The Effect of Glycerol Monostearate on Starch Digestibility of Dough and Its Application in Diacylglycerol Mooncake

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Abstract: The effect of glycerol monostearate on the starch digestibility in diacylglycerol mooncakes was investigated in this study. Various concentrations of glycerol monostearate (0%, 0.3%, 0.6%, 0.9%, 1.2%, 1.5%, and 1.8%) were incorporated into the mooncake dough, and their impact on the formation of starch-lipid complexes was examined. The results indicate that when the glycerol monostearate content was 1.2%, the resistant starch content was 30.52%. The higher resistant starch content was indicated by a complex index, attributed to the formation of complexes between glycerol monostearate and amylose. Glycerol monostearate increased the relative crystallinity from 6.50% to 23.96%, as demonstrated by X-ray diffraction patterns. A significant increase in enthalpy change, from 188.0 J g^{-1} to 328.3 J g^{-1} was observed upon the addition of glycerol monostearate, as revealed by differential scanning calorimetry analysis. Glycerol monostearate enhanced the short-range ordered structure of diacylglycerol mooncake dough that was indicated by fourier-transform infrared spectroscopy spectra. Therefore, incorporating an appropriate amount of glycerol monostearate (1.2%) into diacylglycerol mooncakes can substantially decrease their starch digestibility. This research offers a theoretical foundation for the development of functional foods.

Key words: diacylglycerol; glycerol monostearate; mooncake; starch digestibility

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单硬脂酸甘油酯对面团淀粉消化率的作用及其在甘油二酯月饼中的应用

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摘要：本实验研究了单硬脂酸甘油酯对甘油二酯月饼中淀粉消化率的影响。通过向月饼面团中分别添加不同浓度的单硬脂酸甘油酯（0%、0.3%、0.6%、0.9%、1.2%、1.5%和 1.8%），考察其对淀粉-脂质复合物形成的影响，从而评估其对淀粉消化行为的调控作用。结果表明，当单硬脂酸甘油酯的添加量为 1.2%时，抗性淀粉含量可达 30.52%。复合指数分析显示，抗性淀粉含量的增加主要归因于单硬脂酸甘油酯与直链淀粉之间形成的络合物。X 射线衍射谱分析表明，单硬脂酸甘油酯的加入使相对结晶度从 6.50%显著提高至 23.96%。差示扫描量热分析结果显示，加入单硬脂酸甘油酯后焓变由 188.0 J g^{-1} 增加至 328.3 J g^{-1}。傅里叶变换红外光谱分析进一步表明，单硬脂酸甘油酯增强了甘油二酯月饼体系中的近程有序结构。因此，在甘油二酯月饼中适量添加单硬脂酸甘油酯（1.2%）可显著降低其淀粉消化率，该研究为开发功能性食品提供理论基础。

关键词：甘油二酯；单硬脂酸甘油酯；月饼；淀粉消化率

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Cantonese mooncake, a quintessential Chinese pastry[1], is highly favored by consumers due to its delicate dough, generous filling, tender texture, diverse flavors, and exquisite presentation[2]. Oil is a primary component in mooncake, that significantly influences the overall quality of mooncakes[3]. Peanut triacylglycerol is commonly used in Cantonese mooncakes. However, triacylglycerol may contribute to hyperlipidemia, hypertension, and diabetes. Meanwhile, the high starch digestibility found in Cantonese mooncakes has been identified as a contributing factor to diabetes. The reduction of starch digestibility is a growing concern, an attractive option is to increase resistant starch.

Peanut diacylglycerol is a structural lipid, that one fatty acid in glycerol skeleton is substituted by a hydroxyl group[4]. Diacylglycerol emerged as a potential substitute for triacylglycerol due to diacylglycerol has been shown to potentially suppress obesity and accumulation of abdominal fat. Diacylglycerol not only exhibits health benefits, but also can form lipid-amylose complexes with amylose[5]. It has been discovered that the lipid-amylose complexes can increased the resistant starch content[4]. The increased resistant starch content led to reduced starch digestibility[6]. Fatty acids and glycerol monostearate can also form lipid-amylose complexes with starch. For example, a recent study conducted by Chen et al[5]. demonstrated that the complex formed between starch and glycerol monostearate effectively increased the resistant starch content. Chen et al[7]. found that adding diacylglycerol and glycerol monostearate improved cake texture by enhancing batter aeration, yielding softer and fluffier cakes due to their emulsifying properties. Despite previous study researched on the formation of complex between glycerol monostearate and starch in diacylglycerol cakes in contrast to triacylglycerol cakes. However limited investigations study the influence of glycerol monostearate concentration on starch digestibility and texture of diacylglycerol mooncake.

The objective of this study was to assess the impact of glycerol monostearate concentration on the starch digestibility of diacylglycerol mooncake. Starch digestibility were assessed through resistant starch content, starch hydrolysis and estimated glycemic index. We utilized complex index, X-ray diffraction, differential scanning calorimetry, and fourier transform infrared spectroscopy and Raman to confirm the formation of lipid-amylose complex.

1 Materials and methods

1.1 Materials

The peanut diacylglycerol (DAG) (glyceride composition: 81.27% DAG; fatty acid Composition: 10.95% C16:0, 3.20.6% C18:0, 41.09% C18:1 and 37.55% C18:2) obtained from Guangdong Yueshan Special Medical Nutrition Technology Co., LTD. Stearate monoglyceride (GMS) was obtained from Aladdin Biochemical Technology Co., LTD. (Shanghai, China). Wheat flour with an amylose content ranging from 82%, lotus seed, sugar was purchased from local Wal-Mart stores in Guangdong province of China. All chemical reagents utilized in this study were supplied by Aladdin Biochemical Technology Co., LTD. (Shanghai, China). Distilled water was employed for all recipe preparations.

1.2 Preparation of mooncake

Preparation of mooncake, the production of mooncakes follows the methodology developed by Xie et al[8]. To prepare mooncake dough, 10 g of invert syrup was emulsified with 4.3 g peanut DAG, then homogenized with 16 g wheat flour containing 0%, 0.3%, 0.6%, 0.9%, 1.2%, 1.5% and 1.8% glycerol monostearate. The dough rested for 1.5~2 h. For the filling, steamed lotus seeds were blended with sugar and oil (100:5:1.5 ratio). Each mooncake used 12 g dough wrapped around 33 g filling, forming 45 g blanks that were baked at 150~215 ℃ for 15~18 min. Samples were labeled as DAG, DAG/GMS-0.3, DAG/GMS-0.6, DAG/GMS-0.9, DAG/GMS-1.2, DAG/GMS-1.5, and DAG/GMS-1.8 where the numbers mean the percentage of glycerol monostearate.

1.3. Characteristics of mooncake

1.3.1 Texture analysis

Texture profile analysis (TPA) data were obtained by employing the TPA method proposed by Qiu et al[9]. with certain modifications. The texture of the mooncake samples was evaluated using a Surrey XT Plus texture analyzer from England. A 15×15×15 mm cube, extracted from the central debris of each mooncake, underwent TPA testing. The testing conditions

comprised a compression rate of 2 mm s^{-1}, deformation reaching 60% of the initial cube height, a 5-second interval between each compression cycle, and a trigger force threshold of 2 g. Double compression tests were conducted using an aluminum plate with a diameter of 20 mm. Hardness, cohesiveness, chewability, resilience, colloidality and elasticity parameters were derived from the resulting curves. Three replicates were measured for each mooncake.

1.3.2 Sensory analysis

Sensory evaluation of the mooncakes was performed by adapting methods from a previous study proposed by Starowicz et al[10]. with slight modifications. Thirty trained participants used a 9-point scale to evaluate several sensory characteristics, including appearance, texture, taste, flavor, color, and overall acceptance.

1.4 In *vitro* starch digestibility of mooncake

1.4.1 Total starch content

Total starch content in the cake was obtained by adapting methods from a previous study proposed by Bosmali et al[11]. with minor modifications. Sample (1 g) was taken from finely crushed cake and then mixed with a triple weight of water to acquire a moist sample. Afterwards, this sample was mixed with 8 mL 1 mol L^{-1} NaOH under agitation (25 rad s^{-1}) at 25 ℃ for 35 min. Subsequently, 50 mM phosphate buffer solution (pH 7.4) and 5 mL α-amylase solution (60 U mL^{-1}) were added. The mixtures were agitated at 20 rad s^{-1} in a water bath at 37 ℃ for precisely 45 min. To terminate the enzymatic reaction, 4 mL ethanol (85%, *V/V*) was added, a 200-μL sample was taken for glucose analysis using the GOPOD kit (Megazyme Co., Ltd., Shanghai, China). The conversion factor for starch to glucose was determined as 0.9 based on established protocol[12].

1.4.2 In *vitro* starch digestibility

In *vitro* starch hydrolysis of the cake was analysed by adapting methods from a previous study proposed by Yang et al[13]. with minor modifications. Briefly, cake (1 g) was mixed with 10 mL 0.25 mol L^{-1} acetate-sodium acetate buffer (pH 5.2) followed by adding 0.6 mL amylglucosidase (3 300 U mL^{-1}), 10 mL porcine pancreatic α-amylase (60 U mL^{-1}) and 0.6 mL pepsin (140 U mL^{-1}). The mixture was placed in a water bath shaker at 37 ℃ and 15 rad s^{-1} for 3 h with sampling (1 mL each) at intervals of 0, 20, 60, 90, 120, 150 and 180 min. To terminate the enzymatic reaction, 4 mL ethanol (85%, *V/V*) was added, followed by centrifuging at 3 500×*g* for 5 min. The glucose content in the supernatant was determined using a GOPOD kit (Megazyme Co., Ltd., Shanghai, China). Equations (1 through 4) were used to calculate the content of rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and starch hydrolysis rate.

$$RDS(\%) = \frac{(G_{20} - G_0) \times 0.9}{TS} \times 100 \quad (1)$$

$$SDS(\%) = \frac{(G_{120} - G_0) \times 0.9}{TS} \times 100 \quad (2)$$

$$RS(\%) = 100 - RDS(\%) - SDS(\%) \quad (3)$$

$$S(\%) = \frac{G_t \times 0.9}{TS} \times 100 \quad (4)$$

Where:

G_0——represents the content of glucose present in the cake;

G_{20}——represents the glucose content at 20 min of digestion;

G_{120}——represents the glucose content at 120 min of digestion;

G_t——represents the glucose content at 0, 20, 60, 90, 120, 150 and 180 min in enzymatic hydrolysis;

S——represents starch hydrolysis rate.

According to the formulas (5) and (6), the hydrolysis index (HI) and the estimated glycemic index (eGI) were calculated as described by Goni, GarciaAlonso and SauraCalixto[14].

$$HI = \frac{A \times 0.9}{TS} \quad (5)$$

$$eGI = 39.21 + 0.803 \times HI90 \quad (6)$$

Where:

A——represents the amount of glucose hydrolysis per minute;

TS——represents total starch.

1.5 The complex structure of mooncake

1.5.1 X-ray diffraction

The X-ray diffraction (XRD) patterns for different types of mooncakes were obtained was obtained by adapting methods from a previous study proposed by Ahmed, Xua, Sulieman, Mahdi et al[15]. using an X-ray diffractometer (D8 Advance, Bruker, Germany). Mooncake samples were scanned in the range of diffraction angles (2θ) from 3 to 50 °at a scan speed of 12 °·min^{-1}. The radiation parameters included a scan step size of 0.02 °·s^{-1}, with current and voltage values set at mA=40 and kV=40 respectively. To assess the relative crystallinity (%) associated with starch V-type crystals in these samples, JADE software was used to calculate percentages specifically for areas under diffraction peaks occurring at angle θ=20 °compared to overall peak area measurements.

1.5.2 Differential scanning calorimetry

The thermal characteristics of mooncakes were evaluated using a modified version of the method outlined by Kaur et al[16]. Differential scanning calorimetry (DSC) was used to measure the heating curves of various types of mooncakes with a Q-200 instrument from TA Instruments, USA. The cake was processed in an electric thermostatic blast furnace (DHG-9240A, Shanghai, China) at a temperature of 50 ℃ for a duration of 24 h. Following this drying procedure, the cake was pulverized into a fine powder using a grinder (02A, Guangzhou, China). Subsequently, 8 mg of the powdered cake were mixed with 16 mL of distilled water inside the DSC crucible. After sealing the crucible, it was allowed to equilibrate for 12 h before conducting DSC analysis within a scanning range of 20 to 100 ℃ at a heating rate of 10 ℃·min^{-1}. Key parameters such as initial temperature (T_o), melting temperature (T_m), end temperature (T_c), and enthalpy change (ΔH_m) during melting were recorded.

1.5.3 Fourier-transform infrared spectroscopy

The short-range ordered structure of the mooncake was prepared following the method proposed by Alvarez-Ramirez et al[17], with certain modifications. The Fourier-transform infrared spectroscopy (FTIR) of different types of mooncakes were collected using a Bruker Vertex 70 V infrared spectrophotometer, with a spectral range spanning from 4 000 to 400 cm^{-1} and a resolution of 1 cm^{-1} (acquired over 100 scans). Following acquisition, the infrared spectra were analyzed using Omnic 9.2 software, where a half-peak width of 20 cm^{-1} and an enhancement factor of 3 were applied. The deconvolution integrals in the region from 1 200 to 800 cm^{-1} yielded correction heights at wavenumbers of 1 047, 1 022, and 995 cm^{-1}.

1.6 Data statistics and analysis

The study was conducted at three distinct time intervals, and the mean values were determined. Statistical evaluations were carried out using one-way ANOVA in SPSS software, with subsequent post-hoc comparisons employing Tukey's method. The significance level was set at P<0.05. Data processing and plotting were conducted using ORIGIN software, and significant differences were indicated by different letter labels.

2 Results and discussion

2.1 Characteristics of mooncake

2.1.1 Texture

Table 1 Texture parmeters of DAG and DAG/GMS-based mooncakes. DAG: diacylglycerol oil, GMS: glycerol monostearate

Sample	Hardness/($\times10^3$g)	Cohesiveness	Chewiness/N	Resilience	Gumminess
DAG	17.30±0.05^{a}	0.53±0.004^{a}	0.89±0.002^{a}	0.89±0.002^{a}	980.00±4^{a}
DAG/GMS-0.3	14.70±0.03^{b}	0.39±0.002^{b}	0.10±0.002^{b}	0.10±0.002^{b}	585.00±9^{b}
DAG/GMS-0.6	14.60±0.05^{b}	0.37±0.003^{c}	0.09±0.001^{c}	0.09±0.001^{c}	583.00±9^{b}
DAG/GMS-0.9	14.30±0.05^{c}	0.34±0.001^{d}	0.08±0.001^{d}	0.08±0.001^{d}	575.00±8^{bc}
DAG/GMS-1.2	14.20±0.01^{cd}	0.33±0.002^{e}	0.07±0.003^{e}	0.07±0.003^{e}	561.00±8.^{c}
DAG/GMS-1.5	14.00±0.09^{d}	0.31±0.002^{f}	0.06±0.002^{f}	0.06±0.002^{f}	538.00±7^{d}
DAG/GMS-1.8	13.50±0.02^{e}	0.25±0.003^{g}	0.05±0.001^{g}	0.05±0.001^{g}	523.00±7^{d}

Annotation: Data are expressed as mean±standard deviation (n=3) and different letters show significant difference at the 5% level in Duncan's test (P<0.05).

The texture of mooncakes significantly influences consumer acceptance. The effect of GMS on the texture of DAG/GMS mooncakes is illustrated in Table 1. It was found that the hardness of DAG/GMS mooncakes was significantly reduced compared to that of DAG mooncakes without GMS. The observed reduction in hardness can be attributed to the emulsifying properties of GMS, which enhanced the aeration and structural uniformity of the dough during baking. This results in a softer, fluffier texture, ultimately improving the sensory quality and palatability of mooncakes. This observation aligned with prior studies showing that cake develop a softer texture due to emulsifying properties of GMS[18]. The cohesiveness, chewiness, resilience, and gumminess vulues gradually decreased with the content of GMS increased. These might be due to the emulsifying properties of GMS impacted the internal network of mooncakes. These results were supported by hardness value.

2.1.2 Sensory analysis

Table 2 Sensory analysis of DAG and DAG/GMS-based mooncakes. DAG: diacylglycerol oil, GMS: glycerol monostearate

Sample	Appearance	Texture	Taste	Flavor	Color	Overall acceptance
DAG	8.13±0.68^{a}	8.23±0.77^{ab}	8.20±0.88^{a}	8.46±0.73^{a}	8.50±0.73^{a}	8.30±0.74^{ab}
DAG/GMS-0.3	8.06±0.98^{a}	8.13±1.00^{ab}	8.06±0.98^{ab}	6.76±1.25^{c}	7.96±1.12^{bc}	7.96±0.92^{bc}
DAG/GMS-0.6	8.13±0.68^{a}	7.96±0.49^{b}	8.10±0.92^{ab}	7.60±1.19^{b}	7.80±0.99^{c}	7.80±0.99^{c}
DAG/GMS-0.9	8.50±0.73^{a}	7.96±0.92^{b}	8.36±0.55^{a}	7.66±0.88^{b}	8.33±0.60^{ab}	8.43±0.77^{a}
DAG/GMS-1.2	8.46±0.73^{a}	8.26±0.78^{ab}	8.30±0.74^{a}	7.86±1.07^{b}	8.20±0.88^{abc}	8.66±0.66^{a}
DAG/GMS-1.5	8.40±0.77^{a}	7.96±1.12^{b}	7.70±0.87^{b}	7.86±1.07^{b}	7.83±1.05^{c}	8.40±0.56^{a}
DAG/GMS-1.8	8.33±0.71^{a}	8.50±0.82^{a}	8.40±0.67^{a}	6.96±1.15^{c}	8.40±0.67^{ab}	8.33±0.54^{ab}

Annotation: Data are expressed as mean±standard deviation (n=3) and different letters show significant difference at the 5% level in Duncan's test (P<0.05).

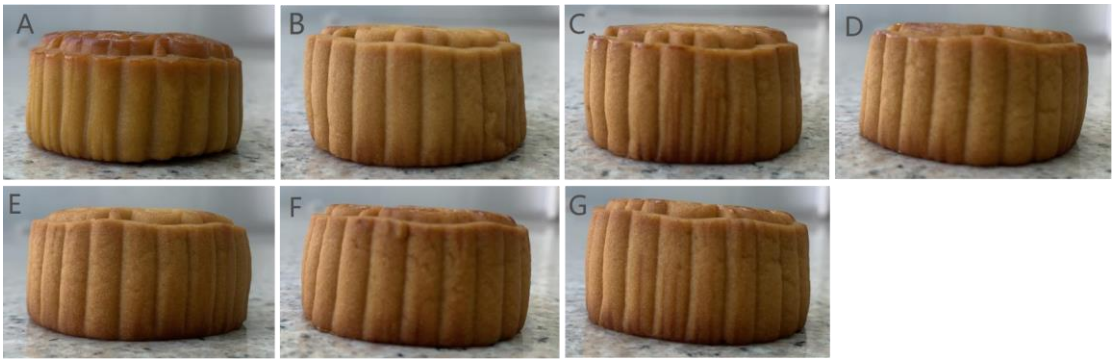

Fig.1 The visual images of DAG and DAG/GMS-based mooncakes

Annotation: A:DAG; B: DAG/GMS-0.6; C: DAG/GMS-0.9; D: DAG/GMS-1.2; E: DAG/GMS-1.5; F:DAG/GMS-1.8. DAG: diacylglycerol oil; GMS: glycerol monostearate.

Sensory evaluation is crucial for gaining a comprehensive understanding of consumer preferences, thereby facilitating the assessment of potential applications for DAG/GMS mooncakes[19]. The results of sensory analysis conducted on DAG/GMS mooncakes are illustrated in Table 2. Among all the assessed criteria, the color and flavor of DAG mooncakes received relatively higher scores compared to DAG/GMS-based mooncakes. And the appearance, texture and taste values of DAG mooncakes and DAG/GMS-based mooncakes were from 7.8 to 8.8. Notably, the overall acceptance values of DAG/GMS mooncakes increased then decreased with the content of GMS from 0.3% to 1.8%. DAG/GMS-1.2 exhibited highest overall acceptability with sensory scores exceeding 8.66. The visual images of mooncakes were displyed in Fig.1.

2.2 In *vitro* starch digestibility of mooncake

2.2.1. Starch components and estimated glycemic index

Table 3 Total starch and starch components of DAG and DAG/GMS-based mooncakes

Sample	Total starch (g/100 g)	RDS (%)	SDS (%)	RS (%)	eGI
DAG	12.32 ± 0.68^{b}	66.05 ± 3.2^{a}	23.15 ± 1.7^{b}	10.79 ± 1.5^{e}	104.20 ± 0.88^{a}
DAG/GMS-0.3	12.31 ± 0.50^{b}	56.60 ± 0.070^{b}	26.35 ± 1.9^{a}	17.04 ± 1.8^{d}	99.17 ± 1.8^{b}
DAG/GMS-0.6	12.60 ± 0.12^{a}	48.05 ± 0.87^{c}	25.88 ± 0.53^{ab}	26.06 ± 1.1^{bc}	92.56 ± 1.2^{c}
DAG/GMS-0.9	12.32 ± 0.62^{b}	44.00 ± 0.73^{de}	27.39 ± 1.9^{a}	28.60 ± 1.5^{ab}	90.29 ± 1.0^{de}
DAG/GMS-1.2	12.35 ± 0.20^{b}	41.09 ± 2.9^{e}	28.38 ± 1.2^{a}	30.52 ± 2.0^{a}	88.25 ± 1.1^{e}
DAG/GMS-1.5	12.41 ± 0.68^{b}	46.42 ± 2.1^{cd}	25.51 ± 1.3^{ab}	28.06 ± 1.0^{ab}	92.05 ± 1.0^{cd}
DAG/GMS-1.8	12.58 ± 0.64^{a}	48.51 ± 0.88^{c}	27.81 ± 1.2^{a}	23.67 ± 1.0^{b}	93.30 ± 1.1^{c}

Annotation: DAG: diacylglycerol oil, GMS: glycerol monostearate, RDS: rapidly digestible starch, SDS: slowly digestible starch, RS: resistant starch, eGI: estimated glycemic index. Data are expressed as mean±standard deviation (n=3) and different letters show significant difference at the 5% level in Duncan's test (P< 0.05).

To quantify the resistant starch (RS) content and evaluate its correlation with digestibility, the starch components of mooncakes was systematically analyzed, with a focus on differentiating between resistant starch (RS), rapidly digestible starch (RDS), and slowly digestible starch (SDS). The resistant starch content of DAG/GMS mooncakes with varying GMS concentrations is presented in Table 3. When GMS concentration increased from 0.3% to 1.2%, the resistant starch content of the mooncakes increased from 10.79% to 30.52% (increased by 182%). However, when the GMS concentration increased to 1.8%, a slight decrease in resistant starch content was observed.

The estimated glycemic index (eGI) serves as a quantitative metric used to assess the effect of mooncakes on blood sugar levels two hours post-consumption[20]. The high GI category consists of foods with a GI value exceeding 70, whereas medium GI foods range from 56 to 69. In contrast, low GI foods are defined as those with a GI value under 55. The effective glycemic index (eGI) of DAG mooncake and DAG/GMS-based mooncakes is displayed in Table 3. Initially, the eGI of all types of mooncakes decreased and subsequently rose as the proportion of GMS increased. The results showed a relationship between the eGI value and the RS content in mooncakes, suggesting that as the RS levels increased in mooncakes, their eGI value tended to decrease. The study conducted by demonstrated that the presence of RS leads to a decline in vulnerability towards enzyme degradation. This could potentially result in a decrease in blood glucose levels, consequently resulting in a reduction of the eGI value, the similar results were supported from previous study by Yun et al[21]. Meanwhile, studies have indicated that excessive intake of GMS may induce insulin resistance, thereby increasing eGI. Therefore, maintaining an appropriate GMS level (1.2%) can effectively minimize eGI[22].

2.2.2 Starch hydrolysis

The digestibility of starch in mooncakes was evaluated through in *vitro* hydrolysis experiments[23]. The starch hydrolysis patterns of different samples are illustrated in Fig.2. A rapid increase in hydrolysis rates was observed in all samples during the initial 20 minutes, followed by the attainment of a near-plateau phase after 90 minutes, during which only minimal further increases were detected. Notably, a significantly higher hydrolysis rate was demonstrated by the

DAG-based mooncake when compared to its DAG/GMS-based counterpart. A notable reduction in starch hydrolysis was observed with the inclusion of GMS, however, when GMS concentration reached 1.8%, a slight increase in hydrolysis was observed. Studies showed that higher resistant starch levels can decrease digestibility[24]. Thus, it was suggested by the findings that the addition of 1.2% GMS optimized starch digestibility in DAG/GMS mooncakes, with this supplemental level being demonstrated to effectively decrease starch digestibility.

Based on the above results, we proposed a hypothesis that when the concentration of GMS was 0%~1.2%, the increased RS content, decreased eGI and decreased starch hydrolysis might be due to an interaction occurred between GMS and starch, resulting in the creation of a lipid-amylose complex within the DAG/GMS mooncakes. Chen et al[7]. reported comparable results, showing that GMS is capable of forming lipid-starch complexes in a DAG environment, which increases the resistant starch content. When the concentration of GMS was 1.2%~1.8%, the decreased RS content, increased eGI and increased starch hydrolysis might be due to hydrophobic interactions between DAG and GMS at elevated concentrations, which could reduce their ability to interact with starch molecules. In line with the findings reported by Chen et al[18], it is crucial to emphasize that GMS can engage in hydrophobic interactions with DAG molecules.

To verify our hypothesis, the complex structure of mooncakes was analyzed using a combination of parameters, including complex index, crystalline structure, gelatinization properties, Fourier-transform infrared (FTIR) spectroscopy, and Raman spectroscopy. We investigated how varying concentrations of glycerol monostearate (0%~1.8%) affect starch digestibility in diacylglycerol-based mooncakes by influencing the formation of starch-lipid complexes.

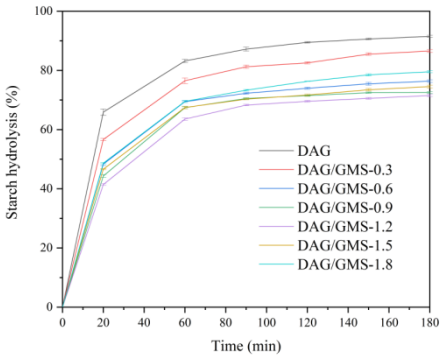

Fig.2 Starch hydrolysis of DAG and DAG/GMS-based mooncakes.

Annotation: DAG: diacylglycerol oil, GMS: glycerol monostearate.

2.3 The complex structure of mooncake

2.3.1 Complex index

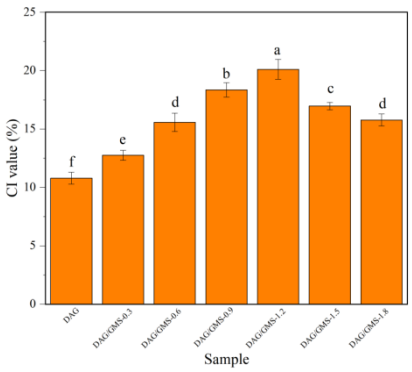

Fig.3 Complex index of DAG and DAG/GMS-based mooncakes

Annotation: DAG: diacylglycerol oil, GMS: glycerol monostearate. Data are expressed as mean±standard deviation (n=3) and different letters show significant difference at the 5% level in Duncan's test (P <0.05).

The complex index (CI) serves as an indicator of the amount of lipid-amylose complexes, and its value was utilized to further investigate the impact of GMS concentration on the formation of lipid-starch complexes in mooncakes. As presented in Fig.3, when the GMS concentration is 0%, the CI value reached its minimum. However, with a gradual increase in GMS concentration to 1.2%, there was a corresponding progressive rise in CI value from 10.78% to 20.11% (increased by 86.54%), which can be attributed to the capacity of GMS to form lipid-starch complexes with starch. Subsequently, upon reaching a GMS concentration of 1.8%, a declining trend in CI value became apparent. It was suggested by this decline that the interactions between GMS and starch were hindered by the presence of DAG, leading to reduced formation of lipid-starch complexes within the mooncakes.

2.3.2 Crystalline structure

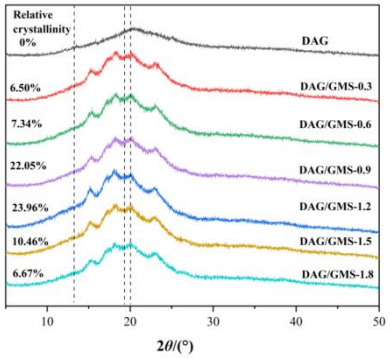

Fig.4 X-ray diffraction (XRD) patterns of DAG and DAG/GMS-based mooncakes

Annotation: DAG: diacylglycerol oil, GMS: glycerol monostearate.

The interaction between amylose in DAG/GMS mooncakes can be analyzed by examining the crystallization patterns in the X-ray diffraction (XRD) analysis[15]. As illustrated in Fig.4, diffraction peak at approximately 20 °was observed in DAG/GMS mooncakes, which differed from those detected in DAG mooncakes, indicating the formation of a lipid-amylose complex. These findings were corroborated by previous research conducted by Ahmed et al[15], in which similar diffraction peaks around 20 °were reported for the lipid-amylose complex.

With an increase in GMS concentration from 0.3% to 1.2%, the relative crystallinity of the mooncakes increased from 6.50% to 23.96% (increased by 268%). It was suggested by this observation that an interaction between GMS and starch was formed. Similar findings were reported by Chen et al[7], demonstrating that GMS can form lipid-starch complexes within a DAG environment, thereby enhancing crystallinity levels. However, when the GMS concentration reached 1.8%, there was a slight decline in mooncake crystallinity, possibly due to the decreaesd lipid-starch complexes formation, the similar results were supported from previous study by Kawai et al[24].

2.3.3 Gelatinization properties

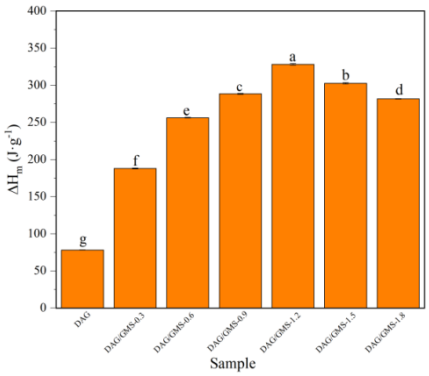

Fig.5 Gelatinization properties of DAG and DAG/GMS-based mooncakes

Annotation: DAG: diacylglycerol oil, GMS: glycerol monostearate, T_o: initial temperature, T_m: melting temperature, T_c: final temperature,

ΔH_m: enthalpy change. Data are expressed as mean ±standard deviation (n=3) and different letters show significant difference at the 5% level in Duncan' s test (P<0.05).

The gelatinization properties of starch in various mooncakes can be investigated through differential scanning calorimetry (DSC) analysis, utilizing parameters such as initial temperature (T_o), melting temperature (T_m), final temperature (T_c), and enthalpy change (ΔH_m). Fig.5 presents the starch gelatinization behavior of DAG/GMS mooncakes with different GMS concentration.

The ΔH_m value serves as a quantitative indicator of the complex and reflects its crystallinity Kawai et al. [24]. With an increase in GMS concentration from 0.3 to 1.2%, the ΔH_m value of DAG/GMS mooncakes tended to significantly increase, rising from 188.0 to 328.3 J g^{-1} (increased by 74.62%) compared to DAG mooncakes. These findings can be attributed to the formation of lipid-amylose complexes between GMS in DAG/GMS mooncakes and amylose in mooncakes. The study conducted by Chen et al[7]. also revealed that DAG collaborates with GMS and starch to form a lipid-starch complex. These results were consistent with those reported by Henning et al[19], who observed that the formation of lipid-starch complexes enhances ΔH_m during starch gelatinization process. However, when the GMS concentration further increased to 1.8%, there was a slight decrease in the ΔH_m value of the mooncake, reduced from 328.3 to 281.7 J g^{-1} (decreased by 14.19%) potentially due to the reduced lipid-starch complexes formation, the similar results were supported from previous study by Chen et al[18]. Kawai et al[24]. demonstrated that an increased ΔH_m value may lead to enhanced resistance against aging for starches. Therefore, it was therefore hypothesized that when supplemented at a level of 1.2%, the starch digestibility in DAG/GMS mooncakes was decreased, which was found to be consistent with XRD analysis.

2.3.4 FTIR spectra

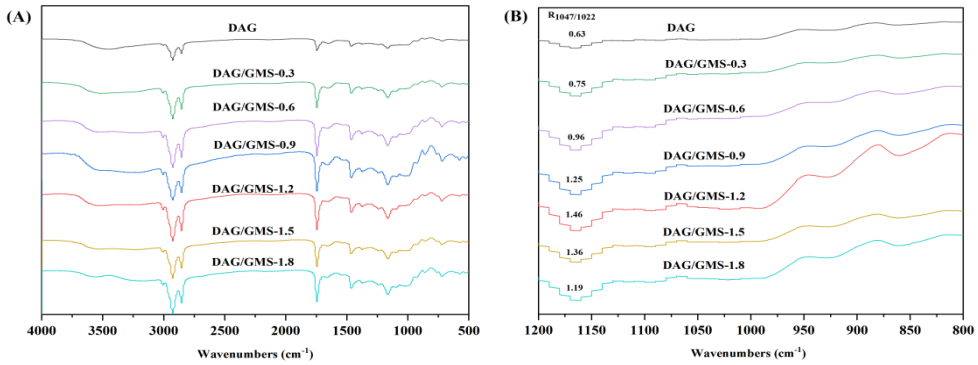

Fig.6 Fourier transform infrared spectra (A) and the fingerprint region (1 200~800 cm^{-1}) (B) of DAG and DAG/GMS-based mooncakes

Annotation: DAG: diacylglycerol oil, GMS: glycerol monostearate, R1 047/1 022 is intensity ratio of the bands at 1 047 and 1 022 cm^{-1}.

The molecular interactions between amylose, DAG and GMS were investigated using Fourier-transform infrared spectroscopy (FTIR) spectra. The FTIR spectra of mooncakes are presented in Fig.6A. Prominent peaks observed within the range of 3 200~3 500 cm^{-1} correspond to N-H bending vibrations of gluten molecules[25]. The absorption peaks at 2 854 and 2 931 cm^{-1} can be attributed to the vibration of C-H bonds in lipids[26]. Additionally, the absorption peak observed at 1 743 cm^{-1} is associated with the vibration of C=O bond[27].

The fingerprint region (1 200~800 cm^{-1}) and peak intensities associated with starch molecules are illustrated in Fig.6B. A crystalline region exhibits a peak at 1 047 cm^{-1}, an amorphous region displays a peak at 1 022 cm^{-1}. The short-range ordered structure of starch is characterized by the absorption ratio $R_{1047/1022}$, where a higher proportion indicates a stronger short-range ordered structure that enhances resistance against amylase digestion[28].

$R_{1047/1022}$ value of 1.25 indicated weak short-range ordered structure in DAG mooncakes, as illustrated in Fig.6B. This observation suggested that the increase in GMS concentration from 0.3% to 1.2% enhanced the short-range ordered structure of mooncakes, as evidenced by an increase in the $R_{1047/1022}$ value from 0.75 to 1.46. This increment can be attributed to the formation of a lipid-starch complex between GMS and starch, which were consistent with previous findings by Chen et al[5]. However, excessive addition of GMS led to a decrease in the $R_{1047/1022}$ value due to the reduced

starch-lipid formation complexes.

A previous study conducted by Wang et al[28]. demonstrated a positive correlation between the degree of short-range ordered structure and the formation of lipid-starch complexes. Consistent with these findings, our FTIR analysis revealed that an increase in GMS concentration to 1.2% resulted in the formation of stronger lipid-starch complexes, as observed in XRD and DSC analysis.

2.3.5 Raman spectra

The half-maximum full width (FWHM) can be employed for investigating the short-range ordered structures of complexes, a lower FWHM value indicates a higher degree of short-range order within the complex[29]. In Fig.7, the results demonstrated that the lowest FWHM value (15.61) was exhibited by the DAG/GMS-1.2 mooncake among all tested samples, indicating that the strongest short-range ordered structure was achieved. This observation may be attributed to DAG's ability to synergistically promote lipid-starch complex formation between GMS and starch, which aligned with the findings reported by Chen et al[5]. Additionally, with an increase in GMS concentration to 1.8%, there was a slight increase in FWHM value, possibly due to weakened short-range ordered structure, which were consistent with FTIR analysis. The preparation of diacylglycerol mooncake and its resistance to amylase digestion are illustrated in Fig.8 through a schematic diagram.

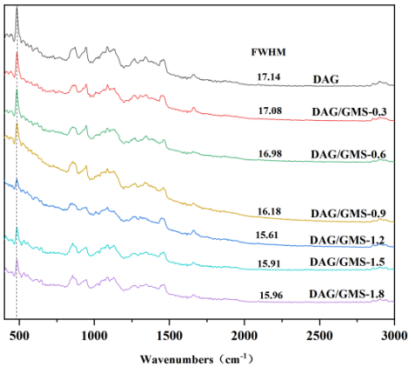

Fig.7 Raman spectra of DAG and DAG/GMS-based mooncakes

Annotation: DAG: diacylglycerol oil, GMS: glycerol monostearate, FWHM is half-maximum full width.

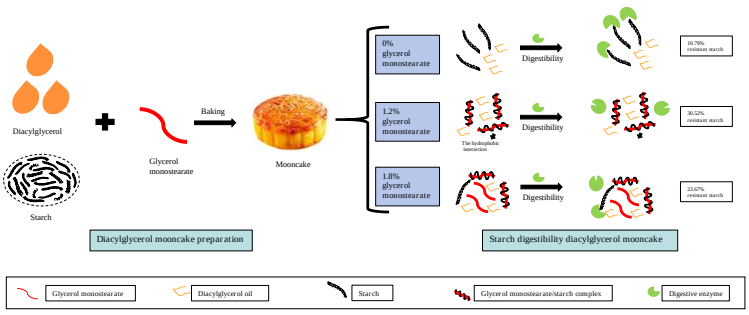

Fig.8 The preparation of diacylglycerol mooncake and its resistance to amylase digestion.

3 Conclusion

This study provides evidence that incorporating an appropriate amount of GMS reduces starch digestibility in diacylglycerol mooncakes, which is attributed to the formation of lipid-starch complexes by GMS. And excessive GMS lead to decreased starch digestibility, possibly due to hydrophobic interactions between DAG and GMS at higher concentrations, which may hinder GMS-starch interaction and lower resistant starch content. The formation of lipid-amylose complexes was promoted by the addition of 1.2% GMS, as evidenced by an increased complex index, higher relative crystallinity, greater enthalpy changes, and enhanced short-range ordered structure. Overall, these findings found that different GMS concentration have different influence on starch digestibility in diacylglycerol mooncake.

Declaration of interests：

The authors declare that they have no known competing financial interests or personal relationships

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