EFFECT OF EXTRUSION ON B-CAROTENE CONTENT AND STORAGE STABILITY OF CORN AND BOVINE LUNG SNACKS*

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ABSTRACT: A β-carotene and iron rich snack was developed aimed at children consumption. β-carotene was added to the snacks, in the flavoring mixture after (A) or before (B) extrusion. Proximate composition, β-carotene content, instrumental color and texture parameters were determined during storage. Both products had low content of lipids and high content of bioavailable iron, β-carotene and proteins. Shear resistance of snack B increased during storage, and the values of B were greater than those of snack A. The color parameter a* measured on snack B was greater than the verified for snack A, whereas the opposite was observed for parameter b*. Initial β-carotene content was higher in the snack A with significant reduction in both snacks during storage. β-carotene reduction from 15 to 60 days was less pronounced in the snack A as compared to snack B. The flavoring and fortification methods affected the shear strength, color parameters and β-carotene contents of the snacks.

KEYWORDS: Storage effect; β-carotene stability; snacks; fortification of food.

INTRODUCTION

Nutritional deficits are still considered a problem of epidemic dimension on the entire World. Iron deficiency anaemia, for example, is a worldwide problem affecting about 2 billion individuals. 16,18,24,27,28 Vitamin A deficiency is also a problem of large magnitude in many countries. 16, 27

Deficit of different micronutrients can occur concomitantly. Vitamin A and hemoglobin levels in the population have been demonstrated to correlate. Iron deficiency is more easily corrected when vitamin A levels are correct, since vitamin A can affect several stages of iron metabolism, which include erythropoiesis and the release of iron from ferritin stores. 12, 25, 29

Different products have been developed with unconventional raw material, in order to obtain products that provide the adequate ingestion of nutrients concomitant to the reduction of their production costs. It includes bovine by-products as lung, which contains a high level of bioavailable iron. 6, 20 There is a great potential for the use of such rich raw materials for production of traditional foods with more adequate composition from a nutritional point of view.

Among these products, there are the snacks, produced by the extrusion process, resulting in a food that presents high acceptability and high nutritional value. The extrusion cooking is a process in which high temperature and short time are employed, modifying the initial raw material (flour) resulting in a structured product. Food extrusion applications are based in altering the food functional properties and increasing the bioavailability of nutrients. 8

The introduction of β-carotene in snacks containing bovine lung is attractive for nutritional reasons and also because it provides an interesting color to the final product. On the other hand, the literature has demonstrated that β-carotene may be easily oxidized. Oxidation losses during processing and storage of foods deserve careful attention when assessing β-carotene values. 14,15

Therefore, the objective of this study was to determine β-carotene in snacks containing iron when this pro-vitamin A is added before and after extrusion, and to assess the stability of these products by following their color, texture and β-carotene contents.

MATERIAL AND METHODS

Snacks Production

The raw material used in the present study for snack production was the mixture of corn grits and the lyophilized fat free flour of bovine lung.

The bovine lung from healthily animals was obtained in a meat store, and the corn was purchased from...
Mercado Cerealista (São Paulo, SP, Brazil). The frozen bovine lung was acquired from ‘Frigorífico Centroeste – Ltd.’ (Guarulhos, SP, Brazil) and sent to liophilization in ‘Nutribrás – Nutrição Brasileira’ (Guarulhos, SP, Brazil). The temperature during transport varied from -7.0°C to -8.5°C. The bovine lung was defatted in our laboratory with 30 L of ethanol per kilogram of lung. The temperature used during the defatting process was about 18°C. Helices coupled to a “Kohlbach 56H” motor (Kohlbach Ltd., Jaraguá do Sul, SC, Brazil) were used in order to mix lung to ethanol during fifteen minutes at 1500 rpm. After this mixing, the suspension was filtered and the solids retained in the filter were dried in a forced-air drier (Quimis Mod. 314M242, Diadema, SP, Brazil) at 50°C during 48 h. The defatted and dried lung was then milled and the smallest fraction (< 0.45 mm) of the resulting milled powder was collected for snacks’ production and further analyses. Corn grits granule size varied from 20 to 40 mesh.”

The proximate composition of the corn grits used was 10.1% of protein, 84.7% of carbohydrates, 2.2% of lipids and 3.0% of ashes, dry basis. Proximate composition of the defatted lung flour used was 94.1% of proteins, 1.1% lipids, 5.8% ashes and traces of carbohydrates. The proportion between corn and bovine lung was 9:1, according to the mixture optimized by Cardoso-Santiago et al. The moisture content of the flour mixture, either containing or not the flavoring solution, was adjusted to 13% (d.s.b). For snack production 4 kg of the flour mixture were used.

The source of iron of the developed snacks was the bovine lung. The β-carotene used for the fortification of the snacks was granted by BASF Company (São Paulo, SP, Brazil) the product assigned Lucarotin 10 CWD F/O. Snacks were produced in a pilot single screw extruder, model RXPQ Labor 24, Inbramaq – Indústria de Máquinas (Ribeirão Preto, SP, Brazil). The extrusion conditions were optimized previously for a series of raw materials, including the corn grits and lung employed in this study. After a series of tests, we observed no effect of β-carotene addition on the final expansion of the extruded snack, and the following conditions were set: 404 rpm screw rotation, 1:1 screw compression ratio, six-hole matrix (1/8” each hole), sub-matrix of 42 holes (1/8” each hole). Temperatures were kept constant in the feed zone (40°C), in the compression-metering zone (80°C), and in the die (120°C). The extruded products were cut using a blade cutter at 123 rpm as they exit the extrusion die.

Snacks presented an expansion ratio of around 2.9 and a cylindrical format. The expansion ratio was determined as the ratio of the extruded product diameter to the diameter of the die hole, both measured using a caliper rule. The value reported was the average of 30 measurements.

Two snacks were produced. The snack A was fortified with β-carotene after the extrusion process, and the snack B was fortified before this process. The fortification of both products was done through a flavoring solution composed by β-carotene, bacon flavor, salt and Arabic gum. The percentages of aroma, salt and Arabic gum in relation to the snack were, respectively, 5.0%, 1.0% and 2.0%. The amount of added β-carotene was 0.2 mg.100 g-1 of snack. The aspersion of the flavoring solution was done onto the surface of the extruded product for the snack A or in the feeding mixture flour for snack B. Both snacks were kept away from the light in laminated packages at room temperature (around 20-25°C) in order to avoid possible interferences in β-carotene content. Each package, closed as conventional snacks, had approximately 30 g of product.

Chemical Composition of Snacks

The proximate composition was determined in triplicate, according to methodology described by the Association of Official Analytical Chemists: moisture by desiccation at 105°C; ash by incineration at 550°C; protein by micro-Kjeldhal technique for nitrogen determination – conversion factor 6.25 x N. The lipids were extracted in Soxhlet extractor with the solution chloroform/methanol in the proportion 2:1 v/v. The total iron contents of both snacks were analyzed after wet digestion with HNO3 and HClO3:3:1 v/v the iron was determined by atomic absorption spectrometry in the polarized equipment Ziemann AAS Hitachi Z-5000 (Tokyo, Japan) with halogen cathode lamps, wavelength 284.3 nm.

Study of Snacks Stability

Shear strength

The parameter chosen for texture measurement was the complete shear strength of the product in a Warner Bratzler cell by using the equipment TAXT2i (TA Instruments, Crawley, West Sussex, England). Moisture of snacks for this test was 6.1% for both snack types.

Fifteen units of each sample, randomly selected from three different packages, were analyzed. Cutting was perpendicular to the snack length and the following parameters were used for this analysis: velocity during the pre-test 2 mm.s-1, velocity during the test 1 mm.s-1, velocity after-test 5 mm.s-1 and distance performed 30 mm (until complete shear). Tests of shear strength were performed seven, fifteen, thirty, sixty, ninety and one hundred and five days after snack production.

Instrumental Color Parameters

Triplicate color tests were performed with an equipment Color Quest XE (Hunter lab, Reston, VA, USA) using the CIE L*a*b* system. The color parameters measured were L* luminosity (black 0/white 100), a* (red+/green-) and b* (yellow+/blue-). The illuminant D65 that corresponds to the natural light of the day was used as the light source.
The total color difference ($\Delta E^{*\text{ab}}$) between snacks were calculated according to the following equation (Sharma, 2003):

$$\Delta E^{*\text{ab}} = [(L^*1 - L^*2)^2 + (a^*1 - a^*2)^2 + (b^*1 - b^*2)^2]^{1/2}$$

The color analyses were performed seven, fifteen and thirty days after extrusion, been periodically repeated each fifteen day until 105 days of storage was completed.

$\beta$-carotene content

$\beta$-carotene was extracted from snack samples at one, seven, fifteen, thirty, forty-five, sixty, ninety-five, and one hundred and five days after snack production. The determination of $\beta$-carotene was performed in snacks A and B. All analysis was done in triplicate, in a dark room. In brief, 300 mg of the milled samples were weighted with 0.2 g of ascorbic acid. A solution containing KOH 11% m/v in ethanol 50% v/v was added (5.5 mL per sample) for the saponification. The samples were shaken for 15 minutes at 65ºC. After cooling at room temperature 3 mL of hexane HPLC grade was added in the samples, and the upper phase was reserved. The addition of hexane and separation of the upper phase was repeated twice, and then the upper phases collected were reunited and dried under nitrogen for posterior dilution in 1 mL of HPLC mobile phase.

HPLC analysis of $\beta$-carotene was carried out in a Shimadzu equipment (Tokyo, Japan) model LC-10A VP equipped with the PDA detector SPD-10A VP and a column Luna of fused silica, 250 mm x 4.6 mm, 5 µm particle (Phenomenex, Torrance, CA, USA). Twenty microliters of the sample extracts were injected into the HPLC column. The isocratic separation of $\beta$-carotene from other substances was accomplished with a mobile phase consisting of hexane: isopropanol (99:1 v/v), at a flow rate of 1 mL per minute. The effluent was monitored at 450 nm. External standard $\beta$-carotene was purchased from Fluka Chemicals (Hauppauge, NY, USA). All samples were quantified through external standard calibration (with a concentration of 1–5 mg all-trans-$\beta$-carotene). The standard solutions were freshly produced every day.

Statistical Analysis

The results were expressed as means and standard deviations. The variables were compared by Student t test or analyses of variance (ANOVA). Significant differences between means were determined by Tukey’s test at a level of P<0.05. The software used was the Statistical Package for the Social Sciences (SPSS) version 13.0 for Windows (Chicago, USA).

RESULTS AND DISCUSSION

Chemical Composition

After extrusion the composition of the snacks with $\beta$-carotene added after (A) and before (B) extrusion was the same (moisture 6.1%, protein 15.5%, lipid 3.80%, ash 1.45%, carbohydrates by difference 73%), resulting in a product with about 1629 kJ per 100 g of product. All results are expressed in dry basis.

The snacks produced in this study have in average 87% less lipids and 18% less energy as compared to three commercial brands marketed in Brazil. Hanley et al. 10 proposed that there is a straight relationship between the junk food consumption and the increase of weight of the young population. Therefore, as snacks are highly consumed by this population, the development of a product with this nutritional composition represents an alternative for a healthier food.

Another important aspect to be emphasized about the proximate composition of these snacks produced in this study is their high protein content, threefold higher than those contained in the commercial snacks. As this nutrient is from animal origin, it presents a good biological quality. 6

Iron content obtained in this study was high after the extrusion process for both snacks (5.1 mg/100 g on a dry basis). The snacks developed in this study can be considered products rich in iron, since 100 g of this product account for 50% of the DRI for children with 4 to 8 years (10 mg/day), or 62% of the DRI for children with 9 to 13 years (8 mg/day). 14,26 Its use in nutritional interventions could be a relevant strategy to control iron deficiency and anemia in areas where this is a major health problem.

The labeling of commercial snacks marketed in Brazil did not present its iron contents. Analyses of our laboratory 6 showed that this nutrient is present in those snacks in concentrations around 1.8 mg/100 mg of product. The snacks developed in this study contain 282% more iron than their commercial counterparts. Another significant characteristic regarding this micronutrient is its bioavailability, since it comes from a raw material of bovine origin. 19 The iron in this product retains its high bioavailability even in severe extrusion conditions with a marked improvement after processing. 6,19,20

Shear Strength

The values of shear strength obtained for snacks A and B are provided in Table 1. Satisfactory correlation between the results of sensory and instrumental texture is usually observed. 23 The higher the shear strength in the instrumental test, the lower is the grade of the sensory crispness evaluation. It is important to mention that the texture acceptability is the most important sensory attribute for snacks.

Snack B showed a significant increase in the shear strength (N) from the beginning to the end of the storage (Table 1). For the snack A it was not observed statistical difference between the initial and final values, probably due to the absence of a linear tendency of increase or decrease of the values, which varied independently of the days of storage.
Table 1 – Shear strength (N) of snacks A (β-carotene added after extrusion), and B (β-carotene added before extrusion) during storage.

<table>
<thead>
<tr>
<th>Storage Time (Days)</th>
<th>Snack A (N)</th>
<th>Snack B (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>32.3b±3.7</td>
<td>39.6a±7.6</td>
</tr>
<tr>
<td>15</td>
<td>26.0&lt;sup&gt;c&lt;/sup&gt;y ± 5.0</td>
<td>41.6&lt;sup&gt;b&lt;/sup&gt;±6.6</td>
</tr>
<tr>
<td>30</td>
<td>39.4&lt;sup&gt;a&lt;/sup&gt;±6.5</td>
<td>46.2&lt;sup&gt;b&lt;/sup&gt;±10.2</td>
</tr>
<tr>
<td>60</td>
<td>24.5±4.1</td>
<td>43.8&lt;sup&gt;b&lt;/sup&gt;±6.7</td>
</tr>
<tr>
<td>90</td>
<td>25.9&lt;sup&gt;a&lt;/sup&gt;±7.3</td>
<td>48.0&lt;sup&gt;b&lt;/sup&gt;±7.7</td>
</tr>
<tr>
<td>105</td>
<td>30.3&lt;sup&gt;b&lt;/sup&gt;±6.8</td>
<td>57.0&lt;sup&gt;b&lt;/sup&gt;±7.6</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation.

Same letters (a,b,c) in the same column mean no statistical differences in time; P < 0.05, n = 3.

Same letters (x,y) in the same line mean no statistical differences between both treatments; P < 0.05, n = 3.

The addition of Arabic gum during extrusion has been reported to help decreasing the mechanical energy while increasing both efficiency and output of the extruder. Such benefits have been observed with addition of 2% to 5% Arabic gum. Optima reduction of electrical intensity, engine torque, and heat build up have been observed with 3.5% Arabic gum. 23 We added 2% of Arabic gum, which may have helped to increase crispiness.

**Color Parameters and β-carotene retention**

Table 2 presents the values of color parameters obtained for both snacks.

Color is an important quality factor that is directly related to the acceptability of food, influencing greatly the physical properties of extruded products. The measurement of the color also may be used as an indirect measurement of food compounds that absorb and reflect the light in visible wavelengths, such as carotenoids. 20 In the presence of β-carotene, it is possible to observe an orange color in food, what can cause an alteration in the final acceptance of the product. 1

In the Ilo et al. 13 study, that evaluated different parameters of extrusion for the production of corn snacks, the color parameter L* varied from 73.0 to 86.2. Capriles & Áreas 4 found luminosity values for corn snacks varying from 76.6 to 77.9. The luminosity results of the studies cited are higher than the ones found in our work probably due to the low standard deviation values obtained in the analysis.

Given that it was not observed a tendency for changes in instrumental color parameters the panelists probably would not notice differences in snacks after storage, but they might be able to differentiate the snacks at any time. This can be seen when the color difference parameter is considered (Figure 1), where there is always a ΔE value above the “just noticeable difference” threshold (ΔE = 2.3) 22 irrespective of the storage time. Because of the results in color parameters, it was not possible to correlate the loss in the β-carotene content during storage with the L*, a*, b* parameters but when color difference is considered it can be noticed a decrease in this value with storage time (Figure 1), which correlates well with the observed decrease in β-carotene content.

The behavior of the β-carotene contents during storage for both products added with this pro-vitamin A as shown in Figure 1 indicate differences, whether the carotene is added after or before the extrusion process.

Initially, the snack B contained about 0.14 mg.100 g<sup>-1</sup> of β-carotene and the snack A contained 0.17 mg.100 g<sup>-1</sup> of β-carotene. This difference was caused by the thermal processing during extrusion. The high content of iron may potentially increase the oxidation rate of the produced snacks. The pro-vitamin, although protects the product from deterioration, is oxidized, losing its physiological function.
Other studies also verified the reduction of this vitamin content caused by extrusion, varying from 27 to 62%. The results from literature suggest that fortification of snacks with vitamin A, especially with β-carotene, must be done after extrusion. Thus, if the carotene is intended to be used for health reasons, the addition of antioxidants such as BHT is necessary to protect the vitamins against the oxidation.

A significant reduction in β-carotene content was found for both snacks analyzed during storage (Figure 1). The β-carotene concentration was somewhat constant until fifteen days of storage for both products, when significant reduction in relation to the first day of storage begins to be observed.

β-carotene concentrations in the snack A were higher than the ones in the snack B from 15th storage day onwards, and this difference was kept until 60 days of storage. These results may be caused by the major interaction of the β-carotene present in the snack B and other minerals present in the raw material, especially iron in bovine lung, that can act as a catalyst, accelerating the degradation of the vitamin. Corrales compared the maintenance of vitamin A after extrusion in snacks elaborated with different raw materials and added of vitamin A before extrusion. The snack made by corn and bovine lung presented a more pronounced reduction in vitamin A contents after extrusion as compared to snacks only made by corn or made by corn and chickpea, a result that is in agreement with our study.

The content on the last forty five days of the study was stable for both snacks. In the end of this study, both snacks presented closer values of this vitamin. An aspect visually observed during the aromatization and fortification after extrusion is the difficulty of homogenizing the synthetic vitamin in the spreading solution, which may impair the dispersion of the vitamin over the final product. Similar difficulty in the dispersion was observed by Chavez-Servin et al. that extracted different kinds of vitamin A from infant milk formulas.

The contents of this vitamin in the raw material that was not fortified, was also evaluated. The mean value obtained for β-carotene content in the corn and bovine lung flour before extrusion and with no addition of the pro-vitamin was 0.04 mg.100 g⁻¹ of product. The fortification therefore increased in about fourfold the content of this vitamin on the product. The initial values of β-carotene in the snack A represented about 4% of DRI for kids from 4-8

### Table 2 – Color parameters of snacks A (β-carotene added after extrusion), and B (β-carotene added before extrusion) during storage.

<table>
<thead>
<tr>
<th>Color Parameters</th>
<th>Storage Time (days)</th>
<th>Snack A</th>
<th>Snack B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminosity (L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>69.9h±0.05</td>
<td>64.4h±0.16</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>69.5h±0.50</td>
<td>62.2h±0.37</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>69.8h±0.01</td>
<td>65.0h±0.48</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>70.4h±0.11</td>
<td>62.6h±0.88</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>68.5h±0.04</td>
<td>62.8h±1.11</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>69.3h±0.18</td>
<td>65.4h±0.04</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>68.7h±0.08</td>
<td>64.7h±0.26</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>68.8h±0.04</td>
<td>64.6h±0.20</td>
<td></td>
</tr>
</tbody>
</table>

| a*               |                     |         |         |
| 7                | 3.9h±0.06           | 5.6h±0.14 |
| 15               | 4.0h±0.12           | 5.5h±0.01 |
| 30               | 3.9h±0.02           | 4.7h±0.08 |
| 45               | 3.7h±0.02           | 4.9h±0.11 |
| 60               | 3.8h±0.01           | 4.8h±0.08 |
| 75               | 3.9h±0.03           | 5.3h±0.06 |
| 90               | 4.1h±0.03           | 5.4h±0.14 |
| 105              | 3.9h±0.03           | 4.9h±0.03 |

| b*               |                     |         |         |
| 7                | 19.7h±0.06          | 19.7h±0.23 |
| 15               | 20.1h±0.01          | 18.3h±0.08 |
| 30               | 20.1h±0.11          | 17.9h±0.14 |
| 45               | 20.0h±0.07          | 17.5h±0.16 |
| 60               | 20.2h±0.12          | 17.6h±0.02 |
| 75               | 20.2h±0.07          | 19.0h±0.32 |
| 90               | 20.0h±0.05          | 19.7h±0.36 |
| 105              | 20.8h±0.03          | 18.3h±0.08 |

Values are mean ± standard deviation.

Same letters (a,b,c,d,e) in the same column for each parameter mean no statistical differences in time; P < 0.05, n = 3. 
Same letters (x,y) in the same line mean no statistical differences between both treatments; P < 0.05, n = 3.
years and 3% for kids from 9-13 years, while values for snack B represented 3% and 2%, respectively. 14,26

CONCLUSION

Iron content of the snacks is not affected by the addition of carotene mixture before or after extrusion. On the other hand, initial β-carotene content of the snacks was lower when this addition occurred before the extrusion process. Concentration of the added β-carotene with storage time for both types of treatment decreased in distinct rates and become similar after 75 days and remained like that for the remaining storage period studied (up to 105 days). The addition of carotene mixture before extrusion increased crispiness, an important parameter for snacks. However, carotene mixture added after extrusion resulted in higher values of luminosity and yellowness than the ones of the snacks with β-carotene added before extrusion, characteristic that may stimulate their consumption by the population.


RESUMO: Um “snack” rico em β-caroteno e ferro foi desenvolvido visando o seu consumo por crianças. O β-caroteno foi adicionado ao lanche na mistura de aromas depois (A) ou antes (B) da extrusão. Os parâmetros: composição centesimal, teor de β-caroteno, cor e textura instrumentais foram determinados durante o armazenamento. Ambos os produtos apresentaram baixo teor de lipídios e alto teor de ferro biodisponível, β-caroteno e proteínas. A resistência ao cisalhamento do snack B aumentou durante o armazenamento, e os valores de B foram maiores que os de A. O valor do parâmetro de cor a* medido no snack-B foi maior que o verificado para o lanche A, enquanto o oposto foi observado para o parâmetro b*. O teor de β-caroteno inicial foi maior no snack A, com redução significativa nos dois lanches durante o armazenamento. A redução de β-caroteno entre 15 e 60 dias foi menos pronunciada no lanche A quando comparado ao lanche B. Os métodos de aromatização e fortificação afetaram a resistência ao cisalhamento, os parâmetros de cor e conteúdo de β-caroteno dos dois tipos de “snack”.

PALAVRAS-CHAVE: Efeito do armazenamento; estabilidade do β-caroteno; snacks; fortificação de alimentos.

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