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Analysis of crystallization phenomenon in Indian honey using molecular dynamics simulations and artificial neural network

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ABSTRACT

Molecular dynamics simulation was performed on sugar profile and moisture content-based mixture systems of six Indian honey samples. Comparative studies were performed to understand the interactive effects of fructose, glucose, sucrose, maltose and water on crystallization. All simulations led to formation of stable crystal but with different interaction energies. Post-simulation analysis showed that Fructose:Glucose of 1.18 formed the most stable crystal with highest van der Waals and electrostatic interactions. The stability of crystal was further validated with least gyration radius (209 ± 1.81 nm^2), accessible surface area (4.09 ± 0.04 nm) and root mean square displacement (3.51 ± 0.00261 nm). Results indicated that not only Fructose:Glucose ratio but also sucrose, maltose and water had a significant effect on the overall crystallization process. The simulation data was used to train the artificial neural network which predicted the stability of honey crystallization depending on Fructose:Glucose and Glucose:Water ratios.

1. Introduction

Honey is a saturated solution of sugars, out of which 75% are monosaccharides and 10–15% are disaccharides (Nayik, Dar, & Nanda, 2016; White, 1975). Such high amount of sugars may result in the crystallization issues which is undesirable as it leads to increased water activity due to reduced interaction of hydroxyl functional group with water molecules (Laos, Kirs, Pall, & Martverk, 2011).

Honey crystallizes in form of α-D-Glucose monohydrate and shows temperature based anomerism above 115 °C with further increase in temperature leading to the melting of dried glucose crystals. (Srisa-nga, Flood, & White, 2006; Venir, Spaziani, & Maltini, 2010). It has already been established that D-Glucose reduces the solubility of sucrose in water while increasing the total sugar content. Likewise, sucrose and fructose are known to reduce the solubility of D-Glucose (Dyce, 1975; Jackson & Silsbee, 1924). Rapidly crystallizing honey has high D-Glucose content (> 28–30%), whereas high fructose levels in honey renders it impossible to form crystals (Jamieson, 1954; Phillips, 1929). Glucose is convenient as an indicator for granulation in honey only when it is in lower (< 28%) or in higher amounts (> 38%) (Manikis & Thrasivoulou, 2001), but the role of other sugars in crystallization has still been a point of interest to many researchers.

Major work on honey crystallization has been carried out in terms of changes in glass transition temperatures of glucose (Dettori, Tappi, Piana, Rosa, & Rocculi, 2018; Nurul Zaizuliana et al., 2017; Venir et al., 2010) but crystallization based on molecular dynamics approach haven't been explored yet in honey. According to Rapaport (2004), molecular dynamics (MD) is a computer simulation technique that predicts the particle interactions in a system wherein the initial conditions as well as interaction parameters are specified and further analysed using the Newtons equations of motion.

Molecular dynamics simulations resolve motion equation provided by Newton for a system of n interacting atoms as:

$$m_i \frac{\delta^2 ri}{\delta t^2} = F_i,\ i = 1 \cdots .n$$

where m_i is mass, r_i is the position of the particle concerning time t, and the forces are the negative derivatives of a potential function V (R_1, R_2..... Rn):

$$F_i = \frac{\delta V}{\delta R_i}$$

The equations are resolved concurrently in trivial steps. The system is trailed for a particular period, wherein the temperature and pressure are kept at the desired values, and the coordinates are inscribed to an output at regular periods (Rapaport, 2004). System positions are

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corelated to the coordinates which is a function of time that represent a trajectory of the system. After initial changes, the system will usually reach an equilibrium state and by averaging over an equilibrium trajectory, many macroscopic properties can be extracted from the output file (Lindahl, Hess, & Spoel, 2001).

MD tools have been used by many researchers to simulate and calculate various complex chemical reactions and energies (Antonija, Pablo, & Modesto, 2019; Bhowmik, Sihn, Varshney, Roy, & Vernon, 2019; Xu, Mandal, Larson, Wang, & Wang, 2019). Post MD processing can provide interaction energies (electrostatic and van der Waals interactions) and radius of gyration which can be significantly utilized to understand the affinity between molecules and significance of crystal compactness during crystallization process in honey (Monajjemi & Oliaey, 2009).

Numerous research works have been undertaken regarding chemical composition, characterization, rheology and storage studies of honey (Dettori et al., 2018; Kabbani, Sepulcre & Wadekind, 2011; Kamal & Klein, 2011; Nayik et al., 2016; Nurul Zaizuliana et al., 2017; Oroian, Amariei, Rosu, & Gutt, 2015; Venir et al., 2010) but very low emphasis has been given to the crystallization mechanism in honey using MD. In the present investigation, MD simulation was carried out on honey samples from India based on its sugar profile and moisture content to understand the crystal stability of each variety. Here the crystal stability refers to the interactive forces that act between the molecules of sugars for which the molecular dynamics simulations were performed. The higher interactive forces acting between the molecules are indicative of the stability of the formed crystals. Further, the stability of crystals can be predicted using Artificial neural networking approach to establish a network based on the sugar and moisture ratios.

Artificial neural networks are created to mimic the working and pattern recognition skills of a human brain and is classified in an orderly assembly to allow its applications in complex data analysis and the processes where manual mathematical models cannot be easily applied (Basheer & Hajmeer, 2000). A properly trained ANN can process and learn patterns efficiently from specimens through iterations without any prior data of associations between variables under investigation (Karimi, Rafiee, & Garavand, 2012; Tarafdar, Shahi, Singh, & Sirohi, 2018). ANN is cost-effective and capacitate optimization algorithms which are due to their capability to model functions with precision during training and validation protocols (Silveira, Belledeli, Soares, Treichel, & Mazutti, 2014).

Till date, ANN has been utilized for various applications and are proving to be valuable tools in fields of quality analysis, food safety, microbial growth modeling, chemical data interpretation, prediction of functional properties of foods during various unit operations (Haugen & Undeland, 2003; Huang, Kangas, & Rasco, 2007; Lou & Nakai, 2001; Martin, Oliveros, Paѵon, Pinto, & Cordero, 2001; Natale et al., 2001; Oroian et al., 2015; Ruan, Almaer, & Zhang, 1995).

As per our knowledge, this is the first investigation using molecular dynamics approach and application of artificial neural network to understand the interactive effect of sugars on the crystallization stability in Indian honey samples. This investigation will help understand the mechanism of crystallization phenomena which can be a boon to honey processing industries as well as for beekeepers.

2. Materials and methods

2.1. Reagents and chemicals

HPLC grade standards of fructose, glucose, sucrose and maltose were obtained from Sigma Aldrich (St. Louis, Missouri, USA). All the reagents and chemicals used for analysis were of analytical grade and procured from LOBA Chemie (Mumbai, India).

2.2. Honey sample collection

Six Indian honey samples were acquired from Royal apiary, Punjab. The samples were diverse in botanical origin in which all the samples were multi-floral except sample H4 and H6. All the samples were heated below 40 °C to melt any pre-formed crystals and stored at a refrigerated temperature in glass containers until further analysis.

2.3. Sugar profile determination

Waters Breeze Chromatographic system (Waters, Milford, Massachusetts, USA) with 2414 RI detector was used for sugar profile determination in which ACQUITY UPLC BEH Amide column (2.1 × 50 mm, 1.7 µm) was the stationary phase. A mixed standard stock solution of glucose, fructose and sucrose were made as mentioned by Xu, Liang, and Zhu (2015) by dissolving, 0.902 g glucose, 1.648 g fructose and 2.970 g sucrose in 100 mL water. The working solutions of sugars were prepared by pipetting 0.4, 0.8, 1.2, 1.6 and 2.0 mL of the above mixed standard stock solution into five different 2 mL amber glass volumetric flasks, and then made up to the mark with water. Maltose solution (10.095 g/L) was prepared by dissolving 2.019 g maltose in 200 mL deionized water. 1 g of each sample was diluted in 5 mL diluent (Water: Acetonitrile, 70:30) and allowed to dissolve. This sample diluent was further diluted by adding its 1 mL to 9 mL of fresh diluent which was then passed through a nylon filter (0.22 µm and 47 mm dia, Sigma Aldrich) and stored in HPLC vials. The final concentration of the sample was 20 mg/mL. For analysis the flow rate was kept at 1.5 mL/min.

2.4. Moisture content

ARICO Abbes refractometer (Advanced Research Instruments, New Delhi, India) was used to determine the moisture content of samples using the refractometric method (IHC-2009). The refractive indices of honey samples were measured at ambient temperature, and the readings were corrected for a standard temperature of 20 °C

2.5. Simulation tools

GROMACS (GROningen MAchine for Chemical Simulation) version 5.14, a molecular dynamics package mainly designed for simulation of various biomolecules was used to explore the crystallization phenomenon. As the study was mainly based on the molecular interactions, the molecule structures of glucose, fructose, sucrose and maltose and their forcefield topologies were obtained from ATB online database (Automated Topology Builder and Repository) version 3 (ATB, 2019; Malde, 2011). GROMOS-54A7 Forcefield was used to describe the interaction behaviour between particles (Poger, Van Gunsteren, & Mark, 2010). The structures obtained were saved as program database files that can be read by GROMACS. For the simulation, a total of 500 molecules were packed as a single mixture database file in context to the quantities obtained from sugar profile and moisture content determination. Total of thirteen MD simulations was conducted out of which one was standard glucose. Among the six Indian honey samples, total subsets were bifurcated into two batches, A and B. Batch A had six honey samples in which glucose, fructose, sucrose, maltose and water were variable components as obtained from the analysis. The later six samples in batch B had only glucose and fructose varied while keeping other elements consistent to the average of sucrose, maltose and water of batch A samples. Comparative analysis among all was carried out to find how these sugars interact and affect the crystallization phenomenon. For each simulation, the unit cell of 8 nm × 8 nm × 8 nm cubic box was defined and restraints for sugar and water were generated and included in topology files of each. Simple Point Charge model (SPC) was used for modelling water molecule behaviour, in agreement with the evaluation carried by Yun et al. (2017). All systems were set in the

Table 1
Sugar composition and moisture content of Indian honey samples. [#] (n = 6).

	H1	H2	H3	H4	H5	H6
Fructose (%)	42.8 ± 0.409[a]	36.8 ± 0.37[d]	33.4 ± 0.312[e]	40.3 ± 0.225[b]	38.6 ± 0.202[c]	35.9 ± 0.203[d]
Glucose (%)	27.9 ± 0.121[c]	31.2 ± 0.449[b]	35.9 ± 0.162[a]	26.1 ± 0.267[d]	31.3 ± 0.431[b]	30.7 ± 0.36[b]
Sucrose (%)	8.56 ± 0.121[a]	4.21 ± 0.201[d]	5.62 ± 0.272[c]	7.58 ± 0.0981[b]	1.8 ± 0.275[e]	6.99 ± 0.389[b]
Maltose (%)	2.71 ± 0.189[b]	2.02 ± 0.0635[b]	1.65 ± 0.311[d]	2.24 ± 0.458[a]	1.34 ± 0.334[c]	1.84 ± 0.181[a]
Moisture (%)	16.8 ± 0.00785[c]	17.9 ± 0.0458[a]	16.3 ± 0.039[e]	17 ± 0.0197[b]	14.8 ± 0.0183[f]	16.4 ± 0.0222[d]
TSS (°brix)	81 ± 0.00976[d]	80 ± 0.0189[f]	82.3 ± 0.0152[b]	80.7 ± 0.0394[e]	83.2 ± 0.012[a]	81.2 ± 0.0268[c]
FG Ratio	1.54	1.18	0.931	1.54	1.23	1.17
GW Ratio	1.65	1.75	2.21	1.53	2.11	1.87

[#] Values are means ± SD Means in a row without a common superscript letter differ ($P < 0.05$) as analysed by one-way ANOVA and the DUNCAN multiple range

same conditions as 298 K of temperature. Bond lengths and angles were fixed using LINCS constraints and equation of motion was integrated using Leapfrog integrator with a time stop of 2 fs. Total steps were 1×10^6 and for output, every coordinate, velocity and energies were saved after every 1 ps. Moreover, the short-range electrostatic cut-off was 1.4 nm and Particle Mesh Ewald (PME) was used for long-range electrostatics to gain the best consequence. Steepest descent algorithm was used to reduce the potential energy of the system. For each system, 200 ps canonical ensemble (NVT) + 200 ps isothermal-isobaric ensemble (NPT) + 30 ns MD simulations were executed under the periodic boundary condition.

2.6. Post MD analysis

GROMACS module VMD (Visual Molecular Dynamics) viewer version 1.9.3 was used to visualize the simulation trajectories. Fluctuations in the structure were determined using Root Mean Square Displacement (RMSD) function. Energetics were calculated using GMX energy function of GROMACS in which the electrostatic, van der Waals interaction energies and total energies of the systems were quantified. Cut-off radii for Coulomb and van der Walls interactions were 1.4 nm. Compactness of the crystal structure was calculated using the radius of gyration. Surface behaviour was computed using the solvent accessible surface area (SASA).

2.7. Statistical analysis

Mean values from analyses were analysed for significant differences at $p \leq 0.05$ by one-way ANOVA and the Duncan Multiple Range Test (DMRT) using web-based software which is freely available online, coded using the R language (Houssein, Zhou, Carroll, & Wu, 2014).

2.8. Artificial neural networking

MATLAB® version R2016a was used which provides an interactive environment for numerical computation, visualization, and programming. Neural network tool was used in which 1000 epoch were used for training the system with data obtained from post MD analysis. The performance of system was measured using plot regression function. Neural network formed included 2 inputs, 10 hidden layers and 1 output layer. Input layers include FG ratio and GW ratio, and output value obtained was interaction energies that could be related to crystallization tendency of honey.

3. Results and discussion

3.1. Sugar profile and moisture content

The results of HPLC analysis of all 6 Indian honey samples are shown in Table 1. Fructose was dominant among all sugars followed by glucose, sucrose and maltose in all the samples. According to Austin (1953) and Escuredo, Dobre, Fernández-González, and Seijo (2014), the crystallization propensity of honey is due to FG ratio and GW ratio, i.e. lower the GW or FG ratio, higher will be the tendency of glucose to crystallize. Every sample demonstrated distinct FG and GW ratios with the FG and GW ratio ranging from 0.93 to 1.54 and 1.53–2.21. This is also in agreement with the literature in which the FG ratio lower than 1.5 showed higher crystallization rate (Draiaia et al., 2015; Manikis & Thrasivoulou, 2001). The amount of sucrose in all samples except H5 was higher than other honey samples studied from India (Saxena, Gautam, & Sharma, 2010). The Moisture content of the sample ranged in between 15 and 18 % which is in accordance to codex standards for honey and was well under the range to avoid spoilage due to yeast.

3.2. Post simulation analysis

The data regarding post MD simulation analysis are presented in Table 2. Results are discussed under the following headings namely visual inspection, basic energetics (kinetic, potential and total energy), interaction energies (electrostatic and van der Waals interactions) and stability (SASA, the radius of gyration, RMSD).

3.2.1. Visual inspection

Every sample run was visualized in VMD 1.9.3 which displays, animates and can analyse the biomolecular systems. Every sample showed similar behaviour in which nucleation and crystal growth was visualized ending with a stable crystal at the end of the simulation (Fig. 1). Initially at 0 ps, the randomized sugar molecules were visibly spread throughout the system, followed by nuclei development which can be seen to enhance further to form a cluster and ending with a crystal. Although there were differences in the time span of crystallization but every sample showed the similar tendency of growth. Similar visualization of 1000 ps was provided in results obtained by Yun et al. (2017).

3.2.2. Basic energetics

Total Energy is the combination of kinetic and potential energies. Potential energy is a forcefield specific quantity so the energy of a same exact configuration of a system under different forces will be distinct whereas the kinetic energy is independent of forcefields so while discussing total energy, its relevance is mainly towards the potential energy (Cramer, 2013). As enlisted in Table 2, the kinetic energy of the system after the MD run of 30000 ps ranged between 6800 KCal/mol with standard glucose showing lowest average kinetic energy throughout its simulation this could be an indication of lower reactivity of system and higher overall stability which was followed by sample H2. Sample H4 showed the highest kinetic energy throughout which could also be indicative of its instability than compared to the remaining samples.

3.2.3. Interaction energies

In molecular mechanics, the atoms are considered as spheres and

Table 2
Post MD simulation analysis.

	Batch A					
	H1	H2	H3	H4	H5	H6
Kinetic energy (KCal/mol)	6820 ± 34.8^{a}	6420 ± 34.1^{d}	6710 ± 34.9^{b}	6780 ± 35.5^{ab}	6530 ± 34.8^{c}	6790 ± 35.6^{ab}
Potential Energy (KCal/mol)	21000 ± 34.1^{d}	19200 ± 50.4^{i}	19800 ± 43.7^{h}	21400 ± 24^{b}	20200 ± 25.1^{f}	21000 ± 23.9^{d}
Total Energy (KCal/mol)	27800 ± 61.3^{c}	25600 ± 47^{h}	26500 ± 43.8^{g}	28100 ± 24.5^{a}	26800 ± 25.8^{f}	27700 ± 23.9^{c}
Electrostatic Interaction (KCal/mol)	−14000 ± 5.83^{b}	−17200 ± 10.3^{g}	−19100 ± 6.07^{k}	−13700 ± 8.76^{a}	−17500 ± 7.51^{i}	−16600 ± 11.2^{f}
van der waals Interaction (KCal/mol)	−358 ± 14.1^{ab}	−487 ± 20de	−532 ± 19.8^{ef}	−328 ± 12.7^{a}	−496 ± 14.2^{de}	−363 ± 14.5^{ab}
SASA (nm^2)	216 ± 2.09^{fg}	213 ± 1.28^{gh}	218 ± 1.7^{fg}	248 ± 1.69^{b}	237 ± 1.16^{d}	268 ± 1.21^{a}
Gyration Radius (nm)	4.14 ± 0.00781^{de}	4.13 ± 0.0436d^{e}	4.46 ± 0.036^{c}	5.9 ± 0.0126^{a}	4.19 ± 0.00981^{d}	3.91 ± 0.0452^{g}
RMSD (nm)	5.55 ± 0.0559^{g}	5.76 ± 0.0295^{e}	5.82 ± 0.0232^{de}	6.86 ± 0.0186^{b}	5.42 ± 0.0117^{h}	5.64 ± 0.0384^{fg}
	Batch B						
	H7	H8	H9	*H*10	*H*11	H12	Glucose
Kinetic energy (KCal/mol)	6580 ± 34.4^{c}	6580 ± 34.5^{c}	6580 ± 34.9^{c}	6580 ± 34.4^{c}	6580 ± 35.1^{c}	6580 ± 34.4^{c}	6090 ± 33.1^{e}
Potential Energy (KCal/mol)	21000 ± 46.5^{d}	20100 ± 42.1^{g}	21700 ± 24.9^{a}	21200 ± 42^{c}	21400 ± 26.5^{b}	20600 ± 43^{e}	14700 ± 47.9^{j}
Total Energy (KCal/mol)	27600 ± 46.6^{d}	26700 ± 42.7^{f}	28300 ± 25.7^{a}	27800 ± 43.8^{c}	28000 ± 27.1^{b}	27100 ± 43.3^{e}	20800 ± 50.8^{i}
Electrostatic Interaction (KCal/mol)	−15200 ± 9.61^{d}	−20000 ± 6.32^{l}	−15600 ± 9.01^{e}	−15100 ± 8.13^{c}	−17300 ± 10.9^{h}	−17900 ± 10.9^{j}	−31200 ± 9.58^{m}
van der waals Interaction (KCal/mol)	−377 ± 14.4^{bc}	−641 ± 18.6^{g}	−422 ± 13.8^{c}	−386 ± 14.3^{bc}	−469 ± 15.6^{d}	−551 ± 17.8^{f}	−2650 ± 11^{h}
SASA (nm^2)	219 ± 1.52^{f}	209 ± 1.81^{hi}	242 ± 1.42^{c}	225 ± 1.37^{e}	252 ± 1.27^{b}	219 ± 1.66^{f}	205 ± 1.29^{i}
Gyration Radius (nm)	5.09 ± 0.0107^{b}	3.51 ± 0.00261^{i}	3.73 ± 0.0394^{h}	4.43 ± 0.0428^{c}	5.19 ± 0.0404^{b}	4.01 ± 0.0416^{f}	4.06 ± 0.0325^{ef}
RMSD (nm)	6.54 ± 0.0390^{c}	4.09 ± 0.045^{j}	4.79 ± 0.0508^{i}	5.73 ± 0.0301^{ef}	5.88 ± 0.00866^{d}	5.53 ± 0.0289^{g}	7.64 ± 0.0406^{a}

Values are means ± SD, means in a row without a common superscript letter differ ($P < 0.05$) as analysed by one-way ANOVA and the DUNCAN multiple range test (DMRT).

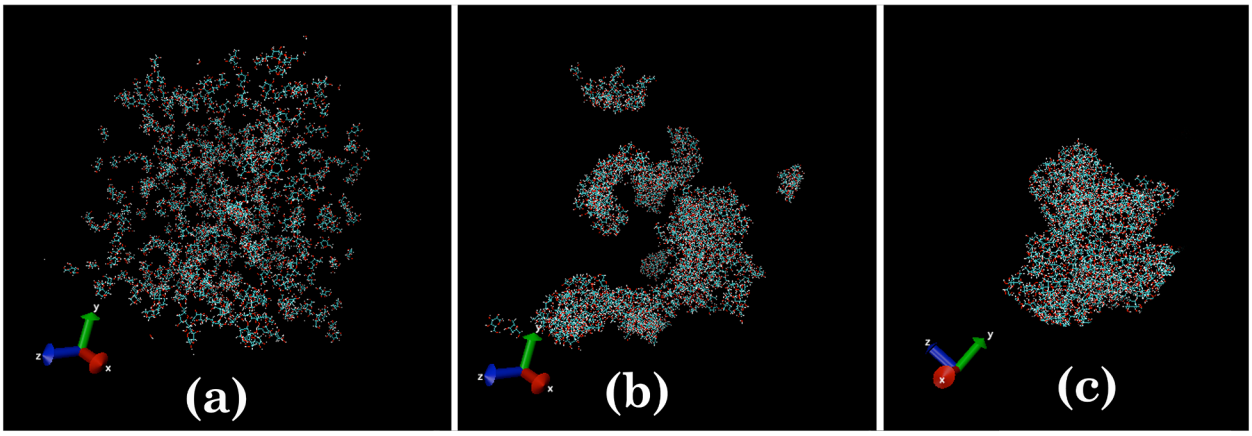

Fig. 1. Visual trajectories of sample H8 with FG ratio of 1.18 at (a) initial, (b) intermediate and (c) end of the simulation.

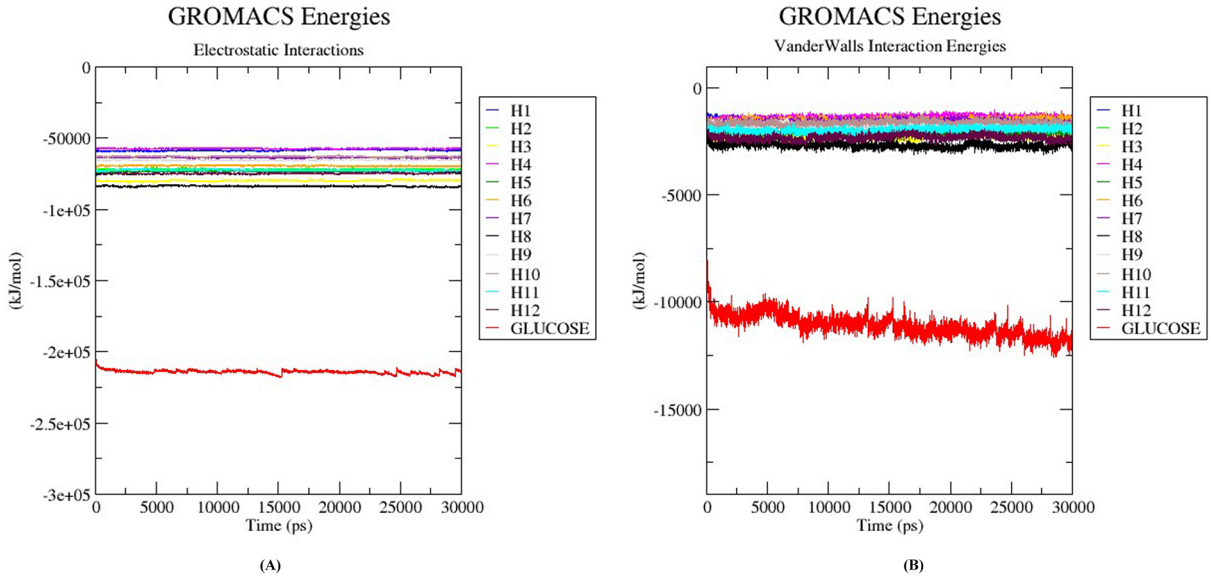

Fig. 2. Interaction energies (A. Electrostatic interaction energy, B. van der Waals interaction energy) with highest being that of glucose followed by H8.

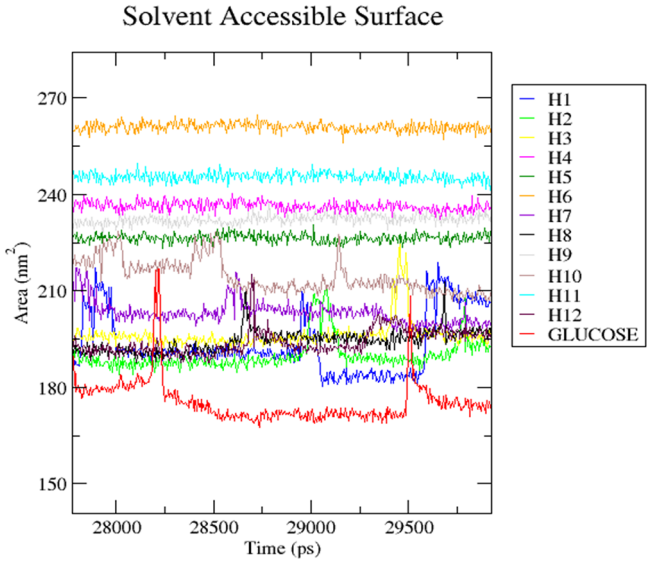

Fig. 3. Solvent accessible surface.

bands as spring. Hence the mathematics of spring behaviour and deformation can be used to describe different types of interaction energies. Electrostatic potentials were calculated by applying coulombs equation and van der Waal interactions between two non-bonded atoms and expressed as the sum of two forces (attractive as well as repulsive). All the results were in negative indicating attractive forces between atoms (Cramer, 2013). From the MD runs, all the interaction energies (both electrostatic interaction-Fig. 2A and van der Waals interaction-Fig. 2B) has been graphed which revealed that glucose followed by sample H8 had the highest interaction energies. It was inferred that electrostatic interactions were more dominant in configuring the crystal structure in all samples. Interestingly, on an average, samples from batch B (H8-12) which had only glucose and fructose components varied showed higher interaction energies as compared to batch A interpreting that the other components were interacting in the crystal formation and might be acting to hinder the crystallization process of glucose. H8 sample also had a higher amount of glucose molecules which supported the theory that main contribution for the interaction was indeed glucose molecules among all other molecules (Yun et al., 2017).

3.2.4. *Stability of structure*

To study the structure stability, root mean square displacement, solvent accessible surface area, and gyration radius was calculated

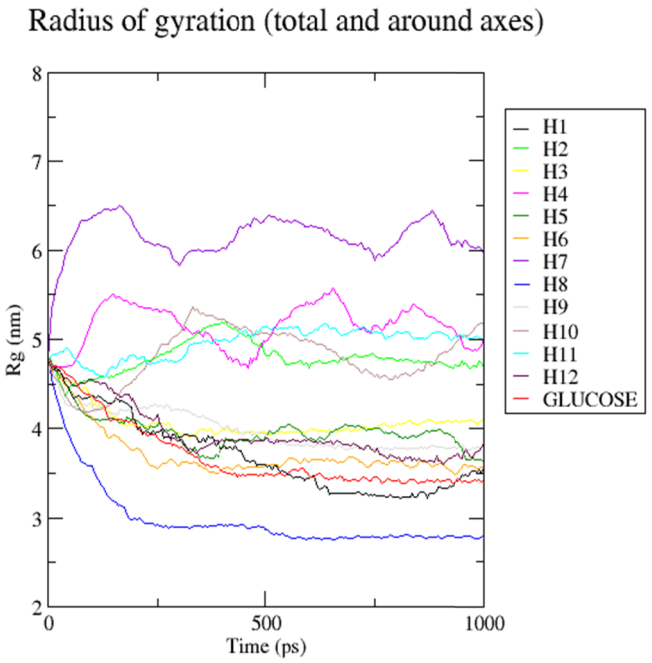

Fig. 4. Radius of gyration (Total and Around Axes).

using GROMACS modules (Table 2). In our investigation, root mean square displacement that provides an overview of fluctuations throughout the simulation inferred that overall least fluctuation in the system structure was seen in sample H8 indicating its higher stability once its crystallization was completed. The FG ratio of 1.18 had shown higher stability than other samples, although the sample H2 that corresponds to the sample H8 did not show similar low fluctuation trend again indicating that the interactions of other components during the crystal structure formation. Double cubic lattice method algorithm provided by Eihsenhaber et al. (1995) was used in GROMACS to obtain SASA. SASA takes into consideration, the van der Waals surface of the atoms that are accessible to contact surface (Fig. 3). It was observed that after standard glucose, the least value was shown by sample H8 followed by H2 which are the samples from the same origin but with different variation in components so on the basis of this observation it has been inferred that the not only fructose and glucose, but also other components were interacting in the crystallization phenomena. The radius of gyration plots (Fig. 4) reflects the molecular compactness of the crystal which was again pointing towards sample H8 with the least gyration radius of 3.51 nm. Sample H11 had highest gyration radius which depicted its lower compactness as well as lower interactions showing its lower stability in terms of gyration radii as compared to other samples. So, from the overall analysis of RMSD, SASA as well as gyration radius it can be concluded that sample H8 showed most stable structure among all and that every component has an interactive effect on crystal formation of the same.

3.3. Artificial neural networking

All the results of post MD analysis were used as targets whereas FG ratio and GW ratio were used as the input variables in nntool function of Matlab software. Individual networks were created with 10 hidden neurons in the network which could provide the interaction energies and stabilities of the samples based on the FG and GW ratios. During

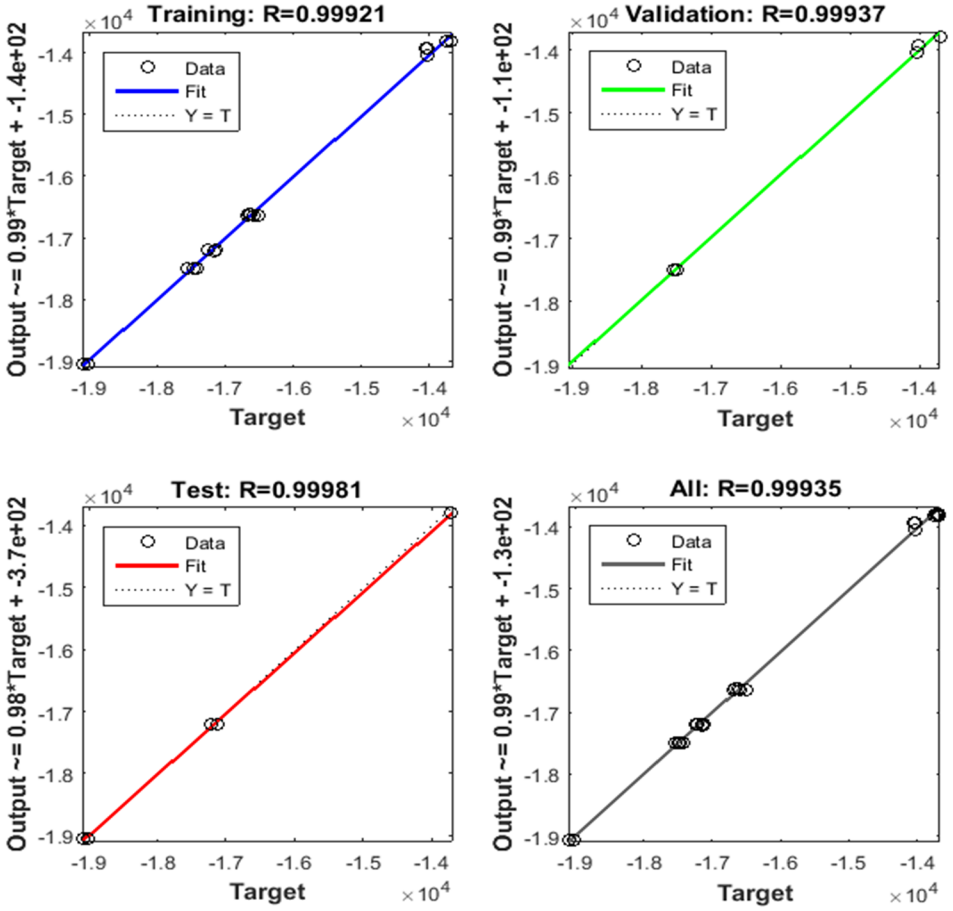

Fig. 5. Regression plot of predicted networks.

neural network training (Fig. 5), the maximum was reached after 1000 epoch and the plot regression of the training (R = 0.9993) was adequate validation (Mhatre, Siddiqui, Dongre, & Thakur, 2015). Validation run completed successfully which means that the network could predict the stability adequately. Further, the validated network successfully predicted the interaction energies (R = 0.9994).

4. Conclusions

The present investigation exhibited the interaction of not only FG or GW ratio but also other constituents like sucrose, maltose and water during crystallization. FG ratio of 1.18 formed the most stable crystal by having most formidable interaction energies, with the most dominant interaction energy being the glucose-glucose electrostatic interaction. Therefore, it can be recommended that this ratio should be kept above 1.18 to avoid crystallization in honey samples. According to our knowledge, this investigation is one of the novel approaches in implementing molecular dynamics to understand the interaction of sugars in honey. The research also confirms to the earlier studies in which various crystallization indicators has been mentioned as a mode to determine the granulation tendency of honey, mainly FG and GW ratio aspects. Application of ANN has successfully demonstrated the potential to predict the stability with high efficacy which is an inimitable approach while analysing different characteristics of honey. The findings of this study can also be implemented in honey processing industries to determine the crystallization tendency of the raw samples of honey and can be beneficial in selection of unit operations required for further processing the honey. More emphasis towards understanding the complexity of honey crystallization is essential and similar computational chemistry can be implemented to shed further light on the mechanisms of various complex biomolecules in food science.

Declaration of Competing Interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125182.

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