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Effect of Thermal Processing on Carotenoids in Fortified Bread †

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Abstract: Bread is a staple food providing essential nutrients to millions of people worldwide, hence its fortification with provitamin A carotenoids can help combat vitamin A deficiencies effectively. This study investigates fortified bread made with *Cucurbita maxima Duch*. fruits through the straight-dough procedure, focusing on changes caused by thermal processing on carotenoids. High performance liquid chromatography (HPLC) with photodiode-array detection was used for sensitive and selective analysis of carotenoids. HPLC showed that lutein, cucurbitaxanthin A, and β-carotene are the major carotenoids in fortified bread, highlighting lutein and cucurbitaxanthin A as the most stable carotenoids during thermal processing. Fortified bread is a functional food that not only can combat vitamin A deficiency and prevent age-related macular degeneration, but also has antioxidant properties with related health effects, offering new market opportunities for producers.

Keywords: bread; fortification; carotenoids; chromatography; HPLC; analysis; functional foods

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1. Introduction

Vitamin A deficiency is a major public health issue in many parts of the world, particularly in low-income countries; because it can lead to serious health problems and can have a profound impact on child development, it was addressed by numerous researches during the last decades, some of them involving food fortification approaches [1,2]. Bread is an ideal food vector for fortification because it is a staple food in many parts of the world, providing an essential source of carbohydrates, proteins and minerals to millions of people [3,4]. In such a context, bread fortification is necessary because bread alone is not sufficient to meet the nutritional requirements of a balanced diet, as it lacks important micronutrients; bread fortification with synthetic or natural provitamin A carotenoids can provide a solution for vitamin A deficiency and *Cucurbita maxima* fruits can be used for this purpose [5–7].

Carotenoids are biologically active compounds widely recognized for their advantageous impact on human health; numerous researches has shown that consuming carotenoid-rich foods is linked to a decreased risk of lung, breast, colon, and prostate cancers, as well as UV-induced skin damage, coronary heart disease, cataracts, and macular degeneration, while certain carotenoids with β-ring end groups can act as provitamins A within the human body [8,9]. Unfortunately these compounds are also sensitive to environmental conditions such as heat, light, extreme pH values, contact with oxidants [10,11], hence their use in technological applications have to be properly handled in order to obtain the desired effects.

The fortification of bread with carotenoids of natural origin is an important factor in ensuring consumer 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 photodiode-array detection was used

as method of analysis, this being the most appropriate analytical technique to date for their analysis [12,13].

The major objective of this study was to investigate the effect of thermal processing on carotenoids from fortified bread, using HPLC as analytical tool, to provide relevant information for food technology, nutritional studies and to support the creation of a functional food that offers health benefits beyond combating vitamin A deficiency.

2. Materials and Methods

2.1. Materials

All solvents for chromatography were HPLC grade purity (Merck) and they were filtered through 0.45 μm Whatman filters, then degassed in an ultrasonic bath, under vacuum, before use. Solvents for extraction were p.a. quality; water was bidistilled, then degassed. Potassium hydroxide was from Merck. The reference carotenoids neoxanthin, violaxanthin, antheraxanthin, lutein zeaxanthin and all-E-β,β-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; prior to injection in HPLC system, all samples were filtered (0.45 μm, Whatman).

2.2. 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 baked at 200^{0}C for 60 min; after cooling, the crust was removed from each slice, these being homogenized in a blender.

A laboratory-scale bread preparation was accomplished using the straight-dough procedure by mixing 300 g commercial wheat flour (Baneasa—000), 5 g dry yeast, 4 g sodium chloride, 150 g water and 100 g + puree; the dough was mixed to optimum development, then was left for fermentation at 25 °C for two hours, being then divided in portions of 100 g which were manually molded. The final dough fermentation was accomplished at room temperature for one hour, then baking was performed at 215 °C for 25 min; the baked breads were allowed to cool on wooden racks at room temperature.

2.3. Sampling and Sample Preparation

Breads were sliced and samples were collected from each bread core; core bread samples of ~1 g were thoroughly homogenized then average samples of ~20 g were weighed and subjected to extraction with 100 mL acetone in a blender, filtered under vacuum on a sintered glass funnel, then the solid material was re-extracted twice with 50 mL acetone.

The combined filtrates were subjected to liquid-liquid extraction in separation funnel in which 100 mL diethyl ether were added and washed five times with 100 mL distilled water. The resulted extract was evaporated to dryness under reduced pressure in a Buchi rotary evaporator at 40°C, re-dissolved in 25 mL diethyl ether, then saponified with 25 mL solution 30% KOH in methanol on a magnetic stirrer (350 rpm, room temperature, 16 h). Carotenoids were next extracted with 50 mL diethyl ether and washed repeatedly with distilled water until free of alkali; the extract was evaporated to dryness under reduced pressure, then the residue was dissolved in 10 mL ethyl acetate, from which an aliquot was filtrated through 0.47 μm membrane filter and subjected to HPLC analysis.

2.4. HPLC Analysis

HPLC analysis were performed according to a procedure published before [14]; carotenoids' identification was completed based on their retention times, HPLC co-chromatography with standards, and visible spectral characteristics compared to reference carotenoids and literature data [15]. Quantifications were achieved using the external standard method [14]

2.5. 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, USA). The provitamin A concentrations were expressed in retinol equivalents (R.E.) according to FAO/WHO requirements [16].

3. Results and Discussion

Reversed-phase HPLC enabled the quantification of three carotenes (all-E-β,β-carotene, 9Z-β-carotene and 15Z-β-carotene) and five xanthophylls (neoxanthin, violaxanthin, cucurbitaxanthin A, lutein and zeaxanthin), highlighting that the major carotenoids from fortified bread were lutein, cucurbitaxanthin A and all-E-β,β-carotene (Figures 1–3).

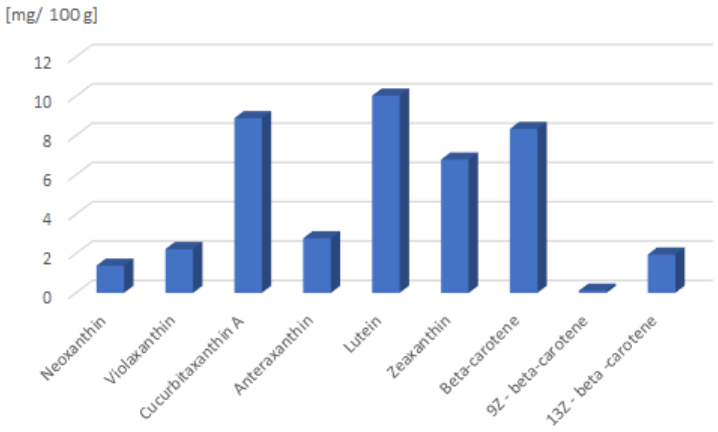
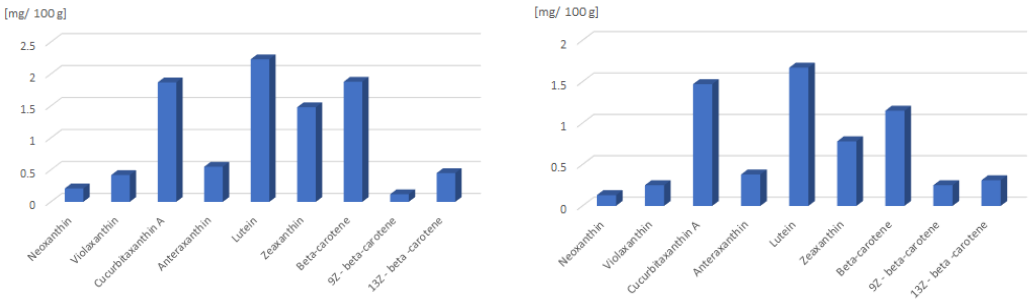


Figure 1. Concentrations' profile of carotenoids in baked *Cucurbita* fruits puree used for fortification.

Since the baked Cucurbita maxima fruits' puree contains considerable amounts of carotenoids (Figure 1), its addition to the dough increased the carotenoid content (Figure 2), the major ones being lutein, β -carotene and cucurbitaxanthin A—the last one being a specific carotenoid for *Cucurbita* genus, with no provitamin A activity.



a **b**

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

Thermal processing caused massive carotenoid degradation, this affecting both xanthophylls and carotenes. The effects of thermal processing on carotenoids is revealed in Figures 2 and 3, which prove zeaxanthin and violaxanthin were the most affected carotenoids, being degraded to the greatest extent, while cucurbitaxanthin A and lutein shoved to be the most stable carotenoids under these circumstances. A special case is that of 9Z-β,β-carotene, a product of all-E-β,β-carotene isomerization, since its overall concentration increased after thermal processing; this is the reason it was not included in Figure 3.

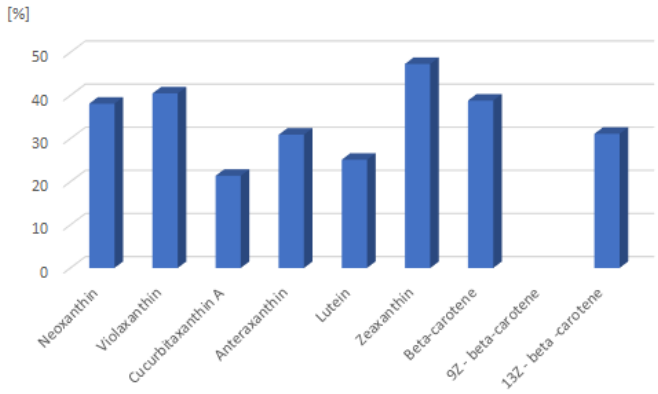


Figure 3. Comparative percentage carotenoids' degradation as a result of thermal processing.

Since thermal processing had an important effect on the most important provitamin A carotenoid from the fortified bread (β-carotene), the retinol equivalent was affected accordingly (Figure 4).

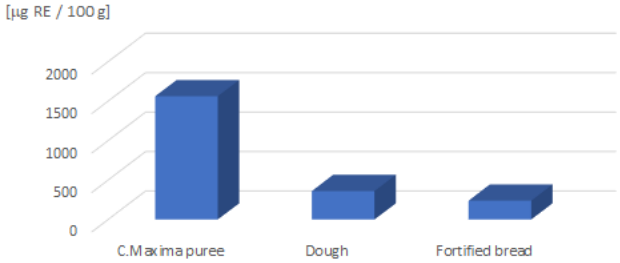


Figure 4. Comparative values for retinol equivalents in the investigated food matrices.

4. Conclusions

- HPLC analysis highlighted that the major carotenoids from fortified bread were lutein, cucurbitaxanthin A and β-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 *Cucurbita* 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 non-fortified 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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