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# Sensory evaluation, textural parameters and microbial analysis of chocolate incorporated with spices 

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#### Abstract

The aim of the present work was to study the effects of incorporation of different spice powders viz., fenugreek, coriander, black cumin and cinnamon in chocolate production. The chocolate was formulated with spice powders at different inclusion levels of $1 \%, 1.5 \%$ and $2 \%$ and compared with control. The developed chocolates were investigated for their sensory characteristics, textural parameters, shelf-life and microbial analysis. The spice powders with $1.5 \%$ incorporation level in chocolate showed higher overall acceptability than others. The hardness values of developed chocolate ranged from 2120.14 to 2613.60 g . The results suggested reduced microbial count in spices incorporated chocolate, which could be attributed to the antimicrobial properties of spices.


Keywords: chocolate, cinnamon, coriander, texture, shelf-life

## 1. Introduction

Chocolate is one of the most desired confectionery product widely consumed by all generations (Liu et al., 2015) ${ }^{[5]}$. Chocolate can be described as an emulsion consisting of nonfat particles (cocoa solids, sugar, and milk powder) dispersed in cocoa butter as a continuous phase (Beckett, 2009) ${ }^{[3]}$ and are categorized into three main varieties based on the composition of cocoa mass namely dark, white and milk chocolate. Cocoa beans are the basic ingredient in chocolate manufacturing, contains a significant amount of fat ( $40-50 \%$ ) as cocoa butter and polyphenols, which constitute about $10 \%$ of a whole bean's dry weight (Rusconi and Conti, 2010) ${ }^{[7]}$. Chocolate is rich in fat, protein, carbohydrates, polyphenols, and other bioactive compounds (Arunkumar and Jegadeeswari, 2019) ${ }^{[2]}$. The present study was undertaken to develop chocolate by incorporating different spices in the form of powders and to evaluate their sensory characteristics, textural parameters, shelf-life and microbial analysis.

## 2. Materials and Methods

The cocoa mass and cocoa butter were purchased from Morde Foods Private Ltd., Pune. Lecithin was purchased from Venus Essence Pvt Ltd., Chennai. Spices viz., coriander, fenugreek, black cumin, cinnamon and sugar were purchased from Sri MRV supermarket, Redhills, Chennai. The present study was carried out in the College of Food and Dairy Technology, Alamathi, a constituent college of Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600052.

### 2.1 Optimization of chocolate incorporated with spices

Chocolate was prepared from cocoa mass incorporated with selected spices viz., coriander, fenugreek, black cumin, and cinnamon. The cocoa mass ( 45 g ), cocoa butter ( 14.5 g ), Sugar $(40 \mathrm{~g})$ and lecithin $(0.5 \mathrm{~g})$ were weighed. Then, the grounded spice powders in different equal proportions i.e., $1 \%, 1.5 \%$ and $2 \%$ were added. Unit operations such as grinding, mixing, refining, conching, tempering and cooling were carried out for the production of chocolate incorporated with spices. The chocolates were then packed and stored at $5^{\circ} \mathrm{C}$.

### 2.2 Sensory evaluation

Sensory evaluation of spice powders incorporated chocolate was carried out using the 9-point hedonic scale (Amerine et al., 2013) ${ }^{[1]}$ by a panel of 20 judges comprising of Post Graduate students and faculty members of College of Food and Dairy Technology, Alamathi, Chennai 600052.

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### 2.3 Texture profile analysis

The textural characteristics of chocolate samples were evaluated instrumentally using TA-XT Plus texture analyzer (Stable Micro System Ltd, Surrey, UK) fitted with a 20 mm diameter cylinder probe, set up to record the force used to penetrate the sample $75 \%$ of their original depth.

### 2.4 Shelf-life studies

The spice powders incorporated chocolate was stored at $5^{\circ} \mathrm{C}$ for six months. The control and samples were analyzed for shelf-life studies based on storage at different time intervals of $0,30,60,90,120,150$, and 180 days by sensory evaluation (Larmond, 1977) ${ }^{[4]}$.

### 2.5 Microbial analysis

The microbial analysis viz., standard plate count, coliform count, yeast and mold count were carried out as per the standard procedure described in BIS: 1981, SP: 18 (Part XI).

### 2.6 Statistical analysis

The results were expressed as Mean $\pm$ SE. The statistical analysis was performed by ANOVA using SPSS $^{\circledR} 20.0$ software for windows to determine the significant differences (Snedecor and Cochran, 2004) ${ }^{[8]}$.

## 3. Results and Discussion

3.1 Sensory analysis of spices incorporated chocolate

The spice powders incorporated chocolate (SPIC) was served to panelists for sensory evaluation. The mean sensory scores
for colour and appearance, texture, flavour, melting quality, taste and overall acceptability of the control and spices incorporated chocolate are shown in Figure 1. The sensory scores of colour and appearance for control, SPIC1, SPIC2 and SPIC3 were found to be $8.05 \pm 0.135,7.50 \pm 0.246$, $7.30 \pm 0.219$ and $7.20 \pm 0.200$ respectively. The texture score for control, SPIC1, SPIC2 and SPIC3 were found to be $8.15 \pm 0.150, \quad 7.05 \pm 0.276, \quad 7.35 \pm 0.244$ and $6.90 \pm 0.270$ respectively. The sensory scores of flavour for control, SPIC1, SPIC2 and SPIC3 were found to be $8.00 \pm 0.162$, $7.00 \pm 0.145,7.25 \pm 0.176$ and $6.85 \pm 0.209$ respectively. The melting quality scores for control, SPIC1, SPIC2 and SPIC3 were found to be $8.10 \pm 0.143,7.35 \pm 0.233,7.15 \pm 0.196$ and $6.80 \pm 0.186$ respectively. The sensory scores of taste for control, SPIC1, SPIC2 and SPIC3 were found to be $8.20 \pm 0.138,7.05 \pm 0.211, \quad 7.10 \pm 0.280$ and $6.75 \pm 0.190$ respectively. The scores of overall acceptability for control, SPIC1, SPIC2 and SPIC3 were found to be $8.30 \pm 0.128$, $7.10 \pm 0.228,7.90 \pm 0.176$ and $6.65 \pm 0.254$ respectively. The statistical analysis revealed that there was a highly significant ( $\mathrm{P}<0.01$ ) difference between the control, SPIC1, SPIC2 and SPIC3 concerning colour and appearance, texture, flavour, melting quality, taste and overall acceptability scores. The addition of spice powders into chocolate ( $1 \%, 1.5 \%$ and $2 \%$ ) significantly influenced the panelist acceptability than the control. The chemical compounds present in spices might produce the specific flavour and colour to the final product and the results are in concordance with Mulato and Suharyanto, (2014) ${ }^{[6]}$.


Fig 1: Sensory evaluation of spice powders incorporated chocolate

### 3.2 Texture profile analysis of spices incorporated chocolate

The textural parameters viz., hardness, adhesiveness, springiness, cohesiveness, gumminess and resilience of the control and spices incorporated chocolate (SPIC) were presented in Table 1. The hardness values for control, SPIC1, SPIC2 and SPIC3 were found to be $2120.14 \pm 5.130$, $2218.42 \pm 4.794, \quad 2411.53 \pm 2.469$ and $2613.60 \pm 3.786 \mathrm{~g}$ respectively. The cohesiveness values for control, SPIC1,

SPIC2 and SPIC3 were found to be $0.614 \pm 0.005$, $0.542 \pm 0.013,0.478 \pm 0.015$ and $0.415 \pm 0.006$ respectively. The gumminess values for control, SPIC1, SPIC2 and SPIC3 were found to be $1314.04 \pm 1.407,1233.96 \pm 2.074,1151.10 \pm 3.496$ and $1090.88 \pm 2.101 \mathrm{~g}$ respectively. The statistical analysis revealed that there was a highly significant ( $\mathrm{P}<0.01$ ) difference between the control, SPIC1, SPIC2 and SPIC3 concerning hardness, adhesiveness, gumminess and chewiness properties.

Table 1: Texture profile analysis of spice powders incorporated chocolate

| Samples | Texture profile analysis |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Hardness (g) | Adhesiveness (g s) | Springiness (mm) | Cohesiveness | Gumminess (g) | Resilience |
| Control | $2120.14 \pm 5.130^{\mathrm{a}}$ | $-1013.19 \pm 1.967^{\mathrm{a}}$ | $0.892 \pm 0.018^{\mathrm{d}}$ | $0.614 \pm 0.005^{\mathrm{d}}$ | $1314.04 \pm 1.407^{\mathrm{d}}$ | $0.267 \pm 0.015^{\mathrm{c}}$ |
| SPIC1 | $2218.42 \pm 4.794^{\mathrm{b}}$ | $-818.70 \pm 2.305^{\mathrm{b}}$ | $0.763 \pm 0.010^{\mathrm{c}}$ | $0.542 \pm 0.013^{\mathrm{c}}$ | $1233.96 \pm 2.074^{\mathrm{c}}$ | $0.240 \pm 0.013^{\mathrm{bc}}$ |
| SPIC2 | $2411.53 \pm 2.469^{\mathrm{c}}$ | $-741.88 \pm 7.013^{\mathrm{c}}$ | $0.650 \pm 0.012^{\mathrm{b}}$ | $0.478 \pm 0.015^{\mathrm{b}}$ | $1151.10 \pm 3.496^{\mathrm{b}}$ | $0.194 \pm 0.006^{\mathrm{ab}}$ |
| SPIC3 | $2613.60 \pm 3.786^{\mathrm{d}}$ | $-638.62 \pm 7.335^{\mathrm{d}}$ | $0.564 \pm 0.016^{\mathrm{a}}$ | $0.415 \pm 0.006^{\mathrm{a}}$ | $1090.88 \pm 2.101^{\mathrm{a}}$ | $0.160 \pm 0.014^{\mathrm{a}}$ |
| F-value | $2736.054^{* *}$ | $893.62^{* *}$ | $98.125^{* *}$ | $64.384^{* *}$ | $1654.169^{* *}$ | $13.949^{* *}$ |

Data are expressed as Mean $\pm$ SE; $\mathrm{n}=6$; ** - Highly significant difference ( $\mathrm{P} \leq 0.01$ )
Different superscripts in the same column indicate that treatments significantly differ.

### 3.3 Shelf-life evaluation of spices incorporated chocolate

The shelf-life studies carried out by sensory evaluation during storage intervals of $0,30,60,90,120,150$ and 180 days at 5 ${ }^{\circ} \mathrm{C}$ for the control and spice powders incorporated chocolate (SPIC) were presented in Table 2. The colour and appearance scores during storage intervals of $0,30,60,90,120,150$ and 180 days for control, SPIC1, SPIC2 and SPIC3 were ranged from $8.37 \pm 0.024$ to $8.70 \pm 0.010,8.28 \pm 0.017$ to $8.41 \pm 0.013$, $8.32 \pm 0.014$ to $8.56 \pm 0.027$ and $7.68 \pm 0.022$ to $8.13 \pm 0.008$ respectively. The texture scores during storage intervals of 0 , $30,60,90,120,150$ and 180 days for control, SPIC1, SPIC2 and SPIC3 were ranged from $8.31 \pm 0.010$ to $8.66 \pm 0.009$, $8.32 \pm 0.020$ to $8.52 \pm 0.015,8.33 \pm 0.010$ to $8.56 \pm 0.011$ and $6.98 \pm 0.254$ to $8.12 \pm 0.027$ respectively. The flavour scores during storage intervals of $0,30,60,90,120,150$ and 180 days for control, SPIC1, SPIC2 and SPIC3 were ranged from $8.34 \pm 0.013$ to $8.63 \pm 0.025,8.44 \pm 0.027$ to $8.66 \pm 0.015$,
$8.11 \pm 0.009$ to $8.31 \pm 0.009$ and $7.01 \pm 0.234$ to $8.05 \pm 0.012$ respectively. The melting quality scores during storage intervals of $0,30,60,90,120,150$ and 180 days for control, SPIC1, SPIC2 and SPIC3 were ranged from $8.27 \pm 0.006$ to $8.43 \pm 0.025,8.29 \pm 0.052$ to $8.54 \pm 0.009,8.07 \pm 0.064$ to $8.39 \pm 0.016$ and $7.30 \pm 0.224$ to $8.04 \pm 0.027$ respectively. The overall acceptability scores during storage intervals of 0,30 , 60, 90, 120, 150 and 180 days for control, SPIC1, SPIC2 and SPIC3 were ranged from $8.35 \pm 0.009$ to $8.63 \pm 0.014$, $8.35 \pm 0.016$ to $8.55 \pm 0.011,8.27 \pm 0.007$ to $8.44 \pm 0.008$ and $7.61 \pm 0.010$ to $8.06 \pm 0.019$ respectively. The developed chocolate stored well at $5{ }^{\circ} \mathrm{C}$ for 180 days. The statistical analysis revealed that there was a highly significant ( $\mathrm{P}<0.01$ ) difference between the control, SPIC1, SPIC2 and SPIC3 concerning sensory characteristics during storage of 0 to 180 days.

Table 2: Effect of storage period on sensory evaluation of spice powders incorporated chocolate at $5^{\circ} \mathrm{C}$

| Attributes | Chocolate variants | Storage days |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 0day | $30^{\text {th }}$ day | $60^{\text {th }}$ day | 90 ${ }^{\text {th }}$ day | 120 ${ }^{\text {th }}$ day | $150{ }^{\text {th }}$ day | 180 ${ }^{\text {th }}$ day | F-value |
| Colour and Appearance | Control | $8.70 \pm 0.010^{\text {dE }}$ | $8.62 \pm 0.008^{\text {dD }}$ | $8.60 \pm 0.011^{\text {dCD }}$ | $8.55 \pm 0.030^{\text {cC }}$ | $8.48 \pm 0.015^{\text {bB }}$ | $8.43 \pm 0.016^{\text {cB }}$ | $8.37 \pm 0.024^{\text {cA }}$ | 41.475** |
|  | SPIC1 | $8.41 \pm 0.013^{\text {bE }} 8$ | $8.39 \pm 0.011^{\text {bDE }}$ | $8.37 \pm 0.012^{\text {bCDE }}$ | $8.36 \pm 0.020^{\text {bCD }}$ | $8.35 \pm 0.010^{\text {bBC }}$ | $8.32 \pm 0.014^{\text {bAB }}$ | $8.28 \pm 0.017^{\mathrm{bA}}$ | 9.680** |
|  | SPIC2 | $8.56 \pm 0.027^{\text {cD }} 8.5$ | $8.52 \pm 0.019^{\text {cCD }}$ | $8.50 \pm 0.011^{\text {cBC }}$ | $8.49 \pm 0.009^{\text {cBC }}$ | $8.48 \pm 0.021^{\text {bBC }}$ | $8.45 \pm 0.013^{\text {cB }}$ | $8.32 \pm 0.014^{\text {bcA }}$ | 19.642** |
|  | SPIC3 | $8.13 \pm 0.008^{\text {ab }}$ | $8.11 \pm 0.011^{\mathrm{aCD}}$ | $8.09 \pm 0.012^{\mathrm{aCD}}$ | $8.03 \pm 0.021^{\text {aC }}$ | $7.94 \pm 0.065^{\text {ab }}$ | $7.87 \pm 0.025^{\mathrm{aB}}$ | $7.68 \pm 0.022^{\mathrm{aA}}$ | 9.968** |
|  | F-value | 228.304** | 290.799** | 351.053** | 118.471** | 51.384** | 240.027** | 267.858** | - |
| Texture | Control | $8.66 \pm 0.009^{\text {cF }}$ | $8.61 \pm 0.014^{\text {dE }}$ | $8.57 \pm 0.011^{\text {cD }}$ | $8.52 \pm 0.013^{\text {cC }}$ | $8.50 \pm 0.014^{\text {cC }}$ | $8.38 \pm 0.024^{\text {bB }}$ | $8.31 \pm 0.010^{\text {bA }}$ | 76.671** |
|  | SPIC1 | $8.52 \pm 0.015^{\text {bF }}$ | $8.49 \pm 0.010^{\mathrm{bEF}}$ | $8.47 \pm 0.012^{\mathrm{bDE}}$ | $8.44 \pm 0.024^{\text {bCD }}$ | $8.40 \pm 0.016^{\mathrm{bBC}}$ | $8.36 \pm 0.012^{\text {bAB }}$ | $8.32 \pm 0.020^{\mathrm{bA}}$ | 21.369** |
|  | SPIC2 | $8.56 \pm 0.011^{\text {bE }}$ | $8.55 \pm 0.012^{\mathrm{cE}}$ | $8.52 \pm 0.015^{\text {bD }}$ | $8.49 \pm 0.014^{\text {bcCD }}$ | $8.46 \pm 0.012^{\text {bcC }}$ | $8.40 \pm 0.009^{\text {bB }}$ | $8.33 \pm 0.010^{\text {bA }}$ | 48.269** |
|  | SPIC3 | $8.12 \pm 0.027^{\text {aC }}$ | $8.07 \pm 0.013^{\mathrm{aC}}$ | $8.01 \pm 0.011^{\text {aC }}$ | $7.87 \pm 0.025^{\text {abC }}$ | $7.71 \pm 0.031^{\text {aB }}$ | $7.60 \pm 0.014^{\mathrm{aB}}$ | $6.98 \pm 0.254^{\mathrm{aA}}$ | 16.308** |
|  | F-value | 193.959** | 400.707** | 439.753** | 245.542** | 355.404** | 598.965** | 27.570** | - |
| Flavour | Control | $8.63 \pm 0.025^{\text {cD }}$ | $8.59 \pm 0.015^{\text {cD }}$ | $8.54 \pm 0.008^{\text {cC }}$ | $8.50 \pm 0.013^{\text {cC }}$ | $8.44 \pm 0.022^{\text {cB }}$ | $8.41 \pm 0.012^{\text {cB }}$ | $8.34 \pm 0.013^{\text {bA }}$ | 39.058** |
|  | SPIC1 | $8.66 \pm 0.015^{\text {cD }}$ | $8.64 \pm 0.006^{\text {dD }}$ | $8.61 \pm 0.011^{\text {dCD }}$ | $8.58 \pm 0.010^{\text {dBC }}$ | $8.53 \pm 0.021^{\text {dB }}$ | $8.46 \pm 0.018^{\mathrm{cA}}$ | $8.44 \pm 0.027^{\mathrm{bA}}$ | 25.224** |
|  | SPIC2 | $8.31 \pm 0.009^{\text {bE }}$ | $8.29 \pm 0.011^{\text {bDE }}$ | $8.28 \pm 0.008^{\text {bDE }}$ | $8.26 \pm 0.018^{\text {bCD }}$ | $8.23 \pm 0.010^{\text {bC }}$ | $8.19 \pm 0.021^{\text {bB }}$ | $8.11 \pm 0.009^{\text {bA }}$ | 29.293** |
|  | SPIC3 | $8.05 \pm 0.012^{\text {ab }}$ | $8.01 \pm 0.009^{\text {aD }}$ | $7.87 \pm 0.024^{\mathrm{aCD}}$ | $7.73 \pm 0.021^{\text {aC }}$ | $7.42 \pm 0.011^{\mathrm{aB}}$ | $7.33 \pm 0.062^{\mathrm{aB}}$ | $7.01 \pm 0.234^{\mathrm{as}}$ | 17.729** |
|  | F-value | 302.068** | 712.084** | 544.372** | 576.190** | 908.506** | 233.848** | 31.056** | - |
| Melting quality | Control | $8.43 \pm 0.025^{\text {bE }}$ | $8.40 \pm 0.022^{\text {bD }}$ | $8.36 \pm 0.017^{\text {bCD }}$ | $8.33 \pm 0.011^{\text {bBC }}$ | $8.32 \pm 0.014^{\text {bAB }}$ | $8.29 \pm 0.019^{\text {cAB }}$ | $8.27 \pm 0.006{ }^{\text {bA }}$ | 11.210** |
|  | SPIC1 | $8.54 \pm 0.009^{\text {cD }}$ | $8.52 \pm 0.014^{\text {cD }}$ | $8.49 \pm 0.010^{\text {cCD }}$ | $8.45 \pm 0.044^{\mathrm{cBCD}}$ | $8.41 \pm 0.028^{\text {cBC }}$ | $8.39 \pm 0.015^{\text {cB }}$ | $8.29 \pm 0.052^{\text {bA }}$ | 8.900** |
|  | SPIC2 | $8.39 \pm 0.016^{\mathrm{bC}}$ | $8.35 \pm 0.010^{\text {bBC }}$ | $8.32 \pm 0.015^{\mathrm{bBC}}$ | $8.27 \pm 0.011^{\text {bB }}$ | $8.25 \pm 0.012^{\text {bB }}$ | $8.12 \pm 0.071^{\text {bA }}$ | $8.07 \pm 0.064^{\text {bA }}$ | 9.955** |
|  | SPIC3 | $8.04 \pm 0.027^{\mathrm{aB}}$ | $7.99 \pm 0.013^{\text {aB }}$ | $7.90 \pm 0.017^{\text {aB }}$ | $7.89 \pm 0.014^{\text {ab }}$ | $7.85 \pm 0.015^{\text {ab }}$ | $7.79 \pm 0.012^{\mathrm{aB}}$ | $7.30 \pm 0.224^{\mathrm{aA}}$ | 8.268** |
|  | F-value | 110.153** | 215.212** | 289.299** | 95.597** | 177.936** | 48.627** | 15.401** | - |
| Taste | Control | $8.70 \pm 0.018^{\text {cD }}$ | $8.68 \pm 0.014^{\text {cD }}$ | $8.63 \pm 0.010^{\text {cC }}$ | $8.60 \pm 0.020^{\text {cC }}$ | $8.59 \pm 0.013^{\text {cBC }}$ | $8.56 \pm 0.011^{\text {caB }}$ | $8.52 \pm 0.009^{\text {bA }}$ | 20.408** |
|  | SPIC1 | $8.53 \pm 0.024^{\text {bF }}$ | $8.49 \pm 0.012^{\mathrm{bEF}}$ | $8.46 \pm 0.027^{\text {bDE }}$ | $8.43 \pm 0.007^{\text {bCD }}$ | $8.40 \pm 0.014^{\mathrm{bBC}}$ | $8.38 \pm 0.013^{\text {bB }}$ | $8.31 \pm 0.016^{\mathrm{bA}}$ | 16.865** |
|  | SPIC2 | $8.49 \pm 0.025^{\mathrm{bD}} 8$. | $8.46 \pm 0.022^{\text {bCD }}$ | $8.43 \pm 0.010^{\text {bCD }}$ | $8.41 \pm 0.009^{\text {bBCD }}$ | $8.39 \pm 0.012^{\text {bBC }}$ | $8.34 \pm 0.014^{\text {bB }}$ | $8.26 \pm 0.059^{\text {bA }}$ | 8.513** |
|  | SPIC3 | $7.88 \pm 0.020^{\mathrm{aB}}$ | $7.81 \pm 0.012^{\text {aB }}$ | $7.74 \pm 0.014^{\text {aB }}$ | $7.67 \pm 0.027^{\text {aB }}$ | $7.63 \pm 0.011^{\mathrm{aB}}$ | $7.58 \pm 0.021^{\text {cB }}$ | $7.18 \pm 0.268^{\mathrm{aA}}$ | 5.069** |
|  | F-value | 268.404** | 590.519** | 551.615** | 556.110** | 1181.129** | 811.867** | 19.642** | - |
| Overall acceptability | Control | $8.63 \pm 0.014^{\text {dG }}$ | $8.58 \pm 0.008^{\text {dF }}$ | $8.54 \pm 0.007^{\text {dE }}$ | $8.50 \pm 0.003^{\text {dD }}$ | $8.46 \pm 0.010^{\text {cC }}$ | $8.41 \pm 0.004^{\text {cB }}$ | $8.35 \pm 0.009^{\text {cA }}$ | 125.997** |
|  | SPIC1 | $8.55 \pm 0.011^{\mathrm{cF}}$ | $8.51 \pm 0.006^{\text {cE }}$ | $8.47 \pm 0.008^{\text {cD }}$ | $8.46 \pm 0.010^{\text {cD }}$ | $8.43 \pm 0.012^{\text {cC }}$ | $8.38 \pm 0.007^{\text {cB }}$ | $8.35 \pm 0.016^{\text {cA }}$ | 43.718** |
|  | SPIC2 | $8.44 \pm 0.008^{\mathrm{bG}}$ | $8.41 \pm 0.007^{\mathrm{bF}}$ | $8.39 \pm 0.006^{\text {bE }}$ | $8.36 \pm 0.004^{\text {bD }}$ | $8.34 \pm 0.002^{\text {bC }}$ | $8.30 \pm 0.009^{\text {bB }}$ | $8.27 \pm 0.007^{\mathrm{bA}}$ | 79.030** |
|  | SPIC3 | $8.06 \pm 0.019^{\text {aF }}$ | $8.00 \pm 0.009^{\mathrm{aE}}$ | $7.94 \pm 0.004^{\text {aD }}$ | $7.92 \pm 0.007^{\text {aD }}$ | $7.86 \pm 0.012^{\text {a }}$ | $7.75 \pm 0.021^{\mathrm{aB}}$ | $7.61 \pm 0.010^{\mathrm{aA}}$ | 139.281** |
|  | F-value | 348.709** | 1143.355** | 1656.389** | 1783.295** | 817.334** | 646.591** | 1025.399** | - |

Data are expressed as Mean $\pm$ SE; $\mathrm{n}=6$; ** - Highly significant difference ( $\mathrm{P} \leq 0.01$ );
Different superscripts in the same column (lowercase) and row (uppercase) indicate that treatments significantly differ.

### 3.4 Microbial analysis of spices incorporated chocolate

The microbial studies by standard plate count, coliform count, yeast and mold count during storage intervals of 0 to 180 days for the control and spice powders incorporated chocolate (SPIC) were shown in Figure 2. The standard plate count during storage intervals of $0,30,60,90,120,150$ and 180 days for the control chocolate was found to be $1.85 \pm 0.016$, $1.88 \pm 0.010,1.91 \pm 0.012,1.93 \pm 0.013,1.94 \pm 0.015,1.95 \pm 0.014$, and $2.02 \pm 0.035$ respectively. The standard plate count during storage intervals of $0,30,60,90,120,150$ and 180 days for SPIC1 was found to be $1.44 \pm 0.010,1.41 \pm 0.011,1.39 \pm 0.006$, $1.38 \pm 0.017,1.36 \pm 0.014,1.34 \pm 0.008$ and $1.29 \pm 0.017$ respectively. The standard plate count during storage intervals of $0,30,60,90,120,150$ and 180 days for SPIC2 was found to be $1.28 \pm 0.005,1.26 \pm 0.012,1.25 \pm 0.013,1.22 \pm 0.011$,
$1.23 \pm 0.010,1.21 \pm 0.009$ and $1.19 \pm 0.019$ respectively. The standard plate count during storage intervals of $0,30,60,90$, 120,150 and 180 days for SPIC3 was found to be $1.22 \pm 0.015$, $1.20 \pm 0.013,1.17 \pm 0.012,1.15 \pm 0.008,1.14 \pm 0.013,1.12 \pm 0.011$ and $1.10 \pm 0.010$ respectively. Statistical analysis revealed that the microbial count in spices incorporated chocolate was reduced, which was due to the antimicrobial properties of spices. The antimicrobial properties of fenugreek, coriander, cinnamon and black cumin might be due to the presence of trigonelline, dodecanol, eugenol and thymoquinone respectively. The coliform and mold count were not found in both the control and spices incorporated chocolates during different storage intervals of 0 to 180 days at $5^{\circ} \mathrm{C}$, which indicates that the hygienic practices were followed during production and storage.


Fig 2: Microbial analysis of spice powders incorporated chocolate

## 4. Conclusion

The chocolate was prepared by incorporating spice powders at varying levels of substitution $(1 \%, 1.5 \%$ and $2 \%$ ) and studied for their sensory attributes, textural parameters, shelf-life and microbial analysis. Based on sensory evaluation, the increased incorporation level of spice powders was the major factor favourably affecting the flavor and taste of the chocolate. The study revealed that the incorporation of spice powders at $2 \%$ significantly enhanced the antimicrobial properties due to the presence of antimicrobial compounds present in the spices and chocolate prepared with incorporation of spices might become a new choice to the consumers.

## 5. Acknowledgements

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## 6. Conflict of interest

The authors declare no conflict of interest pertaining to this manuscript.

## 7. References

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