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Quality Assessment and Comparative Study of Value-added Giblet Pickle Incorporated with Red Calyces of Roselle (*Hibiscus subdariffa*) and Acetic Acid

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ABSTRACT

Background: The present study was carried out to develop a suitable formulation for value added pickle out of chicken giblet by incorporating roselle and evaluate their physico-chemical properties, sensory attributes and shelf-life qualities through microbial count, extend of proteolysis and fat rancidity after packaging in normal food grade polyethylene packaging material stored at ambient temperature.

Methods: The developed value added giblet pickle was standardised with 15% rosella. The comparison was made among the samples incorporated in paste, oven dried powdered and 4% acetic acid.

Result: The addition of 15% roselle in paste form increased the overall acceptability as well as the product yield. The protein content of both the treatments was found to be higher compared to the control. The pH of control (3.06±0.04) ranged to 4.25±.07 in treatment. The water activity did not differ significantly in control and treatments and the value ranged from 0.94±0.12 to 0.95±0.04. Thiobarbituric acid value (TBARS), tyrosine values and microbial count significantly ($p<0.05$) increased during storage days in all the pickle samples. However, the increase in TBARS, tyrosine values and microbial load were within the recommended limits as per FSSAI for pickled meat products. From the above study, it can be conclude that value-added giblet pickle with good nutritive value and higher acceptability can be prepared by incorporating roselle at 15% level.

Key words: Acetic acid, Calyces, Giblet, Roselle, Value added pickle.

INTRODUCTION

Chicken giblets (heart, liver and gizzard) are widely used for direct consumption in most of the developing countries due to their lower cost and shorter preparation time compared to the main edible carcass. From nutritional point as well, edible by-products offers a good source of protein due to the presence of various essential nutrients in it. However, they are highly perishable and possess shorter shelf life compared to the edible carcass, due to which they requires freezing temperature right from the point of slaughter till it reach consumers plate to maintain its optimum quality. Unfortunately due to inadequate processing technology and lack of cold chain facilities these by-products are not properly utilized and often go as waste or sold at very low prices.

Therefore it is need of the hour to develop a shelf-stable, cost effective and convenient ready to eat products using these edible by-products incorporating various locally available herbal bioactive ingredients for its value addition. The overall yield of edible by-products from chicken is 5.0 to 6.0 per cent of the live weight. Out of which the average yield of giblet is 4.36 per cent of the dressed weight. Consumers appreciate chicken based products due to lower price compared to other animal protein sources. Beside there exists no religious sentiment against consumption of chicken based products. Roselle plant (*Hibiscus subdariffa)* is an annual or perennial herb found in abundant in parts of Assam. The tender leaves and the bright red fruit with fleshy calyx are usually prepared with fish and meat by the Assamese people. Various literatures showed that the plant is rich in minerals like potassium and magnesium besides vitamins like ascorbic acid, niacin and pyridoxine. The plant has also been used as herbal tea to treat hypertension, pyrexia and liver damage although the active pharmaceutical components are poorly defined (Hou *et al*. 2005). Olaleye (2007) reported that the aqueous methanolic extract of roselle contained cardiac glycosides, flavonoids, saponins and alkaloids. It also exhibited antibacterial activities against *Staphylococcus aureus, Bacillus stearothermophilus, Micrococcus luteus, Serratiamascences, Clostridium sporogenes, Escherichia coli, Klebsiella, pneumoniae, Bacillus cereus* and *Pseudomonas*

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fluorescence. A shelf stable product incorporated with the calyces of roselle could be developed with the use of hurdle technology. However at present the available scientific literature shows not much work have been done on roselle incorporated with meat/edible by-products for value addition. Therefore, the present study was undertaken to develop and standardized a value-added pickle made from chicken giblet by incorporation of roselle as natural acidulant and compare it with that of acetic acid.

MATERIALS AND METHODS

Research design and sample preparation

Chicken giblet were collected from local butchers (meat shops) hygienically slaughtered for wholesome poultry meat production with proper post mortem inspection. All the birds were culled by halal method allowing maximum bleeding. External fats and fascia adhering on the outer surface of the gizzard were trimmed off, cut open to remove the inner lining along with the gizzard contents. Other non-meat ingredients were procured from local market. Condiments and green chillies used in the formulation were chopped manually on chopping board. The formulation (Table 1) of value added gizzard pickle was standardized by conducting several trials and preparation was done as per procedure shown in Fig 1. Bright red partially matured roselle fruits were plucked from backyard kitchen garden and the seeds were separated manually and only the calyces were used in the preparation. The roselle calyces were minced in a mixer grinder (Bajaj Rex 500W) without addition of water to prepare semi solid paste. And for the second treatment roselle calyces were oven dried at 60°C for 4 hours and grind to powder and used in the preparation of the value added gizzard pickle.

Determination of various parameters

The pH of the value added giblet pickle sample was determined using digital pH meter (Systronics) as per procedure of Troutt *et al*. (1992). The water activity was measured in chamber of Labswift water activity meter (Novasina, Switzerland). The moisture, ether extract, crude fibre and ash content were estimated as per AOAC (1990). Crude protein was estimated as per AOAC (1990) the copper catalyst Kjeldhal method was used for the determination. The analysis was carried out in Kel Plus nitrogen estimation system Pelican Equipment's, India). The total nitrogen estimated was converted to percentage of protein by multiplying with the constant. Crude protein% = 6.25 × Nitrogen%. Total yield per cent was determined by calculating the difference of weight before preparation and the final product as per Berry and Wergin, (1992). Microbiological analysis (Total Plate Count, yeast and mold, *Coliform* count and *Salmonella*) were carried out as per as American Public Health Association, (1984) on the day of preparation, 2nd, 5th, 7th and 9th day of storage at ambient temperature. Thiobarbituric acid (TBA) value was estimated by extraction method described by Witte *et al.* (1970) using spectrophotometer (Systronics-119, UV-visible spectrophotometer, Ahmedabad, India). The Tyrosine values of the samples were estimated as per the method described by Pearson (1968). Sensory attributes were established using 8-point Hedonic Scale (8= extremely acceptable; 1= extremely undesirable) by randomly selected ten semi trained taste panellists as per AMSA (1983) on the day of preparation 3rd, 5th, 7th and 9th day of storage. The sample with acetic acid treatment was used for comparison and the panellists were provided with luke-warm water mouth rinse after each testing. Colour of the pickle samples were determined objectively as per Page *et al.* (2001) using Hunter Lab Mini Scan XE Plus Spectrophotometer (Hunter Lab, Virginia, USA). The data of the colour reading were represented in the values L*, a* and b* respectively, where the L* value gives a measure of the lightness of the product colour from 100 for perfect white to 0 for black, b* denotes redness/greenness and a* gives yellowness/ blueness values, respectively. The cost of production was calculated based on the prevailing market cost of the raw materials used in the preparation. The data obtained with respect to physic-chemical, TBARS, tyrosine values, microbial count were analysed statistically using one way analysis of variance (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Physico-chemical composition

The physico-chemical composition of the roselle calyces used in the preparation of the value-added giblet pickle is shown in Table 2, which revealed that the moisture content

Table 1: Formulary for the preparation of control and value-added gizzard pickle.

Ingredients	Control (%)	**Roselle calyces
*Giblet	100	Treatment-1 (Control + 15% fresh roselle calyces in paste form)
Mustard oil	25	Treatment-2 (Control + 15% oven dried rosells calyces in powdered form)
Garlic	10	
Red chilli powder	3.0	
Green chilli	3.0	**Roselle calyces (paste and oven dried powdered) were added over and above
Curry leaves	1.0	Ginger 10 the quantity of the formulation as suggested by the model design for
Mustard seeds	5.0	development spices 10 of the value added giblet pickle.
Salt	3.0	
4% Acetic acid	15	
Total	100.00	

and yield per cent reduced after drying due to the loss of moisture. Table 3 shows the different physico-chemical parameters on control (with 15% acetic acid) and treatments- T_1 with 15% roselle in paste form and T_2 with 15% dried powdered form. Yield of T_1 significantly ($p<0.05$) increased compared to control and T_2. This increment might be due to the net addition of the roselle as natural acidulant over and above the control formulation (Table 1). Moisture content was 51.17±0.02 for control, 54.16±0.07 for T_1 and 47.14±0.14 T_2 respectively. Treatment T_2 showed significantly ($p<0.05$) lower moisture content which was due to the addition of oven dried roselle which had a moisture content of 16.25±0.25 compared to that of 89.20±0.21 in fresh calyces of roselle. The pH ranged from 3.06±0.04 in control to 4.25±0.07 in treatment. The pH in both the treatments did nt iffer significantly. The water activity of the developed value added gizzard pickle ranged from 0.94±0.12 to 0.95±0.04. Any food that has water activity greater than 0.85 and a pH of 4.6 or below can be categorised as 'pickles' or 'pickled' food (Barron 2007). Proximate parameters (protein, ether extract and fibre) of the developed value-added giblet pickle are shown in Table 3. The result showed that the protein content of control significantly ($p<0.05$) decreased compared to both the treatments, however T_1 and T_2 did not differ significantly. The lower protein content in control (with 15% acetic acid) might be due to more acid denaturation of protein

Separation of the giblet from carcass ← PM inspection

↓

Cooking by moist heat (boiling)

↓

Cool to room temperature and cut into small pieces

↓

Addition of small amount of salt and turmeric powder

↓

Deep fried in mustard oil in low flames till mildly done

↓

Frying of the spices mixture in a separate pan in low flame

↓

Add fried giblet and stir fry till the cooking medium surface

↓

Add 4% acetic acid and mix thoroughly

↓

Cool to room temperature

↓

Package in standing PE pouches

↓

Study of different parameters and shelf life

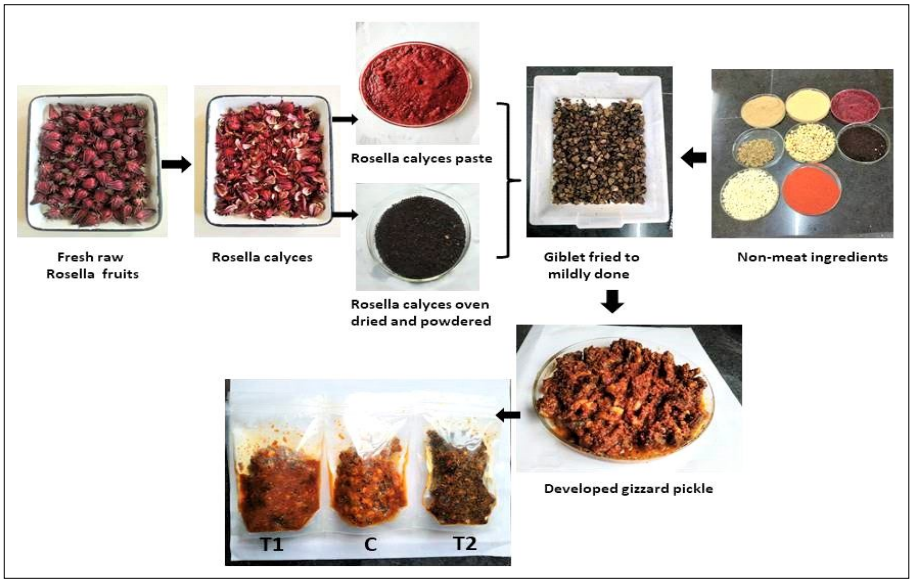

Fig 1: Processing technology.

Table 2: Physico-chemical parameters of the roselle calyces used in the preparation.

Parameters	Fresh roselle calyces	Oven dried roselle calyces
Yield	53.15% (after removing the seeds)	9.0% (after oven dried at 60°C/9 hours)
pH	3.24±0.10	3.73±0.08
Moisture (%)	89.20±0.21	16.25±0.25
a_w	0.993±0.03	0.324±0.01
Roselle calyces (% dry weight basis)		
Protein %		7.15±0.03
Fat %		0.49±0.02
Crude fibre %		12.24±0.05
Ash %		11.21±0.02

Table 3: Effect of roselle on the physico-chemical characteristics of the value added giblet pickle (day of preparation).

Parameter	Control (C)	Treatment (T_1)	Treatment (T_2)
Yield (%)	90.19^a	96.14^b	90.36^a
Moisture (%)	51.17±0.02^a	54.16±0.07^b	47.14±0.14^c
Protein (%)	24.19±0.04^a	25.01±0.08^b	25.42±0.05^b
Ether extract (%)	16.55±0.14	16.16±0.02	16.19±0.01
Crude fibre (%)	2.10±0.04^a	9.98±0.02^b	9.76±0.03^b
pH	3.06±0.04^a	4.25±0.07^b	4.16±0.01^b
a_w	0.94±0.12^a	0.95±0.04^b	0.94±0.05^a

Mean±SE with same superscripts in a row does not differ significantly ($p<0.05$).

(T_1=C + 15% fresh roselle calyces in paste form and T_2= Control + 15% oven dried rosells calyces in powdered form).

Table 4: Colour (Hunter L*a*b*) characteristics of the of the value added giblet pickle (day of preparation).

Parameters	Control	Treatment (T_1)	Treatment (T_2)
L* (Lightness)	30.71±0.12^a	31.25±0.08^b	28.24±0.25^c
a* (Redness)	8.21±0.02^a	14.17±0.05^b	8.87±0.04^a
b* (Yellowness)	22.14±0.03^a	21.27±0.05^b	18.68±0.03^c

Mean±SE with same superscripts in a row does not differ significantly ($P<0.05$).

(T_1=C + 15% fresh roselle calyces in paste form and T_2=Control + 15% oven dried rosells calyces in powdered form).

compared to T_1 and T_2. Ether extract content did not differ significantly in all the pickle samples. Similar results have been observed by Maiti *et al*. (2009) who reported that pickling had no significant effect on proximate composition of chicken gizzard and goat heart. However crude fibre content in T_1 and T_2 increased significantly ($p<0.05$) due to the addition of roselle, which might be due to the higher fibre content in the calyces of roselle. The result was in accordance with Pame *et al*., (2022) who reported that addition of fermented bamboo shoot in pickle prepared from intestine of pig had higher crude fibre contents.

L*a*b* colour value

Colour of any food item is considered as one of the most important quality aspects influencing consumer's acceptance. The results with respect to effect on colour (L*a*b*) values of the controls and the treatments are shown in the Table 4. L* and a* values of T_1 significantly ($p<0.05$) increased, however the L*and b* values were found to be decreased for T_2. An increased in redness (a*) is due to the rich red colour of the roselle calyx due to the presence of anthocyanins and decreased in yellowness (b*) might be due to the conversion of haemoglobin pigments of giblets into methaemoglobin upon cooking and might be due to the presence of more red pigment in the roselle calyces used in the treatments.

Microbial qualities at storage period

Effect of storage at ambient temperature on microbial qualities with respect to total plate count (TPC), yeast and mold, *E.coli* and *Salmonella* on the day of preparation up to 9th day is presented in Table 5. TPC of the control did not differ significantly from 0th day to 7th day of preparation, however it increased significantly ($p<0.05$) on the 9th day of storage. In case of the treatments TPC significantly ($p<0.05$) increased with days of storage. Yeast and mold were not observed initially in all the samples, however the count significantly ($p<0.05$) increased on the 9th days of storage on both the treatment groups. *E.coli* and *Salmonella* were not observed in all the samples on the day of preparation. The count were within the limits as per FSSAI recommendation for pickled type meat products which recommended a maximum permissible number of 500/gm for TPC, 100/gm for yeast and mold and 10/gm for *E.coli* Food Safety and Standards (Food Products Standards and Food Additives) Amendment Regulations, 2015). Relatively lower TPC in treatments as compared to the control might be due to the antimicrobial activity of the roselle. Reports on antimicrobial activity on the mentioned plant were already described by Jung *et al*. (2013).

Sensory attributes

The effect on sensory attributes of the value added giblet pickle presented in Table 6. The appearance and colour score for control did not differ significantly, however in T_1, it significantly ($p<0.05$) increased, which might be due to more red colour pigment (anthocyanin) in paste form, as bright red colour in in food materials are found to be more appealing for most of the consumers. Whereas in T_2 it reduced significantly ($p<0.05$), which might be due to more browning effect on oven drying of the calyses which was unsed in T_2.

Flavour, juiciness and overall acceptability of T_1 significantly ($p<0.05$) increased compared to control and T_2. This is due to the presence of high moisture content of roselle calyces (89.20%), acidic and rhubarb-like flavour. Sourness of control was found to be significantly ($p<0.05$) more, which might be due to difference in pH of acetic acid and roselle used in preparation. However, the overall acceptability of both the treatment groups reduced significantly ($p<0.05$) on 7th day of storage among the panellist, which might be due to the decrease in appearance and colour, flavour and juiciness attributes. This may be due to lipid oxidation and subsequent non-enzymatic browning due to reaction between amino groups with other oxidized compounds formed during storage period. (Che man *et al*., 1995).

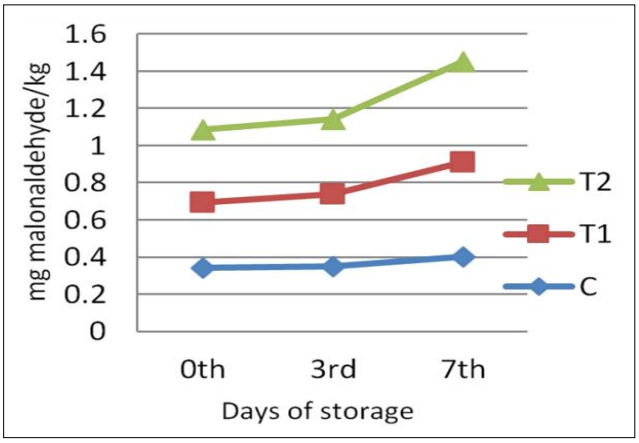

Fig 2: Effect of storage on TBARS.

TBARS and tyrosine value

The TBARS values (Fig 2) significantly increased ($p<0.05$) and the values range from 0.0341±0.12 to 0.401±0.02 for control, 0.351±0.16 to 0.510±0.04 for T_1 and 0.394±0.18 to 0.540±0.05 mg malonaldehyde/kg for T_2 respectively during storage period up to 7th days at ambient temperature. Similar increase in TBARS value was also reported in tenderized gizzard and goat heart pickle Maiti *et al*. (2009). The increase in TBARS values during the storage period was mainly due to the lipid oxidation and rancidity development due to the addition vegetable oil in the formulation. However, the increase in values for T_1 was much slower compared to

Table 5: Effect of storage at ambient temperature on microbial quality.

Days	0th	3rd	7th	9th
Total plate count (log cfu/g)				
C	2.15±0.43^a	2.41±0.06^a	2.39±0.02^a	3.72±0.03^b
T_1	2.39±0.09^a	2.85±0.05^a	3.42±0.05^b	3.72±0.05^b
T_2	2.68±0.06^a	4.42±0.05^b	4.85±0.05^b	4.83±0.05^b
Yeast and mold (log cfu/g)				
C	-	-	-	-
T_1	-	-	-	2.45±0.05^a
T_2	-	-	-	2.74±0.02^a

**E. coli* and *Salmonella* not detected on the day of preparation in all the samples.

Nos. of observations: = 6. Mean±SE with same superscripts in a row does not differ significantly ($p<0.05$). (T_1=C + 15% fresh roselle calyces in paste form and T_2=Control + 15% oven dried rosells calyces in powdered form).

Table 6: Effect of storage on the sensory attributes.

Attributes			Appearance and colour	Flavour	Juiciness	Sourness	Saltiness	Overall acceptability
Storage days	0th	C	7.32±0.25^b	7.24±0.32^b	7.14±0.35^a	8.24±0.37^c	8.65±0.35^c	7.32±0.35^b
		T1	8.25±0.36^b	9.65±0.35^c	8.35±0.95^b	7.25±0.58^a	8.62±0.65^b	8.65±0.02^b
		T2	7.24±0.07^a	6.25±0.08^b	6.52±0.15^a	5.26±0.36^a	7.25±0.25^c	7.68±0.36^c
	3rd	C	7.65±0.39^c	6.95±0.32^b	5.25±0.65^a	7.85±0.25^c	8.65±0.35^d	7.14±0.04^c
		T1	8.25±0.25^b	7.25±0.65^b	7.45±0.61^b	6.25±0.32^a	7.65±0.68^b	6.52±0.05^a
		T2	5.47±0.21^b	4.25±0.64^a	5.24±0.65^b	5.87±0.32^b	6.52±0.08^c	5.21±0.32^b
	7th	C	7.32±0.25^c	7.24±0.32^c	5.14±0.35^a	8.24±0.37^d	7.95±0.35^c	6.32±0.26^b
		T1	8.65±0.22^d	5.14±0.15^c	6.95±0.81	3.25±0.02^a	6.25±0.62^d	4.82±0.02^b
		T2	5.17±0.24^b	4.24±0.84^a	4.24±0.15^a	5.87±0.32^b	6.52±0.08^c	4.91±0.34^a

Number of observations: = 18.

*Sensory attributes of pickles were evaluated on a 8 point Hedonic scale (wherein 1 = Extremely undesirable; 8 = Extremely desirable) Mean±SE with same superscripts in a row does not differ significantly ($p<0.05$).

(T_1 = C + 15% fresh roselle calyces in paste form and T_2 = Control + 15% oven dried roselle calyces in powdered form).

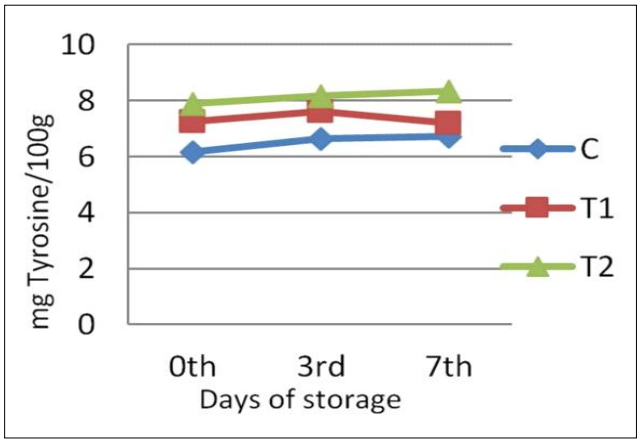

Fig 3: Effect of storage on tyrosine.

control and T_2 which might be due the presence of antioxidant property in the roselle. A gradual increase in tyrosine value (Fig 3) was also observed during storage period. And the values were 6.15±0.12 to 6.71±0.14 for control, 7.25±0.08 to 7.18±0.15 for T_1 and 7.89±0.18 to 8.34±0.13 mg tyrosine/100gm for treatment-2, respectively. Similar increase in tyrosine values were also observed by Gunasekaran *et al*., (2018) in barbecued chicken prepared by application of multiple hurdle technology and stored at ambient temperature. The increase in tyrosine concentration might have occurred due to microbial proteolytic enzymes action on protein substrate present in the giblets.

CONCLUSION

From the study it was found that the value addition of giblet pickle with the incorporation of roselle (15%) preferably in the paste form as a natural preservative can be recommended, since its overall acceptability was found better over that of the synthetic acetic acid. The shelf life studies based on various physic-chemical and microbial evaluation of the product stored at ambient temperature revealed that upto 7th day of storage, it was highly acceptable. Thus, the higher yield and better physico-chemical parameters of roselle added pickle samples is indicative of its good potential for its use in pickle preparation comparing to that of the synthetic acetic acid as a source of natural preservative.

Conflict of interest: None.

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