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ENHANCING OXIDATIVE STABILITY OF RESTRUCTURED BEEF STEAKS USING PUMPKIN SEED AND WHEY PROTEINS: A FUNCTIONAL PROTEIN APPROACH

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I. INTRODUCTION

Restructured meats are gaining increasing popularity due to their quality, safety, convenience, and versatility. These products closely resemble minimally processed meats while allowing for the inclusion of health-promoting ingredients such as walnut, rice fiber, and various proteins. Typically, they are made with salt and phosphate to ensure cohesion, and they require precooking or freezing, which enhances nutritional value and reduces production costs. Pumpkin seed protein (PSP) is nutritionally rich, containing essential amino acids, and exhibits strong antioxidant and angiotensin-converting enzyme inhibitory properties [1]. Whey protein (WP), known for its emulsifying, thickening, and gelling capabilities, serves as a functional alternative to traditional ingredients. Additionally, WP contains bioactive peptides with antioxidant, antihypertensive, and antimicrobial effects [2]. However, to the best of our knowledge, no study has examined the use of PSP and WP as binders in restructured beef steaks. Therefore, the objective of this study was to evaluate the effects of PSP and WP, in combination with MTG (microbial transglutaminase), on the oxidative stability, color parameters, and sensory properties of restructured beef steaks.

II. MATERIALS AND METHODS

In restructured beef steak production, post rigor beef was chilled at +4 °C, cut into 13 mm flakes, and formulated with 90% beef and 10% ice. Additives (NaCl, MTG, PSP, WP) were added on top of this base formulation, resulting in totals exceeding 100%. Ice content was not reduced to allow for additive incorporation. All additive levels were calculated based on the total meat and water content. Beef was tumbled in a vacuum tumbler (50 rpm, <+4 °C, Vakona GmbH, Germany), followed by intermittent tumbling (15 min tumbling-10 min resting-15 min tumbling) after adding salt, ice, and MTG. Subsequently, samples were molded (14×6×6 cm, stainless steel ham molds) and restructured at 3 °C for 24 hours, then sliced (1 cm) using a slicing machine (Berkel Slicers, Italy). The sliced beef steaks were vacuum-packaged and stored at -18 °C for 3 months. Color was assessed with a Konica Minolta CR-200 colorimeter. TBARS, carbonyl, and sulfhydryl contents were measured using methods by Witte et al. [3], Oliver et al. [4], and a modified Ellman [5] method, respectively. Sensory evaluation was performed immediately after product preparation (Day 0). A consumer panel consisting of 20 untrained assessors (50% female, 50% male) participated in the evaluation. A 9-point hedonic scale was used, where 1 = "dislike extremely" and 9 = "like extremely", to assess appearance, color, flavor, juiciness, hardness, texture, and overall acceptability. The evaluation was conducted in accordance with ISO standards [6]. Ethical approval was obtained from Ege University (No: 2442, April 29, 2024). Data were analyzed using ANOVA and Duncan's test (SPSS 23).

III. RESULTS AND DISCUSSION

The color values of the beef steaks are provided in Table 1, and the highest L* values were observed in the M and MP1 groups during the storage period. On Day 0, the use of MTG alone or in combination with PSP or WP increased the a* value ($P < 0.05$). In the 1st month, the highest a* value was observed in MP2 ($P < 0.05$), while in the 2nd and 3rd months, no significant differences were found among the groups ($P > 0.05$). The highest b* value during the storage period was observed in MP1 ($P < 0.05$) due to the color of PSP.

Table 1. Color parameters of restructured beef steaks

Parameter	Storage (Month)	Treatments*			
		C	M	MP1	MP2
L*	0	$36.84^{ab,X}$±1.25	$36.36^{b,Y}$±1.20	$38.25^{a,X}$±0.66	$34.40^{c,X}$±0.49
	1	$36.37^{bc,X}$±1.26	$38.59^{a,X}$±0.88	$37.93^{ab,X}$±0.69	$35.39^{c,X}$±1.23
	2	$33.08^{b,Y}$±0.37	$37.85^{a,X}$±0.86	$38.37^{a,X}$±1.63	$34.48^{b,X}$±1.59
	3	$29.39^{b,Z}$±1.49	$31.96^{a,Z}$±0.87	$30.94^{a,Y}$±0.59	$28.28^{b,Y}$±0.39
a*	0	$9.17^{b,X}$±0.91	$13.74^{a,X}$±0.99	$13.95^{a,X}$±0.94	$14.59^{a,X}$±0.95
	1	$7.20^{b,Y}$±0.50	$7.68^{b,Y}$±0.76	$6.77^{b,Y}$±0.21	$9.18^{a,Y}$±1.45
	2	7.55^{Y}±0.46	7.06^{Y}±0.54	7.27^{Y}±1.19	6.66^{Z}±0.25
	3	4.60^{Z}±0.46	4.95^{Z}±0.52	5.09^{Z}±0.44	5.06^{T}±0.45
b*	0	$7.98^{b,X}$±1.00	$9.02^{b,X}$±0.25	$12.93^{a,X}$±0.69	$8.69^{b,X}$±0.69
	1	$4.51^{d,Z}$±0.92	$8.88^{b,X}$±0.87	$10.52^{a,Y}$±0.79	$5.71^{c,Y}$±0.38
	2	$6.32^{c,Y}$±0.60	$8.91^{b,X}$±0.29	$11.87^{a,X}$±1.07	$4.89^{d,Z}$±0.21
	3	$4.27^{b,Z}$±0.67	$4.48^{b,Y}$±0.15	$6.63^{a,Z}$±0.37	$4.44^{b,Z}$±0.06

*C: Control group with 1% salt, M: Beef steaks with 1% salt and 1% MTG, MP1: Beef steaks with 1% salt, 1% MTG, and 2% PSP, MP2: Beef steaks with 1% salt, 1% MTG, and 2% WP. Additives were added based on the total meat and water content, so the total formulation exceeded 100%. $^{a-d}$:Different letters in the same row indicate a statistically significant difference ($P<0.05$). $^{X-T}$:Different letters in the same column indicate a statistically significant difference ($P < 0.05$).

The TBARS values of the samples are presented in Table 2. During the storage period, the lowest TBARS values were obtained in the MP1 and MP2 groups ($P < 0.05$) due to antioxidant properties of PSP and WP. Except for Day 0, the highest values were observed in the C group, and in the 3rd month of storage, only this group exceeded the limit value (<2 mg MA/kg) determined for red meat [3]. During the storage period, the highest carbonyl value was generally observed in the M, while the lowest value was detected in the MP2 ($P < 0.05$). Similarly, the highest sulfhydryl content was obtained in the MP2 during storage ($P < 0.05$), suggesting that sulfhydryl groups were preserved against oxidation with the addition of WP (Figure 1). The lowest values were found in the C and M groups ($P < 0.05$). The use of MTG alone resulted in sensory scores similar to those of the control group for beef steaks, while the groups in which MTG was combined with PSP or WP were preferred more by the panelists (Table 3).

Table 2. TBARS values of restructured beef steaks

	Storage (Month)	Treatments*			
		C	M	MP1	MP2
TBARS value (mg MA/kg)	0	$0.48^{a, T}\pm0.03$	$0.45^{a, T}\pm0.01$	$0.31^{b, T}\pm0.01$	$0.33^{b, T}\pm0.07$
	1	$0.72^{a, Z}\pm0.02$	$0.69^{b, Z}\pm0.02$	$0.42^{d, Z}\pm0.01$	$0.48^{c, Z}\pm0.01$
	2	$1.20^{a, Y}\pm0.01$	$1.17^{b, Y}\pm0.01$	$0.88^{c, Y}\pm0.01$	$0.74^{d, Y}\pm0.01$
	3	$2.71^{a, X}\pm0.01$	$1.29^{b, X}\pm0.01$	$0.91^{d, X}\pm0.01$	$1.03^{c, X}\pm0.01$

*C: Control group with 1% salt, M: Beef steaks with 1% salt and 1% MTG, MP1: Beef steaks with 1% salt, 1% MTG, and 2% PSP, MP2: Beef steaks with 1% salt, 1% MTG, and 2% WP. Additives were added based on the total meat and water content, so the total formulation exceeded 100%. $^{a-d}$:Different letters in the same row indicate a statistically significant difference ($P < 0.05$). $^{X-T}$:Different letters in the same column indicate a statistically significant difference ($P < 0.05$).

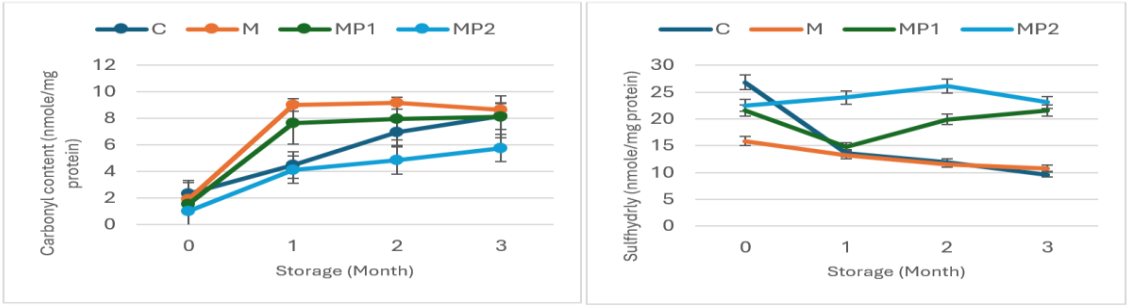


Figure 1. Carbonyl and sulfhydryl contents of restructured beef steaks

C: Control group with 1% salt, M: Beef steaks with 1% salt and 1% MTG, MP1: Beef steaks with 1% salt, 1% MTG, and 2% PSP, MP2: Beef steaks with 1% salt, 1% MTG, and 2% WP. Additives were added based on the total meat and water content, so the total formulation exceeded 100%.

Table 3. Sensory scores of restructured beef steaks

Parameter	Treatments*			
	C	M	MP1	MP2
Appearance	$5.56^{c}\pm0.89$	$7.00^{b}\pm0.71$	$7.22^{ab}\pm0.83$	$7.89^{a}\pm0.78$
Color	$6.33^{b}\pm1.00$	$7.00^{ab}\pm0.87$	$7.33^{a}\pm0.87$	$7.78^{a}\pm0.67$
Flavor	$6.33^{b}\pm0.71$	$6.56^{b}\pm0.53$	$7.33^{a}\pm0.87$	$7.56^{a}\pm1.01$
Juiciness	$6.22^{b}\pm0.97$	$6.00^{b}\pm1.11$	$6.89^{ab}\pm1.27$	$7.67^{a}\pm0.50$
Hardness	$6.56^{b}\pm1.01$	$6.11^{b}\pm1.05$	$7.00^{ab}\pm0.87$	$7.67^{a}\pm0.71$
Texture	$5.89^{b}\pm0.93$	$6.22^{b}\pm0.97$	$6.67^{b}\pm0.71$	$7.56^{a}\pm1.01$
General acceptability	$6.44^{b}\pm0.88$	$6.44^{b}\pm0.73$	$7.00^{b}\pm0.50$	$7.78^{a}\pm0.67$

C: Control group with 1% salt, M: Beef steaks with 1% salt and 1% MTG, MP1: Beef steaks with 1% salt, 1% MTG, and 2% PSP, MP2: Beef steaks with 1% salt, 1% MTG, and 2% WP. Additives were added based on the total meat and water content, so the total formulation exceeded 100%. $^{a-d}$:Different letters in the same row indicate a statistically significant difference (P<0.05).

IV. CONCLUSION

In conclusion, the addition of MTG, particularly in combination with PSP or WP, positively impacted the color, TBARS, and sensory qualities of beef steaks, with the MP2 group showing the best results in terms of antioxidant properties and preservation of sulfhydryl groups. These findings suggest that the use of MTG with PSP or WP can enhance the oxidative quality of restructured beef steaks during storage.

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