Effect of whey protein isolate incorporated with various carbohydrate-based fat replacers on physicochemical and sensorial properties of low-fat chocolate ice cream

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Article history:

Received: 8 September 2021 Received in revised form: 22 October 2021 Accepted: 23 February 2022 Available Online: 10 February 2023

Keywords:

Low-fat chocolate ice cream, Carbohydrate-based fat replacer, Hydroxypropyl distarch phosphate, Pregelatinized starch, Inulin, Whey protein isolate

DOI:

https://doi.org/10.26656/fr.2017.7(1).705

Abstract

The purpose of this research was to study the effects of mixed fat replacers on the physicochemical and sensorial properties of low-fat chocolate ice cream. Fat replacers were the mixtures of whey protein isolate (WPI) and three types of carbohydrate-based fat replacers: pregelatinized starch (Alpha-starch), hydroxypropyl distarch phosphate (ADS-SU 09), or inulin at the ratios of 4:1, 1:1, and 1:4 (w/w). The qualities of the low-fat formulations (2.5% fat) were compared to those of the standard or control (8.5% fat). It was found that samples added with WPI and ADS-SU 09 at a ratio of 1:1 had the lowest melting rate ($p \le 0.05$). The pH of the samples was found to increase when the proportion of carbohydrate-based fat replacers rose. The use of WPI and inulin showed that when the level of inulin increased, the total soluble solid content decreased. The hardness values of all the samples ranged between 72.45 to 105.09 N. The samples incorporated with WPI and ADS-SU 09 at 1:4 ratio and with WPI and Alpha-starch at 1:4 ratio had higher hardness values than the other samples, and the difference was statistically significant ($p \le 1$) 0.05). Our findings displayed that the fat replacer mixtures could reduce the contents of fat and reducing sugar (p≤0.05) as compared to the control, but they had an apparent higher protein content ($p \le 0.05$). Increasing the proportion of carbohydrate-based fat replacers improved some physicochemical and sensory properties of the ice creams. In this case, the properties of low-fat chocolate ice cream incorporated with WPI and Alpha-starch at a ratio of 4:1 were found to have most closely resembled the properties of the standard sample.

1. Introduction

Ice cream is a type of dessert that consists of milk and other ingredients, *viz.* sweetener, stabilizing agent, and emulsifier. It is very popular and loved by people of all ages, especially in Thailand, where the weather is hot all year round. However, most of the ice cream sold results in high energy and cholesterol. It is widely known that milk and butter, the main ingredients of ice cream, are high in fat, which plays a role in increasing blood vessel cholesterol levels. This is the cause of many diseases, such as cardiovascular disease, hypertension, diabetes, and thyrotoxicosis (Akalin *et al.*, 2008; Espinoza *et al.*, 2020).

Nowadays, people are placing more importance on their health, weight control, and reducing disease risks. Therefore, low-fat foods have become more popular (Espinoza *et al.*, 2020). For this reason, the idea of

emerged. However, reducing the amount of fat in ice cream causes it to melt faster and reduces its sensory quality factors that make low-fat ice cream unappealing to consumers (Syed et al., 2018). Fat is an important ingredient in ice cream, it gives viscosity to the ice cream mix, which creates richness in flavour, a smooth texture, stickiness, and a wax coating in the mouth (Goff, 2008). The removal of fat droplets from low-calorie products often has a major influence on their desirable sensory attributes (McClements, 2015). In addition, it was found that the texture of low-fat foods is more important than flavour in determining their overall acceptability (Devereux et al., 2003). Consequently, there have been efforts to improve low-fat ice cream by using fat replacers that could imitate some properties of fat, such as the viscosity it provides to the ice cream liquid mixture, control of ice crystal growth, and water

producing low-fat and low-calorie ice cream has

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separation control (Gibis *et al.*, 2015). Although, the use of inappropriate fat replacers negatively impacts ice cream quality, as fat influences the appearance, flavour, texture, and melting characteristics (Hatipoğlu and Türkoğlu, 2020). Hence, the use of a suitable fat replacer is crucial for creating good quality low-fat ice cream.

Fat replacers can be categorized into three groups according to the raw materials used in production: lipidbased, protein-based, and carbohydrate-based (Tiwari et al., 2015). Each group has different functional properties and can be used alone or as a mixture (Hatipoğlu and Türkoğlu, 2020). Lipid-based fat replacers consist of emulsifiers, medium chain triacylglycerols or surfaceactive structural lipids that can stabilize emulsions. Carbohydrate-based fat replacers consist of modified starch, maltodextrins, cellulose derivatives, inulin, pectin, polydextrose and other dietary fibres (Akbari et al., 2019). Protein-based fat substitutes are generally produced from concentrated whey proteins (Goff and Hartel, 2013). Protein-based and carbohydrate-based fat replacers are preferred for ice cream production (Mahdian and Karazhian, 2013). Akalin et al. (2008) reported that the addition of whey-based fat replacers, such as Simplesse® 100, to low-fat ice cream, causes an increase in mix viscosity compared to that of regular ice cream mix owing to their water-binding capacity. Protein -based fat replacers give the ice cream a smooth texture, softness, a high percentage of overrun, less hardness, and more elasticity. There were many reports of their use in ice cream formulations (Akalin et al., 2008; Mahdian and Karazhian, 2013; Salem et al., 2016; Fuangpaiboon and Kijroongrojana, 2017). Compared to carbohydratebased fat replacers, those that are protein-based have a stronger whey flavour. Carbohydrate-based fat replacers give the ice cream a rough texture and high viscosity (Akalin et al., 2008; Salem et al., 2016), resulting in slow melting and a creamy sensation. Akbari et al. (2016) reported that low-fat ice creams incorporated with inulin had a significantly lower melting rate in comparison with the control. Inulin addition caused the adhesiveness and hardness of the low-fat ice creams to decrease significantly compared with low-fat ice cream without inulin. In this term, modified starches similar to other carbohydrate-based fat replacers can form hydrogen bonds with water molecules and increase ice cream viscosity and as a result improve melting behaviour (Surapat and Rugthavon, 2003).

Modified starch is becoming an increasingly popular additive in dairy products because of its relatively low cost, availability and benefits. Wang and Xu (2018) reported that in present yoghurt production, many manufacturers choose acetylated starch or hydroxypropyl distarch phosphate as a stabilizer. Hydroxypropyl distarch phosphate is a modified resistant starch. It is prepared by treating starch with propylene oxide and phosphoric acid or phosphorus oxychloride. It is stable against heat, acids, alkalis, and starch degrading enzymes, and is used as a thickener, stabilizer, and emulsifier in foods that are freeze-thawed and where better colour and shine are required (Wang and Xu, 2018). Thaiudom et al. (2008) revealed that modified tapioca starches, i.e, octenyl succinic anhydride starch and hydroxypropylated starch, have potential applications in ice cream production. Pregelatinized starches are provided which have a high degree of resistance to a-amylase digestion, fat-like texture and outstanding freeze-thaw stability. The starch products are formed as distarch phosphodiesters that undergo melting of the crystalline phase by heating above their gelatinization temperature. Hajibabaei and Abdolmaleki (2015) found that pregelatinized starch at a concentration of 0.45% and cross-linked starch at a concentration of 0.3% might be selected for the addition to skim milk but milk sample with cross-linked starch was superior due to its acceptable texture and flavour. The pregelatinized starches may be used in various food products, where they lend high dietary fibre, low fat and low-calorie characteristics to the product (Kaur et al., 2019). The physico-chemical properties of starch (such as the pasting, structure, and gelatinization) were influenced by the milk protein ingredients (Cui et al., 2014). As there is more than one type of fat replacer, different fat replacers have been mixed to create better products (Hatipoğlu and Türkoğlu, 2020). Despite many published reports on the use of fat replacers in ice cream, there are limited studies on the development of low-fat chocolate ice cream using mixed fat replacers.

Thus, the objective of this research was to study the effects of combined whey protein isolate (WPI) and carbohydrate-based fat replacers, *viz.* pregelatinized starch, hydroxypropyl distarch phosphate, and inulin, on the physicochemical properties and sensorial acceptability of low-fat chocolate ice cream. The purpose of examining this combination was to develop an alternative ice cream for health-conscious customers and as a guideline for future product development.

2. Materials and methods

2.1 Materials

Pasteurized milk with 0% fat (CP-Meiji Co., Ltd., Thailand), pasteurized cream with 35% fat (CP-Meiji Co., Ltd., Thailand), skim milk powder with 0% fat (ISM Food Products Co., Ltd., Thailand), cocoa powder (Sino-Pacific Trading (Thailand) Co., Ltd., Thailand), sucrose (Thai Multi-Sugar Industry, Thailand), stabilizeremulsifier blend and dark chocolate flavour were received from Miss Icecream Co., Ltd. (Thailand). Dextrose monohydrate, maltodextrin, WPI and inulin were received from Krungthepchemi Co., Ltd. (Thailand). Pregelatinized starch (Alpha-starch) was obtained from Thai Wah Alpha Starch Co., Ltd. (Thailand). Hydroxypropyl distarch phosphate (ADS-SU 09) was acquired from Adinop Co., Ltd. (Thailand).

2.2 Chocolate ice cream preparation

To prepare the fat replacers, WPI was separately mixed with Alpha-starch, ADS-SU 09, and inulin at the ratios of 4:1, 1:1, and 1:4 (w/w) (Rongkom, 2009). The product formulation (2.5% fat content) comprised 67.86% pasteurized milk, 5.22% pasteurized cream, and 1.57% fat-replacer mix. The other dry ingredients were 10.58% sugar, 3.85% cocoa powder, 3.85% maltodextrin, 0.96% dextrose monohydrate, 0.38% stabilizer-emulsifier blend, and 0.1% dark chocolate flavour.

Pasteurized cream was added to the pasteurized milk and the temperature was elevated to 50±2°C using a hot water bath. The thoroughly premixed dry ingredients (skim milk powder, sugar, cocoa powder, maltodextrin, dextrose monohydrate, fat replacer, and stabilizeremulsifier blend) were then transferred into the warmed milk. The mixtures were pasteurized at 80±2°C for 2 mins and homogenized for 2 mins using a high-speed homogenizer (model: W36, Moulinex, France), before cooling down to 4°C in an ice water bath. Following the addition of dark chocolate flavour, all mixtures were aged at 4±2°C for 24 hrs to ensure complete hydration of all ingredients. Ice cream mixes were placed into a hard ice cream maker with a compressor (model: 48845, UNOLD, Germany). Freezing took 45 mins before 50 g portions were transferred into plastic cups and hardened at -20±2°C for 24 hrs before being analyzed.

2.3 Physicochemical analysis

After ageing, the pH of the ice creams was measured using a pH meter (model: Lab 855, SI Analytics, Germany). Total soluble solids (TSS) were evaluated using a hand refractometer (model: N1, Atago, Japan), and viscosity was determined using a viscometer (model: DV-2P R, Anton Paar, Austria) at $10\pm2^{\circ}$ C with a spindle number of R2 and rotation speed of 1,000 rpm.

Overrun was measured by comparing the weight of the ice cream mix and ice cream in a fixed-volume container and determined using the formula of Pon *et al.* (2015).

$$O_n(\%) = 100 (W_m - W_{ic}) / W_{ic}$$
⁽¹⁾

Where O_n (%) is the overrun percentage, W_m (g) is the weight of a given volume of ice cream mix and W_{ic} (g) is

the weight of the same volume of ice cream.

The melting rate of the samples was assessed according to the modified procedure of EI-Zeini *et al.* (2016). In brief, 50 ± 1 g of sample was put on a wire mesh attached to a graduated cylinder and maintained under a controlled temperature of 25°C. The dripped volume was measured at 10 mins intervals for 60 mins. The recorded data were used to calculate the melting rate (100 g/min).

Weight of melting ice cream 100 $g = \frac{Weight of melting ice cream}{starting weight of ice cream} \times 100$ (2)

Hardness measurements were obtained at $25\pm2^{\circ}$ C using a texture analyzer (model: CT3 10, Brookfield, USA) equipped with a 25.4 mm diameter cylindrical probe (TA11). Ice cream samples were tempered to -10° C before analysis. The conditions for analysis were as follows: penetration distance = 10 mm, probe speed during penetration = 2 mm/s, probe speed pre- and post-penetration = 1 mm/s. The ice cream, which remained in the plastic cup, was penetrated at two places on its largest smooth surface.

The colour parameters of the ice cream samples were measured using a colourimeter (model: XE Pluse, MiniScan, USA) with the CIE system, where L^* , a^* , and b^* indicated brightness, reddish, and yellowish, respectively.

For some chemical compositions of the ice creams, fat content was investigated using the Roese-Gottlieb method based on AOAC (2019); method 989.05. Protein content was determined using the Kjeldahl method based on AOAC (2019); method 981.10. Reducing sugar content was evaluated based on AOAC (2019); method 906.03.

2.4 Sensory evaluation

Sensory evaluation of different formulas of low-fat chocolate ice creams was performed by fifty untrained panellists, such as students, academic staff, and others in Phranakhon Si Ayutthaya Rajabhat University. Ice cream samples stored at -20°C were removed from the freezer and tempered for 2 mins at $-2\pm2^{\circ}$ C prior to sensory testing. The sensory characteristics focused on colour, flavour/taste, smoothness, creaminess, gumminess, meltability, and overall acceptability. All low-fat samples were compared to the control (8.5% fat), and the results were recorded as scores ranging from 1 to 9, where 9 = like very extremely, 5 = neither like nor dislike, and 1 = dislike extremely.

2.5 Statistical analysis

Means and standard deviations (means \pm S.D.) of the data were calculated from independent triplicate

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determinations of duplicate samples in the same batch. Analysis of variance (ANOVA) was carried out using SPSS software version 23 (SPSS Inc., Chicago, Illinois, USA). Differences among the treatment means were compared by Duncan's multiple range tests ($p \le 0.05$).

3. Results and discussion

3.1 Physicochemical properties of low-fat chocolate ice creams

As shown in Table 1, the physicochemical properties of low-fat chocolate ice creams revealed that the viscosity of samples incorporated with sole WPI and standard formula were lower than those mixed with other fat replacers, and the difference was statistically significant ($p \le 0.05$). The viscosity values of the sample were found to have increased according to the raising amounts of carbohydrate-based fat replacers. The viscosity of the ice cream that contained WPI and inulin was lower than other carbohydrate-based fat replacers. This might be due to inulin being а heteropolysaccharide, with less ability to absorb water and dilate than ADS-SU 09 and Alpha-starch. ADS-SU 09 and Alpha-starch are hydrocolloids, which could hydrate water better than inulin at the same level of concentration. The high viscosity of ADS-SU 09 and Alpha-starch solutions could be attributed to the more linear conformation of their structure, compared with inulin, resulting in a high free volume of gyration when they rotated themselves in water. This was similar to the report of Fuangpaiboon and Kijroongrojana (2017), who found that among the carbohydrate-based fat replacers, the use of modified starch led to a pronounced increase in the consistency index when compared to the control and the value was significantly different from those of all the other samples, while the addition of inulin and maltodextrin had no significant effect. Marshall et al. (2003) reported that the water holding capacity and degree of polymerization and branching are among the most critical factors influencing the development of viscosity in ice cream mixes. Akalin et al. (2008) revealed that calcium is believed to promote protein association and increase polymer length. Extremely high viscosity in ice cream mixes containing WPI can be sourced from these polymers or large protein networks and also from the higher molecular weight of WPI in comparison to inulin. Therefore, when inulin was combined with WPI, the ice cream became slightly reduced viscous when compared to the ice cream that contained combinations of Alpha-starch and ADS-SU 09 in the same proportion. When the same amount of protein-based fat replacers and carbohydrate-based fat replacers was used, it was found that the sample with ADS-SU 09 was more viscous than those with Alphastarch and inulin. A solution of ADS-SU 09 was more viscous than Alpha-starch even though both of them have emulsifying properties. This may be due to ADS-SU 09 having stronger hydrophilic and hydrophobic groups compared with Alpha-starch, which might have interference from its component ingredients. Furthermore, this finding also displayed that viscosity was evidently related to fat and sugar contents. Akin et al. (2007) revealed that sugar and fat help to increase the amount of solids in the ice cream mixture and improve mix viscosity, air incorporation, body, texture, and melting properties. When fat and sugar content was higher, the level of stickiness increased. Conversely, when the proportions of fat and sugar-reduced, lower viscosity results. Similarly, Balthazar et al. (2017) showed that ice cream becomes stickier when fat content increases.

Overrun in ice cream is mainly the functionality of milk proteins, emulsifiers, and to certain extent milk fat. The emulsion formed during mixing is aerated to induce

Treatment	Fat* (% dry weight basis)	pН	TSS (%Brix)	Viscosity (cP)	Overrun (%)
W (Control)	2.5	$6.60{\pm}0.05^{d}$	35.17±0.41 ^{cd}	393.42±22.27 ^h	16.37±2.29 ^d
W+A1 (4:1)	2.5	6.62±0.01 ^{ed}	$35.33{\pm}0.52^{\circ}$	456.83±19.70 ^g	16.69 ± 0.64^{d}
W+Al(1:1)	2.5	$6.63{\pm}0.00^{cde}$	$36.33{\pm}0.52^{\text{b}}$	563.33±13.48 ^e	14.69 ± 0.05^{cd}
W+Al (1:4)	2.5	6.67 ± 0.02^{bc}	$36.67{\pm}0.52^{ab}$	$912.20{\pm}16.04^{b}$	$14.73 {\pm} 0.17^{cd}$
W+SU (4:1)	2.5	6.62±0.01 ^{ed}	34.67 ± 0.52^{de}	$509.30{\pm}25.46^{\rm f}$	20.88±3.16 ^c
W+SU (1:1)	2.5	6.66 ± 0.03^{bc}	$37.00{\pm}0.00^{a}$	$788.53{\pm}14.45^{c}$	13.85±2.17 ^e
W+SU (1:4)	2.5	$6.69{\pm}0.01^{b}$	$35.33{\pm}0.52^{\circ}$	$1400.53{\pm}27.96^{a}$	$19.38{\pm}2.89^{c}$
W+In (4:1)	2.5	6.64±0.01 ^{cd}	$35.33{\pm}0.52^{\circ}$	$444.93{\pm}12.68^{\rm f}$	15.15±2.31 ^{cd}
W+In (1:1)	2.5	$6.66 {\pm} 0.00^{\rm bc}$	$35.67 \pm 0.52^{\circ}$	$529.60{\pm}15.55^{g}$	15.13±0.64 ^{cd}
W+In (1:4)	2.5	$6.69{\pm}0.01^{b}$	$34.67{\pm}0.52^{de}$	710.31 ± 22.47^{d}	25.56 ± 3.69^{b}
Standard formula	8.5	$6.78{\pm}0.05^{a}$	34.33±0.52 ^e	376.47 ± 12.63^{h}	33.34±2.29 ^a

Table 1. pH, TSS, viscosity and overrun of full fat and low-fat chocolate ice creams incorporated with different fat replacers.

*Calculated from ice cream formula. Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05). W: Whey protein, Al: Alpha starch, SU: ADS-SU 09, In: Inulin

air bubbles in the mix in absence of adequate fat emulsion formation might have been adversely affected. Regarding the relationship between viscosity and overrun (Table 1), it was not possible to draw a definitive conclusion, as the increase in viscosity caused more air holes. However, high viscosity obstructed air absorbance by the ice cream mixture, leading to a decrease in overrun. Sharma et al. (2017) revealed that the lower overrun was correlated with increased viscosity. The overrun of the standard formula was 33.34%, while it was high for other reduced fat ice creams ($p \le 0.05$). The overrun of reduced fat ice creams containing 2.5% fat ranged from 13.83 to 25.56%. The lowest value was observed for the ice cream mix containing WPI and ASD-SU 09 at a ratio of 1:1. Among the reduced fat ice creams, the highest overrun was observed for ice cream with WPI and inulin at a ratio of 1:4. Adapa et al. (2000) concluded that reduced-fat ice cream mixes containing carbohydrate-based fat replacers exhibited a viscous behaviour owing to the capability for imbibing water, which would increase the viscosity of the system. This increased viscosity could have been the primary reason for decreased whipping abilities. Mahdian and Karazhian (2013) also observed a lower overrun for ice cream containing inulin and milk protein concentrate. Yilsay et al. (2006) also reported that the substitution of a 6% whey protein fat replacer (Simplesse®100) for milk fat decreased the overrun in low or fat-free ice cream mixes. A high overrun value corresponds to high foam expansion and foam stability values. When the stickiness is too high or too low, it will the air absorbance of the ice reduce cream (Chansathirapanich et al., 2016). Similarly, Balthazar et al. (2017) showed that formability depends on air cell distribution in ice cream. Air cells can be stabilized on a surface with protein-phospholipid and emulsifier (Yan et al., 2021). Fat has an influence: it prevents formability by replacing protein that holds air cells and has a foam depressant property (Warren and Hartel, 2018).

The melting rates of all samples added with the fat replacers were lower than that of the standard formula (Figure 1, Table 2). Similar results were noticed by Babu *et al.* (2018) and Akbari *et al.* (2016), who found that increasing amounts of fat replacers led to a decrease in the ice cream melting rate. This is because the fat replacers augmented the solid content, which resulted in decreasing water activity. This also led to links between fat replacer molecules, which makes ice cream stickier. This was in agreement with the findings of Pon *et al.* (2015), who showed that all samples with lower solid content had higher melting rates than those with high solid content. Therefore, the standard sample led to having lower solid content and higher melting rates than the samples added with the fat replacers. In addition, similar results were noticed by Goff and Hartel (2013), who reported that ice cream with high viscosity had a slow melting rate. A mixture with a higher water content can create more ice crystals, and heat conduction can occur faster in ice-formatted products. As a consequence, ice cream melts more quickly when there is more water activity in the form of ice (Yan *et al.*, 2021).

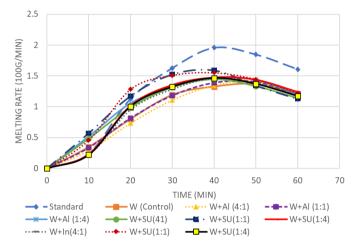


Figure 1. Melting rate of full fat and low-fat chocolate ice creams incorporated with different fat replacers.

The TSS of the samples is also shown in Table 1. The results illustrated that ice cream incorporated with mixed fat replacers had a higher TSS than the standard formula, and the difference was statistically significant (p£0.05). The sample contained a combination of WPI and ADS-SU 09 at a ratio of 1:1 and had the highest TSS, followed by samples added with the combination of WPI and Alpha-starch at a ratio of 1:1 and 1:4, respectively. The samples incorporated with WPI and ADS-SU 09 at 4 :1 ratio and with WPI and inulin at 1:4 ratio were found to have TSS similar to the control. Ice cream with lower TSS may have proportionately more water to freeze, thus contributing to more ice crystal formation, influencing the texture and body of ice cream. Thus, these texture differences will directly influence the consumers' judgment of the ice cream's quality (Pon et al., 2015).

The pH of the samples ranged between 6.60 to 6.70 (Table 1). All reduced fat ice cream samples had higher pH values than standard samples, and the difference was statistically significant ($p \le 0.05$). Our finding indicated that pH most likely increased when the ratio of carbohydrate-based fat replacers rose. In addition, it was found that the use of sole WPI resulted in a lower pH than the others. This was in agreement with the findings of EI-Zeini *et al.* (2016), who showed that a reverse proportion of pH and WPI was obtained for ice cream samples. The pH values of the ice cream mix decreased significantly by substituting milk solids not fat (MSNF) with WPI in the standard formula. Whey protein is

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Table 2. Meiling rate, nargness and colour	parameters of full fat and low-fat chocola	ate ice creams incorporated with different fat
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Treatment	Fat* (% dry weight basis)	Melting (%/min)	Hardness	Colour		
			(N)	L*	L* a*	
W (Control)	2.5	$1.20{\pm}0.01^{cd}$	73.00±6.42°	$28.63{\pm}1.35^{d}$	$8.70{\pm}0.84^{ m bc}$	$10.87{\pm}1.48^{bcd}$
W+Al (4:1)	2.5	1.21 ± 0.03^{bcd}	76.52±4.43°	$28.94{\pm}0.62^{d}$	$9.56{\pm}0.57^{ m abc}$	11.04 ± 1.39^{bcd}
W+Al(1:1)	2.5	$1.19{\pm}0.01^{cd}$	88.04 ± 4.74 ^b	$29.06{\pm}0.91^{d}$	$9.42{\pm}0.28^{abc}$	$10.75 {\pm} 0.97^{bcd}$
W+Al (1:4)	2.5	$1.20{\pm}0.01^{cd}$	$101.07{\pm}10.05^{a}$	$28.69 \pm 1.09^{\circ}$	$7.81 \pm 3.62^{\circ}$	$9.58{\pm}2.35^d$
W+SU (4:1)	2.5	$1.14{\pm}0.04^{ef}$	79.64±4.11 ^{bc}	29.76±1.67 ^d	$9.09{\pm}0.99^{abc}$	10.14 ± 0.22^{bcd}
W+SU (1:1)	2.5	$1.13{\pm}0.02^{\rm f}$	82.02 ± 4.24^{bc}	28.19 ± 3.44^{d}	8.12±1.75 ^c	7.74 ± 1.80^{e}
W+SU (1:4)	2.5	$1.24{\pm}0.01^{b}$	$105.09{\pm}5.26^{a}$	$31.34{\pm}0.23^{bc}$	$10.50{\pm}037^{ab}$	11.17 ± 0.21^{bcd}
W+In (4:1)	2.5	1.21 ± 0.02^{bcd}	75.80±1.48 ^c	$31.82{\pm}0.41^{b}$	$10.81{\pm}0.47^{a}$	13.03±1.06 ^a
W+In (1:1)	2.5	$1.22{\pm}0.03^{bc}$	$76.10 \pm 2.45^{\circ}$	$28.79b{\pm}0.51^{d}$	$9.84{\pm}1.60^{abc}$	$9.74{\pm}1.55^{cd}$
W+In (1:4)	2.5	1.17±0.03 ^e	77.91±3.09°	31.24a±0.41 ^b	$10.87{\pm}0.38^{a}$	11.96 ± 0.35^{ab}
Standard formula	8.5	1.61 ± 0.01^{a}	72.45±3.87 °	38.30±1.38 ^a	8.71±0.36 ^{bc}	11.53±1.19 ^{abc}

*Calculated from ice cream formula. Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05). W: Whey protein, Al: Alpha starch, SU: ADS-SU 09, In: Inulin

produced by acid-mediated coagulation. As a result, products are more acidic when a protein-based fat replacer is used. Additionally, acidity is caused by certain components, such as milk proteins, minerals, and the dissolution of carbon dioxide (Pandiyan *et al.*, 2010).

A texture profile analysis was performed, with texture measured as hardness (N). It was found that samples added with WPI and inulin every ratio had the lowest hardness values than the other carbohydrate-based fat replacers, the hardness was not significantly different from that of the control and standard formula (p>0.05)(Table 2). This was due to Alpha-starch and ADS-SU 09 being modified starches consisting of modified amylose and amylopectin, which can react with water, ice, and other ice cream components. Starch polymer chains in this ice cream might cause ionic gelation, which results in no changes in shape when it touches the tongue. For this reason, it is firmer during sensory evaluation (Yan et al., 2021). The firmness of ice cream is generally related to its structure. Air cells can be affected by the decrease in fat groups, which are the main component of ice cream (Granger et al., 2005). When modified starch is used to reduce fat, firmness is affected-modified starch increases firmness and shrinks ice crystals. While, water absorption by inulin and hence the increase of unfrozen water content and consequently the decrease of the ice crystals in ice cream structure, could probably be the reason for the lower hardness of the inulin-containing low-fat ice cream compared with other carbohydratebased fat replacers (Akbari et al., 2016). Furthermore, when the viscosity and solid content were taken into account, it was observed that samples with higher solid content had higher viscosity and melt slower than samples with lower solid content. It was also found that samples with higher solid content had a firmer and

harder texture than those with lower solid content (Syed *et at.*, 2018).

In terms of colour parameters, the L^* , a^* , and b^* values of the samples were evaluated (Table 2). The L^* values ranged from 28.19 to 38.30. The control ice cream resulted in the highest L^* value (38.20), and the value was significantly higher than those of the others (p£0.05). Despite all the carbohydrate-based fat replacers used being white, the ice cream colour was dark or had a low L^* value due to the inclusion of WPI. The a^* values of the samples ranged from 7.81 to 10.87. Ice cream added with WPI and inulin provided different a^* values than the standard and others, with a statistical significance of p≤0.05. For ice cream with WPI as the only fat replacer, the a^* value was not significantly different from that of the control (p>0.05). The b^* values ranged from 7.74 to 13.03. The sample with WPI and inulin at a ratio of 4:1 had the highest b^* value, but it was not significantly different from that of the standard sample (p>0.05).

For some chemical compositions (Table 3), it was found that the control sample ice creams and the incorporated with mixed fat replacers had higher protein contents than the standard formula, and the difference was statistically significant (p≤0.05). Given that WPI was derived from milk protein, it was not unexpected that the protein content of the samples containing WPI was higher than that of the standard formula. The protein content decreased in samples with WPI and a carbohydrate-based fat replacer as the ratio of carbohydrate-based fat replacers increased. Samples added with sole WPI (control) were found to have the highest protein content (7.82%), following samples added with WPI and Alpha-starch (4:1) (7.67%). It was also found that fat content tended to decrease when the

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Table 3. Chemical compositions of full fat and low-fat chocolate ice creams incorporated with different fat replacers.

1			1	
Treatment	Fat*	Fat	Protein	Reducing sugar
Treatment	(% dry weight basis)	(%)	(%)	(%)
W (Control)	2.5	$2.53{\pm}0.05^{b}$	$7.82{\pm}0.05^{a}$	35.20 ± 0.42^{cd}
W+A1 (4:1)	2.5	$2.70{\pm}0.23^{b}$	$7.67{\pm}0.00^{\rm b}$	$33.56{\pm}0.13^{d}$
W+Al (1:1)	2.5	$2.64{\pm}0.05^{b}$	6.83 ± 0.00^{e}	$34.78{\pm}0.75^{cd}$
W+Al (1:4)	2.5	$2.51{\pm}0.01^{b}$	$6.27{\pm}0.01^{g}$	$33.70{\pm}0.84^{d}$
W+SU (4:1)	2.5	2.80±0.01 ^b	7.49±0.01°	37.41 ± 1.40^{b}
W+SU (1:1)	2.5	$2.73{\pm}0.02^{b}$	$6.54{\pm}0.00^{\rm f}$	34.49 ± 0.89^{cd}
W+SU (1:4)	2.5	$2.54{\pm}0.01^{b}$	$6.17{\pm}0.01^{h}$	36.03 ± 0.91^{bc}
W+In (4:1)	2.5	$2.86{\pm}0.36^{b}$	$7.11 \pm 0.02^{\circ}$	35.86 ± 1.04^{bc}
W+In (1:1)	2.5	$2.76{\pm}0.45^{b}$	$6.26{\pm}0.01^{g}$	$33.16{\pm}0.77^d$
W+In (1:4)	2.5	$2.67{\pm}0.30^{b}$	$6.08{\pm}0.03^{i}$	34.61 ± 0.98^{cd}
Standard formula	8.5	$8.55{\pm}0.05^{a}$	5.99 ± 0.01^{j}	$41.28{\pm}0.76^{a}$
()				

*Calculated from ice cream formula. Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05). W: Whey protein, Al: Alpha starch, SU: ADS-SU 09, In: Inulin

ratio of carbohydrate-based fat replacer increased. The combination with WPI and Alpha-starch resulted in a lower fat content than the combination with WPI and other fat replacers in the same proportion. However, there was no statistically significant difference (p>0.05). Therefore, this revealed that increasing the carbohydrate-based fat replacer ratio can reduce fat content in low-fat chocolate ice cream, especially when using Alpha-starch as a fat replacer. In addition, the reduced sugar contents of all the samples incorporated with the fat replacers were lower than the control (p \leq 0.05).

3.2 Sensorial properties of low-fat chocolate ice creams

The sensory evaluation of the ice cream samples was performed, as shown in Table 4. The colour scores of the low-fat chocolate ice creams ranged from 7.38 to 7.96. This indicated that the consumers were fairly satisfied to very satisfied. The scores acquired were close to those of the standard sample (fat content of 8.55%), which received a colour satisfaction score of 8.10. Flavour preferences were also assessed. Ice cream with WPI only, with WPI and Alpha-starch at a ratio of 4:1, and with WPI and ADS-SU 09 at a ratio of 1:4 and 1:1 received similar scores on chocolate flavour to ice cream with the standard sample (p>0.05). The samples with WPI and inulin at all ratios received different scores on chocolate flavour compared to the standard sample ($p \le 0.05$). Regarding the satisfaction score for chocolate flavour and fat content, it was found that ice cream with WPI and Alpha-starch at a ratio of 1:4 produced the lowest fat content and received the lowest flavour score. This result is expected because the fat provides the transport of many flavour compounds. This finding was similar to the report of Hatipoğlu and Türkoğlu (2020), who found that vanilla-flavoured ice cream with increased fat content received better flavour scores. This is because fat adds flavour to ice cream and creates an additional flavour sensation when ice cream is melting in the mouth. The level of fat content affects the duration of flavour release and tasting (Balthazar et al., 2017). Therefore, when the fat content of the chocolate ice cream formula was decreased, tasters rated it with lower

Table 4. Liking scores of full fat and low-fat chocolate ice creams incorporated with different fat replacers.

	e			1		1
Treatment	Colour	Flavour	Smoothness	Gumminess	Melting	Overall acceptability
W (Control)	$7.96{\pm}0.86^{ab}$	7.50±1.23 ^{abc}	7.56 ± 0.81^{abcde}	$7.36{\pm}1.08^{ab}$	$7.86{\pm}0.88^{ab}$	7.76±1.02 ^{abc}
W+Al (4:1)	$7.80{\pm}1.07^{abc}$	$7.74{\pm}1.05^{ab}$	$7.84{\pm}1.09^{abc}$	$7.36{\pm}1.52^{ab}$	$7.54{\pm}1.34^{abc}$	7.94±1.15 ^{ab}
W+Al(1:1)	7.62 ± 1.12^{abc}	7.52 ± 1.39^{abc}	7.24 ± 1.46^{de}	$7.32{\pm}1.52^{ab}$	7.28 ± 1.49^{bc}	7.46 ± 1.20^{bc}
W+Al (1:4)	7.46 ± 1.42^{bc}	$7.08 \pm 1.28^{\circ}$	$7.50{\pm}1.25^{\text{abcd}}$	$7.20{\pm}1.29^{ab}$	7.24 ± 1.32^{bc}	7.34±1.32°
W+SU (4:1)	$7.64{\pm}0.94^{abc}$	7.48±1.31 ^{abc}	$7.70{\pm}0.99^{bcde}$	$7.44{\pm}1.55^{a}$	$7.50{\pm}1.40^{abc}$	7.52±1.31 ^{bc}
W+SU (1:1)	$7.80{\pm}1.36^{abc}$	7.54 ± 1.51^{abc}	7.68 ± 1.52^{abcd}	$7.48{\pm}1.80^{a}$	$7.52{\pm}1.54^{abc}$	7.62 ± 1.66^{bc}
W+SU (1:4)	7.92 ± 1.12^{abc}	$7.34{\pm}1.36^{bc}$	7.88 ± 1.12^{ab}	$7.64{\pm}1.47^{a}$	$7.42{\pm}1.57^{abc}$	7.56 ± 1.43^{bc}
W+In (4:1)	7.38±1.51°	7.20±1.53 ^{bc}	7.28±1.40 ^{cde}	7.16±1.50 ^{ab}	7.29 ± 1.54^{bc}	7.32±1.38°
W+In (1:1)	7.38±1.34°	7.24 ± 1.38^{bc}	7.28 ± 1.47^{cde}	$7.10{\pm}1.53^{b}$	7.06±1.39°	7.18±1.55°
W+In (1:4)	7.60 ± 1.21^{abc}	7.16 ± 1.52^{bc}	7.06±1.71 ^e	$7.12{\pm}1.35^{ab}$	7.28 ± 1.55^{bc}	7.20±1.32°
Standard formula	$8.10{\pm}0.86^{a}$	7.98±0.91 ^a	$8.08{\pm}0.78^{a}$	7.63±1.30 ^a	$7.98{\pm}1.05^{a}$	$8.18{\pm}0.75^{a}$

Values are presented as mean±SD. Values with different superscript within the same column are significantly different (p<0.05). W: Whey protein, Al: Alpha starch, SU: ADS-SU 09, In: Inulin.

scores for chocolate flavour compared to the standard formula with higher fat content.

In addition fat content influenced ice cream texture by reducing ice crystal size and making the ice cream texture smooth (Espinoza et al., 2020). This was in agreement with the findings of Yan et al. (2021), who stated that the higher fat content in ice cream causes a decrease in water content and shrinks ice crystals. Therefore, ice cream with a lower fat content is flakier and more crumbly, hence less smooth (Balthazar et al., 2017). Reducing the fat content in ice cream makes the texture rougher. In this study, a reduction in the fat content of a chocolate ice cream formula from 8.5% to 2.5% resulted in a smoothness satisfaction score that was decreased but still satisfies consumers in terms of smoothness. The carbohydrate-based fat replacers can form bonds with water, which causes fewer ice crystals (Akbari et al., 2019). For this reason, ice cream still satisfies consumers in terms of smoothness. In terms of the satisfaction score for melting in the mouth, it was found that the score for the sample containing WPI and inulin every ratio had lower scores than other samples and was statistically significant ($p \le 0.05$).

Regarding the results in Table 4, the gumminess scores of all low-fat samples and control were not significantly different (p>0.05). This was because carbohydrate-based fat replacers and microcrystalline cellulose improved product quality and caused slight or no changes in gumminess. In terms of the overall acceptance scores, the standard formula scored the highest at 8.18. This was similar to the scores received by samples with WPI and Alpha-starch at a ratio of 4:1 (7.94) and the control (7.76; p \leq 0.05).

4. Conclusion

This study demonstrated that as the amount of carbohydrate-based fat replacer increased, the viscosity, pH, and TSS of the ice cream rose. The use of WPI together with ADS-SU 09 at a ratio of 1:1 was the best formula for reducing the melting rate. This study also demonstrated that using WPI with carbohydrate-based fat replacer can be used in low-fat ice cream formulations in combination with each other to achieve optimum quality. Low-fat chocolate ice cream incorporated with WPI and Alpha-starch at a ratio of 4:1 was found to have properties that were most similar to those of the standard formula. Therefore, it can be used as an optimal formula for producing low-fat and low-calorie chocolate ice cream in the future.

Acknowledgements

The authors would like to thank Phranakhon Si Ayutthaya Rajabhat University for its financial support. Ethical consideration Before the data collection was undertaken, this research was accepted by the Naresuan University Regional Research Ethics Committee from Naresuan University, Thailand, and the code was COA No. 041/2020.

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