LWT - Food Science and Technology 135 (2021) 110062

Contents lists available at ScienceDirect

LWT

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

Improvement in physical and thermal stability of cloudy ginkgo beverage during autoclave sterilization: Effects of microcrystalline cellulose and gellan gum

Yang Ni, Xiaoshu Tang, Liuping Fan *

State Key Laboratory of Food Science & Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu, 214122, China

ARTICLE INFO

Keywords:
Beverage stability
Ginkgo seeds
Turbiscan
Autoclave sterilization

ABSTRACT

This work aimed to apply microcrystalline cellulose (MCC) and gellan gum to improve the physical and thermal stability of cloudy ginkgo beverages. The stability of each beverage samples was characterized in terms of particle size, size distribution, zeta potential, rheological properties, Turbiscan Stability Index (TSI), and changes in backscattered light intensity (△BS). Our results indicate that an irreversible phase separation occurred in the beverage sample, induced by increased particle size, decreased zeta potential, and decreased viscosity during the sterilization process. Although the addition of either MCC or gellan improved beverage stability by decreasing particle size and increasing zeta potential, this improvement was limited; the aggregation and sedimentation of suspended particles still occurred, as revealed by large △BS and TSI changes. Beverage samples added with a mixture of MCC and gellan exhibited different physical and thermal stability values depending on the ratio of MCC to gellan. The addition of MCC (0.3%, w/w) combined with a small amount of gellan (0.05% or 0.08%, w/w) effectively prevented drastic thermal damage and improved physical and thermal beverage stability in comparison to MCC combined with the larger amount of gellan. This study provides some suggestions for the industrial production of cloudy beverages.

1. Introduction

Ginkgo nuts have been consumed in China for thousands of years due to their plentiful starches, proteins, and vitamins (Li & Hu, 2015; Zhang, Wang, & Xu, 2007). To date, the industrial utilization of ginkgo nuts is still limited, especially in comparison to ginkgo leaves, which are well-known in global markets as a dietary supplement and for their pharmaceutical ingredients (Defeudis & Drieu, 2004; Li & Hu, 2015). Cloudy beverages produced from ginkgo nuts have promising applications for the food industry.

In the current market, vegetable-based beverages attract considerable attention because of the need for flavorful and nutritious of products and a growing demand for non-dairy beverages due to dairy intolerance (Fiocchi et al., 2010). To date, there have been many studies on vegetable-based beverages, including vegetable milk (Bernat, Cháfer, Rodríguez-García, Chiralt, & González-Martínez, 2015; Briviba, Gräf, Walz, Guamis, & Butz, 2016; Smith, Mendonca, & Jung, 2009), cloudy vegetable beverages (Li & Fan, 2020; Ni, Zhang, Fan, & Li, 2019; Yu, Jiang, Cao, Jiang, & Pan, 2016), and vegetable juices (Akkarachaneeyakorn & Tinrat, 2015; Leite, Augusto, & Cristianini, 2014; Lv, Kong, Mou, & Fu, 2017). In beverage production, thermal sterilization is an essential process to prolong shelf life and thus ensure beverage safety. Pasteurization and ultra-high temperature processing sterilization are widely practiced in milk and milk beverages. However, as neutral beverages (pH ranging from 6.5 to 7.5), cloudy vegetable beverages are commonly sterilized using autoclave sterilization. This intense sterilization process can cause severe thermal damage in beverages, leading to flocculation, coalescence, creaming, and eventually complete phase separation (Li & Fan, 2020; Yu et al., 2016). The addition of hydrocolloids is a conventional method to effectively improve stability and to avoid severe thermal damage to cloudy beverages.

According to Stokes's law, relatively high viscosity and small droplet size can improve beverage stability (Li et al., 2016). Therefore, several hydrocolloids are commonly used as beverage additives in order to improve physical stability. These hydrocolloids include xanthan gum (Akkarachaneeyakorn & Tinrat, 2015), guar gum combined with CMC (Lv et al., 2017), and tragacanth gum (Hajmohammadi, Pirouzifard, Shahedi, & Alizadeh, 2016). However, cloudy beverages with a high

* Corresponding author. School of Food Science and Technology, Jiangnan University, Wuxi, 214122, China.
E-mail address: fanliuping@jiangnan.edu.cn (L. Fan).

https://doi.org/10.1016/j.lwt.2020.110062
Received 21 March 2020; Received in revised form 10 August 2020; Accepted 12 August 2020
Available online 15 August 2020

viscosity have been reported to give consumers a sticky and unpleasant sensation (Hajmohammadi et al., 2016). Moreover, cloudy ginkgo beverages contain high levels of insoluble proteins and carbohydrates particles, and the stability of cloudy beverages is closely tied to these particles. Previous studies have reported that microcrystalline cellulose (MCC) and gellan gum (GG) have the ability to suspend insoluble particles and emulsion droplets in lower viscosity beverages (Krawczyk, Venables, & Tuason, 2009; Sworn, 2009). Properly hydrated sols of MCC can form a three-dimensional network based on the electrostatic repulsion of negatively charged cellulose crystals (Krawczyk et al., 2009). This network structure contributes to preventing the precipitation of insoluble particles in cocoa beverages (Kijima, 2006) and to avoiding the coalescence of droplets in curcumin emulsions (Xu, Zhang, Cao, Wang, & Xiao, 2016). GG can form a fluid gel based on the gelation mechanism of the domain model, which assumes the formation of distinct junction zones and disordered flexible polymer chains connecting adjacent junction zones (Grasdalen & Smidsrød, 1987; Sworn, 2009). Moreover, gellan is widely used and known for the ability to form gels at lower concentrations than other stabilizers (Li & Fan, 2020). Vilela and Cunha (2016) employed high acyl gellan (HA) as a stabilizer to obtain a stable oil in water emulsions. Stable gel emulsions can also be prepared using high acyl gellan (Califano, Alicia, Lorenzo, & Zaritzky, 2013).

To the best of our knowledge, there has been very little research on the ability of MCC and GG to improve physical and thermal stability during autoclave sterilization of cloudy beverages composed mainly of carbohydrates and proteins. Therefore, this work aims to evaluate the effect of MCC and GG on the physical and thermal stability of cloudy beverages. Particle size, rheological behavior, backscattered light intensity, and Turbiscan Stability index were evaluated for this purpose. This study provides useful knowledge for the production of stable cloudy beverages containing abundant carbohydrates and proteins in the beverage industry.

2. Materials and methods

2.1. Materials

Ginkgo nuts were purchased from a local market (Jiangsu, China). The MCC (Novagel® GP-3282) was supplied by FMC Trading Co., Ltd. (Shanghai, China) and consisted of 90% MCC (w/w) and sodium 10% carboxymethyl cellulose (CMC) (w/w). Commercial HA gellan powder (Kelcogel LT-100) was obtained from Kelco Biopolymers (San Diego, CA). The ginkgo kernels contained 56.78 g/100 g moisture, 30.67 g/100 g starch, 6.12 g/100 g protein, 1.15 g/100 g crude fiber, and 0.95 g/100 g fat.

2.2. Preparation of cloudy ginkgo beverage

The process used to prepare the cloudy ginkgo beverage was based on a previously published technique (Ni et al., 2019). Briefly, ginkgo seeds were ground with deionized water at a ratio of 1:10 (w/v) using a colloid mill (JM-L50, Qiangzhong, Zhejiang, China). Amylase was used to hydrolyze starches. Hydrocolloids were then added to the beverage system and stirred constantly at a speed of 250 rpm for 20 min at 80 °C. The resulting beverage samples were homogenized at 30 MPa for two cycles using a high-pressure homogenizer (AH-2010, ATS Engineering Inc, China). Finally, the beverage samples were sterilized at 121 °C for 25 min. To investigate the physical changes and beverage stability, differing amounts of MCC and/or gellan were added to samples of the original beverage. Varying polysaccharide formulations were applied as follows: 0.3% MCC (w/w), 0.1% gellan (w/w), F1 (0.3% MCC and 0.05% gellan (w/w)), F2 (0.3% MCC and 0.08% gellan (w/w)), F3 (0.3% MCC and 0.12% gellan (w/w)), and F4 (0.3% MCC and 0.15% gellan (w/w)). The cloudy ginkgo beverage samples (100 mL) were transferred into glass bottles. The bottles were placed into a YXQ-LS-30 sterilizer (Boxun Instrument, Shanghai, China) to conduct the sterilization process (121 °C, 25 min). Three bottles for each formulation were prepared as replicates. After sterilization, beverage samples were left to naturally cool at room temperature.

2.3. Evaluation of physical properties and stability of cloudy ginkgo beverage

2.3.1. Particle size and zeta potential

Particle size and zeta potential were measured based on a method previously described by Ni et al. (2019). Briefly, the particle size (D [4, 3]) of the ginkgo beverage samples was measured using a laser diffraction (Malvern Mastersizer 2000 with Hydro 2000s; Malvern Instruments Ltd., UK), while the zeta potential of beverage samples was measured using a Brookhaven Omni (Nano Brook Omni, Brookhaven Instruments Corporation, USA). All samples were diluted 10 times using deionized water, and these dilutions were used to determine particle size and zeta potential at 20 °C. The final particle size zeta and potential values resulted from analyzing the beverage samples in triplicate.

2.3.2. Rheology

The rheological properties of the cloudy ginkgo beverage samples were measured using a DHR-3 rheometer (Waters, USA) at 25 ± 0.1 °C. Beverage samples (2 mL) were carefully deposited over the plateau of the rheometer, and set a cone and plate geometry (cone diameter 40 mm, angle 2°, gap 0.100 mm) was employed. The shear rate ranged from 0 to 100 s^{-1}, and the apparent viscosity of the system was evaluated at a shear rate of 30 s^{-1}.

2.3.3. Evaluation of beverage stability

A Turbiscan analyzer (Turbiscan MA 2000; Formulaction, France) was used to monitor the movement of suspended particles in the beverage samples and to evaluate beverage stability. Samples were periodically scanned from bottom to top by a pulsed near-infrared light source (wavelength 850 nm). The scanning time was from 0 h to 2 h at intervals of 3 min at 50 °C. The device collected the backscattered profiles of beverage samples and computed the mean values of the delta backscattering intensity based on the TSI formula (Eq. (2)), which can reflect the stability of beverages.

$$TSI = \sum_{i} \frac{\sum_{h} |scan_i(h) - scan_{i-1}(h)|}{H} \quad (2)$$

where: $scan_i(h)$ - average backscattering for each time (i) of measurement, $scan_{i-1}(h)$ - average backscattering for the i-1 time of measurement, and H - sample height.

2.4. Data analysis

All experiments were performed in triplicate. Analysis of variance (ANOVA) was employed to determine significant differences at the $P < 0.05$ level using SPSS 19.0 software. All data are expressed as mean ± standard deviations (SD) of triplicate experiments.

3. Results and discussion

3.1. Changes in particle size and size distribution

As shown in Fig. 1a, the particle size of all beverage samples increased after the sterilization process. This could be due to the denaturation and coagulation of proteins and the aggregation of carbohydrates induced by the high temperature of the sterilization process. Liu, Sun, Xue, and Gao (2016) reported that sterilization (121 °C, over 25 min) led to poor physical stability in walnut beverage emulsions due to the denaturation and coagulation of walnut protein. Fig. 1b showed the change in particle size distribution of beverage samples after

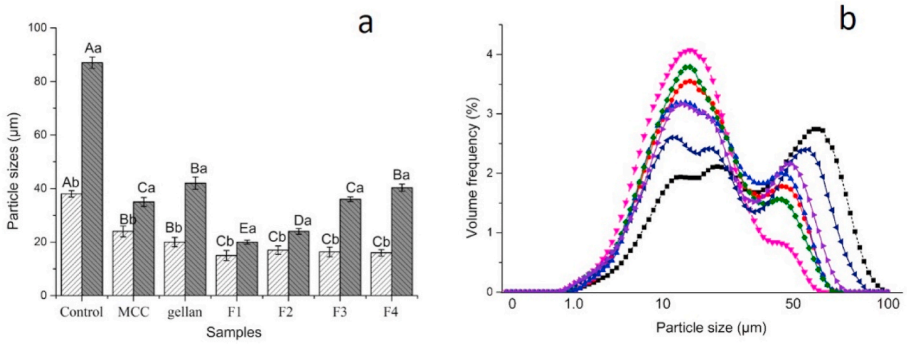

Fig. 1. The particle size of beverages added with different sterilizer before and after sterilization process (a). ( ): Before sterilization; ( ): After sterilization. The particle size distribution curves of beverages after sterilization process (b). ( ): Control; ( ): MCC; ( ): gellan; ( ): F1; ( ): F2; ( ): F3; ( ): F4. Samples designated with different capital letters (A, B, C, D, E) were significantly different ($p < 0.05$) in particle size when compared between beverages added with different formulations (same sterilization treatment). Samples designated with different lower case letters (a, b) were significantly different ($p < 0.05$) when compared between beverages before and after sterilization (same formulation).

sterilization. All beverages exhibited a bimodal distribution after sterilization, with the two modes located at 10 μm and the other approximately 50 μm. This result is consistent with a previous study (Ni et al., 2019). The intense sterilization conditions caused a sharp increase in the peak located at approximately 50 μm for the control beverage, suggesting the appearance of plenty of larger aggregated particles. The addition of MCC and/or gellan was beneficial in inhibiting the aggregation of suspended particles, as evidenced by the lower height of the peak located at approximately 50 μm. Moreover, the particle size values of beverage samples added with hydrocolloids were smaller than those of control sample after sterilization. This protective behavior can potentially be ascribed to the formation of a three-dimensional network structure of MCC (Kijima, 2006) or fluid the gels of gellan (Sworn, 2009). Of note, the particle size of the beverage samples added with only 0.1% gellan increased to 48 μm after sterilization, which was larger than that of the beverages added with 0.3% MCC (37 μm). This result suggests that MCC performs better than gellan at inhibiting particle size change during the thermal sterilization process.

With respect to the beverage samples treated with a mixture of MCC and gellan, the particle size change was closely related to the ratio of MCC to gellan. The beverage samples added with F1 (0.3% MCC and 0.05% gellan, w/w) exhibited the smallest increase in particle size (33%) while the particle size of the beverage added with F4 (0.3% MCC and 0.15% gellan, w/w) increased by 150% after sterilization. This huge increase in particle size of the samples added with F4 was even larger than that of the samples added with only MCC (75%). These results indicate that the appropriate ratio of MCC to gellan is crucial for preventing an increase of particle size.

3.2. Zeta potential changes

As shown in Fig. 2, the zeta potential values for all samples were clearly negative, indicating that there are more negatively charged particles than positively charged particles in the cloudy beverages. This was attributed to both the isoelectric point (pI) of the ginkgo proteins as well as to the properties of added stabilizers. Proteins in ginkgo beverages exhibit negative charge because the pH of beverages (7.0) is above the pI of ginkgo proteins (4.62) (Yang et al., 2011). Furthermore, the absolute value of zeta potential of the beverage samples added with MCC or gellan gum were higher than that of the control. This is because both MCC and gellan are anionic polysaccharides (Sworn, 2009; Xu et al., 2016) and thus provide a certain amount of negatively charged particles. After sterilization, the zeta potential values of all beverages increased, possibly due to a reduction in the effective surface charge of suspended

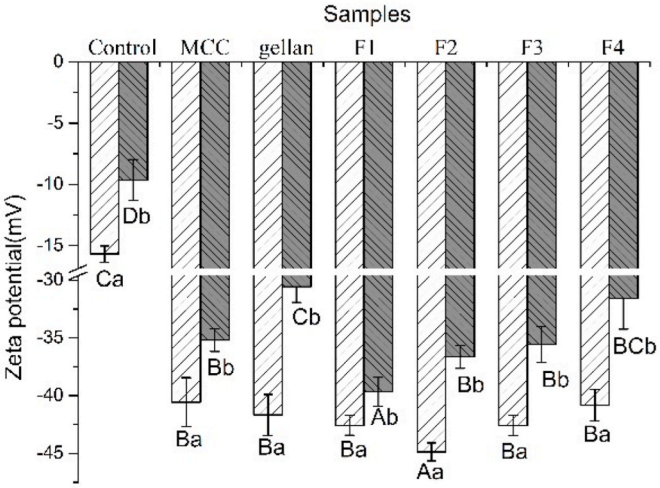

Fig. 2. The zeta potential of beverages added with different stabilizers before and after sterilization process. ( ): Before sterilization; ( ): After sterilization. Samples designated with different capital letters (A, B, C, D) were significantly different ($p < 0.05$) in zeta potential values when compared between beverages added with different formulations (same sterilization treatment). Samples designated with different lower case letters (a, b) were significantly different ($p < 0.05$) when compared between beverages before and after sterilization (same formulation).

particles induced by the particle aggregation. The effective surface charge of suspended particles is a primarily determinant of their dispersion and aggregation (Song, Zhou, Fu, Chen, & Wu, 2013). In addition, the zeta potential of the beverage samples added with only 0.1% gellan increased to −30 mV after the sterilization process, which was a larger change than that in the sample added only 0.3% MCC (−36 mV). This result is consistent with particle size results shown in Fig. 1a. The lowest zeta potential change was observed in the sample added with F1 in which the value after sterilization process reached −40 mV. This implies that the suspended particles in the beverage samples did not easily aggregate together due to strong electrostatic repulsion.

3.3. Rheology properties

The curves of shear stress versus the shear rate of beverage samples following sterilization treatment are shown in Fig. 3. Clearly, all

beverages were typical shear thinning fluids, showing shear stress increases with the increase of shear rate (Ni, Li, & Fan, 2020). Moreover, the Herschel-Bulkley model could be used to fit the stress-shear rate curves due to the observation of a yield stress. The corresponding parameters are tabulated in Table 1. The determination coefficient (R^2) of all beverages was almost equal or higher than 0.96, further confirming the good fitness of this model. All flow behavior index (n) values were lower than 1, suggesting that all beverage samples were pseudoplastic fluids (Li et al., 2019). The observed stress from samples added with MCC or gellan was attributed to the formation of the network structures or fluid gels induced by the entangling effect of polysaccharide (Krawczyk et al., 2009; Sworn, 2009). Interestingly, the yield stress values (τ_0) of samples added with F1 or F2 were higher than those of samples added with only MCC or gellan, while the yield stress values (τ_0) of samples added with F3 or F4 were smaller. This indicates that the network structures in samples added with F1 or F2 were stronger than those of other beverage samples.

Additionally, the apparent viscosity of all samples decreased after the sterilization process, as shown in Table 2. Following sterilization, the reduction in apparent viscosity of the beverage sample added with only gellan (35%) was larger than that of the sample added with only MCC (22%), demonstrating that the fluid gel formed by gellan may be more influenced by sterilization than by the MCC three-dimensional network. This result is consistent with the result related to particle size (Fig. 1) and zeta potential change (Fig. 2). Further, Krawczyk et al. (2009) reported that the MCC system has good thermal stability and that temperature changes have little effect on the functionality and viscosity of MCC dispersions. The viscosity reduction of the samples added with F1, F2, F3, and F4 were 11.24%, 14.56%, 39.02%, and 52.30%, respectively. The result indicates F1 performs better in protecting beverages against viscosity change during sterilization process. It is worth noting that the protection capacity depends on the ratio of MCC to gellan, as evidenced by the differences in viscosity protection of the various formulations. Based on the ratio of MCC to gellan and the corresponding viscosity changes, it was concluded that MCC combined with a small amount of gellan (F1 and F2) may be beneficial to protect beverages against viscosity reduction during sterilization process. In contrast, the combination of MCC and a larger amount of gellan (F3 and F4) could have a negative effect. These results suggest that a small amount of gellan (0.05% and 0.08%) strengthens the thermal stability of the network structure formed by MCC and thus impedes any decrease in beverage viscosity, while more gellan (0.12% and 0.15%) may disturb the formation of MCC network structure due to the tendency of gellan self-gel, resulting in poorer thermal stability of beverages.

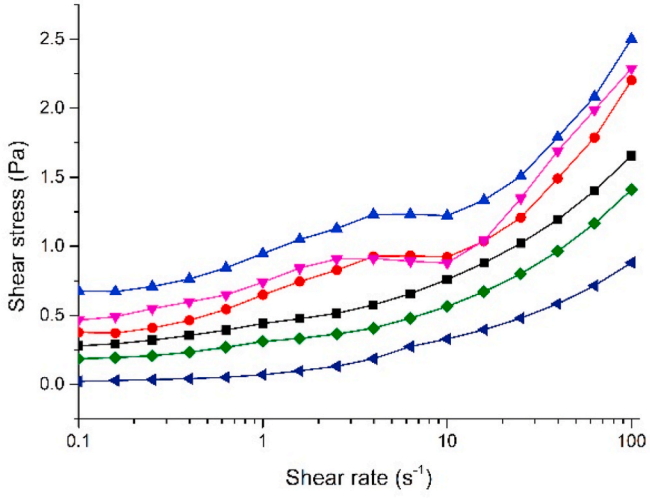

Fig. 3. The shear stress as a function of the shear rate of ginkgo beverages added different stabilizers after sterilization processing. (—■—): MCC; (—●—): gellan; (—▲—): F1; (—▼—): F2; (—◆—): F3; (—◀—): F4.

Table 1
Herschel-Bulkley model parameters of ginkgo beverages added different stabilizers after sterilization treatment.

Samples	τ_0 (Pa)	K (Pa.s^n)	n	R^2
MCC	0.21 ± 0.09^{d}	0.21 ± 0.05^{ab}	0.41 ± 0.02^{c}	0.9995
Gellan	0.34 ± 0.06^{c}	0.25 ± 0.03^{a}	0.43 ± 0.05^{bc}	0.9718
F1	0.64 ± 0.12^{a}	0.25 ± 0.02^{a}	0.43 ± 0.04^{bc}	0.9789
F2	0.51 ± 0.08^{b}	0.18 ± 0.04^{b}	0.53 ± 0.02^{a}	0.9672
F3	0.14 ± 0.02^{d}	0.14 ± 0.01^{c}	0.47 ± 0.03^{b}	0.9993
F4	0.05 ± 0.01^{e}	0.10 ± 0.02^{d}	0.45 ± 0.02^{b}	0.9966

Different letters in the same column indicate significant difference ($P < 0.05$). F1: 0.3% and 0.05%, w/w; F2: 0.3% and 0.08%, w/w; F2: 0.3% and 0.12%, w/w; F2: 0.3% and 0.15%, w/w. After addition of stabilizers, all beverages were sterilized at 121 °C for 25 min.

Table 2
The viscosity change of beverages after sterilization processing.

Samples	Viscosity (mPa.s)	
	Prior to sterilization treatment	After sterilization treatment
control	12.53 ± 1.02^{g}	1.32 ± 0.45^{f}
0.3%MCC	35.12 ± 1.97^{f}	27.14 ± 1.01^{e}
0.1%gellan	70.05 ± 2.11^{e}	46.58 ± 0.83^{d}
F1	89.40 ± 1.34^{d}	79.89 ± 1.73^{c}
F2	103.79 ± 2.74^{c}	88.25 ± 1.49^{a}
F3	132.58 ± 2.04^{b}	80.55 ± 2.14^{b}
F4	185.21 ± 1.21^{a}	89.57 ± 3.47^{a}

Different letters in the same column indicate significant difference ($P < 0.05$) between beverages added different stabilizers. F1: 0.3% and 0.05%, w/w; F2: 0.3% and 0.08%, w/w; F2: 0.3% and 0.12%, w/w; F2: 0.3% and 0.15%, w/w. Sterilization treatment was conducted at 121 °C for 25 min.

3.3.1. *Change in beverage stability*

Fig. 4 shows the change of backscattered light intensity (△BS) of the beverage samples added with different MCC and/or gellan concentrations. The blue and red lines represent the start and the end of scanning, respectively. Prior to the sterilization process, there were no significant differences in the △BS of beverage samples added with MCC or/and gellan. However, after the sterilization process, △BS of beverage samples were different. This was attributed to the changes in particle size, zeta potential, and viscosity induced by the sterilization process. With respect to the control beverage (Fig. 4a), the △BS value of the bottom section (0–13 mm) gradually increased with increased scanning time and △BS of the upper section (13–40 mm) decreased with increased scanning time. This indicates the concentrations of suspended particles in the cloudy beverages underwent considerable changes during the monitoring process (Matsumiya et al., 2014). Cloudy ginkgo beverages contain high levels of carbohydrates and proteins that are suspended in the liquid, thus the movement of these suspended particles (including precipitation and aggregation) is closely related to beverage stability (Ni et al., 2019). The decreased △BS of the upper section (13–40 mm) can be attributed to the precipitation of large suspended particles (86 μm) toward the bottom, resulting in an increased particle concentration near the bottom section (0–13 mm) of the beverages (Ni et al., 2019). In other words, particle precipitation is the main reason for the instability of cloudy ginkgo beverages. In contrast, beverages added with either MCC only (Fig. 4b) or gellan only (Fig. 4c) clearly exhibited smaller △BS values compared to the control beverage. These results indicate that MCC and gellan can improve the physical and thermal stability of couldy beverages due to the ability to form a network structure or self-gelation structure (Kijima, 2006; Vilela & Cunha, 2016). However, this improvement was limited according to the relatively large changes in

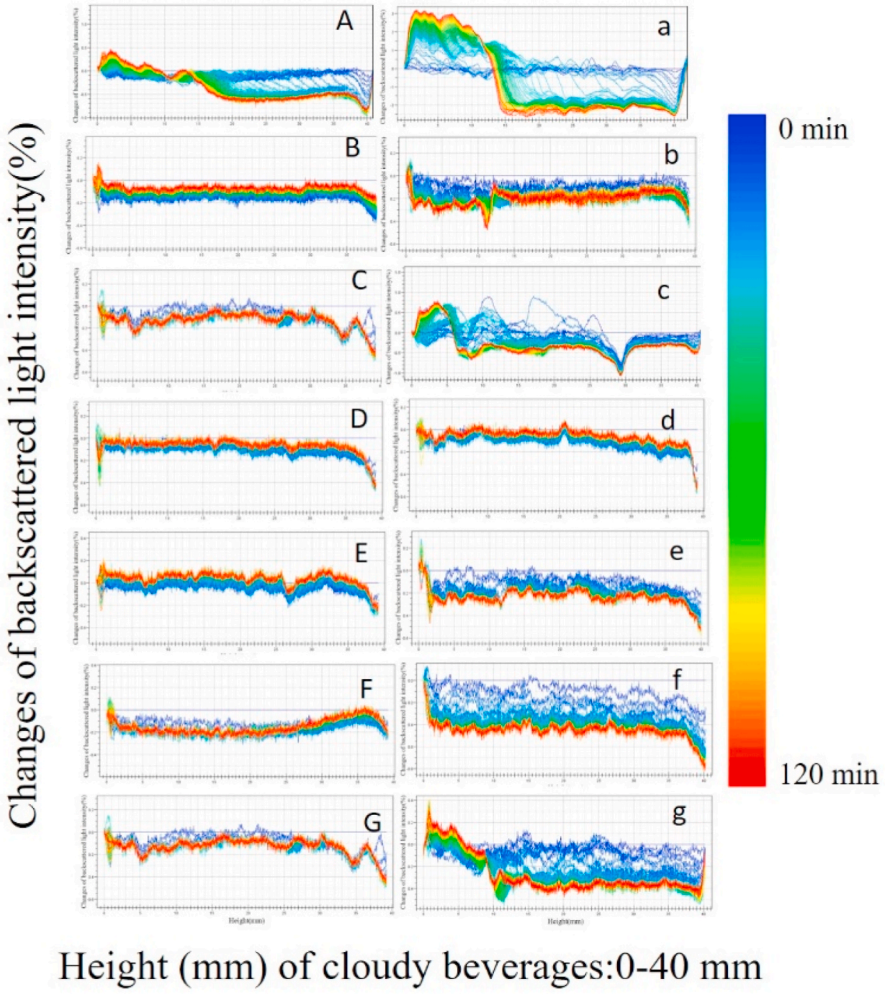

Fig. 4. The change of backscattered intensity light of beverages added different stabilizers before and after sterilization processing. Uppercase letters represent beverages without sterilization processing and lowercase letters represent beverages after sterilization processing.(A, a):0.3% MCC; (B, b):0.1% gellan; (C, c):0.3% MCC and 0.05% gellan; (D, d):0.3% MCC and 0.08% gellan; (E, e):0.3% MCC and 0.12% gellan; (F, f):0.3% MCC and 0.15% gellan (w/w).

□BS shown in Fig. 4b and c. Moreover, it is worth noting that an obvious increased △BS was observed at the bottom (0–15 mm) of beverage samples added with only gellan (Fig. 4c) but not in beverage samples added with only MCC (Fig. 4b). This indicates that, after the sterilization process, some suspended particles still settled to the bottom of beverages added with only gellan. It thus appears that the three-dimensional network formed by MCC has a better ability to resist intense high temperatures in comparison to the self-gelation mechanism of gellan. For beverage samples added with a combination of MCC and gellan, △BS of the beverage samples following sterilization varied according to the ratio of MCC and gellan. The sample added with F1 (Fig. 4d) showed the smallest △BS while the beverage added with F4 (Fig. 4g) exhibited a considerably larger △BS.

To further evaluate the differences in stability among the beverage samples added with differing ratios of MCC and gellan, the (TSI) of the beverage samples as a function of scanning time after the sterilization process was depicted in Fig. 5. A smaller change in TSI means better beverage stability (Ni et al., 2019). It is obvious that beverage samples added with F1 or F2 exhibited a slight TSI change during the monitoring process, indicating that the addition of F1 or F2 could effectively improve the physical and thermal stability of ginkgo beverages by protecting suspended particles from aggregation and precipitation during the sterilization process. It could be confirmed by a lower change in particle size (Fig. 1) and in viscosity (Table 2). However, the addition of F3 or F4 caused light intensity changes that were larger than that of

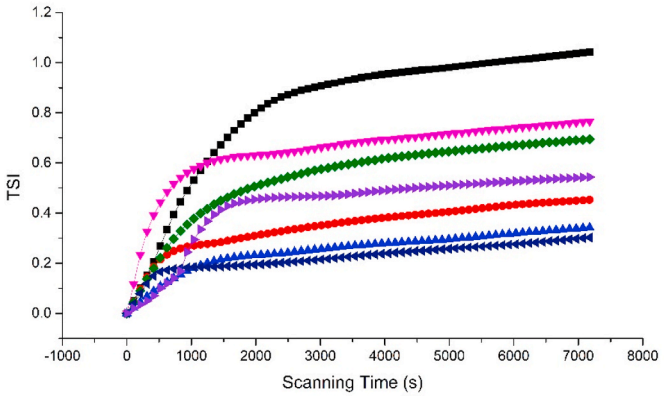

Fig. 5. The TSI of beverages added with different stabilizers after sterilization process. (—■—): Control; (—●—): MCC; (—▶—): gellan; (—▲—): F1; (—◀—): F2; (—◆—): F3; (—▼—): F4.

those observed in the samples added with only MCC or only gellan, suggesting that the addition of F3 or F4 was less effective at improving the thermal stability of beverages. The main difference in the formulations (F1, F2, F3, and F4) was the amount of GG. F1, F2, F3, and F4 contained 0.05%, 0.08%, 0.12%, and 0.15% (w/w) gellan, respectively. Based on the above findings, it can be concluded that the physical and thermal stability improvement of cloudy beverages depends on the amount of gellan added. MCC was beneficial for the improvement of the physical and thermal stability of cloudy ginkgo beverages when combined with a small amount of gellan (0.05% and 0.08%); however, the cloudy beverages showed low thermal stability when higher amounts of gellan were added (0.12% or 0.15%). This is also consistent with the results of our viscosity experiments. We assume that these results reflect the mutual influence of MCC and gellan during the formation of network structures. Both MCC and gellan have the ability to form the network structures. The addition of a small amount of gellan did not appear to affect the network structure formed by MCC. Rather, these results suggest that the GG in F1 and F2 combined synergistically with MCC to strengthen three-dimensional network structures, probably due to the emulsifier role of small amounts of gellan (Vilela & Cunha, 2016). When the content of gellan is increased, gellan molecules are more easily able to form a coil-helix structure through disordered flexible polymer chains connecting adjacent junction zones (Sworn, 2009). However, the network structure of MCC may hinder the connection between the gellan gum molecules, resulting in the formation of an unstable fluid gel structure. The formation process of many fluid gel structures are also able to destroy MCC network structures, leading to the formation of unstable combined network structures that can be easily damaged by sterilization (121 °C, 25 min), thereby decreasing beverage stability.

4. Conclusion

The autoclave sterilization process caused drastic changes in particle size, zeta potential, and viscosity of cloudy ginkgo beverages. Turbiscan analysis revealed that these changes resulted in aggregation and precipitation of suspended particles, causing beverage instability. The addition of MCC and gellan was observed to effectively improve the physical and thermal stability of cloudy ginkgo beverages. However, the protection capacity depended on the ratio of MCC to gellan. MCC combined with a small amount of gellan (0.05% or 0.08%, w/w) exhibited good performance in protecting suspended particles from aggregation and flocculation during the autoclave sterilization process. Moreover, Turbiscan showed that those particles were stably suspended in beverages. In contrast, our results suggest that increasing the gellan concentration to 0.12% or 0.15% (w/w) leads to mutual interference between MCC and gellan during the formation of network or gelation structures, resulting inpoor physical and thermal stability of cloudy ginkgo beverages.

CRediT authorship contribution statement

Yang Ni: Methodology, Data curation, Writing - original draft. **Xiaoshu Tang:** Data curation, Writing - review & editing. **Liuping Fan:** Conceptualization, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

The authors acknowledge financial support of the National Key research & Development Plan (2018YFC1603705-03), Jiangsu Key R & D plan, China (BE2019309), China National Natural Science Foundation (31871840), which has enabled us to carry out this study.

References

Akkarachaneeyakorn, S., & Tinrat, S. (2015). Effects of types and amounts of stabilizers on physical and sensory characteristics of cloudy ready-to-drink mulberry fruit juice. *Food Sciences and Nutrition, 3*(3), 213.

Bernat, N., Cháfer, M., Rodríguez-García, J., Chiralt, A., & González-Martínez, C. (2015). Effect of high pressure homogenisation and heat treatment on physical properties and stability of almond and hazelnut milks. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 62*(1), 488–496.

Briviba, K., Gräf, V., Walz, E., Guamis, B., & Butz, P. (2016). Ultra high pressure homogenization of almond milk: Physico-chemical and physiological effects. *Food Chemistry, 192*, 82.

Califano, Alicia, Lorenzo, & Zaritzky. (2013). Rheological analysis of emulsion-filled gels based on high acyl gellan;gum. *Food Hydrocolloids, 30*(2), 672–680.

Defeudis, F. V., & Drieu, K. (2004). "Stress-alleviating" and "vigilance-enhancing" actions of Ginkgo biloba extract (EGb 761). *Drug Development Research, 62*(1), 1–25.

Fiocchi, A., Brozek, J., Schünemann, H., Bahna, S. L., Berg, A. V., Beyer, K., et al. (2010). *World Allergy Organization (WAO) Diagnosis and Rationale for Action against cow's milk allergy (DRACMA) guidelines: HarperCollinsPublishers*.

Grasdalen, H., & Smidsrød, O. (1987). Gelation of gellan gum. *Carbohydrate Polymers, 7*(5), 371–393.

Hajmohammadi, A., Pirouzifard, M. K., Shahedi, M., & Alizadeh, M. (2016). Enrichment of a fruit-based beverage in dietary fiber using basil seed: Effect of Carboxymethyl cellulose and Gum Tragacanth on stability. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 74*, 84–91.

Kijima, T. (2006). Effects of microcrystalline cellulose on suspension stability of cocoa beverage. *Journal of Dispersion Science and Technology, 27*(7), 941–948.

Krawczyk, G., Venables, A., & Tuason, D. (2009). 27 – microcrystalline cellulose. *Handbook of Hydrocolloids, 44*, 740–759.

Leite, T. S., Augusto, P. E. D., & Cristianini, M. (2014). Using high pressure homogenization (HPH) to change the physical properties of cashew apple juice. *Food Biophysics, 258*(2), 1–12, 18.

Li, Y., & Fan, L. (2020). Comparative studies on the stabilization of Flos Sophorae Immaturus beverages by various hydrocolloids. *Lebensmittel-Wissenschaft & Technologie, 123*, 109117. https://doi.org/10.1016/j.lwt.2020.109117.

Li, Y., & Hu, C. (2015). *Ginkgo biloba L. 银杏 (Yinxing, Baiguo, ginkgo)*.

Liu, S., Sun, C., Xue, Y., & Gao, Y. (2016). Impact of pH, freeze-thaw and thermal sterilization on physicochemical stability of walnut beverage emulsion. *Food Chemistry, 196*, 475–485.

Li, Q., Wang, Y., Wu, Y., He, K., Li, Y., Luo, X., et al. (2019). Flexible cellulose nanofibrils as novel pickering stabilizers: The emulsifying property and packing behavior. *Food Hydrocolloids, 88*, 180–189. https://doi.org/10.1016/j.foodhyd.2018.09.039.

Li, W., Wang, Y., Zhao, H., He, Z., Zeng, M., Qin, F., et al. (2016). Improvement of emulsifying properties of soy protein through selective hydrolysis: Interfacial shear rheology of adsorption layer. *Food Hydrocolloids, 60*, 453–460. https://doi.org/10.1016/j.foodhyd.2016.04.019.

Lv, R., Kong, Q., Mou, H., & Fu, X. (2017). Effect of guar gum on stability and physical properties of orange juice. *International Journal of Biological Macromolecules, 98*, 565–574.

Matsumiya, K., Inoue, T., Niida, J., Katagiri, T., Nishizu, T., & Matsumura, Y. (2014). Evaluation of long-term stability of milk beverages by a novel method for rapid determination of aggregation forces between colloidal particles. *Food Hydrocolloids, 34*(1), 177–183.

Ni, Y., Li, J., & Fan, L. (2020). Production of nanocellulose with different length from ginkgo seed shells and applications for oil in water Pickering emulsions. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2020.01.263.

Ni, Y., Zhang, Z., Fan, L., & Li, J. (2019). Evaluation of physical stability of high pressure homogenization treatment cloudy ginkgo beverages. *Lebensmittel-Wissenschaft & Technologie, 111*, 31–38. https://doi.org/10.1016/j.lwt.2019.05.008.

Smith, K., Mendonca, A., & Jung, S. (2009). Impact of high-pressure processing on microbial shelf-life and protein stability of refrigerated soymilk. *Food Microbiology, 26*(8), 794–800.

Song, X., Zhou, C., Fu, F., Chen, Z., & Wu, Q. (2013). Effect of high-pressure homogenization on particle size and film properties of soy protein isolate. *Industrial Crops and Products, 43*(1), 538–544.

Sworn, G. (2009). 9–Gellan gum. *Handbook of Hydrocolloids, 37*(4), 204–227.

Vilela, J. A. P., & Cunha, R. L. D. (2016). High acyl gellan as an emulsion stabilizer. *Carbohydrate Polymers, 139*, 115–124.

Xu, D., Zhang, J., Cao, Y., Wang, J., & Xiao, J. (2016). Influence of microcrystalline cellulose on the microrheological property and freeze-thaw stability of soybean protein hydrolysate stabilized curcumin emulsion. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 66*, 590–597.

Yang, J. T., Cai, -E. W. U., Ying-Ying, L. I., Jia, S. Q., Fan, G. J., & Peng, F. R. (2011). Identification and purification of an allergic glycoprotein from ginkgo biloba kernel. *Journal of Integrative Agriculture, 10*(4), 631–641.

Yu, Z. Y., Jiang, S. W., Cao, X. M., Jiang, S. T., & Pan, L. J. (2016). Effect of high pressure homogenization (HPH) on the physical properties of taro (Colocasia esculenta (L). Schott) pulp. *Journal of Food Engineering, 177*, 1–8.

Zhang, H., Wang, Z., & Xu, S. Y. (2007). Optimization of processing parameters for cloudy ginkgo (Ginkgo biloba Linn.) juice. *Journal of Food Engineering, 80*(4), 1226–1232.