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Mahendra Kumar Trivedi, *Trivedi Global Inc.*
Alice Branton, *Trivedi Global Inc.*
Dahryn Trivedi, *Trivedi Global Inc.*
Gopal Nayak, *Trivedi Global Inc.*
Khemraj Bairwa, *Trivedi Science Research Laboratory Pvt. Ltd.*, et al.

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Physical, Thermal, and Spectroscopic Characterization of Biofield Energy Treated Potato Micropropagation Medium

Mahendra Kumar Trivedi¹, Alice Branton¹, Dahryn Trivedi¹, Gopal Nayak¹, Khemraj Bairwa², Snehasis Jana², *

¹Trivedi Global Inc., Henderson, NV, USA
²Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, Madhya Pradesh, India

Email address: publication@trivedisrl.com (S. Jana)

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Abstract: Potato Micropropagation Medium (PMM) is the growth medium used for in vitro micropropagation of potato tubers. The present study was intended to assess the effect of biofield energy treatment on the physical, thermal and spectroscopic properties of PMM. The study was attained in two groups i.e. control and treated. The control group was remained as untreated, while the treated group was received Mr. Trivedi’s biofield energy treatment. Finally, both the samples (control and treated) were evaluated using various analytical techniques such as X-ray diffractometry (XRD), differential scanning calorimetry (DSC), thermogravimetric analysis- differential thermal analysis (TGA-DTA), UV-Vis spectrometry, and Fourier transform infrared (FT-IR) spectroscopy. The XRD analysis showed the crystalline nature of both control and treated samples of PMM. The X-ray diffractogram showed the significant increase in the intensity of XRD peaks in treated sample as compared to the control. The XRD analysis revealed 6.64% increase in the average crystallite size of treated PMM with respect to the control. The DSC analysis showed about 8.66% decrease in the latent heat of fusion in treated sample with respect to the control. The TGA-DTA analysis exhibited about 4.71% increase in onset temperature of thermal degradation after biofield treatment with respect to the control, while the maximum thermal degradation temperature (Tmax) was also increased (5.06%) in treated sample with respect to the control. This increase in Tmax might be correlated with increased thermal stability of treated sample as compared to the control. The UV spectroscopic study showed the slight blue shift in λmax of treated sample with respect to the control. FT-IR spectrum of control PMM showed the peak at 3132 cm⁻¹ (C-H stretching) that was observed at higher wavenumber i.e. at 3161 cm⁻¹ in the treated sample. Other vibrational peaks in the treated sample were observed in the similar region as that of the control. Altogether, the XRD, DSC, TGA-DTA, UV-Vis, and FT-IR analysis suggest that Mr. Trivedi’s biofield energy treatment has the impact on physicochemical properties of PMM. This treated PMM might be more effective as a micropropagation medium as compared to the control.

Keywords: Biofield Energy Treatment, Potato Micropropagation Medium, X-ray Diffraction, Differential Scanning Calorimetry (DSC), UV-vis Spectroscopy, Fourier Transform Infrared Spectroscopy

1. Introduction

Micropropagation is the technique of rapidly multiplying the stock plant material to generate a number of progeny plants, using advanced plant tissue culture methods [1, 2]. This method is used to multiply novel plants, such as genetically modified, bred of conventional plant, plants that do not produce seeds etc. [3, 4]. The potato (Solanum tuberosum L.) is one of the vegetable plant belongs to Solanaceae family. Conventionally, potato is propagated using tubers that have low multiplication ratio of about 1:4 [5, 6]. The current progression in tissue culture techniques, specifically micropropagation led to a new method of propagation through in vitro techniques [7]. The micropropagation medium used for propagation of potato is termed as potato micropropagation medium (PMM). It contains ammonium nitrate and potassium nitrate as the source of nitrate; kinetin and indole acetic acid as the plant growth regulators. Apart from this, it also consists with mineral salts, vitamins, and amino acids, which are required
2. Materials and Methods

2.1. Study Design

Potato micropropagation medium (PMM, Table 1) was obtained from HiMedia Laboratories, India. The PMM sample was divided into two groups i.e. control and treated.

Table 1. Chemical composition of potato micropropagation medium.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Mg/Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potassium nitrate</td>
<td>1900</td>
</tr>
<tr>
<td>2</td>
<td>Ammonium nitrate</td>
<td>1650</td>
</tr>
<tr>
<td>3</td>
<td>Calcium chloride. 2H₂O</td>
<td>440</td>
</tr>
<tr>
<td>4</td>
<td>Magnesium sulphate</td>
<td>180.68</td>
</tr>
<tr>
<td>5</td>
<td>Potassium phosphate monobasic</td>
<td>170</td>
</tr>
<tr>
<td>6</td>
<td>Myo-Inositol</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>EDTA disodium salt. 2H₂O</td>
<td>37.3</td>
</tr>
<tr>
<td>8</td>
<td>Ferrous sulphate. 7H₂O</td>
<td>27.8</td>
</tr>
<tr>
<td>9</td>
<td>Manganese sulphate. H₂O</td>
<td>16.89</td>
</tr>
<tr>
<td>10</td>
<td>Zinc sulphate. 7H₂O</td>
<td>8.6</td>
</tr>
<tr>
<td>11</td>
<td>Boric acid</td>
<td>6.2</td>
</tr>
<tr>
<td>12</td>
<td>Glycine (free base)</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>Potassium iodide</td>
<td>0.83</td>
</tr>
<tr>
<td>14</td>
<td>Pyridoxine hydrochloride</td>
<td>0.5</td>
</tr>
<tr>
<td>15</td>
<td>Nicotinic acid (free acid)</td>
<td>0.5</td>
</tr>
<tr>
<td>16</td>
<td>Indole-3-acetic acid</td>
<td>0.5</td>
</tr>
<tr>
<td>17</td>
<td>Thiourea hydrochloride</td>
<td>0.4</td>
</tr>
<tr>
<td>18</td>
<td>Molybdc acid (sodium salt). 2H₂O</td>
<td>0.25</td>
</tr>
<tr>
<td>19</td>
<td>Kinetin</td>
<td>0.04</td>
</tr>
<tr>
<td>20</td>
<td>Cobalt chloride. 6H₂O</td>
<td>0.025</td>
</tr>
<tr>
<td>21</td>
<td>Cupric sulphate. 5H₂O</td>
<td>0.025</td>
</tr>
</tbody>
</table>

The control sample was kept without treatment while the treated sample was handed over in sealed pack to Mr. Trivedi for the biofield energy treatment. Mr. Trivedi provided the biofield energy treatment to the treated group via his unique energy transmission process without touching the sample, under standard laboratory conditions. Subsequently, the treated and control samples were evaluated by various analytical techniques such as XRD, DSC, TGA-DTA, UV-vis, and FT-IR spectroscopy.

2.2. XRD Study

The XRD study of control and treated PMM samples was done on Phillips (Holland PW 1710) X-ray diffractometer. The system was equipped with copper anode and nickel filter while the wavelength was set to 1.54056Å. The percentage change in average crystallite size (G) was calculated with the help of following equation:

\[ G = \left[ \left( G_t - G_c \right) / G_c \right] \times 100 \]

Here, \( G_t \) and \( G_c \) are average crystallite size of control and treated powder samples, respectively.

2.3. DSC Study

The latent heat of fusion and melting temperature of control and treated PMM were determine with the help of Pyris-6 Perkin Elmer differential scanning calorimeter. The analyte samples were heated at the rate of 10°C/min under air atmosphere with flow rate of 5 mL/min. An empty pan,
sealed with cover lid was used as a reference pan. The melting temperature (T<sub>m</sub>) and latent heat of fusion (ΔH) were obtained from the DSC thermogram.

### 2.4. TGA-DTA Analysis

The TGA-DTA analysis of control and treated PMM samples was carried out on Mettler Toledo simultaneous TGA-DTA analyzer. The analytes were heated under air atmosphere from room temperature to 400°C at the heating rate of 5°C/min. The onset temperature of thermal degradation and temperature at which maximum weight loss occur (T<sub>max</sub>) in samples were obtained from the TGA-DTA thermogram.

### 2.5. UV-Vis Spectroscopic Analysis

The spectra of control and treated samples were acquired on Shimadzu UV spectrometer (2400 PC). The spectrometer was equipped with quartz cell of 1 cm, a slit width of 2.0 nm, and the wavelength was set to 200-400 nm.

### 2.6. FT-IR Spectroscopic Characterization

The FT-IR spectroscopy of control and treated samples of PMM was carried out to determine the effect of biofield energy treatment on molecular level like dipole moment, force constant, and bond strength in chemical structure [22]. The samples were prepared by crushing with spectroscopic grade KBr into fine powder and then pressed into pellets. The spectra were obtained from Shimadzu’s Fourier transform infrared spectrometer (Japan) in the frequency region of 500-4000 cm<sup>-1</sup>.

### 3. Results and Discussion

#### 3.1. XRD Analysis

The XRD diffractograms of PMM (control and treated) samples are shown in Fig. 1. The diffractograms showed the sharp and intense peaks of both control and treated samples that indicated the PMM was crystalline nature. The XRD diffractogram of control sample exhibited the peaks at Bragg’s angle (2θ) equal to 18.89º, 23.42º, 23.68º, 29.25º, 32.18º, 32.51º, 33.65º, 33.88º, 40.99º, and 41.65º. Similarly, the XRD diffractogram of treated PMM exhibited the XRD peaks at 2θ equal to 18.95º, 23.50º, 23.75º, 29.34º, 32.31º, 32.62º, 33.58º, 33.77º, 41.08º, and 41.73º. The 2θ values showed the alteration in the 2θ angle of XRD peaks after biofield treatment as compared to the control. It is well reported that values of the 2θ angle might be altered due to presence of internal strain [23]. Based on this, it is presumed that biofield energy induced an internal strain in the treated sample that probably leads to alter its 2θ values with respect to the control sample. The average crystallite size was computed using following equation [24]:

\[
G = \frac{k \lambda}{b \cos \theta}
\]

Here, λ is the wavelength of radiation used; b is full-width half maximum (FWHM); and k is the equipment constant (0.94). The average crystallite size of the control and treated samples were observed as 121.48 nm and 129.55 nm, respectively. The result showed about 6.64% increase in average crystallite size of the treated sample as compared to the control (Fig. 2). It is reported that increase in annealing temperature considerably affects the crystallite size of the compounds. This increase in temperature might lead to reduce the dislocation density and increase the number of unit cell, which ultimately increases the average crystallite size of sample [25, 26].

#### 3.2. DSC Analysis

DSC analysis was carried out to determine the latent heat of fusion (ΔH) and melting temperature of the treated and control samples of PMM. DSC thermograms of control and treated samples are shown in Fig. 3. The melting temperature of treated sample (131.38°C) was observed as very similar to that of control sample (131.85°C). However, the latent heat of fusion corresponding to control and treated samples was observed as 73.57 J/g and 67.20 J/g, respectively (Table 2). The result showed about 8.66% decrease in the latent heat of fusion of treated PMM with respect to the control. It is assumed that biofield energy might supply the energy, which
is stored in the treated sample.

This might lead to increase the $\Delta H$ of treated sample, as it required less energy for phase transition from solid to liquid with respect to the control. Previously, our group has been reported that biofield energy treatment has altered the value of $\Delta H$ in lead and tin powders [10].

![Fig. 2. Average crystallite size of control and treated potato micropropagation medium.](image1)

![Fig. 3. DSC thermograms of control and treated potato micropropagation medium.](image2)

### 3.3. TGA-DTA Analysis

The TGA-DTA thermogram of control and treated PMM samples are shown in Fig. 4, and data is presented in Table 2. The TGA thermogram of control sample showed the onset temperature of thermal degradation at 170°C, which was continued until 245°C (endset temperature). On the other hand, the TGA thermogram of the treated sample showed the onset temperature at 178°C and continued up to 251°C (endset temperature). The result showed about 4.71% increase in the onset temperature of thermal degradation of biofield energy treated sample as compared to the control. During the thermal degradation process, the maximum thermal degradation ($T_{\text{max}}$) was observed at 207.5°C (with 15.14% weight loss) in control and 218°C (with 6.47% weight loss) in the treated sample. This showed about 5.06% increase in $T_{\text{max}}$ with respect to the control sample. Overall, this increase in onset temperature and $T_{\text{max}}$ of treated sample might be due to the alteration in internal energy through biofield energy treatment, which results into enhanced thermal stability of treated sample as compared to the control [27].

### 3.4. FT-IR Spectroscopic Analysis

FT-IR spectra of the control and treated PMM are shown in Fig. 5. The PMM is composed with several mineral salts, vitamins, amino acids etc. Due to these different components, it contains several functional groups such as nitrate, sulfate, acids, aromatic ring etc. The FT-IR spectrum of control sample showed the vibrational peak at 3132 cm$^{-1}$ that might be attributed to aromatic =C-H stretching. The peak was appeared as broad that might be due to overlap with the O-H stretching vibrations. In treated sample, the =C-H peak was observed at slight upstream region i.e. at 3161 cm$^{-1}$. The stretching frequency of any bond is directly proportional to the force constant and inversely proportional to reduced mass [28]. Therefore, it is presumed that biofield energy treatment might increase the dipole moment of =C-H bond as compared to the control sample. As a result, the force constant and bond strength of =C-H bonds might increase as compared to the control.

The FT-IR spectrum of control sample showed the IR peaks at 1760 cm$^{-1}$ that was assigned to N=O bond [29]. This might be due to nitrate (potassium and ammonium) component of PMM. The peaks at 1398 and 825 cm$^{-1}$ were attributed to asymmetric and symmetric vibration of NO$_3$ group, which is also due to the presence of potassium nitrate and ammonium nitrate as a source of nitrogen in PMM. In the FT-IR spectrum of treated sample, these vibrational peaks for N=O stretching, asymmetric NO$_3$ and symmetric NO$_3$ stretching appeared at 1760, 1400, and 825 cm$^{-1}$, respectively. The result showed the similar pattern of the vibrational frequency of nitrate group in both control and treated samples. The IR peaks at 1679 and 1141 cm$^{-1}$ in control sample were attributed to C=O stretching and C-O stretching, respectively may be because of glycine present in PMM [30]. These peaks were appeared at the same frequency.
region in treated sample. The vibrational peak at 1622 cm\(^{-1}\) in both control and treated sample might assigned to aromatic C=C stretching.

The IR peaks at 956-1004 cm\(^{-1}\) were assigned to S=O stretching, which might be due to presence of different sulfates salts such as zinc sulphate, cupric sulphate magnesium sulphate, manganese sulphate, ferrous sulphate etc. in PMM [31]. The peaks due to S=O bond in different sulfates are appeared at similar region i.e. 954-1001 cm\(^{-1}\) in the treated sample. The IR peaks at 603-663 cm\(^{-1}\) region were might be due to the Ca-Cl stretching in both control as well as treated samples of PMM [32]. Overall, the FT-IR study showed shifting of wavenumber of =C-H vibrations that might be due to increase of the force constant and bond strength of =C-H bonds in treated sample as compared to the control. The reset of vibrational peaks were observed at the similar region of IR frequency in both control and treated samples.

Fig. 4. TGA-DTA thermograms of control and treated potato micropropagation medium.
Fig. 5. FT-IR spectra of control and treated potato micropropagation medium.

Fig. 6. UV spectra of control and treated potato micropropagation medium.
3.5. UV-Vis Spectroscopy

UV spectra of the control and treated PMM are shown in Fig. 6. The UV spectrum of control sample showed the absorbance maxima ($\lambda_{\text{max}}$) at 201.0 nm. Similarly, the UV spectra of treated sample showed the $\lambda_{\text{max}}$ at 198.0 nm in treated sample. The result showed slight blue shift in the absorbance maxima in the treated sample as compared to the control.

The compound absorbs UV waves due to transition of electrons from stable state \textit{i.e.} highest occupied molecular orbital (HOMO) to excited state \textit{i.e.} lowest unoccupied molecular orbital (LUMO). When the HOMO-LUMO gap was altered, the $\lambda_{\text{max}}$ was also altered correspondingly [22]. Based on this, it is presumed the HOMO-LUMO gap in treated sample has been increase, which required higher energy to overcome the electronic transition and thus the $\lambda_{\text{max}}$ was shifted to higher energy (blue shift) as compared to the control.

4. Conclusion

In conclusion, the XRD study showed the crystalline nature of PMM in both the samples (control and treated). The intensity of XRD peaks and average crystallite size (6.64%) were increased after biofield energy treatment as compared to the control. The DSC study revealed the significant decrease in the latent heat of fusion (8.66%) in treated sample with respect to the control. The TGA-DTA study showed the slight increase in onset temperature as well as $T_{\text{max}}$ by 4.71% and 5.06%, respectively with respect to the control. This showed the increase in thermal stability of treated PMM. The UV analysis revealed the slight blue shift in $\lambda_{\text{max}}$ of treated sample with respect to the control. Moreover, the FT-IR analysis showed the increase in wavenumber of $\equiv\text{C-H}$ stretching after biofield treatment from 3132 cm$^{-1}$ (control) to 3161 cm$^{-1}$ (treated).

Overall, data suggest that Mr. Trivedi’s biofield energy treatment showed the considerable impact on the physical, thermal and spectroscopic properties of PMM. Based on this, it is expected that Mr. Trivedi’s biofield energy treatment can modulate the physicochemical properties of PMM so that it could be utilized as a better growth medium for the micropropagation of potato.

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