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Effects of soy flour formulation and pretreatment on the properties of gluten-free cookies: A comprehensive study from flour, dough, to baked products

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ABSTRACT

Grain-based gluten-free cookies are often nutritionally inferior owing to their low protein content. This study aimed to enhance the nutritional value of gluten-free cookies by incorporating soy flour and to investigate the effects of different types of modified soy flour on the properties of gluten-free dough and cookies. Results indicate that all types of modified soy flour significantly decreased water absorption capacity ($p < 0.05$) and protein molecular weight while significantly increasing free sulfhydryl groups and free amino group content ($p < 0.05$). Adding modified soy flour significantly reduced the mixograph peak time from 7.26 min to less than 1.9 min ($p < 0.05$). Incorporating 30 % cysteine-modified soy flour significantly increased the cookie spread ratio from 9.2 to 22.8 ($p < 0.05$). Moreover, adding modified soy flour maintained the moderate hardness and fracturability of gluten-free cookies and achieved a more desirable color.

1. Introduction

Gluten proteins are present in wheat, barley, and rye and provide dough its distinctive viscoelasticity when hydrated. Gluten-related disorders are triggered in individuals with a genetic and/or immunologic predisposition when they consume gluten-containing foods. Celiac disease is an immune-mediated systemic disorder and is the most common form of gluten sensitivity, characterized by an autoimmune reaction in the small intestine that develops upon the intake of gluten. Until now, the only treatment for people with celiac disease has been adherence to a gluten-free (GF) diet, i.e., completely avoiding gluten to allow the gut to heal and resolve nutritional deficiencies and other symptoms (Jnawali et al., 2016).

Common wheat-based products are important sources of energy and nutrients for humans, including gluten proteins. For instance, cookies, a widely enjoyed cereal-based food, contain certain ingredients such as soft wheat flour, making them unsuitable for individuals with gluten sensitivity. According to current studies, various types of gluten-free flour (GFF) and starches (rice, sorghum, buckwheat, cassava, etc.) can be used to prepare GF cookies (Indrianingsih et al., 2024). However, the grain-based GFF contains low protein content and lacks essential amino acids, which has low nutritional value. From a nutritional point of view, soy flour (SF) is a good source of protein and bioactive compounds without gluten. Thus, partially replacing grain GFF with SF can compensate for the low nutritional value of GF cookies.

Moreover, it should be noted that gluten removal can have detrimental effects on the quality of bakery goods because the dough-forming process is associated with gluten interaction and formation (Schopf & Scherf, 2021). Note that merely incorporating SF does not guarantee the quality of GF cookies. Therefore, modifying SF and then partially replacing GFF may improve the quality of GF cookies; however, such research has not been reported yet.

Various modifiers have shown potential in improving protein properties. Among them, glutathione (GSH) is a tripeptide comprising glutamic acid, cysteine, and glycine residues. During the dough mixing process, GSH is involved in sulfhydryl (–SH)/disulfide exchange reactions and the cleavage of interchain disulfide bonds in glutenin, thereby affecting the rheological properties of the dough (Li et al.,

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2004). In addition, an early study by Faris et al. (2008) showed that the cleavage of disulfide bonds reduces the molecular weight of the protein, thereby improving the digestibility of soy protein. However, there are differences in the composition and properties of SF and wheat flour, and the effect of GSH on SF protein has not been reported, requiring further exploration.

Another modifier, cysteine, cleaves the disulfide bonds of proteins. In addition, cysteine promotes the formation of interchain disulfide bonds via the thiol/disulfide exchanges of protein molecules (Yang et al., 2021). A recent study by Jiang et al. (2023) showed that the disulfide bonds in soy protein isolate (SPI) subunits can be cleaved by cysteine and adding cysteine reduced the solubility of SPI film. Apparently, cysteine acts as an SF modifier by acting on disulfide bonds; however, studies on the effects of cysteine-modified SF on the quality of GF cookies have not been reported. Sodium sulfite is a common reducing agent that reduces disulfide bonds to free —SH groups. Moreover, relevant data indicate that sodium sulfite improves soy protein solubility and protein dispersibility index, thereby improving the quality of GF cookies (Abtahi & Aminlari, 1997).

In addition, enzymatic hydrolysis is also an effective way to modify SF. However, partial hydrolysis of some proteins, particularly soy protein, could produce a strong bitter taste. At present, a mixture of endopeptidases and exopeptidases has been used to reduce the bitterness of the hydrolysate (Lee et al., 2021). Flavourzyme comprises aminopeptidases, peptidases, medium-sized endopeptidases, and exopeptidases, reducing the bitterness owing to the addition of soy hydrolysate to GF cookies. Moreover, previous studies have shown that compared with other enzyme treatments, SF treated with Flavourzyme has a higher degree of hydrolysis and better gelling quality and foaming performance (Novozym, Alcalase, etc.; Hrčková et al., 2002). The study by Knežević-Jugović et al. (2023) demonstrated that adding soy protein hydrolyzed by Flavourzyme to wheat flour reduced moisture adsorption and, to some extent, prolonged the dough formation. This effect can be attributed to the weakening of the gluten network, which also decreases the starch regeneration rate, ultimately extending the shelf life of baked products. Moreover, this provides a reference for this study to add SF protein hydrolyzed by Flavourzyme to improve the quality of GF cookies.

Based on the research background presented, we hypothesize that incorporating modified SF could enhance the quality and formulation of GF cookies. This study investigated how these modifications (SF modified by cysteine, GSH, sodium sulfite, and Flavourzyme) influence SF characteristics, such as free-SH group and free amino group concentrations, as well as protein molecular weight. In addition, this study further evaluated the effects of modified SF on GF flour properties, such as dough viscosity, cookie spread ratio, hardness, fracturability, color, and moisture content. The findings provide a comprehensive reference to improve the quality of GF cookies.

2. Materials and methods

2.1. Materials

Commercial GF flour blend (12.12 % moisture content) comprising garbanzo bean flour, potato starch, tapioca flour, whole grain sorghum flour, and fava bean flour was obtained from Bob's Red Mill (Milwaukie, OR, USA). Defatted SF (50 % protein content) was provided by Cargill (Wayzata, MN, USA). Sugar, shortening, salt, and dextrose were purchased from a local grocery store. Sodium bicarbonate, sodium dodecyl sulfate (SDS), sodium sulfite, cysteine, GSH, and Flavourzyme were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. SF modification

The modifier (cysteine, GSH, and sodium sulfite, 1.0 % based on SF weight) was added into 10 % SF suspension (SF/water, *w/v*) and stirred for 2 h at room temperature (25 °C) to promote the reaction. Flavourzyme (1.0 % based on SF weight) was added into 10 % SF suspension and mixed in a water bath (50 °C) for 10 and 30 min, respectively. Subsequently, the tube was transferred into boiling water to deactivate the enzyme. The pretreated SF suspensions were lyophilized using a freeze dryer (FreeZone 4.5 L, Labconco Corporation, Kansas City, MO, USA) at −40 °C and 60 Pa for 48 h, achieving a final moisture of <5 %.

2.3. Flour water absorption capacity and mixing properties

Water absorption capacity (WAC) was evaluated according to the method of Quinn and Paton (1979) with some modifications. First, 1 g of flour sample (GFF, unmodified SF (UMSF) or modified SF) was added into 10 mL of distilled water in a 15 mL tube and then vortexed for 30 s. Subsequently, the tube was transferred into a water bath at 30 °C for 30 min. Sample tubes were centrifuged at 3000 ×*g* for 20 min. The sediment was collected and weighed. WAC was calculated as follows:

$$WAC(g\,H_2O/g\,flour) = (W_2 - W_1)/W_1 \tag{1}$$

where W_2 is the sediment weight (g) and W_1 is the sample weight (g).

Flour mixing properties were measured using mixograph (National Manufacturing Co. Lincoln, NE, USA) following the AACC method (50–40.01). The SF was premixed with GFF (30 g SF/100 g total flour) in a KitchenAid mixer before the Mixograph test. The tests were conducted in duplicate.

2.4. Free —SH concentration and free amino group analysis

Free —SH concentration was determined according to a previous method with some modifications (Shen et al., 2021). Approximately 30 mg of UMSF or modified SF was accurately weighed and added to 6 mL of reaction buffer containing 0.05 M sodium phosphate, 2 % SDS, 3 M urea, and 1 mM ethylenediaminetetraacetic acid at pH 6.5, vortexed for 30 s, and mixed for 1 h. The sample was centrifugated at 13,600*g* for 10 min. Subsequently, 300 μL of Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid)) (0.1 %) was added into 3 mL supernatant, vortexed for 30 s, and allowed to react for 45 min. The absorbance was determined at 412 nm. The free –SH content was calculated using $C_{SH} = A/\varepsilon b$ (where A is the absorbance, ε is the extinction coefficient of 13,600, and b is the cell path length). The free amino group was determined according to the method reported by Gujral and Rosell (2004) with some modifications. First, 30 mg of UMSF or modified SF was mixed with 6 mL of distilled water and then vortexed thoroughly, and the supernatant was collected. Then, 1.0 mL $NaHCO_3$ (4 %) and 1.0 mL TNBS (0.1 %) were added into 1.0 mL sample solution and vortexed for 30 s. The tube was then transferred to a water bath at 40 °C for 2 h. Subsequently, 1 mL SDS (10 %) was added to the sample, followed by the addition of 0.5 mL of HCl (1 N) and cooled at room temperature (20 °C–25 °C) for 15 min. The absorbance was measured at 340 nm. Results were determined against an L-leucine standard curve and tested in duplicate. Each test was performed in triplicate using three separate flour samples.

2.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE of the protein samples was conducted under nonreducing conditions following a literature method with small modifications (Sechi & Beavis, 2002). The UMSF or modified SF sample was dispersed into distilled water (5 mg/mL), vigorously mixed overnight, and then centrifuged at 10,000*g* for 5 min at room temperature. A PowerPac 1000 (Bio-Rad, USA) was used to run the electrophoresis. In addition, the GelAnalyzer 23.1 (BootstrapMade) was used to analyze the intensity of electrophoresis bands of different molecular weights. Moreover, the Precision Plus Protein™ Dual Color Standards (BIO-RAD, #1610394) with a molecular weight of 10–250 kDa was used for the

SDS-PAGE analysis.

2.6. Cookie dough preparation and stickiness properties

Cookie dough was prepared according to the formulation presented in Table 1 by mixing GFF, water (same or twice the amount), shortening (same or 1.5 times the amount), and/or sugar (same or 1.5 times the amount). In addition, SF or different types of modified SF were added to replace 30 % of the GFF. Cookie dough stickiness, adhesiveness, and cohesiveness were tested using the TA-XTplus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with an SMS/Chen-Hoseney Dough Stickiness rig according to a previous method (Chen et al., 2020). During the test, the probe was lowered to a speed of V1 (2.0 mm/s) to contact the dough during the bonding phase. Once the compression force reached a predetermined value, the movement of the probe was controlled to maintain this force for a fixed duration. Following the bonding phase, which recorded negative forces, the probe was then increased at a speed of V2 (9.6 mm/s) to separate from the dough sample during the debonding phase. Each test was conducted in triplicate by three separate cookie doughs.

2.7. Preparation of cookies

Cookies were prepared using the AACC method 10–50.05. Table 1 presents the cookie formulation. To investigate the SF effect, 30 % of the GFF was replaced by SF based on the combination formula (i.e., 2 times water, 1.5 times shortening, and 1.5 times sugar). GFF cookies were baked at 205 °C for 10 min.

2.8. Cookie analytical methods

The cookie spread ratio was calculated as a ratio of the average diameter to the average height of the cookies according to a previous report (Handa et al., 2012). Cookie hardness and fracturability were determined using the TA-XTplus Texture Analyzer, according to a previous study (Chen et al., 2020). Color analysis was conducted using a CIE-LAB color system (XITIAN machine equipment Co., Ltd., Huizhou, China), and the results were presented as L* (lightness), a* (redness-greenness), and b* (yellowness-blueness). Baking loss was calculated as the ratio of the weight loss of the cookies to the initial weight of the dough before baking. Each treatment was performed in triplicate by three cookie samples.

2.9. Statistical analysis

The data were analyzed using SAS statistical software (SAS Institute, Cary, NC, USA). Tukey's multiple comparison test was used to determine significant differences between individual data. A significant level was defined at $p < 0.05$. The parameters obtained from the flour, dough, and cookie tests were visualized using principal component analysis (PCA). This analysis generated biplots that illustrate the impact of each characteristic on the principal components and the individual sample scores. Moreover, PCA was conducted using GraphPad Prism 10 (GraphPad Software Inc., San Diego, CA).

Table 1
Formulations of different types of GF cookies (based on g per 100 g flour).

Formulation	GFF, g	SF, g	Shortening, g	Sugar, g	Water, g
GFF	100	0	28.44	57.78	7.11
30 % UMSF	70	30	28.44	57.78	7.11
30 % UMSF-2 × water	70	30	28.44	57.78	14.22
30 % UMSF-1.5 × sugar	70	30	42.66	57.78	7.11
30 % UMSF-1.5 × shortening	70	30	28.44	86.67	7.11
Combination	70	30	42.66	86.67	14.22
30 % SF-Cys	70	30	42.66	86.67	14.22
30 % SF-GSH	70	30	42.66	86.67	14.22
30 % SF-SS	70	30	42.66	86.67	14.22
30 % SF-H10	70	30	42.66	86.67	14.22
30 % SF-H30	70	30	42.66	86.67	14.22

Note: GFF = gluten-free flour; UMSF = unmodified soy flour; SF-Cys = soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS = soy flour-sodium sulfite; SF-H10 = soy flour-hydrolysis, 10 min; SF-H30, soy flour-hydrolysis, 30 min.

3. Results and discussion

3.1. Effects of pretreatment on SF properties

3.1.1. Effects on WAC of SF

The WAC of flour plays a crucial role in determining the processibility of dough and the qualities of baked goods. Fig. 1 presents the results of WAC of different flours. The results revealed a significant difference ($p < 0.05$) in the WAC between commercial GFF (0.71 g of water per g of flour) and SF (1.63 g of water per g of flour). According to relevant reports, the presence of soy protein could quickly absorb and trap water during the mixing process (Srikanlaya et al., 2018), thereby contributing to the higher WAC. These results were consistent with those of Park et al. (2015) who found that okara with added SF increased the WAC by 47.5 %. An earlier report has shown that with the same amount of water added, flour with lower WAC would result in drier and more brittle cookies, indicating that the addition of SF reduced the brittleness of GF cookies (Mcwatters, 1978). Moreover, the amount of water available for gelatinization depends on how much water the dough can absorb. This absorption is influenced by the presence of hydrophilic groups in the flour that attract and bind water molecules. Therefore, flour with higher water absorption capability provides more water for gelatinization, making it easier to cook with. In this study, the WAC of modified SF or UMSF was slightly higher than that of GFF, which may benefit their baking and other applications.

SF modification using cysteine (SF-Cys), GSH (SF-GSH), sodium sulfite (SF-SS), or enzymatic hydrolysis (SF-H10, SF-H30) significantly reduced the WAC compared with the UMSF ($p < 0.05$). According to relevant reports, the reduction in WAC of SF modified by reducing agents or Flavourzyme was due to the modifier causing disulfide bond cleavage, tertiary structure denaturation, or peptide bond cleavage of soy protein, thereby affecting the interaction between SF and water molecules (Shen et al., 2021).

3.1.2. Free —SH concentration and free amino group content

The quality of baked products is heavily influenced by the cross-linking of proteins within the dough, a process primarily determined by the concentration of free –SH groups during protein network formation (Jia et al., 2022). Fig. 1 presents the free —SH content of different pretreated SF. Results indicate that the free —SH concentration of SF significantly increased ($p < 0.05$) after modification with cysteine, GSH, and sodium sulfite, whereas Flavourzyme hydrolysis significantly reduced ($p < 0.05$) the free —SH concentration of SF. Specifically, the SF-Cys group significantly increased the free —SH content from 4.18 to 31.42 mmol/g flour ($p < 0.05$). Although the addition of cysteine itself increased the content of free —SH groups, a previous study reported that the cleavage of disulfide bonds caused by cysteine was also an important reason for the increase in free —SH groups (Li & Lee, 1998). Compared with cysteine, SF-GSH and SF-SS groups contained less free —SH, corresponding to 17.61 and 24.20 mmol/g, respectively, which were significantly higher than the SF-H10 (2.19 mmol/g) and SF-H30 (2.03 mmol/g) group. This is closely related to the effect of modifiers on the properties of SF proteins. Sodium sulfite is a potent reducing agent that can unfold proteins by breaking intermolecular and intramolecular disulfide bonds, leading to a higher concentration of free —SH (Zhu et al., 2016). GSH is actively involved in —SH/disulfide bond exchange reactions within proteins, consequently altering the levels of free —SH groups (Joye et al., 2009). The free —SH group content changes with the

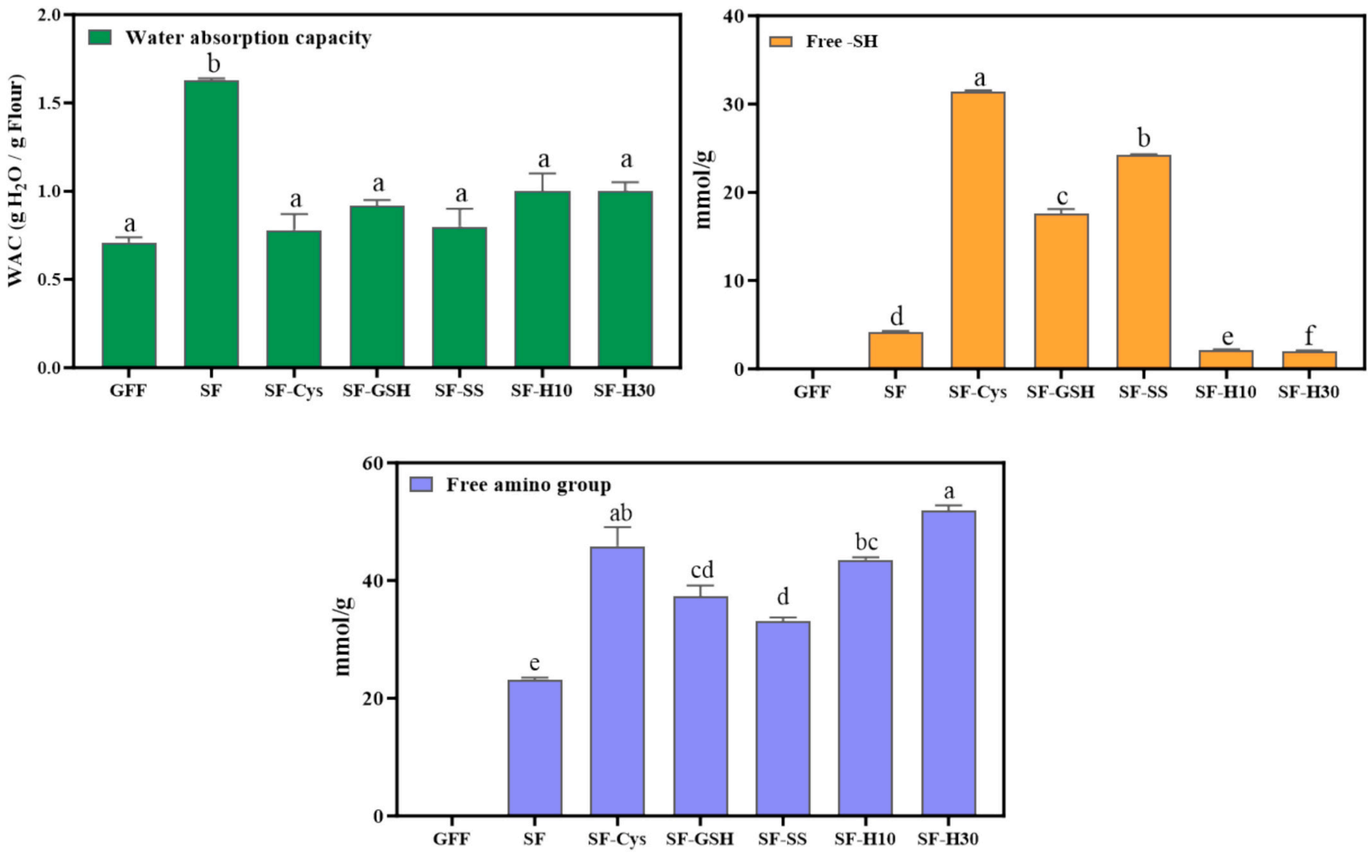

Fig. 1. Effects of different modifiers on the soy flour properties. Note: WAC = water absorption capacity; GFF = gluten-free flour; SF = soy flour; SF-Cys = soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS, soy flour-sodium sulfite; SF-H10, soy flour-hydrolysis, 10 min; SF-H30 = soy flour-hydrolysis, 30 min. Different letters within each property denote significant differences ($p < 0.05$); same letters within each property denote no significant differences ($p > 0.05$). The abbreviated information in the experimental results below is consistent with this figure.

breaking of covalent bonds (disulfide bonds) and the formation of new bonds. The crosslinking of proteins is usually confirmed by a change in the content of free sulfhydryl groups, affecting the quality of the final baked product (Jia et al., 2022). In this study, because the enzymatic hydrolysis was conducted at 50 °C and the enzyme was inactivated in boiling water (i.e., 100 °C), the free —SH groups exposed at high temperatures were more likely to undergo disulfide crosslinking at high temperatures, which led to a decrease in free –SH groups.

The free amino groups in SF significantly increased after modification ($p < 0.05$), particularly in the 30 % SF-Cys (45.88 mmol/g) and enzyme hydrolysis groups (SF-H10, 43.59 mmol/g; SF-H30, 51.93 mmol/g), nearly twice as much as untreated SF (23.15 mmol/g). The increased concentration of free amino groups indicates the exposure of lysine side chains and amino termini owing to protein unfolding and peptide bond hydrolysis (Shen et al., 2022). In fact, some free amino groups in lysine residues in SF are hidden in the hierarchical structure of the protein, and reducing agents such as cysteine and sodium sulfite may promote the exposure of free amino groups in lysine residues. Enzymatic hydrolysis could cleave peptide bonds in the primary structure and release free amino groups; thus, the amount of free amino groups significantly increased ($p < 0.05$) as the hydrolysis time increased, with an increase of 19.1 % from 10 to 30 min.

3.1.3. SDS-PAGE profiles

In addition to demonstrating the effectiveness of SF modification through the increase in free —SH groups and free amino groups, SDS-PAGE was used to further analyze the occurrence of protein cleavage. Fig. 2 presents the SDS-PAGE profiles of proteins in UMSF and modified SF. This study showed that under nonreducing conditions, it was evident that proteins modified with cysteine, GSH, and sodium sulfite had lower overall molecular weight than UMSF groups. In particular, the introduced modifiers have weaker band intensity over 100 kDa but higher band intensity at 25–37 kDa, which suggests that these modifiers partially cleave intermolecular disulfide bonds, thereby reducing the average molecular weight of the protein. Similarly, sodium sulfite reduces the molecular weight of pea proteins (Shen et al., 2022). In addition, another study showed that cysteine could cleave the disulfide bonds of α and α' subunits, reduce the molecular weight of soy protein, and reduce the viscosity of SPI-based FFS (Jiang et al., 2023). In this study, the addition of GSH promoted the cleavage of SF protein and reduced the molecular weight of SF protein, which was related to the fact that GSH also contained cysteine.

According to relevant reports, the 7S globulin (β-conglycinin) and 11S globulin (glycinin) are the two major components in soy protein (Petruccelli & Anon, 1994). β-Conglycinin is formed by three subunits, namely, α', α, and β, with molecular weights of 72, 68, and 52 kDa, respectively. Glycinin is formed by acidic (A) subunits (34–43 and 10–15 kDa) and basic (B) subunits (18–25 kDa). The current study showed that SF-Cys group had higher 11S (A, 34–43 kDa) and 11S (B, 18–25 kDa) band intensities than SF-GSH or SF-SS groups. The results indicated that cysteine modification produced more small-molecule proteins than GSH or sodium sulfite, which was also consistent with the results showing higher free —SH content and free amino content in cysteine-modified SF. However, a study by Abtahi and Aminlari (1997) showed that sodium sulfite was more effective than cysteine because it broke soy protein disulfide bonds, resulting in the loss of larger molecular weight bands and the appearance of new bands of smaller molecules. This difference depends on several factors, such as the level of

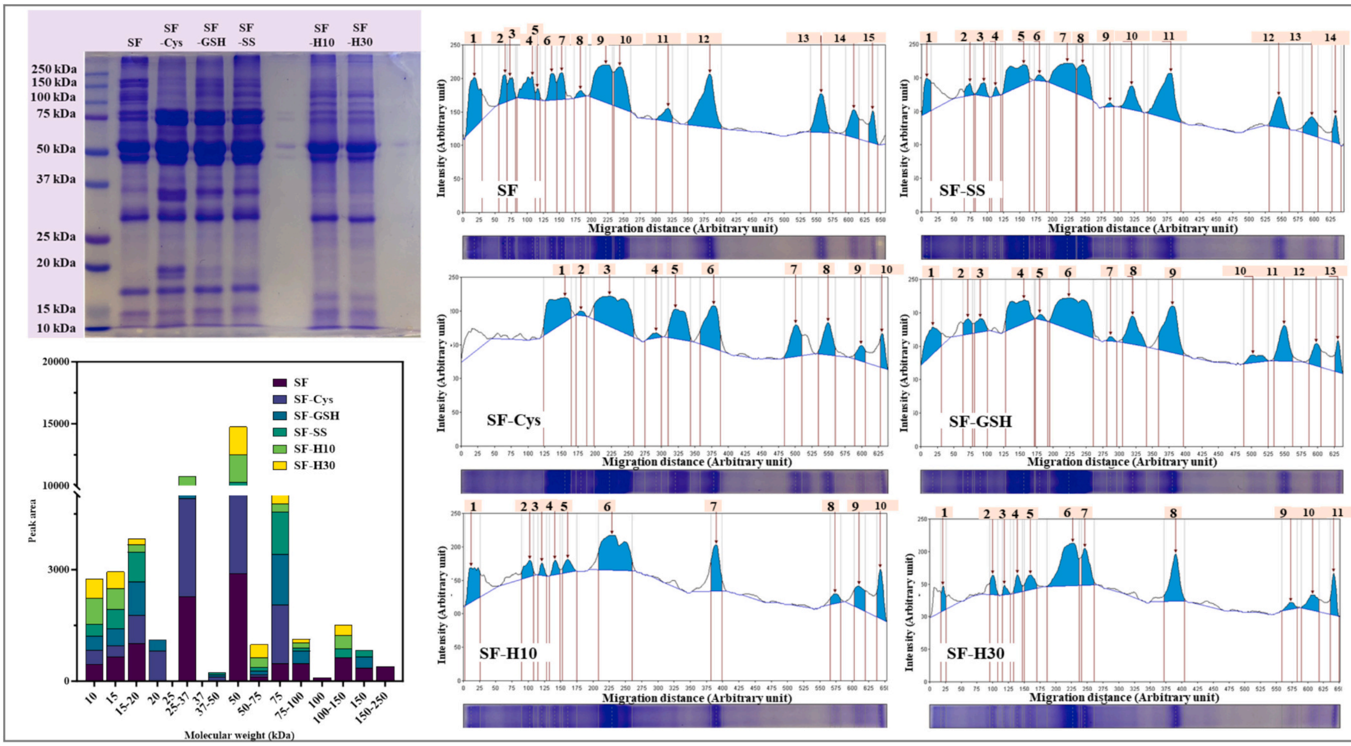

Fig. 2. SDS-PAGE of unmodified SF or modified SF by cysteine, GSH, sodium sulfite, and Flavourzyme. Note: SF = soy flour; SF-Cys = soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS, soy flour-sodium sulfite; SF-H10 = soy flour-hydrolysis, 10 min; SF-H30, soy flour-hydrolysis, 30 min.

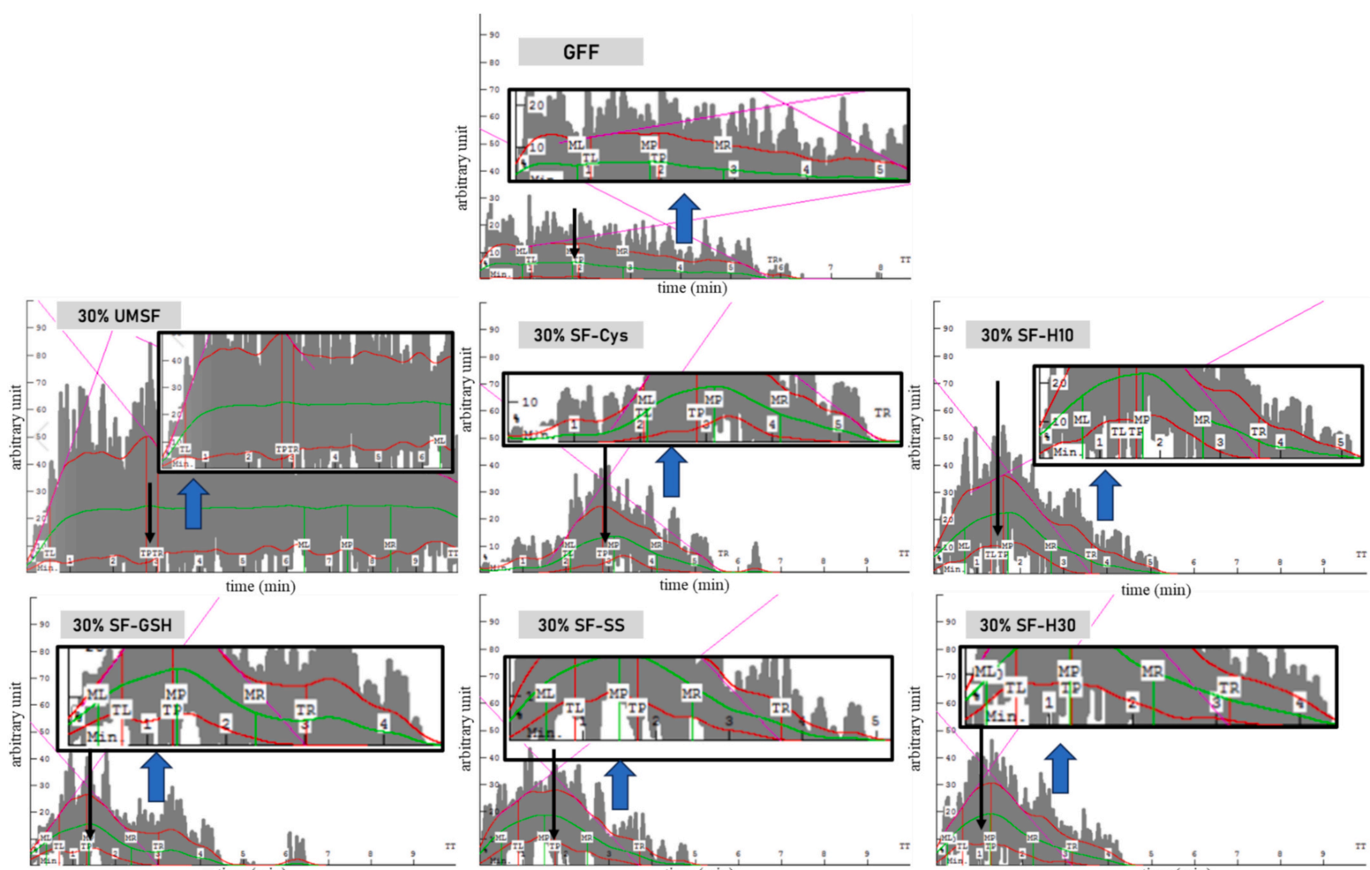

Fig. 3. Mixographs of GF cookie dough with different modified SF. Note: GFF = gluten-free flour; UMSF = unmodified soy flour; SF-Cys, soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS = soy flour-sodium sulfite; SF-H10 = soy flour-hydrolysis, 10 min; SF-H30 = soy flour-hydrolysis, 30 min.

added modifiers, protein type, and reaction conditions, indicating that effective modification methods should be selected for different raw materials. The protein in SF-H group exhibited low molecular weight bands, with the lowest molecular weight band obtained after 30 min of hydrolysis. Following hydrolysis by Flavourzyme for either 10 or 30 min, there was a notable decrease in the band intensity ranging from 15 to 37 kDa and greater than 50 kDa, accompanied by an increase in bands of 10 and 15 kDa. These results are expected given that Flavourzyme has endopeptidase and exopeptidase activities, which can accelerate protein hydrolysis to produce small molecular weight proteins or peptides (Yang et al., 2020). Overall, compared with different modifiers, Flavourzyme is more efficient in hydrolyzing soy protein, and more small-molecule proteins are obtained after 30 min of hydrolysis. This may favor GFF in forming a more uniform dough structure and improving protein digestibility.

3.2. Effects of SF on GF dough properties

3.2.1. Mixing properties

The mixing process plays a crucial role in dough-based product manufacturing, facilitating the incorporation of flour, water, and any additional ingredients into a unified mass. Fig. 3 presents the mixographs of GF cookie dough with different modified SF. In addition, Table 2 presents the dough strength, peak time, and peak value of the doughs. The mixing peak time can reflect the mixing tolerance of the dough. Results presented in Table 2 show that GFF containing 30 % UMSF exhibited a high peak time value (7.26 min), indicating strong dough consistency and stability, which might have a negative impact on the quality of the cookies because it could limit the spread of the dough (Park et al., 2015). A significant decrease ($p < 0.05$) in the peak time of dough was observed upon the addition of modified SF. These results signified a reduction in dough consistency and stability during the mixing process, which reflected a diminished ability of the dough to withstand mechanical stress and maintain its structural integrity during the mixing process. Srikanlaya et al. (2018) explored the effects of soy protein on the mixing properties of GFF. In addition, their study showed that high peak time values were mainly related to strong protein interactions caused by high protein content. This relationship is further supported by the observed peak time difference between SF-H30 (1.85 min) and SF-H10 (0.92 min), indicating a longer peak time associated with stronger protein interactions.

The mixograph 1 min right of the peak represents the behavior of the dough immediately after reaching its maximum resistance during mixing, the width value in mixograph analysis is the shear resistance of the dough during mixing, and the integral value refers to the area under the curve, which is a measure of the total energy required for mixing the dough over time. Results indicate that adding UMSF achieved the highest width and integral values, whereas adding modified SF exhibited a significant reduction ($p < 0.05$) in width and integral values. This indicated that the shear resistance and total energy required for mixing were reduced when SF was subjected to various treatments, which was closely related to the pretreatment effects on soy protein. In particular, cysteine reduced disulfide bonds between proteins and weakened the network of the dough, thereby reducing the elastic (solid-like) component of the dough, helping it relax and reducing mixing time (Angioloni & Dalla Rosa, 2007). GSH was believed to participate in the –SH group/disulfide bond exchange reaction, which caused the dough to exhibit reduced elasticity and weakened structure (Joye et al., 2009). In addition, sodium sulfite could destroy disulfide bonds and induce the formation of free —SH groups, thus weakening the dough structure (Schmid et al., 2017).

Moreover, when comparing the different treatments of SF, it was found that apart from the SF-H10 and SF-GSH groups, which showed significant differences ($p < 0.05$) in peak time, peak value, and integral, there were no significant differences ($p > 0.05$) observed among the other groups in terms of WAC and mixing properties. In addition, this indicated that the duration of Flavourzyme treatment of SF was an important parameter to consider. The current study only examined treatment durations of 10 and 30 min, and future research could explore more refined hydrolysis times to optimize the SF modification process.

Table 2
Mixing properties of GFF containing different SF profiles.

	Mixograph peak		Mixograph 1 min right of the peak	
	Time, min	Value, %	Width, %	Integral, % Tq*Min
GFF	–	–	–	–
30 % UMSF	7.26 ± 0.23^{a}	25.42 ± 0.82^{a}	37.29 ± 0.90^{a}	187.02 ± 4.98^{a}
30 % SF-Cys	1.63 ± 0.51^{bc}	16.54 ± 3.31^{bc}	13.05 ± 0.61^{b}	28.95 ± 5.06^{c}
30 % SF-GSH	0.65 ± 0.13^{c}	11.16 ± 2.14^{c}	11.94 ± 1.98^{b}	13.97 ± 4.11^{c}
30 % SF-SS	0.95 ± 0.16^{bc}	15.60 ± 1.35^{bc}	12.12 ± 2.84^{b}	22.96 ± 2.99^{c}
30 % SF-H10	1.85 ± 0.20^{b}	21.88 ± 1.08^{ab}	20.16 ± 3.21^{ab}	50.03 ± 4.43^{b}
30 % SF-H30	0.92 ± 0.01^{bc}	15.66 ± 0.11^{bc}	15.04 ± 1.79^{b}	22.56 ± 0.21^{c}

Note: GFF = gluten-free flour; UMSF = unmodified soy flour; SF-Cys = soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS = soy flour-sodium sulfite; SF-H10 = soy flour-hydrolysis, 10 min; SF-H30, soy flour-hydrolysis, 30 min. Different letters within each property denote significant differences ($p < 0.05$), same letters within each property denote no significant differences ($p > 0.05$).

3.2.2. GF dough texture

Textural parameters can be further used to estimate the dough quality and rheological properties (Liu et al., 2021). Dough stickiness, cohesiveness, and adhesiveness determine the subsequent processing characteristics. This study analyzed the effects of different formulas and SF on the stickiness, cohesiveness, and adhesiveness properties of GF dough, and results are presented in Table 3.

Table 3
GF cookie dough texture properties with soy flour incorporation from different formulations and pretreatments.

Formulation	Stickiness, N	Adhesiveness, N•s	Cohesiveness
GFF	0.362 ± 0.0135^{a}	0.0159 ± 0.0008^{a}	0.68 ± 0.06^{a}
30 % UMSF	0.207 ± 0.0222^{d}	0.0054 ± 0.0007^{d}	0.35 ± 0.02^{b}
30 % UMSF-2 × water	0.267 ± 0.0060^{c}	0.0072 ± 0.0006^{d}	0.44 ± 0.05^{b}
30 % UMSF-1.5 × sugar	0.179 ± 0.0188^{d}	0.0045 ± 0.0004^{d}	0.31 ± 0.02^{b}
30 % UMSF-1.5 × shortening	0.184 ± 0.0199^{d}	0.0048 ± 0.0006^{d}	0.34 ± 0.04^{b}
Combination	0.291 ± 0.0072^{bc}	0.0103 ± 0.0007^{c}	0.62 ± 0.05^{a}
30 % SF-Cys	0.343 ± 0.0190^{ab}	0.0144 ± 0.0014^{ab}	0.75 ± 0.13^{a}
30 % SF-GSH	0.348 ± 0.0122^{a}	0.0150 ± 0.0009^{ab}	0.74 ± 0.07^{a}
30 % SF-SS	0.326 ± 0.0051^{ab}	0.0129 ± 0.0006^{bc}	0.63 ± 0.01^{a}
30 % SF-H10	0.348 ± 0.0274^{a}	0.0143 ± 0.0017^{ab}	0.73 ± 0.08^{a}
30 % SF-H30	0.357 ± 0.0249^{a}	0.0149 ± 0.0013^{ab}	0.68 ± 0.03^{a}

Note: GF = gluten-free; GFF = gluten-free flour; UMSF = unmodified soy flour; SF-Cys = soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS = soy flour-sodium sulfite; SF-H10 = soy flour-hydrolysis, 10 min; SF-H30, soy flour-hydrolysis, 30 min. Different letters within each property denote significant differences ($p < 0.05$), same letters within each property denote no significant differences ($p > 0.05$).

Dough stickiness is a serious and common problem during bakery product production, indicating the adhesion of the dough when it contacts the surface (Basri et al., 2020). Results presented in Table 3 indicate that the addition of SF in any form reduced the stickiness of GF dough compared with that of the control group (100 % GF). When 30 % UMSF was added to GFF, an increase in moisture content resulted in a significant increase in dough stickiness ($p < 0.05$). However, increasing shortening and sugar content had no significant ($p > 0.05$) effects on dough stickiness, indicating that moisture plays a dominant role in influencing GF dough stickiness.

The formula of the combination group (2× water, 1.5× shortening, and 1.5× sugar) was the same as that of the modified SF; therefore, the effects of the modifiers on the stickiness of GF dough could be compared. Compared with the combination group (2× water, 1.5× shorting, and 1.5× sugar), results indicate that the use of GSH and Flavourzyme significantly increased GF dough stickiness ($p < 0.05$). Relevant reports have shown that the water content in dough affected its stickiness (Hamed et al., 2015). In this study, the cysteine-, GSH-, and sodium sulfite–modified SF changed the WAC value of GF dough to varying degrees, which was an important factor in its stickiness.

In the bakery industry, the adhesion of dough is a critical factor, influencing various aspects of production and product quality. In particular, it is crucial to manage the adhesive characteristics of dough during kneading to prevent excessive sticking of the dough to the equipment. Results indicate that the addition of 30 % UMSF reduced the adhesiveness of GF dough compared with that of the 100 % GFF group. Further changes in a single variable (water, sugar, or shortening content) had no significant effect on the cohesiveness of GF dough in the 30 % UMSF addition group ($p > 0.05$). However, changing the contents of three variables (water 2×, sugar 1.5×, and shortening 1.5×) at the same time significantly increased the stickiness of GF dough ($p < 0.05$), indicating an interaction between water, sugar, and shortening. In addition, from the results, based on the combined formula (2× water, 1.5× shortening, and 1.5× sugar), adding modified SF further increased the stickiness of GF dough. Cohesiveness describes the difficulty with which the internal structure is broken down (Mamat & Hill, 2014). Compared with the 100 % GF group, the addition of 30 % UMSF (except the combination group) significantly reduced ($p < 0.05$) the dough cohesiveness, indicating a more fragile dough formed (Anggraeni et al., 2024). Compared with the 30 % UMSF group, changing the proportion of water (2×), sugar (1.5×), or shortening (1.5×) alone had no significant effect ($p > 0.05$) on dough cohesiveness. Compared with the combination group, adding modified SF had no significant effect ($p > 0.05$) on GF dough cohesiveness. Generally, adding 30 % UMSF significantly ($p < 0.05$) reduced the stickiness, adhesiveness, and cohesiveness of GF dough, whereas adding modified SF had almost no significant ($p > 0.05$) effect on the stickiness, adhesiveness, and cohesiveness of GF dough.

3.3. Effects of SF on GF cookie properties

3.3.1. Baking loss and moisture content

We further analyzed the baking quality of GF cookies, such as baking loss, cookie moisture content, spread ratio, hardness, and fracturability, and the results are presented in Table 4. Results indicate that adding 30 % modified or UMSF reduced baking loss compared with the GFF group. When adding 30 % UMSF, further using two times water resulted in an increased baking loss, whereas adjusting the proportion of sugar or shortening in flour did not affect baking loss, indicating that baking loss was primarily caused by water evaporation. However, the modification of SF increased baking losses to varying degrees (particularly in the 30 % SF-SS and 30 % SF-H30 groups, $p < 0.05$), which was related to the modifier that reduced the WAC of SF. In addition, the moisture content of cookies showed a similar trend to that of baking loss, i.e., adding 30 % UMSF or modified SF increased or decreased the moisture content of GF cookies.

Table 4
GF cookie baking loss, spread ratio, hardness, and fracturability.

	Baking loss, %	Cookie moisture content, %	Spread ratio	Hardness, N	Fracturability, mm
GFF	13.1 ± 0.4^{a}	4.6 ± 0.0^{d}	9.2 ± 0.1^{f}	38.62 ± 2.14^{cd}	46.6 ± 0.3^{ab}
30 % UMSF	8.8 ± 0.3^{cde}	7.3 ± 0.0^{b}	5.5 ± 0.1^{h}	57.70 ± 5.85^{b}	46.6 ± 0.4^{ab}
30 % UMSF-2 × water	11.0 ± 0.3^{abcd}	8.2 ± 0.0^{a}	5.7 ± 0.1^{h}	48.13 ± 3.51^{bc}	46.0 ± 0.5^{b}
30 % UMSF-1.5 × sugar	8.2 ± 0.3^{e}	5.7 ± 0.1^{c}	6.5 ± 0. 1^{g}	99.31 ± 6.05^{a}	46.7 ± 0.2^{ab}
30 % UMSF-1.5 × shortening	9.0 ± 0.1^{bcde}	5.9 ± 0.1^{c}	6.7 ± 0.1^{g}	40.66 ± 4.60^{c}	47.3 ± 0.3^{a}
Combination	8.6 ± 0.7^{de}	6.9 ± 0.1^{b}	10.1 ± 0.1^{e}	12.26 ± 2.07^{f}	42.3 ± 0.5^{c}
30 % SF-Cys	9.9 ± 0.9^{bcde}	3.8 ± 0.2^{e}	22.8 ± 0.3^{a}	22.83 ± 2.46^{ef}	40.8 ± 1.0^{d}
30 % SF-GSH	11.2 ± 0.9^{abcd}	3.5 ± 0.1^{ef}	21.7 ± 0.3^{b}	18.79 ± 1.44^{ef}	41.6 ± 0.2^{cd}
30 % SF-SS	11.4 ± 0.7^{abc}	3.2 ± 0.1^{fg}	19.1 ± 0.0^{cd}	28.23 ± 2.22^{de}	41.9 ± 0.2^{cd}
30 % SF-H10	10.1 ± 1.7^{bcde}	3.4 ± 0.1^{ef}	18.7 ± 0.1^{d}	26.83 ± 5.58^{e}	42.1 ± 0.1^{c}
30 % SF-H30	11.6 ± 1.9^{ab}	2.8 ± 0.2^{g}	19.4 ± 0.1^{c}	26.30 ± 3.31^{e}	42.3 ± 0.1^{c}

Note: GF = gluten-free; GFF = gluten-free flour; UMSF = unmodified soy flour; SF-Cys = soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS = soy flour-sodium sulfite; SF-H10 = soy flour-hydrolysis, 10 min; SF-H30, soy flour-hydrolysis, 30 min. Different letters within each property denote significant differences ($p < 0.05$), same letters within each property denote no significant differences ($p > 0.05$).

3.3.2. Spread ratio and cookie texture

The spread ratio determines the quality of the cookies, and generally high-spread ratio cookies are desired by consumers because they correspond to larger diameters (Anggraeni et al., 2024). The results presented in Table 4 indicate that adding 30 % UMSF significantly reduced ($p < 0.05$) the spread ratio of GF cookies. However, adding 30 % modified SF significantly increased ($p < 0.05$) the spread ratio of GF cookies, which was more than twice that of 100 % GF cookies. Obviously, this can be attributed to the nature of the dough. In dough mixing tests, we found that adding modified SF significantly reduced ($p < 0.05$) the dough toughness and consistency, whereas 30 % UMSF group showed an extremely high mixing peak time (7.26 ± 0.23 min), which affected dough expansion.

Hardness is the maximum force necessary to break the cookies (Gan et al., 2023). Results indicate that adding 30 % SF significantly increased ($p < 0.05$) the hardness of cookies. In addition, adjusting the flour formula could increase the hardness of GF cookies, particularly when the amount of sugar was increased by 50 %, the hardness value reached as high as 99.31 N ($p < 0.05$). However, results indicate a significant decrease ($p < 0.05$) in hardness when the amounts of water (2×), sugar (1.5×), and shortening (1.5×) were changed at the same time, indicating that these three raw materials significantly impact the hardness of GF cookies. A moderate hardness can give consumers a good sensory experience, whereas a hardness that is too high will make it difficult to chew, and a hardness that is too low will negatively affect the taste of the cookies. Results indicate that after adding modified SF, the hardness of GF cookies was significantly reduced ($p < 0.05$) compared with the 30 % UMSF group but increased compared with the combination group, indicating that adding modified SF can maintain the hardness of cookies in a moderate state, which was conducive to improving the quality of cookies. The effect of adding SF on the hardness of GF cookies could be attributed to increased protein content because similar studies have shown that incorporating high-protein ingredients into cookies, such as concentrated protein and emulsifier mixtures and buckwheat flour, could significantly reduce ($p < 0.05$) the hardness of GF cookies (Sarabhai et al., 2015). Moreover, different modification methods have no significant ($p > 0.05$) effects on the hardness of GF cookies.

Fracturability is the tendency of cookies to fracture when applying a relatively small amount of force or impact (Gan et al., 2023). Results indicate that adding UMSF or changing the formula of a single raw material had no significant impacts ($p > 0.05$) on the fracturability of GF cookies, whereas changing the formula of three raw materials at the same time or adding modified SF significantly reduced ($p < 0.05$) the fracturability of GF cookies. High fracturability of cookies typically causes challenges in industrial processing such as packaging and distribution (Gan et al., 2023); therefore, this study showed that the addition of SF may address this concern. Comparing the effects of different modified SF on the fracturability of GF cookies, the results indicate that the fracturability of the 30 % SF-H30 group was significantly higher than that of the 30 % SF-Cys group ($p < 0.05$), whereas there were no significant effects between other groups ($p > 0.05$). These results could be attributed to the moisture content of GF cookies because the moisture content of the 30 % SF-H30 group was lower than that of the 30 % SF-Cys group, resulting in the higher fracturability of the 30 % SF-H30 cookies.

3.3.3. Cookie color

Cookie color is a vital component of attraction for consumers and is primarily developed during the later stages of baking. The L* value is the level of lightness or darkness of the cookies. L* = 0 represents black, while L* = 100 indicates white (Yang et al., 2022). Results presented in Fig. 4 indicate that adding 30 % UMSF had no significant effect ($p > 0.05$) on L* and a* of GF cookies but significantly increased ($p < 0.05$) the b* value. On this basis, further changing the water content significantly increased the L* value, whereas changing the sugar and shortening content had no effect ($p > 0.05$) on the color of GF cookies. This indicates that water content had an important impact on the color of GF cookies. According to relevant research, browning occurs during cookie baking when the water activity reaches ~0.7. Therefore, increased water content in cookie dough requires more time for the cookies to reach the water activity value corresponding to the maximum Maillard reaction rate, thus delaying the occurrence of browning and increasing the L* value (Yang et al., 2022).

After adding the modified SF, the L* value of GF cookies decreased to varying degrees. Particularly, the 30 % SF-H30 group exhibited the lowest L* value and b* value and the highest a* value, which showed a browner cookie (Fig. 4). During the baking process, the surface color of cookies changes owing to the Maillard reaction between reducing sugars and amino acids, as well as starch dextrinization and sugar caramelization (Tamanna & Mahmood, 2015). The Maillard reaction occurs between a carbonyl group (reducing sugar, aldehyde, or ketone) and an amino compound (protein, peptide, or amino acid) (Ni et al., 2022). According to relevant studies, higher a* values and lower L* values are indicators of Maillard browning (Lara et al., 2011), indicating that adding 30 % SF-H30 might promote the Maillard reaction during the baking process. Compared with the combination group, adding 30 % SF-Cys, SF-GSH, and SF-SS also generally reduced the L* and b* values and slightly increased the a* value, which made the GF cookies more caramel colored. Overall, the obtained results indicate that the addition of SF promoted baking coloration, which increased the sensory attractivity of GF cookies.

3.4. PCA

PCA was conducted to further understand the associations among

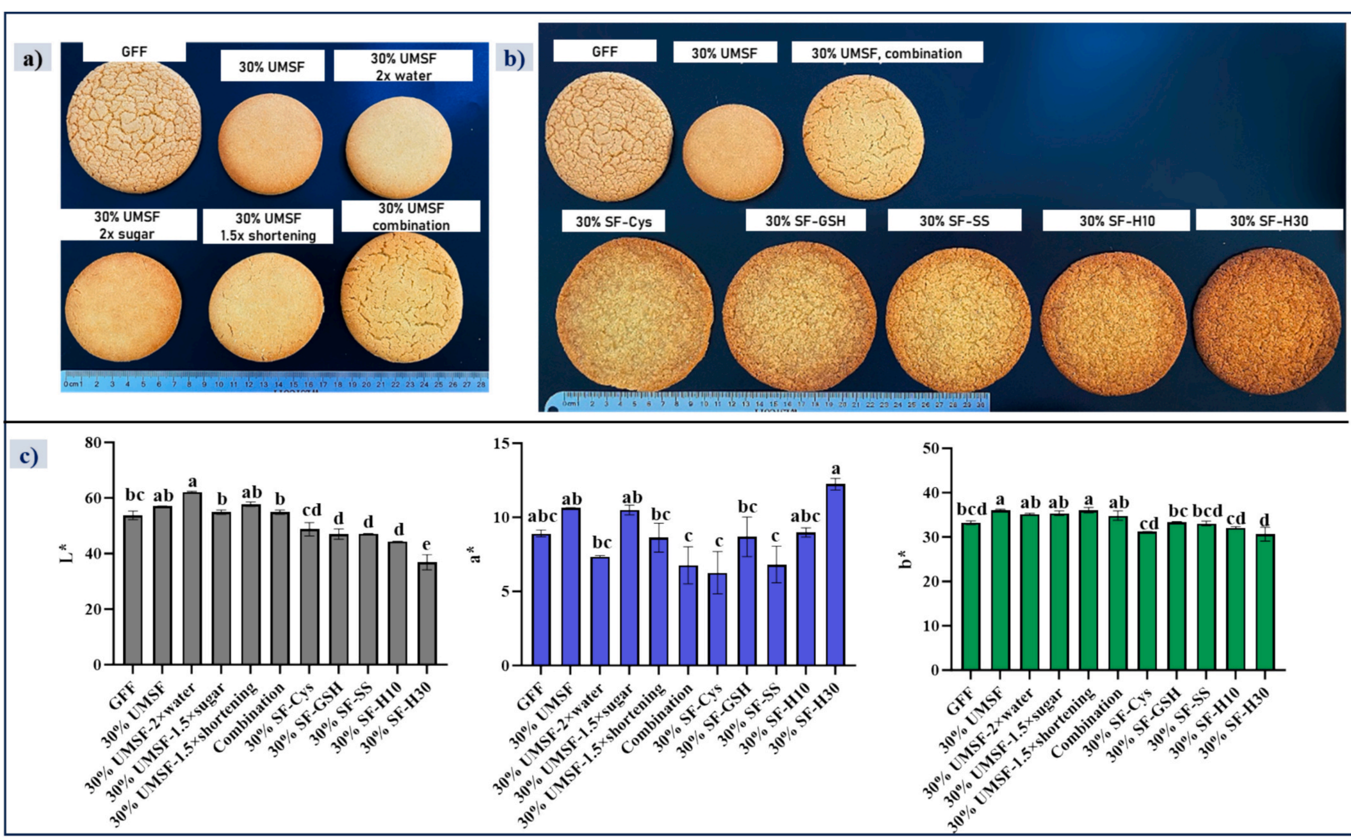

Fig. 4. Appearance and L*, a*, and b* values of GF cookies. Note: Different letters within each property denote significant differences ($p < 0.05$); same letters within each property denote no significant differences ($p > 0.05$). Note: GFF = gluten-free flour; UMSF = unmodified soy flour; SF-Cys = soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS = soy flour-sodium sulfite; SF-H10 = soy flour-hydrolysis, 10 min; SF-H30, soy flour-hydrolysis, 30 min.

dough and cookie physicochemical properties and SF with different modifiers. As shown in Fig. 5, The principal component 1 and the principal component 2 interpreted 68.56 % and 14.14 % of the variability, respectively, with a total of 88 %. The PCA results further confirmed that the addition of 30 % modified SF (by cysteine, sodium sulfite, and GSH) is closely related to the properties of GF dough and cookies, which are strongly positively related to dough cohesiveness, stickiness, and adhesiveness and negatively related to GF cookie fracturability and hardness. The light color of cookies is positively correlated with UMSF, whereas the dark color is strongly correlated with enzymatic SF. In addition, 100 % GF shows the strongest positive correlation with baking loss, indicating that adding SF effectively reduces the baking loss. Further, a Pearson correlation coefficient test was conducted to analyze the correlation between cookie quality test parameters, and the results are presented in Table S1. Results indicate a highly significant positive correlation ($p < 0.05$) between the cookie dough stickiness ($r = 0.98341$), adhesiveness ($r = 0.96637$), and cohesiveness ($r = 0.97097$). Stickiness, adhesiveness, and cohesiveness of the cookie dough are positively correlated with cookie baking loss and spread ratio ($p < 0.05$) and negatively correlated with cookie moisture content, hardness, fracturability, L*, a*, and b* ($p < 0.05$). Moreover, the results indicate that the quality of dough formation determines the cookie quality to the greatest extent, whereas the baking loss during the baking process has no significant correlation with the cookie quality (hardness, crispness, and L*, a*, and b*; $p > 0.05$). Cookie color (L* and b*) is closely related to various indicators ($p < 0.05$), such as stickiness, adhesiveness, cohesiveness, moisture content, spread ratio, and fracturability, whereas the a* value has no significant correlation with these indicators ($p > 0.05$).

Overall, incorporating modified SF into GF cookies improved their quality compared with other options. Specifically, modified SF enhanced the extensibility of GF cookies, maintained a moderate level of hardness, reduced fracturability, and improved their color. Among the modified SFs tested, 30 % SF-Cys was the most effective. This addition not only preserved the desirable hardness and color of GF cookies but also enhanced the spread ratio and minimized fracturability. Thus, 30 % SF-Cys is the most suitable choice for achieving the best-quality GF cookies.

4. Conclusions

This study investigated the impact of modifying SF with cysteine, GSH, sodium sulfite, and Flavourzyme, as well as variations in raw material formulations (water, shortening, and sugar) on the properties of GF dough and cookies. Incorporating 30 % modified SF (via cysteine, GSH, sodium sulfite, and Flavourzyme) into GF flour enhanced dough properties, which can be attributed to alterations in SF protein properties, such as the free –SH groups, free amino groups, and protein molecular weight. Among the modified SFs, incorporating 30 % SF-Cys significantly increased the cookie spread ratio, which increased from 9.2 to 22.8 ($p < 0.05$). In addition, using 30 % hydrolyzed SF substantially reduced baking loss from 13.1 % to 10.1 % ($p < 0.05$). Moreover, the addition of modified SF maintained a moderate range of hardness (18.79–28.23 N) and fracturability (40.8–42.3 mm) in GF cookies while

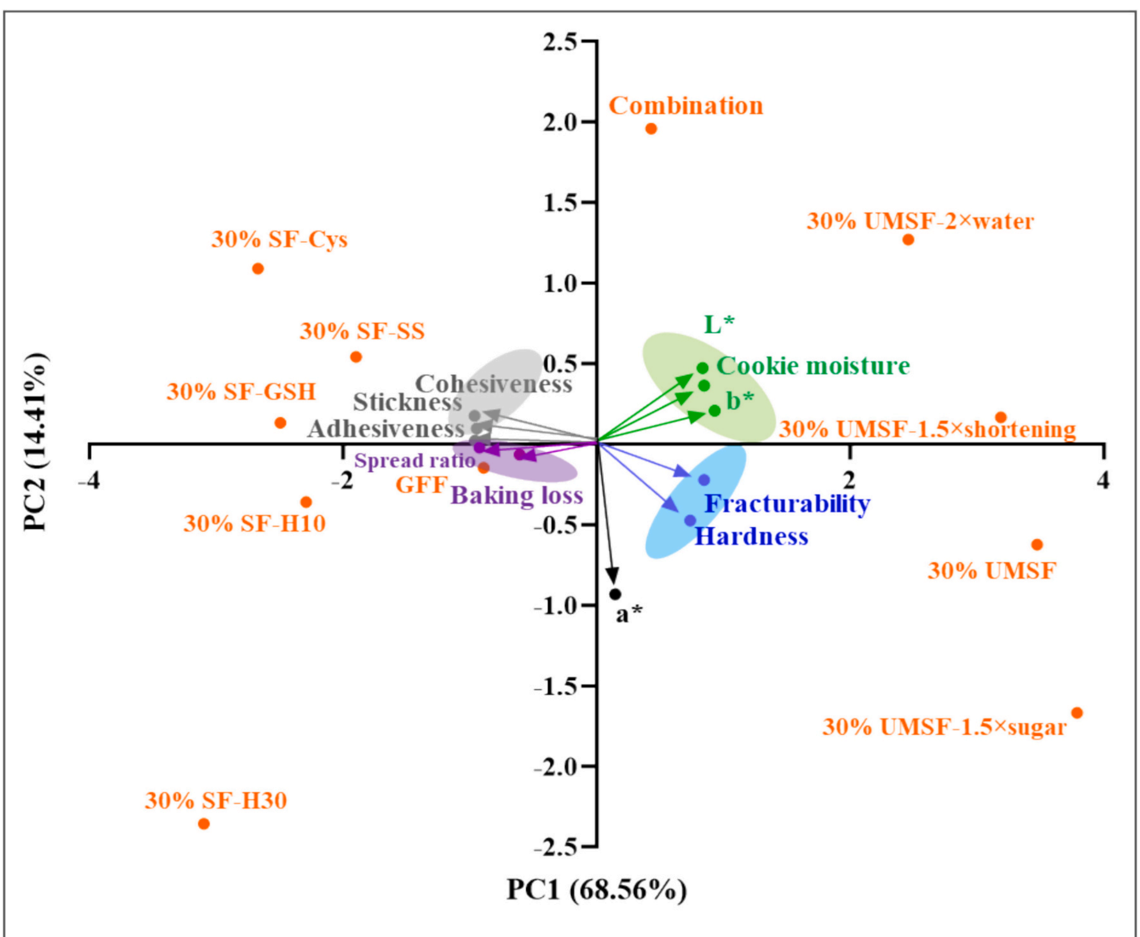

Fig. 5. Principal component analysis (PCA) describing relationships between GF dough and cookie physicochemical properties and different formulations. Note: GFF = gluten-free flour; UMSF = unmodified soy flour; SF-Cys = soy flour-cysteine; SF-GSH = soy flour-glutathione; SF-SS = soy flour-sodium sulfite; SF-H10 = soy flour-hydrolysis, 10 min; SF-H30, soy flour-hydrolysis, 30 min.

enhancing their color, thus markedly improving their overall quality. This study provides important insights into the enhancement of GF cookies. Future work should focus on refining the modification process for SF by exploring a broad range of modifiers and methods, as well as optimizing the proportions of these additives. Considering the potential influence of modified SF on the cookie flavor, sensory evaluations of GF cookies will be conducted in future work, which is crucial to produce GF cookies with high acceptability.

CRediT authorship contribution statement

Yiqin Zhang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Jianjun Zhou:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Wenfei Tian:** Writing – review & editing, Investigation. **Yijie Gui:** Writing – review & editing, Investigation. **Yonghui Li:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Upon the authors' agreement, data can be made available upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.142481.

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