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Efficacy of fruits (red grapes, gooseberry and tomato) powder as natural preservatives in restructured chicken slices

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Abstract

In view of present national and international barriers regarding the use of chemical food additives in food processing and preservation, search for biological and plant origin food additives have notably increased for the production of high-quality, ready-to-cook or ready-to-eat products in recent years. So present study was envisaged with the objectives to augment the shelf life of restructured chicken slices by incorporating fruits (Red grapes, gooseberry and tomato) powder (1%) as natural preservatives and to assess their effect on microbiological and sensory attributes of the product under refrigerated (7 ± 1^oC) storage. The products added with gooseberry and red grapes powder had significantly ($P < 0.01$) lower standard plate counts (SPC) than control and reference [BHT (200 ppm) added] products except on 6th day of storage where increase in the SPC for BHT added products and control were not significant. No significant differences were existed in the SPC counts of tomato and red grapes powder added products throughout the storage period. Yeast and mould counts were not detected in all the products and control throughout the storage period. Sensory evaluation scores showed that restructured chicken slices incorporated with fruits powder were equally acceptable as reference product and rated good to very good for appearance, flavour, juiciness, texture and overall acceptability. Restructured chicken slices with fruits powder can be stored safely without much loss in its quality even upto 20 days under refrigerated condition.

Introduction

Poultry meat is the fastest growing component of global meat production, consumption, and trade with developing economy playing a pivotal role in its growth due to its enormous potential to bring rapid economic growth with low investment. Different reformulation strategies are being carried out to confer meat as functional food by modifying its lipid and fatty acid content, and/or by incorporating a series of functional ingredients like fiber, vegetable proteins, phytochemicals, prebiotics and other natural antioxidants (Fernandez *et al*., 2004). Considering the need of extending the storage stability of poultry meat in perspective of its marketing potentiality, various additives with antioxidant and antimicrobial properties have been tried with varying degrees of success. The use of natural preservatives to increase the shelf-life of meat products is a promising technology since many herbs, plants, vegetable and fruits extracts or powders have antioxidant and antimicrobial properties (Biswas *et al*., 2012).

Red grapes (*Vitis vinifera*) are rich source of polyphenolic compounds. It mainly includes anthocyanins, flavonols, stilbenes and phenolic acids. Flavonoids and other plant phenolics reported to have antioxidant activity (Frankel *et al*., 1995) and antimicrobial activity (Renaud and Longeril, 1992). Gooseberry (*Emblica officinalis*) is the richest source of antioxidants like vitamin C, emblicanin A and B, punigluconin, pedunculagin, superoxide dismutase, catalase, gluthathione peroxidase (Bhattacharya *et al*., 2000; Rajak *et al*., 2004), tannin, trigalloyl, polyphenol, flavonoids, ellagic acid and phyllembic acid (Anilakumar *et al*., 2004). Gooseberry has also been reported to possess antifungal, antibacterial and antiviral activities (Godbole and Pendse, 1960; Dutta *et al*., 1998; Rani and Khullar, 2004). Tomato (*Solanum lycopersicum*) and tomato based products contain phytochemicals such as lycopene, folate, vitamin C, phenolics and flavonoids which are having health beneficial effects (Beecher, 1998; Agarwal and Rao, 2000). Lycopene is the major carotenoid present in tomato and a highly potent antioxidant (Dimascio *et al*., 1989; Rao and Agarwal, 1999).

Plant sources can bring new natural products into the food industry with safer and better antioxidants that provide ample protection against oxidative damage occurring both in the human body and in the processed food. It is observed that oxidation and colour fading are the major constraints in the retail acceptance of restructured meat products (Pearson and Dutson, 1987). Many technological advantages on processing combination have been used in the

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preparation of restructured meat products to acquire sensory characteristics somewhere between ground meat and intact muscle steaks. A highly acceptable restructured chicken slice without addition of extra fat has been developed as a novel meat product (Mandal *et al*., 2002). This product was found to be acceptable only upto 10th day of storage under refrigeration. Sensory scores of the restructured chicken block decreased significantly (P < 0.05) on the 10th day of storage (Sudheer *et al*., 2011). On 15th day, product had pale appearance and unpleasant odour, so it was not subjected for sensory evaluation. Hence, the present study is envisaged with the objectives to augment the shelf life of restructured chicken slices by incorporating fruits (Red grapes, gooseberry and tomato) powder powder as natural preservatives and to assess their effect on microbiological and sensory attributes of the product under refrigerated (7±1°C) storage.

Materials and Methods

Chicken meat

Heavy weight broiler chickens were selectively procured from the market and slaughtered under hygienic condition in the semi-automatic poultry dressing unit in the Department of Livestock Products Technology (LPT), Rajiv Gandhi College of Veterinary and Animal Sciences (RAGACOVAS). All the carcasses were deboned manually and cut into small chunks and stored in freezer (-18±1°C) till further use.

Preparation of restructured chicken slices

Restructured chicken slices were prepared by using deboned broiler chicken meat and curing ingredients such as salt, sugar, sodium monobasic phosphate, nitrite and water following the procedure and recipe standardized in the Department of LPT, RAGACOVAS (Mandal *et al*., 2002). The standardized recipe for the product includes deboned meat (100%) with salt 2%, sugar 1%, phosphates 0.4%, nitrite 150 ppm and water 10% on meat weight basis.

The frozen deboned meat was thawed by keeping the meat in the refrigerator (7±1°C) overnight. The thawed meat was minced by passing through 8 mm plate in a meat mincer (Mado Shop Mincer Junior, Germany). The minced meat was mixed manually with curing ingredients viz., salt, sugar, phosphate, nitrite and water as per the recipe and then tumbled in home mixer grinder (Sumeet Machines Ltd, Mumbai). The tumbled mass was filled into clean stainless steel mould lined with food grade aluminium foil and covered with stainless steel lid and then kept overnight in refrigerator (7±1°C) for the curing reaction to take place. Next day the moulds were cooked in water at 90°C for 45 minutes followed by cooling under tap water and chilling overnight in the refrigerator (7±1°C) for setting. Next day, the moulds were removed from the refrigerator and opened to get the restructured chicken block. Products were sliced to 3 mm thickness using meat slicer (Sirmon SPA, Italy).

Fruits powder as natural preservatives in restructured chicken slices

Three different fruits viz red grapes, gooseberry and tomato were selected for the study. For each fruit preliminary trials were conducted to select the mode of use such as paste, crude extract and powder at different levels of incorporation in the recipe of restructured chicken block. Based on their effect on sensory and physicochemical properties, powder form of fruits at the level of 1.0% was selected for conducting the actual experiment.

Preparation of fruits powder

Red grapes and tomato

Fresh tomatoes and red grapes were procured from local market at Kurumbapet, Puducherry. After washing and dicing, paste from fruits were prepared by grinding in home mixer grinder separately. Fruit paste (1 Kg) was later mixed with sunflower oil (30 ml) and drying was carried out at 50°C for 72 hours by using hot air oven. The dried fruits were then pulverized using home mixer and sieved through a fine mesh. The resulting fruits powder were then sealed in glass bottles and kept in freezer for further use.

Gooseberry

Fresh gooseberries were purchased from the local market at Kurumbapet, Puducherry. It was washed thoroughly under running tap water to remove extraneous matters. After slicing, it was dried under the shade for 8 hours followed by final drying carried out at 50°C for 2 hours in hot air oven. The dried gooseberries were ground mechanically by home mixer grinder and sieved through a fine mesh. Later it was stored in a bottle and kept in freezer for further use.

Incorporation of fruits powder in restructured chicken slices

Powder of all the three fruits at 1.0% level were incorporated in the standard recipe of the restructured chicken block separately. Similar to experiment I, a reference with BHT and control product were prepared. After preparation of the test products and control, they were sliced to 3mm thickness and packed in low density polyethylene (LDPE) bags (200 gauge) and stored under refrigeration (7±1°C). The samples were taken on 1, 6, 10, 14, 17 and 20th day to assess physicochemical, organoleptic and microbiological qualities. Control product were subjected only for physico-chemical and microbiological analysis.

Analytical procedures

Microbiological quality

Microbiological parameters viz, standard plate count and yeast and mould counts were determined by following the procedures recommended by APHA (1984).

Preparation of serial dilutions

Ten gm of samples were weighed aseptically and transferred to a sterile mortar containing 90 ml of sterile 0.1% peptone water. The samples were homogenized for two minutes using a sterile pestle to make 10^{-1} dilution. Further serial dilutions 10^{-2} and 10^{-3} were made using sterile peptone water 0.1% as diluent. Preparation of samples and serial dilutions were made near flame in a horizontal laminar air flow (Model: Clean air systems) observing all possible aseptic conditions.

Standard plate count

Plate count agar (Hi-Media Laboratories Pvt. Ltd. Mumbai) of 23.5 g was suspended in 1000 ml of distilled water and boiled to dissolve the medium completely. It was sterilized at 15 lb pressure (121°C) for 15 minutes using an autoclave. The pour plate technique was followed for plating. One ml of each serial dilution was taken in duplicate in respective plates and 15 to 20 ml molten media (around 45°C) was poured in the petridish. The plates were mixed and allowed to solidify and were incubated at 37°C for 24 – 48 hours in an inverted position. The number of colonies were multiplied by the reciprocal of the respective dilution and expressed as log10 cfu/g.

Yeast and mould counts

Potato dextrose agar of 39 g was suspended in 1000 ml of distilled water. It was then boiled to dissolve the medium completely and sterilized at 15 lbs pressure at 121°C for 15 minutes using an autoclave. In order to obtain pH of 5.7, the sterile cooled medium was acidified with 10 ml of sterile 10% tartaric acid. Precaution was taken not to heat the medium after addition of acid. One ml each of serial dilution was inoculated into sterile petridishes in duplicates and 15 to 20 ml of molten medium at 45°C was poured into the plates. The plates were mixed and allowed to solidify. The petridishes were incubated at 25°C for 5 days by maintaining relative humidity of 85%. The number of colonies obtained were multiplied by the reciprocal of the respective dilution and expressed as log10 cfu/g.

Sensory evaluation

A semi-trained panel consisting of faculty and postgraduate students of RAGACOVAS was used for the sensory evaluation of the product. The panelists were explained about the nature of experiment without disclosing the identity of the samples. They were requested to record their preferences on an 8 point hedonic scale (8 = like extremely, 1 = dislike extremely) for appearance, flavour, juiciness, texture and overall acceptability as given in the score sheet.

Statistical analysis

Each experiment was replicated thrice and each parameter was analyzed in duplicate. The data recorded were analyzed using SPSS version 17.0 (SPSS, Chicago, III, and U.S.A). Two way analysis of variance was applied and the data were tabulated. The level of significant effects were tested by comparing mean values using the least significant difference (LSD) test at 1 and 5% level (Snedecor and Cochran, 1967).

Result

Gooseberry powder added products were found to have significantly lower SPC counts (3.14±0.11-3.77±0.02 log10 CFU/g) compared to control (3.40±0.07 - 4.20±0.17), BHT (3.39±0.07 - 4.18±0.21) and tomato (3.31±0.04 - 3.99±0.04) powder added products throughout the storage period (Table 1). The products added with gooseberry and red grapes powder had significantly ($P < 0.01$) lower SPC than control and BHT added products except on 6th day of storage where increase in the SPC for BHT treated products and control were not significant (Table 1). No significant differences were noticed between red grapes and gooseberry powder added products during storage except on 14th day, where products treated with red grapes powder showed significantly ($P < 0.01$) higher microbial counts (3.73±0.04 log10 CFU/g). While for tomato and red grapes powder added products, no significant differences were existed in the SPC values throughout the storage period. Yeast and mould counts were not detected in

Table 1. Effect of different fruit powders on the standard plate counts (log10 CFU/g) of restructured chicken slices during refrigerated storage (Mean±SD)

	Parameters				
Storage period (days)	Control	T1	T2	T3	T4
1	3.40±0.07^{cA}	3.39±0.07^{cA}	3.26±0.05^{abA}	3.14±0.11^{aA}	3.31±0.04^{bcA}
6	3.53±0.04^{bA}	3.52±0.14^{bA}	3.38±0.05^{abA}	3.27±0.09^{aB}	3.44±0.05^{bB}
10	3.82±0.04^{dB}	3.80±0.04^{cdB}	3.58±0.17^{abB}	3.47±0.08^{aC}	3.66±0.02^{bcC}
14	3.95±0.04^{cBC}	3.94±0.07^{cBC}	3.73±0.04^{bC}	3.59±0.01^{aD}	3.81±0.07^{bD}
17	4.09±0.09^{cCD}	4.06±0.19^{cCD}	3.84±0.04^{abCD}	3.68±0.03^{aDE}	3.90±0.05^{bcE}
20	4.20±0.17^{cD}	4.18±0.21^{cD}	3.94±0.03^{abD}	3.77±0.02^{aE}	3.99±0.04^{bcF}

n = 6; Control = Restructured chicken slices with standard recipe, T1= added with BHT, T2 = added with Redgrapes powder, T3 = added with Gooseberry powder, T4 = added with tomato powder

Means with different superscripts (capital letters in the same column and small letters in the same row) differ significantly (P < 0.01)

Table 2. Effect of different fruit powders on the sensory qualities of restructured chicken slices during refrigerated storage (Mean±SD)

	Storage period (days)					
Parameters	1	6	10	14	17	20
Appearance						
T1	7.29±0.69	7.25±0.73	7.25±0.73	7.22±0.65	7.20±0.97	6.79±0.97
T2	7.41±0.65	7.33±0.63	7.22±0.65	7.16±0.70	7.12±0.79	6.83±0.96
T3	7.25±0.60	7.20±0.65	7.20±0.70	7.02±0.78	6.95±0.69	6.72±0.94
T4	7.62±0.49	7.58±0.71	7.56±0.49	7.50±0.58	7.41±0.65	7.35±0.63
Flavour						
T1	7.20±0.83	7.12±0.74	7.12±0.79	7.10±0.77	7.08±0.82	6.91±1.21
T2	7.08±0.65	6.91±0.71	6.89±0.80	6.87±0.81	6.83±0.76	6.79±0.83
T3	7.08±0.71	7.00±0.78	6.91±0.73	6.85±0.65	6.79±0.97	6.64±0.81
T4	7.25±0.60	7.20±0.81	7.16±0.81	7.14±0.77	7.12±0.67	7.02±1.02
Juiciness						
T1	7.33±0.76	7.31±0.71	7.29±0.62	7.27±0.73	7.16±0.76	7.08±0.82
T2	7.12±0.67	7.12±0.79	7.10±0.77	7.06±0.81	7.00±0.88	6.83±0.81
T3	7.33±0.73	7.16±0.76	7.14±0.74	7.02±0.78	6.95±0.75	6.81±0.70
T4	7.50±0.72	7.37±0.64	7.35±0.72	7.25±0.79	7.12±0.74	7.10±0.77
Texture						
T1	7.33±0.81	7.20±0.77	7.16±0.81	7.06±0.81	7.00±0.83	6.95±0.99
T2	7.29±0.69	7.04±0.69	7.00±0.88	6.93±0.98	6.89±0.97	6.79±0.72
T3	7.29±0.55	7.02±0.75	6.97±0.81	6.91±0.84	6.87±0.79	6.52±0.82
T4	7.41±0.71	7.25±0.67	7.22±0.77	7.12±0.85	7.08±0.77	6.97±0.81
Overall acceptability						
T1	7.29±0.69	7.16±0.76	7.12±0.79	7.08±0.81	6.97±0.89	6.91±0.71
T2	7.25±0.79	7.08±0.63	7.04±0.69	6.97±0.69	6.95±0.87	6.83±1.12
T3	7.04±0.75	6.97±0.72	6.91±0.60	6.89±0.82	6.85±0.77	6.72±0.73
T4	7.41±0.65	7.37±0.76	7.35±0.77	7.29±0.80	7.22±0.77	7.14±0.80

n = 6; Control = Restructured chicken slices with standard recipe, T1 = added with BHT, T2 = added with Red grapes powder, T3 = added with Gooseberry powder, T4 = added with tomato powder

all the test products and control samples throughout the storage period.

No significant differences were observed for all the sensory attributes of restructured chicken slices formulated with both fruits powder and BHT throughout the storage period, though a gradual decrease in the sensory scores were noticed during the entire storage. Sensory scores for appearance were numerically higher for tomato powder (7.35-7.62) followed by BHT (6.79-7.29), red grapes powder (6.83-7.41), and gooseberry powder (6.72-7.25) added products (Table 2). The flavour scores ranged from 6.64 to 7.25 where numerically higher scores recorded for tomato powder (7.02±1.02 - 7.25±0.60) and the lower for gooseberry powder(7.08±0.71 - 6.64±0.81) added products. Sensory scores for juiciness were not affected significantly during storage and the scores in ascending order were red grapes powder (6.83-7.12) followed by gooseberry powder (6.81-7.33), BHT (7.08-7.33), and tomato powder (7.10-7.50) added products (Table 2). Texture scores of the products ranged from (6.52 to 7.41) indicating good to very good in the hedonic scale where numerically higher scores recorded for tomato powder (6.97±0.81 - 7.41±0.71) added products. Overall acceptability scores were numerically higher for tomato powder (7.14-7.41) followed by BHT (6.91-7.29), red grapes (6.83-7.25) and gooseberry powder (6.72-7.04) added products and ranged between 6.72 to 7.41 indicating good to very good overall acceptability throughout the storage period (Table 2).

Discussion

Gooseberry is reported to possess high antibacterial activity against *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aerogenosa* and *Salmonella paratyphi* (Saeed and Thariq, 2007). Antimicrobial activity of aqueous and methanolic extract of gooseberry was tested against different microorganisms by Vimela *et al.* (2009). Anastasiadi *et al.* (2009) suggested that high concentration of flavonoids and their derivatives such as stilbenes and phenolic acids in grapes were responsible for its antimicrobial activity. Rotava *et al.* (2009) reported that phenolic compounds from defattened grape (*Vitis vinifera*) seed extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli.* Similar to the findings of the present study, the total plate counts of pork sausages at 1.2 and 1.5% levels of tomato powder incorporation were significantly (P < 0.05) lower than control during refrigerated storage (Kim *et al*., 2010).

Yeast and mould counts were not detected in all the test products and control samples throughout the storage period which were in conformity with the findings of Sudheer *et al.* (2011) who could not detect any yeast and mould in restructured chicken slices incorporated with gizzard. Xie *et al*. (2010) reported that phenolic compounds in grape like resveratrol displayed potent antifungal activity against the *Candida albicans.*

Trend of comparatively higher appearance scores in the tomato powder added products were probably due to the imparting of attractive red colour of the tomato powder to the products. Findings of the present study are in agreement with Kim *et al*. (2010) who reported that sausages with 1.2 and 1.5% tomato powder had significantly (P < 0.05) higher values for redness, yellowness, chroma and hue and were more attractive to panelists than control during storage. He also reported that there were no significant (P < 0.05) differences for flavour, tenderness and juiciness scores of pork sausages during thirty days of storage under refrigeration, but the flavour scores were higher in tomato powder added sausages at every stages of storage. Similar to the present findings, Candogan (2002) reported that tomato paste addition at the level of 10 and 15% increased the juiciness of beef patties. Kim *et al.* (2010) reported that addition of tomato powder did not cause any negative effect in the texture properties of pork sausage. He also reported that the scores of overall acceptability in tomato powder (0.8, 1.2 and 1.5%) added sausages were higher and it was significantly ($P < 0.05$) higher in 0.8% tomato powder added samples compared to others during 30 days of refrigerated storage.

Based on the finding of present study, it may be concluded that addition of fruits (red grapes, gooseberry and tomato) powder did not have any undesirable effects on the microbiological and sensory attributes of restructured chicken slices. Fruits powder have potent natural preservative effects to augment the shelf life of restructured chicken slices from ten days to twenty days and could replace the synthetic antioxidants effectively. Utilization of these natural preservatives in restructured chicken slices have not only increased the storage stability, but will replace the toxic effect of chemical preservatives and also work as functional additives by providing adequate health promoting effect in the human body system.

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