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Effect of Wheat Germ on the Properties of Vegetarian Sausage

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Wheat Germ (WG) is a by-product that is rich in nutritional value but is facing the problem of food loss and waste. This study utilised WG from the wheat mill to process vegetarian sausage according to traditional processing methods to investigate the effect of added WG ratio on the quality of vegetarian sausage, including structural properties, sensory value and nutritional value at different mixing ratios. Additional WG rates were examined at 0, 4, 5, 6, 7, and 8 % (% wt). Evaluate the textural properties using the TPA method on the Textural analyzer, SEM microstructure, retained water, pH value, and colour parameter combined with sensory value analysis. The results showed that WG can improve hardness, adhesiveness, gumminess and chewiness, but cohesiveness and springiness were not significant. The pH index gradually decreased, the water separation ability changed clearly, and the colour values also changed, especially the a* and b* values, which decreased when adding WG. Although there are still some low outliers, the sensory scores of the 6 and 7 % added vegetarian sausage have an average score of about 5.5, comparable to the control sample. Besides, WG has also been proven to improve nutritional value without adding artificial sweeteners, especially protein composition. This work can be considered for utilizing WG to develop products rich in plant protein.

1. Introduction

Food waste is a significant problem that touches every stage of the food supply chain and has implications for global hunger. If the amount of food lost was reduced by a quarter, it could be enough to feed people facing hunger globally. The current production and consumption of food need to focus on optimizing resource use and minimizing waste to respond circular economy trend. WG is a valuable by-product originating from the rice milling industry, attracting the attention of many researchers because of its ability to provide important nutritional components such as essential amino acids and fatty acids, polyunsaturated fats, minerals, vitamins B and E, fibre and phytochemicals such as flavonoids and phytosterols (Khosroshahi and Razavi 2023). Marti et al. (2014) pointed out that WG has a high sugar content of 17.8 % - 19.6 % (g/100 g db). Therefore, in this study, WG used a highly sweet ingredient without adding artificial sweeteners. WG has been added to beef sausage preparation (Elbakheet et al., 2018), mixed with other ingredients in food formulations or used as a stand-alone food (Mahmoud et al., 2015).

Recently, there has been extensive research on meat substitutes aimed at building a more sustainable food system, in addition to providing health benefits. Keerthana et al. (2022) studied the textural, physicochemical and sensory properties of vegan sausage ("Chả chay" in Vietnamese) using banana floret and jackfruit as meat substitutes. Vegetarian sausage (VS) is a familiar dish that is often prepared manually with the traditional packaging material of banana leaves. Banana leaves are one of the popular materials used to make environmentally friendly, easily degradable food so they have been used to wrap many traditional Vietnamese foods. Furthermore, banana leaves also have antibacterial and antifungal properties and are rich in antioxidants, protecting human health from infectious diseases from the environment and food (Kora, 2019). This study used WG, a nutritious by-product source, to create structure and natural sweeteners to replace artificial sweeteners to ensure the nutritional security trend. This study was carried out with the following objectives: 1) To assess the effect of WG on textural properties, 2) To evaluate the impact of WG on sensory attributes, and 3) To determine the nutritional value of VS.

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2. Materials and Methods

2.1 Materials

The Wheat Germ ASW - Australia Standard White germ was a product supplied by the Mekong Flour Mills Co., Ltd. It had the following nutritional components: energy 384.10 kcal; protein 31.02 %; fat 8.27 %; carbohydrates 44.65 %; fibre 1.45 %; ash 3.42 %; and moisture 10.74 %. Mung beans had a moisture content of 11.30 %; ash of 3.86 %; fibre of 3.01 %; carbohydrates 56.25 %; fat 0.84 %; protein 24.75 %; and energy 337.50 kcal. Tofu skin 111, a product of Lac Viet Vegetarian Food Joint Stock Company, had the following specified components: energy 253 Kcal; protein 20.13 %; fat 18.10 %; carbohydrates 2.30 %; fibre 0.34 %; ash 1.61 %; and moisture 57.49 %. Wheat gluten was a product of Anhui Ante Food Co., Ltd.
Pepper mill and freshly minced garlic were products of Vipep, located at A18, 58 Street, Dong Thanh Thiems Residential Area, Binh Trung Dong Ward, Thu Duc District, Ho Chi Minh City.
Musa sinensis L. was supplied by the online store Ngoc Anh Herbal Medicine, which has a warehouse at 577/103A Vuon Lai Street, An Phu Dong Ward, District 12, Ho Chi Minh City.

2.2 The process of making vegetarian rolls

60 g of fresh tofu were ground with a power of 800 W. 27 g of mung beans were cooked with 170 g of water on a stove (Gen Electric model GE – A03, 400 W) for 25 min at 100 °C (verified with a thermometer). When the beans boiled, they were stirred continuously until soft, and then the stove was turned off to cool. During the waiting time for the mung beans to cool, banana leaves were cut as follows: tip leaves (4 leaves): 18.5 * 5 cm, inner wrapping leaf (1 leaf): 20 * 24.5 cm, outer wrapping leaf (1 leaf): 25 * 29.5 cm. The banana leaves were steamed with 400 g of water on a stove with a power of 800 W for 1 min at 100 °C. Afterwards, supplementary ingredients were weighed: 8 g of wheat gluten, 1.5 g of ground pepper, 1 g of salt, 2.5 g of garlic, and 15 g of oil. The WG were measured according to each formula in Table 1 and blended with 60 g of ground tofu, 27 g of cooked mung beans, and the supplementary ingredients using a hand blender for 15 min, then roughly shaped. The mixture was allowed to rest in the refrigerator compartment at 9 °C for 30 min before packaging. Finally, the mixture was steamed for 15 min at 100 °C with 600 g of water, cooled to room temperature, and stored in the refrigerator compartment for 24 hrs before proceeding with further experiments.

Table 1: Mixing formula ingredients of the examined VS formulas

Ingredients	Control sample	FM 0	FM 1	FM 2	FM 3	FM 4	FM 5	FM 6
Wheat germ	-	-	3 %	4 %	5 %	6 %	7 %	8 %
Mung beans	27 g	27 g	27 g	27 g	27 g	27 g	27 g	27 g
Tofu skin	60 g	60 g	60 g	60 g	60 g	60 g	60 g	60 g
Wheat gluten	8 g	8 g	8 g	8 g	8 g	8 g	8 g	8 g
Pepper powder	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
Salt	-	1 g	1 g	1 g	1 g	1 g	1 g	1 g
FMG	-	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g
Oil	15 g	15 g	15 g	15 g	15 g	15 g	15 g	15 g
Refine sugar	1 g	-	-	-	-	-	-	-
MSG	1.25 g	-	-	-	-	-	-	-
SP	1.25 g	-	-	-	-	-	-	-

Noted: FMG: fresh minced garlic, MSG: Monosodium glutamate, SP: seasoning powder, (-): no added

Determination of textural properties: based on the TPA method by Noguerol et al. (2022) with slight adjustments. The structural analyzer (Brookfield, CT3) and a cylinder probe TA25/1000 with a diameter of 50.8 mm and a length of 20 mm were used. The VS samples were cut into lengths of 20 mm and diameters of 35 mm at room temperature (24 – 25 °C). The VS samples were compressed with 41.145 N force, a depth of 5 mm at a test rate of 1 mm/s. The parameters were recorded in Table 2.
Determination of pH values: The pH was directly determined by a handheld pH meter (Hanna, HI981036, Italy). VS samples were cylinders with lengths of 20 mm and diameters of 35 mm, pierced obliquely at room temperature 37 ± 1 °C to a depth of 2 cm. The pH meter probe was inserted and the measurements were started.
Determination of the retained water: based on the method of Noguerol et al. (2022). Filter paper was dried in a drying cabinet set at 105 °C for 30 min and allowed to cool in a desiccator to room temperature. The filter paper, along with two thin plastic films, was weighed. 3 g of sample were placed on the filter paper, and the portion containing the sample was sandwiched between the two thin plastic films. Finally, a plate containing 2 kg of

pebbles was placed on top and left undisturbed for 5 min. After the time elapsed, the sample was carefully removed from the filter paper surface, and the damp filter paper and plastic films were reweighed. The retained water (RW) was calculated according to Eq(1):

$$\% \text{ RW} = \frac{(m_1 - m_2)}{m_0} \times 100 \quad (1)$$

where m_1: mass of filter paper and wet plastic film (g); m_2: mass of filter paper and plastic film (g); m_0: sample mass (g).

Determination of colour parameters: The VS samples were guaranteed to be chilled in the refrigerator compartment and stored in banana leaves in plastic containers just before measuring. A Konica Minolta CR-400/CR-410 colourimeter was used for determining L*, a*, and b*.

Determination of the microstructure: using Scanning Electron Microscopy (SEM) according to Noguerol et al. (2022) with slight modifications. The VS samples were sent to the High Technology Center (District 9, Ho Chi Minh City, Viet Nam) for taking the SEM pictures.

Sensory evaluation technique: based on Mousavi et al. (2019) with slight adjusted. The VS samples for sensory evaluation were cut into blocks of 2 cm thickness at 24 - 25 ºC. After eating a sample, participants were asked to rinse their mouths with water and wait 1 - 2 min before eating the next sample, the participant repeated this action until all the samples were evaluated.

Determination of nutritional ingredients: according to TCVN 4335:1986, ash content according to TCVN 4070: 2009, fibre according to TCVN 4998:1989, protein according to TCVN 10791: 2015, lipid according to TCVN 8103: 2009. Carbohydrate content was determined according to FAO guidelines as Eq(2):

$$\text{Carbohydrate (\%)} = 100\ \% - (\%\ \text{Moisture} + \%\ \text{Lipid} + \%\ \text{Protein} + \%\ \text{Ash} + \%\ \text{Fiber}) \quad (2)$$

Table 2: Effect of WG on the textural properties of VS

Samples	Hardness 1 (N)	Adhesiveness (mJ)	Hardness 2 (N)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
Control	36.17±0.18 ^{c}	0.31±0.09 ^{a}	33.34±0.27 ^{c}	0.77±0.03 ^{bcd}	4.30±0.20 ^{a}	27.90±1.00 ^{cd}	119±9 ^{bc}
FM 0	41.30±0.80 ^{de}	0.30±0.30 ^{a}	39.00±1.00 ^{d}	0.80±0.03 ^{d}	4.51±0.01 ^{c}	33.00±1.60 ^{fg}	148±8 ^{ef}
FM 1	28.50±0.50 ^{a}	0.56±0.18 ^{a}	25.80±0.80 ^{a}	0.74±0.02 ^{abc}	4.43±0.12 ^{bc}	21.00±0.80 ^{a}	93±6 ^{a}
FM 2	34.00±1.00 ^{b}	0.80±0.40 ^{a}	30.80±0.70 ^{b}	0.73±0.01 ^{ab}	4.37±0.07 ^{abc}	24.80±0.70 ^{b}	110±5 ^{b}
FM 3	42.30±0.90 ^{ef}	0.60±0.30 ^{a}	38.70±1.40 ^{d}	0.75±0.02 ^{abc}	4.26±0.09 ^{a}	31.40±1.30 ^{ef}	140±7 ^{de}
FM 4	35.90±1.80 ^{c}	0.30±0.20 ^{a}	32.70±1.70 ^{bc}	0.72±0.01 ^{a}	4.29±0.05 ^{ab}	25.80±1.70 ^{bc}	112±10 ^{b}
FM 5	42.60±1.50 ^{ef}	1.10±0.90 ^{a}	39.00±2.00 ^{de}	0.75±0.04 ^{abc}	4.36±0.06 ^{ab}	32.00±3.00 ^{fg}	141±15 ^{de}
FM 6	40.00±1.40 ^{d}	3.70±1.80 ^{b}	37.00±2.00 ^{d}	0.72±0.02 ^{ab}	4.33±0.06 ^{ab}	29.00±1.40 ^{de}	130±5 ^{cd}

Different letters in the same column represent a statistically significant difference according to ANOVA analysis (α = 0.05)

3. Results and Discussions

3.1 Effect of WG on the textural properties of VS

The results indicated that Hardness 1 increased from 36.17 ± 0.18 N in the control to a peak of 42.60 ± 1.50 N in FM 5 (Figure 1g). According to Mazumder et al. (2023), the main components of the VS were green peas and WG, along with fresh tofu, but increasing the protein content from WG compared to green peas and fresh tofu resulted in a firmer structure than the control. The adhesiveness significantly increased from 0.31 ± 0.09 mJ (control, Figure 1a) to 3.70 ± 1.80 mJ in FM 6 (Figure 1h), possibly due to WG producing gelatinized compounds or polysaccharides, enhancing the surface's adhesion capability. Hardness 2 increased could be explained based on the interaction between proteins and carbohydrates in the VS, creating a sustainable structural network. Adhesiveness values fluctuated slightly from control to FM 0 (Figure 1b) and decreased at FM 4 (Figure 1f), and springiness also had only a small change from control FM 0. It varied slightly in other formulations, indicating that WG had minimal effect on the springiness and cohesiveness of the VS. This work showed that WG did not significantly affect the springiness and cohesiveness of the VS. Gumminess increased from the control to FM 0 and peaked in FM 5, then decreased in FM 6, demonstrating that WG improved the tensile strength of the VS. However, when the amount of WG was too high, flexibility decreased due to an imbalanced structure. Chewiness also increased from the control to FM 5, then slightly reduced in FM 6, indicating that WG increased the product's chewiness to a certain extent. Gumminess and chewiness increased when WG was added because WG protein is richer in essential amino acids, especially lysine and methionine, which are considered key amino acids in protein structure formation.The pH values of the vegetarian roll samples showed a gradual decrease when increasing the content of added WG (see Table 3). The control sample (CS) had the highest pH of 6.51 while the FM 1 had the lowest pH of 6.20. Samples from FM 3, FM 4, FM 5 and FM 6 had pH ranging from 6.22 - 6.27 showing a slight decrease compared to the control. The CS had a high pH index due to lower protein content than the sample supplemented with WG.

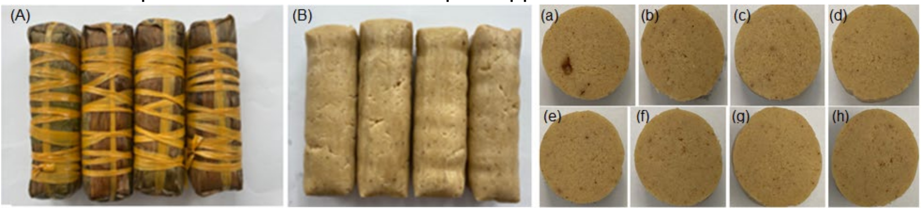

Figure 1: Illustration of VS: (A) wrap by banana leaves, (B) after steaming and peeling the banana leaves, and preparation samples for determining textural properties (a) Control; (b) FM 0; (c) FM 1; (d) FM 2; (e) FM 3; (f) FM 4; (g) FM 5; (h) FM 6

Table 3: Determination of physical properties of vegetarian rolls

Samples	pH	% RW	Colour parameters		
			L *	a *	b *
Control	6.51±0.02 e	6.59±0.08 d	58.10±0.70 d	28.27±0.34 de	27±0.70 e
FM 0	6.40±0.04 d	6.80±0.10 d	58.20±0.60 d	28.30±0.20 e	27.46±0.36 f
FM 1	6.20±0.08 bc	5.77±0.16 c	57.60±0.90 cd	28.25±0.24 de	27.40±0.50 f
FM 2	6.25±0.06 c	4.61±0.08 b	58.00±0.90 d	28.23±0.23 de	27.50±0.40 f
FM 3	6.27±0.03 c	3.75±0.08 a	55.30±0.50 a	27.80±0.10 ab	27.34±0.34 ef
FM 4	6.26±0.04 c	4.35±0.14 b	57.36±0.36 bc	28.13±0.10 cde	27.20±0.27 ef
FM 5	6.24±0.02 c	4.50±0.40 b	56.80±0.70 b	28.30±0.30 e	25.90±0.40 c
FM 6	6.22±0.04 bc	4.46±0.08 b	59.10±0.60 e	28.30±0.20 e	25.30±0.30 b

Different letters in the same column represent a statistically significant difference according to ANOVA analysis (α = 0.05)

According to Liu et al. (2020) regarding WG, the pH of WG was usually in the range of 6 - 7. This protein pH was to be consistent with the protein level in WG. Therefore, the relationship between pH index and high protein content in WG could create a valuable and viable alternative to synthetic emulsifiers and could be used as a nutritional supplement for security nutrition. Sample FM 0 had the highest % RW of 6.80 %, while sample FM 3 had the lowest % RW of 3.75 %. This showed that WG can significantly affect the water retention capacity of VS. According to Bakhsh et al. (2021) VS samples without WG often have lower water holding capacity (the RW higher) than samples supplemented with WG due to the interaction between water and fat with the protein component in WG. Regarding colour, the L* (brightness) value showed heterogeneous changes. The FM 6 model had the highest brightness of 59.10 while the FM 3 model had the lowest brightness of 55.30. Other samples fluctuated around 57 - 58 similar to the control. The a* value (redness) decreased slightly when adding WG. with the CS being 28.27. Sample FM 3 had the lowest a * value of 27.80 showing a clear decrease in red colour. The b* value (yellowness) also decreased slightly when adding WG. The CS was 27 while samples FM 5 and FM 6 have the lowest b* values of 25.90 and 25.30 showing a clear decrease in yellow colour. The colour results found that L* tended to decrease in brightness. The a* had a decrease in redness and b had a decrease in yellowness. In summary, adding WG proved that the corresponding product had a gradually decreasing pH index, a clear change in water separation ability and a change in colour values, especially a* and b* values decreased when increasing WG content.

The CSs had uneven surfaces with numerous visible gaps and voids (Figure 1a), indicating a lack of adhesion between components. Without the addition of WG, they lacked fibre and natural binding compounds, resulting in a rough and brittle surface. FM 4 (Figure 1b) showed significant improvement in surface structure homogeneity. Gaps and voids became smaller and smaller due to the WG adding fibre and polysaccharides, creating a smoother and more uniform surface structure. FM 5 (Figure 1c) maintained surface uniformity with fewer voids compared to the control, showing some areas where adhesion may have peaked and begun to decline slightly. This could be due to an excessive amount of WG causing over-dense structure, disrupting component bonding. FM 6 (Figure 1d) exhibited the presence of spherical structures and small voids, leading to unwanted gaps in the structure, reducing uniformity and potentially affecting the sensory quality of the product. These conclusions are drawn from detailed observations from SEM images. WG play a crucial role in enhancing the product's mechanical properties, such as hardness, adhesiveness, cohesiveness, and springiness. According to Esbroeck et al. (2024), plant proteins in the initial stages absorb and retain more water, making the product more porous but dependent on varying moisture levels and WG supplementation rates. Lower WG content results in lower protein levels, so lower water-binding capacity and relatively fewer surface voids. The water absorption and retention capabilities of WG significantly contribute to the structure of the product.

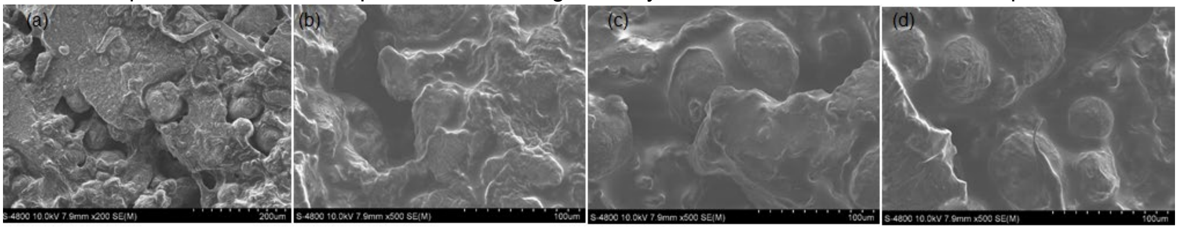

Figure 2: The Scanning Electron Microscope of samples a) Control. b) FM 4. c) FM 5 and d) FM 6

3.2 Effect of WG on the sensory evaluation of the VS

Based on the results obtained, the control scored a total of 87 points, which was the lowest among the samples, while FM 4 achieved the highest score of 127. Anova analysis showed a P-value < 0.05, indicating a statistically significant correlation at the 95 % confidence level. This suggests there was a difference in the preference level among participants for the products. Figure 3 illustrates the collected data.

Based on the chart in Figure 3, the CS scored from FM 0 to FM 6. The CS had the highest average score, around 5.5, and the widest range of scores, from approximately 2 to 9, indicating diverse sensory evaluations. Samples FM 0 and FM 1 had average scores around 5, with a fairly even distribution from 3 to 7, suggesting higher uniformity in evaluations, meaning assessors tended to agree more on the quality of these samples. Samples FM 2 and FM 3 had lower average scores, around 4.5, with scores ranging from 3 to 6, indicating less diversity in evaluations and possibly less popularity. Samples FM 4 and FM 5 had average scores around 5.5, similar to the CS. This result proved that adding WG in FM 4 and FM 5 created a sweetness equivalent to sugar and MSG in the CS. Sample FM 6 had an average score of around 5, with a wide range from 2 to 8, showing diversity in sensory evaluations. Similar to the CS, this indicated highly varied opinions on the quality of sample FM 6. Overall, the CS, and FM 4 and FM 5 samples had higher average sensory scores compared to others, but the CS had the widest range of scores, indicating greater diversity in sensory evaluations. FM 0 and FM 1 samples had more uniform sensory evaluations, with narrower score ranges and no outliers, indicating stable quality and consistent evaluation by participants. In summary, fewer participants rated sample FM 6 favourably, while sample FM 4 was preferred by many due to the balanced WG supplementation ratio creating uniformity in quality.

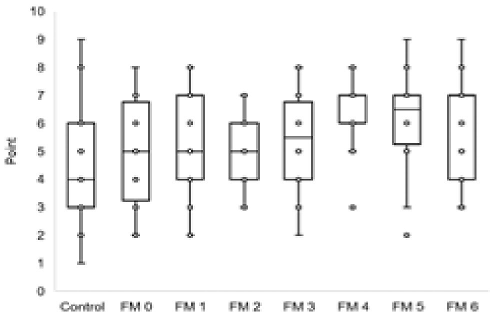

Figure 3: Chart of the VS's sensory evaluation

3.3 Effect of WG on the nutrition values of the VS

WG was considered the most important nutritional part of the wheat grain, providing 381 calories/100 g, with 54 % of energy from carbohydrates, 23 % from protein and 23 % from lipids (Boukid et al., 2018). Results Table 4 proved that the energy-generating components, including protein (4 kcal), carbohydrates (4 kcal) and lipids (9 kcal), would lead to an increase in the total energy of VS. However, what was worth discussing was that the nutritional value of VS increases when the content of WG increases. According to Boukid et al. (2018), WG was a rich source of vitamin E with α-tocopherol, which contains thiamine, riboflavin, niacin, phytosterols, polycosanol and antioxidants mainly phenolics. In addition, WG also contains coenzyme Q10 (ubiquinone) and PABA (para-aminobenzoic acid) (Kumar et al., 2011). Table 3 could be a valuable source of information for reference when mixing depending on the energy needs of consumers.

Table 4: Effect of WG on the nutrition values of the VS

Samples	Protein (g)	Lipid (g)	Carbohydrate (g)	Fibre (g)	Ash (g)	Moisture (g)	Energy (kCal)
Control	25.20±0.40 [a]	26.30±0.50 [a]	12.90±0.50 [a]	3.57±0.06 [a]	3.65±0.16 [a]	46.60±0.50 [b]	400±5 [ab]
FM 0	25.37±0.35 [a]	26.30±0.50 [b]	13.43±0.25 [b]	3.60±0 [a]	5.50±1.20 [c]	37.77±0.22 [c]	398±5 [a]
FM 1	26.30±0.40 [b]	26.50±0.60 [b]	13.83±0.15 [bc]	3.70±0 [b]	4.11±0.27 [ab]	45.10±0.80 [a]	407±6 [abc]
FM 2	26.60±0.40 [bc]	26.60±0.60 [c]	14.03±0.06 [c]	3.80±0 [c]	4.90±1 [bc]	44.55±0.13 [a]	410±6 [bcd]
FM 3	26.90±0.40 [bcd]	26.70±0.50 [d]	14.37±0.12 [c]	3.80±0 [c]	4.26±0.03 [ab]	44.72±0.14 [a]	413±7 [cde]
FM 4	27.20±0.40 [cde]	26.80±0.50 [d]	15±0.60 [d]	3.87±0.06 [d]	5.40±0.60 [c]	44.78±0.17 [a]	416±7 [cde]
FM 5	**27.50±0.40 [de]**	**26.90±0.50 [e]**	**16.10±0.30 [e]**	**3.90±0 [d]**	**4.50±0.10 [abc]**	**44.70±0.20 [a]**	**420±7 [de]**
FM 6	27.80±0.40 [e]	27±0.50 [f]	17.10±0.30 [f]	3.90±0 [d]	4.40±0.90 [abc]	44.61±0.09 [a]	423±7 [e]

Different letters in the same column represent a statistically significant difference according to ANOVA analysis (α = 0.05)

4. Conclusions

This study proved that WG significantly improves the quality properties of VS, reduced the pH value and increased the water-holding capacity of VS. Sensory evaluation showed that adding WG was well accepted by consumers. If combined with the results of structural properties and sensory evaluation, FM 4 and FM 5 were two mixing formulas worth considering for developing this product. Further research can focus on developing VS products supplemented with WG combined with other ingredients to diversify plant food products. Besides, it is necessary to evaluate the effectiveness of using banana leaves in packaging VS since it is a traditional method that can bring both environmental and health benefits for the global nutritional security trend.

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