Research Paper

Evaluation of Double-modified High Amylose Rice Starch and Applications in Food Models

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Abstract

Rice starch is an important carbohydrate widely used in food and non-food industries. Native rice starch has some drawbacks that prevent direct use. These limitations include poor thermal stability, inadequate gelling properties, a high degree of retrogradation, and syneresis, which exacerbates coagulation/ hardening problems and ultimately results in a reduction in water content. To overcome these limitations, a series of modifications were undertaken to improve the functional characteristics of high-amylose native rice starch through a two-step modification process. Initially, it was subjected to Octenyl Succinic Anhydride (OSA) modification, followed by subsequent cold water granulation and pre-gelatinization. Furthermore, an imitation cheese food model was used to assess the performance of the modified starches. Pre-Gelainized (PG) starch PG-OSA exhibited greater oil/ water absorption capability and improved cold-water solubility compared to both Granular Swelling (GS)-OSA and native OSA. This enhancement was attributed to the fragmentation of its starch granules, leading to increased exposure of hydrophilic and hydrophobic regions. Starch granules of GS-OSA and OSA- native were more intact which restricted the binding capacity. The rheological properties and textural properties of imitation cheese probed the higher elastic properties, melting temperature, and hardness value in the control sample (native OSA starches) followed by PG starches substituted cheese. The gel-sol transition temperature of imitation cheese was used as a quality parameter for the meltability of the cheese. The melting temperature or cross–over temperature (TC) of OSA-native and PG-OSA were higher than GS-OSA. The GS produced more soft cheese compared to PG starch-substituted cheese. These results indicate that the double modification process was performed to produce more tender imitation cheese, highlighting its significance for food processors in achieving the desired quality in imitation cheese production.

Keywords: Cold water granular starch, Imitation cheese, Pre-gelatinized starch, Rheological properties, Rice starch

Introduction

Starch is an important carbohydrate widely used in the food industry as a gelling agent, stabilizer, pickering emulsifier, thickener, and bulking agent. However, native rice starch has functional deficiencies such as solubility, heat tolerance, and texture adhesion that are essential properties of raw materials for food and non-food industrial applications. These drawbacks could be overcome through a modification to obtain desirable chemical, physical, and enzymatic functionalities. The Octenyl Succinic Anhydride (OSA) modification is widely used to impart hydrophobic properties by weakening the internal bonding that keeps granules together (Bhosale & Singhal, 2007). OSA-modified starches are Generally Recognized As Safe (GRAS) for use in food, the upper limiting amount of OSA per weight of starch is 3%, and they are extensively used as food emulsifiers (No *et al*., 2019). In an aqueous medium, the hydroxyl groups of glucose molecules are substituted by OSA groups, resulting in esters with both hydrophilic and hydrophobic molecules, giving them amphiphilic properties (Agama-Acevedo & Bello-Perez, 2017). Although OSA starch exhibits favourable functional properties, it lacks the ability to dissolve in cold-water, a crucial characteristic for numerous food applications. Starch can undergo two modifications known as Pre-gelatinization (PG) and cold-water granular swelling (GS), both of which result in increased solubility in cold water at room temperature.

PG starches are produced by drum drying or extrusion techniques, which cause a grainy texture and are less flexible for food applications such as instant foods due to the disintegration of starch granular structure and the retrogradation of starch gel (Rajagopalan & Seib,1992). However, GS produced viscous, soft, and smoother textures in their gels. Morphologically, PG starch does not have any granular structure, whereas GS possesses a swollen and deformed granular structure, in some cases with Maltese crosses (Li *et al*., 2014; Hedayati *et al*., 2016). The PG and GS are commonly used including heat sensitive produced at low temperatures and instant foods. Chen & Jane (1994) reported that these two types of starch are used to produce cold deserts, pie fillings, gravies, soups, and sauces.

The incorporation of starch into imitation cheese experienced a significant increase. Numerous studies have explored the potential of incorporating various forms of native and modified starch to substitute either protein or fat. The imitation cheese is produced in partial replacement of caseinate by PG starch or modified high amylose starch (Mounsey & O'Riordan, 2008; Noronha *et al*., 2008). Mounsey and O'Riordan (2001) revealed that replacing protein (caseinate) and fat with different starches resulted in reduced meltability, variation in texture, and microstructure of cheese.

The objectives of this study were to investigate the functionality of OSA-modified PG and GS starches, followed by the development of a prototype of an imitation cheese with the substitution of sodium caseinates, to find the influence of OSA-modified PG and GS incorporated imitation cheese on their rheological and textural properties and the influence of type of modified starch and amylose content on the rheological and textural properties. It was hypothesized that GS would produce good emulsifying properties and softer imitation cheese than PG incorporated cheese. The structural components, such as starch, protein, and fat, create the microstructure, which influences the textural, rheological, and melting properties significantly. These findings would be beneficial for the future development of imitation cheese for producers as well as end users.

Materials and Methods

High amylose (27.13%) rice starch was obtained from Watcharewan green farm, Thailand. Sodium caseinate (Excellion EM7, friesland campina) from Friesland company and Sunflower oil (Zonne Bloem) was obtained from Netherlands respectively. Rice grains were milled and stored in a refrigerator at 4 °C.

Rice starch extraction

The milled rice was soaked overnight in a 1:3 ratio of distilled water at room temperature. The cleaned soaked rice was ground using a supermass colloidal machine (Aerosia interpac Co., Ltd, Thailand), producing a starch slurry that was centrifuged by a basket centrifuge (Wasino Co., ltd, Thailand). The rice cake was dried overnight at 45 °C to reach a moisture content of approximately 10-12 %. The dried flour was ground to a fine powder using a laboratory grinder (used 100 mesh sieve). The alkali steeping method was used to extract the starch (Wijesinghe and Gunathilake, 2020). Starch (100 g) was dissolved in a 0.2% NaOH solution (1:4 ratio) overnight with continuous overhead stirring.

The solution was centrifuged at 4000 rpm for 15 minutes and the resulting precipitate was dissolved in the same solution with the same ratio for another 3 hours. The starch slurry was centrifuged after being filtered through cotton wool to remove any starch fiber materials. The upper top brown layer was scrapped out using a spatula to obtain whitish starch. The resultant starch was washed twice with distilled water, neutralized with 0.1 M HCl solution, and washed with distilled water. The starch was dried overnight in a dryer at 45 °C to obtain moisture content of 10-12%. The dry starch was ground and sieved using a 100-mesh sieve and stored in a refrigerator.

Octenyl Succinic Anhydrite -Starch modifications : Starch (dry basis) was dissolved in 1:2 ratio in distilled water, and pH was adjusted to 8.5-9.0 using 1M NaOH. Then OSA at a concentration of 3% based on the dry weight of starch was added stepwise in 4 equal portions at 15 minutes intervals. Throughout the modification process, the pH of the starch solution was maintained using 1M NaOH. The reaction was allowed to run for 6 hours followed by neutralization using 1M HCl. The modified starch solution was washed twice with distilled water and dried overnight at 50 °C. The dried starch was ground and sieved through a 100-mesh sieve, packed, sealed, and stored in a desiccator untill further testing. The prepared sample was coded as high amylose -OSA-native (HA-OSA-N).

Cold water granular swelling (GS) and Pre-gelled (PG) starch preparation : The cold water granular was produced according to Chen & Jane (1994) with slight modification. The OSA-modified starch (10 g db) was suspended in 70 g of 40% alcohol and 27 g of 3M NaOH was slowly added over 15 minutes while stirring continuously. After stirring for 15 minutes, 40% ethanol was added and stirred for another 10 minutes. The solution was then allowed to precipitate for swollen starch granules and supernatant was discarded. The starch was resuspended in 40% ethanol and neutralized with 3M HCl in absolute alcohol. The starch was washed with 60%, 95%, and absolute ethanol followed by drying at 80 °C for 3 hrs. The dried starch was ground and sieved through a 100-mesh sieve and packed and stored in a desiccator. The samples were coded as HA-OAS-GS.

The pre-gelled starch was prepared using a 5% (V/V) starch concentration heated in a water bath at 100 °C for two and half hours. After cooling the starch paste was poured into half of the plastic cups, covered and stored at -20 °C for one day before lyophilizing. The pre-gelled starch was lyophilized using a Freeze dryer (Christ Alpha 2-4 LD plus, Germany) for 4 days. The lyophilized pre-gelled starch was ground, packed and stored in a sealed bag in a desiccator. The samples were coded as HA- OSA-PG.

Degree of substitution : The degree of substitution of modified starch (only native HA-OSA) was measured using the chemical method following a procedure of Song *et al*. (2006). The starch was treated with a few drops of 95% ethanol, followed by mixing with 25 ml of 0.1M HCl for 30 min. The solution was centrifuged at 4000 rpm for 10 min and washed with 25 ml of 95% ethanol, followed by two times with distilled water. The starch pellet was added to 150 ml of distilled water and heated in a water bath at 90 °C for 10 min. The starch paste was cooled rapidly to 25 °C by dipping it in an ice bath and titrating the solution using 0.1 M NaOH solution to reach pH of 8.3. The degree of substitution was calculated using the following equation.

$$DS = \frac{0.162 \times V \times M/W}{1\text{-}(0.210 \times V \times M/W} \qquad (1)$$

Where: V is the titration volume of NaOH solution (ml), M is the molarity of NaOH solution, and W is the dry weight (g) of the OSA starch.

Morphology of starch : The morphology of native, GS, and PG starch particles was visualized using a light microscope (zeissaxioskop 2 plus, Jena, Germany) at × 50 and × 20 magnification, respectively. A starch concentration of 0.1% was used to prepare a starch suspension with milli-Q water. The solution was vortexed for 2 min before the measurements. A drop of starch solution was poured onto the clean glass slide and covered with a coverslip without trapping the air bubbles.

Water solubility : The cold-water solubility was determined following the procedure of Chen *et al*. (2020), with some modifications. Precisely measured distilled water (100 ml) was added to the laboratory blender, and 1 g of starch sample was added when the blender worked at slow speed for 30 sec. Then, the starch mixture was run at high speed for 2 min. The mixture was transferred into a 250 ml centrifuge bottle and centrifuged at 3000 g for 15 min. The supernatant (25 ml) was transferred to a pre-weighted stainless steel moisture evaporating dish. The mixture was dried in an oven at 120 °C until it gets constant weight. The water solubility was determined using the following equation.

$$\text{CWS \%} = \frac{(\text{g of solid in supernatant} \times 4)}{\text{g of sample}} \times 100 \qquad (2)$$

The oil/water absorption of the modified samples was determined following the procedure of Ahmad *et al* (2020), with some modifications. The starch samples (1 g) were mixed with 10 ml of sunflower oil /distilled water separately and continuously stirred for 30 min at room temperature. The slurry was centrifuged (Beckman Coulter, Allerga X-22R) at 3000 g for 10 min. The supernatant was decanted, and centrifuged tubes turned upside down to drain off free water and oil.

$$\text{Oil/ water absoption capacity} \left(\frac{g}{g}\right) = \frac{(m_2 - m_1)}{m_2} \qquad (3)$$

Where: m_2 is the residual weight, m_1 is the dry starch weight

Preparation of imitation cheese and physical properties

The prototype of imitation cheese preparation was done using four main ingredients, *i.e.* Sodium caseinate, water, sunflower oil, and starch. The starch amount requirement was matched with the commercial cheddar cheese using five concentrations of native OSA-modified starch to find out the exact G' of the imitation cheese. Then, based on the starch concentration, imitation cheese was developed using different modified starch. In the production of imitation cheese, sunflower oil was heated at 100 °C for 2 min. Starch, water, and oil were mixed in a Waring blender (Waring 7010BU, USA) to form the emulsion, followed by adding sodium caseinate. The imitation cheese mixture was heated at 80 °C for 10 minutes, followed by vacuuming to remove trapped air. These imitation cheeses were cooled, moulded, sealed, and stored in a refrigerator at 4 °C for 24 hrs before analysis. The imitation cheeses were coded as HA-OSA-N, HA-OSA-PG, and HA-OSA-GS.

Melting property

Based on the method of Balkir *et al*. (2011) with modifications, a cylindrical-shaped piece of cheese was bored (10 × 10 mm) and loaded into a glass tube (30 mm diameter × 200 mm height) and covered with a porous rubber stopper. The sample was kept horizontally in a preheated oven at 180 °C for 10 min. The distance travelled was recorded as an indicator of meltability.

Small Amplitude Oscillatory Shear measurements

The rheology was measured using a stress-controlled rheometer (MCR302, Anton Paar, Graz, Austria). The imitation cheese was bored to a diameter of 25 mm and a thickness of approximately 2 to 3 mm. The dynamic rheology measurements were carried out using a serrated parallel-pate geometry (P25/P2, SN78336) to prevent slippage over the melting of fat. The measurements were performed with constant shear force at 0.25 N throughout the experiment at 25 °C temperature. The gap between the two plates was covered with a low-density silicon oil layer. The LVR was determined using an amplitude sweep test and found that 1-10% strain within the region

The frequency test was performed at 0.1 to 20 Hz at 1% constant strain, followed by an amplitude sweep test from 1 to 1000% at 1 Hz constant frequency at. The dynamic rheological properties of storage modules and viscous modulus were recorded.

Temperature sweep test

A temperature sweep was performed to determine the temperature dependency with a constant strain of 1% and a constant frequency of 1 Hz, starting from 20 °C to 95 °C at the rate of 5 °C/ min. The circle shape cheese (25 mm diameter, 2 mm thickness) was bored and placed on the Peltier plate and the gap was covered with low-density silicon oil followed by covering with a moisture trap to ensure the prevention of moisture evaporation. The dynamic modules of storage and viscous modulus as well as loss tangent were determined. The gel-sol transition (cross-over point) temperature was determined to predict an indicator of meltability or structural stability.

Textural property

The texture parameters of imitation cheese during storage at 4 °C were evaluated using a TA-XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming Surrey, UK). Imitation cheeses were placed at room temperature to equilibrate at 25 °C for 1 hour before analysis. The imitation cheeses were cut in a cylindrical shape using a cheese corer with a diameter and height of 15 mm x 10 mm, respectively. The experiment was conducted using a 5 kg load cell and a P/35 Al probe, with total deformation of 50% at a 2 mm/sec test speed and 5.0 g trigger point load. The textural parameters, including hardness and cohesiveness, were calculated using the software (Texture Expert Excede Version 1.0, Stable Micro System software). All treatments were conducted in triplicates.

Statistical analysis

All results were reported as the mean and standard deviation of three replicates. Analysis of variance (ANOVA) was performed using a completely randomized design. The mean difference was determined by Duncan's Multiple Range Test ($P<0.05$) (IBM SPSS Statistics for Windows, Version 26.0, IBM Corp., NY, USA).

Results and Discussion

Degree of substitution of starch and Morphology of starch

The titration method revealed that HA-OSA-N starch exhibited a Degree of Substitution (DS) of 0.012±0.001. Previous research has indicated that amorphous materials are more readily substituted compared to crystal materials (No *et al*., 2019). These OSA substituted rice starch were further modified into cold water granular and pre-gelled starch.

The microscopic visualization of HA-OSA-N, HA-OSA-GS and HA-OAS-PG starch is shown in Figure 1. The OSA modified native starch appeared in a clear polygonal shape without any deformation (Figure 1-A1). However, it was clear that alcoholic-alkaline treatment produced swollen, deformed starch granules. HA-OSA- GS surfaces were rough with several fissures and wrinkles due to the internal fragmentation and leaching of amylose from starch granules (Chen & Jane, 1994). The starch granule shrinkage resulted in the evaporation of water and alcohol during the drying process (Figure 1-A3). Similar observations were reported for GS maize, sago, potato, and buckwheat starches (Chen & Jane, 1994; Li *et al*., 2014; Singh & Singh 2003). In the PG starch concern, they were produced by heating at 100 °C for two and half hours followed by freeze-drying. The PG starches lost their granular integrity and presented irregular laminar structures (Hedayati *et al*., 2016), implying total gelatinization during heating (Figure 1-A2). This observation was in accordance with the micrographs obtained for PG maize, wheat, and buckwheat starches (Majzoobi *et al*., 2011; Li *et al*., 2014). However, the PG's smooth surface indicated uniform starch molecules packing during freeze-drying.

Oil and water absorption and cold-water solubility

The oil absorption capacity of OSA-native, PG, and GS starches is illustrated in Figure 2A. There were significant differences among the starches. HA-OSA-N starch showed the lowest oil absorption of 0.26±0.03 g of oil/g. HA-OAC-PG starch showed the highest oil absorption of 4.24±0.04 g of oil/g of dry starch. In this study, it was clear that GS showed less oil absorption capacity than PG starch. This difference might be due to PG starch completely separating and fragmenting its starch molecules, which facilitate oil absorbance due to the many active sites.

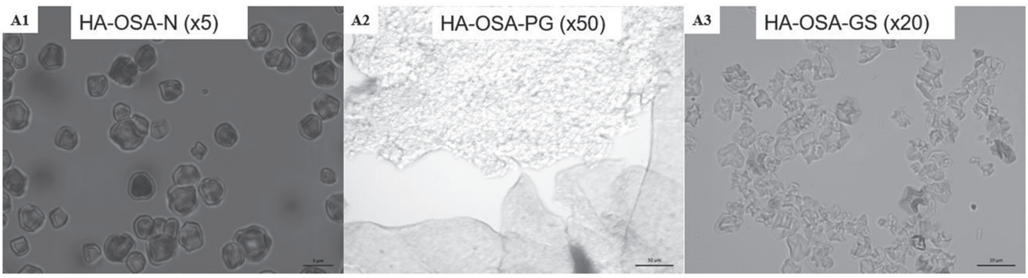

Figure 1. Light microscopic images; (A1) HA-OSA-N, (A2) HA-OSA-PG, (A3) HA-OSA-GS, magnification of x5, x50 and x20, respectively

In GS, an intact starch granule restricted the binding of oil. This proved the PG to be more hydrophobic sites than the GS. HA-OSA-GS oil absorption capacity was lower than the PG, accounting for 1.66±0.05 g oil/g dry starch.

The water absorption capacity of starch relates to its water-holding ability, which is a processing parameter that reflects viscosity (Figure 2B). Significantly higher water absorption was found in PG starches (10.04±0.08) than in their counterpart, GS (8.58±0.00) and HA-OSA-N (3.29±0.14). The PG starches' granular structure has broken down into their molecular components and fragments, which can easily absorb more water and have a higher solubility than the GS. Past investigations indicated that PG starches readily form associations with acid and water molecules through amylose and amylopectin due to the absence of starch granules that could impede these reactions whereas intact granules of GS and native starch hinder such reactions (Montesinos-Herroro *et al*., 2006). However, previous research found that GS starches exhibited higher water absorption than PG starch, in which the size of GS granules increases with the conversion of a double helical structure into a single structure creating a more open structure to absorb more water molecules (Li *et al*., 2014).

The water solubility was significantly different (p=0.05) among all the modified starches. The solubility rankings were in the following order, HA-OSA-PG starch (73.61±0.78), HA-OSA- GS (53.34±1.06) and HA-OSA-N (3.65±0.51) (Figure 2C). The cold-water solubility is influenced by molecular weight, degree of branching, and the ratio of amylose to amylopectin (Singh & Kaur, 2004). During the gelatinization process, complete fragmentation of starch granules occurs, leading to enhanced water absorption and increased soluble components compared to HA-OSA-GS

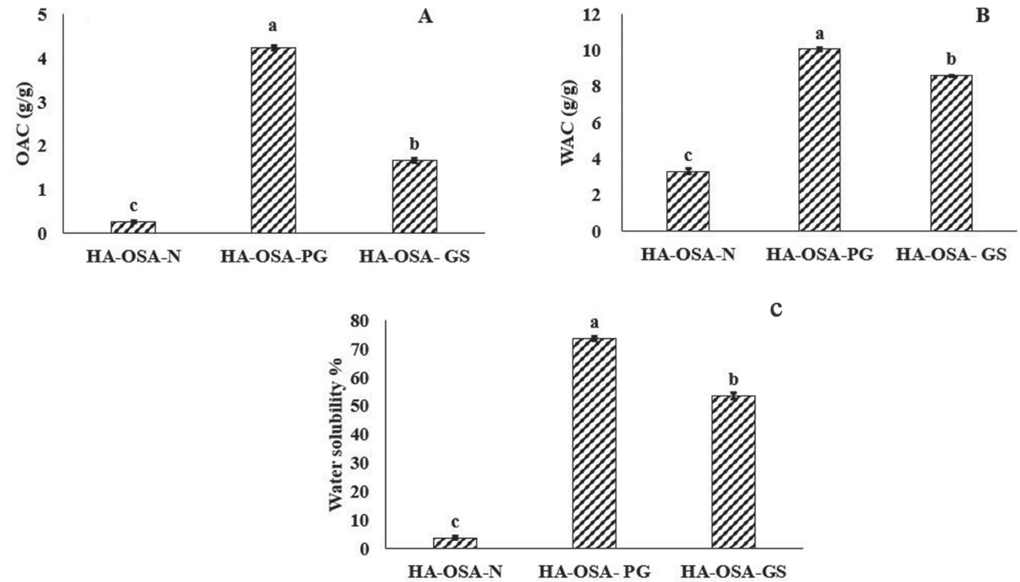

Figure 2. PG and GS starches; (A) oil absorption capacity, (B) water absorption capacity, (C) water solubility

and HA-OSA-N. Previous research found that cold-water solubility is influenced by amylose content (Jivan *et al*., 2014).

Starch concentration and morphology of imitation cheese

The application of PG and GS starch in the food model was performed by producing imitation cheese compared with the control treatment of HA-OSA-N starch. Based on the preliminary studies of HA-OSA-N starch, the G' was matched with commercial cheddar cheese (Figure 3A). Based on that, the starch concentration of 6%, which matched with the G' of the cheddar cheese, was used for the rest of the studies. As other ingredients, sodium caseinate (20%), water (49%), and sunflower oil (25%), were used for imitation cheese production. The appearance of developed imitation cheese shown in figure 3B.

Meltability

Melting behaviour is an important physical property of imitation cheese, which displays its microstructure (Table 1). The starch addition changes the texture of the cheese from soft to brittle and significantly decreases the melting property due to the lack of free water. Moreover, starch disrupted the continuity of the protein matrix (Noronha *et al*., 2008). Mounsey and O'Riordan (2001) observed that starch addition to the imitation cheese identified irregular shape particles as evidence of disruption of the protein matrix. The addition of fat replacers also influences the melting behaviour such as inulin, which improves gelling properties by absorbing free water and increasing flowability (Hennelly *et al*., 2006). In this study, the meltability of imitation cheese differed significantly, with the highest value recorded for HA-OSA- GS (27.5 mm), HA-OSA-PG (25.5 mm), and HA-OSA-N (24.5 mm). These results confirmed that higher flowability or melting behaviour depends on the availability of free water. The meltability of GS is higher than that of PG and OSA-N, possibly due to a greater free moisture content that weakened the internal microstructure of imitation cheese and caused discontinuity and easy flowing (Hennelly *et al*., 2005). The OSA native starch had the least meltability related to less free water due to competing with the protein caseinate. In addition, when heated, the starch granules take more energy to melt than the GS and PG. These results are in agreement with the results of SAOS G' and G'' and temperature sweep measurements.

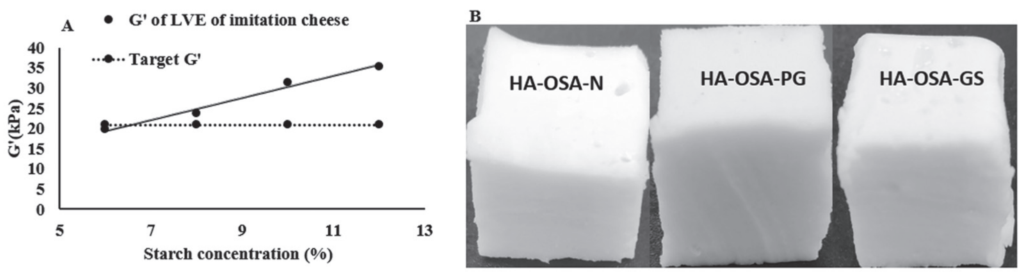

Figure 3. (A) Starch concentration for matching G' with cheddar cheese, (B) Images of casein substituted imitation cheese (IC) with modified starches.

Table 1. Meltability and textural properties of imitation cheese substituted casein by different starch types.

Types of imitation cheese	Meltability (distance mm)	Hardness (kg)	Cohesiveness
HA-OSA-N	24.5±0.1^{b}	0.91±0.02^{a}	0.49±0.12^{a}
HA-OSA-PG	25.5±0.1^{ab}	0.61±0.01^{b}	0.56±0.22^{a}
HA-OSA-GS	27.5±0.1^{a}	0.42±0.13^{c}	0.53±0.01^{a}

$^{a\text{-}c}$ *different letters in the same column indicated significant differences (p < 0.05).*

Small Amplitude Oscillatory Shear (SAOS) measurements of imitation cheese (IC)

The data of dynamic oscillatory measurements represents the internal structure of the sample from the elastic and viscous modulus, G' and G'', respectively (Figure 4). The viscoelastic properties of imitation cheese were measured in SAOS under the Linear Viscoelastic (LVE) region, which involves probing the structure of the sample in the non-destructive stage (Kealy, 2006). All the imitation cheese samples had greater G' than the G'', indicating elastic dominant properties and more energy was stored than dissipated during shearing. Under the frequency sweep measurements (0.1 to 50 Hz), G' increased with the increasing frequency (Figure 4A), indicating frequency dependence and a positive slope. Therefore, imitation cheese behaved as a weak viscoelastic material. All samples did not show a cross-over point between G' and G'', indicating elastic properties with strong bonds in the imitation cheeses (Sharma *et al*., 2016). Tan δ (G''/G') of the imitation cheese below 1, indicating all the samples dominated more solid-like behaviour over viscous behaviour.

According to the amplitude sweep test (Figure 4B), the highest G' (kpa) was recorded in HA-OSA-N starch (20.08±0.64 kpa) substituted imitation cheese, followed by HA-OSA-PG (17.67±0.67 kpa) and HA-OSA-GS (13.70±0.39 kpa). The lowest G' was recorded in GS, where the water absorbed by the intact granules makes a weaker structure and prevents the hydration of caseinate protein. This condition might create a discontinuity in the protein matrix and a weaker gel structure. HA-OSA-N had the highest G', which is related to the higher amylose content, and retained the integrity of swollen starch granules more than others.

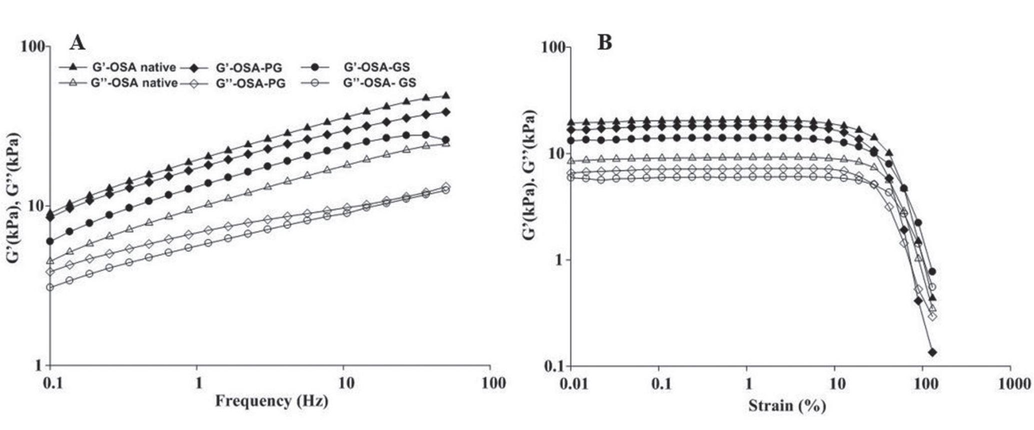

Figure 4. SAOS measurements, (A) Frequency sweep, (B) Amplitude Sweep

Amylose molecules developed a three-dimensional network as a filler in the imitation cheese, and retrogradation also influenced the rigid texture (Biliaderis, 2009). In this study, OSA native showed the highest elastic properties, followed by PG starches and GS starch. This might be due to the present of higher intact starch granules in native starch than the other two modified starches. PG starches displayed higher elasticity than GS, this might be due to higher molecular interactions, which resulted in a rigid structure.

Temperature sweep test

The rheological study is important to provide valuable information relating to the temperature induced changes in the viscoelastic imitation cheese that can be worth enough for the producer as well as the end-user. The moduli G' and G'' decreased with increasing temperature from 20 to 95 °C at the rate of 5 °C per minute (Figure 5A), showing that the matrix began to melt or soften. This agrees with the previous studys' results of similar work on natural cheese (Rosengerg *et al*., 1995) and imitation cheese (Mounsey & O'Riordan, 1999). The temperature at which tan δ is equal to 1 (G'=G''/ cross-over point), has been considered an indicator of the melting point of imitation cheese (Mounsey & O'Riordan, 1999; Montesinos-Herrero *et al*., 2006; Noronha *et al*., 2007; Ye & Herwitt, 2009). The gel-sol transition temperature of imitation cheese is used as a quality parameter for the meltability of imitation cheese (Guggisberg *et al*., 2007). When imitation cheese is heated to a higher temperature, the bonds become weaker drastically, which is indicated by a decline of G' and G'' or an increase of tan δ.

This condition is known to be the viscous nature of cheese at the higher temperature (Figures 5 A-C). In this study, the decrease of G' and G'' of the imitation cheese over the temperature range observed at 20-40 °C (slow decrease) and 40 -65 °C (rapid decrease) corresponded with the melting of fat globules and protein matrix, respectively (Butt *et al*., 2020) The reasons for this incidence would be due to the melting of the vegetable fat, weakened protein-protein interactions, and softening of the protein matrix. The G' of all products considerably dropped as the measurement temperature increased from 20 °C to 95 °C (Mounsey & O'Riordan, 1999, 2001).

According to the findings of this study, the G' and G'' were highest in HA-OSA-N, which could be attributed to improper starch solubility with insufficient water or incomplete gelatinization due to the time-temperature effect (Mounsey & O'Riordan, 2001). The melting temperature (cross-over temperature [Tc]) of the HA-OSA-N was 53.9±1.4 °C. The HA-OSA suggests the interference of starch granules with the continuous protein matrix (Butt *et al*., 2019). In addition, there is no free water to plasticize the imitation cheese microstructure, imitation cheese made with native starch melts at a noticeably higher temperature (Mcmahon *et al*., 1996). Previous research found that imitation cheese containing native corn starch presents smaller oil droplets than non starch. These fat globules, and protein form a tight, continuous matrix in imitation cheese (Mounsey & O'Riordan, 2001).

PG starch-substituted imitation cheese displayed the second highest melting temperature and both moduli were higher than the GS starches. The Tc was highest in HA-OSA-PG (49.1±0.0 °C), followed by HA-OSA-GS (45.3±0.0 °C). The Tc of GS is less than that of PG, possibly due to a greater free moisture content that weakened the internal microstructure of imitation cheese and caused discontinuity and easy flow (Hennelly *et al*., 2005). In GS, intact starch granules with fragmented internal structures result in more spaces to retain water and a weaker structure which leads to the plasticizing of the protein molecules during heating, resulting in lower G' compared to PG and OSA native starch. This agrees with the explanation of Montesinos-Herrero *et al*. (2006), which status that bound water reduces the available water to plastizise the matrix with higher G' and G'' compared to the GS starch (Montesinos-Herrero *et al*., 2006). The PG starch exhibited higher oil absorption, water solubility, and water absorption capacity (Figure 2) compared to the GS starch. This can be attributed to the absence of free water, resulting in the formation of a rigid microstructure. The OSA native starch added imitation cheese had a lower loss tangent at 20 °C than the PG and GS starch (Figure 5C), showing higher elastic behaviour which is related to presence of intact starch granules. The HA-OSA-PG starch-added imitation cheese had a second lowest loss tangent at 20 °C than the GS starch (Figure 5C), showing higher elastic behaviour related to better fat emulsification and better water molecules distribution due to high hydrophilicity.

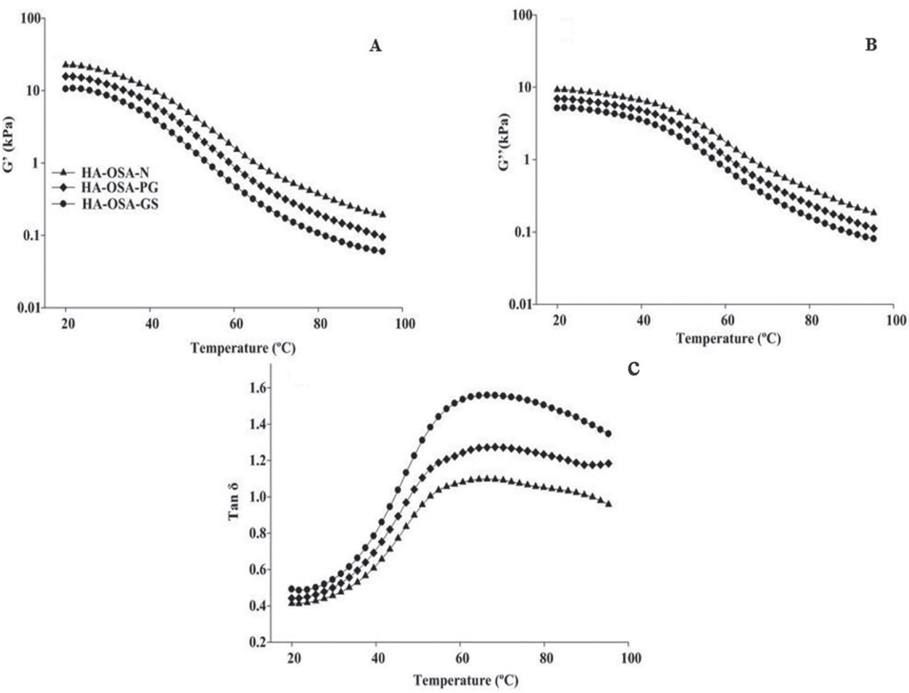

Figure 5. Temperature sweep of imitation cheese; (A) Storage modulus, (B) Loss modulus, (C) Loss tangent

All imitation cheese displayed a lower tangent value below 1 at 20 °C, representing elastic dominant properties. The HA-OSA-N sample showed lower tan δ at 20 °C as compared with PG and GS. The tan δ value depends on the starch properties substituted such as retrogradation, emulsifying properties, and dehydration of the protein network, as reported by Sharma *et al*., 2016. Increasing temperatures from 20 to 95 °C, resulted in the melting of imitation cheese and an increase in tan δ was observed. All the imitation cheese, except HA-OSA-N, lost tangent started to increase faster at a temperature of 31 °C whereas HA-OSA-N started at 37 °C. This difference might be due to the starch granule's higher in amylose content. At the above temperatures, the imitation cheese started melt, melting the fat globules of the matrix. HA-OSA-N reached a maximum tan δ value of 1.10±0.04 at 66.41°C, whereas the maximum tan δ values were for HA-OSA-PG (1.27±0.01 at 68.33 °C), and HA-OSA-GS (1.56±0.04 at 66.41 °C), respectively. These results suggested that the weak microstructure of GS creates more flowability than other imitation cheeses. After reaching a maximum tan δ, all imitation cheeses showed a levelling off and a slight decline. This behaviour was previously recorded by Mounsey and O'Riordan, (2001) and Butt *et al*. (2019). Imitation cheese melting is a technical process in which fat melting and mobilization of protein moieties occur which causes a reduction of the casein-casein interaction at the lower temperature.

Textural property of imitation cheese

The data measured by the texture analyser are presented in Table 1. Results revealed that there are significant differences ($p<0.05$) among the tested samples in hardness and cohesiveness. One of the major components is substituted with starch, resulting in changes in their properties. Imitation cheese substituted with HA-OSA-N starch had the highest hardness value of 0.91±0.0 kg, followed by HA-OSA-PG (0.61±0.0 kg) and HA-OSA-GS (0.42±0.1 kg) (Table 1). During the storage of imitation cheese, starches with a high amylose-to-amylopectin ratio tend to retrograde and gel more quickly than those with lower amylose content (Guinee *et al*., 2004). It is revealed from the results that GS and PG starches containing imitation cheese samples were softer than the control ones (HA- OSA-N). The findings of this study are consistent with the previous findings that PG-incorporated imitation cheese had lower hardness than the control (Mounsey & O'Riordan, 2008). This might be due to the softening effect created by a less hydrated protein matrix and the impairment of the melting of large fat globules (Mounsey & O'Riordan, 2008). Figures 4A, 4B and 5A showed that a higher G' of OSA native starch substituted imitation cheese, suggesting a harder structure requiring greater energy to deform (Rinaldoni *et al*., 2014). PG starch binds water molecules more tightly than GS because of its granular structure, which produces a tighter matrix and results in a rigid or harder structure (Noronha *et al*., 2008).

The degree of cohesion reveals how strong the cheese's matrix internal bonds are. In earlier studies, it was confirmed that imitation cheese containing rice starches was softer; hence, less stress was developed, and ultimately the product resisted fracture, resulting in high cohesiveness as compared to the control (Mounsey & O'Riordan, 2008). This study confirms previous findings that no fracture point was found. However, higher cohesiveness was reported in PG and GS starches than in OSA native, indicating that double modified OSA starch contained more cohesive force.

Conclusions

The morphological and functional characteristics of pre-gelled and cold-water granular starch differ significantly from those of OSA native starch. PG starches exhibit higher OAC, WAC, and water solubility compared to GS and OSA native starches, indicating their greater amphiphilic nature The substitution of casein partially with modified high amylose groups of rice starches revealed that the imitation cheeses have changed their rheological and textural properties. Imitation cheeses with HA-OSA-N starch substitution display elevated G' and G'', melting temperature, and hardness compared to other modified starches. This suggests that OSA native starch is well-suited for producing hard thermoplastic cheese. Conversely, PG starches exhibit higher cohesiveness, moderate hardness values, and modulus, making them suitable for producing semi-hard imitation cheeses. The cold-water-soluble starch can be used to produce more soft cheese.

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