ULTRASONIC STUDIES OF HONEY AT DIFFERENT MOISTURE CONTENT AND TEMPERATURES

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
PHYSICS
(Minor Subject: Mathematics)

By
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CERTIFICATE I

This is to certify that the thesis entitled **"Ultrasonic studies of honey at different moisture content and temperatures"** submitted for the degree of **M.Sc.** in the subject of **Physics** (Minor subject: **Mathematics**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Gurpreet Kaur (L-2014-BS-313-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled, "**Ultrasonic studies of honey at different moisture content and temperatures**" submitted by **Gurpreet Kaur** (Admn. No. **L-2014-BS-313-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.** in the subject of **Physics** (Minor subject: **Mathematics**) has been approved by the Student's Advisory Committee after an oral examination on the same.

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ABSTRACT

The composition of natural honey present in the world is different and India too produces honey from various floral sources with wide variations in physicochemical and rheological characteristics. From, last decades honey has been a prime target of adulteration for economic gain. These artificial honey which can be made poorer in quality by adding amounts of sucrose, commercial glucose, water and other substances, often have similar taste and physical appearance as natural honey, but they lack the medicinal and nutritional properties of natural honeys. Detection of adulteration in honey is difficult, but some physicochemical properties of honey that can be easily determined which is helpful for comparison of different honey. Ultrasound is a versatile non-destructive technique and used in the food industry in both for the analysis and modification of foods. In the present study, the physicochemical properties including ultrasonic velocity, density, electrical conductivity and total soluble solids (TDS) in honey was investigated at different temperatures (20,30,40,50,60 and 70°C) and concentrations (100, 90, 80, 70 and 60%) to have the idea of effect of water adulteration and effect of heat on these properties. The ultrasonic velocity was found with ultrasonic interferometer at frequency of 1 MHz and density is measured with specific gravity bottle method. The electrical conductivity and TDS in honey was recorded from digital conductivity and TDS meter. Some thermodynamics properties of honey like surface tension, adiabatic compressibility, acoustic impedance, bulk modulus and intermolecular free length was also calculated from the observed parameters which will provide a fundamental insight on its structural organization. All the observed parameters except density were significantly vary with the temperature and dilution. The ultrasonic velocity, density, surface tension, acoustic impedance and bulk modulus was found to be decreases with temperature and dilution of honey while electrical conductivity, TDS, adiabatic compressibility and intermolecular free length was increases with the applied conditions. A significant correlation was observed between electrical conductivity and TDS at all temperatures and concentrations.

Keywords: Ultrasonic velocity, density, honey, electrical conductivity, TDS, thermodynamic parameters.

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Signature of Major Advisor **Signature of the Student**

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

CONTENTS

CHAPTER-I

INTRODUCTION

Honey is a natural sweet food produced by Apis mellifera bees from the nectar of blossoms called nectar honey or from the secretions of living plants or excretions of plant sucking insects of the living part of plants called honeydew honey (Alvarez *et al* 2010). Nectar is a thin, easily spoiled sweet liquid that is changed (ripened) by the honey bee to a stable, dense and high energy food. The natural honey is a supersaturated sugar solution, with sugars and water making up 99% of most honeys where sugars are its main constituents comprising about 95% of its dry weight, while glucose and fructose are the dominant constituents; about 25 different sugars have been detected (Siddiqui 1970, Doner 1977, Fallico *et al* 2003, Alvarez *et al* 2010). The sugar concentration depends on different climatic factors such as temperature, soil humidity and season. Along with sugar and water, natural honey contains 200 substances, which consists of the complex mixture of other saccharides, amino acids, carbohydrates, aroma constituents, dextrin, peptides, enzymes, proteins, organic acids, polyphenols, and carotenoid like substances, vitamins, mineral pollen and other substances (White 1975, Sato and Miyata 2000, Gheldof *et al* 2002).

Honey is a biological product used as food since ancient time. The special status of honey among consumers is due to its natural image and to its purported health benefits including unique nutritional and medicinal properties. Honey produced by honey bees has been traditionally recognized as valuable source of energy which contains antimicrobial and antioxidant characteristics. It is a unique sweetening agent that can be used by humans without processing.

The composition of honey produced in the world is variable. Although the major constituents of honey are nearly the same in all honey samples, the precise chemical composition and physical properties of natural honeys differ according to various factors like the plant species on which the bees forage, geographical, seasonal, floral source, plant origin, harvest prior to complete maturation and storage conditions (Serrano *et al* 2004, Nanda *et al* 2003, James *et al* 2009, Cantarelli *et al* 2008, Ciappini *et al* 2008, Omafuvbe and Akanbi 2009, Ebenezer and Olubenga 2010). Honey is classified on basis of packaging and processing as crystallized honey, pasteurized honey, raw honey, strained honey, filtered honey, ultrasonicated honey, creamed honey, dried honey, comb honey, chunk honey and honey decoctions.

Adulteration usually refers to mixing other substance of an inferior and sometimes harmful quality with food or drink intended to be sold. With companies concerned about the bottom line, the temptation to cheat is considerable, and unfortunately, the adulteration of food products is a serious economic and regulatory problem. Fluid foods, like, milk, honey,

alcohol, fruit juices and other beverages fall easy prey to adulteration as a number of cheap liquids are easily miscible with them. Sometimes, they are highly toxic and when mixed unscrupulously with liquids for human consumption, may cause serious health problems. Honey has been a prime target for adulteration to get economic gain. Honey can be made poorer in quality by adding amounts of sucrose, commercial glucose, starch, chalk, gelatins, water and other substances. These artificial honeys often have similar taste and physical appearance as natural honeys, but they lack the medicinal and nutritional properties of natural honeys, because of the absence of the minor constituents that are present in natural honeys. Although the adulteration of honey is not injurious to health, problems of honey fraud negatively influence market growth by damaging consumer confidence (Cabanero *et al* 2006). It seems quite necessary that preparing an overall review of the applied procedures by researchers in detecting honey adulteration would be useful and serve as a good source in oncoming works. Singh and Dwivedi (1995) reported changes in the physical properties of honey such as density, viscosity and homogeneity, which were accompanied by changes in ultrasound velocity due to adulteration.

Detection of adulteration in honey is difficult, because of the large natural variability of honey, such as differences in species, maturity, environment, processing and storage technique (Cordella *et al* 2003). This informs the need for characterization of honey samples. Although, the tasks involved for complete analysis of a complex product like honey is not only laborious but also expensive. However, some physicochemical properties of honeys that can be easily determined have been found to be helpful for comparison of natural honey samples from different locations and also serves as important indicators that can help to distinguish natural honey from artificial honey.

Moisture content of honey which is defined as the percentage of water present in it comes from the nectar. Moisture content can be considered the most important parameter of honey quality as it determines its stability and resistance towards microbial spoilage during storage above the maximum permitted level which affects its sensory properties (Bogdanov *et al* 1999). The higher the water content in honey, the greater possibility of yeast fermentation and thus the change of the flavor and color of honey. The analysis of moisture content and acidity of honey play an important role in determining the overall characteristic of honey and final assessment of its quality (Nadezda *et al* 2014). Because of its unique composition and chemical properties, honey is suitable for long-term storage and is easily assimilated even after long preservation. Honey and objects immersed in honey, have been preserved for decades and even centuries. The key to preservation is limiting access to humidity. If exposed to moist air, its hydrophilic properties will pull moisture into the honey, eventually diluting it to the point that fermentation can begin. It is frequently necessary to heat honey to prevent fermentation by sugar tolerant yeasts and to keep it liquid as long as possible (Fallico *et al*

2003).

Ultrasound is a versatile non-destructive technique and highly useful for the investigation of various physical properties of different food. Ultrasound techniques are relatively cheap, simple, energy saving, and thus became an emerging technology to be used for food processing. These techniques find use in the food industry in both for the analysis and modification of foods. Microbial and enzyme inactivation are other applications of ultrasound in food processing. The principle aim of this technology is to reduce the processing time, save energy and improve the shelf life and quality of food products (Chemat *et al* 2011). This technology involves the passage of ultrasonic waves through the given sample. Ultrasonic waves are defined as sound waves with a higher frequency than the maximum that can be sensed by the human ear. The frequency range of ultrasonic waves consists of very high frequencies between 20 KHz to 200 MHz.

Ultrasonic wave velocity is usually correlated with density fluctuations of pure liquid thereby it is closely related to compressibility, which is a response function of the density to the mechanical pressure. Ultrasonic waves will passes easily through low viscosity fluids causing cavitations. The low frequency ultrasound will be better in penetrating viscous products such as honey than high frequency ultrasonic waves, since high frequency ultrasonic waves will be dispersed in the solution causing a reduction in the overall intensity of the input energy. Ultrasonic velocity can be used with other properties by thermodynamic relationship, results many important thermodynamic properties (Singh and Singh 2011).

The effect of ultrasonic on honey causes reduction of the crystal size. Initially it was thought that ultrasound treatment of the D-glucose monohydrate crystals in honey would shatter them, thereby reducing their size. However, the use of an image analyzer has shown that rather than reducing the crystal size through breakup of the crystals, the crystal size is reduced through partial melting or dissolution of the D-glucose monohydrate crystals. The clean, sharp crystal structures (plates) are replaced by irregular shaped plates, indicating that some glucose molecules on the edge of the crystal structure dissolve into the surrounding liquid. Thus the size of the crystals in the seed honey can be reduced by ultrasound treatment. Kaloyereas (1955) reported that frequency sound waves (9 kHz) eliminated the existing crystals and retarded further crystallization in honey. Ultrasound processing destroyed most of the yeast cells that were present in the honey, and those that survived had lost their ability to grow. No crystals were observed in ultrasound treated honey and inhibited granulation for a period (15 months at 16°C) comparable to heating the honey (Kaloyereas *et al* 1958). None of the ultrasound treatments enhanced the level of D-glucose monohydrate crystals relative to the untreated honey. Propagation of ultrasonic waves through the fluid foods was studied because mixing of adulterants in them changes their physical properties, such as density, viscosity and homogeneity, etc. These have direct impact on the velocity of ultrasonic waves

passing through these media. This study would help in easy and quick detection of adulteration in fluid foods thereby resulting in better health care.

Electrical conductivity (EC) is one of the important factors in the determination of the physical characteristics of honey, which can be determined by relatively inexpensive instrumentation. The EC of an aqueous solution is the measure of its ability to carry an electrical current by means of ionic motion. It is affected by the type and number of ions in the solution and by the viscosity of the solution itself. Honey contains organic acids, minerals, ash content, proteins, complex sugars and mineral salts, these compounds are chemically called ionizable that is when in solution, they have the property to conduct electric current; also the higher their content, the higher the resulting EC (Bogdanov 2002, Terrab *et al* 2003, Szczesna and Rybak 2004). The measurement of EC was introduced a long time ago (Vorwohl 1964). This parameter shows great variability according to the floral origin and can be used for differentiating between honey samples (Nigussie *et al* 2012).

Total Dissolved Solids (TDS) is a measure of the combined content of all inorganic and organic substances in honey in the molecular, ionized or micro-granular (colloidal solution) suspended forms. There is a good correlation between EC and TDS, indicating that both parameters can be used to determine honey purity (Khalil *et al* 2012).

In general, honey is acidic in nature irrespective of its variable geographical origin. The acidity of honey is due to the presence of organic acids, particularly the gluconic acid and the inorganic ions such as phosphate and chloride (Ouchemoukh *et al* 2007, Ajlouni and Sujirapinyokul 2010). The presence of organic acids in honey contributes to its flavor and its stability against microbial spoilage. The pH of honey samples is important during the extraction process because it affects the texture of honey as well as its stability and shelf life (Terrab *et al* 2002). The pH and the free acidity of honey can influence honey stability and its storage conditions. They also give information on honey origin.

The rheological and physicochemical properties of honey provide the parameters for characterization and classification of honeys which includes important qualities that influence the sensory quality of the product, acceptability of honey and also affect a number of technological operations, such as honey heating, mixing, filtering, hydraulic transport and bottling (Yanniotis *et al* 2006, James *et al* 2009). Thermodynamic parameters such as adiabatic compressibility, acoustic impedance, intermolecular free length and relative association number, etc have been considered as an analytical tool to provide fundamental insight on the structural organization of any food (Tadkalkar *et al* 2011). Surface tension and viscosity are the important parameters to characterize the material. Similar to surface tension and viscosity, ultrasonic velocity is also an important parameter for materials' characterization such as honey. The International Honey Commission (IHC) has therefore proposed certain constituents like moisture content, sugar, acidity and viscosity as the quality

criteria for honey. The effect of honey on light can also be useful for determining the type and quality of product. Variations in the moisture content of honey alter its refractive index. Moisture content can easily be measured with a refractometer.

Plenty of literature is available on physicochemical, rheological and quality aspects of honey produced in different countries. A vast country like India too produces honey from various floral sources with wide variations in physicochemical, functional and rheological characteristics. Only a limited amount of information is available on rheological and thermal properties of Indian honey. These properties are to be noted over wide range of temperatures to know the effect of temperature on them during processing and storage of this liquid food. Therefore, it is proposed to conduct and investigate the properties of honey with following objectives:

- To study the ultrasonic velocity and density of honey at different moisture content and temperatures.
- To evaluate the thermodynamic parameters such as adiabatic compressibility and surface tension from the measured data.

CHAPTER-II

REVIEW OF LITERATURE

Honey is a biological product used as food since ancient time. To prevent the honey adulteration, it is necessary to study the various physicochemical properties or characteristics of honey as its composition is variable in the world. The major research work done for physicochemical and other properties of honey in different nations and in India will be discussed in this chapter.

According to White (1975), honey is a hygroscopic product that absorbs moisture from the air, making it susceptible to fermentation. Most honeys contain more fructose than glucose, and fructose is a hygroscopic carbohydrate. The gain or loss of moisture in honey, when exposed to the air, depends on temperature, moisture content and relative humidity of the air.

Honey is considered as Newtonian food but non-Newtonian behaviour had been reported by Munro (1943). He showed the effect of temperature on viscosity of honey from three different states. The greatest decrease in viscosity occurs with cold honey heated to room temperature, later heating reduces the viscosity of honey and heating above 30°C has a low practical value.

Yanniotis *et al* (2006) studied the effect of moisture content on the viscosity of honey at different temperatures. It was found that viscosity of honey varies with temperature, moisture content and its botanical origin. Viscosity was measured in two honeydew honey (pine and fir) and four unifloral nectar honey (thymus, orange, helianthus and cotton) at their initial moisture content as well as at 17, 19 and 21% water content at 25, 30, 35, 40 and 45°C. Viscosity varied between 0.421 and 23.405 Pas. Viscosity was time independent. Arrhenius equation was used to describe satisfactorily the variation of viscosity with temperature. The activation energy decreased as the moisture content increased varied between 70.8 and 96.3 kJ/mol, indicated that the viscosity is more sensitive to temperature changes at low moisture content and all the samples showed Newtonian behaviour.

Zaitoun *et al* (2001) investigated the rheological properties of selected apple, besromia, citrus, and ziziphus types of light colored Jordanian honey. The types of honey used were identified via assessing the source of nectar using pollen analysis. The moisture content of honey samples was indirectly accessed via measuring the refractive index of the sample using a refractometer. A rotational, concentric cylinder viscometer was used to measure rheological properties of honey samples. The apparent viscosity was measured and Newton's law of viscosity was found to adequately describe the flow behavior of honey samples. The apparent viscosity was found to decrease with temperature, and the temperature dependency of viscosity was found to follow the Arrhenius model. Moreover, the viscosity was also found to decrease with moisture content of honey.

Popek (2002) developed a new procedure to identify a honey type. The physicochemical parameters of quality of honeys were determined in 73 honey samples. A special dependence exists among the specific electrical conductance, the total ash content, and the dynamic viscosity, a nearly correct classification (98.67%) was achieved using these parameters. The high specific electrical conductance of honey resulted from the high content of total ash in them. For the relationship between specific electrical conductance and dynamic viscosity, it was confirmed that a high specific electrical conductance honey was connected with a low level of their dynamic viscosity. It was found that the dynamic viscosity showed comparable values for all investigated honey samples from various type and variety groups. It was analyzed that the lowest acidity of honey was connected with the fact that dissociated organic acids, contained these honey varieties. At the same time, with regard to sugars contained in each of the 73 investigated honey samples, an interesting inverse proportionality was stated between the sugar content and water content in honey. Thus discriminant function analysis based on the determination of the five honey quality parameters which are EC, total acidity, ash content, sucrose, and reducing sugars will enable the identification of types and varieties of honey.

Conforti *et al* (2006) studied honey from different regions of Buenos Aires. The honey samples were stored at −20°C, and factors that affect crystallization were analyzed. Crystals were observed by light microscopy. Firmness, adhesivity and viscosity of the samples were measured. Honey was characterized by determining the water activity, turbidity, moisture, fructose, and glucose contents. Results obtained shows that the viscous characteristics of the samples depend on the number, size, and disposition of crystals. Various honey samples exhibited Newtonian, pseudo plastic and thixotropic behaviors. Crystallization was favored at higher moisture contents, suggesting that the parameters that affect honey crystallization at room temperature have a different effect at freezing temperatures. Honey that presented higher values of firmness had moisture content lower than 17%. Therefore, sugars in honey are responsible for properties such as viscosity, hygroscopy, specific rotation, crystallization phenomena and energy value. The ratio of glucose to fructose partially determines the crystallizations speed of honey.

Some selected Brazilian honeys produced in the northeast region were analyzed by Costa *et al* (2013) for determination of the rheological, thermal and some other physical-chemical properties including the water activity, pH, soluble solids content (°Brix) and moisture content. Twelve honey samples were taken for study, two samples were melipona subnitida and ten other samples were of Apis mellifera. Viscosity curves were obtained using a rheometer (25°C, 0-100 s^{-1}). Thermal analyses were performed on a differential scanning calorimeter, with heating rate of 10°C/min (-100 to 100°C). The water content and the pH of the honey samples varied from 17.2 to 27.9% and from 3.2 to 4.2 respectively and the water

activity of the samples varied from 0.57 to 0.74. Two samples were out of specification with respect to water content, according to Brazilian laws. In relation to rheology, all honey samples showed Newtonian behaviour. The viscosity varied as an exponential function of the moisture content. The highest viscosity was obtained for the sample with lower values of moisture content and water activity. Also, the analysis revealed that the floral source and honey bee species affected the quality parameters by affecting the moisture of the samples, with exception for the pH and the soluble solids. Each honey type was shown to have properties that could be expressed as a function of its water content.

Mehryar *et al* (2013) investigated several physicochemical, thermal and rheological characteristics of six Iranian honey samples from various floral sources. The studied parameters were included moisture content, water activity, water insoluble solids, diastase activity, pH, free acidity, total sugar content, reducing sugar, fructose to glucose ratio, sucrose content, ash, fat, total nitrogen content, hydroxyl methyl furfural (HMF), density, specific weight, color, EC, glass transition temperature, viscosity, stickiness and equilibrium moisture content. In order to have a precise evaluation of honey samples, some of its physical properties such as density and viscosity were investigated at three temperature levels. Most of the investigated physicochemical indices were in the range of related standards and were similar to the values found by other researchers. The relationships between some physical and chemical properties were also determined. All of the samples showed non-Newtonian behavior. Equilibrium moisture content of the samples was increased by increasing water activity but the inversion effect has appeared at a constant water activity (>0.5) among different temperatures of the samples. Results of the physicochemical analysis of honey samples suggest that the floral sources of honey are the most important factor in its variety.

Pryzybylowski and Wilcznska (2001) reported the contents of Zn, Cd and Pb in fifteen honey samples from the Pomeranian region by atomic absorption spectrometer. The mean values for Zn, Cd and Pb were 7.76, 0.015 and 0.048 mg/kg respectively. The other properties of honey samples invert sugar, sucrose, HMF, diastase activity, pH, EC, moisture and mechanical pollutions were also determined. Pomeranian honeys were of good quality, but they were not free of heavy metals. Results suggested that honey may be useful for assessing the presence of environmental contaminants. Therefore, honey can be used as a environmental marker which makes it even more interesting to compares the mineral contents of honeys produced in different areas of the world.

For the study of Indian honey Ahmed *et al* (2007) analyzed some selected Indian honey samples and revealed that floral source has important role in quality parameters pertaining to processing and storage. Physico-chemical, thermal, rheological and dielectric characteristics of seven Indian honey samples obtained from various floral sources were investigated. This study demonstrated that honey collected from same environmental and

geographical location significantly varied in rheological, thermal and dielectric properties. The samples were found to differ from each other in pH, ash content and visual color values. Contrary to most of the reports, some honey samples exhibited non-Newtonian behaviour with yield stress. Honey obtained from neem floral source exhibited maximum total solids, higher consistency index and lower glass transition temperature compared to rest of the honey samples. Dielectric properties were found to be function of water and ash content of honey samples and varied significantly among studied samples.

Gairola *et al* (2013) studied some honey samples from Uttarkashi district of Uttarakhand state in India. They evaluated and compared the physicochemical properties of Apis cerana indica honey samples from Uttarkashi. They analyzed the 11 honey samples for their physico-chemical properties including viscosity, specific gravity, moisture content, HMF, total reducing sugars (TRS), fructose, glucose and sucrose. The results showed that the moisture percentage ranged from 19-25 percent. Fructose and glucose represented the major sugars in all honey samples. The fructose percentage ranged from 37.27-40.51, whereas values for glucose were 35.12-38.04 percent. They found that out of 11 honey samples, six showed lower granulation tendency and all the honey samples have good quality. The study indicated that all the honey samples had good quality and it will be important for the understanding local honey properties and commercialization of regional honey.

Iftikhar *et al* (2014) determined the quality of honey samples from local and imported brands available in the Rawalpindi and Islamabad markets. The physicochemical analysis of twenty four honey samples was done including the properties moisture content, pH, acidity, sucrose, total sugars and EC. The ranges of different parameters are 17.0-19.0% moisture, 3.24-6.5 pH, 7.0-25.0 acidity, 7.60-8.70% sucrose, 75.0-83.0% total sugars and 0.08-0.80 mS/ cm EC. These parameters are within the normal ranges. Some of the samples showed high HMF and low diastase number which confirm low quality of the samples. One sample had highest value of 95.0 mg/kg of HMF while two of samples had 79.0 mg/kg and 75.0 mg/kg HMF. These are higher than the recommended ranges. These high HMF value indicated that these three samples were of low quality honey.

The physicochemical properties of fifteen commercial honey samples from EDO state, Nigeria was evaluated by Oshomah *et al* (2015). Physicochemical properties such as pH, colour, acidity, ash content, moisture content, EC, refractive index, total sugar concentration, HMF and viscosity were examined. Results of physicochemical properties shows that the studied parameters were within recommended limits with the exception of moisture and HMF contents for which recorded values were beyond the maximum recommended limits for some of the studied samples. Moisture content ranged between 15.40±0.02 to 24.96±0.02 while the values observed for HMF contents were within the range of 1.99±0.43 to 71.73±0.78mg/kg. This situation could be attributed to the incorrect processes

applied by honey producers in extraction, processing, storage and preservation. The presence of mould and bacteria in some of the samples may be attributed mainly to contamination due to poor handling at harvest, packaging or storage. Honey obtained from the local markets was contaminated with fungi and bacteria and coliform organisms indicating inadequate hygiene conditions.

Awada and Elgornazib (2016) studied the honey samples from two different areas of Libya. Ten honey samples were collected from Kasr Khiar and Garaboli (Tripoli Area) in west of Libya. The physicochemical properties of honey samples collected during raining season (November 2014 to March 2015) from Kasr Khiar area were compared with honey samples obtained from Garaboli area (West of Libya) and properties of both samples were compared with International standards. The physicochemical properties like moisture content, pH value, specific conductivity, TDS, specific gravity, acid equivalent, sucrose content, maltose content and mineral composition of honey samples was calculated. The results showed that the average value of moisture, pH value, specific conductivity, TDS, specific gravity, acid equivalent, sucrose content, and maltose content of samples collected from Kasr Khiar area were found to be 18.722 %, 3.77, 587.8 μS/cm, 674 ppm, 1.368, 23.33 m Eq./kg, 1.38 % and 9.02 % respectively, while for Garaboli samples the average values, for the above parameters, were found to be 18.11 %, 4.09, 777 μS/cm, 1255 ppm, 1.38, 16.66 m Eq./kg, 1.68 % and 8.95 % respectively. The maltose content of honey samples collected from Kasr Khiar and Garaboli areas were ranged respectively from 6.24 to 10.98% and 7.98 to 9.87. Comparing the results with International standards it was found that all these samples were agreed within the specification of purity and they have high quality and they unspotted from mixed. There was no significantly difference on pH value and specific gravity between the honey samples of two areas and these results fall within international standard. The ionic organic and inorganic substance in honey samples were measured as TDS. There was a clear correlation between TDS and EC, indicating that both parameters can be used to determine honey purity.

Sohaimy *et al* (2015) investigated the physicochemical characteristics of different honey samples from different origins to confirm its economical and nutritional quality. The samples of honey taken were Alexandria, Egypt, Yemeni, Saudi and Kashmiri honey. The characteristics of these honey samples like moisture content, ph, EC, colour analysis, colour intensity, optical density, ash content, total protein content and sugar content was calculated. There is no significant difference in colour remarked between all studied samples of honey. All investigated types of honey were acidic and were within the standard limit. All studied types of honey were within the standard limit of moisture content. A correlation coefficient was found between ash content of honey and Kashmiri honey showed the highest protein content followed by Yemeni and Saudi, while the lowest value of protein content was

registered in Egyptian honey. The high sugar content of the investigated honey samples could be attributed to its high acidity and low moisture content, which inhibits the formation of HMF from sugars, especially glucose and fructose. Thus, the quality and physicochemical properties of honey were varied based on the geographical and botanical origins, handling, transportation and storage conditions.

According to Bhandari *et al* (1999) there are diverse methods to stop crystallization of honey which is stored at freezing temperature (- 40°C), which includes the heat treatment to dissolve crystals and crystal nuclei, removal of air bubbles, dust and pollen particles by filtration of honey, filling at higher temperatures (> 45°C) to avoid air bubbles incorporation during filling, addition of inhibitors in honey such as isobutiric and ascorbic acid, and adjusting the glucose to fructose ratios or water content.

Helena (2007) examined the six samples of honey and changes undergoing in the composition of honey during a half-year storage time of that product at temperatures of 20°C ±2°C and 4°±2°C were determined. The samples were also checked for any possible changes that could have been brought about by the thermal stabilization treatment which was performed at a temperature of 100°C (in a boiling water bath) for 15 minutes. The study indicates that a honey sample can be protected against changes in carbohydrate contents for half a year by storing it in a refrigerator (in this particular experiment at 4°C±2°C). At room temperature, during a half-year storage period of honey samples the greatest changes were recorded in sucrose content. Sucrose content dropped by as much as 79% compared to its initial value. Thermal stabilization process itself did not bring about any change in the content of the sugars tested. The stabilization of honey samples to be examined for sugar contents proved to be warranted if the samples were to be exposed to uncontrolled ambient temperatures during storage and transport. Storage of honey samples to be examined for sugar contents and as well as that of check samples should also performed at low temperature (4°C).

Karabournioti and Zervalaki (2001) studied the effect of heat treatment on five Greek honeys with different botanical origins at temperatures of 35, 45, 55, 65 and 75°C for 24 hours. These heat treated honey samples were analyzed for their HMF content and invertase activity, as these are considered as the main parameters for evaluating the freshness and storage history of honey. The increase in HMF content and decrease in invertase activity were observed after heating. It was found that the heat resistance is different according to the botanical origin of honey, with the pine honey sample being the most resistant. Further, a decrease in invertase activity started at a temperature of 35°C, and the HMF content was less than the international standard of 40 mg/kg even at 55°C. The combination of both invertase and HMF showed an analytical criteria and safer way in order to detect the exposure to heat of honey, as invertase can detect gentle heating and HMF gives information about overtime heating.

Slawomir (2007) measured the rheological properties of Polish honeys. The influence of temperature and water content on the rheological properties of honey samples was analyzed. It was based on the description of honey viscosity using two parameters: absolute temperature and water content in terms of mass fractions. This lead to the origin and chemical composition of the honey investigated. The measurements were conducted within a wide temperature range from 260 K to 330 K. The water mass fraction in all the investigated honey samples was 0.146-0.20 g/g. All the investigated honey samples showed the Newtonian behaviour. It was found out that the temperature was the parameter that had the most significant effect on honey viscosity. An exponential dependence between honey viscosity and temperature and water content was determined. Making use of multiple regressions it was possible to create a mathematical model to describe honey viscosity in the function of temperature and water content.

The processing of honey was studied by Subramanian *et al* (2007). It was explained that the microwave heating can be effectively used for thermal processing of honey, as it provides a rapid heating to achieve the desired level of yeast reduction with reduced thermal damage which results long-term storage of honey. Thermal processing of honey eliminates the microorganisms responsible for spoilage. Microwave heating, infrared heating, ultrasound processing, and membrane processing have been explored as alternatives to conventional heat processing. Infrared heating was not as rapid as microwave heating but desired results were achieved in a relatively shorter duration (3 to 4 minutes) when compared to the conventional method. It was explained that the area of microwave and infrared heating of honey needed to establish the relationship between various processing conditions and honey quality. Ultrasound processing destroyed most of the yeast cells presents in the honey, besides eliminating the existed crystals and it retarded further crystallization in honey. Microfiltration and ultrafiltration could be employed to produce enzyme-enriched honey besides clarified honey.

Saxena *et al* (2014) characterized Indian honey samples for their rheological properties under the effect of temperature and gamma radiation. The seven samples of commercial Indian brands were taken from the market. Viscosity of all the honey samples belonging to different commercial brands was found to decrease with increase in temperature (5-40°C) and their sensitivity towards temperature varied significantly as explained by calculating activation energy based on Arrhenius model and ranged from 54.0 to 89.0 kJ/mol. The viscosity of honey samples remained unaltered upon gamma radiation treatment. Honey was known to contain pathogenic microbial spores and gamma radiation was found to be effective in achieving microbial decontamination of honey. The effect of gamma radiation on rheological properties of honey was assessed, and it was found to remain unchanged upon radiation treatment. The glass transition temperatures of these honey samples were analyzed

by differential scanning calorimetry varied from -44.1 to -54.1°C and remained unchanged upon gamma radiation treatment. The results provided the information about some key physical properties of commercial Indian honey. Radiation treatment was useful for ensuring microbial safety of honey samples and does not alter these properties. All the commercial Indian honey brands evaluated showed Newtonian behaviour.

Sancho *et al* (1991) studied the EC and the ash content of the honey. In this study thirty honeys of the Basque country (N Spain) originating from the chief honey production areas was taken and linear relationships have been found between EC, measured at 20°C in solutions containing 10 g of honey dissolved in 75 ml of water, and total and sulphated ash contents. The EC measurements were taken with a Radiometer conductimeter with a 1-cm radiometer electrode. Sample temperature was maintained at 20°C using an ultra thermostat. An infrared lamp and an oven were used to find ash content of honey samples. To find the sulphated ash contents the infrared lamp described above was used to heat the samples on a hot plate until they were well carbonized and evaporated after the second addition of sulphuric acid. The straightforwardness of EC measurements in honey makes these relationships useful means of calculating ash content.

Serrano (2004) gave the characterization of two types of Andalusian unifloral honey. Characterization of honey samples was carried out on the basis of their physicochemical properties like moisture, HMF, diastase activity, pH, free acidity, lactone acidity, EC, glucose, fructose, sucrose, proline, invertase, glucose-oxidase, water activity and insoluble solids. All the data were statistically to classify the honey samples and identifying the most significant parameters in the classification. It was verified that these properties were different, depending on the type of honey. Of the six main factors obtained with a variance percentage of 78.95, it was the first one (free acidity, water, invertase, total sugars, EC and solids) which explained the greater part of the variability (22.9%). The variables with the greatest discriminatory power were water activity and EC with discrimination coefficients of –22.367 and 11.739, respectively. The overall proportion of accurately arranged samples was 96.6%. Therefore, the EC can be used as an additional method for determining the source of the honey which depends on depends on the content of minerals, organic acids, proteins and some sugars.

Bogdanov *et al* (2004) reviewed all the known physico-chemical methods used for the determination of the botanical origin of honey. This review gave a critical evaluation of all physico-chemical methods, which have been used for the classification of unifloral honeys. The review is divided into two parts. The first one provides a general survey of classical physico chemical methods. These methods are mostly validated and widely used in the routine control of honey. In the second part of review gave the utility of new methods for authentication of botanical origin. In most cases, these methods are not yet harmonized and validated for routine use. In case of acidity of honey, it was found that honey is deceptively

acidic, as the high sugar content tends to mask the acidity in the taste. Honey is a buffer, which means that its pH does not change by the addition of small quantities of acids and bases. The buffer capacity is due to the content of phosphates, carbonates and other mineral salts.

Acquarone *et al* (2007) gives the pattern of pH and EC upon honey dilution as a complementary tool for discriminating geographical origin of honeys. Nineteen nectar honey samples of different floral origin and from several regions of Argentina were analyzed for seven physicochemical parameters (water content, ash, water activity, free, lactone and total acidity, pH and EC). The pattern of EC and pH upon honey dilution were also studied with increasing honey concentration, the pH values decreased exponentially. The dependence of specific EC (j) on honey concentration was characterized by a maximum at a j value (j_{max}) corresponding to a dry solids of honey concentration of 30-35% (w/w). For a given geographical region, ash and acidity values were useful for discriminating honeys of different floral origins. The variation of pH and EC with honey dilution provides more information than does the determination at a fixed concentration, and was observed to depend on the soil characteristics of the geographical regions.

Yadata (2014) evaluated the EC and acidity of honey from different areas of Tepi region, Ethopia and compared these physicochemical properties of honey samples in summer and winter seasons. The instrumentation and apparatuses used in the work were conductometry and titration apparatuses. Honey samples obtained from four different areas Korcha, Mexi, Sheko and Gobito of Tepi town have been analyzed. The honey solutions were prepared by dissolving 20g of each anhydrous honey sample in 100mL of deionized water in order to prepare 20% (w/v) solution to measure conductivity. The acidity was detected after dissolving 10g of each honey sample in 75mL of deionized water. The conductivity in summer honey determined to be 0.097mS/cm, 0.153mS/cm 0.117mS/cm and 0.337mS/cm in Korcha, Mexi, Sheko and Gobito respectively. Acidity was found to be 17, 28.67, 29 and 25miliequi/Kg. The conductivity in winter honey was detected to be 0.123, 0.186, 0.103 and 0.246mS/cm. The acidity in winter honey was 3.36-4.26 and 18.3-25.3meq/Kg from pH meter and titration respectively. The conductivity in summer season is compared with winter season and the results become almost fall in similar range since the honey product detected was floral and no more components is significantly added with season change. The conductivity of darker honey was slightly greater than lighter honey, which indicated that the darker honey has more mineral content and almost darker honey exhibited slightly high acidity.

The influence of temperature and concentration on the evolution of different physical properties of honey was given by Oroian (2013). Density, viscosity, surface tension and ultrasonic velocity of seven different honey types with different concentration (expressed as °Brix) and source type, were measured at different temperatures 20, 25, 30, 35, 40, 45 and

50°C. Concentrations and temperature influenced significantly the magnitude of the physical properties. Temperature influenced negatively all the physical properties of honey. The regression equations were obtained for predicting the density, viscosity, ultrasonic velocity and surface tension with temperature and concentration. A good correlation between density, viscosity, ultrasonic velocity and surface tension of honey samples using linear and polynomial equations was observed which leads to obtain a master curve for predicting these parameters with high accuracy. Also a good correlation between density, ultrasonic velocity and surface tension for all the honey samples was obtained.

James *et al* (2009) evaluated the physical characterization of some honey samples from North-Central Nigeria. The physicochemical properties (moisture content, sugar content, viscosity, pH and conductivity) of four honey samples taken from Kwara State in North-Central Nigeria was determined and used to evaluate their behaviour in comparison with other reported honey sample around the globe. Four different solutions (20, 40, 60 and 80 (w/w)) of each sample of the honey were prepared, and the exact water content was determined. The moisture content and sugar content varied within the range of (18.22-36.82%) and (63.82-80.25%) respectively. The pH increased with increase in moisture content and the conductivities of the samples had correlation with proportion of minor constituents in the honey samples. The temperature dependence of viscosity was evaluated with Arrhenius model, the activation energy with value of 70.07 kJ/g is fairly unaffected by moisture content. It was found that the percentage moisture content was an important parameter used to access quality of honey samples and could serve as an indicator to detect an artificial honey sample disguised as a natural honey sample.

According to Ercan and Soysal (2013) ultrasound is considered as emerging technology in the food industry. They discussed the various methods of generation of ultrasound and their applications in the food preservation. There are numerous advantages of using ultrasound for food processing such as effective mixing, minimizing flavor loss, saving energy, enhanced quality, reduced chemical and physical hazards, and is environmentally friendly. Due to the elimination of microorganisms and enzymes without destroying nutrients of food, ultrasound can be used as an alternative method to thermal treatments in the food preservation. When ultrasound was applied with pressure and temperature its efficiency increases but cautions needed to determined and control nutritional loss. Also, the process parameters and applied material change under application of temperature and pressure. Thermosonication method (combined effect of ultrasound and heat) produced a greater effect on inactivation of microorganisms in food than heat alone.

Chandrapala (2015) studied the low intensity ultrasound for its applications on food systems. The effect of low intensity ultrasonic waves was noticed on fruits, vegetables, bread, honey, fish and meat. The changes to the physical properties of ultrasound such as scattering,

attenuation and acoustic velocity caused by food materials have been used in food quality assurance applications and were discussed for selected food systems which are of importance to Australian export industry. The ultrasonic velocity increased with the increase of honey amount in the mixture. Though the flavour and taste did not change even up to the adulteration level of 50%, ultrasonic velocity was found to be affected by addition of sugar even in little amounts in honey.

Kaloyereas (1955) reported that sound waves of frequency 9 kHz eliminated the existing crystals and retarded further crystallization in honey. Ultrasound processing destroyed most of the yeast cells that were present in the honey, and those that survived had lost their ability to grow. No crystals were observed in ultrasound treated honey and inhibited granulation for a period (15 months at 16°C) comparable to heating the honey.

Thrasyvoulou *et al* (1994) investigated the effects of ultrasonic waves on the quality of honey focusing on some of the chemical characteristics. Ten samples of crystallized honey were divided into 3 equal parts. The first part was liquified by ultrasonic waves at 23 kHz, the second by heating at 60°C for 30 minutes and the third part remained untreated. Some physicochemical properties including diastase activity, HMF value, moisture content, EC, pH and time of recrystallization of the honey samples were noted. The time that was needed for the liquefaction of honey varied from 18 to 25 min, the energy from 0.1056 to 0.1466 kWh, and the temperature from 76 to 82°C. The quality of honey was affected by increasing HMF levels from 6.5±0.8 ppm to 11.4±1.4 ppm and decreasing diastase activity from 20.8±1.8 DN to 17.4±1.5 DN. Significant differences were found among the HMF averages of samples liquified by ultrasonication and samples liquified by heating. There were no differences found among diastase activity, moisture, EC, pH and time of recrystallization among ultrasonic and heat treatment.

Ultrasonic methods were used by Ratajski *et al* (2010) to identify the honey types. They determined the correlations between the viscosity and temperature of honey and the velocity of ultrasonic wave propagation, and to investigate the use of ultrasonic methods in the identification of different honey types. Two types of honey oilseed rape honey and mixed honey comprising multifloral honey and buckwheat honey at a 1:1 ratio were used for study. Honey was harvested in 2004 in a private apiary kept by one of the authors. To reduce the degree of crystallization, the studied honey was stored for 10 hours in an incubator at a temperature of 50°C. The rheological properties of each sample were analyzed included viscosity and ultrasonic velocity. Measurements were performed at four honey temperatures 25, 28, 33 and 39°C, using a heating circulator with a water bath. Significant correlations between the velocity of ultrasonic wave propagation and the viscosity of honey were determined within the analyzed temperature range. The results of their study suggested that if the difference in the viscosity of honey analyzed at a temperature of 25°C reaches

approximately 1.1 Pa s, then the viscosity could be used as a factor discriminating between various types of honey.

In the last decades some physical and rheological properties of honeys from different origins have been investigated by other scientists. Only limited amount of information is available on rheological and thermal properties of Indian honeys. But no other studies regarding the influence of temperature on density, surface tension and ultrasonic velocity of Indian honey samples at different moisture content have been reported in the literature yet.

CHAPTER-III

MATERIALS AND METHODS

In this chapter the equipment and experiment procedure followed during the course of the study have been discussed in detail. Raw honey was selected to study the effect of temperature on ultrasonic velocity, EC, TDS and density of honey at different concentrations. The various thermodynamic parameters like surface tension, adiabatic compressibility, intermolecular free length, acoustic impedance and bulk modulus are calculated. The experiments were performed at the PG research laboratory of Department of Mathematics, Statistics and Physics, Punjab Agricultural University, Ludhiana.

3.1 Selection of honey sample

Raw honey was preferred over the processed honey because, the pure processed honey will be standardized already with given standards of honey commission or it might contain some adulteration which will not give the exact ultrasonic velocity and other properties of honey. Raw honey from the Brassica (Rapeseed) plant was taken for study. Raw honey straight from hives was believed to be pure honey.

3.2 Preparation of different honey concentrations

To evaluate the effect of temperature on different parameters of honey at different moisture content, the honey concentrations were prepared, which contains 100, 90, 80, 70 and 60 (w/w)% of honey with distilled water and these concentrations are heated at selected temperatures 20, 30, 40, 50, 60 and 70°C for about 20-25 minutes. The 100% honey concentration was the pour raw honey itself. To prepare 90% honey concentration, 10g of water is added in 90g of honey and stirred properly to obtain a uniform mixture of honey with distilled water. Similarly 80, 70 and 60% honey concentrations were prepared by adding 20, 30 and 40g of distilled water in 80, 70 and 60g of honey respectively. The selected parameters ultrasonic velocity, EC, TDS and density of these honey concentrations was recorded at selected temperatures in months of July and August. The experiment for each property was performed with replications of thrice and the mean value of replications is considered as the final value.

3.3 Descriptions of instruments used in study

The brief description of the instruments used for measurements are discussed in following sections.

3.3.1 Ultrasonic interferometer

Ultrasonic interferometer is a device to determine the velocity of ultrasonic waves in a liquid medium. The apparatus contains an ultrasonic cell, which is double walled cell with chromium plated surfaces having capacity of 10 ml. The double wall allows circulation of water around the experimental medium to maintain the known constant temperature of the liquid medium. The ultrasonic waves are prepared by the piezoelectric crystal placed at the

bottom of measuring cell as shown in Fig. 3.1.

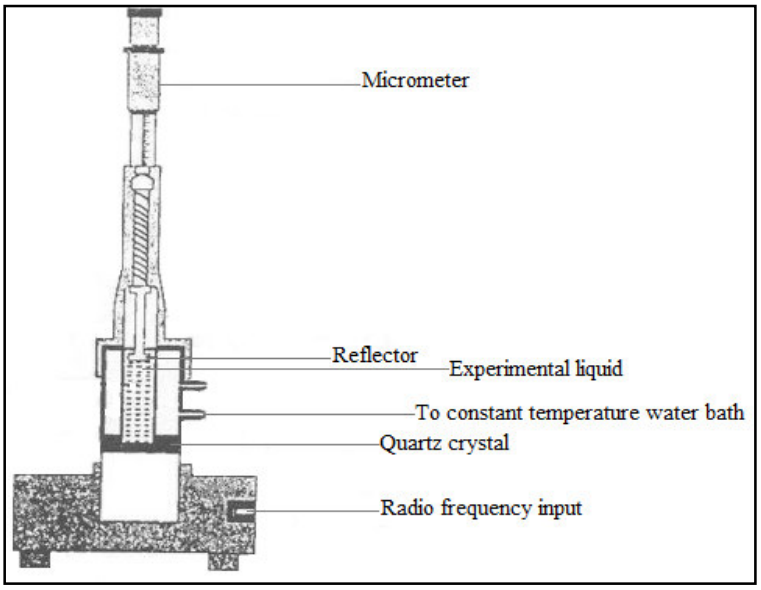

Fig. 3.1 Measuring cell of ultrasonic interferometer

In an ultrasonic interferometer, the ultrasonic waves are produced by the piezoelectric method. An oscillatory circuit is used to provide the electromotive force (EMF) and tuning is obtained by variable condenser. Q is piezoelectric crystal sliced between A and B as shown in Fig. 3.2. This combination forms a parallel plate condenser with crystal as dielectric. The metal plates are connected to primary of transformer which is coupled to oscillatory circuit of triode valve. If the natural frequency of triode valve coincides with the crystal frequency, the resonance will occur and the crystal is set to mechanical vibrations due to piezoelectric effect. With a quartz crystal, ultrasonic of frequencies 540 KHz can be produced. To produce higher frequencies, the plate has to be very thin and strong so that it may stand the strain. In a fixed frequency variable path interferometer, the wavelength of the sound in an experimental liquid medium is measured, and from this one can calculate its velocity through that medium.

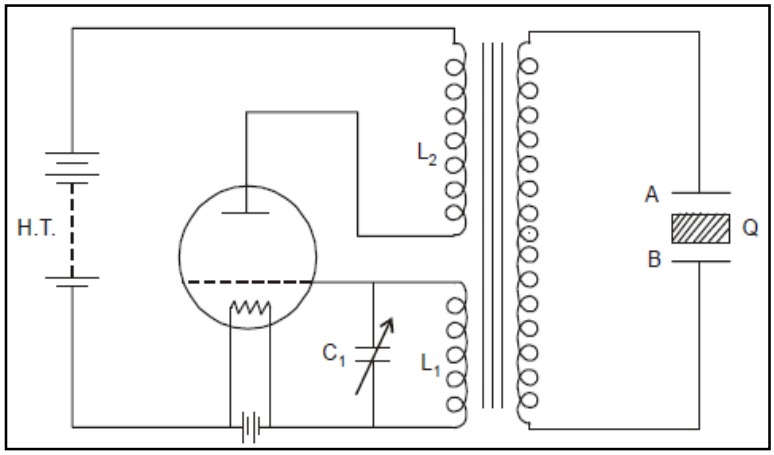

Fig. 3.2 Oscillatory circuit in interferometer

Ultrasonic waves of known frequency are produced by a quartz crystal which is fixed at the bottom of the cell. There is a movable metallic plate parallel to the quartz plate, which reflects the waves. The waves interfere with their reflections, and if the separation between the plates is exactly an integer multiple of half-wavelengths of sound, standing waves are produced in the liquid medium. Under these circumstances, acoustic resonance occurs. The resonant waves are a maximum in amplitude, causing a corresponding maximum in the anode current of the piezoelectric generator.

If we increase or decrease the distance by exactly one half of the wavelength (λ/2) or an integer multiple of one half wavelength, the anode current again becomes maximum. If d is the separation between successive adjacent maxima of anode current, then,

$$d = \lambda/2$$

We have, the velocity (v) of a wave is related to its wavelength (λ) by the relation,

$$v = \lambda f = 2df$$

3.3.2 Conductivity and TDS meter

The DiST (Dissolved Solids Tester) by Hanna instruments (HI 98311) is an advanced, easy and affordable meter to measure the EC and TDS of liquids as shown in Fig. 3.3. It is one of the more expensive portable and bench top meters and digital instrument with large multi level LCD display that shows the standard value EC and TDS along with temperature of the liquid simultaneously. This tester includes features such as: a replaceable graphite electrode, adjustable TDS ratio, battery level indicator at start up, stability indicator to alert the user when a stable reading has been obtained, automatic shut-off and automatic calibration and a hold button to freeze readings on the display. The graphite conductivity electrode offers greater accuracy by resisting contamination by salt deposits in the sample. If meter shows the conductivity reading with temperature, the TDS reading can taken at same temperature by pressing mode button. It has the property of automatic temperature compensation i.e. all readings are compensated for variations in temperature and temperature can displayed in $^{\circ}$C or $^{\circ}$F along with EC and TDS reading.

Fig. 3.3 DiST (Dissolved Solids Tester) by Hanna instruments

3.3.3 Abbe refractometer

The Abbe instrument is the most convenient and widely used refractometer shown in Fig. 3.4. It can be used to measure both refractive index of liquids and solids. In both cases refractive index of the substance must be lower than the refractive index of the glass used to make measuring prism. Its high accuracy is achieved with the use of even a few drops of liquid under test present a convenient and economical method of testing. Its technical specifications involve refractive index range 1.300-1.700 and refractive index accuracy 0.0002.

Fig. 3.4 Abbe refractometer for measurement of refractive index

The upper (measuring) prism is firmly mounted on a bearing that allows its rotation by means of the side arm. The lower (illuminating) prism is hinged to the upper to permit separation for cleaning and for introduction of the sample. The liquid sample which must be non corrosive is put as a thin layer (~0.1mm) between two prisms - measuring and illuminating. Light enters sample from the illuminating prism, gets refracted at critical angle at the bottom surface of measuring prism, and then the telescope which is provided with crosshairs in making a measurement is used to measure position of the border between bright and light areas as shown in Fig. 3.5. Telescope revert the image, so the dark area is at the bottom, even if we expect it to be in the upper part of the field of view. Knowing the angle and refractive index of the measuring prism it is not difficult to calculate refractive index of the sample. Surface of the illuminating prism is matted, so that the light enters the sample at all possible angles, including those almost parallel to the surface.

Refractive index of a substance is a function of a wavelength. If the light source is not monochromatic light gets dispersed and shadow boundary is not well defined, instead of seeing sharp edge between white and black, blurred blue or red border is seen, which causes the measurements either very inaccurate or even impossible. To prevent this dispersion, two compensating Amici prisms is used that can be rotated with respect to another serve to collect

the divergent critical angle rays of different colors into a single white beam, that corresponds in path to that of the sodium D ray.

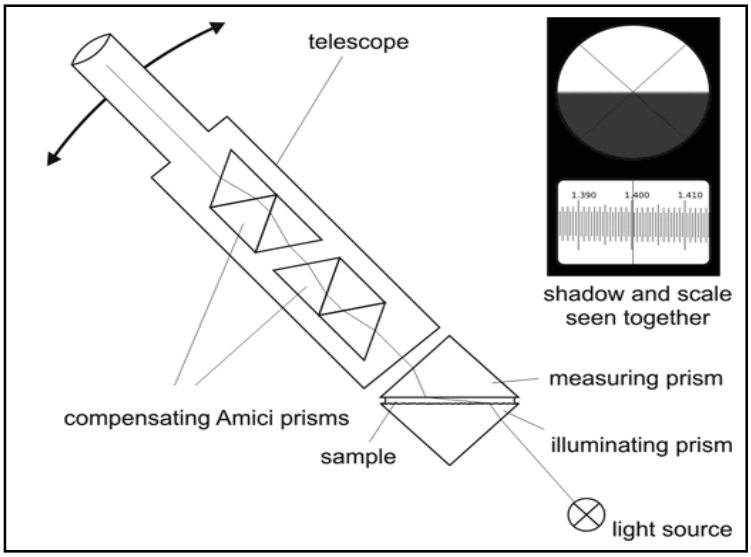

Fig. 3.5 Working of Abbe refractometer

The refractive index of material changes with temperature, for a correct result of refractive index measurements we have to either use thermostated sample or after measuring the refractive index, measure temperature and apply the temperature correction.

3.3.4 Hand refractometer

Hand held refractometer is one of the most popular analytical devices (shown in Fig. 3.6). They are very simple in use and give almost instant result, without tedious and costly laboratory procedures. They are made with a variety of different scales to measure dissolved solids in solution and require only an outside light source to be functional. Hand held refractometer is in most cases critical angle refractometer, not much different from the Abbe refractometer. °Brix hand refractometer is developed for sugar-related liquids such as fruit juices, honey, soft drinks, and wines. These units are used to help monitor and control the sugar concentrations of products in the food.

Fig. 3.6 Hand refractometer for measurement of °Brix

Light bends as it passes through glass of water. If sugar is added to it, the light will

bend more. The refractometer takes advantage of this effect to measure the amount the bending (refraction) which indicates the amount of sugar in the sample. Most refractometer uses a prism and a light source to illuminate the sample.

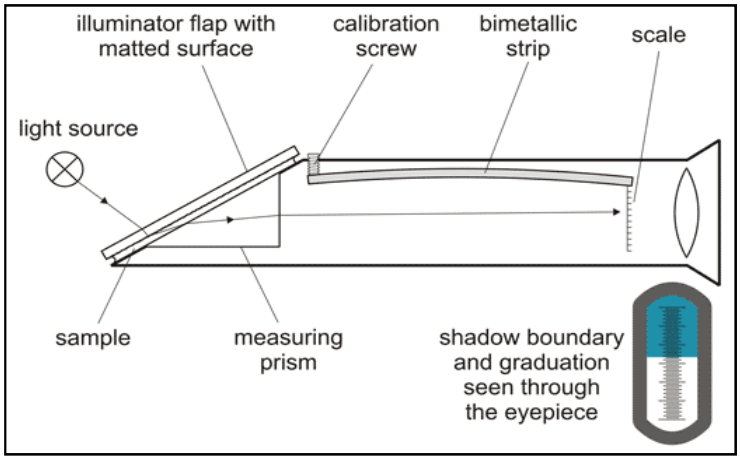

Fig. 3.7 Description of hand refractometer

As shown in Fig. 3.7, hand held refractometer have an illuminator flap which produces a diffused light at a grazing angle and helps to keep the sample in place. Light passes through the sample, enters the measuring prism and possibly other lenses, and finally falls on the measuring scale where it can be read. Depending on the reason for using the refractometer, its scale can be graduated in °Brix degrees.

3.3.5 pH meter

The Hanna pH waterproof tester having pH range is from -2.0 to 16.0, is an advanced meter that has many features found in more expensive portable and bench top meters (shown in Fig. 3.8). This ergonomic meter features automatic one or two point calibration to known buffer, automatic temperature compensation; battery percent level indicator at start up, large multi level LCD display that shows both pH and temperature simultaneously and a stability indicator to alert the user when a stable reading has been obtained. The pH tester also features a replaceable pH probe that has a unique extendable cloth junction to clear any clogging that occurs.

Fig. 3.8 pH meter by Hanna instruments

It has the property of automatic temperature compensation i.e. all readings are compensated for variations in temperature and temperature can displayed in $^{\circ}$C or $^{\circ}$F along with pH reading of sample.

3.4 Measurement of ultrasonic velocity

The ultrasonic velocity in honey concentrations was measured by using ultrasonic interferometer (Batra Trading Company, Timber Market, Ambala, Dual frequency 1 and 3 MHz) with constant frequency of 1 MHz and with least count of micrometer 0.01 mm. The honey sample of prepared concentration was poured in the measuring cell and reading was noted from the micrometer for each maximum of anode current in the ammeter with counts of three maxima. The correct final reading was then calculated by subtracting the first maxima from the third maxima and dividing the result by three. The experimental setup used for ultrasonic velocity measurement is shown in Fig. 3.9.

Fig. 3.9 Experimental setup for measurement of ultrasonic velocity

The ultrasonic velocity in honey was measured at different temperatures (20, 30, 40, 50, 60 and 70°C). Constant temperature was maintained using the water bath. To achieve the particular temperature water at that temperature was circulated for 20 minutes before taking the reading.

The ultrasonic wave velocity was not obtained for 100% honey concentration at temperatures 20, 30 and 40°C and, for 90% and 80% honey concentrations at 20°C. There was not any change observed in anode current of ultrasonic interferometer at frequency input of 1 MHz, for these concentrations at given specified temperatures. It can be because of the incompressibility of honey samples of these concentrations at specified temperatures, so that the honey samples had infinitely large value of modulus of elasticity and hence had infinitely high ultrasonic wave velocity will be propagated through the honey samples which was not measurable.

3.5 Measurement of EC and TDS

EC and TDS of selected honey concentrations were measured. The calibration of meter was done each time before taking the reading with distilled water. After calibration, the

meter in dipped into the beaker containing honey sample and value of EC and TDS was measured at temperatures 20, 30, 40, 50 and 60°C. EC and TDS were measured at constant temperature using water bath. Measurement was taken directly from the screen when honey attained the desired temperature. EC, TDS and temperature were simultaneously measured. Three observations were taken for particular concentration and at particular temperature and their mean value was taken as actual reading.

3.6 Measurement of density

The density of selected honey concentrations was determined by using specific gravity method. The specific gravity bottles of 25 ml were used and weighted using electronic balance with accuracy 0.0001 g. To evaluate the effect of temperature, the gravity bottles were placed in the temperature controlled water bath with temperature regulation for 20 minutes at each temperature, so the honey samples will acquired the selected temperature.

3.7 Measurement of refractive index

The refractive index or index of refraction (n) of a material is a dimensionless number that describes how light propagates through that medium. It is defined as,

$$n = c/v$$

Where c is the speed of light in vacuum and v is the phase velocity of light in the medium. For example, the refractive index of water is 1.333 means that light travels 1.333 times slower in a water than it does in vacuum. This implies that vacuum has a refractive index of 1 and that the frequency of the wave is not affected by the refractive index. In the present study the refractive index of selected honey concentrations was calculated by using the Abbe refractometer. The reading of refractive index was noted from scale by adjusting the cross wire of telescope of Abbe refractometer on region distinguishing the dark and light region at room temperature and then a temperature correction factor 0.00023 per °C for temperature greater than 20°C was applied to calculate the final value of refractive index for each honey concentration.

3.8 Measurement of °Brix

°Brix is defined as the percent by weight of sugar solids in a pure sucrose solution and is expressed as in terms of degree brix. It is the ratio of Total Soluble Solids (TSS) to water in solution. One degree °Brix is 1 gram of sucrose in 100 grams of solution and it represents the strength of solution as percentage by mass, for example 25 °Brix is 25% TSS by weight i.e. 25 grams of solids to 75 grams of water.

The °Brix of selected honey and its different concentrations was measured by °Brix refractometer (with °Brix scale 58-92% and 28-62%). The few drops of honey sample to be examined were placed on the measurement prism surface by opening the illuminator flap which is connected to the device by a small hinge. After that the flap was closed and reading of the °Brix was directly noted from the result scale through the eyepiece by keeping the

refractometer in the direction of some light source like in sunlight direction.

3.9 Measurement of pH

pH was measured with Hanna pH meter (Model HI73127). The calibration of sample was done before each reading with the standard buffer solutions (Buffer 4 and 7). After calibration, the pH meter was dipped into the honey sample of particular concentration in such a way that bulb of pH meter was completely immersed into the sample. Press the cal button and then screen display the reading of pH up to two decimal places. The pH was recorded for five different concentrations at room temperature with three replications and their mean value was taken as actual reading.

3.10 Determination of other thermodynamic parameters

Some other physicochemical properties of honey samples were also determined using the values of measured properties. The effect of temperature on some other thermodynamic properties of the honey was determined by using the measured values of ultrasonic velocity and density of honey concentrations which are given in following sections.

3.10.1 Determination of moisture content

The moisture content of honey samples of various concentrations were determined from the recorded values of refractive index (n) of the honey samples using the relation given by Abu-Jdayil *et al* (2002).

$$\text{Moisture } \% = 608.277\text{-}395.743 \times n$$

3.10.2 Determination of surface tension

Surface tension of a liquid is its elastic tendency which makes it to acquire the least surface area possible. It describes the behavior of the outermost layer of molecules of a liquid at the liquid/gas interface and is a much simpler concept. It is characterized by what appears to be an elastic sheet on a liquid's surface. The water molecules inside the liquid are attracted to one another through cohesive forces which balance out to zero. The molecules at the liquid's surface, however, do not have as many molecules to attract to and are thus pulled inward causing the surface of a liquid to behave like an elastic sheet such that if we try to pull a molecule from the surface an attractive restoring force due to cohesive forces acts on the molecule and if we slightly depressed a surface molecule, then the molecule experiences a restoring force. At liquid air interfaces, surface tension results from the greater attraction of water molecules to each other (cohesion) than to the molecules in the air (adhesion). The surface tension was computed using the equation given by,

$$\sigma = 6.33 \times 10^{-10}\, \rho v^{1.5}$$

Where v is the ultrasonic velocity in m/s, σ is the surface tension in N/m and ρ is the density in kg/m^3.

3.10.3 Determination of bulk modulus

The bulk modulus is a material property characterizing the compressibility of a fluid.

It is a numerical constant that describes the elastic properties of a solid or fluid when it is under pressure on all surfaces. The applied pressure reduces the volume of a material, which returns to its original volume when the pressure is removed. Sometimes referred to as the incompressibility, the bulk modulus is a measure of the ability of a substance to withstand changes in volume when under compression on all sides. It is equal to the quotient of the applied pressure divided by the relative deformation. A large bulk modulus indicates a relative incompressible fluid. All materials, whether solids, liquids or gases, are compressible, i.e. the volume of a given mass will be reduced when a force is exerted uniformly all over its surface. If the force per unit area of surface increases, the relationship between change of pressure and change of volume depends on the bulk modulus of the material.

Bulk modulus (K) = (change in pressure) / (volumetric strain)

Volumetric strain is the change in volume divided by the original volume. Therefore, the concept of bulk modulus is mainly applied to liquids, since for gases the compressibility is so great that the value of bulk modulus is not a constant. Because the denominator, strain, is a ratio without dimensions, the dimensions of the bulk modulus are those of pressure, force per unit area. The bulk modulus may be expressed in units (N/m^2) or Pascal. The relation of bulk modulus in terms of ultrasonic velocity (v) and density of liquid is,

$$K = \rho \times v^2$$

3.10.4 Determination of adiabatic compressibility

Adiabatic compressibility of a liquid medium or material is ratio of its relative volume change as a response to a pressure change. Compressibility is defined as the reciprocal of bulk modulus. A substance that is difficult to compress has a large bulk modulus but a small compressibility. A substance that is easy to compress has a high compressibility but a low bulk modulus. It is usually denoted by a Greek symbol, beta (β). The adiabatic compressibility of the material or liquid sample can be calculated by using the relation,

$$\beta = \frac{1}{\rho v^2}$$

Where ρ is the density of the liquid medium and v is the ultrasonic velocity in that medium. The adiabatic compressibility of liquid is measured in unit m^2N^{-1}.

3.10.5 Determination of acoustic impedance

The resistance that a material offered to the propagation of sound waves is known as acoustic impedance. When sound waves are emitted from the transducer, most of the sound waves are reflected at the interface of the face of the transducer, the couplant and the front surface of the material resulting in a little amount propagating into the material. This results in some portion of energy being reflected at the boundaries of the material. The reflected energy is received by the receiver and then converted into signals. The intensity of energy reflected and transmitted at a boundary is dependent on the acoustic impedance of the media involved.

Mathematically, the expression for acoustic impedance in terms of acoustic velocity or ultrasonic velocity (v) in material and density (ρ) of material is given by,

$$Z = v \times \rho$$

The units for acoustic impedance are $kgm^{-2}s^{-1}$ or Ns/m^3.

3.10.6 Determination of intermolecular free length

Intermolecular free length is defined as the distance between the surfaces of two neighbouring molecules and was used to investigate the exact nature of the molecular interactions. It can be derived using relation,

$$L = K_J \times \beta^{1/2} \text{ Å}$$

Where β is the acoustic impedance and K_J is the Jacobson's temperature dependent constant and its value is given in Table 3.1

Table 3.1 Values of Jacobson's constant in c.g.s units.

Temperature (in °C)	0	10	20	25	30	40	50
Log K_J	6.543	6.778	7.097	7.274	7.459	7.820	8.181

The value of Jacobson's constant can also be found by using the relation,

$$K_J = (93.875+0.375\ T) \times 10^{-8}$$

Where T is the absolute temperature and this constant is independent of the nature of liquid used as given by Ernest and Kavitha (2011).

3.11 Statistical analysis

The results obtained from the experiments were significantly tested using one way analysis of variance (ANOVA) by the software SPSS version 20.0. The regression analysis of obtained data was done with mathematically of fitting the observed properties of different concentrations with temperatures.

CHAPTER-IV

RESULTS AND DISCUSSION

The experimental results of effect of various temperatures on the ultrasonic velocity, conductivity, TDS, density, surface tension, bulk modulus, adiabatic compressibility, acoustic impedance and intermolecular free length of honey at different moisture content are discussed in this chapter. Statistical analysis of recorded data was performed to check its significance. Results of the study for each property and their relationship are presented and discussed in following sections.

4.1 Physicochemical parameters measured

Some physicochemical properties of honey sample like refractive index, °Brix and moisture content of different honey concentrations under study are given in Table 4.1.

Table 4.1 Physicochemical parameters of honey samples.

Parameter	Honey concentrations ((w/w) %)				
	100	90	80	70	60
Refractive index	1.488	1.465	1.452	1.438	1.411
°Brix	78.2	69.6	62.0	56.0	50.1
Moisture content (%)	19.41	28.52	33.66	39.20	49.89
Sugar + moisture content %	97.61	98.42	95.66	95.20	99.99
pH	3.30	3.35	3.28	3.33	3.34

The refractive index of honey samples with different moisture content varied from 1.488 to 1.411, °Brix (sugar percent) from 78.2 to 50.1 and moisture content from 19.41 to 49.89 percent. With the increase in moisture content of honey samples the refractive index and °Brix decreases. It implies that the light propagates approximately 1.488 times slower through medium honey then that in vacuum. The passage of light through honey becomes faster with increase in moisture content. The sugar content in honey decreases with increase moisture content which results in reducing the bending of light. The overall sum of the moisture and sugar content constitute (95-99) % and the remaining (5-1) % could be attributed to non sugar components of these concentrations. The pH value obtained for honey concentrations is consistent with literature data (Acquarone *et al* 2007) and it is not showing any considerable change because of high acidity.

Table 4.2 Grade of honey based on moisture content as per the USDA standard.

Type of Grade	Moisture content (%)	Total sugar (%)
Good	≤ 18.6	> 81.0
Reasonably good	18.6 – 20.0	80.0 – 81.0
Low	> 20.0	< 75.0

The United States Department of Agriculture (USDA) classification of grading the honey based on moisture content and total sugar content is given in Table 4.2. Therefore, according to the USDA classification, the selected pure raw honey i.e. 100% concentration of honey falls in reasonably good category whereas other concentrations falls in the low category.

4.2 Variations of ultrasonic velocity at different temperatures and concentrations

The measured values of ultrasonic velocity in honey samples as a function of selected temperatures and concentrations is expressed in the form of graphs between ultrasonic velocity versus temperature at different concentrations and ultrasonic velocity versus honey concentration at different temperatures which is shown in Fig. 4.1 and 4.2. The ultrasonic velocity in honey samples is ranged from 2311.86 to 1728.24 m/s for all concentrations. However, for distilled water it varies from 1491.12 to 1555.56 m/s. The ultrasonic velocity of honey samples having different concentrations was higher than water. It was highly influenced by concentration and temperature of the honey sample and varies quadratically as a function of temperature and concentration. The ultrasonic velocity in honey samples decreases with the increase in temperature. The decrease was more for 100% honey concentration and becomes low for other concentrations with 60% concentration having least decrease. The ultrasonic velocity in honey also deceases with increase in the quantity of distilled water in samples. The same variation of ultrasonic velocity with temperature and concentrations was reported by Oroian M (2013) and Ratajski *et al* (2010) in various honeys and several researchers (Dikko *et al* 2015, Narendra *et al* 2014, Rao *et al* 1986 and Srinivas *et al* 2013) in case of various organic liquids. For distilled water, the ultrasonic velocity increases with increase in temperature.

The ultrasonic velocity in honey samples decreases with the increase in temperature because the intermolecular free length increases with the increase of thermal energy as temperature increases (Dikko *et al* 2015). Molecules at higher temperatures have more energy, thus they can vibrate faster and ultrasonic waves can travel more quickly. The association of the interacting molecules varies with the temperature of the ultrasonic waves, the decreasing magnitude of interactions results in the loose packing of the molecules inside the liquid. The cohesive force as well as internal pressure increases with the increase of temperature which tends to decrease in value of the ultrasonic velocity.

The decrease in ultrasonic velocity of honey with increase in quantity of the distilled water can be explained due to the presence of strong attraction between water and honey molecules after mixing (Srinivas *et al* 2013). In the 100% honey concentration, the molecules of honey are self associated, but with addition of distilled water the interactions between water and honey molecules causes increase in intermolecular free length and hence the compressibility in the honey samples increases. Therefore, increase in the value of compressibility of molecules results the decrease in ultrasonic velocity.

In the case of distilled water, the behaviour of ultrasonic velocity obtained was totally different, it increases with increase in temperature. Because of the decrease in density of water as temperature increased up to 70°C, the ultrasonic wave velocity increases. Similar results were reported by Ghaedian *et al* (1998) and Benedito *et al* (2004).

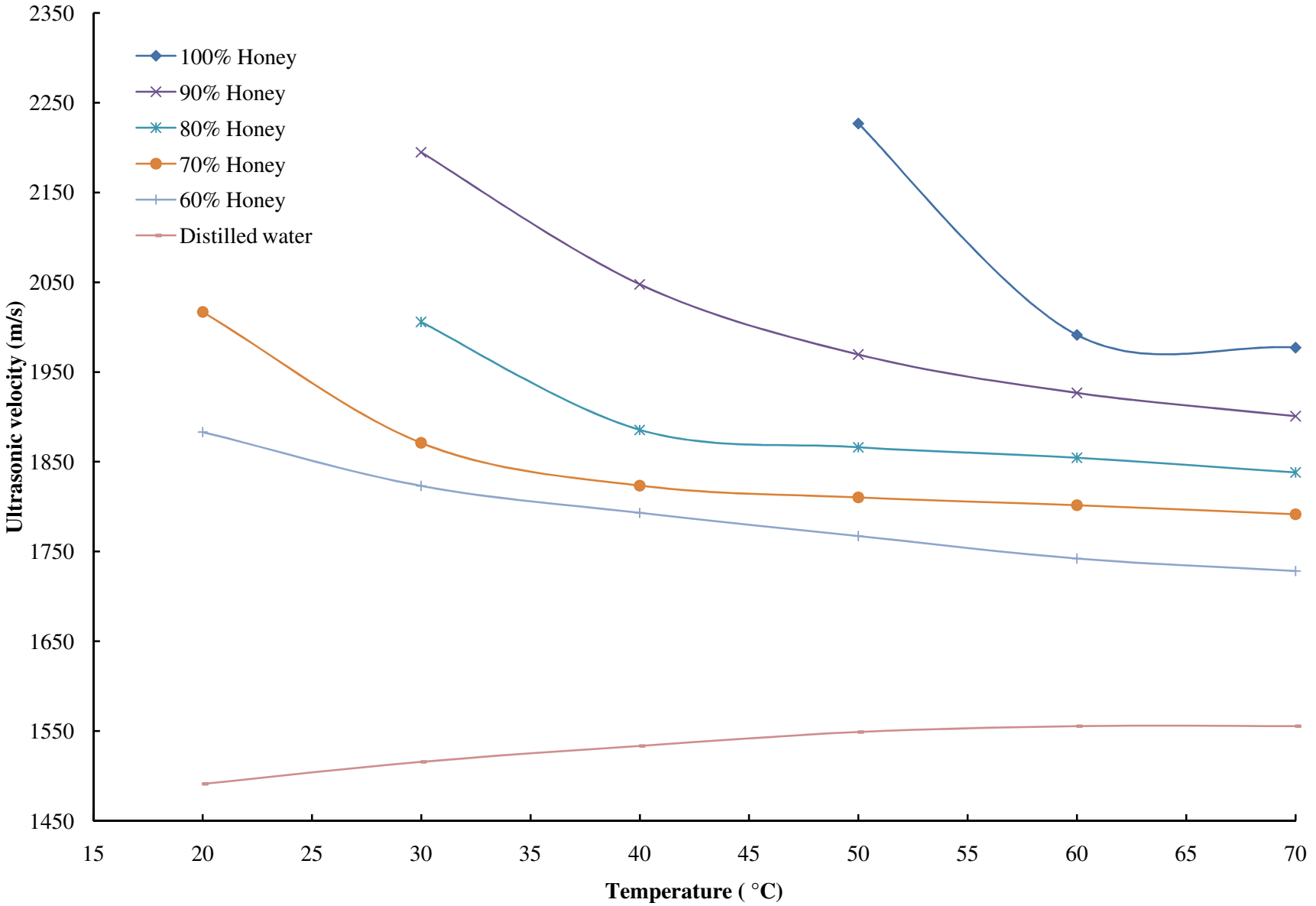

Fig. 4.1 Variations of ultrasonic velocity at different temperatures.

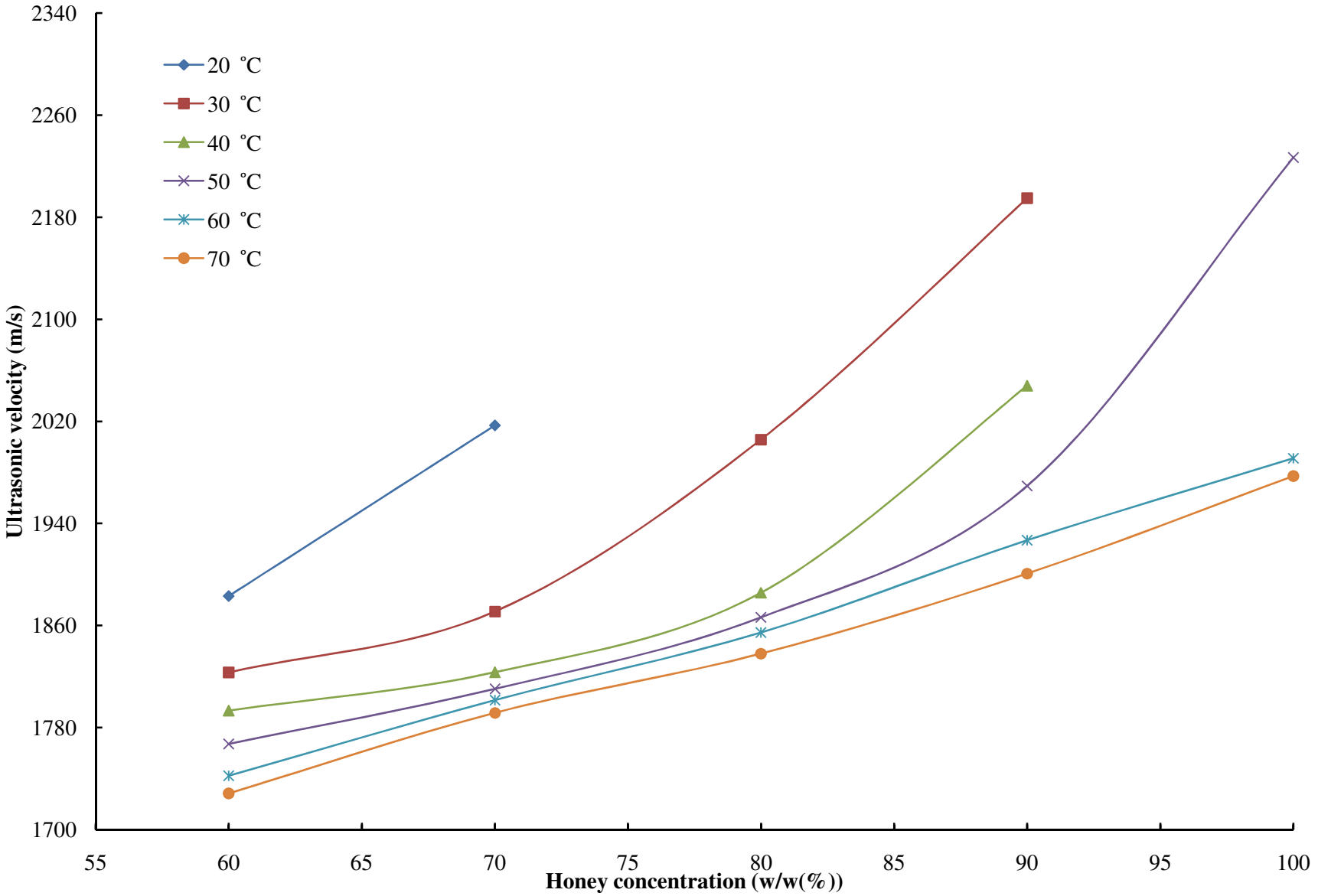

Fig. 4.2 Variations of ultrasonic velocity at different concentrations.

The correlations were made using the ANOVA at 5% level of significance, to allow the prediction of ultrasonic velocity in honey samples as a function of temperature and concentration by means of quadratic fitting for the experimentally obtained data. The plot for honey concentration at 20°C was only fitted linearly as it shows linear trend. The effect of honey concentration and temperature on ultrasonic velocity was also found to be significant. Table 4.3 and 4.4 shows the statistical analysis of ultrasonic velocity with temperature and concentration.

Table 4.3 Ultrasonic velocity at different temperatures.

Temperature (°C)	Ultrasonic velocity (m/s)				
	100%	90%	80%	70%	60%
20	-	-	-	2016.73^{a}	1882.98^{a}
30	-	2194.90^{a}	2005.67^{a}	1870.95^{b}	1823.06^{ab}
40	-	2047.77^{b}	1885.55^{b}	1823.34^{b}	1793.18^{bc}
50	2312.00^{a}	1969.40^{c}	1866.21^{b}	1810.17^{b}	1767.10^{bc}
60	1991.33^{b}	1926.67^{cd}	1854.33^{b}	1801.41^{b}	1742.13^{bc}
70	1977.00^{b}	1900.74^{d}	1837.95^{b}	1791.49^{b}	1728.24^{c}

The figures followed with different superscripts in table are significantly different ($p < 0.05$)

Table 4.4 Ultrasonic velocity at different concentrations.

Honey concentrations (w/w (%))	Ultrasonic velocity (m/s)					
	20°C	30°C	40°C	50°C	60°C	70°C
100	-	-	-	2312.00^{a}	1991.33^{a}	1977.00^{a}
90	-	2195.00^{a}	2047.67^{a}	1969.33^{b}	1926.67^{ab}	1901.00^{b}
80	-	2005.33^{b}	1885.67^{b}	1866.00^{bc}	1854.00^{c}	1837.67^{c}
70	2016.73^{a}	1871.33^{c}	1823.33^{bc}	1810.00^{c}	1801.67^{c}	1791.33^{c}
60	1882.98^{b}	1823.00^{c}	1793.33^{c}	1767.00^{c}	1742.33^{d}	1728.33^{d}

The figures followed with different superscripts in table are significantly different ($p < 0.05$)

The fitted correlation of ultrasonic velocity on temperature is given below,

$$v = aT^2 + bT + c$$

Where v = ultrasonic velocity of honey in m/s

T = temperature in °C

The value of regression coefficient a, b and c, its coefficient of determination R^2 are given in Table 4.5.

Table 4.5 Regression coefficients for ultrasonic velocity with temperature ($v = aT^2 + bT + c$).

Honey concentration ((w/w) %)	a	b	c	R^2
100	1.532	-200.550	8510.333	0.898*
90	0.199	-27.019	2820.790	0.979*
80	0.153	-18.980	2425.352	0.817*
70	0.149	-17.252	2283.871	0.829*
60	0.045	-7.026	2001.595	0.796*

* Significant at 5% level of significance ($p < 0.05$)

The correlation of ultrasonic velocity with concentration is given by the equation,

$$v = aC^2 + bC + c$$

Where v = ultrasonic velocity of honey in m/s

C = concentration in (w/w) %

The value of regression coefficient a, b and c, its coefficient of determination R^2 are given in Table 4.6.

Table 4.6 Regression coefficients for ultrasonic velocity with concentration ($v = aC^2 + bC + c$).

Temperature (°C)	a	b	c	R^2
20	-	13.333	1083.333	1.000*
30	0.353	-40.500	2979.500	0.958*
40	0.330	-41.247	3083.500	0.951*
50	0.462	-61.411	3809.210	0.928*
60	0.022	2.687	1502.086	0.967*
70	0.031	1.156	1551.895	0.968*

* Significant at 5% level of significance ($p < 0.05$)

4.3 Variations of EC at different temperatures and concentrations

The observed values of EC in honey at different temperatures and concentrations is expressed in a form of EC versus temperature at various honey concentrations and EC versus concentration at various temperatures graphically as shown in Fig. 4.3 and 4.4. The graph between EC and temperature revealed that EC increased with the increase in the temperature as expected and is consisted with the literature data (Guo *et al* 2011 and Szczesna *et al* 2004). The increase was more in case 60% and least for 100% honey concentration increase. Also, the EC values increases rapidly at higher value of temperature. The value of EC was also found to be increases with increase in the quantity of water in the honey concentration. The increase in EC with concentration was also explained by Acquarone *et al* (2007). The increase was found to be linear with temperature and concentration. The values of EC vary from 2 to 614 $\mu S\ cm^{-1}$.

The EC of liquid depends on the number of ions that can move freely in the liquid. So, the EC in honey samples could be enhanced by the increased ionic movement as the samples temperature was raised. The molecules of the honey concentrations acquired the kinetic energy from the temperature applied, hence increase in their movement.

The values of EC depend on the concentration and mobility of ions present in the honey solution. In the concentrations corresponding to the more diluted honey samples, the EC increased as a result of increasing ion concentration up to a maximum value. However, simultaneously, the ionic mobility decreases as a result of increasing viscosity of the solution. This latter effect predominates in the descending region of the curve with high honey concentration (Acquarone *et al* 2007). Thus, results the increase in conductivity.

The EC in honey was significantly affected at different temperatures and concentrations, when analyzed with ANOVA software at 5% level of significance. Table 4.7 and 4.8 shows the statistical analysis of EC with temperature and concentration.

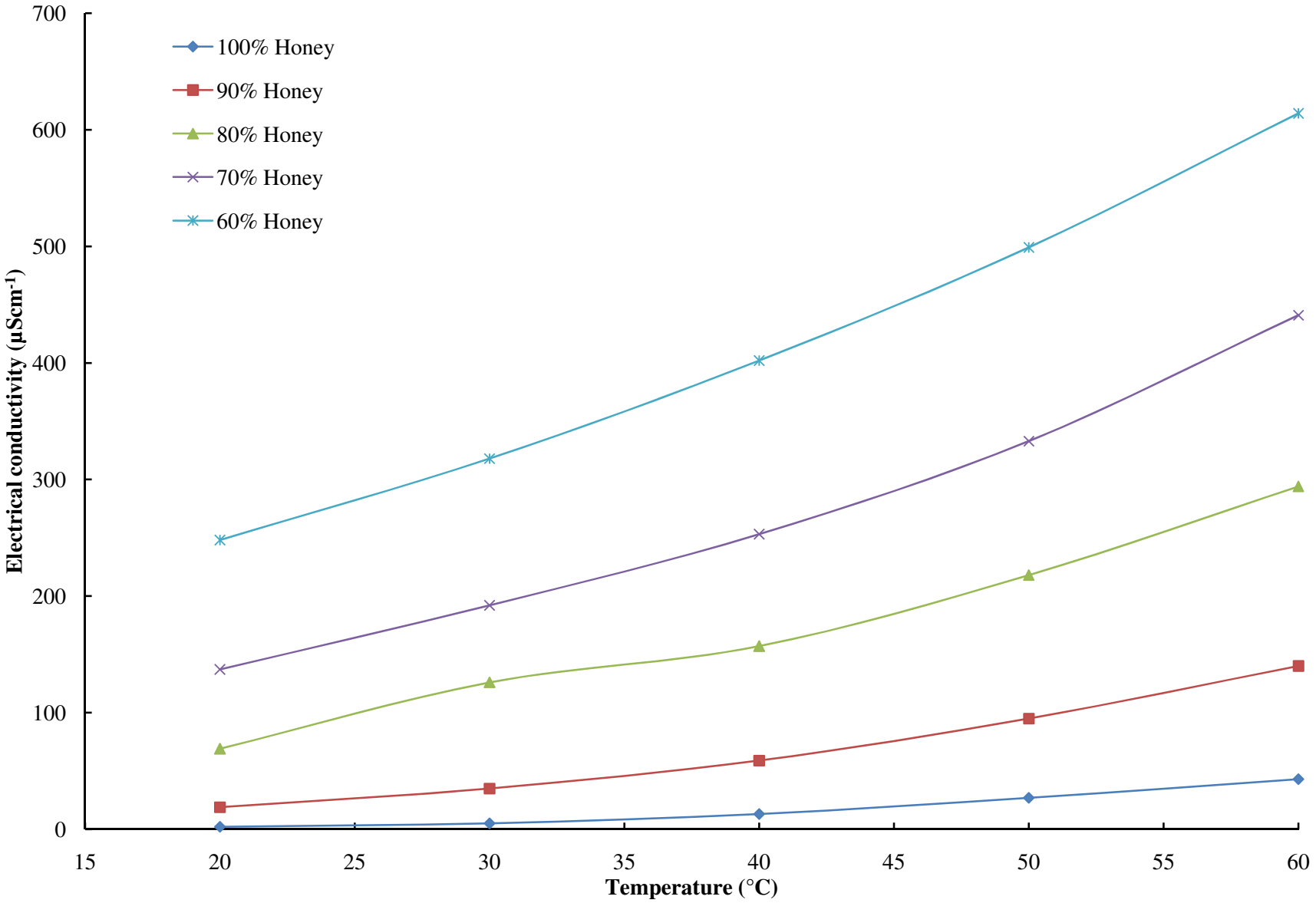

Fig. 4.3 Variations of EC at different temperatures.

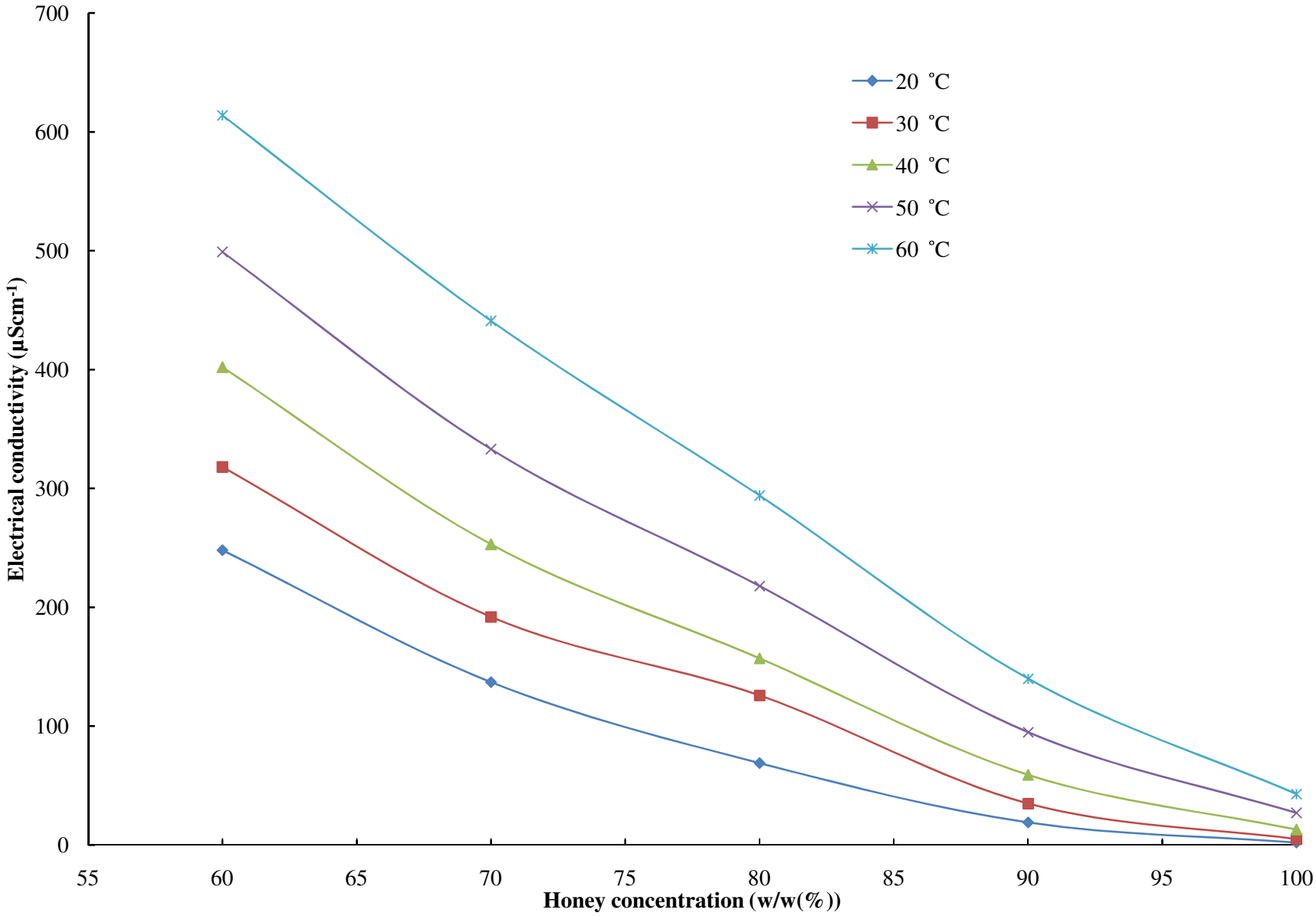

Fig. 4.4 Variations of EC at different concentrations.

Table 4.7 EC honey at different temperatures.

Temperature (°C)	EC (μScm^{-1})				
	100%	90%	80%	70%	60%
20	1.67^{a}	9.33^{a}	34.33^{a}	137.33^{a}	248.33^{a}
30	5.33^{a}	16.33^{b}	62.33^{b}	192.00^{ab}	319.00^{b}
40	12.67^{b}	29.67^{c}	79.00^{c}	253.33^{bc}	401.67^{c}
50	26.67^{c}	47.33^{d}	108.33^{d}	333.33^{c}	499.33^{d}
60	42.67^{d}	70.33^{e}	146.67^{e}	440.67^{d}	609.33^{e}

The figures followed with different superscripts are significantly different ($p < 0.05$)

Table 4.8 EC honey at different concentrations.

Honey concentration ((w/w) %)	EC (μScm^{-1})				
	20°C	30 °C	40°C	50°C	60°C
100	1.67^{a}	5.33^{a}	12.67^{a}	26.67^{a}	42.67^{a}
90	18.67^{a}	34.67^{a}	59.33^{a}	94.67^{b}	139.67^{b}
80	69.33^{b}	126.33^{b}	157.00^{b}	218.00^{c}	293.67^{c}
70	137.33^{c}	192.00^{c}	253.33^{c}	333.33^{d}	440.67^{d}
60	248.33^{d}	319.00^{d}	401.67^{d}	499.33^{e}	609.33^{e}

The figures followed with different superscripts are significantly different ($p < 0.05$)

The dependency of EC on temperature was analyzed with regression analysis. The dependency of EC on temperature was significantly linear in nature as given below,

$$\kappa = a + bT$$

Where κ = EC of honey in μScm^{-1}

T = temperature in °C

Temperature had significant influence on the EC of honey. The value of regression coefficient a and b, its coefficient of determination R^2 are given in Table 4.9.

Table 4.9 Regression coefficients for electrical conductivity with temperature ($\kappa = a + bT$).

Honey concentration ((w/w) %)	a	B	R^2
100	-23.533	1.033	0.920*
90	-51.400	3.020	0.956*
80	-43.267	5.403	0.977*
70	-27.867	7.480	0.907*
60	54.600	9.023	0.983*

*Significant at 5% level of significance ($p < 0.05$)

The concentration of honey is correlated with the EC using regression analysis. Significant correlation is obtained with fitting,

$$\kappa = a + bC$$

Where κ = EC of honey in μScm^{-1}

C = concentration in (w/w) %

The value of regression coefficient a and b, its coefficient of determination R^2 are given in Table 4.10.

Table 4.10 Regression coefficients for electrical conductivity with concentration ($\kappa = a + bC$).

Temperature (°C)	a	b	R^2
20	584.667	-6.120	0.920*
30	763.200	-7.847	0.953*
40	954.400	-9.720	0.957*
50	1181.600	-11.840	0.973*
60	1452.667	-14.343	0.980*

*Significant at 5% level of significance ($p < 0.05$)

4.4 Variations of TDS at different temperatures and concentrations

The patterns for the variations of TDS for honey samples are plotted graphically, TDS versus temperature at different concentrations and TDS versus concentration at different temperatures as shown in Fig. 4.5 and 4.6. It is observed from the graph that number of TDS in honey samples increases with increase in temperature of the sample. The increase in the TDS value was high at higher temperature (40, 50 and 60°C) as compared to low temperatures (20 and 30°C). The 100% honey concentration had lowest TDS value and it increases very slowly with temperature increase. While for the 60% honey concentration the value of TDS rapidly increases with the temperature increase. Also, it was found that the TDS in honey samples is increases as the dilution of honey samples with distilled water increases. The highly diluted honey sample, 60% concentration had higher value of TDS as compared to other honey concentrations at any temperature. Therefore, 100% honey concentration had least value of TDS. The increase in TDS was obtained linear both for the temperature and concentration changes. The values of TDS in honey samples vary from 1-307 ppm in all the concentrations.

It was observed that the pure honey sample i.e. 100% honey concentration contain very low value of TDS and as the temperature of sample was increased the TDS value increased. The increase in the TDS value can be explained due to breaking of the bonds in the honey molecules by acquiring the thermal energy supplied externally in the form of temperature or heat. As, a result of temperature increase, the concentrations of the ions or the total soluble salts in the sample increases which can be measured in the form of increase in the value of TDS. Similarly, at higher temperatures more energy was supplied and acquired by molecules so that the TDS value was increased rapidly as compared at low temperatures.
The TDS value in the honey samples was increased with dilution. This variation in the value of TDS as the function concentration can be because when honey sample was mixed with the distilled water, due to interaction of honey molecules with water molecules a number of free molecules or ions were liberated in the solution. Also, as the dilution was increased to form various honey concentrations the liberation of free molecules in the solution was increased which was observed in the form of increase in the TDS value of various concentrations.

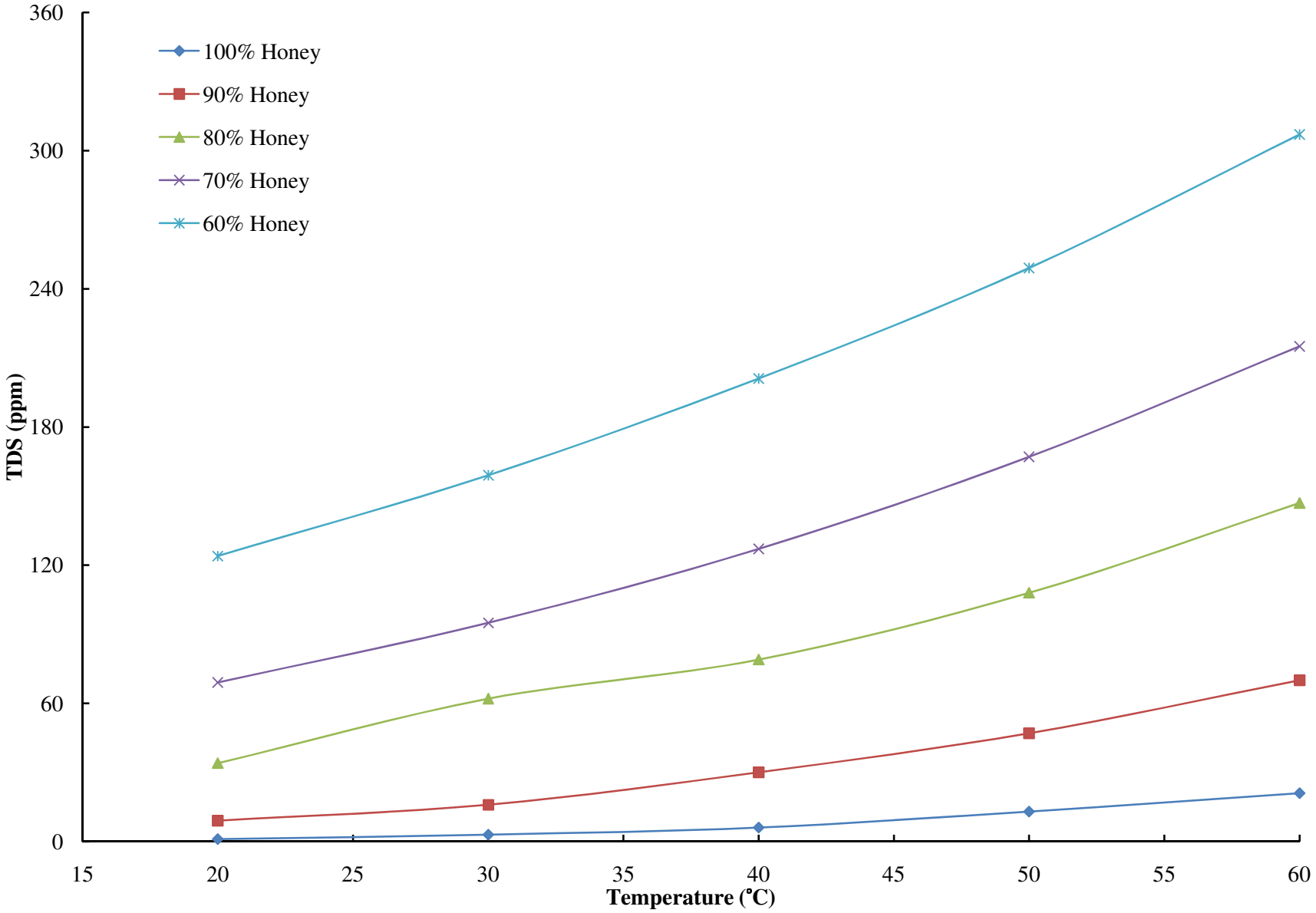

Fig. 4.5 Variations of TDS at different temperatures.

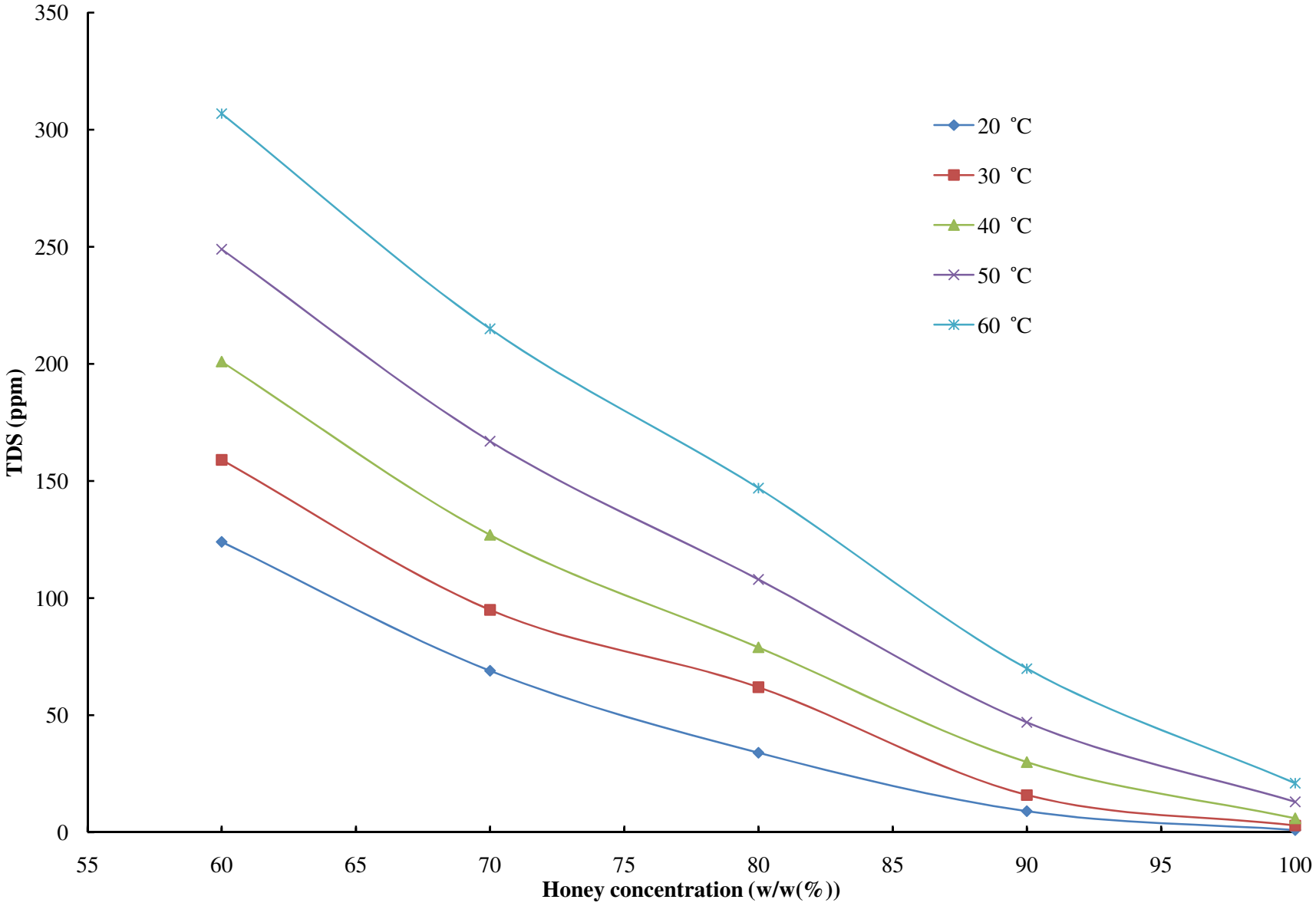

Fig. 4.6 Variations of TDS at different concentrations.

Analysis of variance was performed to check the significance of the differences between the TDS values for different temperatures and concentrations at 5% level of significance, the ANOVA tables recorded is given as Table 4.11 and 4.12.

Table 4.11 TDS at different temperatures.

Temperature (°C)	TDS (ppm)				
	100%	90%	80%	70%	60%
20	0.67^{a}	9.33^{a}	34.33^{a}	68.67^{a}	124.67^{a}
30	2.67^{a}	16.33^{b}	62.33^{b}	95.33^{ab}	159.33^{b}
40	6.00^{b}	29.67^{c}	79.00^{c}	126.67^{bc}	201.00^{c}
50	12.67^{c}	47.33^{d}	108.33^{d}	166.67^{c}	249.33^{d}
60	21.00^{d}	70.33^{e}	146.67^{e}	215.33^{d}	304.67^{e}

The figures followed with different superscripts are significantly different ($p < 0.05$)

Table 4.12 TDS at different concentrations.

Honey concentration ((w/w) %)	TDS (ppm)				
	20°C	30°C	40°C	50°C	60°C
100	0.67^{a}	2.67^{a}	6.00^{a}	12.67^{a}	21.00^{a}
90	9.33^{a}	16.33^{a}	29.67^{a}	47.33^{b}	70.33^{b}
80	34.33^{b}	62.33^{b}	79.00^{b}	108.33^{c}	146.67^{c}
70	68.67^{c}	95.33^{c}	126.67^{c}	167.67^{d}	215.33^{d}
60	124.67^{d}	159.33^{d}	201.00^{d}	249.33^{e}	304.67^{e}

The figures followed with different superscripts are significantly different ($p < 0.05$)

Regression analysis of TDS with temperature results that the TDS in honey samples had significant dependency on temperature and is linear in nature as given below,

$$TDS = a + bT$$

Where TDS of honey samples in ppm (parts per million)

T = temperature in °C

The value of regression coefficient a and b, its coefficient of determination R^2 are given in Table 4.13.

Table 4.13 Regression coefficients for TDS with temperature (TDS = a + bT).

Honey concentration ((w/w) %)	a	b	R^2
100	-11.667	0.507	0.919*
90	-26.600	1.530	0.954*
80	-22.133	2.707	0.980*
70	-11.333	3.647	0.925*
60	27.800	4.500	0.982*

*Significant at 5% level of significance ($p < 0.05$)

The correlation of TDS with concentration was significant and is linear in nature as,

$$TDS = a + bC$$

Where TDS of honey samples in ppm (parts per million)

C = concentration in (w/w) %

The value of regression coefficient a and b, its coefficient of determination R^2 are given in Table 4.14.

Table 4.14 Regression coefficients for TDS with concentration (TDS = a + bC).

Temperature (°C)	a	b	R^2
20	293.400	-3.073	0.919*
30	381.067	-3.923	0.951*
40	478.067	-4.870	0.958*
50	591.000	-5.927	0.974*
60	721.467	-7.123	0.984*

*Significant at 5% level of significance ($p < 0.05$)

4.5 Variations of density at different temperatures and concentrations

The change in density value for honey samples recorded in the experimental work is plotted as density versus temperature at various honey concentrations and density versus concentration at various temperatures graphically which is shown in Fig. 4.7 and 4.8. The density of honey decreases non significantly with the increase in temperature and concentration. The density of honey samples varies within the range 1525.11 to 1251.90 kg/m^3 for all the concentrations. The distilled water had density value from 977.75 to 998.20 kg/m^3 on the specified temperatures. The density of distilled water decreases with the temperature increase, but its density value was smaller than all honey concentrations.

The density in different honey concentrations are not significantly affected with the temperature and concentration change at 5% level of significance when analyzed with ANOVA software. Table 4.15 and 4.16 shows the statistical analysis of conductivity with temperature and concentration.

Table 4.15 Density at different temperatures.

Temperature (°C)	Density (kg/m^3)				
	100%	90%	80%	70%	60%
20	1525.11^a	1464.33^a	1382.96^a	1321.87^a	1270.01^a
30	1524.47^a	1463.89^a	1382.32^a	1321.28^a	1269.63^a
40	1521.99^a	1463.32^a	1381.81^a	1316.67^a	1268.21^a
50	1518.41^a	1457.31^a	1378.92^a	1311.33^a	1265.52^a
60	1505.73^a	1450.38^a	1374.26^a	1306.75^a	1257.38^a
70	1498.20^a	1439.72^a	1367.96^a	1301.83^a	1251.90^a

The figures with same superscripts are non significant

Table 4.16 Density at different concentrations.

Honey concentration ((w/w) %)	Density (kg/m^3)					
	20°C	30 °C	40 °C	50 °C	60 °C	70°C
60	1269.67^a	1269.67^a	1268.00^a	1265.33^a	1257.33^a	1252.00^a
70	1322.00^a	1321.00^a	1316.67^a	1311.33^a	1307.00^a	1302.00^a
80	1383.00^a	1382.33^a	1381.67^a	1378.67^a	1374.33^a	1368.00^a
90	1464.33^a	1463.67^a	1463.33^a	1457.33^a	1450.33^a	1439.67^a
100	1525.33^a	1524.33^a	1522.00^a	1518.33^a	1506.00^a	1498.00^a

The figures with same superscripts are non significant

Correlations were made to allow the predictions of density in the honey samples as a function of temperature by means of linear fitting using the experimental results obtained in

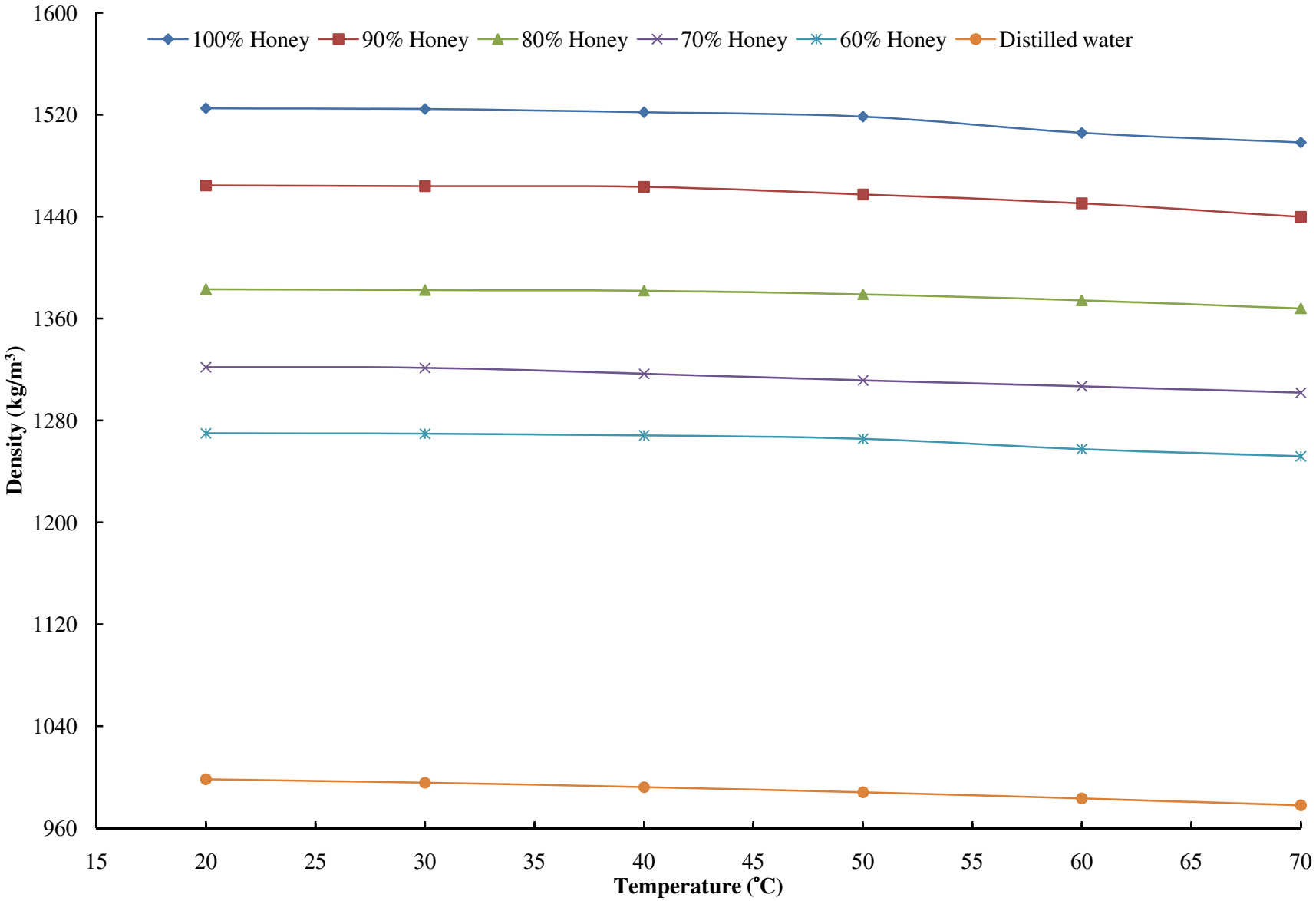

Fig. 4.7 Variations of density at different temperatures.

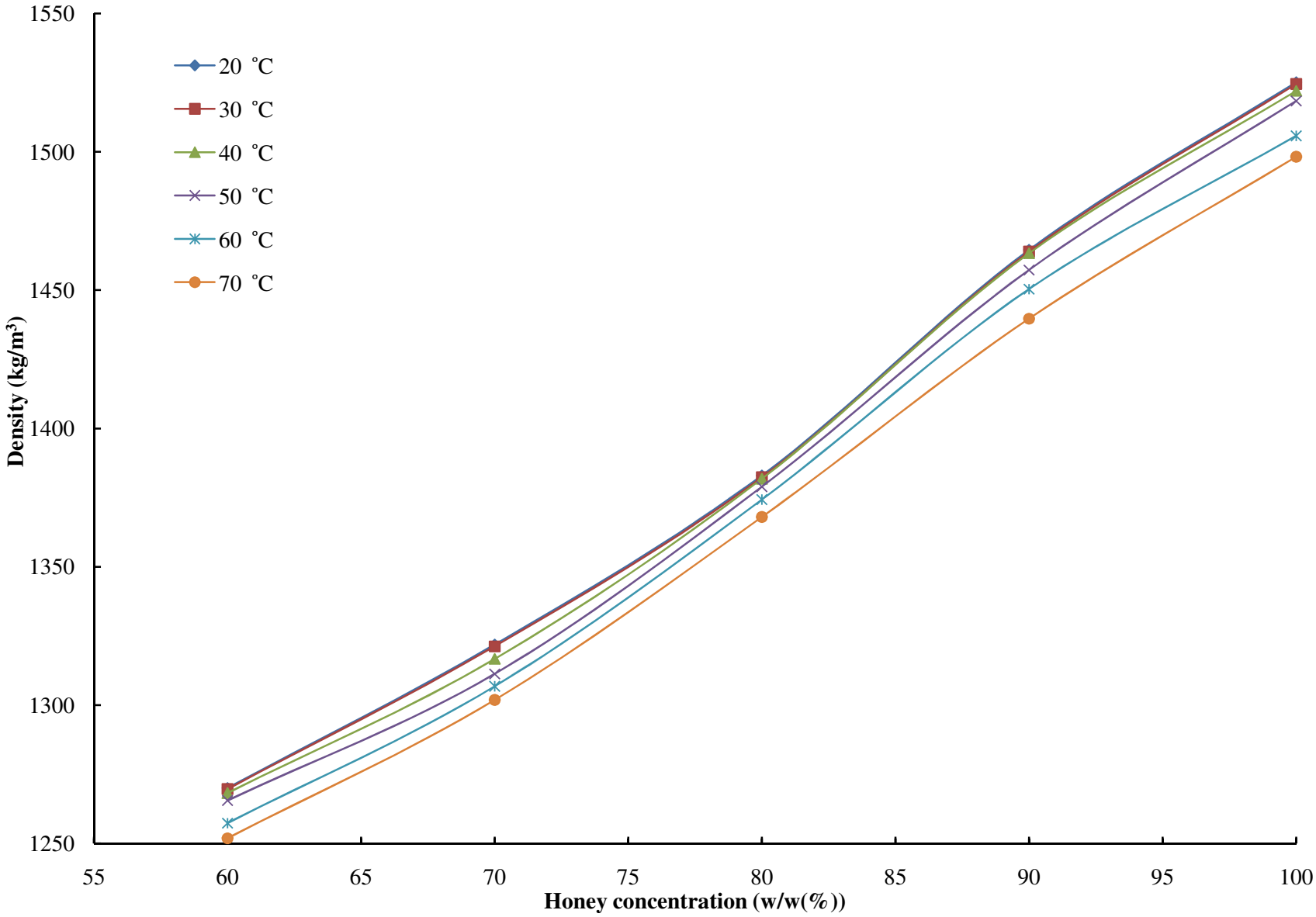

Fig. 4.8 Variations of density at different concentrations.

the experimental work. The fitted correlations use the following equation,

$$d = a + bT$$

Where d = density of honey in kg/m^3

T = temperature in °C

The value of regression coefficient a and b, its coefficient of determination R^2 are given in Table 4.17.

Table 4.17 Regression coefficients for density with temperature (d = a + bT).

Honey concentration ((w/w) %)	a	b	R^2
100	1540.641	-0.555	0.076^{NS}
90	1478.216	-0.484	0.066^{NS}
80	1377.530	-0.235	0.033^{NS}
70	1332.276	-0.421	0.004^{NS}
60	1280.124	-0.366	0.003^{NS}

The figures with superscripts NS are non significant

Density in the honey samples as a function of concentration by means of linear-fitting using the obtained experimental data. The fitted correlations use the following equation,

$$d = a + bC$$

Where d = density of honey in kg/m^3

C = concentration in (w/w) %

The value of regression coefficient a and b, its coefficient of determination R^2 are given in Table 4.18.

Table 4.18 Regression coefficients for density with concentration (d = a + bC).

Temperature (°C)	a	b	R^2
20	869.933	6.537	0.512*
30	870.600	6.520	0.510*
40	866.600	6.547	0.507*
50	864.600	6.520	0.501*
60	866.467	6.407	0.504*
70	868.200	6.297	0.498*

*Significant at 5% level of significance ($p < 0.05$)

4.6 Effect of temperatures and moisture content on parameters of honey derived from ultrasonic velocity and density

The other thermodynamic parameters of honey like surface tension, bulk modulus, adiabatic compressibility, acoustic impedance and intermolecular free length was derived from ultrasonic velocity and density of honey concentrations with varying temperatures by using relations given in Chapter-3 and are discussed in the following sections.

4.6.1 Effect of temperature and moisture content on surface tension

The surface tension values calculated from ultrasonic velocity and density are given in Table 4.19. Surface tension for honey samples varies from 10.68×10^{-2} to 5.69×10^{-2} N/m in all concentrations at selected temperatures. The obtained results are plotted graphically as

surface tension versus temperature at different concentrations and surface tension versus concentration at different temperatures shown in Fig. 4.9 and 4.10.

Table 4.19 Surface tension of honey at different temperatures and concentrations.

Temperature (°C)	Surface tension (σ in N/m) × 10^{-2}				
	100%	90%	80%	70%	60%
20	-	-	-	7.58	6.57
30	-	9.53	7.86	6.77	6.25
40	-	8.58	7.16	6.49	6.09
50	10.68	8.06	7.04	6.42	5.95
60	8.47	7.76	6.95	6.32	5.79
70	8.34	7.55	6.82	6.25	5.69

Surface tension of honey samples decrease with increase in temperature as can be analyzed from the graphs. The decrease in surface tension value was more for 100% honey concentration with temperature and 60% honey concentration had least decrease in surface tension values. The decrease in surface tension is more dominant at higher temperatures as compared at low temperatures. It was also found that surface tension in honey samples increases with increase in the concentration of honey. The change in surface tension with temperature and concentration was quadratic and agrees with results observed by Oroian (2013) in various honey samples.

Surface tension is influenced by mineral constituents and all other active minor honey constituents which may be surface active. Substances which distribute themselves uniformly throughout solution raise the surface tension. The decrease in the value of surface tension with temperature increase shows that the molecules present at the surface do not behaves as the inner molecules present inside the honey samples i.e. the surface molecules are less tightly bound as compared to inner honey molecules with the increase in temperature.

The decrease in value of surface tension with increase in the dilution of honey samples indicates the increase in the intermolecular separation in the honey sugar molecules which is more in case of the surface molecules as compared to inner honey sugar molecules. Also, the sugar molecules are attracted more strongly by the liquid molecules. If this attraction exceeds that between the liquid molecules among themselves, it reduces the surface energy, resulting in a decrease in the surface tension of the solution (Shinde *et al* 2015).

Temperature dependency of surface tension was found by using regression analysis. The correlation obtained as given under,

$$\sigma = aT^2 + bT + c$$

Where σ = surface tension of honey in N/m

T = temperature in °C

The value of regression coefficients a, b and c with the determination coefficient R^2 is shown in Table 4.20.

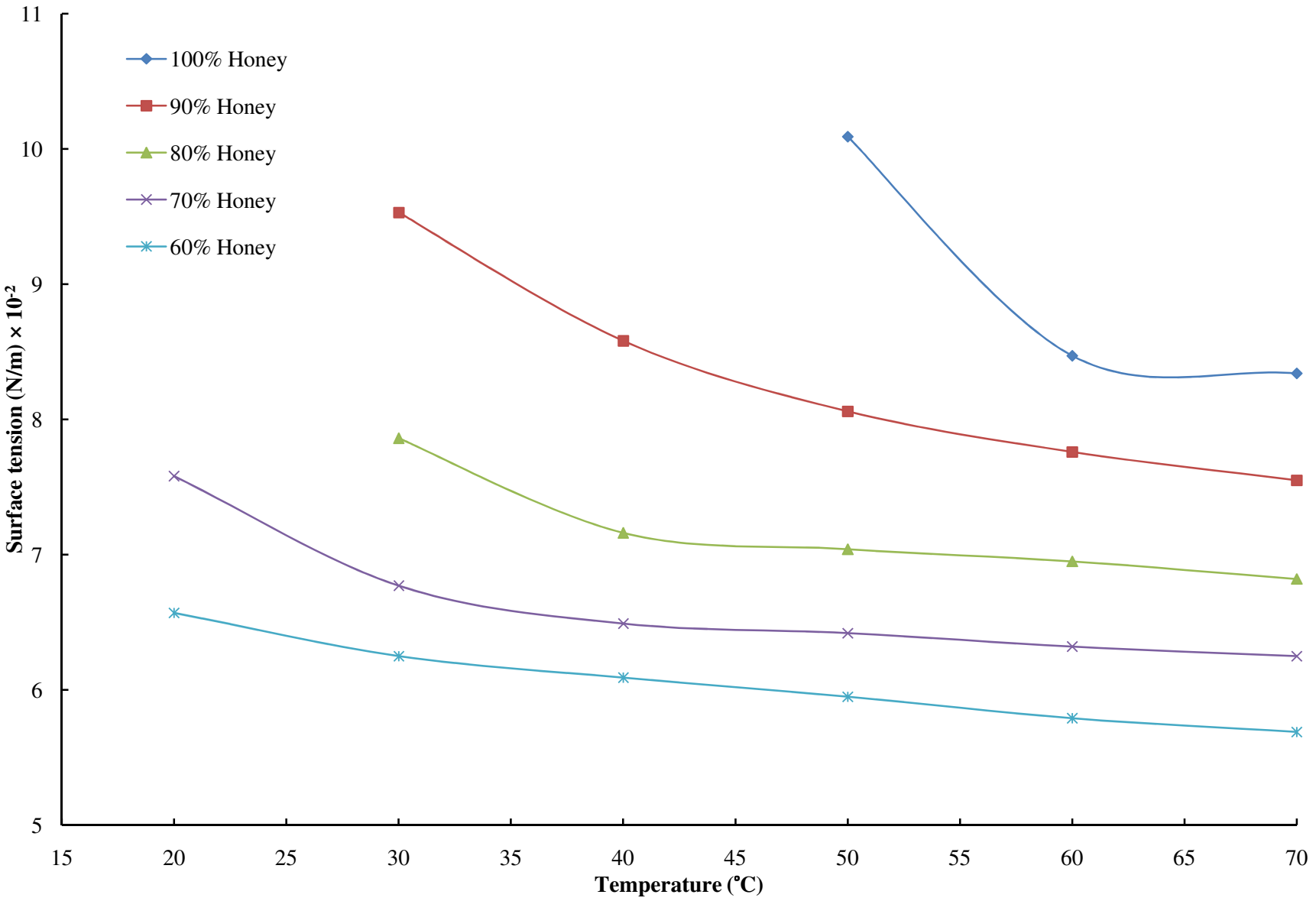

Fig. 4.9 Variations of surface tension at different temperatures.

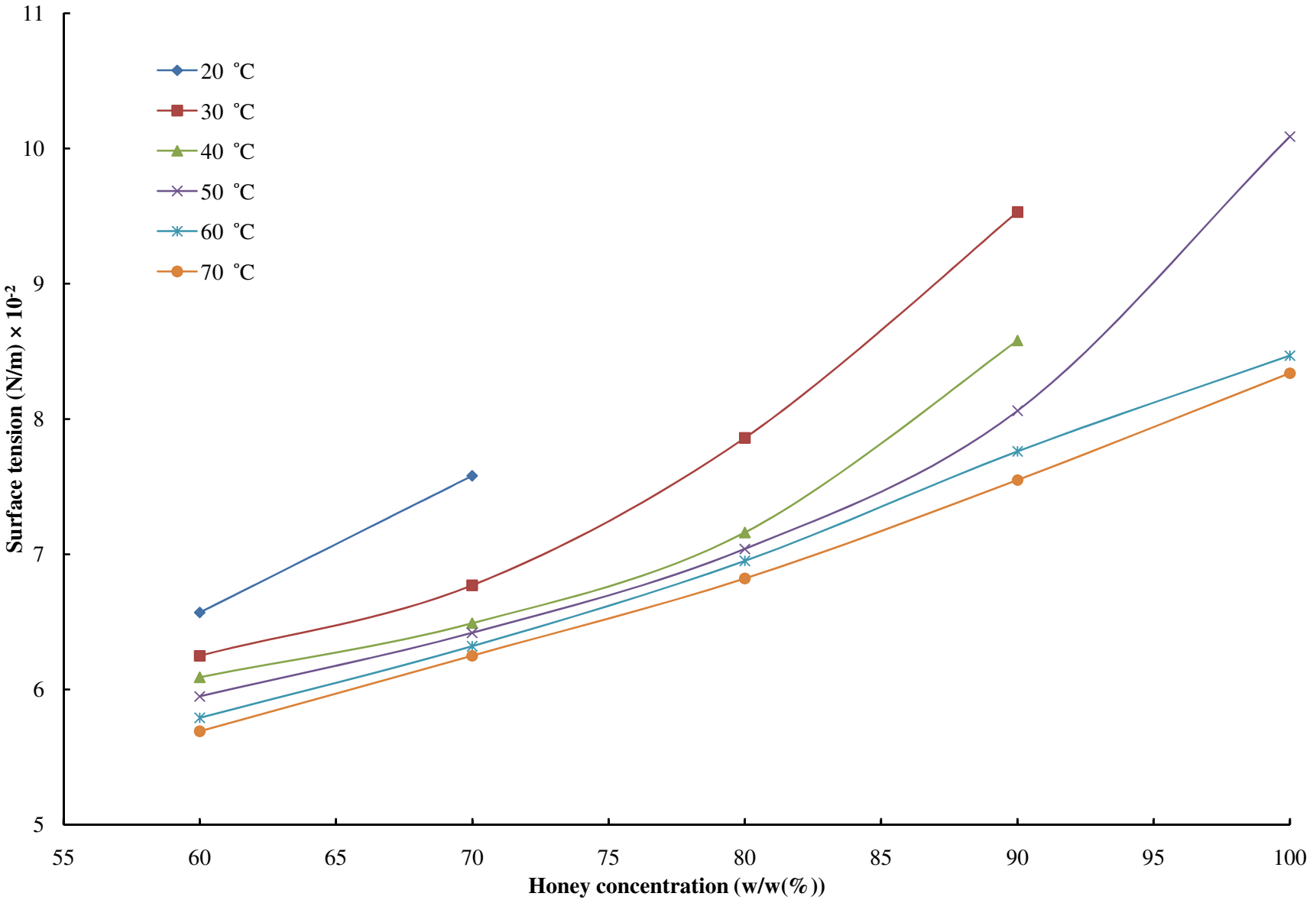

Fig. 4.10 Variations of surface tension at different concentrations.

Table 4.20 Regression coefficients for surface tension with temperature ($\sigma = aT^2 + bT + c$).

Honey concentration ((w/w) %)	a	b	c	R^2
100	0.010	-1.365	52.930	1.000*
90	0.001	-0.169	13.479	0.995*
80	0.001	-0.106	10.233	0.937*
70	0.001	-0.094	9.044	0.949*
60	0.000	-0.035	7.158	0.993*

*Significant at 5% level of significance ($p < 0.05$)

The surface tension variations with concentrations was quadratic with concentration and its relation is,

$$\sigma = aC^2 + bC + c$$

Where σ = surface tension of honey in N/m

C = concentration in (w/w) %

The value of regression coefficients a, b and c with the determination coefficient R^2 is shown in Table 4.21.

Table 4.21 Regression coefficients for surface tension with concentration ($\sigma = aC^2 + bC + c$).

Temperature (°C)	a	b	c	R^2
20	0.000	0.101	0.510	1.000*
30	0.003	-0.322	15.217	1.000*
40	0.003	-0.301	15.000	0.997*
50	0.003	-0.426	19.564	0.984*
60	0.000	0.006	4.009	0.999*
70	0.000	-0.005	4.396	1.000*

*Significant at 5% level of significance ($p < 0.05$)

4.6.2 Effect of temperature and moisture content on bulk modulus

The value of bulk modulus varies from 81.15×10^8 to 37.39×10^8 N/m^2 for honey at different temperatures and concentrations, given in Table 4.22. The plots of bulk modulus versus temperature with different concentrations and bulk modulus versus temperature with different concentrations are shown in Fig. 4.11 and 4.12.

Table 4.22 Bulk modulus of honey at different temperatures and concentrations.

Temperature (°C)	Bulk modulus (K in N/m^2) × 10^8				
	100%	90%	80%	70%	60%
20	-	-	-	53.76	45.03
30	-	70.52	55.61	46.25	42.20
40	-	61.36	49.13	43.77	40.78
50	81.15	56.52	48.02	42.75	39.52
60	59.69	53.84	47.25	42.40	38.16
70	58.56	52.01	46.21	41.78	37.39

Bulk modulus of honey samples decreased with increase in temperature as observed from plot. The decrease in the value of bulk modulus was more at high temperatures as compared to decrease at low temperatures. Also, it can be seen that bulk modulus decreases

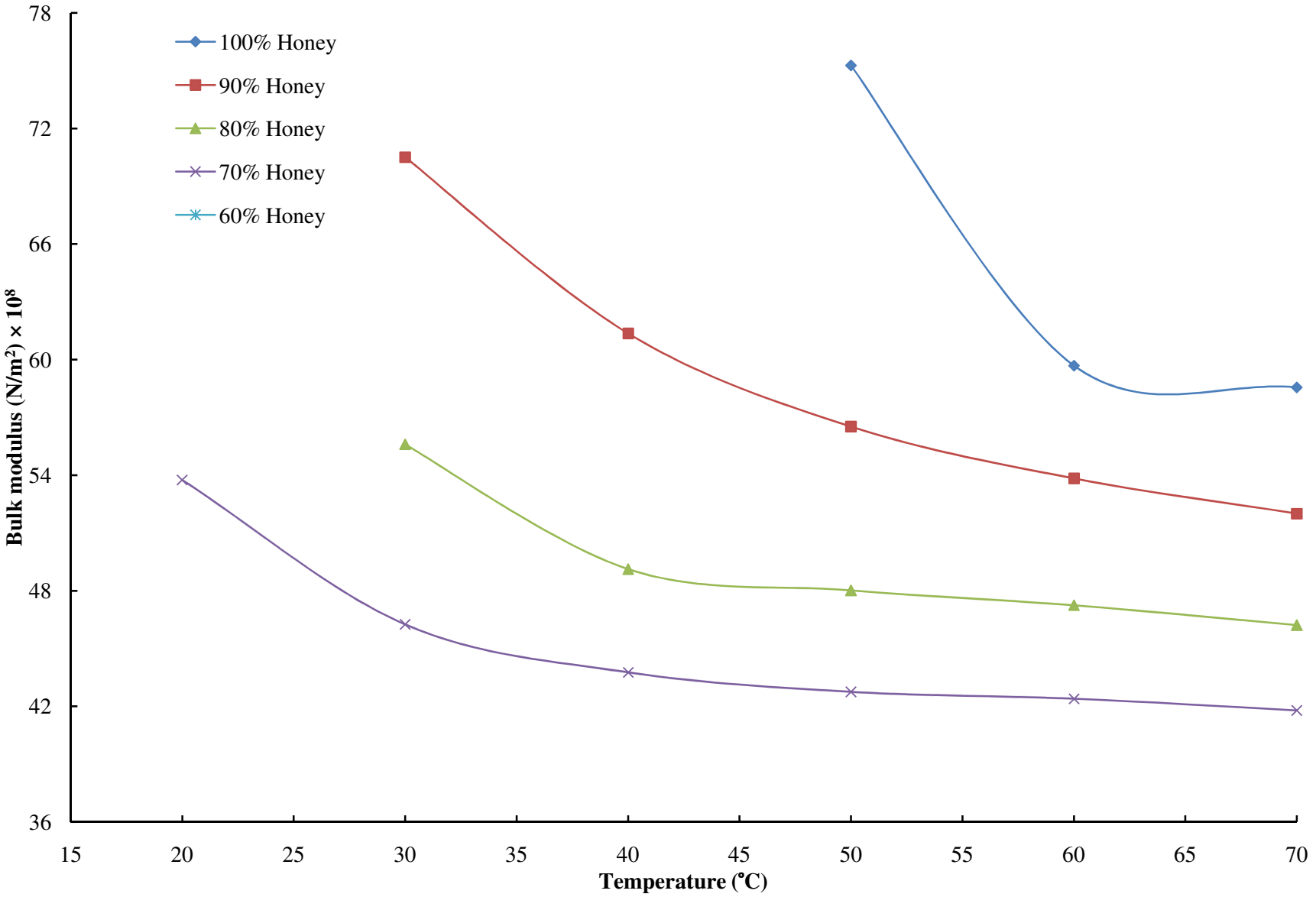

Fig. 4.11 Variations of bulk modulus with different temperatures.

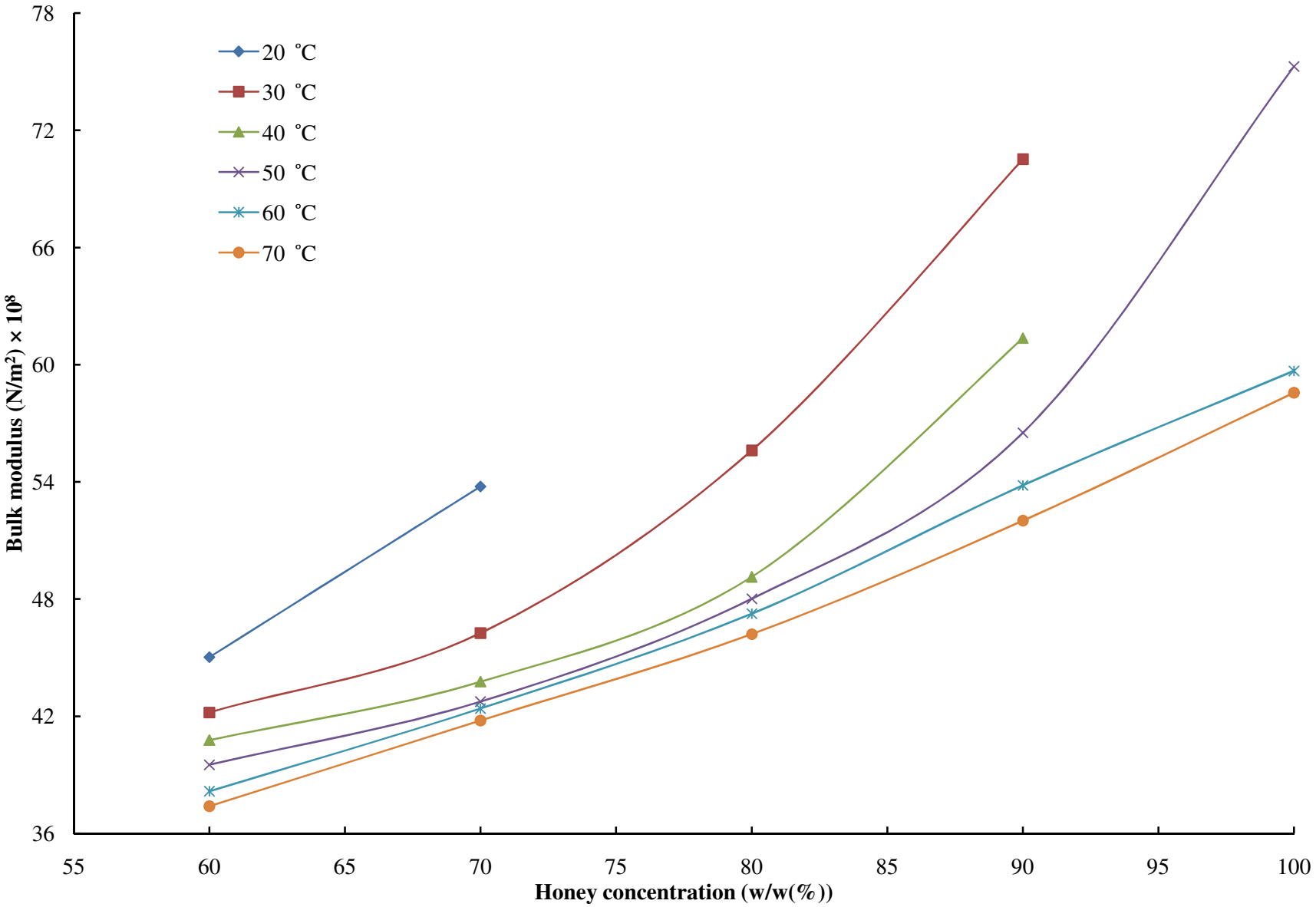

Fig. 4.12 Variations of bulk modulus at different concentrations.

with increase in the dilution of honey with 100% concentration had large values of bulk modulus as compared to other concentrations. The similar trend of bulk modulus with temperature and concentration was investigated by Singh (2014) and Balaji *et al* (2014). The variation of bulk modulus was obtained as quadratic in nature.

Bulk modulus is a measure of fluid's resistance to compressibility. The decrease in the values of bulk modulus with increase in temperature of sample signifies the increase in the compressibility of the honey samples which in turns decrease the value of ultrasonic velocity in the samples. The compressibility of the honey samples increases with temperature increase because of increase in intermolecular length in sugar molecules of honey samples, the molecules thus vibrate easily with respect to their mean positions, hence resultant bulk modulus decreases in the temperature increase.

The decrease in bulk modulus with increase in quantity of the distilled water is due to the presence of strong attraction between water and honey molecules after mixing (Balaji *et al* 2014). With the addition of distilled water in honey, the interactions between water and honey molecule causes increase in intermolecular free length and hence the compressibility in the honey samples increases. Therefore, increase in the value of compressibility of molecules results the decrease in bulk modulus of the honey concentrations.

The correlation of bulk modulus with temperature is defined by following equation,

$$K = aT^2 + bT + c$$

Where K = bulk modulus of honey in N/m^2

T = temperature in °C

The value of regression coefficients a, b and c with the determination coefficient R^2 is shown in Table 4.23.

Table 4.23 Regression coefficients for bulk modulus with temperature ($K = aT^2 + bT + c$).

Honey concentration ((w/w) %)	a	b	c	R^2
100	0.102	-13.327	493.400	1.000*
90	0.012	-1.648	108.772	0.995*
80	0.008	-1.007	77.981	0.937*
70	0.008	-0.898	67.736	0.952*
60	0.002	-0.317	50.407	0.993*

*Significant at 5% level of significance ($p < 0.05$)

The bulk modulus dependency on concentration is given as under,

$$K = aC^2 + bC + c$$

Where K = bulk modulus of honey in N/m^2

C = concentration in (w/w) %

The value of regression coefficients a, b and c with the determination coefficient R^2 is shown in Table 4.24.

Table 4.24 Regression coefficients for bulk modulus with concentration ($K = aC^2 + bC + c$).

Temperature (°C)	a	b	c	R^2
20	0.000	0.873	-7.368	1.000*
30	0.027	-3.129	132.230	1.000*
40	0.023	-2.794	125.485	0.996*
50	0.033	-4.290	179.815	0.980*
60	0.004	-0.023	26.684	0.999*
70	0.004	-0.125	30.333	1.000*

*Significant at 5% level of significance ($p < 0.05$)

4.6.3 Effect of temperature and moisture content on adiabatic compressibility

The adiabatic compressibility values calculated for honey at different temperatures and concentrations are given in Table 4.25. To know its variation, graphs were plotted as adiabatic compressibility versus temperature at different concentrations and adiabatic compressibility versus concentration at different temperatures (shown in Fig. 4.13 and 4.14).

Table 4.25 Adiabatic compressibility of honey at different temperatures and concentrations.

Temperature (°C)	Adiabatic compressibility (β in m^2/N) $\times 10^{-11}$				
	100%	90%	80%	70%	60%
20	-	-	-	18.60	22.21
30	-	14.18	17.98	21.62	23.70
40	-	16.30	20.35	22.84	24.52
50	12.32	17.69	20.82	23.27	25.30
60	16.75	18.57	21.16	23.58	26.20
70	17.08	19.22	21.64	23.93	26.74

Adiabatic compressibility lies in range 12.32×10^{-11} to 26.74×10^{-11} m^2/N. It increases with increase in temperature with 60% concentration had large value of compressibility at all temperatures. The adiabatic compressibility also increases as concentration of honey decreases. Its variation is consistent with the data obtained by Dikko *et al* (2015) and Rao *et al* (1985). Also, the inverse relation of adiabatic compressibility with density and ultrasonic velocity shows its increase with temperature and dilution of honey samples.

Adiabatic compressibility increased with increase in temperature, indicating that the free volume of the mixture increased as the internal pressure decreased with increase in temperature of mixture. This is because of the loose packing of the molecules inside the shield, which may be brought about by the decreasing magnitude of interactions with increase in temperature (Dikko *et al* 2015). Therefore, the increase in the intermolecular free length results the easy movements of molecules and hence increase in the compressibility with the temperature increase.

The change in adiabatic compressibility with concentration is because of the interaction of the distilled water and honey molecules after mixing. With the addition of distilled water in honey, the interactions between water and honey molecule causes increase in intermolecular free length and hence the adiabatic compressibility increases.

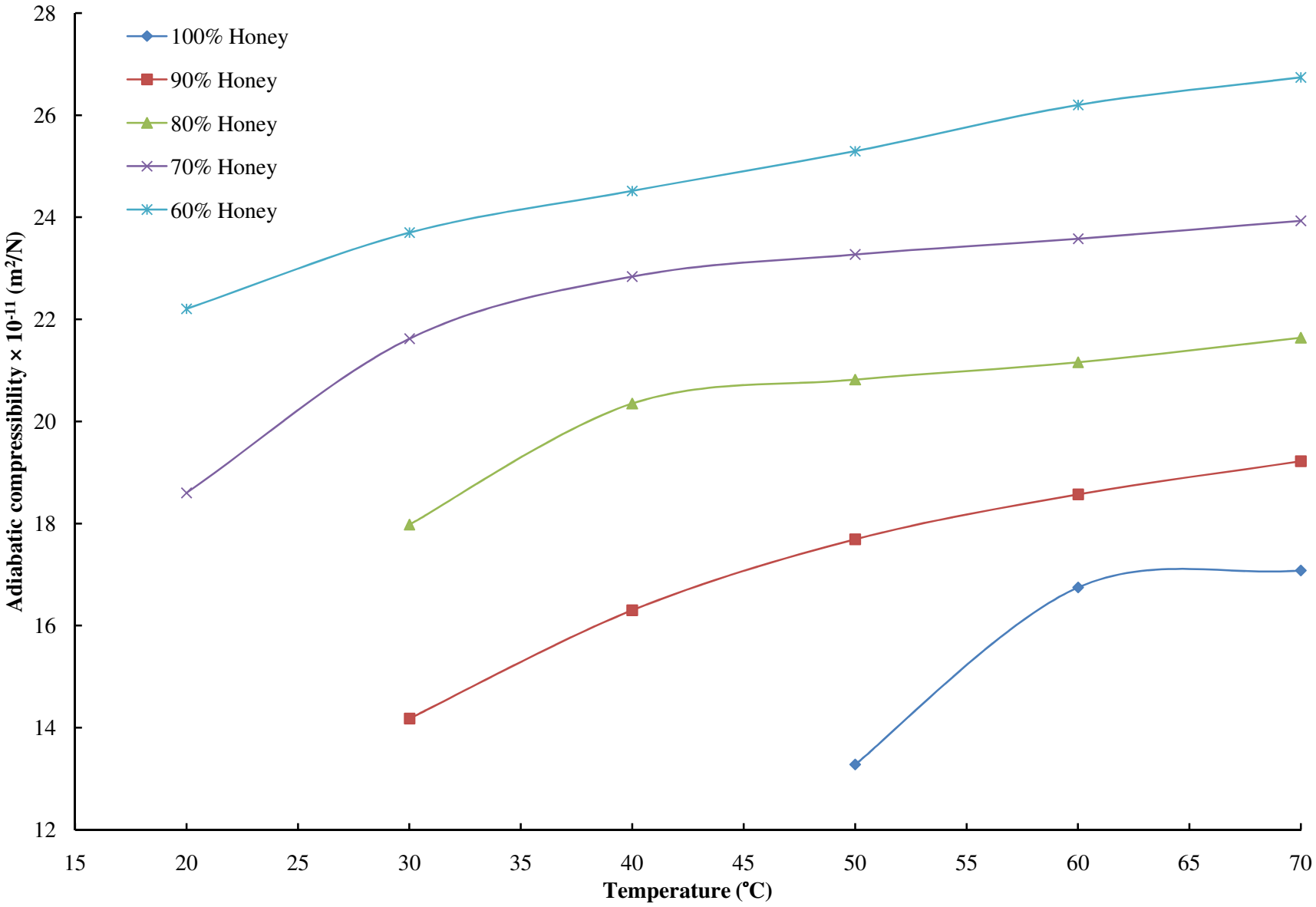

Fig. 4.13 Variations of adiabatic compressibility at different temperatures.

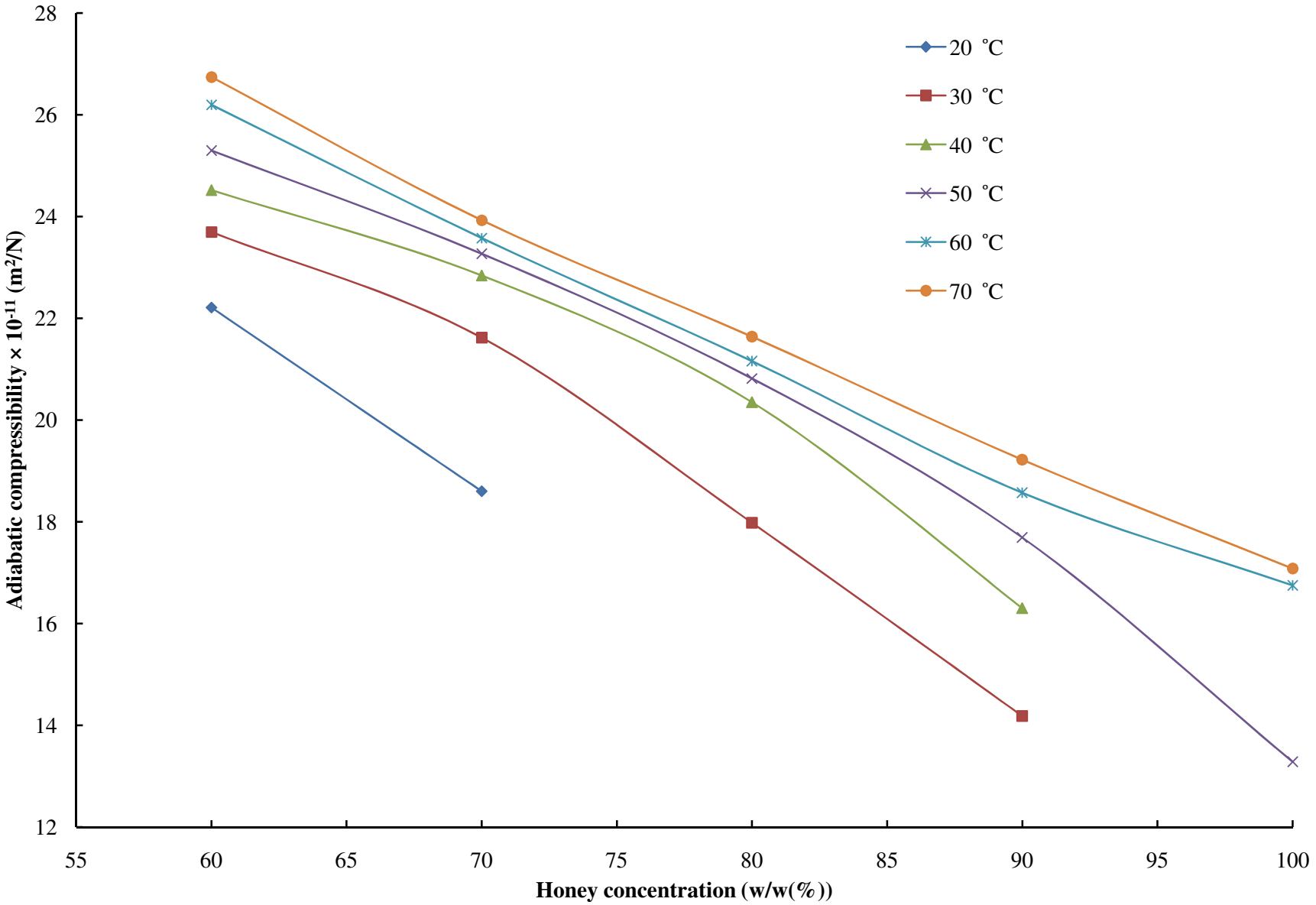

Fig. 4.14 Variations in adiabatic compressibility at different concentrations.

The trend of increase of adiabatic compressibility was quadratic with temperature as given below,

$$\beta = aT^2 + bT + c$$

Where β = adiabatic compressibility in m^2/N

T = temperature in °C

The value of regression coefficients a, b and c with the determination coefficient R^2 is shown in Table 4.26.

Table 4.26 Regression coefficients for adiabatic compressibility with temperature ($\beta = aT^2 + bT + c$).

Honey concentration ((w/w) %)	a	b	c	R^2
100	-0.020	2.698	-71.330	1.000*
90	-0.002	0.370	5.349	0.998*
80	-0.003	0.361	9.901	0.945*
70	-0.003	0.367	12.810	0.963*
60	-0.001	0.160	19.430	0.995*

*Significant at 5% level of significance ($p < 0.05$)

Correlation of adiabatic compressibility with concentration is given as,

$$\beta = aC^2 + bC + c$$

Where β = adiabatic compressibility in m^2/N

C = concentration in (w/w) %

The value of regression coefficients a, b and c with the determination coefficient R^2 is shown in Table 4.27.

Table 4.27 Regression coefficients for adiabatic compressibility with concentration ($\beta = aC^2 + bC + c$).

Temperature (°C)	a	b	c	R^2
20	0.000	-0.361	43.870	1.000*
30	-0.0004	0.323	19.870	0.998*
40	-0.006	0.617	8.778	0.999*
50	-0.005	0.526	12.518	0.997*
60	0.001	-0.403	46.713	0.999*
70	0.001	-0.379	46.305	1.000*

*Significant at 5% level of significance ($p < 0.05$)

4.6.4 Effect of temperature and moisture content on acoustic impedance

The acoustic impedance at different temperatures and concentrations are plotted graphically as acoustic impedance versus temperature at various concentrations and acoustic impedance versus concentration at different temperatures which are shown in Fig. 4.15 and 4.16. The calculated value varies from 3.51×10^6 to 2.16×10^6 Ns/m^3 in all the concentrations are given in Table 4.28.

It can be seen that acoustic impedance decreased with increase in temperature. The decrease in acoustic impedance value with temperature increase was more pronounced at high

Table 4.28 Acoustic impedance of honey at different temperatures and concentrations.

Temperature (°C)	Acoustic impedance (Z in Ns/m^3) × 10^5				
	100%	90%	80%	70%	60%
20	-	-	-	26.66	23.91
30	-	32.13	27.72	24.72	23.15
40	-	29.96	26.05	24.01	22.74
50	35.10	28.70	25.73	23.62	22.36
60	29.98	27.94	25.48	23.54	21.90
70	29.62	27.36	25.14	23.32	21.63

temperatures then at low temperatures. It can also reveal that it decreases with concentration increase. The similar trend of acoustic impedance with temperature at different concentration of polymer polyvinyl acetate (PVA), an organic liquid was observed by Singh and Bhatt (2010). In the case of variation is quadratic with temperature and concentration.

The decrease in acoustic impedance might be due to the decrease in resistance with temperature increase. This is in agreement with requirement as both ultrasonic velocity and density decreased with increase in temperature, as these parameters as directly related. The decrease in ultrasonic velocity value with temperature indicates the easy flow of sounds waves through the honey sample and hence the acoustic impedance decreases with temperature increase (Singh and Bhatt 2010).

The decrease in acoustic impedance of honey samples decreases with the dilution of samples, which gives indication of the effective distilled water and honey interactions after mixing of both liquids. The increase in intermolecular free length after mixing results increase in adiabatic compressibility and hence the acoustic impedance decreases (Balaji *et al* 2014).

The acoustic impedance showed the following correlation with temperature,

$$Z = aT^2 + bT + c$$

Where Z = acoustic impedance in Ns/m^3

T = temperature in °C

The value of regression coefficients a, b and c with the determination coefficient R^2 is shown in Table 4.29.

Table 4.29 Regression coefficients for acoustic impedance with temperature ($Z = aT^2 + bT + c$).

Honey concentration ((w/w) %)	a	b	c	R^2
100	0.024	-3.130	132.100	1.000*
90	0.003	-0.378	41.044	0.996*
80	0.002	-0.252	33.374	0.944*
70	0.002	-0.238	30.415	0.965*
60	0.000	-0.081	25.308	0.994*

*Significant at 5% level of significance ($p < 0.05$)

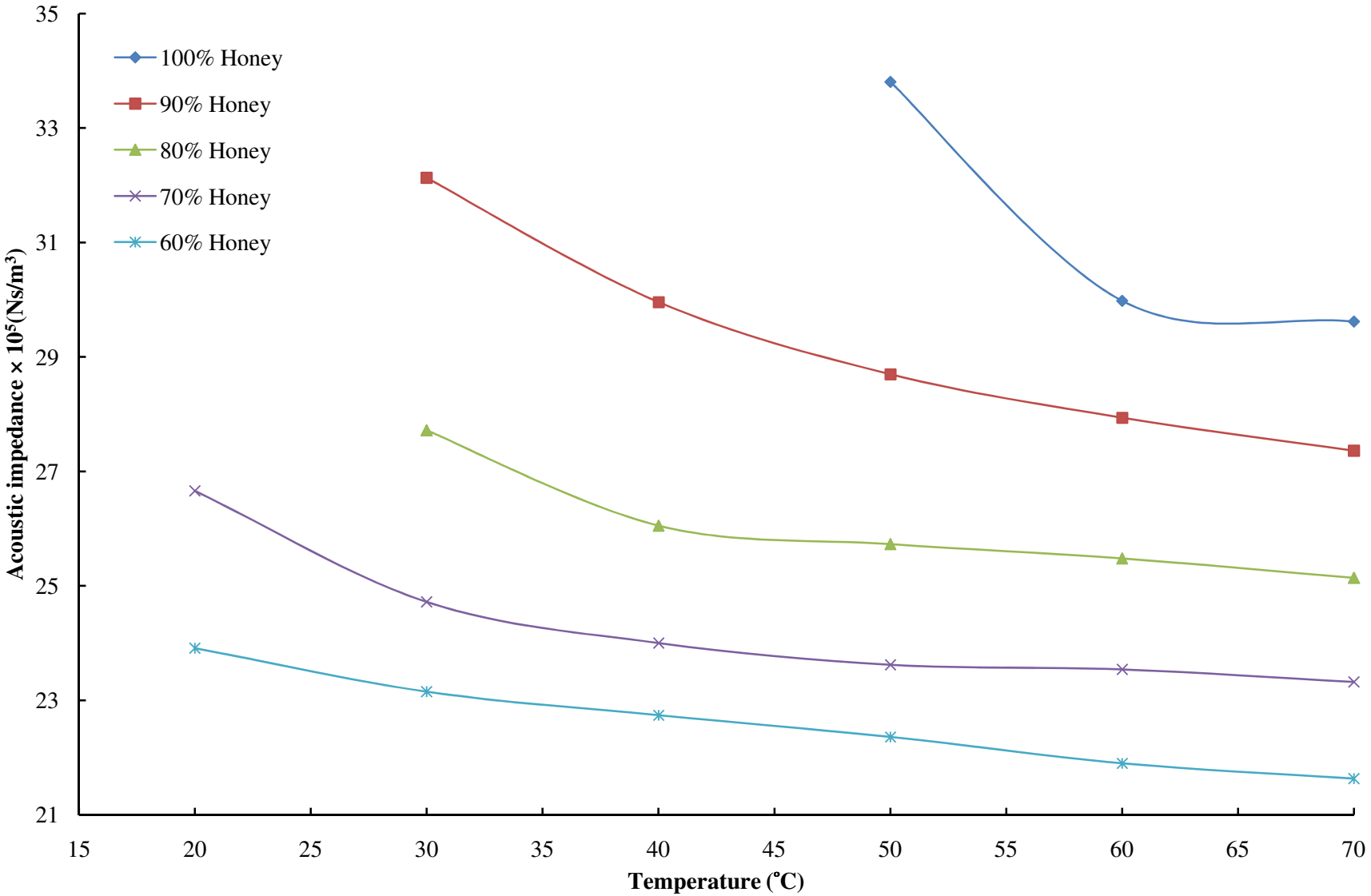

Fig. 4.15 Variations of acoustic impedance at different temperatures.

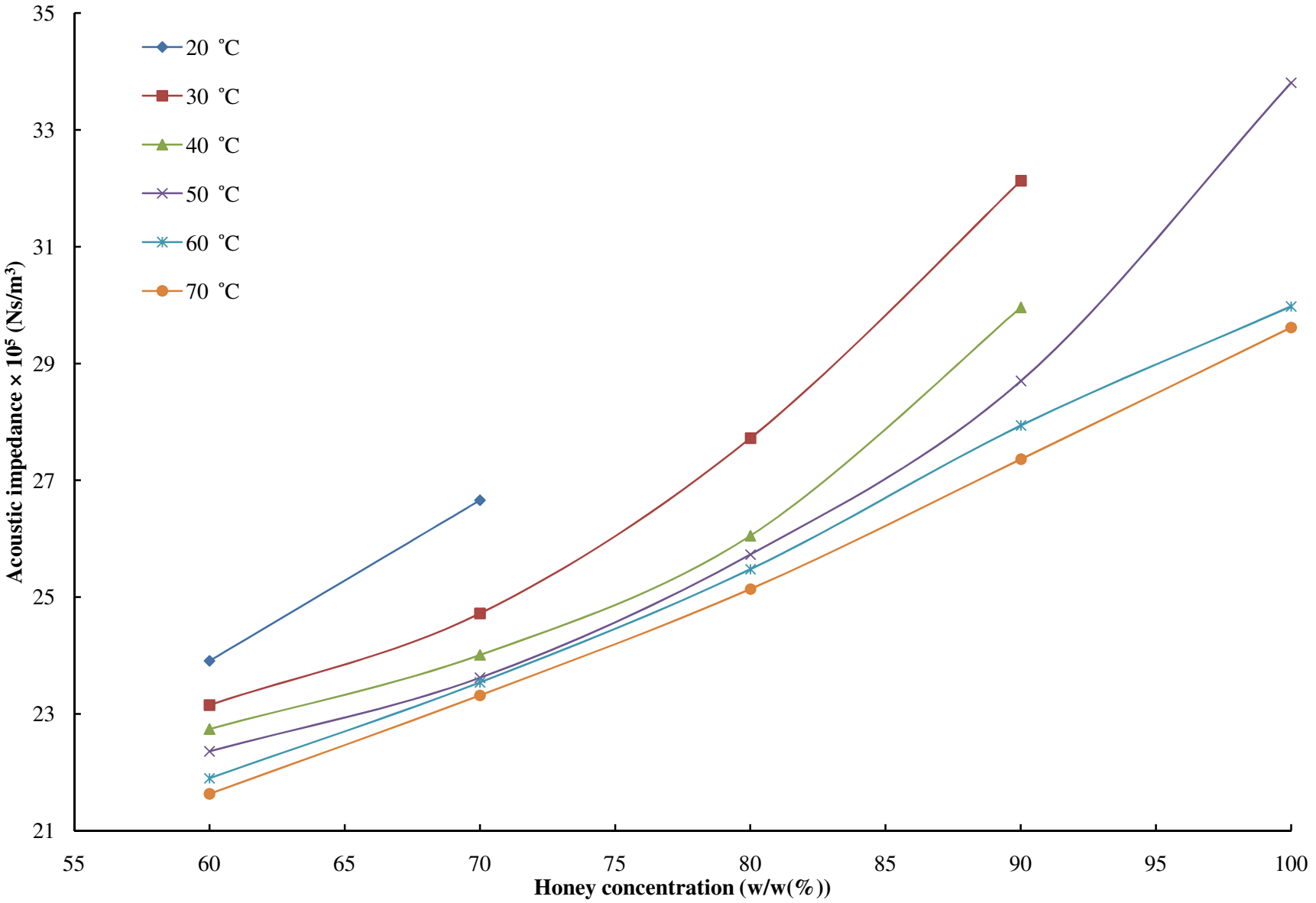

Fig. 4.16 Variations of acoustic impedance at different concentrations.

The acoustic impedance showed quadratic behaviour with concentration predicted as,

$$Z = aC^2 + bC + c$$

Where Z = acoustic impedance in Ns/m^3

C = concentration in (w/w) %

The value of regression coefficients a, b and c with the determination coefficient R^2 is shown in Table 4.30.

Table 4.30 Regression coefficients for acoustic impedance with concentration ($Z = aC^2 + bC + c$).

Temperature (°C)	a	b	c	R^2
20	0.000	0.274	7.470	1.000*
30	0.007	-0.766	43.525	1.000*
40	0.007	-0.753	44.215	0.998*
50	0.008	-0.968	51.988	0.993*
60	0.001	0.055	15.166	0.998*
70	0.001	0.024	16.218	1.000*

*Significant at 5% level of significant ($p < 0.05$)

4.6.5 Effect of temperature and moisture content on intermolecular free length

The value of intermolecular free length obtained is expressed in form of intermolecular free length versus temperature and intermolecular free length versus concentration graphically which is shown in Fig. 4.17 and 4.18. The change in intermolecular free length was quadratic with temperature and concentration. It is revealed from the graph that free length interactions of honey samples are both concentration and temperature dependent. Table 4.31 shows its calculated value varies from 0.24 to 0.36 Å.

Table 4.31 Intermolecular free length of honey at different temperatures and concentrations.

Temperature (°C)	Intermolecular free length (L in Å)				
	100%	90%	80%	70%	60%
20	-	-	-	0.28	0.30
30	-	0.25	0.28	0.30	0.32
40	-	0.27	0.30	0.32	0.33
50	0.24	0.28	0.31	0.33	0.34
60	0.28	0.30	0.32	0.33	0.35
70	0.29	0.31	0.33	0.34	0.36

The intermolecular free length values increased with increase in temperature. Also, the intermolecular free length increases with the increase in dilution of honey. The trend of intermolecular free length was consisted with literature (James *et al* 2009 and Rao *et al* 2015).

Intermolecular free length is the distance between the surfaces of the neighboring molecules. The influence of temperature increase can be attributed to ease of breaking of sugar bonds in the honey samples. The sugar molecules of the sample acquired the thermal energy from the applied temperature and hence which results in increasing intermolecular free length of the samples.

The increase in free length is more for diluted concentrations of honey because of the

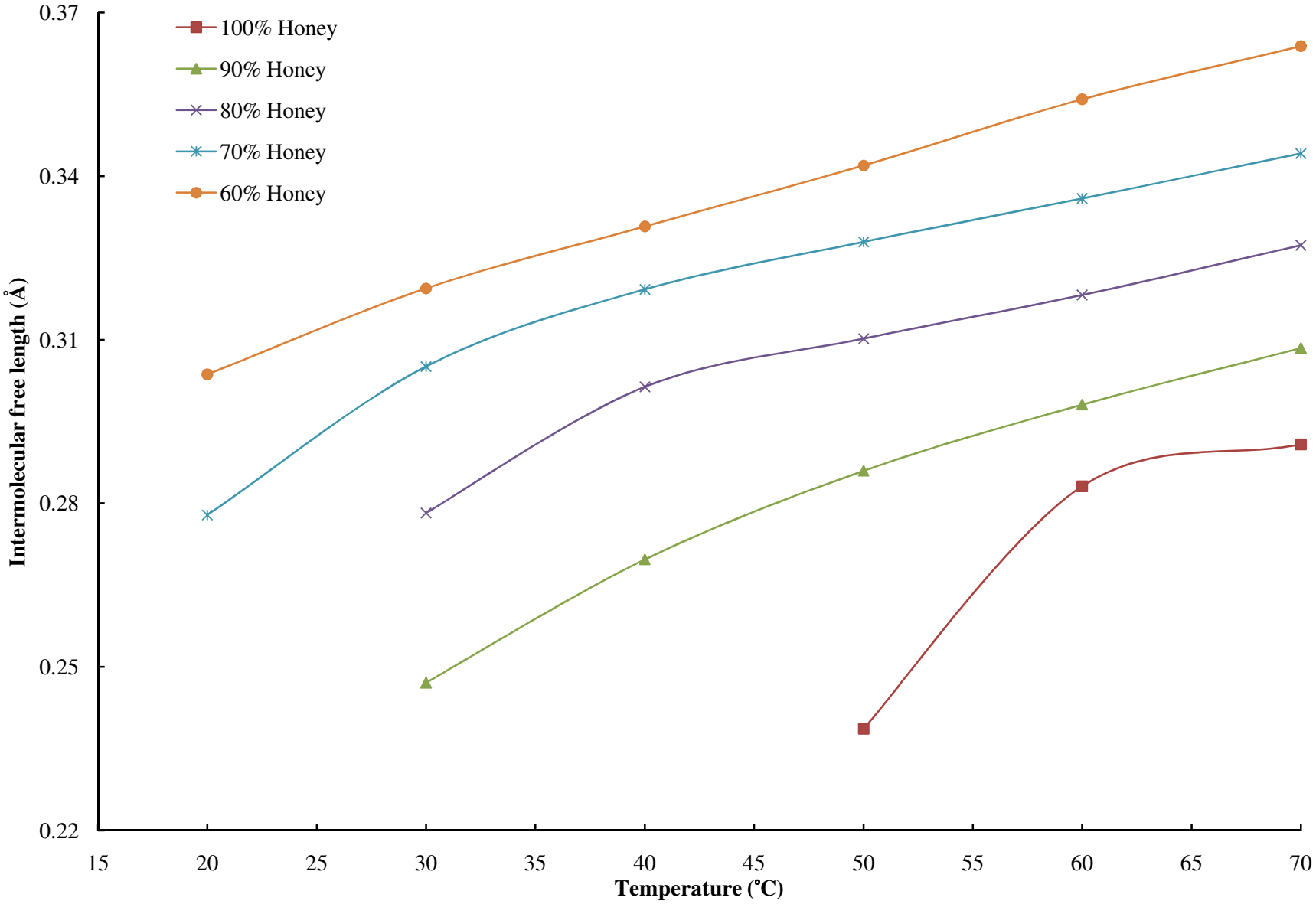

Fig. 4.17 Variations of intermolecular free length at different temperatures.

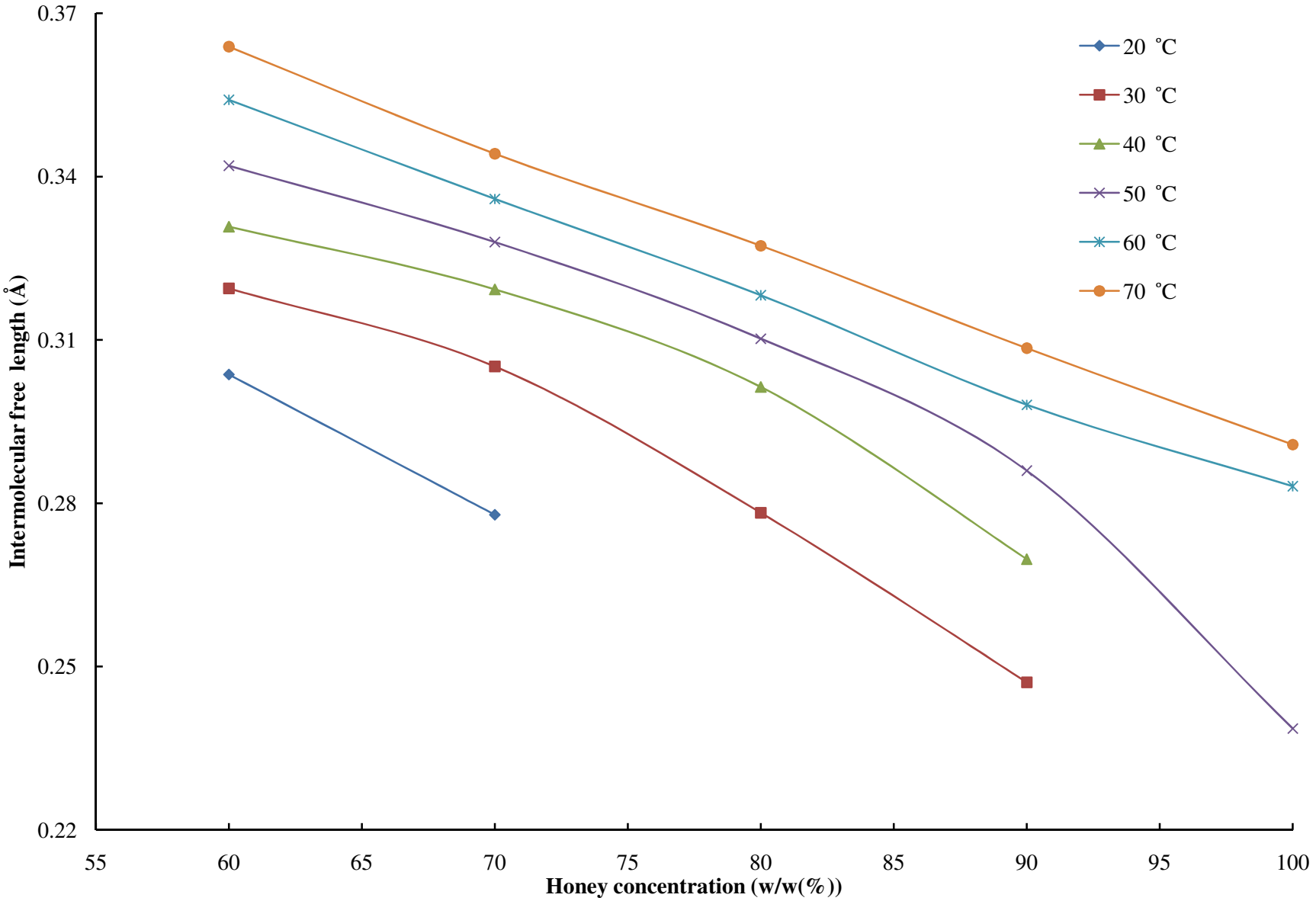

Fig. 4.18 Variations in intermolecular free length at different concentrations.

direct relation with adiabatic compressibility which also increases with dilution of honey samples. The intermolecular free length between the sugar molecules is small at low percentage of water. Addition of water to honey brings about exchange of the sugar-sugar hydrogen bonding with sugar-water hydrogen bonding (James *et al* 2009). As the dilution of honey samples increases, the molecules were getting apart, thereby increasing the intermolecular free length

The dependence of intermolecular free length on temperature was can be written as,

$$L = aT^2 + bT + c$$

Where L = intermolecular free length in Å

T = temperature in °C

The value of regression coefficients a, b and c with the determination coefficient R^2 is given in Table 4.32.

Table 4.32 Regression coefficients for intermolecular free length with temperature ($L = aT^2 + bT + c$).

Honey concentration ((w/w) %)	a	b	c	R^2
100	0.000	0.000	-0.410	1.000*
90	-7.143E-006	0.002	0.191	0.990*
80	-1.429E-005	0.003	0.215	0.992*
70	-2.321E-005	0.003	0.225	0.982*
60	-8.929E-006	0.002	0.266	0.992*

*Significant at 5% level of significance ($p < 0.05$)

Correlation of intermolecular free length with concentration is,

$$L = aC^2 + bC + c$$

Where L = intermolecular free length in Å

C = concentration in (w/w) %

The value of regression coefficients a, b and c with the determination coefficient R^2 is given in Table 4.33.

Table 4.33 Regression coefficients for intermolecular free length with concentration ($L = aC^2 + bC + c$).

Temperature (°C)	a	b	c	R^2
20	0.000	-0.002	0.420	1.000*
30	-2.500E-005	0.001	0.323	0.998*
40	-5.000E-005	0.005	0.180	1.000*
50	-5.000E-005	0.006	0.190	1.000*
60	-7.143E-006	-0.001	0.408	0.992*
70	-7.143E-006	-0.001	0.418	0.992*

*Significant at 5% level of significance ($p < 0.05$)

4.7 Correlation between EC and TDS

TDS is the measure of all organic and inorganic substances dissolved in a given liquid, revealing the proportion of different solids. Since the EC is a measure to the capacity

of liquid to conduct electrical current, it is directly related to the concentration of salts dissolved in liquid, and therefore to TDS. It can be observed the EC and number of TDS in honey samples increases with decrease in honey concentration and increase in temperature of the sample. Since EC and TDS of honey samples show the same behaviour with respect to temperature and concentrations, results demonstrate that there is a good correlation between EC and TDS, indicating that both parameters can be used to determine honey purity. The positive correlation between EC and TDS were noticed by Khalil *et al* (2012) in case of Algerian honeys, Moniruzzaman *et al* (2013) in case of various Malaysian honeys and Hamed *et al* (2014) in some kinds of Iraqi honeys of local markets. The correlation coefficient for EC and TDS is found to be 1.000 and it is significant at 1% level of significance. Hence, these two parameters are significantly correlated and graphical variation of conductivity with TDS of honey samples in shown in Fig. 4.19.

The dependence of EC on TDS was found to be linear and is,

$$\kappa = a + b\ (TDS)$$

Where κ = EC in μScm^{-1}

TDS = total soluble solids in ppm

The value of regression coefficients a and b with the determination coefficient R^2 is given in Table 4.34.

Table 4.34 Regression coefficients for intermolecular free length ($\kappa = a + b\ (TDS)$).

a	b	R^2
0.498	-0.022	0.999*

*Significant at 5% level of significance ($p < 0.05$)

4.8 Conclusions

It was observed that many of honey parameters (ultrasonic velocity, EC, TDS, surface tension, bulk modulus, adiabatic compressibility, acoustic impedance and intermolecular free length) except density showed significant variations with change in moisture content and temperature. Hence, moisture content and temperature could be serving as an indicator to detect an artificial honey sample distinguished from a natural honey sample. The variation in the physicochemical properties with honey dilution provides more information than does at a fixed concentration. The percentage of moisture content was an important parameter used to access quality of honey samples with temperature.

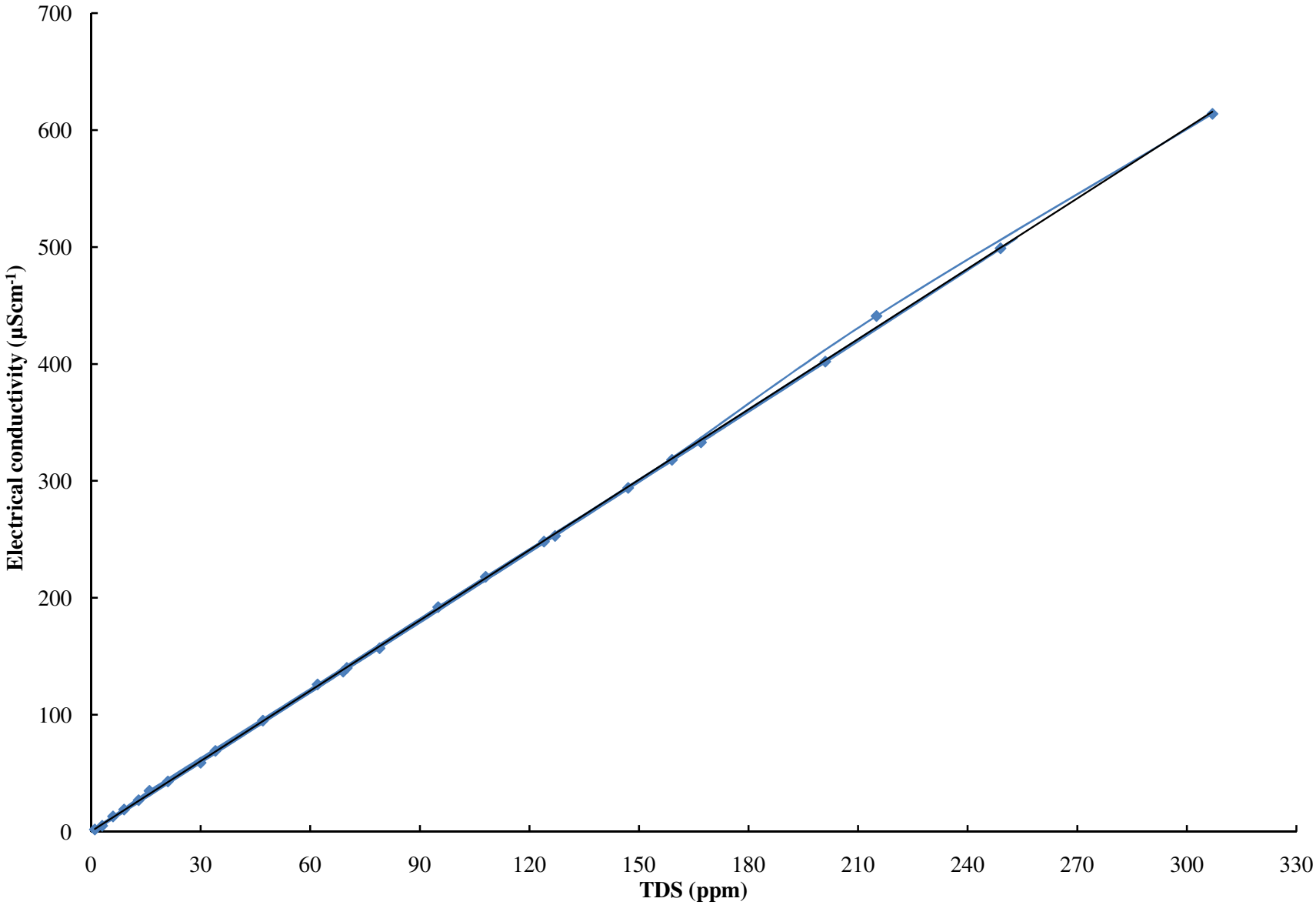

Fig. 4.19 Correlation of TDS with EC for all honey concentrations.

CHAPTER-V

SUMMARY

Honey is the sweet and purest form of food known to man since ancient times consumed because of its medical and domestic needs for its high nutritional values and beneficial effects on human health. Honey and beekeeping have a long history in India. With raising demand for antioxidant in the food, honey is becoming the trendy source of antioxidant. It is the excellent source of employment for the rural population. The chemical composition of honey is complex, containing sugars, proteins, moisture, vitamins, minerals, hydroxymethylfurfural (HMF), enzymes, flavonoids, phenolic acids and volatile compounds. Composition of honey is variable and primarily depends on the floral source, seasonal and environmental factors and processing steps and conditions.

Honey is a prime target of adulteration for economic gain and can be made poorer in quality by adding amounts of sucrose, commercial glucose, starch, chalk, gelatins, water and other substances. These artificial honeys often have similar taste and physical appearance as natural honeys, but they lack the medicinal and nutritional properties of natural honeys. It is necessary to prepare certain procedures for detecting honey adulteration. Detection of adulteration in honey is difficult because of the large natural variability. Some physicochemical properties have been found helpful for comparison of natural honey samples from different locations and also can help to distinguish natural honey from artificial honey which includes surface tension, ultrasonic velocity and viscosity. Many parameters such as adiabatic compressibility, acoustic impedance, intermolecular free length, electrical conductivity (EC) etc. can be used to compare honey.

The present study comprises: measurement of ultrasonic velocity, EC, total soluble solids (TDS) and density of honey at different moisture contents and temperatures and determination of thermodynamic parameters like surface tension, bulk modulus, adiabatic compressibility, acoustic impedance and intermolecular free length from the measured parameters and establishing relation between these properties at different temperatures.

The literature reviewed on the study of honey in India and other various countries of world are discussed in the chapter II. The various studies include the physicochemical and rheological properties of honey and other fluids. The literature reviewed shows that honey properties such as ultrasonic velocity, surface tension, viscosity, EC etc. vary with the origin and floral source and thus can be used as a tool to distinguish the honey from different geographical origins. The physicochemical properties of honey vary depending upon the type of flora used to produce it, moisture level, temperature and the proportions of various sugars contained in it. The literature also revealed about pH, refractive index, color, acidity and other various antioxidant and microbiological properties of honey. The literature reviewed involved

the honey properties of Algeria, Australia, Brassica, Ethopia, Egypt, Iran, India, Jordanian, Nigeria, Pakistan, Libya honey. There is a significant correlation between EC and TDS of honey as observed in various research papers which can be used to discriminating honey types.

The material and methods used for measurement of physicochemical properties such as ultrasonic velocity, EC, TDS, density, refractive index and pH of honey at different moisture content and temperatures are discussed in chapter III. The experimental setups used for measurements at specific conditions have been discussed in this chapter with appropriate equations/relations used to derive thermodynamic parameters of honey. The other thermodynamic parameters such as surface tension, bulk modulus, adiabatic compressibility, acoustic impedance and intermolecular free length are calculated from the measured properties.

In the fourth chapter, the results obtained in the study are presented in form of tables and graphs. The chosen honey sample falls under the reasonably good category as per described by USDA grading of honey in terms of moisture content. The ultrasonic velocity and density in honey samples decreases with the increase in temperature and decrease in honey quantity in concentration. The decrease in ultrasonic velocity was higher for 20 to 50°C temperature increase and quadratic in behaviour but density decrease was not very high and linear with increase in temperature and decrease in honey quantity in sample. On the other hand, EC and TDS in honey samples were found to be increases linearly with the increase in temperature and decrease in honey quantity in concentration. The surface tension, bulk modulus and acoustic impedance of honey were also found decreases with temperature increase and decrease in honey for various concentrations, however; adiabatic compressibility and intermolecular free length increases. The significant correlation between EC and TDS of honey was observed.

The results presented in the fourth chapter were supported by using the statistical tools. To check the significance of results obtained one way analysis of variance (ANOVA) and Tukey's test was applied by using the software SPSS version 20.0. The correlation of a particular physicochemical property with evolution of temperature was established using the regression analysis. Honey parameters except density showed significant variations with change in moisture content and temperature. Hence moisture content and temperature could be serving as an indicator to detect an artificial honey sample distinguished from a natural honey sample. The moisture content and temperature can also be used as complementary variables for discriminating the botanical origin of honeys of a given geographic region. In this preliminary research, a set of complementary techniques is proposed that does not require chemical reactants or expensive equipment, to improve the characterization of honeys from regions with different soil characteristics.

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