Effect of Interesterified *Moringa oleifera* Oil on Physico-Chemical Characteristics and Storage Stability of Ice Cream

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**ABSTRACT**

Partial replacement of milk fat with liquid vegetable oils in ice cream decreases its melting time, use of solid fats in the same adds enormous amount of undesirable saturated fatty acids that can fade away the objective of replacement. This study aimed to investigate the effect of high melting point interesterified *Moringa oleifera* oil with substantial amount of unsaturated fatty acids on physical-chemical and oxidative stability characteristics of ice cream. Of the 10% fat in ice cream, 30% was replaced by interesterified *Moringa oleifera* oil at three levels i.e. 10, 20 and 30% (T1, T2 and T3, respectively). Oleic acid increased from 26.55% to 31.69%, 36.94% and 42.15% in T1, T2 and T3 with no effect on melting time, compositional attributes and free fatty acid content of ice cream (P>0.05). Supplementation of ice cream with interesterified *Moringa oleifera* oil inhibited the antioxidation process in ice cream during 3-months storage period (P<0.05). The loss of oleic and linoleic acid in the fresh and 3-months stored control and T2 was 26.55%, 24.15%, 26.39% and 1.93%, 1.24% and 1.79%, respectively. Peroxide value of three months stored control and T3 was 1.12 and 0.39 (meqO₂/kg). The overall acceptability score of T2 was 80% of the total score (9). 20% Interesterifed *Moringa oleifera* oil can be used in the formulation of functional, extended shelf life ice cream with reasonable sensory characteristics.

**Keywords:** *Moringa oleifera* oil, interesterification, over run overall acceptability

**INTRODUCTION**
Milk fat contains plenty of atherogenic fatty acids which have a great deal of contribution in the enhancement of harmful LDL cholesterol. In addition to the saturated fatty acids, milk fat also contains higher extent of the dietary cholesterol; these factors make it the first choice of criticism for the nutritionists and dieticians (Williams, 2000). The deadly reputation of milk fat is widely spread and understood, about 44% of the US population has shown negative opinion against the milk and dairy products particularly, fat rich dairy products, people are becoming more health conscious (Hansel et al. 2007; Honda et al. 2007). The increased rate of mortalities form cardiovascular disease has led to the development of large number of modification and replacement strategies. Partial replacement of milk fat with vegetable oils results in weak body and lower melting resistance in ice cream (Nadeem et al. 2010). This problem can be ameliorated by using lipids having higher melting points and melting resistance, extent of saturated fatty acids and melting points are usually correlated with each other. It is quite difficult to find the fats which possess both the desired healthful and functional characteristics simultaneously. In our other study we observed that melting point of Moringa oleifera oil can be substantially increased through the random rearrangement of esters (Nadeem et al. 2012). Interesterified Moringa oleifera oil can fulfill the above mentioned quality and functionality characteristics simultaneously (Nadeem et al. 2012). Interesterification is an effective way to increase the slip melting point and solid fat content of vegetable oils without generation of undesirable trans isomers (Osman and Idris, 1999). Moringa oleifera is widely grown in tropics, quality of oil is almost similar to olive oil (Mohammed et al. 2003) The oil content of seeds ranges between 30-40%, oil is edible and rich in monounsaturated fatty acids (Tsakinis et al. 1998). Moringa oleifera oil improved the oxidative stability of vegetable oils and butter oil (Anwar et al. 2007; Nadeem
et al. 2013b). The antioxidant potential of *Moringa oleifera* oil on the oxidative stability of ice cream has not been studied so far. This study aimed to evaluate the suitability of interesterified *Moringa oleifera* oil to partially replace milk fat in the formulation of functional ice cream to find out the suitability of chemically modified *Moringa oleifera* oil in blends with milk fat on the basis of some physico-chemical, sensory and oxidative stability characteristics.

**MATERIALS AND METHODS**

**Chemical Interesterification:** *Moringa oleifera* oil was extracted by laboratory scale expeller, oil was stored in clean and dry pet bottles, sealed, stored at -18°C till further usage. Melting point of *Moringa oleifera* oil and milk fat was 19.8 and 35.2°C. In our another study on the interesterification of *Moring oleifera* oil, we observed that melting point increased after the rearrangement of the esters. Chemical interesterification of *Moringa oleifera* oil was performed to improve the melting resistance of ice cream. After rearrangement reaction melting point of *Moringa oleifera* oil was 35.6°C. Samples were dehydrated in a flask, under reduced pressure, fitted with a vacuum pump in a water bath at 95°C. The portions were mixed with 0.2% (w/w) of sodium methylate. Interesterification reaction was performed under reduced pressure at 70°C in a 1000 mL stoppered flask in a water bath with constant agitation for one hour. To stop the interesterification reaction, 5-mL distilled water was added in the flask. Treated blend was washed with hot water twice for the complete removal of catalyst (Sreenivasan, 1978).

**Experimental Plan:** Milk fat was partially replaced with interesterified *Moringa oleifera* oil at 10, 20 and 30% levels (*T*₁, *T*₂, *T*₃, respectively). The formulation of control comprised of 10% milk fat, 11% MSNF, 15% sucrose, 0.5% cremodan with no added color and flavor.
Analysis: Fat was extracted from ice cream by following the recommended method of AOAC (1997). Fatty acids were analyzed as fatty acid methyl esters using sodium methyleate as transesterifying agent (Qian, 2003). Fatty acid methyl esters were injected into Gas Chromatograph model Shimadzu, Japan 17-A, fitted with a methyl lignoserate-coated (film thickness 0.25µm), SP-2330 (SUPELCO Inc. Supelco Park Bellefonte, PA 16823- 0048, USA) polar capillary column (30m x 0.32mm) and a flame ionization detector. Fatty acids were identified and quantified by the FAME-37 internal standards (Sigma Aldrich, UK). Fat, protein, ash, lactose content, total solids, pH and acidity was determined by following the recommended methods of AOAC (2000). The viscosity readings were taken after ageing the mix at 4°C for 24 hours. Melting time was determined by the method of Abdou et al. (1996). Peroxide and anisidine values were determined as per methods of AOCS (1995). The ice creams were tempered to -18°C for 2 hours before serving. All the samples were coded with a 3 digit random number, and all orders of servings were completely randomized. Evaluation was performed by 10-trained judges on a 9-point Hedonic scale (1the worst, 9 the best) as prescribed by Larmond (1987). The statistical analysis of the triplicate experiment was performed by one way and two way analysis of variance techniques as prescribed by Steel et al. (1997) in a completely randomized design, the significant difference among the treatments was made by using Duncan’s Multiple Range Test.

RESULTS AND DISCUSSION

Characterization of Moringa oleifera Oil: Preliminary characterization of cold pressed Moringa oleifera oil revealed; free fatty acids 0.19% (oleic acid), moisture content 0.22%, color on Lovibond Tintometer scale Red 2.1 and Yellow 3.2, unsaponifiable matter 1.29%, peroxide
value 0.22 (meqO₂/kg), iodine value 69.4 and slip melting point 19.8°C. The free radical scavenging activity, flavonoid content and total antioxidant activity of *Moringa oleifera* oil 74.19%, 35.14 mg/100-grams and total antioxidant capacity was 37.68%, respectively.

**Fatty Acid Profile:** Fatty acid composition of milk fat, *Moringa oleifera* oil and their blends has been presented in Table-1. Marked changes were recorded in fatty acid composition of the blends after the formulation of blends particularly the content of short chain fatty acids declined from 10.65% (T₀) to 8.67, 7.49 and 6.68% in T₁, T₂ and T₃, respectively, which was 19.6, 29.68 and 37.28% less than the control. Oleic acid increased from 26.55% (T₀) to 31.69, 36.94 and 42.15% in T₁, T₂ and T₃, respectively. Oleic acid progressively increased as a function of higher levels of *Moringa oleifera* oil. Lim et al. (2010) studied the effect of partial replacement of milk fat with flaxseed oil and observed progressive increase in the content of unsaturated fatty acids with increasing increments of flaxseed oil. Nadeem *et al.*, (2010) incorporated rape seed oil into ice cream and recorded higher concentration of oleic acid in the blends. Anwar *et al.*, (2007) supplemented sunflower oil by *Moringa oleifera* oil and observed major difference in fatty acid composition of the blends. Muhammad *et al.*, (2003) characterized *Moringa oleifera* oil with the objective to explore its potential as future’s oil and reported that major fatty acid was oleic acid (78.9%). Fatty acid composition of *Moringa oleifera* oil was almost similar to olive oil. Mariod *et al.*, (2005) studied the effect of blending on fatty acid composition of sunflower and *Sclero caryabirrea* oil. Oleic acid increased by 41.3% and linoleic acid decreased by 51% in the blend when blended in 60:40 ratios. Sunflower, palm oil and ground nut oils were blended in various proportions; fatty acid composition of blends was significantly distinguishable from the substrate oils.
**Composition:** The results of chemical composition of functional ice cream containing interesterified *Moringa oleifera* oil is given in Table-2. The addition of *Moringa oleifera* oil at all three levels did not have any effect on pH, acidity, fat, protein, ash content and total solids of ice cream. The reason could be the identical formulation and non-variation in the ingredients. Chemical composition of ice cream formulated from milk fat, palm kernel oil, cotton seed oil, dalda vanaspati, ground nut and rape seed oil was not different from the standard ice cream (Miglani et al. 1987; Adhikari and Arora 1994; Abdou et al. 1996; Nadeem et al. 2010).

**Viscosity:** Viscosity of ice cream mix has a direct connection with the whipping process; higher viscosities are associated with better whipping ability (Lim et al. 2010). Viscosity of ice cream mixes supplemented with interesterified *Moringa oleifera* oil was not different from the control. Partial replacement of milk fat with vegetable oils had a pronounced effect on the viscosity of ice cream mix (Adhikari and Arora, 1994; Im and Marshall, 1998). The result of our study is different from the previously conducted research works, although we also used the vegetable oil, yet it was chemically modified with different physical characteristics which definitely contributed in the improvement of the physical characteristics of ice cream.

**Overrun:** 30% replacement of milk with *interesterified Moringa oleifera* oil did not reveal any effect on overrun of ice cream, significant changes were observed beyond this level. Replacement of milk fat with corn and rape seed oils decreased the overrun in mellorine (Rodriquez et al. 1991; Nadeem et al. 2010). The result of this investigation are in partial confrontation of other researchers. These results can be justified by two; replacement level was not too high and secondly the fat used in this investigation was chemically modified having higher solid fat index and higher melting point.
Melting Time: Melting time of all the experimental samples were at par with the control. Other studies on milk fat replacement with vegetable oils revealed that melting time markedly decreased as the milk fat was replaced with liquid vegetable oils. The contradiction in the results of this investigation from the literature is due to the usage of interesterified *Moringa oleifera* oil which had greater melting point 35.6°C than milk fat 34.6°C, higher melting point improved the melting resistance. Adhikari and Arora (1994) while studying the replacement of butter fat with unmodified ground nut oil observed that replacement of milk fat significantly (P<0.05) decreased the melting time. Melting time of the ice cream is directly dependent on the melting point of fats, fats having higher melting point will take more time to melt, the fats with lower melting point will melt quickly (Flack, 1988). For ice cream the fats having melting point in the range of 34-36 °C may give the optimum melting time. The fat used in this study was wisely interesterified to increase the melting point from 18 to 34.6°C which contributed in the melting resistance of ice cream and confer the melting time to be almost similar to milk fat at medium level of fat replacement ($T_2$).

Changes in Fatty Acid Composition: Determination of fatty acid composition of three months stored ice cream exhibited two different trends; saturated fatty acids increased whereas, unsaturated fatty acid decreased as a function of storage period. The increase or decrease in the extents of saturated and unsaturated fatty acids could be attributed to the better oxidative stability of saturated fatty acids and break down of unsaturated fatty acids into the oxidation products. The changes in the fatty acid composition during 3-months storage period was used as an indication of the oxidative stability of fat in ice cream, as accelerated oxidation tests are normally not recommended to determine the oxidation resistance of ice cream. Supplementation of milk
fat with *Moringa oleifera* oil at all three levels tended to inhibit the autoxidation process in the stored ice cream. The extent of breakdown of unsaturated fatty acids in the three months stored ice cream was in the order of $T_3 > T_2 > T_1 >$ control. The strong inhibition of the oxidative breakdown in the supplemented ice creams could be attributed to the presence of higher concentration of polyphenolic compounds in *Moringa oleifera* oil. The concentration of unsaturated fatty acids decreased in ice cream supplemented with flax seed oil during the course of 42-days storage period (Lim et al. 2010). Long term storage of butter at refrigeration and freezing conditions increased the extents of oxidation products (Krause et al. 2008). In our another study relate to the determination of oxidative stability of olein based ice creams we also observed the enhancement of saturated fatty acids and decline of unsaturated fatty acids during 6-months storage (Nadeem et al. 2013a).

**Peroxoide Value:** Supplementation of milk fat with interesterified *Moringa oleifera* oil at all concentrations considerably inhibited the formation of primary oxidation products ($P<0.05$). Enhancement of unsaturated fatty acids in ice cream either by using modified milk fat or vegetable oils results in development of oxidized flavor in ice cream during the storage process (Gonzalez et al. 2003; Lim et al. 2010). In this study, the use of *Moringa oleifera* oil offered a unique benefit of enhancing the beneficial oleic acid without posing a problem of rancid flavor. The lower extents of primary oxidation products could be attributed to the lower oxidation susceptibility of oleic acid and presence of phenolic compounds. Enhancement of the oxidative stability of soybean and sunflower oils by mixing with *Moringa oleifera* oil has been reported by Anwar et al. (2007). We also observed a strong inhibition of the autoxidation process when butter oil was blended with *Moringa oleifera* oil (Nadeem et al. 2013b). Flavor score and
peroxide value were highly correlated, determination intervals showing higher peroxide value revealed lower flavor score (Fig. 1). Generation of oxidation products decreased the flavor score of olein based ice creams (Nadeem et al. 2013a).

**Anisidine Value:** Anisidine value measures the magnitude of secondary oxidation products generated in fats and oils during the course of autoxidation, higher values usually anticipate poor storage stability (Pritchard, 1991). Ice creams supplemented with *Moringa oleifera* oil exhibited better storage stability over the control due to the strong inhibition of free radical mechanism lead to the formation of secondary oxidation products. Enhancement of the unsaturated fatty acids in ice cream by the manipulation of the cow’s ration with unsaturated fatty acids and flaxseed oil in ice cream rendered it vulnerable to the oxidative breakdown (Gonzalez et al. 2003; Lim et al. 2010). Olein based ice creams yielded the higher extents of secondary oxidation products during storage of 6-months (Nadeem et al. 2013a).

**Free Fatty Acids:** The free fatty acids of the control and all the treatments steadily increased during 3-months storage period and were at par with each other. Supplementation of ice cream with *Moringa oleifera* oil was ineffective to stop the generation of free fatty acids during the storage period. Free fatty acids in food systems are produced due to the hydrolytic activities of lipases, presence of moisture, metal ion contamination etc. and phenolic compounds do not have an mechanism to stop their formation. Free fatty acids are associated with the generation of off-flavors in foods; higher values can have a negative influence on the quality of the stuffs (McSweeney and Fox, 2003). The free fatty acid content of three months stored ice creams were within the acceptable limits. Free fatty acids of six months stored olein based ice creams and control were not different from each other (Nadeem et al. 2013a).
Sensory Evaluation: The results of sensory evaluation of fresh ice cream disclosed that the addition of interesterified *Moringa oleifera* oil at 20% level did not impart any effect on color score (P>0.05). Milk fat is unique among the edible fats for having appreciable amount of short chain fatty acids which are responsible for characteristics flavor of milk and milk products (Marshall et al. 2003). Replacement of milk fat up to 20% level created non distinguishable difference in flavor of ice cream. As the replacement level increased to 30% flavor score decreased from 7.1 ($T_0$) to 6.5 ($T_3$). The decline in flavor score could be attributed to the lower concentration of short chain fatty acids in $T_3$. The texture score of the control and $T_3$ were at par with each other (P>0.05). The results of our investigation are not in line with the findings of Nadeem et al. (2010) who reported lower texture score when milk fat was replaced with rape seed oil. The deviation in texture score was due to the difference in the type of edible oil, we used interesterified *Moringa oleifera* oil which had higher melting point than the milk fat, which definitely contributed in the texture of the ice creams.

CONCLUSIONS

The addition of interesterified *Moringa oleifera* oil did not have any effect on compositional attributes, overrun, viscosity and melting time, rather it improve the texture and storage stability of ice cream. Interesterified *Moringa oleifera* oil can be used in the formulation of functional ice cream.
REFERENCES


AOCS. 1995. Official Methods and Recommended practices of the American Oil Chemists’ Society, 4th ed. AOCS, Champaign, IL, USA.


<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>$T_0$</th>
<th>$T_1$</th>
<th>$T_2$</th>
<th>$T_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4:0</td>
<td>4.47±0.09d</td>
<td>4.10±0.03b</td>
<td>3.57±0.11c</td>
<td>3.12±0.01c</td>
</tr>
<tr>
<td>C6:0</td>
<td>2.28±0.12a</td>
<td>2.04±0.05b</td>
<td>1.84±0.02c</td>
<td>1.56±0.04d</td>
</tr>
<tr>
<td>C8:0</td>
<td>1.34±0.04a</td>
<td>1.21±0.01a</td>
<td>1.06±0.01b</td>
<td>0.95±0.07b</td>
</tr>
</tbody>
</table>
C10:0  2.56±0.07a  2.32±0.04b  1.85±0.07c  1.75±0.11d  
C12:0  2.66±0.15a  2.36±0.06b  2.14±0.02c  1.84±0.04d  
C14:0  9.24±0.19a  8.35±0.09b  7.44±0.05c  6.46±0.15d  
C16:0  25.84±0.23a  23.95±0.12b  22.05±0.18c  18.15±0.10d  
C18:0  14.65±0.11a  13.45±0.02b  12.34±0.07c  11.14±0.06d  
C18:1  26.55±0.16c  31.69±0.14c  36.94±0.23b  42.15±0.12a  
C18:2  1.93±0.12d  2.14±0.17c  2.34±0.09b  2.58±0.22a  
C18:3  1.31±0.05c  1.17±0.05b  1.03±0.03c  0.82±0.09d  
C20:0  0.15±0.02d  0.37±0.01c  0.61±0.05b  0.87±0.02a

Means of triplicate experiment; means sharing same letter in same row are statistically non significant

Table 2 - Effect of replacing milk fat with interesterified Moringa oleifera oil on composition of ice cream

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fat%</th>
<th>Protein%</th>
<th>Ash%</th>
<th>Total Solids%</th>
<th>pH</th>
<th>Acidity%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>9.84±0.06</td>
<td>4.05±0.47</td>
<td>0.73±0.17</td>
<td>36.18±0.10</td>
<td>6.70±0.05</td>
<td>0.17±0.04</td>
</tr>
<tr>
<td>T₁</td>
<td>9.85±0.09</td>
<td>4.01±0.42</td>
<td>0.70±0.17</td>
<td>36.15±0.10</td>
<td>6.68±0.03</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>T₂</td>
<td>9.94±0.16</td>
<td>3.95±0.21</td>
<td>0.72±0.09</td>
<td>36.09±0.10</td>
<td>6.71±0.01</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>T₃</td>
<td>9.78±0.11</td>
<td>3.81±0.56</td>
<td>0.71±0.09</td>
<td>36.03±0.05</td>
<td>6.75±0.05</td>
<td>0.16±0.08</td>
</tr>
</tbody>
</table>

Refer to Table-1 for the detail of treatments

Table 3 - Effect of replacing milk fat with interesterified Moringa oleifera oil on physical characteristics of ice cream

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Viscosity (CP)</th>
<th>Overrun (%)</th>
<th>Melting Time (Minutes)</th>
<th>MP (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>67.38±1.55a</td>
<td>82.2±1.23a</td>
<td>6.33±0.90a</td>
<td>34.8±0.2a</td>
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<tr>
<td>T₁</td>
<td>65.98±1.47a</td>
<td>81.9±1.45a</td>
<td>6.15±1.71a</td>
<td>34.6±0.1a</td>
</tr>
<tr>
<td>T₂</td>
<td>64.29±1.34a</td>
<td>70.3±1.99a</td>
<td>5.98±0.95a</td>
<td>35.0±0.3a</td>
</tr>
<tr>
<td>T₃</td>
<td>63.18±1.32a</td>
<td>77.5±1.79a</td>
<td>5.70±1.10a</td>
<td>35.2±0.2a</td>
</tr>
</tbody>
</table>

Means of triplicate experiment; means sharing same letter in column are statistically non significant

Refer to Table-1 for the detail of treatments

MP: Slip Melting Point (°C)

Table 4 - Changes in major fatty acids of fresh and 3-months stored ice cream

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fresh</th>
<th>T₀-3M</th>
<th>T₁-3M</th>
<th>T₂-3M</th>
<th>T₃-3M</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>25.84±0.23b</td>
<td>27.42±0.82a</td>
<td>25.36±1.16b</td>
<td>25.51±0.92b</td>
<td>25.72±0.54b</td>
</tr>
<tr>
<td>C18:0</td>
<td>14.65±0.11b</td>
<td>15.89±0.73a</td>
<td>15.12±0.38b</td>
<td>15.79±0.61b</td>
<td>15.93±0.71b</td>
</tr>
</tbody>
</table>
C18:1 26.55±0.16b 24.15±0.44a 25.94±0.75b 26.17±0.97b 26.39±0.58b
C18:2 1.93±0.12b 1.24±0.11a 1.65±0.08b 1.74±0.16b 1.79±0.07b

Means with different letter in a row are not different
3M: Three Months Stored Ice Cream at -18°C
Refer Table-1 for the detail of treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Peroxide Value</th>
<th>Anisidine Value</th>
<th>FFA%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh 3M</td>
<td>Fresh 3M</td>
<td></td>
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<tr>
<td>T₀</td>
<td>0.25±0.03a</td>
<td>1.12±0.08a</td>
<td>3.58±0.12a 13.59±0.43a 0.11±0.1a 0.16±0.02a</td>
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<tr>
<td>T₁</td>
<td>0.28±0.02a</td>
<td>0.72±0.06b</td>
<td>3.62±0.18a 9.72±0.24b 0.10±0.01a 0.16±0.02a</td>
</tr>
<tr>
<td>T₂</td>
<td>0.26±0.03a</td>
<td>0.54±0.05c</td>
<td>3.68±0.07a 6.65±0.30c 0.11±0.01a 0.15±0.01a</td>
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<tr>
<td>T₃</td>
<td>0.21±0.01a</td>
<td>0.39±0.02d</td>
<td>3.52±0.04a 5.18±0.22d 0.11±0.02a 0.16±0.01a</td>
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</table>

Means denoted by different letter in a column are different
3M: Three Months Stored Ice Cream at -18°C
Peroxide Value (MeqO₂/Kg)
FFA: Free Fatty Acids

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Color</th>
<th>Flavor</th>
<th>Texture</th>
<th>Overall acceptability</th>
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<tbody>
<tr>
<td>T₀</td>
<td>7.4±0.25a</td>
<td>7.1±0.41a</td>
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<td>T₁</td>
<td>7.2±0.31a</td>
<td>7.0±0.05a</td>
<td>7.6±0.19a</td>
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<td>T₂</td>
<td>7.2±0.45a</td>
<td>6.9±0.43a</td>
<td>7.4±0.24a</td>
<td>7.2±0.31a</td>
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<tr>
<td>T₃</td>
<td>6.9±0.34a</td>
<td>6.5±0.17b</td>
<td>7.5±0.14a</td>
<td>6.7±0.28b</td>
</tr>
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</table>

Means of triplicate experiment; means sharing same letter in a column are non significant
Refer Table-1 for the detail of treatments
Fig. 1: Correlation Between PV and Flavor Score

$R^2 = 0.9963$