

Physicochemical Characterization of Biofield Energy Treated Hi VegTM Acid Hydrolysate

Mahendra Kumar Trivedi¹, Alice Branton¹, Dahryn Trivedi¹, Gopal Nayak¹, Ragini Singh², Snehasis Jana^{2,*}

¹Trivedi Global Inc., Henderson, USA

²Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, Madhya Pradesh, India

Email address:

publication@trivedisrl.com (S. Jana)

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Abstract: The hydrolysed vegetable proteins are acidic or enzymatic hydrolytic product of proteins derived from various sources such as milk, meat or vegetables. The current study was designed to evaluate the impact of biofield energy treatment on the various physicochemical and spectra properties of Hi VegTM acid hydrolysate *i.e.* a hydrolysed vegetable protein. The Hi VegTM acid hydrolysate sample was divided into two parts that served as control and treated sample. The treated sample was subjected to the biofield energy treatment and its properties were analysed using particle size analyser, X-ray diffraction (XRD), surface area analyser, UV-visible and infrared (FT-IR) spectroscopy, and thermogravimetric analysis. The results of various parameters were compared with the control (untreated) part. The XRD data showed the decrease in crystallite size of treated sample from 110.27 nm (control) to 79.26 nm. The particle size was also reduced in treated sample as 162.13 μm as compared to the control sample (168.27 μm). Moreover, the surface area analysis revealed the 63.79% increase in the surface area of the biofield treated sample as compared to the control. The UV-Vis spectra of both samples *i.e.* control and treated showed the absorbance at same wavelength. However, the FT-IR spectroscopy revealed the shifting in peaks corresponding to N-H, C-H, C=O, C-N, and C-S functional groups in the treated sample with respect to the control. The thermal analysis also revealed the alteration in degradation pattern along with increase in onset temperature of degradation and maximum degradation temperature in the treated sample as compared to the control. The overall data showed the impact of biofield energy treatment on the physicochemical and spectroscopic properties of the treated sample of Hi VegTM acid hydrolysate. The biofield treated sample might show the improved solubility, wettability and thermal stability profile as compared to the control sample.

Keywords: Protein Hydrolysate, Acid Hydrolysis, Hi VegTM Acid Hydrolysate, Hydrolysed Vegetable Protein

1. Introduction

The protein hydrolysates are derived through the acid or enzymatic hydrolysis of the proteins. The protein molecules are hydrolysed and degraded into the small fragments by the cleavage of peptide bond, hence they are also termed as peptides or peptones [1]. The protein hydrolysate can be derived from various sources such as milk, casein, animal derived (meat, collagen) and vegetable origin (wheat gluten, rice protein, pea protein, soy protein, *etc.*) [2]. The protein hydrolysates are used to improve the taste of food products. For example, soy and other vegetable proteins are used in the production of hydrolysed vegetable protein (HVP) that is commonly used in flavouring the meat products, sauces, and

soups [3]. The hydrolysed vegetable protein is traditionally produced by acid hydrolysis using the hydrochloric acid (HCl). The process includes hydrolysing in 10-20% HCl at atmospheric or elevated pressure followed by neutralization with NaOH [4]. The reason behind use of HCl is that the process is fast with high product yield and high aromatic profile [5]. However, some problems might occur in this process such as partial destruction or loss of amino acids and toxic by-product formation [6]. The acid hydrolysate of proteins is available in the form of pastes, liquids, granules, or powders and mainly composed of amino acids, small peptides, and salts [7]. The main differences between the acid hydrolysed and enzyme hydrolysed product are the colour and aromatic flavour. The acid hydrolysed products

possess strong aromatic flavour and dark brown in colour; whereas, the enzymatic products are much less meaty flavour and lighter in colour [8]. The hydrolysed vegetable proteins sometimes produced the flavours through the process of Maillard reaction and free amino acids and peptides are mainly responsible for the flavour production [9]. Beside, their use in food ingredients, some researches also reported their effect in increasing the plasma insulin response in case of both, the healthy subjects and type-II diabetes patients [10, 11]. The response was reported due to the impact of amino acids in the blood on alpha and beta pancreatic cells [12]. The literature reported the commercial use of hydrolysed vegetable protein directly by controlling the overall characteristics or after some processing [13, 14]. Besides, acid hydrolysis may reduce the reaction time but this process have many disadvantages as after hydrolysis the removal of residual acid is difficult and time consuming. In this work, the Hi VegTM acid hydrolysate *i.e.* a hydrolysed vegetable protein was given the biofield energy treatment and the impact on various physicochemical properties were analysed.

The concept of biofield originates from the biological energy field that is produced from the physiological processes and thoughts of the human beings [15]. Moreover, the living organisms can exchange this energy from the environment to maintain their health [16]. It is also taken under the healing therapies and recently known for its impact in reducing the anxiety and pain related problems [17]. The practitioners of these therapies channel the energy from the environment and sent it towards certain object [18]. Hence, a human can harness the energy from the environment and send it to any living or non-living object. After absorbing this energy, the object will respond in a better way; this process is termed as the biofield energy treatment. Mr. Trivedi is also known for his unique biofield energy treatment (The Trivedi Effect[®]) thereby causing alterations in various plants [19], microbes [20], metals [21], organic products [22], *etc.* The main aim of this study was to subject the Hi VegTM acid hydrolysate with the biofield energy treatment and evaluate the impact on its properties using several analytical techniques, such as particle size analyser, x-ray diffraction, surface area analyser, UV-visible spectroscopy, Fourier transform infrared spectroscopy, and thermogravimetric analysis.

2. Materials and Methods

The Hi VegTM acid hydrolysate was procured from HiMedia Laboratories, India and divided into two equal parts. For treatment, one part of the sample was handed over to Mr. Trivedi in a sealed pack; the other part was kept untreated and regarded as the control. In treatment methodology, Mr. Trivedi provided the biofield energy treatment to the treated part through his unique energy transmission process, without touching the sample. Further, the treated sample was characterized for various properties such as particle size, crystallite size, surface area, spectral properties and thermal

stability profile with respect to the control sample for analysing the impact of biofield energy treatment.

2.1. Particle Size Analysis

The Sympatec Helos-BF laser particle size analyser was used to determine the particle size of the control and treated samples. The instrument was having a detection range of 0.1 μm to 875 μm .

2.2. X-ray Diffraction (XRD) Study

The X-ray powder diffraction study was done using Phillips Holland PW 1710 X-ray diffractometer. The wavelength of radiation was chosen as 1.54056Å. The X-ray generator was operating at 35kV and 20mA and equipped with a copper anode with nickel filter. The diffractograms of control and treated samples obtained from the study were analysed and compared with respect to the Bragg's angle (2θ) and the average crystallite size.

2.3. Surface Area Analysis

The Brunauer–Emmett–Teller (BET) surface area analyser, Smart SORB 90 was used to calculate the surface area of the control and treated samples.

2.4. UV-visible (UV-Vis) Spectroscopic Characterization

The UV-Vis spectroscopic analysis was carried out using Shimadzu UV-2400 PC series spectrophotometer. The spectra of the control and treated samples were recorded using 1 cm quartz cell that has a slit width of 2.0 nm. The wavelength range was selected from 190 nm-400 nm.

2.5. Fourier Transform-Infrared (FT-IR) Spectroscopic Characterization

The FT-IR spectra of the control and treated samples were recorded using Shimadzu's Fourier transform infrared spectrometer (Japan). The frequency range was selected from 4000-450 cm^{-1} . The spectra were obtained in the form of wavenumber ($1/\text{cm}$) vs. percent transmittance (%T). The peaks were assigned according to the functional groups present in the sample and the frequency of peaks in the treated sample was compared with the control for determining the impact of biofield energy treatment.

2.6. Thermogravimetric Analysis/ Derivative Thermogravimetry (TGA/DTG)

The thermal degradation pattern of sample was analysed using Mettler Toledo simultaneous thermogravimetric analyser (TGA/DTG) in the temperature range from room temperature to 400°C. The analysis was carried out under air atmosphere with a heating rate of 5°C/min. The impact of biofield treatment was analysed by comparing the pattern of degradation, onset and maximum degradation temperature of the treated sample as compared to the control.

3. Results and Discussion

3.1. X-ray Diffraction (XRD)

The X-ray powder diffraction studies of control and treated samples of Hi VegTM acid hydrolysate showed a series of peaks in their diffractograms (Fig. 1). The XRD diffractogram of the control sample showed the peaks at 2θ equal to 31.71° , 45.45° , 45.59° , and 56.46° . Besides, the treated sample showed the peaks at 2θ equal to 32.07° , 45.75° , and 56.77° . The data showed that peaks of the treated sample were slightly shifted towards higher angle as compared to the control. The internal strain produced within any compound can alter the positions and shape of X-ray diffraction peaks [23]. Hence, it is assumed that the biofield energy treatment might produce

some internal strain within the treated sample. Moreover, the average crystallite size of the samples was determined using Scherrer equation [24]. The results showed that the crystallite size of control and treated sample was 110.27 and 79.26 nm, respectively. The crystallite size data also supported the presence of internal lattice strain as the increase in lattice strain may reduce the crystallite size of the sample [25, 26]. Thus, the XRD data showed that biofield energy treatment might induce some lattice strain within the treated sample that caused the fracturing of grains into sub grains and resulted in 28.12% decreased crystallite size (Fig. 2) of the treated sample with respect to the control. The decreased crystallite size of the treated sample might improve its solubility when used in food ingredients as compared to the control [27].

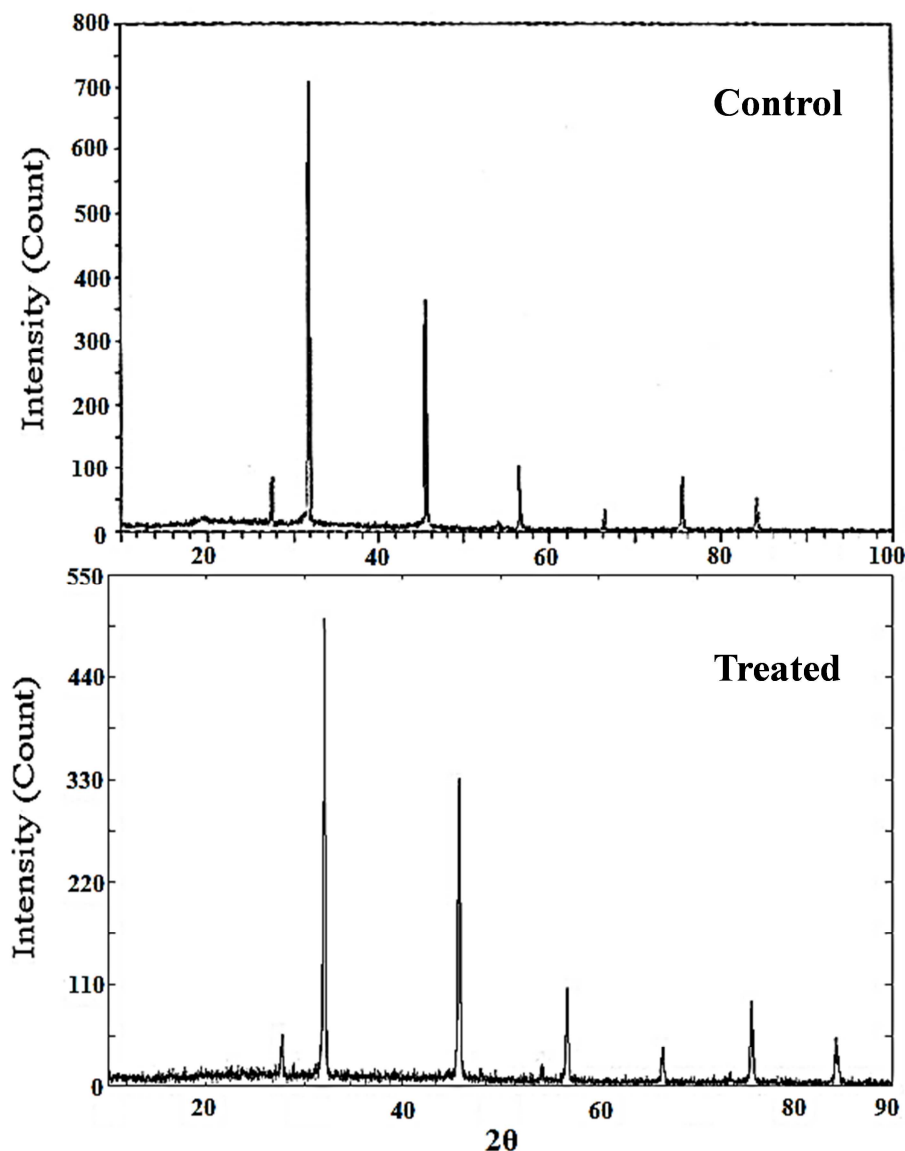


Fig. 1. X-ray diffractograms of control and treated samples of Hi VegTM acid hydrolysate.

3.2. Particle Size Analysis

The particle size analysis revealed that the control sample showed the d_{99} (particle size below that 99% particles are

present) of $168.27 \mu\text{m}$ while the treated sample showed a d_{99} of $162.13 \mu\text{m}$. The result showed a slight decrease (3.65%) in the particle size of the treated sample as compared to the control (Fig. 2). It was also supported by the crystallite size

analysis as both are directly related to each other [28]. Besides, the wettability and dispersibility are the important factors in the food formulations that depend on the polarity, texture, area and the size of the protein particles. The particle size of proteins has its impact on the colour, appearance, and mouth feel of the food material in which it is used [29]. Hence, the biofield treatment of the sample may increase its wettability due to reduction in particle size that further help to improve the appearance and mouth feel of the food material.

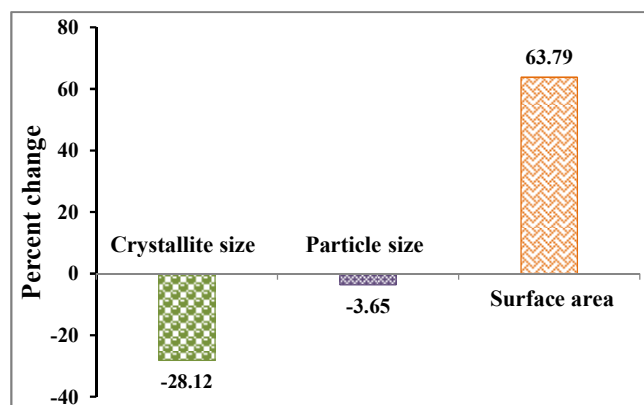


Fig. 2. Percent change in crystallite size, particle size, and surface area of the treated Hi VegTM acid hydrolysate as compared to the control.

3.3. Surface Area Analysis

The surface area of control and treated samples of Hi VegTM acid hydrolysate was investigated and the data reported that the treated sample showed a surface area of 0.095 m²/g as compared to the control sample (0.058 m²/g). It showed that the surface area of the treated sample was increased by 63.79% (Fig. 2) after the biofield energy treatment. Some studies had reported the impact of altered crystallite size and particle size on the surface area [30]. Hence, the resultant increase in the surface area of treated sample might be due to the decrease in crystallite size and particle size after the biofield treatment. Moreover, the increased surface area helped in increased interaction between the protein molecules and water that resulted in increased swelling and viscosity of the protein dispersion. It was reported that the gelling ability of protein provides a base for holding the water, sugar, flavours, and food ingredients [29]. Thus, the biofield treated sample with increased surface area may be more useful in the food industry as compared to the control.

3.4. UV-Vis Spectroscopic Analysis

The absorption peak is described by the excitation of one electron from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). LUMO represents the ability to accept an electron, whereas HOMO represents the ability to donate an electron [31]. The UV spectra of the control and treated samples are shown in Fig. 3. The spectrum of control sample showed the absorption peak at λ_{\max} equal to 265 nm. Moreover, the biofield treated sample also showed peak at same wavelength *i.e.* at 267 nm. Hence, it is hypothesized that biofield energy treatment might not affect

this HOMO→LUMO transition due to which the peak was observed at same position in the treated sample as compared to the control sample.

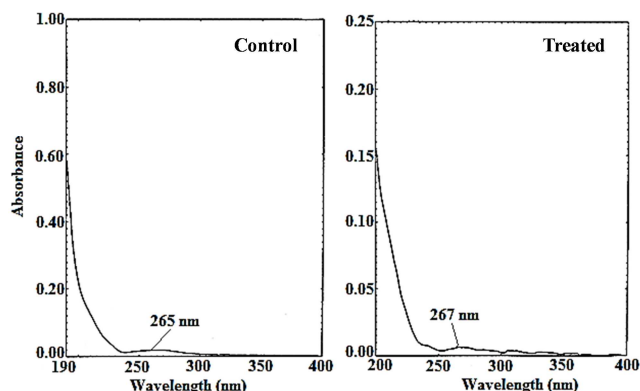


Fig. 3. UV-Vis spectra of control and treated samples of Hi VegTM acid hydrolysate.

3.5. FT-IR Spectroscopic Analysis

The FT-IR spectra of Hi VegTM acid hydrolysate (control and treated samples) are shown in Fig. 4. The compound mainly contains amino acids and carbohydrates along with some volatile components that are responsible for the flavouring properties. These include 2-butanone, dimethyl disulphide, furfural, 3-methyl thiopropanal, *etc.* [32]. Hence, the major vibration peaks observed (Table 1) were assigned to the functional groups present in these components. The peak at 3226 cm⁻¹ in the control sample was assigned to the N-H stretching peak of the amino acids. However, in the treated sample the corresponding peak was observed at higher frequency *i.e.* at 3411 cm⁻¹. The peak at 3080 cm⁻¹ in the control sample was assigned to NH₃⁺ antisymmetric stretching of amino acids; however the peak might get merged with the aromatic C-H stretching peaks [33]. Besides, in treated sample the peak was shifted to a higher frequency at 3101 cm⁻¹. Similarly, the C-H stretching peaks of carbohydrates and other volatile components were appeared at 2893 and 2815 cm⁻¹ in the control sample, whereas, in the treated sample, the peaks were appeared at 2898 and 2833 cm⁻¹, respectively. Besides, the overtone of C-H bending of aldehyde group present in volatile substances was assigned to the peak at 2767 cm⁻¹ in control sample; however the corresponding peak was not observed in the treated sample. Similarly, the C=O stretching of furfural was assigned to the peak at 1762 cm⁻¹, which was not observed in the treated sample [34]. Moreover, the C=O stretching of amide group was observed at 1635 and 1622 cm⁻¹ in the control and treated sample, respectively. The NH₃ deformation peak and CH₃ asymmetric bending peak were assigned to peaks at 1577 and 1438 cm⁻¹, respectively in the control sample; however, the corresponding peaks were not observed in the treated sample. The O-H bending peak (carboxylic acid) and C-N stretching peak (amide) were merged and appeared at 1400 and 1406 cm⁻¹ of control and treated samples, respectively. The aldehydic C-H bending peak was observed at 1336 cm⁻¹ in the control sample while

1348 cm^{-1} in the treated sample. The peaks at 1161 and 1068 cm^{-1} in the control sample were assigned to C-O stretching and C-N stretching, respectively; however, the corresponding peaks were observed at 1143 and 1085 cm^{-1} in the treated sample. The NH_2 wagging peak was observed at same frequency in both, the control and treated samples *i.e.* at 844 cm^{-1} [33]. The C-S stretching (sulphide) of dimethyl disulphide and C-C-CHO bend of aldehydes was assigned to the peak at 655 cm^{-1} in the control while 640 cm^{-1} in the treated sample [35]. Besides, the C-CO-C bending of ketone was assigned to peak at 561 cm^{-1} in the control and 526 cm^{-1} in the treated sample.

The observation showed the alterations in the frequency of several peaks in the treated sample such as N-H, C-H, C=O, C-N, C-S, *etc.* as compared to the control. Hence, it was assumed that the biofield energy treatment might affect the treated sample with respect to the bond length, bond angle, or dipole moment of the corresponding functional groups.

Table 1. Vibration modes observed in *Hi VegTM* acid hydrolysate.

S. No.	Functional group	Wavenumber (cm^{-1})	
		Control	Treated
1	N-H stretching	3226	3411
2	NH_3^+ stretching	3080	3101
3	C-H stretching	2893, 2815	2898, 2833
4	C-H bending (overtone)	2767	ND
5	C=O stretching (carbonyl)	1762	ND
6	C=O stretching (amide)	1635	1622
7	NH_3 deformation (amino acids)	1577	ND
8	CH_3 bending (aliphatic)	1438	ND
9	O-H bend (carboxylic acid), C-N stretching (amide)	1400	1406
10	C-H bend (aldehyde)	1336	1348
11	C-O stretching	1161	1143
12	C-N stretching	1068	1085
13	NH_2 wagging	844	844
14	C-S stretching (sulphide), C-C-CHO bend	655	640
15	C-CO-C bending (ketone)	561	526

ND: Not detected

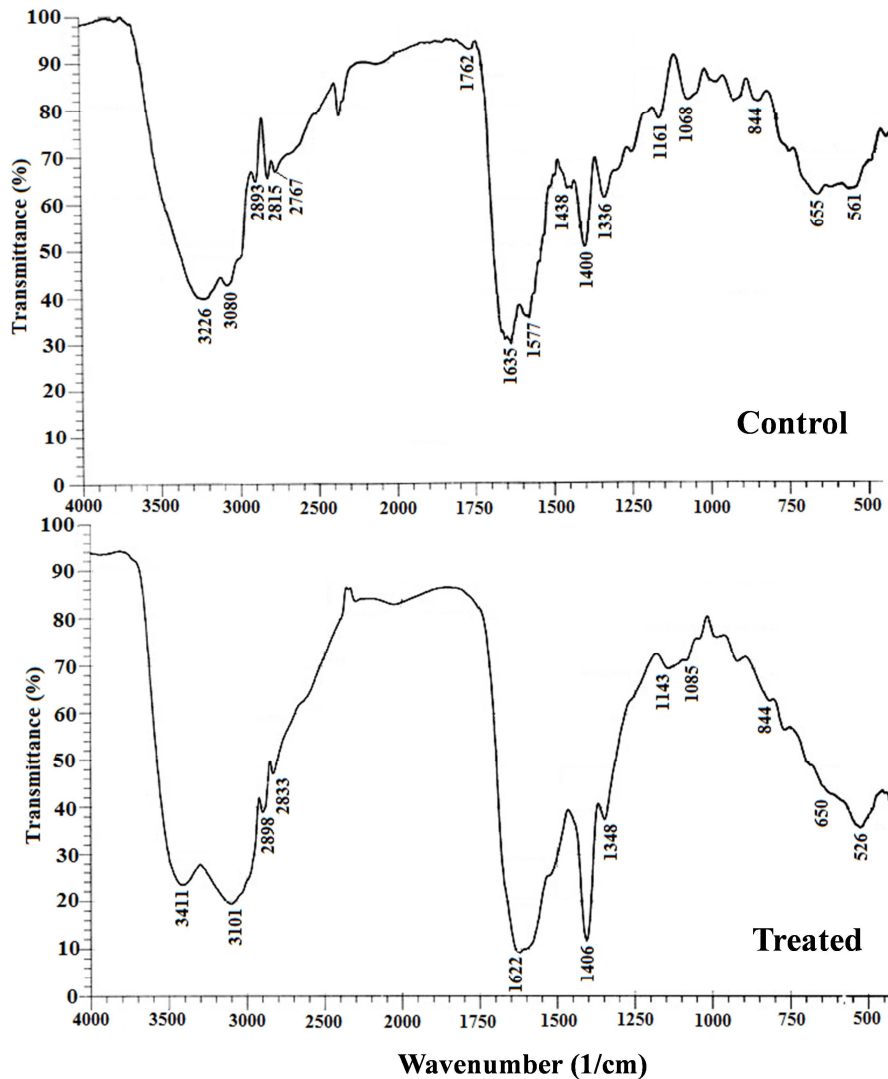


Fig. 4. FT-IR spectra of control and treated samples of *Hi VegTM* acid hydrolysate.

3.6. TGA/DTG Analysis

The TGA/DTG studies analyse the thermal degradation behaviour as well as the thermal stability of the compound. The analysis was done by evaluating the temperature at which weight loss of the sample occurs along with the maximum degradation temperature. The TGA/DTG thermograms of the control and treated samples of Hi VegTM acid hydrolysate are reported in Fig. 5. The TGA thermogram of control sample showed the presence of three-step degradation. The first step was observed as a slight reduction in weight at 54°C followed by second and third step at 192°C and 260°C, respectively. However, the treated sample showed a single step of degradation that started at 173°C and ended at 261°C. Besides, the DTG thermogram data showed that T_{max} was observed at 204.33°C in the control sample while 214.14°C in the treated sample. The thermal study data indicated that the pattern of degradation of the treated sample was changed from three-step

(control) to single step. Also, the onset temperature of degradation was shifted from 54°C (control) to 173°C in the treated sample. The T_{max} was also delayed by 10°C in the treated sample as compared to the control. Hence, the overall studies suggest an increase in the thermal stability of the treated sample due to increase in onset temperature followed by the T_{max} value. Besides, during long term storage and cooking of food materials, some physicochemical changes might occur in the protein content that further lead to change in the colour and texture [29]. The change may also include decreased digestibility and loss of some reactive amino acids. A common example is Maillard reaction that causes browning reaction by involving the reducing sugar and amino groups of proteins [4]. Hence, the biofield treatment might be used as an effective measure to increase the thermal stability, thereby increasing the quality and shelf-life of the food materials containing these compounds.

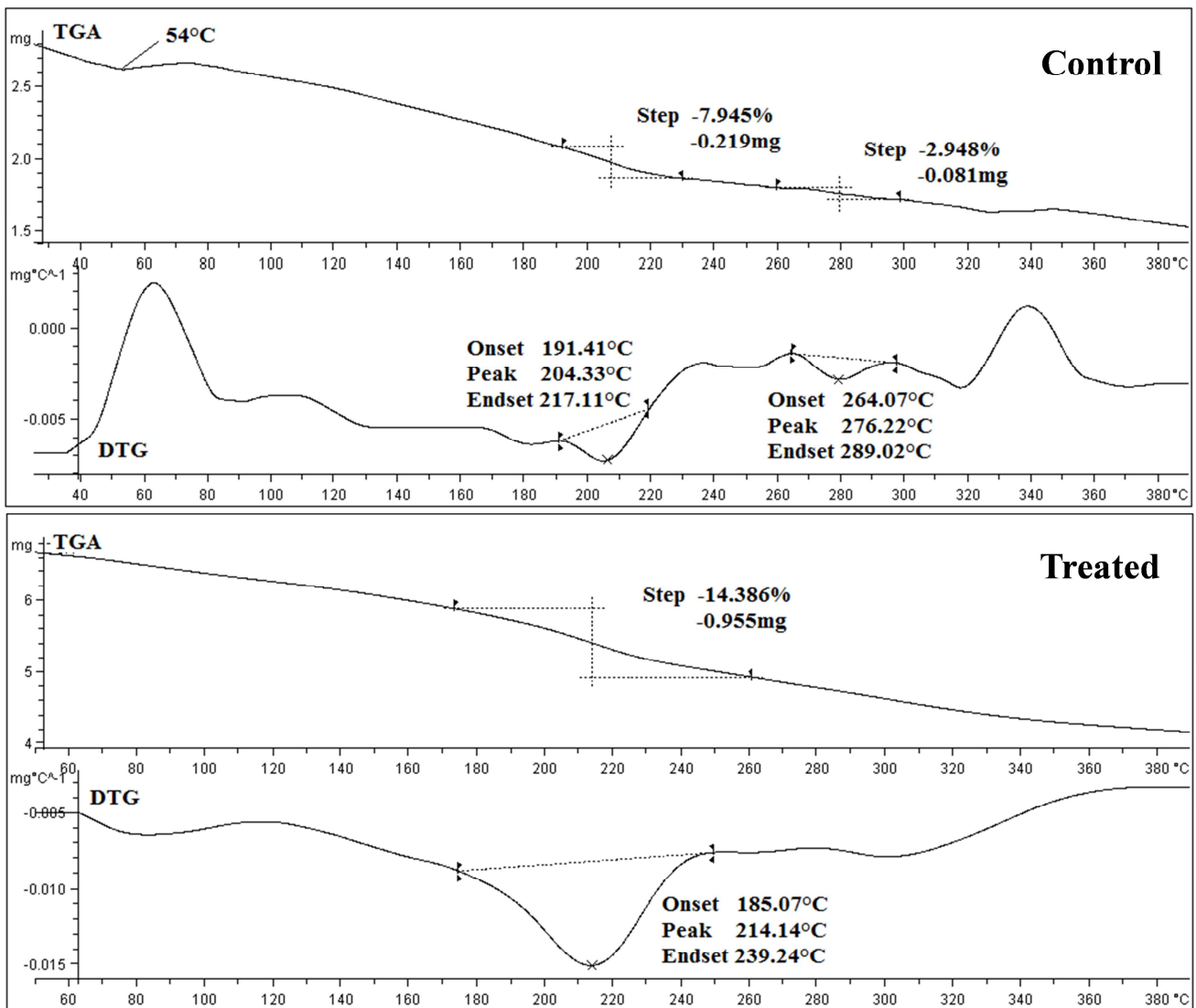


Fig. 5. TGA/DTG thermograms of control and treated samples of Hi VegTM acid hydrolysate.

4. Conclusions

The biofield treated sample showed a significant decrease in crystallite size (28.12%) and particle size (3.65%) that suggested the presence of internal strain within the molecules. The surface area was found increased by 63.79% in the treated sample that also supported the data of crystallite size and particle size. Moreover, the FT-IR spectroscopy revealed the alteration in the frequency of peaks corresponding to various functional groups in the treated sample such as N-H, C-H, C=O, C-N, C-S, *etc.* The thermogravimetric analysis revealed the increase in onset temperature of degradation and T_{max} that suggested the increased thermal stability of the treated sample as compared to the control. The overall data revealed the impact of biofield energy treatment on the physical, spectroscopic and thermal properties of the Hi VegTM acid hydrolysate sample. The biofield treated sample might show good solubility, wettability and gelling ability along with improved heat stability when used in food industry.

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