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Antimicrobial Efficacy and Preservative Quality of Essential Oil Blends with Chitosan in Sweet and Sour Chicken Meat Spread

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ABSTRACT

Background: Synthetic preservatives in food are associated with lot of consumer health risks considering their negative health impact with increasing awareness towards natural products, present study evaluated the effects of natural antimicrobial and antioxidant additive essential oil blends (EOBs) with Chitosan (Ch) for preserving quality and microbial safety of sweet and sour chicken meat spread (SSMS) applying hurdle concept.

Methods: Concentration EOs in blends were decided based on screening for MIC and sensory acceptability essential oils (EOs) of *Origanum vulgare*, *Cinnamomum aromaticum*, *Thumus vulgaris, Syzygium aromaticum*, *Ocinum Tenuiforum*, *Trachyspermum ammi L* and *Piper betle* EOs). EOs blends were further screened and selected blends (0.125% each) with Ch (0.5%) were incorporated in SSMS and products quality was evaluated for extended storage at refrigeration temperature.

Result: Storage studies revealed that treatments Ch + blend A had significantly ($P<0.05$) lower pH and TBARS values as compared to blend B products whereas, total phenolic content and DPPH activity were significantly ($P<0.05$) higher for blend A followed by blend B, Ch and Control. TPC Staphylococcus (SC), Psychrophilic (PC) and Yeast and Mold(Y andM) count were significantly ($P<0.05$) lower in with Ch+EOBs. Highest ($P<0.05$) antimicrobial and antioxidant activity was exhibited by Ch + EOBs. Although initially slightly lower sensory score was observed for EOs blend added product however significantly ($P<0.05$) higher sensory quality of the containing EOBs treatments was found during storage. Results suggested that chitosan with essential oil blends exhibited potential antioxidant and antimicrobial preservative effect during extended storage so it could be applied as potential natural preservative combination for formulating enhanced shelf life natural antioxidant enriched meat food products.

Key words: Antimicrobials, Antioxidant, Chicken meat spread, Chitosan, Essential oil blends, Hurdle treatments, Minimum inhibitory concentration.

INTRODUCTION

Food borne illness are a significant concern for consumers moreover problem of using synthetic preservatives for food application are associated with lot of risks which are again significant issues for consumers, the food industry and food safety authorities. EOs are natural volatile liquids extracted from certain plants and are complex mixture of secondary metabolites with varying degree of biological activity (Falleh *et al.,* 2020; Singh *et al.,* 2012) which are incorporated as antimicrobial and antioxidant additives (Ribeiro-Santos *et al.,* 2017) in food to replace the synthetic chemical preservatives and have wide applications in food industry (Ravindran, *et a.l,* 2025). Mechanism of bacterial inhibition involves its ability to interact with electron transport, ion gradients, protein translocation, phosphorylation and other enzyme-dependent reactions associated with the phospholipid bilayer resulting into increased bacterial cell membrane permeability (Lambert *et al.,* 2001). Chitosan is a major component of the shells of crustacean such as crab, shrimp and crawfish. Due to its antimicrobial activity against a wide range of food borne filamentous fungi, yeast and bacteria (Sagoo *et al.,* 2002), biocompatibility, biodegradability and non-toxicity properties

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it is recommended as natural antimicrobial (Everendilek 2015, Sharma *et al.,* 2022)It is a modified, natural carbohydrate polymer derived by deacetylation of chitin [poly-β-(1→4)-N-acetyl-D-glucosamine] in presence of alkali (Sánchez-Gonzalez *et al., 2010*). Its food application is versatile (Gupta *et al.,* 2021) due to its unique rheological and physiological characteristics and antimicrobial/ antifungal properties. Antimicrobial activity of chitosan is contributed by its ability to change cell permeability due to interactions between the polycationic chitosan and the electronegative microbial cell surfaces leading to the leakage of intracellular electrolytes and cell constituents (Alizadeh Behbahani *et al.,* 2020; No *et al.,* 2002). The antimicrobial activity of chitosan is affected by its deacetylation degree, molecular weight, pH of the medium, temperature and presence of food components. Gram-negative bacteria are found to be more susceptible while the sensitivity of the Gram-positive bacteria is highly variable (Zareie *et al.,* 2020).

Essential oils are potential food preservatives their antimicrobial activity has already been demonstrated against various food borne pathogens Al-Maqtari, *et al.,* 2021). Major concern in their a food application is their pungent flavour which could be overcome by using in combination or with there in preservatives so that they each will lower concentration but in combination extort potential antioxidant and antimicrobial effects so considering these points present study is designed by Essential oil blends which is combined with Chitosan for applying as natural preservative in food.

Most of the stable food based on the hurdle concept applying thermal treatments, pH, water activity, low temperature and preservatives (Padhan, 2018). Concept of present study is to use various EOS with chitosan for enhancing the safety aspect without any synthetic preservative. Objectives of the present study formulation of EOs blends and combining these blends with chitosan for improving quality and stability of sweet and sour chicken meat spread (SSMS) at refrigeration temperature (4±1°C). Sensory acceptability of essential oils (EOs) in food is limited owing to its pungent flavor volatiles so formulation of EOS blends and combining with Ch for enhancing microbial quality safety and oxidative stability of the product.

MATERIALS AND METHODS

Standard culture of *Staphylococcus* and *E. coli* were obtained from Hi-media Laboratories Pvt. Ltd., Mumbai (Code: MM043) India and maintained in tryptose soy agar (Hi-media,Germany) slant at 4°C with regular sub- culturing. EOs and chitosan were purchased from reputed commercial firms. All the chemicals and media used in the study were of analytical grade and obtained from Hi media® Mumbai and Merck®Mumbai.

Formulation of essential oil blends (EOBs)

Minimum inhibitory concentration(MIC) against standard culture of *Staphylococcus* AICC15597 and *E coli* ATCCBAA977and Sensory screening of EOs of oregano; *Origanum vulgare (OV)*, cassia; *Cinnamomum aromaticum (CA)*, thyme (*Thumus vulgaris* Cinnamon; *Cinnamomum zeylanicum (CZ)*; clove *Syzygium aromatic (SA)* and holy basil (*Ocinum Tenuiforum* (*OT*)*, Ajowan; Trachyspermum ammi L* (*TA*) and *betle Piper betle (PB)*. (Table 3 Fig 1) was carried based on these preliminary trial followings EOBs (Each with different type and concentration of essential oils) were formulated (Table 1).

Those above 5 EOBs were again screened for MIC against standard culture of *Staphylococcus* and *E. coli* and sensory acceptability (Table 4). Finally 2 EOB EOB1 and EOB 4 were selected for incorporation in product (as blend A and blend B) with chitosan (Ch).

Level of Chitosan was decided based on MIC studies and sensory acceptability in SSMS.

Table 1: Formulation of EOBs.

EOs (%)	EOB1	EOB2	EOB-3	EOB4	EOB5
OV	40	75	_	_	_
CA	30	25	40	40	20
TV	10	_	20	40	15
SA	10	_	20	20	20
OT	10	_	20	_	25
TA	_	_	_	_	10
PB	_	_	_	_	10

OV: Origanum vulgare EOs; CA: Cinnamomum aromaticum EOs; TV: Thumus vulgaris EOs; SA: Syzygium aromaticum EOs; OT: Ocinum Tenuiforum EOs; TA: Trachyspermum ammi L EOs; PB: Piper betle EOs.

Table 2: Ch-EOBs for formulation (a) of treatments and control SSMS.

Test ingredients (w/w)	Control	Ch	Ch + BlendA	Ch + BlendA
Ch	-	0.5	0.5	0.5
EOB 1			0.125%)	
EOB 4	-			0.125%)

Preparation of sweet and sour chicken meat spread (SSMS) incorporated with Ch-EOBs

Standardized formulation of Arya *et al.* (2017) was used for preparation of SSMS (Fig 1). Treatments EOB A and EOB B (Table 2) products were subjected to storage analysis along with control (without Chitosan and EOBs) at refrigeration temperature (4±1°C).

Determination of MIC

Stock inoculums of Test bacterial culture *Staphylococcus aureus* AICC15597 and *E. coli* ATCCBAA977 were prepared using 0.5 Mcfarland standards and MIC of EOs and chitosan were calculated by tube dilution method (Wiegand *et al.,* 2008) including a positive and negative control. Chitosan was dispersed in 1% acetic acid prior to assay (No *et al.,* 2002).

Determination of storage quality attributes

pH was measured as per the procedure of AOAC (1995) using digital pH meter (Cyberscan®, pH510, Eutech Instruments, Singapore). Water activity was measured with the help of a water activity meter (Hygrolab 3®, Rotronics, Switzerland).

Antioxident quality

TBARS value was determined by using the distillation method described by Tarladgis *et al.* (1960). DPPH assay was taken using a method used by Tepe *et al.* (2005) with slight modifications. 0.1 g of the sample and 5ml of 0.004% DPPH in methanol were added to a test tube. The samples were subjected to homogenization for 30 sec using Ultra Turrax tissue homogenizer (Model IKA®T 18, Janke and Kenkel, IKA Labor Technik, Germany) and left for 30 mins at room temperature with constant mixing. Absorbance was measured using spectrophotometer (Model: Beckman DU 640, USA) at 517 nm, using methanol as a blank.

$$\text{DPPH activity \%} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Total phenolic contents were determined by the Folic-Ciocalteau method (Ebrahimzadeh *et al.,* 2010; Nabavi *et al.,* 2008). Sample extract were prepared by the method of Naveena *et al.,* (2008). Calibration curve was prepared using 0, 50, 100, 150, 200 and 250 mg/ml solutions of tannic acid in methanol: water (50:50 v/v). OD was measured at 765 nm and quantification of total phenolics was expressed as tannic acid equivalent (mg/g of dry mass).

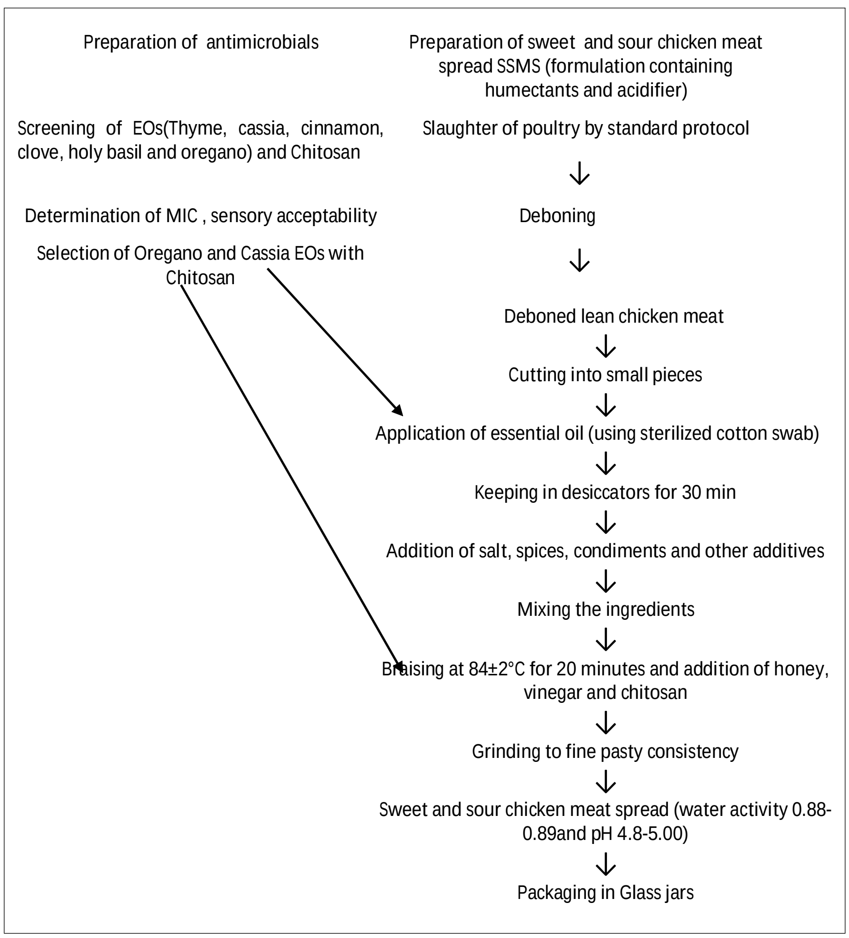

Fig 1: Preparation of sweet and sour chicken meat spread (SSMS) incorporated with Chitosan essential oil blends as bio-preservatives.

Microbiological quality

Microbiological quality was analyzed following the methods described by American Public Health Association (APHA, 2000).

Statistical analysis

Experiment was repeated three times and duplicate samples were drawn for each parameter (n=6) except sensory evaluation where 7 number of panel judges were evaluating the experiment in three trials (N=21). The data were analysed by statistical method of ANOVA and using SPSS Statistics 20.0 software package. Means and standard de*via*tions were calculated and when F-values were significant at the P<0.05 level, mean differences were separated using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Optimization and selection of blends

MIC studies (Table 3) revealed that SA (*Syzygium aromaticum*), OT (*Ocinum Tenuiforum)* and OV (*Origanum vulgare*) EOs showed the highest inhibition against both the test bacterial culture whereas, CZ (*Cinnamomum zeylanicum*) EOs showed inhibition at higher concentration. OV, CA and OT were has shown minimum value foe MIC at tested concentration against *Escherichia coli* and *Staphylococcus aureus.*

Table 3: MIC of essential oils and chitosan against test bacterial culture.

Essentials oils/ Chitosan	*Escherichia coli*	*Staphylococcus aureus*
OV	0.05	0.03
CA	0.04	0.10
TV	0.13	0.25
CZ	0.23	0.25
SA	0.04	0.06
OT	0.06	0.04
TA	0.12	0.01
PB	0.18	-
Ch	0.16	0.16

OV: *Origanum vulgare* EOs; CA: *Cinnamomum aromaticum* EOs; Cinnamon; *Cinnamomum zeylanicum* (CZ); TV: *Thumus vulgaris* EOs; SA: *Syzygium aromaticum* EOs, OT: *Ocinum Tenuiforum* EOs; TA: *Trachyspermum ammi* L EOs; PB: *Piper betle* EOs; Ch: Chitosan.

Table 4: MIC of EOBs against test bacterial culture.

Essentials oils/ Chitosan	*Staphylococcus aureus*	*Escherichia coli*
EOB 1	0.06	0.03
EOB 2	0.02	0.04
EOB 3	0.07	-
EOB 4	0.13	0.11
EOB 5	0.18	0.23

Berthold-Pluta *et al.* (2019) observed higher effectiveness of *Thumus vulgaris*, *Cinnamomum zeylanicum* and *origanum marjorana* whereas moderate effectiveness of *Syzygium aromaticum, Foeniculum vulgare* and *Cuminum cyminum* EOs for inhibition. However, Alizadeh Behbahani *et al.* (2020) suggested that EOs of Cinnamomum zeylanicum was more effective for inhibiting *Staphylococcus aureus* and *Bacillus cereus*. Considering the MIC values 4 blends (Table 4), EOB 1 and EOB 4 showed highest antimicrobial activity.

MIC value of chitosan (Table 3) was observed as 0.11 and 0.16% for the test bacterial culture *Staphylococcus aureus* and *Escherichia coli* respectively. Islam et al (2011) also obtained inhibitory activity of chitosan against the same bacterial culture Sanchez-Gonzalez *et al.* (2011) demonstrated that pure chitosan films presented a significant antimicrobial activity against L. *monocytogenes* and *E. coli*.

Based on antimicrobial and sensory screening (Table 3, 4) EOB 1 and EOB 4 at 0.125% level were selected with Chitosan @0.05% concentration (Table 3) for incorporation in SSMS.

Quality of selected EOBS-Ch incorporated SSMS

pH and Water activity (a_w)

pH of treatments as well as control increased throughout the storage although the rate of increase was slower in treatments (Fig 2). pH increased significantly (P<0.05) from 7th day onwards in control, 14th day onwards in Ch and 21st day onwards in Ch + Blend A and Ch + Blend B whereas, Ch + Blend A showed significantly (P<0.05) lower values. pH is the key indicator of meat freshness (Chen *et al.,* 2019; Zang *et al.,* 2019) and increase in pH is might be due to propagation of spoilage aerobic microorganisms (Wang, *et al.,* 2017) resulting in higher protein degradation and accumulation of metabolites of bacterial action. Yaghoubi *et al.* (2021) also reported decreased pH in fresh chicken meat with Ch coating containing *Antimisia fragrans* EO. Findings of Bharti *et al.* (2020) also suggested the lower pH values of EOs chicken wrapped in starch edible film nuggets containing EOs.

Addition of chitosan and essential oils did not significantly affected aw of the product throughout the storage period. Non-significant increase, might be related to proteolysis or pyrolysis of amino acids during storage.

Antioxidant quality

TBARS value

Treatments showed significantly (P<0.05) lower TBARS value than control throughout the storage period and among treatments, Ch had significantly (P<0.05) higher values from 28th day onwards. Non-significantly (P>0.05) lower values were obtained for Blend A during the entire storage period (Fig 2). Increase in TBARS value was due to increased lipid oxidation (Vilarinho *et al.,* 2018) and strong antioxidant activity of and EOBs might have resulted reduced TBARS in treatments during the storage. Vieira *et al.* (2019) also observed significantly reduced TBARS values with use of Ch- clove EOs for preventing lipid oxidation of Tambaqui (*Colossoma macrospora*) fillets. Farsanipour *et al.* (2020) also obtained lower TBARS value of *Scomberoides commersonnianus* fillets with coating containing Ch-whey protein isolate incorporated with t*erragon Artemisia daracunculus* EO.

DPPH activity

As the storage period progressed, DPPH activity significantly (P<0.05) decreased in control and treatments. DPPH activity significantly (P<0.05) reduced on 14th day in control as well as treatments except for Ch + Blend A in which significant (P<0.05) reduction was observed from 21st day onward (Fig 2). Considerably higher DPPH (% inhibition) of treatments probably attributed to presence of antioxidant compound of EOs and Ch. Results of the study are in agreement to that of Kalaikannan *et al.* (2023) in Eos incorporated chicken patties and Hafsa *et al.* (2016) who reported that DPPH free radical scavenging capacities of chitosan incorporated with *Eucalyptus globulus* (EG) EOs significantly increased (P<0.05) with increasing EG essential oil. Antioxidant activity of the chitosan contributed by formation of stable flourosphere with volatile aldehyde (Wang *et al.,* 2017) further combination with EOs might have produced synergistic antioxidant activity for controlling lipid oxidation (Zhang *et al.,* 2019; Basavegowda and Baek, 2021).

Total phenolics

Total phenolic content decreased significantly (P<0.05) during storage however, the rate of decrease was slower in treatments throughout the storage period total phenolic content differed non-significantly till 14th day whereas from day 21 onwards significantly (P<0.05) (Fig 3). The findings of the study are in agreement of Bazargani-Gilani *et al.,* (2015) who observed that pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* essential oil coatings reduces phenolic degradation of chicken breast meat during refrigeration storage. Among treatments Ch + Blend A showed highest value. Higher total phenolic content of sliced carrot coated with Ch containing microencapsulated Thyme essential oil was also observed during refrigeration storage by Viacava *et al.* (2022).

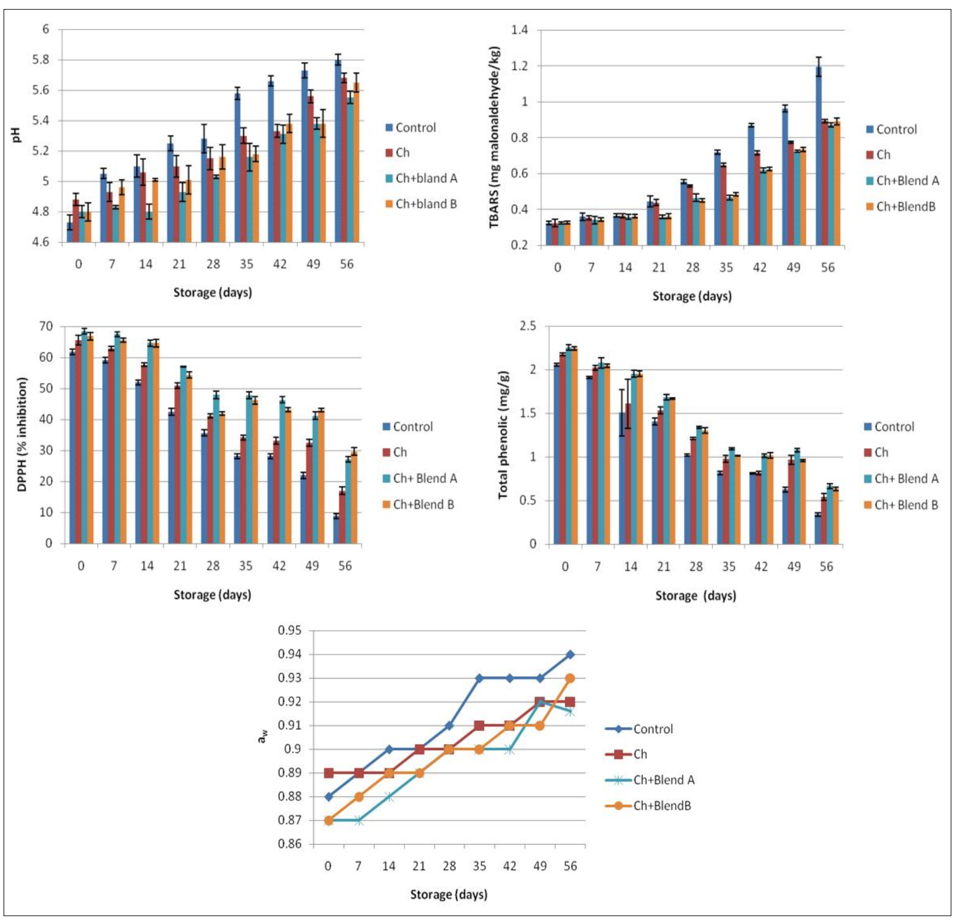

Fig 2: Effect of chitosan-EOBs on pH, TBARS (mg malonaldehyde/kg), DPPH (% inhibition), total phenolic content (mg/g) and water activity of sweet and sour chicken meat spread.

Microbial Characteristics

Total plate count

During storage, total plate count (TPC) decreased initially up to 7th day in control, 14th day in treatments and thereafter increased significantly (P<0.05) in control and treatments (Fig 3). Lowest count in Ch + Blends A (Fig 3) might be due to the synergistic interaction of antimicrobials. TPC of all the products always remained below permissible limit up to 35th day of storage in control, 42th day in Ch and 49th day in Ch-EOBs which was indicative of unacceptability of cooked meat products. The composition, structure as well as functional groups of the EOs determine the antimicrobial activity (Yuan *et al.,* 2016). Increased antimicrobial activity in treatments with acidic pH of the product further produced additional effect for retarding the growth of microorganisms (Aguilar-Veloz *et al.,* 2020, Bharti *et al.,* 2020).

Staphylococcus count (SC)

The absence SC during initial storage days might be because of cooking of the products, humectants and acidifier and combination antimicrobials. SC was inhibited up to 14th day of storage in treatment while their growth appeared afterwards. Significant increase (P<0.05) in SC was observed with storage period in control as well as treatments throughout the storage period whereas in case of treatment products significantly (P<0.05) lower count was observed (Fig 3) were observed by Naseri *et al.* (2020) by using gelatine-Ch film containing *Ferulago angulate* EOs for prolongation of turkey meat shelf life.

Yeast and mold count (Y and M)

Y and M were not detected up to 7th day in control and up to 14th day in treatment might be due to destruction of cells during cooking and added bio-preservatives. Recovery of injured micro-organisms and their multiplication during subsequent storage might have contributed to increased count during storage. However, the counts were significantly (P<0.05) lower for the treatments containing EOBs during the entire storage (Fig 3). The antifungal potential of Ch + blend A was found highest among all treatments followed by Ch + blend B. Treatment product Ch and Ch with EOBs retained acceptable count up to 42th day and 49th day whereas, upto 35th day in control. Behbahani *et al.* (2020) reported antifungal activity of EOs incorporated in edible coating for improving microbial stability whereas, Ayon Reyna *et al.* (2022) and Yuan *et al*. (2016) also observed antifungal activity of Ch in edible packaging. EOBs component with Ch might have produced synergistic antifungal activity (De Souza *et al.,* 2020; Hossain *et al.,* 2019) which might have contributed to the reduced yeast and mold count of EOBs containing treatments during entire storage.

Psychrophilic counts (PC)

Psychrophilics were not detected up to 21st day of storage in control and treatments which could be due to destruction of psychrophiles because of cooking, humectants, acidifiers and antimicrobials. Nonsignificant (P>0.05) difference was observed among Ch + Blend A and Ch + Blend B, except on 49th day where significantly (P<0.05)

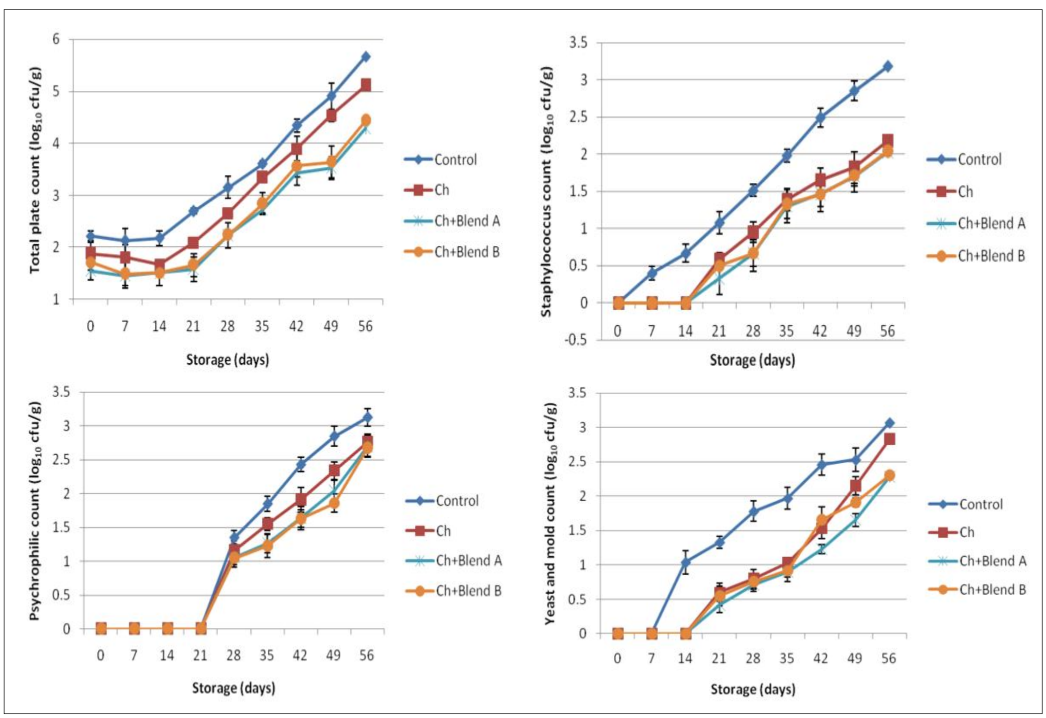

Fig 3: Effect of Chitosan-EOBs on total plate count ((log cfu/g), *Staphylococcus* count (log cfu/g), psychrophilic count (log cfu/g) and Yeast and mold count (log cfu/g) values of Sweet and sour chicken meat spread.

lower values were observed for Ch + Blend B (Fig 3). Lower counts in treatment could be attributed to inhibitory effects of chitosan to psychrophilic microorganism and phenolic groups present in essential oil having antimicrobial activity. Enhancement of psychrophilic microbes during the storage has also been reported in sliced chicken breast treated by dipping in sodium lactate and potassium sorbate by Kenawi *et al.* (2007).

No *et al.* (2002) revealed that antimicrobial activity of chitosan is inversely affected by pH value that is higher activity at lower pH range. pH range of the treatment product (4.5-5.6 during storage) might also have facilitated antimicrobial activity in SSMS.

CONCLUSION

Essential oil blends with chitosan was proven as effective preservative enhancing shelf life of sweet and sour chicken meat spread in the studyas natural additive for meat products. Chitosan-EOBs combination could significantly improved the microbial quality and oxidative stability of the product during extended storage. The hurdle concept used in the study *i.e.* acidic pH, reduced water activity of original product and refrigeration storage along with combination antimicrobials exhibited significant antioxidant and antimicrobial effect for extending the shelf life and could be a potential alternative for synthetic preservatives. The concept of combination antimicrobials using natural ingredients chitosan and essential oils with additional hurdles can be successfully employed for extended shelf life product manufacturing for commercial application. These type and other processed products could be formulated using Essentail oil blends-chitosan without compromising food safety.

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Author contribution

Anita Arya conducted all the experiments; Sanjod Kumar Mendiratta designed the research and objectives. Ravi Kant Agrawal provided essential oils, Chitosan and test bacterial culture and also helped in microbiological analysis. Sanjay Kumar Bharti figured out the statistical analysis and data interpretation. Pramila Umarao, Jyoti Palod and Sumit Gangwar helped in manuscript preparation and proofing.

Conflict of interest

Authors have no conflict of interest to declare.

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