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Effect of *Kappaphycus Alvarezii* in Development of Functional Based Reformulated Chicken Sausages Utilizing Spent Hen Meat

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ABSTRACT

Development of functional based reformulated chicken sausage is a newer challenge since this type of product not only contributes functional health attributes but also improves or at least retains product's quality. So, a research study was undertaken to develop a functional chicken sausage with incorporation of *Kappaphycus alvarezii* (KP-A) powder. KP-A was added in the formulation at 0 (Control), 0.5 (T_1), 0.75 (T_2) and 1.0 (T_3) % levels, mixed separately, encased in casings and finally, water cooked. The products were evaluated for different physicochemical quality, antioxidant activity, instrumental color and sensory quality parameters. Result showed that cooking yield was significantly ($P<0.05$) higher in T_3 sample than other treatments. Similar observations were found for emulsion stability and emulsion and cooked product pH. The WBSFV increased with the increasing in the level of KP-A and it was highly significant in T_2 sample than control. In case of Lovibond tintometer colour, there were non-significant ($P>0.05$) differences in redness (*a*-value), yellowness (*b*-value) Hue and Chroma values amongst the treated samples and control. However, incorporation of KP-A in the product formulations showed only numerical increase in $ABTS^+$ and DPPH activity. Sensory evaluation parameters revealed that T_1 and T_3 samples scored nearly similar but the scores for T_2 sample were higher for all attributes than control, T_1 and T_3. Thus, it was concluded that addition of KP-A has greater role in improving functional health attributes of chicken sausage besides its contribution in improving product quality.

Keywords: *Kappaphycus alvarezii, Functional chicken sausage, Reformulated meat product, Antioxidant activity, Meat color*

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INTRODUCTION

In the recent years, it has been reported that aquatic plants especially sea weeds have diverse functional activities in variety of processed food products. Amongst all the sea weed varieties, the red sea weed that is family Rhodophyta are most important. They are widely distributed around the world including Philippines, Indonesia and India. Some red sea weeds are economically important as delivered many structural and functional benefits towards food use. *Kappaphycus alvarezii* commonly called Irish moss or carrageen moss is eukaryotic algae comprised of a soft mucilaginous body which contains many functionally active compounds including polysaccharide (55%), protein (10%) and minerals (15%). The mineral matters include sodium, potassium, calcium, magnesium; iodine and sulfur are notably abundant.

Sausage items though are widely distributed amongst the vast array of population but their quality improvement using *Kappaphycus alvarezii* (KP-A) as a functional ingredient only little exploited. Sausages are emulsion based meat products encased in casings and then processed in variety of ways. Production of biogenic amines during fermentation, degradation of bioactive components due to heat processing or bioactive molecule's unwanted association with many organic molecules in meat are some of the major challenges in development of meat sausages. However, sound and innovative technological adoption may offset some of these detrimental effects. Biswas *et al.* (2018) reported that use of KP-A in the chicken meat bite processing substantially improved functional activity of finished product but reported findings yet to be validated through *in-vivo* human / animal model trials. While Necas and Bartosikova (2013) reported that kappa carrageenan (a product of KP-A) had vast array of bioactivities

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such as anticoagulant, antiviral, cholesterol-lowering effects, immunomodulatory activity, and antioxidant activity. Kappa carrageenan possesses promising functional activity both *in-vitro* and *in-vivo*, with promising potential to be developed as therapeutic agents. Mandal *et al.* (2019) reported that use KP-An in hens diet improved productive performances and egg quality traits. Similarly, Quadri *et al.* (2019) observed that use of KP-An in broiler chicken diet improved physicochemical and microbiological characteristics of meat. But, there are no other literature reports on use of KP-A as functional ingredients in development of chicken meat products though other processed forms such as kappa carrageenan are under used for technological improvement i.e., increase of cooking yield, hardness and bind strength of low-fat sausages, although these effects were less pronounced at higher salt concentrations (Xiong *et al.* 1999). Trius *et al.* (1994a) reported that kappa and lambda carrageenan when combined with NaCl or KCl, in low-fat beaker sausage lowered cooking losses compared to controls. Further, Trius *et al.* (1994b) observed that the addition of lamda carrageenan produced softest texture in beaker sausage while kappa and iota carrageenans improved water retention. Thus, the present work was conducted to develop a suitable chicken sausage with the incorporation of KP-A powder in the formulation.

MATERIALS AND METHODS

Sources of raw materials

Source of meat: Spent chicken of above 50 weeks age group of both the sexes were selected from Experimental layer and broiler farms of ICAR-Central Avian Research Institute, Izatnagar, and Bareilly, India. The birds were slaughtered as per standard slaughtering techniques in the Experimental Poultry Processing Plant of Division of Post-Harvest Technology, ICAR-Central Avian Research Institute, Bareilly, India. Immediately after slaughtering operations were over, the carcasses were shifted to the laboratory for processing. For this, skin of the carcasses was removed – off, hot deboned, packed in self-sealing LDPE bags, labeled and finally, kept at refrigeration temperature (4 ± 1 ºC) for overnight. The meat was then shifted to deep freezer and stored at -18 ± 2ºC until further use. The spice mix, condiments and other ingredients used in the experiments were procured from local market of Bareilly, Uttar Pradesh, India.

Spice mix: The spices were procured from local market of Bareilly, India. After removal of extraneous matters, the spices were oven dried at 50 ± 2°C for 2 h. The ingredients were ground mechanically and sieved through a fine (U.S.S. #30) mesh screen. The powders so obtained were mixed in suitable proportion to obtain a spice mix for marinade. The spice mix was stored in a PET (polyethylene terephthelet) jar for subsequent use.

Condiments (green curry stuff): Condiments used in the study (onion: garlic: ginger = 3:1:1; w/w/w) were purchased from local market of Bareilly, India. After peeling off the external coverings of fresh and clean onion, garlic and ginger, they were chopped-off into small pieces and ground in a mixture grinder to make fine paste of each component, separately. All the ingredients were mixed well in the above mentioned proportion for use in the experiments.

Table salt, sodium tri-polyphosphate, sodium nitrite etc.: Table salt (Tata Chemicals Ltd., Mumbai, India), sodium tri-polyphosphate, citric acid, sodium ascorbate, sodium nitrite etc. were procured from SRL Pvt. Ltd., New Delhi, India, wheat flour and other ingredients used in preparation of emulsion.

Chemicals and reagents: All the chemicals and reagents used in the study were analytical and molecular biology grades. Chemicals include Tris – HCl, 2-2-azinobis-3-ethylbenthiazoline-6- sulfonic acid cation ($ABTS^+$), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), potassium per-sulfate and ethanol. ABTS, DPPH and standards used in this study were procured from Sigma Aldrich, USA. Other chemicals were procured from reputed firms like SRL Pvt. Ltd., New Delhi, India, s. d. Fine Chemicals, Mumbai, India etc.

***Processing of chicken sausage*:** For processing of chicken sausage, about 4.0 kg of lean meat was ground through a meat grinder (Hobart, USA) using an 8 mm grinding plate (sieve). It was then divided into four different batches of 1.0 kg each. The *Kappaphycus alvarezii* (KP-A) were added @ 0. 0.5, 0.75 and 1.0% level for control, T_1, T_2 and T_3, respectively. The added ingredient of all treatment groups was replaced with the lean meat. All batches of samples were mixed manually and kept for 30 min at 4 ±1ºC. These were then mixed separately with the curing ingredients, fillers, binders and other seasonings in a Hobbart paddle type mixer for 8 min. The sausage batters were encased in artificial cellulose casings and then were subjected to heat process by water cooking. All the products were subjected to evaluation for different quality parameters.

Analytical Procedures

Physicochemical quality: For quality analysis of chicken sausage, it was evaluated for emulsion stability (Baliga and Madaiah 1970), emulsion and cooked product pH (Trout *et al.* 1992), cooking yield and W-B shear force value (WBSFV) (Berry and Stiffler 1981). For determination of emulsion stability, accurately 20 g of meat sample was taken in polyethylene bag and heated in a thermostatically controlled water bath at 80ºC for 20 min. Then the exudate was wiped out and the cooked mass was weighed. The percentage of cooked mass was expressed as emulsion stability. Cooking loss of a product is inversely proportional to its emulsion stability. For determination of pH, meat homogenates were prepared by blending 10 g sample with 50 ml distilled. PH of homogenates was recorded by digital pH meter (Eutech, pH 2700). The cooking yield was calculated using the formula- Cooking yield (%) = weight of cooked product after cooling/ (weight of raw product) x 100. The WBSFV of sausage was determined by placing the fully cooked chicken sausage (diameter 1.2 cm) in the blade attached to the Warner-Bratzler shear force apparatus (Model 81031307, G.R. Elect. Mfg. Co. USA). Ten observations were recorded for each sample to obtain the value of shear force in kg/cm^2.

Lovibond tintometer color*:* The colour profiles of cooked sausage were measured using Lovibond tintometer (Model F, Greenwich, U.K.). Four different samples (n=12) of each product were analyzed. The sample colour was matched by adjusting red (*a*) and yellow (*b*) units and the corresponding colour units were recorded. The 'Hue' and 'Chroma' values were determined by using formula, $(\tan^{-1})$ b/a and $(a^2 + b^2)^{1/2}$ (Froehlich *et al.* 1983) respectively, where a, red unit; b, yellow unit.

Antioxidant activity (AOA): The AOA of cooked sausage sample was determined by evaluating ABTS$^+$ (2-2-azinobis-3ethylbenthiazoline-6-sulphonic acid) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) activity. Both these parameters were determined following spectrophotometric methods as mentioned by Biswas *et al*. (2015). This method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of standard antioxidants while the activity of DPPH was determined by evaluating the ability to scavenge free radicals in aqueous or ethanol solution. The absorbance of DPPH was taken at 517 nm. The free radical scavenging activity was calculated as a decrease of absorbance from the equation as developed after calibration of standard curve. Gallic acid (200-600 mM/ml) was used as a standard antioxidant.

Sensory quality: A seven members experienced panel of judges consisting of teachers and postgraduate students of Poultry Science Discipline, ICAR-IVRI, Izatnagar were evaluated the samples for the attributes of appearance and colour, texture, flavour, juiciness and overall acceptability using an 8 point descriptive scale (Keeton 1983), where 8=extremely desirable and 1=extremely undesirable. Three sittings (n=21) were conducted for each replicate and at each storage time on samples warmed in a microwave oven for 20 sec. Tap water at room temperature was provided to each panel member to rinse the pallet before tasting of each sample.

Statistical analysis: Experimental data were analyzed statistically using standard software packages version SPSS-16 as developed by Snedecor and Cochran (1994). Duplicate samples were drawn for each parameter and the experiment was replicated thrice (n=6). Sensory evaluation was performed by a panel of seven members judges three times, so total observations being 21 (n=21).) The Lovibond tintometer colour was determined by taking four sub-samples from each sample and thus total observations were 12 (n=12). Data generated for the experiments was analyzed using one-way ANOVA, homogeneity test and Tukey's Test (Tukey 1949) for comparing the means to find the effects between treatments. The statistical significance was expressed at $P < 0.05$.

RESULTS AND DISCUSSIONS

Physicochemical parameters**:** Data (Table 1) revealed a significant effect ($P<0.05$) on cooking yield, emulsion stability, emulsion pH and WBSFV but the product pH did differ significantly. It was observed that cooking yield was significantly higher in T_3 sample than other treatments and the values were increased with the increase of *Kappaphycus alvarezii* added levels. Emulsion stability, an index of loss of water, fat and solids from meat emulsion on heating, increased significantly ($P<0.01$) with the incorporation of *Kappaphycus alvarezii*. Similar observations were reported by Biswas *et al.* (2016) who demonstrated that cooking yield of chicken nuggets was found to be increased due to addition of carrageenan. While in this study, it was found that inclusion of KP-A powder could have greater role in improvement of cooking yield. It could be due to the ability of KP-A to form complexes with water and protein (Egbert *et al.,* 1991) which improved water retention and cooking yield. However, higher emulsion stability of treated samples could be due to presence of high ionic strength soluble protein present in KP-A (He and Sabranek, 1996). The pH of emulsions containing different levels of *Kappaphycus alvarezii* differ significantly (P<0.01) and maximum pH obtained in T_3 sample. Cooked sausage showed comparatively higher pH than raw emulsion without compromising any treatment groups. The WBSFV increased with the increasing in the level of *Kappaphycus alvarezii* and it was highly significant in T_2 sample than control. The significant increase in WBSFV was due the reduction of fat level and increase in added water level which gives soft mushy texture of the product (Keeton 1994).Further, the pH has dominant role in the physical and biochemical changes during muscle to meat conversion that taking place during storage (Devine, 1993). Changes of pH in muscle also directly affect the WHC of meat proteins by effecting the protein structure and subsequent hydration properties (Petracci *et al.*, 2001). Polysaccharides' gums are often used to hold water and enhance the texture properties of low fat meat products (Garcia-Cruz and Scamparini, 1992). Similar observations were reported by Nayak *et al*. (2015) who observed that there was a significant (P<0.05) difference in pH values between the control and carrageenan treated chicken nuggets. The higher W-BSF values in T_3 samples could be due to compact and tight binding with the increase level of KP-A leads to harder products therefore achieved greater values (Biswas *et al.*, 2018).

Table 1: Effect of *Kappaphycus alvarezii* on physicochemical quality of chicken sausages

Parameters	Control	T_1	T_2	T_3	SEM	P Value
Physicochemical parameters						
Cooking Yield (%) #	88.64^a	94.19^a	94.44^{bc}	95.90^c	0.33	0.001
Emulsion Stability (%)	86.29^a	93.05^b	93.071^b	93.70^b	0.58	0.001
Emulsion pH	6.36^a	6.45^b	6.54^{bc}	6.58^c	0.14	0.001
Product pH	6.63	6.50	6.60	6.64	0.04	0.197
W-BSFV (kg/cm²)	0.27^a	0.33^{ab}	0.39^b	0.37^{ab}	0.02	0.001

n=3, Mean with different superscript row-wise differ significantly at ($p < 0.05$) T1= 0.5%, T2=0.75% and T3=1.0 % of *Kappaphycus alvarezii* Lovibond *tintometer color and antioxidant activity*

The result in Table 2 showed that there were non-significant (P>0.05) differences in redness (*a*-value), yellowness (b-value) hue and chroma among the treated samples and control. Further, it was also observed that with the increase of inclusion level of KP-A up to 1.0% there was no change in color values for any color co-ordinates. But contradictory findings were reported by Biswas *et al.* (2018) in chicken meat bites formulated with KP-A. They reported that dilution of color pigment in chicken meat bites could be due to addition of yellow component and also compensatory water in the formulation of low fat chicken bites. Fat reduction results in darker sausages with less red color, but has no effect on yellowness (Hughes *et al.*, 1998). Addition of water in low-fat meat products resulted in a higher dilution of color and consequently less red color (Morin *et al.*, 2002).

However, antioxidant activity of chicken sausage was little affected with the inclusion of KP-A since this product showed only a numerical increase in ABTS+ and DPPH activity. This indicates KP-A could have very little or no antioxidantive effect on cooked chicken sausage. The control sample showed antioxidant effect due to presence some other food ingredients added in the formulation. Little higher but non-significant ABTS activity of treated samples could be attributed to the presence of polysaccharides and some other bioactive compounds in KP-A (Matanjun *et al.*, 2008). The contribution of other phenolic compounds cannot be ignored (Song *et al.* 2010) which have different antioxidant capacity (Tatiya *et al.*, 2011). The antioxidant activity of kappa- carrageenan was reported by Necas and Bartosikova (2013). All the samples showed very poor DPPH activity.

Table 2: Effect of *Kappaphycus alvarezii* on instrumental colour and antioxidant activity of chicken sausage

Parameters	Control	T_1	T_2	T_3	SEM	P Value
Lovibond tintometer colour#						
Redness(*a*-value)	2.47	2.50	2.50	2.52	0.04	0.882
Yellowness(*b*-value)	6.65	6.60	6.68	6.60	0.04	0.461
Hue	1.22	1.21	1.21	1.21	0.01	0.763
Chroma	7.09	7.06	7.14	7.06	0.04	0.516
Antioxidant activity						
$ABTS^+$	59.74	65.50	66.48	68.07	2.30	0.151
DPPH	16.01	18.20	16.88	16.76	0.98	0.507

n=6, #n=12, Mean±SE with different superscript row-wise differ significantly at ($p < 0.05$) T_1= 0.5%, T_2=0.75% and T_3=1.0 % of *Kappaphycus alvarezii*

Sensory evaluation: Sensory evaluation parameters revealed that treated samples showed highest scores for all attributes *viz*., color and appearance, flavor, texture, juiciness and overall acceptability. Amongst the treatment groups, T_1 $_3$ samples scored nearly similar but the scores of T_2 sample were comparatively higher than that of scores received for other two treated samples for all attributes. T_2 sample showed greatest scores for all attributes and differed significantly (P<0.05) from control but non-significantly (P>0.05) from T_1 $_3$. The texture and juiciness scores were differed non-significantly (P>0.05) amongst control, T_1 and T_3. Thus, it was attributed that increasing of concentration of KP- A up to 0.75 % increased sensory scores for all attributes. Similar observation was reported by Biswas *et al.* (2018) in the color and appearance score of KP-A treated chicken bites. These findings are also in the accordance with the results of Indumathi *et al.* (2011) in low-fat chevon patties. Further, Biswas *et al.* (2016) reported comparatively higher sensory scores for appearance and color, texture and flavor attributes in functional chicken nugget incorporated with 2% broken wheat and 1% carrageenan. Huffman *et al.* (1991) also reported the similar findings in low fat beef patties. Thus, it was found that KP-An addition up to 0.75% level was very much desired in development of functional chicken sausage.

Table 3: Effect of *Kappaphycus alvarezii* on sensory quality of chicken sausage

Parameters	Control	T_1	T_2	T_3	SEM	P Value
Sensory evaluation*						
Colour & appearance	6.45^a	7.12^b	7.19^b	7.05^b	0.10	0.001
Flavour	6.48^a	7.14^b	7.05^b	7.07^b	0.12	0.001
Texture	6.40^a	7.02^b	7.10^b	6.83^{ab}	0.12	0.002
Juiciness	6.55^a	7.10^{ab}	7.29^b	6.88^{ab}	0.12	0.001
Overall Acceptability	6.51^a	7.21^b	7.17^b	7.18^b	0.11	0001

n=21,*Based on 8-point descriptive scale (where 8=extremely desirable and 1= extremely undesirable). Mean±SE with different superscript row-wise differ significantly at ($p < 0.05$) T_1= 0.50%, T_2=0.75% and T_3=1.0 % of *Kappaphycus alvarezii*

CONCLUSION

It was found that addition of 0.75% of *Kappaphycus alvarezii* in development of chicken sausage could help in improvement of physicochemical quality besides its beneficial effects antioxidant activity, color stability and sensory acceptability of finished products.

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CONFLICT OF INTEREST: The authors declare that they have no competing interests.

ETHICS STATEMENT: Not applicable

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