit
(Carica papaya L.)
Papaya
chips (PC)
Freeze-dried
papaya (FDP)
mixing
+ H2O
PC-S
PC-P
FDP+S
FDP+P
mixing
+ H2O+ oils (S or P)
Papaya mixtures
Oral
administration
Microscopy
analyses
samples
liver
plasma
Carotenoid
absorption/biocoversion
Retinyl palmitate content (µg/g liver)
250
200
150
100
50
0
a
ab
b
c
c
Control
PC-S
PC-P
FDP+S
FDP+P
relationship
Processed food
microstructure
A
PC-S
B
PC-P
C
FDP+S
D
FDP+P