



Honey Crystallization, Myth and Microscopical Characterization

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Abstract: Honey is a sweet liquid food with high nutritional value, and provides immense health benefits. It is highly concentrated with sugar and contains mostly glucose and fructose. Pollens are also embedded naturally in the honey, and pollen contains protein and amino acids. These proteins and amino acids are essential for life-saving. The presence of pollens within honey samples may be a sign of natural characteristics and further, we can differentiate and define mono and multi-floral origin. Crystallization is a mass transfer phenomenon that leads to creation of a solid-liquid interface and results in a positive contribution to free energy of the nucleation. Crystallization of honey will affect its quality appearance, as well as consumers' acceptability. This research was conducted with the aim to study the microscopic characteristics and crystallization behavior of different honey. About 50 out of 500 honey samples were studied for the presence and percentage of Mustard (*Brassica* spp) pollen and other types of pollen grains. Out of 50 honey samples, 41 were recorded with Mustard pollens, and 18 were recorded with high crystallization, 11 were in the initiation phase, 03 are non-crystallized and the rest 09 honey samples were recorded without crystallization. Interestingly, 09 samples that were recorded without Mustard pollen are also non-crystallized. Significantly, 09 honey samples were recorded without Mustard pollens. This study has suggested that the crystallization behavior is a natural process influenced by some types of nectar and pollens.

Keywords: Nectariferous honey • Pollens • Nucleation • Crystallization • Perception • Mitigation

Introduction

Honey is a supersaturated natural sweet substance produced by bees (*Apis* spp) from nectar and secretions of living parts/sap and dew of the plant species (Bahadur et al., 1986; Upadhyay et al., 2014). An enzyme invertase is added into nectar in bee's salivary glands. Invertase converts the nectars, primarily a sucrose to mainly levulose and dextrose solution by dehydration and maturation in the honeycomb (Masierowska, 2003). Carbohydrates are the largest constituent of the honey with protein content depending on nectariferous plants (Bahadur et al., 1986). Honey contains pollen derived antioxidant p-

coumaric acid (Ferreira et al., 2009; Saxena et al., 2010). Majorly, it contains more than 70-80% sugar and less than 15-25% water. This means that the water in honey contains more sugar than it should naturally hold. An overabundance of sugar makes honey unstable due to supersaturation. Thus, honey crystallizes and it is now established as a natural phenomenon (Escaredo et al., 2014). Fructose (fruit sugar) and glucose (grape sugar) are the principal sugars in honey. Generally, fructose ranges from 30-44% and glucose from 25-40%. The balanced ratio of these two major sugars is the factor which primarily decides the crystallization of honey (**Images A-B**).

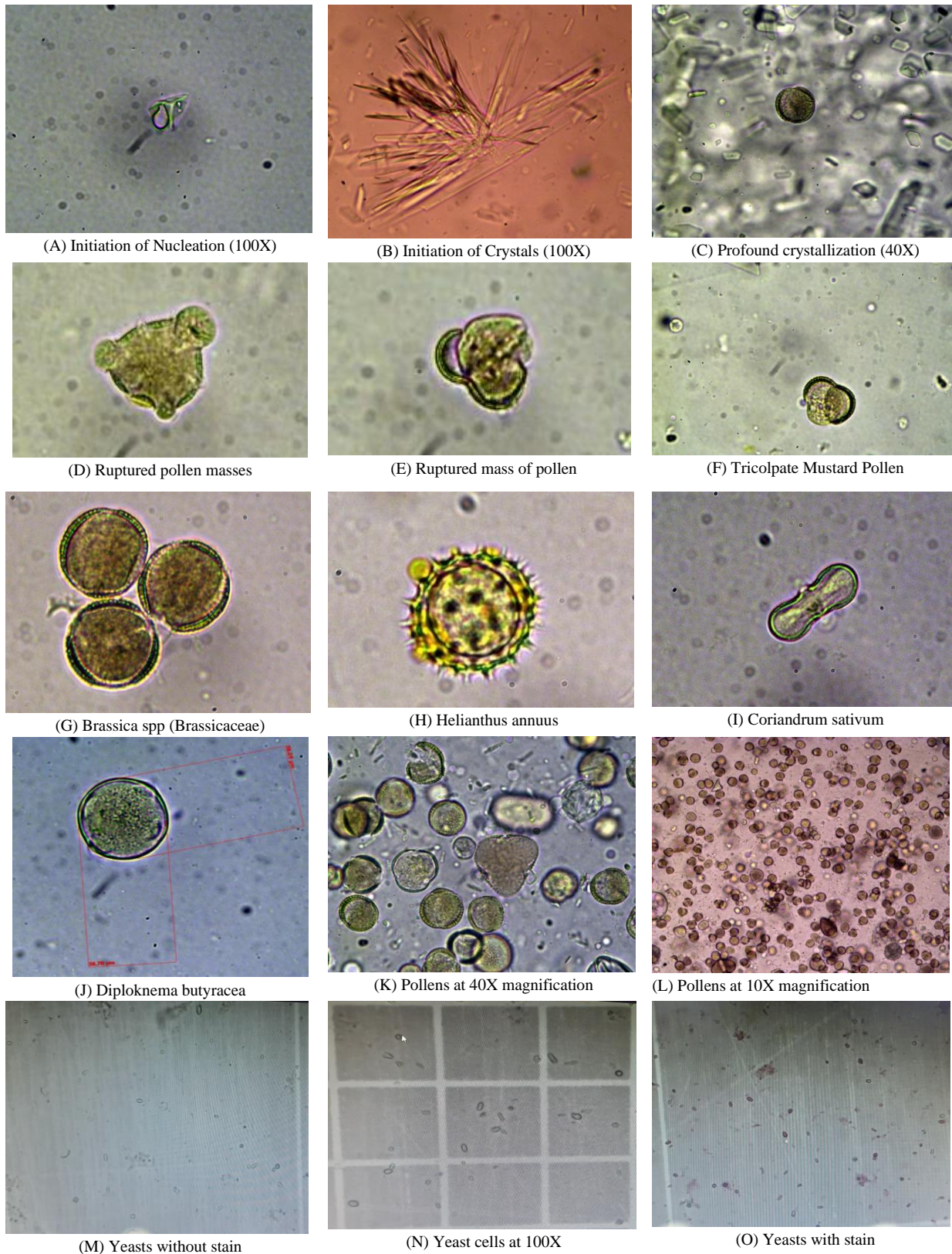


Figure 1. Microscopic images of the pollen, crystal and yeasts within Honey sample



The content of fructose and glucose in honey varies from one type of honey to the others depending on its phyto-geographical collection/floral availability (Wang et al., 2010; Escaredo et al., 2014). Individually relative percentages determine whether it will crystallize rapidly or slowly (Escaredo et al., 2014; Gregrova et al., 2015). Glucose crystallizes due to its lower solubility. When glucose crystallizes, it separates from water and takes the form of tiny microscopic crystal. Fructose is more soluble in water than glucose and will remain fluid (Gleiter et al., 2006; Zamora and Chirife, 2006; Shafiq et al., 2014). As the crystallization progresses, more glucose crystallizes and these crystals spread throughout the bottom to upper surface if honey is placed in a container (McPherson, 2019). The liquid solution changes to a stable saturated form and ultimately the honey becomes profoundly crystallized (Gleite, et al., 2006; Laos et al., 2011; Costa et al., 2015; Zaizuliana et al., 2017; Amariei et al., 2020; Krisnan et al., 2021; Peirong et al., 2023). Honey crystallization might be thermodynamic, chemical, physical and physico-chemical (Manikis, 2011). In addition, it may be occur biologically (due to nectar types and types of yeasts and pollens).

Pollen grains are male microgametophytes of Angiospermic plants. Individual pollen grains are very small and require enough magnification to see its detail, except aquatic plants (**Images C-L**). Pollens have a double wall and the vegetative and generative cells are surrounded by a thin delicate wall of unaltered cellulose called intine, and a tough resistant outer cuticularized wall composed largely of sporopollenin called exine. The exine often bears warts or is variously sculptured, and the character of the markings is often of value for identifying a particular plant (Mbagwu et al., 2009; Chwil, 2015). Apertures of pollen are regions of the

pollen wall that may involve exine thinning or a significant reduction in exine thickness. They allow shrinking and swelling of the grain caused by changes in moisture content. Elongated apertures or furrows in the pollen grain are called colpi or sulci. Apertures that are more circular are called pores. Colpi, sulci and pores are major features in the identification of pollen grains (Bazarragchaa et al., 2012; Upadhyay et al., 2014; Saikat, 2016).

Pollens are studied in Palynology and pollens with honey studied in Melissopalynology. Pollen contains about 22,7% of protein on average, including 10,4% of amino acids such as methionine, lysine, threonine, histidine, leucine, isoleucine, valine, phenylalanine, and tryptophan. These protein elements and amino acids derived from pollen grains are life essential and the organism cannot synthesize them by itself. So the presence of pollen grains within honey is a sign of its nature and quality too. If a honey comprises pollens it means honey is purely natural or nectariferous instead of sap and dew honey as well as that it is a sign of mono or multi-floral honey characteristics on the basis of percentage of particular pollen grains (45% as per FSSAI) and their geography (forests/domestic/orchards) too. Cytoplasmic mass of the pollen generally comes through an aperture which may be an adequate reason for the Nucleation (**Images A-F**).

The commencement of a phase transition in a tiny region, such as the creation of semisolid to solid crystal from normal liquid solution, is referred to as nucleation (Marangoni and Wesdrop, 2013). Nucleation is the total of two types; primary and secondary nucleation. Primary nucleation is the initial creation of a crystal in the absence of other crystals or in the presence of crystals that have no influence on the process (Vekilov, 2010). This can happen in two ways. The first is homogeneous nucleation, which is nucleation that is not impacted by



solids in any way. These solids include the crystallizer vessel's walls and any foreign substances. The second classification is heterogeneous nucleation. This occurs when the presence of solid particles of foreign substances causes an increase in the rate of nucleation that would not otherwise be observed (Vekilov, 2010). The development of nuclei due to the effect of existing tiny crystals is known as secondary nucleation. Secondary nucleation occurs when crystal growth is triggered by interaction with other pre-existing crystals or spores (Vekilov, 2010). Nucleation is the process by which atoms distributed in the solvent begin to congregate into microscopic clusters (elevating solute concentration in a narrow region) that become stable, and these stable clusters form the nuclei (Vekilov, 2010). Therefore, the clusters has reached to a critical size and such critical size is dictated and can be amplified by the factors of microfiltration, temperature and supersaturation (Laos et al., 2011; Shafiq et al., 2014; Costa et al., 2015; Zaizuliana et al., 2017; Amariei et al., 2020; Krisnan et al., 2021; Peirong et al., 2023).

Crystal growth is a dynamic process which occurs in an equilibrium where an atom precipitates out of solution, and dissolves back into solution (Vedantam and Ranade, 2013). Super saturation is one of the driving forces of crystallization, as the solubility of a species is an equilibrium quantified process (Vedantam and Ranade, 2013). Crystallization is (natural or artificial) the process in which a solid form, where the atoms or molecules are highly organized into a structure known as a crystal (Mutafschiev, 1993; Venir et al., 2010; Vekilov, 2010; Marangoni and Wesdrop, 2013; Vedantam and Ranade, 2013). Honey crystallization occurs in two major steps, the first is nucleation and the second step is known as crystal growth, which is an increase in the size of particles and leads to a solid crystal state (Laos et al., 2011; Marangoni

and Wesdrop, 2013; Shafiq et al., 2014; Costa et al., 2015; Zaizuliana et al., 2017; Amariei et al., 2020; Krisnan et al., 2021; Peirong et al., 2023). Keeping all the above facts in mind, the present study has been carried out with Microscopical characterization of multi-floral and mono-floral honey and to find possible causes of crystallization and pollen identification to mitigate, revamp the myth (scientific and people perception) which impart on people acceptability and needs of industrial business consistency.

Material and Methods

Sample Analysis: Honey samples were collected from the market and different vendors during the years 2017-2022. Two drops of each honey have been taken from the upper and bottom surface of a collection jar or boxes. A drop of the honey is placed over the Micro slide and covered with a cover slip instead of Glycerin or any other reagents. For the total pollen count, 10, 05, 01 gram honey sample was taken and centrifuged as per FSSAI guidelines. Pollen grains within honey samples have been identified with the help of published literature on palynology and in-house standards of pollens; those were collected direct from anthers of the fresh flowers. Determination of total count of pollens and plant elements in mono-floral and multi-floral honey has been done based on the basis of the STP and SOP followed by (IS 4941:1994; Von Der Ohe et al. 2004; DN10760).

Microscopical Evaluation: Slides were observed under Axiostar plus Microscope and captured images at 10X, 40X and 100X magnifications using the camera AxioCam ICc5.

Observations: It was done for different honey samples with percentage of Mustard pollens in particulars and using internationally accepted material and methods (Von Der Ohe et al. 2004; IS 4941:1994, Reaffirmed, 2019) and stage of Nucleation for the initiation of



crystallization using microscopic images at 100X.

Results and Discussion

A total of 50 honey samples were studied out of 500 random raw honey samples for the presence and percentage of Mustard pollen grains in particular and for the determination of botanical origin of honey along with crystallization. About 41 samples are recorded with Mustard pollen grains. Out of 41 Mustard honey samples, 18 are recorded with crystallization, 11 are in initiation phase, 03 are non-crystallized and rest 09 samples are without crystallization. Significantly, 09 samples those are recorded without the presence of Mustard pollens are also found to be non-crystallized. Interestingly, 19 honey samples were identified to having yeasts. Honey with mustard pollen from 45% to 75% are recorded with profound crystallization.

Honey samples with mustard pollen from 15% to 30% are only in the initiation phase. Honey samples with mustard pollen below 10% are recorded without crystallization. No crystals and no initiations are observed in case Mustard pollens are usually absent or having a percentage below to 10 percent in the respective honey sample. Exceptionally, three honey samples are also found crystal free having Mustard pollen grains which gives strong perception about causes of honey crystallization. There are so many others unidentified pollen grains, needs further investigation which may be also responsible for the crystallization. Mustard, Eucalyptus, Acacia, Litchi, Callistemon, Coriander, Chinaberry, Tagetes, Guava, Holly basil, Bombax, Sunflower, Acacia, Mango, Butternut, Rutaceae, Rosaceae, Lamiaceae, Asteraceae and Poaceae types and so many other unidentified pollens are also observed (**Table 1**).

Table 1. Microscopy of the different (Multi and Mono-floral) honey samples

Pollen Type	Mustard Pollen%	Identification of Pollen Grains	Crystallization
07	Absent	Poaceae type pollen, Bombax and others unidentified pollen grains	Absent
05	60 %	Mustard, Eucalyptus, yeasts and others unidentified pollens grains	Crystallized
15	6%	Acacia, Litchi, Coriander, Asteraceae type, Mustard and others unidentified pollen grains	Absent
16	6%	Coriander, Eucalyptus, Mustard, Tagetes, Acacia and others unidentified pollen grains	Absent
06	Absent	Acacia, Asteraceae type and others unidentified pollen grains	Absent
07	54%	Mustard, Eucalyptus, Coriander, Guava, yeasts and others unidentified pollen grains	Crystallized
05	60%	Mustard, Coriander, Guava, yeasts and others unidentified pollens	Non crystallized
05	Absent	Eucalyptus, Poaceae types and others unidentified pollen grains	Absent
08	45%	Mustard, Eucalyptus, Bombax, Coriander and others unidentified pollens	Initiation
04	65%	Mustard, Asteraceae types, yeasts and two unidentified pollen grains	Crystallized
11	9%	Mustard, Sunflower, Coriander and others unidentified	Absent



		pollen grains	
08	52%	Mustard, Acacia, Coriander, Asteraceae types and others unidentified pollen grains	Initiation
02	Absent	Asteraceae types and unidentified pollen grains	Absent
08	47%	Mustard, Coriander, Bombax and others unidentified pollen grains	Initiation
08	48%	Mustard, Eucalyptus and others unidentified pollen grains	Initiation
05	15%	Mustard, Eucalyptus and others unidentified pollen grains	Non crystallized
06	56%	Mustard, Bombax, Asteraceae types, Eucalyptus, yeasts and others unidentified pollen grains	Crystallized
09	25%	Mustard, Asteraceae types and others unidentified pollen grains	Initiation
14	Absent	Acacia, Eucalyptus, Asteraceae, Poaceae types and unidentified pollens	Initiation
08	20%	Asteraceae types, Coriander, Mustard and others unidentified pollens	Initiation
09	15%	Mustard, Coriander, Eucalyptus and others unidentified pollen grains	Initiation
10	10%	Asteraceae type, Mustard, Guava and others unidentified pollen grains	Absent
10	10%	Mustard, Cyperaceae types and others unidentified pollen grains	Absent
04	60%	Mustard, yeasts and others unidentified pollen grains	Crystallized
06	45%	Mustard, Lamiaceae types, yeasts and others unidentified pollen grains	Crystallized
10	Absent	Coriander, Acacia and others unidentified pollen grains	Absent
08	46%	Mustard, Eucalyptus and others unidentified pollen grains	Initiation
06	49%	Asteraceae, Cyperaceae types Mustard, Eucalyptus, yeasts and others unidentified pollen grains	Crystallized
05	63%	Mustard, Eucalyptus, yeasts and others unidentified pollen grains	Crystallized
10	Absent	Asteraceae types, Bombax, Acacia and others unidentified pollen grains	Absent
10	10%	Mustard, Asteraceae Cyperaceae types, Eucalyptus, Sunflower and others unidentified pollen grains	Absent
10	10%	Mustard, Asteraceae types, Eucalyptus, Guava and others unidentified pollen grains	Absent
15	06%	Mustard and others unidentified pollen grains	Absent
05	64%	Mustard, yeasts and others unidentified pollen grains	Crystallized
09	Absent	Asteraceae type, Eucalyptus and others unidentified pollen grains	Absent
07	46%	Asteraceae type, Mustard, Eucalyptus, yeasts and others unidentified pollen grains	Crystallized
07	47%	Rosaceae, Asteraceae types, Mustard, Eucalyptus, yeasts and others unidentified pollen grains	Crystallized
04	Absent	Eucalyptus, Mango and others unidentified pollen grains	Absent
05	59%	Mustard, yeasts and others unidentified pollen grains	Crystallized
10	10%	Asteraceae type, Mustard, Eucalyptus and others	Absent



		unidentified pollens	
12	08%	Acacia, Asteraceae type, Cyperaceae types, Mustard, Eucalyptus and others unidentified pollen grains	Absent
04	66%	Eucalyptus, Asteraceae types, Mustard, yeasts and others unidentified pollen grain	Crystallized
04	67%	Mustard, Asteraceae types, yeasts and others unidentified pollen grains	Crystallized
03	33%	Mustard, yeasts and others unidentified pollen grains	Crystallized
07	45%	Rosaceae, Asteraceae, Lamiaceae type, Mustard, yeasts and others unidentified pollen grains	Crystallized
09	20%	Mustard, Litchi, Eucalyptus, Bombax, Coriander and others unidentified pollen grains	Initiation
09	20%	Mustard, Litchi, Asteraceae, Guava and others unidentified pollen grains	Initiation
04	66%	Mustard, Eucalyptus, yeasts and others unidentified pollen grains	Crystallized
03	75%	Mustard, yeasts and an unidentified pollen grain	Crystallized
03	10%	Diploknema butyracea, Mustard and Asteraceae types pollen grains	Non crystallized

Transmission electron microscope (TEM), scanning electron microscope (SEM) and light microscope (LM) are useful for Microscopic characterization (Rana et al., 2018, 2020; Rana et al., 2022). Microscopic techniques are able to detect suspicious foreign particles in the raw materials, including pollen identification, honey crystallization, an adulteration and alternates (Rana et al., 2023). Honey adulteration could not be possible manually. It could be happen when beekeepers fed raw sugar to honey bees in winter and rainy seasons or in harsh conditions, when the natural flora declines or at the time of migration of the bee boxes in different localities (Agila and Barringer, 2013; Blanka and Lenka, 2015). Generally natural feed is not available to bees during decline of flowering plants in autumn. It might be Sugarcane (*Saccharum officinarum*), Sugar Beet (*Beta vulgaris*), Rice (*Oryza sativa*), wheat (*Triticum vulgare*) and Maize (*Zea mays*) syrup respectively. However, based on their carbon metabolism, these things can easily be detected now through C3 and C4 study (Blanka and Lenka, 2015). Now a days, liquid chromatography-mass spectrometry (LC-

MS), isotope-ratio mass spectrometry (IR-MS), high resolution mass spectrometry (HR-MS), and nuclear magnetic resonance (NMR) are able to detect any foreign sugar particles at PPM level.

More recently, Vekilov (2010) has observed that formation of crystalline nanoparticles starts with a nucleation. The crystallization process consists of two major events, nucleation and crystal growth, which are driven by thermodynamic as well as physio-chemical properties. The most significant of these are the two-step mechanism, according to which the crystalline nucleus appears inside pre-existing metastable clusters of size, which consist of dense liquid and are suspended in the solution (Vekilov, 2010). The applicability of this mechanism for small-molecule organic and inorganic materials, colloids, and bio-minerals (Vekilov, 2010; Vedantam and Ranade, 2013). After being extracted from honeycomb, honey tends to crystallize much faster than in hives due to temperature variability in the geographical conditions. The most important phenomenon for honey crystallization was assumed to be the ratio of the fructose/glucose (Yun Ma, et al., 2017;



Peirong et al., 2023). The content of fructose and glucose is dependent on the different types of Honey (DeSilva et al., 2016). Generally, fructose ranges from 30 to 44% and glucose from 25 to 40% (Wang et al., 2010). The balance between these two basic monosaccharide compositions is the main reason for crystallization of honey. And it determines whether a certain type of honey would crystallize faster or at a slower proportion. When glucose crystallizes, it separates from water and turns into crystal initiation or nucleation (Marangoni and Wesdrop, 2013). As a matter of facts, glucose is the underlying reason of honey crystallization because of its lower solubility compare to fructose having better solubility and formation of monohydrate glucose crystals (Zamora and Chirife, 2006; Laos et al., 2011; Gregrova et al., 2015). Nearly all types of honey crystallize but the time period depends on pollens and nectar types (Shafiq et al., 2014). F/G ratio also can't be used as a predictor indicator for crystallization. Higher glucose concentration can initiate crystallization in honey. However, the higher percentage of glucose contained in honey will distinguish the rate of crystallization (Zaizuliana et al., 2017). An influence of temperature and homogenization was observed, especially in relation to the crystal size distribution. Different honeys have recorded different percentages of fructose and glucose that will determine the crystallization rate (Yun Ma, et al., 2017). Agila and Barringer (2013) have suggested that storing honey at room temperature (25°C) may delay the formation of crystallization. And temperature of honey in the honeycomb may be varied to 20°C -40°C respectively. Zaizuliana et al., (2017) has observed that Hutan honey is very sensitive to cold temperature compared to other types of honeys, and the greatest crystal formation was found to occur at the storage temperature of 4°C. Costa et al., (2015) has also

concluded that all the honey samples stored at 15°C were uniform and fully crystallized but samples stored at 25°C, less than half the volumes were crystallized. Acacia honey can be stored at low temperature (-20°C) and room temperature (4°C) meanwhile, Gelam (*Melaleuca* spp) honey should be avoided from being stored at low temperature (-20°C) to prevent crystallization (Costa et al., 2015; Zaizuliana et al., 2017; Amariei et al., 2020). The percentage of the intact pollen grains may also be varied and it may be mono-floral or multi-floral honey. Mustard (*Brassica* spp.), Butter nut (*Diploknema butyracea*), Litchi (*Litchi chinensis*), Jamun (*Syzygium cumini*), Sundarbans (*Heritiera fomes*, *Excoecaria agallocha*, *Ceriops decandra*, *Sonneratia apetala*) are the example of mono-floral honey those are having different trend of crystallization.

Crystallization is also affected by presence and a number of crystallization centers, mainly pollen grains (Gregrova et al., 2015). Filtered honey has a significantly lower tendency to crystallization (Gregrova et al., 2015) but it will also depend on types of nectar and pollens. If there are some particular floral pollen grains that get into the honey, the whole process could be accelerated. Honey varieties with a low fructose to glucose ratio, such as Dandelion (*Taraxacum* spp), Aster (*Aster* spp), Clover (*Trifolium* spp), and Rata (*Metrosideros* spp) honey crystallize swiftly in days and weeks, while honey varieties with a high fructose to glucose ratio e.g., Tupelo (*Nyssa sylvatica*), Acacia (*Acacia* spp), Longan (*Dimocarpus longan*), Eucalyptus (*Eucalyptus* spp), Leatherwood (*Eucryphia lucida*) and Honeydew (*Cucumis* spp) crystallize slowly and stay liquid for the years (Escuredo et al., 2014). Butter nut (*D. butyracea*), Litchi (*L. chinensis*), Jamun (*S. cumini*) and Sundarbans honey have also low tendency of crystallization. It is found during our recent study for the determination of



botanical origin of honey and total count of pollen and plant elements as per IS- 4941:1994, (Reaffirmed, 2019) and Von Der Ohe et al (2004). Meanwhile, in the present study, almost all honey sample possess Mustard (*Brassica* spp) pollens in particular and other pollens in general (*Eucalyptus* spp., *Acacia* spp., *Litchi chinensis*, *Citrus* spp., *Callistemon botrytis*, *Coriandrum sativum*, *Melia azedarach*, *Tagetes* spp., *Psidium guajava*, *Ocimum* spp., *Bombax ceiba*, *Helianthus annuus*, *Syzygium cumini*, and *Pongamia pinnata*). Half of them were found with profound crystallization and rest honey samples were found in the initiation phase. High percentage of the Mustard pollens are also plays a major role in honey crystallization. When mustard pollen percentages were found below 10% there was not a sign of initiation of crystallization. When the percentage of Mustard pollens increased certainly crystallization got started. Definitely it supports as stated above pollen types and variation of F/G ratio of a particular type of honey is responsible for the possible causes of honey crystallization.

Melissopalynology, qualitative and quantitative pollen counts are mainly used to determine botanical and geographical origin of honey (Von Der Ohe et al., 2004; Petersen and Bryant, 2011; Song et al., 2012). Almost all honey, either mono-floral or multi-floral, contains sugar tolerant yeasts. The yeast cells present in air, water or even on the nectariferous and non nectariferous flowers get access to honey through nectar, pollen and rain water, which are collected by bees (Anonymous). These yeasts also get access to honey through unclean extractors, knives, strainers and containers (**Images M-O**). The presence of yeast content can give a hint towards a fermentation or a stopped fermentation. Yeasts are most commonly found in processed sugars, gluten-based foods. Yeasts are eukaryotic, single-celled microorganism classified as members of the

fungus kingdom. Yeast cells vary enormously in size and vary between 2 and 4 micrometers. *Zygosaccharomyces* are among the most problematic spoilage yeasts found in high-sugar foods. Four types namely, *Z. harkeri*, *Z. mellis*, *Z. nussbaumeri* and *Z. richgeri* of yeasts from fermented honey that all belong to the genera *Zygosaccharomyces* (Peter, 2022).

Conclusion

Honey crystallization is a rarely understood or rather mistaken perception. The crystallization of honey is a harsh reality and an uncontrollable natural process. Interestingly, nectar disparity of FGS (fructose, glucose, and sucrose) ratio, temperature change, and the presence of Tricolpate pollen of mustard, all play important roles in nucleation and crystallization inside honey. Pollen sac may break during maturation, increasing the likelihood of pollen mass distribution inside honey solution. Many previous studies had suggested the same things. It is unquestionably an adequate foundation for the commencement of nucleation for honey crystallization. According to the literature, all varieties of honey have a tendency to crystallize over time, and the numerous elements stated above either support or worsen this process of crystallization. However, crystallization is not a marker to define the quality of any honey. Most consumers equate crystallized honey that has turned coarse and gritty in texture with table sugar and believe it is artificial, contaminated, or of inferior quality, which is not supported by scientific evidence. Some people also believe that crystallized honey is rotten and should be thrown. Because natural sugar crystals have been destroyed by pasteurization and any suspended particles, such as pollen grains, which stimulate crystal initiation, have been controlled throughout the raw honey filtration process. Processed honey generally remains in liquid form on market shelves for a longer time. Pollen



identification and quantification within honey may indicate natural nectariferous and non-nectariferous (sap and dew) honey. Yeasts are among the most troublesome spoiling organisms found in high-sugar meals. Identification and measurement of yeasts may potentially be a key marker of storage honey determination and deterioration. The yeast content can indicate whether or not a fermentation is taking place.

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