Anti-oxidant and anti-microbial properties of mutton nuggets incorporated with blends of essential oils

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Abstract The present study was carried out to evaluate effect of natural anti-oxidants on the quality of mutton nuggets. Different blends of essential oil were evaluated for incorporation in mutton nuggets and it was found that Blend-1 had significantly higher sensory scores. Then, Blend-1 was tried at 0.25, 0.5 and 0.75% levels and product containing 0.25% level received significantly higher sensory scores. Thereafter, two combinations of flaxseed flour and 0.25% Blend-1 were tried viz., 4% flaxseed flour + 0.25% Blend-1 and 8% flaxseed flour + 0.25% Blend-1. Evaluation of sensory and physico-chemical properties were done in mutton nuggets incorporated with 0.25% Blend-1 (T-1) and selected combination (4% flaxseed flour + 0.25% Blend-1) (T-2). T-2 had significantly higher dietary fiber and crude fiber than T-1 products. These products were then assessed for quality changes during storage at refrigerated temperature for 30 days at 5 days interval. Significantly lower TBARS values were recorded for treatment products than control at each interval of storage period. T-2 product showed significantly higher DPPH value than other products. Microbial count remained within the permissible limit of $\log_{10}4$ cfu/g for TPC, PC, yeast and mould count up to 15th day, 25th day and 30th day for control, T-1 and T-2 products, respectively.

Essential oil and their combination incorporated mutton nuggets had about 10 days longer shelf life than control.

Keywords Mutton nuggets · Essential oil · Flaxseed flour · Physico-chemical characteristics · Shelf life · Sensory attributes and microbiological quality

Introduction

Minced and cooked processed meat products are relatively more susceptible to lipid oxidation at an accelerated rate and this is considered as one of the main reasons for loss of physico-chemical and microbial quality of meat products (Singh 1996). Oxidation in meat products causes the degradation of lipids present and thus, produces off-flavour, poor texture and unpleasant colour of displayed meat products. Thus, prolonging the shelf life of meat products either by retarding the lipid oxidation or by checking the microbial spoilage is the need of an hour for related meat processors (Sharma et al. 2017a). In this regard, use of antioxidants and antimicrobial agents has been tried by different workers. Synthetic additives like BHA, BHT and TBHQ are mainly avoided by the consumers because of the associated carcinogenic properties. This has led to notable increase in use of natural additives, especially of plant origin (Burt 2004).

Flaxseed is considered to be the valuable food sources of the secoisolariciresinol diglucoside (SDG), plant lignan and exhibit the strong antioxidant activity. SDG along with lignin metabolites (enterodiol and enterolactone) are responsible for anti-oxidant behavior of flaxseed (Rubilar et al. 2010). Flaxseed is an excellent source of fiber which is responsible for the cholesterol-lowering effects. Many herbs, spices and their extract, such as oregano (*Origanum*

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vulgare L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.) have been studied by several authors and they have reported the improved sensory scores and extended shelf life of herb incorporated foods. (Hernández-Ochoa et al. 2014; Arfat et al. 2015; Chandran et al. 2017; Maskan and Horuz 2017). These herbs, spices and their extracts owe their antioxidant property to the phenolic compounds (including volatile compounds) present, which are also known as essential oils. They have high antioxidant activity through three mechanisms: (a) free-radical scavenging activity; (b) transition-metal-chelating activity; and/or (c) singlet-oxygen quenching capacity (Mukai et al. 2005). Different methods employed to estimate the antioxidant capacity of vitamins and phenolic compounds indicates that some natural antioxidants have a high antioxidant activity over the others (Velasco and Williams 2011). Main constituents of thyme consists of thymol which acts as anti-microbial by disrupting vesicles and cell membranes, and impairing ergosterol biosynthesis which ultimately, affects cell membrane integrity (Ahmad et al. 2011). Eugenol is considered to be the major component of clove oil which alters cell membrane and cell wall structures of proliferating resulting in the release of cellular content (Bennis et al. 2004).

Although number of studies have been carried out on development and quality characteristics of meat nuggets prepared from various sources of meat including mutton but the effect of essential oils and flaxseed on the quality characteristics of mutton nuggets have not been studied so far. Therefore, sensing the need to replace synthetic antioxidant and antimicrobial preservatives with natural ones and also to evaluate their effect on the quality characteristics of meat products, the present study was attempted to study the effect of natural antimicrobial and anti-oxidants viz., essential oils and flaxseed on the quality and shelf life of mutton nuggets.

Materials and methods

Procurement of mutton

Adult sheep (18–24 months) were slaughtered according to traditional Halal method and dressed in Experimental Abattoir of Livestock Products Technology Division, IVRI, Izatnagar. Meat samples were hot deboned and separable fat, tendons and connective tissue were trimmed off. The samples were then stored in deep freezer (Blue Star, FS345, Denmark) for storage at -18 ± 2 °C, until further use.

Food ingredients and other additives

Spice mix, refined salt, refined wheat flour (maida) and flaxseed were purchased from local market. Flaxseeds were grounded in the grinder (REMI MIXIE at speed setting 2) to form flour. Before incorporation in the mutton nuggets, it was hydrated (1:1) with equal amount (w/w) of distilled water. For condiments, onion and garlic paste were used in the ratio 3:1. All the chemicals used were of analytical grade and obtained from standard firms (Qualigen, Hi-Media, Sdefine etc.). Food grade essential oils were procured from commercial suppliers.

Low density polyethylene (LDPE) pouches (200 gauge) were used for aerobic packaging of product during storage.

Processing of mutton nuggets

The formulation of control and treatment mutton nuggets, having optimum levels of essential oil and combination of flaxseed flour and essential oil is given in Table 1. Frozen meat was thawed for approx. 16–18 h at 4 ± 1 °C (temperature reaching between -3 and -5 °C). Deboned mutton in a semi frozen state was cut into chunks of 3–4 cm³ size. Essential oil blends were applied on surface of thawed meat cubes with the help of sterilized cotton swab and kept for half an hour in a desiccator. Then the meat for control sample and treated samples were double minced separately with Electrolux meat mincer. Meat emulsion was prepared in a bowl chopper (Seydelmann K20, Ras, Germany). Pre-weighed quantity (as given in Table 1) of minced mutton, salt, sodium tripolyphosphate, and sodium nitrite were added and chopped for few minutes. Chopping was continued for further 2 min after adding ice flakes. Refined vegetable oil was incorporated slowly while chopping. After complete dispersion of refined oil in the batter, other non-meat ingredients such as refined wheat flour, condiments, spice mix were added and chopped for 2 more minutes. Chopping was continued (3–4 min) till uniform dispersion of all the ingredients and fine viscous consistency of the emulsion was achieved. Weighed quantity of emulsion was taken and filled in stainless steel mould (11 × 18 × 3.5 cm). Steam cooking of the product was done for 40 min. Mutton block so obtained was cooled, sliced and cut into pieces to get nuggets.

Experimental details

In the present study, different blends of essential oils (in different ratio) were incorporated in mutton nuggets by replacing lean meat in pre-standardized formulation. Individual essential oils for the blends were selected on the basis of review of literature and results obtained for their

Table 1 Formulation of control and treated mutton nuggets

Ingredients (%)	Control	T-1 (blend of essential oil)	T-2 (combination of essential oil and 4% flaxseed flour)
Mutton	72	72	68
Ice flakes	10	10	10
Essential oil	–	0.25	0.25
Sodium chloride	1.6	1.6	1.6
Sodium nitrite (ppm)	150	150	150
STPP	0.4	0.4	0.4
Vegetable oil	8	8	8
Flaxseed flour	–	–	4
Refined wheat flour	3.0	3.0	3.0
Condiment mix	3.5	3.5	3.5
Spice mix	1.5	1.5	1.5

in vitro anti-microbial activity (Sharma et al. 2017b). Concentration of different essential oils in different blends is given below. These three blends were incorporated in mutton nuggets at 0.25% level.

Selection of blend of essential oils

Essential oils (%)	Blend-1	Blend-2	Blend-3
Cassia oil	20	25	30
Clove oil	15	20	30
Holybasil oil	25	30	40
Thyme oil	20	25	–
Ajowan oil	10	–	–
Beetal oil	10	–	–

On the basis of product yield and sensory evaluation, one blend of essential oils was selected for further studies. In next step, three different incorporation levels of selected blend of essential oils (0.25, 0.50 and 0.75%) were standardized and on the basis of sensory evaluation and cooking yield, 0.25% of incorporation level of essential oil was selected for further studies. Further, two different levels of combination of essential oil and flaxseed flour viz., 4% flaxseed + 0.25% essential oil and 8% flaxseed + 0.25% essential oil were tried and one level was selected for final study.

Analytical methods

The product yield was obtained by measuring weight of mutton nuggets for control as well as each treatment and calculating the ratio of cooked weight and raw weight and expressed as percentage. pH of mutton nuggets was recorded by immersing combined glass electrodes of

digital pH meter EL 68 of ELICO pH meter (Model LI-120) in homogenate (Troutt et al. 1992). Moisture, protein, fat and ash contents of mutton nuggets were determined by standard methods using hot air oven (Acumen labware Pvt Ltd., Haryana, India), Kjeldahl assembly (VELP Scientifica, Italy), Soxhlet extraction apparatus (Garg Process Glass India Pvt Ltd) and muffle furnace at 550 °C (MTI Corp Ltd. India) respectively as per AOAC (1995). The ratio between moisture and protein has also been calculated. Shear force value (expressed in kg/cm²). was determined as per Berry and Stiffler (1981), as force required for shearing 1 cm square block measured in kg on Warner–Bratzler Shear Press (81031307 GR Elec. MFG. Co. USA). Water activity of mutton nuggets were measured by a_w meter (Hygrolab 3, Rotronics, Switzerland). The procedure of Tarladgis et al. (1960) was followed to estimate the TBARS (thio-barbituric acid reacting substances) number and was expressed as mg malonaldehyde/kg of sample. The DPPH stable free radical scavenging assay was performed as described by Hu and Kitts (2000) with slight modifications. Textural properties were determined using the Texture Profile Analytical procedure of Bourne (1967).

Colour analysis

The colour of mutton nuggets was measured using a Lovibond Tintometer (Model F, Greenwich, UK). The sample colour was matched by adjusting the red (a*) and yellow (b*) units, while keeping the blue unit fixed at 0.1. The corresponding colour units were recorded. The hue and chroma values were determined by using the formulae, $\tan^{-1}(b/a)$ (Little 1975) and $(a^2 + b^2)^{1/2}$ (Froehlich et al. 1983), respectively where a = red unit, b = yellow unit.

Microbiological evaluation

All the microbiological parameters of mutton nuggets were determined as per the method described by ICMSF (1996). Ready made media from Hi-media laboratories (P) Ltd, Mumbai, were used for the enumeration of different microbes. The plates for Total Plate count (Code No. M 091) were incubated at $37 \pm 1^\circ\text{C}$ for 48 h and plates showing 30–300 colonies were counted. For psychrophilic counts, the plates were prepared similarly but incubated at $7 \pm 1^\circ\text{C}$ for 5–7 days. Violet Bile Red Agar (Code No. M 049S) was used for coliform count and the plates were incubated at $37 \pm 1^\circ\text{C}$ for 48 h. The average numbers of colonies were multiplied with reciprocal of the respective dilutions and expressed as $\log_{10}\text{cfu/g}$.

Sensory analysis

Sensory evaluation method using 8 point descriptive scale (Keeton 1983) was followed, where 8 = like extremely; 1 = dislike extremely. The samples were rejected if the scores were below 5. The sensory panel consisted of seven assessors (trained scientists and post graduate students of the division). Taste panel were organized around 3.30–4.00 pm every time. The sensory panelists were asked to rate their preference on 8 point descriptive scale on the sensory evaluation proforma for general appearance, flavour, juiciness, texture, binding and overall acceptability. Plain water was provided to rinse the mouth in between the samples.

Statistical analysis

The statistical design of the study was 3 (treatments) * 3 (replications) randomized block design. The data generated from various trials under experiment were pooled, processed and analyzed by statistical method of one way-ANOVA and Mean $\pm$ SE using SPSS software package developed as per the procedure of Snedecor and Cochran (1989). Critical difference was determined at the 5% significance level.

Results and discussion

Standardization of the incorporation of essential oils and selection of the optimum level in mutton nuggets

There was no significant difference ($P > 0.05$) in product yield between control and treatment groups (Table 2). This confirmed the findings of Dzudie et al. (2004) in Ginger essential oil and Basilica essential oil treated beef patties. Sensory scores for flavour, after taste and overall

acceptability showed significant difference ($P < 0.05$) between control and treatment groups. Among treatment products, Blend-1 had significantly higher ($P < 0.05$) sensory scores than other two blends. Thus, on the basis of sensory scores blend having more number of essential oils (Blend 1) was found to be more acceptable by the sensory panelists which might be due to reduction in flavour intensity of each individual essential oil with increasing number of essential oils in a particular blend.

Selected blend (Blend 1) of essential oils was incorporated in pre-standardized formulation of mutton nuggets at three different levels viz., 0.25, 0.50 and 0.75% (Table 2). Incorporation of blends of essential oils in mutton nuggets did not cause any significant difference ($P > 0.05$) between product yields of control and treatment groups. Treatment products had significantly lower general appearance scores ($P < 0.05$) than control. Among treatments, products containing 0.25% level observed significantly higher ($P < 0.05$) sensory scores than other two levels of incorporation. Foda et al. (2010) also found that odour, taste and overall acceptability scores of cheese decreased significantly ($P < 0.05$) by higher concentration of spearmint essential oil. Thus, 0.25% concentration of blend of essential oils was adjudged as optimum and selected for further studies.

Standardization of the incorporation of combination of flaxseed flour and essential oils in combinations and selection of the optimum levels in mutton nuggets

No significant difference ($P > 0.05$) was observed in product yield of control and treatment products (Table 3). General appearance, flavour, binding and juiciness scores did not differ significantly ($P > 0.05$) between T-2 (0.25% E.O + 4% flaxseed flour) and T-3 (0.25% E.O + 8% flaxseed flour) products, although scores were marginally higher for control and T-2. Least scores were observed for T-3 products which might be due to incorporation of flaxseed flour replacing lean meat in formulation. Bilek and Turhan (2009) also reported that sensory scores for flavor, tenderness, juiciness and overall acceptability were significantly improved ($P < 0.05$) by increased level of flaxseed flour. Hence, combination of 0.25% E.O + 4% flaxseed flour was selected for further experiments.

Physico-chemical analysis

Chemical composition

Significantly lower ($P < 0.05$) moisture content of T-2 products than control might be due to the addition of flaxseed flour which resulted in the increased dry matter in

Table 2 Sensory characteristics and product yield of mutton nuggets incorporated with different blends of essential oils and different concentrations of selected blend of essential oils (mean $\pm$ SE)

Parameters	Control	Different blends of essential oils (0.25%)		
		Blend-1	Blend-2	Blend-3
Product yield (%)	97.42 $\pm$ 0.21	97.41 $\pm$ 0.26	97.13 $\pm$ 0.24	97.40 $\pm$ 0.27
Sensory attributes				
General appearance	7.32 $\pm$ 0.023	7.30 $\pm$ 0.028	7.28 $\pm$ 0.019	7.29 $\pm$ 0.027
Flavour	7.33 $\pm$ 0.019 ^a	7.04 $\pm$ 0.043 ^b	6.82 $\pm$ 0.049 ^{bc}	6.65 $\pm$ 0.053 ^c
Texture	7.40 $\pm$ 0.018	7.39 $\pm$ 0.034	7.38 $\pm$ 0.038	7.40 $\pm$ 0.035
Binding	7.41 $\pm$ 0.019	7.40 $\pm$ 0.021	7.38 $\pm$ 0.018	7.39 $\pm$ 0.028
Juiciness	7.30 $\pm$ 0.035	7.26 $\pm$ 0.139	7.25 $\pm$ 0.042	7.24 $\pm$ 0.024
After taste	7.31 $\pm$ 0.022 ^a	7.09 $\pm$ 0.023 ^b	6.71 $\pm$ 0.079 ^c	6.49 $\pm$ 0.052 ^d
Overall acceptability	7.32 $\pm$ 0.018 ^a	7.09 $\pm$ 0.069 ^b	6.85 $\pm$ 0.060 ^c	6.61 $\pm$ 0.064 ^d
Concentration of blends of essential oils				
		0.25%	0.50%	0.75%
Product yield (%)	97.12 $\pm$ 0.22	97.63 $\pm$ 0.19	97.19 $\pm$ 0.20	96.83 $\pm$ 0.51
Sensory attributes				
General appearance	7.32 $\pm$ 0.016 ^a	7.28 $\pm$ 0.024 ^{ab}	7.25 $\pm$ 0.024 ^b	7.22 $\pm$ 0.028 ^b
Flavour	7.33 $\pm$ 0.022 ^a	7.10 $\pm$ 0.034 ^b	6.40 $\pm$ 0.066 ^c	5.49 $\pm$ 0.082 ^d
Texture	7.34 $\pm$ 0.013	7.33 $\pm$ 0.022	7.31 $\pm$ 0.033	7.30 $\pm$ 0.024
Binding	7.33 $\pm$ 0.037	7.32 $\pm$ 0.028	7.30 $\pm$ 0.017	7.30 $\pm$ 0.027
Juiciness	7.31 $\pm$ 0.020 ^a	7.28 $\pm$ 0.026 ^a	7.25 $\pm$ 0.025 ^{ab}	7.20 $\pm$ 0.030 ^b
After taste	7.32 $\pm$ 0.025 ^a	7.07 $\pm$ 0.023 ^b	6.50 $\pm$ 0.052 ^c	5.40 $\pm$ 0.047 ^d
Overall acceptability	7.28 $\pm$ 0.019 ^a	7.09 $\pm$ 0.039 ^b	6.30 $\pm$ 0.050 ^c	5.62 $\pm$ 0.079 ^d

Mean $\pm$ SE with different superscripts in a row differ significantly ($P < 0.05$)

n = 21 for each treatment

Table 3 Sensory characteristics and product yield of mutton nuggets incorporated with combination of two different levels of hydrated flaxseed flour and selected blend of essential oils (mean $\pm$ SE)

Parameters	Control	Different combinations of flaxseed flour and blend of essential oils	
		T-2	T-3
Product yield (%)	97.09 $\pm$ 0.18	97.50 $\pm$ 0.28	97.40 $\pm$ 0.29
Sensory attributes			
General appearance	7.30 $\pm$ 0.018 ^a	7.27 $\pm$ 0.022 ^{ab}	7.22 $\pm$ 0.021 ^b
Flavour	7.30 $\pm$ 0.024 ^a	7.13 $\pm$ 0.028 ^b	7.09 $\pm$ 0.016 ^b
Texture	7.32 $\pm$ 0.025 ^a	7.30 $\pm$ 0.028 ^a	7.22 $\pm$ 0.025 ^b
Binding	7.30 $\pm$ 0.021 ^b	7.33 $\pm$ 0.025 ^{ab}	7.37 $\pm$ 0.027 ^a
Juiciness	7.31 $\pm$ 0.022 ^a	7.27 $\pm$ 0.022 ^{ab}	7.23 $\pm$ 0.030 ^b
After taste	7.32 $\pm$ 0.024 ^a	7.13 $\pm$ 0.024 ^b	7.07 $\pm$ 0.017 ^c
Overall acceptability	7.30 $\pm$ 0.020 ^a	7.15 $\pm$ 0.021 ^b	7.00 $\pm$ 0.023 ^c

Mean $\pm$ SE with different superscripts in a row differ significantly ($P < 0.05$)

n = 21 for each treatment; T-2 = 0.25% essential oil + 4% flaxseed flour and T-3 = 0.25% essential oil + 8% flaxseed flour

the nugget formulation (Table 4). Similar results were observed by Turhan et al. (2005) for beef burgers incorporated with hazelnut pellicle. Addition of flaxseed flour increased fat content of product, which was obvious due to

significantly higher fat content of T-2 product (Puvirajah 2014) than control and T-1 products. T-2 product had significantly higher ($P < 0.05$) ash content than other products indicating higher ash in products with higher level

Table 4 Physico-chemical characteristics of control and treatment mutton nuggets (mean $\pm$ SE)

Parameters	Control	T-1	T-2
Product yield (%)	97.25 $\pm$ 0.31	97.20 $\pm$ 0.278	97.34 $\pm$.22
Moisture (%)	69.24 $\pm$ 0.03 ^a	69.593 $\pm$ 0.13 ^a	67.57 $\pm$ 0.13 ^b
Fat (%)	12.26 $\pm$ 0.17 ^b	12.59 $\pm$ 0.21 ^b	13.15 $\pm$ 0.11 ^a
Ash (%)	2.42 $\pm$ 0.03 ^b	2.43 $\pm$ 0.02 ^b	2.47 $\pm$ 0.01 ^a
Dietary fibers (%)	0.48 $\pm$ 0.003 ^b	0.48 $\pm$ 0.004 ^b	0.57 $\pm$ 0.002 ^a
Crude fibers (%)	0.71 $\pm$ 0.004 ^b	0.70 $\pm$ 0.004 ^b	1.15 $\pm$ 0.012 ^a
Protein (%)	15.12 $\pm$ 0.08	15.15 $\pm$ 0.08	14.78 $\pm$ 0.10
Moisture protein ratio	4.58 $\pm$ 0.02	4.59 $\pm$ 0.03	4.57 $\pm$ 0.04
pH	6.32 $\pm$ 0.040	6.34 $\pm$ 0.025	6.33 $\pm$ 0.003
a _w	0.979 $\pm$ 0.0002 ^a	0.979 $\pm$ 0.0003 ^a	0.977 $\pm$ 0.0001 ^b
Shear force value (kg/cm ²)	0.28 $\pm$ 0.01	0.28 $\pm$ 0.0	0.29 $\pm$ 0.015
Textural properties			
Hardness (N/cm ²)	52.49 $\pm$ 0.67 ^b	52.38 $\pm$ 0.73 ^b	55.06 $\pm$ 0.55 ^a
Adhesiveness (Ns/gms)	− 0.10 $\pm$ 0.004 ^b	− 0.10 $\pm$ 0.005 ^b	− 0.12 $\pm$ 0.004 ^a
Springiness (cm/mm)	0.79 $\pm$ 0.02 ^a	0.80 $\pm$ 0.01 ^a	0.74 $\pm$ 0.01 ^b
Cohesiveness	0.41 $\pm$ 0.01	0.43 $\pm$ 0.02	0.42 $\pm$ 0.01
Gumminess (N/cm ²)	23.56 $\pm$ 0.48 ^a	22.86 $\pm$ 0.48 ^a	20.68 $\pm$ 0.31 ^b
Chewiness	14.04 $\pm$ 0.11 ^a	13.77 $\pm$ 0.20 ^a	11.35 $\pm$ 0.12 ^b
Colour values			
Redness	2.45 $\pm$ 0.04 ^c	3.10 $\pm$ 0.03 ^b	3.88 $\pm$ 0.05 ^a
Yellowness	3.52 $\pm$ 0.05 ^a	3.13 $\pm$ 0.03 ^b	3.43 $\pm$ 0.06 ^a
Hue	55.12 $\pm$ 0.78 ^a	50.91 $\pm$ 0.31 ^b	41.47 $\pm$ 0.28 ^c
Chroma	4.29 $\pm$ 0.03 ^c	4.92 $\pm$ 0.04 ^b	5.18 $\pm$ 0.07 ^a

Mean $\pm$ SE with different superscripts in a row differ significantly ($P < 0.05$)

n = 6 for each treatment; T-1 = 0.25% selected blend of E.Os and T-2 = 0.25% selected blend of E.Os + 4% hydrated flaxseed flour

of flaxseed flour. Similar results were reported in low fat beef patties (Turhan et al. 2005). Dietary fibre and crude fibre percentage also recorded similar trend in control and treatment products. T-2 products has significantly higher ($P < 0.01$) dietary fiber and crude fiber than T-1 product which was obvious as flaxseed contains 30% dietary fibre. These results were in accordance with Kumar et al. (2017). No significant difference ($P > 0.05$) was recorded in protein percentage between various products. However, gradual decline in protein content of treatment products was noticed with incorporation of flaxseed flour and was expected as a result of lean meat replacement with fat rich ingredient (Puvirajah 2014).

There was no significant difference ($P > 0.05$) in the moisture –protein ratio and pH of control and treatment products. Bilek and Turhan (2009) also observed similar results and reported no effect on pH of beef patties by incorporation of flaxseed flour. Water activity of T-2 product were significantly ($P < 0.05$) lower than control and T-1 product which could be explained by less moisture content in mutton nuggets with increase in flaxseed level (Turhan et al. 2005). The slightly higher shear force

value of T-2 product than control could be present due to gel formation by flaxseed. Chen et al. (2004) had reported about high viscosity of flaxseed gum in aqueous solutions.

Texture profile analysis

Results showed that hardness for control and T-1 product was significantly lower ($P < 0.05$) than T-2 products (Table 4). The increasing hardness in T-2 products might be due to increased shear force and binding strength of the product. Huang et al. (2005) had also reported improved hardness of product due to increase in carbohydrate content in pork meatballs. Springiness was observed with significantly higher values ($P < 0.05$) for control and T-1 products than T-2 products indicating the decrease in springiness of the product with incorporation of flaxseed flour. The decreased springiness can be justified as non-meat ingredients were being diluted in meat protein system and thus, resulting in reduced friction or binding among meat particles (Farouk et al. 2000). Similar trend was noticed for gumminess and chewiness of mutton nuggets

with significantly higher values ($P < 0.05$) for control and T-1 products.

Colour values

Colour values revealed highly significant difference ($P < 0.01$) in redness, yellowness, hue and chroma values for control and treatment products (Table 4). Redness value recorded significantly higher value ($P < 0.01$) for T-2 product followed by T-1. Higher values for T-2 product might be due to synergistic colour effect produced by flaxseed flour and essential oil. Seow et al. (2014) reported that essential oils can react with iron present in meat product and form dark pigmentation. Yellowness was observed significantly lower ($P < 0.01$) in T-1 than other products. Higher value for T-2 product was expected due to incorporation of flaxseed flour in the product (Sharma et al. 2014). Mean values for hue showed significantly higher values ($P < 0.05$) for control, although chroma values recorded similar trend as with redness value in control and treatment products.

Storage study

pH and water activity

Results showed that the pH values increased significantly ($P < 0.01$) from 10th day onwards for control and T-1 product and from 15th day for T-2 products (Table 5). The marginal increase in pH may be due to degradation of NPN substances and thus, liberation of their metabolites. It was also found that control product had significantly higher value ($P < 0.01$) than T-1 and T-2 products from 10th day onwards. It might be due to presence of anti-microbial constituents in treatment products which led to reduction in protein breakdown by bacteria and thereby, decreased production of amines. (Jay 1996). Among treatments, no significant difference ($P > 0.05$) was observed in pH values till 25th day of storage, but on 30th day, T-2 had significantly higher value ($P < 0.01$) than T-1 due to presence of flaxseed in T-2. Thus, pH value showed increasing trend in both control and treatment products, however increase was more in control followed by T-1 and T-2. Kumar et al. (2017) and Sharma et al. (2017a) had also reported increasing trend in pH in flaxseed incorporated mutton nuggets and essential oil incorporated fresh chicken sausages, respectively.

Water activity values showed decreasing trend with increase in storage for control as well as treatment products (Table 5). T-2 showed significant decrease ($P < 0.01$) in water activity from 15th day onwards. Control and T-1 products showed significantly higher ($P < 0.01$) values than T-2. Decreased water activity of T-2 at the end of

storage period might be because of the presence of less moisture content in mutton nuggets with increase in flaxseed level (Turhan et al. 2005).

TBARS value and DPPH free radical scavenging activity

TBARS values increased significantly ($P < 0.05$) for control and treatment products at each interval of storage period (Table 5). The increase TBARS was possible due to accelerated oxidation of lipids which produced volatile metabolites during aerobic storage (Modi et al. 2003). However, the values remained below 1 mg/kg of meat which is considered as minimum threshold value (Tarladgis et al. 1960). During storage period, treated groups showed generally lower lipid oxidation ($P < 0.05$) than the control. These results are in agreement with Sharma et al. (2014). Lower TBARS of treatment groups could be due to strong antioxidant activity of EOs attributed to their phenolic diterpene structure which interferes with free radical propagation process. Moreover, several plant phenolic compounds also act as metal chelators and singlet O_2 quenchers. However, T-2 showed lesser increase in TBARS than T-1 which might be explained by synergistic effect produced by combination of flaxseed flour and essential oil providing the product with more anti-oxidant activity than essential oil alone (Gaafar et al. 2013; Khanjari et al. 2013).

DPPH values of both control and treatment products decreased significantly ($P < 0.05$) with increase in storage period (Table 5). DPPH was found to be significantly higher for T-2 products followed by T-1 which might be due to combined anti-oxidant activity of flaxseed flour (Kitts et al. 1999) and phenolic content in essential oil (Velasco and Williams 2011; Gaafar et al. 2013). Higher DPPH activity in treatment products could be correlated to the presence of various phenol compounds (eugenol, thymol) in blend of essential oil which were able to reduce stable free radical DPPH to non radical form DPPH-H (Sharma et al. 2017b).

Microbiological quality

Total plate count

Total plate count showed significant increase ($P < 0.01$) in both control and treatment products with increase in storage period (Table 5). However, the counts observed lied within the permissible limit i.e. $\log_{10}$ 4 cfu/g for cooked meat products in control and T-1 products till 15th day and 25th day of storage period, respectively (Jay 1996). Counts were below the permissible limits even after 30th day of storage in T-2, indicating the synergistic antimicrobial effect of flaxseed flour and essential oil. Increase in total

Table 5 Effect of refrigerated storage on physico-chemical, anti-oxidant and anti-microbial activity of aerobically packaged control and treatment mutton nuggets (mean $\pm$ SE)

Treatments	Refrigerated storage period (days)						
	0	5	10	15	20	25	30
pH							
Control	6.32 ± 0.042 ^e	6.35 ± 0.005 ^e	6.42 ± 0.032 ^d	6.48 ± 0.005 ^{c1}	6.56 ± 0.008 ^{b1}	6.65 ± 0.006 ^{a1}	6.71 ± 0.007 ^{a1}
T-1	6.32 ± 0.014 ^e	6.34 ± 0.002 ^{de}	6.38 ± 0.013 ^d	6.42 ± 0.031 ^{c2}	6.46 ± 0.022 ^{c2}	6.51 ± 0.009 ^{b2}	6.59 ± 0.002 ^{a2}
T-2	6.34 ± 0.005 ^f	6.35 ± 0.004 ^{ef}	6.37 ± 0.002 ^e	6.41 ± 0.007 ^{d2}	6.44 ± 0.0016 ^{c2}	6.51 ± 0.002 ^{b2}	6.56 ± 0.014 ^{a3}
Water activity(a _w)							
Control	0.979 ± 0.0007 ^{a1}	0.977 ± 0.0008 ^{ab1}	0.976 ± 0.0008 ^{bc}	0.975 ± 0.0005 ^{c1}	0.973 ± 0.0030 ^{d1}	0.970 ± 0.0003 ^e	0.968 ± 0.007 ^{f1}
T-1	0.978 ± 0.0006 ^{a1}	0.976 ± 0.0008 ^{ab1}	0.975 ± 0.0008 ^b	0.974 ± 0.0007 ^{bcd12}	0.972 ± 0.0004 ^{cd1}	0.970 ± 0.0005 ^d	0.966 ± 0.0016 ^{e1}
T-2	0.976 ± 0.0002 ^{a2}	0.975 ± 0.0005 ^{a12}	0.974 ± 0.0004 ^{ab}	0.973 ± 0.0002 ^{b2}	0.970 ± 0.0006 ^{c2}	0.968 ± 0.0012 ^d	0.964 ± 0.0002 ^{e12}
TBARS values (mg malonaldehyde/kg product)							
Control	0.18 ± 0.001 ^g	0.32 ± 0.004 ^{f1}	0.40 ± 0.001 ^{e1}	0.49 ± 0.002 ^{d1}	0.64 ± 0.003 ^{c1}	0.83 ± 0.008 ^{b1}	0.98 ± 0.002 ^{a1}
T-1	0.17 ± 0.005 ^g	0.19 ± 0.001 ^{f2}	0.22 ± 0.002 ^{e2}	0.26 ± 0.003 ^{d2}	0.31 ± 0.004 ^{e2}	0.35 ± 0.002 ^{b2}	0.41 ± 0.002 ^{a2}
T-2	0.17 ± 0.003 ^e	0.18 ± 0.002 ^{e3}	0.21 ± 0.002 ^{d3}	0.22 ± 0.002 ^{d3}	0.25 ± 0.001 ^{c2}	0.32 ± 0.016 ^{b2}	0.37 ± 0.002 ^{a2}
DPPH free radical scavenging activity							
Control	15.03 ± 0.05 ^{a3}	11.59 ± 0.58 ^{b3}	7.52 ± 0.32 ^{c3}	3.96 ± 0.11 ^{d3}	1.93 ± 0.08 ^{e3}	0.94 ± 0.04 ^{f3}	0.16 ± 0.09 ^{g3}
T-1	82.54 ± 0.38 ^{a2}	76.08 ± 0.88 ^{b2}	71.43 ± 0.65 ^{c2}	65.64 ± 0.83 ^{d2}	60.06 ± 0.71 ^{e2}	54.13 ± 0.75 ^{f2}	50.00 ± 0.60 ^{g2}
T-2	91.05 ± 0.38 ^{a1}	85.20 ± 0.74 ^{b1}	81.12 ± 0.96 ^{c1}	76.37 ± 0.42 ^{d1}	72.66 ± 0.82 ^{e1}	67.28 ± 0.74 ^{f1}	60.68 ± 0.96 ^{g1}
Total plate count (log ₁₀ cfu/gm)							
Control	1.48 ± 0.12 ^{g1}	2.15 ± 0.08 ^{f1}	3.07 ± 0.06 ^{e1}	3.96 ± 0.04 ^{d1}	4.83 ± 0.07 ^{c1}	5.87 ± 0.06 ^{b1}	6.98 ± 0.04 ^{a1}
T-1	0.85 ± 0.27 ^{g2}	1.34 ± 0.08 ^{f2}	1.93 ± 0.04 ^{e2}	2.52 ± 0.06 ^{d2}	3.10 ± 0.07 ^{c2}	3.77 ± 0.07 ^{b2}	4.12 ± 0.04 ^{a2}
T-2	0.80 ± 0.26 ^{g2}	1.31 ± 0.07 ^{f2}	1.65 ± 0.05 ^{e3}	2.31 ± 0.06 ^{d3}	2.98 ± 0.05 ^{e2}	3.52 ± 0.03 ^{b3}	3.95 ± 0.04 ^{a3}
Yeast and mould count (log ₁₀ cfu/gm)							
Control	ND	ND	1.20 ± 0.06 ^{e1}	1.45 ± 0.11 ^{d1}	1.83 ± 0.04 ^{c1}	2.60 ± 0.02 ^{b1}	3.53 ± 0.02 ^{a1}
T-1	ND	ND	ND	ND	1.15 ± 0.07 ^{c2}	1.58 ± 0.13 ^{b2}	1.89 ± 0.04 ^{a2}
T-2	ND	ND	ND	ND	1.05 ± 0.22 ^{c2}	1.45 ± 0.07 ^{b2}	1.65 ± 0.06 ^{a3}
Psychrophilic count (log ₁₀ cfu/gm)							
Control	ND	ND	2.18 ± 0.09 ^{e1}	2.92 ± 0.03 ^{d1}	3.39 ± 0.04 ^{c1}	3.97 ± 0.06 ^{b1}	4.59 ± 0.09 ^{a1}
T-1	ND	ND	ND	1.73 ± 0.07 ^{d2}	1.99 ± 0.04 ^{c2}	2.33 ± 0.08 ^{b2}	2.71 ± 0.06 ^{a2}
T-2	ND	ND	ND	1.52 ± 0.10 ^{d3}	1.83 ± 0.05 ^{c3}	2.23 ± 0.04 ^{b2}	2.64 ± 0.15 ^{a2}

Mean $\pm$ SE with different superscripts row wise (alphabet) and column wise (numeral) differ significantly ($P < 0.05$)

n = 6 for each treatment; T-1 = 0.25% selected blend of E.Os, and T-2 = 0.25% selected blend of E.Os + 4% hydrated flaxseed flour

Coliforms were not detected during entire storage period

plate count with increase in storage period has also been reported by Sachindra et al. (2005), Kumar and Tanwar (2011) and Sharma et al. (2015). During the entire storage period, increase in TPC for control was significantly higher ($P < 0.05$) than treatment products and it was obvious due to presence of essential oils, active principle of which (eugenol, carvacrol etc.) is known to inhibit development of spoilage and pathogenic organisms (Fратиanni et al. 2010). T-2 showed least counts which could be due to combined effect of natural anti-microbials (lignan component of flaxseed flour and phenolic compounds in blend of essential oils), thus inhibiting the growth of bacteria.

Yeast and mould count

Yeast and mould counts were not detected up to 5th day of storage period in control and up to 15th day of storage in T-1 and T-2 products (Table 5). Counts increased significantly ($P < 0.05$) for control product at each interval of storage period, however, in treatment products, significant increase ($P < 0.05$) was observed from 20th day onwards. Similar trend of increase in yeast and mould count have been reported by Sharma et al. (2017a, b) in fresh chicken sausages and Mishra et al. (2015) in chicken meat rings. During the entire storage period, yeast and mould counts remained significantly lower ($P < 0.05$) for T-2 products followed by T-1 and then control, which might be attributed to combined effect of natural anti-microbial (lignan component of flaxseed flour and phenolic compounds in blend of essential oils) which inhibited the growth of fungi. The maximum permissible limits of yeast and mould counts has been reported as $\log_{10} 4$ cfu/g (Jay 1996) and was exceeded only in control product on 30th day of storage. Bang et al. (2000) had reported that cinnamaldehyde inhibits enzymes which synthesize cell wall in *S. cerevisiae* by acting as a non-competitive inhibitor of β -(1,3)-glucan synthase and a mixed inhibitor of chitin synthase isozymes.

Psychrophilic count

Psychrophilic counts (PC) increased significantly ($P < 0.05$) from 10th day onwards in control products (Table 5) and from 15th day onwards in treatment products. Control product had significantly higher ($P < 0.01$) count than treatment products on 10th and 15th day of storage. Initial periods of storage were observed with absence of psychrophiles due to the sudden change in physical environment resulting in reduced metabolic rate, thus prolonging the log phase of bacteria. Similar results in early phase of aerobic refrigerated storage were reported by Thomas et al. (2006); Gadekar et al. (2014) and various others authors. However, the count always remained below

$\log_{10} 4$ cfu/g, that is reported to cause microbiological spoilage of stored meat product (Jay 1996).

Coliforms

Absence of Coliforms was noticed during the entire storage period in all the samples due to cooking of product to an internal temperature of 72 °C, much above their lethal temperature of 57 °C. Denis et al. (2006) found that coliform species could not survive heat treatment of 60 °C for 2 min. Further, good hygienic and sanitation practices followed while handling, processing and packaging of the cooked products could also account for the absence of coliforms. Similar results were reported by Sharma et al. (2013) in restructured mutton chops and Kumar et al. (2015) in meat spread.

Sensory quality

Sensory scores for general appearance decreased significantly ($P < 0.01$) with increase in storage period after 5th day of storage period for all the products (Table 6). Treatment products obtained good sensory scores even on 30th day of storage. Significantly lower ($P < 0.05$) score for general appearance in T-2 products than T-1 might be attributed to darkening of the product due to incorporation of essential oil (Seow et al. 2014). Regarding the flavor, significant decrease ($P < 0.01$) in scores was recorded from 10th day onwards in all products. Decreased flavor of the product could also be correlated to increased TBARS values and free fatty acids and decreased anti-oxidant activity in the meat products with advancement in storage period under aerobic conditions (Thomas et al. 2006). Texture scores of T-1 and T-2 products decreased significantly ($P < 0.01$) from 10th day and 15th day onwards respectively. Decline in texture might be attributed to proteolytic and disulphide bonds changes (Santamaria et al. 1992) taking place with progress of storage period. Mean scores for binding also recorded similar trend as in texture of the product. The increasing trend of binding scores on addition of flaxseed might be due to increased viscosity of samples (Wang et al. 2011).

Control products showed significant decrease ($P < 0.01$) in juiciness at each interval of storage period and scores of T-1 and T-2 products were significantly lower ($P < 0.05$) from 10th day onwards of storage period to respective initial score of these products. Loss of moisture occurring during aerobic storage of the product might have resulted in decreased juiciness of products. Similar results were reported by Ahamed et al. (2007), Sharma et al. (2015) and many others. After taste score declined significantly ($P < 0.01$) from 5th day onwards. Least scores for T-1 products might be attributed to

Table 6 Effect of refrigerated storage on sensory attributes of aerobically packaged control and mutton nuggets incorporated with optimized level of selected blend of essential oils (mean $\pm$ SE)

Treatments	Refrigerated storage period (days)						
	0	5	10	15	20	25	30
General appearance							
Control	7.32 $\pm$ 0.034 ^a	7.26 $\pm$ 0.031 ^{a1}	7.16 $\pm$ 0.031 ^{b1}	6.80 $\pm$ 0.041 ^{c12}	–	–	–
T-1	7.30 $\pm$ 0.031 ^a	7.25 $\pm$ 0.039 ^{ab1}	7.18 $\pm$ 0.030 ^{b1}	6.89 $\pm$ 0.039 ^{c1}	6.76 $\pm$ 0.047 ^{d1}	6.63 $\pm$ 0.052 ^e	6.51 $\pm$ 0.0038 ^f
T-2	7.27 $\pm$ 0.027 ^a	7.21 $\pm$ 0.029 ^{ab12}	7.12 $\pm$ 0.034 ^{b12}	6.78 $\pm$ 0.053 ^{c12}	6.70 $\pm$ 0.034 ^{cd12}	6.60 $\pm$ 0.037 ^d	6.46 $\pm$ 0.029 ^e
Flavour							
Control	7.31 $\pm$ 0.025 ^{a1}	7.24 $\pm$ 0.028 ^{a1}	7.14 $\pm$ 0.043 ^{b1}	6.80 $\pm$ 0.035 ^c	–	–	–
T-1	7.06 $\pm$ 0.028 ^{a2}	7.01 $\pm$ 0.026 ^{a2}	6.90 $\pm$ 0.050 ^{b2}	6.71 $\pm$ 0.048 ^c	6.60 $\pm$ 0.035 ^c	6.39 $\pm$ 0.024 ^d	6.20 $\pm$ 0.043 ^e
T-2	7.14 $\pm$ 0.027 ^{a2}	7.09 $\pm$ 0.023 ^{ab12}	7.01 $\pm$ 0.034 ^{b12}	6.74 $\pm$ 0.040 ^c	6.62 $\pm$ 0.035 ^d	6.43 $\pm$ 0.033 ^e	6.25 $\pm$ 0.033 ^f
Texture							
Control	7.32 $\pm$ 0.029 ^a	7.24 $\pm$ 0.027 ^b	7.14 $\pm$ 0.027 ^b	7.08 $\pm$ 0.034 ^c	–	–	–
T-1	7.31 $\pm$ 0.029 ^a	7.22 $\pm$ 0.033 ^{ab}	7.16 $\pm$ 0.030 ^{bc}	7.10 $\pm$ 0.066 ^c	7.07 $\pm$ 0.030 ^{c1}	6.90 $\pm$ 0.034 ^{d1}	6.71 $\pm$ 0.043 ^{e1}
T-2	7.29 $\pm$ 0.030 ^a	7.19 $\pm$ 0.032 ^b	7.12 $\pm$ 0.023 ^{bc}	7.05 $\pm$ 0.028 ^c	6.92 $\pm$ 0.045 ^{d2}	6.73 $\pm$ 0.032 ^{e2}	6.63 $\pm$ 0.040 ^{f2}
Binding							
Control	7.30 $\pm$ 0.033 ^{a2}	7.21 $\pm$ 0.036 ^{b12}	7.10 $\pm$ 0.028 ^{c12}	7.00 $\pm$ 0.041 ^d	–	–	–
T-1	7.29 $\pm$ 0.035 ^{a2}	7.22 $\pm$ 0.039 ^{ab12}	7.11 $\pm$ 0.030 ^{bc12}	7.02 $\pm$ 0.037 ^c	6.80 $\pm$ 0.056 ^d	6.68 $\pm$ 0.058 ^e	6.51 $\pm$ 0.040 ^f
T-2	7.34 $\pm$ 0.031 ^{a1}	7.26 $\pm$ 0.031 ^{ab1}	7.18 $\pm$ 0.039 ^{b1}	7.06 $\pm$ 0.034 ^c	6.85 $\pm$ 0.035 ^d	6.72 $\pm$ 0.041 ^e	6.55 $\pm$ 0.036 ^f
Juiciness							
Control	7.33 $\pm$ 0.030 ^a	7.23 $\pm$ 0.024 ^b	7.14 $\pm$ 0.036 ^c	7.01 $\pm$ 0.032 ^d	–	–	–
T-1	7.31 $\pm$ 0.026 ^a	7.22 $\pm$ 0.034 ^{ab}	7.12 $\pm$ 0.029 ^b	7.00 $\pm$ 0.054 ^c	6.83 $\pm$ 0.048 ^d	6.65 $\pm$ 0.042 ^e	6.43 $\pm$ 0.037 ^f
T-2	7.29 $\pm$ 0.030 ^a	7.20 $\pm$ 0.032 ^{ab}	7.10 $\pm$ 0.070 ^b	6.95 $\pm$ 0.039 ^c	6.76 $\pm$ 0.042 ^d	6.58 $\pm$ 0.045 ^e	6.40 $\pm$ 0.024 ^f
After taste							
Control	7.31 $\pm$ 0.041 ^{a1}	7.24 $\pm$ 0.034 ^{a1}	7.13 $\pm$ 0.039 ^{b1}	7.01 $\pm$ 0.035 ^{c1}	–	–	–
T-1	7.05 $\pm$ 0.037 ^{a2}	7.01 $\pm$ 0.042 ^{a2}	6.90 $\pm$ 0.035 ^{b2}	6.81 $\pm$ 0.049 ^{b2}	6.70 $\pm$ 0.046 ^b	6.55 $\pm$ 0.060 ^c	6.40 $\pm$ 0.048 ^d
T-2	7.11 $\pm$ 0.032 ^{a2}	7.05 $\pm$ 0.034 ^{a12}	6.80 $\pm$ 0.061 ^{b2}	6.85 $\pm$ 0.048 ^{b2}	6.76 $\pm$ 0.047 ^b	6.61 $\pm$ 0.043 ^c	6.48 $\pm$ 0.045 ^d
Overall acceptability							
Control	7.30 $\pm$ 0.030 ^{a1}	7.21 $\pm$ 0.041 ^{b1}	7.12 $\pm$ 0.034 ^{c1}	6.80 $\pm$ 0.046 ^{d1}	–	–	–
T-1	7.08 $\pm$ 0.029 ^{a2}	7.0 $\pm$ 0.021 ^{a2}	6.93 $\pm$ 0.046 ^{b2}	6.65 $\pm$ 0.039 ^{c2}	6.52 $\pm$ 0.031 ^d	6.40 $\pm$ 0.028 ^e	6.25 $\pm$ 0.034 ^f
T-2	7.14 $\pm$ 0.024 ^{a2}	7.07 $\pm$ 0.030 ^{a2}	7.00 $\pm$ 0.036 ^{ab12}	6.70 $\pm$ 0.045 ^{c1}	6.58 $\pm$ 0.045 ^d	6.44 $\pm$ 0.034 ^e	6.31 $\pm$ 0.030 ^f

Mean $\pm$ SE with different superscripts row wise (alphabet) and column wise (numeral) differ significantly ($P < 0.05$)

n = 21 for each treatment; T-1 = 0.25% selected blend of E.Os. and T-2 = 0.25% selected blend of E.Os + 4% hydrated flaxseed flour

pungent aroma perceived by panelists which was partially masked in T-2 products. Overall acceptability of control and treatment products decreased significantly ($P < 0.05$) at each interval of storage period. Decrease in overall acceptability of product during storage was expected due to increased degradation of lipids in the presence of oxygen, pigment oxidation and fat and protein oxidation present in mutton nuggets. Overall acceptability scores for treatment products did not differ significantly ($P > 0.05$) throughout storage period. The decrease in overall acceptability scores of the products during storage had also been reported by various authors such as Radziejewska et al. (2008) in poultry meat products, Biswas et al. (2011) in duck patties.

Conclusion

Flaxseed flour and blend of essential oils have been evaluated for their anti-microbial and anti-oxidant efficacy in mutton nuggets. Based on present study, it was found that control products, mutton nuggets incorporated with 0.25% Blend-1 and combination of 4% flaxseed flour + 0.25% Blend-1 retained acceptable microbiological counts and good to very good sensory ratings up to 15th day, 25th day and 30th day, respectively during aerobic storage in LDPE pouches under refrigeration at 4 ± 1 °C. Therefore, it can be concluded that essential oil and combination of flaxseed flour and Blend-1 incorporated mutton nuggets had about

10 days longer shelf life than control during aerobic storage under refrigeration at $4 \pm 1^\circ\text{C}$.

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