



PLS-R modeling and sensory optimization of functional brownies formulated with spinach and modified cassava flour

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Abstract

This study investigates the complex sensory dynamics of functional brownies formulated with wheat flour, modified cassava flour (mocaf), and spinach powder. The novelty of this research lies in the application of Partial Least Squares Regression (PLS-R) to decode the non-linear interactions and structural trade-offs within a gluten-free matrix, a departure from traditional linear sensory analysis. Formulations varied in flour composition (70-0% wheat, 25-95% mocaf, and 5-10% spinach powder), and were evaluated by 70 untrained panelists using a 7-point hedonic scale. Statistical modeling was performed using R Studio (v2024.12.1+563) with the *pls*, *tidyverse*, and *plsVarSel* packages. Results revealed that the PLS-R model effectively identified wheat flour as the primary driver for texture (VIP=1.343) and aroma (VIP=1.300). Despite low explanatory power ($R^2 < 1.5\%$), the model successfully mapped the antagonistic relationship of spinach antioxidants towards flavor formation and the significant textural compromises in high-mocaf substitutions. These findings provide a multivariate framework for optimizing functional bakery products, emphasizing that wheat remains essential for sensory integrity at substitution levels above 40%.

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INTRODUCTION

Consumer demand for functional foods and locally sourced ingredients has increased significantly, driven by growing awareness of health benefits and environmental sustainability, as well as interest in foods offering nutritional advantages (Baker et al., 2022; Bigliardi & Galati, 2013). This trend has accelerated the development and consumption of functional food products (Alba et al., 2021; Vrgović et al., 2022). Modified cassava flour (Mocaf), a gluten-free alternative, shows potential for replacing wheat flour in various food products such as biscuits, cakes, and noodles, while supporting local economies and reducing wheat imports (Widanti et al., 2021). Mocaf is increasingly used in bakery and pastry products due to its safety for individuals with celiac

disease, autoimmune disorders, memory impairments, and autism, offering broader health benefits compared to gluten-containing wheat flour (Hastati et al., 2024; Matita et al., 2024).

Studies have demonstrated that substituting 10–20% Mocaf in soft rolls improves crust color, porosity, aroma, and elasticity, with 10% substitution yielding optimal overall quality (Nurfiantoro et al., 2023). Similarly, in biscuits, a 65:35 ratio of Mocaf to carrot pulp produced the best sensory and chemical properties, including 5.25% moisture content and 8.83% protein content (Mamentu et al., 2013). However, in steamed bread, substituting wheat flour with Mocaf and adding butterfly pea flower puree affected shape, color, flavor, and texture, with optimal results achieved at 40% Mocaf substitution and 40% puree addition (Nisa' et al., 2024).

In addition to Mocaf-based innovations, incorporating spinach (*Amaranthus* spp.) into bakery products can substantially enhance their nutritional profile and functional properties. Spinach is a source of iron, vitamins A, C, K, and B, as well as minerals such as calcium, magnesium, and zinc (Czarnowska-Kujawska et al., 2022). Research suggests that incorporating heat-stable spinach constituents, such as beta-carotene, zeaxanthin, and lutein, enhances the antioxidant profile of wheat-based products (Junejo et al., 2021). The inclusion of 10–20% spinach in bread recipes not only augments protein, fiber, and mineral levels but also positively affects texture and porosity (Jyoti & Jood, 2020; Prasad et al., 2024). When applied to brownies, fortification seeks to convert a calorie-dense treat into a more healthful option. However, adding spinach powder to brownies poses certain challenges, as it can alter color and flavor, potentially compromising sensory attributes and affecting the development of Maillard reaction products (Melini et al., 2024; Račkauskienė et al., 2019). Furthermore, excessive incorporation of spinach may decrease product acceptability (Jyoti et al., 2022). Consequently, optimizing spinach concentration is crucial to achieve nutritional benefits while preserving preferred sensory characteristics.

While prior studies have explored Mocaf- and spinach-based bakery products, their statistical approaches remain limited to univariate and bivariate analyses (Junejo et al., 2021; Nisa' et al., 2024; Prasad et al., 2024). Notably, the relationship between ingredient composition and consumer preference is inherently multivariate and complex (Kumar et al., 2024; Vabø & Hansen, 2014). Partial Least Squares Regression (PLS-R) is an effective method for predicting consumer sensory responses based on food product composition, as evidenced by its widespread application in food science. PLS-R combines principal component analysis and multiple regression, enabling efficient dimensionality reduction and prediction with complex response data (Cook & Forzani, 2024). In food product research, PLS-R has successfully predicted consumer preferences, ranked sensory variables, and guided production processes (Bi et al., 2019). However, its application in optimizing brownie formulations using local ingredients remains underexplored.

This study aims to (1) analyze the correlation between flour composition (wheat, Mocaf, and spinach powder) and hedonic sensory scores (color, flavor, aroma, texture) using PLS-R, and (2) identify key predictors of panelist acceptability.

METHOD

Sample Preparation

Brownie samples were created using four different formulas (Table 1), with spinach powder concentrations of 5% and 10% chosen based on the findings of Prasad et al. (2024) and Jyoti et al. (2022), which indicated that enrichment within these parameters optimises nutritional value and sensory appeal. The integration of Mocaf as a substitute for wheat flour is observed in the suggestions for functional, gluten-free bakery products provided by Widanti et al. (2021) and

Hastati et al. (2024). Ingredient percentages were determined based on the total weight of the base formulation (Table 2), while the preparation technique referred to the method of Masriani & Fatima (2020) with slight modifications to suit the specific experimental design.

Table 1. Brownie Treatment Formulations

Treatment	Wheat Flour (% w/w)	Spinach Powder (% w/w)	Mocaf (% w/w)
T70B5	70	5	25
T70B10	70	10	20
T0B5	0	5	95
T0B10	0	10	90

Table 2. Base Formulation for Brownie Preparation

Ingredient	Amount (g)
Sugar	314
Cocoa powder	126
Chocolate milk	50
Baking soda	6
Cooking oil	94
Water	94
Eggs	314
Total	1000

Organoleptic Testing

A hedonic test was conducted with 70 untrained panelists, predominantly university students aged 19-23, of diverse gender, chosen to reflect the target consumer demographic for functional snack products. In accordance with the protocol established by Pertuzatti et al. (2015), brownie samples were produced as uniform cubes (2 x 2 x 2 cm) and presented at ambient temperature on plain white plates. Each sample was assigned a randomized three digit code to eliminate bias. The samples were served to each panelist in a randomized sequence, and mineral water was supplied as a palate cleanser between assessments. Panelists evaluated four sensory attributes color, flavor, aroma, and texture utilizing a 7-point scale (1 = 'very dislike' to 7 = 'highly like').

Statistical Analysis

Partial Least Squares Regression (PLS-R) was used for statistical analysis to model the complex relationship between flour ingredient composition and sensory outcomes. PLS-R is a statistical method that identifies patterns in data by finding new variables, called components, that capture the most important relationships between the input data and the output results. The methodology followed that of Bi et al. (2019). PLS-R was chosen for its ability to address multicollinearity, which is inherent to percentage-based formulations. The predictor matrix (X) included standardized percentages of wheat flour, spinach powder, and mocaf. The response matrix (Y) contained standardized hedonic scores for color, flavor, aroma, and texture.

The modeling was performed in RStudio (v2024.12.1+563) using R-Package, namely the *pIs*, *tidyverse*, and *pIsVarSel*. To avert overfitting, a 10-fold cross-validated model was created, with the optimal number of latent components determined by minimizing the Root Mean Squared Error of Prediction (RMSEP) and maximizing Q^2 . Subsequently, the relevance of variables was evaluated

using Variable Importance in Projection (VIP) scores, with values >1 indicating a substantial contribution of a particular flour component to the sensory qualities. Biplots and loading plots were then utilized to highlight predictor-response linkages, thereby facilitating the identification of clustering and correlations. Finally, the model's dependability was further validated through residual analysis, Hotelling's T^2 , and DModX for outlier detection, as well as sensitivity testing via wheat-flour replacement simulations.

RESULTS AND DISCUSSION

Predictive Performance of PLS-R in Modeling Formulation-Sensory Response Relationships

The PLS-R model's predictive capacity indicated a complex functional matrix in the brownie formulation. The extremely low R^2 values (Table 3), each below 1.5%, observed across all sensory attributes, indicate that the interactions among wheat, Mocaf, and spinach powder are not straightforward. This inadequate model fit arises because conventional PLS-R assumes additive effects among components, an assumption that is frequently violated in complex food systems (Kvalheim et al., 2024). Substituting wheat with Mocaf and incorporating spinach powder can engender nonlinear interactions that may manifest as synergistic or antagonistic effects. Studies have shown that the texture, color, and sensory acceptance of wheat-Mocaf blends often exhibit optimal compositions rather than straightforward linear relationships (Prayitno et al., 2018; Yuniartini & Dwiani, 2021). Spinach powder contributes to these intricate transformations by affecting water uptake, fiber concentration, and the Maillard reaction, depending on ingredient proportions (Julaya et al., 2025; Jyoti & Jood, 2020). From a mechanistic perspective, these observed effects likely arise from the nonlinearity of starch gelatinization and the formation of protein-lipid complexes throughout the baking process. This results in a system that simple linear modeling cannot adequately represent (Li et al., 2025). Because the models don't include quadratic or specific cubic terms, interactions are mixed up. This prevents the model from accurately finding the best combinations in the mixture (Dingstad et al., 2004; Squeo et al., 2021). Consequently, these results underscore the need to employ nonlinear methodologies to comprehensively elucidate the intricate effects in highly substituted gluten-free formulations (Squeo et al., 2021).

Table 3. Validation and Cross-Validation for Each Component and Sensory Attribute

Component	Color		Flavor		Aroma		Texture	
	R^2	Q^2	R^2	Q^2	R^2	Q^2	R^2	Q^2
1	0.0026	-0.0043	0.0008	-0.0031	0.0113	0.0002	0.0014	-0.0052
2	0.0052	-0.0054	0.0011	-0.0057	0.0122	0.0020	0.0014	-0.0090

Interestingly, while most attributes exhibited negative Q^2 values, aroma showed a marginal positive predictive power with Q^2 values ranging from 0.0002 to 0.0020. This suggests a more organized and quantifiable relationship between the formulation ratios and the release of volatile compounds from the spinach matrix, as well as the formation of Maillard products. Unlike physical characteristics, volatile Maillard profiles are frequently modeled and linked to sensory odor descriptors and consumer preferences. Consequently, this establishes a more definitive quantitative correlation between chemical precursors and perceived olfactory quality (Cui et al., 2022; Diez-Simon et al., 2019). In this instance, Maillard chemistry is responsible for the brownie's aroma, which is more closely associated with ingredient ratios than with its structural composition (El Hosry et al., 2025; Starowicz & Zieliński, 2019).

The model's inability to explain texture differences ($R^2 = 0.14\%$) shows that rheology shifts greatly when Mocaf is added at high levels (95–100%). At these levels, a complete breakdown of the gluten network and changes in the amylose-amylopectin ratio affect the dough's viscoelasticity (Sun et al., 2023). The link between Maillard chemistry and texture acceptance is less direct and weaker than that between aroma and chemical-to-sensory progression. This is because textural changes, such as protein cross-linking and water binding, are often less noticeable than large structural disruptions (Starowicz & Zieliński, 2019). As a result, the texture of highly substituted gluten-free brownies is shaped by complex ingredient interactions that are poorly documented and hard to quantify with linear predictive models.

Moreover, the negative Q^2 values associated with color, flavor, and texture indicate substantial evaluator noise and intrinsic fluctuations in sensory evaluation (Ayele et al., 2022). This variability is primarily attributed to the subjective perspectives of untrained panelists, who often struggle to develop consistent sensory terminology and frequently use imprecise descriptors when evaluating unconventional formulations (de Kock & Magano, 2020; Laignier et al., 2022). Such challenges become even more pronounced with spinach-enriched brownies, where the considerable departure from the wheat-based standard, characterized by an unusual green coloration and a denser crumb structure, introduces a level of variance that exceeds the model's predictive capabilities. These panels frequently demonstrate low repeatability, as preconceived notions and unfamiliarity with gluten-free (GF) profiles can obscure genuine sensory distinctions or reflect consumer bias rather than inherent quality (Louis et al., 2019; Pešić et al., 2023). Consequently, the sensory quality of highly substituted GF products appears to be influenced by complex synergistic interactions that linear modeling cannot adequately address.

The interaction among spinach, protein denaturation, and Mocaf starch retrogradation forms a complex system in which ingredients compete for water during gelatinization (Aussanasuwannakul & Singkammo, 2025). Notably, the unpredictability in sensory evaluation described earlier is also rooted in such complex material interactions. The heat-induced unfolding of spinach proteins creates a viscoelastic network that integrates with the starch gel, significantly affecting the crumb's elasticity and its breaking behavior (Bresciani et al., 2022; Li et al., 2025). Furthermore, while some proteins can slow retrogradation and maintain softness, high substitution levels without a balanced hydrocolloid system often lead to excessive hardness and rapid staling (Bi et al., 2019; Ziobro et al., 2016). Addressing these multi-faceted changes in material flow and the potential for off-flavors associated with concentrated plant proteins thus requires non-linear analytical methodologies. These methodologies encompass descriptive analysis utilizing trained panels and machine learning models, which are employed to thoroughly investigate the relationship between structural properties and sensory attributes (Ayres et al., 2023; Ha et al., 2025; Hesso et al., 2015).

Functional Roles of Flour Ratios in Sensory Response Profiles

Texture (VIP=1.343) and aroma (VIP=1.300) (Table 4) were the primary drivers of sensory differentiation across the brownie formulations, as indicated by the PLS-R model. This distinction is inherently connected to the presence or absence of a functional gluten matrix. Variable Importance in Projection (VIP) scores supported the structural and sensory dominance of wheat flour, with all hedonic attributes exceeding a score of 1.0. The biplot's first component (Figure 1), accounting for 70% of the variance, further clarified this, showing strong positive loadings (0.5–1.0) for wheat, which led to the T70B5 and T70B10 formulations clustering together. This clustering emphasizes wheat's essential role in forming elastic gluten networks and promoting consistent crumb structures through starch-lipid interactions (Martínez et al., 2025).

Tabel 4. Predictor Importance Rankings for Hedonic Attributes

Variable	Color	Flavor	Aroma	Texture
Wheat flour	1.005	1.163	1.300	1.343
Spinach powder	0.994	0.805	0.556	0.444

Conversely, the analysis reveals a significant trade-off between functional improvement and sensory acceptability. The wheat-free formulations (T0B5/T0B10) were located in the negative quadrants of the biplot, suggesting textural inadequacies and less desirable flavor profiles. While spinach powder contributes to color acceptances (VIP=0.994), its use at levels exceeding 10% demonstrated a negative correlation with flavor and aroma. This contrasting positioning suggests that the antioxidants in spinach may inhibit the formation of volatile Maillard compounds, thereby reducing flavor and color deterioration (Melini et al., 2024; Račkauskienė et al., 2019). Furthermore, the increase in moca and spinach concentrations was associated with a roughly 60% reduction in dough strength, thereby supporting the idea that enhanced nutritional density often compromises structural integrity and consumer preference (Herdiana et al., 2024).

These integrated findings support a strategic approach to the development of functional brownies. Optimizing the ratios of spinach and moca is crucial for achieving a balance between nutritional value and consumer acceptance (Asghari-pour et al., 2020), potentially through methods like moca pre-gelatinization to restore functional properties (Hong & Liu, 2023). From a methodological perspective, the interplay between VIP scores and biplot visualization highlights the importance of interdisciplinary methods in food analysis. VIP scores quantify the contribution of each flour component, whereas biplots elucidate hidden interactions, thereby facilitating the exploration of underutilized compositional areas. This integrated analytical approach not only confirms the sensory importance of wheat but also offers a framework for the strategic application of alternative flours in the development of sustainable food products.

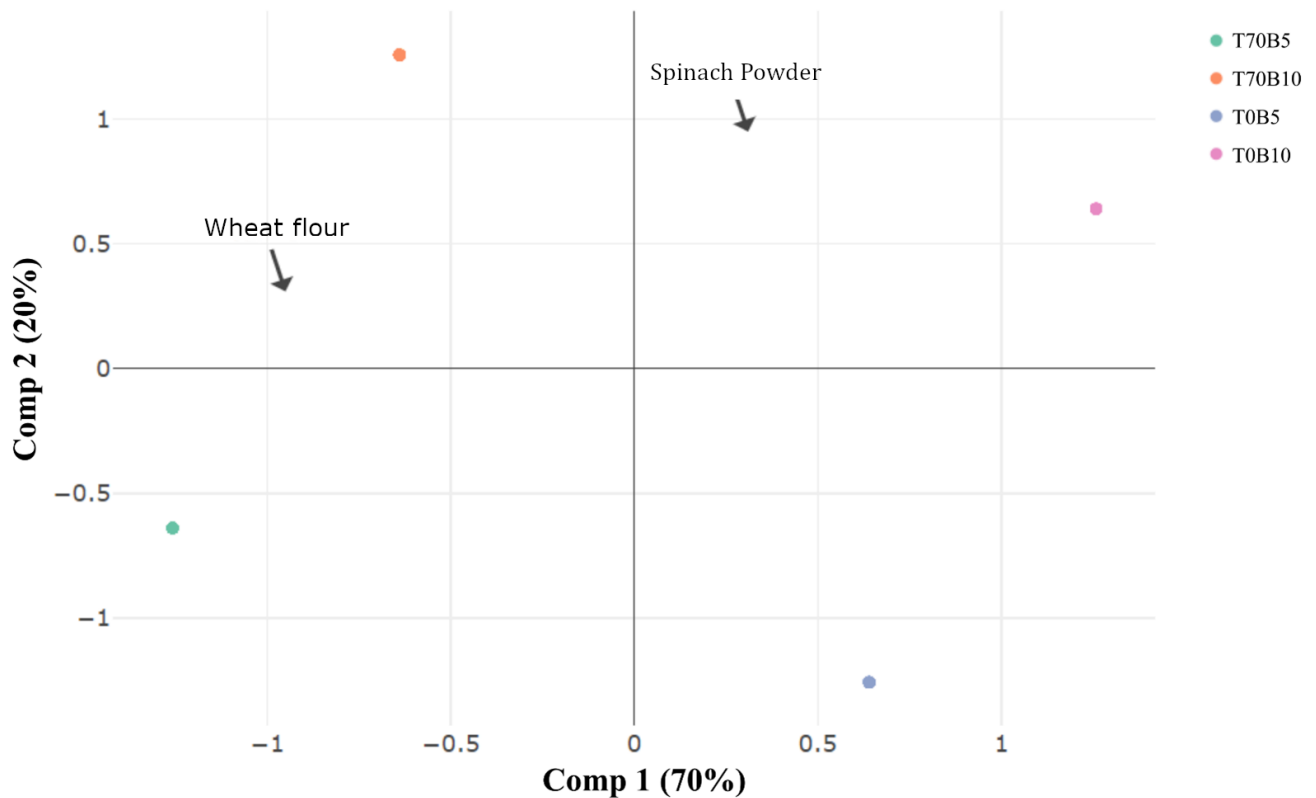


Figure 1. PLS Component 1 vs. Component 2 biplot showing: (a) Formulations (T70B5, T70B10, T0B5, T0B10) as points, (b) Sensory attributes (color, flavor, aroma, texture) as vectors, and (c) Percentage variance explained per axis

CONCLUSION

Partial Least Squares Regression (PLS-R) helps explain how composite flour formulations interact with sensory perceptions in functional brownies. The very low explanatory power ($R^2 < 1.5\%$) and negative predictive relevance (Q^2) suggest a strong nonlinearity in gluten-free foods. These results show that the mix of spinach chlorophyll, mocafl starch, and protein creates a complex system that cannot be easily modeled by simple linear methods. Wheat flour emerges as the primary predictor of sensory quality, particularly for texture (VIP = 1.343) and aroma (VIP = 1.300). A significant trade-off exists between functional enrichment and hedonic acceptance; specifically, spinach powder inclusion above 10% and high levels of mocafl substitution are consistently associated with reduced structural integrity and inhibition of Maillard reactions. The integration of VIP scores with biplot visualization offers a strategic framework for establishing optimal substitution thresholds. For future development, a multidimensional approach is recommended, such as employing mocafl pre-gelatinization to mitigate textural degradation while maintaining the product's nutritional profile.

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