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Application of camellia oil-based diacylglycerol and its solid fractions in soft ice cream

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Abstract

Ice cream products are sweet and rich in taste, which make them popular desserts among consumers. However, the high-fat and high-sugar contents in ice creams may result in multiple health conditions after prolonged consumption. Camellia oil-based diacylglycerol (CD) oil can reduce the body fat accumulation due to their different metabolic pathways from triacylglycerol (TAG) oil. It is believed that the long-term consumption of CD can improve human's health by lowering the plasma TAG and blood sugar levels. Nevertheless, CD is unsuitable for direct application in ice cream products due to its low solid fat content (SFC). Therefore, in the present work, fractionation was attempted to increase the SFC of the CD. Subsequently, camellia oil, CD, and its solid fractions were characterised and further incorporated in ice cream formulations. It was found that fractionation significantly ($p < 0.05$) increased the SFC of the CD. The solid fractions of CD exhibited similar physicochemical properties with the oils/fats commonly used for ice cream production, namely palm olein and milk fat. At 0°C, the SFC of the CD solid fractions was 29.33%; whereas at 37°C, the SFC decreased rapidly, which enabled them to melt quickly in the mouth. At the same time, CD solid fractions were rich in β'-form crystals which contributed to the rich, delicate, and smooth texture for ice cream products. The ice cream formulated with CD solid fractions showed better overrun (48.24%) and hardness (594.18 g) as compared to the ice creams prepared with camellia oil (with the overrun and hardness of 41.27% and 524.36 g, respectively) and CD (with the overrun and hardness of 39.77% and 284.31 g, respectively). The substitution of TAG with CD solid fractions made the formulated ice cream product a healthier dessert, and at the same time provided similar organoleptic properties as conventional ice creams.

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Introduction

Lipid plays an important role in influencing the flavour and texture of ice cream (Choi and Row, 1999). There are two types of ice cream namely hard and soft ice cream, which contain 8 - 16% and 3 - 8% lipid contents (Feizi *et al.*, 2021), respectively. Generally, the higher proportions of lipid incorporation in ice cream formulation aid in lowering its melting rate by reducing the thermal diffusivity and preventing heat transformation (Hossain *et al.*, 2021). However, ice cream is often related as high-fat and high-sugar product which can possibly lead to some health issues upon high and frequent intake, for instance obesity, diabetes mellitus, and non-alcoholic fatty liver disease (Zeng *et al.*, 2012). Therefore, it is high time to develop healthier ice cream product with reduced fat, or with healthier lipid incorporation in the formulations.

Various research works have attempted to develop healthier ice cream formulations by incorporating healthier lipids, oleogels, hydrocolloids (*e.g.*, pea protein isolate, modified starch), as well as fat substitute (*e.g.*, olster, oatrim) (Subroto *et al.*, 2020; Silva-Avellaneda *et al.*, 2021; Yang *et al.*, 2022). However, not all developed healthier ice cream formulations yielded desirable organoleptic properties. For instance, Aime *et al.* (2001) applied modified pea starch as fat replacer in preparing low-fat and fat-free ice creams, but the products showed low viscosity, smoothness, and mouth coating properties. The choice of fat substitutes/replacers

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often impacted the taste, crystal formations, as well as the imparted flavour, which could be less acceptable among the consumers (Mao *et al.*, 2018). Therefore, the selection of the type of lipids or fat replacers is relatively important in formulating an ice cream product.

The main component of typical edible oils is triacylglycerols (TAGs). Diacylglycerol (DAG) is also naturally present in edible oil, and generally possesses similar organoleptic properties like regular TAGs. However, it is worth mentioning that DAG possesses different metabolic pathway from TAG, which results in reduction of body fat accumulation upon long term consumption of DAG (Cho *et al.*, 2006). Recently, camellia oil has gained popularity as a health-promoting oil as it is rich in monounsaturated fatty acids (~80%, mainly oleic acid), and contains low level of saturated fatty acids (~15%, mainly palmitic acid and stearic acid). Study shows that the consumption of camellia oil is effective in lowering the low-density lipoprotein level, and reduces the risk of atherosclerosis (Lee *et al.*, 2007). Due to the health benefits of DAG over TAG, the incorporation of camellia oil-based diacylglycerol (CD) in ice cream products is of particular interest. However, the low melting point of CD poses a limitation in producing frozen products. Dry fractionation is a useful method in separating the solid and liquid fractions of the oil. It is often conducted to improve the quality of oil by preventing the crystallisation of liquid oil (Luo *et al.*, 2020). In the present work, the dry fractionation process was applied on CD, and the CD solid fractions obtained were subsequently incorporated into ice cream formulations. It is believed that the CD solid fractions could yield better ice cream texture and overrun as compared to the unfractionated CD or camellia oil, due to its higher solid fat content (SFC) and melting point.

The present work thus aimed to investigate the physicochemical properties of camellia oil, CD, and the CD solid fractions for better understanding of their applications in ice cream production. Besides, the overrun, texture, and polymorphism of ice creams produced using camellia oil, CD, and solid fractions of CD were also evaluated and compared.

Materials and methods

Chemicals and reagents

Camellia oil and CD were supplied by Guangdong Yue-Shan Special Nutrition Technology Co. Ltd. (Guangdong, China). Skimmed milk powder was purchased from a local supermarket (Guangdong, China). Maltodextrin, carboxymethyl cellulose, xanthan gum, pectin, and xylitol were purchased from Sigma-Aldrich (Shanghai, China). Vanilla essence was purchased from Anhui Zhonghong Biological Engineering Co. Ltd. (Anhui, China). Triolein, diolein, monoolein, and fatty acid methyl ester standards were purchased from Aladdin (Shanghai, China). Isopropanol, isooctane, formic acid, and *n*-hexane were of HPLC-grade, and purchased from Aladdin (Shanghai, China).

Dry fractionation of camellia oil-based DAG

The DAG fractionation process was conducted following the method described by Luo *et al.* (2020). CD was first melted completely at 50°C for 2 h. The initial temperature of dry fractionation was 28.6°C, and was gradually cooled down to 20.0°C within 6.5 h. Subsequently, the same temperature was maintained for 3 h to increase the hardness of the solid fraction. Brinell funnel was used to separate the solid and liquid fractions. The physicochemical properties of solid fractions were analysed prior to their incorporation into ice cream formulations. The fractionated CD was referred as the solid fractions of the CD in the following sections.

Characterisation of camellia oil, camellia oil-based DAG, and its solid fractions

Acylglycerol profile

The acylglycerol profile of CD and its solid fractions were determined using HPLC (Waters Corporation, Milford, MA, USA) (Li *et al.*, 2019). Briefly, 45 µL of samples were mixed with 1 mL of mobile phase (*n*-hexane:2-propanol:formic acid; 18:1:0.003; v/v/v) and small amount of anhydrous sodium sulphate. After centrifugation at 10,000 *g* for 3 min, the supernatant was filtered and injected into HPLC. A Luna column (250 × 4.6 mm i.d., 5 µm particle size; Phenomenex Corporation, Torrance, CA, USA) was used to analyse the samples, and the mobile phase flow rate was fixed at 1 mL/min. The proportions of TAG, DAG, monoacylglycerol (MAG), and free fatty acid (FFA) were calculated based on area normalisation method.

Fatty acid compositions

Gas chromatography (GC) coupled with a flame ionisation detector (FID; Agilent 7890A, Agilent Technologies, CA, USA) were adopted to

determine the fatty acid compositions of the oil samples. Firstly, 300 µL of sample and 6 mL of methanolic sodium hydroxide solution (2%, w/v) were mixed and heated at 60°C for 30 min. Then, 3 mL of methanolic boron trifluoride solution was added to the mixture and further heated at 60°C for 5 min. After the mixture was cooled down to room temperature, 5 mL of isooctane and 10 mL of saturated sodium chloride solution were added and mixed thoroughly. The mixture was allowed to stand for a 5 min. The upper layer was collected and added with small amount of anhydrous sodium sulphate for water removal. After centrifugation at 10,000 *g* for 3 min, the supernatant was injected into the GC. The fatty acid compositions were quantified using the area normalisation method.

Solid fat content (SFC)

The SFC of the oil samples was determined using a pulsed nuclear magnetic resonance (p-NMR) spectrometer (Minispec-mq20, Bruker, Karlsruhe, Germany) (Podchong *et al.*, 2018). Briefly, 3 mL of sample were transferred into an NMR tube, and incubated at 90°C to eliminate the existing crystals. Then, sample was incubated at 0°C for 90 min before the SFC determination. The SFC of the oil samples was determined from 0 to 40°C (with 5°C interval).

Polymorphism analysis

The polymorphism analysis was determined using an X-ray diffractometer (X'pert 3 Powder, Panalytical, Almelo, Netherlands) (Cho *et al.*, 2006). The oil samples were heated at 80°C for 2 h to ensure complete melting, then subsequently cooled down to -18°C gradually, and kept at -18°C for 1 h. The data was analysed using the "X'pert Data Collector" software.

Ice cream preparation

The ice cream was prepared following the method proposed by Feizi *et al.* (2021). Firstly, the aqueous phase was prepared by mixing skimmed milk powder, xylitol, maltodextrin, carboxymethyl cellulose, pectin, xanthan gum, and water using blender at room temperature for about 10 min (Table 1). Then, the camellia oil, CD, or fractionated CD was added, followed by the addition of vanilla essence. The mixture was then pasteurised by heating at 70 - 80°C for 15 min. The resulting pasteurised mixture was first homogenised using the high shear homogeniser at 6,000 rpm for 10 min (FJ200-SH, Huxi Industrial Co. Ltd., Shanghai, China), followed by the high-pressure homogeniser at 200 bar for one cycle (AH-MINI, ATS Industrial System Co., Ltd., Canada). The emulsion was cooled to 4°C in refrigerator for at least 2 h before analysis.

Table 1. Ice cream formulation.

Ingredient	Percentage (%, w/w)
Skimmed milk powder	9.24
Maltodextrin	2.07
Xylitol	4.55
Camellia oil/CD/fractionated CD	5.11
Carboxymethyl cellulose	0.41
Xanthan gum	0.17
Pectin	0.12
Vanilla essence	1.01
Water	77.32

CD: camellia oil-based diacylglycerol.

Overrun

Overrun is the mass difference of ice cream emulsion before and after frozen at the same volume condition (Spence *et al.*, 2019). The ice cream overrun was calculated using Eq. 1:

$$\text{Overrun (\%)} = \frac{\text{weight of ice cream emulsion - weight of frozen ice cream}}{\text{weight of frozen ice cream}} \times 100 \quad \text{(Eq. 1)}$$

Hardness

The TA-XT2i texture analyser (Stable Micro Systems Ltd., Surrey, UK) was used to determine the hardness of the formulated ice cream (Suebsiri *et al.*, 2019). A spherical P/1SP probe (25.4 mm or 1 inch in diameter) and a 5 kg load cell were adopted. The probe was set to penetrate 20 mm into the sample with 2 mm/s crosshead speed. The hardness values were obtained from the curves. The analysis was performed in triplicate.

Statistical analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) with two-tailed Student's *t*-test ($p < 0.05$) was performed to determine the differences among the measured values.

Results and discussion

Acylglycerol profile

Based on Table 2, the acylglycerol profiles of the camellia oil, CD, and fractionated CD were significantly different ($p < 0.05$). TAG was the main component found in camellia oil which comprised up to 98.78%, while DAG only consisted about 1.19%. On the other hand, majority of the acylglycerol composition in CD was dominated by DAG (85.11%), with only a small amount of TAG (14.27%). The DAG content of the fractionated CD further increased to 87.27%, while this led to a decrease in TAG content to only 12.40%. This phenomenon also explained the increase in melting point of the fractionated CD following the dry fractionation process. With the same fatty acid composition, the melting point of DAG was actually higher than TAG; therefore, DAG would remain in the solid fraction during the dry fractionation process (Shi *et al.*, 2020).

Although there were insignificant ($p > 0.05$) difference between the DAG profiles of CD and fractionated CD, their melting points were significantly ($p < 0.05$) different, with fractionated CD's melting point increased markedly. This might have been attributed to the acyl transfer reaction which took place during the fractionation process (Luo *et al.*, 2020). The free fatty acid content for camellia oil and fractionated CD fell within the safety limit (< 4 mg KOH/g) prescribed by the CODEX standard for vegetable oils (Codex Alimentarius, 2019), except CD, whereby the FFA level was slightly higher than the safety limit (0.5%). MAG was not detected in all oil samples which also indicated the acceptable quality of all oil samples with only minor impurities.

Fatty acid composition

The fatty acid compositions of all the analysed oil samples are shown in Table 3. The predominant fatty acid found in camellia oil was oleic acid (C18:1; 79.74%), followed by linoleic (C18:2; 8.54%), palmitic (C16:0; 8.32%), stearic (C18:0; 2.15%), and lastly a small amount of linolenic acid (C18:3; 0.35%). CD and fractionated CD exhibited the similar fatty acid profiles as the camellia oil. It is worth mentioning that the production and dry fractionation processes of CD did not modify the fatty acid compositions of the camellia oil. The results were in accordance with the fatty acid composition of refined

Table 2. Acylglycerol profiles and melting points of camellia oil, CD, and fractionated CD.

Parameter	Camellia oil	CD	Fractionated CD
TAG (%)	98.78 ± 0.11^{a}	14.37 ± 0.26^{b}	12.40 ± 0.34^{c}
DAG (%)	1.19 ± 0.12^{b}	85.11 ± 0.25^{a}	87.27 ± 0.31^{a}
MAG (%)	N.D.	N.D.	N.D.
FFA (%)	0.03 ± 0.01^{b}	0.50 ± 0.03^{a}	0.33 ± 0.04^{a}
Melting point (°C)	$< 0^{c}$	15.20 ± 0.40^{b}	32.70 ± 0.60^{a}

Means followed by different lowercase superscripts within the same row are significantly different ($p < 0.05$). CD: camellia oil-based diacylglycerol; TAG: triacylglycerol; DAG: diacylglycerol; MAG: monoacylglycerol; FFA: free fatty acid; and N.D.: not detected.

Table 3. Fatty acid compositions of camellia oil, CD, and fractionated CD (%).

Fatty acid	Camellia oil	CD	Fractionated CD
C16:0	8.32 ± 0.16^{a}	8.42 ± 0.12^{a}	8.18 ± 0.10^{a}
C18:0	2.15 ± 0.08^{a}	2.17 ± 0.15^{a}	2.28 ± 0.16^{a}
C18:1	79.74 ± 0.20^{a}	79.62 ± 0.15^{a}	79.58 ± 0.05^{a}
C18:2	8.54 ± 0.23^{a}	8.62 ± 0.06^{a}	8.77 ± 0.11^{a}
C18:3	0.35 ± 0.04^{a}	0.34 ± 0.02^{a}	0.38 ± 0.03^{a}
Other	0.89 ± 0.12^{a}	0.84 ± 0.14^{a}	0.81 ± 0.06^{a}

Means followed by different lowercase superscripts within the same row are significantly different ($p < 0.05$). CD: camellia oil-based diacylglycerol; C16:0: palmitic acid; C18:0: stearic acid; C18:1: oleic acid; C18:2: linoleic acid; and C18:3: linolenic acid.

camellia oil reported by Shi *et al.* (2020). The abundance of oleic acid is beneficial as it can lower the cholesterol, triglyceride, and low density-lipoprotein levels in human body (Lee *et al.*, 2007).

Solid fat content

The SFC in lipid is an important physical parameter which can influence their applications in food formulations (Saberi *et al.*, 2011). The SFC analysis is of paramount importance as it can provide valuable insights regarding the suitability of camellia oil, CD, and fractionated CD to be incorporated in ice cream formulations.

The SFC contents of camellia oil, CD, and fractionated CD are shown in Figure 1.

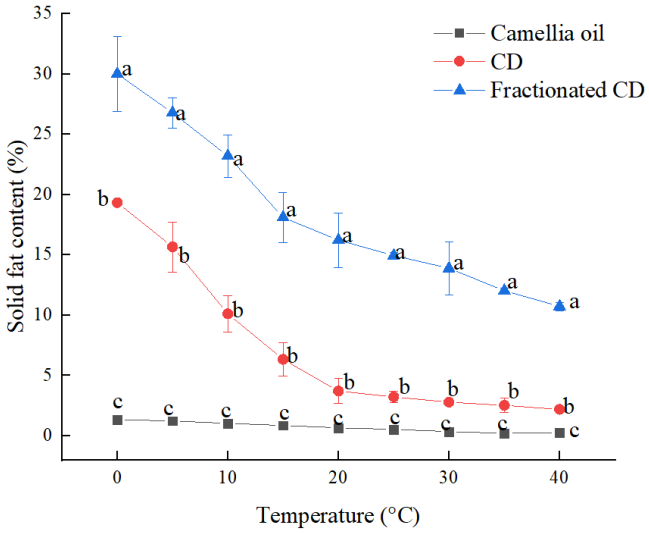

Figure 1. Solid fat contents of camellia oil, CD, and fractionated CD at temperature range of 0 to 40°C. Different lowercase letters indicate significant differences among the mean values at the same temperature ($p < 0.05$). CD: camellia oil-based diacylglycerol.

The SFC of the fractionated CD was significantly ($p < 0.05$) higher than camellia oil and CD throughout the analysed temperature range of 0 to 40°C, which justified its suitability to be incorporated into ice cream formulations as compared to camellia oil and CD. At the initial temperature of 0°C, the SFC of fractionated CD was 29.71 ± 2.87%, which subsequently decreased tremendously when the temperature was increased. When the temperature was close to 40°C, the SFC was 8.97 ± 0.36%, and could achieve a good melt in human mouth. Whereas for CD, the initial SFC at 0°C was 18.87 ± 0.35%, and 2.63 ± 0.02% at 20°C. The SFC of CD was rather low, thus unsuitable to be incorporated into frozen food production as the ice cream would melt too easily or remained liquid at room temperature. Based on past researches, for an oil to be applied in food products, it should possess sufficient level of SFC, which is higher than 10% at 20°C to limit the oiling-off phenomenon, and improve the texture of the resulting product (Wassell and Young, 2007; Podchong *et al.*, 2018).

Polymorphism analysis

The polymorphism properties of lipids pose significant impact on the functionality of lipids, and also play a vital role especially in the sensory aspects of the resulting product. The polymorphic forms of samples were analysed based on the following information: for α-form, the 2θ value was approximately 21° and the short spacing was 4.15 Å; for β'-form, the 2θ values was approximately 20.8° and 23.0°, while short spacings were 4.2 and 3.8 Å, respectively; for β-form, the 2θ value was approximately 19.1°, with a corresponding short spacing of 4.6 Å (Miklos *et al.*, 2013). The α-form crystals are the most unstable crystals, and unsuitable to be incorporated into food formulations. This is because the α-form crystals can be easily transformed into other crystal forms during storage, which will eventually lead to quality deterioration of the products. On the other hand, although β' and β-forms crystals are relatively stable, both of them are also significantly different in terms of physical properties. The β-form crystal is mainly rough and dimmed, and unsuitable for formulating specialty fats, while β'-form crystals exhibit a smoother texture, which is why this form of crystals is widely adopted in food products such as chocolates and ice creams (Svenstrup *et al.*, 2005).

The polymorphic properties of CD and fractionated CD are shown in Figure 2. It was obvious that the fractionated CD contained more β'-form crystals, and could be better applied in frozen products such as ice creams. According to Chawla and Deman (1994), generally more β'-form crystals could be observed in lipids which contained higher level of DAG. As compared to CD, fractionated CD actually contained higher amount of DAG, although from statistically point of view, they were insignificantly ($p > 0.05$) different. The huge differences in terms of the polymorphic forms

between these two oils might have been attributed to some molecular rearrangement which required further research.

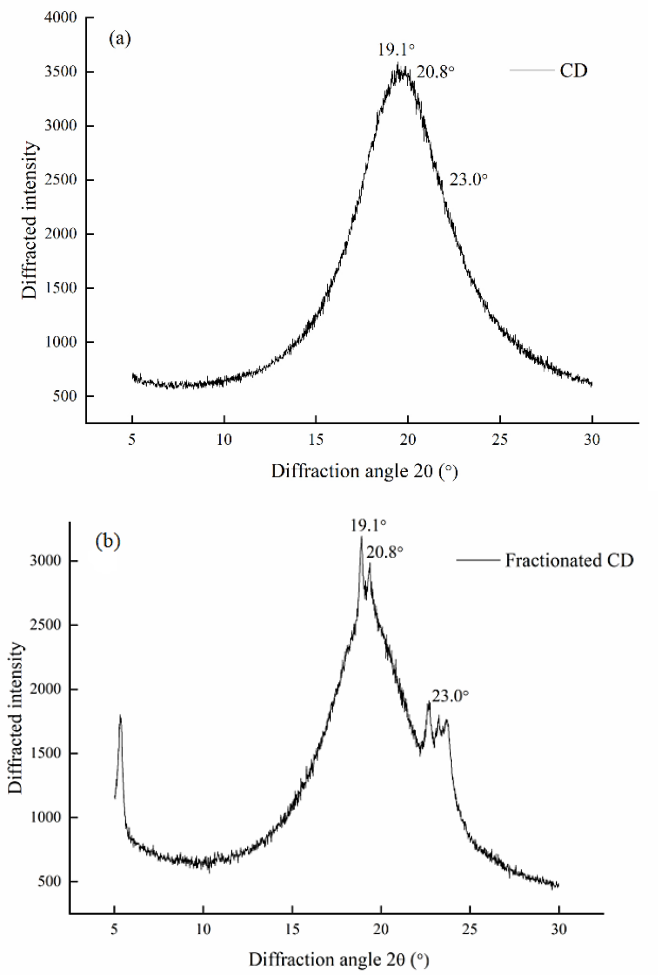

Figure 2. X-ray diffraction spectrum of **(a)** CD, and **(b)** fractionated CD.

Overrun and texture analysis of different ice cream formulations

The overrun and hardness are both important parameters for ice cream production as they significantly influence the quality attributes of the resulting products. A higher overrun means that more air is incorporated into the products during the emulsion preparation. Based on Table 4, the ice creams prepared by all oil samples showed significantly ($p < 0.05$) different overrun, with the range of 39.77 to 48.24%. The overrun values were similar to a research done by BahramParvar and Goff (2013) who prepared the low-fat ice creams with the overrun of 42.7 to 46.5% using the basil seed gum as stabiliser. The hardness of ice cream is often influenced by many factors which include ice phase volume, ice crystal size, overrun, freezing point depression of the mix, fat destabilisation, and total solids (Feizi *et al.*, 2021). The ice cream formulated with fractionated CD was the hardest (with the hardness value of 594.18 ± 10.02 g) as compared to ice cream prepared with CD (424.36 ± 5.11 g) and camellia oil (284.31 ± 5.72 g). Other than the difference in SFC, the significant differences of the ice cream hardness might have also been attributed to the interactions of the camellia-based DAG with the other ice cream ingredients (Goraya *et al.*, 2021).

Table 4. Overrun and hardness of ice cream prepared by camellia oil, CD, and fractionated CD.

Lipid	Overrun (%)	Hardness (g)
Camellia oil	39.77 ± 1.69^{b}	284.31 ± 5.72^{c}
CD	41.27 ± 0.97^{a}	424.36 ± 5.11^{b}
Fractionated CD	48.24 ± 2.17^{a}	594.18 ± 10.02^{a}

Means followed by different lowercase superscripts within the same column are significantly different ($p < 0.05$). CD: camellia oil-based diacylglycerol.

Conclusion

Dry fractionation significantly ($p < 0.05$) increased the SFC of the fractionated CD as compared to the unfractionated CD. Fractionated CD showed sufficient SFC (~30%) at 0°C, and the SFC was maintained at slightly above 10% when the temperature approached 37°C (close to the human body temperature), which indicated that the fractionated DG could melt well when placed in a human's mouth. Besides, based on the X-ray diffraction spectrum, fractionated CD contained higher level of β'-form crystals which could endow the ice cream product with a smooth texture. The hardness value of ice cream prepared with fractionated CD was also significantly ($p < 0.05$) higher as compared to the one prepared with camellia oil and CD. All the analysed physicochemical properties demonstrated better suitability of fractionated CD to be applied in ice cream formulation as compared to camellia oil and CD. The present work thus provided useful insights regarding the possibility of developing a healthier ice cream by fractionated CD incorporation without compromising the organoleptic properties of the ice cream.

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