Effect of pH and Sucrose on Physical Properties of Drinking Yoghurt Stabilized by Whey Protein Concentrate

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ผลของพีเอชและซูโครสต่อสมบัติทางกายภาพของโยเกิร์ตพร้อมดื่มที่เพิ่มความคงตัวด้วยโปรตีนเยลลี่แบบเยมยัน

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บทคัดย่อ

งานวิจัยนี้ทำการศึกษาผลของพีเอช (3.5, 4.0 และ 4.5) และรูโตรส (0.0 และ 1.0%, W/W) ต่อสมบัติทางกายภาพของโยเกิร์ตพร้อมเลมที่เพิ่มความคงตัวด้วยโปรตีนเยลลี่แบบเยมยัน (Whey Protein Concentrate) ที่ 0.0, 0.5, 1.0, 1.5 และ 2% (W/W) โดยนำยีน์สชันโยเกิร์ตพร้อมเลมมาทำกาพัฒนาชัวและโยเกิร์ตข้าวไทยในช่วงความค้นตูลสูงแบบสิ้นเปลือง (Single-stage High Pressure Valve Homogenizer) สงคราม จากนั้นนำยีน์สชันที่ได้มาวิเคราะห์ความเฉลี่ยโดยการเกิดครีม (Creaming Stability) ความสามารถ และความคงตัวปรากฏ จากผลการทดลองพบว่าโปรตีนเยลลี่แบบเยมยันสามารถทำให้ระบบยีน์สชันมีความคงตัวมากกว่าระบบยีน์สชันที่ไม่มีการเติมโปรตีนเยลลี่แบบเยมยัน โดยพิจารณาจากค่า L* ที่สูงชิ้น ค่าความเหนียวปรากฏที่ลดลง และค่าดัชนีการเกิดครีม (Creaming Index) ที่ลดลง โดยระบบยีน์สชันที่พีเอช 4.5 มีความคงตัวน้อยที่สุด การเติมรูโตรส
Abstract

The influence of pH (3.5, 4.0 and 4.5) and sucrose (0.0 and 1.0%, W/W) on physical properties of drinking yoghurt stabilized by whey protein concentrate (WPC) at 0.0, 0.5, 1.0, 1.5 and 2.0% (W/W) was investigated. Drinking yoghurt was then pasteurized and passed twice through a single-stage high pressure valve homogenizer. The obtained emulsions were measured and creaming stability, lightness, and apparent viscosity were determined. In the presence of WPC, the emulsions were more stable to droplet flocculation than those not stabilized by WPC which was attributed to high L* value, low apparent viscosity, and low creaming index. Emulsion instability was observed at pH 4.5. Addition of sucrose to the emulsions showed a negative significant effect on the creaming stability of emulsion ($p<0.05$). This study has important implications for the formulation and production of protein-stabilized drinking yoghurt.

Keywords: pH, Sucrose, Drinking Yoghurt, Whey Protein, Creaming Stability

Introduction

Drinking yoghurt is categorized as stirred yoghurt of low viscosity, and this product is consumed as a refreshing drink. Many types of yoghurt can be considered to be oil-in-water emulsions (McClements, 2004a; Tamime and Robinson, 1985). The aqueous phase of yoghurt contains a three-dimensional network of aggregated casein and whey proteins, which gives yoghurt its characteristic textural attributes (McClements, 2004a). Normally emulsions are thermodynamically unstable systems that tend to breakdown during storage through a variety of physicochemical mechanisms, including creaming, flocculation, coalescence and Ostwald ripening (Dickinson, 1992; Friberg and Larsson, 1997). Therefore, the production of high quality food emulsions that can remain kinetically stable for a required period of time will depend on the understanding of food manufacturers to prevent or retard these breakdown mechanisms. In general,
emulsifiers are needed for stabilizing emulsions because they decrease the interfacial tension between the oil and water phase and form a protective coating around the droplets which prevents them from coalescing with each other (McClements, 2004a). Many proteins are surface-active molecules that can be used as emulsifiers because they are able to facilitate the formation, improve the stability, and produce desirable physicochemical properties in oil-in-water emulsions (McClements, 2004b). Whey protein ingredients commonly used as emulsifiers are amphiphilic molecules and their ability to form and stabilize oil-in-water emulsions is required for emulsion formation (Dickinson, 1997). The proteins in these ingredients facilitate the formation of small oil droplets during homogenization by lowering the interfacial tension, and they increase the stability of the droplets formed to aggregation by increasing the repulsive colloidal interactions between them (Demetriades, Coupland, and McClements, 1997a, 1997b; Surh, Ward, and McClements, 2006).

Food emulsions are usually compositionally complex with many ingredients that contribute to their stability, taste, texture, and appearance depending on solution conditions including pH. The pH of the aqueous phase plays an extremely important role in determining the physicochemical, microbiologic, and organoleptic properties of food emulsions (McClements, 2004a). In practical applications, food emulsions stabilized by proteins are highly sensitive to pH because the interfacial membranes formed by proteins are usually relatively thin and electrically charged, hence, the major mechanism preventing droplet flocculation in protein-stabilized emulsions is electrostatic repulsion, rather than steric repulsion. They tend to flocculate at pH values close to the isoelectric point of the adsorbed proteins because the electrostatic repulsion between the droplets is no longer sufficiently strong to overcome the various attractive interactions, e.g. van der Waals, hydrophobic, or depletion forces (Thepkunya Harnsilawat, Rungnaphar Pongsawatmanit, and McClements, 2006; Rungnaphar Pongsawatmanit, Thepkunya Harnsilawat, and McClements, 2006). A number of food emulsion products that contain whey proteins as functional ingredients also contain sugars. There are two roles that sucrose plays in determining the thermal stability of whey proteins in emulsions and gels. First, its ability to stabilize the globular state of the protein means that it is necessary to heat the system to higher temperatures before the protein molecules unfold. Second, its ability to increase the strength of protein-protein interactions means
that once the protein molecules have unfolded, there is an increased attraction between protein molecules, which leads to stronger gels and more droplet flocculation (Kulmyrzaev, Bryant, and McClements, 2000). The incorporation of sucrose would be expected to alter the aggregation stability of protein-stabilized emulsions by modifying the conformation stability and intermolecular interactions of the globular proteins, as well as the kinetics of droplet-droplet collisions (Kim, Decker, and McClements, 2003; Kulmyrzaev, Bryant, and McClements, 2000).

The main objective of this study is to examine the influence of pH and sucrose on the physical properties of drinking yoghurt stabilized by whey protein concentrate. The results of this study will have important implications for the formulation and production of protein stabilized drinking yoghurt.

**Materials and Methods**

1. **Materials**

Commercial stirred yoghurt (Danon) and sucrose (MITR PHOL) were purchased from a local supermarket. Spray-dried whey protein concentrate (WPC) was kindly provided from Davisco Foods International Co. (lot JE 030-3-578, Le Sueur, MN, USA). As stated by the manufacturer, the total protein content of the WPC powder was 78.0% (W/W) and the moisture content was 4.8% (W/W). Analytical grade citric acid and sodium azide were purchased from the Sigma Chemical Co. (St. Louis, MO). Distilled water was used for the preparation of all solutions.

2. **Methods**

2.1 **Drinking Yoghurt Preparation**

Whey protein solution was prepared by dispersing the desired amount (0.0-2.0%, W/W) of WPC powder into distilled water and stirring for 2 hrs. at room temperature to ensure complete dissolution. WPC solution was then mixed with sucrose (0.0, 1.0%) and 0.02% (W/W) sodium azide (as an antimicrobial agent). The amount (353.0 grams) of commercial yoghurt was added and stirred for 10.0 min. The pH of the emulsions was adjusted to pH 3.5, 4.0, and 4.5 using 1 M citric acid. The drinking yoghurt emulsions (1.0% (W/W) milk fat, 0.0-1.0% (W/W) WPC, and 0.0 or 1.0% (W/W) sucrose) were then pasteurized (72°C for 20 sec.) and passed through a single-stage high pressure valve homogenizer (Armfield model FT9, UK) twice at 1000 psi. The obtained drinking yoghurt emulsions were stored at ambient temperature for 24 hrs. before being analyzed.

2.2 **Creaming Stability Measurement**

Ten grams of drinking yoghurt emulsion were transferred into a test tube (internal
diameter of 13 mm, height of 150 mm), tightly sealed with a plastic cap. The creaming stability was measured by visual observation of the emulsions for ten hours. After storage, emulsions were separated into an optically opaque ‘cream’ layer at the top and a transparent (or turbid) ‘serum’ layer at the bottom. We defined the serum layer as the sum of the turbid and transparent layers. The total height of the emulsion (HE) and the height of the serum layer (HS) were measured. The extent of creaming was characterized by creaming index (%) = 100x(HS/HE). The creaming index provided indirect information about the extent of droplet aggregation in an emulsion: the faster the creaming, the higher the creaming index, and the larger the particle size (Surh, Ward, and McClements, 2006; Rungnaphar Pongsawatmanit, Thepkunya Harnsilawat, and McClements, 2006).

2.3 Viscosity Measurement

The viscosity of the drinking yoghurt was determined at 25°C at various shear rates (from 2 to 285 s⁻¹) with the aid of a Brookfield DV-II, LV viscometer (Brookfield Engineering Laboratories, USA), equipped with concentric cylinder geometry. The flow curves giving viscosity η (mPa.s) as a function of shear rate (s⁻¹) were the characteristic of shear-thinning behaviour.

2.4 Lightness Measurement

The lightness of the drinking yoghurt emulsion was measured using a Hunter Lab Mini-Scan XE Plus (Reston, VA). The device had a 2.54 cm port and was standardized using a black tile and a white tile. Readings were taken ten times and the average of the readings for L* was recorded. Illuminate A and a 10° standard observer were used.

2.5 Statistical Analysis

Results are presented as mean value ± standard deviation (at least three replicate experiments). Statistic analysis among treatments was determined at the significance level of p<0.05.

Results and Discussion

1. Influence of pH on the Physical Properties of Drinking Yoghurt

The purpose of these experiments was to examine the influence of pH (3.5 to 4.5) on the physical properties of drinking yoghurt emulsions stabilized by WPC. The creaming stability, viscosity, and color of emulsions were measured after preparation. However, only the viscosity and color of emulsions were measured during storage at 60°C for 7, 14, and 21 days. The color of the emulsions was determined using a colorimeter and
represented in L* value as shown in Table 1. The L* values, or ‘lightness’, of the emulsions decreased with increasing storage time, indicating that the droplet size of emulsions increased which was due to the particle size dependence of the scattering efficiency (Wiroj Chantrapornchai, Clydesdale, and McClements, 1998). The spectral reflectance of emulsions decreased with increasing droplet size of emulsion with diameter from 0.5 to 26 μm (Wiroj Chantrapornchai, Clydesdale, and McClements, 1999). The size of the particles in oil-in-water emulsions might change appreciably over time due to coalescence, Ostwald ripening, or flocculation which would be expected to alter their color (McClements, 2004a). It appeared from our observation that the L* values of the drinking yoghurt emulsions containing WPC 1.0% at pH 4.5 were significantly lower than those of pH 4.0 and 3.5 for day 0 and 7 (Table 1), whereas the L* values of 1.0% WPC emulsion had changed during storage for a period longer than 7 days. It showed that the L* values of emulsion from the same condition (pH and WPC concentration) were slightly different at days 14 and 21. These results indicated that the droplet size of these emulsions at pH 4.5 was larger than those at pH 4.0 and 3.5 for the first period of storage. Initial droplet sizes were in the range of 0.36 to 0.42 μm (data not shown), after which the droplet sizes were too large resulting in not being able to distinguish L* values due to the effect of size. Each L* value of the control (WPC 0.0%) and other concentrations (WPC 0.5, 1.5 and 2.0%) showed no significant difference at day 0. This implies that the optimum concentration of WPC would be 1.0%. Typically, the interfacial membranes formed by proteins are usually relatively thin and electrically charged, hence, the major mechanism preventing droplet flocculation in protein stabilized emulsions is electrostatic repulsion (McClements, 2004a). Therefore, the electrostatic repulsions of droplets with low concentrations of WPC were no longer sufficiently strong enough to overcome the various attractive interactions between droplets. On the other hand, the high concentrations of proteins might be dragged along the interface, leaving some regions of depleted emulsifier. Emulsifier-depleted regions on two different droplets can come into close proximity during a droplet-droplet encounter (McClements, 2004a).

The shear viscosity of drinking yoghurt emulsions stabilized by WPC at different pH’s after preparation was measured using a viscometer. Figure 1 is the shear rate dependence of the steady shear viscosity of drinking yoghurt emulsion at different pH
values (3.5 to 4.5) at a constant temperature (25°C). The size of oil droplets and the interfacial characteristics of the emulsions have been found to have an effect on viscoelastic properties due to the protein films formed at the o/w interface (Dickinson, 1992) and to the amount of protein adsorbed to the interface (Logaraj, et al., 2008). Conventionally, the viscosity-related properties of liquid are assessed by measuring their resistance to flow. In this experiment, the shear rate increased from 2 to 57 s⁻¹. All drinking yoghurt emulsions exhibited shear-thinning flow behavior in this studied range with the viscosity decreasing with increasing shear rate, referring to the flocculated emulsion system. The result was consistent with Demetriades, Coupland, and McClements (1997a). Figure 2 shows the apparent viscosity of drinking yoghurt emulsions at a specified shear rate (28.5 s⁻¹). It has been reported that this selected shear rate is in the range of common process of chewing and swallowing processes (101 to 102 s⁻¹) (Steffe, 1996). At pH 4.5, in the present of sucrose, the apparent viscosities of emulsions containing 1.5 and 2.0% WPC were higher compared to those of other pH and concentrations, suggesting extensive flocculation could have occurred in the systems. Demetriades, Coupland, and McClements (1997b) reported that droplet flocculation near the isoelectric point of whey protein (pH ≈ 4.8) caused a significant change in rheological properties.
Table 1  L* value of drinking yoghurt emulsion in the absence of sugar at various pH during storage for
21 days

<table>
<thead>
<tr>
<th>pH</th>
<th>WPC (%)</th>
<th>Storage times (days)</th>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>3.5</td>
<td>0.0</td>
<td>80.08±0.21d</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>80.68±0.31d</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>85.16±0.26i</td>
</tr>
<tr>
<td></td>
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<td>80.61±0.41d</td>
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<td></td>
<td>2.0</td>
<td>77.54±0.19bc</td>
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<td>75.34±0.19b</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
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<td>2.0</td>
<td>70.40±0.29a</td>
</tr>
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</table>

Note: Data followed by different letters within each column are significantly different according to Duncan’s multiple range test at p<0.05. Data obtained from at least three replicates.

Figure 1  Shear rate dependence of the steady shear viscosity of drinking yoghurt emulsions at constant temperature 25°C; (a) pH = 3.5, (b) pH = 4.0, and (c) pH = 4.5
Normally, creaming occurs during the storage of emulsion because the continuous oil droplets coalesce to larger oil droplets and tend to migrate towards the top due to the density difference. Finally, an oil layer is produced at the top of an emulsion. Measurement of the creaming stability of drinking yoghurt emulsions indicated that they were highly unstable to creaming at pH 4.5, but relatively stable at pH 3.5 and 4.0 (Figure 3). This result provided indirect information about the extent of droplet aggregation in an emulsion: the faster the rate of creaming, the higher the creaming index, and the larger the particle size which is in agreement with the L* value. The emulsion formed at pH 4.5 undergoes rapid creaming on standing, and within 3 hours, the creamed layer separates leaving a clear solution at the bottom. This is due to a pH close to the isoelectric point of protein (Surh, Ward, and McClements, 2006; Verheul, et al., 1999). Das and Kinsella (1989) reported that emulsifying properties of β-lactoglobulin which is the major protein in the whey fraction obtained from milk are dependent on pH. Emulsion droplets around pH 4.0-5.0 contained coarse, large droplets and underwent rapid creaming.
2. Influence of Sucrose on the Physical Properties of Drinking Yoghurt

Food emulsions contain a wide variety of different constituents, including sucrose. The physicochemical and organoleptic properties of a product depend on the type of constituents present, their physical location, and their interactions with each other. Therefore, the influence of sucrose on the physical properties, including viscosity and creaming stability, of drinking yoghurt emulsions was investigated in this study.

The apparent viscosity of drinking yoghurt emulsions, at a shear rate of 28.5 s⁻¹, in the absence or presence of sucrose (1%) after preparation is shown in Figure 4. Sucrose did not affect the apparent viscosity of drinking yoghurt emulsions in the range of pH between 3.5–4.5, while the apparent viscosity slightly increased when whey protein concentrate was increased. This is consistent with observations on O/W emulsions by Maskan and Göğüş (2000). They reported that sucrose concentrations less than 2% did not have an effect on the viscosity. In the presence of sucrose, the creaming index of all drinking yoghurt emulsions decreased compared to those of in the absence of sucrose (Figure 5), indicating a lesser extent of droplet aggregation in emulsions containing sucrose. The ability of sucrose to retard droplet aggregation in the emulsions might be due to the change of the dielectric constant, refractive index, and interfacial tension of the aqueous solution surrounding the emulsion droplets. The presence of sucrose can influence the strength of the attractive van der Waals and hydrophobic interactions and the repulsive electrostatic interactions between the droplets (McClements.

Figure 3 Creaming stability of different WPC concentrations stabilized drinking yoghurt emulsions at (a) pH = 3.5, (b) pH = 4.0 and (c) pH = 4.5.
Sucrose might have altered the delicate balance of attractive and repulsive interactions between the droplets, thus changing their propensity to aggregate.

**Figure 4** The apparent viscosity at shear rate 28.5 s\(^{-1}\) of drinking yoghurt emulsions (a) in the absence of sucrose and (b) in the presence of sucrose.

In 2% WPC stabilized drinking yoghurt, it was found that large droplet aggregation occurred at pH 4.0 and 4.5; therefore, it was not possible to measure the creaming and viscosity of emulsion samples.

**Figure 5** Dependence of creaming index of drinking yoghurt emulsions on WPC concentration at different pH values after storage for 10 hours (a) in the absence of sucrose and (b) in the presence of sucrose.
Conclusion

This study revealed that drinking yoghurt emulsion stabilized by WPC was more stable to droplet aggregation than those without WPC. The physical properties of drinking yoghurt stabilized by whey protein concentrate depended on pH. At pH 4.5, the emulsions lost their stability due to the pH near to the isoelectric point of the adsorbed protein molecules. Addition of sucrose to the emulsions showed an effect on the creaming stability of emulsion. These results will be useful for the formulation and production of a protein stabilized drinking yoghurt.

Acknowledgement

This study is partially supported by a grant from the University of the Thai Chamber of Commerce (UTCC).

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