Effect of reaction solvent on physico-chemical properties, microstructure and digestive properties of starch-fatty acid complexes

ZHANG JIAYAN – XIONG JIANWEN – CHEN ZHENGPEI – CUI NA

Summary
The starch-fatty acid complex is of great significance for digestibility, nutrition and production of starchy foods. To further broaden the application range of starch-fatty acids, rice starch in this paper was complexed with fatty acids using deionized water, sodium sulfate solution, acetone and ethanol to investigate the effects of solvents on physico-chemical properties, microstructure and digestive properties of starch-fatty acid complexes. Results on physico-chemical and digestive properties showed that organic solvents could improve the complex's ability to resist digestion, while the water content could assist in regulating the rigidness of the complex, thereby increasing the degree of insertion of the guest substance into the helical structure of amylose. The complex prepared in an aqueous solution loses the granular structure of natural rice starch, while the composite particles prepared in an organic solvent largely retain the shape of the original rice starch. The study showed that these differences might be due to the different solubilities and affinities of different solvents. Choosing a suitable solvent and adjusting the ratio of the solvents can help improve the degree of complexation and decrease the digestibility of the complex.

Keywords
starch-fatty acid complex; reaction solvent; microstructure; affinity coefficient; digestion

Amylose and some linear segments of the side chains of amylopectin can interact with linear chain molecules and form V-type helical inclusion complexes [1], in which the guest molecules are confined in the helical cavities of amylose [2]. Starch-lipid complexes have many functions, such as delaying starch retrogradation [3], slowing enzymatic digestion [4], inhibiting microbial growth [5], enhancing the tensile strength of starch-based films [6] and carrying biologically active substances to improve their bioavailability and slow down their release in the intestines [7].

Many papers have discussed the factors influencing the properties of starch-guest complexes, such as the type of starch [8, 9], the structure of the guest [10], temperature[11], pH [12], moisture content [13] or order of addition of the guest substances [14]. Starch with pores or channels, high amylose content and proper depolymerization could facilitate the formation of starch-lipid complexes [8]. Conversely, a fatty acid with *cis* double bonds [9], hydrophobic chains that are too short or too long, and inappropriate ligand concentrations can lead to fewer crystalline complexes [10]. In addition, physical modification technology, such as high-pressure homogenization [15], microfluidization [16] or annealing treatments [17], was reported to increase V-type crystallization and the resistant starch content. Other ingredients in food, such as NaCl, can promote the complexation of medium-chain fatty acids (such as lauric acid) and starch but have little effect on long-chain fatty acids (such as palmitic acid or stearic acid) [18]. In contrast to NaCl, triglycerides promote the formation of complexes between starch and long-chain fatty acids (C16:0 and C18:0) but hinder the formation of complexes between starch and medium-chain fatty acids (C12:0 and C14:0) [19]. In addition, the hydrophilic-lipophilic balance values (HLB) and head size of the emulsifier also significantly affect the degree of complexation [3].

Three main solvents, namely, dimethyl

Zhang Jiayan, Xiong Jianwen, Chen Zhengpei, Cui Na, Department of Food and Chemical Engineering, Liuzhou Institute of Technology, Xinliu Street 99, Liuzhou 545616, China; City Key Laboratory of Plant-derived Ingredients of Liuzhou river snails rice noodle, Xinliu Street 99, Liuzhou 545616, China.

Correspondence author:
Zhang Jiayan, e-mail: zhangjiayan0707@163.com

sulfoxide (DMSO), alkali solutions and high-temperature water, are used to induce the formation of inclusion complexes [20]. Precipitation from an ethanol solution can also induce the formation of single helixes and V-type crystals of starch. Dries found that the thermal stability of V_H crystals was affected by the ethanol concentration [21]. ZHONG [22] used methanol, ethanol, *n*-propanol, isopropanol, and *tert*-butanol to induce the formation of V-type crystals of starch, which were used to encapsulate ethylene. The encapsulation efficiency of the complex prepared with ethanol for ethylene was found to be higher than those of the complexes prepared with other solvents. KONG and ZIEGLER [20] used ethanol to precipitate DMSO-dissolved starch to prepare „empty" V-amylose, and the crystallinity of V-starch was found to be significantly improved by annealing in a high-temperature ethanol solution.

The above results showed that the solvent has a substantial influence on the formation of starch-lipid complexes. However, there is little systematic information on the effects of the solvent on formation of starch-lipid complexes. Therefore, the influence of solvent on the properties of starch-fatty acid complexes was investigated in this study. Rice is a common staple food, but its glycemic index is high [23], so we used rice starch complexed with long-, medium- and short-chain fatty acids (stearic acid, lauric acid and hexanoic acid, respectively) in four different reaction media (deionized water, sodium sulfate aqueous solution, ethanol and acetone) under otherwise constant reaction conditions, and the starch-fatty acid complexes were characterized by measuring their physico-chemical and structural properties. This study aimed to investigate the effects of various types of solvents on the properties of complexes formed by starch and various chain length fatty acids to improve the efficiency of starch-fatty acid complex formation and to reduce digestibility of the complex.

MATERIALS AND METHODS

Materials

Rice starch was obtained from Wuxi Jinnong Biotechnology (Wuxi, China; 8.50 % moisture, 21.36 % amylose, 0.75 % protein and 0.10 % lipids). Stearic acid and lauric acid were obtained from Aladdin Industrial (Shanghai, China), hexanoic acid was obtained from Xiya Reagent (Chengdu, China), pancreatin from porcine pancreas (EC 232-468-9, 15 $U \cdot mg^{-1}$) and amyloglucosidase (EC 3.2.1.3, 300 $U \cdot ml^{-1}$) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Sodium sulfate, ethanol and acetone were obtained from Sinopharm Chemical Reagent (Beijing, China). All the chemicals used in this study were of analytical grade.

Preparation of starch-fatty acid complexes

The starch-fatty acid complexes were prepared according to the method of MARINOPOULOU et al. [11]. First, rice starch (dry basis) was dispersed in deionized water (15 $mg \cdot ml^{-1}$), heated at 90 °C for 30 min and cooled down to 70 °C. The fatty acid (10% on weight basis relative to the dry starch) was dissolved in different solvents (distilled water, 0.1 $mol \cdot l^{-1}$ sodium sulfate solution, acetone or 95% ethanol), and the suspension was heated at 70 °C in a water bath for 30 min, the vessel with the acetone solution being equipped with a reflux condenser. The two solutions were mixed and stirred at 70 °C for 30 min to obtain a precipitate. The starch-fatty acid complex was separated from the suspension by centrifugation (3 580 ×*g*, 30 min). The precipitate was washed 7 times with an ethanol-water mixture (50 : 50, v/v) and centrifuged to remove free fatty acid residues. The complex was transferred to a Petri dish and dried at 25 °C for approximately 24 h until the moisture was reduced to approximately 10 %. Then, the solid was milled into a powder and filtered through a 100 μm sieve.

Starch-fatty acid complexes were also prepared using ethanol at ratio ranging from 20 % to 80 % (w/w) by following the above described procedure. The complexes prepared in distilled water, 0.1 $mol \cdot l^{-1}$ sodium sulfate solution, acetone and 95% ethanol are referred to as RFW, RFS, RFA and RFE, respectively.

Analysis

The starch-lipid complex (0.4 g) was weighed into a 50 ml centrifuge tube and distilled water was added to a total weight of 5 g. The suspension was vortexed and then heated in a boiling water bath and shaken occasionally for approximately 20 min until the starch was completely gelatinized. After the solution was cooled down to room temperature, 25 ml of distilled water was added to the gelatinized sample. The samples were then vortexed for 2 min and centrifuged at 3 580 ×*g* for 15 min. Then, 500 μl of the supernatant was mixed with 15 ml of distilled water and 2 ml of iodine solution, and the sample was thoroughly mixed. The absorbance was measured at 690 nm, three parallel experiments being performed. The control sample only contained distilled water and other operations were the same as above [24].

The iodine solution was prepared by dissolving

2 g of potassium iodide and 1.3 g of iodine in distilled water to a total volume of 100 ml.

$$CI = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where *CI* is the complex index (in percent), A_c is the absorbance of control and A_s is the absorbance of samples.

Solubility and swelling power

The solubility (*S*) and swelling power (*SP*) were determined according the methods described by Li et al. [10]. *S* and *SP* of each sample were calculated as follows:

$$S = \frac{W_d}{W_s} \times 100 \quad (2)$$

$$SP = \frac{W_w \times 100}{W_s} \times (100 - S) \quad (3)$$

where *S* is solubility (in percent), *SP* is swelling power, W_d is the weight of dried supernatant, *Ws* is the dry weight of starch, W_w is the weight of wet sediment.

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) of the starch samples was done using an IRAffinity-1S spectrometer (Shimadzu, Kyoto, Japan) in the spectral range of 400–4 000 cm^{-1} with a resolution of 4 cm^{-1} using 32 scans per sample.

X-ray diffraction

The formation of the V-starch complex was confirmed using a D8 Advance diffractometer (Bruker, Bremen, Germany) with a Cu target and Kα radiation (λ = 0.15406 nm) at 40 kV and 40 mA. The starch powder was scanned between 5° and 40° (2θ) in steps of 2° per minute at room temperature. The crystal size *(CS)* is the crystalline grain diameter perpendicular to the crystal plane, and was calculated by Scherrer's equation [11].

$$CS \approx \frac{\lambda}{(FWHM \times \cos\theta)} \quad (4)$$

where λ is wavelength, *FWHM* is the full width at half-maximum, and θ is diffraction angle.

The relative crystallinity (*RC*) of the samples was calculated according to the Hermans's formula [11]:

$$RC = \frac{Sc}{(Sc + Sa)} \times 100 \quad (5)$$

where *Sc* is the crystallinity area, *Sa* is the amorphous area.

Scanning electron microscopy

The starch samples were attached to double-sided tape under an infrared lamp. After removing the excess starch, the samples were placed in a SU8020 model scanning electron microscope (SEM) SU8020 (Hitachi, Tokyo, Japan) for observation. The acceleration voltage was 20 kV.

Determination of sample digestibility

The in vitro digestibility of each sample was determined according to the methods of Englyst et al. [25] and Liu et al. [15] with some modifications. Briefly, 1.0 g of sample was dispersed in 20 ml of 0.1 $mol \cdot l^{-1}$ sodium acetate buffer (pH 5.2) and mixed thoroughly with heating in a 37 °C water bath for 20 min, and then 5 ml of enzyme solution containing 150 $U \cdot ml^{-1}$ porcine pancreatin and 3 $U \cdot ml^{-1}$ of amyloglucosidase was added. The mixture was heated in a water bath at 37 °C with magnetic stirring to ensure sufficient mixing. After heating for 20 min and for 120 min, 0.5 ml of the hydrolysis solution was removed and mixed with 2.5 ml of 95% ethanol to terminate the enzymatic hydrolysis reaction. The hydrolysis solution was centrifuged for 10 min (3 580 ×*g*) and the glucose concentration in the supernatant was measured by the glucose oxidase and peroxidase method using GOPOD reagent (Megazyme, Bray, Ireland). Each sample was analysed in triplicate.

Resistant starch (*RS*), slowly digestible starch (*SDS*) and rapidly digestible starch (*RDS*) in the samples were calculated using the following formulas.

$$RDS = \frac{T_{20} \times 0.9}{TS} \times 100 \quad (6)$$

$$SDS = \frac{(T_{120} - T_{20}) \times 0.9}{TS} \times 100 \quad (7)$$

$$RS = \frac{(TS - RDS - SDS)}{TS} \times 100 \quad (8)$$

where T_{20} is the glucose content after 20 min of enzymatic hydrolysis, T_{120} is the glucose content after 120 min of enzymatic hydrolysis and *TS* is the total starch content in the samples.

Statistical analysis

The effects of solvents on the physico-chemical properties of the starch-fatty acid complexes were analysed by one-way ANOVA using SPSS statistical software (IBM, Armonk, New York, USA). Differences were considered significant at $p < 0.05$ as determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

Complex index analysis

The *CI* value indicates the degree of complexation between starch and the fatty acid. The complexation ability and efficiency between starch and the guest were affected by solubility of the reactant and the affinity coefficient, which was influenced by hydrophobic forces, hydrogen bonding and concentration gradients [26].

CI values of the samples are shown in Fig. 1A. They were found to be different in individual solvents, indicating that the solvent significantly influences the complexation degree and reaction efficiency. The *CI* values of the complexes prepared in organic solvents were higher than those of complexes prepared in aqueous solvents. However, when ethanol was mixed with water, the *CI* values increased with a decrease in alcohol concentration (Fig. 1B), reaching a maximum at an ethanol concentration of 40 %, and then decreased as the ethanol concentration was further decreased, indicating that the concentration of ethanol has a significant effect on the *CI* value. This effect may be due to water increasing the mobility of the starch molecular chains, as water can disrupt the intermolecular interactions among the starch molecules by reducing the intra- and intermolecular hydrogen bonding density. Thus, a moderate water content could lower the rigidity of the starch chains, facilitating insertion of guests into the single helix [27]. However, increasing the water content decreases the reactant solubility, so when the ethanol concentration is less than 40 %, the effect of the decrease in solubility was greater than the effect of the increase in the affinity coefficient and the *CI* value decreased with a decrease in ethanol concentration. This result is similar to those of previous studies. Kong and Ziegler [20] found that moderate concentrations (40–60 %) of ethanol afforded suitable solvents for precipitation of V-type structures, as the starch was flexible enough to provide ample space for guests.

The *CI* values of RFE were higher than those

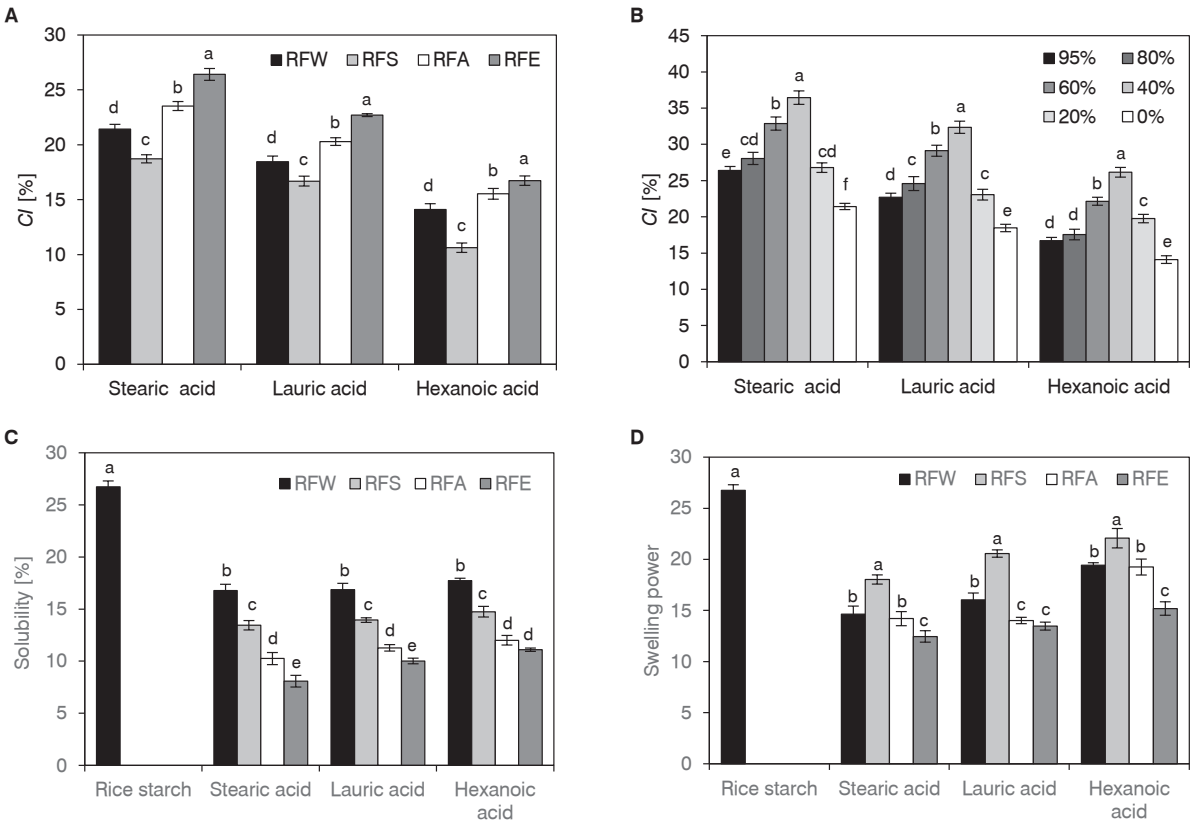

Fig. 1. Complex index values, solubility and swelling power of starch-fatty acid complexes.

A – complex index values of samples in various solvent media, B – complex index values of samples in different concentrations of ethanol, C – solubility of samples in various solvent media, D – swelling power of samples in various solvent media.
Different letters in superscript indicated significant differences in each column at $p < 0.05$.
CI – complex index, RFW – complex prepared in distilled water, RFS – complex prepared in 0.1 mol·l^{-1} sodium sulfate solution, RFA – complex prepared in acetone, RFE – complex prepared in 95% ethanol.

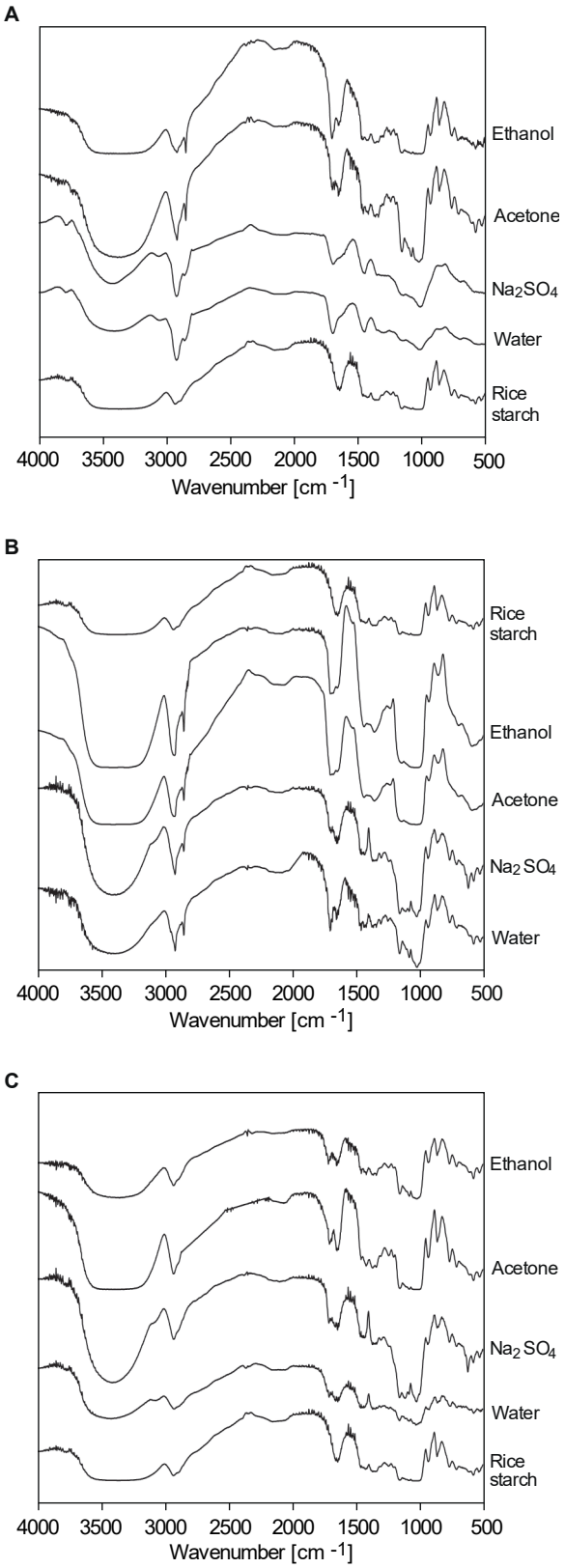

Fig. 2. Fourier transform infrared spectra of starch-fatty acid complexes prepared in various solvent media.

A – complexed with stearic acid, B – complexed with lauric acid, C – complexed with hexanoic acid.

of RFA (Fig. 1A), which can be attributed to the differences in both the solubility and affinity coefficients. Fatty acids are more soluble in ethanol than in acetone, and the solubility decreases with increasing chain length. The affinity coefficients decrease as the polarity of the system decreases [23–26], and acetone is less polar than ethanol [28]. Fatty acids are less soluble in sodium sulfate solution than in water due to the salting-out effect [29], which leads to RFW having a higher *CI* value than RFS.

Solubility and swelling power

S and *SP* of each sample are shown in Fig. 1C and Fig. 1D. Compared with those of native rice starch, *S* and *SP* of the complexes were significantly lower. The decrease may be due to the higher crystallinity of the complexes, which makes it difficult for water to enter the starch molecules [10]. In addition, fatty acids can effectively prevent the leaching of amylose and delay gelatinization, thereby reducing the swelling capacity [30]. Moreover, previous studies showed that amylose-fatty acid complexes can form hydrophobic films on the surfaces of starch particles, reducing their ability to bind water molecules [31].

Compared with those of native starch, *S* and *SP* of RFE were the lowest, followed by RFA, RFW and RFS. This can be explained by the fact that more starch-fatty acid complexes were formed from the organic solvent system (Fig. 1A), so those complexes have a greater capacity to limit the entry of water and concomitant expansion. Similar results were found by Li et al. [10] and Vasiliadou et al. [32].Their results indicating that solubility and swelling power decreased with increasing *CI* value due to the tighter crystal packing in the complexes.

FTIR analyses

Fig. 2 shows the FTIR spectra of the native rice starch and the starch-fatty acid complexes. Each spectrum of each sample has an absorption peak at approximately 3 400 cm^{-1}, which is derived from the stretching vibrations of the hydroxy groups. This peak does not notably change with a change in solvent. The peak at 2 935 cm^{-1} in the spectrum of native rice starch was attributed to asymmetric stretching vibrations of the methylene group in the glucose ring [33]. The corresponding peaks in the spectra of the complexes prepared in different solvents are all shifted to lower wavenumbers (approximately 2 850 cm^{-1}), indicating that the added fatty acids promote intermolecular interactions involving the methylene groups, thereby favouring hydrophobic interactions between the starch and

Tab. 1. Absorbance ratios of rice starch fatty acid complexes.

Fatty acid	Absorbance ratio	Solvent medium			
		Water	Na_2SO_4	Acetone	Ethanol
Stearic acid	A_{1045}/A_{1022}	1.032 ± 0.005 c	1.021 ± 0.005 c	1.58 ± 0.008 b	1.959 ± 0.010 a
	A_{1022}/A_{995}	0.993 ± 0.003 a	1.014 ± 0.007 a	0.538 ± 0.005 c	0.621 ± 0.006 bc
Lauric acid	A_{1045}/A_{1022}	1.168 ± 0.006 b	1.133 ± 0.009 b	2.459 ± 0.011 a	2.517 ± 0.012 a
	A_{1022}/A_{995}	0.857 ± 0.003 a	0.871 ± 0.006 a	0.124 ± 0.004 b	0.110 ± 0.003 b
Hexanoic acid	A_{1045}/A_{1022}	1.023 ± 0.008 d	1.121 ± 0.006 cd	1.266 ± 0.005 b	1.791 ± 0.009 a
	A_{1022}/A_{995}	0.966 ± 0.004 a	0.865 ± 0.003 b	0.438 ± 0.001 c	0.372 ± 0.001 d

Data are mean ± standard deviation (n = 3). Different letters in superscript within the same row with different letters are significantly different ($p < 0.05$).
A_{1045}/A_{1022} – ratio of absorbance at 1 045 cm^{-1} and 1 022 cm^{-1}, A_{1022}/A_{995} – ratio of absorbance at 1 022 cm^{-1} and 995 cm^{-1}.

Tab. 2. The in vitro digestibility of starch-fatty acid complexes prepared in various solvent media.

Fatty acid	Digestibility	Solvent medium			
		Water	Na_2SO_4	Acetone	Ethanol
Stearic acid	*RDS* [%]	59.0 ± 2.8 bc	63.6 ± 3.2 a	56.9 ± 2.6 c	51.5 ± 1.8 d
	SDS [%]	19.4 ± 1.5 b	17.2 ± 2.6 c	20.1 ± 1.9 b	22.9 ± 1.8 a
	RS [%]	21.6 ± 1.8 b	19.3 ± 2.1 c	23.0 ± 2.3 b	25.7 ± 1.6 a
Lauric acid	*RDS* [%]	58.4 ± 3.4 b	65.1 ± 3.0 a	57.6 ± 2.9 b	54.3 ± 2.1 c
	SDS [%]	20.7 ± 1.2 b	15.4 ± 1.0 c	20.9 ± 2.2 b	22.5 ± 2.9 a
	RS [%]	21.0 ± 2.3 bc	19.4 ± 2.1 c	21.5 ± 2.2 b	23.1 ± 2.3 a
Hexanoic acid	*RDS* [%]	67.5 ± 3.5 b	72.6 ± 4.1 a	62.6 ± 3.7 c	59.1 ± 2.8 d
	SDS [%]	15.1 ± 0.9 c	10.7 ± 0.7 d	18.4 ± 2.2 b	20.2 ± 1.5 a
	RS [%]	17.4 ± 1.1 bc	16.8 ± 2.0 c	19.0 ± 2.8 b	20.6 ± 1.8 a

Data are mean ± standard deviation (n = 3). Different letters in superscript within the same row with different letters are significantly different ($p < 0.05$)
RDS – rapidly digestible starch, *SDS* – slowly digestible starch, *RS* – resistant starch.

the fatty acids [34]. This shift was more obvious in the spectra of the stearic acid and lauric acid complexes than in the spectrum of the hexanoic acid complex, which can be explained by the weaker hydrophobic interactions between the rice starch and hexanoic acid, as the molecular chain of hexanoic acid is shorter.

The complexes exhibited a -C=O stretching vibration resulting in an absorption peak at 1 700 cm^{-1}, and this peak was mainly attributable to the carbonyl vibrations in the fatty acids. Compared with the carbonyl vibration peak of native rice starch (1 652 cm^{-1}), the carbonyl vibration peaks of the complexes were shifted to higher frequencies because the hydrogen bonds of the fatty acids were broken during the crystallization stage and new hydrogen bonds were formed between the carbonyl group of the fatty acid and the hydroxy groups of amylose [35]. The stretching absorption peak for -CH_3 and -CH_2 appeared at 2 800 cm^{-1}, and this was attributed to the methyl and methylene groups in the fatty acids. The appearance of peaks at 1 700 cm^{-1} and 2 850 cm^{-1} indicates that the fatty acids were successfully encapsulated into starch. The peaks of the complexes prepared in organic solvents were more intense than those of the complexes prepared in aqueous solvents (Fig. 2), indicating that the complexation ability between starch and fatty acids might be stronger in organic media. This result is consistent with the *CI* values of the starch-fatty acid complexes listed in Fig. 1A.

Previous studies [31–34] showed that the absorbance bands at 1 045 cm^{-1} and 1 022 cm^{-1} are related to the amorphous and crystalline structures of starch, respectively, so the absorbance ratios of 1 045 cm^{-1}/1 022 cm^{-1} (A_{1045}/A_{1022}) and 1 022 cm^{-1}/995 cm^{-1} (A_{1022}/A_{995}) can be used as indicators of the short-range order in the helix. The values of of these ratios are shown in Tab. 1. The A_{1045}/A_{1022} ratio of the complex prepared in an organic solvent was high, while the A_{1022}/A_{995} ratio was lower, indicating that starch-fatty acid complexes prepared in organic solvents have a higher degree of short-range molecular order than do the complexes prepared in aqueous solvents. The complexes formed in ethanol had the highest ratio (1.96), implying that organic solvents pro-

mote the formation of a more ordered structure in starch. For inorganic solutions, no significant differences in the A_{1045}/A_{1022} and A_{1022}/A_{995} ratios were observed among the complexes (Tab. 1). This is probably due to the salt solution promoting retrogradation of starch, which increases the overall short-range molecular order in the complexes. In addition, higher short-range order in the helical structure makes it more resistant to enzymatic hydrolysis and thus results in a higher resistant starch content (Tab. 2).

X-ray diffraction analysis

The X-ray diffraction patterns of rice starch and the starch-fatty acid complexes prepared in various solvents are shown in Fig. 3, and the relative crystallinity (*RC*) and crystal size (*CS*) of starch-fatty acid complexes prepared in various solvent media, calculated based on the X-ray diffraction data, are shown in Tab. 3. Rice starch has strong diffraction peaks at 2θ values of 15°, 17°, 17.8° and 23°, which are typical of A-type crystal structures [36]. The complexes prepared in ethanol, acetone and water showed diffraction peaks at approximately 7.6°, 12.8° and 19.8°. These peaks are typical diffraction peaks of V-type crystalline structures, indicating the formation of starch-lipid complexes [37]. As the chain length increases, the diffraction intensity of the characteristic V-type peaks also increases. This is because the number of hydrophobic sites on the fatty acid chains increases as the chain length increases, so fatty acids can bind to the hydrophobic sites of starch with more hydrophobic interactions. The complexed samples obtained from organic solvents presented stronger V-type diffraction peaks at 12.8° and 19.8° (2θ) than did the samples prepared in aqueous solvents, implying that more regularly arranged helical cavities formed in organic solvents. The starch-fatty acid inclusion complexes prepared in aqueous sodium sulfate solution showed a mixture of V-type and B-type patterns with diffraction peaks at 2θ values of 17° and 22.8°. V-type crystals can be completely transformed into B-type structures during crystallization or during recrystallization after dissolution as long as sufficient water is present [38]. However, while RFS showed a B-type pattern, RFW did not, suggesting that certain salt ions can promote the transformation of the starch crystal pattern. This may be because sulfate

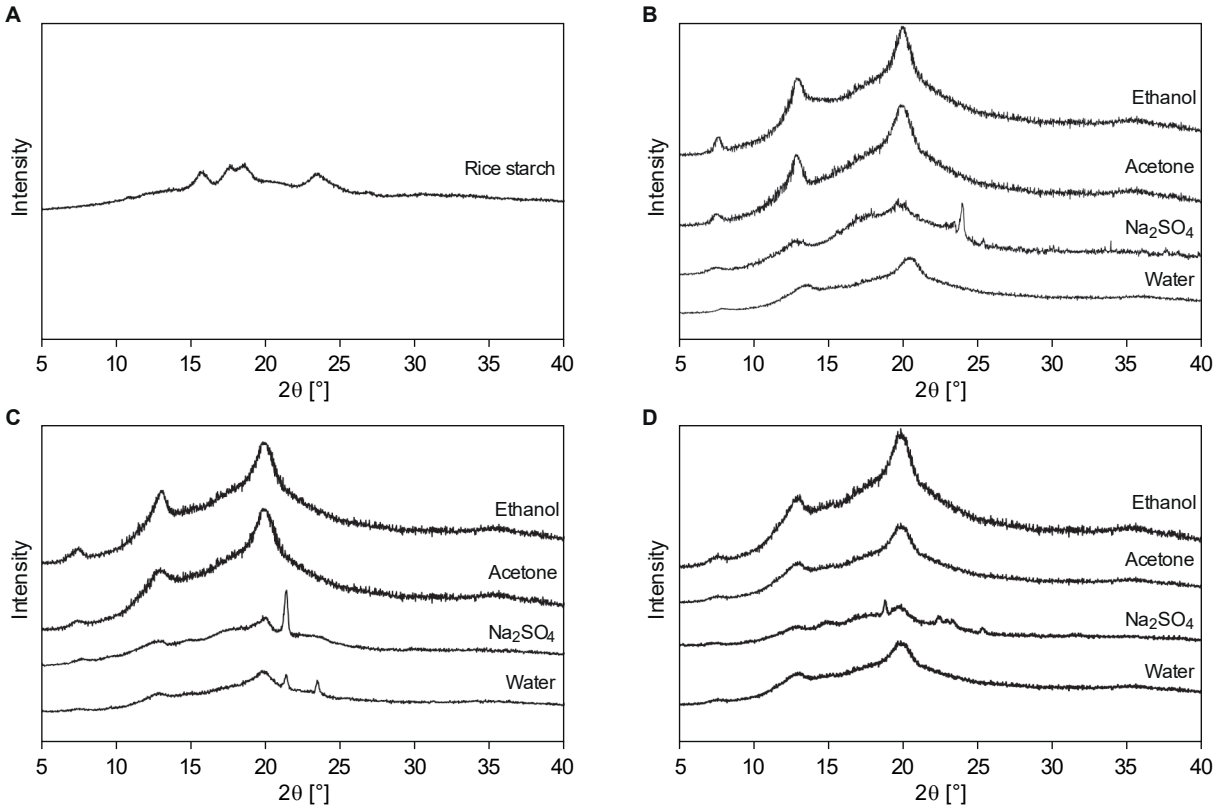

Fig. 3. X-ray diffraction patterns of samples.

A – rice starch, B – starches complexed with stearic acid, C – starches complexed with lauric acid, D – starches complexed with hexanoic acid.

Tab. 3. Relative crystallinity and crystal size of starch-fatty acid complexes prepared in various solvent media.

Fatty acid		Solvent medium			
		Water	Na_2SO_4	Acetone	Ethanol
Stearic acid	*RC* [%]	27.5 ± 0.8^{c}	24.6 ± 0.3^{d}	28.8 ± 0.8^{bc}	36.0 ± 1.0^{a}
	CS [%]	10.9 ± 0.7^{c}	12.1 ± 0.5^{b}	12.5 ± 0.3^{b}	14.4 ± 0.1^{a}
Lauric acid	*RC* [%]	24.5 ± 0.6^{b}	22.2 ± 0.4^{c}	24.5 ± 0.6^{b}	30.1 ± 0.8^{a}
	CS [%]	9.7 ± 0.2^{c}	11.9 ± 0.8^{b}	11.7 ± 0.3^{b}	13.8 ± 0.2^{a}
Hexanoic acid	*RC* [%]	21.0 ± 0.6^{cd}	20.9 ± 0.8^{d}	25.8 ± 0.4^{b}	27.5 ± 0.7^{a}
	CS [%]	8.6 ± 0.2^{c}	10.4 ± 0.3^{ab}	9.0 ± 0.2^{b}	11.3 ± 0.4^{a}

Values reflect the ability of the reaction solvent to promote formation of the complex. The crystal size of starch-fatty acid complexes was calculated from the diffraction peaks at 2θ values 7.6°, 12.8°, and 19.8°.
Data are mean ± standard deviation ($n = 3$). Different letters in superscript within the same row with different letters are significantly different ($p < 0.05$).
RC – relative crystallinity, *CS* – crystal size.

radicals have radial symmetry and a low surface charge, so they tend to protect the hydrogen bonds among starch molecules and between starch and water molecules. Therefore, they promote the rearrangement of starch molecules during the cooling stage, which causes the V-type structures to partially convert into B-type structures after complexation.

The diffraction peaks at 22° and 24° were from aggregates of uncomplexed fatty acids trapped between the helices of the crystallized complexes [16, 23]. Among the three fatty acids, the aggregation of uncomplexed hexanoic acid was the least obvious. This can be explained by hexanoic acid having a short chain (C6) and good fluidity as well as flexibility, which make it easy to be removed by washing with an ethanol-water mixture (50 : 50). This result is similar to the results of MARINOPOULOU [11], who also found that, compared to that of long-chain fatty acids, the fatty acid aggregation peak of capric acid (C10) is much weaker. When complexed with the same fatty acid, the peaks at 22° and 24° in the pattern of the complexes generated in an aqueous solvent were stronger than those in the pattern of the complexes generated in an organic solvent. This was most notable for the material prepared in a sodium sulfate solution, and these results indicate that one of the reasons for the stronger V-type diffraction peaks of the complexes from organic solvents was the difference in solubility of the fatty acids in different solvents.

The crystallities of the complexes prepared in aqueous media were found to be smaller than those of the complexes prepared in organic media (Tab. 3). This can be explained by the fact that water and sodium sulfate solutions have higher heat transfer rates than the organic solvents. The higher heat transfer rate results in faster nucleation, favouring formation of more small crystallites [23]. Moreover, absolute ethanol is more viscous than water, and migration of the molecules is slower in high viscosity systems, which slows down nucleation [24], causing formation of fewer but larger crystallites in ethanol.

The differences in thermal conductivity and viscosity between water and 0.1 mol·l^{-1} sodium sulfate solution were small, which indicates that the sizes of the crystallites obtained from these solutions were similar. As the length of the fatty acid chains increased, the crystal size increased. When the chains were short, the formed crystals were small.

Scanning electron microscopic analysis

Fig. 4 shows the SEM micrographs of the native rice starch and the starch-fatty acid complexes. Most of the native rice starch granules were pentagonal or hexagonal, and a few were irregular in shape.

The rice starch-fatty acid complexes prepared in different solvents were morphologically quite distinct. The starch complexes prepared in aqueous solvents lost the granular structure of the native rice starch and presented a dense irregular structure. The larger structures had an amorphous layer with rough surfaces, complexes were formed on the surface of the layer phase [16] and complexes appeared either in the form of spherulites that protruded from the surface of the particles (Fig. 4B, 4C, 4E and 4F) or in the form of lamellae (Fig. 4C, 4F, and 4G), which had cylindrical shapes and were closely aggregated in parallel to each other [23]. At the same time, there were some protrusions on the surface of the starch granules, which were caused by leaching of amylose from within the starch granules (Fig. 4B, 4C, 4D, 4E and 4F), and the amylose protruding from the surface of the granules allowed the formation of complexes with the fatty acids [32].

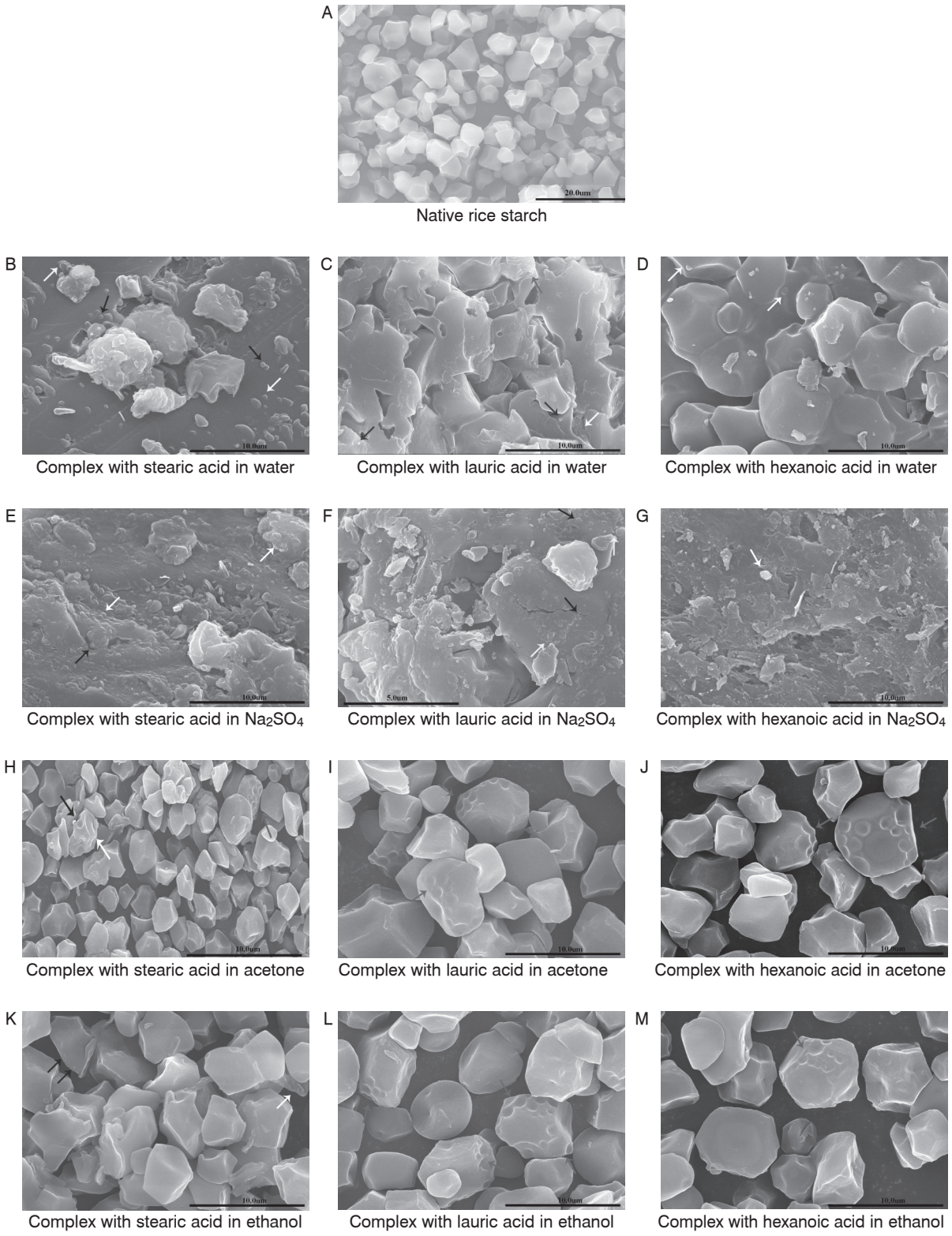

Fig. 4. Scanning electron microscopic images of starch-fatty acid complexes prepared in various solvent media.

The complex granules prepared in organic solvents largely retained the morphology of native rice starch. ZHONG [22] used the alcohol-alkali method to prepare V-shaped crystals from potato starch. The results showed that the V-shaped crystalline starches prepared from five alcohols all retained the structure of the original starch particles, and higher degrees of complexation were associated with smoother particle surfaces and less starch particle breakage. It was speculated that the alcoholic solutions can inhibit expansion of the starch granules, so the starch complexes maintain the morphology of the original starch granules. Although the starch complexes prepared in organic solvents maintained the starch granule shape, spherulites (Fig. 4H and 4K), lamellae (Fig. 4F, 4H and 4I) and protrusions (Fig. 4H and 4K) appeared on the surface of the starch granules, and the shape of the lamellae was different from that of the complexes prepared in aqueous solvents, which were semielliptical and closely aggregated in parallel on the surface. The SEM results showed that organic solvents may be more suitable for maintaining the complete particle morphology of starch.

Digestibility of the complexes

Tab. 2 shows the *RDS*, *SDS* and *RS* contents of the starch-fatty acid complexes. The digestibility of the complexes prepared in different solvents varied dramatically in accordance with their contents of *RS*, *SDS* and *RDS*. The inclusion complexes prepared in organic solvents appeared to be more resistant to enzymatic attack and showed lower *RDS* contents. This indicates that organic solvents are more conducive to formation of *SDS* and *RS*. The resistance of amylose inclusion complexes to enzymatic digestion depends on the structure of the inclusion complex, which can be expressed in terms of its crystallinity and thermal stability. The stability of the complex depends on the structure of the guest compound and the method used to form the complex. For example, higher saturation of the included ligand and longer hydrophobic chains lead to higher resistance to enzymatic digestion because lipids with longer hydrocarbon chains form more hydrophobic interactions with the hydrophobic helical cavity of amylose, resulting in higher crystallinity and thermal stability [34]. Organic solvents can improve the ability of the complex to resist digestion, which might be attributable to fatty acids being more soluble in organic solvents, facilitating the insertion of the fatty acids into the helical structures. Therefore, the crystallinities of the complexes prepared in organic solvents were higher, and they had higher RS contents because the dense crystalline region makes it difficult for digestive enzymes to enter the starch granules, indicating that the reaction media notably influence digestibility of starch-fatty acid complexes.

CONCLUSIONS

The study shows that the reaction solvent has a significant effect on properties and digestibility of starch-fatty acid complexes, probably because the solvents affect the solubility of fatty acids and the affinity coefficient of the reaction system. This study deepens the understanding of how starch and fatty acid complexes are affected by reaction solvents. It also provides an essential reference for further improving the complexation degree and reducing digestibility of starch-lipid complexes.

Acknowledgement

This work was supported by Basic Research Ability Improvement Project for Young Teachers in Guangxi Higher Education Institutions (Grant No: 2019KY1109), Scientific Research and Innovation team Project of Liuzhou Institute of Technology (2018LSTD02).

REFERENCES

1. Chao, C. – Yu, J. – Wang, S. – Copeland, L. – Wang, S.: Mechanisms underlying the formation of complexes between maize starch and lipids. Journal of Agricultural and Food Chemistry, *66*, 2018, pp. 272–278. DOI: 10.1021/acs.jafc.7b05025.
2. Obiro, W. C. – Sinha, R. S. – Emmambux, M. N.: V-amylose structural characteristics, methods of preparation, significance, and potential applications. Food Reviews International, *28*, 2012, pp. 412–438. DOI: 10.1080/87559129.2012.660718.
3. Yamashita, S. – Matsumiya, K. – Kogo, Y. – Takamatsu, K. – Matsumura, Y.: Emulsifiers efficiently prevent hardening of pancakes under refrigerated conditions via inclusion complexes with starch molecules. Food Hydrocolloids, *100*, 2020, article 105432. DOI: 10.1016/j.foodhyd.2019.105432.
4. Wang, M. – Shen, Q. – Hu, L. – Hu, Y. – Ye, X. – Liu, D. – Chen, J.: Physicochemical properties, structure and in vitro digestibility on complex of starch with lotus (*Nelumbo nucifera* Gaertn.) leaf flavonoids. Food Hydrocolloids, *81*, 2018, pp. 191–199. DOI: 10.1016/j.foodhyd.2018.02.020.
5. Hay, W. T. – Fanta, G. F. – Rich, J. – Evans, K. O. – Skory, C. D. – Selling, G. W.: Antimicrobial properties of amylose-fatty ammonium salt inclusion complexes. Carbohydrate Polymers, *230*, 2020, article 115666. DOI: 10.1016/j.carbpol.2019.115666.
6. Hay, W. T. – Behle, R.W. – Ruiz-Vera, U. M. – Fanta, G. F. – Selling, G. W.: Use of novel film form-

ing starch complexes to directly and indirectly reduce insect damage to plants. Crop Protection, *130*, 2019, article 105048. DOI: 10.1016/j.cropro.2019.105048.
7. Jiang, M. – Hong, Y. – Gu, Z. B. – Cheng, L. – Li, Z. F. – Li, C. M.: Preparation of a starch-based carrier for oral delivery of Vitamin E to the small intestine. Food Hydrocolloids, *91*, 2019, pp. 26–33. DOI: 10.1016/j.foodhyd.2019.01.021.
8. D'Silva, T. V. – Taylor, J. R. N. – Emmambux, M. N.: Enhancement of the pasting properties of teff and maize starches through wet–heat processing with added stearic acid. Journal of Cereal Science, *53*, 2011, pp. 192–197. DOI: 10.1016/j.jcs.2010.12.002.
9. Nuessli, J. – Sigg, B. – Conde-Petit, B. – Escher, F.: Characterization of amylose-flavour complexes by DSC and X-ray diffraction. Food Hydrocolloids, *11*, 1997, pp. 27–34. DOI: 10.1016/s0268-005x(97)80007-0.
10. Li, X. – Gao, X. – Lu, J. – Mao, X. – Wang, Y. – Feng, D. – Cao, J. – Huang, L. – Gao, W.: Complex formation, physicochemical properties of different concentration of palmitic acid yam (*Dioscorea opposita* Thunb.) starch preparation mixtures. LWT – Food Science and Technology, *101*, 2019, pp. 130–137. DOI: 10.1016/j.lwt.2018.11.032.
11. Marinopoulou, A. – Papastergiadis, E. – Raphaelides, S. N.: An investigation into the structure, morphology and thermal properties of amylomaize starch-fatty acid complexes prepared at different temperatures. Food Research International, *90*, 2016, pp. 111–120. DOI: 10.1016/j.foodres.2016.10.035.
12. Yotsawimonwat, S. – Sriroth, K. – Kaewvichit, S. – Piyachomkwan, K. – Jane, J. L. – Sirithunyalug, J.: Effect of pH on complex formation between debranched waxy rice starch and fatty acids. International Journal of Biological Macromolecules, *43*, 2008, pp. 94–99. DOI: 10.1016/j.ijbiomac.2008.03.006.
13. Chang, F. – He, X. – Huang, Q.: Effect of lauric acid on the V-amylose complex distribution and properties of swelled normal cornstarch granules. Journal of Cereal Science, *58*, 2013, pp. 89–95. DOI: 10.1016/j.jcs.2013.03.016.
14. Exarhopoulos, S. – Raphaelides, S. N.: Morphological and structural studies of thermally treated starch-fatty acid systems. Journal of Cereal Science, *55*, 2012, pp. 139–152. DOI: 10.1016/j.jcs.2011.10.011.
15. Liu, K. – Chi, C. – Huang, X. – Li, X. – Chen, L.: Synergistic effect of hydrothermal treatment and lauric acid complexation under different pressure on starch assembly and digestion behaviors. Food Chemistry, *278*, 2019, pp. 560–567. DOI: 10.1016/j.foodchem.2018.11.097.
16. Chen, B. – Guo, Z. – Miao, S. – Zeng, S. – Jia, X. – Zhang, Y. – Zheng, B.: Preparation and characterization of lotus seed starch-fatty acid complexes formed by microfluidization. Journal of Food Engineering, *237*, 2018, pp. 52–59. DOI: 10.1016/j.jfoodeng.2018.05.020.
17. Shi, L. – Zhang, B. – Li, C. – Fu, X. – Huang, Q.: Annealing improves the concentration and controlled release of encapsulated ethylene in V-type starch. International Journal of Biological Macromolecules, *141*, 2019, pp. 947–954. DOI: 10.1016/j.ijbiomac.2019.08.244.
18. Niu, B. – Chao, C. – Cai, J. – Yan, Y. – Copeland, L. – Wang, S. – Wang, S.: The effect of NaCl on the formation of starch-lipid complexes. Food Chemistry, *299*, 2019, article 125133. DOI: 10.1016/j.foodchem.2019.125133.
19. Li, X. – Luo, S. – Hou, Y. – Liu, Y. – Hu, X. – Liu, C.: Effect of triglyceride on complexation between starch and fatty acid. International Journal of Biological Macromolecules, *155*, 2019, pp. 1069–1074. DOI: 10.1016/j.ijbiomac.2019.11.072.
20. Kong, L. – Ziegler, G. R.: Molecular encapsulation of ascorbyl palmitate in preformed V-type starch and amylose. Carbohydrate Polymers, *111*, 2014, pp. 256–263. DOI: 10.1016/j.carbpol.2014.04.033.
21. Dries, D. M. – Gomand, S. V. – Goderis, B. – Delcour, J. A .: Structural and thermal transitions during the conversion from native to granular cold-water swelling maize starch. Carbohydrate Polymers, *114*, 2014, pp. 196–205. DOI: 10.1016/j.carbpol.2014.07.066.
22. Zhong, L.: Preparation of V-starch and its application in ethylene encapsulation and controlled release. [Master thesis.] Guang Zhou : South China University of Technology, 2019. <http://cdmd.cnki.com.cn/Article/CDMD-10561-1019620837.htm> In Chinese.
23. Frei, M. – Siddhuraju, P. – Becker, K.: Studies on the in vitro starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. Food Chemistry, *83*, 2003, pp. 395–402. DOI: 10.1016/s0308-8146(03)00101-8.
24. Lee, H. S. – Kim, K. H. – Park, S. H. – Hur, S. W. – Auh, J. H .:Amylose-lipid complex as a fat replacement in the preparation of low-fat white pan bread. Foods, 9, 2020, article 194. DOI: 10.3390/foods9020194.
25. Englyst, H. N. – Kingman, S. M. – Cummings, J. H.: Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition, *46*, 1992, Supplement 2, pp. S33–S50. ISSN: 0954-3007.
26. Loftsson, T. – Brewster, M. E.: Cyclodextrins as functional excipients: methods to enhance complexation efficiency. Journal of Pharmaceutical Sciences, *101*, 2012, pp. 3019–3032. DOI: 10.1002/jps.23077.
27. Kong, L. – Perez-Santos, D .M. – Ziegler, G. R.: Effect of guest structure on amylose-guest inclusion complexation. Food Hydrocolloids, *97*, 2019, pp. 352–361. DOI: 10.1016/j.foodhyd.2019.105188.
28. Chunin, N. – Phooplub, K. – Kaewpet, M. – Wattanasin, P. – Kanatharana, P. – Thavarungkul, P. – Thammakhet-Buranachai, C.: A novel 3D-printed solid phase microextraction device equipped with silver-polyaniline coated pencil lead for the extraction of phthalate esters in cosmeceutical products. Analytica Chimica Acta, *1091*, 2019, pp. 30–39. DOI: 10.1016/j.aca.2019.09.036.
29. Schwier, A. – Mitroo, D. – McNeill, V. F.: Surface

tension depression by low-solubility organic material in aqueous aerosol mimics. Atmospheric Environment, *54*, 2012, pp. 490–495. DOI: 10.1016/j.atmosenv.2012.02.032.
30. Raphaelides, S. N. – Georgiadis, N.: Effect of fatty acids on the rheological behaviour of amylomaize starch dispersions during heating. Food Research International, *41*, 2008, pp. 75–88. DOI: 10.1016/j.foodres.2007.10.004.
31. Richardson, G. – Langton, M. – Bark, A. – Hermansson, A .M.: Wheat starch gelatinization - the effects of sucrose, emulsifier and the physical state of the emulsifier. Starch - Stärke, *55*, 2003, pp. 150–161. DOI: 10.1002/star.200390029.
32. Vasiliadou, E. – Raphaelides, S. N. – Papastergiadis, E.: Effect of heating time and temperature on partially gelatinized starch-fatty acid interactions. LWT – Food Science and Technology, *60*, 2015, pp. 698–707. DOI: 10.1016/j.lwt.2014.10.026.
33. Dankar, I. – Haddarah, A. – Omar, F. E. L. – Pujolà, M. – Sepulcre, F.: Characterization of food additive-potato starch complexes by FTIR and X-ray diffraction. Food Chemistry, *260*, 2018, pp. 7–12. DOI: 10.1016/j.foodchem.2018.03.138.
34. Sun, S. – Hong, Y. – Gu, Z. B. – Cheng, L. – Li, Z. F. – Li, C. M.: An investigation into the structure and digestibility of starch-oleic acid complexes prepared under various complexing temperatures. International Journal of Biological Macromolecules, *138*, 2019, pp. 966–974. DOI: 10.1016/j.ijbiomac.2019.07.166.
35. Lay Ma, U. V. – Floros, J. D. – Ziegler, G. R.: Formation of inclusion complexes of starch with fatty acid esters of bioactive compounds. Carbohydrate Polymers, *83*, 2011, pp. 1869–1878. DOI: 10.1016/j.carbpol.2010.10.055.
36. Wongprayoon, S. – Tran, T. – Gibert, O. – Dubreucq, E. – Piyachomkwan, K. – Sriroth, K.: Pullulanase debranching of various starches upgrades the crystalline structure and thermostability of starch-lauric acid complexes. Starch - Stärke, *70*, 2018, article 1700351. DOI: 10.1002/star.201700351.
37. Godet, M. C. – Bouchet, B. – Colonna, P. – Gallant, D. J. – Buleon, A.: Crystalline amylose-fatty acid complexes: Morphology and crystal thickness. Journal of Food Science, *61*, 1996, pp. 1196–1201. DOI: 10.1111/j.1365-2621.1996.tb10959.x.
38. Le Bail, P. – Bizot, H. – Pontoire, B. – Buléon, A .: Polymorphic transitions of amylose-ethanol crystalline complexes induced by moisture exchanges. Starch - Stärke, *47*, 1995, pp. 229–232. DOI: 10.1002/star.19950470608.

Received 23 May 2021; 1st revised 14 August 2021; accepted 14 September 2021; published online 22 October 2021.