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Honey crystallization: Mechanism, evaluation and application

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Abstract
Crystallization of honey is a natural phenomenon, and it also gives the authenticity of honey. But most of the consumers consider crystallization as adulteration of honey. Crystallization process will not cause any change in nutritional value if honey is properly crystallized, but improper crystallization will lead to increase in water activity and thus leading to fermentation. It is a desired phenomenon in the production of creamed honey, a spread that is becoming popular among the consumers. The factors that influence crystallization include F/G ratio, G/W ratio, presence of crystallization centres and storage temperature. There are several methods to evaluate crystallization in honey including DSC, NMR, molecular dynamics etc. Crystallization being an undesired phenomenon, there are several methods to prevent it such as heating honey or storing at low temperature, ultrasound treatment, filtration, ultrafiltration. There are latest studies focussing on the addition of certain food additives also lowers crystallization rate.

Keywords: Honey, crystallization, creamed honey, DSC, NMR, molecular dynamics

1. Introduction

Honey is the most important product of bee keeping and is the natural sweet substance produced by honeybees from the nectar of flowering plants which is transformed by them in the honeycomb (Codex Alimentarius, 2001) [12]. Honey is an intermediate moisture food (IMF) having a moisture content in the range of approximately 16-18% and water activity (a_w) of 0.6 (Fig 1), these properties along with the high osmotic environment do not support microbial growth thus making honey shelf stable (Machado De-Melo *et al*., 2018) [39].

Any solute present in a solvent above its saturation will crystallize out, same is the case of honey (Shafiq *et al*., 2012) [55]. Crystallization is a natural process because of honeys supersaturated nature. The supersaturated solution is mainly composed of a complex mixture of carbohydrates (Saxena *et al*., 2010) [53]. Glucose is the principal component that crystallizes in honey as it exists in a supersaturated state (Costa *et al*., 2015) [14]. Glucose precipitates as glucose monohydrate during crystallization (Berk *et al*., 2021; Shafiq *et al*., 2012; Zamora and Chirife, 2006) [6, 55, 0]. The process in which crystalline lattice structure is formed from the liquid phase is crystallization. Crystallization involves four steps which includes generation of supersaturated or supercooled state, nucleation–formation of crystalline lattice structure, growth-increase in the size of nuclei until the equilibrium phase volume is reached and recrystallization–reorganization of the crystalline structure to lower the free energy further (Bund and Hartel, 2010; Hartel, 2013) [10, 26].

Nucleation is of two types: primary and secondary. In primary nucleation, there is absence of pre-formed crystals and for the formation of new nuclei, it must overcome an energy barrier whereas secondary nucleation occurs only in the presence of pre-formed crystals. The guided or induced static crystallization follows secondary nucleation principle i.e., seed crystals are introduced to the system to act as primary crystallization nuclei. The problem with this type is that it can lead to unpredictable changes in texture and cause crystallization defects (Dettori *et al*., 2018; Tappi *et al*., 2019) [15, 61]. Dynamic crystallization is the process of performing guided crystallization with slow manual or automatic stirring. This process will produce a spreadable and creamy product of honey (Tappi *et al*., 2019, 2021) [60, 61].

Crystallization of honey affects the shelf life as the non-crystallized portion of honey will contain higher moisture content, which makes it vulnerable to yeast growth. The rate of nucleation and crystal growth is dependent on temperature. Crystallization also occurs faster at lower temperatures.

Honey stored at very low i.e. -20°C and close to ambient (20°C)temperatures results in fine crystals and coarse grains, respectively. While storing it in mild temperature range (4-10°C) results in mixed size crystals. Lower temperature resulted in small crystal sizes due to limited mobility of the molecules (Costa *et al*., 2015) [14]. Zamora and Chirife (2006) [0] reported that the optimum temperature for crystallization is between 10 and 15°C.

The crystallization process will not alter the chemical or nutritional value, it is still less valued and to retard crystallization honey is heated. Uncontrolled crystallization during storage makes the product cloudy and is not desired by consumers (Conforti *et al*., 2006) [13]. The higher the heating temperature longer the crystallization will be under control. But heating influences the taste and the enzyme content (Costa *et al*., 2015) [14]. Crystallization is an undesirable property in handling, processing, and marketing, except for the production of creamed honey. Controlled crystallization helps in attaining product of better quality and extended shelf-life (Hartel, 2013) [26]. In honey, controlled crystallization is carried out to improve sensory and physical properties of the regular (natural) honey thus attaining spreadable character (Suriwong *et al*., 2020) [59]. This product is called as creamed honey (Conforti *et al*., 2006; Subramanian *et al*., 2007) [13, 58]. The process of crystallization is shown in Fig 2.

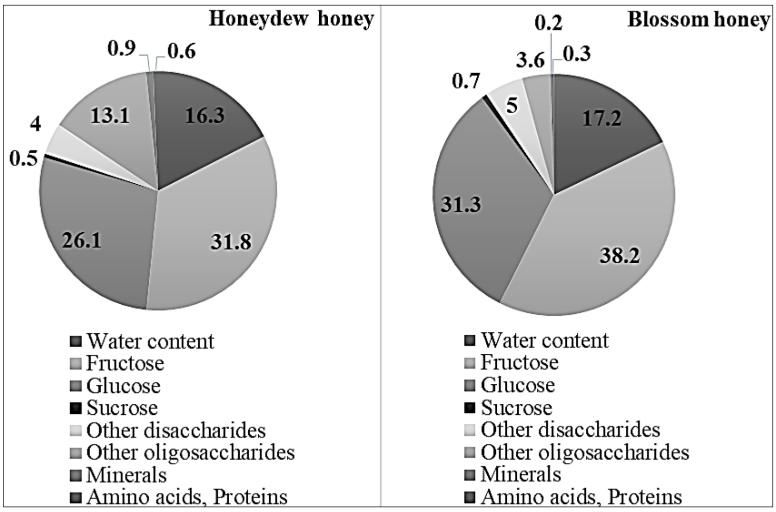

Fig 1: Composition of a) Honeydew honey b) Blossom honey (Bogdanov, 2016) [9]

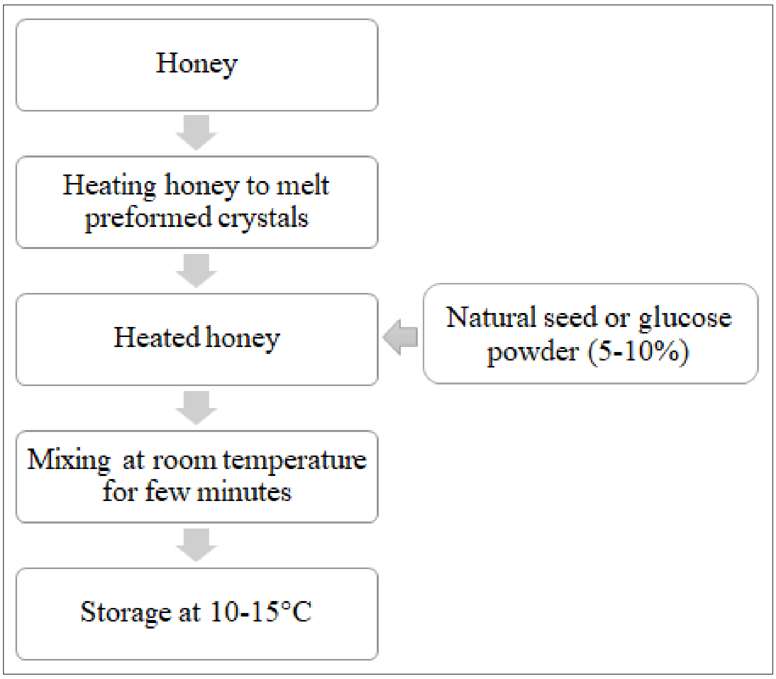

Fig 2: Process of induced honey crystallization (Dettori *et al*., 2018; Dyce, 1931) [15, 17]

An interesting point to note is that in normal circumstances, honey does not crystallize in the comb. According to certain literature, in the capped cell of the comb, honey crystallization is inhibited because the comb provides an environment that protects it from moisture, dust and other contaminants (Bhandari *et al*., 1999) [7]. This review focusses on the factors that affect crystallization, methods for evaluating crystallization, various methods to prevent crystallization along with other aspects of crystallization.

2. Factors affecting crystallization

Crystallization of honey is a complex phenomenon and is a property of interest among beekeepers, processors, and honey handlers. Parameters that influence this property is the composition and rheology of various honey (Bhandari *et al*., 1999; Conforti *et al*., 2006) [7, 13]. Based in the floral source, climatic region, environmental conditions and bee handling practices, the composition of honey varies from sample to sample. Crystallization occurs faster when the honey is distributed i.e., by shaking, stirring, and agitation (Grégrová *et al*., 2015; Rybak-Chmielewska, 2003) [24, 52]. Faster crystallization rate was reported for sunflower, cotton and clover honey; slow rate for citrus, chestnut and thyme and very slow rate for vetch honey (Hamdan, 2010) [25]. Table 1 shows certain factors affecting crystallization.

Table 1: Various factors affecting crystallization

Factors	No granulation	Slow granulation	Fast granulation	References
Glucose (%)	<27.7	-	>35	Bhandari *et al*. (1999) [7]
G/W ratio	>1.58	>1.33	<1.11	Escuredo *et al*. (2014) [21]; Dobre *et al*. (2012) [16]; Manikis and Thrasivoulou, (2001) [40]
F/G ratio	-	<1.17	>2.0	Escuredo *et al*. (2014) [21]; Dobre *et al*. (2012) [16]; Venir *et al*. (2010) [64]; Smanalieva and Senge, (2009) [56]
Temperature	-	-	At -20°C moisture content influenced the crystallization rate	Conforti *et al*. (2006) [13]
			10-15°C	Berk *et al*. (2021) [6]; Dettori *et al*. (2018) [15]; Elhamid and Abou-Shaara, (2016) [20]; Costa *et al*. (2015) [14]; Zamora and Chirife, (2006) [0]

2.1 Fructose: Glucose ratio (F/G)

The time for honey crystallization is dependent mainly upon the F/G ratio. Samples having the ratio greater than 1.58 has no tendency for crystallization (Venir *et al*., 2010) [64], and that with value greater than 1.33 does not crystallize for a long time (slow crystallization) (Dobre *et al*., 2012) [16], whereas samples with the ratio less than 1.11 crystallizes quickly (Escuredo *et al*., 2014; Smanalieva and Senge, 2009) [21, 56]. Due to higher content of less soluble glucose blossom honey crystallize faster. In honeydew honey crystallization is slow this is due to the presence of higher fructose and less glucose. But some honeydew honey has higher amount of crystal forming trehalose and melezitose (Grégrová *et al*., 2015; Dobre *et al*., 2012) [16, 24]. Fructose has higher solubility and it stays in the solution whereas glucose crystallizes fast and is converted to glucose monohydrate (Laos *et al*., 2011) [34]. Al-jouri *et al*. (2019) [2] found that F/G ratio was the major factor influencing crystallization in Syrian honey (Southern and Central region).

Escuredo *et al*. (2014) [21] found that faster crystallization takes place when the honey has a lower F/G ratio and water content. The results from the study demonstrated that the main factors that influence crystallization are fructose, glucose, moisture content and sugar ratio (F+G, F/G and G/W). The botanical source of honey also influences its F/G ratio. Rape and sunflower honey had the highest reducing sugar content (F+G), honeydew honey had the lowest. A high glucose content and low F/G ratio will make honey crystallize more rapidly as in case of rape and sunflower honey. Honey with a higher F/G ratio (i.e., containing less than 30% glucose) crystallizes quite slowly, like acacia, eucalyptus, honeydew, bramble, heather, and chestnut honeys. In the study on Estonian honey, Laos *et al*. (2011) [34] found that F/G ratio is the most important parameter that influence rate of crystallization. They also concluded that the due to the release of water after glucose crystallization will result in an increase in water activity (a_w). Due to the formation of crystal structures the viscosity of honey increases.

2.2 Glucose: Water ratio (G/W)

Rate of glucose crystallization depends on G/W ratio. Higher the glucose and lower the water content faster is crystallization. Honey samples having G/W ratio less than 1.7 exhibits slow crystallization, but when the ratio was greater than 2.0, crystallization was fast and complete (Escuredo *et al*., 2014; Dobre *et al*., 2012; Manikis and Thrasivoulou, 2001) [16, 21, 40]. Physical properties of honey such as viscosity, rheological properties, crystallization etc. are affected by the water content. Sunflower honey has the highest moisture content. G/W ratio of rape honey was 2.0 which is higher than that of eucalyptus, honeydew, heather, and chestnut honey. The ratio G/W is one of the most useful index to predict granulation as it gave accurate prediction in 68% of international and 93% of Greek honeys (Manikis and Thrasivoulou, 2001) [40]. Thus, from many studies it was found that G/W ratio is a better measure of honey crystallization (El Sohaimy *et al*., 2015; Dobre *et al*., 2012; Amir *et al*., 2010; Manikis and Thrasivoulou, 2001) [4, 16, 19, 40].

2.3 Presence of crystallization centres

The presence of crystallization centres in honey mainly pollen grains also helps in crystallization to occur. Even though crystallization is influenced by many factors, study by Grégrová *et al*. (2015) [24] found that absolute pollen count (a qualitative parameter) positively correlated with the crystallization degree. The crystal size is determined by the number of crystallization centres present. When crystallization is faster, presence of higher number of nuclei, higher will be the crystals with less size. Slow granulation leads to the production of thick crystals with non-compact structure and a fast crystallization leads to fine crystallization with compact structure (Machado De-Melo *et al*., 2018) [39].

3. Products of honey crystallization

A variety of honey-based products are available such as granulated honey, creamed honey, co-crystallized honey etc. which can be a replacer of natural honey. These are also the products of honey crystallization which are accepted by the consumers (Umesh Hebbar *et al*., 2008) [63]. If honey is properly crystallized even at room temperature it spreads like butter and it does not drip. The pasteurization and proper control of temperature during crystallization creates properly crystallized honey (Dyce, 1975) [18].

3.1 Granulated honey

Controlled crystallization can be employed to produce granulated honey. Dyce method is used to produce fine honey crystals having smooth consistency. Honey is heated twice at 49 and 66°C and starter nuclei is added. After processing granulated honey with spreadable consistency is obtained. Various methods employing new nuclei have been developed for producing granulated honey. The crystals must be small such that it cannot be detected by the tongue and it must be stable when kept at room temperature. By Dyce method, it is generally prepared by the addition of seed crystals i.e., properly ground granulated honey 5-10% (w/w) which initiates crystal growth. The sample was kept at 14°C until complete crystallization (Chen *et al*., 2009; Dyce, 1975) [11, 18].

Chen *et al*. (2009) [11] developed crystallized honey by using a new seed material. The new nuclei were 0.1% (w/w) glucose powder instead of the natural seed. Litchi honey was pasteurised at 60°C for 30min and then cooled to room temperature under running water for 1hr. Anhydrous D-glucose powder was added to the pasteurised sample at a concentration of 0.1% (w/w) and blended. The sample was stored in glass jar at 14°C until it is completely crystallized and after crystallization the honey was kept at room temperature. To maintain proper spreadability, the sample must be stored at 11-30°C.

3.2 Creamed honey

Controlled crystallization of honey produces fine crystals and the product formed is creamed honey (Umesh Hebbar *et al*., 2008) [63]. The creamed honey is a desired product of honey crystallization which can be used as a spread. When honey was crystallized by the addition of 0.1% glucose powder, it produced good quality creamed honey with desired spreadability (Chen *et al*., 2009) [11].

Elhamid and Abou-Shaara, (2016) [20] developed creamed honey from cotton and clover honey by using glucose powder as the seed at a 5°C. The glucose powder was added at concentration of 0.1, 0.3, 0.6, 1.2, 1.8 and 2.4% (w/w). It was found that creamed honey was produced after 2 weeks at a temperature of 5°C. The desired glucose concentration was 1.2-2.4% (w/w). The creamed honey was developed as a feed for honeybee. Suriwong *et al*. (2020) [59] performed induced granulation to produce creamed honey with proper texture. Glucose powder was added, and the honey was stored at 10-15°C for crystallization. Highest crystallization rate was found after the addition of 2.0% (w/w) glucose addition. During storage intensity of yellow colour reduced.

3.3 Co-crystallized honey

The process of incorporating an active ingredient into the conglomerate of crystals is co-crystallization (Bhandari *et al*., 1998; Umesh Hebbar *et al*., 2008) [8, 63]. The primary ingredient for co-crystallization is sucrose. The processing steps include crystallization of supersaturated sucrose syrup, then the active ingredient is added so that it gets incorporated into the void spaces of the agglomerate of crystals. The advantages of the co-crystallized products are its better flowability, low hygroscopic nature and dispersion property. This is similar to encapsulation as it helps to prevent the losses in sensitive compounds (Bhandari *et al*., 1998; Umesh Hebbar *et al*., 2008) [8, 63].

Bhandari *et al*. (1998) [8] performed the co-crystallization of honey with sucrose. Different sucrose: honey proportion were selected (90:10, 85:15 and 80:20). First the sucrose syrup was heated to 128°C before adding honey and then cooled to 60°C. After cooling the mix was transferred to a watch glass and dried in an oven at 40°C overnight. Better product of desired property was produced from 90:10 and 85:15 combination. Another study on co-crystallization of honey with sucrose was done by Maulny *et al*. (2005) [41]. Sucrose water was mixed with water and was heated to 128°C, then honey was added to the mix at sucrose: honey ratio of 90:10, 85:15 and 80:20 (w/w). Nucleation occurred spontaneously and then the product was cooled to room temperature. In this study, after cooling two methods were applied. One was overnight drying in oven at 40°C (Bhandari *et al*., 1998) [8] and the other was centrifugal filtration to remove agglomerate crystals. The solid part after centrifugal filtration was transferred to watch glass and oven dried at 40°C. The products by both the process were ground finely using a mortar and pestle and was stored in airtight glass bottles. The moisture content of the product increased with increase in honey concentration. The co-crystalline product produced by centrifugal filtration had a maximum of 2% honey and had a lower moisture content when compared to the other product. The relative flowability was lower for the unfiltered product. From the DSC analysis exhibited an overall decrease in crystallinity of the product with increase in honey concentration. The filtered product was found to be better than the unfiltered one in terms of flowability and moisture content.

4. Evaluation of honey crystallization

Various studies related to evaluation of honey crystallization have been dealt in this section and is summarised in Table 2.

Table 2: Methods to evaluate honey crystallization

Methods	Parameters analysed	Conclusion	Advantage	References
Molecular dynamics	Morphology of crystals.	Sugar composition of honey played an important role in formation of stable crystals.	Powerful tool to analyse phenomenon at molecular level.	Ma *et al*. (2017) [38]
Molecular dynamics and artificial neural network (ANN)	Crystal stability of the samples were studied.	The sample with F/G ratio 1.18 formed the most stable crystal and this sample had the highest glucose-glucose electrostatic interaction.	ANN mimics the working and pattern recognition by human brain.	Naik *et al*. (2019) [43]
Electrical impedance	Crystallization process increases the impedance.	Change in impedance colour and water activity can be used to identify crystallization.	Measuring electrical parameters is a rapid and effective alternative to the costly chemical analysis.	Łuczycka *et al*. (2016) [35]
Absorbance	Increase in absorbance intensity is an indication of granulation.	The turbidity of crystallized honey increased, and the intensity of yellow colour was found reducing.	Absorbance measurement at 660nm is an easy method.	Suriwong *et al*. (2020) [59]; Lupano, (1997) [37]
Differential scanning calorimetry (DSC)	Crystal growth and type of crystals formed.	F/G ratio influenced crystallization rate and storage at lower temperature can prevent crystallization.	Avrami model can be used to explain crystallization kinetics and DSC determines crystallization kinetics.	Suriwong *et al*. (2020) [59]; Dettori *et al*. (2018) [15]; Lupano, (1997) [37]; Nurul Zaizuliana *et*

				al. (2017) [47]
Time domain-nuclear magnetic resonance (TD-NMR)	Magic Sandwich Echo (MSE) monitored honey crystallization. Crystallization was induced by adding glucose and relaxation time was measured at the storage temperature.	The results of induced crystallization in TD-NMR system and DSC followed consistent kinetics.	Easy analytical method to detect honey crystallization. Do not require sample preparation. MSE solves dead time problem in conventional NMR.	Berk *et al*. (2021) [6]

Ma *et al*. (2017) [38] used molecular dynamic simulation to evaluate the crystallization process in honey. Molecular dynamics (MD) is a powerful tool to analyse the phenomenon at molecular level. Environment scanning electron microscopy (ESEM) was used to analyse the crystals and was found that the honey crystals had an irregular shape with smooth edge and that of glucose crystal exhibited plate-like morphology with flattened edge. Conformational changes in the crystal were analysed using molecular dynamic simulation. It was concluded that the sugar composition plays an important role in the formation of stable honey crystals. The G/F ratio of 2.5:1 is considered the critical ratio for honey crystallization.

Naik *et al*., 2019 [43] molecular dynamics simulation of six Indian honey samples were performed on sugar profile and moisture content-based mixture systems. The interaction of other constituents such as water, sucrose, and maltose along with F/G and G/W ratio was studied. From the post simulation analysis most-stable crystal was formed when F/G ratio was 1.18 and it had highest van der Waals and electrostatic interaction. Artificial neural network (ANN) is created in such a way that it mimics human brain in working and pattern recognition skills. The glucose-glucose electrostatic interaction energy was found to be most dominant. F/G ratio should be greater than 1.18 to avoid crystallization in honey samples. The study also concluded that water, sucrose, and maltose in honey affected the crystallization process. Electrical parameters such as impedance can be used for the measurement of honey crystallization. Crystallization process increases the impedance (Łuczycka *et al*., 2016) [35]. The change in colour and water activity can also be used as a measure to evaluate honey crystallization (Kuroishi *et al*., 2012) [32].

The increase in absorbance intensity is considered a valid measure of granulation (Suriwong *et al*., 2020; Lupano, 1997) [37, 59]. The crystal growth and type of crystal formed in honey stored at different temperature was analysed using differential scanning calorimeter (Lupano, 1997) [37]. Honey with few crystals were stored at -20, 4, 10, 20°C. DSC was done along with measurement of absorbance at 660nm and light microscopy. There was an increase in turbidity with increasing granulation, thus the absorbance at 660nm increased. The enthalpy of melting (T_m) and absorbance had a linear relationship. Coarse crystals were formed in honey stored at 20°C and had a T_m between 45 and 65°C. finely grained honey was formed at -20°C whereas big and small crystals were formed at 10 and 4°C, which showed intermediate property comparing with -20 and 20°C storage temperature (Lupano, 1997) [37]. Lupano (2007) [36] specified that with increase in the storage time the crystal size of honey increases, whereas the storage temperature affects the crystallization degree and the crystal size (Patrignani *et al*., 2018) [49].

Absorbance measurement at 660nm was performed to evaluate crystallization behaviour of sunflower and longan honey by the addition of glucose. After the addition of glucose powder at concentrations 1.0, 1.5, 2.0, 2.5% (w/w), the samples were stored at 10-15°C. Microstructure and colour of crystallized honey was also analysed by Suriwong *et al*. (2020) [59]. Crystallization kinetics was explained using Avrami model and the highest crystallization rate was found at 2.0% w/w concentration. The turbidity of crystallized honey increased, and the intensity of yellow colour was found reducing. With increasing rate of granulation, absorbance intensity at 660nm also increased. Avrami equation was used to explain the crystallization kinetics in honey samples and DSC was used to determine crystallization kinetics. F/G ratio was specific for the three honey samples and 5% crystals have been added before storing the sample at 14°C and was kept for crystallization. The Avrami equation properly described the crystallization kinetics. The crystallization rate of honey samples were proportional to the F/G ratio (Dettori *et al*., 2018) [15].

DSC was done to analyse crystallization in Malaysian honey stored at different temperature for different storage times. The temperature of storage was 25, 4 and -20°C at different storage times of 0, 5, 14, 30, 60 and 180 days. The Hutan honey had the highest crystallization at 4°C when stored for 14 days whereas acacia and kelulut honey did not show any significant peak in its thermogram when stored at 4 and -20°C. Gelam honey crystallized when stored at -20°C, so in order to prevent its crystallization it should not be stored at lower temperature. At a temperature of 25°C, crystallization was delayed (Nurul Zaizuliana *et al*., 2017) [47].

Berk *et al*. (2021) [6] used time domain nuclear magnetic resonance (TD-NMR) through magic sandwich echo (MSE). Honey samples were heated to remove crystals present and then seeded using glucose powder, this addition increased the G/W ratio to 2.27. The TD-NMR analysis was done using NMR system of frequency 20.34 MHz. This was found to be an easy analytical method for analysing honey crystallization.

Tappi *et al*. (2021) [60] found that the traditional static method will create crystallized product that has pronounced differences based on initial composition whereas the dynamic method helped to obtain crystallized honey having similar colour and structural properties as that of the initial product. The formation of microcrystals by stirring produced product having low viscosity and hardness. The time for crystallization was also reduced by the dynamic method.

5. Effects of honey crystallization

Crystallization of honey has many advantages along with certain disadvantage. Honey crystallization lowers the glucose concentration in the liquid phase leading to an increase in water activity (Zamora and Chirife, 2006) [0]. Thus, it causes yeast proliferation and leading to honey fermentation. Natural yeast present in honey (osmotolerant) will act on the glucose and fructose producing ethanol and CO_2. Ethanol also get converted to acetic acid and water which produces the sour taste (Shafiq *et al*., 2012) [55]. A study by Ozkok and Silici (2018) [48] showed that there was reduction in antioxidant property of honey during long storage irrespective of fast or slow crystallization. Excessive heating of honey to retard crystallization will cause loss of the functional property and quality degradation. Consumers without knowing the principle behind crystallization often perceive it to be artificially produces or adulterated. Sensory parameters such as flavour, odour, colour, texture etc., were reduced by crystallization and thus its acceptability also decreased. The firm texture and turbidity as a result of crystallization are the major factor for reduced acceptance. Repeated melting of honey can cause an unstable state which reduces the shelf-life (Srinual and Intipunya, 2009) [57].

Differential scanning calorimetry (DSC), time domain nuclear magnetic resonance (TD-NMR) along with water activity measurements were performed to evaluate the water state during induced crystallization by both static and dynamic manner in honey (Tappi *et al*., 2019) [61]. Honey samples with specific F/G ratio 1.05 (fast crystallization), 1.20 (medium crystallization) and 1.40 (slow crystallization) were selected. Samples were heated at 50°C to remove pre-formed crystals and the absence of any crystals were confirmed using optical microscopy. Finely granulated crystals from citrus honey were added to the three samples. The stirring provided (dynamic crystallization) helped in achieving crystallization faster when compared to the static one. This study there was increase in a_w, but the value did not increase above the threshold level for microbial growth (Tappi *et al*., 2019) [61].

6. Methods to prevent honey crystallization

Crystallized honey will make the people believe that honey is adulterated. But the fact is crystallization guarantee the purity of honey. Crystallization has many disadvantages even though it is a natural process there are problems during handling and processing (Nurul Zaizuliana *et al*., 2017) [47] and portioning, casting of honey becomes difficult. The presence of crystal makes it unacceptable by children.

Crystallized honey is not accepted by children because of the presence of crystals. Honey is a food that has to be included in the diet of children due to its miscellaneous properties such as antioxidant, prebiotic, antibacterial, anticancer and antifungal (Naik *et al*., 2019; García-Tenesaca *et al*., 2018; Quintero-Lira *et al*., 2017; Nayik *et al*., 2016; Nayik and Nanda, 2015, 2016; Kamboj *et al*., 2013; Saxena *et al*., 2010) [23, 29, 43-46, 50, 53]. The acceptability criteria of honey include colour, degree of crystallization and flavour (Patrignani *et al*., 2018) [49]. Honey not only influences acceptance but also it is a criterion for recognition. The changes in these factors along with difficulty in handling and processing caused by crystallization creates a need to prevent this process (Amariei *et al*., 2020) [3].

6.1 Heat treatment at high or very low temperatures

Thermal processing of honey helps in melting the pre-formed crystals, it also reduces the microbial load and moisture content that leads to fermentation of honey (Subramanian *et al*., 2007; Tappi *et al*., 2021) [58, 60]. Crystallization can be controlled by heating honey or storing at proper conditions. According to certain literatures, during bottling if honey is kept at 40-71°C, then crystallization can be reduced. During mild heating, the crystals melt and heating it at a temperature of 60-71°C, the crystals dissolve and expand leading to expulsion of the trapped air which again can stimulate crystallization process (Eshete and Eshete, 2019; Grégrová *et al*., 2015; Subramanian *et al*., 2007) [22, 24, 58]. Aydogan-Coskun *et al*. (2020) [5] studied the effect of heat treatment for pasteurisation and liquefaction of honey. The effect of pasteurization treatment done at 90°C for 15 sec was higher when compared to liquefaction treatment done at 55°C for 2hrs. Due to the lower temperature for liquefaction, its impact was less on the honey samples.

Consumer acceptance was not affected when honey was melted at low temperature and partial separation of honey crystals were performed. But repeated melting can lead to quality degradation and reduce soluble solids by crystal formation which lead to an unstable state reducing the shelf-life (Srinual and Intipunya, 2009) [57].

Freezing temperature maintained the freshness of honey. The storage at freezer temperature reduced granulation, but at a temperature of 13 and 15.5°C granulation was accelerated (Kędzierska-Matysek *et al*., 2016) [30]. Treatment of honey at -40°C crystallization was prevented but it is a costly process and difficult for honey producers (Amariei *et al*., 2020; Subramanian *et al*., 2007) [3, 58].

6.2 Ultrasound

Heating of honey negatively affect the quality. It leads to increase in 5-Hydroxymethyl furfural (HMF) and the bioactive compounds in honey are also lost. The use of ultrasound is a non-thermal processing method, which is effective in reducing microbial load and it also helps in destruction of the crystalline network and thus inhibit crystallization (Quintero-Lira *et al*., 2017) [50]. Other advantage of this method is HMF formation does not take place (Mortaș and Yazıcı, 2013) [42]. Ultrasonic waves when passed through liquid medium will cause mechanical and thermal changes and also changes in unicellular organisms present (Subramanian *et al*., 2007; Thrasyvoulou *et al*., 1994) [58, 62]. According to many research, ultrasonic waves were found to destroy crystals in honey, and it inhibited crystallization for a longer period. From these studies it is evident that the quality of honey is less affected by liquefaction of honey using ultrasound.

Ultrasonic bath and Bain-Marie heat treatment method was performed on honey to study its effect on crystallization/recrystallization (Akyol and Güneşdoğdu, 2019) [1]. The samples were subjected to heat treatment at 25 and 50°C for 2hr. The 8.75% was the average crystallization rate of honey subjected to ultrasonic heat treatment and that with bain-marie method was found to be 31.25%. The control sample had an average crystallization rate of 90%. At 50°C, ultrasonic bath treatment for 2hrs was found to be effective to avoid crystallization.

Optimised power ultrasound treatment was found reduce the effects on HMF, colour parameters, diastase activity another and other quality parameters. Ultrasonically treated honey was found to have lower count of aerobic mesophilic bacteria when compared to thermally processed honey samples (Janghu *et al*., 2017) [27]. Liquefaction of crystallized honey he was performed in an ultrasonic bath of 40 kHz at a temperature of 40 to 60°C at different time of 20, 40 and 60 minutes. Ultrasound treatment at temperature below 50°C was found to increase the rate of liquefaction process. This process conducted at low temperature was found to preserve honey quality and helped to reduce energy consumption. It also reduced the crystals found in the sample initially (Kabbani *et al*., 2011) [28]. The high cost of the procedure is a disadvantage (Amariei *et al*., 2020) [3].

6.3 Filtration and ultrafiltration

Filtered honey has lower tendency to crystallization. Filtration process removes the crystallization centres such as pollen grains, glucose crystals, wax particles and other compounds that stimulates crystallization (Amariei *et al*., 2020; Grégrová *et al*., 2015) [3, 24]. Filtration process has increased the exposure to light, air etc., which influences its quality, and it also reduces the antibacterial property of honey. Ultrafiltration process requires filter materials with pore size less than 80µm (Amariei *et al*., 2020) [3]. The desirable enzymes α-amylase and α-glucosidase are removed by ultrafiltration (Subramanian *et al*., 2007) [58].

6.4 Addition of food additives

The use of additives such as isobutyric acid and sorbic acid to prevent crystallization have been followed in many countries. But the European Union bans the use of this process (Subramanian *et al*., 2007) [58]. Several research and patented processes have been developed to prevent crystallization by modifying the F/G ratio through the removal of glucose. In order to prevent crystallization a new method by the use of trehalose have been developed by (Amariei *et al*., 2020) [3]. Trehalose is a non-reducing disaccharide found in a large amount in acacia honey, which crystallizes slowest. Trehalose has two glucose units connected by α, α-1,1-glycosidic bond. It helps plants and microorganisms during stress condition thus playing a protective role (Kosar *et al*., 2019) [31]. It is also a GRAS (generally recognised as safe) additive to be used in consumer product (Richards *et al*., 2002) [51]. To 500g of honey sample, 1.2-1.5mL of 2% trehalose solution was added and it was stored at 14-16°C. Through the DSC analysis of trehalose added honey, it was clear that crystallization did not occur. Its addition prevented the crystallization process and the sample remained in its liquid state with no change in colour.

7. Crystallization for detecting adulteration in honey

Crystallization of honey is a natural process and it guarantees honey authenticity (Scripca and Amariei, 2018) [54]. Honey crystallization, also called granulation, which was considered a defect is not always undesirable. Honey adulteration can be determined using crystallization and rheological properties. Kurt *et al*. (2020) [33] attempted a first study to determine adulteration by crystallization based on seeding sunflower honey with different levels of crystallized honey. The crystallization rate was analysed using rheology and the physiochemical, microstructural and FTIR spectroscopy of honey was carried out. The natural samples were named A, D, and E. For indirect adulteration, the bees were fed with bee feeding syrup (BFS; 100L/colony). Invert syrup of 75°Brix was used as adulterant, sample B. The honey obtained from the indirectly fed colony was named sample C. Pure honey crystals were produced by keeping the samples A, D, and E stored at 14°C for 1 week. At a concentration of 1%, 5%, and 10% (w/w) the seeds of sample A were inoculated to samples B, C, and natural honey samples; then vortexed for 10min at 20°C and was stored at 20°C. By mixing 15g each of both the samples mixture of sample AB and AC was produced. It was vortexed for 30min at 20°C and seed crystals at concentration of 5% was added, rest of the process was same as above. The adulterated and pure samples differed in their rheological behaviour i.e., the natural honey had different visco-elastic and flow behaviour from adulterated ones. Faster and homogeneous crystallization was observed in natural honey (A). The industries can utilise this simple method for determining adulteration in honey.

8. Conclusion

Honey crystallization is a natural phenomenon occurring due to its composition. Most of the consumers prefer liquid honey and consider crystallization an undesired process. Glucose is the principal component that crystallizes in honey due to its supersaturated state and fructose is more soluble than glucose. The rate of crystallization will be higher if the glucose content is high. Many studies reveal the optimum temperature of crystallization as 10-15°C, but there are some studies conducted at lower temperature which successfully produced crystallized honey. Depending upon the F/G ratio the crystallization rate varies i.e., samples having the ratio greater than 1.58 has no tendency for crystallization and that with value greater than 1.33 show slow crystallization, whereas samples with the ratio less than 1.11 crystallizes quickly. Some studies suggest G/W ratio as a better method to predict honey crystallization. Samples with ratio less than 1.7 exhibits slow crystallization and ratio greater than 2.0 exhibits fast crystallization. Honey crystallization is a desired process in the production of creamed honey. The crystallization can be monitored by differential scanning calorimetry (DSC), molecular dynamics, artificial neural network, nuclear magnetic resonance (NMR) etc. These methods are effective in determining honey crystallization. Being an undesired process there are several methods developed to prevent it. The prevention methods include heating, storing at low temperature, filtration, ultrafiltration, ultrasound treatment and latest methods include addition of additives. Heating is normally done to reduce the risk of crystallization, but it increases hydroxymethyl furfural content (HMF) and it also reduces the amount of certain desired enzymes. So, addition of GRAS additives is a better method. Thus, honey crystallization has both negative and positive aspects.

9. References

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