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ORIGINAL ARTICLE

Formula Optimization of Marinade to Tenderize Beef Meat and its Effect on Physicochemical and Sensory Properties

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KEYWORDS

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ABSTRACT: Meat tenderness has been considered one of the most effective features of meat and meat products, influencing consumers' acceptance and satisfaction. Hence, the proteolytic enzymes of fruits/vegetable origin were identified for postmortem meat tenderization. The application of plant-derived proteolytic enzymes has recently become more popular due to their safety, non-thermal and cost-effective properties. In this research, Response Surface Methodology (RSM) was employed to optimize the combined effects of broccoli (10-30%), asparagus (10-30%), and ginger (10-30%) extracts as well as low pH ingredients such as soy sauce (1-5%) and white vinegar (1-5%) on five sensory characteristics parameters of beef meat. According to response surface analysis, sensory properties of beef meat were affected by the concentration of broccoli, asparagus, ginger extracts, soy sauce, and vinegar ingredients in the formulation of marinade treatments. The optimized formula estimated by the models was combined of 26.5% of broccoli extract; 13.52% of asparagus extract; 26.26% of ginger extract; 3.68% of soy sauce, 5% of vinegar concentration, and 25.04% of distilled water. In general, there was a significant increase ($p<0.05$) in cooking loss, water-holding capacity, and collagen solubility and a significant reduction ($p<0.05$) in Warner-Bratzler shear force and pH values in treated samples compared to the control group during storage. Therefore, the formulated marinade can be utilized as an effective beef meat tenderizer at both industrial and retail levels. Also, optimized marinade formula could be consumed as food additives to improve other physicochemical and textural properties of tough beef meat.

INTRODUCTION

The tenderness of meat has been known as the major determinant of consumers' eating-satisfaction. Meat tenderness can be associated to connective tissues and myofibrillar proteins [1]. Many techniques have been used to enhance meat tenderness and enzymatic hydrolysis while the treatment of meat by proteolytic enzymes is the most popular method [1-5]. There is growing interest in using new proteases from various plants for this goal [5]. Proteolytic enzymes such as bromelain, ficin and papain have been widely used as meat tenderizers in most parts of the world [4-8]. Plant-derived protease enzymes mostly are premier to bacterial proteolytic enzymes because of safety concerns [8] such as pathogenicity [9-11]. These exogenous enzymes have been utilized in various forms such as marinades [12], pre-slaughter injection into the animal's vascular system [13], and incorporation into various spices as meat tenderizers [14-16]. One such promising enzyme, protease from broccoli plants, has been reported to have proteolytic activity [17]. These proteases were divided into several groups, depending on the active site [18], and the most commonly identified were cysteine proteases [4]. Another such plant proteolytic enzyme called ''zingibain'' was extracted from ginger. Ginger is employed typically as a flavoring agent for meat products [19-20]. Its proteolytic activity on both collagen and actomyosin was reported to produce more tender meat [5]. According to Tsai et al. (2012), ginger extract

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could improve the tenderness of duck breast muscle [20, 6]. The tenderization effect of ginger extract mainly results from its degradation of myofibrillar proteins such as myosin heavy chain (MHC), troponin T, α-actinin, and desmin of duck breast muscle [8, 21]. The hydrolyzing activity of proteases derived from asparagus toward meat proteins is reported by Ha et al. (2013) [7, 22]. The proteolytic activity of green asparagus juice was quite evident on both high and low-molecular-weight proteins. The action of asparagus cysteine protease was markedly affected by the 220 and 50 kDa bands associated with MHC and desmin respectively [8, 23-24].

Nevertheless, these proteolytic enzymes have very broad specificities and, therefore, generally indiscriminately degrade the myofibrillar proteins and connective tissue which may cause unwanted attributes i.e. mushy-textured product. Besides, acidic marination includes meat immersion in vinegar or fruit juice. The mechanism of the acidic tenderization of marinades is linked to involve various parameters such as the weakening of structures due to swelling of the meat, enhanced conversion of collagen to gelatin at low pH during cooking, and increased proteolysis activity by cathepsins [25-26]. Zochowska-Kujawska et al. (2017) reported that vinegar could be used for flavoring tender meat with an intense aroma to elucidate the mechanism of meat tenderization with low pH marinades such as roe deer meat [9, 27].

The proteolytic activity of these ingredients i.e. exogenous enzymes and acidic conditions is still a matter of discussion. The same enzyme can show differing results in activity levels, optimal pH, and temperature ranges depending on the substrate [8]. Despite several studies on meat texture alteration [1-9,22,28], there needs to be more research on adding novel ingredients to the formulation of meat tenderizers that may affect the final quality of meat products. Based on this information, we employed RSM as a strong statistical and mathematical technique to simultaneously optimize and analyze multiple factors and measure their linear, quadratic, and interaction effects [10, 29-30]. Accordingly, RSM analyzes differences in meat product quality when component levels in the formulation are altered [11, 31-32]. Thus, it was estimated which level of applied ingredients is more appropriate to prevent over-softening and improve meat samples' textural and sensory characteristics.

This study aimed to determine the optimal formulation of five natural marinade ingredients (broccoli, asparagus, ginger, soy sauce, and vinegar) for tenderizing beef meat. The influence of these ingredients' addition at different marinade times on beef meat's physicochemical and sensory attributes was also investigated.

MATERIALS AND METHODS

Materials

Top round beef muscles (*Bos taurus Linnaeus*) were obtained in a butcher in the town of Gorgan, Iran, likewise all ingredients including ginger (*Zingiber officinale roscoe*), broccoli (*Brassica oleracea* L.), asparagus (*Asparagus officinalis* L.), white vinegar, and soy sauce (Kikkoman brand, Walworth) used to marinades formulations were collected from local market. All the other chemicals and reagents were of analytical grade and were purchased from Merck Chemicals Co. (Darmstadt, Germany) or Sigma-Aldrich Co (St Louis, Mo, USA).

Preparation of extract from broccoli, asparagus and ginger

With slight changes, the Amid et al. (2012) method was used to generate the crude enzyme extract from the samples.With slight changes, the Amid et al. (2012) method was used to generate the crude enzyme extract from the samples.[12]. Prior to being mixed with 100 mL of sodium phosphate buffer (0.1 M, pH=7.5) at 4°C for two minutes, samples were first ground by a mixer (D70, Moulinex, Germany).. This mixture was filtered through cheesecloth and centrifuged at 15,000×g at 4°C for 15 min by a Beckman Coulter centrifuge (5XP, Mervue, USA). The supernatants were recovered as enzyme extract and kept at -40°C until further use.

Optimizing formulation of marinade ingredients

RSM was used to measure the ideal experimental circumstances and to produce an optimization formulation because it is a powerful statistical approach. In accordance with the findings of the single-factor experiment, Box-Behnken Design (BBD) was used to optimize the formulation variables and access their interactions. [10]. The factors for the formulation of marinade ingredients were evaluated at three different levels of low (1), medium (0), and high (+1), namely the broccoli concentration (%The samples were first ground in a mixer (Moulinex D70, Germany) before being combined with 100 mL of sodium phosphate buffer (0.1 M, pH7.5) at 4 °C for 2 min.) (X_1), asparagus concentration (%) (X_2), ginger concentration (%) (X_3), soy sauce concentration (%) (X_4), and vinegar concentration (%) (X_5). The response variables were five sensory parameters of meat quality after cooking. By keeping two variables at their center levels and making 3D plots of two factors, the Design Expert 10.0.3 program was used to assess the results. The second-order polynomial model was achieved from data analysis of the responses and formulation variables as demonstrated in Equation:

$$\mathrm{Y} = b_0 \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i<j=1}^{n} b_{ij} X_i X_j$$

Where, Y is the predicted response, Xi and Xj are the independent variables, b0 shows the constant coefficient bii is the quadratic coefficients, bi is the linear coefficient, bij is the interaction coefficient, and n is the number of independent variables.

Marination of meat by optimized formulation

Top round beef muscles were trimmed of all visible fat and connective tissues, then cut into slices of 2×2×2 cm. Each treatment involved the use of 30 pieces of beef. The samples were sprayed with either 30% w/v of optimized marinade formulation (26.5% of broccoli extract; 13.52% of asparagus extract; 26.26% of ginger; 3.68 % of soy sauce, 5% of vinegar concentration, and 25.04% of distilled water) for 0, 3 and 24 h. After the marination, the meat chunks were cooked on an electric grill. The cooked samples were evaluated for sensory properties as responses (chewiness, juiciness, taste, cutting, and odor).

Sensory Evaluation

An instructed 15-member panel conducted sensory analyses in an environmentally controlled room partitioned into stalls. Two pieces from each run were cooked as previously explained, and allowed to equilibrate to room temperature. Each sample had 3-digit numbers randomly chosen. The chewiness, juiciness, taste, cutting, and odor of the cooked meat were evaluated using 9-points hedonic test. The score for overall acceptability were collected from 0 to 9, where 1 illustrated dislike extremely and 9 demonstrated like extremely by considering some terms related to the sensory characteristics of meat [2].

Cooking Loss

The percentage of cooking loss was calculated using the Akwetey and Knipe (2012) technique [13]. 20g of beef samples were formed into a loop shape. The sample's weight was assessed both before and following cooking in an electric grill.

Water Holding Capacity (WHC)

WHC was determined according to Naveena et al. (2004) [2, 33]. Twenty grams of minced meat were added to a centrifuge tube that contained thirty milliliters of 0.6 M NaCl and swirled for one minute. After 15 minutes at 4 degrees Celsius, the tube was swirled once more and centrifuged at 3000 g (R-24, Remi Instruments, India) for 25 minutes. WHC was calculated as a percentage after measuring the supernatant.

Shear Force

After equilibration at room temperature, shear force values of cooked samples were assessed using an Instron 4301 Universal testing apparatus. Samples were cut at a right angle to the direction of the muscle fibers using a Warner-Bratzler shearing machine equipped with a flat blade attachment that was 1 mm thick. There was a 50 mm/min cross-head speed. One KN was the load cell used. Records were made of the amount of force needed to shear the samples (N cm-2) [1,32].

Determination of pH

Using calibrated electrodes designed specifically for meat products, a Crison 507 pH meter (Alella, Barcelona, Spain) was used to measure the pH of the samples.

Collagen Solubility

The procedure outlined by Mahendrakar et al. (1989) was used to test collagen solubility [14]. Five grams of muscle tissue were placed in a 250 mL beaker, covered with a Petri plate, and submerged in a water bath. After reaching boiling, the water bath was maintained for 30 minutes. The cooked beef was then removed from the beaker, diced, and blended for two minutes at 4 °C with 50 mL of distilled water. The extract was then centrifuged for 30 minutes at 1500 g. The soluble hydroxyproline was determined after hydrolyzing aliquots of cooked-out juice for 18 hours in a centrifuge. Collagen solubility was calculated as Collagen solubility (%) =7.14×hydroxyproline solubilized (%)

Statistical Analysis

Design-Expert 10.0 software was used to carry out experimental designs according to the Box-Behnken center combination principle [12]. In addition, all experiments were repeated at least 3 times. The obtained data were analyzed using a one-way analysis of variance test carried out with SPSS software (version 23.0, IBM, Chicago, USA). A Duncan's Multiple Range Test was employed to calculate the significance of differences among results ($p<0.05$).

RESULTS AND DISCUSSION

Effect of marinades factors on responses

The findings of 43 experiments utilizing the Box-Behnken Design are provided in Table 1, along with experimental and projected values for each of the five reactions (chewiness, juiciness, taste, cutting, and odor) for each trial. Table 2 illustrates the regression coefficients, determination coefficients, and significances of the models for the response variables of sensory properties including chewiness, juiciness, taste, cutting, and odor. All models to predict these variables were very significant ($P<0.001$), and R^2 ranged between 0.8 and 0.945 implying that these models were suitable for estimating the responses to changes in broccoli, asparagus, ginger, soy sauce, and vinegar levels in the formulation of marinade ingredients. The R^2 value was employed to judge the adequacy of models, while a value near one indicates a perfect fit. A coefficient of variance (CV) below 10 illustrates that variation in the mean value is low and satisfactorily develops an adequate response model [11]. All models' CV ranged from 2.49 to 5.27. Table 2 demonstrates that, with the exception of ginger's effect on juiciness, taste, and odor (p0.05), all linear coefficients of broccoli, asparagus, gingand er concentrations on five responses are significant with extremely tiny p-values. Due to their higher p-values ($p>0.05$), the other coefficients are not significant. When ginger was added to the sample, flavor-producing processes that take place during cooking were enhanced. In comparison to the control, the juiciness scores were improved with ginger extract treatment. Syed Ziauddin et al. (1995) [15] also found an improvement in the look, flavor, and juiciness of buffalo meat samples treated with ginger extract and organic acids. The observed increases in protein and collagen solubility and the reported improvement in eating pleasure of cooked camel meat chunks treated with ginger extract, particularly samples treated with 30 percent ginger extract, are consistent. The connective tissue-derived hydrolyzed collagen has a good capacity to bind water and can increase the softness of cooked meats [16].

Optimization of marinade ingredients' formulation

Formulation optimization of marinade ingredients was estimated based on maximum score of chewiness, juiciness, taste, cutting, and odor in constant range according to obtained results of control sample. The optimum formulations were as follows: 26.5% of broccoli extract; 13.52% of asparagus extract; 26.26% of ginger; 3.68% of soy sauce, 5% of vinegar concentration, and 25.04% of distillated water. In these circumstances, the optimized values of all responses were presented in Table 3.

Table 1. Box–Behnken design matrix with coded variables and measured and predicted values

Run	Independent Variable					Responses									
	Broccoli (X_1) (%)	Asparagus (X_2) (%)	Ginger (X_3) (%)	Soy sauce (X_4) (%)	Vinegar (X_5) (%)	Chewiness		juiciness		Taste		Cutting		Odor	
						Act	Pred	Act	Pred	Act	Pred	Act	Pred	Act	Pred
1	30	30	20	3	3	8.10	7.81	7.90	7.88	6.40	6.56	8.30	8.09	6.80	6.93
2	30	20	20	3	1	7.70	7.76	7.40	7.41	7.00	7.25	7.90	7.83	7.20	7.61
3	20	30	20	3	5	7.90	8.06	7.90	8.02	7.10	6.97	8.20	8.26	7.50	7.23
4	10	20	20	3	5	6.70	6.59	7.10	6.83	7.20	7.14	6.50	6.36	7.60	7.31
5	20	20	30	1	3	8.00	7.99	7.40	7.62	6.50	6.77	8.20	8.16	7.00	7.16
6	20	30	20	5	3	7.80	7.92	7.90	7.85	6.20	6.56	8.20	8.26	6.10	6.72
7	10	20	30	3	3	6.90	7.02	7.00	7.10	6.10	6.29	7.00	6.98	6.50	6.83
8	20	10	20	5	3	7.50	7.35	7.40	7.10	7.60	7.55	7.70	7.33	8.20	8.14
9	30	20	20	5	3	7.80	7.86	7.50	7.55	7.50	7.44	8.00	8.09	8.10	7.80
10	10	20	10	3	3	6.20	6.39	6.40	6.73	6.40	6.35	5.70	5.88	6.50	6.57
11	20	20	20	3	3	7.70	7.67	7.30	7.13	8.20	8.23	8.00	7.93	8.10	8.17
12	30	20	30	3	3	8.20	8.14	8.00	7.76	7.00	7.00	8.10	7.91	7.20	7.31
13	20	20	10	3	5	7.90	7.72	8.00	7.97	7.20	7.55	8.20	8.05	7.40	7.67
14	20	10	30	3	3	7.90	7.83	7.50	7.56	7.70	7.31	8.10	7.88	8.20	7.85
15	20	20	20	3	3	7.70	7.67	7.00	7.13	8.30	8.23	7.90	7.93	8.20	8.17
16	20	10	20	1	3	7.40	7.16	7.80	7.49	6.50	6.53	7.50	7.17	6.70	6.71
17	20	20	20	1	1	7.50	7.71	7.10	7.37	6.90	6.91	7.80	7.81	7.60	7.26
18	20	20	20	1	5	7.60	7.67	7.80	7.91	7.80	7.41	8.00	7.97	8.10	7.89
19	10	10	20	3	3	5.70	5.81	6.20	6.36	6.50	6.33	5.50	5.49	6.80	6.63
20	20	20	20	3	3	7.60	7.67	7.10	7.13	8.20	8.23	7.90	7.93	8.20	8.17
21	30	20	20	3	5	7.90	7.92	8.20	8.25	7.60	7.80	8.30	8.14	8.30	8.29
22	20	20	20	5	5	7.90	7.86	7.50	7.57	8.60	8.03	8.10	8.08	8.20	8.23
23	10	20	20	1	3	6.50	6.54	6.80	6.78	6.30	6.22	6.00	6.35	6.50	6.54
24	20	30	10	3	3	7.80	8.08	8.00	8.05	7.00	6.88	8.00	8.45	7.50	7.41
25	10	30	20	3	3	7.50	7.13	7.50	7.41	5.80	6.05	7.40	6.81	6.30	6.45
26	20	10	10	3	3	6.90	7.06	7.20	7.30	6.40	6.47	6.80	6.98	6.40	6.64
27	20	20	30	3	5	8.20	8.19	7.90	7.78	7.50	7.59	8.10	8.35	8.00	8.08
28	10	20	20	3	1	6.60	6.53	7.20	6.90	6.50	6.49	6.40	6.35	6.90	7.04
29	20	20	10	3	1	7.80	7.61	7.40	7.33	6.90	7.05	8.10	7.79	7.40	7.64
30	30	20	10	3	3	7.80	7.82	8.00	8.00	7.30	7.07	8.20	8.21	7.80	7.64
31	20	30	20	3	1	8.00	8.00	7.80	7.84	7.20	6.82	8.30	8.39	7.80	7.25
32	20	20	10	1	3	7.80	7.67	7.90	7.76	6.80	6.58	8.00	7.76	7.40	7.05
33	30	10	20	3	3	7.50	7.68	7.60	7.83	7.50	7.25	7.10	7.46	7.90	7.70
34	20	30	30	3	3	8.20	8.26	7.90	7.91	6.50	5.92	8.30	8.35	6.80	6.12
35	20	10	20	3	5	7.30	7.39	7.60	7.67	7.40	7.91	7.40	7.58	7.80	8.20
36	20	20	10	5	3	7.70	7.56	7.60	7.37	7.30	7.34	8.00	7.88	7.90	7.68
37	20	20	30	5	3	8.20	8.18	7.50	7.63	6.50	7.03	8.20	8.28	7.20	7.50
38	30	20	20	1	3	7.70	7.72	8.00	7.94	7.00	6.93	7.50	7.68	7.50	7.51
39	10	20	20	5	3	6.40	6.48	6.70	6.79	6.80	6.73	5.90	6.16	7.50	7.23
40	20	20	20	5	1	7.50	7.60	7.10	7.33	7.50	7.33	7.90	7.92	8.00	7.90
41	20	30	20	1	3	8.00	8.04	7.90	7.84	6.10	6.55	8.10	8.19	6.50	7.18
42	20	20	30	3	1	8.10	8.08	7.80	7.64	7.00	6.89	8.20	8.29	7.10	7.16
43	20	10	20	3	1	7.30	7.22	7.10	7.09	6.60	6.86	6.90	7.12	7.10	7.23

All models were significant at $p<0.001$.

Table 2. Analysis of variance for the regression model of sensory properties

	Chewiness		juiciness		Taste		Cutting		Odor	
	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value
Model	7.67	<0.0001	7.13	<0.0001	8.23	<0.0001	7.93	<0.0001	8.17	0.0006
X_1- *Broccoli*	0.64	<0.0001	0.48	<0.0001	0.36	0.0006	0.81	<0.0001	0.39	0.0006
X_2-*Asparagus*	0.36	<0.0001	0.27	<0.0001	-0.24	0.0122	0.49	<0.0001	-0.24	0.0233
X_3-*Ginger*	0.24	<0.0001	0.031	0.5731	-0.031	0.7296	0.20	0.0103	-0.019	0.8492
X_4-*Soy sauce*	0.019	0.6932	-0.094	0.1002	0.26	0.0089	0.056	0.4386	0.24	0.0203
X_5-*Vinegar*	0.056	0.2432	0.19	0.0018	0.30	0.0028	0.081	0.2668	0.24	0.0233
X_1X_2	-0.30	0.0042	-0.25	0.0321	-0.100	0.5811	-0.17	0.2328	-0.15	0.4497
X_1X_3	-0.075	0.4326	-0.15	0.1836	0.000	1.0000	-0.35	0.0225	-0.15	0.4497
X_1X_4	0.050	0.5994	-0.10	0.3700	0.000	1.0000	0.15	0.3043	-0.10	0.6130
X_1X_5	0.025	0.7924	0.22	0.0515	-0.025	0.8899	0.075	0.6042	0.10	0.6130
X_2X_3	-0.15	0.1241	-0.100	0.3700	-0.45	0.0195	-0.25	0.0935	-0.62	0.0041
X_2X_4	-0.075	0.4326	0.100	0.3700	-0.25	0.1754	-0.025	0.8625	-0.47	0.0233
X_2X_5	-0.025	0.7924	-0.100	0.3700	-0.23	0.2208	-0.15	0.3043	-0.25	0.2129
X_3X_4	0.075	0.4326	0.10	0.3700	-0.12	0.4912	0.000	1.0000	-0.075	0.7041
X_3X_5	0.000	1.0000	-0.12	0.2649	0.050	0.7821	-0.050	0.7292	0.23	0.2607
X_4X_5	0.075	0.4326	-0.075	0.4996	0.050	0.7821	0.000	1.0000	-0.075	0.7041
X_1^2	-0.51	<0.0001	-0.035	0.6857	-0.83	<0.0001	-0.82	<0.0001	-0.58	0.0011
X_2^2	-0.046	0.5430	0.27	0.0045	-0.86	<0.0001	-0.15	0.1911	-0.66	0.0003
X_3^2	0.19	0.0192	0.30	0.0023	-0.73	<0.0001	0.13	0.2569	-0.50	0.0036
X_4^2	-0.004	0.9557	0.16	0.0699	-0.58	0.0005	-0.044	0.7017	-0.32	0.0505
X_5^2	0.046	0.5430	0.25	0.0089	-0.24	0.1095	0.056	0.6228	-0.027	0.8621
C.V. %		2.49		2.92		5.08		3.74		5.27
R^2		0.9459		0.8879		0.8391		0.9292		0.8000

All models were significant at p<0.05.

Table 3. Desirability specifications and predicted values for different parameters in optimum formulation.

Name	Goal	Low limit	High limit	Predicted optimum level
Broccoli (X_1) (%)	in range	10	30	26.50
Asparagus (X_2) (%)	in range	10	30	13.52
Ginger (X_3) (%)	in range	10	30	26.26
Soy sauce(X_4) (%)	in range	1	5	3.68
Vinegar (X_5) (%)	in range	1	5	5.00
Chewiness	maximize	5.7	8.2	8.2
juiciness	maximize	6.2	8.2	8.1
Taste	maximize	5.8	8.6	8.1
Cutting	maximize	5.5	8.3	8.2
Odor	maximize	6.1	8.3	8.7
Desirability	-	-	-	0.950

Effect of formulation optimization on physicochemical properties of beef meat

The physicochemical properties of beef meat marinated with the optimized formulation of marinade ingredients during different times are presented in Table 4. The amount of cooking loss is a crucial factor since it affects the quality of the meat, such as its tenderness and juiciness, as well as the final yield of the product. Beef with a smaller cooking loss is typically considered to be juicier. Table 4 shows that there was no significant different between the unmarinated control sample and the beef that had been marinated for 0 and 3 hours ($p<0.05$). However, the 24 hour marinated sample had much greater cooking loss values ($p<0.05$). This might have happened as a result of the myofibrillar protein or connective tissue degrading, which would have caused the muscle structure to loosen and increased water evaporation during cooking, which would have increased cooking loss [17]. In previous research, it was discovered that beef steak treated with protease experienced greater cooking loss than a control sample. Pietrasik and Shand (2011) discovered that samples treated with microbial protease showed a greater cooking loss [18]. Similarly, the cooking loss of buffalo meat treated with 2% Kachri extract also increased. Yousefi et al. (2018) reported that the marinade pH affected the cooking loss of chicken breast fillets [19].

The marination treatment of meat increased water-holding capacity and also by increasing marination time, water-holding capacity increased reciprocally. This was due to the use of phosphate buffer which gave the excess negative charges at high pH [20]. The pH of the unmarinated sample was significantly higher than the other three samples. Marination of all muscle samples led to an increase in acidity in all samples. The lower pH of the treated samples probably caused the low pH of the optimized formulation marinade (pH= 4.89). The effect of marination on water-holding capacity could be explained as the protonation of carboxyl groups in muscle protein or as the difference in osmotic pressure [21]. Van Laack and Lane (2000) indicated that the protein solubility of chicken muscles decreased at a lower pH value of 5.4 compared with pH= 6.5 due to protein denaturation [22].

In comparison to the control, all marinated samples showed higher collagen solubility values. According to Thompson et al. (1973), who reported a considerable increase in collagen solubility of ovine B. femoris muscle with ginger extract treatment, the improved collagen solubility in marinated samples in the current study was consistent with their findings [23]. They found that ginger protease had substantially stronger proteolytic activity on collagen than actomyosin, and that the combined proteolysis of these two muscle proteins produced meat that was noticeably more soft. Increases in collagen solubility brought on by the expansion of meat protein in these investigations could be used to explain the effects of marinating. Additionally, Kong et al. (2008) found a negative correlation between chicken breast collagen solubility and shear force [24]. All samples treated with enzyme had much lower shear forces than the control sample. When marination time was extended, the amount of shear force needed to cut samples was dramatically reduced ($p<0.05$). Numerous investigations have been made to determine the effectiveness of different plant crude extracts for tenderizing meat, including ginger extract, Kachri extract [2], kiwi fruit extract [25], and pineapple extract [20]. After treating the meat with these plant extracts, they observed a decrease in the Warner-Bratzler shear force. The weakened muscle proteins caused by proteolysis as well as the solubility of collagen may be to blame for the reduction in shearing force of marinated samples.

Table 4. Physico-chemical properties of beef meat marinated with optimized formulation of marinades ingredients during different times

Name	Control (unmarinated)	Marinating time (h)		
		0	3	24
Cooking loss (%)	37.5 ± 2.1^b	36.9 ± 1.4^b	37.9 ± 3.6^b	40.4 ± 2.3^a
Water holding capacity (%)	26.2 ± 1.7^d	30.8 ± 3.1^c	32.4 ± 3.2^b	35.6 ± 1.6^a
Collagen solubility (%)	1.84 ± 0.05^c	2.35 ± 0.12^b	2.58 ± 0.08^{ab}	2.79 ± 0.06^a
pH	6.15 ± 0.42^a	5.74 ± 0.25^b	5.68 ± 0.36^b	5.65 ± 0.37^b
Shear force (N cm^{-2})	79.15 ± 2.36^a	78.19 ± 3.44^a	67.61 ± 2.24^b	60.81 ± 1.87^c

* Different capital letters in columns indicate a significant difference of at least 0.05.

CONCLUSIONS

The sensory properties of beef meat were affected by the concentration of broccoli, asparagus, ginger, soy sauce, and vinegar in the formulation of marinade ingredients. The optimized formula estimated by the models combined 26.5% of broccoli extract; 13.52% of asparagus extract; 26.26% of ginger; 3.68% of soy sauce, 5% of vinegar concentration, and 25.04% of distilled water. There was a significant increase in cooking loss, water-holding capacity, collagen solubility, and a significant reduction in shear force values and pH in marinated samples compared to the control.

Conflict of interests

No conflict.

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