



Effect of ultrasound amplitude on the complexation of green banana starch with two types of fatty acids

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Abstract

This work aimed to investigate the influence of different ultrasound amplitudes (20%, 40%, 60%, 80%, 100%) on the complexation of green Saba banana starch with two types of fatty acids (lauric acid, C12:0 and stearic acid, C18:0). The complex index (CI), swelling power, solubility and *in vitro* digestibility were determined. In general, ultrasonication promoted more starch-fatty acid complexes with shorter chain lauric acid (LA) than stearic acid (SA). Increasing ultrasound amplitudes from 20% to 40% promoted higher CI, but higher amplitudes caused severe starch degradation that reduced the complexing efficiency. Significant correlations were found between CI and swelling power ($r = -0.848$, $p < 0.01$), solubility ($r = -0.754$, $p < 0.01$), rapidly digestible starch ($r = -0.745$, $p < 0.01$) and resistant starch ($r = 0.947$, $p < 0.05$). This study concludes that selecting the optimal amplitude is crucial for successfully complexing green Saba banana starch with fatty acids and reducing its digestibility. While current results demonstrate key functional and digestibility effects, further research is required to evaluate structural and thermal properties to confirm this phenomenon definitively.

Keywords: Digestibility, Green banana starch, Resistant starch, Starch-fatty acid complex, Ultrasonication

1. Introduction

Starch is the main staple carbohydrate for humans. Amylose and amylopectin molecules are two major constituents of starch. Amylose molecules are predominantly linear and significantly smaller than amylopectin with a degree of polymerization (DP) ranging from approximately 1000 to 5000, depending on the source of the starch [1]. The association of excessive intake of starch with several diet-related non-communicable diseases, such as type 2 diabetes, obesity, cardiovascular and cerebrovascular diseases [2] has drawn appreciable research attention to mitigate the digestibility of starch preventing rises in postprandial blood glucose levels [3]. Amylose possesses two unique types of helical structures; a parallel double stranded right-hand helix and a single helix with hydroxyl groups protruded externally creating a hydrophobic inner core [4]. When amylose is dispersed in aquas medium, the water compatible surface permits its existence as single helix. The internal cavity of the helix is readily interacting with hydrophobic ligands such as iodine, fatty acids, peptides, glycerol and so on to form V-type complexes. The amylose-inclusion complexes with fatty acids are referred to as type 5 resistant starch (RS5) which is fermented by the microorganisms in the large intestine to form short-chain fatty acids that offers significant gut health benefits, improves metabolism of cholesterol and fats, improved blood sugar control and insulin sensitivity that are beneficial for managing type 2 diabetes, promotes satiety for effective weight management and reduces inflammation [5]. Other alterations of starch functionalities reported by the formation of starch-fatty acids (SFA) complexes include retarded gelatinisation, delayed retrogradation, decreased paste viscosity, weakened swelling power and reduced solubility [6].

The promising health benefits of RS5 have received considerable growing attempts to promote its formation through interaction of amylose with fatty acids. The formation of SFA complexes including their properties such as the yield, structural order and stability have been reported to be influenced by the characteristics of the fatty acids (lipid types, chain lengths, saturation, configuration of the double bond, form of carboxyl group, etc.) and the characteristics of amylose (chain length, degree of polymerisation, molecular weight, botanical source, crystal form and short-range order, etc.) [6-8]. The degree of unsaturation and chain length of fatty acids are known to influence the formation, structure and functionality of starch-lipid complexes due to differences in hydrophobicity and spatial fit. Unsaturated fatty acids are generally having lower complexation ability than saturated fatty acids due to their steric hindrance and reduced hydrophobicity [9]. Shorter chain lauric acid required lower activation energy to complex with starch as compared to longer chain fatty acids such as stearic acid [10]. Ultrasonication is a cost-effective physical modification method that promotes the SFA complexes by using cavitation to release linear starch chains, thereby enhancing their integration with guest fatty acid molecules. Ultrasonication also improves lipid dispersibility in the gelatinised starch solution to facilitate the complexation process [9]. The extent of influence on formation of SFA complexes by ultrasound is depending on the treatment conditions such as the amplitude or power density, frequency, treatment time, treatment temperature and the application sequence [11-15]. Understanding the interplay between the raw materials for complexation and treatment conditions are essential for successful application of ultrasound technology in promoting SFA complexes.

Driven by the potential of ultrasound, research on ultrasound-assisted SFA complexation has expanded rapidly across diverse botanical sources to induce RS5 formation. Among them include root and tuber starch such as cassava [16], elephant foot yam [17], sweet potato [15], potato [18]; cereal starch such as rice [11], corn [13], wheat [14]; and various other non-conventional starch such as water caltrop [12], arrowhead [19] and faba bean [20]. However, limited work has been reported on banana starch. Recently, Santo *et al.* [21] examined the development of RS5 in green banana flour (*Musa x paradisiaca*) without ultrasonication using virgin coconut oil instead of fatty acids. Another work utilised ultrasound to promote starch-resveratrol complexes in green banana starch (Cavendish subgroup cv. Nan tian huang), which successfully reduced its *in vitro* digestibility [22].

Saba banana (ABB triploid hybrid) originated from the Philippines is widely cultivated in the state of Sabah, Malaysia. The green banana flour contains 68.9% of type 2 resistant starch [23] that offers attractive health benefits. It is commonly used as a cooking banana to prepare various local dishes and snacks. Since starch from different botanical sources have varying granular sizes, crystalline structures, and amylose content, their susceptibility to ultrasound-induced V-type complexes varies. Till date, there is no study reporting ultrasound-induced SFA complexes in green Saba banana starch. This study aimed to investigate how ultrasound amplitude affects the formation of SFA complexes in green Saba banana starch with two types of saturated fatty acids: lauric acid, a medium-chain fatty acid (C12:0) and stearic acid, a long-chain fatty (C18:0). Saturated fatty acids were chosen because they are known to form complexes more efficiently than unsaturated options. We also aimed to examine how chain length of fatty acids impacts RS5 formation during ultrasonication. The alteration of swelling power, solubility, and *in vitro* digestibility of the starch by ultrasonicated-induced fatty acids complexation were determined. This work seeks to identify the optimal ultrasound amplitude that could effectively enhance the SFA complexes in green Saba banana starch.

2. Materials and methods

2.1 Materials

Saba banana (*Musa acuminata* x *Musa balbisiana*) which purchased from a local orchard in Papar, Kota Kinabalu, Sabah, Malaysia. Only matured, unripe bananas with total green peel without any defect were used in the study. Lauric Acid, C12:0 (W261416), Stearic Acid, C18:0 (W303518), pancreatic porcine α -amylase (A3176) and amyloglucosidase (A7095) and invertase (I-4505) were purchased from Sigma-Aldrich (St. Louis, MO). Glucose oxidase peroxidase (GOPOD) assay kit (K-GLUC) from Megazyme International Ireland Ltd. was used. Other chemicals were of reagent grade and distilled water was used.

2.2 Extraction of green banana starch

Extraction of starch from green banana was carried out according to Lee *et al.* [24]. Briefly, the sliced pulp was dried (50 °C, 24 h), milled and sieved (100-mesh). The flour obtained was suspended in 0.05 N NaOH (1:5 ratio), homogenized, and centrifuged. The sediment was washed 4 times with distilled water, with the pH adjusted to 7.0 before the final centrifugation. The purified starch was dried at 50 °C, ground to pass a 200-mesh sieve, and stored in an airtight container until further used.

2.3 Preparation of starch-fatty acid complexes

Preparation of starch-fatty acid (SFA) complexes was according to the method of Wang *et al.* [18] with slight modification. Fatty acid (2% of the starch weight) was pre-dissolved in absolute ethanol and mixed with 60 mL 10% (w/w) starch suspension. The suspension was heated at 60 °C with continuous stirring for 60 min and then subjected to ultrasonication (Qsonica Sonicator Q 500, 500 W, 20kHz equipped with a 12.7 mm diameter titanium probe designed for sample volumes between 20 – 250 mL) for 5 min (with 4 s on and off cycles). The starch suspensions were treated with five amplitudes: 20%, 40%, 60%, 80% and 100%. After ultrasonication, the suspension was immediately cooled down, washed three times with 50% alcohol and centrifuged at 3000 rpm for 15 min. The sediment was dried (50 °C) and ground to powder for further tests. Starch suspension without addition of fatty acid and without ultrasound treatment were used as control of experiment. Starch without addition of fatty acid is denoted as NONFA; whilst starch added with lauric acid (C12:0) and stearic acid (C18:0) are denoted as LA and SA respectively. The figures in the abbreviations (0, 20, 40, 60, 80, 100) represent the amplitudes of ultrasonication used in the treatment.

2.4 Determination of complex index

The complex index (CI) of modified starch was determined according to Raza *et al.* [19] with slight modification. Briefly 2 g of dried sample was dispersed into 20 mL of distilled water and heated for 20 min at 95 °C with continuous mixing. 5 g of starch paste was centrifuged (3000 rpm, 20 min), after that, 500 µL supernatant was mixed with 15 mL distilled water and 2 mL of iodine solution (2.0% KI and 1.3% of I₂ in deionized water). The mixture was thoroughly mixed, and the absorbance (Abs) was measured at 690 nm (Lambda 35 UV/Vis Spectrophotometer, PerkinElmer). The CI value was determined using the following equation:

$$CI (\%) = 100 \times (Abs_{Reference} - Abs_{Complex}) / Abs_{Reference}$$

The absorbance of starch pastes without addition of fatty acid and ultrasonication was used as Abs_{Reference}.

2.5 Swelling power and solubility

Starch dispersions (10 mL, 1% w/w) were heated at 90 °C for 30 min with continuous stirring followed by centrifugation at 1600 rpm for 15 min. Each supernatant was dried at 105 °C to a constant weight. The solubility (%) was calculated as the ratio of the dried supernatant weight to the dry starch weight. While swelling power was calculated as the weight of the centrifuged precipitate (g) per gram of the dry starch [25].

2.7 In vitro digestibility

The method of Ng *et al.* [26] was followed to determine the fractions of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in the samples. Glucose oxidase peroxidase (GOPOD) assay kit was used to measure the glucose content of the enzyme hydrolysed samples.

2.8 Statistical analysis

Triplicate measurements were used for all the measurements. The data were subjected to one-way ANOVA and Duncan's Multiple Range Test ($p < 0.05$) using the SPSS 29.0 software (SPSS, IBM, Chicago, IL, USA). Pearson correlation test was used to quantify the strength and direction of the linear relationship between relevant variables.

3. Results and discussion

3.1 Complex index

Figure 1 shows the complex index (CI) of the untreated and treated starch samples. The complex index indicates the extent to which starch, primarily amylose, has formed inclusion complexes with fatty acids [27]. The NONFA samples show negative values and a declining trend from 20% to 100% ultrasound amplitude (CI values of 0.00% to -31.98%). The calculation of CI is based on comparison of the ability of amylose to bind with iodine between the untreated banana starch and ultrasonicated samples. Since no fatty acid was added in this group of samples, the CI values reflected the complexation with iodine molecules instead. The negative values obtained from the NONFA samples suggested that ultrasonication induced disintegration of swollen starch granules. This process released additional amylose molecules from the inner structure of the starch [28], which then interact with

iodine, resulting in a higher iodine binding capacity than that of native banana starch. The drop in CI values (negative values) is directly related to the effects of ultrasonic cavitation which broke the starch granule structure [29].

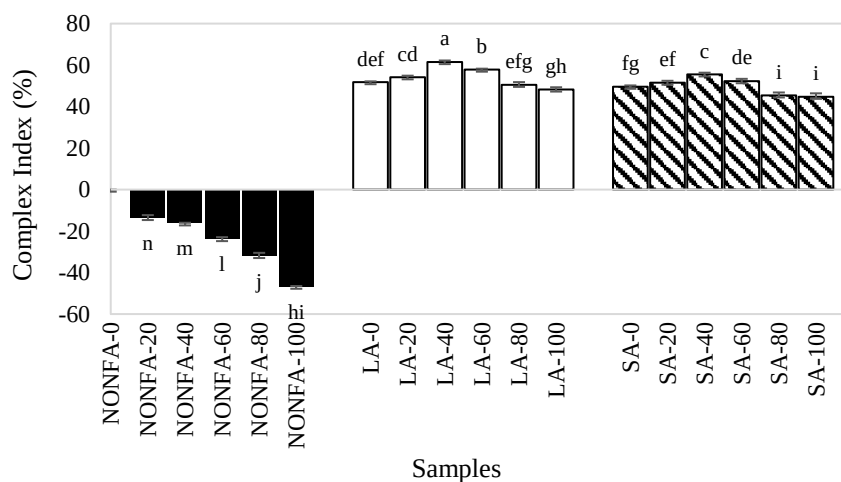


Figure 1 Effect of various ultrasonic amplitudes on the complex index (CI) of green banana starch treated with and without fatty acid. Values shown are means of triplicate (n=3). Values with different alphabet indicate significant differences ($p < 0.05$).

Results obtained show that ultrasound is effective in improving the complexation of green banana starch with both LA and SA. Ultrasound increased the CI from 20% amplitude to the maximum CI value at 40% amplitude (LA-40 and SA-40); but began to drop from 60% to 100% amplitude. This observation suggests that ultrasonication at 20% and 40% amplitude produced more amylose-like linear fragments [30] with appropriate size for inclusion with fatty acids. Nonetheless, higher amplitude from 60% and beyond resulted in excessive amylose molecules damage to the extent of losing their complexation ability with fatty acids. Similar findings were reported by Chumsri *et al.* [11] where ultrasound amplitude higher than 20% was found to decrease the CI value of brown rice-butyric acid complexes. They postulated excessive ultrasonic intensity may have degraded the amylose helical structure or the final complex structure that negatively affected the fatty acid inclusion into the amylose helix. The CI values of both sonicated LA samples and SA samples display similar trends in response to the ultrasound amplitudes, but LA samples generally showed higher CI than SA counterparts. With longer chain length than LA, SA required larger cavities of the amylose helices to fit in, hence it was more difficult to enter the starch spiral structure [31] and lower the chances of forming V-complexes. At the same time, shorter chain fatty acids like SA can be better dispersed within starch molecules for complexation. Whereas longer chain fatty acids have higher tendency to self-aggregate (higher hydrophobicity), reducing the probability of collision with starch molecules [30]. The highest CI for LA-40 and SA-40 were reported to be 61.48% and 55.52%, respectively. Meanwhile, for LA and SA samples without sonication (LA-0 and SA-0), the CI values were 51.79% and 49.58% respectively; suggesting the enhancement of SFA complexes in banana starch by 40% ultrasound amplitude was more effective by using LA (9.69% increase) as opposed to SA (5.94% increase). The CI values for both types of fatty acid complexes were affected by ultrasound amplitude in the descending order of $40\% > 60\% > 20\% \geq 0\% \geq 80\% \geq 100\%$; implying using too high ultrasound amplitude (in this case 80% and beyond) is incapable of enhancing formation of fatty acids complexes in green banana starch.

Two distinctive trends were observed between the effects of ultrasound amplitudes on the complexation with iodine molecules and fatty acids. Increasing ultrasound amplitude up to 100% appeared to promote more complexation with iodine (indicated by the increasing negative values of CI in NONFA samples), but the maximum complexation with fatty acids was found maximum at the medium amplitudes of 40% for both types of fatty acids. The molecular weight of amylose may influence its behavior during guest inclusion [32]. The molecules of iodine are known to be smaller than fatty acids, therefore the smaller amylose molecules produced by severe fragmentation of starch molecules at 80% and 100% ultrasound amplitudes were still able to complex with iodine; these amylose molecules were however, too small and less compatible for complexation of larger fatty acid molecules like LA and SA, hence lowered the CI values of LA-80, LA-100, SA-80 and SA-100. Results suggested that the extent of complex formation is influenced by the dimensional compatibility between the green banana amylose helix and the guest molecules [33], either iodine or fatty acid in this case.

3.2 Swelling power and solubility

The swelling power and solubility of NONFA-0, which is also the native green banana starch (not added with fatty acids and not subjected to ultrasound treatment) is the highest ($p < 0.05$) among all tested samples (Table 1). The ultrasonic cavitation generated shear forces and shock waves that damaged the starch granules by tearing off the microscopic starch particles and breaking polymeric chains; at the same time, creating more mechanical collisions between starch granules and the wall of container to destroy the starch structure [29, 34, 35]. Typical increase in swelling power and solubility of starch by ultrasonication [29] was not observed in this study, rather reduction of swelling power and solubility with increasing ultrasound amplitude in NONFA samples was apparent. Probably the condition used in ultrasonication of this work caused extremely severe starch disruption that destroyed its functionalities. Decrease of swelling power and solubility has been reported for prolonged ultrasonic treatment that induced the erosion of the crystals in the starch granules [36]. Acoustic cavitation during ultrasonication may also cause temperature spikes of starch suspension which could potentially result in premature gelatinization hence alter the swelling power and solubility of the samples. To minimise this effect, 4 s on and off cycles ultrasonication was performed instead of continuous sonication to allow the suspension to cool down between pulses followed by immediate cooling after sonication. The confounding effect of ultrasound-induced heating is neutralized in this experiment by using an identical control group, i.e. NONFA counterparts.

Though without specific trends, SFA complexes (both LA and SA samples) showed lower swelling power and solubility than the NONFA counterparts ($p < 0.05$), except 100% amplitude where lowest swelling power was observed for all the three samples. Significant negative correlation between CI and swelling power ($r = -0.848$, $p < 0.01$) and solubility ($r = -0.754$, $p < 0.01$) were observed. The complexation between amylose and fatty acid molecules caused the starch structure to be more compact and hence inhibited the swelling of starch granules and limited the leaching of starch molecules [30]. Generally, the solubility of SA samples was lower than the LA counterparts, which is aligned with the findings of Hao *et al.* [30] whereby longer chain length fatty acid resulted lower solubility of ultrasonicated maize starch-fatty acid complexes than shorter chain length fatty acid.

Table 1 The swelling power and solubility of banana starch-fatty acids complexes treated with various ultrasonic amplitudes.

Sample	Swelling power (g/g)	Solubility (%)
NONFA-0	15.85 ± 0.70 ^a	15.02 ± 0.62 ^a
NONFA-20	12.05 ± 0.55 ^c	11.92 ± 0.26 ^b
NONFA-40	12.98 ± 0.37 ^b	11.00 ± 0.23 ^{cd}
NONFA-60	12.27 ± 0.48 ^{cd}	11.45 ± 0.23 ^{bc}
NONFA-80	11.24 ± 0.69 ^d	11.05 ± 0.48 ^{cd}
NONFA-100	9.33 ± 0.55 ^{fg}	7.47 ± 0.37 ⁱ
LA-0	8.98 ± 0.11 ^{gh}	9.85 ± 0.12 ^{fg}
LA-20	10.15 ± 0.18 ^{de}	10.83 ± 0.07 ^{cde}
LA-40	7.37 ± 0.36 ⁱ	10.19 ± 0.05 ^{ef}
LA-60	11.22 ± 0.62 ^e	9.70 ± 0.26 ^{fg}
LA-80	10.79 ± 0.30 ^{gh}	11.27 ± 0.05 ^{bc}
LA-100	9.48 ± 0.62 ^{gh}	10.34 ± 0.10 ^{def}
SA-0	8.33 ± 0.30 ^h	9.26 ± 0.17 ^g
SA-20	9.30 ± 0.29 ^{fg}	6.71 ± 0.20 ^j
SA-40	7.47 ± 0.40 ^j	4.39 ± 0.23 ^l
SA-60	9.20 ± 0.67 ^{gh}	5.62 ± 0.33 ^k
SA-80	10.08 ± 0.19 ^{ef}	8.03 ± 0.06 ^{hi}
SA-100	8.72 ± 0.28 ^{gh}	8.57 ± 0.50 ^h

Different superscript alphabet within the same column indicates significant differences ($p < 0.05$).

3.3 In vitro digestibility

The *in vitro* digestibility of the starch was determined by measuring the quantity of RDS, SDS and RS in the samples (Table 2). Without addition of fatty acids, the digestibility of ultrasonicated banana starch was dependent on the amplitude of the ultrasound wave, whereby 20% and 40% amplitude reduced the RDS but increased the RS ($p < 0.05$). Conversely, the starch became more digestible with increasing of RDS and reducing of SDS when the ultrasound amplitude was raised to 60%, 80% and 100%. The decrement of RS is remarkable at 100% amplitude ($p < 0.05$). Significant correlations between ultrasound amplitude with RDS ($r = 0.842$, $p < 0.01$) and SDS ($r = 0.950$, $p < 0.01$) were noticed. The alteration of starch fractions was more prominent at 100% amplitude, in agreement with the findings of swelling power, solubility and thermal behaviour.

Formation of ultrasonicated-induced SFA complexes further lowered starch digestibility by converting RDS to RS, as evident by the significant correlations between CI and %RDS ($r = -0.745$, $p < 0.01$) and %RS ($r = 0.947$, $p < 0.05$). Conforming to higher CI values, the digestibility of LA samples was relatively lower than the SA counterparts. Starch complexation with fatty acids produced more compact structure that restricted enzyme access and slowed down the digestion process [30]. Comparatively, the highest amount of RS was found in LA-40

(57.24%), followed by SA-40 (55.71%) ($p < 0.05$). Ultrasonication induced more than 5% of RS increment in these two samples compared to the counterparts without ultrasound treatment (LA-0 and SA-0). The formation of SFA complexes is known to positively affect the short-range ordered molecular structure of starch, resulting in the increase of RS content which in turn reduces the rate of starch digestibility [37, 38]. Sun et al. [22] also reported increase of more than 2% of RS in green banana starch (Cavendish subgroup cv. Nan tian huang) using ultrasound-assisted complexation with resveratrol.

Table 2 The rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) content of banana starch-fatty acids complexes treated with various ultrasound amplitudes.

Sample	RDS (%)	SDS (%)	RS (%)
NONFA-0	18.54 ± 0.20 ^e	32.44 ± 0.58 ^d	49.02 ± 0.41 ^g
NONFA-20	14.53 ± 0.06 ^{fg}	34.01 ± 0.37 ^c	51.55 ± 0.43 ^e
NONFA-40	14.95 ± 0.61 ^{fg}	30.97 ± 0.23 ^e	54.08 ± 0.38 ^c
NONFA-60	22.24 ± 1.17 ^d	27.60 ± 0.54 ^f	50.16 ± 0.65 ^f
NONFA-80	25.04 ± 1.02 ^c	24.89 ± 0.98 ^g	50.07 ± 0.85 ^f
NONFA-100	29.29 ± 0.29 ^a	22.94 ± 0.41 ^h	47.77 ± 0.15 ^h
LA-0	14.03 ± 0.48 ^g	34.04 ± 0.58 ^{de}	51.93 ± 0.23 ^{de}
LA-20	14.57 ± 0.34 ^{fg}	31.79 ± 0.66 ^{gh}	53.65 ± 0.35 ^c
LA-40	7.80 ± 0.53 ⁱ	34.96 ± 0.79 ^c	57.24 ± 0.70 ^a
LA-60	11.00 ± 1.36 ^h	33.27 ± 0.50 ^{ef}	55.73 ± 0.90 ^b
LA-80	15.76 ± 0.59 ^f	33.07 ± 0.37 ^f	51.17 ± 0.44 ^e
LA-100	26.63 ± 0.66 ^b	23.74 ± 0.49 ^k	49.63 ± 0.55 ^{fg}
SA-0	13.81 ± 0.66 ^g	36.01 ± 0.38 ^b	50.18 ± 0.31 ^f
SA-20	15.67 ± 0.57 ^f	31.91 ± 0.47 ^h	52.42 ± 0.35 ^d
SA-40	6.77 ± 0.98 ^j	37.52 ± 0.57 ^a	55.71 ± 0.70 ^b
SA-60	14.01 ± 0.92 ^g	33.33 ± 0.48 ^{ef}	52.66 ± 0.35 ^d
SA-80	15.69 ± 1.07 ^f	34.29 ± 0.64 ^{cd}	50.02 ± 0.44 ^f
SA-100	26.48 ± 0.66 ^b	25.70 ± 0.24 ⁱ	47.82 ± 0.55 ^h

Different superscript alphabet within the same column indicates significant differences ($p < 0.05$).

4. Conclusion

Results of complex index suggest that ultrasonication is useful to promote complexation of green Saba banana starch with fatty acids in an amplitude-dependent manner. The most effective ultrasound amplitude to induce SFA complexes and lower the digestibility of green banana starch was identified as 40%. Higher amplitude caused severe disintegration of starch, reducing the efficiency of complexing. Ultrasonication-induced SFA complexation caused structural modification of the banana starch, hence the quantity of SFA complexes formed (CI value) showed significant correlations with the swelling power, solubility, %RDS and %RS of the starch. Comparatively, shorter chain LA formed SFA complexes more readily than SA under ultrasound treatment. While these findings provide strong functional and digestibility indicators of ultrasound-induced SFA complexation, direct structural and thermal verification remains necessary. Future studies utilizing X-ray diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FTIR), and thermal characterisation are required to definitively confirm complex formation. Such insights are vital for validating and precise control of ultrasound technology to promote the formation of SFA complexes.

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6. Author Contribution

Conceptualization: JSL; Data curation: JSL, AM; Formal analysis: AM; Investigation: AM; Methodology: JSL, PTC; Project administration: JSL, PTC; Resources: ALH, AAJ; Supervision: JSL; Validation: CKS, AAJ; Visualization: JSL, PTC, AM; Writing – original draft: JSL, AM; Writing – review & editing: JSL, ALH, CKS.

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