Effect of Clove and Holybasil Essential Oil on Sensory and Microbiological Quality of Fresh Chicken Sausages

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Abstract

The present study was aimed to study the anti-microbial effect of clove and holybasil essential oils and their effect on sensory attributes in fresh chicken sausages. Different levels of incorporation viz., 0.125%, 0.25%, 0.5% and 1% were tried in product for both oils. Essential oil incorporated products were analysed for total plate count and sensory attributes and optimum level of incorporation was identified. Total plate count was observed significantly lower (P<0.05) in essential oils incorporated products than control. 1% clove oil and holy basil oil incorporated products showed approx 1.5 log reduction in TPC when compared to control. However, sensory panelists gave satisfactory scores to lower level of essential oil products. Thus, based on total plate count and sensory scores, 0.25% and 0.125% were adjudged as optimum level of incorporation in fresh chicken sausages. Proximate composition revealed no significant difference in moisture, fat, protein, ash of treatment products and control, however pH was significantly decreased (P<0.05) in case of oil incorporated products.

Keywords: Fresh chicken sausages, Clove oil, Holybasil oil, Microbial quality, Sensory attributes.

1. Introduction

Poultry meat is a very popular food commodity around the world and its consumption has increased over the last decades in many countries. India ranks fifth in chicken meat in the world with production figure of 2.19 MT of chicken meat (DAHDF, 2012). Availability of high biological value of animal proteins, essential amino acids and fatty acids, vitamins and other nutrients are the other reasons to ensure its popularity among masses (Biesalski, 2005). However, high amount of polyunsaturated fatty acids in poultry meat make it a highly perishable food commodity providing an almost perfect medium for microbial growth including both spoilage and pathogenic microorganisms (Jay et al., 2005).

Sausage is one of the oldest known forms of processed meat products and is very popular in many areas. Fresh sausage is a sausage “made from selected cuts of fresh meat (not cooked or cured) and must be stored in a refrigerated (or frozen) state prior to being consumed.” (Liu et al., 2009). However, one difficulty associated with storage is the oxidative rancidity which is one of the most important quality defects of chicken meat or meat product during storage. Another important factor causing spoilage of fresh products is microbial growth (Petrou et al., 2010). According to USDA, sausage makers should ensure that their products are not contaminated by pathogens such as Listeria, Escherichia coli O157:H7, Salmonella, Trichinea and Staphylococcus enterotoxin.

To extend the period of refrigerated storage, which is the most common method used for preserving fresh meat and meat products, many synthetic additives have been used over the years. However, synthetic additives have been accused for some carcinogenic and toxic properties. In this respect, many naturally occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage organisms and pathogens (Dorman and Deans, 2000; Dhanze et al., 2013). They have been classified as GRAS (Generally Recognized as Safe).

Syzygium aromaticum (L.) (clove) and Ocimum sanctum (Holy Basil) are widely used plant in tropical countries. These two plants have been used for many purposes since ancient times in various food applications. Clove and Holybasil oil have a wide
spectrum of actions not only antibacterial, antiviral, antifungal and antiprotozoal, but also have beneficial
effects on the cardiovascular and immune system
(Harris et al., 2001; Maheshwari et al., 2012). The anti-
microbial and anti-oxidant activity of essential oils is
attributed to number of monoterpenoids (carvone, cineole, fenchone, geraniol, linalool, myrcene and thujone), sesquiterpenoids (caryophyllene and farnesol), a triterpenoid (ursolic acid), and flavonoid (apigenin) present in them (Pirbalouti et al., 2013). Keeping the above view, present study was planned to study the anti-microbial activity of essential oils viz.,
clove oil and holybasil oil and their effect on sensory
attributes in fresh chicken sausage.

2. Materials and Methods

2.1 Raw Materials

Spent hens were procured from Central Avian
Research Institute (CARI), Izatnagar within 4 h of
slaughter. These carcasses were manually deboned in
experimental abattoir of LPT Division, IVRI, Izatnagar. Chicken meat thus obtained was packaged in
clean low density polyethylene bags and kept for
conditioning in a refrigerator at 4°C for about 24 h.
Food grade essential oils and cellulose casings were
purchased from commercial suppliers. Refined salt was
purchased from local market of Bareilly. Food
additives (sodium nitrite, sodium tripolyphosphate etc)
were of food grade quality and procured from reputed
firms.

2.2 Processing of Fresh Chicken Sausages

Chicken meat was cut into intact pieces
weighing 500g each for control and treatments.
Individual essential oils (clove oil and holybasil oil)
were applied on surface of thawed meat pieces in
treatment samples (sterile distilled water in case of
control samples) with the help of autoclaved cotton
swab and kept for half an hour in a desiccators. Then
the meat samples along with salt (1.6%), sodium nitrite
(150ppm) and sodium tripolyphosphate (0.4%)
dissolved in chilled water were processed and formed
into meat mix. The mix was then stuffed into casings
using manual sausage filler and linked at about certain
intervals.

In the present study, clove and holybasil
essential oils were used at four different levels viz.,
0.125%, 0.25%, 0.5% and 1% in fresh chicken
sausages to study their anti-microbial activity and their
effect on sensory attributes. The product was analyzed
for total plate count and sensory attributes and based on
the results; most acceptable products from both the
treatments were assessed for proximate composition
and compared with control.

2.3 Microbiological Analysis

Total plate count was determined by following
standard methods of APHA (2001). About ten grams of
sample was aseptically weighed and transferred to a
sterile plastic container containing 90 ml of sterile
0.1% peptone (Hi-Media®) water. The sample was
homogenized for 2 minutes using Ultra Turrax tissue
homogenizer (Model IKA®T 18, Janke and Kenkel,
IKA Labor Technik, Germany) under sterile condition.
Serial dilutions were made as per requirement by
transferring 1ml of this 10² dilution to sterile test tubes
containing 9 ml of sterile 0.1% peptone water and
mixed uniformly to get dilutions 10⁻², 10⁻³ and so on.
100 µl from each dilution was poured into sterile
petriplates and spread gently with the help of
autoclavable sterile spreader. The plates were
incubated at 35±2°C for 48 h. Plates showing 30-300
colonies were counted. The number of colonies was
multiplied by the reciprocal of the dilution and
expressed as log₁₀ cfu/g.

2.4 Sensory Analysis

Sensory scores were given by the experienced
panelists on the basis of 8-point hedonic scale where
8=extremely liked and 1= extremely disliked (Keeton,
1983), drawn from scientists and P.G students of LPT
division.

2.5 Proximate Composition

Moisture, crude fat, protein and ash of fresh
chicken sausages were determined by standard
procedures of Association of Official Analytical
Chemists (AOAC, 1995) by using hot air oven, Soxhlet
apparatus, Kjeldhal apparatus and Muffle furnace
respectively.

2.6 Statistical Analysis

The data generated from various trials under
experiment were pooled, processed and analyzed by
statistical method of one way-ANOVA and Mean±SE
using SPSS software package developed as per the
procedure of Snedecor and Cochran (1995) and means
were compared by using Duncan’s multiple range test
(Duncan, 1955).

3. Results and Discussion

3.1 Effect of Clove Oil on Microbial and
Sensory Attributes in Fresh Chicken
Sausages

Clove essential oil was incorporated at four
different levels viz., 0.125%, 0.25%, 0.5% and 1% in
fresh chicken sausages and it was found that there was
significant difference (P<0.05) in total plate count -
Table 1: Effect of different concentrations of clove oil on total plate count and sensory attributes in fresh chicken sausages

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Clove oil (0.125%)</th>
<th>Clove oil (0.25%)</th>
<th>Clove oil (0.5%)</th>
<th>Clove oil (1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Plate Count</td>
<td>3.21±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.85±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.50±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.16±0.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.69±0.10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Appearance</th>
<th>Flavour</th>
<th>Binding</th>
<th>Texture</th>
<th>Juiciness</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.31±0.03</td>
<td>7.10±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.34±0.03</td>
<td>7.30±0.04</td>
<td>7.28±0.04</td>
<td>7.16±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.29±0.03</td>
<td>6.98±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.32±0.04</td>
<td>7.31±0.04</td>
<td>7.27±0.04</td>
<td>7.05±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.32±0.03</td>
<td>6.87±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.34±0.03</td>
<td>7.32±0.04</td>
<td>7.27±0.05</td>
<td>6.91±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.31±0.04</td>
<td>6.69±0.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.31±0.04</td>
<td>7.32±0.04</td>
<td>7.25±0.05</td>
<td>6.69±0.08&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.29±0.04</td>
<td>6.38±0.07&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.32±0.04</td>
<td>7.29±0.06</td>
<td>7.27±0.07</td>
<td>6.42±0.08&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*Mean± S.E. with different superscripts in a row differ significantly (P<0.01); n=6 for each treatment and n=21 in case of sensory attributes</sup>

Table 2: Effect of different concentrations of holy basil oil on total plate count and sensory attributes in fresh chicken sausages

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Holybasil oil (0.125%)</th>
<th>Holybasil oil (0.25%)</th>
<th>Holybasil oil (0.5%)</th>
<th>Holybasil oil (1%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Plate Count</td>
<td>3.24±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.94±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.63±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.30±0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.85±0.07&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Appearance</th>
<th>Flavour</th>
<th>Binding</th>
<th>Texture</th>
<th>Juiciness</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.29±0.04</td>
<td>7.30±0.04</td>
<td>7.28±0.04</td>
<td>7.28±0.04</td>
<td>7.27±0.04</td>
<td>7.11±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.14±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.94±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.72±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.58±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.34±0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.95±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.31±0.03</td>
<td>7.29±0.04</td>
<td>7.31±0.04</td>
<td>7.32±0.05</td>
<td>7.32±0.04</td>
<td>6.78±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.32±0.05</td>
<td>7.30±0.03</td>
<td>7.30±0.03</td>
<td>7.31±0.03</td>
<td>7.32±0.04</td>
<td>6.59±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.29±0.05</td>
<td>7.27±0.04</td>
<td>7.29±0.06</td>
<td>7.27±0.07</td>
<td>7.28±0.08</td>
<td>6.33±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*Mean± S.E. with different superscripts in a row differ significantly (P<0.01); n=6 for each treatment and n=21 in case of sensory attributes</sup>

Table 3: Proximate composition of fresh sausages incorporated with optimized level of clove oil and holybasil oil.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Clove oil (0.25%)</th>
<th>Holybasil oil (0.125%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (%)</td>
<td>81.47±0.93</td>
<td>81.53±0.63</td>
<td>81.94±0.49</td>
</tr>
<tr>
<td>pH</td>
<td>6.16±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.11±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.14±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>72.61±1.17</td>
<td>70.85±0.74</td>
<td>71.18±0.57</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>2.97±0.22</td>
<td>2.55±0.16</td>
<td>2.77±0.17</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>18.95±0.73</td>
<td>19.23±0.94</td>
<td>19.35±0.79</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.69±0.16</td>
<td>2.98±0.15</td>
<td>2.83±0.21</td>
</tr>
</tbody>
</table>

<sup>*Mean± S.E. with different superscripts in a row differ significantly (P<0.01); n=6 for each treatment</sup>

Eugenol is a major component (approximately 85%) of clove oil (Farag et al., 1989) which is identified as the major chemical components responsible for exerting antimicrobial activity (Krishnan et al., 2014). Significant reduction (P<0.05) in total plate count was observed with increase in concentration of essential oil from 0.125% level to 1% level of clove oil. Sivropoulou et al. (1996) had reported that the antimicrobial effect of EOs is concentration dependent. With respect to the use of clove oil, present results are in agreement with those of (Zengin and Baysal, 2014) who had reported that clove oil treatment restricted the growth of artificially inoculated Salmonella typhimurium and native coliforms in the ground beef. Hoque et al. (2008) studied the effect of clove oil in...
chicken patties and reported that clove essential oil at 10% reduced all *Listeria monocytogenes* cells to an undetectable level in ground chicken meat within 1 day of exposure. Membrane disruption by phenolics and thus inhibiting the growth of microorganisms is generally considered as the mechanism of action for the antimicrobial activity of essential oil (Cowan, 1999). Some researchers have reported that phenolic compounds from different plant sources could inhibit various food-borne pathogens, and the total phenolic content have been highly correlated with antibacterial activity (Shan et al., 2009). Sensory scores for general appearance did not significantly differ (P>0.05) between control and treatment products which might be due to inhibition of met-myoglobin formation by eugenol present in clove (Djenane et al., 2003) thus, retarding the change in colour of sausage. Non-significant difference (P>0.05) was also observed for binding and texture scores in control and treatment products. Sausages with 0.125% and 0.25% incorporated clove oil had good sensory ratings, however flavour scores for treatment products were significantly lower (P<0.05) than control products. The decrease in flavour of products incorporated with essential oil might be due to pungent and condensed aroma given by volatile constituents present in it (Tiwari et al., 2009). Overall acceptability scores were comparable (P>0.05) for control and treatment products with 0.25% clove oil. The score of the flavor and general acceptability decreased with an increase in the rate of incorporation of clove essential oil in beef patties had also been reported by Siewe et al. (2015). The sensory panel gave the expected results: sausages with 0.125% clove essential oil was acceptable followed by satisfactory results for 0.25% level. Thus, only EO concentrations lower than or equal to 0.25% could be organoleptically acceptable, not exceeding of the flavour acceptability threshold observed by the panellists, so they could be used as preservatives/food additives.

3.2 Effect of Holybasil Oil on Microbial and Sensory Attributes in Fresh Chicken Sausages

Incorporation of holybasil oil at four different levels viz., 0.125%, 0.25%, 0.5% and 1% revealed the significant decrease (P<0.05) in total plate count of treatment products with increase in each level of essential oil. Strong anti-bacterial activity in holybasil had been reported by Maheswari et al. (2012). Methyl chavicol, methyl cinnamate, methyl eugenol, citral, and linalool are generally the main chemotypes in basil which are identified with anti-microbial properties (Chalchat and Ozcan, 2008; Carovic-Stanko et al., 2010). Dzudie et al. (2004) investigated the effect of addition of ginger EO and basilica EO at a 0.2% level on the quality and stability of beef patties and revealed significantly lower standard plate counts in treatments. Concentration dependent decrease in total plate count was noticed in holybasil incorporated products as well (Sivropoulou et al., 1996).

Sensory scores revealed that general appearance, binding and texture of product was not significantly (P>0.05) affected by the incorporation of oil in fresh chicken sausages. However, significant difference (P>0.05) was observed in flavour and overall acceptability scores of holybasil oil incorporated products. Liu et al. (2009) have reported that use of rosemary at higher concentration of 1500 ppm resulted higher off-odour scores than control in fresh chicken sausages. Overall acceptability scores of control and 0.125% holybasil oil incorporated product did not differ significantly (P>0.05), which indicated the satisfactory acceptance of product by sensory panellists at this level. However, above 0.125%, significantly lower (P<0.05) scores were obtained at each increasing level of essential oil and the product was unacceptable at the highest incorporation level. A marked odour of spices was discernible in the bolognas containing the EO oils (thyme and oregano) had been reported by Viuda-Martos et al. (2009). Thus, the fresh chicken sausages to which 0.125% of holybasil essential oil had been added were best appreciated by the panellists.

Therefore, on the basis of total plate count and sensory scores, optimum level of incorporation of clove and holy basil essential oil was adjudged as 0.25% and 0.125%. Further, comparison of proximate composition of control product with optimized level of clove and holybasil oil was carried out. It was found that all the parameters except pH were not significantly different (P>0.05) between control and treatment products. Similar results had been reported by Dzudie et al. (2002). pH was significantly higher in treatment products than control and our results were in agreement with Naveena et al. (2008) who had reported the significant reduction in pH of Butyl hydroxyl toluene (BHT) incorporated patties compared to the others.

4. Conclusion

Acceptable fresh chicken sausages can be prepared with incorporation of 0.25% clove essential oil and 0.125% holybasil essential oil. Fresh chicken sausages incorporated with these levels had better sensory scores than higher level of essential oils, however the anti-microbial effect of essential oils was found to be concentration dependent. Reduction in total plate count of treatment products was observed than control. Therefore, clove and holybasil oil can be successfully incorporated in meat products with much
efficient anti-microbial and anti-oxidative activities, although some new methodologies need to be devised

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