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Rushikesh A Kantale
Department of Livestock Product Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Rajesh V Wagh
Department of Livestock Product Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Manish K Chatli
Department of Livestock Product Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

OP Malav
Department of Livestock Product Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Corresponding Author
Rushikesh A Kantale
Department of Livestock Product Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Assessment of *Spinacia oleracea* leaves (SOL) extract as natural antioxidant in chicken meat sausages

Rushikesh A Kantale, Rajesh V Wagh, Manish K Chatli and OP Malav

Abstract
Chicken meat sausages are a famous functional meat product in Western European countries, and it is also becoming progressively popular in India. Presently protein rich meat and meat products are essential components of any human diet. Chicken meat is widely accepted by consumers in India and throughout the world with minimum religious taboo. Lipid oxidation is a prime concern in the meat industry, and sausage has a higher fat content. Plant phenolic compounds are the most common source of natural antioxidants. *Spinacia oleracea* also known as spinach is an antioxidant-rich plant with beneficial phytonutrients and polyphenols. The goal of the study was to determine the best level of *Spinacia oleracea* leaves extract inclusion as a natural antioxidant in chicken meat sausages. Three distinct amounts of *Spinacia oleracea* leaves extract (T-1: 1.0 percent, T-2: 1.5 percent, and T-3: 2.0 percent) were included individually in chicken meat sausages and analysed for changes in physico-chemical, textural, and sensory analysis, as well as a control (without extract). The incorporation of 2 percent *Spinacia oleracea* leaves (SOL) extract was shown to be the most suitable for the manufacture of chicken meat sausages rich in natural bioactive phyto-extracts based on physico-chemical, sensory evaluation, and instrumental color, texture analysis parameters.

Keywords: Chicken meat sausages, natural antioxidants, *Spinacia oleracea* leaves (SOL) extract

Introduction

Meat, in general, holds a special place in the diet due to its delectable flavor, texture, and nutritional value. In addition to customer demand for high-quality ready to eat or convenient meals, food safety has become an increasingly critical public health issue (Wagh *et al.*, 2017) [17]. Slaughter meat has also short shelf life, the principal causes of meat deterioration during cold storage due to lipid oxidation and inclination of microbiological growths. Adoption of alternative preservation methods, such as hurdle technology, has been shown to be particularly effective in limiting foodborne viruses and maintaining food quality throughout storage (Wagh *et al.*, 2017) [17].

Poultry meat holds a special place in diet because of its high nutritional value. Poultry meat is the major meat consumed in India and has wide regional acceptance, compared to the mutton, pork, beef, fish with minimum religious taboo (Devi *et al.*, 2014) [4]. Poultry meat is also considered as a healthy, nutritious, safe and affordable choice among the available meat source (Pouta *et al.*, 2010) [12]. Poultry is one of the fastest growing sector of India's livestock industry, providing a large percentage of the protein supplements from egg and meat (Abd El-Ghany *et al.*, 2021) [1]. Poultry meat is in high demand around the world since it is one of key source of protein for human growth and development. Poultry meat has a number of advantages, including enough nutrition, a delightful taste, a relatively low price, ease of availability, and acceptance by people from all worldwide (Abd El-Ghany *et al.*, 2021) [1].

Several researcher reports, poultry play a vital role in the livestock revolution, contributing significantly to our economy while also providing employment to farmers (Wynn *et al.*, 2021) [18]. The effective and proper utilization of this Chicken meat has become one of the most demanding areas in the chicken meat processing business due to huge expansion in the layer sector. Consumers in India have a high level of acceptance for chicken meat. Poultry meat is popular throughout the country because of its distinct flavor and texture, and it is marketed at a premium price without any religious restrictions. Fresh eggs and chicken flesh are commonly available. However, with the introduction of cold storage facilities and the introduction of branded growing facilities, demand & momentum for processed or preserved meat foods is increasing day by days (Vikram *et al.*, 2020) [15].

Sausage is a famous comminuted meat product in Developed nations, and it is also becoming increasingly popular in India. Since 1500 BC, the Babylonians and the Chinese have utilized sausages as the oldest kind of processed cuisine (Pearson and Gillette, 1996) [11]. Comminuted meat products have a shorter shelf life than whole cuts due to the larger surface area of the meat particles and frequent handling, which might promote microbial development. Furthermore, because sausage has a larger fat content, lipid oxidation is higher (5 to 30 percent).
Indian plants contain a variety of substances called ‘‘Phytochemicals’’ (Pratt, 1992) [13], that owe to naturally occurring components present in plants. The phytochemical preparations with dual functionalities in preventing lipid oxidation and antimicrobial properties have tremendous potential for extending shelf life of food products. Plant phenolic chemicals (flavonoids, phenolic acids, alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid, and carotenoids are the most abundant sources of antioxidants (Jagtap *et al.*, 2019) [8]. Phytochemicals such as carotenoids, flavonoids, and other phenolic compounds are rich in fruits and vegetables (Miller *et al.*, 2000) [6]. These phytochemicals, particularly polyphenols, have been shown in several research that also have substantial free-radical scavenging action, lowering the risk of chronic disease, cancer, and age-related neurological degeneration. As a result, eating a diet rich in antioxidants, such as fruits and vegetables, is critical for reducing the negative consequences of oxidative stress (Wagh *et al.*, 2017) [17].
Spinacia oleracea (Family-Chenopodiaceae) commonly known as "Spinach" is a native to South-West Asia and cultivated through the world as vegetable. Several parts of this plant are used in traditional Indian medicine for numerous therapeutic effects like laxative, diuretic, carminative etc. It is a rich source of Vitamins A, C, E, K, B_6, B_{12} and minerals like magnesium, manganese, folate, betaine etc. (Castenmiller *et al.*, 2002). *Spinacia oleracea* is also known to be packed with a number of anti-oxidants like polyphenols, flavonoids and carotenoids, which have shown to possess anti-inflammatory and anti-neoplastic effect (Sheikh *et al.*, 2020) [14]. Even though its extract has a great potential to be used as a natural antioxidant, very little is known about the optimal level to prevent oxidative changes of meat products while maintaining other sensory and physico-chemical qualities.
Since, very limited research work has been carried out on suitability of *Spinacia oleracea* as a natural antioxidant in the functional meat industry as well as chicken meat sausages, the present research study work was therefore, planned accordingly.

Material Methods

Source of meat

The University Poultry Farm, Department of Livestock Production Management, Guru Angad Dev, and Veterinary Animal Sciences University, Ludhiana, provided spent hen chicken meat for research study. The animals were slaughtered in the experimental slaughter house of the Department of Livestock Products Technology according to standard protocol, with animal welfare and ethical considerations properly. Those dressed carcass were immediately brought to the laboratory and chilled at (4±1ºC) for 12-18 hours then being deboned physically. Boneless meat was recovered after the skin, external fascia, fat, and all separable connective tissues were removed.
The boneless meat and fat were packed individually in 1 kg unit packs in low density polyethylene (LDPE) bags and then stored in a deep freezer at (-18±1ºC) until required. A needed quantity of frozen meat packs was removed from the freezer and thawed overnight in a refrigerator (4±1ºC) before use.

Spice mix

All of the spices for the spice mixes were purchased in a local bazaar in Ludhiana, Punjab. After removing any foreign stuff, the spices were oven dried for 2 hours at 45±2ºC. The components were mechanically ground (grinding machine) and sieved through a fine mesh (Grinding machine-Inalsa, Wonder axie plus, Delhi, India). To make a spice mix, the different spice powders were blended in a standard ratio (Table 1). The spice blend was kept in moisture-free Polyethylene Terephthalate (PET) jars until it was needed again.

Table 1: Composition of spice mixture

Name of ingredients	Percentage (w/w)
Aniseed (Soanf)	10.00
Black pepper (Kalimirch)	10.00
Caraway seeds (Ajwain)	10.00
Capsicum (Mirch powder)	8.00
Cardamom dry (Badi Elaichi)	5.00
Bay leaves (Tej pata)	2.00
Cinnamon (Dalchini)	5.00
Cloves (Laung)	5.00
Coriander (Dhania)	18.00
Cumin seeds (Zeera)	15.00
Mace (Javitri)	1.00
Nutmeg (Jaifal)	2.00
Dry Ginger Powder (Soanth)	8.00
Cardamom (Choti Elaichi)	1.00
Total	100

Condiments

Onion, ginger, and garlic were used to make the condiment paste. The onion, ginger, and garlic outermost layers were peeled and cut into small pieces, and a fine condiment paste was formed by blending onion, ginger, and garlic in a 3:1:1 ratio in a grinder (Inalsa, Wonder maxie plus, Delhi, India) with use an appropriate blade.

Salt, tetra sodium polyphosphate and sodium nitrite

Table salt (Tata Chemicals Ltd., Mumbai, India), STPP (Hi-media Laboratories Pvt. Ltd., Mumbai, India), and sodium nitrite (Central Drug House Pvt. Ltd., New Delhi, India) were utilized in this study.

Packaging Materials

For aerobic packing, low density polyethylene (LDPE 100-120 gauge) bags with a volume of 1 kg were utilized.

Table 2: Formulations of the chicken meat sausages

S. No.	Ingredients	Percentage (w/w)
1.	Spent hen chicken meat	78
2.	Ice/Chilled water	5
3.	Vegetable oil	3
4.	Condiments	3
5.	Salt	1.5
6.	Refined wheat flour	3.5
7.	Dry spices	1.5
8.	STPP	0.2
9.	Sugar	0.25
10.	Nitrite	100 ppm
11.	Egg	5
12.	Chicken fat	5

Preparation of Chicken meat sausages

Partially thawed Chicken flesh was chopped into small cubes and double minced in a meat mincer with a 4 mm plate (Mado Eskimo Mew-714, Mado, Germany). A bowl chopper was used to make the meat emulsion (Seydelmann K20, Ras, Germany).

Frozen meat at -18 ˚C
↓
Thawing of meat at 4±1˚C for 24 hrs
↓
Mincing through 8 mm sieve plate
↓
Chopping of meat in bowl chopper with salt, STPP and sodium nitrite with ice flakes
↓
Addition of oil
↓
Chopping (2-3 mins)
↓
Addition of refined wheat flour, spices, condiments and other ingredients
↓
Chopping to obtain uniform dough
↓
Filling into sausage filler machine (natural goat casings)
↓
Hot air oven for 30 mins at 115 ˚C (internal core temp. 72 ˚C)
↓
Cooling to ambient temperature, cutting, packaging and storage at 4±1˚C
↓
Sensory evaluation and sample analysis

Flow diagram for preparation of Chicken meat sausages

The incorporation of three distinct levels of *Spinacia oleracea* leaves (SOL) extract in the development of chicken sausages.

After substituting the lean meat in the pre-standardized chicken meat sausage formulation, three different levels (1%, 1.5%, 2%) of *Spinacia oleracea* leaves (SOL) extract were added individually in chicken meat sausage. The physico-chemical parameters (pH, cooking yield), instrumental colour analysis, texture profile analysis, and sensory evaluation of chicken meat sausage fortified with three different doses of (SOL) *Spinacia oleracea* leaves extract, were all investigated.

Table 3 represents the results of physicochemical parameters, proximate composition, instrumental colour analysis, texture profile analysis, and sensory evaluation of chicken meat sausages with three different amounts of (SOL) *Spinacia oleracea* leaves extract.

Table 3: Effect of different levels of *Spinacia oleracea* leaves (SOL) extract on the physico-chemical, color, texture parameters and sensory analysis of chicken sausages.

Parameters	C	T-1 (1.0%)	T-2 (1.5%)	T-3 (2.0 %)
Physio-chemical				
pH	6.13±0.04^{A}	6.14±0.01^{A}	6.14±0.04^{A}	6.15±0.02^{A}
Cooking yield	85.80±0.01^{A}	85.81±0.02^{A}	85.82±0.03^{A}	85.86±0.04^{A}
Color Parameters				
Lightness (*L**)	50.25±0.03^{A}	50.39±0.04^{A}	50.45±0.03^{A}	50.53±0.01^{A}
Redness (*A**)	10.79±0.04^{A}	10.51±0.03^{A}	10.63±0.02^{A}	11.06±0.02^{B}
Yellowness (*B**)	9.62±0.03^{A}	9.54±0.04^{A}	9.56±0.01^{A}	9.59±0.03^{A}
Texture Profile				
Hardness (N)	25.45±0.04^{A}	25.49±0.01^{A}	25.51±0.01^{A}	25.53±0.11^{A}
Springiness(mm)	12.30±0.02^{A}	12.32±0.03^{A}	12.34±0.04^{A}	12.36±0.01^{A}
Stringiness(mm)	19.48±0.03^{A}	19.62±0.03^{A}	19.51±0.03^{A}	19.36±0.03^{A}
Chewiness (J)	269.74±0.02^{A}	269.71±0.01 ^{A}	269.16±0.04^{A}	269.47±0.02^{A}
Gumminess (N)	7.42±0.04^{B}	7.43±0.04^{B}	7.44±0.02^{B}	6.48±0.02^{A}
Resilience	1.54±0.01^{A}	1.55±0.04^{A}	1.56±0.02^{A}	1.57±0.03^{A}
Sensory Analysis				
Color & Appearance	7.14±0.04^{B}	6.72±0.04^{A}	6.74±0.03^{A}	7.27±0.02^{C}
Flavor	7.03±0.02^{B}	6.79±0.03^{A}	6.94±0.06^{B}	7.28±0.01^{C}
Texture	6.95±0.01^{A}	6.77±0.02^{A}	6.85±0.04^{A}	7.12±0.03^{B}
Juiciness	7.11±0.01^{B}	6.56±0.01^{A}	7.03±0.03^{B}	7.18±0.04^{C}
Overall acceptability	7.14±0.03^{B}	6.91±0.02^{A}	7.09±0.02^{B}	7.32±0.02^{C}

n=6, C: Without extracts, T-1: *Spinacia oleracea* leaves extract (1.0%), T-2: *Spinacia oleracea* leaves extract (1.5%), T-3:*Spinacia oleracea* leave extract (2.0%) *Mean ± SE. with different superscripts row wise (Capital alphabets) differ significantly (p<0.05).

Result and Discussion

Physico-chemical properties pH

Digital pH metre (SAB 5000, LABINDIA, Mumbai) with a combination glass rod to determine the pH of chicken meat sausages. For one minute, ten grammes of sample were homogenized in 50 ml distilled water. Making use of (T-25D S22 digital ultra-TURRAX Germany). A combination glass electrode was dipped into the suspension to record the pH.

Three distinct amounts of *Spinacia oleracea* leaves extract (SOL) were used in the formulation of standardized chicken meat sausages: 1.0 percent, 1.5 percent, and 2.0 percent. The pH values of treated chicken sausages decreased significantly (P<0.05) as the amount of *Spinacia oleracea* leaves (SOL) extract in the formulation increased. Because the amount of incorporation was so little, the pH values for all of the treated products and the control exhibited no significant variation (Jagtap *et al.*, 2019) [8].

Cooking yield

The weight of each sausage was measured before and after cooking. The cooking yield was computed by dividing the cooked product weight by the raw uncooked weight and multiplying by 100. The cooking yield was calculated using a formula and then expressed as the percentage.

$$\text{Cooking Yield (\%)} = \frac{\text{Wt. of cooked chicken meat sausage}}{\text{Wt. of raw chicken meat sausage}} \text{ X } 100$$

For all of the treatment and control (meat products), the cooking yield of chicken meat sausages was comparable. Except for the control group (No Extract), the cooking yield of meat products, such as Chicken meat sausages, demonstrates that Phyto-extract integrated T-1: 1.0 percent, T-2: 1.5 percent, and T-3: 2.0 percent shows comparative increased cooking yield in Chicken meat sausages. Similar findings in cooking yield were reported by (Fang *et al.*, 2019) [5]. The cooking yield improved non-significantly (P>0.05) as the level of SOL incorporation increased in treatment products, which could be owing to the increasing carbohydrate content in *Spinacia oleracea* leaves (SOL) extract.

Color Analysis

The colour profile was determined using a CR-400 Konica Chroma metre (Konica Minolta, Japan) set to 2o of cool white light (d65) and referred to as the 'l', a, and b values. Representation of '*l*' value (brightness 100) or lightness (0), *a* (+ redness/- greenness), *b* (+ yellowness/-blueness). The light trap (black hole) and white tile that came with the device were used to calibrate it. Then the color characteristics listed above were chosen. At three different sites, the instrument was placed directly on the surface of chicken sausages.

Incorporation of SOL non-significantly affected the lightness (L*) and yellowness value (b*) of chicken meat sausages, while redness showed elevated values in T-3 when compared to other treatments. However, there was no discernible difference in lightness or yellowness in chicken sausages as the level of additional Phyto-extract was increased. Researcher (Kumar *et al.* 2015) [10] reported simillar findings.

Texture Profile

Texture analyzer was used to perform instrumental texture profile analysis (TPA) (TMS-PRO, Food Technology Corporation, USA). A size of the sample of 1.0cm, 1.0cm, 1.0cm was exposed to a double compression cycle with a load cell of 2500 N at pre-test speed (30mm/sec), post-test speed (100mm/sec), and test speed (100mm/sec). As a probe, a 25 mm compression platform was used. The TPA was carried in according to Bourne's, (1978) specifications.

The preloaded software in the equipment calculated parameters including hardness, gumminess, springiness, resilience, chewiness, and cohesiveness automatically from the force-time plot. On samples, all instrumental texture investigations were carried out at room temperature (precooked or cooked sausages). Two measurements were taken twice for each replication, and mean values for each treatment were provided. Sausage texture profile analysis (TPA) was investigated (Bourne, 1978; Brennan and Bourne, 1994) [2-3].

Table 3 shows the textural profile features of chicken meat sausages incorporating three different quantities of SOL (*Spinacia oleracea* leaves) extract. The texture profile metrics hardness, springiness, chewiness, gumminess, and resilience of chicken sausages combined with three different quantities of (*Spinacia oleracea* leaves) SOL showed a non-significant difference. The addition of (*Spinacia oleracea* leaves) SOL to treatment products resulted in a non-significant decline in stringiness. For both treatments and controls, the values for all texture profile measures, such as hardness, springiness, stringiness, chewiness, gumminess, and resilience, were found to be incomparable.

Sensory Analysis

The chicken meat sausages were rated on an 8-point descriptive scale, where 8=extremely desirable and 1=extremely undesirable (Keeton, 1983) [9] for look and color, texture, flavor, juiciness, and overall acceptability at (COVS) College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

Before serving to the sensory panelists, the samples were warmed in a microwave oven for 20 seconds. The panelists were seated in a noise-free environment. Sensory samples with codes were prepared and served to panelists while still warm. Water is provided as a palate and mouth rinse between each sample.

Table 3 and Fig 1 show the mean sensory scores of chicken meat sausages including three different degrees of (*Spinacia oleracea* leaves) SOL extracts. T-3 had a non-significantly higher appearance score (P<0.05) than the control. T-2 and T-3 had non-significantly higher (P<0.05) flavor scores than control, while T-3 had a score that was comparable to control. T-1 and T-2 had significantly reduced texture and juiciness scores (P<0.05) than control, although the sensory panelists gave T-3 the highest score out of the control and other treatment products.

The drop in juiciness scores of treatment items (P<0.05) could be attributable to a fall in pH and moisture loss during (Meat Product) cooking. The overall acceptability scores for the T-1 and T-2 were substantially lower (P0.05) than the control, which is mirrored of other sensory characteristics' values. However, the T-3's overall acceptance was equivalent to the control and had a very good (7.0) sensory rating. Meat scientist (Wagh *et al.*, 2017) [17] was observed similar research findings.

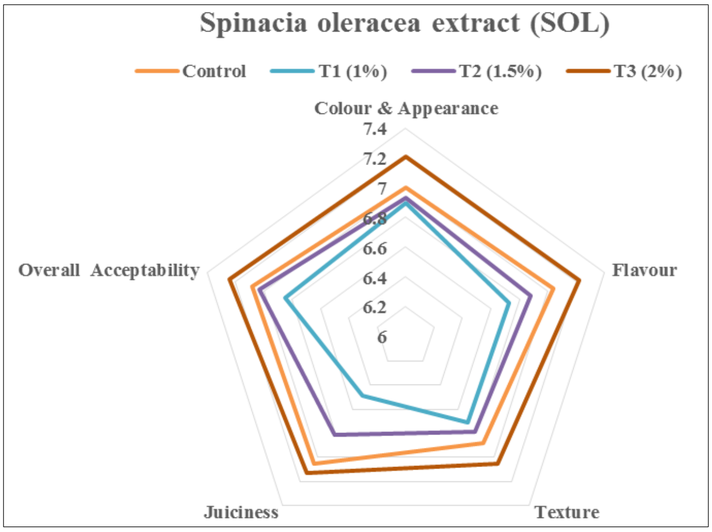

Fig 1: Effect of three different levels of *Spinacia oleracea* leaves extract (SOL) onsensory analysis of chicken sausages (C-without SOL, T1-1%, T2- 1.5% and T3- 2%)

Conclusions
The current study was designed and carried out with a focus of consumer demands for human healthy meat food as well as a primary concern issue in the meat industries. Based on the research findings of physico-chemical analysis, instrumental colour, texture profile, sensory analysis, and overall acceptability, the incorporation of *Spinacia oleracea leaves* (SOL) extract at a concentration of 2% was found to be the most acceptable for the development of chicken meat sausages. The sausages made from chicken meat added with bioactive SOL can be used to propose the prospect of employing them as functional meat products, which will surely improve the economic and health status of functional meat and meat products in meat industry.

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