Development of Antioxidant Rich Functional Dairy Product and Study their Total Antioxidant Status Measured by DPPH Assay

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Present investigation has featured the capability of functional dairy product fortified with essential oil has bearing antiradical properties that decolorize the stable DPPH radical. The antioxidant activity of *Syzygium aromaticum* (clove buds) and *Cinnamomum verum* (cinnamon) has found to possess inhibitory effect against reactive oxygen species (ROS) due to their high oxygen radical absorption capacity (ORAC) value. The target of the investigation is to set up the useful dairy item "coagulated cream", with the fortress of basic oils from *S. aromaticum* and *C. Verum* and blend of both the oils. In addition, determination of the total antioxidant activity of the product by DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay in three different types of clotted cream sample prepared along with varying concentrations of *S. aromaticum* and *C. Verum* and combination of both the oils. Additionally, to compare the proximate quality and overall acceptability of the final product were analysed antioxidant analysis of the product reveals that the maximum DPPH antioxidant activity percentage was observed in combination of both essential oils from the *S. aromaticum* and *C. Verum* before treatment at 0.25% focus for example 70.9% while the IC50 esteem is 0.029ml at 0.75% focus shows a powerful antioxidant product in correlation with every single other samples.

**Keywords:** *Syzygium aromaticum, Cinnamomum verum, Antioxidant, functional dairy product, free radicals scavenging, clotted cream, CLO (clove oil)*

**INTRODUCTION**

In Japan, the term functional food was first coined in 1984. Functional foods are defined that provide positive physiological effects with the nutrients. Food and food products can only be considered functional if together with the basic nutritional impact it has beneficial effects on the mankind thus improving the general and physical conditions and decreasing the risk of development of diseases (Danik and Emma, 2018). Functional food contains the proper balance of ingredients which aid us to function better and effectively in many aspects of our lives, as well as help us directly in the prevention and treatment of illness and disease. The benefits of a particular food component for the human health that people should struggle to consume a wide variety of food such as to assure the ingestion of compound such as carotenoids, flavonoids, fibers, specific fatty acids, minerals, phytoestrogens, prebiotics and probiotics, soy proteins and vitamins, among others, in order to reduce the risk of disease. Pakistan is the one of the top milk producers according to FAO 2010 in 2007-2008 approximately 42.17 million tons of milk was produced. Majorly about 90-95% of milk is produced in rural and check -urban regions by three or four milking animals. Major milk producing animals are buffalos and cows with 62% and 34% respectively.

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Province of Punjab and Sindh are leading milk producers with the production of 25.62 million and 9.35 million liters respectively. Khyber Pakhtoon Khwah produces 4.88 million liters per annum while Baluchistan estimated produces 0.81 million liters (PDDC, 2006). Clotted cream can be a value-added product and produce with the greatest demand in the milk producing districts. Despite the fact that the product is majorly consumed in European countries, but it would really contribute in the country’s exports. Furthermore, it would be an industry returning an adequate profit to the farmer or the man who undertakes to pioneer and substantiate the production. It is quite feasible to prepare the cream on a small scale particularly on farms, cottage industry or pilot plants. The global market of functional food promises to have a bright future according to its compound annual growth rate of 6% between the periods 2011 to 2015, there is no universally accepted definition a functional food but according to the international food information Council functional food or components of food better provide benefit beyond basic nutrition. Popular types of product worldwide include vitamins and daily supplements, functional beverages such as Sports and energy drinks as well as products fortified with prebiotics.

Clotted cream, also called scalded, clouted Devonshire or Cornish. It is rich, thick and yellow in color it has cooked or scalded flavour, created by heating milk which has not been pasteurized up to the point when a thick creamy layer appears on its top. Cream contents rises to the surface during this time and form clots. The milk gets cooled afterwards and the top layer gets removed. Clotted cream has grainy or soft crusted surface, with sometimes oily globules appearance on top. It has a rich cooked milk or nutty flavour. Although its origin is exciting, the production of cream is commonly associated with Dairy farm in south west England and in particular the counties of Cornwall and Devon the current largest commercial producer in the UK is Roddas at Scorrier, Redruth, Cornwall, which can produce about 25 tons (25000 kg, 55,000 lb) clotted cream a day in 1998. Since it has higher fat content so the consumption of clotted cream on regular basis is thought to be not suitable for health. Although some researchers have proved the dairy fat is beneficial in moderate amount. Syzygium aromaticum commonly called as clove belongs to the family Myrtaceae. Clove are also known as the ultimate protective herb and have been used in essential oil blends such as on Guards and Thieves. Moreira, et al.,2004 and Ali et al., (2005), reported that the essential oils from clove possess antimicrobial and anti fungal activities. Ground cloves has the astounding ORAC value of 290, 283 units (Kaefer et al., 2008). The strong antifungal activity of clove oil is due to its high phenolic content which vaporizers easily (Plaza, et al., 2004). Some experiments proved that phenols exhibited stronger antifungal activity compared to other components in essential oil such as alcohol, aldehydes, ketones and esters. Eugenol is the main component which is present in S. aromaticum possessed antifungal properties was reported by (Can et al., 2010) Antioxidant activity of clove and clove oil are higher than the standard butylatedhydroxytoluene (BHT). Gallic acid and eugenol (Kramer,1985) and eugenyl acetate has been identified from the S. aromaticum and exhibited a antioxidant activity. Eugenyl acetate and eugenol possessed similar antioxidant activity with natural antioxidant, alphatocopherol (Vitamin-E).

It is a mild antioxidant when compared with other Essential oil .It has an ORAC value of 10, 340 micro TE/100 g Cinnamon essential oils ability to fight cancer (Kaul et al., 2003) several studies have reported that the cinnamaldehyde from Cinnamomum verum ability to inhibited the tumor cell proliferation Via Trigger cancer cell apoptosis (programmed cell death). The widespread appreciation for healing properties of cinnamon is due to its medicinal applications. Extracts of cinnamon oil contains a variety of antimicrobial properties. Studies showed that the bark extract of Cinnamomum verum were active against 27 strains of Vibrio cholera and also against Shigella (Islam et al., 1990). Cinnamon bark oil has fungicidal and insecticidal properties and also prevents aflatoxin production.

Clove oil is the most imperative essential oils used for flavouring every kind of food product, such as sauces, confectioneries, baked goods, etc. Perfumery and pharmacy also use clove oil. Particularly clove bud oil used in pharmaceuticals and perfumery. Also, pickles confectionery bakery products and sauces. The natural antioxidants contained in the plant play a vital role in the treatment of numerous diseases, including diabetes, atherosclerosis, heart disease and cancer. Secondary metabolites of plants are essential oils obtained from flowers, leaves, roots, bark seeds or pericarp. The main constituent of essential oils is terpenes/Terpenoids aromatic and aliphatic compounds which are characterize as a low molecular weight aroma chemical. Generally, essential oils are comprised of two or three main component in relatively high concentration (20 to 95%) and other component present in trace levels that they may be extracted by steam distillation or hydro distillation (Majda et al., 2019). At ambient temperature afforded an essential oil yield of 1.13 %. Upon infrared drying at 45 °C of Essential oil was lowered by 31%, while convective drying at 45 °C or 65 °C led to losses of up to 83% as compared to the essential oil amount that is extracted from the raw material and dried at ambient temperature. Following convection drying, essential oil exhibited a loss of monoterpenic hydrocarbons, which was probably due to chemical rearrangement of compounds or their volatility with steam. The drying process reduced the content of monoterpenic esters about 13% and increase the amount of monoterpenic alcohols about 4% that this is probably due to the activation of enzyme belonging to the group of hydrolysis and hence the change in the quantitative
compostion of essential oils. Microwave vacuum drying lead to Products with a favourable color and an aroma better than that obtained by conventional methods. The drying method affects not only the amount of essential oil, but also often their chemical composition (Nowacka et al., 2012).

Clotted cream is typically used for flavouring and garnishing in our food. Fortification of clotted cream with clove and cinnamon essential oil increase the antioxidant level that will good for cancer patient because eugenol in clove can protect the liver against disease. It was also observed that eugenol reserves cellular oxidation which causes aging and also inflammation. The rationale behind the present study is to prepare a functional dairy product, determining antioxidant levels determined by DPPH assay and comparison of proximate quality and overall acceptance. DPPH is stable organic nitrogen radical. The method is simple yet sensitive for determining the antioxidant compounds in natural products (Ilecholubo et al., 2017; Nayan et al., 2013). Hydrogen donor is an antioxidant in the DPPH assay. Radical scavenger compounds are measured by DPPH. 1,1-Diphenyl-2-picrylhydrazyl converts its color from deep blue-violet to yellow when scavenged. The scavenging activity of radicals between DPPH and an antioxidant (H-A) can be written as:

\[ \text{Scavenging activity} = \frac{\text{A} - \text{F}}{\text{A}} \times 100 \]

MATERIALS AND METHODS

Commercially prepared cream, essential oils of cinnamon and clove, sucrose, were purchased from the local super market of Karachi. Distilled water, methanol, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Activated Silica gel Formalin solution 35%, Methanol, Sodium hydroxide, sulphuric acid. All chemicals and reagents used were of analytical grade while food grade oils were used to conduct the study.

Preparation of clotted cream

The present study was undertaken in the period from July 2017 to January 2018 in the Department of Food Science & Technology, Jinnah University for women, Karachi, Pakistan. Commercially prepared cream was used for the preparation of clotted cream. The preparation of clotted cream involves the heat treatment up to 88 °C (190°F) described by Wilfrid Sadler, 1917] for 8hrs along with subsequent cooling for 24 hrs. Prior to heat treatment, a total of 10 samples were prepared. All samples were prepared on laboratory scale and preserved at 4-5°C.

Preparation and formulation of clotted cream fortified with essential oils

Three different concentrations; 0.25%, 0.5% and 0.75% of cream with essential oils of clove, cinnamon and their combinations were prepared, along with one control sample. For 0.25%, 250µl clove and Cream with 2gm of sucrose cinnamon essential oils were added separately in each container having 50g cream with 2g of sucrose. 0.5% was prepared by adding 500µl of each essential oil separately with constant amount of cream and sucrose. For 0.75%, 750µl clove and cinnamon essential oil were added. Three different concentrations made with combinations of clove and cinnamon essential oil. For combination of 0.25%, 125µl clove +125µl cinnamon essential oil was added in constant amount of cream and sucrose. For the combination of 0.5% 250µl clove + 250µl for cinnamon oil were added in constant amount of cream and sucrose. Third combination samples i.e. 0.75%, 375µl clove+375µl cinnamon essential oils were added along with the constant amount of cream and sucrose.

Chemical analyses

The chemical tests were carried out in triplicates for fat, protein, carbohydrates, moisture and ash.

Fat content

Fat content was determined by Gerber method described by Kohler’s measuring method.

Protein content

Protein content was determined by Formol titration method (AACC, 46-13.01). The protein content was calculated as follows:

\[ \text{Protein} = \frac{(V_1 - V_2) \times 1.94}{W_1 - W_0} \]

Where:
- \( V_1 \) = burette reading of sample
- \( V_2 \) = burette reading of blank
- \( W_1 \) = weight of ash
- \( W_0 \) = weight of sample

Carbohydrate content

The carbohydrate content was determined by subtracting the summed-up percentage compositions of moisture, protein, lipid, fibre and ash contents from 100 (Lawal et al., 2014). It was calculated as follows:

\[ \text{Carbohydrate} = 100 - \% \text{moisture} - \% \text{protein} - \% \text{lipid} - \% \text{ash} \]
Antioxidant Capacity Assay

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method was used to assay the free radical scavenging ability of the fortified samples of clotted cream (Ilecholubo et al., 2017).

Preparation of sample solution for measurement of antioxidant level

Briefly, 1g of each prepared samples of clotted cream (before heat treatment and after heat treatment) was accurately weighed in centrifuge tubes and homogenized on vortex with 10 ml of aqueous methanol for about 10 min. After mixing, the samples were centrifuged at 2000 rpm for 10 minutes and the supernatant was carried out and used for further analysis.

Experimental procedure for the measurement of free radical scavenging activity for DPPH

1mM of methanolic DPPH solution was prepared by adding 0.04gm and make up with methanol accurately in 100 ml volumetric flask. For the DPPH assay, 6 different dilutions of sample extracts were prepared in the following order i.e. 0 ml, 0.2ml, 0.4ml, 0.6ml, 0.8ml & 1ml with 1ml (300µl) of DPPH solution. Each dilution then further makes up-to 2ml by using methanol. The solutions were mixed thoroughly and were allowed to stand for 30 min in the dark at room temperature (28±2°C). The absorbance of the resulting mixture was measured at 517nm using UV-visible spectrophotometer. The control was prepared by adding only methanol to DPPH reagent and the analysis followed as described above. The results were expressed as percent inhibition (I %) using Equation 3.1

\[ \% \text{ Inhibition} = \frac{[A_{\text{Control}} - A_{\text{Test}}]}{[A_{\text{Control}}]} \times 100 \]

Where, \( A_{\text{Control}} \): tube containing only methanol; \( A_{\text{Test}} \): tube containing sample extract

Sensory evaluation

Fifteen panellists were selected from among students, staff and faculty at the Jinnah University for women, Karachi to evaluate the color, flavour, aroma and overall acceptability of the functional dairy product in comparison to the control. They scored the sample on the basis of 9 points hedonic scale, where 9 =like extremely through 5 = neither like nor dislike to 1 = dislike extremely. The Panelists were adequately trained to avoid any biasing during the evaluation of the sample.

Statistical analysis

Analysis of variance of proximate data were done with the help of Analysis Tool pak program under Microsoft excel 2007. On-way ANOVA for proximate analysis was used to compare the effects of heat treatments. Means were compared by Fisher's Least Significant Test at a significance level of P> 0.05.

DISCUSSION

Proximate analysis of clotted cream prepared with different concentrations:

Proximate analyses of each prepared samples of clotted cream were carried out using conventional methods. The determination of proximate constituents is necessary for assessing nutritional levels of newly developed functional product, the analysis revealed important findings.

Data in table 1 shows the proximate composition of clotted cream prepared with different concentrations of you have to change and mentioned like S. aromaticum and C. verum and combination of both oils. The crude fat content ranged from 40.258 ± 0.114% to 41.823 ± 1.345%. The lowest fat content was found in concentration 0.75% CLO while the highest fat content was calculated in concentration 0.5% CIN sample. (Belgen and Irfan, 2009) analyzed fat content in 16 kinds of different samples of clotted cream prepared on a large scale. They analyzed the fat content in samples ranged from 58.97% - 66.59%. The original milk cannot have any considerable amount of the percentage of fat in finished clotted cream. Collectively considerable amount of fat content was found in clotted cream prepared with cinnamon oil with all the three concentrations (0.25%, 0.5% and 0.75%), while minimal fat content apparently was found in clotted cream prepared with clove oil with all the three concentrations (0.25%, 0.5% and 0.75%). The significant variations on the basis of sample concentrations. Since (p> 0.05) therefore, no significant difference was found on the fat content by changing the concentration of oils. The protein content ranged from 2.153 ± 0.605% to 3.113 ± 0.220%. The lowest protein content was found in concentration 0.25% COMB while the highest protein content was found in concentration 0.5% CIN sample. The considerable amount of protein content was found in clotted cream prepared with clove oil with all the three (0.25%, 0.5% and 0.75%), while minimal protein content apparently was found in clotted cream prepared with combination oil with all the three concentrations (0.25%, 0.5% and 0.75%). The carbohydrate content was found to be in the range from 4.455 ± 0. 627 3% to 5.707 ± 0.143%. The lowest carbohydrate content was found in concentration 0.75% CIN while the highest carbohydrate content was calculated in concentration 0.5% CLO sample. Respectively the collectively considerable amount of ash content was found in clotted cream prepared with clove and combination oil with all the three concentrations (0.25%, 0.5% and 0.75%), while minimal carbohydrate content apparently was found in clotted cream prepared with cinnamon oil. The moisture content of samples ranges from 62.362 ± 2.055% to 67.588 ± 1.531%. The lowest moisture content was found in concentration 0.5% CIN while the highest moisture content was determined in concentration 0.25% CLO sample. Moisture content was found to be higher in all samples with minimum variations between the samples of all the three concentrations (0.25%, 0.5% and 0.75%). The ash content was found to

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be in the rage from 0.748 ± 0.577% to 1.832 ± 0.262%. The lowest ash content was found in concentration 0.5% CLO while the highest ash content was found in concentration 0.75% CLO sample. Collectively considerable amount of ash content was found in clotted cream prepared with combination oil with all the three concentrations (0.25%, 0.5% and 0.75%), while minimal ash content apparently was found in clotted cream prepared with cinnamon oil with all the three concentrations (0.25%, 0.5% and 0.75%). In contrast the majority of compositional measures no significant variation were found between the replicates of clotted cream with variant concentrations of cinnamon, clove and combination oils.

Free Radical scavenging activity (DPPH) of clotted cream

The antioxidant activity by DPPH assay of clotted cream fortified with essential oils of clove, cinnamon and the combination of both oils were examined by comparing it with the control product that contain no essential oil and the %SCV and IC 50 of product is examined before and after thermal treatment. Ascorbic acid, which is a natural antioxidant, is used as standard to compare the antioxidant potential (Can et al., 2010).

As the standard substance, Ascorbic acid showed maximum scavenging capacity of 98.790% at 50 μg/ml concentration and has 6.345 IC50 (Table.2) while clotted cream fortified with cinnamon oil at a concentration 0.75% showed maximum scavenging capacity of 68.023% before thermal treatment while after thermal treatment the percentage is reduced to 61.279%. The clotted cream fortified with clove oil at a concentration 0.50% showed maximum scavenging capacity of 62.209% before thermal treatment while after thermal treatment the percentage is reduced to 59.069%. The clotted cream fortified with combination of both oils at a concentration 0.25% showed maximum scavenging capacity of 70.930% before thermal treatment while after thermal treatment the percentage is reduced to 70.581%. Comparison of antioxidant activity against DPPH Free radical scavenging shows that sample having 0.50% cinnamon essential oil is potent antioxidant before thermal treatment (IC50=0.063 ml decline to 0.339ml ) after thermal treatment as compared to sample having 0.25 % cinnamon essential oil before thermal treatment (IC50=0.132 ml decline to 0.346 ml) after thermal treatment and 0.75%( IC50=0.150 ml to 0.214ml) after thermal treatment (Table.2). Comparison of antioxidant activity against DPPH Free radical scavenging shows that sample having 0.75%clove essential oil is potent antioxidant before thermal treatment (IC50=0.054 ml decease into 0.196ml ) after thermal treatment as compared to sample having 0.50 % clove essential oil before thermal treatment (IC50=0.191 ml to 0.432 ml) after thermal treatment and 0.25%( IC50=0.216 ml to 0.403ml) after thermal treatment. The 0.75% combination of both essential oils is potent antioxidant before thermal treatment (IC50=0.029 ml decline to 0.369ml) after thermal treatment as compared to sample having 0.25 % combination of both essential oils before thermal treatment (IC50=0.194 ml decline to 0.412 ml) after thermal treatment and 0.50% (IC50=0.286 ml decline to 0.412ml) after thermal treatment. Hence the IC50 value of the clotted cream containing combination of both essential oils at 0.75% concentration before thermal treatment is found to be best antioxidant because of the lowest IC50 value i.e.0.029 ml followed by the sample that containing 0.75% clove oil and cinnamon oil having IC50 0.054ml and 0.063ml respectively. The observed increase of interest in antioxidant has provided a stimulus for scientists to search for new drying methods, preserving the highest amount of biologically active compounds possible, as well as minimizing changes in the appearance, taste, color, and texture of the materials (Kaefer and Milner 2008; Milene et al., 2013). Present study have highlighted the potential of functional dairy product fortified with essential oil has bearing antiradical properties that decolourized the stable DPPH radical. Antioxidant analysis of the product reveals that the maximum DPPH antioxidant activity percentage was observed in combination of both essential oils before heat treatment at 0.25% concentration i.e. 70.9% while the IC50 value is 0.029ml at 0.75% concentration indicates a potent antioxidant product in comparison with all other samples.

Sensory Evaluation of clotted cream

Based on mean results of hedonic scores of clotted creams prepared with different concentrations of oils representing in figure 3.3.1, four sensory attributes; color, flavour, aroma and overall acceptability clotted cream prepared with different concentrations of oils were evaluated. The flavour of the cinnamon 0.50% and clove 0.25% were most acceptable. While the most unlike flavour was recorded for control, followed by cinnamon 0.75%. Cinnamon 0.25%, clove 0.50% and 0.75%, combination 0.50% and 0.75% was moderately acceptable essential oils are natural antioxidants, and not only impart sensory qualities (flavor, aroma) to food, but also improve its microbiological stability (Wargovich et al., 2001). Most liked aroma was recorded for cinnamon 0.25% and 0.75%. While less acceptable aroma was recorded with the samples of control, clove 0.50%, 0.75% and combination 0.50%. Moderate aroma was found to likeable in combination 0.25%. Color was not much affected by the oil concentrations, so no visible variations were found in the samples. Overall cinnamon 0.50%, clove 0.25% followed by combination 0.75% was categorized in most preferable range, while control lies in least acceptable category. Cinnamon 0.75%, combination 0.25, combination 0.50% lies in moderate acceptable category showed in table .3.

CONCLUSION

From the above study concludes that the scavenging activity of all essential oil is greater before thermal
treatment and after thermal treatment scavenging % is reduced, similarly IC50 value of all essential oil is less before heat treatment and after heat treatment IC50 value after treatment higher, the maximum scavenging % observed in clotted cream fortified with combination of both oils at a concentration 0.25% i.e. 70.930% before thermal treatment and after thermal treatment maximum scavenging % observed in clotted cream fortified with combination of both essential oils at a concentration 0.25% i.e. 70.581%. IC50 value of the clotted cream containing combination of both essential oils at 0.75% concentration before thermal treatment shows higher antioxidant potential as compared to others.

REFERENCES


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APPENDIX

Figure 1: DPPH free radical scavenging activity of clotted cream of control sample

Figure 2: Percentage inhibition of standard at various concentrations

Table 1: Proximate analysis of clotted cream prepared with different concentrations

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>CHO (%)</th>
<th>Crude Fat (%)</th>
<th>Crude Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>63.486 ± 1.527</td>
<td>1.105 ± 0.133</td>
<td>6.486 ± 1.527</td>
<td>40.621 ± 0.266</td>
<td>2.976 ± 0.256</td>
</tr>
<tr>
<td>Sample B (CIN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25%</td>
<td>66.020 ± 1.002</td>
<td>1.235 ± 0.353</td>
<td>5.296 ± 0.049</td>
<td>41.731 ± 1.282</td>
<td>2.616 ± 0.662</td>
</tr>
<tr>
<td>0.5%</td>
<td>64.617 ± 2.450</td>
<td>1.384 ± 0.08402</td>
<td>5.161 ± 0.883</td>
<td>41.823 ± 1.345</td>
<td>3.113 ± 0.220</td>
</tr>
<tr>
<td>0.75%</td>
<td>62.362 ± 2.055</td>
<td>1.079 ± 0.33094</td>
<td>4.455 ± 0.627</td>
<td>41.661 ± 1.314</td>
<td>2.500 ± 0.636</td>
</tr>
<tr>
<td>Sample C (CLO)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25%</td>
<td>67.588 ± 1.531</td>
<td>1.210 ± 0.292</td>
<td>5.121 ± 0.711</td>
<td>41.662 ± 1.163</td>
<td>2.937 ± 0.915</td>
</tr>
<tr>
<td>0.5%</td>
<td>64.485 ± 2.632</td>
<td>0.748 ± 0.577</td>
<td>5.707 ± 0.143</td>
<td>41.559 ± 0.271</td>
<td>2.631 ± 0.498</td>
</tr>
<tr>
<td>0.75%</td>
<td>64.574 ± 2.284</td>
<td>1.832 ± 0.262</td>
<td>4.693 ± 0.624</td>
<td>40.258 ± 0.114</td>
<td>2.538 ± 0.486</td>
</tr>
<tr>
<td>Sample D (COMB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25%</td>
<td>65.989 ± 1.809</td>
<td>1.556 ± 0.113</td>
<td>5.040 ± 0.284</td>
<td>41.343 ± 0.692</td>
<td>2.153 ± 0.605</td>
</tr>
<tr>
<td>0.5%</td>
<td>46.561 ± 1.075</td>
<td>1.719 ± 0.334</td>
<td>5.313 ± 0.990</td>
<td>42.075 ± 1.328</td>
<td>2.593 ± 0.147</td>
</tr>
<tr>
<td>0.75%</td>
<td>65.632 ± 1.805</td>
<td>1.333 ± 0.127</td>
<td>5.521 ± 0.350</td>
<td>41.699 ± 1.062</td>
<td>2.758 ± 0.244</td>
</tr>
</tbody>
</table>

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Figure 3.1: DPPH free radical scavenging activity of clotted cream containing CLO 0.5%

Figure 3.2: DPPH free radical scavenging activity of clotted cream containing clove oil 0.25%

Figure 3.3: DPPH free radical scavenging activity of clotted cream containing clove oil 0.75%

Figure 4.1: DPPH free radical scavenging activity of clotted cream containing cinnamon oil 0.25%

Figure 4.2: DPPH free radical scavenging activity of clotted cream containing cinnamon oil 0.50%
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Figure 4.3: DPPH free radical scavenging activity of clotted cream containing cinnamon oil 0.75%

Table 2: Data represents values of IC₅₀ of different prepared concerns of Clotted Cream

<table>
<thead>
<tr>
<th>S.no</th>
<th>Sample Conc.</th>
<th>Std.Conc.</th>
<th>IC₅₀</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascorbic Acid</td>
<td>Before IC₅₀</td>
<td></td>
<td>0.986</td>
</tr>
<tr>
<td>1.</td>
<td>Clove 0.25%</td>
<td>0.215</td>
<td>6.345</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Clove 0.50%</td>
<td>0.190</td>
<td></td>
<td>0.432</td>
</tr>
<tr>
<td>3.</td>
<td>Clove 0.75%</td>
<td>0.053</td>
<td></td>
<td>0.195</td>
</tr>
<tr>
<td>4.</td>
<td>Cinnamon 0.25%</td>
<td>0.132</td>
<td></td>
<td>0.345</td>
</tr>
<tr>
<td>5.</td>
<td>Cinnamon 0.50%</td>
<td>0.063</td>
<td></td>
<td>0.339</td>
</tr>
<tr>
<td>6.</td>
<td>Cinnamon 0.75%</td>
<td>0.150</td>
<td></td>
<td>0.213</td>
</tr>
<tr>
<td>7.</td>
<td>Combination 0.25%</td>
<td>0.193</td>
<td></td>
<td>0.412</td>
</tr>
<tr>
<td>8.</td>
<td>Combination 0.50%</td>
<td>0.286</td>
<td></td>
<td>0.327</td>
</tr>
<tr>
<td>9.</td>
<td>Combination 0.75%</td>
<td>0.028</td>
<td></td>
<td>0.368</td>
</tr>
</tbody>
</table>

Table 3: Sensory evaluation of the clotted cream with different concentrations of oils.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color</th>
<th>Flavour</th>
<th>Aroma</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.50 ± 0.707</td>
<td>5.50 ± 0.707</td>
<td>4.00 ± 1.414</td>
<td>5.50 ± 0.707</td>
</tr>
<tr>
<td>CIN 0.25%</td>
<td>7.50 ± 0.707</td>
<td>7.50 ± 0.707</td>
<td>8.50 ± 1.414</td>
<td>7.00 ± 1.414</td>
</tr>
<tr>
<td>CIN 0.50%</td>
<td>6.50 ± 0.707</td>
<td>8.50 ± 0.707</td>
<td>8.00 ± 1.414</td>
<td>8.00 ± 1.414</td>
</tr>
<tr>
<td>CIN 0.75%</td>
<td>7.00 ± 1.414</td>
<td>5.50 ± 0.707</td>
<td>6.00 ± 1.414</td>
<td>8.00 ± 1.414</td>
</tr>
<tr>
<td>CLO 0.25%</td>
<td>6.50 ± 0.707</td>
<td>8.50 ± 0.707</td>
<td>7.50 ± 1.414</td>
<td>8.00 ± 1.414</td>
</tr>
<tr>
<td>CLO 0.50%</td>
<td>7.50 ± 0.707</td>
<td>7.50 ± 0.707</td>
<td>6.00 ± 1.414</td>
<td>7.50 ± 0.707</td>
</tr>
<tr>
<td>CLO 0.75%</td>
<td>6.50 ± 0.707</td>
<td>8.00 ± 1.414</td>
<td>6.00 ± 1.414</td>
<td>8.00 ± 1.414</td>
</tr>
<tr>
<td>COMB 0.25%</td>
<td>7.00 ± 1.414</td>
<td>8.00 ± 1.414</td>
<td>8.00 ± 1.414</td>
<td>6.50 ± 3.536</td>
</tr>
<tr>
<td>COMB 0.50%</td>
<td>7.50 ± 0.707</td>
<td>7.00 ± 1.414</td>
<td>5.50 ± 1.414</td>
<td>6.50 ± 2.121</td>
</tr>
<tr>
<td>COMB 0.75%</td>
<td>6.50 ± 0.707</td>
<td>7.50 ± 0.707</td>
<td>8.50 ± 1.414</td>
<td>6.00 ± 1.414</td>
</tr>
</tbody>
</table>