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Characterization of ternary blends of water chestnut starch, amino acids and fatty acids for their physicochemical properties

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Abstract

The influence of incorporating amino acids and fatty acids into water chestnut starch (WS) to form ternary blends was examined in this research. The effects of methionine (Met), histidine (His), oleic acid (C-18:1), and linolenic acid (C-18:3) on the physicochemical characteristics of WS were assessed. The ternary blends containing amino and fatty acids showed reduced swelling power, solubility index and paste clarity, but enhanced freeze-thaw stability. The complex index % indicated the highest affinity for blends of methionine and oleic acid with WS. This study underscores how various amino and fatty acids, along with their interactions with WS to form complexes, affect starch properties. These findings have significant implications for the application of ternary blends in food science and related fields, offering invaluable insights that could drive innovation and optimization in the development of novel food products with enhanced functional properties and stability.

Keywords: Ternary blends, Water chestnut starch, amino acids and fatty acids, Physicochemical properties

Introduction

The interplay among the various components found in plant-based foods, including starch, proteins, and lipids, plays a crucial role in shaping the quality characteristics of the final food products. These interactions are pivotal during food processing, influencing attributes like flavor, texture, mouth-feel, and digestibility, all of which are significant for human nutrition and health (Mariscal-Moreno *et al.*, 2019 [14]; Qin *et al.*, 2019) [16]. For more than a half-century, researchers have focused on complexes generated by starch and lipids, but the study of starch-lipid-protein complexes or ternary blends is a relatively new domain with little insights acquired so far. The development of these complexes has the potential to significantly affect the physicochemical and functional properties of finished food products in terms of flavour, texture, and shelf life (Wang *et al.*, 2020) [26]. Lipids, including fatty acids, lysophospholipids, and monoglycerides, primarily associate with the amylose component of starch, forming complexes. These natural amylose-lipid complexes are present in native starch and are also generated during food processing, particularly during the cooling phase (Godet *et al.*, 1995) [5]. The development of starch-lipid complexes modifies the functional characteristics of the starch by reducing swelling capacity (Li *et al.*, 2019) [11], raising gelatinization temperature, delaying retrogradation, and decreasing susceptibility to enzymatic hydrolysis. Proteins may also interact with lipids through covalent bonding, hydrophobic interactions, electrostatic interactions, and hydrogen bonds (Sponton *et al.*, 2014) [20]. Certain proteins have lipid-binding sites that are particular (Liu *et al.*, 2017) [12]. Additionally, protein, lipids, and starch can interact under certain circumstances to form ternary complexes (Zhang & Hamaker, 2003 [29]; Zhang *et al.*, 2003) [30].

Previous studies have shown that fatty acids can form complexes with starch and protein Compared to the equivalent starch-lipid complexes, these novel complexes show more short-range molecular order and a higher level of relative crystallinity (Wang *et al.*, 2017 [27]; Zheng *et al.*, 2018) [31]. Furthermore, in comparison to starch-lipid blends, these new complexes exhibit unique functional characteristics such higher paste viscosity, lower gel strength, and lower *in vitro* enzymatic digestibility (Wang *et al.*, 2020; Wang *et al.*, 2017 [27]; Zhang & Hamaker, 2003 [29, 30]; Zheng *et al.*, 2018) [31]. Starch is generally believed to have a preference for interacting with lipids, leading to the creation of starch-lipid complexes. These complexes then bind with proteins to form starch-lipid-protein complexes. It has been suggested that negatively charged fatty acids serve as the link between starch and protein. Numerous studies have concentrated on examining how the sequence of adding fatty acids to starch/protein systems influences the creation of starch-amino-fatty acid complexes, aiming to understand the impact of interactions between starch and lipids (Chao *et al.*, 2018 [4]; Wang *et al.*, 2017 [27]; Zheng *et al.*, 2018) [31]. Proteins as well as starch can independently engage in lipid interactions, as indicated by previous research findings suggesting that the interplay among lipid and protein influences the development of starch-lipid-protein complexes. Lipid structure as well as the nature of proteins/amino acids has a major impact on how starch-lipid-protein complexes are formed. Shorter chain fatty acids and fewer unsaturated bonds tend to promote the ternary complex formation. Conversely, as the length of the fatty acid chain increases, the thermal stability of these complexes rises, while greater unsaturation decreases stability (Zheng *et al.*, 2018) [31]. Regarding amino acids, charged ones can influence starch by altering its overall charge. Additionally, neutral amino acids can impact starch by weakly interacting with its molecules, through less noticeable electrical forces (Xu *et al.*, 2022) [28]. However, the exploration of starch-amino-fatty acid interactions or ternary complexes is relatively recent and lacks in-depth understanding. This area requires further investigation to provide detailed insights into the mechanisms at play. Hence, the objective of this research was to investigate the impact of amino acids (Methionine and Histidine) and fatty acids (Oleic and Linolenic) on the physicochemical attributes of water chestnut starch (WS). The primary aim was to enhance our understanding of how the interactions between amino and fatty acids shape the physicochemical properties of WS.

Materials and methods

1. Material

The fresh tubers of water chestnut (*Trapanatans* L. var. *Bispinosaroxburgh*) were procured from the local market in Rohtak (India). Two essential fatty acids, namely oleic acid (MUFA, C-18:1) and linolenic acid (PUFA, C-18:3), along with two essential amino acids, methionine (Met) and histidine (His), with a high purity level ≥ 99% were procured from Sigma-Aldrich (3050 Spruce Street, St. Louis, USA). All the other chemicals and reagents used in the analysis were of analytical grade.

2. Isolation and chemical analysis of water chestnut starch (WS)

The method of Singh *et al*. (2009) [18] was used to separate the starch from water chestnut fruit. The moisture, protein, fat, and ash content of the isolated starch were determined using the standard methods of AACC (2000) [1]. The method of Hoover & Ratnayake (2001) [6] was used to calculate total and apparent amylose content of isolated starch.

3. Preparation of ternary blends composed of starch-amino-fatty acid

The ternary blends were prepared using the method of Zang & Hamaker (2003) [29] with slight modifications. Starch (2.00 g), fatty acids (100 mg), and amino acids (200 mg) were used for the preparation of ternary blends. The heating cycle involved heating of starch slurry at 50˚C for 1 min followed by ramping of temperature to 95°C during a period of 3 min 42s and holding at 95°C for 2 min. Following a 2 min period, the temperature was permitted to decrease to 50° C in an additional 3 min and 48 sec period, staying there for a 2 min period while the device rotated continuously at 160 rpm. The sample pastes were freeze-dried, grounded, sieved and stored for further analysis. The blends prepared were denoted as WS+Met+Oleic, WS+Met+Linolenic, WS+His+Oleic, and WS+His+Linolenic.

4. Water and oil absorption capacity (WAC and OAC)

The water absorption capacity (WAC) and oil absorption capacity (OAC) of ternary blends was determined using the method of Sosulski *et al*. (1976) [19] with few modifications.

$$\text{Water absorption capacity (g/g)} = \frac{Weight\ of\ wet\ sediment\ \ (g)}{Dry\ weight\ of\ starch(g)} \quad (1)$$

$$\text{Oil absorption capacity (g/g)} = \frac{Weight\ of\ wet\ precipitate\ \ (g)}{Dry\ weight\ of\ starch(g)} \quad (2)$$

5. Complex index (CI %)

CI% was used to calculate the extent of starch-amino-lipid complex formation. The Wang *et al*. (2018) [24] method was used to assess the CI of ternary blends. Compared to native starch, the technique measured the amount of iodine bound to the amylose part of starch-amino-fatty acid blends (Meng *et al*., 2014) [15]. The CI was determined using the following equation-

$$\text{CI (\%)} = \frac{Abs_{Ref} - Abs_{ternary\ \ blends}}{Abs_{Ref}} \times 100 \quad (3)$$

Where, Abs_{Ref} is the absorbance of native starch solution, $Abs_{ternary\ blends}$ is the absorbance of the ternary blends.

6. Swelling power and solubility index (SP and SI)

Using the Wang & Copeland (2012) [25] method, the SP and SI of ternary blends were determined in response to a temperature increase from 55 to 95°C with an interval of 10°C increment. The SP and SI were calculated using the subsequent equation:

$$\text{Swelling capacity (g/g)} = \frac{Weight\ of\ swollen\ \ granules}{Dry\ weight\ of\ a\ \ sample} \quad (4)$$

$$\text{Solubility index (g/g)} = \frac{Weight\ of\ solubles}{Dry\ weight\ of\ a\ sample} \quad (5)$$

7. Freeze-thaw stability

The samples were assessed for freeze-thaw stability using the method described by Lutfi *et al*. (2017) [13] with minor changes. With constant stirring, the sample solution (6% w/v) of ternary blends was heated to 95°C for 20 minutes. The gel was formed and stored at -18°C for 24 hours. It was then thawed for 6 hours at 30°C. The percentage of syneresis was determined by weighing the gel before and after thawing at a specific time, and comparing the weights in terms of the amount of water that exuded, which was measured by centrifugation for 10 min at 3000×g. Five freeze-thawing cycles were used to evaluate the stability of the freeze-thaw process.

8. Paste clarity

With a few minor modifications, the method of Waliszewski *et al*. (2003) [23] was used to assess the paste clarity of the samples. The 4% w/w sample suspensions of ternary blends were heated to 95°C for 30 min while being shaken intermittently. After that, they were cooled to room temperature and stored for five days at 30°C and 6°C, respectively. With a PC-based double beam spectrophotometer 2202 (Systronics India, New Delhi), the % transmittance was determined at 650 nm at 24h intervals.

9. Statistical analysis

The results were expressed as the mean ± standard deviation (SD), with each measurement being made in triplicate. An analysis of variance (one-way ANOVA) and a Tukey's HSD test ($p<0.05$) were conducted using SPSS (version 19.0).

Results and discussion

1. Physicochemical characteristics

The findings presented in Table 1 demonstrate that the incorporation of specific amino acids and fatty acids led to a notable reduction in the WAC of WS, with statistical significance ($p < 0.05$). This change in WAC of WS on account of addition of fatty acids and amino acids can be attributed to the formation of ternary blends that comprised interaction of the non-polar end of fatty acids with starch that form inclusion compounds of starch and fatty acids, while the negatively-charged carboxyl moiety of the fatty acid is believed to engage with amino acid, resulting in the formation of a ternary complex involving starch, amino acid, and fatty acid (Bhopatkar *et al*., 2015 [3]; Chao *et al*., 2018) [4]. The OBC of WS after the addition of amino acids and fatty acids also showed a declining trend. This can be explained by the high CI % between amino and fatty acids with starch that resulted in the formation of more rigid globular structure. As the value of percent CI increased, the ability of starch to bind with oil diminished owing to the development of starch/amino acid/fatty acid blends (Kaur & Singh, 2000) [8].

The WS+Met+Oleic blend exhibited the highest complex index (CI %) at 58.10%, indicating a substantial propensity for forming complexes (Table 1). Conversely, the WS+His+Linolenic (32.39%) blend demonstrated the lowest CI%. The high number of double bonds in linolenic acid hindered its ability to penetrate the helical structure of starch, thereby decreasing the likelihood of complex formation and resulted in a lower CI % (Kong & Ziegler, 2014) [9]. The blend of WS+Met+Oleic acid formed stable complexes with amylose. This stability was attributed to the presence of a single unsaturated bond in oleic acid, which enhances the compatibility and interaction between the components, thereby increasing the CI% (Kro'likowska *et al*., 2022) [10]. The single double bond in oleic acid created a more flexible molecular structure compared to linolenic acid, facilitating its incorporation into the starch helix and promoted more extensive complex formation. This increased flexibility and compatibility in oleic acid's molecular structure allowed it to interact more effectively with both amylose and the amino acid in the blend, leading to a higher degree of complexation.

Table 1: The WAC, OAC and complex index of ternary blends.

Samples	WAC	OAC	Complex index (%)
WS	2.96±0.90^{b}	2.82±0.47^{b}	Reference
WS+Met+Oleic	1.13±0.43^{a}	1.08±0.02^{a}	58.10±1.57^{c}
WS+Met+Linolenic	1.29±0.35^{a}	1.10±0.14^{a}	47.12±2.03^{b}
WS+His+Oleic	2.62±0.56^{b}	2.40±0.17^{b}	44.10±1.86^{b}
WS+His+Linolenic	2.75±0.43^{b}	2.22±0.10^{b}	32.39±2.66^{a}

The values are expressed as the mean ±SD of three independent determinations.

Values in the same column with different superscripts are significantly different ($p<0.05$).

Where, Where, WS= Water chestnut starch, S+Met+Oleic= Starch-Methionine-Oleic, WS= Water chestnut starch, WS+Met+Oleic= Water chestnut starch-Methionine-Oleic, WS+Met+Linolenic= Water chestnut starch -Methionine-Linolenic, WS+His+Oleic= Water chestnut starch -Histidine-Oleic, WS+His+Linolenic= Water chestnut starch -Histidine-Linolenic, WAC=Water absorption capacity and OAC= Oil absorption capacity.

2. Swelling power and solubility index (SP and SI)

The addition of fatty acids and amino acids significantly ($p<0.05$) decreased the SP of WS (Fig.1a). The introduction of ternary blends significantly impacted the osmotic flow of water into starch granules, resulting to a drop in SP of WS. Among the blends examined, WS+Met+Oleic showed a more pronounced decrease in SP compared to others. This could be attributed to various interactions such as hydrogen bonding and hydrophobic interactions that disrupted the potential of starch molecules to effectively imbibe water during gelatinization. As a result, competition for available water molecules between starch and the amino/fatty acids limited the swelling of starch granules (Xu *et al*., 2022) [28]. The non-polar and hydrophobic nature of methionine, blended with the single unsaturated bond in oleic acid, contributed to the reduction in SP of WS. This effect was also observed in our study on binary blends containing these specific amino (Attri *et al*., 2024) [2] and fatty acids. The hydrophobic side chain of methionine interacted favorably with the non-polar regions of starch, thus decreasing the ability of starch to absorb water and swell (Attri *et al*., 2024) [2]. Similarly, the presence of a single double bond in oleic acid facilitated its incorporation into the starch matrix, enhancing hydrophobic interactions (Tang & Copeland, 2007) [21] and further limited water uptake. These interactions collectively resulted in a more compact and less hydrated starch structure, thereby reducing the swelling power of the starch. The combination of these molecular characteristics in methionine and oleic acid influenced the hydration properties of WS, ultimately leading to a decrease in its ability to swell in the presence of water. Furthermore, the molecules presented in ternary blends might have hindered the movement of water molecules into the starch granules due to steric hindrance (Scott & Awika, 2023) [17]. This obstruction restricted hydration and subsequent swelling of starch, contributing to a reduced swelling power. Additionally, as the temperature rose (from 55 to 95°C), the SP of WS and ternary blends increased dramatically, reaching at 19.21 g/g for native WS, 8.16 g/g for WS+Met+Oleic blend, and 11.21 g/g for WS+Met+Linolenic blend.

A substantial increase in SP of WS occurred during the temperature sweep from 55 to 65°C succeeded by a further noteworthy increase in SP during temperature sweep from 85 to 95°C. Nevertheless, with ternary blends, the SP elevated sharply over a temperature interval from 55 to 75°C. Additionally, introducing could have induced structural changes within the starch gel network, resulting in a more compact and less porous structure that was less susceptible to swelling as indicated by the observations of MD simulations study. Furthermore, the formation of ternary component blends could additionally affected the hydration kinetics and swelling behavior of the starch granules (Zhang & Hamaker, 2003) [29]. In line with SP, the SI of WS also exhibited a decreasing pattern when ternary blends were introduced (Fig.1b). The interaction among starch, amino acids, and fatty acids formed complexes that impeded the dispersal of starch molecules in water, resulting in reduced solubility. These findings aligned with earlier research on sorghum starch combined with whey protein isolate/palmitic/oleic/linoleic acid (Zhang & Hamaker, 2003) [29] where a decrease in starch solubility following the addition of these components was noted.

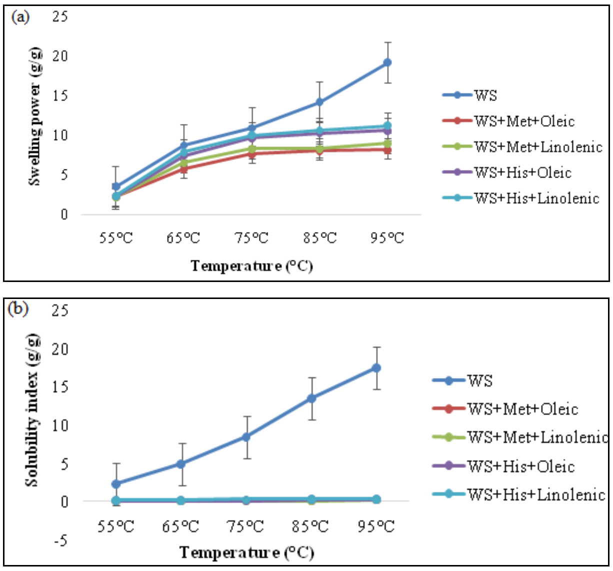

Fig 1: **(a)** Swelling power, and **(b)** solubility index of ternary blends.

3. Freeze-thaw stability

The stability of starch in freeze-thawing holds significance in formulating frozen and refrigerated foods. During the cycle of freezing and thawing, minimizing water exudation is crucial for enhancing the freeze-thaw stability of starch. Therefore, assessing water exudation serves as a valuable indicator for evaluating starch freeze-thaw stability (Li *et al.*, 2019) [11]. The results demonstrated a gradual decline in water exudation from starch gels as time progressed (Fig.2a). This suggested an increase in the stability of WS gel with the passage of time. The incorporation of ternary blends caused a decline in the amount of % syneresis, thereby leading to a greater stability in the gels. This improvement can be attributed to the creation of complexes between starch/fatty acid/amino acid molecules, reinforcing the starch structure and reducing water exudation during freezing and thawing. The restricted availability of water, as evidenced in our study, resulted in diminished absorption, thereby causing the development of smaller crystals of ice during the process of freezing (Jiménez *et al.*, 2012) [7]. These smaller ice crystals were less likely to cause structural damage to the starch matrix upon thawing, thereby improving overall stability. Additionally, the decreased swelling of starch granules and reduced water content resulting from complex formation mitigate % syneresis – the expulsion of water from the gel structure upon thawing. This helps the starch-based system retain its moisture content and structural integrity, resulting in reduced syneresis and improved stability (Li *et al.*, 2019) [11]. Moreover, complex formation aids in maintaining the structural integrity of the starch matrix during freeze-thaw cycles by minimizing excessive expansion and contraction of starch granules. This contributes to the blends ability to withstand freeze-thaw stress without significant damage.

4. Paste clarity

Incorporating fatty acids and amino acids resulted in a decrease % transmittance of native WS, leading to a negative effect on paste clarity (Fig.2b and 2c). Fig. 2b showed that initially, the paste clarity values for WS were significantly higher than those observed in the ternary blends up to 48 hours at 6°C. However, after 48 h, the paste clarity values of WS began to deteriorate drastically, mirroring the trend observed in the ternary blends. On the other hand, Fig.2c illustrated the % transmittance at 30°C which demonstrated a consistent decline in paste clarity with ternary blends similar to Fig.2c. However, it was more apparent at this elevated temperature that the presence of ternary blends noticeably impacted paste transparency. The interactions among the components of ternary blends led to a reduction in the swelling behavior of starch granules, as evidenced by our research findings. The presence of intact granules or aggregates within the gel matrix exacerbated light scattering, contributing to increased opacity or cloudiness of the gel and further compromising its clarity and transmittance (Li *et al.*, 2019) [11]. In essence, the decreased % transmittance of WS reflected the intricate interplay of molecular interactions and structural modifications induced in ternary blends during gelatinization, highlighting the complex nature of starch gel behavior. These results suggested that paste clarity was impacted by a variety of factors, including presence of amino acid/fatty acid, amylose aggregation, amylose-to-amylopectin ratio, chain lengths, intra- or intermolecular bonding, granule swelling, granule remnants, starch paste consistency, interfering substances, and granule size (Li *et al.*, 2019[11]; Tessema & Admassu, 2021) [22].

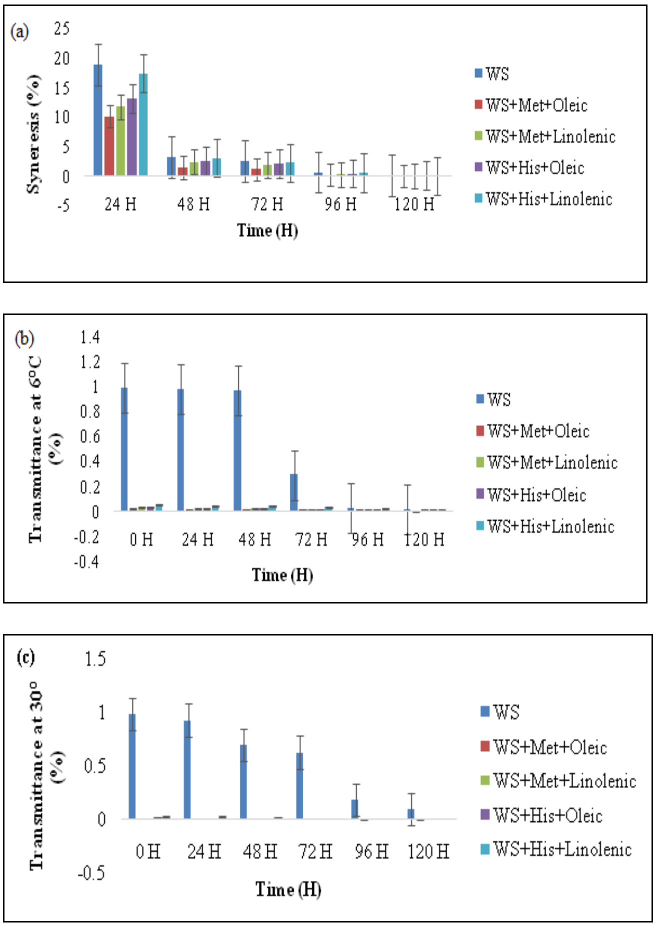

Fig 2: **(a)** % syneresis, **(b)** % transmittance at 6°C, and **(c)** % transmittance at 30°C of ternary blends.

Conclusion

This research delved into exploring the impact of ternary blends on the physicochemical and rheological properties of WS through complexation. The findings underscored the significant influence of specific amino and fatty acids in forming complexes. Notably, the WS+Met+Oleic blend exhibited the highest complex formation index, while the presence of WS+His+Linolenic, characterized by the imidizole ring in His and a higher number of double bonds in linolenic acid, hindered stable complex formation. Overall, the formation of ternary blends led to a reduction in water binding capacity, solubility, and swelling power, while improving freeze-thaw stability and paste clarity. The varied applications of these findings on ternary blends span across the food industry. They provide valuable insights into customizing food products, optimizing nutritional as well as novel food formulations, assisting in processing techniques, improving product stability, and contributing to the development of health-focused foods and supplements.

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