**Improvements in Emulsion Stability of Dairy Beverages Treated by High Pressure
Homogenization: A Pilot-scale Feasibility Study**

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Abstract

The impact of high pressure homogenization (HPH) on physical characteristics and emulsion stability of nutritional formulations were evaluated on a pilot scale HPH unit. Two products of industrial interest were used for these experiments. Manufactured formulations were analyzed using material characterization methods as particle size distribution, microscopy and dynamic mechanical spectra, compared to a control formula manufactured with conventional homogenization and heat treatments. Dynamic rheological measurements as strain and frequency sweeps exhibited increased product stability in parallel to increased homogenization pressures. Homogenization at elevated pressures yielded more shear thinning fluids evidenced by increased viscosities at low shear rates, ten fold greater compared to the control treatment, further evidence to increased physical stability. Experimental findings revealed the existence of a threshold pressure (100-150 MPa) for both formulations exhibiting significant correlation with product quality and physical stability. The study outcomes presents an opportunity for reducing stabilizer concentration in dairy beverages; in addition to shelf life and physical stability improvement for formulations treated at increased homogenization pressures.

Keywords: High pressure homogenization, pilot-scale equipment; dynamic mechanical spectra; particle size distribution; clean label; dairy beverages.

1. Introduction

Health-conscious consumers are looking for liquid beverages formulated with healthy ingredients to satisfy their daily nutritional needs. Such beverages are ideal transport system for many beneficial bioactive compounds as they can be dissolved, suspended, encapsulated or emulsified (Piorkowski and McClements, 2013). Most of the healthy ingredients are hydrophobic in nature and their incorporation into aqueous/stable colloidal dispersion is a challenging process.

Industrially processed beverages are formulated with a variety of processing ingredients/aids in order to provide desirable texture, flavor, and shelf-life (Wahlgren et al., 2015). During the manufacture of formulated beverages, numerous unit operations are involved including dissolving, mixing, emulsification, homogenization and thermal treatment. Emulsification is one of the key processing steps in which fat droplets are finely dispersed into an immiscible continuous phase (Walstra, 1993). Emulsion formation is aimed to prepare stable dispersions of hydrophobic compounds (oil, oil soluble vitamins, and bioactive compounds) that otherwise would separate from the aqueous medium (Piorkowski and McClements, 2014). Emulsification is a dynamic phenomenon that involves deformation and break-up of liquid fat droplets, forming smaller droplets of narrow size range. Concomitantly, the newly produced droplets are rapidly adsorbed by surfactant as proteins and emulsifiers, creating a colloidal dispersion. Such a fine dispersion is kinetically active due to colloidal and hydrodynamic interactions leading to inter-droplet collisions which may results in the formation of larger droplets. This is known as coalescence and it is avoided by the addition of water-soluble polymers, called stabilizers. Adsorption and coalescence occur within short time scales and the final droplet size range of the colloidal dispersion and its stability is governed by the input of

mechanical energy, type and concentration of stabilizer, and emulsifier to stabilizer ratio (Lee et al., 2013).

In general, making stable colloidal dispersions requires use of high concentration of surfactants and/or high input of mechanical energy. In the last decade, consumers oriented with health and wellness life style have perceived synthetic emulsifier agents (emulsifiers, stabilizers and thickeners) as undesirable ingredients. Recently, scientific evidence have related emulsifying agents commonly used in beverages with disruption of microbiota host relationship, promoting chronic inflammatory diseases (Cani, 2015; Cani and Everard, 2015; Chassaing et al., 2015). To address such perceptions and emerging scientific challenges, food processors are interested in identifying cost-effective alternative technological solutions that improve emulsification and potentially minimize the use of stabilizers.

Technological strategies leading to improve emulsion stability and microbial inactivation have become a topic of industrial interest. Approaches under current investigation include sonication (Zinoviadou et al., 2015), pulsed electric fields (Barba et al., 2015), and high pressure (Barba et al., 2012). In this study we evaluated the application of high pressure homogenization (100-400 MPa, HPH) on the emulsion stability (droplet break-up, adsorption and coalescence). During HPH, the fluid is forced to pass through a minute orifice or gap under the action of a constant force, which hydrostatically pressurizes the fluid. When the pressurized fluid exits the gap and enters an area of lower pressure, the kinetic energy of the fluid is dissipated into energy that is absorbed, redistributed and lost through heat (Balasubramaniam et al., 2015). Depending on the differential pressure, the fluid experiences great shear, cavitation and turbulence and such mechanical effects are put to work for mixing, dispersion, particle size reduction, and

emulsification. Systematic studies on the role of HPH during fat emulsification are scarce. Desrumaux and Marcand (2002) evaluated the effect of HPH on emulsion properties and reported significant changes in the structure of the oil in water emulsion at increased pressures. Auty et al. (2011) reported enhanced fat-protein interactions during yogurt manufacture with applied high pressures. Images of this study from confocal scanning laser microscopy showed a highly interconnected fat/protein network with nano-clusters. Such network was not observed in control samples. Donsì et al. (2011) has pointed out that HPH stabilized droplets by preventing coalescence after droplet disruption. Although encouraging experimental results have been reported in the literature, applying HPH for emulsification on a commercial scale presents many significant challenges. The objective of this work is to systematically evaluate the role of HPH on emulsion stability using pilot scale equipment. The emulsion stability was characterized in terms of particle size distribution, microstructure, and rheology.

2. Material and Methods

2.1. Beverage formulation

Two different formulations of industrial interest were used to assess the effect of HPH on emulsification during the manufacture of dairy-based beverages. The emulsion stability of the formulations is well documented, and thus help us to detect any changes induced by HPH. **Table 1** shows the composition of both formulations. First, blends of soy and milk proteins at approximately 90:10 and 75:25 ratios for formulation 1 and 2, respectively, were dissolved in deionized water for 30 min at 50-60°C under constant agitation. Secondly, slurries of different carbohydrate systems consisting of sucrose and maltodextrin for formulation 1 (60:40 sucrose to maltodextrin ratio), and sucrose, maltodextrin and sucromalt for formulation 2 (40:52:8 sucrose

to matodextrin to sucromalt ration), minerals (Ca, P, Na, K, F, Cu, Mg, Mn, and Zn) premixes added with stabilizers: a carrageenan, (Viscarin) in formulation 1, and cellulose gum (Avicel) in formulation 2 were prepared in deionized water for 15 min at 60-65°C. Then, the slurry was transferred into the main kettle where it was mixed with the protein blend. Finally, oil blends consisting of soy, canola and corn oils supplemented with vitamin A, D, E and K with combinations of carageenans (Viscarin and Seakem RLC) and cellulose gum (Avicel) were added and mixed for additional 10 min at 60°C. Each experimental run consisted of 60 L of formulated product. Each formulation was tested with and without added stabilizer. Total solids of the final formulation were 30% and adjusted to neutral pH. All ingredients were kindly donated by Abbott Nutrition (Columbus, OH).

2.2. *High pressure homogenization*

The experiments were conducted using a pilot scale high pressure homogenizer (FPGP11300, Stansted Fluid Power Ltd. Essex, UK) coupled with a custom fabricated fluid handling system (Brim Process Equipment, Columbus, OH) and glove box for semi-aseptic packaging (**Fig 1**). The pilot scale system consists of a feed tank, heat exchanger, and pressure intensifier, two homogenization valves for first and second homogenization stage, cooling system, and glove box packaging system. The whole system operated at flow rate of $90 \pm 3 \text{ L h}^{-1}$ and at homogenization pressure of up to 320 MPa. The fluid temperatures in different processing steps (feed tank, heat exchanger, compression, first stage, second stage homogenization valves, and cooling) were monitored during experiments using digital RTD gauge and K-type thermocouples (Omega Engineering Inc., Stamford, CT). To minimize heat loss, pipes, fitting and heat exchange surfaces within high pressure homogenizer were thermally insulated. The

flow rate was measured at the outlet of the HPH using a turbine flow meter (GPI A1, Instrumart Inc., South Burlington, VT). The homogenization pressure was measured using a flow transducer valve (Stansted Fluid Power Ltd, Essex, UK). The processed samples were manually filled in 250 mL beverage bottles (Nalgene Low Density Polyethylene, VWR, PA). All the processed samples were refrigerated until analysis, within a week.

2.3. *Experimental design*

Two sets of experiments were performed to evaluate the effect of HPH on emulsification of formulated beverages. The first set of experiments consisted on the evaluation of HPH conditions on emulsion stability of formulations 1 and 2. The second set of experiments, 3-week storage at 6°C, of emulsion shelf life and physical stability of Formulation 2 was studied.

2.4. *Analytical determination*

2.4.1. *Particle size determination*

Size distributions of formulated beverages was determined by laser light-scattering method in a Microplus diffractometer analyzer (Malvern, Worcestershire, UK), equipped with a He-Ne (633 nm) light source and a convergent beam arrangement. An obscuration value of 30% was used and samples outside this range were diluted accordingly. For each sample, size distribution was obtained in percentage of volume as function of droplet diameter in the range of 0.05-600 μm . The size distribution histogram was analyzed with polydisperse analysis to calculate mean droplet diameter and percentiles.

The mean particle size (d_{50}) was used to characterize the maximum droplet size as function of the energy density (E_v), according to Equations (1) and (2):

$$\Delta p [Pa] = E_v [J m^3] \quad (1)$$

$$d_{50} = a_1 \cdot E_v^{-b_1} \quad (2)$$

Where Δp is the pressure drop, a_1 and b_1 are regression coefficients obtained through non-linear regression analysis performed using the Solver option in Excel Microsoft.

2.4.2. *Dynamic rheological measurements*

The dynamic rheological characterization of formulated beverages was performed in an ARES LS1 rotational rheometer with stainless steel couette with 32 mm bob and 34 mm cup geometry (TA instruments, NEW Castle, DE). Samples were tested at 25°C. Three types of tests were performed to characterize the rheological behavior of formulated beverages: dynamic modula as a function of strain and frequency, and equilibrium shear viscosity at shear rates of 13 and 0.5 s⁻¹.

2.4.2.1. *Strain sweep*

Dynamic modulus is recorded as a function of strain. The linear range in this data corresponds to elastic modulus wherein change as a function of strain is insignificant. The line fit is performed to identify the plateau elastic modulus. A strain out of the linear range is selected to conduct the frequency measurement. The elastic modula responses and the linear ranges in these measurements are used to compare emulsion shelf life and physical stability of the products.

2.4.2.2. *Frequency sweep*

A strain out of the linear ranges in strain sweep measurements is selected and used to conduct the frequency sweep measurements. The elastic and viscous moduli obtained in frequency sweep measurements represent equilibrium conditions of these products; hence, also used to evaluate the emulsion shelf life and physical stability, emulsion and suspension, of these products.

2.4.2.3. *Step rate*

Shear viscosities of products were measured at 0.5 and 13 s⁻¹ after products were calibrated for shear history. Products were sheared at 100 s⁻¹ for 120 s to break all the structures formed, and then following the products were relaxed for 200 s prior to shear viscosity measurements. Shear viscosity values are averages of 120 s at these shear rates. Measured viscosities are used to understand the impact of processing on shear thinning flow behavior; hence, product stability.

2.4.3. *Microstructure*

The microstructure of treated and control liquid samples was evaluated using confocal scanning laser microscopy. Dual labelling of liquid foods was used to visualize fats and proteins (Ciron et al., 2010). A drop of sample was transferred to a glass slide and stained with 100 µL of Nile Red-Fast Green mixture (3:1). Images were taken using plan different magnification with a Zeiss LSM510 META laser scanning confocal microscope (Carl Zeiss, Jena, Germany) in The Ohio State Campus Microscopy and Imaging Facility.

2.4.4. *Quality parameters*

2.4.4.1. Color and pH

Color in beverage samples was measured using a colorimeter (CR210, Minolta, Osaka, Japan). The colorimeter was calibrated using a white standard plate ($L^* = 97.83$, $a^* = 0.43$, $b^* = 1.98$). Hunter-lab L^* , a^* and b^* values are represented as the indicators of lightness, redness and yellowness, respectively. The pH of formulated beverages was measured using a pH meter (Accumet XL 15, Fisher Scientific, Mississauga, ON, Canada).

3. Results and discussion

3.1. Temperature increase

Fig 2a shows the temperature changes in the test beverage during a typical HPH treatment. The treatment starts with formulation and mixing of different ingredients in the feeding tank, where the temperature used was between 55-65°C (T_u), depending on the formulation. The formulated beverage was heated at 90°C (T_{in}) using a plate heat exchanger before entering the high pressure intensifier, where the temperature (T_{comp}) increased due to heat of compression. Subsequently the fluid was depressurized via two-stage homogenization valves. At the end of homogenization, the temperature of the beverage increased ~18°C per 100 MPa (T_p) due to dissipation of kinetic energy upon discharging pressure. The length of the pipe that connects the distance between first and the second homogenization stage was considered as the holding tube and it provides a residence time of less than 1 s. Thus, under such test conditions, the product temperature did not change between first and second homogenization stage. After second homogenization stage, the fluid is cooled to a refrigeration temperature (T_c). Residence times of 0.24, 0.48 and 0.7 s have been reported in literature for pilot scale HPH units (Pereda et al., 2007; Georget et al., 2014; Peng et al., 2015; Toro-Funes et al., 2015). It is worth to mention

that the demonstration of commercial sterility was not a goal of the current study. However, similar to what is practiced in aseptic processing industry, by adjusting the high pressure homogenization process conditions (initial and process temperature of the beverage, target homogenization pressure), and/or having a hold tube section of adequate length, it may be possible to produce beverages with commercial sterility.

Fig 2b exemplifies the increase in temperature during HPH (~20°C per 100 MPa). The temperature increases via adiabatic heating through hydrostatic compression and dissipation of energy of a compressed fluid through pressure discharge (Balasubramaniam et al., 2015). Interestingly, the increase in product temperature due to dissipation of kinetic energy did not differ from the increase in temperature observed for di-ionized water. This observation suggests that the design of the homogenization valve, geometry and configuration, plays a major role on how the energy is dissipated and therefore the resulting increase in temperature. A detailed description of temperature increase during HPH is of a great relevance to facilitate comparison and interpretation of experimental findings.

3.2. Particle size distribution

Fig 3 illustrates the effect of HPH conditions on the particle size distribution of formulation 1. For the control sample with added stabilizer, two peaks of similar broadness were observed that spanned from <10 to 1 µm and from <1 to 0.1 µm, respectively. Both peaks showed a maximum size at ~6 % of volume distribution (2.88 µm and 0.31 µm, respectively). In the case of HPH treated beverages with added stabilizer (**Fig 3a**), the curves showed a consistent and systematic shift in the bimodal distribution of particles towards large particles with increased

homogenization pressures. In addition, higher pressures (> 200 MPa) yielded products with larger particle sizes (between 10 to 100 μm). The same was true for pressures lower than 100 MPa. This generalization was observed within pressure levels up to 160 MPa. **Fig 3b** shows the particle size distribution of formulation 1 without stabilizer. For the control sample without added stabilizer, a single and large peak was observed having a broad tail that spanned from 10 to 0.1 μm . The particle size distribution for pressures up to 200 MPa vary between 0.1 to >100 μm with two to three major peaks. The distribution of particles at these pressures also shows primary peak's distribution <1 μm .

The effect of HPH on particle size distribution of formulation 2 is shown in **Fig 3c** and **3d**. For formulation 2 with added stabilizer, a bimodal distribution was observed when homogenization pressure ranged from 40 to 102 MPa (**Fig 3c**). The application of homogenization pressures higher than 100 MPa yielded a broader distribution of large particle size. Similarly, samples without stabilizer (**Fig 3d**) showed a bimodal distribution within pressure levels of 50 to 108 MPa while the application of homogenization pressure higher than 108 MPa resulted in a broad distribution of large particles. Similar observations were reported by Desrumaux and Marcand (2002) who evaluated the effect of increasing homogenization pressure from 50 to 350 MPa on the Sauter diameter in oil-in-water emulsion. These authors found that the Sauter diameter decreased exponentially with pressure from 20 to 100 MPa while the application of pressure levels from 100 to 300 MPa increased the Sauter diameter.

A broader distribution of particles may be due to coalescence of newly formed droplets. In order to evaluate the effect of pressure on particle droplet, the mean particle size (d_{50}) was used as an indicator for the maximum droplet size. **Fig 4a** shows the mean particle size as function of homogenization pressure for formulation 1. In general, the mean particle size

decreases with increasing pressure (103-160 MPa) reaching minimum values of 1.8-2 and 0.06-0.12 μm for samples with and without stabilizer, respectively. The application of homogenization pressure levels from 160 to 288 MPa gradually increases the mean particle size up to 7.5 μm for both samples. Similar trend was observed for formulation 2 (**Fig 4b**) where the mean particle size decreases with pressure (from 40 to 102 MPa) reaching a minimum value of 1.4-2.1 and 2.0-2.6 μm for samples with and without stabilizer, respectively. Increasing the homogenization pressure higher than 102 MPa yielded larger mean particle size for both samples. The effects of processing conditions (90-150°C and 40-300 MPa) on protein stability cannot be ignored. Marco-Molés et al. (2012) showed protein insolubility in those samples treated at 250 MPa, causing coalescence and subsequent separation.

A close examination of the d_{50} versus pressure curves obtained for both formulations reveals the existence of a threshold pressure. Below such pressure value, the reduction of d_{50} follows the Kolmogorov's theory and it can be represented by Equation (2). Contrary, pressures above the threshold value increases the d_{50} . Similar observation was previously reported by Marco-Molés et al. (2012) who identified two distinctive effects of pressure on the stability of oil-in-water emulsions. These authors found stable emulsions within the pressure range of 70 to 150 MPa, while destabilization of the emulsions was observed when increasing the pressure up to 250 MPa. The occurrence of coalescence has been thought to be dependent on collision frequency and probability of collision, which are function of physical properties of the emulsion and operating conditions, energy density and flow conditions (Tesch and Schubert, 2002; Jafari et al., 2008). High input energy during emulsification will increase the collision frequency and therefore the probability of coalescence. Flourey et al. (2004) performed a numerical simulation of the velocity profiles for water within a HPH unit operated at constant flow rate of 10 L h⁻¹ and

maximum pressure of 350 MPa. These authors predicted that the fluid velocity dramatically increases with pressure, reaching values up to 200 m s^{-1} at the operating pressures of $>300 \text{ MPa}$. After developing a parabolic velocity profile, the velocity of the fluid rapidly decreases over a short distance with velocities of 0.6 m s^{-1} . It seems reasonable to assume that such circumstances (increasing particle velocity and decreasing over short distance) favor the occurrence of coalescence over adsorption.

It is well known that the droplet size is strongly influenced by the flow conditions, namely laminar, transitional and turbulent regime (Finke et al., 2014; Kelemen et al., 2014; Schlender et al., 2015). In this study, the calculated Reynolds number at the homogenization gap reveals a turbulent regime within the experimental conditions ($2000 \leq \text{Re} \leq 5000$). Different forms of equation (2) have been used to describe the droplet size within turbulent regime (Finke et al., 2014). **Table 2** shows the coefficients calculated for both formulations within the pressure range of 0.1-160 MPa and 0.1-102 MPa for formulation 1 and 2, respectively. The values of parameter a are rather large and its value depends on specific set of experimental conditions and the formulation itself (Schultz et al., 2004). On the other hand, the values of parameter b were 0.24 and 0.39 for formulation 1 and 0.42 and 0.44 for formulation 2 (with and without stabilizer, respectively). Similar values of parameter b (0.3-0.5) has been reported radial diffuser annular valves and axial-flown through orifice valves (Schultz et al., 2004). Parameter b has been used as an indicator for turbulent fragmentation (Hakansson, 2015) since it combines the Kolmogorov's theory with dissipation rate of turbulent energy. The prediction of mean particle size at pressure levels below the threshold value was fairly well described by a power law fashion according to Kolmogorov's theory. At high pressure levels, velocity gradients of large magnitude and protein stability may shift the balance of adsorption and coalescence.

3.3. Strain sweep measurements

Fig 5 shows storage module (G') as function of strain amplitude for formulation 1. For those samples with added stabilizer (**Fig 5a**), two different regions were observed linear viscoelastic region where G' was constant and nonlinear region in which G' started to decrease with increasing strain. Within the linear viscoelastic region, the G' values were independent of the applied strain meaning that the samples behaved like a viscoelastic solid (**Fig 5a**). The length of the linear section is closely related to product stability since it is thought to account for the formation of physical entanglements between proteins and carbohydrates at the oil/water interface providing structure to the liquid (Franco et al., 1995). Increasing the homogenization pressure resulted in higher values of G' when compared with the control sample, meaning that HPH increased the strength of the network. This was evidenced both by the increased magnitude of G' plus extended linear range indicated by the critical strain which is determined as 85% of G' at the linear range. The highest strength of the network judging by the magnitude of G' values was obtained at 45 ± 3 MPa/92°C and the application of higher homogenization pressures (78 to 286 MPa) did not increase the strength of the network (**Fig 5a**). The yielding point or critical strain is associated with the breakdown of the secondary network of particles and it marks the transition from viscoelastic solid to viscoelastic liquid (Hesarinejad et al., 2014). In the case of samples without added stabilizer, the G' values as function of strain amplitude were too low and rather scattered regardless of the HPH conditions. Magnitude of the G' for products without stabilizer was one logarithmic scale lower. The linear ranges were not as easily visible due to the detection limits of the instrument; although, some exhibited better linear ranges.

Strain sweep graphs for formulation 2 are given in **Fig 5b**. The magnitude of the G' for the HPH treated samples with added stabilizer was larger and exhibited longer linear ranges.

Increasing the homogenization pressure not only extended the length of the viscoelastic region but also yielded higher values of G' compared with the control sample (1.94 and 0.11 Pa, respectively). For formulation 2 without added stabilizer, the strain sweeps for HPH treated revealed a relatively short viscoelastic region for all samples spanned from 1.3 to 46.3 % within a relatively low G' values, ranging from 0.15 to 0.22 Pa.

Elastic module measured as a function of strain (**Fig 5**) indicated increased product stability with pressure of 50 MPa for both formulations 1 and 2 with added stabilizers. No further increased product stability was observed at elevated pressures; however, further experiments would be needed to confirm the exact pressures between 50 and 100 MPa as 100 MPa is the next pressure tested. HPH changed fluid characteristics significantly, showing characteristics of strong gel in samples with added stabilizer.

3.4.Frequency sweep measurements

The information obtained from strain sweep analysis was used to define the limits of the viscoelastic region and determine the mechanical spectra for each sample. Frequency sweep plots are commonly used to characterize the mechanical spectra of dispersions (Steffe, 1996). **Fig 6** shows the storage module (G') and loss module (G'') as a function of frequency for formulation 1 with added stabilizer. Formulation 1 with added stabilizer had typical behavior of dilute solution where G' and G'' slightly increased with frequency and G'' was always higher than G' within the experimental domain, meaning that the viscous behavior dominates (Steffe, 1999). Similar behavior has been reported for surfactant suspensions (Shukla et al., 2015), colloidal gels (Chan and Mohraz 2012), and whey protein solutions (Paraskevopoulou et al., 2013). For formulation 1 without added stabilizer (see supplemental data), the elastic modula at

low frequencies indicate increase in suspension stability with pressures up to 200 MPa. This relates to improved overall product stability.

Fig 7 shows the changes in G' and G'' as a function of frequency for formulation 2 with added stabilizer. For the control samples, the values of G' and G'' slightly increased with frequency and showed a typical behavior of dilute solution ($G' < G''$). A remarkable increase in both G' and G'' was observed when samples were treated with HPH. It seems that homogenization pressure favors the formation of increasingly complex structure when compared to the control samples. More importantly, G' becomes greater than G'' indicating a clear tendency to form macromolecular networks. This means that formulation 2 treated with HPH shows a weak gel behavior and their structure is not affected by further increasing the homogenization pressure. This type of behavior has also been reported for gel made of potato starch (Yoneya et al., 2003), k-carrageenan (Eleya and Turgeon, 2000), *Lepidium perfoliatum* seed gum (Hesarinejad et al., 2014), xanthan gum (Mermet-Guyennet et al., 2015), and collagen (Oechsle et al., 2015).

For formulation 2 without added stabilizer, the mechanical spectra showed that G'' was always higher than G' , indicating a typical behavior of dilute solution. In general, the type of formulation and HPH conditions play a relevant role in determining the mechanical spectra of the formulated beverages. The analysis of $\log G'$ vs $\log \omega$ can be used to infer possible structuring mechanisms for liquid-like fluids (Ferry, 1980). A relation was found between G' and frequency according to Equation (3) and their parameters are given in **Table 3**. The parameter b_2 is used to determine the strength and nature of liquid (Ferry, 1980). For the conventionally prepared formulation 1 with added stabilizer, $b_2 > 1$ (1.40) meaning that the formulated beverage has characteristics of a viscous gel. Interestingly, the application of HPH yielded values of b_2

ranging from 0.86-1.13, indicating that the formulated beverage showed characteristics of elastic gel (Hesarinejad et al., 2014). In the case of formulation 2 with added stabilizer, the formulated beverage showed gel strength increasing up to 200 MPa which then started regressing judging by b_2 values. For formulation 2 without added stabilizer, the b_2 values (>1) revealed that HPH induced structural changes such that increased pressures induced more elasticity for the viscoelastic liquid.

3.5.Step rate measurements

Table 4 shows the effect of HPH on the viscosity of formulation 1 and 2. For formulations added with stabilizer, HPH induced significant changes in the flow profile. In the low shear spectrum (0.5 s^{-1}), HPH yielded an increase in the viscosity up to 2.44- and 31-fold for formulation 1 and 2, respectively. At higher shear rates (up to 100 s^{-1}), the increase in viscosity becomes less pronounced. Imparted viscosities at medium and high shear rates were 1.65- and 11-fold (formulation 1 and 2, respectively) greater compared to the control treatment. For formulated beverages without added stabilizers, the flow profile was not affected by HPH. The viscosity ranges from 3 to 7 and from 16 to 31 cP for formulation 1 and 2 without added stabilizer, respectively, regardless of the shear rate spectrum. These observations exemplify the complexity of interactions between different ingredients during HPH.

3.6.Microstructure

Confocal laser microscopy was used to observe the microstructure of formulated beverages. **Fig 8** shows the gross morphology of the HPH treated formulations. Overall, all samples showed a porous and heterogeneous microstructure, comprising of dense aggregates of protein particles dispersed in a continuous phase. Individual fat emulsions could not be observed in the confocal analysis (**Fig 8**) due to the presence of a highly dense protein network. No differences

in the microstructure and morphology was observed between the different samples. During HPH treatment, thermal and mechanical effects (shearing, turbulence and cavitation) may disrupt the network, making it difficult to detect with the adopted confocal methodology.

3.7.Storage study

Strain and frequency sweep data indicated that HPH enhanced the formation of stronger network for formulation 2 compared to that of formulation 1. Thus, formulation 2 with and without added stabilizer was chosen to evaluate the effects of HPH conditions on the emulsion stability up to 3-week of storage at 6°C. Two combinations of homogenization pressure and product temperature for each formulation were selected: 1) with added stabilizer, 62 ± 4 MPa/103°C and 245 ± 10 MPa / 145 °C; and 2) without added stabilizer, 56 ± 6 MPa / 100 °C and 252 ± 7 MPa / 147 °C.

Fig 9a shows the strain sweep analysis of formulation 2 after 3 weeks of storage at 6°C. For those samples with added stabilizer, the application of HPH not only extended the LVR but also yielded a stronger network compared with those samples without added stabilizer. The strain sweep of samples without added stabilizer is comparable to that obtained for samples conventionally prepared. In general, increasing pressure from 56-62 to 245-252 MPa did not further improve the stability or it was negatively impacted.

The frequency sweep analysis of formulation 2 after 3 weeks of storage is given in **Fig 9b**. Interestingly, HPH did not change the mechanical spectra of samples without added stabilizer, showing a behavior of dilute solution ($G'' > G'$) over the entire frequency range. On the other hand, HPH induced significant changes in samples with added stabilizer, showing a weak gel behavior ($G' > G''$).

The effect of HPH on viscosity of formulation 2 during storage is shown in **Table 5**. HPH dramatically increased the viscosity depending on the addition of stabilizer and pressure applied and such changes were more notorious at low shear spectrum. For instance, the viscosity increased up to 3.43-fold in samples without added stabilizer, while an increase of up to 88-fold was observed in samples with added stabilizer. This is an important consideration in relation to food safety and quality since changes in viscosity might alter the residence time distribution as well as the uniformity of the thermal treatment. Considering the results from dynamic rheological measurements, it seems that HPH can be used as a technological tool for product development. Alongside with the changes in the dynamic rheological behavior one should consider the impact of HPH on selected quality parameters. **Table 6** shows the effect of HPH on color parameters (L^* , a^* and b^*) and particle size distribution. Product color changed with the application of HPH, showing an increase in lightness with more saturated in red and more intense yellow. This can be explained by the complex interactions between ingredients and the by change in the mechanical spectra.

4. Conclusions

The analysis of particle size distribution, viscosity and rheology data indicate that there is an opportunity to reduce the concentration of stabilizers in dairy beverage formulation. In addition, significant stability and emulsion shelf life improvement was achieved by processing the beverage below pressure threshold value when compared with beverages conventionally homogenized. The product lightness increased after HPH; however, consumer evaluation would be needed to confirm if this attribute is desirable. Our experimental findings suggest that HPH can be used as technological tool for clean label process development, adding another dimension

to traditional product formulation and development. The information generated in this study may help to develop strategies for the formulation and manufacture of beverages with reduced concentration of stabilizers.

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Figure captions

Fig 1. Schematic diagram of high-pressure homogenization pilot plant located at The Ohio State University. T_u – temperature of untreated product; T_{in} – inlet temperature; p – pressure; T_{com} – temperature rise due to hydrostatic compression; T_p – product temperature or temperature after first homogenization stage; T_{2st} – second homogenization stage temperature; T_c – cooling temperature.

Fig 2. Representative changes in temperature during high pressure homogenization. (a) Temperature history for formulation 1 with stabilizer at different homogenization pressures, inlet temperature 90°C. T_u – temperature of untreated product; T_{in} – inlet temperature; p – pressure; T_{com} – temperature rise due to hydrostatic compression; T_p – product temperature or temperature after first homogenization stage; T_{2st} – second homogenization stage temperature; T_c – cooling temperature. (b) Comparison of temperature increase for formulation 1 with stabilizer and deionized water. ΔT_{com} – increase in temperature due to hydrostatic compression; ΔT_{diss} – increase in temperature due to dissipation of kinetic energy. T_{in} – inlet temperature 90°C.

Fig 3. Effect of the high pressure homogenization conditions on the particle size distribution of formulated beverage: (a) formulation 1 added with stabilizer; (b) formulation 1 without stabilizers; (c) formulation 2 added with stabilizer; and (d) formulation 2 without stabilizers. Control sample refers to conventionally homogenized (30 MPa) and UHT treated formulation.

Fig 4. Mean particle size as function of homogenization pressure for different formulation with and without the addition of stabilizer. (a) formulation 1 and (b) formulation 2.

Fig 5. Strain sweep dependency of storage module (G') for: (a) formulation 1 with added stabilizer and (b) formulation 2 with added stabilizer. Frequency = 10 rad s^{-1} .

Fig 6. Frequency sweep analysis for formulation 1 with added stabilizer.

Fig 7. Frequency sweep analysis for formulation 2 with added stabilizer.

Fig 8. Confocal micrograph of Formulation 1 with added stabilizer (a), Formulation 1 without added stabilizer (b), Formulation 2 with added stabilizer (c), and Formulation 2 without added stabilizer (d). The protein matrix is represented in green while the fat phase is represented in blue. Scale bar = $40\mu\text{m}$.

Fig 9. Strain and frequency sweep for formulation 2 with and without added stabilizer after 3-week of storage at 6°C . (a) Strain sweep analysis. Frequency = 10 rad s^{-1} . (b) Frequency sweep analysis. P1-Stabilizer – $62 \pm 4 \text{ MPa}/103^\circ\text{C}$; P2-Stabilizer – $245 \pm 10 \text{ MPa}/145^\circ\text{C}$; P1-Wihtout stabilizer – $56 \pm 6 \text{ MPa}/100^\circ\text{C}$; P2-Wihtout stabilizer – $252 \pm 7 \text{ MPa}/147^\circ\text{C}$.

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1 Table legend

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3 **Table 1.** Formulation of beverages used for high pressure homogenization experiments.

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5 **Table 2.** Estimated parameters for the evaluation of mean particle size (d_{50}) as function of energy
6 density.

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8 **Table 3.** Relationship between storage module (G') and frequency (ω) for formulated beverages.

9

10 **Table 4.** Effect of high pressure homogenization on viscosity (cP) of formulated beverages with
11 and without added stabilizer.

12

13 **Table 5.** Changes in viscosity of formulation 2 after 3 weeks of storage at 6°C.

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15 **Table 6.** Impact of high pressure homogenization on color parameters and particle size
16 distribution for formulation 2after 3 weeks of storage at 6°C.

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Table 1

Ingredient	Concentration g per 100 g of beverage	
	Formulation 1	Formulation 2
Protein blend	5.31	9.42
Carbohydrate blend	21.52	29.27
Mineral blend	0.57	0.74
Oil blend	2.98	5.23
Stabilizer	0.05	0.51

Table 2

Parameters	Formulation 1			
	With stabilizer		Without stabilizer	
	Value	CI95%	Value	CI95%
a	238.81	0.17	1854.87	0.04
b	0.24	5.7 x 10 ⁻⁵	0.39	1.8 x 10 ⁻⁶
R ²	0.969		0.981	
Parameters	Formulation 2			
	With stabilizer		Without stabilizer	
	Value	CI95%	Value	CI95%
a	1.8 x 10 ⁴	775.5	2.3 x 10 ⁴	853.8
b	0.42	0.03	0.44	0.03
R ²	0.998		0.998	

CI95% — 95% confidence interval; R² — coefficient of determination; a and b — regression coefficients of Eq. (2)

Table 3

Formulation 1 with added stabilizer			
Treatment	a₂	b₂	R²
Control	0.001	1.40	0.947
45 ± 3 MPa/92°C	0.006	1.13	0.992
78 ± 7 MPa/96°C	0.006	1.07	0.991
103 ± 7 MPa/105°C	0.007	1.07	0.991
159 ± 3 MPa/115°C	0.009	0.93	0.981
210 ± 6 MPa/130°C	0.012	0.86	0.994
286 ± 16 MPa/150°C	0.006	1.02	0.976
Formulation 2 with added stabilizer			
Treatment	a₂	b₂	R²
Control	0.04	0.59	0.712
55 ± 6 MPa/92°C	0.65	0.46	0.977
80 ± 8 MPa/100°C	0.96	0.38	0.971
102 ± 8 MPa/112°C	1.08	0.38	0.969
148 ± 6 MPa/120°C	1.15	0.35	0.978
214 ± 7 MPa/133°C	0.85	0.41	0.968
237 ± 4 MPa/140°C	0.87	0.38	0.944
Formulation 2 without added stabilizer			
Treatment	a₂	b₂	R²
53 ± 7 MPa/95°C	0.003	0.985	0.895
79 ± 10 MPa/101°C	0.004	1.004	0.974
108 ± 10 MPa/108°C	0.004	1.101	0.964
151 ± 7 MPa/117°C	0.004	1.073	0.982
203 ± 8 MPa/130°C	0.001	1.403	0.962
222 ± 8 MPa/138°C	0.005	1.031	0.981
Parameters estimated using Equation (3); R ² coefficient of determination			

Table 4

Formulation 1 with added stabilizer				Formulation 2 with added stabilizer			
Treatment	Shear rate (s ⁻¹)			Treatment	Shear rate (s ⁻¹)		
	0.5	13	100		0.5	13	100
Control	50.34±3.77	26.31±1.97	18.22 ± 1.37	Control	48.62±3.65	22.66±1.70	14.35±1.08
45±3 MPa/92°C	122.11±9.16	43.83±3.29	24.41 ± 1.83	55±6 MPa/92°C	1151.77±96.46	209.56±15.72	95.18±7.14
78±7 MPa/96°C	82.68±4.62	36.08±2.71	21.47 ± 1.61	80±8 MPa/100°C	1421.35±106.58	241.01±18.12	104.66±7.85
103±7 MPa/105°C	118.97±8.92	42.24±3.17	26.01 ± 1.96	102±8 MPa/112°C	1517.51±113.78	255.79±19.18	109.63±8.22
159±3 MPa/115°C	56.47±3.23	27.90±2.09	19.30 ± 1.45	148±6 MPa/120°C	1444.73±108.30	249.22±18.69	109.43±8.21
210±6 MPa/130°C	52.13±2.88	27.89±3.01	20.23 ± 1.52	214±7 MPa/133°C	1208.82±90.61	232.10±17.41	105.14±7.89
286±16 MPa/150°C	41.58±1.39	24.61±1.85	18.15 ± 1.36	237±4 MPa/140°C	1329.83±99.68	246.53±18.49	111.82±8.39
Formulation 1 without added stabilizer				Formulation 2 without added stabilizer			
Treatment	Shear rate (s ⁻¹)			Treatment	Shear rate (s ⁻¹)		
	0.5	13	100		0.5	13	100
Control	6.43±0.48	6.23±0.47	6.38±0.48	53±7 MPa/95°C	21.69±1.63	17.77 ± 1.33	16.25±1.22
54±8 MPa/92°C	6.10±0.46	6.22±0.47	7.17±0.54	79±10 MPa/101°C	31.40±2.36	21.82 ± 1.64	17.96±1.35
81±8 MPa/101°C	4.91±0.37	6.09±0.46	6.82±0.51	108±10 MPa/108°C	29.78±2.23	22.52 ± 1.69	19.19±1.44
107±7 MPa/102°C	3.65±0.27	7.38±0.55	7.13±0.53	151±7 MPa/117°C	31.19±2.34	22.60 ± 1.70	18.54±1.39
155±5 MPa/123°C	5.18±0.39	5.91±0.44	6.71±0.50	203±8 MPa/130°C	32.56±2.44	22.01 ± 1.65	18.72±1.40
203±5 MPa/132°C	3.49±0.26	5.89±0.44	6.63±0.50	222±8 MPa/138°C	36.16±2.71	24.20 ± 1.82	19.89±1.49
287±12 MPa/148°C	5.24±0.39	6.27±0.47	7.01±0.53				

Table 5.

Treatment	Shear rate (s ⁻¹)		
	0.5	13	100
Control-Stabilizer	48.62 ± 3.64	22.66 ± 1.69	14.35 ± 1.07
P1-Without stabilizer	165.08 ± 12.81	91.21 ± 6.84	64.09 ± 4.81
P1-Stabilizer	4270.09 ± 320.25	527.66 ± 39.51	198.92 ± 14.91
P2-Without Stabilizer	103.94 ± 7.79	76.38 ± 5.75	60.86 ± 4.56
P2-Stabilizer	2657.43 ± 199.31	435.09 ± 32.63	184.32 ± 13.86
P1-Stabilizer – 62 ± 4 MPa/103°C; P2-Stabilizer – 245 ± 10 MPa/145°C; P1-Wihtout stabilizer – 56 ± 6 MPa/100°C; P2-Wihtout stabilizer – 252 ± 7 MPa/147°C			

Table 6.

Treatment	Color		
	L*	a*	b*
Control-Stabilizer	66.34 ± 6.91	-6.87 ± 0.63	5.82 ± 1.88
P1-Without stabilizer	77.76 ± 2.29	-7.16 ± 0.07	7.59 ± 0.77
P1-Stabilizer	75.44 ± 0.69	-7.52 ± 0.31	7.41 ± 0.09
P2-Without Stabilizer	79.46 ± 1.01	-6.86 ± 0.22	9.86 ± 0.54
P2-Stabilizer	69.91 ± 4.01	-6.86 ± 0.25	6.54 ± 1.05
Treatment	Particle size distribution		
	d _[v,0.1]	d _[v,0.5]	d _[v,0.9]
Control-Stabilizer	0.18 ± 0.02	1.96 ± 0.35	7.51 ± 0.89
P1-Without stabilizer	0.21 ± 0.01	2.41 ± 0.21	25.75 ± 1.25
P1-Stabilizer	0.18 ± 0.02	1.91 ± 0.20	56.71 ± 20.52
P2-Without Stabilizer	0.31 ± 0.01	6.91 ± 0.52	34.45 ± 1.47
P2-Stabilizer	0.23 ± 0.02	3.53 ± 0.27	51.18 ± 11.25
P1-Stabilizer – 62 ± 4 MPa/103°C; P2-Stabilizer – 245 ± 10 MPa/145°C; P1-Without stabilizer – 56 ± 6 MPa/100°C; P2-Without stabilizer – 252 ± 7 MPa/147°C			