Food Research International 106 (2018) 487–494

Contents lists available at ScienceDirect

Food Research International

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

High pressure homogenization combined with pH shift treatment: A process to produce physically and oxidatively stable hemp milk

Qingling Wang[a], Jiang Jiang[a], Youling L. Xiong[a,b,*]

[a] State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, China
[b] Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40546, United States

ARTICLE INFO

Chemical compounds studied in this article:
Nile red (PubChem CID: 65182)
Fluorescein isothiocyanate (PubChem CID: 18730)
Acetic acid (PubChem CID: 176)
Trichloromethane (PubChem CID: 6212)
2-Thiobarbituric acid (PubChem CID: 2723628)
Trichloroacetic acid (PubChem CID: 6421)

Keywords:
Hemp milk
pH shift
Particle size
Viscosity
Emulsion stability
Oxidation stability

ABSTRACT

Hemp milk, an emerging beverage with high nutritional value and low allergenicity, is an attractive alternative to dairy, soy, and nut milks. To obtain a non-thermally processed, physically and oxidatively stable hemp milk, high pressure homogenization (HPH) combined with pH shift treatment was investigated. For hemp milk (4% protein, 5% fat) without pH shift, increasing the homogenization pressure (up to 60 MPa) resulted in a more uniform distribution of emulsion droplets (2.2–2.7 μm). When pH shift was applied prior to HPH, large clusters and aggregates of oil droplets (3.5–8.2 μm) were formed. Interestingly, hemp milk with such interactive structures was remarkably stable, showing negligible phase separation within 3-day storage at 4 °C. Moreover, hemp milk made by combined pH shift and HPH exhibited delayed hydroperoxides (expressed as peroxide value, PV) and malondialdehyde (expressed as thiobarbituric acid-reactive substances, TBARS) production, suggesting the resistance of such emulsion cluster structures to radicals. On the other hand, a significant reduction of microbial population was observed in hemp milk prepared by pH shift combined with HPH. The results indicate that the pH shift + HPH combination treatment may potentially be employed for the production of non-thermally processed hemp milk.

1. Introduction

The consumption of hemp products has increased in recent years (House, Neufeld, & Leson, 2010) due to the recognized nutritive value and low allergenicity of hemp seeds. Hemp seeds contain about 30% oil and 25% protein (Callaway, 2004). The oil is rich in polyunsaturated fatty acids (PUFAs), especially linoleic (ω-6) and α-linolenic (ω-3) acids with a desirable ratio between 2:1 and 3:1 for optimal health (Bartkiene et al., 2016; Da Porto, Decorti, & Natolino, 2015). The protein fractions (65% edestin and 35% albumin) have a high digestibility and a well-balanced amino acid composition (Girgih, Udenigwe, & Aluko, 2010). Arginine is particularly abundant in hemp protein (Bartkiene et al., 2016).

Hemp milk has been produced from whole hemp seeds to deliver the nutritional benefits. Hemp milk contains most of the original nutrients and is considered highly nutritious. Studies have shown that the consumption of hemp milk could lead to the reduction of serum triacylglycerols, cholesterol, and thyroid hormones (Chichłowska, Kliber, Kozłowska, Biskupski, & Grygorowicz, 2009). Besides, being lactose-free and low in allergenicity, hemp milk is considered as an attractive alternative to dairy, soy, and nut milks.

A typical hemp milk is processed by the homogenization of ground seeds in water (1:5 w/v), and the filtered milk containing approximately 4% protein and 5% fat is usually heated for shelf-life and safety. However, thermal treatments not only damage heat-sensitive nutrients, particularly ω-3 fatty acids and vitamins, but also reduce the product freshness. Nevertheless, similar to the non-thermal production of many emerging vegetable-based beverages, such as 'cold-pressured-without-heat-pasteurized' juices (cold-pasteurization technique to kill potentially harmful microorganisms), it is possible to prepare hemp milk without heating treatment to preserve its nutrients and original clean flavor.

As with other milk alternatives made from plant seeds (soybean, almond, etc.), hemp milk as an oil-in-water (O/W) emulsion system is unstable and has a tendency to flocculate, coalesce, and cream (unpublished result). This will cause a loss of quality and shorten the shelf-life, limiting its acceptance by the consumer. To obtain a stable product, exogenous emulsifiers or stabilizers are commonly added to improve the kinetic stability of emulsions. However, the use of additives will not only increase the production cost but also lead to health concerns. For

* Corresponding author.
E-mail address: ylxiong@uky.edu (Y.L. Xiong).

https://doi.org/10.1016/j.foodres.2018.01.021
Received 30 September 2017; Received in revised form 6 January 2018; Accepted 11 January 2018
Available online 12 January 2018
0963-9969/

example, some studies have shown that long-term consumptions of synthetic emulsifying agents could induce chronic inflammatory diseases, obesity-associated diseases, and metabolic disorders (Cani & Everard, 2015; Chassaing et al., 2015). Therefore, the demand for cost-effective alternative technologies and additive-free food products is on a constant rise.

Hemp protein is known for its low emulsifying capacity (Yin, Tang, Wen, & Yang, 2009) due to its compact structure and poor water solubility. There have been attempts to modify the native structure of hemp protein for improving the emulsifying capacity, for example, enzymatic hydrolysis (Yin et al., 2008) and acylation (Yin et al., 2009). An alternative method to modify the structure of proteins is pH shift, namely, to expose native protein to an extreme pH condition that induces partial unfolding followed by incubation in a neutral pH environment to allow partial refolding. Such a process disrupts the tertiary structure and generates a molten globule conformation that has a markedly enhanced amphiphilicity. Hence, pH shift has been successfully applied to the treatments of legume proteins, including soy protein (Jiang, Chen, & Xiong, 2009; Matsudomi, Sasaki, Kato, & Kobayashi, 1985) and pea protein (Jiang, Zhu, Liu, & Xiong, 2014) for improved emulsifying properties.

High pressure homogenization (HPH) has also been introduced in recent years to aid in the preparation of stable O/W food emulsions (Fernández-Ávila, Escriu, & Trujillo, 2015). HPH mechanically disperses oil into fine droplets resulting in an increased total surface area and a uniform size distribution with an improved stability (Briviba, Graf, Walz, Guamis, & Butz, 2016; Lee, Lefèvre, Subirade, & Paquin, 2009). Moreover, low-level HPH (up to 50 MPa) can effect distress on microorganisms, therefore, is valuable for cold pasteurization (Patrignani & Lanciotti, 2016). Jiang et al. (2014) tested the efficacy of combined pH shift and HPH to prepare pea protein–sunflower oil emulsions. The emulsion produced with pH_{12}-treated pea protein under a 70 MPa high pressure exhibited an optimum physical and oxidative stability.

The objective of the present study was to apply pH shift and HPH process to produce additive-free hemp milk with a focus on its physical and oxidative stability. Emulsifiers and heat treatments were avoided in an attempt to maintain the original flavor and palatability of the finished product.

2. Materials and methods

2.1. Materials

Raw hemp seeds were purchased from Yunnan Industrial Hemp Co., Ltd. (Yunnan, China). All chemicals were purchased from Sigma Chemicals (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA) and were minimally reagent grade.

2.2. Hemp milk preparation

Hemp milk was freshly prepared in the lab. To ensure minimal microbial contamination, all containers were thoroughly cleaned. Hemp seeds were thoroughly washed (3 times), ground in 5 volumes of deionized water with a DS-1 blender (Shanghai Jingke Industrial Co. Ltd., Shanghai, China) at 12000 rpm for 3 min, then recirculated in a colloid mill (JMS, Kunjieyucheng Machinery Co. Ltd., Beijing, China) twice to ensure thorough pulverization. The mixture was filtered through eight layers of cheese cloth to remove particles. The crude milk (approximately 4% protein and 5% fat) was subsequently homogenized in a two-stage homogenizer (AH-BASIC, ATS Engineering Inc., Shanghai, China) at 30 or 60 MPa for two passes. For pH shift treatment, the milk was adjusted to pH 12 with 2 N NaOH and held at room temperature for 1 h, then neutralized to pH 7.0 with 1 N HCl and held for 1 h (Jiang et al., 2009) before or after homogenization. The crude hemp milks prepared by blending without the HPH and pH shift treatment (defined as 0 MPa), and with pH shift only, was used for comparison. Because HPH treatment (after two passes) caused a temperature rise of the hemp milk from 22.3 °C to 31.4 °C at 30 MPa and to 38.8 °C at 60 MPa, all prepared samples were quickly chilled in an ice slurry before analysis. Salt concentration of all hemp milk samples was determined by using a HI 98360 EC/TDS/NaCl Meter (Hanna Instruments, Woonsocket, RI, USA).

2.3. Microstructure

Hemp milk was subjected to light microscopy and confocal laser scanning microscopy (CLSM) to observe the oil droplet morphology. Light microscopic images were taken at 400× magnification using a ZEISS Axiostar Plus Light Microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany) and processed using Infinity Capture Application v4.5 software (Lumenera Corporation, Ontario, Canada). For CLSM, a Leica TCS SP8 microscope (Leica Microsystems Inc., Heidelberg, Germany) with a 40× objective lens was applied to more intuitively visualize the microstructure of the emulsions. Fluorescein isothiocyanate dye (FITC) and Nile Red, both at the concentration of 0.1% (w/v), were used to stain protein (colored green) and oil (colored red), respectively. Excitation wavelengths of FITC and Nile Red were set at 552 nm and 488 nm, respectively.

2.4. Droplet size

All light scattering experiments were performed at 25 °C using Brookhaven NanoBrook Omni Particle Size Analyzer (Brookhaven Instruments Corp., Holtsville, NY, USA) with a detection angle of 90° (standard) and 173° (backscatter) to measure protein hydrodynamic radius. Hemp milk samples were diluted approximately 200 times with 50 mM phosphate buffer (pH 7.0) before analysis. The effective (hydrodynamic) diameter of emulsion droplets was calculated according to the Stokes-Einstein equation, and the mean diameter was the average of effective diameters (sum divided by number of runs). The intensity distribution is naturally weighed according to the scattering intensity of particles in different sizes.

2.5. Viscosity

Steady shear viscosity of hemp milk was characterized using a Discovery Hybrid Rheometer-3 (DHR-3, TA Instruments, New Castle, DE, USA) with a cone-plate (diameter 40 mm, cone angle 4°) at 25 °C. The gap between plates was set at 102 μm. Apparent viscosity was tested with shear rates ranging from 0.1 to 100 s^{-1}. A time-waiting process of 60 s was applied before each test.

2.6. Physical stability

The physical stability of hemp milk was evaluated using a Turbiscan Lab™ machine (Formulaction, Toulouse, France), which measures the phase separation tendency of dispersions (Kaombe, Lenes, Toven, & Glomm, 2013). A hemp milk sample was transferred to a cylindrical glass cell up to the height of holder immediately after preparation and scanned by a pulsed near infrared (wavelength 880 nm) light source. Two synchronous detectors received light transmitted through the sample and light scattered backward by the sample, respectively. The sample was scanned every 3 min for 60 min at 30 °C. Data were analyzed by using TurbiSoft Ver 2.1.0.52. Turbiscan stability index (TSI), a parameter based on sample transmission (T) and backscattering (BS) intensity changes, was calculated to reflect the destabilization of hemp milk emulsion using the following formula:

$$\mathrm{TSI} = \sqrt{\frac{\sum_{i=1}^{n} (x_i - x_{BS})^2}{n-1}} \quad (1)$$