Interactive effects of genotype, nitrogen and sulfur fertilization on buckwheat starch properties: Composition, functionality and gelatinization

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Abstract: This study investigated the effects of genotype (G), nitrogen (N) and sulfur (S) fertilizers as well as their interactions on buckwheat starch properties. Five varieties were evaluated under varying N (0, 45 and 90 kg N ha^{-1}) and S (0 and 45 kg SO_3 ha^{-1}) levels to investigate the main and interactive effects. The results showed that G played a dominant role in determining color characteristics and functional properties of buckwheat starch. Among different genotypes, significant variations were observed in amylose (23.86-30.00%), amylopectin (53.92-60.39%) and total starch (83.86-84.97%) content. N fertilizer was the most influential factor for improving starch composition, pasting behavior and thermal characteristics. Meanwhile, S fertilizer exhibited moderate but significant effects on the evaluated parameters. As for the interaction, there was significant impact among G, N and S across the evaluated parameters, with two-way interactions (G x N, G x S and N x S) exhibiting stronger influences than three-way interaction (G x N x S). These findings indicated the complex relationship between genetic and agronomic factors, providing insights for optimizing buckwheat starch properties through targeted breeding and fertilization strategies. This research provides a foundation for improving functional foods based on buckwheat starch and developing sustainable cultivation practices.

Keywords: buckwheat; starch; nitrogen; sulfur; fertilization; interactive effects

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench), a widely cultivated pseudocereal, is valued for its nutrition and health benefits [1]. Buckwheat grains are rich in bioactive compounds, amino acids and minerals. Starch, accounting for approximately 70% of the dry weight in buckwheat grain, plays a crucial role in its utilization [2]. Buckwheat starch is characterized by unique granule size, shape and molecular structure, distinguishing it from other cereal starch [3-5]. These characteristics have drawn attention for potential use in food processing, pharmaceutical formulation and biodegradable materials [6-8]. While current research primarily investigates the general characteristics and applications of buckwheat starch, the specific influences of genotype (G) and fertilization strategies on its relative properties remain underexplored.

Nitrogen (N) is an essential element for plant growth and development [9]. Studies have shown that N fertilizer plays a crucial role in regulating starch structure and physicochemical properties in buckwheat. For example, Zhang et al. [10] reported that increased N application in Tartary buckwheat enhanced water solubility, relative crystallinity and pasting temperature while reducing amylose content and starch granule size. Similarly, Lei et al. [11] found that common buckwheat starch under medium N levels exhibited higher pasting temperature and gelatinization enthalpy. Wan et al. [12] further revealed that higher N application rate enhanced starch composition (amylose, amylopectin and total starch) in common buckwheat. These effects are largely attributed to N-mediated regulation of key enzymes involved in starch synthesis, as evidenced by previous study showing increased enzyme activity leading to enhanced starch accumulation in Tartary buckwheat [13]. Sulfur (S), another crucial nutrient element, plays a vital role in protein synthesis and enzyme activity, particularly in S-containing amino acids of proteins such as cysteine and methionine [14, 15]. S fertilizer has been shown to increase total phenolic acid content and yield but decrease protein content in buckwheat kernels [16, 17]. However, its impact on buckwheat starch properties remains largely unexplored. N is essential for the synthesis of amino acids, proteins and nucleic acids, while S is essential for specific amino acids and coenzymes, reflecting their interdependence in biochemical pathways [18]. This interdependence suggests that their combined application has the potential to optimize nutrient utilization efficiency [19] and change starch properties. However, there is a lack of systematic studies on the interactions of N and S fertilizers in buckwheat starch characteristics. G is another crucial determinant of starch structure and functionality, primarily through its regulation of enzyme activity and carbohydrate metabolism [20]. However, limited research has explored the interactive effects of G with N and S fertilizers on buckwheat starch properties. It is hypothesized that these interactions are essential for optimizing agronomic practices to obtain desirable starch characteristics.

Some work has been carried out on the effects of the above factors on buckwheat quality. One study explored the interaction of G, N and S fertilizers on the nutritional and functional properties of buckwheat flour [17]. Another study focused on the physicochemical properties of buckwheat starch under combined N and S fertilizer treatments, but the main emphasis was on N and S fertilizer interaction, without a comprehensive evaluation of genetic differences [21]. Although these studies provided important insights, there are still unresolved questions about the interactive effects of G, N and S fertilizers on buckwheat starch properties, especially when incorporating wider genetic diversity.

To address these gaps, this study investigated the effects of G, N and S fertilizers on the composition, color characteristics, functional properties, pasting behavior and thermal properties of buckwheat starch. The main and interactive effects were systematically explored by selecting five buckwheat varieties on the basis of food or feed resource potential and setting three N levels and two S levels. Unlike previous studies, this study introduced a larger range of genetic diversity and integrated the interactions of multiple factors, thus providing deeper insights into how genetic and agronomic factors combine to influence the starch properties of buckwheat. The results of this study may contribute to future buckwheat production and field management practices, facilitating the development of buckwheat grains with desirable starch characteristics for diverse applications.

2. Materials and methods

2.1 Experimental design

The field trials were conducted in 2022 at the Ghent University research farm in Bottelare, Belgium (50.97N, 3.78E). The average monthly temperature at the site was 17.2°C and the average monthly precipitation was 50.3 mm. Five common buckwheat varieties widely grown in European countries were selected for this study. Among these varieties, Devyatka (B1), Panda (B3) and Kora (B5) are mainly used for grain production, while Drushina (B2) and Noiaj (B4) are commonly used as fodder due to their high yields and suitability for rough processing. Specific details about selected buckwheat varieties are shown in Table S1. These varieties were grown under three contrasting N levels: N0 = 0 kg N ha^{-1}, N1 = 45 kg N ha^{-1} and N2 = 90 kg N ha^{-1}. At each level, two S levels (S0: 0 kg SO_3 ha^{-1}; S1: 45 kg SO_3 ha^{-1}) were applied. N and S fertilizers were applied as ammonium nitrate (27%) and patentkali (30% K2O, 10% MgO and 42.5% SO3), respectively. All fertilization rates were set following according to the recommendations of the National Soil Service of Belgium. The field experiment followed a randomized complete block design with four replications, where each block contained all 30 treatments randomly assigned to individual plots. The experimental plot was 1.5 m wide and 9.2 m long with a total of 120 subplots. Soil properties before fertilization are summarized in Table S2. Seeds were sown on 10 May and harvesting was completed in mid-August. More details about sample information, field experimental setup and fertilization can be found in a previous study [17].

2.2 Starch extraction

Seeds harvested from the four replicates of each treatment were thoroughly mixed. The mixed seeds were dehulled with a Spelt huller (KMPP 300, JK Machinery, Czech Republic) and then ground into wholemeal flour using a Hammertec mill (Foss, Hilleroed, Denmark). Buckwheat starch was extracted following the previously established method [2] with modifications to improve the removal of non-starch components. Specifically, buckwheat wholemeal flour (500 g) was immersed in a 0.2% (w/v) NaOH solution at a 1:3 ratio (flour to solution) and incubated at room temperature (25°C) for 16 h to promote the dissociation of proteins from the starch granules. The resulting slurry was passed through a 150 μm sieve while distilled water was added until no more starch was released. After centrifugation at 4000 xg for 10 min, the supernatant and yellow layer were discarded. The precipitate was resuspended in distilled water and centrifuged repeatedly under the same conditions (at least three times) until the supernatant became clear. To further purify starch and remove residual lipids, the resulting starch precipitate was immersed in anhydrous diethyl ether at a ratio of 1:3 (w/v) and gently stirred for 30 min. The starch was then filtered, and the process was repeated twice with a fresh solvent. After filtration, the starch was placed in a fume hood to allow complete evaporation of the residual solvent. The purified starch was dried overnight in an oven (Heraeus type UT12, Germany) at 40°C, grounded to powder and stored in polythene bottles at room temperature for subsequent analysis.

2.3 Determination of starch composition

Total starch (TS) and apparent amylose (AM) content were determined using the Amylose/Amylopectin Assay Kit (REF: K-AMYL; SKU: 700004262) (Megazyme Ltd., Wicklow, Ireland) following the manufacturer's instructions. Briefly, starch samples were subjected to Con A precipitation to separate the amylopectin (AP) (formation of precipitate) from the soluble AM. For the TS content determination, samples were hydrolyzed to D-glucose using α-amylase and amyloglucosidase. The glucose was then oxidized by glucose oxidase to produce hydrogen peroxide (H_2O_2) and D-gluconate. In the presence of peroxidase, H_2O_2 reacted with *p*-hydroxybenzoic acid and 4-aminoantipyrine to form quinonimine, and the AM content was determined colorimetrically. The AP content was calculated as the difference between the TS and

AM content.

2.4 Determination of nitrogen and sulfur content

N content was measured by VarioMax C/N (Elementar Analysesystemin, Langenselbold, Germany) [22]. The inductively coupled plasma-optical emission spectrometry (ICP-OES, IRIS Intrepid II XSP, Thermo Scientific, Waltham, MA, USA) was used for S content determination. Samples were mineralized in a muffle furnace (500°C, 4 h) and digested with hydrochloric acid (3 mol/L). After filtration with Whatman filter paper, the resulting solution was introduced into the ICP torch for analysis [23].

2.5 Color characteristics

The Konica Minolta Spectrophotometer CM-700d (Konica Minolta, Tokyo, Japan) was calibrated with a white standard plate before being used to measure color parameters of starch samples. L^* (lightness to darkness), a^* (redness to greenness) and b^* (yellowness to blueness) were recorded [24]. Polar coordinate chroma (ΔC) was calculated as follows:

$$\Delta C = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2} \qquad (1)$$

2.6 Functional properties

Water absorption capacity (WAC) and oil absorption capacity (OAC) of starch samples were determined following the method of Beuchat [25] with some modifications. A 50 mL centrifuge tube containing 2 g of starch sample (m_1) was filled with 20 mL of distilled water or culinary corn oil (purchased from a local shop) and homogenized for 1 min. After standing at room temperature (25°C) for 30 min, the mixture was centrifuged at 5000 xg for 10 min. The supernatant was then carefully removed, and the residue was weighed (m_2). WAC and OAC were expressed by dividing the residue weight by the initial sample weight:

$$\text{WAC/OAC (g/g)} = \frac{m_2}{m_1} \qquad (2)$$

Water absorption index (WAI), water solubility (WS) and swelling power (SP) of starch samples were measured as described by Abebe et al. [26] with minor modifications. Briefly, buckwheat starch (2 g, w_o) was dispersed in a 50 mL centrifuge tube containing 20 mL of distilled water. The mixture was then heated (95°C, 15 min), cooled to room temperature (25°C) and centrifuged (4000 xg, 10 min). The supernatant was poured into a pre-weighed evaporation dish to determine its solids content, and the sediment was weighed (w_s). The supernatant was dried overnight at 105°C to obtain the weight of dry solids (w_d). WAI, WS and SP were calculated using the following equations:

$$\text{WAI (g/g)} = \frac{w_s}{w_o} \qquad (3)$$

$$\text{WS (\%)} = \frac{w_d}{w_o} \times 100 \qquad (4)$$

$$\text{SP (g/g)} = \frac{w_s}{w_o - w_d} \qquad (5)$$

2.7 Pasting properties

The pasting behavior of buckwheat starch were determined using the MCR 102 Rheometer (Anton Paar GmbH, Graz, Austria) equipped with a starch pasting cell containing six blades [23]. A 6% (w/v) starch-water suspension (adjusted to the moisture content) was prepared and vigorously shaken for 15 s. Measurement started after setting the gap to 1 mm and covering the sample holder to prevent evaporation. Briefly, the suspension was heated to 50°C while stirring at 960 rpm during the pre-shearing phase.

A holding phase of 1 min at 50°C was followed by a heating phase to 95°C at a rate of 5°C/min. Subsequently, a second holding period at 95°C for 5 min was conducted followed by a gradual cooling phase (rate equal to the heating stage). The determination was completed by a final holding phase at 50°C for 2 min. The parameters derived from the pasting curves included peak viscosity (PV), holding strength (HS), final viscosity (FV), breakdown (BD), setback (SB) and pasting temperature (PT).

2.8 Thermal properties

The thermal characteristics of starch samples were measured using a differential scanning calorimetry (DSC) (Q2500, TA instruments, New Castle, DE, USA) according to a previous study [27]. Starch (3 mg) was weighed and mixed with distilled water (9 μL) in an aluminum pan. The mixture was then stored overnight at 4°C. Samples were equilibrated at 30°C for 5 min and then scanned over a range of 30-120°C at a heating rate of 10°C/min. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and gelatinization enthalpy (ΔH) were obtained from the DSC thermograms using the TA instruments TRIOS software.

2.9 Statistical analysis

All measurements were performed in triplicate. A three-way analysis of variance (ANOVA) was conducted using IBM SPSS Statistics 22.0 software (IBM Corp., Armonk, USA) to determine the main and interactive effects of G, N and S fertilizers. The data normality was assessed using the Shapiro-Wilk test before adopting a mixed model analysis. Differences between means were compared for significance by Tukey's HSD post hoc test ($p < 0.05$). Pearson's correlation test was performed to determine the relationships between the evaluated parameters at $p \leq 0.05$. Colors and shapes indicated the direction and strength of the correlation. Blue and red ellipses represented negative correlations and positive correlations, and darker colors indicated stronger correlations. Elongated ellipses indicated a strong correlation, while ellipses close to a circle indicated a weak or no correlation. Hierarchical cluster analysis was conducted to classify the 30 treatments based on group averaging. All data were standardized using Z-scores and normalized to a range between 0 and 1. Principal component analysis (PCA) was used to understand the variability of the dataset for different treatments. The partial least squares structural equation model (PLS-SEM) was developed using SmartPLS 4 software (SmartPLS GmbH, Germany) to analyze the direct and indirect effects of the three factors on buckwheat starch properties. All data were plotted using Origin software (version 2024, OriginLab, USA).

3. Results

3.1 Starch composition

The ANOVA results showed that all factors (G, N and S) had significant effects ($p < 0.001$) on starch composition (Table 1). N fertilizer had the greatest impact on TS and AM content, followed by G and S fertilizer. However, G played a dominant role for AP content, followed by N and S fertilizers. As for interactions, G x N, G x S and N x S had significant effects on TS, AM and AP content. However, G x N x S exhibited weaker but still significant effects ($p < 0.05$) on TS and AM content, while there was no significant effect ($p > 0.05$) on AP content. The mean results further showed the variation in buckwheat starch composition under different treatments (Table 3). There were significant differences in starch composition among the genotypes, with B4 having the highest TS (84.97%) and AP content (60.39%), while B1 had the highest AM content (30.00%). Conversely, B1 had the lowest TS (83.86%) and AP (53.92%) content, while the lowest AM (23.59%) content was found in B5. N fertilizer had a significant impact on buckwheat starch composition. As N level increased from N0 to N2, the TS and AM content decreased by 1.34% and 16.32%, respectively. Conversely, AP content increased by 5.78%. Similarly, higher S level (S1) reduced TS and AM content by 0.27% and 5.79%, respectively, but enhanced AP content by 2.16%.

3.2 Nitrogen and sulfur content

The ANOVA results indicated that G, N and S all significantly ($p < 0.001$) affected N and S content (Table 1). N fertilizer was the main factor affecting N content, followed by G and S fertilizer. However, S content was primarily influenced by N fertilizer, followed by S fertilizer and G. The interactions of G x N, G x S, N x S and G x N x S all showed significant ($p < 0.001$) effects on N and S content. The mean results of N and S content under different treatments are displayed in Fig. 1. Significant differences in N and S content were observed among the genotypes, with B4 having the highest N content (420.09 mg/kg) and B2 the lowest (239.11 mg/kg) (Fig. 1a), while B1 exhibiting the highest S content (41.33 mg/kg) and B3 the lowest (33.41 mg/kg) (Fig. 1d). As N level increased from N0 to N2, the N and S content increased by 115.56% (Fig. 1b) and 38.80% (Fig. 1e), respectively. Similarly, there was a 24.05% increase in N content (Fig. 1c) and an 8.49% increase in S content (Fig. 1f) with the application of S fertilizer.

3.3 Color characteristics

As shown in Table 2, G had highly significant ($p < 0.001$) effects on all color parameters (L^*, a^*, b^* and ΔC). In contrast, N fertilizer showed a significant ($p < 0.001$) effect only on a^* and not on L^*, b^* and ΔC ($p > 0.05$). S fertilizer significantly affected all color parameters, with the greatest ($p < 0.001$) effect on b^* and ΔC. In terms of interactions, G x N had a significant ($p < 0.001$) effect on a^*, b^* and ΔC but not on L^* ($p > 0.05$). G x S showed significant ($p < 0.001$) effects across all color parameters, particularly for a^*, b^* and ΔC. Similarly, N x S significantly influenced a^*, b^* and ΔC but had no effect ($p > 0.05$) on L^*. In addition, G x N x S showed minor but significant effects on a^*, b^* and ΔC. Fig. 2 displays the mean values of buckwheat starch color as affected by G, N and S fertilizers. Among the genotypes, B2, B3 and B5 exhibited higher L^* values (approximately 95), while B1 and B4 had significantly lower L^* values (92.19 and 91.59, respectively) (Fig. 2a). As for a^*, B1 had the highest value (0.25), while B2 and B3 showed the lowest value (0.11 and 0.10, respectively) (Fig. 2d). Similar trends were observed for b^* (Fig. 2g) and ΔC (Fig. 2j), with B1 and B4 exhibiting the highest values, while the lowest values were observed in B2 and B3. Regarding the effects of N fertilizer, there was no significant impact on L^* (Fig. 2b), b^* (Fig. 2h) and ΔC values (Fig. 2k). However, a^* value under N2 level increased by 42.86% compared to N0 level (Fig. 2e). In contrast, as S level increased from S0 to S1, all color parameters showed an increase: L^* (0.35%) (Fig. 2c), a^* (13.33%) (Fig. 2f), b^* (4.19%) (Fig. 2i) and ΔC (4.17%) (Fig. 2l).

3.4 Functional properties

The effects of G, N and S fertilizers on functional properties of buckwheat starch are summarized in Table 2. For the main effects, both G and N fertilizer exhibited highly significant ($p < 0.001$) effects on all functional properties. G emerged as the dominant factor for OAC, WAI, WS and SP, while N fertilizer played a primary role in WAC. In contrast, S fertilizer significantly influenced WAC ($p < 0.05$), OAC ($p < 0.001$) and WS ($p < 0.001$), but its effects on WAI and SP were not significant ($p > 0.05$). As for the interactions, G x N was significant across all the evaluated parameters. G x S significantly affected OAC ($p < 0.05$) and WS ($p < 0.01$), with no significant effects on other parameters. Similarly, N x S only influenced OAC ($p < 0.001$) and showed no significant ($p > 0.05$) impact on the remaining functional properties. The three-way interaction (G x N x S) exhibited minor but significant effects ($p < 0.05$) on OAC and WS, with no significant ($p > 0.05$) effects on other parameters. In terms of mean comparison (Table 3), notable differences were observed among different genotypes, with B3 having the highest WAC and OAC and B4 having the lowest values, B5 exhibiting the highest WAI and SP but the lowest WS. Regarding N fertilizer, increasing N levels enhanced the functional properties, with N2 level producing the highest values. Similarly, S fertilizer (S1) significantly improved WAC by 1.65%, OAC by 3.94% and WS by 21.43% as compared to S0 level.

3.5 Pasting properties

The ANOVA results revealed that G, N and S had significant ($p < 0.001$) effects on starch pasting behavior (Table 4). Regarding the main effects, N fertilizer was the dominant factor influencing PV, HS, FV, SB and PT, while BD was primarily influenced by S fertilizer. In terms of interactions, G x N significantly ($p < 0.001$) influenced all pasting parameters. G x S was significant for PV ($p < 0.05$), FV ($p < 0.05$), BD ($p < 0.001$) and PT ($p < 0.001$), but had no significant ($p > 0.05$) effects on HS and SB. N x S had significant effects on all parameters except SB. Notably, G x N x S was highly significant ($p < 0.001$) for all pasting parameters. Mean comparisons further demonstrated the impact of G, N and S fertilizers on buckwheat starch pasting properties (Table 5). Among the genotypes, B1 exhibited the highest values for PV (764.94 mPa·s), HS (748.07 mPa·s), FV (1494.44 mPa·s) and SB (746.37 mPa·s), while B2 showed the lowest values for these parameters (PV: 608.58 mPa·s, HS: 562.03 mPa·s, FV: 1234.96 mPa·s and SB: 626.37 mPa·s). On the contrary, B2 had the highest BD (46.57 mPa·s), while the lowest value (16.87 mPa·s) was found in B1. PT values varied slightly among the genotypes, with B1 showing the lowest (69.32°C) and B2 the highest (69.69°C). Regarding the effects of N fertilizer, increasing N levels significantly enhanced PV and FV, with N1 and N2 showing higher values (PV: 711.06 and 718.32 mPa·s; FV: 1449.43 and 1422.10 mPa·s, respectively). However, the PT slightly decreased (0.42%) under the N2 level compared to N0 level. Similarly, the increasing S level from S0 to S1 led to significant increases in PV (5.76%), HS (3.97%), FV (3.98%) and BD (68.89%) but a slight decrease (0.09%) in PT.

3.6 Thermal properties

The ANOVA results revealed that G, N and S had significant ($p < 0.001$) effects on all thermal parameters (Table 4). Among the main effects, G was the dominant factor for T_c, while N fertilizer played a leading role in T_o, T_p and ΔH. Regarding the interactions, both G x N and G x S exhibited highly significant ($p < 0.001$) effects across all parameters. N x S significantly influenced T_p ($p < 0.01$) and T_c ($p < 0.001$) but had no significant ($p > 0.05$) effects on T_o and ΔH. The three-way interaction (G x N x S) significantly affected T_p, T_c and ΔH, though its impact on T_o was not significant ($p > 0.05$). In terms of mean comparison (Table 6), there were significant differences among various genotypes. B2 exhibited the highest T_o (62.63°C), followed by B3, while B1 had the lowest value (62.36°C). The highest T_p (68.28°C) and T_c (78.62°C) were observed in B3 and B2, respectively. In contrast, B1 showed the highest ΔH (7.24 J/g), while B5 exhibited the lowest value (6.62 J/g). In terms of N fertilizer, increasing N levels significantly decreased gelatinization temperatures. Compared to N0 level, T_o, T_p and T_c under N2 level reduced by 0.69%, 0.89% and 1.40%, respectively. Conversely, ΔH increased by 9.26%. Similarly, higher S level (S1) slightly decreased T_o (0.22%), T_p (0.19%) and T_c (0.36%), while resulting in a 2.20% increase in ΔH.

3.7 Correlation analysis

As displayed in Fig. 3a, the correlation matrix provides a comprehensive overview of the relationships among the evaluated variables of buckwheat starch properties. Specifically, TS showed significant positive correlations with AM, PT, T_o, T_p, T_c but significant negative correlations with WAC, PV, FV and ΔH. AM was significantly positively correlated with b^*, ΔC and T_c, whereas the opposite trend was observed between AP and these parameters. Both NC and SC had strong positive correlations with color parameters (a^*, b^* and ΔC), WAC, viscosities (PV, HS, FV and SB) and ΔH but significant negative correlations with PT, T_o, T_p and T_c. L^* was significantly positively correlated with OAC, WAI, SP, BD and PT, while opposite trends were observed for a^*, b^* and ΔC. WS was significantly positively correlated with PV, HS, FV, ΔH but negatively correlated with gelatinization temperatures. Both WAI and SP showed strong positive correlations with BD and PT but negative correlations with PV, HS, FV, SB and ΔH. Pasting properties (PV, HS, FV and SB) displayed significant negative correlations with gelatinization temperatures (T_o, T_p and T_c) but strong positive correlation with ΔH. In addition, significant negative correlation was observed between gelatinization temperatures and ΔH.

3.8 Hierarchical cluster analysis

Fig. 3b shows the hierarchical cluster analysis of the 30 treatments based on starch composition, nitrogen and sulfur content, color characteristics, functional properties, pasting behavior and thermal properties. The treatments were divided into four main clusters (green, red, blue and purple branches), reflecting different patterns influenced by G, N and S fertilizers. Specifically, cluster 1 (green) primarily consisted of treatments with low (N0), moderate (N1) and high (N2) N levels combined with varying S levels (S0 and S1) and genotypes (B2, B3 and B5). These treatments showed a high similarity degree in terms of overall characteristics. Cluster 2 (blue) comprised treatments with the specific variety of B4, including N1S1B4, N2S0B4 and N2S1B4. These treatments showed different patterns in the measured parameters, which made them cluster together. This indicated that the interaction of high N and S inputs made these treatments distinct from the others regarding general starch properties. Cluster 3 (red) mainly included treatments comprising a particular variety of B1 associated with various N and S levels. Similar to cluster 2, cluster 4 also included treatments of a specific variety of B4, but combined with low N levels (N0S0B4, N0S1B4 and N1S0B4).

3.9 Principal component analysis

The PCA was performed to determine the relationships between treatments and buckwheat starch properties. PCA score plot (Fig. 3c) showed the distribution of 30 treatments based on the first two principal components (PC1 and PC2), explaining 63.43% of the total variation (PC1 = 38.98%, PC2 = 24.45%). All treatments were well separated into different quadrants, reflecting their diverse impacts on buckwheat starch properties. For example, treatments of N2S1B1 and N2S1B4 (high N and S levels) were located in the upper-right quadrant, indicating their distinct contribution to specific variables. Conversely, treatments such as N0S0B4, N0S0B1 and N0S0B2 (low N and S levels) distributed in the lower-left quadrant, suggesting different effects compared to the high fertilizer treatments. There were several treatments clustered around the origin, implying minimal deviations or similar response under certain combinations. PCA loading plot highlighted the contribution of individual starch-related properties to the principal components (Fig. 3d). The directions and magnitudes of the arrows indicate the strength and relationships among the variables. Both PC1 and PC2 were positively correlated with N and S content, functional properties (WAC and WS), viscosities (PV, FV and SB) and ΔH, but negatively correlated with TS and PT, T_o, T_p and T_c. AM and color parameters (a^*, b^* and ΔC) were positively correlated with PC1 but negatively correlated with PC2. However, AP, L^* and functional properties (OAC, WAI and SP) were negatively correlated with PC1 but positively correlated with PC2.

3.10 PLS-SEM establishment

The PLS-SEM was developed to explore the effects of G, N and S fertilizers on buckwheat starch properties (Fig. 4). As shown in Fig. 4a, G was positively correlated with color characteristics as well as functional, pasting and thermal property, but negatively correlated with starch composition. N fertilizer had significant positive correlations with color (path coefficient = 0.829) and pasting property (path coefficient = 0.898), but significant negative correlations with starch composition (path coefficient = -0.662) and thermal property (path coefficient = -0.871). S fertilizer was positively correlated with color and pasting property but negatively correlated with starch composition and thermal property. The total (Fig. 4b) and indirect effects (Fig. 4c) of the influencing factors were further analyzed based on the path analysis plot. The total effect of all factors on starch composition was negative, with N fertilizer exhibiting the largest effect (-0.662). The total effect of G on color and pasting property was negative, while it was positive for N and S fertilizers. The total effect of all factors on functional property was positive with the maximum effect of G (0.593). The total effect of G on thermal property was positive, whereas that of N and S fertilizers was negative. All factors had no indirect effects on starch composition, positive indirect effects on functional property and negative indirect effects on color and pasting property. The indirect effect of S fertilizer on thermal property was relatively limited. Overall, the PLS-SEM analysis highlighted the dominant role of N fertilizer in influencing functional, pasting and thermal properties, while G significantly affected starch composition and color parameters. The role of S fertilizer in specific pathways was minor compared to other factors.

4. Discussion

4.1 Effects of genotype on buckwheat starch properties

This study emphasized the critical role of genotype (G) in influencing buckwheat starch properties. Significant genotypic variations were observed in starch composition, with B4 exhibiting the highest TS and AP content, while B1 had the highest AM content. Notably, the AM content in this study (23.59-30.00%) was higher than the values reported for Tartary buckwheat starch (20.53-22.23%) by wang et al. [28], but lower than the range found in our previous research on common buckwheat starch (29.55-36.13%) [2]. These results aligned with previous findings that suggested genetic background significantly influenced starch composition in buckwheat [28]. The observed genotypic variations in starch composition were closely related to the activity of key enzymes involved in starch biosynthesis [29]. Granule bound starch synthase (GBSS) is essential for amylose synthesis, while starch branching enzymes (SBE) and soluble starch synthase (SSS) regulate amylopectin biosynthesis [29]. The application of N fertilizer likely enhanced the activities of ADP-glucose pyrophosphorylase (AGPase) and SSS, thereby promoting amylopectin accumulation and reducing AM content, especially in B2 and B5 varieties. Starches from different genotypes differ in functionality [30]. In this study, functional properties of buckwheat starch further reflected the genetic influence. Specifically, B3 and B5, characterized by higher WAC and OAC, suggested superior ability to interact with polar and nonpolar molecules. This finding indicated that starch granules of these varieties had higher porosity and surface hydrophilicity, making it an ideal candidate for applications requiring moisture retention of flavor encapsulation, such as baked goods and emulsified products [31, 32]. In contrast, the lower WAC of B1 and B4 indicated a denser granule structure, which could be advantageous for specific applications requiring reduced water absorption [33]. Significant differences were also observed in starch pasting behavior. Starch from B1 exhibited the highest PV, indicating greater swelling capacity and susceptibility to shear during heating [34]. Conversely, the lowest PV observed in B2 reflected the influence of higher AM content, which restricted starch granule swelling and reduced paste viscosity due to its structural rigidity [35]. In addition, thermal characteristics further underlined the genetic differences. B4 and B5 showed lower gelatinization temperatures (T_o, T_p and T_c), implying less crystalline structure, which may be due to the higher AP content and increased SSS activity. However, B1 exhibited the highest gelatinization enthalpy (ΔH), which can be explained by its higher AM content.

4.2 Effects of nitrogen fertilizer on buckwheat starch properties

Nitrogen (N) fertilizer significantly influenced buckwheat starch properties, making it a critical agronomic factor. For starch composition, the observed decrease in TS and AM content, along with an increase in AP content under higher N levels, can be attributed to the role of N in regulating carbohydrate metabolism. N availability impacts carbon distribution in plants and promotes the allocation of photosynthetic products to the starch synthesis pathways [36]. AGPase, a key enzyme controlling the initiation of starch biosynthesis, is known to be upregulated by N availability, promoting increased starch synthesis [29]. However, excessive N may prioritize protein synthesis over carbohydrate storage, thereby reducing overall starch accumulation [37]. The variations in AM content may also be linked to N-mediated regulation of granule-bound starch synthase (GBSS) and starch branching enzyme (SBE), which control amylose and amylopectin biosynthesis, respectively. A shift in the balance between these enzymes due to N fertilization may explain the observed results. Functional properties such as WAC, OAC, WS and SP showed significant improvements under higher N levels, probably due to the reduced AM content. N fertilizer also significantly influenced starch pasting behavior. The observed increase in starch viscosities (PV, HS, FV, BD and SB) with higher N levels suggested that buckwheat starch granules became more susceptible to water infiltration and swelling, potentially due to enhanced amylopectin biosynthesis [4]. This finding was consistent with previous research on potato and sweet potato starch [38, 39], whereas different trends were reported on rice starch [40]. Furthermore, reduced gelatinization temperatures (T_o, T_p and T_c) under high N levels indicated a less crystalline structure, which was consistent with previous finding [27]. However, the increased ΔH suggested stronger intramolecular binding within the starch granules, leading to enhanced thermal stability. These findings can provide valuable information on how N fertilizer can be strategically applied to improve buckwheat starch properties for industrial and nutritional applications.

4.3 Effects of sulfur fertilizer on buckwheat starch properties

The effect of sulfur (S) fertilizer, although relatively weaker than N fertilizer, still significantly affected buckwheat starch properties. The increase in AP content after S application may be due to S-mediated enzyme regulation and protein interactions affecting carbohydrate metabolism [41]. S is essential for the synthesis of S-containing amino acids like cysteine and methionine, which are involved in the activation of starch biosynthesis enzymes [42]. This suggested that S fertilizer may enhance the activity of key enzymes, such as AGPase, GBSS and SBE, thereby influencing buckwheat starch composition. In addition, the increase in b* and ΔC values following S application may be attributed to the synthesis of secondary metabolites such as polyphenolic compounds and carotenoids [43], which are critical for food quality and consumer preference. Furthermore, the slight increase in viscosities (PV, FV and BD) and ΔH, along with reduced gelatinization temperatures (T_o, T_p and T_c) indicated that S fertilizer may influence starch granule organization and stability. These findings indicate that S fertilizer can play a role in changing buckwheat starch properties to enhance its functionality in food and industrial applications.

4.4 Interactions among genotype, nitrogen and sulfur fertilizers

The interactions between genotypes, N and S fertilizers significantly influenced buckwheat starch properties, emphasizing the interplay between genetic background and agronomic strategies. The G x N interaction significantly enhanced starch composition, functional and pasting properties, likely due to the role of N in promoting protein synthesis, which indirectly affects starch biosynthesis by regulating carbon and nitrogen partitioning in plants. The G x S interaction strongly influenced starch color parameters, functional properties (OAC and WS) and thermal properties. S is essential for the synthesis of S-containing amino acids, which contribute to protein-starch interactions [14, 15]. These interactions can modify the structural integrity of starch granules, altering their hydration properties. The N x S interaction showed notable effects on starch composition and pasting properties. The combined application of N and S fertilizers likely enhanced starch granule swelling and viscosity by influencing starch-protein interactions and intra-starch molecular bonding. Previous study on wheat starch has demonstrated that the simultaneous application of N and S fertilizers can increase swelling power and viscosity [44], possibly due to enhanced amylopectin synthesis and granule hydration properties. The G x N x S interaction, while relatively weaker, still had a significant impact on key starch properties, including TS, AM, a^*, b^*, viscosity and ΔH. These findings suggested that buckwheat starch characteristics may be dominated by a complex interplay between genetic factors and nutrient availability, where optimal N and S levels regulate the expression of genes encoding key starch metabolic enzymes. For example, genotypes like B1 and B4 exhibited improved pasting behavior under higher N and S levels, indicating enhanced gelatinization and thickening properties suitable for food applications requiring high viscosity. In contrast, genotypes such as B2 and B5, which showed greater thermal stability and lower AM content under reduced fertilization levels, may be preferable for industrial applications where heat resistance is critical. In addition, previous research has demonstrated that the significant influence of genotype-by-environment interactions on starch content and physicochemical properties in Tartary buckwheat [45]. Integrating such findings into our study highlights the necessity of optimizing fertilization strategies in conjunction with genotype selection to optimize starch functionality in buckwheat.

5. Conclusions

This research investigated the effects of genotype (G), nitrogen (N) and sulfur (S) fertilizers on buckwheat starch properties. The results showed that G had the greatest impact on starch color and functional properties, N fertilizer predominantly influenced starch composition, pasting and thermal properties, while S fertilizer exhibited moderate but significant effects across multiple characteristics. Regarding the interactions, G x N strongly affected starch functionality and gelatinization, G x S significantly influenced color and thermal properties, while N x S showed significant effects on starch composition. The G x N x S, though weaker, influenced specific characteristics such as starch composition, color and pasting behavior. These findings provide valuable insights for optimizing buckwheat starch properties through targeted breeding and agronomic strategies.

Notes

The authors declare no competing financial interest.

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Fiugure captions

Fig. 1. Effects of genotype (G), nitrogen (N) fertilizer and sulfur (S) fertilizers on N and S content of buckwheat starch (a, d: G; b, e: N fertilizer; c, f: S fertilizer).

Fig. 2. Effects of genotype (G), nitrogen (N) fertilizer and sulfur (S) fertilizers on color characteristics of buckwheat starch (a, d, g, j: G; b, e, h, k: N fertilizer; c, f, i, l: S fertilizer).

Fig. 3. (a): Pearson's correlation matrix of determined variables for buckwheat starch under different genotype (G), nitrogen (N) and sulfur (S) fertilizer treatments. (b): Hierarchical cluster analysis; principal component analysis (PCA) with the score plot (c) and loading plot (d).

Fig. 4. Establishment of the least squares structural equation model (PLS-SEM) to determine the direct and indirect effects of genotype (G), nitrogen (N) and sulfur (S) fertilizers on buckwheat starch properties. (a): Path coefficients diagram. Significant relationships in the PLS-SEM model are indicated by arrows, with thicker arrows indicating stronger correlations. The solid black line and dashed red line represent positive and negative correlation, respectively. Numbers next to the arrows were path coefficient. (b): Total effects plot. (c): Indirect effects plot.