Innovative Food Science and Emerging Technologies 47 (2018) 161–169

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Changes in the shelf life stability of riboflavin, vitamin C and antioxidant properties of milk after (ultra) high pressure homogenization: Direct and indirect effects

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ARTICLE INFO

Keywords:
(Ultra) high pressure homogenization
Milk
Riboflavin
Vitamin C
Antioxidative capacity

ABSTRACT

Changes in the stability during shelf life of riboflavin, vitamin C and the antioxidant capacity of (ultra) high pressure homogenized ((U)HPH) milk were explored. With increasing pressures, a decrease of up to 50% in the degradation rate of riboflavin was observed, likely due to an indirect effect induced by (U)HPH leading to increased scattering and absorbance of the wavelengths related to riboflavin's photosensitized oxidation. Such mechanism was also confirmed in a model system. Vitamin C presented minimal decrease in concentration immediately after (U)HPH treatment, yet it quickly degraded during shelf life. The antioxidant capacity of (U)HPH treated milk measured by ORAC showed a higher value when compared to pasteurized milk, yet no effect was observed by the ABTS method. The observed changes during shelf life, that can be related to direct and indirect processing effects suggest that (U)HPH can assist in possible improvement of milk nutritional quality.

Industrial relevance: Milk is a highly consumed product worldwide due to its high nutrient content. Novel innovative methods are constantly researched to improve the nutritional qualities of foods including milk. High or Ultra high pressure homogenization ((U)HPH, depending on the maximal pressure) is a novel technology that can possibly provide microbial inactivation in addition to numerous physicochemical changes in the product. The aim of this project was to evaluate the direct and indirect effect of (ultra) high homogenization pressures on nutritional qualities of milk exposed to fluorescent light during shelf life, conditions encountered in retail stores. A better fundamental understanding of the outcomes of novel processing technologies such as (U)HPH on quality parameters such as vitamin content and antioxidant properties can promote further development and acceptance of those technologies by the industry and by the consumers.

1. Introduction

Milk is a highly consumed product worldwide due to its nutrient content containing valuable macro and micro-nutrients such as carbohydrates, proteins, fat, minerals and some vitamins. While milk is not a major source of the recommended daily intake (RDI) of vitamin C it is an important source of the B group vitamins and especially for B_{12} and B_2 (riboflavin) (Claeys et al., 2013). Deficiencies of riboflavin in the general population are associated with several symptoms of anemia, cataracts, and developmental abnormalities (Powers, 2003). In recent years an increasing, yet controversial (Ndhlala, Moyo, & Van Staden, 2010), interest emerges in the anti-oxidant capacity of food products including milk, leading to expanding research in this field (Gülçin, 2012). Foods containing anti-oxidative compounds were suggested to support the prevention of illnesses such as cancer, atherosclerosis and many others (Virtanen, Pihlanto, Akkanen, & Korhonen, 2007). Beyond the presence of the valuable macro and micro nutrients, milk also contains different groups of antioxidant factors such as naturally occurring vitamins (E and C), β-carotene and enzymatic systems, leading to a complex set of possible pro- and anti-oxidative reactions (Calligaris, Manzocco, Anese, & Nicoli, 2004; Lindmark-Månsson & Åkesson, 2000; Pihlanto, 2006; Toyosaki & Mineshita, 1988; Zulueta, Esteve, Frasquet, & Frígola, 2007).

The pH and water activity of milk make it an ideal medium for development of microbial contaminations, therefore thermal treatment is usually being used to insure microbial safety (Rysstad & Kolstad, 2006), and novel methods for ensuring microbial safety are constantly researched. B group vitamins were shown to decrease by 10–20% due to heat treatment (pasteurization and Ultra High Temperature processing (UHT)) and a continuous decrease in their concentration was observed during packed storage. Vitamin C was also shown to be significantly degraded during pasteurization (Michalski & Januel, 2006),

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https://doi.org/10.1016/j.ifset.2018.02.014
Received 21 November 2017; Received in revised form 21 January 2018; Accepted 15 February 2018
Available online 17 February 2018

some studies reported degradation during storage of heat treated milk, while others suggested that no notable loss can be detected (Lindmark-Månsson & Åkesson, 2000). Light exposure and high oxygen permeability negatively affected vitamin C content during shelf life of UHT milk (Chotyakul et al., 2014). While riboflavin is relatively heat stable it is very sensitive to light (Michalski & Januel, 2006), therefore packaging is an important factor in minimizing the oxidation of riboflavin, with pigmented high density polyethylene (HDPE) and pigmented polyethylene terephthalate (PET) bottles presented various levels of riboflavin degradation during storage under commercial conditions (Zygoura et al., 2004). In addition to packaging, the effects of other parameters on riboflavin degradation in milk such as milk fat and solids content and light exposure intensity were studied (Gaylord, Warthesen, & Smith, 1986). When exposed to light riboflavin can induce both Type I and Type II photosensitized oxidation that might result in the formation of free radicals (Jung, Kim, & Kim, 1995), thus negatively affecting antioxidant balance. Therefore, it is clear that minimization of the photo-oxidation of riboflavin is important both to conserve its biological activity as a vitamin and to prevent the pro-oxidative chain reaction triggered by its photo-oxidation. While some information exists regarding the effects of heat on the antioxidant capacity of milk immediately after processing, in-depth information regarding changes occurring during shelf life of pasteurized milk is scarce, yet it was suggested that the anti-oxidant capacity of milk diminishes during storage, possibly due to the oxidation of riboflavin and vitamin C (Smet et al., 2008). Similarly, unpasteurized human milk showed a clear decrease in the antioxidant activity during both cold and frozen storage (Hanna et al., 2004).

(Ultra) high pressure homogenization ((U)HPH), a method that includes a high pressure pump that pumps a product towards a valve, has already been suggested as a possible novel milk processing technique-combining advantages of homogenization and pasteurization in one single process and reducing, at least partially, some negative aspects of thermal treatment. The pressure limit separating HPH (high pressure homogenization) from UHPH (ultra high pressure homogenization) is not clearly defined and in literature both terms can be found (pressures above 200 MPa more often named UHPH (Michalski & Januel, 2006), while for pressures between 100 and 200 MPa HPH is more commonly used). The efficiency of (U)HPH for the destruction of microorganisms is due to a combined effect of short time temperature increase and the mechanical forces created during the homogenization process, contributing to the microbial reduction (Dumay et al., 2013; Hayes & Kelly, 2003; Pereda et al., 2008). The required effect is obtained by homogenization at high pressures of up to 400 MPa, compared to currently used 20–50 MPa used in commercial homogenization processes of milk, needed to reduce creaming and increase emulsion stability. During (U)HPH temperature increase ranging from 16.6 to 19.5 °C per 100 MPa was reported (Zamora & Guamis, 2014), with a recent review suggesting that pressures of about 200 MPa and higher (Tin = 24 °C) result in similar microbial stability to the one obtained by pasteurization (Dumay et al., 2013). Slightly lower efficacy of microbial inactivation was reported in a different study, also showing a difference in microbial inactivation during shelf life between pasteurized and UHPH treated samples (Pereda, Ferragut, Quevedo, Guamis, & Trujillo, 2007).

It was previously suggested that a deeper understanding of the complex interplay of pro- and antioxidants in milk may lead to an optimized milk handling and processing, possibly resulting in better health promotion of large populations (Lindmark-Månsson & Åkesson, 2000). Immediately after processing, UHPH was shown to preserve vitamin C better than thermal treatments in milk (Amador-Espejo, Gallardo-Chacon, Nykänen, Juan, & Trujillo, 2015) and in fruit juices (Zamora & Guamis, 2014). UHPH was also shown to result in a lower riboflavin degradation compared to pasteurization and UHT in bovine milk (Amador-Espejo et al., 2015), and in no degrading effect (compered to untreated) in almond milk (Briviba, Gräf, Walz, Guamis, & Butz, 2016). Yet no information exists regarding riboflavin and vitamin C stability during shelf life after the (U)HPH treatment of milk. One of the major consequences of UHPH on whole milk is significantly smaller milk fat globules, compared to regularly homogenized milk (d_{4-3} 3.81 for 0.1 MPa compared to 0.189 for 300 MPa) (Picart et al., 2006). Reduced degradation of riboflavin during shelf life due to regular homogenization (compared to non-homogenized) was reported before and explained by an increased scattering of light in the wavelengths most detrimental to the stability of the vitamin (420–550 nm) (Hettiarachchy & Ziegler, 1994; Saidi & Warthesen, 1995). Yet, the optimal light scattering was suggested to occur when particle sizes are about a quarter of the wavelength incident of light (Saidi & Warthesen, 1995). The goal of the current study was to test the hypothesis that by manipulation of the particle size by (U)HPH, we can better control riboflavin degradation (an indirect effect of (U)HPH) during storage exposed to light (as can occur in retail stores). In addition, we hypothesized that when comparing to regular pasteurization, due to a low thermal effect of this method vitamin C will be conserved (a direct effect) and the combined effects will result in a better preservation of antioxidant capacity (compared to regular homogenization and pasteurization).

2. Materials and methods

2.1. Materials

Fresh raw bovine milk (~3.5% fat) was generously provided by local Israeli farms and stored at 4 °C in dark conditions prior processing (after less than 24 h). Riboflavin > 99.8% was purchased from Fluka analytical, Ascorbic acid 99% (#A15613) was purchased from Alfa Aesar (UK). Methanol absolute 99.8%, dichloromethane 99.9% and sodium chloride were purchased from Bio-Lab Ltd. (Israel). DL-Dithiothreitol 99%, Trolox, meta-Phosphoric acid, BioXtra, > 33.5%, Fluorescein sodium salt, AAPH, were purchased from Sigma Aldrich (USA).

2.2. Methods

2.2.1. Ultra high pressure homogenization and pasteurization

Prior to homogenization, raw milk was preheated to room temperature and pre-homogenized using a Omni general laboratory homogenizer (OMNI International Inc., Waterbury, CT, USA) operated for 2 min at ~10,000 RPM.

(U)HPH treatment of raw milk was preformed using a Stansted high pressure homogenizer (model FPG 12800, Stansted Fluid Power Ltd., Essex, UK). The homogenizer design includes one Y-shape HP-valve, one piston, 9 ml cell, external cooling jacket and external heat exchanger to avoid a long-term high outlet temperature. Homogenization pressure range of 40–250 MPa was used at T_{in} = 25 °C in one cycle, unless mentioned otherwise. The HP valve and the milk outlet stream was cooled using circulating water at 4 °C. For the pasteurization process, milk samples homogenized at 40 MPa were heated for 15 s at 72 °C and cooled immediately. The thermal pasteurization was performed by forming a thin layer (< 3 mm, 20 ml of milk in a plastic bag of 15 × 10 cm) immersed in a water bath. In such conditions the time required to reach the pasteurization temperature was found to be < 1 s.

2.2.2. β-lactoglobulin and oil model

Mixture of 84% ammonium acetate buffer (pH = 6.7, 20 mM), 15% soy oil and 1% β-lactoglobulin (w/w) was prepared. Riboflavin [1.5 μg/g] was added to the solution and homogenized into a pre-emulsion using a hand blender (PRO 200, BIOGEN series pro scientific, Oxford CT, USA) operated for 1 min at ~35,000 RPM. The solution was homogenized using a high pressure homogenizer (Emulsiflex, Avestin Inc., Canada) at different pressure of 35–170 MPa (3–5 cycles) at initial temperature of 25 °C, aiming to reach particle distributions similar to ones obtained for the (U)HPH treated whole milk. The cooling step was