EFFECT OF WHEY PROTEIN ISOLATE ON THE OXIDATIVE STABILITY OF VITAMIN A

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"THE ONE WHO CAN DISCIPLINE THEIR MIND CAN STEP OUT OF THIS ENDLESS CYCLE OF DESIRE AND AVERSIONS."

- GAUTAMA BUDDHA

ABSTRACT

The purpose of this study was to investigate the possible protective effect of whey protein isolate (WPI) on the oxidative stability of vitamin A in an aqueous phase. The first part of the study focused on the development of a reliable method to extract retinol from the samples for analysis. Direct solvent extraction and saponification were both tested, with saponification, which converts retinol acetate into retinol, chosen for the experimental work. Extracted retinol was quantified using reversed phase HPLC, to obtain the degradation trends of the samples that had been subjected to various conditions.

During the second part of the study, the degradation trends were obtained for the samples of retinol acetate in the presence and absence of WPI, when subjected to fluorescent light, pasteurisation, UHT treatment or storage at 5 or 40 °C. Samples exposed to fluorescent light at 4 °C showed exponential degradation of retinol acetate. Within 48 hours of light exposure, almost 60% of the retinol acetate had degraded regardless of the initial concentration. However, samples containing WPI retained slightly more retinol acetate at initial retinol acetate concentrations > 25 μg/ml.

The presence of WPI had a protective effect on retinol acetate during pasteurisation at 72 °C for 15 seconds. This protective effect appeared to be associated with the WPI concentration. However, there was no difference between samples with or without WPI after UHT treatment at 144 °C for 3-4 seconds, presumably due to the denaturation of the whey proteins.

The effect of WPI on retinol acetate during storage was minimal at 40 °C, where total degradation of retinol occurred within 48 hours of storage regardless of the initial retinol acetate concentration. In contrast, WPI showed a significant protective effect on retinol acetate at 5 °C, especially when the initial retinol acetate concentration was > 25mg/ml.

Overall, the presence of WPI and higher initial retinol acetate concentrations showed better stability than the control samples when exposed to light, stored at 5°C or during pasteurisation.

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CHAPTER 1

INTRODUCTION

If vitamin A is to be incorporated into a food or beverage then the vitamin must be sufficiently stable to ensure adequate potency in the product throughout the shelf-life. This is an issue due to the vitamin A losses that occur mainly through oxidation or geometric isomerisation. While oxidation results in total degradation of vitamin A, isomerisation reduces the vitamin A activity. The focus of this study was on stabilising vitamin A against the oxidation.

The main factors affecting vitamin A stability are light, storage and processing temperature. Ultraviolet (UV) rays can either result in photo-oxidation or photo-isomerisation. Thermal stresses triggered by elevated storage temperature and high processing temperature also result in the oxidation and isomerisation of vitamin A. Much research has focused on stabilising retinol in the presence of these conditions using physical barriers such as colour pigmented packaging, UV absorbers and wavelength transmitting filters (Sattar *et al.*, 1977; Fanelli *et al.*, 1985; Cladman *et al.*, 1998). Recently, physico-chemical approaches such as the use of emulsion systems, encapsulation and vitamin A carriers have also been reported (Zahar *et al.*, 1986; Halbout *et al.*, 1997; (Beaulieu *et al.*, 2002). It has also been suggested a carrier molecule that can bind to vitamin A and thus reduce its reactivity may be able to improve its stability.

Whey protein isolate (WPI) comprises β-Lg, α-La, BSA and immunoglobulins, with β-Lg representing approximately 50 % of the total protein. Preliminary studies on WPI have shown that retinol as well as retinol acetate bind to WPI in an aqueous environment (Schofield, 1999).

Vitamin A has been shown to bind to certain whey proteins. Retinol is known to bind to the most abundant whey protein, β-lactoglobulin (β-Lg) as well as to α -

lactalbumin (α-La) and bovine serum albumin (BSA). Bovine β-Lg consists of eight anti-parallel β-sheets that form a calyx, which is flanked on one side by an α-helix, forming a hydrophobic pocket (Dufour *et al.*, 1991). It has been shown that retinol binds in this hydrophobic central calyx (Kontopidis *et al.*, 2002). In addition, retinol is also known to bind at an external binding site that is located on the surface of the β-Lg molecule (Monaco *et al.*, 1987). Futterman *et al.* (1972) has reported retinol binds to BSA and forms water-soluble complexes. Therefore, whey proteins appear to have the potential to be used as carrier molecules to stabilise vitamin A in an aqueous environment. Most importantly, the potential use of β-Lg as a carrier molecule for lipophilic fragments, such as retinol, has been patented (Swaisgood *et al.*, 1998).

Vitamin A naturally present in foods is known to be associated with the lipid components. There are many fat-free or low fat food products currently available, which are usually also low in fat-soluble vitamins. Although many other vitamin A carrier emulsion systems have been reported in the literature (Zahar *et al.*, 1986), they contain fat components (such as butter, coconut and peanut oil) and thus affect the total fat composition of the product. The advantages in the use of WPI to stabilise vitamin A in low-fat food products include its ability to solubilise the vitamin without the addition of fat. However, if vitamin A is to be incorporated into a food or beverage product using WPI, it must be ascertained that the added vitamin A remains above a minimum level during the shelf –life of the product.

The purpose of this study was to gain a better understanding of any possible protective effect of WPI on vitamin A under a range of conditions. This involved finding appropriate methodologies for extracting and quantifying the added retinol acetate and the studying of degradation trends of the added retinol in the presence of the light, storage and processing temperatures.