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Set yoghurt processing with eggs as milk replacements, and improvement of texture, rheology, and microstructure by formulation design and optimisation

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Abstract

Eggs are suitable for the processing of set yoghurt as milk replacements, but there is no yoghurt mainly made from eggs. In the present work, production formula was designed and optimised for high-quality egg-based yoghurt processing by improving water holding capacity, aroma, texture, and taste using single factor experiment and D-optimal mixture design. Results showed that with optimised formula (whole liquid egg, 100 g; water, 180 g; sucrose, 30.89 g; diacetyl tartaric acid ester of mono(di)glycerides, 0.28 g; gelatine, 0.112 g; gellan gum, 0.14 g; and β-cyclodextrin, 0.56 g), the product showed high sensory evaluation score, fine viscosity, as well as preferable hardness and suitable fracturability. SDS-PAGE electrophoretogram indicated that the proteins in egg-based yoghurt was degraded during fermentation, which might have contributed to the improvement of gel structure and taste. Based on the SEM images, the prepared egg-based yoghurt had smoother, stronger, and more compact gel network microstructure when compared with milk-based yoghurt.

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Introduction

Lactic acid bacteria (LAB) are a common category of Gram-positive bacteria that can convert sugars to lactic acid. It has been demonstrated that LAB have a series of beneficial effects on health, including enhancing the flavour and taste of foods (Barcenilla *et al.*, 2022), regulating the balance of intestinal flora (Liu *et al.*, 2021) and the function of the immune system (Sjofjan *et al.*, 2021), promoting the absorption of nutrients (Wang *et al.*, 2021), reducing allergenicity of allergic food (Pi *et al.*, 2021), preventing diarrhoea, lowering cholesterol, as well as improving lipid metabolic disorder (Jitpakdee *et al.*, 2021). Consequently, they are widely used in the food, pharmaceutical, and feed industries. There are various LAB-fermented food products such as fermented dairy, bean, vegetable and fruit, and meat. During fermentation, carbohydrates and proteins are hydrolysed gradually, meanwhile a wide variety of nutrients, flavour compounds, and active components are produced, for example organic acids, amino acids, polypeptides, vitamins, polysaccharides, and antibiotics. Therefore, these foods possess multiple health benefits, good taste, and unique flavour, thus becoming an important part of people's diet. A number of deep-processing products from the highly nutritious eggs have been developed in recent years such as keto foods, sports nutrition, and flexitarian diets (Sun *et al.*, 2004; Martinez *et al.*, 2019; Molnár and Pal, 2022), and high-protein healthy foods like yoghurts are still in great demand.

Yoghurt is one of the most common LAB-fermented dairy products widely favoured by consumers all over the world due to its high quality proteins, unique texture and flavour, as well as many kinds of healthy functions. Similar to milk, eggs contain various nutrients like proteins, fats, phospholipids, vitamins, and minerals, and have always been considered a perfect food (Yang, 2021). Furthermore, proteins account for about 12% in eggs, which are almost four times that in milk. Great

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abundance and various functional properties of protein such as foaming, emulsification, adhesion, and gelation make eggs a very important material in food processing (Yja *et al.*, 2022). In terms of the main compositions, eggs are very nourishing, have the ability to form protein gel, and theoretically suitable for the production of set yoghurt. However, nowadays there is no such yoghurt mainly made from eggs on the market, and few reports are available on egg-based yoghurt. Our team has creatively conducted investigations on egg-based set yoghurt processing, and there were two critical obstacles in the development of raw material replacement. Firstly, egg-based set yoghurt usually had a coarse gel texture with certain numbers of pinholes inside upon high heat treatment. This might be explained by the poor thermal stability (Singh *et al.*, 2020) of egg proteins as compared to milk proteins, as egg proteins began to denature and agglomerate at around 56°C, and completely set at 80°C, whereas heat treatment over 56°C was usually unavoidable in egg-based yoghurt processing. Secondly, the prepared egg-based yoghurt often had unpleasant smell, which was hard to remove. The difficulties in removing the unpleasant egg odour consequently reduce consumers' acceptance.

To solve the aforementioned two obstacles, egg-based yoghurt formula was designed and optimised through the single factor experiments and D-optimal mixture design in the present work. The effects of water and addition amount of sucrose, DATEM, gelatine, gellan gum, and β-CD on the physicochemical and sensory properties of egg-based yoghurt were comprehensively investigated to obtain a high-quality egg gel product. Furthermore, using WHC, pH, texture, viscosity, SDS-PAGE, microstructure, and sensory quality as indicators, the quality of egg-based yoghurt was fully evaluated with the yoghurt made from milk as a control.

Materials and methods

Materials

Fresh eggs were obtained from local food market (Nanchang, China). Commercial frozen yoghurt starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was obtained from Angel Yeast (China) Co., Ltd. Sucrose was obtained from Nanhua Sugar Co., Ltd. (Laibin, China). β-CD and anhydrous citric acid were obtained from Nuoyi Biotechnology Co., Ltd. (Quzhou, China). Gelatine and gellan gum were obtained from Meikang Trading Co., Ltd. (Nanchang, China). Diacetyl tartaric acid ester of mono(di) glycerides (DATEM) was obtained from Honest Food Co., Ltd. (Nanchang, China). Salt was obtained from Zhongyan Xingan Salt Chemical Co., Ltd. (Nanchang, China). Butter was obtained from Mengniu Dairy Co., Ltd. (China). Flavouring agents were obtained from Yuwanbang Management Co., Ltd. (Shenzhen, China). In addition, all additives were food-grade, and other chemicals were analytical grade, and obtained from Solarbio Science Technology Co., Ltd. (Beijing, China).

Processing technology of egg-based yoghurt

The processing technology and main parameters were as follows: (a) fresh eggs were rinsed thoroughly with clean water, surface-sterilised by 75% (v/v) ethanol, and then air-dried fully; (b) clean and sterilised eggs were manually broken, and egg white and yolk were collected and poured into a stainless steel container, then stirred evenly; (c) whole liquid egg was diluted with 1.6 times water; (d) additives, including sucrose, β-CD, DATEM, gelatine, gellan gum, salt, and butter were successively added following the designed formula, and then mixed evenly with diluted whole liquid egg; (e) the mixtures were heated at 100°C for 20 min; (f) the pH of mixtures was adjusted to 6.0 ± 0.05 with anhydrous citric acid; (g) the mixtures were fully ground in a colloid mill (JM-L, Shanghai, China) until the particle sizes was 100 ± 10 μm; (h) a total of 0.02% yoghurt starter (w/w) and 0.4% flavouring agents (w/w) were added and well-mixed; (i) the mixtures with fermentation starter were sealed and then incubated at 42°C (SPX-70BE, Shanghai, China) for 7 - 10 h until pH reached 4.0 ± 0.05; (j) the fermented egg-based yoghurt was cooled down in a refrigerator at 4 - 6°C, and the samples were collected at the 24^{th} hour after fermentation for further analyses.

Scheme of single-factor experiments for egg-based yoghurt production

Based on our previous studies on egg-based yoghurt, we identified six factors namely water, sucrose, DATEM, gelatine, gellan gum, and β-CD as significant factors that have notable effects on the quality of egg-based yoghurt. To achieve high-quality egg-based yoghurt, single-factor experiments were conducted.

Mixture design experiment scheme for optimisation of egg-based yoghurt production

To further improve the egg-based yoghurt quality, the formula was optimised by D-optimal mixture design, with sensory evaluation, pH, viscosity, and water holding capacity (WHC) as evaluating indicator. The design was conducted using Design-Expert 13.0 software.

Sensory

Sensory evaluation was conducted according to Nikzade *et al.* (2012) with some modifications. Briefly, five sensory indicators, including appearance, texture, aroma, taste, and overall acceptance were used to comprehensively evaluate the quality of egg-based yoghurt. The sensory evaluation panel was composed of five trained men and women aged from 20 to 25.

pH

The collected egg-based yoghurt sample was stirred evenly, and then its pH was detected directly using a portable pH meter (pH-10, Shanghai, China).

Water holding capacity

The method described by Nguyen *et al.* (2017) was adopted to determine the water holding capacity (WHC) with some modification. Briefly, a total of 10 g of yoghurt sample was collected and centrifuged at 3,000 *g* for 15 min at 5°C. The supernatant was then drained for 10 min, and the remaining precipitate was accurately weighed. The WHC value was calculated and presented as the percentage of precipitate weight in the sample.

Texture

Texture profile analysis (TPA) of yoghurt samples was conducted with a P/0.5 cylindrical probe (12.7 mm in diameter) using a TA-XT plus texture analyser (Stable Micro Systems, Surrey, UK) according to Godoi *et al.* (2021) with some modifications. Briefly, the determination was performed with a test speed of 0.5 mm/s, a trigger force of 1 g, and a 20 mm test distance.

Rheology

According to Nguyen *et al.* (2017) and Fu *et al.* (2018), rheological behaviours of egg-based yoghurt were detected in a shear rate-controlled rheometer (Discovery Hybrid Rheometer, TA Instrument, USA). Briefly, the samples (in 50 mL glass container) were equilibrated at 22 - 25°C for 1 h, and then gently stirred with a stainless-steel spoon to eliminate any phase separation before measurement. Viscosity of the samples was measured under steady state shear conditions using 60 mm stainless steel parallel plates at 1,000 µm gap, with shear rate ranging from 0.1 to 1,000 s^{-1}. In addition, oscillation test was performed within the linear viscoelastic range (LVR) of each sample. Frequency sweep (0.01 - 100 Hz) was conducted at the optimal deformation condition (1 % strain). The storage modulus (G') and loss modulus (G") were all recorded automatically.

Electrophoresis

SDS-PAGE was used to characterise the protein patterns of the yoghurt samples with a vertical electrophoresis unit (Bio-Rad, Richmond, CA, USA), according to Wang *et al.* (2020). Briefly, soluble proteins were mixed (1:1, v/v) with SDS-PAGE sample buffer (100 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β-ME, and 0.2% bromophenol blue), and boiled for 5 min. A total of 10 µL of prepared samples and standard protein marker were loaded for gel electrophoresis. The electrophoresis was conducted at a constant voltage of 120 V in the resolving gel. After the electrophoresis was complete, the gels were stained with 0.125% Coomassie brilliant blue R-250 in 25% methanol and 10% acetic acid. Destaining was performed using 25% ethanol and 8% acetic acid.

SEM detection

The microstructure of egg-based yoghurt was observed with a scanning electron microscope according to Martin *et al.* (2006) with some modifications. Briefly, the collected egg-based yoghurt samples were fixed in 2.5% glutaraldehyde solution of pH 7.2 at 4°C for 8 - 10 h. Subsequently, the fixed samples were washed with 0.1 M phosphate buffer of pH 6.8, and then subjected to a dehydration cycle with increasing concentrations of alcohol (15 min 30%, 15 min 50%, 15 min 70%, 15 min 90%, and 30 min 100% alcohol) (Espírito-Santo *et al.*, 2013). Afterwards, the dehydrated samples were lyophilised in a lyophiliser (SCIENTZ-10N/A, Scientz, China) for 1 - 1.5 h at -80°C. After coating with gold-palladium, the microstructure of the prepared sample was observed under a scanning electron microscope (FEI Quanta 250, FEI, USA).

Statistical analysis

All the determinations and assays were performed in triplicate, and the data were presented as mean ± standard deviation. Statistical analyses were conducted using SPSS for Windows (SPSS Inc., Chicago, IL, U.S.A.). One-way analysis of variance (ANOVA) was carried out, and means were compared using Duncan's multiple range tests. Significant differences were determined at $p < 0.05$. Correlation analysis was conducted using a two-tailed test.

Results

Effect of water addition amount on physicochemical and sensory properties of egg-based yoghurt

Figure 1a shows that WHC was negatively correlated with the water addition amount, which decreased from 84.32 to 74.30% when the water addition amount was increased from 120 to 200%. However, no significant difference ($p > 0.05$) in pH was observed among the egg-based yoghurt samples with different amounts of water addition (Figure 1a). The prepared egg-based yoghurt displayed a typical shear thinning characteristics (as showed in Figure 2b). With the increase in water addition amount, the viscosity of egg-based yoghurt showed significant downward trend, especially at low shear rate stage. When 200% water was added, remarkable decrease in viscosity was recorded, which might have further led to an obvious sensory quality degradation of egg-based yoghurt (Figures 2a, 3a, and 4a).

With regard to the sensory quality of egg-based yoghurt, water addition amount had no obvious effect ($p > 0.05$) on appearance and aroma, but made significant impact ($p < 0.01$) on the texture and taste (Figures 3a and 4a). With the increase in water addition amount, the sensory score of texture and flavour increased first and then decreased, and both peaked at 180%. When the water addition amount was lower than 180%, egg-based yoghurt showed a light flavour and rough texture. On the contrary, high levels of water led to fragile texture and light taste. Overall, the optimal water addition amount was 180%.

Effect of sucrose addition amount on physicochemical and sensory properties of egg-based yoghurt

As shown in Figure 1b, sucrose addition amount (4 - 12%) had no obvious effect on ($p > 0.05$) pH, but showed significant impact ($p < 0.05$) on WHC, viscosity, and sensory quality. It can be seen from Figure 1b that WHC displayed a continuous upward trend when the sucrose addition amount was increased from 4 to 10%, and peaked (83.16%) at 10%. WHC of the egg-based yoghurt with 12% sucrose addition amount lowered slightly when

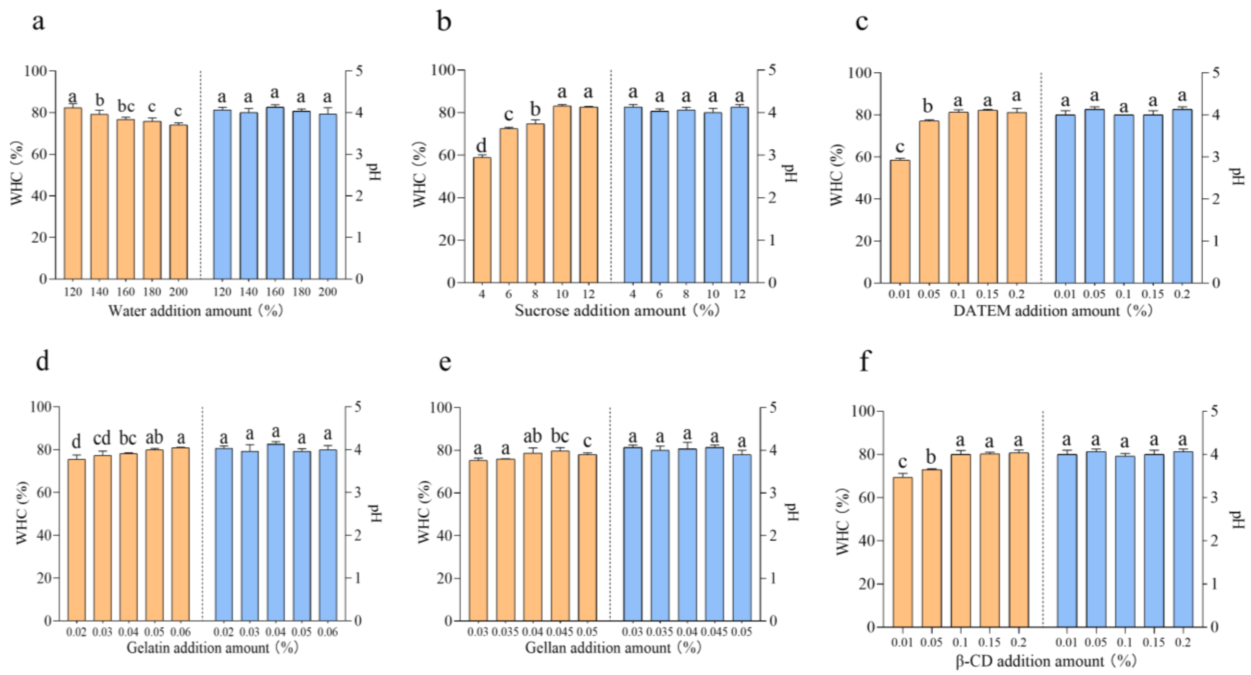

Figure 1. Effects of addition amounts of water **(a)**, sucrose **(b)**, DATEM **(c)**, gelatine **(d)**, gellan gum **(e)**, and β-CD **(f)** on pH and WHC of set egg-based yoghurt.

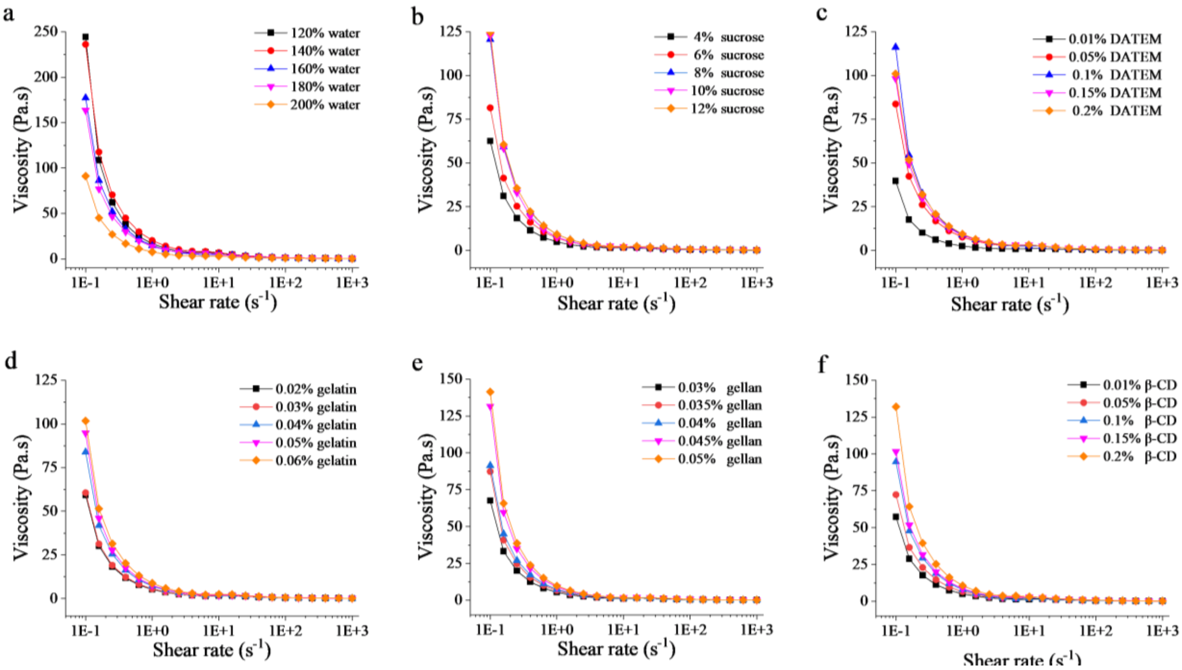

Figure 2. Effects of addition amounts of water **(a)**, sucrose **(b)**, DATEM **(c)**, gelatine **(d)**, gellan gum **(e)**, and β-CD **(f)** on viscosity of set egg-based yoghurt.

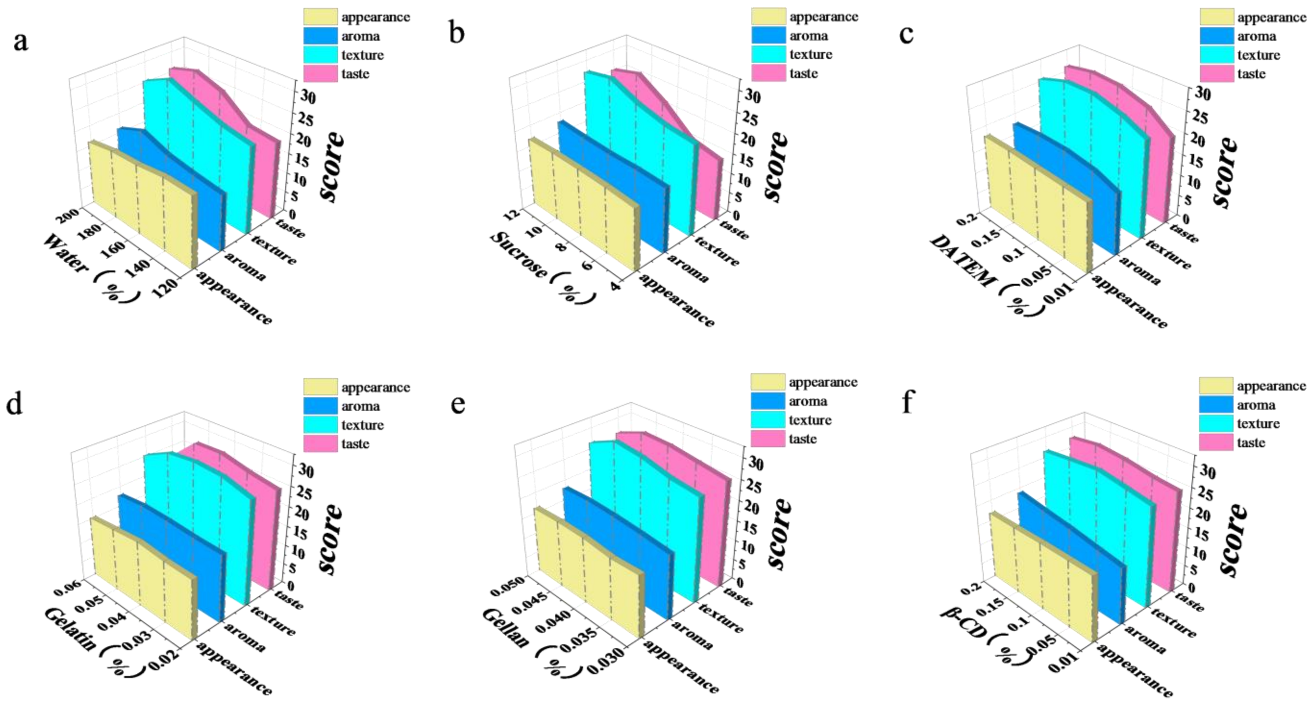

Figure 3. Effects of addition amounts of water **(a)**, sucrose **(b)**, DATEM **(c)**, gelatine **(d)**, gellan gum **(e)**, and β-CD **(f)** on single sensory evaluation score of set egg-based yoghurt. Full score of appearance, aroma, texture, and taste was set as 20, 20, 30, and 30, respectively.

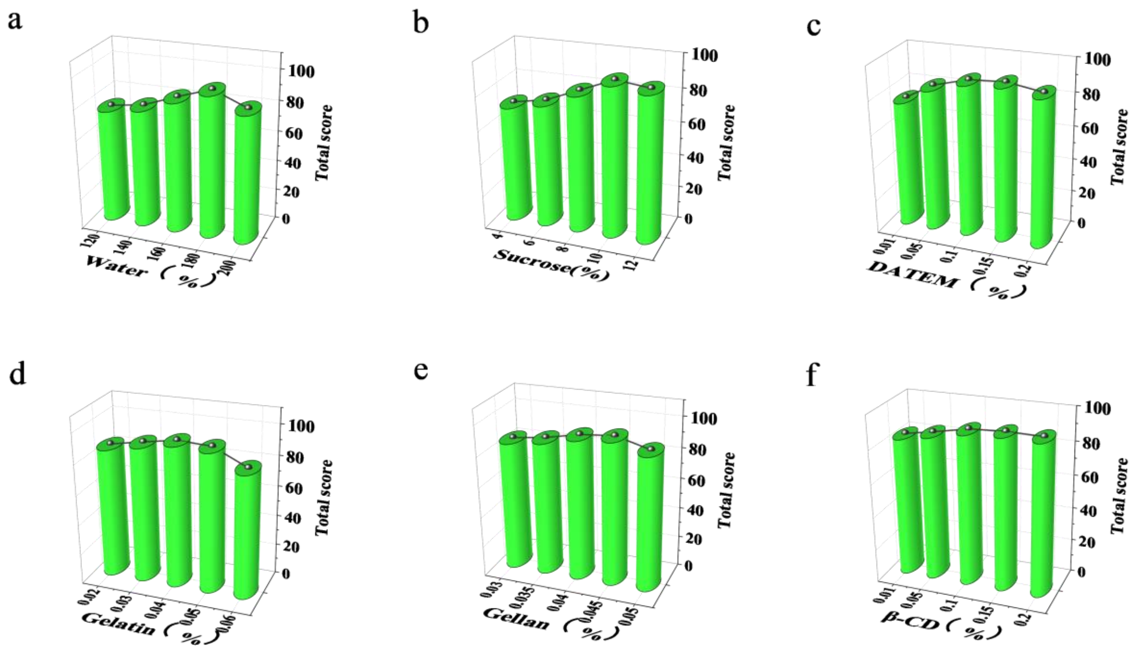

Figure 4. Effects of addition amounts of water **(a)**, sucrose **(b)**, DATEM **(c)**, gelatine **(d)**, gellan gum **(e)**, and β-CD **(f)** on total sensory evaluation score of set egg-based yoghurt. Full score of total sensory score was set as 100.

compared with that at 10%, but no significant difference (p > 0.05) was observed. In general, the viscosity of egg-based yoghurt increased with the increase in sucrose concentration, and remained unchanged when sucrose addition amount was higher than 8%, which could be seen clearly at the stage of low shear rate, but the difference almost disappeared when the shear rate exceeded 10 s^{-1} (Figure 2b). It could be seen from Figure 4b that the highest total sensory score appeared at 10% sucrose addition amount. In terms of single sensory indicators, sucrose concentration had little effect (p > 0.05) on the appearance and aroma, but exhibited notable impact (p < 0.05) on the texture and taste, which also peaked at 10% (Figure 3b).

Effect of DATEM addition amount on physicochemical and sensory properties of egg-based yoghurt

Overall, the addition amount of DATEM (from 0.01 to 0.2%) had no obvious effect (p > 0.05) on the pH of egg-based yoghurt, while significantly affected (p < 0.05) the WHC, viscosity, and sensory quality (Figures 1c, 2c, 3c, and 4c). As shown in Figure 1c, WHC increased firstly and then decreased with the increase in DATEM concentration, and the highest WHC appeared at 0.15%. However, there was no obvious difference among that of 0.1, 0.15, and 0.2%. The viscosity also presented a similar change profile with the WHC at low shear rate when the concentration of DATEM was increased from 0.01 to 0.2%, but the difference disappeared at high shear rate (Figure 2c). The change profiles of WHC and viscosity under different concentrations of DATEM might have resulted in similar change trends of total sensory, taste, texture, and aroma score, with a peak score of 90.6, 27.3, 27.6, and 18.1 at 0.15, 0.10, 0.15, and 0.15%, respectively (Figures 3c and 4c). Overall, the optimal addition amount of DATEM was 0.15%.

Effect of gelatine addition amount on physicochemical and sensory properties of egg-based yoghurt

Gelatine was used to improve the gel quality of egg-based yoghurt in the present work. Results obtained suggested that gelatine had significant influence (p < 0.05) on the physicochemical and sensory quality of egg-based yoghurt within the experimental concentration range except pH (as shown in Figures 1d, 2d, 3d, and 4d). As the gelatine addition amount was increased, both WHC and viscosity at low shear rate generally showed an upward trend, while all of the total sensory, appearance, aroma, texture, and taste scores increased at first and then decreased, and peaked at 0.04, 0.04, 0.05, 0.04, and 0.04%, respectively (Figures 3d and

4d). Although moderate gelatine concentration could provide smooth taste and fine texture, excessive gelatine addition might lead to a deterioration in the quality of the egg-based yoghurt gel. Overall, the optimal addition amount of gelatine was 0.04%.

Effect of gellan gum addition amount on physicochemical and sensory properties of egg-based yoghurt

In the present work, gellan gum ranging from 0.03 to 0.05% was added, and its effects on the physicochemical and sensory properties of egg-based yoghurt were comprehensively investigated. Results obtained demonstrated that the addition amount of gellan gum had little effect ($p > 0.05$) on pH, but it significantly influenced ($p < 0.05$) WHC, viscosity, and sensory quality (Figures 1e, 2e, 3e, and 4e). When the gellan gum addition amount was increased from 0.03 to 0.05%, the WHC enhanced gradually and peaked at 0.045% (Figure 1e). However, the highest viscosity was observed at 0.045% rather than 0.05% (Figure 2e). All the sensory indicators, including total sensory, appearance, aroma, texture, and taste scores, peaked at 0.045% (Figures 3e and 4e). Therefore, the optimal addition amount of gellan gum in the egg-based yoghurt processing was 0.045%.

Effect of β-CD addition amount on physicochemical and sensory properties of egg-based yoghurt

β-cyclodextrin (β-CD) is commonly used in many food products as a masking agent and stabiliser (Marques, 2010). Eggs and egg-based foods usually have unfavourable egg smell, which can reduce consumers' satisfaction to a certain extent. Consequently, β-CD was utilised to mitigate the unfavourable smell, and further improve the sensory quality of egg-based yoghurt in the present work. Overall, β-CD had no significant effect ($p > 0.05$) on the pH, while having notable impact ($p < 0.05$) on WHC, viscosity, and sensory quality (Figures 1f, 2f, 3f, and 4f). Results obtained showed that WHC linearly increased when the concentration of β-CD was increased from 0.01 to 0.1%, and it remained around 80% when the β-CD concentration exceeded 0.1% (Figure 1f). Meanwhile, β-CD enhanced the viscosity of egg-based yoghurt at low shear rate, with dose-effect relationship increased (Figure 2f). In addition, results demonstrated that β-CD significantly improved total sensory evaluation score with a peak of 90.3 at 0.15%. From the results of single sensory evaluation, β-CD showed no effect on the appearance, but markedly elevated the score of aroma, texture, and taste, which peaked at 0.2, 0.15, and 0.15%, respectively (Figures 3f and 4f). From Figure 3f, it could be seen that β-CD with a concentration over 0.15% had a negative impact on the texture and taste, which might be caused by the excessive high hardness and viscosity.

Optimisation results of mixture design

Based on the results of single factor experiments, the addition amounts of sucrose, DATEM, gelatine, gellan gum, and β-CD were further optimised using the D-optimal mixture design experiment.

Experimental design and results

Based on the D-optimal mixture design principle, the optimal experiment was designed using Design Expert 13.0. The results (Table 1) indicated that all the sensory scores of the 25 experimental groups were over 83, among which four were even more than 90, thus suggesting that the selected range of each formula component based on single factor test was relatively appropriate, and therefore all the egg-based yoghurts produced with the 25 formulations showed good quality. Group 11 displayed the best quality with a score as high as 90.9. Further analysis suggested that when the addition amount of sucrose, DATEM, gelatine, gellan gum, and β-CD were within 9.8 - 11.6, 0.13 - 0.20, 0.039 - 0.041, 0.043 - 0.05, and 0.11 - 0.16%, respectively; the egg-based yoghurt would achieve high sensory evaluation score.

Model establishment and variance analysis

Using multiple regression fitting, regression equation (Eq. 1) was developed as following:

$$Y = -5.12A + 87.73B - 341.82C - 54.09D + 86.76E + 87.29AB + 727.23AC + 354.38AD + 88.66AE + 547.14BC + 106.84BD - 11.82BE + 606.06CD + 497.90 CE + 209.37DE \quad \text{(Eq. 1)}$$

Variance analysis of the results from the mixture design experiment showed that the developed model had extremely significant difference ($p < 0.01$), while the lack of fit was not significant ($p = 0.9190 > 0.05$), thus indicating that the developed model had good fitting degree with a small error, thus could be used to predict the sensory score of the egg-based yoghurt. Furthermore, both the coefficient of determination (R^2) and corrected coefficient of determination (R^2_{adj}) were over 0.9, reaching 0.9789 and 0.9495 respectively. Moreover, it could be seen that the interactions between A and C, B and C, and C and D were all extremely significant ($p < 0.01$), meanwhile all the interactions between A and B, A and D, A and E, and C and E were significant ($p < 0.05$).

Table 1. Sensory scores of egg-based yoghurts of mixture design experiment.

Number	A (%)	B (%)	C (%)	D (%)	E (%)	Score (Y)
1	11.587	0.135	0.040	0.050	0.160	90.3 ± 0.94
2	8.289	0.147	0.020	0.050	0.200	86.2 ± 1.02
3	10.807	0.153	0.060	0.041	0.138	87.3 ± 0.55
4	9.865	0.200	0.040	0.043	0.118	89.5 ± 1.12
5	11.750	0.170	0.026	0.040	0.147	86.2 ± 0.98
6	11.185	0.130	0.023	0.036	0.200	85.4 ± 0.71
7	11.587	0.135	0.040	0.050	0.160	90.1 ± 1.21
8	10.002	0.168	0.020	0.030	0.181	83.8 ± 0.52
9	8.000	0.182	0.055	0.050	0.133	86.9 ± 0.79
10	12.000	0.103	0.060	0.049	0.169	87.9 ± 0.30
11	9.865	0.200	0.040	0.043	0.118	90.9 ± 0.69
12	8.575	0.200	0.020	0.030	0.164	84.5 ± 1.20
13	12.000	0.078	0.060	0.042	0.200	87.1 ± 1.06
14	11.985	0.174	0.060	0.050	0.096	88.3 ± 0.62
15	9.658	0.093	0.060	0.050	0.200	87.4 ± 1.00
16	8.000	0.200	0.060	0.030	0.130	87.6 ± 0.67
17	8.000	0.165	0.055	0.030	0.169	86.7 ± 0.57
18	10.112	0.113	0.056	0.030	0.200	87.1 ± 0.83
19	8.000	0.140	0.042	0.038	0.200	89.8 ± 0.77
20	10.807	0.153	0.060	0.041	0.138	87.9 ± 0.57
21	9.865	0.200	0.040	0.043	0.118	90.7 ± 0.97
22	12.000	0.187	0.044	0.030	0.119	89.6 ± 1.11
23	12.000	0.200	0.060	0.050	0.070	88.5 ± 0.68
24	8.000	0.140	0.042	0.038	0.200	88.9 ± 0.69
25	12.000	0.200	0.020	0.046	0.114	83.1 ± 0.92

A-E: sucrose, DATEM, gelatine, gellan gum, and β-CD, respectively. All designs are fitted automatically in Design Expert 13.0.

Validation test of raw material ratio

The developed fitting model was further used to predict the optimal parameters and sensory score accordingly, and the results suggested that the optimal theoretical score could reach 91.6198 when the addition amount of sucrose, DATEM, gelatine, gellan, and β-CD were 11.2604, 0.0952361, 0.04216, 0.05, and 0.2%, respectively. Considering the convenience and operability, the addition amounts were respectively adjusted to 11, 0.1, 0.04, 0.05, and 0.2%, respectively, in the following validation test. The validation test results showed that the sensory score of the egg-based yoghurt was as high as 91.3, which was basically consistent with the predictive value of the developed model.

Texture comparison between egg- and milk-based yoghurts

In this section, hardness and fracturability were compared to characterise the gel texture difference between non-optimised egg-based yoghurt (NOEY), optimised egg-based yoghurt (OEY), and milk-based yoghurt (MY). Both hardness and fracturability were significantly enhanced through optimisation, increasing from 13 and 10.1 to 18.3 and 14.6 g, respectively, thus suggesting that great improvement had been obtained through optimisation. Furthermore, as compared to MY, OEY showed higher hardness and fracturability; the fracturability was 39.05% higher than that of MY, thus indicating that OEY possessed a preferable gel quality, which could have been attributed to the appropriate addition amount of gelatine and gellan gum.

Comparative analysis of rheological behaviour between egg- and milk-based yoghurts

The determination results of viscosity indicated that NOEY, OEY, and MY exhibited typical pseudoplastic behaviour. Generally, the viscosity of OEY was higher than that of NOEY at low shear rates, which could have been attributed to the thickening effect of gelatine and gellan gum in OEY. Both NOEY and OEY showed a significant higher viscosity as compared to MY at low shear rates, which could have been attributed to the differences in protein types and contents in the yoghurts. Furthermore, the viscosity of NOEY, OEY, and MY tended to converge at high shear rates.

By comparing the change profiles of G' (storage modulus), G" (loss modulus), and tan∂ (G"/G'), it was concluded that NOEY and MY had the common characteristics of viscoelasticity, and therefore could be classified into the same category fluid, while OEY displayed another greatly different kind of viscoelasticity. When the angular frequency increased from 0.16 to 62.83 rad/s, G' and G" of NOEY and MY all increased very slowly, meanwhile G' was always notably higher than G" during the frequency scanning, and therefore tan∂ was always less than 1, which indicated that NOEY and MY mainly presented elastic behaviour. However, with regard to OEY, G' was significantly lower than G" when applying an angular frequency no more than 3.96 rad/s, while G' and G" were almost the same within angular frequency from 6.28 to 99.58 rad/s. On the contrary, G' was higher than G" when the angular frequency was greater than 99.58 rad/s, meanwhile tan∂ decreased from 26.65 to 0.49 when angular frequency increased from 0.16 to 157.83 rad/s. These suggested that OEY primarily displayed viscous behaviour at low angular frequency, but exhibited elastic behaviour at high angular frequencies, which also demonstrated that our optimisation of the processing formulation successfully changed the rheological behaviour of egg-based yoghurt.

SDS-PAGE analysis of egg- and milk-based yoghurts

The electrophoretogram of fresh egg liquid displayed three main protein bands, OVN, OVT, and OVA, with molecular weights of 150, 77.9, and 45 kDa, respectively. However, in the electrophoretogram of the egg-based yoghurt, only a narrow OVA band was clearly observed, while the OVN and OVT bands were almost undetectable. This indicated that significant degradation of proteins occurred during fermentation of the egg-based yoghurt.

Microstructure comparison of egg- and milk-based yoghurts

The microstructure of the egg- and milk-based yoghurts were observed using scanning electron microscopy. Comparatively speaking, the texture of egg-based yoghurt looked more compact, denser, and smoother with fewer cavities, while milk-based yoghurt texture had a looser and coarser with more cavities.

Discussion

LAB fermentation is the fundamental principle of yoghurt processing. The production of lactic acid during fermentation lowers the pH to around 4.0, thus leading to significant changes in texture and the formation of the gel structure. Therefore, pH serves as an important indicator for LAB fermentation and yoghurt quality. According to Mahomud *et al.* (2017), pH is one of the key factors influencing the WHC of yoghurt. In protein gel foods, water is generally divided into the following three categories: bound, immobilised, and free water (Li *et al.,* 2021). Bound water has minimal impact on WHC, while immobilised water, which constitutes the majority, strongly influences both WHC and sensory quality (Qayum *et al.,* 2021). Therefore, the embedding of water molecules in gel networks, and the adsorption of water molecules by proteins are critical to keeping water in gel (Jose *et al.,* 2016), which have been heavily affected by the pH, pore size, and uniformity of gel network structure (Martin *et al.*, 2016; Urbonaite *et al.*, 2016). Our results (Figures 1 - 4) showed that all the addition amounts of water, sucrose, DATEM, gelatine, gellan gum, and β-CD had no significant effect ($p > 0.05$) on pH of egg-based yoghurt. However, these additions had notable impact ($p < 0.05$) on WHC, viscosity, and sensory quality. Therefore, it was reasonable to believe that the difference in WHC, viscosity, and sensory quality might be mainly caused by the different microstructure rather than pH of the gel when different amounts of water, sucrose, DATEM, gelatine, gellan gum, and β-CD were added. On the other hand, the pH of all our egg-based yoghurt samples could reach about 4.0 after 7 - 10 h fermentation, thus indicating that LAB could grow very well under the setting addition amounts of water, sucrose, DATEM, gelatine, gellan gum, and β-CD.

Furthermore, results also showed that egg-based yoghurt had an excellent WHC of up to 86.73%, which reached or exceeded that of the mainstream milk-based yoghurt in the current market (Fu *et al.*, 2018). We speculated that there might be two main reasons for the better WHC of egg-based yoghurt: Firstly, following LAB fermentation, the pH of our egg-based yoghurt reached the isoelectric point, which ranged from 3.9 to 4.2, and had no obvious difference from that of milk-based yoghurt (Mahomud *et al.*, 2017). Suitable pH is an important prerequisite for the formation of good texture and WHC of egg-based yoghurt. Secondly, the optimised formulation of egg-based yoghurt by adding water, sucrose, DATEM, gelatine, gellan gum, and β-CD significantly improved the gelation properties, which further enhanced the WHC of egg-based yoghurt.

Texture formation plays a crucial role in set yoghurt processing, which will be affected by many factors, such as water content, protein content, and the addition of sucrose and hydrophilic glues. According to Hao *et al.* (2016), the presence of sucrose reinforced the protein-protein interactions, which further significantly improved the hardness of protein gel. It had been already demonstrated that the increase in sugar concentration contributed to the OVA and OVT stability in egg white protein gels (Mohammadi Nafchi *et al.*, 2013). Gelatine and gellan gum are two common hydrocolloids in food, and now have been widely used to improve the texture of the protein gel products. Supavititpatana *et al.* (2008) found that gelatine improved yoghurt stability by decreasing syneresis. According to Mudgil *et al.* (2018), the syneresis of camel-milk-based yoghurt was reduced significantly with the addition of gelatine. Kiani *et al.* (2010) also suggested that network structure was formed by electrostatic attachment of gellan gum to fragments of acid-casein gel, and therefore strengthened the yoghurt structure by increasing the WHC. Based on the performed analyses, the appropriate amount of water, sucrose, DATEM, gelatine, gellan gum, and β-CD were added to improve the texture of egg-based yoghurt with hardness and fracturability as main indicators. Our results showed that the texture of the produced egg-based yoghurt with optimised formula was greatly improved when compared with that of the control, and the hardness and fracturability increased from 13 and 10.1 to 18.3 and 14.6 g, respectively. These results were well consistent with the microstructure images from SEM, which showed that the texture of the egg-based yoghurt with optimised formula looked more compact, denser, and smoother, and there were less cavities inside, while milk-based yoghurt texture was looser and coarser with more cavities, which might have been caused by the addition of hydrophilic glues and the different protein kinds and contents between egg- and milk-based yoghurts. Therefore, we deduced that the added gelatine and gellan gum might have complemented and enhanced the aggregation of three-dimensional network of egg proteins, which then contributed to well-defined rearrangement of the protein network, and immobilised more amount of egg proteins. Similar research was reported by Matumoto-Pintro *et al.* (2011).

Conclusion

Egg-based yoghurt formula was designed and optimised through the single factor experiments and D-optimal mixture design to obtain a high-quality egg gel product. The optimised egg-based yoghurt formula was as follows: whole liquid egg, 100 g; water, 180 g; sucrose, 30.89 g; DATEM, 0.28 g; gelatine, 0.112 g; gellan gum, 0.14 g; and β-CD, 0.56 g. Using the optimised formula, the produced egg-based yoghurt exhibited good textural, rheological, and microstructural properties, with high sensory score of 91.3. In comparison to milk-based yoghurt, our egg-based yoghurt presented a more compact, denser, and smoother texture, which might be mainly caused by the different kinds and contents of proteins in the two yoghurts, as well as the addition of gelatine and gellan gum.

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