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Combinations of hydrocolloids show enhanced stabilizing effects on cloudy orange juice ready-to-drink beverages

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ABSTRACT

Cloudy ready-to-drink beverages have to be stabilized by hydrocolloids in order to prevent turbidity loss and undesirable sedimentation during storage time. The present work focuses on the stabilizing effects of pectin, guar gum and locust bean gum and the combinations of two of them on ready-to-drink beverages containing 7% of orange juice. The application and the potential combined effects of the mentioned hydrocolloids in these drinks has not been reported yet. The particle size distribution, zeta potential, viscosity, turbidity and sedimentation behaviour of beverages were measured to evaluate the impact of the different concentrations and combinations of the stabilizers. Applying 2.5 g pectin and 2.5 g guar gum per kg base was identified to be most suitable for stabilizing the turbidity over five weeks of beverages with orange juice concentrate, which contained large juice particles ranging from 0.6 to 200 μm. In contrast, 2 g pectin as well as the application of both 2.5 g pectin and 2.5 g locust bean gum per kg base maintained the storage stability of beverages containing juice concentrate, which was composed of smaller juice particles (0.5–120 μm). When locust bean gum and guar gum were applied without pectin being involved, negative effects on particle size and zeta potential could be observed leading to increased cloud loss and sedimentation rates compared with the other pectin-containing samples. The results indicated that the combination of hydrocolloids showed greater effects on long-term storage stability of the ready-to-drink beverages compared to the application of individual stabilizers.

1. Introduction

A major challenge in the food industry is the maintenance of turbidity in fruit juices over storage time. The stabilization of citrus-based cloudy beverages is necessary for sensory and consumer acceptance, as sedimentation of pulp in bottled drinks is considered a quality defect (Ellerbee & Wicker, 2011). The specialty of ready-to-drink non-carbonated drinks is that the dilution takes place at the producer's site instead of the costumer's, while their formulations are similar to those of dilutables (Brennan, 2016).

Flavor, color and aroma of citrus juices are attributed to the cloud particles, which is why removing the cloud mechanically would leave an undesirable watery material (Klavons, Benett, & Vannier, 1991).The clarification of orange juice beverages is commonly thought to be influenced by the action of the enzyme pectin methylesterase (PME) (Wicker, Ackerley, & Hunter, 2003). PME naturally occurs in citrus juices and causes cloud loss during storage by the blockwise de-esterification of methoxylated pectin. Pectin, an anionic polysaccharide in the cell wall of fruits, is composed of α-(1–4)-linked D-galacturonic acid (GalA) units (Aghajanzadeh, Kashaninejad, & Ziaiifar, 2018). Pectin may be methyl-esterified on C6 and acetylated on C2/C3, varying in its molecular weight, degree of esterification and the presence of sugar side chains at the galacturonic acid backbone (Wicker et al., 2003). If the degree of esterification (DE) is higher than 50%, it is

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classified as high-methoxyl pectin, whereas low-methoxyl pectin has a DE below 50% (Pegg, 2012).

Conventionally, elevated pasteurization temperatures are used for the inhibition of PME, but excessive heating has a negative impact on flavor and color of orange juice (Ibrahim et al., 2011). Moreover, there is still residual enzyme activity found after the pasteurization process (Rothschild & Karsenty, 1974). For the inhibition of the thermostable PME-isozyme, temperatures of 90 °C have to be applied to juices (Chen & Wu, 1998). Other treatments like dynamic high pressure homogenization are also applied instead in the juice industry (Lacroix, Fliss, & Makhlouf, 2005). However, as the inactivation of PME is not sufficient to prevent cloud loss in citrus juices completely, other factors might contribute to the process of clarification. In a previous study it was reported that divalent cations, such as calcium, could accelerate cloud loss in orange juice (Wicker et al., 2003). This result is in agreement with the findings of Ellerbee and Wicker (2011) about the influence of calcium and pH on orange juice cloud stability. They found that calcium ions form insoluble calcium pectate by crosslinking pectin chains resulting in large conglomerates that sediment along with the cloud particles. Furthermore, they reported that at pH 7 no cloud loss occurred, whereas the juices at pH 3–4 were the least stable. The authors suggested that this phenomenon is linked to protein coagulation and flocculation (Ellerbee & Wicker, 2011).

Aside from enzyme inactivation, particle size of the juice plays an important role in the stability of cloudy beverages (Tiwari, Muthukumarappan, Donnell, & Cullen, 2009). Orange juice cloud consists of complexes of proteins, lipids, cellulose, hemicellulose and pectin with particle sizes ranging from 0.4 to 5 μm (Genovese & Lozano, 2001). Cloud particles of 2 μm and smaller form more stable suspensions than large particles and are less likely to sediment during storage (Baker & Cameron, 1999). This can be explained by Stokes Law (Equation (1)), which states that the rate of sedimentation is based on the size of juice particles:

$$V_0 = \frac{\left(d_p{}^2\left(P_s - P_f\right)g\right)}{18V_f} \quad (1)$$

where V_0 is the settling velocity of particles [cm/s], d_p is the diameter of particles [cm], P_s is the density of particles [g/cm^3] and P_f of the fluid [g/cm^3]. V_f is the viscosity of the fluid [Pa] and g is the acceleration of gravity [9.8 m/s^2] (Lv, Kong, Mou, & Fu, 2017).

Homogenization is the leading process used in industry to decrease the particle size of fluids and to produce emulsions. It also influences the structure of pectin in fruit juices and reduces its de-esterification by PME, leading to extended shelf life (Aghajanzadeh, Ziaiifar, & Kashaninejad, 2017).

Another approach to improve the long-term stability of ready-to-drink beverages is the application of hydrophilic colloids (hydrocolloids or food gums). Hydrocolloids are polysaccharides used in the food industry for their water-holding capacity and their ability to modify rheological properties of solutions (Sinchaipanit, Kerr, & Chamchan, 2013). They are applied to food products like sauces, ice cream, instant foods and beverages functioning as thickeners, gelling agents, stabilizers or providers of a certain texture and mouthfeel (Karaman et al., 2014). Some hydrocolloids are negatively charged, like pectin or carboxymethylcellulose, leading to the stabilization of juice particles by increasing the inter-particle repulsive forces, since juice particles also have a negative electrical charge (Genovese & Lozano, 2001). The surface charge or zeta potential of hydrocolloids defines their stabilizing function (Wan et al., 2019). The larger the zeta potential (positive or negative) of particles, the more they will repel each other within the dispersion. Generally, dispersions with potentials exceeding +30 or −30 mV are considered to be stable (Genovese & Lozano, 2001).

Another mechanism of stabilization is steric or polymeric repulsion, a phenomenon which occurs when gum macromolecules, such as guar gum or locust bean gum, form a layer around juice particles and keep them suspended (Croak & Corredig, 2006). According to Stokes' law (Equation (1)), the sedimentation rate of particles is related to the density difference between serum and pulp particles. As a consequence, an increase in viscosity of the continuous phase leads to lower sedimentation rates (Sinchaipanit et al., 2013). Guar gum is a galactomannan, consisting of a chain of β-1,4 linked D-mannose and α-1,6 linked D-galactose units. The ratio between galactose and mannose units is 1:2 and is decisive for the rheological properties of the gum solutions (Hussain et al., 2015). Guar gum solutions show shear-thinning, non--Newtonian behaviour, where the viscosity decreases with increasing shear rate (Torres, Hallmark, & Wilson, 2014). Locust bean gum differs from guar gum in its carbohydrate-ratio of 1:3.5 and shows low solubility in cold water. It has to be heated above 80 °C to achieve maximum solubility, while guar gum is soluble at cold and hot temperatures (Pegg, 2012). Solutions of locust bean gum also showed non-Newtonian shear-thinning behaviour in viscosity studies (Karaman et al., 2014).

Although there is a wide range of hydrocolloids, the present work will focus on pectin, locust bean gum and guar gum, as they are commonly used in the beverage industry as additives. These stabilizers provide a positive public image due to their natural occurrence and are therefore suitable for "clean labelling". However, as far as the authors are aware, no published data is available concerning the application of the mentioned hydrocolloids in cloudy ready-to-drink beverages containing orange juice. Which parameters are decisive for maintaining the cloud stability and which stabilizers in which concentration and combination are useful in these specific drinks has not been systematically reported yet. Due to the different composition high methoxyl pectin and locust bean gum or guar gum stabilize beverages via different mechanisms. While pectin might be partially negatively charged in aqueous solutions, guar gum and locust bean gum are considered to be non-ionic in beverages. We hypothesize that a combination of steric and electrostatic repulsion might lead to an enhanced cloud stability.

The objective of this work was to investigate both, the effect of the individual stabilizers and their combination on the beverages' storage stability by means of turbidity, sedimentation, viscosity and zeta potential measurements. Furthermore, it was aimed to evaluate if long-term stability studies may be accelerated and replaced by using the LUMiFuge, an analytical centrifuge, which assesses the phase separation behaviour of fluids (Kuentz & Röthlisberger, 2003). So far, the applicability of named device was studied mainly for other food products such as emulsions and milk products (e.g. (Jin et al., 2017) (Kelleher et al., 2019)), and non-food products like coatings (Lacruz et al., 2021) which implies that there is a lack of information concerning ready-to-drink beverages with juice content. The results obtained may contribute to the optimization of stabilizers' application to ensure product quality that is appealing to consumers.

2. Materials and methods

2.1. Materials

Two types of orange juice concentrates (OJC1 and OJC2) were used for preparing the samples.OJC1 (Citrosuco, Sao Paulo, BRA) was a frozen concentrated orange juice low in pulp made from ripe Brazilian oranges (*Citrus sinensis* varieties). It contained a maximum of 2.5% pulp, had 65.5–66.5 °Brix and a pH of 3.7. It contained 1585 mg pectin per kg.

The second OJC (F.lli Branca Spa, Terme Vigliatore, IT) was a frozen orange juice concentrate low in pulp from ripe Italian oranges (*Citrus sinensis* (L.) Osbeck), containing a maximum of 1.0% pulp. According to the manufacturer, it had 59.0–61.0 °Brix and a pH of 3.0–3.6. OJC2 contained 1230 mg pectin per kg.

Pectin, guar gum (GG) and locust bean gum (LBG) were selected for evaluating the effect of adding stabilizers to ready-to-drink (RTD) beverages.

Pectin brown ribbon high viscosity was a pure, high methoxyl pectin (DE: 69–76%, calculated; method: 4.4.01.04.01.11–1) extracted from apple and citrus fruits, which is standardized to a defined viscosity of 350–600 mPa x s (2% solution, 25 °C), as stated by the manufacturer (Naturex AG, Bischofszell, CH). Its ash- and moisture-free polygalacturonic acid content is 65–100% (calculated; method: 4.4.01.04.01.11–4). The moisture is <12.0% and the pH (1% in water) is 2.8–3.8,

Guar gum VIDOGUM G200 I (UNIPEKTIN Integredients AG, Eschenz, CH), extracted from the endosperm of the guar plant seed (*Cyamopsis tetragonoloba)* had a viscosity of 3600–4500 (cold) and 4500–5300 (hot) mPa x s. According to the product specification, the minerals and ash content is <1.5% and the moisture is <13.5%. The pH value (1% in water) is 4.5–7.0. The molecular weight of guar gum was 50 000–8 000 000.

Locust bean gum VIDOGUM L200 (UNIPEKTIN Integredients AG, Eschenz, CH) had a hot viscosity of 1700–2300 mPa x s and was extracted from the endosperm of the locust bean seed (*Ceratonia siliqua)*. As stated by the manufacturer, it contained <1.2% minerals and ash and the moisture was <13.5%. The pH (1% in water) was 4.5–6.7. The molecular weight of locust bean gum was 50 000–3 000 000.

To investigate the effect of oil components on turbidity, orange oil (OO) (Ungerer Limited, Chester, UK) was added to one of the beverage bases. The oil was an orange to red liquid with a characteristic odour and a flash point of 43 °C.

Fanta Orange (Coca-Cola GmbH, Vienna, AT) acted as reference sample for RTD beverages because of its well-known cloud stability. It contained 5.3% orange juice from orange juice concentrate and guar gum as stabilizer.

2.2. Sample preparation

In order to prepare the beverage bases (Table 1) the OJC was mixed with water and 10% sodium benzoate solution (Emerald Kalama Chemical, LLC, US) and placed on a magnetic stirrer at room temperature for 15 min. The stabilizers or still water for the blanks were added while stirring continuously for 30 s at level 4.5 with a Turrax dispersing machine (Ultra-Turrax T 50, Janke & Kunkel IKA-Labortechnik). After adding ascorbic acid (Ningxia Qiyuan Pharmaceutical Co, Ltd., CN) and citric acid (monohydrate solution 50%, Sunshine Biotech International Co., Ltd., THA) the bases were pasteurized for 20 min in a water bath to 85 °C (Heizbad MB28/P, LHG). Then they were cooled down to room temperature and homogenized in two stages at 20 and 180 bar (Homo Lab 60-10, APV Gaulin).

The prepared bases with or without stabilizers were diluted to 1 l RTD beverages and adjusted to an orange juice content of 7.0%, 7.0 °Brix and a pH of approx. 3.6. This was achieved by adding still water, preservatives (10 %-solutions of sodium-benzoate and potassium-sorbate (Ningbo Wanglong Technology Co., Ltd, Zhehijang, CN)), sugar-solution (65%), ascorbic acid and citric acid (monohydrate solution 50%) (see Table 2). The samples were stored in 1 l PET bottles and a second sample preparation was filled in 1 l Imhoff cones. The final beverages contained 0.03–0.5% stabilizers.

Table 1
Different formulations of beverage bases.

Type of OJC	Amount of stabilizer per kg beverage base	Water (g)	Sodium-benzoate 10% (g)
OJC1	2 g pectin | GG | LBG	279.8	1.2
	5 g pectin | GG | LBG	502.0	1.2
	10 g pectin | GG | LBG	735.4	1.2
	combinations of 2.5 g + 2.5 g: pectin + GG | pectin + LBG | GG + LBG	502.0	1.2
	combinations of 2.5 g + 2.5 g: pectin + GG | pectin + LBG | GG + LBG + 3 g OO	502.0	1.2
OJC2	2 g pectin | GG | LBG	225.3	1.2
	combinations of 2.5 g + 2.5 g: pectin + GG | pectin + LBG | GG + LBG	465.3	1.2
	combinations of 3.0 g + 3.0 g: pectin + GG | pectin + LBG | GG + LBG	466.3	1.2

2.3. Particle size distribution

A Malvern Mastersizer 3000 instrument + Hydro LV (Malvern Instruments Ltd, Worcesterhire, UK) was used to determine the particle size distribution of the beverage bases by laser diffraction, before and after homogenization (ISO 13320:2020). The Hydro LV is an automated dispersion unit used for liquid samples. The sample was added manually till a light-shading value between 10 and 15% was achieved. Three independent measurements, lasting 30 s each, were carried out per sample. The data was analyzed using the Mastersizer 3000 software.

2.4. Viscosity

Rheological properties of the RTD beverages were analyzed using the controlled-stress Rheometer MCR300 (Anton Paar GmbH, Graz, AT), with a cone-plate configuration (cone diameter 25 mm, gap: 0.048 mm) (DIN 53019–1:2008–09). The apparent viscosity was measured within a linearly increasing shear rate range from 1 to 100 s^{-1} with ten measuring points at a shear rate of 100 s^{-1} at constant temperature (25 °C). Three independent measurements were made per sample. The data analysis was performed using the Rheoplus Software.

2.5. Zeta potential

The zeta potential of the samples (mV) was determined by using a ZetaView PMX-120 (ParticleMetrix GmbH, Munich, DE) (ISO 13099–1:2012). The RTD beverages were diluted 1:800 with water and the temperature was kept at 22 °C during the measurement. The sample volume injected was 1000 μl. The following parameters were optimized for the samples and set to (arbitrary units): shutter: 100, sensitivity: 80, framerate: 30, resolution: "medium", minimum brightness: 20, min area: 20, max area: 10000, tracelength: 15; Three independent profile measurements were performed for each sample and average values were calculated to assess the zetapotential.

2.6. Turbidity

The turbidity of samples stored in 1 l PET bottles at RT for a period of five weeks (35 days) was analyzed with a Turbiquant 1100 IR (Merck KGaA, Darmstadt, DE) by nephelometry (ISO 7027–1:2016). The sampling was carried out in triplicate within the same sample at two positions of the bottle (at 800 and 100 ml) after 0, 2, 7, 14, 21, 28 and 35 days to evaluate the progress of the turbidity loss from top to bottom. Constant attention was paid in avoiding the movement of samples to prevent the potential influence on turbidity.

2.7. Sedimentation

The RTD beverages were stored in 1 l Imhoff cones (Nalge Nunc Inc., New York, US) at RT for five weeks (35 days) to monitor their sedimentation behaviour. In the Imhoff cone test the sedimentable parts settle down to the base of a calibrated conical vessel, where their apparent volume can be transcribed (Ignatyeva, 2016). The sedimentation volume was read off after 0, 2, 7, 14, 21, 28 and 35 days.Sedimentation (%) was calculated using the following equation:

$$Sedimentation\ (\%) = \left(\frac{V_s}{V_t}\right) x\ 100 \qquad 2$$

where V_s is the sedimentation volume [ml] and V_t is the total volume of the beverage [ml] (Yu et al., 2021).

Table 2
Different formulations of RTD beverages.

RTD beverages	OJC1				OJC2		
Amount of stabilizer per kg beverage base	**2 g**	**5 g**	**10 g**	**2.5:2.5 g**	**2 g**	**2.5:2.5 g**	**3.0:3.0 g**
Water (g)	916.06	908.06	883.06	908.06	916.06	908.06	908.06
Sodium-benzoate 10% (g)	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Potassium-sorbate 10% (g)	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Ascorbic acid	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Citric acid 50% (g)	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Sugar	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Beverage base (g)	17.0	25.0	50.0	25.0	17.0	25.0	25.0

2.8. Accelerated long-term stability

Near infrared (NIR) transmission profiles of the RTD beverages were measured with a LUMiFuge 1514-85 (LUM GmbH, Berlin) to compare the long-term stability of different samples in the presence of accelerated gravitational force (ISO/TR 13097:2013). This device uses the STEP (Space and Time resolved Extinction Profiles) technology, where the transmitted light intensity is displayed as a function of time and position over the sample length during centrifugation. With the software SepView an instability index was calculated, which provides quantitative information about the clarification based on particle size and separation at a given time under accelerated conditions, divided by the total transmission increase (Yerramilli & Ghosh, 2017). Eight sample tubes (2 ml) were centrifuged per measurement cycle for 4 h at 4000 rpm, while 380 transmission profiles were recorded. Three independent measurements were carried out for each sample.

2.9. Statistics

Three independent measurements were performed with controls. Values are expressed as means ± standard deviations. Analysis of variance (ANOVA) was carried out using Sigmaplot 13.0 statistical software. Differences were evaluated for significance based on mean values and areas under the curve (AUC) and calculated at a significance level of $p < 0.05$ using the Holm Sidak method.

3. Results and discussion

3.1. Particle size distribution of the beverage bases and orange juice concentrates

For determining the particle size distribution, the beverage bases instead of the ready-to-drink beverages were measured, as the particle size did not change during the dilution process and to ensure that the sample's light-shading value was within the measuring range. Before measurement of the particle size distribution of the beverage bases the particles of the juice concentrates were analyzed. Diameters at 10% (Dv10), 50% (Dv50) and 90% (Dv90) of the volume distribution were calculated and presented as the particle size distribution. The differences between the groups were tested for significance based on AUC. Without homogenization the particles of OJC1 were significantly larger than that of OJC2 and Fanta Orange ($p < 0.05$), whereas the particle sizes of OJC2 and Fanta Orange were similar ($p > 0.05$) (AppendixFigure s1). After homogenization no significant difference between the juice concentrates and Fanta Orange could be determined ($p > 0.05$) (Appendix Figures s1A, s7 D, s15 D).

3.1.1. OJC1

The particle size of the OJC1 beverage bases with 2 g stabilizers per kg did not change during the homogenization process ($p > 0.05$) (Appendix Figure s2vs. s7). Before homogenization, significant differences in particle size could only be measured between the blank as well as the base containing pectin vs. the base containing GG ($p < 0.05$), where GG showed the largest particles (Appendix Figure s2D vs. B & A vs. B). Previously, a significant effect of guar gum on the cloud stability of orange juice compared to CMC (carboxymethylcellulose) hydrocolloid base component was reported (Lv et al., 2017). In another study, it could be demonstrated that a low concentration (0.1%) of guar gum influenced significantly the viscosity and the cloud stability of Kinnow juice (Aggarwal, Kumar, Yaqoob, Kaur, & Babbar, 2020). Even after homogenization the particle sizes of the bases with pectin and GG remained dissimilar ($p < 0.05$) (Appendix Figure s7A vs. B). Using higher concentrations of the stabilizers (5 g/kg and 10 g/kg), before processing, the particles of the bases with GG were the largest, followed by LBG, pectin and the blank ($p < 0.05$) (Appendix Figures s3 & s4). The homogenization process led to a decrease in particle size and the differences were no longer significant afterwards ($p > 0.05$) (Appendix Figures s8 & s9). The higher the concentration of GG and LBG in the base, the larger was the particle size, although the difference between 2 g and 5 g of LBG per kg was not statistically significant ($p > 0.05$) (Appendix Figures s2 C vs. s3 C). An increase of the pectin concentration in the base did not affect particle size significantly ($p > 0.05$) (Appendix Figures s2, s3, s4 A). Combining 2.5 g pectin with 2.5 g GG or LBG per kg base led to an enlargement of particles compared to beverages stabilized with pectin alone ($p < 0.05$) (Appendix Figures s3 A vs. s5 A & B). However, after homogenization the difference was no longer significant between 5 g/kg pectin and the combination of 2.5 g/kg pectin and 2.5 g/kg LBG ($p > 0.05$) (Appendix Figures s8 A vs. s10 B), Combining GG and LBG (Appendix Figure s5 C) led to an increase in particle size in comparison to all other formulations ($p < 0.05$), except from the application of 10 g GG, which still resulted in larger particles, even after homogenization ($p < 0.05$) (Appendix Figure s4 B & s9 B). In the same study of Lv et al. (2017) it could be shown that the combination had a significant effect. In case of combination of the guar gum with CMC (carboxymethylcellulose), guar gum with agar, and guar gum with xanthan gum, the particle size distributions were significantly increased compared with the particle size distribution of the control group with no hydrocolloid. However, the impact of homogenization on the particle size and cloud stability was not analyzed but was considered in the current study. The addition of OO to the OJC1 bases containing combinations of 2.5 g/kg stabilizers led to larger particles ($p < 0.05$) (Appendix Figure s6). This kind of enlargement was also observed in a study of Mirhosseini et al. (2008) where they found that orange oil affected the particle size in orange beverage emulsions (Mirhosseini et al., 2008). However, this effect could be overcome by homogenization in the current study (Appendix Figure s11).

3.1.2. OJC2

Particle sizes differed between the OJC2 beverage bases with 2 g stabilizers per kg ($p < 0.05$) (Appendix Figure s12). Again, the addition of GG as well as LBG led to the largest particle sizes, followed by pectin and the blank. Aggarwal et al. (2020) found that juice and beverages containing CMC and sodium alginate showed significantly higher cloud stability than other hydrocolloids. After reducing the particle size by homogenization, the differences were no longer significant ($p > 0.05$) (Appendix Figure s15). When the stabilizers were combined in concentrations of 2.5 g each per kg significant differences could be determined between all OJC2 bases ($p < 0.05$), apart from those with pectin & GG vs. GG & LBG ($p > 0.05$) (Appendix Figure s13). This variability in particle size remained after the homogenization process (Appendix Figure s16). HPH processing has the potential to significantly alter the structural characteristics of suspended particles, which could therefore have an impact on the beverage's overall physical characteristics (Yu, Jiang, Cao, Jiang, & Pan, 2016). Ni, Zhang, Fan, and Li (2019) found that the particle size and zeta potential decreased gradually when homogenization pressure increased due to the disruption of particles in taro pulp products.

An increase in the stabilizers' amount to combinations of 3 g each per kg did not change the particle size significantly compared to the beverages bases with combinations of 2.5 g each per kg ($p > 0.05$) (Appendix Fig. s14). It is assumed that the rise in concentration was too small to show an effect. These beverages were excluded from zeta potential and viscosity measurements.

The results of the particle size measurement showed that the homogenization process, the type and concentration of stabilizer as well as the orange juice concentrate had an influence on the particle size distribution of the beverage bases. The authors reported that in case of combining pectin and CMC, the cloudiness of orange beverage emulsions has significantly decreased during six months storage depending on the type and concentration of hydrocolloid as well as storage time (Mirhosseini et al., 2008). LBG and GG led to an enlargement of particles of the bases, while in beverage bases containing pectin only small differences of particle sizes could be observed compared to the blank. Homogenization of the beverage bases decreased the size of particles in all samples and often reduced the extent of variability between the particle sizes of bases with different stabilizers. The reduction of particle sizes of orange juice concentrate by homogenization which was observed in this study has been described before, although higher pressures (250–1500 bar) were applied (Leite, Augusto, & Cristianini, 2016). In watermelon juice the correlation between particle size, homogenization, rheology and the addition of xanthan was investigated, where they found that the xanthan-induced enlargement of particles led to higher viscosity and that the homogenization process enhanced the repulsion forces between the negative charged juice particles and xanthan (Aghajanzadeh et al., 2017). The addition of LBG and GG showed a visible viscosity enhancing effect on the beverage bases even after being homogenized. Thus, the impact of stabilizers on the rheology of final beverages is discussed in the following.

3.2. Rheological properties of ready-to-drink beverages

The rheological measurements (https://doi.org/10.25365/phaidra.342) did not show significant differences in apparent viscosity between the RTD beverages with different stabilizers and orange juice concentrates ($p > 0.05$). As the beverage bases showed visibly diverse viscosities, it was assumed that the final concentration of stabilizers in the RTD beverages (0.03–0.5%) was too low to influence the solutions' rheological properties. Results from literature on gum and pectin solutions confirmed that in concentrations of 5–20 g/l these solutions resemble each other in their shear-thinning non-Newtonian behaviour, but differ in their apparent viscosity (Owens, Lotzkar, Merrill, & Peterson, 1944) (Elfak, Pass, & Phillips, 1979) (Torres et al., 2014) (Yin, Sim, James, & Jun, 2017).

3.3. Zeta potential of particles (mV) in ready-to-drink beverages

As expected, due to its negative net charge, the addition of pectin to RTD beverages led to an increase in the negative zeta potentials compared to the blank (Table 3), but the differences were only statistically significant in beverages containing OJC1 with 2 and 5 g/kg pectin ($p < 0.05$). The results showed that higher concentrations of pectin had no effect on the magnitude of the zeta potential ($p > 0.05$).

The zeta potential of Fanta Orange (−39.25 ± 1.60 mV) was more negative than that of OJC1 in case of combining hydrocolloids without

Table 3
Zeta potential of RTD beverages with different concentrations of pectin, GG and LBG.

RTD	zeta potential (mV)
Fanta Orange	−39.25 ± 1.60
OJC1 2 g/kg Blank	−29.01 ± 2.26[a]
OJC1 2 g/kg Pectin	−45.92 ± 2.15[b]
OJC1 2 g/kg GG	−33.87 ± 2.32[a]
OJC1 2 g/kg LBG	−33.46 ± 0.57[a]
OJC1 5 g/kg Blank	−28.67 ± 2.21[a]
OJC1 5 g/kg Pectin	−45.51 ± 2.80[b]
OJC1 5 g/kg GG	−35.93 ± 1.34[a]
OJC1 5 g/kg LBG	−36.16 ± 3.39[a]
OJC1 10 g/kg Blank	−40.03 ± 3.00[a]
OJC1 10 g/kg Pectin	−43.93 ± 1.35[a]
OJC1 10 g/kg GG	−32.69 ± 1.74[b]
OJC1 10 g/kg LBG	−21.76 ± 1.53[c]
OJC1 2.5 + 2.5 g/kg Blank	−35.46 ± 2.89[a]
OJC1 2.5 + 2.5 g/kg Pectin & GG	−40.24 ± 2.07[a]
OJC1 2.5 + 2.5 g/kg Pectin & LBG	−38.34 ± 1.09[a]
OJC1 2.5 + 2.5 g/kg GG & LBG	−28.95 ± 1.10[b]
OJC1 2.5 + 2.5 g/kg Blank + OO	−33.17 ± 0.56[a, b]
OJC1 2.5 + 2.5 g/kg Pectin & GG + OO	−35.63 ± 1.26[b]
OJC1 2.5 + 2.5 g/kg Pectin & LBG + OO	−40.86 ± 0.99[c]
OJC1 2.5 + 2.5 g/kg GG & LBG + OO	−31.67 ± 0.94[a]
OJC2 2 g/kg Blank	−40.64 ± 0.19[a]
OJC2 2 g/kg Pectin	−42.76 ± 1.54[a]
OJC2 2 g/kg GG	−43.29 ± 1.17[a]
OJC2 2 g/kg LBG	−30.06 ± 0.48[b]
OJC2 2.5 + 2.5 g/kg Blank	−45.00 ± 3.34[a]
OJC2 2.5 + 2.5 g/kg Pectin & GG	−38.86 ± 0.53[a, b]
OJC2 2.5 + 2.5 g/kg Pectin & LBG	−39.58 ± 1.30[a, b]
OJC2 2.5 + 2.5 g/kg GG & LBG	−35.49 ± 1.05[b]

mean values ± standard deviations; differences between groups were evaluated for significance based on mean values ($n = 3$) and calculated at a significance level of $p < 0.05$; different small letters indicate significant difference between the zeta potential of beverages with different hydrocolloids of the same concentration.

OO where the values ranged between (−28)-(-40)mV ($p < 0.05$), but similar to the zeta potential of OJC2 when the hydrocolloids were combined ((−35)-(-45)mV; $p > 0.05$). It could be reduced when adding or combining two hydrocolloids such as xanthan gum and CMC due to increasing the electronegativity depending on the type and the concentration of the hydrocolloids (Genovese & Lozano, 2001). For the current study, it can be suggested that the smaller juice particles of Fanta and OJC2 led to the larger negative zeta potentials. This effect was also observed in a study of Nakatuka, Yoshida, Fukui, and Matuzawa (2015) where they found a correlation between the size of spherical silica particles and their zeta potential by the use of the sedimentation method. According to the former studies, mixed emulsifiers or hydrocolloids could enhance the electrostatic repulsion and the absorption of protective layers at oil-water interfaces of the beverage emulsions (Liu, Sun, Xue, & Gao, 2016). Similarly, in the current study, the combination of pectin and LBG in the case of OJC2 led to a zeta potential as low as in Fanta Orange, which was not observed when LBG was applied alone.

Adding stabilizers to bases with OJC2 had no significant effect on the zeta potential of the beverages ($p > 0.05$), except the ones with LBG, which led to a weaker negative zeta potential than the blank, possibly because of the LBG-induced particle size enlargement ($p < 0.05$). The application of 10 g LBG per kg base to OJC1 had the same decreasing effect on the negative zeta potential compared to the blank ($p < 0.05$).

Since GG and LBG are neutral polysaccharides, the negative zeta potential of the RTD beverages with these stabilizers was solely caused by the negative charge of the orange juice particles. The stabilization mechanism of steric instead of electrostatic repulsion by GG was shown in zeta potential measurements of carrot juice in a previous study (Qin, Xu, & Zhang, 2005). Consequently, the application of GG and LBG did not increase the absolute value of negative zeta potential of the beverages in comparison to the blank ($p > 0.05$). As described, in some cases the negative zeta potential was even decreased due to larger particle sizes, although not significantly by the addition of GG ($p > 0.05$). When pectin was combined with either GG or LBG in the OJC1 beverages the effect on the zeta potential was lower than in beverages with pectin alone ($p < 0.05$), which may also be explained by the neutral charge of GG and LBG.

OO as an additive did not significantly affect the zeta potential compared to the samples containing 2.5 g and 2.5 g stabilizers without OO ($p > 0.05$). This may be due to the low dosage of the oil, as OO in higher concentrations led to negative zeta potentials in emulsions in a former study (Acedo-Carrillo et al., 2006).

The concentration of stabilizers within the ready-to-drink beverages did not have an effect on the magnitude of the zeta potential, which was shown in polymer solutions of pectin, LBG and GG in 0.1 M HCl before (Barbosa, Abdelsadig, Conway, & Merchant, 2019). The addition of pectin led to an increase in the absolute value of the negative zeta potential of ready-to-drink beverages containing OJC1, while the zeta potential of the beverages with OJC2 did not change significantly ($p > 0.05$). As mentioned before, the electrostatic repulsion between negatively charged hydrocolloids and the juice particles may lead to a stable cloud by influencing the turbidity and sedimentation behaviour as reported for other types of juice beverages (Genovese & Lozano, 2001) (Udomsup, Therdthai, & Harnsilawat, 2011) (Babbar, Aggarwal, & Oberoi, 2015) (Aghajanzadeh et al., 2017).

3.4. Turbidity stability of the RTD beverages

While some beverages clarified to varying degrees from top to bottom ($p < 0.05$), others showed steady turbidity loss within the bottle. The latter was observed in the RTD beverages with pectin & GG 2.5 g + 2.5 g/kg (OJC1), LBG 5 g/kg (OJC1), GG 5 g/kg (OJC1) and pectin & LBG 2.5g + 2.5 g/kg (OJC2) (Fig. 1 A & Appendix Figures s20 B & C, s25).

Fanta Orange showed higher turbidity than the blank with OJC1 and OJC2 (Appendix Figures s18 vs. s19 D & s24 D). It is assumed that this was caused by the colourant carotene. The blank with OJC2 was more turbid than with OJC1, which can be explained by the smaller juice particles, as there is an inverse relationship between particle size and turbidity (Wu, Jiang, & Wheatley, 2009).

3.4.1. OJC1

In the RTD beverages with a stabilizer content of 2 g per kg base, lower turbidity loss could be measured compared to the blank ($p < 0.05$) (Appendix Figure s19). However, undesirable clarification still occurred in all samples and the beverages' visual appearance was unstable over storage time.Increasing the amount of stabilizers to 10 g per kg base had a positive impact on the turbidity stability of the RTD beverages (Appendix Figure s21). On the other hand, the sedimentation volume was also increased (as shown in Fig. 3 C) and led to visible deposits on the bottom of the bottle. When 5 g stabilizers per kg base had been added, the clarification process was delayed and the turbidity difference between the top and the bottom position was reduced to a minimum. The turbidity of the RTD beverages stabilized by hydrocolloids was significantly higher than of the blank ($p < 0.05$) (AppendixFigure s20).

In Fig. 1 it is shown that the combination of pectin & GG had the most stabilizing effect on the cloud of RTD beverages compared to the other samples. When pectin was combined with LBG, turbidity was also stable, however, at the end of storage time, lower than in the combination with GG ($p < 0.05$) (Fig. 1 A vs. Appendix Figure s22). GG & LBG in combination led to higher sedimentation amounts (Fig. 3 D) and unsteady clarification from top to bottom (Fig. 1 C) in comparison to the other stabilizers ($p < 0.05$).

Adding OO led to a significant increase in turbidity ($p < 0.05$) in all formulations (Appendix Figure s23), an effect described in a previous study in orange beverage emulsions (Mirhosseini et al., 2008).Although turbidity had been high in the beginning, rapid clarification occurred over the 5 weeks, particularly in the formulations with GG & LBG. This is in accordance with the sedimentation behaviour of the RTD-beverage stabilized by GG & LBG, where it showed the highest sedimentation volumes (Fig. 3 E).

3.4.2. OJC2

When 2 g stabilizers had been added to the bases with OJC2, pectin provided the highest turbidity-stabilizing effect on the RTD beverages followed by GG, LBG and the blank ($p < 0.05$) (Appendix Figure s24). In the Imhoff test, 2 g of pectin also showed lower sedimentation volumes than GG and LBG (see Fig. 4 A), which suggests that pectin is suitable for the stabilization of beverages with OJC2. As described before, the negatively charged pectin particles might keep the juice particles in suspension through electrostatic repulsion. This effect could be confirmed by the increase of the absolute value of the negative zeta potential after the addition of pectin (Table 3), although not statistically significant ($p > 0.05$).

Pectin combined with GG showed lower turbidity than pectin alone, while combined with LBG higher levels were achieved ($p < 0.05$) (Fig. 2

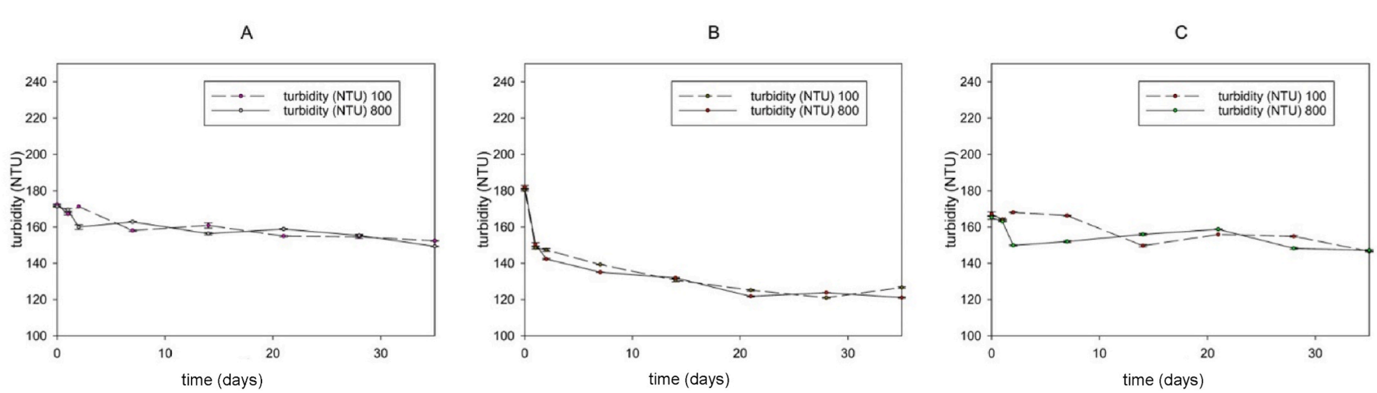

Fig. 1. Turbidity stability of RTD beverages with OJC1 stored for 35 days at RT in 1 l PET-bottles; A: 2.5 g pectin +2.5 g GG per kg base, B: blank, C: 2.5 g GG + 2.5 g LBG per kg base; the dotted line shows the turbidity in NTU (nephelometric turbidity units) at 100 ml and the continuous line at 800 ml of the bottle; mean values ± standard deviations; differences between groups were evaluated for significance based on mean AUC (n = 3) and calculated at a significance level of $p < 0.05$.

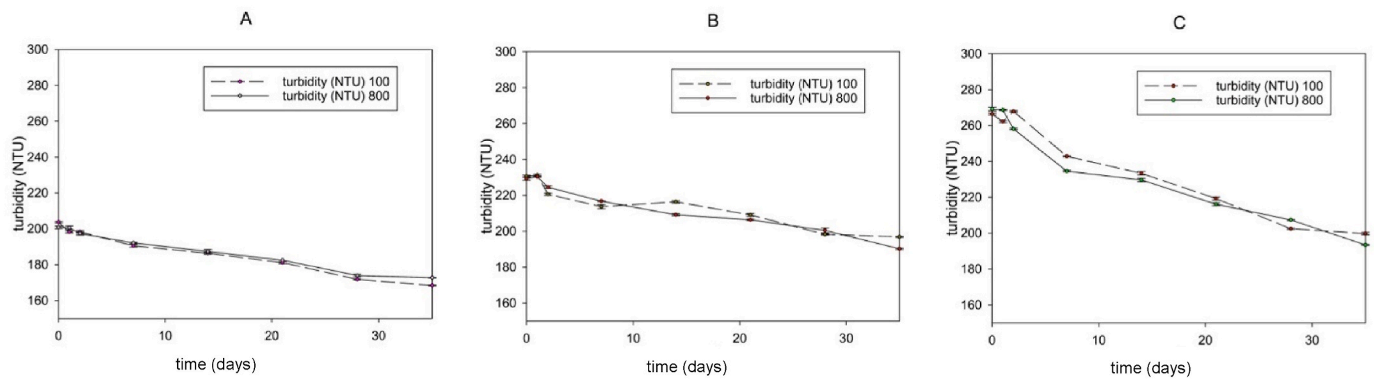

Fig. 2. Turbidity stability of RTD beverages with OJC2 stored for 35 days at RT in 1 l PET-bottles; A: 2.5 g pectin +2.5 g GG per kg base, B: blank, C: 2.5 g GG + 2.5 g LBG per kg base; mean values ± standard deviations; differences between groups were evaluated for significance based on mean AUC (n = 3) and calculated at a significance level of $p < 0.05$.

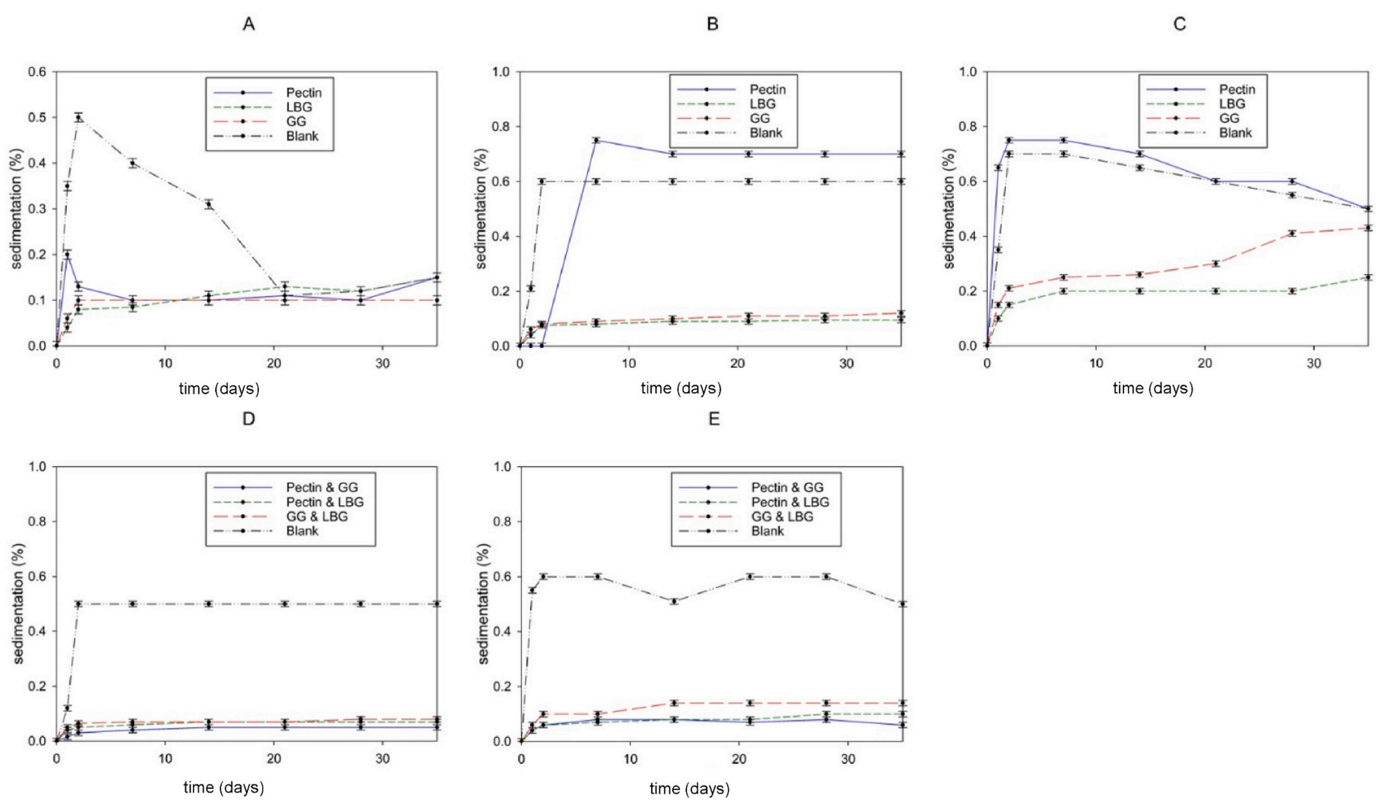

Fig. 3. Sedimentation in RTD beverages with OJC1 stored for 35 days at RT in 1 l Imhoff cones; A: 2 g stabilizer per kg base, B: 5 g stabilizer per kg base, C: 10 g stabilizer per kg base, D: combinations of 2.5 g + 2.5 g stabilizers, E: combinations of 2.5 g + 2.5 g stabilizers + 3 g OO; mean values ± standard deviations; differences between groups were evaluated for significance based on mean AUC (n = 3) and calculated at a significance level of $p < 0.05$.

A vs. Appendix Figures s24 A vs. s25). GG and LBG in combination showed the highest turbidity in the beginning but then rapid cloud loss occurred over storage time (Fig. 2), an effect which may be related to the high sedimentation rate of the larger particles (Fig. 4 B). Increasing the concentration of stabilizers to a combination of 3 g each per kg base led to a significant decrease in turbidity ($p < 0.05$) resulting in the lowest cloud stability (Appendix Figure s26).

In conclusion, the cloud of RTD beverages with OJC1 was most stable over 5 weeks when a combination of 2.5 g pectin and 2.5 g GG had been added to the beverage bases. In contrast, the highest stability of the turbidity in beverages with OJC2 was achieved by adding pectin alone or in combination with LBG.

The turbidity loss of beverages may be directly linked to the sedimentation of cloud particles, an effect that has been observed in cloudy apple juice before (Genovese & Lozano, 2001).

3.5. *Sedimentation of cloud particles within the RTD beverages*

3.5.1. *OJC1*

The addition of 2 g stabilizers to the bases already led to decreased sedimentation in comparison to the blank ($p < 0.05$) (Fig. 3 A). Nevertheless, between the stabilizers themselves no significant differences could be measured ($p > 0.05$). Increasing the amount of pectin to 5 g or 10 g per kg base generated higher sedimentation amounts in Imhoff cones compared to GG, LBG and the blank, suggesting that the dosage of pectin should not exceed 2 or 2.5 g per kg base (Fig. 3 B & C). In contrast, beverages with GG and LBG showed similar lower sedimentation volumes compared to the blank ($p < 0.05$).

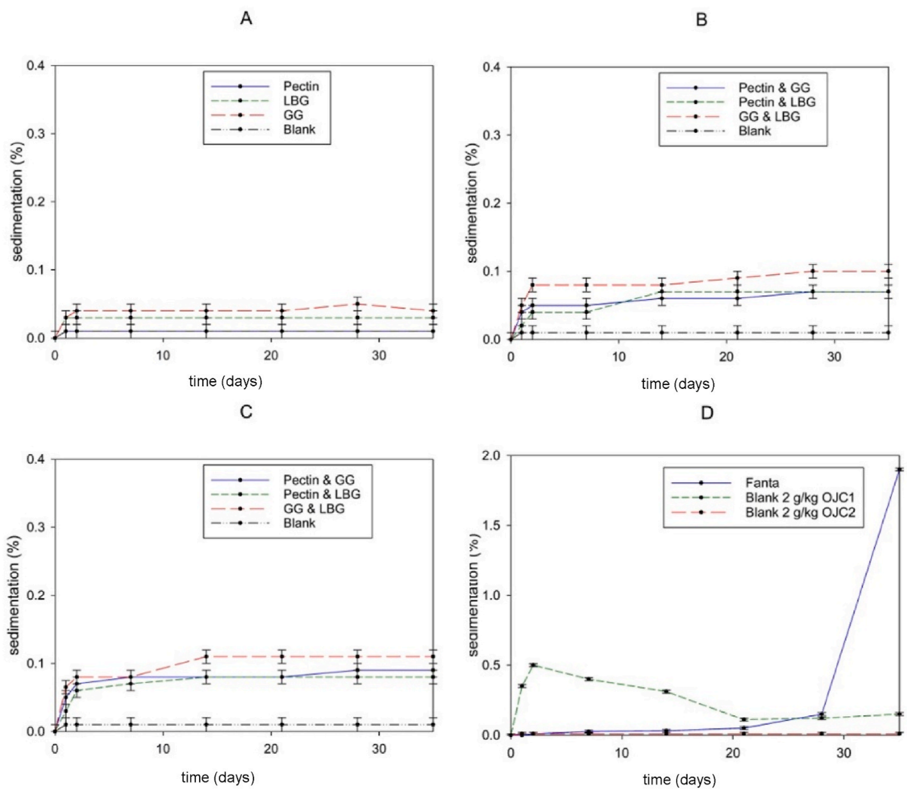

Fig. 4. Sedimentation in RTD beverages with OJC2 and Fanta Orange vs. blanks stored for 35 days at RT in 1 l Imhoff cones; A: 2 g stabilizer per kg base, B: combinations of 2.5 g + 2.5 g stabilizers, C: combinations of 3 g + 3 g stabilizers, D: Fanta vs. blank OJC1 and OJC2; mean values ± standard deviations; differences between groups were evaluated for significance based on mean AUC (n = 3) and calculated at a significance level of $p < 0.05$

When the stabilizers were combined in a concentration ratio of 2.5:2.5 g/kg the lowest sedimentation amount was achieved by pectin & GG, followed by pectin & LBG and GG & LBG (Fig. 3 D). However, only the difference between the combination of pectin & GG and GG & LBG was significant ($p < 0.05$). It may be concluded that pectin could be either used with GG or LBG to decrease the beverage's sedimentation amount. All combinations showed significantly lower sedimentation than the blank ($p < 0.05$).

Adding OO to the beverage bases only increased the sedimentation amount for the blank and the combination of GG & LBG (Fig. 3 E). This could be explained by the fact that pectin may stabilize the juice particles by electrostatic repulsion and as OO is also negatively charged (Table 3), it may be kept in suspension in the presence of pectin.

3.5.2. OJC2

An overall trend was observed in the blank, which showed the lowest sedimentation for all samples. This may be due to the fact that the particles of OJC2 were smaller and more negatively charged than those of OJC1 and the RTD beverages were therefore stable without stabilizers. This hypothesis could be confirmed by the lower sedimentation of the blank for OJC2 over time than for OJC1 and Fanta Orange (Fig. 4 D). In Fig. 4 A it is shown that the addition of 2 g GG and LBG per kg base led to higher sedimentation compared with pectin and the blank, however sedimentation was only significantly increased by GG ($p < 0.05$).

Because of the sudden rise in sedimentation of Fanta Orange at the end of the observational study it might be assumed that there would occur clarification at the same time within the PET bottle. However, such turbidity loss could not be measured (Appendix Figure s18). This might be explained by the fact that no additional preservatives were applied to the reference sample in contrast to the other RTD beverages, leading to rapid turbidity loss within the Imhoff cone. These assumptions agree with the results of a previous study, where the lack of preservatives had an impact on physicochemical parameters of coconut beverages, including turbidity (Pereira & Faria, 2013).

The addition of 2 g pectin to the base did not change the sedimentation behaviour over storage time compared to the blank ($p > 0.05$) (Fig. 4 A) as it had no significant impact on size or zeta potential of the particles in the processed beverage (Figure s12 A and Table 3). Because of the increase in particle size by GG, sediments tended to form more easily. For that reason, in the RTD beverages stabilized by 2.5 g GG and 2.5 g LBG per kg base, the highest sedimentation amount was measured because the larger particles settled to the bottom of the cone (Fig. 4 B). The inverse relationship between particle size and sedimentation stability was shown for carrot juice in a previous study of Reiter, Neidhart, and Carle (2003). However, no stabilizers were applied to these samples (Reiter et al., 2003).

Increasing the amount of hydrocolloids from 2.5 g each to 3 g each per kg base did not have a significant impact on the sedimentation of particles within the RTD beverages ($p > 0.05$) (Fig. 4 C).

While the addition of pectin, GG and LBG to the beverage bases with OJC1 in concentrations up to 5 g/kg led to lower sedimentation volumes for the RTD beverages than the blank, beverages with OJC2 showed less sedimentation even without stabilizers because of their smaller juice particles. However, as described before, the lack of stabilizers led to undesirable turbidity loss in the OJC2 samples (Fig. 2 B & Appendix Figure s24 D, s26 D). The combination of pectin & GG showed the most stabilizing effect on the beverage with OJC1 in turbidity and sedimentation studies.

3.6. Accelerated long-term stability of RTD beverages

Fig. 5 inserted.

RTD beverages containing OJC1 and LBG were the least stable in all concentrations in comparison to those with pectin, GG and the blank (Fig. 5A–C). The higher the concentration of LBG, the less stable was the RTD beverage. Similar results were found in a previous study, where

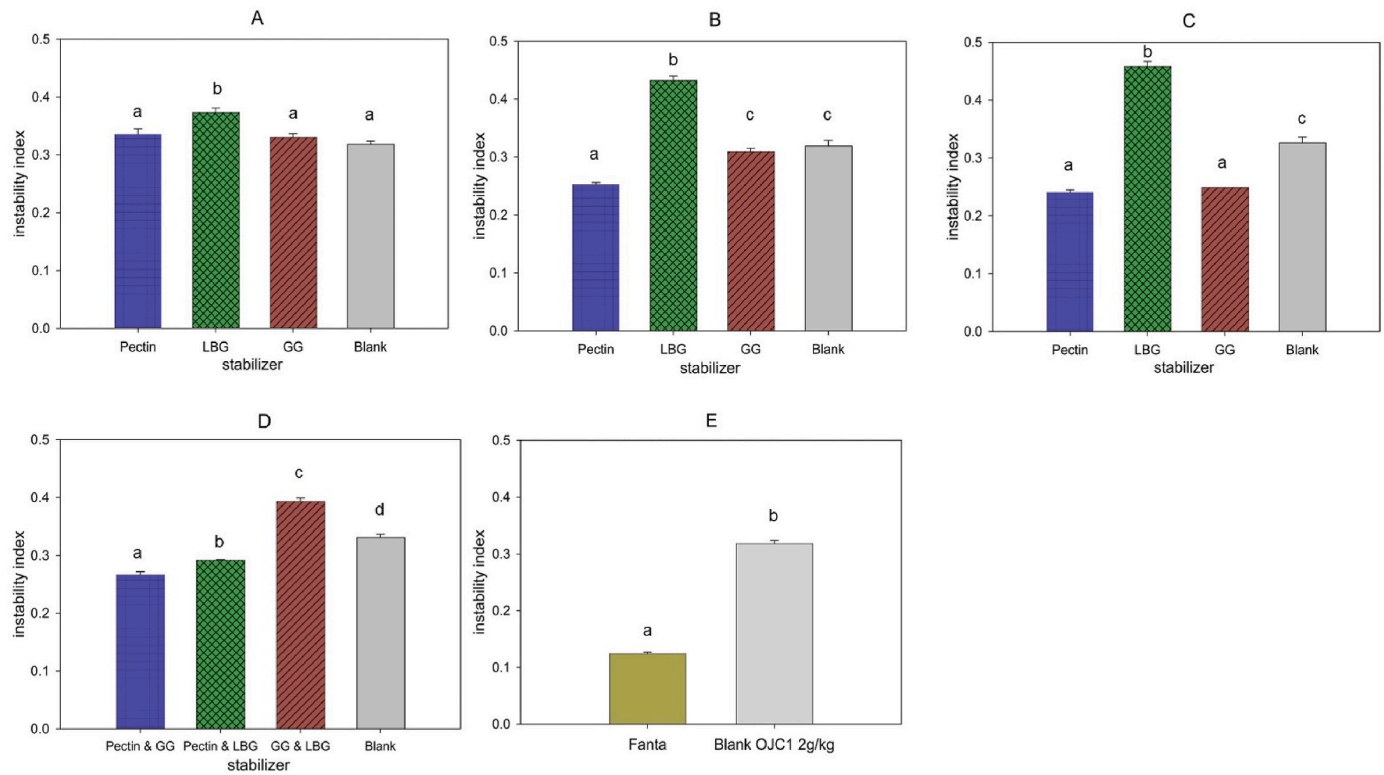

Fig. 5. Instability indices of RTD beverages with OJC1 and Fanta Orange measured with the LUMiFuge under accelerated conditions; A: 2 g stabilizer per kg base, B: 5 g stabilizer per kg base, C: 10 g stabilizer per kg base, D: combinations of 2.5 g + 2.5 g stabilizers per kg base, E: Fanta Orange vs. blank; mean values ± standard deviations; differences between groups were evaluated for significance based on mean values (n = 3) and calculated at a significance level of $p < 0.05$; different small letters indicate significant difference. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

higher concentrations of xanthan gum in water led to increasing clarification within the sample (Kuentz & Röthlisberger, 2003). When LBG was combined with pectin, a lower instability index could be achieved, which provided a significantly better stability than the blank and the combination of GG & LBG ($p < 0.05$) (Fig. 5 D). The latter even had a negative impact on the stability of the RTD beverages, which was also observed in the Imhoff test, where high sedimentation volumes were measured (Fig. 3 D).

In contrast, Fanta Orange had the lowest instability index compared with all other samples ($p < 0.05$) (Fig. 5 E), which is in agreement with the turbidity measurements (Appendix Figure s18).

The instability indices of the beverages with 2 g pectin and GG per kg base were not significantly different from the blank ($p > 0.05$), in contrast to RTD beverages with LBG ($p < 0.05$) (Fig. 5 A). The results were confirmed by the turbidity measurements, where the RTD beverages with 2 g stabilizers could not prevent cloud loss (Appendix Figure s19). The application of 5 and 10 g pectin per kg base led to lower instability indices compared to the blank ($p < 0.05$) (Fig. 5 B & C), although the sedimentation amounts were higher (Fig. 3 B & C). It is suggested that the low instability index can be attributed to the fact that the value only considers the sedimentation velocity but does not quantify the sedimentation volume itself (Hoffmann, 2016). While a concentration of 5 g GG per kg base had no impact on the stability of RTD beverages ($p > 0.05$), an amount of 10 g GG showed a significantly lower instability index than the blank ($p < 0.05$) (Fig. 5 B & C).

In analogy to the sedimentation and turbidity tests, the combination of 2.5 g pectin and 2.5 g GG per kg base showed the most effective stabilization effect compared to the other stabilizers and the blank ($p < 0.05$) (Fig. 5 D). While the current results suggest LUMiFuge being a promising alternative to long-term stability studies, further measurements should be considered with other types of beverages to confirm the possibility of accelerating and replacing storage tests by using the LUMiFuge.

A study investigated the impact of some hydrocolloid blends, such as pectin and carboxymethylcellulose, on the viscosity and sensory properties of raspberry juice-milk; the viscosity of juice-milk in case of blend gums was increasing gradually in parallel with increasing the concentration of the blend gums at certain limited level. Also, the sedimentation was inversely proportional to the viscosity changing state, which can be explained by the mixing effect of the blend gums and their ability of strong water absorption (Abedi, Sani, & Karazhiyan, 2014). Likewise, combining methoxyl pectin and locust bean gum or guar gum showed similar results in terms of cloudiness stability and other rheological properties in the current study, most likely due to the steric and electrostatic repulsion elicited only by applying both hydrocolloids simultaneously. Guar gum might even interact with the hydrophobic moieties of the high methoxyl pectin chain, which might also explain the improved cloud stability when both hydrocolloids were combined.

4. Conclusions

The present work revealed that the addition of 2.5 g pectin and 2.5 g GG per kg base significantly ($p < 0.05$) improved cloud stability in RTD beverages with OJC1 within 5 weeks of storage at room temperature. For OJC2, an application of 2 g of pectin or the combination of 2.5 g pectin and 2.5 g LBG per kg base were both capable of stabilizing the beverages regarding turbidity and sedimentation. OJC2 revealed to be more stable than OJC1 due to its smaller particles and the higher negative zeta potential. The well-known stabilization mechanisms of the hydrocolloids are particle-to-particle repulsion by pectin and steric repulsion by GG and LBG. Nevertheless, GG and LBG alone and combined adversely affected the beverages' stability because the enlargement in particle size tended to offset their stabilizing ability and led to higher sedimentation amounts compared to pectin and the blank. This comprehensive analysis reveals the advantages of combining stabilizers in cloudy ready-to-drink beverages and contributes to a more effective and profitable application of hydrocolloids.

Author contributions

Lisa Staubmann: Validation, Formal Analysis, Writing – Original Draft, Visualization, Investigation; Agnes Mistlberger-Reiner: Software, Methodology, Writing – Review & Editing; El Mehdi Raoui: Writing – Review & Editing; Gertrude Brunner: Conceptualization, Methodology, Resources, Project Administration; Lisa Sinawehl: Software, Methodology, Writing – Review & Editing; Marion Winter: Conceptualization, Resources, Project Administration, Funding acquisition; Robert Liska: Writing – Review & Editing; Marc Pignitter: Writing – Review & Editing, Supervision.

Declaration of competing interest

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Data availability

I have shared the link to my data in the manuscript in the section appendix A supplementary data

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2022.108436.

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