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RESEARCH ARTICLE

Combined Effect of Non Meat Proteins and Different Binders on Low Salt Poultry Meat Systems

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Abstract

There is a growing concern over health for non vegetarian population because of high intake of high salt meat products. Development of low salt meat products is a challenge because of the reduction of desirable functional properties conferred by salt. In the present study effect of 6 different binders namely corn flour, rice flour, bengal gram flour, black gram flour, pectin and maltodextrin in combination with two non meat protein, whey and soy protein on low salt poultry model meat systems was evaluated. Parameters such as water and salt soluble proteins concentration, water holding capacity (WHC), cooking loss and texture profile were estimated to find the most suitable binder-protein combination. WHC was found to be highest for corn flour-whey protein incorporated systems. Black gram flour-whey protein combination had the highest extracted water and salt soluble protein concentrations. Texture profile of the systems was significantly influenced by the non meat protein and binders added. Addition of whey protein resulted in harder texture when compared to soy protein. Numerical optimization using factorial design was carried out to select the best non meat protein-binder combination for low salt meat systems and black gram flour-whey protein combination was found to show the highest desirability at 0.89 against the constraints.

Introduction

Common salt is a vital ingredient in the development of meat products especially restructured and reformed meat products like sausages, steaks, roll, meatballs, nuggets, patties etc. There are growing health concerns over addition of salt to food as common salt is the major contributor to sodium in the diet. High intake of sodium is correlated to incidence of cardiovascular diseases including hypertension (Frost et al., 1991; Sasaki et al., 1995; He et al., 1999). Since salt content in processed meat products is relatively high (2-6%), proper efforts are required to develop reduced salt products with acceptable quality. This is not easy because salt imparts a number of desirable properties like flavor, water binding, tenderness and juiciness to meat products. Although a number of low salt products have been developed they fail to attain the sensory quality and functional characteristics of products having higher salt content (Verma and Banerjee, 2012). Salt provides the necessary ionic strength for the myofibrils to swell and disintegrate; releasing the myofibrillar proteins to meat surface which on heat coagulation help in binding of adjacent meat pieces together (Hamm, 1986; Offer and Knight, 1988; Ruusunen and Puolanne, 2005). Moreover, salt brings about conformational changes in myofibrillar proteins, which ultimately enhances the ability of protein to bind water (Hamm, 1960; Offer and Trinick, 1983; Puolanne et al., 2001; Terrel, 1983). Therefore reduction of salt will greatly affect properties like water holding capacity, cooking loss, texture etc. It is suggested to use non meat proteins and carbohydrates like starch flours and polysaccharides to compensate for losses owing to reduction of salt in meat products (Verma and Banerjee, 2012). Non meat proteins like soy protein and whey protein can improve water binding, cooking yield and texture of meat products (Thompson et al., 1982; El Magoli et al., 1996; Chin et al., 1999; Hongsprabhas and Barbut, 1999; Ulu, 2004; Andres et al., 2006; Pietrasik et al., 2007; Szerman et al., 2007, 2008, 2012). Cereal flours like corn flour and rice flour are commonly used binders by the meat industry. Several studies have reported increase in water retention, reduced cooking loss and improved texture when using corn flour and rice flour as binders in meat products (Serdaroglu and Degirmencioglu, 2004; Ahmed et al., 2007; Yi et al., 2012; Ikhlas et al., 2011; Nag et al., 1998; Kumar and Sharma, 2005). Legume flours like bengal gram flour and black gram flour have been reported to increase moisture retention and yield in meat products (Serdaroglu et al., 2005; Modi et al., 2003; Bhat and Pathak, 2011). Maltodextrin was found to improve texture and functional properties of low fat frankfurters (Crehan et al., 2000). Pectin improves cooking yield and water binding in low fat burgers (Troy et al., 1999). Although several researches have been carried out to evaluate the effect of binders and non meat proteins in non modified meat products as well as low fat products, studies on their combined effect in low salt meat systems have not been undertaken so far. The aim of the present study was to investigate the influence of non meat proteins namely whey protein concentrate and soy protein isolate and binders like corn flour, rice flour, bengal gram flour, black gram flour, pectin and maltodextrin on reduced salt model poultry meat systems in order to find the best combination of non meat protein and binder that can be employed for the development of economical low salt meat products.

MATERIALS AND METHODS

Preparation of meat batter

Fresh deboned and deskinned chicken meat was purchased from local meat suppliers. Whey protein concentrate was purchased from Pristine Organics, Bangalore and maltodextrin from Mudra Enterprises, Bangalore. All other ingredients were procured from local market. Meat was trimmed off heavy connective tissue and external fat and then ground through a 5mm plate (Sirman Mincer, Italy). Twelve different treatments along with a control, all having low salt (1%) content were formulated as shown in Table 1. Ground lean meat was blended with the 1% salt and half of the ice at low speed setting for 30 seconds, followed by blending at high speed setting for 30 seconds. This was followed by 1 minute resting time for protein extraction. Non meat protein and binder were evenly sprinkled on top of the meat mixture. Remaining ice was added and blending continued at high speed setting for another 2 minutes till a viscous batter was formed. Final batters temperature did not exceed 15^0C. Random samples of raw batter from each formulation were taken for analyses of water and salt soluble proteins, water holding capacity and cooking loss. The remaining batter (75g) from each formulation was stuffed into aluminium molds of (75 mm diameter) and steam cooked without pressure to core temperature of 80^0C measured using a digital thermometer (HTA Instrumentation Pvt. Ltd., Bangalore). Cooked meat systems were removed on reaching the designated end point temperature, drained and placed in chiller (2^0C) for subsequent analyses.

Water and salt soluble proteins extraction

Ten grams of homogenized samples were blended with 20 ml of refrigerated double distilled water at low speed. The slurry was centrifuged at 9000 rpm for 10 minutes at 2^0C in centrifuge. The supernatant was filtered and collected to estimate water soluble proteins. The precipitated pellet was dissolved in 20 ml of cold 2% NaCl (containing 0.5% Sodium tripolyphosphate) solution followed by centrifugation at 15000 rpm at 4^0C for 1hour. The supernatant containing salt soluble proteins was collected. The water and salt soluble proteins from supernatant solutions were estimated using the Biuret method (Gornall et al., 1949).

Estimation of water holding capacity

The water holding capacity as percentage bound water of raw and cooked batters were determined by the method described by Lianji and Chen (1991). 10g raw batter was placed in glass jar and heated at 90^0C in water bath for 10 minutes. After heating the samples were cooled to room temperature, weighed, wrapped in cotton cheese cloth and placed in centrifuged tubes which had been one third filled with absorbent cotton wool. The samples were centrifuged for 15 minutes at 10,000 rpm at 4^0C. After centrifugation, cheese cloth was removed and sample weight was recorded. Cooked samples were directly centrifuged in similar way and weights noted. The percentage water holding capacity was calculated by the following formula;

$$\%\text{ water holding capacity } = 1 - \frac{T}{M} \times 100 = 1 - \frac{B - A}{M} \times 100$$

Where,
T= total fluid loss during cooking and centrifugation in g
M = total water content in the sample in g
B = weight of the sample before centrifugation in g

A = weight of sample after centrifugation in g

Estimation of cooking loss

Cooking loss was measured by the procedure followed by Youssef and Barbut (2011). The released juice from cooked batter of each mold was decanted and the weight of the fluid was measured. Percent cook loss was calculated as the ratio between liquid expelled (g) and raw batter weight (g).

$$\text{Cooking loss (\%)} = \frac{\text{weight of liquid expelled}}{\text{weight of raw batter}} \text{ x } 100$$

Texture profile analysis

The texture profiles of the samples were reported using Texture Analyser (TA Plus, Lloyd Instruments, Hampshire, UK) by the method described by Bourne (1978). Samples were compressed twice to get an imitation of mastication which includes first and second bite. 5mm cylindrical probe was used, connected to the moving crosshead which was cycled at a pre and post test speed of 30 mm/ min. The maximum clearance between the moving cross head and sample was maintained at 3mm throughout the study with compression ratio of 85%. The measured and derived parameters like hardness (N) springiness (mm), cohesiveness, gumminess (N) and chewiness (Nmm) were estimated using Nexigen software.

Statistical Analysis

All experiments were conducted with three replicates and Student Newman Keuls test was used to evaluate the statistical significance at $p<0.05$ (Coplot:2003. CoStat version 6.204) using ANOVA. Optimization of the multiple responses was conducted using factorial design and numerical optimization technique of the statistical software Design Expert 6.0.10. Both the factors namely non meat proteins and binders were kept within range while the responses were either maximized or minimized. The desirability function was established and the system with highest desirability was obtained from the numerical solution.

RESULTS AND DISCUSSIONS

Water and salt soluble protein extraction

Extraction of water and salt soluble proteins is an important factor in the processing of meat products especially emulsion and restructured type products. Table 2 shows the concentrations of water and salt soluble proteins extracted from raw and cooked meat systems. Significant differences ($p\leq0.05$) were observed between the systems for the amount of water and salt soluble proteins extracted. Combination of whey protein with black gram flour had the highest water and salt soluble protein concentration. Maltodextrin whey protein system had the lowest concentration of salt soluble protein at 4.48 mg/ml. Extracted protein concentrations were higher for raw meat systems than cooked meat systems. This is due to the loss of water soluble proteins as well as heat denaturation of proteins (Totosau et al., 2002). Present study showed that the systems with higher amounts of proteins extracted exhibited greater capacity to hold and retain water, consequently reducing the losses during cooking. This is due to the solubilisation of myofibrillar proteins in the presence of salt, which on heat coagulation and activation help in increasing the water binding capacity (Desmond, 2006). Moreover, extraction of salt soluble protein is necessary for stabilizing fats and increasing the emulsion stability, thus, retaining water and fat during processing operations to a greater extent (Hamm, 1986). Lin and Mei (2000) also found similar relation between protein extraction and water holding capacity in reduced fat meat batters.

Water holding capacity

The ability of meat products to retain moisture during cooking and processing is an important characteristic that significantly influences the quality and yield of the end product. One of the main functional properties conferred by salt in meat systems is water binding. The water holding capacity of raw and cooked meat systems estimated in the present study are presented in Table 3. All treatments had higher water holding capacity than control sample. Corn flour-whey protein meat system was found to have the highest water holding capacity in both raw and cooked state, followed by black gram flour and whey protein based meat system. Others such as Serdaroglu and Değırmencioglu (2004) and Ahamad et al. (2007) reported increased water retention in meat products with corn flour added as binding agent. Hongsprabhas and Barbut (1999) reported that addition of 2% preheated whey protein isolate can improve water binding in low salt (≤ 1.5% NaCl) poultry meat batters. Soy protein when added at 1-3 % improved water binding in reduced salt frankfurter (Whiting, 1984, Su et al., 2000)

There was significant difference ($p\leq0.05$) between the water holding capacity of the meat systems, with all whey protein containing systems reporting higher values than soy protein based systems irrespective of the binder used. Ulu (2004) reported higher water retention in whey protein added meatballs than soy protein added samples. In contrast Hong et al. (2006) reported higher water holding capacity in restructured pork meat containing isolated soy protein than whey protein. Another trend observed was the higher water holding property shown by cooked systems when compared to raw systems. This might be due to the lower moisture content of cooked systems resulting from loss of water during cooking. Hence the amount of water lost under the application of external pressure will be comparatively low.

Cooking loss

Cooking loss estimation is important to understand the effect of non meat ingredients on the cooking behavior of a product. Addition of salt restricts losses during cooking and increases tenderness and juiciness of the products. Lee et al. (1998) had reported that the addition of sodium salts like sodium phytate, sodium pyrophosphate and sodium tripolyphosphate along with sodium chloride increased cooking yield in restructured beef. In low sodium meat systems, this important function of salt is greatly compromised. In the present study cooking loss varied significantly ($p \leq 0.05$) among the systems ranging from 6.09% in whey protein concentrate-corn flour system to 14.05% in whey protein-maltodextrin system, with control system reporting the highest percentage of cooking loss (Figure 1.). Szerman et al. (2012) reported improved yield in reduced salt (1.25% NaCl) sous vid cooked beef muscles with the addition of whey protein concentrate. Other studies though not on low sodium meat systems have nevertheless, indicated that incorporation of binders and non meat proteins can help to increase hydration and retain water during cooking, resulting in lower cook losses. Modi et al. (2003) reported highest cooking yield in buffalo meat burgers containing black gram flour compared to burgers prepared with other legume flours such as Bengal gram, green gram and soya bean. Addition of corn flour in buffalo meat cutlets resulted in higher cooking yield than the addition of refined wheat flour, tapioca and potato starch (Ahamed et al., 2007).

The present study shows that systems having higher water holding capacity were found to resist losses during cooking to a greater extent than samples with lower water holding property, indicating that water holding capacity had a direct impact on cooking loss. This agrees with Youssef and Barbut (2011) who reported that non meat proteins like whey and soy proteins improved water holding capacity of meat batters and thus, reduced cooking loss. The same trend was seen in sausages extended with increasing levels of common bean flour (2.5, 5, 7.5 and 10 %) as cooking loss decreased with increasing water holding capacity (Dzudie et al., 2002). Pietrasik and Chan (2002) also reported that combined addition of non meat proteins and binders favored water binding in beef gels and thereby reduced cooking loss. It was observed in present study that higher the amount of salt soluble proteins extracted from the systems, lower was the cooking loss owing to greater retention of water within the protein structure. Systems containing black gram flour and corn flour showing higher concentration of salt soluble protein suffered from lower losses during cooking.

Texture Profile Analysis

Texture plays an important role in determining the consumer acceptability of any food. Texture of meat systems is primarily influenced by muscle proteins and their interaction with other non meat ingredients. Texture parameters namely hardness, springiness, cohesiveness, gumminess, and chewiness were obtained from Texture Analyser using Nexigen software and the results are reported in Table 4. Hardness was significantly influenced by the kind of non meat protein and binder used. Incorporation of corn flour resulted in least hardness in both whey protein and soy protein based systems when compared to other binders. Ikhlas et al. (2011) had reported low hardness score for corn flour and soy flour incorporated quail meat ball than those for meatballs containing other flours like potato and wheat flour. Control sample and maltodextrin incorporated systems were found to have the high scores for hardness. The harder texture could possibly be attributed to greater moisture release during cooking. The hardness values of soy protein systems were lower than whey protein incorporated systems. Youssef and Barbut (2011), who also obtained similar results between soy and whey protein treated meat batters, suggested that the weaker molecular interactions of soy-meat proteins than those of the whey-meat protein could be responsible for the lower hardness values for soy protein treated samples. A previous study had reported increase in the hardness of low fat, reduced salt ground beef patties with the addition of whey protein concentrate, although it did not affect its acceptability by sensory panellists (El-Magoli et al., 1996). Non meat proteins can combine with muscle proteins to form mixed gels. Whey protein in association with muscle proteins give rise to a much stronger gel with increased hardness (Foegeding, 1988).

Springiness and cohesiveness did not show significant difference ($p > 0.05$) between the meat systems except in the case of soy protein-pectin system for which both the parameters recorded lowest values. Gumminess and Chewiness varied significantly ($p \leq 0.05$) between the samples with whey protein treated samples generally showing higher values for the both the parameters than soy protein treated samples. This could be due to the difference in the gel matrices formed by these two proteins in the meat system and the difference in the viscosity of the meat batter on their addition (Youssef and Barbut, 2011). These results were in agreement with findings by Ulu (2004), who reported higher scores for hardness, gumminess and chewiness in cooked meat balls containing whey protein concentrate than those containing soy protein isolate. This effect may be due beta-lactoglobulin present in whey

protein which is found to increase hardness of meat products (Hayes et al., 2005). Previous studies have reported that addition of whey protein to low salt meat products helped to improve textural properties (Szerman et al., 2008, 2012).

From the texture profile analysis results it is evident that the interaction between non meat proteins and binders can influence the gel formation in low salt meat systems and thereby affect the textural parameters like hardness, gumminess and chewiness.

Optimization of data for selection of the best protein-binder combination

Numerical optimization was carried out for low salt meat systems for obtaining the best non meat protein and binder combination. Two factors were considered namely non meat protein (two levels; whey protein concentrate and soy protein isolate) and binder (six levels; corn flour, rice flour, bengal gram flour, black gram flour, pectin and maltodextrin) and their interactions were studied. Constraints were imposed on different factors and responses like water soluble protein concentration, salt soluble protein concentration and water holding capacity were kept maximized, whilst hardness and cooking loss were minimized. Highest desirability was established from the numerical solution obtained against the constraints (Figure 2). Black gram flour- whey protein system having the maximum desirability at 89% was adjudged as the best binder- non meat protein combination for low salt meat systems.

Table 1. Formulation of low salt poultry meat systems

Treatment	Lean meat (%)	Ice (%)	Salt (%)	Non meat Protein (%)		Binder (%)					
				WPC	SPI	Corn flour	Rice Flour	Bengal gram flour	Black gram flour	Pectin	Maltodextrin
1	80	15	1	1	-	3	-	-	-	-	-
2	80	15	1	1	-	-	3	-	-	-	-
3	80	15	1	1	-	-	-	3	-	-	-
4	80	15	1	1	-	-	-	-	3	-	-
5	80	15	1	1	-	-	-	-	-	3	-
6	80	15	1	1	-	-	-	-	-	-	3
7	80	15	1	-	1	3	-	-	-	-	-
8	80	15	1	-	1	-	3	-	-	-	-
9	80	15	1	-	1	-	-	3	-	-	-
10	80	15	1	-	1	-	-	-	3	-	-
11	80	15	1	-	1	-	-	-	-	3	-
12	80	15	1	-	1	-	-	-	-	-	3
Control	80	15	1	-	-	-	-	-	-	-	-

WPC- Whey Protein Concentrate, SPI- Soy Protein Isolate

Table 2. Water and salt soluble protein concentration of low salt meat systems

Treatment	Water soluble protein concentration (mg protein/ml)		Salt soluble protein concentration (mg protein/ml)	
	Raw	Cooked	Raw	Cooked
1	10.71 ± 0.47[c]	4.83 ± 0.59[cd]	6.57 ± 0.01[c]	1.84 ± 0.02[d]
2	9.67 ± 0.21[d]	4.37 ± 0.22[de]	6.52 ± 0.04[c]	1.58 ± 0.08[de]
3	11.53 ± 0.06[b]	5.67 ± 0.27[b]	7.23 ± 0.23[b]	2.59 ± 0.01[bc]
4	12.46 ± 0.13[a]	6.72 ± 0.19[a]	8.24 ± 0.58[a]	2.92 ± 0.14[a]
5	6.77 ± 0.26[f]	3.03 ± 0.91[g]	5.50 ± 0.49[d]	1.72 ± 0.22[de]
6	6.28 ± 0.25[f]	2.34 ± 0.49[h]	4.48 ± 0.08[f]	1.61 ± 0.24[de]
7	9.91 ± 0.21[d]	3.96 ± 0.19[ef]	5.35 ± 0.03[de]	1.79 ± 0.03[de]
8	8.32 ± 0.52[e]	3.62 ± 0.13[fg]	5.17 ± 0.04[de]	1.43 ± 0.13[e]
9	11.81 ± 0.24[b]	5.11 ± 0.27[bc]	7.97 ± 0.54[a]	2.49 ± 0.24[c]
10	11.63 ± 0.04[b]	5.47 ± 0.18[bc]	7.18 ± 0.26[b]	2.79 ± 0.10[ab]
11	6.69 ± 0.34[f]	1.82 ± 0.05[hi]	4.83 ± 0.02[ef]	1.72 ± 0.19[de]
12	6.20 ± 0.22[f]	1.72 ± 0.10[hi]	5.22 ± 0.06[de]	1.46 ± 0.07[e]
Control	5.06±0.06[g]	1.36± 0.49[i]	4.28±0.04[f]	1.04± 0.04[f]

Values are means±SD, n=3

Values with different superscripts within a column differ significantly ($p \leq 0.05$)

Table 3. Water holding capacity and cooking loss of low salt meat systems

Treatment	Water holding capacity (%bound water)	
	Raw	Cooked
1	50.31 ± 0.57^{a}	65.84 ± 1.65^{a}
2	47.22 ± 2.96^{ab}	56.59 ± 0.96^{c}
3	45.32 ± 2.28^{b}	54.96 ± 1.03^{cd}
4	48.11 ± 1.77^{ab}	61.43 ± 0.61^{b}
5	34.21 ± 1.52^{d}	54.40 ± 1.13^{cd}
6	28.84 ± 1.94^{e}	52.56 ± 0.86^{de}
7	47.48 ± 1.77^{ab}	62.33 ± 1.17^{b}
8	41.01 ± 0.88^{c}	55.60 ± 1.16^{cd}
9	33.34 ± 2.38^{d}	51.28 ± 0.92^{ef}
10	42.18 ± 0.94^{c}	56.67 ± 1.05^{c}
11	32.62 ± 1.73^{d}	54.77 ± 0.99^{cd}
12	28.06 ± 1.44^{e}	49.14 ± 1.14^{f}
Control	24.89 ± 0.21^{f}	45.11 ± 2.25^{g}

Values are means±SD, n=3

Values with different superscripts within a column differ significantly ($p \leq 0.05$)

Table 4. Texture profile analysis of low salt meat systems

Treatment	Hardness (N)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (Nmm)
1	8.48±0.84^{g}	0.38±0.04^{a}	8.96±0.50^{ab}	3.47±0.49^{gh}	35.28±1.42^{e}
2	11.65±1.16^{f}	0.39±0.02^{a}	10.05±0.17^{a}	4.45±0.22^{e}	45.10±4.28^{d}
3	14.28±0.48^{d}	0.39±0.01^{a}	9.99±0.14^{a}	5.64±0.22 ^{c}	55.72±2.76^{c}
4	13.39±0.26^{de}	0.41± 0.02^{a}	10.34±0.15^{a}	5.45±0.30^{cd}	56.41±3.77^{c}
5	9.60±0.57^{g}	0.36±0.02^{a}	9.73±0.12^{a}	3.44±0.14^{gh}	33.48±1.22^{e}
6	20.93±0.97^{a}	0.40±0.01^{a}	9.98±0.54^{a}	7.68±0.15^{a}	77.63±4.71^{a}
7	9.17±0.58^{g}	0.41±0.01^{a}	10.59±0.29^{a}	3.75±0.27^{fg}	39.72±2.64^{de}
8	11.21±0.57^{f}	0.38±0.02^{a}	10.60±0.24^{a}	4.26±0.45^{ef}	44.73±1.92^{d}
9	12.32±0.64^{ef}	0.37±0.02^{a}	9.75±0.35^{a}	4.79±0.58 ^{de}	46.85 ± 3.79^{d}
10	12.72±0.33^{ef}	0.41±0.01 ^{a}	10.18±0.18^{a}	5.20±0.28^{cd}	52.99±3.22^{c}
11	9.57±0.96^{g}	0.30±0.03 ^{b}	8.48±1.71^{b}	2.83± 0.39^{h}	23.55±1.48^{f}
12	16.89±0.23^{b}	0.38±0.01^{a}	9.97±0.26^{a}	6.52±0.18^{b}	64.69±2.67^{b}
Control	15.65±0.21^{c}	0.38± 0.01^{a}	9.57± 1.10^{a}	6.31 ± 0.03 ^{b}	63.19± 1.01 ^{b}

Values are means±SD, n=3

Values with different superscripts within a column differ significantly ($p\leq0.05$)

Table 5. Solutions for optimum conditions

Solution number	Non meat protein	Binder	Desirability
1	Whey protein	Black gram flour	0.89
2	Whey protein	Corn flour	0.73
3	Soy protein	Black gram flour	0.69
4	Whey protein	Bengal gram flour	0.56
5	Soy protein	Bengal gram flour	0.55
6	Soy protein	Corn flour	0.54
7	Whey protein	Rice flour	0.52

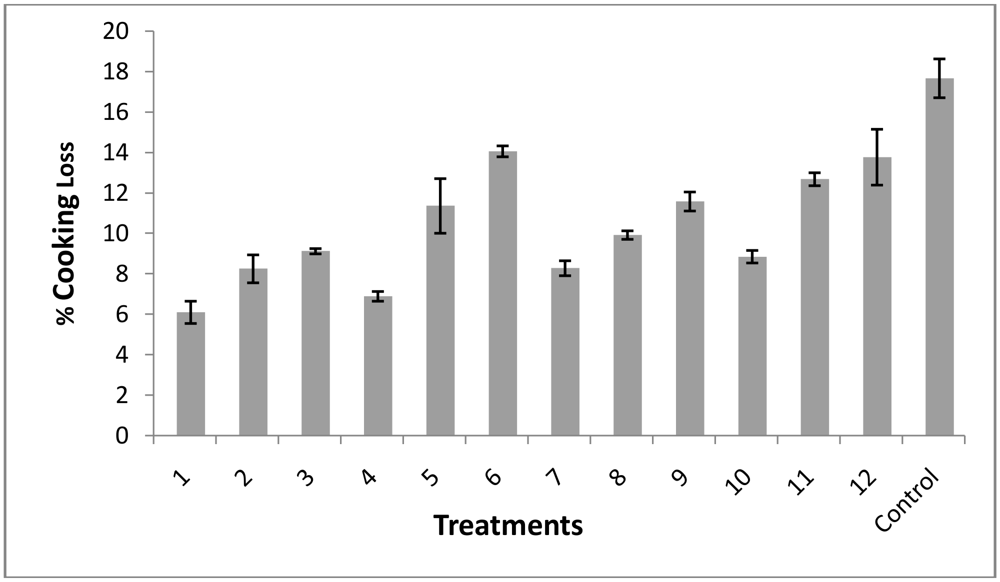

Fig.1. Cooking loss of low salt meat systems.

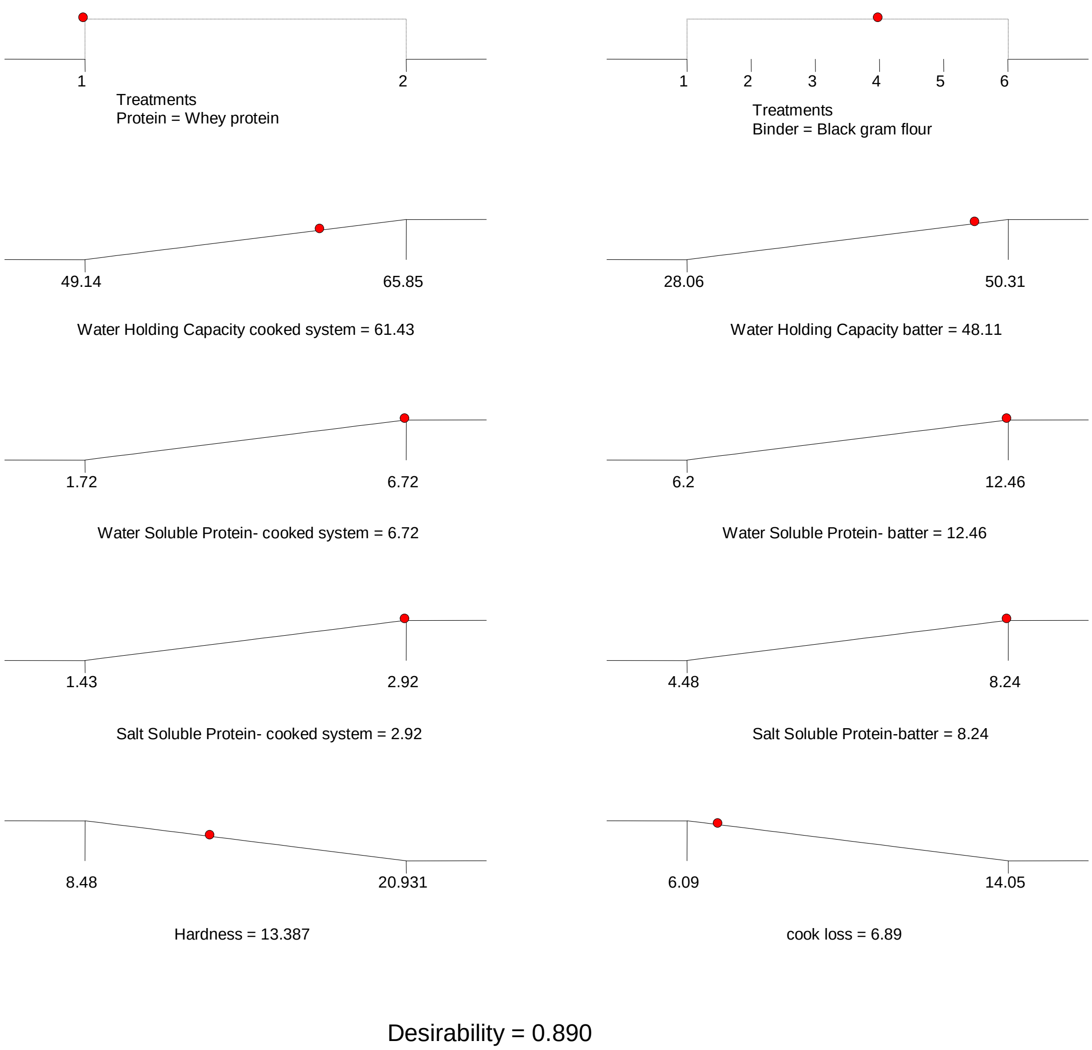

Fig. 2. Solution for the numerical optimization of low salt poultry meat system

CONCLUSIONS

Incorporation of non meat proteins and binders in low salt poultry meat systems can improve functional properties like moisture retention and binding that could be due to the interaction between the non meat proteins and binders to form stable complexes. Addition of proteins and binders resulted in greater extraction of water and salt soluble proteins, improved water holding capacity and thereby, reduced cooking losses. The textural properties varied depending on the type of non meat protein and binder used. Whey protein and black gram flour incorporated system showed the highest desirability at 89%, thus being selected as the most suitable non meat protein-binder combination for promising applications in low salt meat products.

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