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### Effect of Thermal Processing on Carotenoids in Fortified Bread



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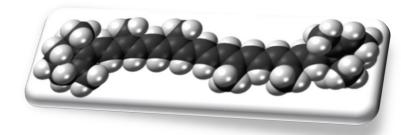
### Introduction



- Vitamin A deficiency is a major public health issue in many lowincome countries.
- Bread is an ideal food vector for fortification because it is a staple food in many parts of the world
- Bread fortification with synthetic or natural provitamin A carotenoids can provide a solution for vitamin A deficiency.



### Introduction (cont.)



- Carotenoids are biologically active compounds with numerous health benefits, such as reducing the risk of lung, breast, colon, and prostate cancers, as well as UV-induced skin damage, coronary heart disease, cataracts, and macular degeneration.
- Carotenoids with β-ring end groups can act as provitamins A within the human body
- Challenges involving carotenoid fortification:
  - Carotenoids are sensitive to environmental conditions such as heat, light, and contact with oxidants.
  - Proper handling is necessary to obtain the desired effects.

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### Introduction (cont.)



- Bread fortification with carotenoids of natural origin is an important factor in ensuring consumers' acceptance and compliance, hence this study targets fortified bread obtained through the straight-dough procedure, in which a puree made from *Cucurbita maxima Duch*. fruits was added.
- Because carotenoids are heat-sensitive biomolecules, high performance liquid chromatography (HPLC) with photodiodearray detection was used as method of analysis, this being the most appropriate analytical technique for their analysis.





### **Research objectives**

- To investigate the effect of thermal processing on carotenoids from fortified bread using HPLC as analytical tool.
- To provide relevant information for food technology and nutritional studies.
- To support the creation of a functional food that offers health benefits beyond combating vitamin A deficiency.



### **Material and methods**



#### Materials

- All solvents for chromatography were HPLC grade purity (Merck) and they were filtered (0.45 μm Whatman filters) then degassed in an ultrasonic bath, under vacuum, before use; solvents for extraction were p.a. quality, freshly distilled; water was bidistilled, then degassed.
- Potassium hydroxide was from Merck.
- $\circ$  Reference carotenoids neoxanthin, violaxanthin, antheraxanthin, lutein zeaxanthin and all-E- $\beta$ ,  $\beta$ -carotene were from CaroteNature GmbH (Lupsingen, Switzerland).
- All the analytical operations were carried out in reduced light, avoiding samples heating at more than 40°C.
- $\circ$  Prior to injection in HPLC system, all samples were filtered (0.45  $\mu$ m, Whatman).

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### Material and methods (cont.)

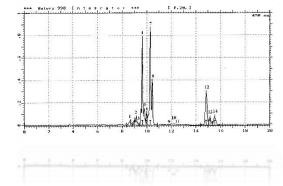


#### Biological material & bread fortification

- Mature Cucurbita maxima Duch. fruits were harvested from the experimental field of the University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, Romania; fruits were sliced, the seeds and the placental tissues were removed, then the resulted slices were baked at 200°C for 60 minutes; after cooling, the crust was removed from each slice, these being homogenized in a blender.
- A laboratory-scale bread preparation was developed using the straight-dough procedure by mixing 300 g commercial wheat flour, 5 g dry yeast, 4 g sodium chloride, 100 g water and 100 g puree; the dough was mixed to optimum development, then was left for fermentation at 250C for two hours, being then divided in portions of 100 g which were manually molded. Final dough fermentation was accomplished at room temperature, then baking was performed at 215°C for 25 minutes; the baked breads were allowed to cool on wooden racks for one hour at room temperature.

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### Material and methods (cont.)



#### Sampling, sample preparation and HPLC analysis

• Bread sampling, sample preparation and HPLC analysis were performed according to a procedure published before [Muntean et al., 2004]

#### Data processing

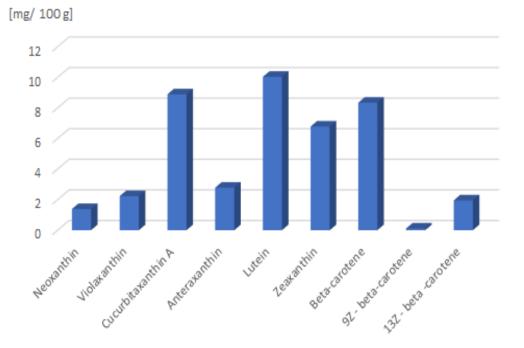
 Chromatographic data analysis was accomplished using Waters 990 software (Waters Corporation, Milford, MA, USA), then chromatographic data were further processed using Excel (Microsoft Corporation, Redmond, WA, USA). The provitamin A concentrations were expressed in retinol equivalents (R.E.) according to FAO/WHO requirements [WHO].

Muntean, E.; Muntean, N.; Modoran, C.; HPLC analysis of carotenoids in bread. Scientiffic Researches – Agroalimentary Processes and Technologies **2004**, X (1), 186-190.

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Reversed-phase HPLC enabled the quantification of three carotenes ( $\beta$ -carotene, 9Z- $\beta$ -carotene and 15Z- $\beta$ -carotene) and eight xanthophylls (neoxanthin, violaxanthin, cucurbitaxanthin A, lutein and zeaxanthin), highlighting that the major carotenoids from fortified bread were lutein, cucurbitaxanthin A and  $\beta$ , $\beta$ -carotene.

*Figure 1.* Concentrations' profile of carotenoids in baked *Cucurbita maxima* fruits puree used for fortification.

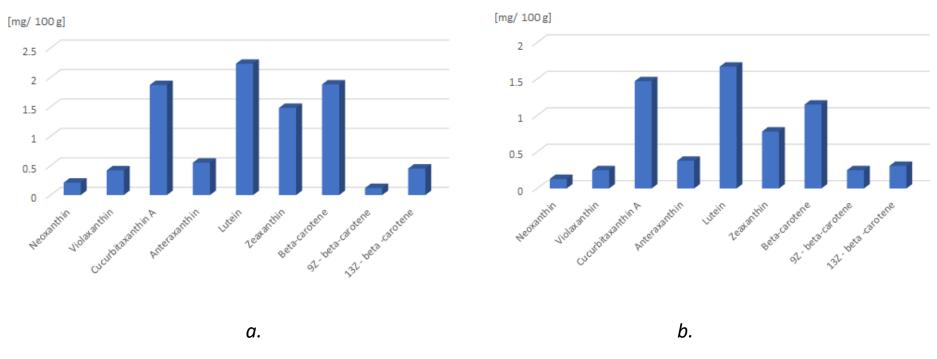






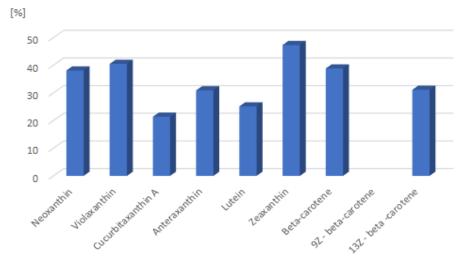
- Since the baked Cucurbita maxima fruits' puree contains considerable amounts of carotenoids, as can be seen in figure 1, its addition to the dough increased the carotenoid content (figure 2), the major ones being lutein, β -carotene and cucurbitaxanthin A.
- Thermal processing caused massive carotenoid degradation, this affecting both xanthophylls and carotenes (figures 2 and 3); zeaxanthin and violaxanthin were the most affected carotenoids, being degraded to the greatest extent, while cucurbitaxanthin A and lutein proved to be the most stable carotenoids under these circumstances.
- A special case is that of 9Z-β,β-carotene, a product of β,β-carotene isomerization, since its overall concentration increased after thermal processing; this is the reason it was not included in figure 3.





*Figure 2.* Concentrations' profile of carotenoids in the initial dough (a) and in the fortified bread (b)

> Because thermal processing had an important effect on the most important provitamin A carotenoid from the fortified bread ( $\beta$ -carotene), the retinol equivalent was affected accordingly (figure 4).



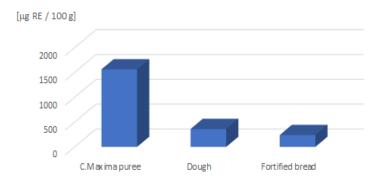


Figure 3. Comparative carotenoids' degradation as a result of thermal processing

Figure 4. Comparative values for retinol equivalents in the investigated food matrices

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### Conclusions



- > HPLC analysis highlighted that the major carotenoids from fortified bread were lutein, cucurbitaxanthin A and  $\beta$ , $\beta$ -carotene, as well as the stability of the targeted compounds and the change in provitamin A activity as a result of thermal processing during baking; the most stable carotenoids proved to be lutein and cucurbitaxanthin A.
- The behavior of the involved carotenoids can provide relevant information for nutritional studies.
- Fortified bread with baked pumpkin is not only a possible solution for combating vitamin A deficiency, but also a true functional food.
- Bread products fortified with naturally occurring carotenoids may be of interest for consumers because of health benefit of the antioxidant effect, as compared with nonfortified products. The use of natural sources of carotenoids, such as *Cucurbita maxima* fruits, can also increase consumer acceptance and compliance.
- Fortified bread can also open new market opportunities for producers; being a solution to a serious public health issue, it can be used as a selling point for health-conscious consumers.

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### Many thanks for your attention!



# Please send your questions to edimuntean@yahoo.com!

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