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Development a monoclonal antibody-based enzyme-linked immunosorbent assay for screening carotenoids in eggs

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ABSTRACT

In this study, a monoclonal antibody (mAb) with broad-specificity against several carotenoid analogs with equal or similar efficacy was prepared. The obtained mAb C11, with the IgG1 isotype, showed cross-reactivity (CR) with canthaxanthin (100%), β-ionone acid (140.4%), β-carotene (92.9%), capsanthin (90.1%), β-apo-8′-carotenal (92.7%), and xanthophyll (95.8%). Using the mAb C11, a highly sensitive and inexpensive indirect competitive enzyme linked immunosorbent assay (ic-ELISA) was developed with a simple sample preparation procedure for the simultaneous detection of these carotenoid compounds in eggs. The limit of detection of the various carotenoids ranged from 1.31 mg kg^{-1} to 1.48 mg kg^{-1}. Recoveries from egg yolks spiked with the above carotenoids ranged from 91.8% to 113.3%, with coefficients of variation (CVs) of less than 14.8%. These results suggest that the developed ic-ELISA is a sensitive, specific, accurate, and inexpensive method that is suitable for the screening of carotenoid residues in routine monitoring.

1. Introduction

Color is a key factor for the consumer acceptance of many foods. The red fillet color caused by the deposition of carotenoid pigments (e.g., astaxanthin and canthaxanthin) in the muscular tissue is an important quality criteria for consumer acceptance and willingness to pay in salmonid fishes (Caballo, Costi, Sicilia, & Rubio, 2012; Dissing, Nielsen, Ersbøll, & Frosch, 2011; Folkestad et al., 2008). In eggs, a golden yellow yolk color, which is also caused by the deposition of carotenoid pigments (e.g., canthaxanthin, β-carotene, β-apo-8′-carotenal, and capsanthin) in the yolk, is associated with health and quality (Furusawa, 2011; Grashorn & Steinberg, 2002; Ren & Zhang, 2008). However, neither salmonid fishes nor egg-laying hens can produce carotenoids, and they have to obtain these pigments from dietary sources.

Although more than 600 carotenoids have been defined in nature, only a few of them are used in animal feed, pharmaceuticals, cosmetics and food coloring (Kop & Durmaz, 2008; Ong & Tee, 1992). In fish and poultry farming and food processing, several carotenoids, including canthaxanthin, β-carotene, β-apo-8′-carotenal, capsanthin, and xanthophyll (Fig. 1A), are frequently used as colorings and feed additives to pigment the eggs or meat of hens, broilers, salmon, and trout to make food more attractive and appetizing (Caballo et al., 2012; Fujii, Shimizu, & Nakamura, 2001; Furusawa, 2011; Ong & Tee, 1992).

However, some of these substances pose a potential risk to human health, especially if they are excessively consumed. For example, canthaxanthin has been reported to cause liver injury and an eye disorder called canthaxanthin retinopathy, the formation of yellow deposits on the retina (FDA, 2003). Although no adverse effect of high-dose oral β-carotene supplementation was observed in several standard toxicological studies in various experimental animals (rat, mice, rabbits) (Grenfell-Lee, Zeller, Cardoso, & Pucaj, 2014; Woutersen, Wolterbeek, Appel & Berg, 1999), intervention trials with large doses of β-carotene found adverse effect on the incidence of lung cancer in smokers and workers exposed to asbestos (Omenn et al., 1996). In addition, there is evidence that both β-apo-8′-carotenal and β-carotene at high doses could significantly enhance DNA strand breaks and lipid peroxidation and impair mitochondrial functions (Siems et al., 2005; Yeh & Wu, 2006).

For these reasons, safety data, such as acceptable daily intakes, based on toxicological studies with experimental animals and human clinical studies have been repeatedly determined and evaluated by the Food and Agricultural Organization (FAO) and World Health Organization (WHO) (Minioti, Sakellariou, & Thomaidis,

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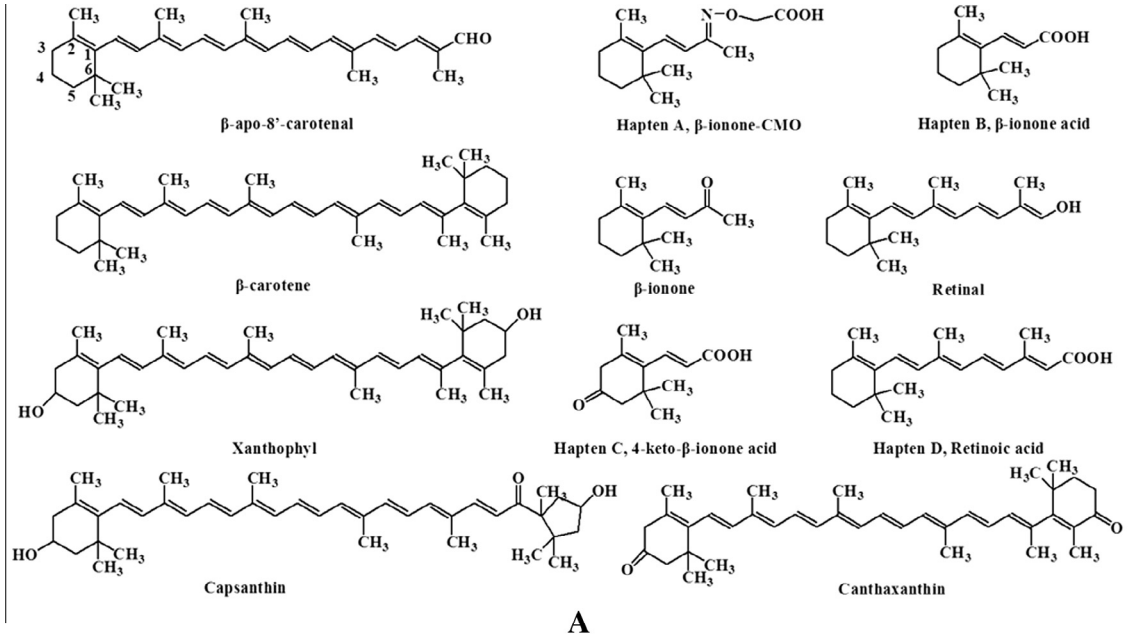

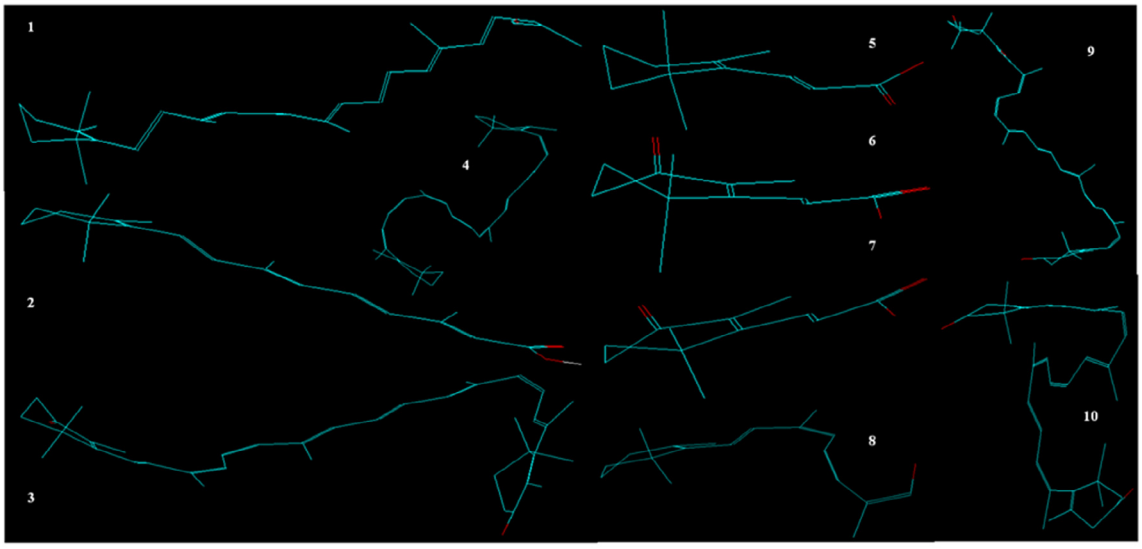

Fig. 1. The structure of some β-carotenoid pigment: A, the structure of some β-carotenoid pigment and haptens; B, stereochemical structure of some β-carotenoid pigment, 1, β-apo-8′-carotenal; 2, retinoic acid; 3, canthaxanthin; 4, β-carotene; 5, β-ionone acid; 6, β-ionone; 7, 4-keto-β-ionone acid; 8, retinol; 9, capsanthin; 10, xanthophyl.

2007). The European Commission adopted a directive in 2003 to reduce the authorized level of canthaxanthin in animal feed. Recently, the maximum residue limits (MRLs) of canthaxanthin in animal tissues and feeds were set by the European Food Safety Authority (EFSA) and the Japanese Ministry of Health, Labour and Welfare, respectively (Furusawa, 2011). Therefore, monitoring the presence of such carotenoids in edible animal tissues and products is necessary to ensure the safety and appropriateness of products for human consumption, satisfactory product quality and adequate production costs.

Numerous analytical methods, such as spectrophotometry (Schoefs, 2002), thin layer chromatography (Hayashi et al., 2003) and high performance liquid chromatography (HPLC) (Akhtar & Bryan, 2008; Barba, Hurtado, Mata, Ruiz, & Tejada, 2006; Breithaupt, 2004; Caballo et al., 2012; Hu, Lin, Lu, Chou, & Yang, 2008; Minioti et al., 2007; Ren & Zhang, 2008), have been used for the determination of carotenoids in a variety of food matrices. These methods, however, are not suitable for the routine monitoring of carotenoids because they involve expensive instrumentation and are time consuming. This has in turn created a demand for better analytical methods that are inexpensive, rapid, and robust for the detection and quantification of such carotenoids in a variety of food matrices. Indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) is the most popular method for detecting drugs in animal tissues due to its high sensitivity, low cost, and ability to screen large numbers of samples. Production of antibodies against carotenoids or development of ic-ELISA methods for the analysis of carotenoids in edible animal tissues have not been reported.

Therefore, this study aimed to prepare a monoclonal antibody (mAb) with broad-specificity against several carotenoid analogs and to develop a highly sensitive and low cost ic-ELISA for the simultaneous detection of these carotenoid compounds in edible animal tissues and products with a simple sample preparation procedure.