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Carotenoid and color changes in traditionally flaked and extruded products

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ABSTRACT

The objective of this study was to evaluate the impact of process and formulation on individual carotenoid loss in traditionally prepared cornflakes and those prepared by extrusion. The first step in the traditional process (maize grits cooking) promoted a 60% lutein content reduction and 40% in zeaxanthin loss, showing lutein more susceptibility to isomerization and decomposition. After toasting, the last step, the total loss averaged 80% for both compounds. The extruded maize in a plain formulation showed a 35% lutein and zeaxanthin reduction. However, in samples containing quinoa the decrease reached 60%, and the major loss (80%) was found in chia-containing formulations. Correlations between the color coordinate b*, total and individual carotenoid content, were obtained. It is of a major importance that the efforts to increase carotenoid content in raw materials are complemented with attempts to reduce the losses during processing.

1. Introduction

Cereals are among the main ingredients selected for breakfast in all parts of the world. The different presentations involve precooked products, flakes, extrusion products, cereal bars, cereal mix, among others (Rada-Mendoza, Sanz, Olano, & Villamiel, 2004; Rufián-Henares, Delgado-Andrade, & Morales, 2006). They are usually selected as a source of energy because of their high content in complex carbohydrates, and also as a protein source. In the case of maize, aside from starch, there is also the possibility to incorporate a good quantity of antioxidant pigments in the form of carotenoid compounds.

Much work has to be done in terms of producing better snacks, enhancing their nutritional value, since they play an important role in humans daily intake (Obradovic et al., 2015; Potter, Stojceska, & Plunkett, 2013; Singh, Gamlath, & Wakeling, 2007). Previous works have studied the amount of carotenoids present in different varieties of maize, and have been able to isolate and quantify the main ones (Amaya & Kimura, 2004). The major carotenoids present in maize are lutein, zeaxanthin, β-cryptoxanthin and β-carotene and their proportions depend on the maize variety (Kimura, Kobori, Rodriguez-Amaya, & Nestel, 2007). Dietary lutein and zeaxanthin are selectively taken up into the macula of the eye, where they absorb up to 90% of blue (short wavelength) light and help to protect eye from light-induced oxidative damage (Krinsky, Landrum, & Bone, 2003).

Extrusion process is a convenient alternative to the traditional flake-toasting processing since it is much easier to change formulations favouring the development of new products, one of the main objectives in any industry. It combines multiple unit operations, providing the flexibility of controlling temperatures, mechanical shearing and product shaping, which is ideal for manufacturing ready-to-eat snack foods (Brennan, Derbyshire, Tiwari, & Brennan, 2013). Some research dealing with the influence of extrusion on carotenoids has been recently reported (Emin, Mayer-Miebach, & Schuchmann, 2012; Waramboi, Gidley, & Sopade, 2013). In order to obtain the best quality products, a complete understanding of the processing must be achieved. Waramboi et al. (2013) stated that examination of carotenoids stability is of immense interest in the overall research on processing-carotenoid in sweet potato extruded products and the same concept can be applied to cereal products.

Thermal decomposition is the most likely cause for losses of bioactive compounds. These degradative processes depend on the chemical structure of the phytochemicals, being molecules with

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unsaturated covalent bonds prone to degradation (Oliveira, Pintado, & Almeida, 2012; Rawson et al., 2011).

Therefore, the objective of this study was to evaluate the impact of formulation on individual carotenoid loss for several formulations of extruded products and to compare them to the loss occurred during the traditional flake-toasting process.

2. Materials and methods

2.1. Traditional flaked and extruded samples

At the beginning of the traditional corn flake processing, the grits are cooked under saturated steam. During this process, the starch is gelatinized and non-enzymatic browning takes place (Farroni & Buera, 2012). Cooked grits are cooled, flaked and then toasted in a stream of air at high temperature (~230 °C) for a short time (few minutes) to obtain the final product.

Samples of five different stages from the same processing batch, of the traditional flaking-toasting process, were provided by a local company (Buenos Aires, Argentina) and stored frozen (−26 °C) until analysis. The production process included raw grits, cooked grits, dried grits, flaked grits, and toasted cornflakes.

To evaluate the carotene loss during the extrusion process five mixtures of maize semolina with natural purple corn extract (AO), chia (C) and/or quinoa (Q) were prepared as described in Table 1. A conical, counter-rotating twin-screw extruder (CM45-F Cincinnati Milacron, Austria) was used. The general screw geometry was: length 1000 mm, diameter from 90 to 45 mm, channel depth 8.5 mm, calender gap 0.5 mm, and flight gap 0.2 mm. The screw configuration had five conveying sections and three drossel zones (SK-1552-300, Cincinnati Milacron, Austria). A 4.5 mm circular die was fitted in the die plate. The feed moisture of all mixtures was set to 14% prior to extrusion cooking and no additional water was added during the process. All extruder parameters were set equal for all blends in order to maintain constant the specific mechanical energy input: feed rate 45 kg/h, screw speed 82 RPM, torque 80%, temperature profile: 50 °C zone 1, 80 °C zone 2, 100 °C zone 3, 150 °C zone 4, and 130 °C screw temperature.

Samples were then collected, cooled to room temperature under natural convection conditions and stored for further use in sealed polyethylene bags inside a controlled chamber.

2.1.1. Natural antioxidant extraction

Anthocyanin rich powder was obtained from purple corn cobs. These were bought in a local market in Buenos Aires, cut, milled with a knife grinder and mixed with mili Q water in the proportion 1:1. An ultrasonic-assisted aqueous extraction of purple corn was performed using a Hielscher UP100H equipment, with a7S probe. Working amplitude was 100% with a 0.5 s pulse time. Extraction time was 5 min. The aqueous extract was then centrifuged at 15400 RCF for 30 min at 4 °C (centrifuge 5804 R, Eppendorf, Germany) and filtered through a 0.45 μm filter paper. Maltodextrin (MD) DE15 was added to the aqueous purple solution to obtain a final MD concentration of 20%. Then, the extract was spray dried in a laboratory-scale Mini Spray Dryer Büchi B290 (Flawil, Switzerland). The operation conditions during the drying process were: inlet air temperature 175 ± 3 °C, outlet air temperature 83 ± 3 °C, flow rate 8 mL·min^{-1}, air pressure 3.2 bar and nozzle diameter 1.5 mm.

2.2. Carotenoid extraction

Before analysis, regular milling for 30 s (M-11basic IKA-Werke GmbH & Co. KG, Staufen, Germany) was performed until the entire sample passed throw a 420 μm mesh screen.

Total carotenoid content may show differences according to the different assay method employed. The spectrophotometric method has been reported to over-estimate the total carotenoid content, compared to the more specific HPLC method (Waramboi et al., 2013).

Most of the extraction methods for carotenoids include a first extraction with ethanol, a short step of saponification and a change to a less polar phase with hexane. The qualitative and quantitative carotenoid composition of foods varies considerably and a single method for determining carotenoid composition cannot be developed for all crops. Indeed, the method needs to be adapted to each carotenoid composition and food matrix (Kimura et al., 2007).

In this work, different extraction methods were assayed. When extracted with ethanol followed by a phase change to hexane, it was not possible to extract all the carotenoid pigments quantitatively on the less polar phase. This could be due to the nature of maize carotenoids, presenting certain polar characteristics due to their OH groups. When using methanol as primary solvent after evaporation, the starch particles encapsulated the pigments. As a result, it was difficult to solubilize the carotenoids initially extracted for HPLC analysis. Thus, the procedure proposed by Amaya and Kimura (2004), which involves a primary extraction with acetone, and a phase change to petroleum ether, was the most appropriate to accurately quantify carotenoids in the studied samples.

Aliquots of 3 g of samples were weighed and rehydrated with 10 mL of water for 30 min. Then 20 mL of acetone were added. After 15 min, the samples were filtered through 0,45 μm (Milipore filters). At least 2 more extractions with 50 mL of acetone were performed or until all color was removed. One third of the extract was placed over 20 mL of petroleum ether in a separatory funnel and gently 300 mL of water were added without shaking. The water was discarded and this procedure was repeated 3 times. Samples were dried with anhydrous sodium sulfate and evaporated in a vacuum dryer system. Dry samples where solubilized in 2 mL of acetone:MeOH (50:50) to minimize the volume change while assuring that all compounds where correctly dissolved. The vials were closed after flushing the surface with N_2. In order to minimize carotenoid degradation all procedures were done under yellow light and0.5% butylated hydroxyanisole (BHA) was added to the solvents. All results were expressed in a dry basis after measuring water content. Humidity was measured gravimetrically by drying the milled samples (passing 0.420 μm mesh) for 4 h at

Table 1
Composition of the blends used in the extrusion process.

Sample	maize	quinoa	chia	Antioxidant (purple corn extract)	Estimated Total fat %[a]
1	100%				2.00
2	98%			2%	1.96
3	80%	20%			2.80
4	95%		5%		3.40
5	93%		5%	2%	3.36

[a] These data were extracted from United States Department of Agriculture, National Nutrient Database for Standard Reference, and was used as an estimated quantity for preparing the blends.

130 °C under forced air current until constant weight (±0.0002 g), all samples were run in triplicate.

2.3. HPLC

A HPLC Waters Alliance 2576 model with a C18 reverse phase column and a PDA detector was employed. Flow rate was 1 mL/min and column temperature was

25 °C. Elution gradient started with 93% MetOH, 7%water, and in 30 min reached 100% MetOH using a convex model curve. Total run time was 60 min for each sample including re-equilibration. MetOH (Sintorgan) and water were HPLC grade. Characteristic UV/Vis spectra were determined from 420 to 460 nm wavelength, and identification was done at 450 nm.

Calibration curves for lutein, zeaxanthin, βcryptoxanthin and βcarotene were made from commercially analytical standards (Extrasynthese, Lyon Nord, France), range of concentrations being 0.03–5 μg/ml (R^2 = 0.989), 0.06–3 μg/ml, (R^2 = 0.999), 0.2–2 μg/ml (R^2 = 0.984) and 0.4–4 μg/ml (R^2 = 0.987) respectively. 20 μL of samples or standards were injected. In each day run, one random solution of each standard was injected to check for reproducibility. Limit of detection (LOD) and limit of quantification (LOQ) where 0.05 and 0.15 ppm, calculated after the signal to noise relation.

2.4. CIELAB color parameters

Color changes were determined by image analysis. The computer vision system consisted on a standard gray box (luminance 50 of the Munsell scale) with a D65 illumination system located in the upper part, designed to simulate daylight (Lawless & Heymann, 1998). A digital camera (EOS 40D, Canon, Inc., USA) was used to obtain the images, located at an angle of 45° with the sample plane (Francis & Clydesdale, 1975), at 60 cm distance from the lens. The obtained color coordinates were calibrated with a photocolorimeter (CR-A-70, Japan). Image acquisition was performed by a computer program using the remote capture Eos Utility (Canon Inc., USA). The camera settings were: shutter speed 1/8 seconds (no zoom, no flash), macro focus mode, opening f = 6.3 and ISO 100. The images were obtained with a resolution of 3888 × 2592 pixels and saved in JPEG format. The obtained photographs were processed using Adobe PhotoShopCS4 software and color parameters were analyzed according to the procedure described by Yam and Papadakis (2004). The samples were placed in plastic capsules of 1.5 cm height and 3 images were made from each of them, rotating the samples 90° in between. Color changes were assessed throughout the CIELAB variables L^*, a^* and b^*.

2.5. Surface fluorescence

Surface fluorescence spectrum was measured by back scattering using an Ocean Optics spectrofluorimeter (Ocean Optics, Dunedin, USA), with an excitation wavelength of 340 nm (Matiacevich, Santagapita, & Buera, 2005). Excitation and emission light were conducted by a dual way optic fiber (Ocean Optics, Dunedin, USA). The incident angle was exactly at 45°, in order to maintain the excitation beam reflection away from the detector lens. The irregular topography of the samples affected the beam backscattering and invalidated the fluorescence measurements on the intact samples. Thus, in order to avoid the surface texture effects, the samples were ground and manually compressed into tablets using a IR screw press.

2.6. Statistical analysis

ANOVA with Tukey post hoc test was conducted to establish statistical differences. Infostat InfoStat versión 2012 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina) was used for statistical analysis and GraphPad Prism version 5.00 for Windows, (San Diego California USA) was used for graphical analysis. All samples were run in triplicate and results averaged. For surface color five replicates were measured.

3. Results and discussion

3.1. Carotenoid during traditional process

HPLC chromatogram revealed the presence of at least four carotenoid compounds, identified as lutein, zeaxanthin, β-cryptoxanthin and β-carotene, according to the standards. A good resolution was obtained for all compound peaks with retention times of 17.5; 19; 28.9 and 38.8 min respectively.

Fig. 1 shows the obtained lutein and zeaxanthin content in samples from the five stages of the traditional cornflakes production process. In the raw grits, all four carotenoids were identified, showing lutein (0.96 ppm) and zeaxanthin (0.78 ppm) the highest values for all samples. A high decrease of these compounds was observed after the steam cooking stage, which conducted to the complete loss of β-cryptoxanthin and β-carotene. Thus, although LOQ was low for all the carotenoids, the only carotenoids that could be quantified in all the processed samples were lutein and zeaxanthin, which are the main protecting pigments of the eye tissue, as stated in the introduction section.

During the following two processing stages (drying and flaking), lutein and zeaxanthin content remained constant at about 0.33 and 0.45 ppm, respectively. In the subsequent drying stage, cooked grits were placed under hot air current (110–120 °C) to reduce moisture content to about 28–30%db. The dried grits were allowed to settle for 24 hours to homogenize humidity after which they were flaked. The flaking rollers were cooled to maintain the temperature under 40 °C. This flaking step implies a very short time (fractions of seconds), just enough to go throw the rollers.

It was interesting that in the drying stage, after cooking, even though drying temperatures were high (120 °C), no further loss was observed. It can be possible that the spheroid geometry of grits, previous to flaking, and the high humidity gained during cooking, had helped to protect the carotenoids that are beneath the grit surface. On the other hand, some antioxidant compounds that are formed as a result of Maillard reaction during cooking and also during the drying stage (Farroni & Buera, 2012) could also protect these pigments from oxidative degradation. These last authors have reported that non-enzymatic browning and fluorescence development occur greatly during cooking, drying and toasting stages, where the conditions of the cooking and drying stages are most appropriate for the generation of Maillard Reaction Products (MRP) with antioxidant capacity (Malgorzata, Konrad, & Zielinski, 2016; Yilmaz & Toledo, 2005). It has been previously shown that those antioxidant MRP are related to fluorescence development (Morales & Jiménez-Pérez, 2001).

In our samples, a significant increase of surface fluorescence index was found after the cooking stage (Fig. 2). These early Maillard products could help protect remaining carotenoids from oxidation, explaining the constant values during drying and flaking. Antioxidant capacity of MRP undergoes important reductions at temperatures over 120 °C (Malgorzata et al., 2016; Yilmaz & Toledo, 2005), therefore, a higher temperature could further degrade both carotenoid pigments and MR antioxidant products in spite of the short time involved.

The toasting stage, which develops at higher temperatures (230 °C, 90 s), results in additional carotenoid degradation. At the end of the traditional process, corn flakes showed 0.17 ppm of lutein and 0.2 ppm of zeaxanthin.

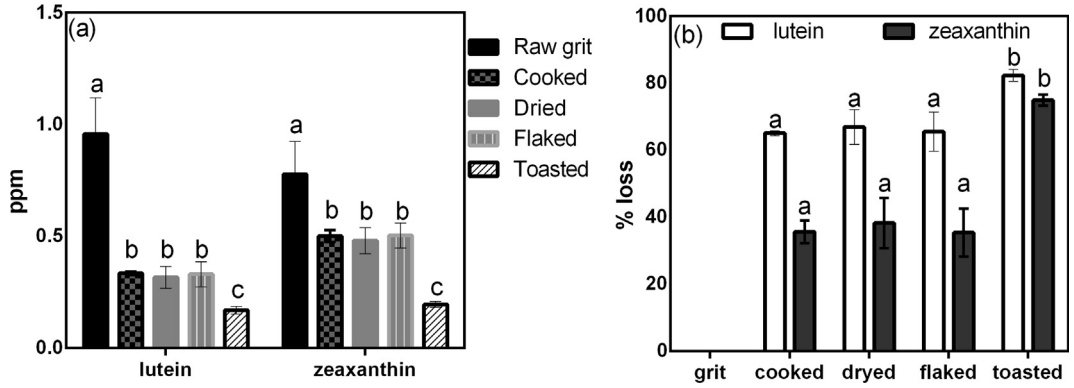

Fig. 1. (a) Individual carotenoid content and (b) Individual% loss of lutein and zeaxanthin through the 5 steps of traditional production of cornflakes. β-Cryptoxanthin and β-Carotene were below limit of quantification. Error bars represent the standard deviation. Different letters indicate statistically significant difference.

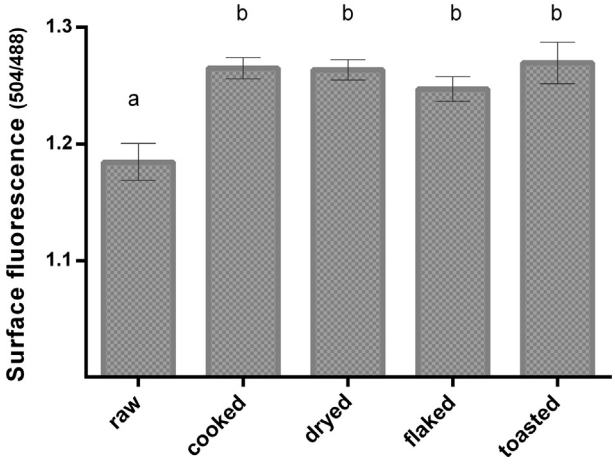

Fig. 2. Surface fluorescence index (504/488) for the 5 processing stages of traditional processing. Different letters indicate significant statistical difference. Bars represent mean of three replicates with standard deviation.

Individually, during the cooking stage lutein was more susceptible to isomerization and decomposition than zeaxanthin as it is shown in the% of loss: 60% for lutein and 40% for zeaxanthin after steam cooking (Fig. 1b). In accordance with this result, Burt, Grainger, Young, Shelp, and Lee (2010) reported a larger loss of lutein in relation to zeaxanthin during storage and drying (90 °C) of maize grains. This significant difference was maintained until toasting was performed, after which the total loss averaged 80% for both compounds. The highest losses corresponded to the cooking and toasting steps, as expected, due to the combination of high temperature- processing time employed. In this last step, the% of loss of zeaxanthin was similar to the percent of loss for lutein, showing that in the final product both major pigments suffered similar losses.

3.2. Carotenoid content during extrusion

Maize semolina used for extrusion cooking had an initial content of 0.49 ppm lutein and 0.26 ppm zeaxanthin (Fig. 3a and b). The formulation that included purple corn extract showed a small difference being 0.57 ppm and 0.36 ppm for these compounds. The dried purple corn extract showed an anthocyanin content of 52.6 ppm expressed as equivalents of β-cyanidin, and 12.3 mg/g equivalents of galic acid were measured in these samples through the Folin-Ciocalteu method (Shui & Leong, 2006).

During extrusion, lipids are released from cells owing to the high temperature and physical disruption of tissue, favoring the exposure of carotenoid compounds.

The carotenoid content reduction during extrusion varied for the different formulations. The added purple corn extract showed no protective effect on carotenoids during processing. A mixture with chia (at a 5% ratio) showed the greatest losses in spite of the intrinsic natural antioxidants present in chia (Valdivia-López & Tecante, 2015), that could not overcome the influence of the high extrusion temperature and shearing forces. It has been observed that carotenoid pigment degradation is enhanced in an oil medium containing high proportions of unsaturated fatty acids (Achir, Randrianatoandro, Bohuon, Laffargue, & Avallone, 2010). Chia seeds have a high proportion of non-saturated lipids along with comparatively low level of tocopherol (Ayerza, Coates, & Lauria, 2002; Ixtaina et al., 2011) and thus under thermal treatment they can be easily oxidized (Achir et al., 2010). In addition to this, there is also a higher total lipid content in this formulation (Table 1), which can also favor the production of peroxides and other free radicals that can react with the carotenoid compounds thus contributing to their loss (Frankel, 1991).

The addition of quinoa did not show any direct effect on the initial carotenoid content (considering that quinoa addition was at a 20% level) nor on their final retention.

Fig. 4 shows the percentage of loss for all 5 formulations after extrusion. It can be noted that for the simple corn mixture individual retention of carotenoids was much higher in extruded samples than in the traditional processing. In the latter, the cooking stage promoted the loss, with a value that doubles the one found after the extrusion process. Due to the high temperature, high exposure to ambient oxygen and low water content of samples at the toasting stage of the traditional process, the relative carotenoid losses are even higher if this step is taken into account. In accordance to this, Rodríguez-Huezo, Pedroza-Islas, Prado-Barragán, Beristain, and Verno-Carter (2004) found that a maximum degradation rate of a combination of carotenoids encapsulated in hydrocolloids in spray-dried multiple emulsions occurred at 64% R.H and Prado, Buera, and Elizalde (2006) found that the kinetic constant for carotene degradation increased as water content decreased.

3.3. Color variables

Color coordinates L^*, a^* and b^* were studied in relation to the pigment loss. Fig. 5(a) shows the relationship of b^* (yellowness) and the total carotenoid content for extruded samples, where a linear relationship can be observed, with a R^2 value of 0.89. A good linear relation between b^* and total carotenoid content was previ-

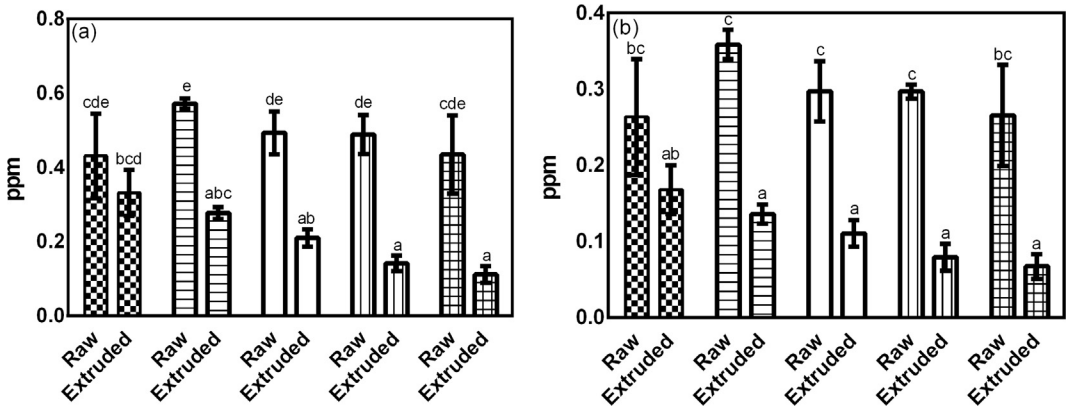

Fig. 3. (a) lutein and (b) zeaxanthin individual carotenoid content (ppm) for the five formulations on raw (R) and extruded (E) samples (maize ▩, maize + AO ▤, maize + Quinoa □, maize + Chia ||||, maize + Chia + AO ▦). Bars present the main values of triplicates with the corresponding standard deviation, different letters indicate statistically significantly differences ($p < 0.05$)

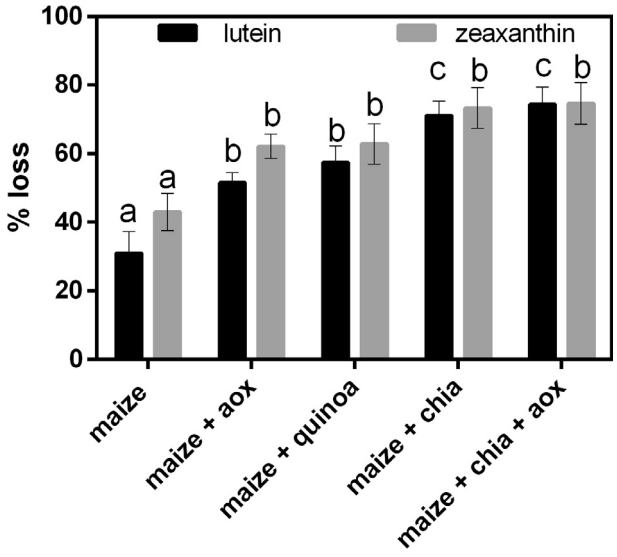

Fig. 4. Individual % loss of lutein and zeaxanthin for the 5 different blends used in the extrusion process. Averaged % of loss values of 3 different replicates were plotted. Error bars represent the standard deviation of the % loose. Different letters indicate statistically significant difference.

ously reported in olive oil by Moyano, Meléndez-Martínez, Alba, and Heredia (2008).

There was also a linear relationship between b^* and lutein (R^2 0.89), and between b^* and zeaxanthin (R^2 0.87), as is shown in Fig. 5(b). This behavior was expected, since the absorption maxima of both pigments are very close and thus affect the sample color in a similar way. Since their absorption maxima are in the visible spectrum zone that corresponds to the blue color (around 440 nm), with the higher energy and damage potential, both pigments reflect in the yellow-yellow-red zone. The chromatic coordinate b^* varies between blue and yellow so the loss of these pigments has a direct impact on the color variable representing yellowness, and therefore the slope of the correlation between b^* and carotenoid content is positive. It has to be mentioned that these correlations were obtained from extruded samples, that present small variations in their structure. In raw sample mixtures other factors affected sample color such as particle size, geometry and degree of mixture, and therefore it was not possible to extend the same relationship to the raw blends.

On the contrary, variables a^* (redness) and L^* (luminosity, clarity) did not show any direct correlation with the individual carotenoid content, with R^2 of 0.032 and 0.52 respectively.

For the traditional process, variable b^* and carotenoid content did not show any apparent correlation. This was because, on one

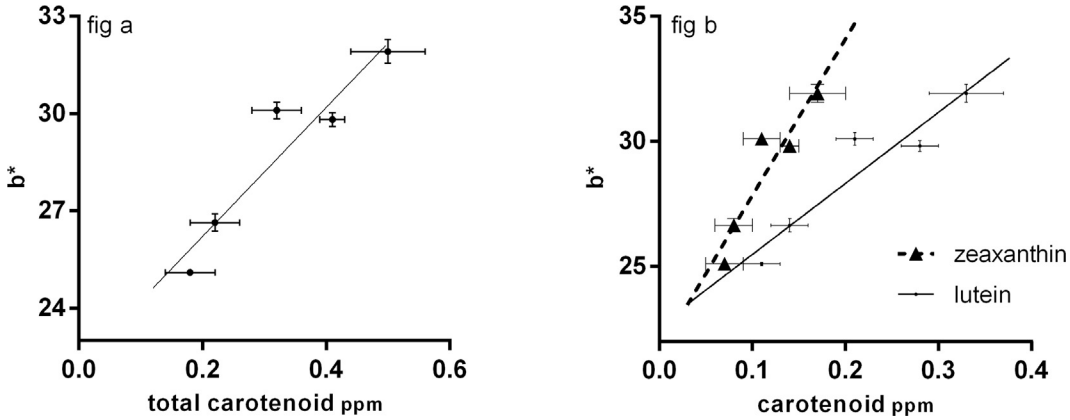

Fig. 5. Correlation between CIELAB coordinate b^* and total (a) and individual (b) carotenoid content of lutein or zeaxanthin. Mean values with their standard deviations are plotted in both axes. R^2were 0.89 for total carotenoids, and 0.89 and 0.87 for lutein and zeaxanthin respectively.

hand, the development of browning drastically modified the color variables (L*, a* y b*), masking the changes due to pigment loss. On the other hand, variations in surface texture and microstructure that take place during the different processing stages affect the reflectance measurements (Farroni & Buera, 2012), making it even harder to obtain a correlation.

4. Conclusions

As much as 80% of lutein and zeaxanthin, the two main carotenoids of corn which are involved in the eye tissue protection, were lost during extrusion or flaking. In the case of extrusion, for the simple corn mixture individual retention of carotenoids was higher. In the latter, the carotenoid loss depended on initial formulation, the addition of chia reduced carotenoid retention probably due to its high unsaturated lipid content. In these samples, their parallel oxidation possibly favoured carotenoid destruction.

The addition of a small amount (2%) of an anthocyanin-rich purple corn extract did not improve the preservation of carotenoid compounds during extrusion.

Lutein and zeaxanthin losses showed higher differences between them in the first stages of traditional cooking-flaking process. After toasting, both losses achieved similar values.

For extruded samples linear correlations between b* and total carotenoid, and b* and individual carotenoids content were obtained. Thus, a potential method for the non destructive measurement of carotenoids may be developed through image analysis, which is valid for samples comparison in which no structure changes or browning reactions are involved.

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