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# Effects of lipid type and complexation temperature on the formation and digestibility of sweet potato starch-lipid complex

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**Abstract**
Amylose-lipid complexes (resistant starch type 5) were prepared by using sweet potato starch and different type of lipids including lauric acid (LA), oleic acid (OA), and glycerol monolaurate (GML) under varied complexation temperatures of 60ºC and 90ºC. The complexing index (CI) and *in vitro* digestion were carried out to study the complexation ability and the digestibility of the formed starch-lipid complexes. The CI values in both complexation temperatures followed the order of GML > LA > OA, suggesting that monoglyceride formed more complexes with starch than fatty acids. The formation of starch-lipid complexes enhanced the resistant starch content. The present study confirmed that considering the lipid type and complexation temperature are crucial factors in resistant starch type 5 formation.

**Keywords:** sweet potato starch, amylose-lipid complex, complexation index, digestibility

## Introduction

Starch is the primary carbohydrate in cereal, tuber, and root plant, which plays a critical role in the quality attributes of many food products [1, 2]. The linear amylose fraction as the substantial polysaccharide in starch is widely known can form inclusion complexes with lipids, generally known as amylose-lipid complexes (ALCs) or V-amylose [3-6]. It has been proposed as a novel source of resistant starch type 5 [7]. The ALCs has been indicated to play the essential roles in altering the performance of starchy food, reportedly to develop the pasting properties (inhibiting the gelatinization progression and retarding the retrogradation) [8], to produced novel resistant starches [9], to create a new vehicle to shield and deliver sensitive ligand such as unsaturated fatty acids [10, 11], and even to implement in starch-based film [12].

Depend on the melting point of the crystalline form, the ALCs exist in two distinct polymorphic forms, called type I and type II. Type I complex is dissociated below 100ºC and formed through rapid nucleation of amylose at temperature <60º. Type II is above 100ºC and produced during the slow nucleation at a higher complexation temperature (>90ºC) [13-15]. Type I complexes consist of less ordered structure with no distinct crystalline networks. When type I complexes are packed into lamellae and crystallized collectively resulted in different crystalline/semicrystalline structures, they are identified as type II complexes [16]. Crystalline type II consisted of two subunits, IIa and IIb, which is IIb, has a slightly higher melting temperature. Type IIa and IIb are varied slightly in crystallinity and lattice organization [14, 17].

The inclusion complexes formed between amylose and lipid depends upon many factors such as amylose content [18, 19], degree of polymerization [20], debranching treatment [7, 9, 21], lipid concentration [18], and complexation reaction condition [20, 22, 23]. In the case of lipid as a guest ligand, the V-amylose formation is highly relying on the lipid characteristics include carbon chain length [24], the degree and position of unsaturation [22, 25], and the lipid amount [26].

Many authors have extensively studied the production of ALCs between amylose and a specific group of lipids. However, limited studies investigated the formation of ALCs between amylose and different groups of lipids. It was reported that brown lentil starch complexed with the structurally different lipid type (including saturated fatty acids and triglycerides) formed amylose-lipid complexes which were resistant to enzymatic digestion [27]. The same phenomenon was also reported in previous research [28], on heating of some starches such as tapioca, normal maize and high amylose maize starches in the existence of corn oil, soy lecithin, saturated fatty acids, and unsaturated fatty acids. However, a little impact on the enzymatic hydrolysis was found in waxy maize starch. Other research [29] investigated the degree of complexes formation between native maize starch and palmitic acid and its corresponding mono-, di-, and tripalmityl glycerols. Their results revealed that monoglyceride generated more starch complexes than a fatty acid with a less stable structure confirmed by DSC. Only few complexes were formed between starch and diglycerides or triglycerides.

Although the impact of different lipid structures (free fatty acid, monoglyceride, diglyceride, or triglyceride) has been known to influence the properties of ALCs, [27, 29, 30], the effects of those lipids in different complexation temperatures, to the best of our knowledge, have not been investigated. Lauric acid (LA, C12:0) contains a straight-chain structure, whereas oleic acid (OA, C18:1) possesses a bent-chain structure. Glycerol monolaurate (GML) is a sort of polar lipid derived from lauric acid. This work aimed to understand the effect of the different structures of lipids and complexation temperatures on the complexation formation and digestibility of sweet potato starch-lipid complexes. Hence in this study, the resulting complex between starch and fatty acids (lauric acid, oleic acid) and monoglyceride (glyceryl monolaurate; GML), reacted at 60ºC and 90ºC were identified.

## Materials and Methods

### Materials

Sweet potato (variety Tainung 57) was obtained from

Chiayi Agricultural Experiment Station, Chiayi, Taiwan. Sweet potato starch was isolated as previously described [31]. Pullulanase microbial (E.C.3.2.1.41, activity 1498 NPUN/g, 1.2 g.ml) and porcine pancreatic α-amylase type VI-B (E.C.3.2.1.1, activity 10 units/mg) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Total starch assay kit, glucose oxidase-peroxidase reagent (GOPOD) kit, and amyloglucosidase (3260 U/ml) were purchased from Megazyme International Ltd. (Co. Wicklow, Ireland). Lauric acid (LA) was purchased from Koch-Light Ltd. (Haverhill, UK). Oleic acid was purchased from Show Chemical Co. Ltd. (Akita, Japan). Glycerol monolaurate was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). All other reagents and chemicals used were of analytical grade.

**Preparation of starch-lipid complexes**

The starch used in the preparation of the starch-lipid complex is the debranched form.The debranched starch-lipid complex was prepared from the previous method [9] with some modifications. The debranching process was carried out by incubating the gelatinized starch with a pullulanase (180 NPUN/g of starch) at 60ºC for 12 hours. Then the debranching process was deactivated, followed by the complexation step with lipids (10%, w/w) for 30 minutes at different temperatures of 60ºC and 90ºC. The starch-lipid complexes were obtained by centrifugation and then washed using hot water and 50% ethanol before undergoing the oven drying and milling process. Later in this paper, the debranched starch-lipid complexes will be referred to as starch-lipid complexes.

**Complexing index (CI) of starch-lipid complexes**

The complexing index (CI) of starch-lipid complexes was measured[24]. A reference sample containing only starch was also prepared. The absorbance (Abs) values of the sample and a reference were measured at 690 nm with a UV-VIS spectrophotometer. The CI was calculated as:

$CI\ (\%) = 100 \times (Abs_{reference} - Abs_{sample})/Abs_{reference}$

***In vitro* digestibility of starch-lipid complexes**

The *in vitro* starch digestibility was carried out using the previous methods [32, 33]. The amount of hydrolyzed starch, which classified into rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS) was measured using the GOPOD kit [32]. The calculations were as follows:

$RDS\ (\%) = [(G_{20} - FG)/TS] \times 0.9 \times 100$

$SDS\ (\%) = [(G_{120} - G_{20})/TS] \times 0.9 \times 100$

$RS\ (\%) = [(TS\text{-}RDS\text{-}SDS)/TS] \times 100$

Where $G_{20}$ and $G_{120}$ are the glucose content released at 20 and 120 min of hydrolysis, respectively; FG is free glucose; TS is total starch.

**Statistic Analysis**

Data were subjected to one-way ANOVA, followed by Duncan's multiple range test ($p<0.05$) accomplished using IBM SPSS 25.0 (SPSS, Inc., Chicago, IL, USA).

**Results and discussion**

**Complexing index (CI)**

The complexing index (CI) is an indicator that indirectly calculated the rate of starch-lipid complex formation based on the reduction in the iodine binding capacity with amylose [34, 36]. The effects of various lipids and different complexation temperatures on the CI of starch-lipid complexes are shown in Fig 1. Regardless of the complexation temperature, the starch-GML complex showed the highest CI, followed by starch-LA and starch-OA complexes. The higher complexation of starch-monoglycerides compared to starch-fatty acid complexes was also reported [29, 36]. It is explained by the greater solubility and better emulsifying characteristic of monoglyceride [29]. In contrast, fatty acids have reduced water solubility, thus resulting in poor dispersiveness in cooked starch [26]. The CI value, which appears to be smaller on starch-OA than starch-LA, indicated that double bonds in oleic acid would impede the complex formation. Hence, the amount of carbon used by complex formation is smaller than that of saturated fatty acids [26, 35]. Therefore, it is thought that saturated fatty acids are more preferred for complex formation, defined by a higher degree of amylose-lipid binding. Other researchers have found similar results [12, 26]. The reaction temperature for complex formation influenced the complexation index for starch-GML and starch-OA samples, whereas its effect on the CI of starch-LA was not significant. The CI value decreased as the complexation temperature increased. The effect of different complexation temperature on the CI value were significant on starch-OA (39.2% at 60ºC and 26.8% at 90ºC). These CI value results supported that saturated fatty acid and monoglyceride were more favorable to make complexes with amylose than unsaturated fatty acid.

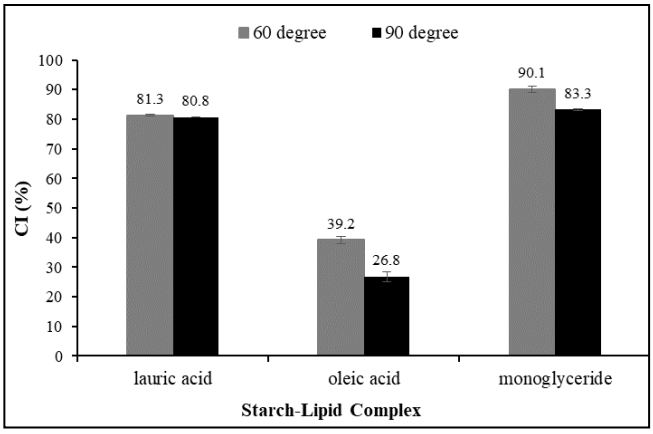


**Fig 1:** Complex indices of starch by different lipids and different complexation temperatures

***In vitro* digestibility of starch-lipid complex**

Starch can be categorized into three groups based on the rate and extent of starch digestion include rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Among those starches, resistant starch is of great interest because it might be fermented in the colon, producing the beneficial short-chain fatty acid instead of digested and absorbed in the intestine [32]. The RDS, SDS, and RS contents in the debranched sweet potato starch (as a control) and starch-lipid complexes are presented in Table 1. The RDS, SDS, and RS contents in control were 46.7%, 38.8%, and 14.4%, respectively. On the other hand, the RS contents of all starch-lipid complexes were higher than that of their control. The addition of lipids to the starches considerably increased the RS and decreased the RDS. The RS contents varied based on the type of lipid and complexation temperature. The complex samples including

starch-LA and starch-GML formed from complexation temperatures of 90° produce more resistant starch compared to complexation temperature of 60°C.

RS content of free fatty acid-complexed starches such as starch-LA and starch-OA was higher than that of starch-GML. Interestingly, although starch-GML showed functional formation capacity in starch-lipid complex formation based on the CI results, its RS content presented the lowest among samples. These results agreed with the previous findings [37] that the complexation using GML (short monoglyceride) provides a slight decrease in enzymatic hydrolysis relative to pure amylose at the beginning of incubation period, following 2h incubation, the hydrolysis rate was closer to pure amylose. The enzyme resistance [37] and thermal resistance [38] of the monoglyceride-amylose complex were found to be largely dependent on monoglyceride chain length. It is indicating the complex stability to enzymic hydrolysis seems not to be related to the complex-forming ability.

**Table 2.** RDS, SDS and RS content of starch-lipid complexes by different lipids and different complexation temperature[a]

| Sample | Temperature (°C) | RDS (%) | SDS (%) | RS (%) |
|---|---|---|---|---|
| Control | - | $46.7 \pm 0.1^{e}$ | $38.8 \pm 0.1^{c}$ | $14.4 \pm 0.2^{a}$ |
| Starch-LA | 60 | $38.5 \pm 3.5^{c}$ | $32.5 \pm 0.4^{b}$ | $28.9 \pm 4.0^{d}$ |
| | 90 | $29.2 \pm 1.1^{a}$ | $25.2 \pm 1.7^{a}$ | $45.5 \pm 1.8^{e}$ |
| Starch-OA | 60 | $26.5 \pm 0.3^{a}$ | $45.2 \pm 1.2^{e}$ | $28.3 \pm 0.9^{d}$ |
| | 90 | $33.2 \pm 0.3^{b}$ | $42.7 \pm 2.2^{de}$ | $24.1 \pm 2.6^{cd}$ |
| Starch-GML | 60 | $43.3 \pm 1.3^{de}$ | $39.8 \pm 1.4^{cd}$ | $16.9 \pm 0.1^{ab}$ |
| | 90 | $41.3 \pm 0.1^{cd}$ | $39.1 \pm 1.9^{cd}$ | $19.6 \pm 1.8^{bc}$ |

The data are mean of three determinations with standard deviation.

Means with different letter within the same column differed significantly ($p < 0.05$).

[a] RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch

## Conclusions

The lipid types showed different effects on the complexing index and *in vitro* digestibility of starch-lipid complexes. The saturated fatty acid was more favored for the formation of the starch-lipid complex and the resistant starch production, indicated by the higher complexing index value and higher resistant starch content. The higher complexation temperature was favorable to the formation of resistant starch. The interactions between starch and lipids could be useful to produce starchy foods with a lowered glycemic index.

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