EFFECT OF ULTRASOUND AND HIGH HYDROSTATIC PRESSURE (HHP) ON LIQUEFACTION AND QUALITY PARAMETERS OF SELECTED HONEY VARIETIES

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EFFECT OF ULTRASOUND AND HIGH HYDROSTATIC PRESSURE (HHP) ON LIQUEFACTION AND QUALITY PARAMETERS OF SELECTED HONEY VARIETIES

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ABSTRACT

EFFECT OF ULTRASOUND AND HIGH HYDROSTATIC PRESSURE (HHP) ON LIQUEFACTION AND QUALITY PARAMETERS OF SELECTED HONEY VARIETIES

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Heat treatment (around 50°C) is a major step in honey filling and packaging that is applied before filtration to decrease viscosity, reduce the moisture level, to destroy yeasts, liquefy crystals and delay crystallization. As a result, formation of Hydroxy Methyl Furfural (HMF), decrease in enzymatic activity, color deterioration, decrease in viscosity and many other structural changes are observed. HMF is produced as a result of Maillard reaction and/or hexose dehydration -which is undesirable-, practically, it is found in fresh honey in low levels, and increases due to heat treatment, storage temperature, pH (acidity) and sugar concentration of honey. HMF level and diastase number are important quality parameters and shelf life indicators of honey. Alternatives of

heat treatment may be the use of ultrasound and high hydrostatic pressure (HHP) to decrease viscosity, liquefy honey and thus minimise adverse affects of heat treatment. Therefore, the aim of this study is to evaluate the effect of HHP (220-330 MPa, 50-60°C, time) and ultrasound (24 kHz) on liquefaction and quality parameters (HMF, diastase number, color and viscosity) of different honey varieties (sunflower, cotton and canola) and to compare the changes with heat treated (50°C and 60°C, time) and untreated honey. Based on the results of the chemical and physical analysis, for HHP treatment the best treatment combination was determined as 220 MPa, 50°C, 106 min. For ultrasound treatment the best treatment combinations were determined as 7 mm probe- 0.5 cycle (batch) applications. On this basis the study points out that Ultrasound and HHP can be suggested as alternative methods to traditional thermal treatment for the liquefaction of honey crystals. When compared to thermal treatment, Ultrasound is advantageous in shorter application times, slight changes in quality parameters and ease in operation. HHP treatment is also an alternative method with shorter application times and lower HMF values.

Keywords: ultrasound, high hydrostatic pressure, honey, HMF, diastase number

ÖZ

ULTRASON VE YÜKSEK HİDROSTATİK BASINÇ (YHB) UYGULAMALARININ DEĞİŞİK BAL TÜRLERİNDE KRİSTALLERİN ÇÖZÜLMESİ VE BALLARIN KALİTE PARAMETRELERİ ÜZERİNE ETKİSİ

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Gıda endüstrisinde bal üretiminin önemli bir parçası viskoziteyi azaltmak, nem miktarını düşürmek, maya inhibisyonu, kristalleri çözmek ve kristalizasyonu geciktirmek amacıyla filtrasyon aşamasından önce uygulanan ısıl işlemdir (50°C civarında). Isıl işlem sonucu balda istenmeyen hidroksi metil furfural (HMF) oluşumu, mevcut enzim aktivitelerinde azalma, renk değişimi ve viskozite düşüşü gibi birçok yapısal değişiklik gözlemlenmektedir. HMF, Maillard reaksiyonu ve/veya hekzos dehidrasyonu sonucu oluşan istenmeyen bir üründür. Taze balda pratik olarak HMF düşük miktarlardadır ve uygulanan ısıl işlem, depolama sıcaklığı, balın pH'sı (asitlik) ve şeker konsantrasyonuna bağlı olarak HMF yükselir. HMF düzeyi ve diastaz sayısı balda önemli kalite

parametreleri ve raf ömrü indikatörleridir. Ultrasonik teknikler ve yüksek hidrostatik basınç uygulaması balda kristalleri çözmek, viskoziteyi düşürmek amaçlı kullanılabilecek ısıl işleme alternatif metotlardandır. Bu nedenle, bu çalışmanın amacı, yüksek hidrostatik basınç (220-330 MPa, sıcaklık (50-60°C) ve zaman) ve ultrason (24 kHz) uygulamalarının değişik bal türlerinde (ayçiçek, pamuk ve kanola) kristallerin çözülmesi ve kalite parametreleri (HMF, diastaz sayısı, renk ve viskozite) üzerine etkisini araştırıp, ısıl işlem gören (50°C ve 60°C, zaman) ve işlenmemiş bal ile karşılaştırmaktır. Kimyasal ve fiziksel analizlerden elde edilen sonuçlar baz alındığında, 220 MPa basınç-50°C sıcaklık-106 dakika en iyi YHB kombinasyonu olarak belirlenmiştir. Ultrason uygulaması için ise 7 mm prob- 0,5 cycle (kesikli) uygulamaları en iyi kombinasyonlar olarak belirlenmiştir. Bu çalışma sonucuna göre, balın kristallerini çözme işleminde Ultrason ve YHB uygulamaları geleneksel ısıl işleme alternatif metotla olabilir. Isıl işlemle karşılaştırıldığı zaman Ultrason, daha kısa uygulama süresi, balın kalite parametrelerinde kayıplara neden olmaması ve kullanım kolaylığı avantajlıdır. YHB uygulaması ise daha kısa uygulama süresi, ve düşük HMF değerleri elde edilmesinden dolayı avantajlıdır.

Anahtar kelimeler: ultrason, yüksek hidrostatik basınç, bal, HMF, diastaz sayısı.

to my parents, my friends and beloved…

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INTRODUCTION

1.1 What is honey?

Honey is defined as the natural sweet substance produced from nectar of plants, from secretions of living plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect and transform by combining with specific substances of their own. These nectars are then deposited, dehydrated, stored left in the honey comb to ripen and mature (Codex Alimentarius, 1998).

The biological definition is as follows:

Honey is a substance produced by honeybees and some other social insects from nectar or honeydew collected from living plants. They transform the nectar or honeydew by evaporating water and by the action of enzymes they secrete. As a rule, honeybees seal the finished honey in cells of their comb (Crane, 1990).

Bee honey is broadly categorised as blossom honey and honeydew honey. Blossom honey is formed from the nectar of plants. Honeydew honey is produced from honeydew which consists of excretions of plant sucking insects (Codex Alimentarius, 1998; Clemson, 1985; Doner, 1977).

Table 1.1 Honey nutritional facts

	Average (%)	**Range**
Fructose	38.4	30.9 - 44.3
Glucose	30.3	22.9 - 40.8
Minerals (Ash)	0.2	0.02 - 1.03
Moisture	17.2	13.4 - 22.9
Reducing Sugars	76.8	61.4 - 83.7
Sucrose	1.3	0.3 - 7.6
pH	3.9	3.4 - 6.1
Total Acidity, meq/kg	29.1	8.7 - 59.5
True Protein, mg/100g	168.6	57.7 – 567.0
a_w	0,59	0.56-0.62

1.1.1 Effect of heat on honey

1.1.1.1 Hydroxymethylfurfural (HMF) formation

HMF can be formed by hexose dehydration in acid media or by the Maillard reaction (Feather et al., 1982; Hoseney, 1984). This process is reported to be enhanced by heat or storage under elevated temperatures. Bogdanov et al. (1997) reported that practically there is HMF in low levels in fresh honeys, but the level increases during storage and heating depending on the pH of honey and the storage and heating temperature.

Honey is heated at different stages of its processing to reduce viscosity, destroy yeast, and dissolve crystals. However, such heat treatments increase the HMF content of honey. Therefore, HMF content can be used as an indicator to detect the heat damage and shelf life behavior of honey. Further, it has been reported that the HMF content of honey increases during storage in the warm climates of tropical and subtropical countries (D'Arcy, 2007). The latest Codex standards for the HMF content of honey is set as less than 40 mg/kg after processing and/or

blending of honey (Codex Alimentarius, 2001, Revised codex standard for honey).

1.1.1.2 Inactivation of Enzymes in honey

Enzymes are other important constituents of honey because they play an important role in honey production from the nectar of the plant. Further, enzymes are heat sensitive and extra low levels indicate that honey has been overheated. Further, their activities are decreased during storage and used as indicators of the freshness of honey (D'Arcy, 2007).

One of the important enzymes in honey is diastase (amylase). The diastase activity is expressed as ml of 1% starch hydrolyzed by the enzyme in 1 g of honey in 1 h, called the diastase number (Küçük et al., 2007). Minimum level of diastase number is 8 (TS 3036- Turkish honey standards). Thermal treatment, applied to honey, produce a simultaneous decrease in diastase activity.

1.1.1.3 Color changes of honey

One of the effects of thermal treatment is non-enzymatic browning reactions including Maillard reaction in honey (Turkmen at al., 2006).

Heat processing can darken the natural honey color (as a result of browning reactions).

The color of honey depends on the floral source and its mineral content (Anon, 2003b). Perez-Arquillue et al. (1994) reported that honey with a higher mineral content is darker in colour. Rodgers (1976) reported that the colour of honey sourced from the same plants also depends on the climatic factors and the honey ripening temperature in the hive. Published work reported that honey color is a temperature sensitive parameter, and honey can become darker as a result of different storage conditions (Anon, 2003b).

1.1.2 Physical Characteristics of honey

1.1.2.1 Viscosity of Honey

Viscosity is an important property in handling and processing of honey. The flow properties of honey depend on the composition, moisture content, and temperature. Generally, honey samples with high moisture contents have low viscosity (D'Arcy, 2007).

Knowledge of the rheology of honey is necessary in its production, processing and storage (Juszczak & Fortuna, 2006).

In most published work, honey is reported to be a Newtonian fluid (White, 1978; Junzheng & Changying, 1998; Mossel, 2002; Juszczak L. and Fortuna T, 2006). However, there are some reports in the literature, as cited in Mossel et al. (2000), for non-Newtonian behaviour.

1.1.2.2 Crystallisation of Honey

Crystallisation is a natural phenomenon in honey, which is a supersaturated sugar solution. The supersaturated state occurs because honey contains more than 70% sugars and less than 20% water (D'Arcy, 2007).

As honey is a supersaturated sugar solution containing glucose and fructose, glucose loses water and crystallises as D-glucose monohydrate at room temperature. These monohydrate crystals serve as seeds for the crystallisation process. In addition, other small particles such as dust, pollen and air bubbles serve as nuclei for crystallisation. The water released by glucose during crystallisation increases the moisture content of honey, making it more susceptible to fermentation. The tendency of honey to crystallise depends on its composition and moisture content. Honey with glucose content less than 30%

rarely crystallises and those with 35% glucose are naturally crystalline (Assil et al., 1991).

1.2 Minimal Processing Technologies

The term 'minimal processing' has been defined in various ways, for example very broadly as 'the least possible treatment to achieve a purpose' (Manvell, 1996). A more specific definition which addresses the question of purpose describes minimal processes as those which 'minimally influence the quality characteristics of a food whilst, at the same time, giving the food sufficient shelf-life during storage and distribution (Huis in't Veld, 1996).

Food processing technologies which are being widely used in the food industry for preservation and shelf-life extension, such as heat treatments, pasteurization and else can cause reduction in the quality of food because of the decrease of nutrients, vitamins, proteins or sensory characteristics such as aroma, flavor, color and else; therefore, over the last decade, there occurred a demand by the consumers for the foods that are minimally or not processed but are compatible with the processed foods in terms of safety, suitability and consumability. This gave rise to the development of minimal processing methods that preserve foods produced by treatments involving reduced or mild degrees of temperature, so as to prevent the loss of nutritional and sensory quality of foods due to the heat applications.

For many years, the traditional preservation methods that need little or no heat treatment such as fermentation, curing or insalination were being used. More recently, research and development studies were focused on several minimal processing methods like pulsed electric fields (PEF), high hydrostatic pressure (HHP) processing, high intensity light and ultrasound, irradiation, ozone

treatments, controlled and modified atmosphere. Consequently, those recent processes began to take part for the preservation of several food stuffs. However, none of those preservation items were enough by itself for permitting adequate safety and palatability. Novel approach in minimal processing technologies involves the extensions or combinations of one or more minimal processing methods. This also supported the adaptation of the hurdle concept which brings together the combined effect of more than one minimal processing method and each preservation method in this concept constitutes a hurdle to be beaten by the physical, chemical or microbial agents and other contaminants within the food. The resulting products have higher quality and consumer appeal in markets where the retention of nutritional sensory characteristics can command premium prices (Fellows, 2000).

1.2.1 High Hydrostatic Pressure (HHP) in Food Processing

There has been growing interest in using high hydrostatic pressure processing as a non-thermal food preservation technique. Its primary advantage is that it can inactivate microorganisms and degradative enzymes at substantially lower treatment temperatures (as compared to conventional thermal processing) that result in processed foods possessing sensory and nutrient qualities closely resembling the original fresh or raw product (Hoover, 1993; Smelt, 1998).

1.2.1.1 General Principle and Mechanism of HHP

By subjecting foods to high pressures in the range 3000-8000 bars, microorganisms and enzymes can be inactivated without the degradation in flavour and nutrients associated with traditional thermal processing.

The HHP process is non-thermal in principle, even if the pressure increase in itself causes a small rise in temperature. HHP affects all reactions and structural changes where a change in volume is involved, as in the gelation of protein or starch. The mechanism behind the killing of microorganisms is a combination of such reactions, the breakdown of non-covalent bonds and the permeabilisation of the cell membrane. Vegetative cells are inactivated at about 3000 bars at ambient temperature, while spore inactivation requires much higher pressures (6000 bars or more) in combination with a temperature rise to 60-70°C. Certain enzymes are inactivated at 3000 bars, while others are very difficult to inactivate at all within the pressure range that is practically available today (Hoogland et al., 2001).

HHP is a three-variable process consisting of pressure, time and temperature. For effective use of this method in food preservation it is necessary to study the interaction of these factors and determine the minimum conditions to obtain desirable levels of microbial destruction while maintaining a maximum degree of sensory and nutritional quality (Alpas et al., 1998).

1.2.1.2 History of commercial use of HHP

Over the past 15 years, HHP has emerged as a commercial alternative to traditional thermal processing methods for some foods, e.g., jams, fruit juices, guacamole, and fresh whole oysters (Hoover, 1993).

The technology was first commercialised in Japan in early 1990s for the pasteurisation of acid foods for chilled storage. The first commercial products were fruit juices such as orange and grape juices, fruit jams such as apple, kiwi, strawberry, raspberry; fruit toppings' salad dressings and tenderized meat. In 1995, orange juice produced by HHP was commercialized in France. Following

that, in 1999 HHP was discovered to be effective on the shucking of oysters, which initiated the introduction of HHP treated oysters in the US market by Motivatit Sea Foods Inc. as Gold Band Oysters (Knorr, 1999; Duchene, 2001). The approach was followed by Nisbet Oyster Company in 2001 by introducing pressurized oysters (Kuriloff, 2003). In spite of massive research efforts, particularly in Europe and the USA, commercial development outside Japan has been slow so far, mainly because of the very high investment and processing costs of high pressure processing as well as regulatory problems in regions such as Europe.

1.2.1.3 HHP Equipment and Operation

Most pressure vessels are made from a high tensile steel alloy 'monoblocks' (forged from a single piece material), which can withstand pressures of 400-600 MPa. For higher pressures, pre-stressed multilayer or wire-wound vessels are used (Mertens, 1995). Vessels are sealed by a threaded steel closure; a closure with an interrupted thread, which can be removed more quickly; or by a scaled frame which is positioned over the vessel. In operation, after all air has been removed, a pressure-transmitting medium (either water or oil) is pumped from a reservoir into the pressure vessel using a pressure intensifier until the desired pressure is reached. This is termed 'indirect compression' and requires static pressure seals. Another method, termed 'direct compression', uses a piston to compress the vessel, but this requires dynamic pressure seals between the piston and internal vessel surface.

Temperature control in commercial operations can be achieved by pumping a heating/cooling medium through a jacket that surrounds the pressure vessel. This is satisfactory in most applications as a constant temperature is required.

Two methods are available for the processing of foods in high pressure vessels: in-container processing and bulk processing. The former is generally performed as a batch process while the latter provides a semi-continuous processing. In bulk processing, the food is elevated by pumps and pipes through the pressure vessel.

1.2.2 Ultrasound in Food Processing

Ultrasound techniques find use in the food industry in both the analysis and modification of foods. Microbial and enzyme inactivation are other applications of ultrasound in food processing. The use of ultrasound on its own in the food industry for bacterial destruction is currently unfeasible; however, the combination of ultrasound and pressure and/or heat shows considerable promise. The future of ultrasound in the food industry for bactericidal purposes lie in thermosonication, manosonication, and manothermosonication, as they are more energy-efficient and result in the reduction of microbial and enzyme activity when compared to conventional heat treatment (Demirdöven&Baysal, 2009).

1.2.2.1 General Principle and Mechanism of Ultrasound

Ultrasound is defined as sound waves with a frequency greater than that of human hearing range. Human hearing is in the frequency range of 0.016 to 18 kHz, and the power intensity of normal conversation is about 1 W/cm^2 (Leadley &Williams, 2002; Mason, 1998; McClements, 1995).

Ultrasound waves with frequencies more than 18 kHz are generated by the application of a vibration force to the surface of a material. When the vibration force is applied to the surface of a material, it is transmitted through the bonds

within molecules. Further, each of the molecules transmits the motion to an adjoining molecule before returning to approximately its original position in this process. If ultrasound is applied perpendicular to the surface of the material, then a compression wave is generated within the material. Similarly, a shear wave is generated by the application of ultrasound parallel to surface. The ultrasound waves cause the layers in the material to oscillate in their original positions at the same frequency as the ultrasound waves. Thus, displacement of a fixed position in the material varies sinusoidal with time, and the time difference between two maximum positions is the period of oscillation (McClements, 1995).

The application of ultrasound to a liquid creates compressions. Thus, sound waves with sufficient high amplitude produce bubbles or cavities, and this incident is called 'cavitation'. These cavitation bubbles have a limited lifetime and break up into smaller bubbles or completely disappear. There are two types of cavitation; stable or transient. Stable cavitation occurs due to the oscillation created by ultrasound waves, which forms small bubbles in the liquid. It takes so many oscillatory cycles for the bubbles to increase their size in a stable cavitation. As the ultrasound waves pass through the liquid, they vibrate these bubbles and strong current is produced in the surrounding liquid. Further, it attracts the other small bubbles into the sonic field and microcurrents are created in the liquid. This effect is called microstreaming, which provide a substantial force causing the cells to shear and breakdown without the collapse of bubbles. The shear force created by this process is one of the actions that lead to disruption of cells. In transient cavitation, the bubble size changes in a few oscillatory cycles and it collapses with different intensities. The larger bubbles eventually collapsed producing high pressures of up to 100 MPa and high

temperatures up to 5000 °K instantly. The pressure produced during bubble collapse is also sufficient to disrupt cell walls and eventually lead to cell disruption. Application of ultrasound to a liquid also leads to the formation of free radicals by sonolysis of water due to these high pressures and temperatures (Leadley & Williams, 2002; Sala et al., 1995; Scherba et al., 1991; Suslick, 1988).

1.2.2.2 History of Ultrasound Application on Honey

Kaloyereas (1955) reported that high 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 at al., 1958). One disadvantage of this method was that exposure times of 15 to 30 minutes were required with cost implications.

Liebl (1977) proposed an improved method for preventing the granulation by exposing the honey to ultrasound waves of a much higher frequency (18 kHz) that drastically reduced the liquefaction time to less than 30 seconds. This patented process was designed to work at lower processing temperature (33°C) facilitating greater retention of aroma and flavor along with huge savings on cost of energy compared to the conventional processing involving heating and cooling steps. Studies were carried out at a considerably higher scale (liquefaction of ~1500 kg of honey/h) to demonstrate the claims on the cost effectiveness of the process.

Thrasyvoulou et al. (1994) studied the effects of ultrasonic waves on the quality of honey focusing on some of the chemical characteristics. Crystallized honey

samples were liquefied by ultrasonic waves at 23 kHz and compared with conventionally heated (water bath heating; 60°C for 30 minutes) and untreated samples. The complete liquefaction of honey required 18 to 25 minutes by ultrasound processing, while it is 30 minutes in thermal heating. Accordingly the energy required for liquefaction varied from 0.1056–0.1466 kWh, and the maximum temperature attained by the samples ranging from 76–82°C for ultrasound applications. The variation in the time required for liquefaction was attributed to the original granulated condition and the nature of samples.

The combined effect of temperature and processing time resulted an increase in HMF level. The average increase in HMF content was significantly low (86%) in samples liquefied by sonication compared to samples liquefied by heating (129%). Ultrasonic energy negatively affected the diastase activity of samples. The average decrease of diastase activity was 16% after ultrasonic treatment and 23% after heat treatment (ultrasonic waves at 23kHz, water bath heating; 60°C for 30 minutes). Factors other than sonication or heat and typical behavior of individual samples could also affect diastase activity (Thrasyvoulou et al., 1994). Moisture content, electrical conductivity and pH were not significantly affected by ultrasonic treatments. The ultrasonic and heat treated samples were stored at 25 ± 4°C and there was no significant difference in their recrystallization time. The ultrasound treated samples remained in the liquefied state for 344 ± 39 days and heat treated samples for 282 ± 86 days (Thrasyvoulou et al., 1994).

1.2.2.3 Ultrasound Equipment and Operation

Most devices used for the generation of ultrasound are based on electroacoustic systems. Piezoelectric transducers are mostly used in these systems. The equipment required to convey ultrasound energy to a liquid system consists of

following three parts: (i) a generator to convert mains electricity into high frequency alternating current to drive the transducer assembly; (ii) a transducer element that converts the high frequency alternating current into mechanical vibrations; (iii) a delivery system that conveys the vibration to the liquid.

The first step in the use of high-power ultrasound is to derive a method for generating acoustic energy. A transducer is the device used to convert mechanical or electrical energy into sound energy. Three main types of transducers namely, liquid driven transducers, magnetostrictive transducers, and piezoelectric transducers are available. Liquid driven transducers consist of a liquid whistle, where a liquid is forced across a thin metal blade causing it to vibrate at ultrasound frequencies (Leadley & Williams, 2002; Mason, 1998; Mason & Lorimer, 1988). The rapidly alternating pressure and the effect of cavitation generates a high degree of mixing in the liquids. As it involves pumping a liquid, processing applications are limited to mixing and homogenisation. Magnetostrictive transducers are devices that use the magnetostriction effect of some ferromagnetic materials such as iron or nickel. Magnetostriction is the change of dimension of the materials on the application of magnetic field. A magnetostrictive transducer is in the form of a rod acting as the magnetic core within a solenoid. The core is an assembly of layers of thin nickel plates forming a closed square loop and coils wound around two opposite sides of it. The application of current to the coil results in a reduction of the dimensions of the core, and a reduction in the dimensions of the transducer thereafter. The maximum frequency generated by these transducers is limited to 100 kHz, while the energy efficiency is about 60%. However, these transducers can withstand long exposure to high temperatures. Piezoelectric transducers are the most commonly used transducers for the generation of ultrasound. The

shape and dimensions of a piezoelectric transducer is dependent on its working frequency. A 20 kHz transducer has twice the length of 40 kHz transducer. The transducer is attached to the upper fixed horn to connect it to the delivery system. Further, the tip of the horn can become eroded, with the overall horn length being reduced after prolonged use. Hence, replaceable screw-threaded tips are made to fix at the end of the horn. The availability of power through a transducer is inversely proportional to the square of frequency. Therefore, lower frequencies are selected for high power applications (Leadley & Williams, 2002; Mason, 1998).

1.3 Objectives of the Study

The study consists of four parts;

- Apply HHP to crystallised honey samples;
- Apply ultrasound to crystallised honey samples;
- Apply thermal heating to crystallised honey samples;
- Make physical, chemical and microbiological analysis to compare minimal processing techniques with traditional heat treatment and to choose the best HHP and ultrasound conditions individually as measured with quality parameters such as HMF formation, diastase number, color and viscosity in selected (sunflower, cotton and canola) honey varieties.

The objective of the first, second and third part of this study was to evaluate the effect of ultrasound (24 kHz) (time-temperature combinations) and HHP (pressure 220-330 MPa, temperature 50-60°C and time) on complete liquefaction

time of crystals in honey and to compare the required liquefaction time with heat treated honeys.

The objective of the fourth part was to compare ultrasound and HHP treated honey samples' physical and chemical characteristics with heat treated and fresh honey characteristics and to choose best HHP and ultrasound conditions.

The main goal is to understand whether HHP and ultrasound technology can be used as an alternative to traditional heat treatment for the liquefaction of crystals and for retention of physical and chemical properties of different honey varieties.

CHAPTER 2

MATERIALS AND METHODS

2.1 Samples

Wholly crystallized and untreated honey samples (sunflower, cotton, canola) were obtained from Balparmak Honey Company (Ümraniye, İstanbul, Turkey). In order to eliminate any possible honey fake, related analysis (which is required to understand the honey is natural or not), were completed in the company. The honey samples were kept at room temperature (up to 2 weeks at most) in Food Engineering Department-METU, until used for the experiments. Throughout the HHP, ultrasound and thermal treatments, samples from the same main stock were used so as to avoid the possible mistakes that can occur due to differing initial conditions. The properties of honey samples were given in table 2.1. These informations were given by the company. Initial HMF level and diastase numbers were also analyzed.

Table 2.1 Properties of honey samples

Honey Samples	**Region**	**HMF (ppm)**	**Diastase number**	**Moisture (%)**	**Fructose/Glucose ratio**
Sunflower	Thrace	2,30	13,9	18,1	1,12
Cotton	Urfa	1,10	13,9	17,9	1,18
Canola	Thrace	0,90	13,9	19,7	1,01

2.2 Sample Preparation

For Ultrasound treatment, about 150 g of crystalised honey was weighed in a 150 ml glass beaker. For HHP treatment 4 ml cryovials (Simport Plastic, Canada) were fully filled with honey samples.

2.3 Treatments

The samples were treated with HHP, ultrasound and heat in a water bath. Treated samples were stored in deep-freeze for HMF, diastase number and color analysis, stored at room temperature for viscosity analysis. The analysis duration did not exceed two weeks.

2.3.1 HHP application

HHP equipment in the Middle East Technical University Non-Thermal Food Processing Laboratory with the capacity of 30 cm^3 and maximum pressure level of 330 MPa was used for the pressure treatments (Fig. 2.1).

Increase and release times of pressure were detected approximately as 5 and 10 seconds for the designed system, respectively. Motor oil was used as the pressure transmitting medium. The equipment consists of 4 main parts:

- Pressure chamber,
- Pressure pump,
- Hydraulic unit,
- Temperature control device.

Pressure chamber is a cylindrical vessel, equipped with two end closures for loading and unloading and a means for restraining the end closures. The vessel

is made up of hot galvanized carbon steel. Before the HHP treatment, the vessel is filled with the pressure transmitting fluid where the samples were exposed to high pressure and the air is retained out of the vessel. The pressure pump controls the hard chrome plated piston, polished to mirror finish (steel type heat treated special K). Hydrostatic pressure is supplied directly by the compression of the pressure transmitting fluid via piston. The hydraulic unit is for the generation of the high pressure by compression. For sustaining constant temperature throughout the treatment, a temperature control device is connected to the equipment. The pressure transmittance fluid within the vessel was heated prior to pressurization to the desired temperature by an electrical heating system surrounding the chamber. Pressurization time reported in this study did not include the pressure increase and release times.

Figure 2.1 HHP unit

2.3.2 Ultrasound Application

Ultrasound equipment (Dr Hielscher UP400S ultrasound processor) in the Middle East Technical University Non-Thermal Food Processing Laboratory used for this study has a frequency of 24 kHz, 400 W power, amplitude adjustment 20% to 100% and is designed for both batch and continuous application to process fluid or solid media on a laboratory scale.

Figure 2.2 Ultrasound unit

The processor has an effective output power of 400 W in liquid media. The efficiency of the processor is more than 85%. Ultrasonic waves generated by the processor are transmitted to the media by sonotrodes that emit from the front face. Sonotrodes are composed of titanium alloy and screwed to the electroacoustic transducer of the processor. There were two sonotrodes with diameters of 7 and 40 mm available for testing. These sonotrodes have different maximum amplitudes, maximum immerse depths and maximum sonic power densities (Table 2.2).

Table 2.2 Technical details of ultrasound sonotrodes

Sonotrode diameter (mm)	**Maximum amplitude (μm)**	**Maximum immerse depth (mm)**	**Maximum sonic power density (W/cm^2)**
7	175	90	300
40	12	20	12

2.3.3 Thermal Treatment

Temperature controlled waterbath equipment (Wisecircu, WCB-6, Germany) in the Middle East Technical University Non-Thermal Food Processing Laboratory was used for this study.

2.3.4 Experimental Design

2.3.4.1 HHP Treatment

Sunflower honey samples were pressurized at 220 and 330 MPa at 50 and 60°C for required time for complete liquefaction of crystals. The pressure treatments employed in this study were chosen according to maximum pressure capacity of the equipment. The temperatures were selected according to thermal liquefaction temperatures used in industry. The samples were dispensed in 4 ml portions in cryovials, avoiding as much air as possible and placed inside the pressurization chamber for the HHP application. The chamber was fully filled with preheated motor oil according to the temperature studied. Untreated samples were used as controls.

HMF (Hydroxy methyl furfural) in ppm was detected for each process condition in order to determine a best combination (Table 2.3).

Table 2.3 HHP treatments

Parameters Studied	HHP Treatment	
	220 MPa	
	50°C-106 min	60°C-23 min
HMF	+	+
	330 MPa	
	50°C-106 min	60°C-23 min
HMF	+	+

In order to compare the effect of pressure on HMF formation, the same liquefaction times were used for HHP applications and thermal treatment. The complete liquefaction of crystals was not expected for these thermal treatments (50°C-106 min and 60°C-23 min). HMF values were measured for these samples also.

2.3.4.2 Ultrasound Treatment

For sunflower honey samples, 7 and 40 mm diameter ultrasonic sonotrodes were used at 20, 40, 60, 80 and 100% of maximum amplitudes of 175 and 12 μm and cycle 1 (continuous) and cycle 0,5 (batch) for this experiment. For cotton and canola honey samples the 7 mm diameter ultrasonic sonotrode was used at 80% and 100% of maximum amplitudes of 175μm and cycle 0,5 (batch). The samples were weighed about 150 g in 150 ml glass beakers.

According to Table 2.4, HMF (Hydroxy methyl furfural) in ppm, diastase number, viscosity (Pa.s) and color values (Hunter L*a*b color scale) were detected for each process condition in order to determine a best combination.

Table 2.4 Ultrasound Treatments

			Cycle 100% (continuous)				Cycle 50% (batch)			
Sample	**Amplitude**	**Sonotrode (mm)**	**HMF**	**Diastase number**	**Viscosity**	**Color**	**HMF**	**Diastase number**	**Viscosity**	**Color**
Sunflower	100%	7	+	+	+	+	+	+	+	+
		40	+	+	+	+	+	+	+	+
	80%	7	+	+	+	+	+	+	+	+
		40	+	+	+	+	+	+	+	+
	60%	7	+	+	+	+	+	+	+	+
		40	+	+	+	+	+	+	+	+
	40%	7	+	+	+	+	+	+	+	+
		40	+	+	+	+	+	+	+	+
	20%	7	+	+	+	+	+	+	+	+
		40	+	+	+	+	+	+	+	+
Cotton	100%	7	-	-	-	-	+	+	+	+
	80%	7	-	-	-	-	+	+	+	+
Canola	100%	7	-	-	-	-	+	+	+	+
	80%	7	-	-	-	-	+	+	+	+

(+) indicates performed analysis

2.3.4.3 Thermal Treatment

To compare with HHP treatment, 50 and 60°C thermal treatments were applied to honey samples until complete dissolution of crystals. To compare with ultrasound treatment, 60°C was applied. According to table 2.5, HMF (Hydroxy methyl furfural) in ppm, diastase number, viscosity (Pa.s) and color values (Hunter L* a* b color scale) were detected for each process condition to compare with HHP and ultrasound treated samples.

Table 2.5 Thermal treatments

		As HHP referance				**As ultrasound referance**			
Sample	**Temp. (°C)**	**HMF**	**Diastase Number**	**Viscosity**	**Color**	**HMF**	**Diastase number**	**Viscosity**	**Color**
Sunflower	50	+	-	-	-	-	-	-	-
	60	+	-	-	-	+	+	+	+
Cotton	50	-	-	-	-	-	-	-	-
	60	-	-	-	-	+	+	+	+
Canola	50	-	-	-	-	-	-	-	-
	60	-	-	-	-	+	+	+	+

(+) indicates performed analysis

2.3.4.4 Storage

Treated samples were stored at deep-freeze (-18°C) for HMF, diastase number and color analysis. They were kept at room temperature (about 25°C) for viscosity analysis. All the samples were analyzed within two weeks.

2.4 Physical, Chemical and Microbiological Analysis

The physical and chemical analysis were encountered by taking five quality parameters into consideration. Those were HMF (Hydroxy methyl furfural)

content, diastase number (DN), rheologic behaviour (viscosity), color (Hunter L*a* b) values and microbial count of the untreated and treated honey samples. From the samples collected, duplicate measurements were performed and average results are presented.

2.4.1 Determination of HMF content

The International Honey Commission (IHC, Stefan Bogdanov, 2002) recommends three methods for the determination of HMF. These methods include two spectrophotometric methods which are Bisulfite White and Winkler, and a chromatographic method, HPLC.

According to Zappala et al. (2005) HPLC method seems to be the more appropriate for HMF determination in honey, because the presence of substances, probably derived by heat or storage damage, which interfere with the UV methods did not reveal. Therefore the experiment was performed by HPLC.

2.4.1.1 Scope

The method can be applied to all honey samples.

2.4.1.2 Definition

The method determines the concentration of Hydroxymethylfurfural (HMF). The result is expressed in ppm.

2.4.1.3 Procedure

Preparation of samples

The honey samples were diluted with distilled water 1:10 (w/w).

Determination

The sample was injected onto an Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a quaternary pump, an autosampler, a diode array

detector and a temperature-controlled column oven. The chromatographic separations were performed on an Atlantis dC18 column, using the isocratic mixture of 0.1% aqueous acetic acid solution and acetonitrile (90:10, v/v) at a flow rate of 1.0 ml/min at 40°C. Data acquisition was performed, acquiring chromatograms at the detection wavelength of 285 nm.

Stock solution of HMF was prepared at a concentration of 1.0 mg/ml in distilled water. Working standards were prepared daily by diluting the stock solution to concentrations of 0.05, 0.10, 0.25, 0.50 and 1.0 g/ml with distilled water.

2.4.1.4 Calculation and Expression of Results

The HMF content of the samples was calculated by comparing the corresponding peak areas of the sample and those of the standard solutions, taking into account the dilution. There is a linear relationship between the concentration and the area of the HMF peak. Results are expressed in ppm.

2.4.2 Determination of Diastase Number

This experiment was performed by method described in Turkish Standards (TS 3036, Honey Standard).

2.4.2.1 Definition

The diastase activity is expressed as ml of 1% starch hydrolyzed by the enzyme in 1 g of honey in 1 h, called the diastase number (Küçük et al., 2007).

2.4.2.2 Principles

Starch solution was mixed with honey solution and then put in waterbath at a constant temperature of 48°C. By the action of diastase enzyme in honey, starch hydrolysis occurs. After starch hydrolysis, retained starch content gives blue color complex with iodine solution. By visual observation of blue color

formation, starch solution volume which can completely hydrolyse 1 grams of honey was calculated.

2.4.2.3 Reagents

- Iodine solution, 0,1 N. (Merck, Germany)
- Phosphate / Citrate buffer (Citric acid monohydrate solution ($C_6H_8O_7.H_2O$) and disodium hydrogen phosphate dihydrate solution ($Na_2HPO_4.2H_2O$) (Merck, Germany)
- Hydrochloric acid solution, 0,5 N. (Merck, Germany)
- Sodium Hydroxide solution, 0,5 N. (Merck, Germany)
- Sodium Chloride solution, 0,1 N. (Merck, Germany)
- Starch solution, 1%. (Merck, Germany)
- Starch solution and buffer solution mixture

2.4.2.4 Procedure

- 10 g of sample is weighed.
- Dissolved in approximately 40-50 ml distilled water.
- Compeleted to 100 ml in volumetric flusk.
- Twelve different volumes of honey solution, distilled water and starch-buffer solution added to test tubes according to Table 2.6. Each tube volume must be 18 ml.

Table 2.6 Volumes of honey solution and reagents in diastase number analyse.

Sample No	Honey solution (mL)	Distilled water (mL)	Starch – Buffer solution	Total volume (mL)	Diastase number
1	10,0	5,33	2,67	18,0	1,0
2	10,0	3,3	4,7	18,0	2,5
3	10,0	0	8,0	18,0	5,0
4	7,7	2,3	8,0	18,0	6,5
5	6,0	4,0	8,0	18,0	8,3
6	4,6	5,4	8,0	18,0	10,9
7	3,6	6,6	8,0	18,0	13,9
8	2,8	7,2	8,0	18,0	17,9

Table 2.6 (continued)

9	2,1	7,9	8,0	18,0	23,0
10	1,7	8,3	8,0	18,0	29,4
11	1,3	8,7	8,0	18,0	38,5
12	1,0	9,0	8,0	18,0	50,0

- After 1 hour thermal treatment, test tubes are put in ice, and cooled.
- By adding one drop of 0,1 N iodine solution to each tube, blue color formation is observed.
- Diastase number is determined according to Table 2.6.

2.4.3 Determination of viscosity

This experiment was performed by using TA Instruments AR2000 ex Rheometer. Viscosity profiles of honey samples were obtained versus shear rate (1/s). Viscosity values were measured in Pa.s.

2.4.3.1 Equipment

The rheometer design includes;

- Ultra low inertia drug cup motor
- porous carbon air bearings
- direct strain
- controlled rate performance

The equipment is appropriate for a wide variaty of applications including characterization of delicate structures in fluids of any viscosity, polymer melts, solids and reactive materials.

2.4.4 Determination of color

This experiment was performed by using AVANTES, AvaSpec-2048 model spectrophotometer.

2.4.4.1 Equipment

Color measurements of objects and thick fluids can be done in different setups, e.g. using reflection probes or an integrating sphere. For the different applications different probes can be used. Probe for solid and semi-solid materials was used in measurement. The wavelength range of spectrophotometer was 200-1100 nm.

2.4.5 Determination of moisture and sugar concentration

This experiment was performed by using Abbe refractometer.

2.4.6 Determination of microorganisms

This experiment was performed in microbiological analysis laboratory in Balparmak Honey Company.

2.4.6.1 Procedure

10 g of sample is weighed in sterile conditions. 90 ml buffer peptone water is added and mixed with sample for 15-20 minutes. This solution is 10^{-1} stock solution. 1 ml is taken from this stock solution and inoculated to 3M Petrifilm (dry rehydratable film). The petrifilm is incubated at 35 ± 1 ^{o}C for 48 ± 2 hours for total aerobic bacteria, 35 ± 1 ^{o}C for 24± 2 hours for total coliform, 35 ± 1 ^{o}C for 48±2 hours, 20-25^{o}C for 3-5 days for yeasts and moulds. After incubation, the colonies are counted. The results are expressed in cfu/g.

2.4.7 Statistical Analysis

The results of the study were analyzed by Analysis of Variance (ANOVA). The data evaluated for the HMF level of the HHP treated sunflower honey and for the HMF level, diastase number and color values of the ultrasound treated

honey samples were analyzed with one-way ANOVA with a probability limit of $p<0.05$. Throughout the analysis, differences at $p<0.05$ were considered as significant. Throughout the statistical analysis, Microsoft Excel 2003 and SPSS 10.0 for Windows were used. Experiments and measurements were duplicated on separate days, in order to justify the data obtained and averages are reported.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Chemical Analysis in HHP treated samples

Chemical analysis was based on the Hydroxy metyhyl furfural (HMF) content. Before the analysis, the initial HMF contents of untreated honey samples were determined.

The effect of HHP treatment on the honey samples analyzed is presented in figure 3.1. The calculations and the relevant statistical analysis are given in the Appendix A.

3.1.1 Hydroxy methyl furfural (HMF) Analysis in HHP treated sunflower honey samples

In the first part of the study, the effect of HHP on HMF content of sunflower honey was studied and was measured as 2,30 ppm. The results of HMF content after HHP treatment at 220 and 330 MPa and thermal treatment at 50 ^{0}C and 60°C are presented in figures 3.1 and 3.2. To compare the effect of pressure on HMF formation, the same liquefaction times were used for HHP applications and thermal treatment.

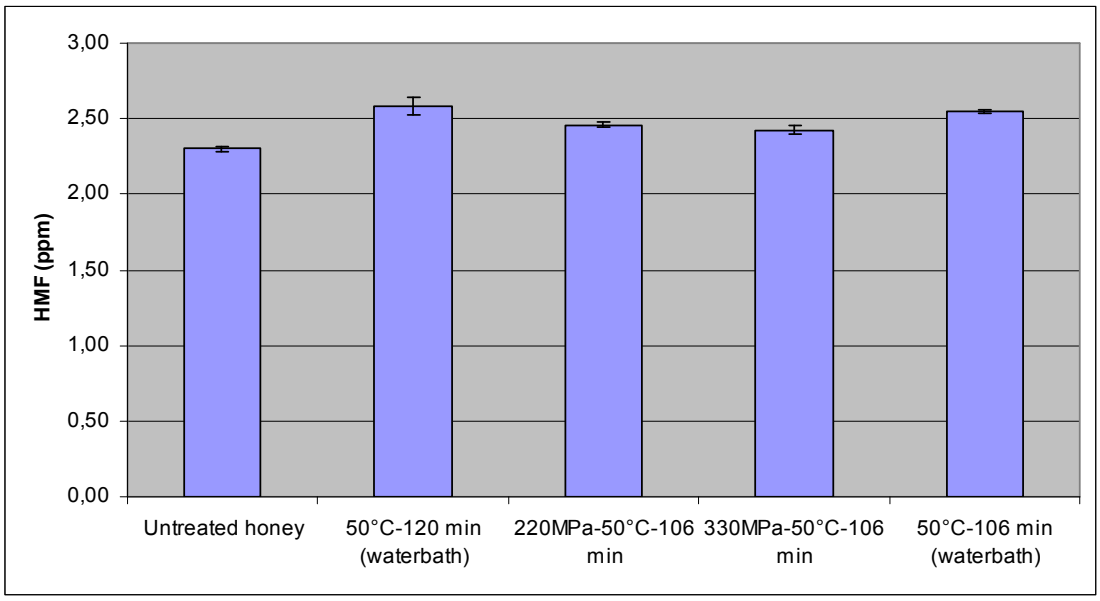

Figure 3.1 HMF content (ppm) for sunflower honey treated at 220 and 330 MPa at 50°C for 106 minutes and 50°C thermal treatment for 120 and 106 minutes. The error bars indicate the standard deviations of the measurements.

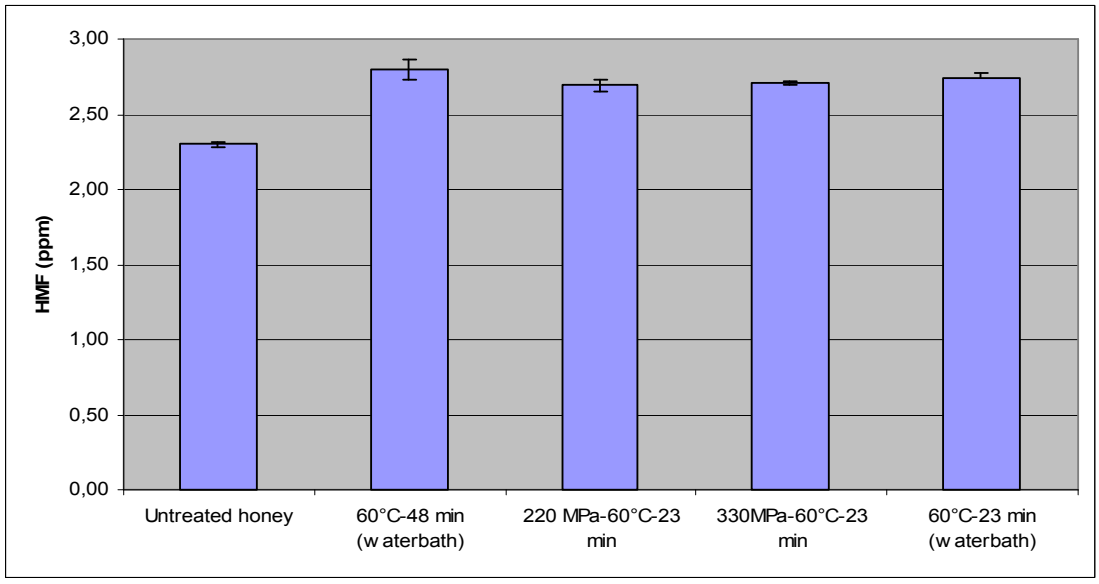

Figure 3.2 HMF content (ppm) for sunflower honey treated at 220 and 330 MPa at 60°C for 23 minutes and 60°C thermal treatment for 48 and 23 minutes. The error bars indicate the standard deviations of the measurements.

In the HHP applications of 220 and 330 MPa pressure at 50°C, crystals dissolved in 106 minutes. In 50°C thermal treatment, time to dissolve crystals was 120 minutes. When HHP was applied at 60°C, crystals dissolved in 23 minutes. In 60°C thermal treatment by itself, time to dissolve crystals was 48 minutes. Pressure increase has no significant effect on liquefaction time ($p>0.05$). On the other hand, when compared with thermal treatment, the liquefaction times are shorter in HHP treatment at corresponding temperatures. As a result, the processing time to liquefy crystals decrease with pressure application in addition to temperature.

As seen from figure 3.1 and 3.2, HMF levels of thermal treated samples were higher than HHP treated samples at the same temperatures. The HMF content of thermal treated sample at 50°C was 2,59 ppm, the values of HHP treated samples at 220 and 330 MPa (at 50°C) were 2,46 ppm and 2,42 ppm, respectively. The HMF level increase was 12,6% in thermal treatment, whereas it was 6,96% and 5,21% in 220 and 330 MPa treated samples, respectively.

The HMF level of thermal treated sample at 60°C was 2,80 ppm, the values of HHP treated samples at 220 and 330 MPa (at 60°C) were 2,69 ppm and 2,71 ppm. The HMF level increase was 21,74% in thermal treatment, whereas it was 16,96% and 17,83% in 220 and 330 MPa treated samples, respectively.

The HMF level at 50°C after 106 minutes was 2,55 ppm, the HMF level at 60°C after 23 minutes was 2,74 ppm. HMF level increase was 10,87% in 50°C, 106 minutes thermal treatment and 19,13% in 60°C, 23 minutes thermal treatment. The results stated that, the heat treated samples' HMF values are higher than that of HHP treated samples all compared with control.

The reason of obtaining higher HMF at 60°C treatments than 50°C treatments was due to increase of HMF content of honey due to heat (D'Arcy, 2007). As applied temperature increases, the HMF formation accelerates. Increasing pressure has no significant effect on HMF formation ($p>0.05$). It was observed that, pressure had a positive effect on liquefaction time of honey and a negative effect on HMF formation of sunflower honey compared to heat alone ($p<0.05$). Probably, pressure has an effect on Maillard reaction. The findings of Komthong et al. (2003) also support this hypothesis. They prepared model solutions with fructose and glucose, and investigated the effect of HHP (at 100 MPa) combined with pH (6.0, 7.0, and 8.0) and temperature (80 and 90°C) on Maillard reaction. It was stated that, Maillard reaction was suppressed under HHP, but accelerated by high pH value and high temperature. HMF content was also enhanced with the increase in temperature, but inversely, decreased with high pressure treatment.

3.1.2 Summary of the effect of HHP treatment on HMF formation

Formation of HMF decreased with HHP treatment for the pressure levels applied in this study compared to heat treatment. Both pressure levels seem to be appropriate for the treatment when based on HMF formation. The HMF levels and processing times for decrystallization were not affected by pressure level. Processing times were affected mainly by temperature. Therefore selecting lower pressure levels would be cost effective.

Lower application temperatures resulted with lower HMF formation. HHP at 50°C resulted even lower HMF values, than 60°C HHP applications. HMF content increased with the increase in temperature, but decreased with HHP treatment.

According to the results obtained, 220MPa/50°C/106 minute combination can be suggested as the best combination.

3.2 Chemical and Physical Analysis of Ultrasound Treated Sunflower Honey

In the second part of the study, best combination of ultrasound application was evaluated based on the chemical and physical analysis of sunflower honey. According to the selected combination, canola and cotton honeys were also analysed.

Chemical analysis was based on the HMF content and diastase number. Physical analysis was based on color, viscosity and moisture. Before the analysis, the initial HMF content, diastase number and initial temperature of samples were measured. During ultrasound application, honey samples were stirred in certian periods and temperature was measured.

The temperature profiles were analyzed in section 3.2.1. The results of the effect of ultrasound treatment applied to different honey samples were analyzed in sections 3.2.2, 3.2.3, 3.2.4 and 3.2.5. The best combination was given in section 3.2.6. The calculations and the statistical analysis were given in Appendix A.

3.2.1 Temperature Profiles and Application Times of Ultrasound and Thermal Treated Sunflower Honey

The initial temperatures of untreated samples were measured and ultrasound treatment was applied with selected combinations (with 7 and 40 mm diameter ultrasonic sonotrodes at 20, 40, 60, 80 and 100% of maximum amplitudes of 175 and 12 μm and cycle 1 (continuous) and cycle 0,5 (batch). The application times were recorded when complete liquefaction was achieved. The liquefaction times and final temperatures were given in Table 3.1.

Table 3.1 Liquefaction times and final temperatures of ultrasound and thermal treated sunflower honey samples.

Sample	Final Temp.(°C)	Liquefaction time (min)
100% amp.-7 mm probe-Cycle 1	88	5
80% amp.-7 mm probe-Cycle 1	84	6
60% amp.-7 mm probe-Cycle 1	83	8
40% amp.-7 mm probe-Cycle 1	79	12
20% amp.-7 mm probe-Cycle 1	78	18
100% amp.-7 mm probe-Cycle 0,5	80	12
80% amp.-7 mm probe-Cycle 0,5	84	18
60% amp.-7 mm probe-Cycle 0,5	78	22
40% amp.-7 mm probe-Cycle 0,5	73	30
20% amp.-7 mm probe-Cycle 0,5	72	42
100% amp.-40 mm probe-Cycle 1	70	10
80% amp.-40 mm probe-Cycle 1	67	12
60% amp.-40 mm probe-Cycle 1	61	16
40% amp.-40 mm probe-Cycle 1	56	24
20% amp.-40 mm probe-Cycle 1	56	44
100% amp.-40 mm probe-Cycle 0,5	62	26
80% amp.-40 mm probe-Cycle 0,5	64	45
60% amp.-40 mm probe-Cycle 0,5	62	56
40% amp.-40mm probe-Cycle 0,5	60	88
20% amp.-40 mm probe-Cycle 0,5	58	105
60°C thermal treatment	59	100

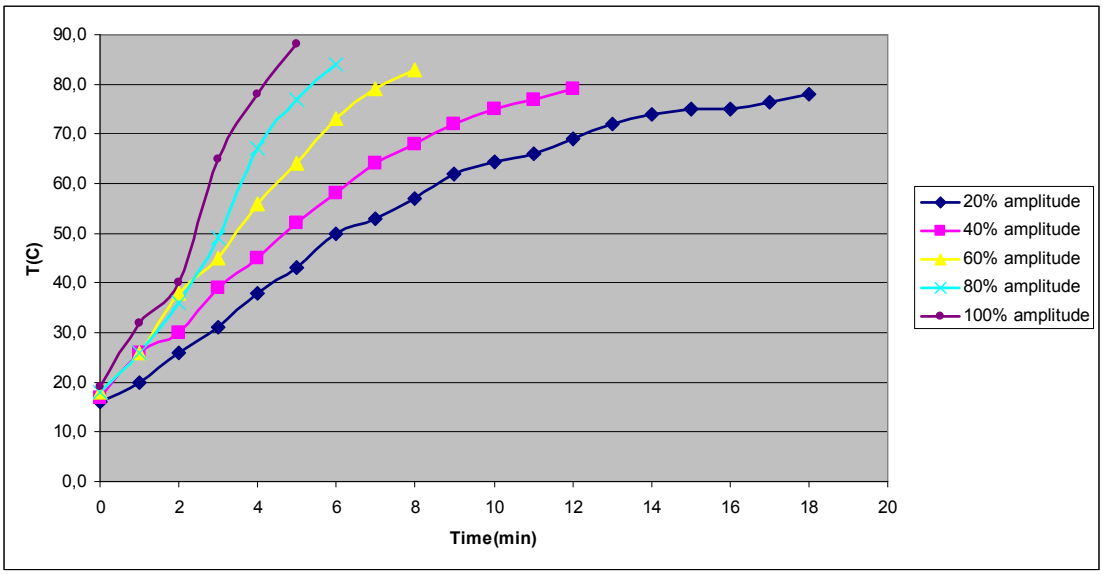

Figure 3.3 Temperature profile of ultrasound treated sunflower honey (7 mm probe, cycle 1).

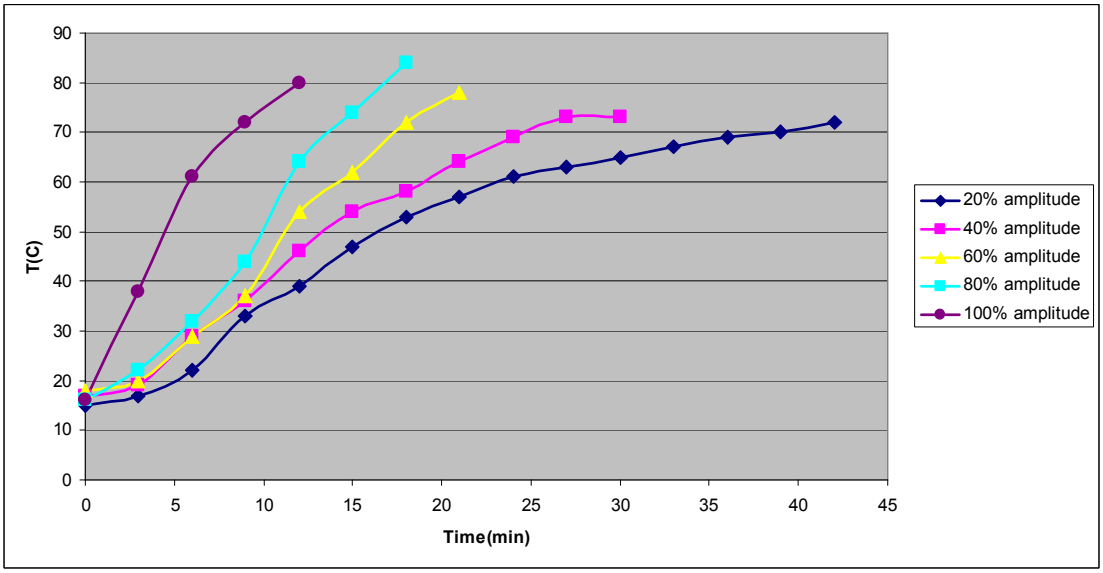

Figure 3.4 Temperature profile of ultrasound treated sunflower honey (7 mm probe, cycle 0,5).

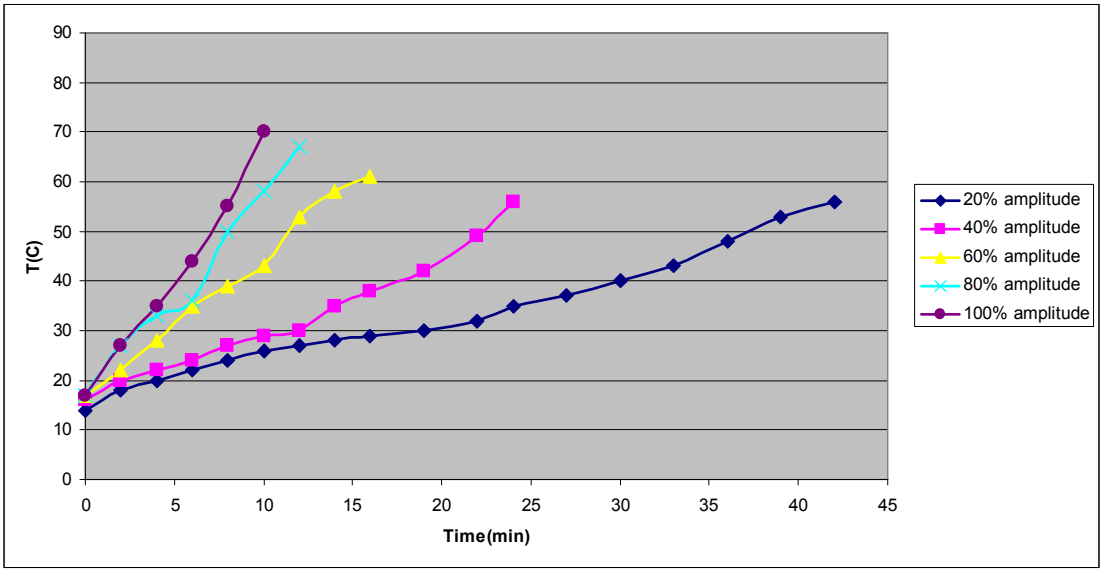

Figure 3.5 Temperature profile of ultrasound treated sunflower honey (40 mm probe, cycle 1).

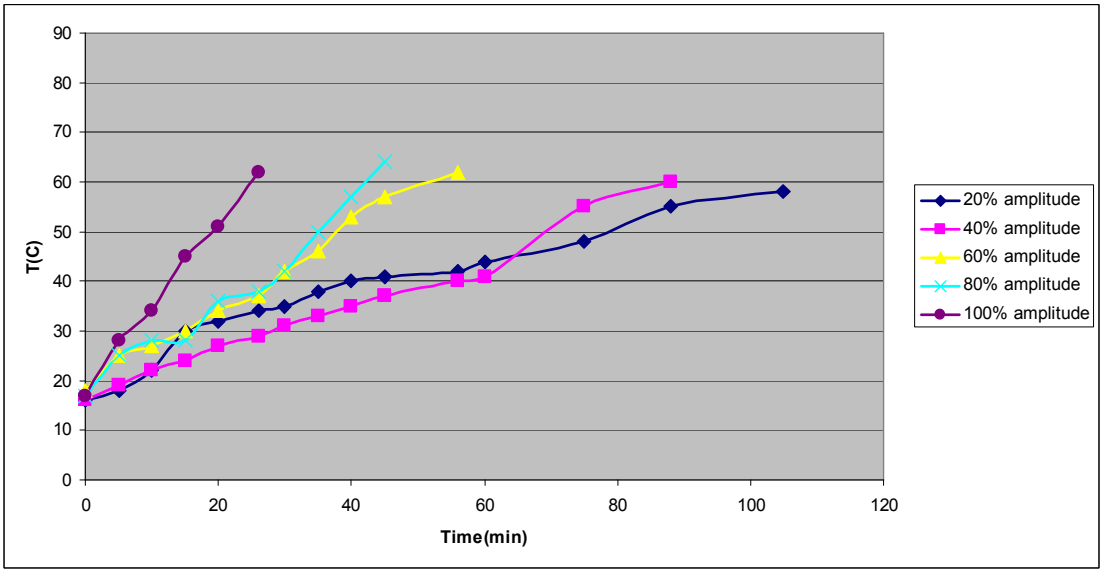

Figure 3.6 Temperature profile of ultrasound treated sunflower honey (40 mm probe, cycle 0,5).

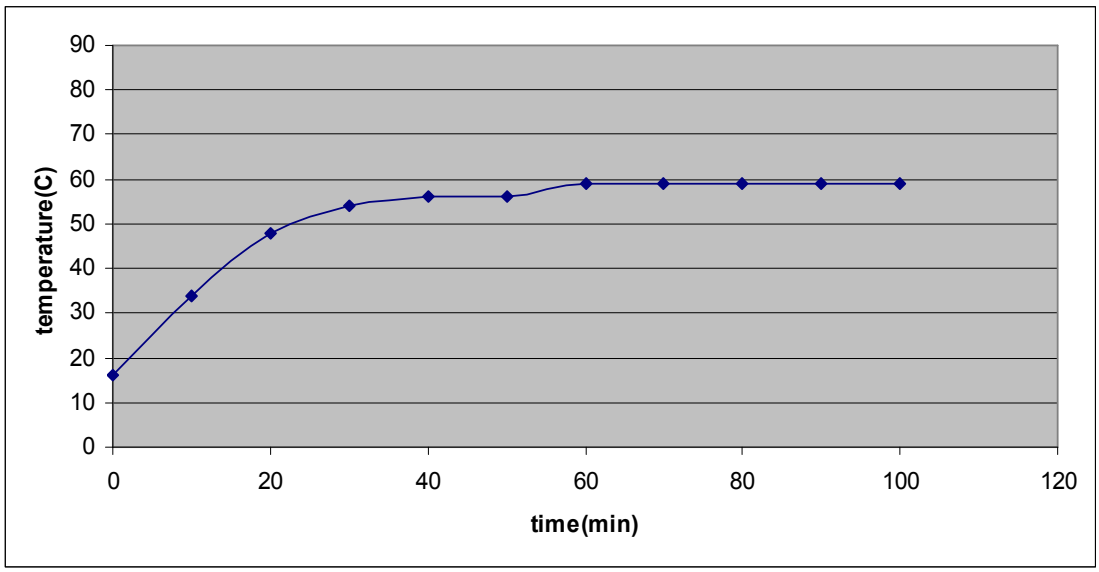

Figure 3.7 Temperature profile of heat treated sunflower honey (60°C).

As shown in Figure 3.3 the shortest liquefaction time (5 minutes) and the highest final temperature (88°C) was obtained at 100% amplitude-7 mm probe-1 cycle combination. The reason for that was the maximum power given with this combination.

The longer application times were obtained in 20% amplitude, 40 mm probe, 0,5 cycle combination and in 60°C thermal application. The reason for long time in ultrasound was the lowest power given among ultrasound applications.

According to statistical analysis of ultrasound treated samples, probe size and cycle have significant effect on liquefaction time ($p<0.05$), amplitude has no significant effect ($p>0.05$). Probe size has a significant effect on final temperature of honey ($p<0.05$), amplitude and cycle have no significant effect ($p>0.05$).

Our results showed that, with ultrasound treatment, crystals were dissolved quicker than thermal treatment (except 20% amplitude, 40 mm probe, 0,5 cycle combination).

3.2.2 Hydroxy methyl furfural (HMF) Analysis in Ultrasound treated sunflower honey

The effect of ultrasound on HMF content of sunflower honey until the complete liquefaction of crystals was measured. The initial HMF content of sunflower honey was measured as 2,37 ppm. The results of HMF content after ultrasound treatment for selected conditions and thermal treatment are represented in figure 3.8.

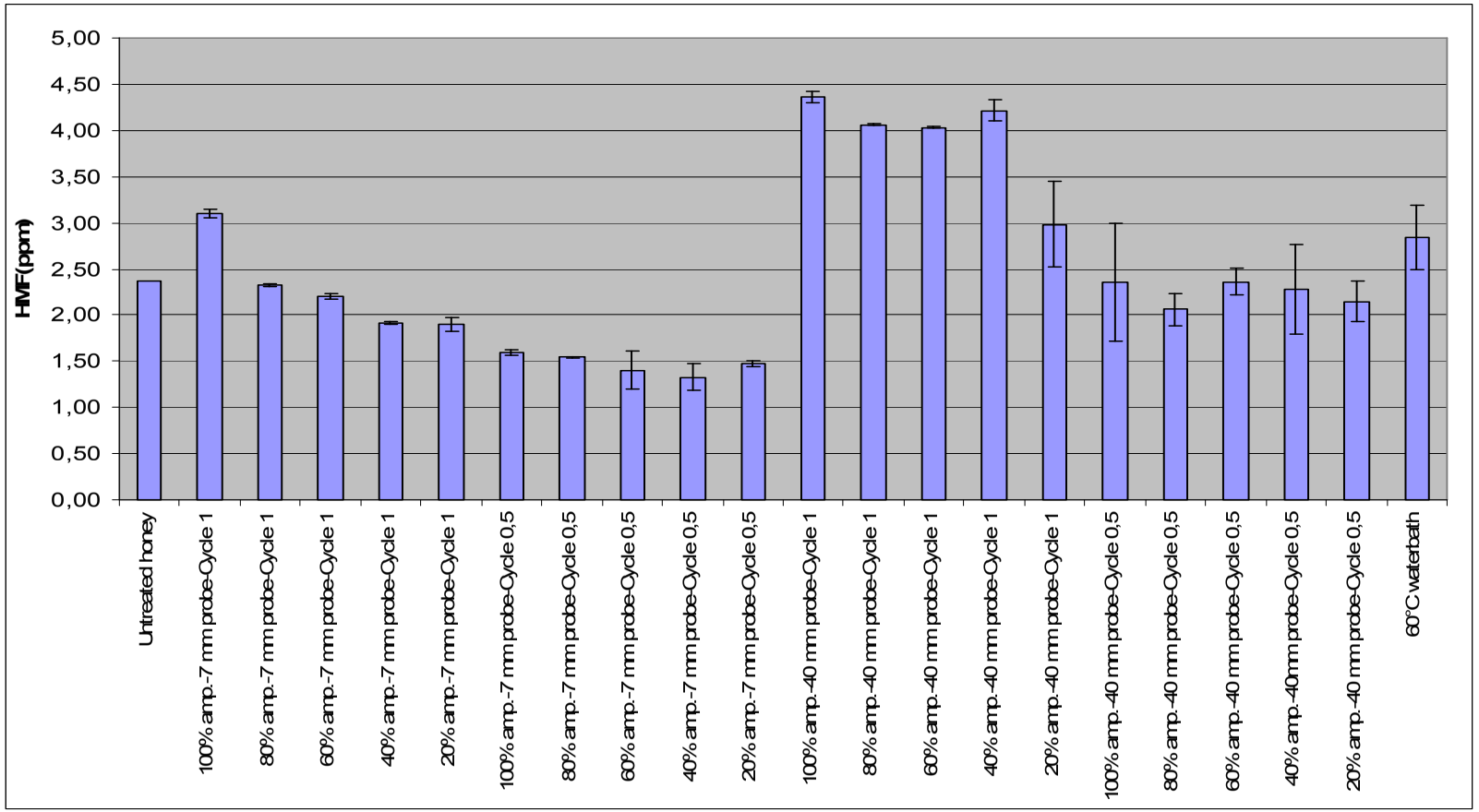

Figure 3.8 HMF levels (ppm) of ultrasound and thermal treated sunflower honey samples. The error bars indicate the standard deviations of the measurements.

The results showed that the lowest HMF values were measured with 7 mm probe- 0,5 cycle combinations. The HMF levels were also lower than untreated sample in 7 mm probe – 0,5 cycle combinations. (1,60 ppm in 100% amplitude, 1,55 ppm in 80% amplitude, 1,40 ppm in 60% amplitude, 1,32 ppm in 40% amplitude, 1,48 ppm in 20% amplitude). Ultrasound waves have an effect on Maillard reaction.

Vercet et al., (2001) studied manothermosonication (MTS) (117 μm amplitude - 20 kHz frequency - 92°C , 102°C and 111°C) in milk and orange juice resembling systems and analysed the free and bound HMF produced as a result of Maillard reaction. It was reported that free HMF production by heat (92°C, 102°C and 111°C) was faster than by MTS. These rate differences could occur for very different reasons. Ultrasound could affect the free HMF formation or destruction rate or even both. The temperature dependence of both reactions could be also very different, which together with the well-known fact that ultrasound intensity diminished at higher temperatures, would explain the different free HMF formation rates by heat and MTS treatments at different temperatures. MTS application changed the behavior of nonenzymatic browning. No formation of free HMF was detected in fruit juice model systems after MTS treatment. For bound HMF the production rate was lower by MTS than by heat treatment. Bound HMF was the direct HMF involved in reactions with proteins. Bound HMF levels were lower after MTS treatments under all of the experimental conditions tested. This could be related to the well-known effect of MTS on proteins. Enzyme inactivation or protein degradation could diminish the availability of lysine groups of protein to react with glucose, reducing in this way bound HMF. Ultrasound could i) promote reactions of sugars with other compounds, reducing also their availability to react with

proteins; ii) promote reactions of sugars and proteins without any bound HMF formation; iii) destroy bound HMF after it had been formed.

In a further work, Yong et al.,(2009) studied effect of Ultrasound on glycin-glucose solution and measured absorbance at 294 nm wavelength to understand the effect of ultrasound on formation of intermediate reaction products in Maillard reaction. The A_{294} of the glycin–glucose solution after being treated by the ultrasonic intensity at 17.83 W/cm^2 showed a significant increase within the first 30 min. At the ultrasonic intensity of 15.29 W/cm^2, the A_{294} of Maillard reaction products increased from approximately 0 to 1,38, 2,39, and 2,39 as the treatment time increased from 30, 40 to 50 min, respectively, and at 17.83 W/cm^2, the A_{294} increased from approximately 0 to 2,38, 2,39, and 2,40 at 30, 40, and 50 min, respectively. On the other hand, at lower ultrasonic intensities (i.e., 10.19 and 12.74 W/cm^2), the A_{294} changes were not significant. The results suggested that Maillard reaction products were produced to a great extent at higher ultrasonic intensities (i.e., 15.29 and 17.83 W/cm^2). Also with extended reaction time, some intermediate products might polymerize resulting in only a small amount of intermediate products. For example, little change of the A_{294} was observed when the treatment time was longer than 30 and 40 min at 15.29 and 17.83 W/cm^2, respectively and at 50 min, the A_{294} became the same. It appeared that when the treatment time was longer than 30 min (at ultrasonic intensity of 17.83 W/cm^2), some intermediate products turned into new polymers, leaving a reduced amount of the intermediate products. Meanwhile, glycin and glucose continued to react, producing new intermediate products. The rates of formation and polymerization of the intermediate products might become equal. Thus, the change of A_{294} was slight after 30 min treatment at 17.83 W/cm^2 or 40 min at 15.29 W/cm^2. The mechanism and the rate of the Maillard reaction

products formation affected by the ultrasonic intensity need further study according to Yong et al (2009).

The results presented in this study are in aggrement with those reported by Vercet et al. (2001) where ultrasound suppressed Maillard reaction by preventing aminoacid and carbohydrate reaction which was the essential components starting Maillard reactions and could destroy bound HMF after it had been formed. This hypothesis could explain the decrease of HMF in 7 mm probe – 0,5 cycle combinations and lower HMF levels in Ultrasound treated honey samples than thermal treated ones in this study. The effect of ultrasound on Maillard reaction pathway was investigated by Yong et al. (2009) and it was reported that the intermediate products could form other components. Ultrasound waves could promote HMF degradation which was present in fresh honey.

In another study about HMF production rate comparison between Ultrasound and thermal treatment, it was reported that the combined effect of temperature and processing time resulted with an increase in HMF level (Thrasyvoulou *et al.*, 1994). Crystallized honey samples were liquefied by ultrasonic waves at 23 kHz and by heating at 60°C for 30 mins. The average increase in HMF content was significantly low (86%) in samples liquefied by sonication compared to samples liquefied by heating (129%). As a result of this study, the HMF increase in heat treatment was higher than ultrasound treatment in honey.

D'Arcy (2007) stated that, as a small amount of HMF is present in fresh honeys, this amount increases according to the temperature and duration if honey is heated. D'Arcy, (2007) also stated that the HMF concentrations in the honeys treated with ultrasound (40 mm probe–100% amplitude–cycle 1) were significantly lower than the HMF concentrations in the heat-treated honeys (at

70°C temperature). According to the results of this study 40 mm probe-cycle 1 combinations showed higher HMF levels, but our temperature was 60°C (lower than 70°C). HMF formation would be higher with increasing temperature from 60°C to 70°C.

In addition to studies discussed above, Bath and Singh (1999) also investigated HMF change in *Helianthus annuus* and *Eucalyptus lanceolatus* honey by convective (50, 60, 70 and 80°C for 15, 30, 45 and 60 min in oven) and microwave (70, 140, 210 and 280 W power levels for 30, 90, 150, 210 and 270 s) heating. Initial HMF contents of *Helianthus annuus* honey was 4.45 mg/100 g and for *Eucalyptus lanceolatus* honey, it was 1.23 mg/100 g. Heating at 70°C for 60 min resulted in an increase in HMF formation from 4.45 to 7.66 mg/100 g, in *Helianthus annuus* honey and from 1.23 to 3.1 mg/100 g under similar conditions in *Eucalyptus lanceolatus* honey. The study showed that HMF formation varied linearly with temperature and time of heating in both honey types.

Water activity of honey is about 0.6, rendering it a suitable medium for Maillard reaction as the optimum water activity for this reaction is in the range of 0.5–0.8 (Labuza and Baisier 1992).

Increase in HMF level in honey could be the result of hexose dehydration in acidic media in higher temperature processes. Due to the low pH level (3,4 – 6,1) and high sugar concentration of honey (around 85 %), hexose dehydration could be another reason for HMF formation besides Maillard reaction. HMF formed by hexose dehydration especially at pH 5 or lower, or by the Maillard reaction (Fennema, 1996). The composition of honey has a role on the HMF formation kinetics (Singh and Bath, 1997). Glucose and fructose together correspond for 85-95% of honey carbohydrates and their amounts depend on the honey source

(Cavia et al., 2002); subsequently, composition of honey as well as storage conditions affects both crystallization and HMF formation.

Our results were also in aggrement with data reported in literature. For 100% amplitude with 7 mm probe - 1 cycle application, the HMF level was higher than untreated honey (3,10 ppm), mainly due to the highest power intensity applied and accordingly highest temperature reached. Higher temperatures accelerate rate of HMF formation.

The highest HMF levels were obtained with 40 mm probe-1 cycle combinations, (except 20% amplitude). A continuos process could accelerate the HMF formation. In a batch process, there was no significant change in HMF level with respect to untreated honey. 7 mm and 40 mm probe-0,5 cycle applications support this argument.

Amplitude has no significant effect on HMF level ($p>0,05$), but applying different probe and cycle (batch or continuous process) have statistically significant effect on HMF level ($p<0,05$).

40% amplitude-1 cycle applications could increase HMF formation because the treatment time was higher than 7 mm probe-1 cycle applications. Thermal treatment at 60°C increased HMF formation more as compared to the above mentioned ultrasound conditions (7 mm probe – 1 cycle (except 100% amplitude), 7 mm probe – 0,5 cycle and 40 mm probe – 0,5 cycle).

According to studies above and our findings, batch ultrasound processes provided lower HMF levels. As compared to untreated honey, ultrasound applications with 7 mm probe could decrease HMF level (except 100% amplitude-1 cycle application). As processing temperature increases, HMF formation rate increases. Ultrasound waves have a suppression effect on Maillard reaction and could break down HMF molecules (Vercet et al., 2001).

Further studies could be performed to understand if ultrasound can actually break down HMF molecules or not and to understand the different effects of batch and continuous Ultrasound processes on HMF formation.

3.2.3 Diastase Number Analysis in Ultrasound treated sunflower honey samples

The effect of ultrasound on diastase number of sunflower honey was measured after complete liquefaction of crystals. The initial diastase number of sunflower honey was 13,9. The results of diastase number after ultrasound treatment for selected conditions and thermal treatment are represented in figure 3.9.

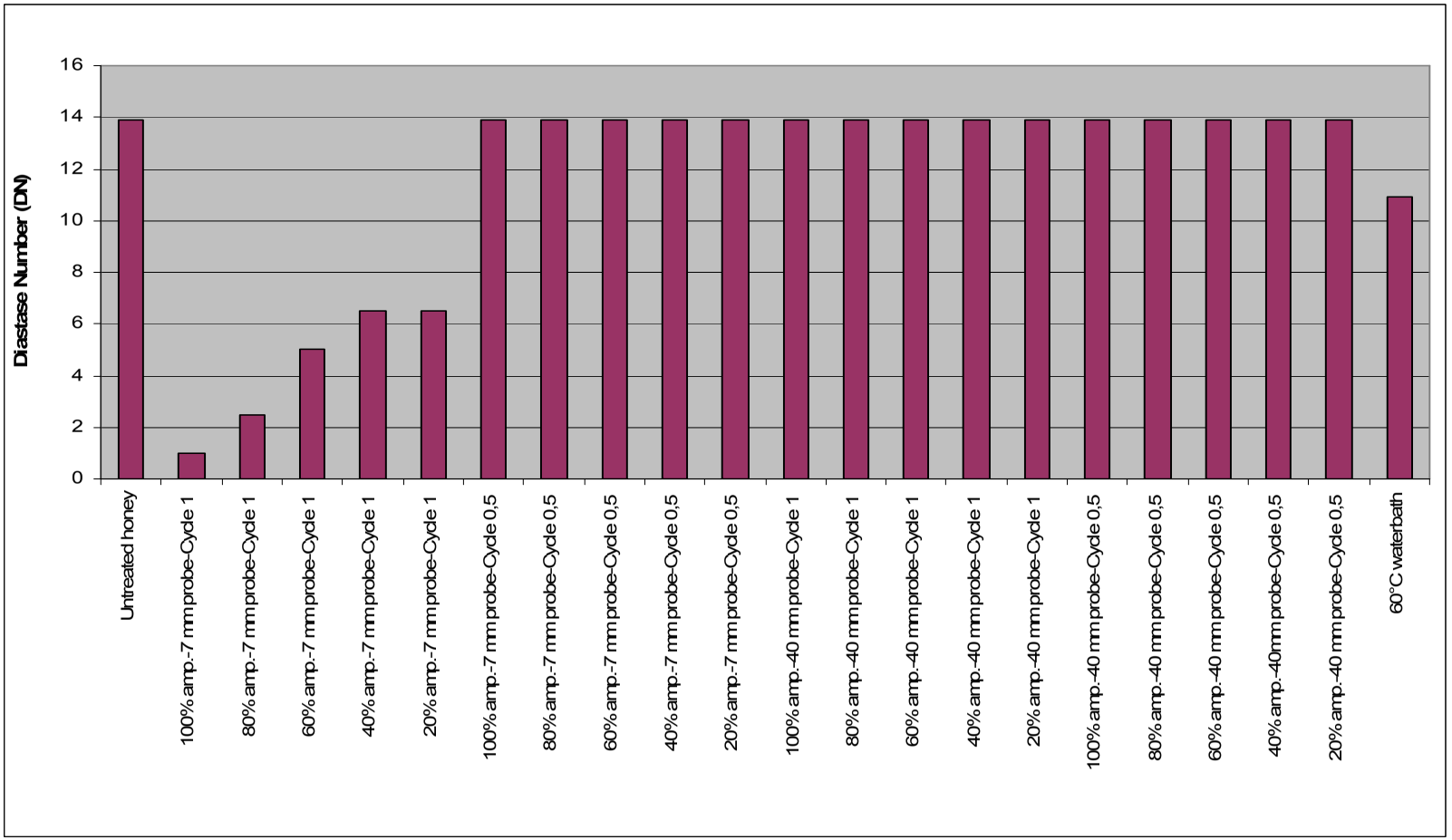

Figure 3.9 Diastase numbers of ultrasound and thermal treated sunflower honey samples. Standart deviations of the measurements were zero, no error bars shown.

The results of ultrasound and thermal applications showed that with 7 mm probe-1 Cycle combination, the diastase number decreased sharply. The maximum decrease was measured at 100% amplitude where diastase number decreased to 1. As the amplitude percentage increased from 20 to 100%, the diastase number decreased from 6,5 to 1. The main reason is attributed to the degradation of amylase in honey. As power decreased, diastase number decrease was lower. As shown in Table 3.1, the diastase number decrease can also be correlated with final temperatures reached. For example, for 7 mm probe – cycle 1 – 100% amplitude the diastase number was 1 and final temperature was 88°C, for 80% amplitude diastase number was increased to 2,5 and final temperature decreased to 84°C. For the other applications, there was no change in diastase number. For 60°C thermal application, diastase number decrease slightly (10,9). The statistical analysis of ultrasound treatments revealed that, amplitude has no significant effect on diastase number ($p>0,05$), however using different probe size and cycle (batch or continuous process) have statistically significant effect ($p<0,05$).

As a result, thermal treatment degrades enzymes more than ultrasound treatment. It was reported that the effect of the ultrasound treatments (40 mm sonotrode – 100% amplitude) on diastase enzyme activity in honey is negligible as compared to heat-treated (70°C) samples (D'Arcy, 2007). No detrimental effect on diastase activity in honey from any ultrasound treatment was reported. Our results supported literature data generally except sharp degradation in diastase activity observed with 100% amplitude-7mm probe-1 cycle application. Thraysvoulou et al, (1994), reported a slight change in diastase number decrease between ultrasound and thermal treated samples. The average decrease of

diastase activity after ultrasonic treatment (23 KHz frequency) was 16.2% and after heat (60°C for 30 minutes) 23.1 %.

3.2.4 Color Analysis of Ultrasound Treated Sunflower Honey

The effect of ultrasound on color of sunflower honey after the complete liquefaction of crystals was measured. L, a, b values after ultrasound treatment for selected conditions and thermal treatment are represented graphically in figure 3.10.

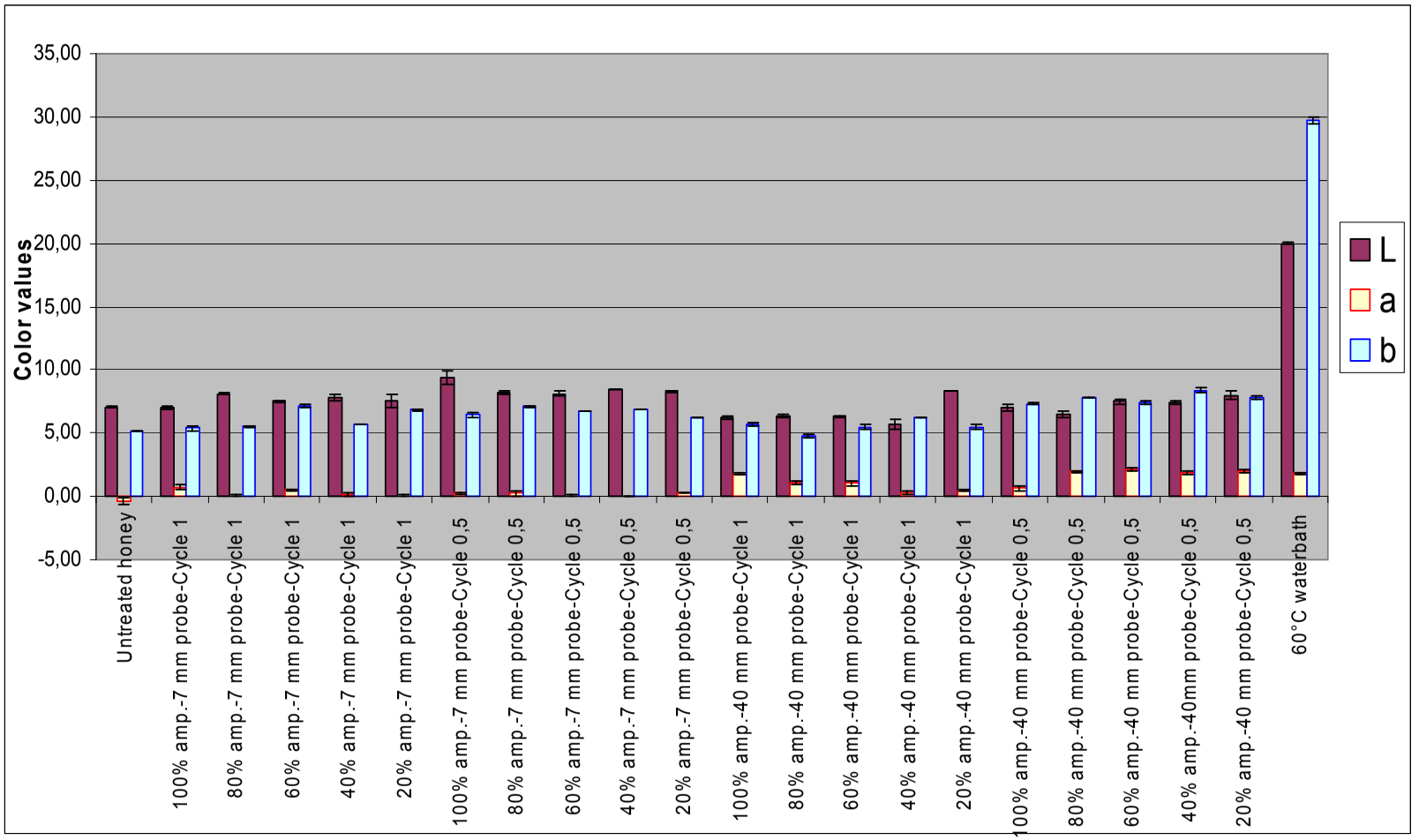

Figure 3.10 L*a*b values of ultrasound and thermal treated sunflower honey samples. The error bars indicate the standard deviations of the measurements.

The statistical analysis reveal that L and a values changed significantly between 7 and 40 mm probes ($p<0.05$). b values significantly changed with change in cycle ($p<0.05$). Amplitude has no significant effect on L, a, b values. There is no significant difference between untreated and ultrasound treated samples. However, L and b values changed in thermal treatment.

3.2.5 Viscosity and Sugar Analysis of Ultrasound Treated Sunflower Honey

The change in viscosity with shear rate was measured in untreated and treated honey samples. The measurements were performed at 25°C.

The results of viscosity values after ultrasound treatment for selected conditions and thermal treatment are represented graphically in figures 3.11 to figure 3.16.

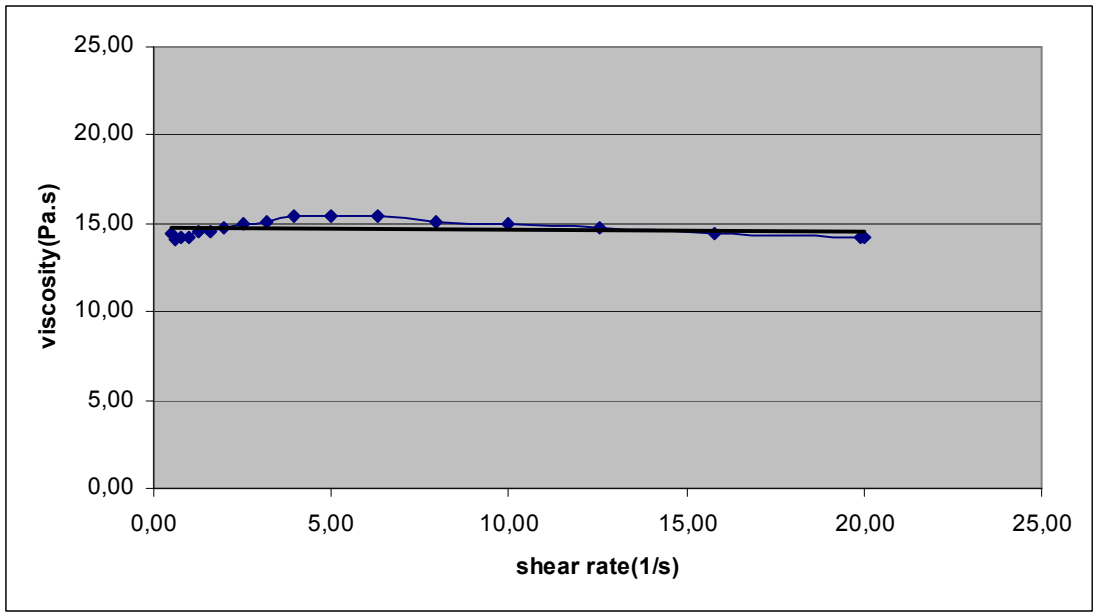

Figure 3.11 Viscosity profile of 100% amplitude- 7 mm probe- cycle 1 ultrasound treated sunflower honey in Pa.s versus shear rate (1/s).

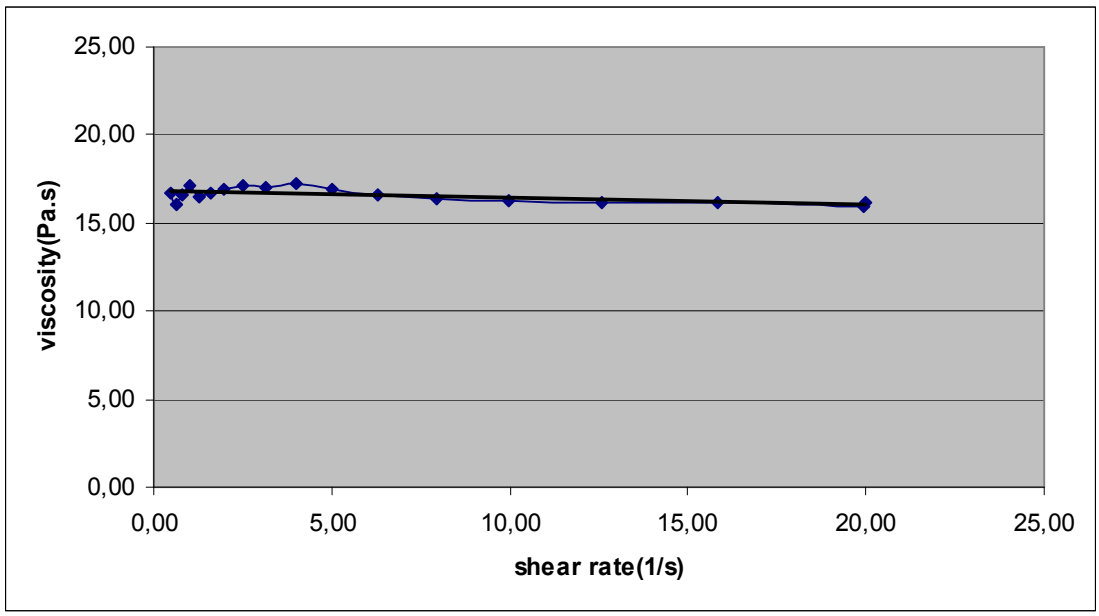

Figure 3.12 Viscosity profile of 100% amplitude- 7 mm probe- cycle 0,5 ultrasound treated sunflower honey in Pa.s versus shear rate (1/s).

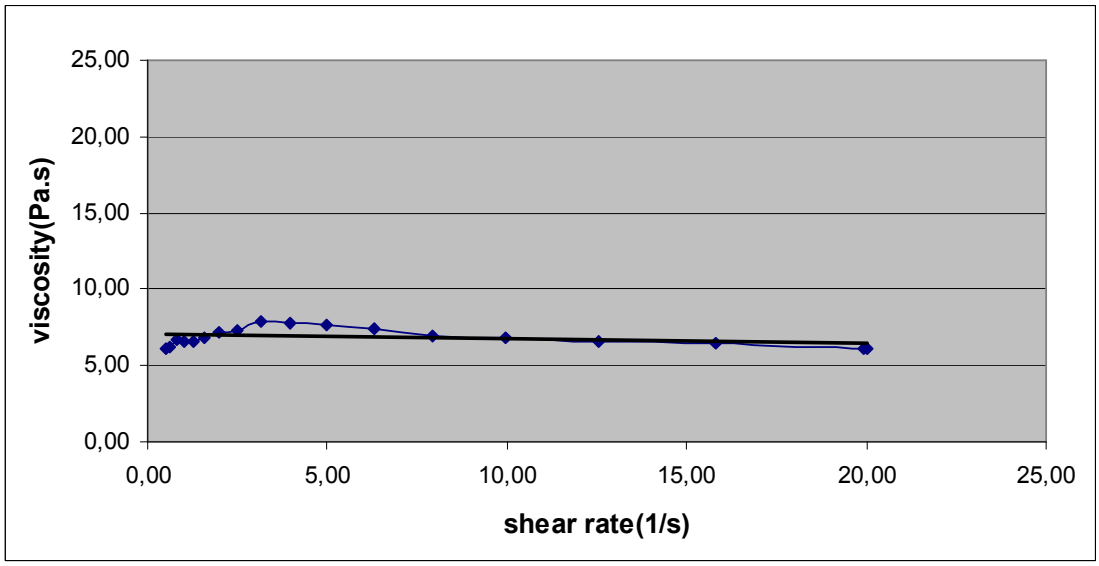

Figure 3.13 Viscosity profile of 100% amplitude- 40 mm probe- cycle 1 ultrasound treated sunflower honey in Pa.s versus shear rate (1/s).

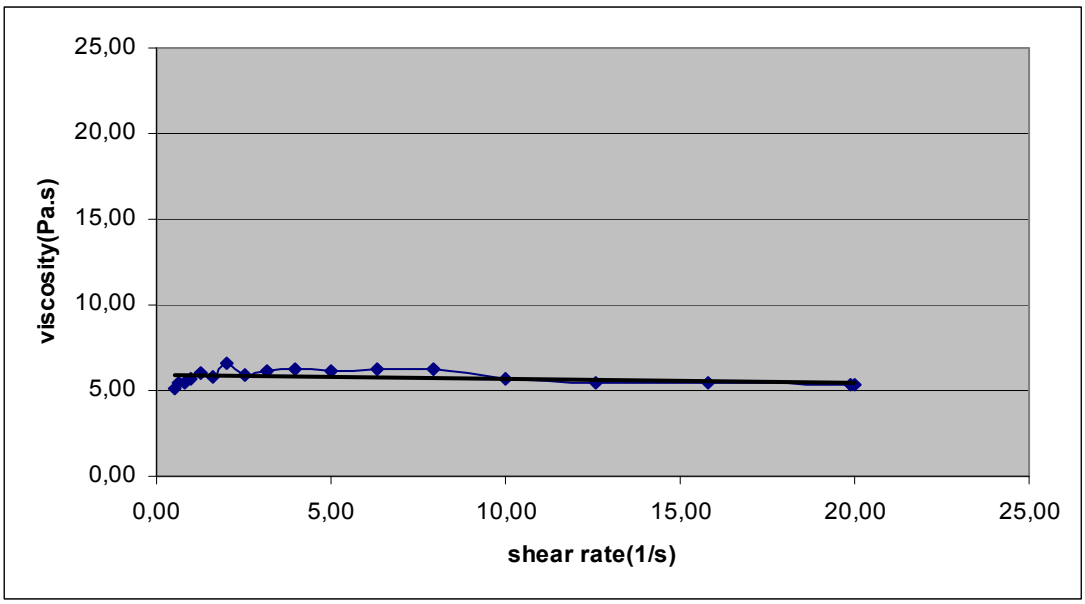

Figure 3.14 Viscosity profile of 100% amplitude- 40 mm probe- cycle 0,5 ultrasound treated sunflower honey in Pa.s versus shear rate (1/s).

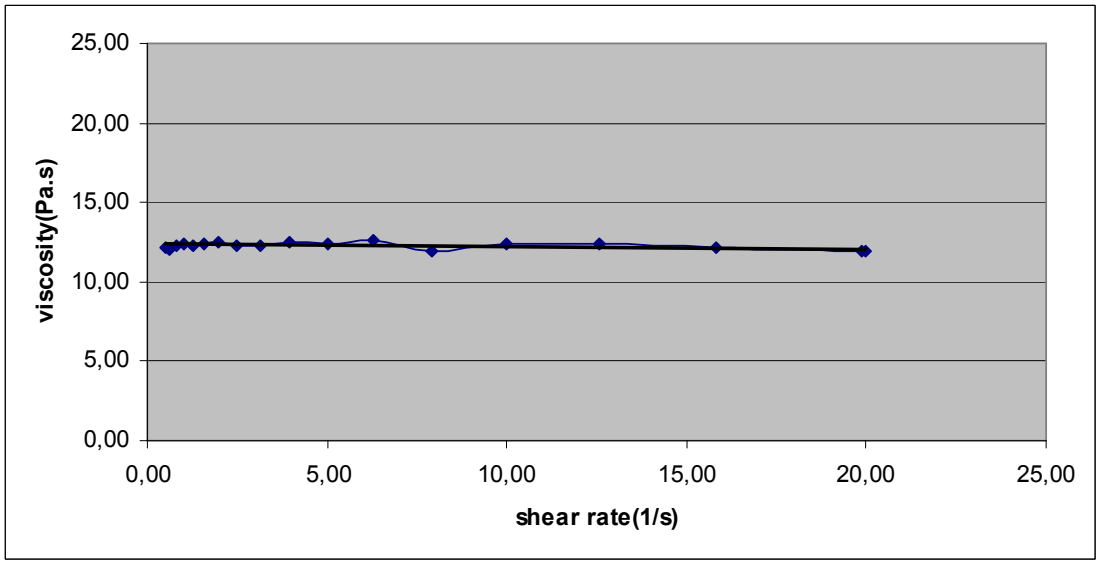

Figure 3.15 Viscosity profile of heat treated (60°C) sunflower honey in Pa.s versus shear rate (1/s).

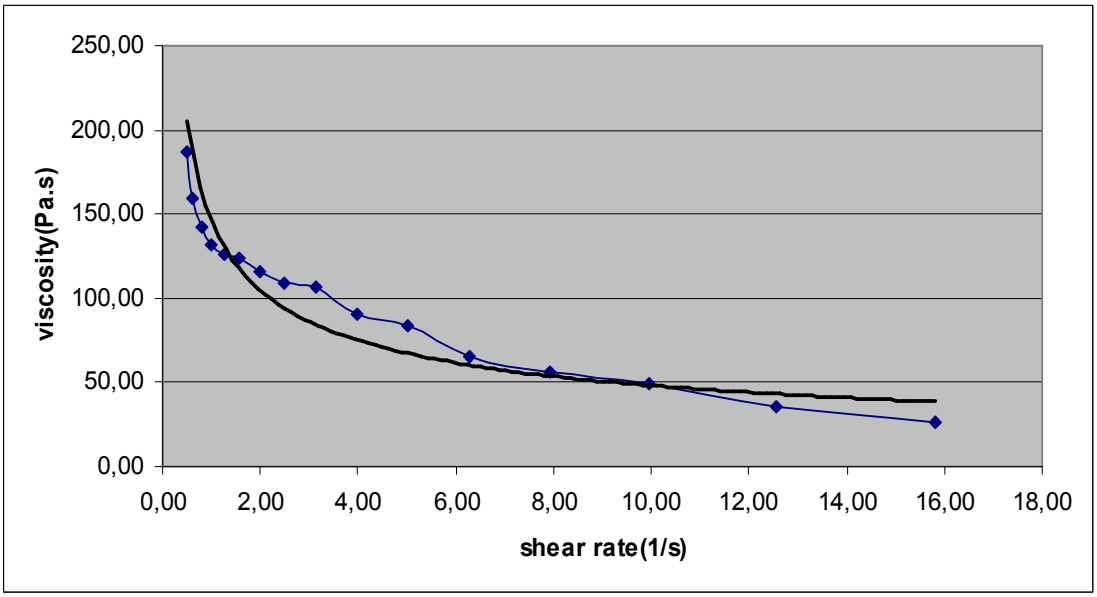

Figure 3.16 Viscosity profile of untreated crystal honey in Pa.s versus shear rate (1/s).

As shown in figure 3.16, crystal sunflower honey showed non-Newtonian behaviour. It had a shear-thinning structure. Viscosity values decreased as shear rate increased.

The ultrasound treated and thermal treated honey samples had no or slightly low crystals. That's why, they showed Newtonian behavior. In the literature, honey was reported to be a Newtonian fluid (White, 1978; Junzheng and Changying, 1998; Mossel, 2002; Juszczak and Fortuna, 2006). Juszczak and Fortuna, (2006) reported that the selected Polish honeys showed Newtonian behaviour, no thixotropy or dilatancy were observed. The viscosity of the samples depended upon the kind of honey and the temperature of measurement.

To understand the effect of honey composition on viscosity, dry matter (sugar content) measurements were performed. The results were represented in Table 3.2.

Table 3.2 The sugar and viscosity relationship in ultrasound and thermal treated sunflower honey samples.

Honey sample	**Sugar(%)**	**Viscosity(Pa.s) (Mean values)**
60°C thermal treatment	86,8	12,25
100% amp-7 mm probe-1 Cycle	86,5	14,71
100% amp-7 mm probe-0,5 Cycle	86,7	16,59
100% amp-40 mm probe-1 Cycle	84,5	6,86
100% amp-40 mm probe-0,5 Cycle	83,9	5,80

As shown in Table 3.2 it could be said that as sugar content of treated honey samples increased, the viscosity values increased.

Honey's viscosity depends upon the amount of water and the type and amount of sugar it contains. Because the two properties are closely connected, it is possible to construct mathematical models correlating them (White, 1975; Zaitoun et al., 2001). The viscosity and sugar concentration differences could be due to the difference in ultrasound conditions.

The low viscosity values measured in this study is an advantage in handling and processing of honey.

3.2.6 Summary of Ultrasound Treatment

According to the chemical and physical analysis, the best ultrasound applications were selected as 7 mm probe - 0,5 cycle combinations.

When treatment times, HMF and diastase number were analysed; it could be seen that the lower HMF values were obtained with 7 mm probe-0,5 cycle and 40 mm probe-0,5 cycle applications. Batch ultrasound processes provided lower HMF levels.

When the application times were compared, 40 mm probe-0,5 cycle application times were longest among all combinations because of the lowest power given to honey to liquefy the crystals. Longer treatment times will cause an additional cost. That's why, long processing times are undesired. As compared to untreated honey samples, 7 mm probe applications decreased the HMF level (except 100% amplitude-1 cycle application).

When diastase numbers were analyzed, the fastest decrease was in 7 mm probe-1 cycle applications. That's the result of the highest power given which inactivates amylase enzyme in honey. The results of other combinations were the same and caused no decrease in diastase number.

In terms of physical properties; lower viscosity values and higher moisture contents were observed with 40 mm probe applications. If sunflower honey has no or slightly low crystals, it shows non-Newtonian behaviour. The change in color values was insignificant.

When the important quality indicators of honey was evaluated (HMF and diastase number), the higher diastase numbers and lower HMF values were obtained with 7 mm probe- 0,5 cycle applications. The processing times were also short. The amplitude has no effect on chemical and physical parameters, it only affects application time. Amplitude can be determined according to the application time preferred and power could be spent.

According to the above selected combinations, 7 mm probe–0,5 cycle ultrasound treatments were selected and applied to cotton and canola honeys. 100 and 80%

amplitudes were applied. The same physical and chemical analysis was performed. For diastase number analysis, 7 mm probe- Cycle 1 combinations were applied in addition to the best combination.

3.3 Chemical and Physical Analysis of Ultrasound Treated Cotton and Canola Honey

Chemical analysis was based on the HMF content and diastase number. Physical analysis was based on color and viscosity.

Before the analysis, the initial HMF content and diastase numbers of untreated honey samples were measured. In addition, initial temperature of samples was measured. During ultrasound application, honey samples were stirred in certain periods and temperature was measured.

The temperature profiles were analyzed in section 3.3.1. The results of the effect of ultrasound treatment applied to honey samples were detailed in section 3.3.2, 3.3.3, 3.3.4 and 3.3.5. The calculations and the statistical analysis were given in Appendix A.

3.3.1 Temperature Profiles and Application Times of Ultrasound and Thermal Treated Cotton and Canola Honey

The initial temperature of untreated samples was measured and ultrasound treatment was applied at selected combinations. The application times were recorded for complete liquefaction.

The temperature profiles for selected ultrasound combinations and thermal treatment are represented graphically in figures 3.17 to 3.18.

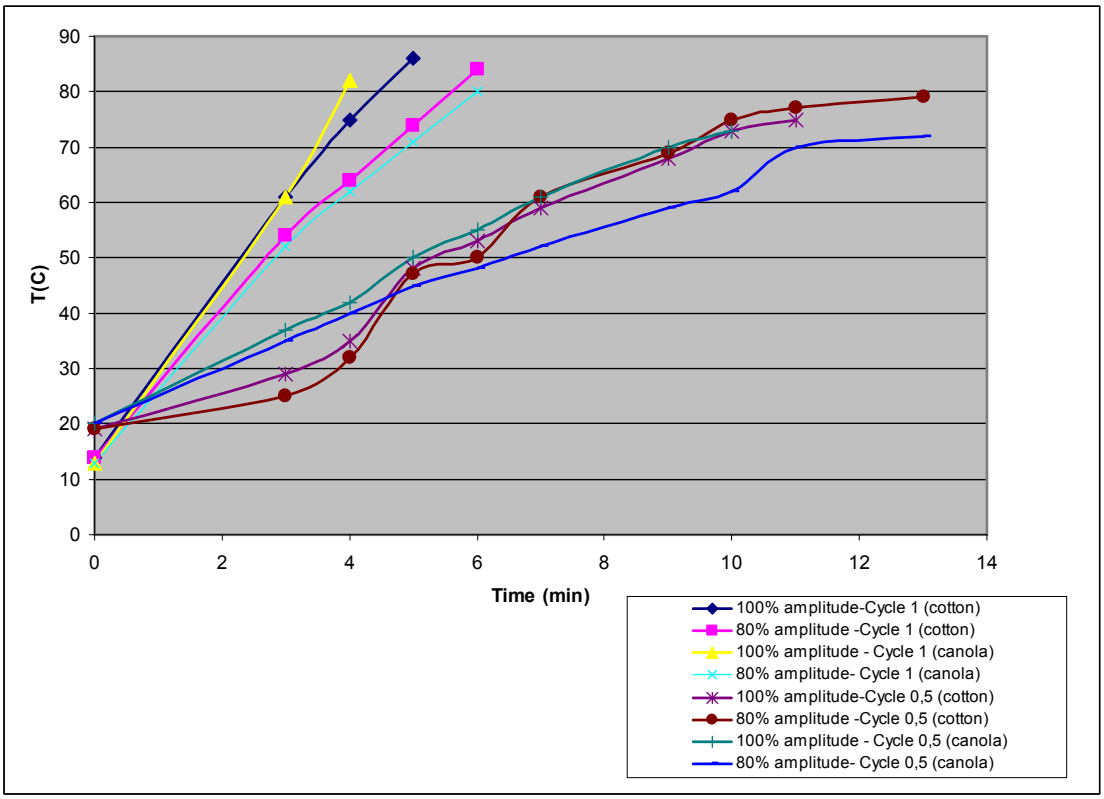

Figure 3.17 Temperature profile of ultrasound treated cotton and canola honeys for 7 mm probe applications.

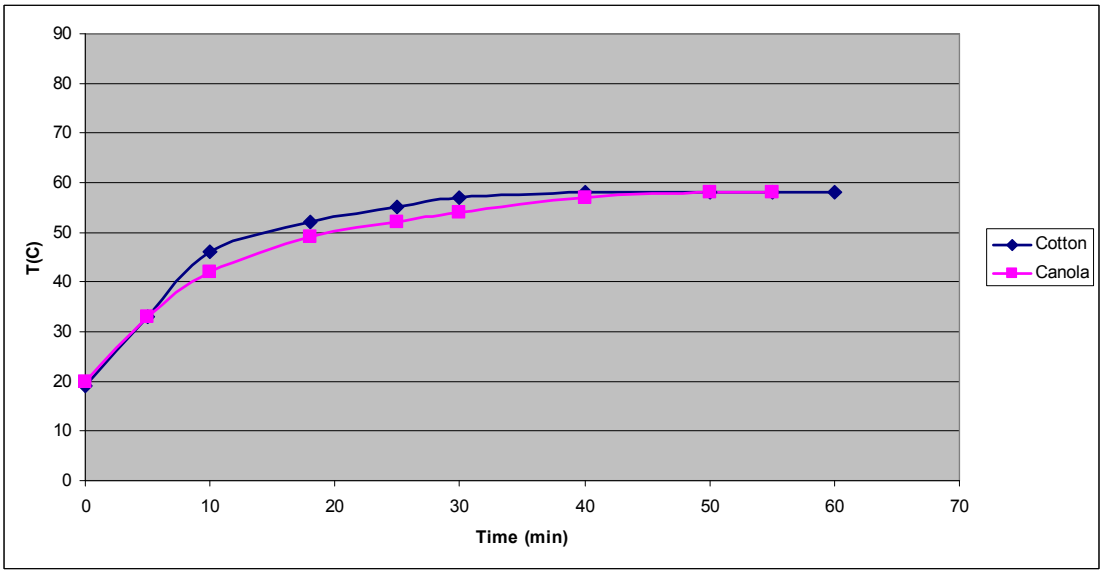

Figure 3.18 Temperature profile of heat treated (60°C) cotton and canola honeys.

As shown in figures above, the shortest application time was in 100% amplitude-7 mm probe-cycle 0,5 application and the longest application time was in thermal treatment in both cotton and canola honey samples as expected from sunflower honey results. The application times and final temperatures are represented in Appendix A.

3.3.2 Hydroxy methyl furfural (HMF) Analysis in Ultrasound treated cotton and canola honey

The effect of ultrasound on HMF content of cotton and canola honeys until the complete liquefaction of crystals were measured. The initial HMF content of cotton and canola honeys were measured as 1,10 and 0,9 ppm, respectively.

The results of HMF content after ultrasound treatment for selected conditions and thermal treatment are represented in figures 3.19 and 3.20.

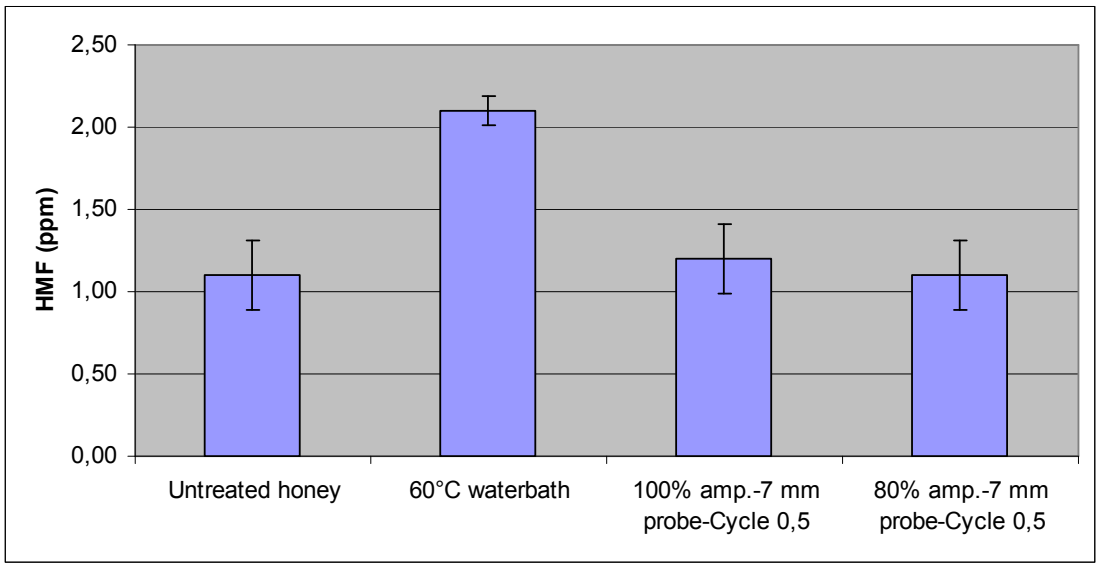

Figure 3.19 HMF levels (ppm) of ultrasound and thermal treated cotton honey. The error bars indicate the standard deviations of the measurements.

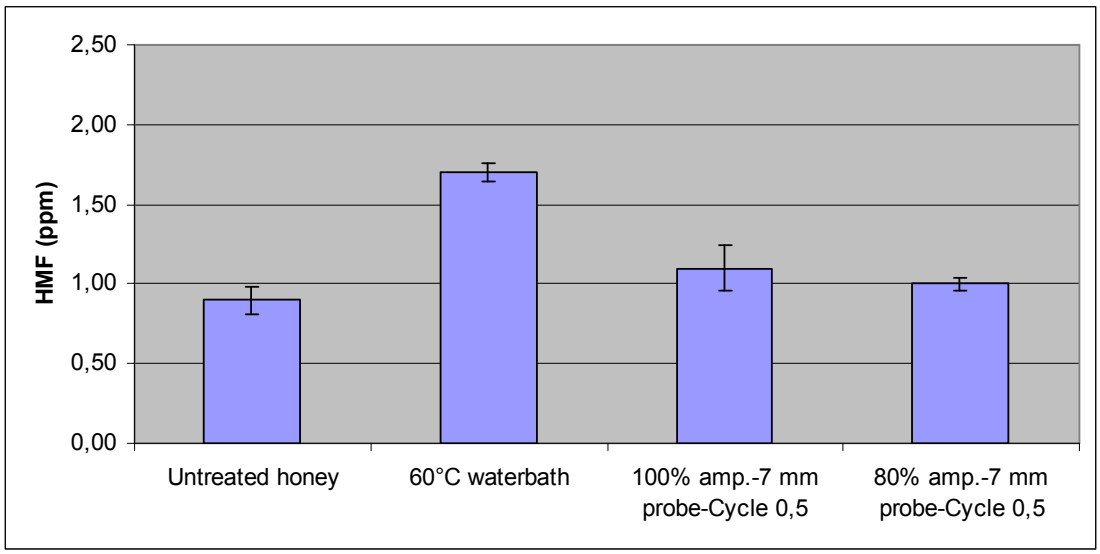

Figure 3.20 HMF levels in (ppm) of ultrasound and thermal treated canola honey. The error bars indicate the standard deviations of the measurements.

The results showed that the HMF values were lower in ultrasound applications than thermal applications for both honey types. For cotton honey, in 100% amplitude-7 mm probe-cycle 0,5 application, the HMF level was 1,2 ppm and in 80% amplitude-7 mm probe-cycle 0,5 application the HMF level was 1,1 ppm. For canola honey, in 100% amplitude-7 mm probe-cycle 0,5 application, the HMF level was 1,1 ppm and in 80% amplitude-7 mm probe-cycle 0,5 application the HMF level was 1 ppm. For thermal treatments, HMF formation was higher (cotton honey, 2,1 ppm, canola honey, 1,7 ppm)

As a result, the HMF analyses for cotton and canola honey samples support our analysis for sunflower honey. For sunflower honey, the 7 mm probe – cycle 0,5 analyse HMF results were also lower than untreated sample (1,60 ppm in 100% amplitude, 1,55 ppm in 80% amplitude, 1,40 ppm in 60% amplitude, 1,32 ppm in 40% amplitude, 1,48 ppm in 20% amplitude). Heat treatment at 60°C increased

HMF formation more as compared to the selected ultrasound condition (7 mm probe – 1 cycle).

3.3.3 Diastase Number Analysis in Ultrasound treated cotton and canola honey

The effect of ultrasound on diastase number of cotton and canola honey after the complete liquefaction of crystals was measured. The initial diastase numbers of honeys were the same (13,9).

The results of diastase number after ultrasound treatment for selected conditions and thermal treatment are represented graphically in figure 3.21 and 3.22.

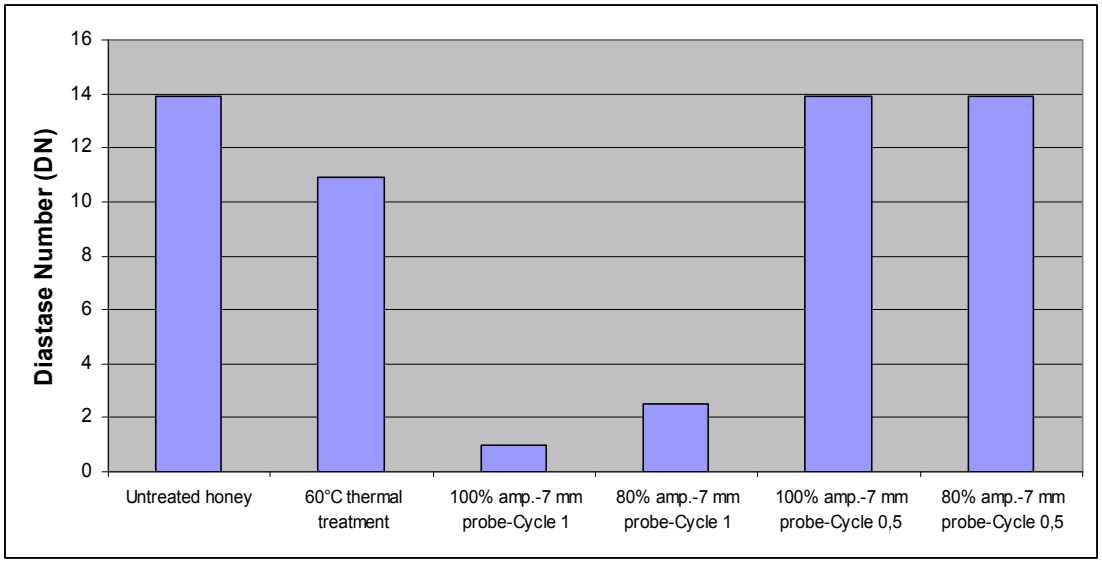

Figure 3.21 Diastase numbers of ultrasound and thermal treated cotton honey. Standart deviations of the measurements were zero, no error bars shown.

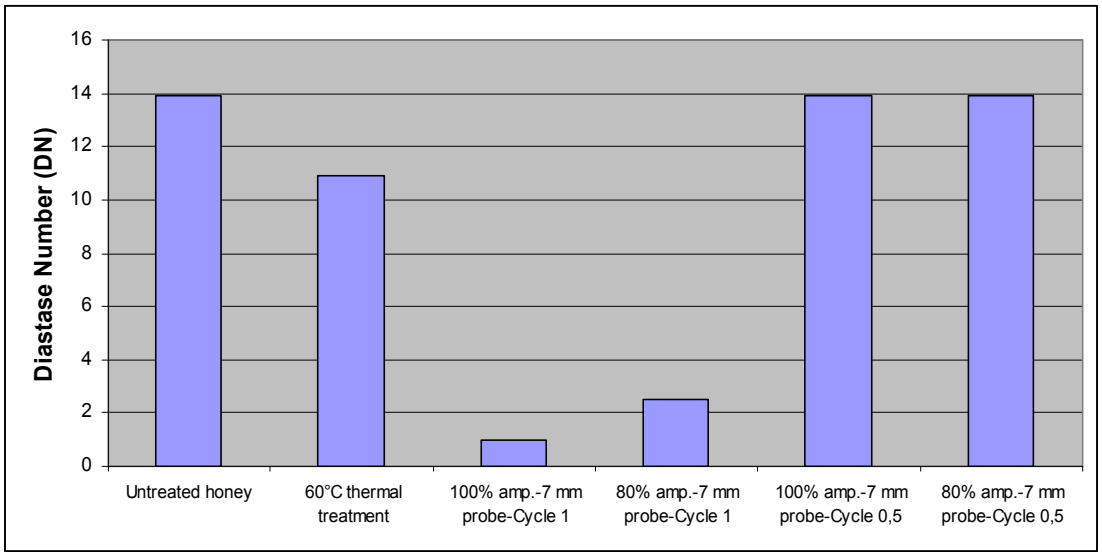

Figure 3.22 Diastase numbers of ultrasound and thermal treated canola honey. Standart deviations of the measurments were zero, no error bars shown.

In both honey samples, the same results were obtained. In thermal treatment diastase number was decreased to 10,9. The sharp decrease for 7 mm probe-cycle 1 combinations in sunflower honey was observed in both cotton and canola honeys, too. In the best ultrasound combinations, the values remained the same as untreated honey. In addition, the values were the same as that of sunflower honey.

3.3.4 Color Analysis in Ultrasound Treated Cotton and Canola Honey

The results of L, a, b values after ultrasound treatment for selected conditions and thermal treatment are represented in figure 3.23 and 3.24.

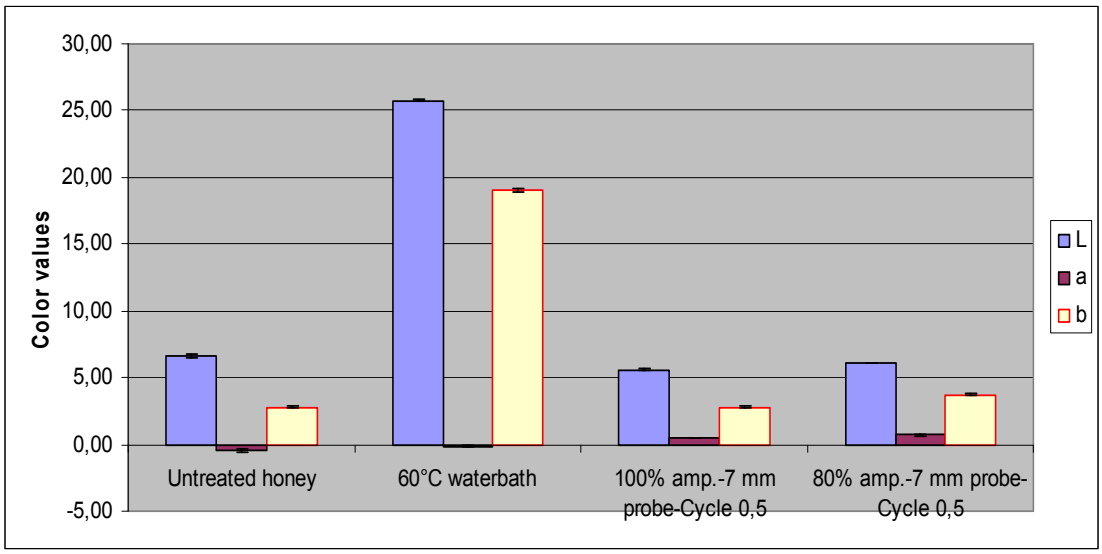

Figure 3.23 L*a*b values of ultrasound and thermal treated cotton honey. The error bars indicate the standard deviations of the measurements.

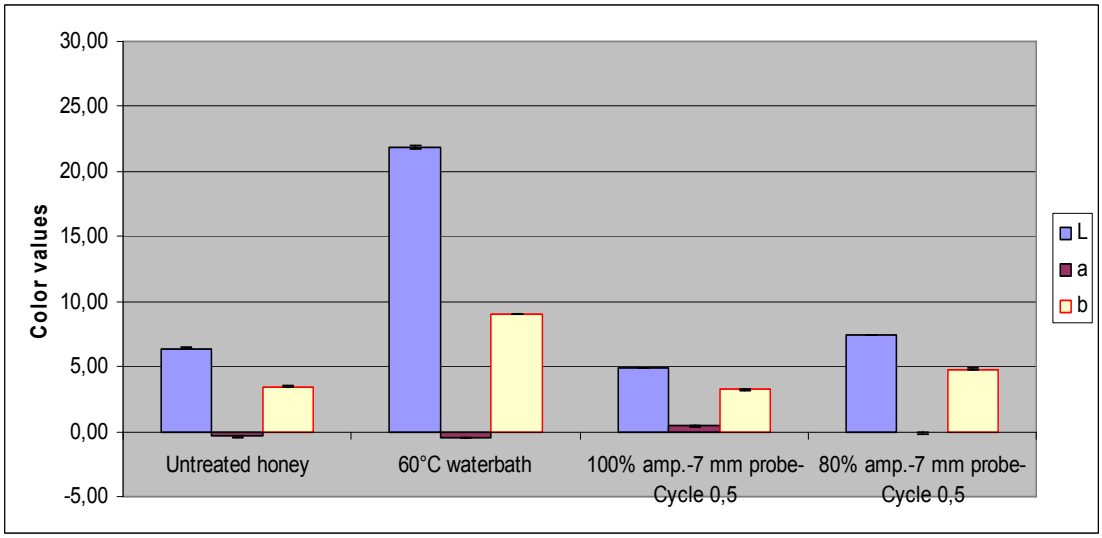

Figure 3.24 L*a*b values of ultrasound and thermal treated canola honey. The error bars indicate the standard deviations of the measurements.

The L, a, b values between ultrasound treated samples did not significantly change ($p>0.05$). In 60°C treatment L and b values were increased. As a result, ultrasound application did not have an effect on L*a*b values of cotton and canola honeys when compared to thermal treatment. L and b values changed in thermal treatment. This is also in agreement with sunflower honey.

3.3.5 Viscosity Analysis in Ultrasound Treated Cotton and Canola Honey

The results of viscosity values after ultrasound treatment for selected conditions and thermal treatment are represented graphically in Figures 3.25 to 3.30.

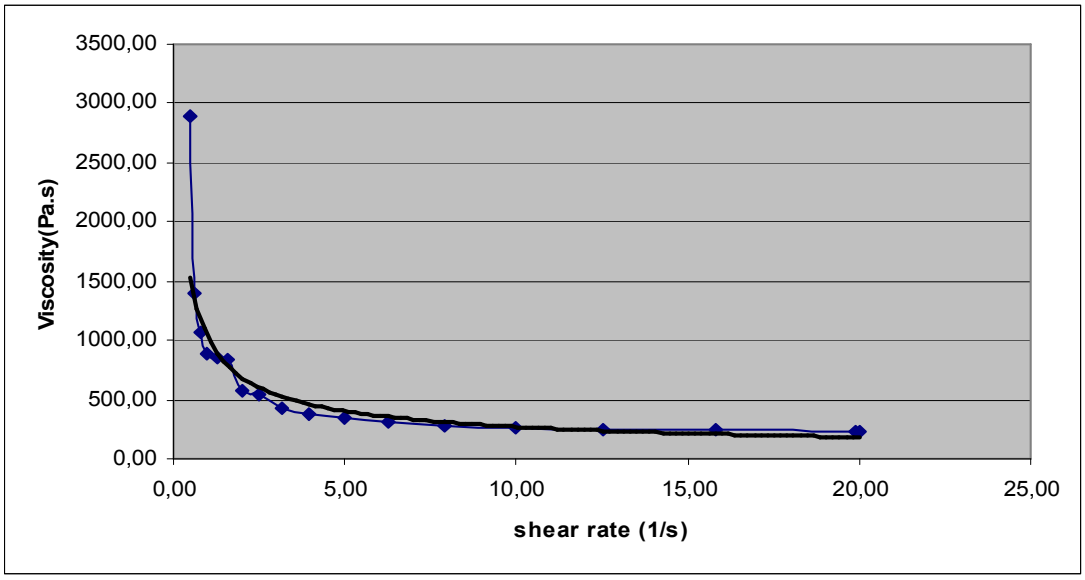

Figure 3.25 Viscosity profile of untreated cotton honey in Pa.s versus shear rate (1/s).

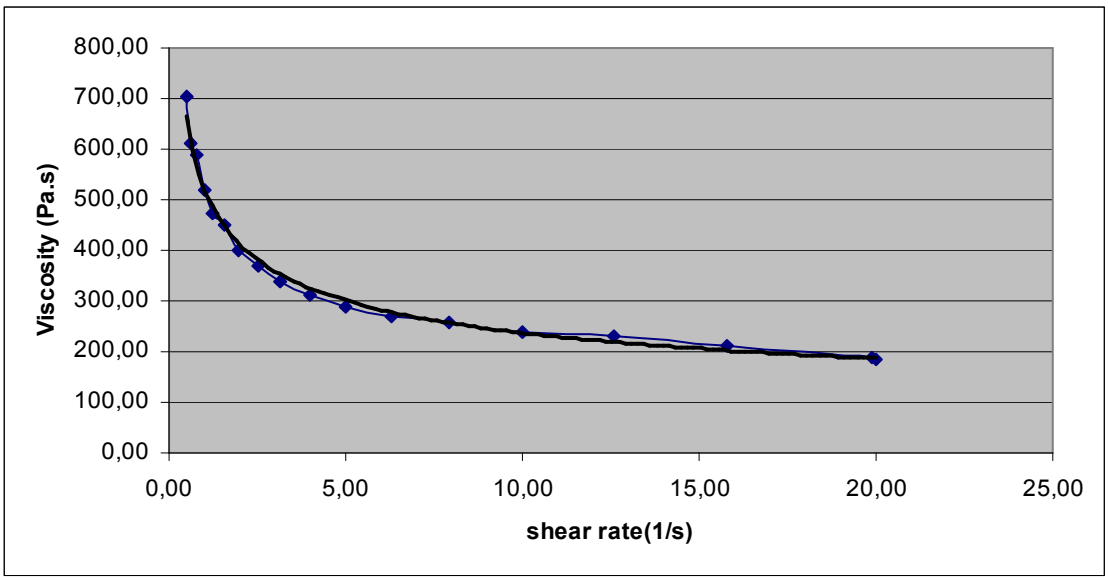

Figure 3.26 Viscosity profile of untreated canola honey in Pa.s versus shear rate (1/s).

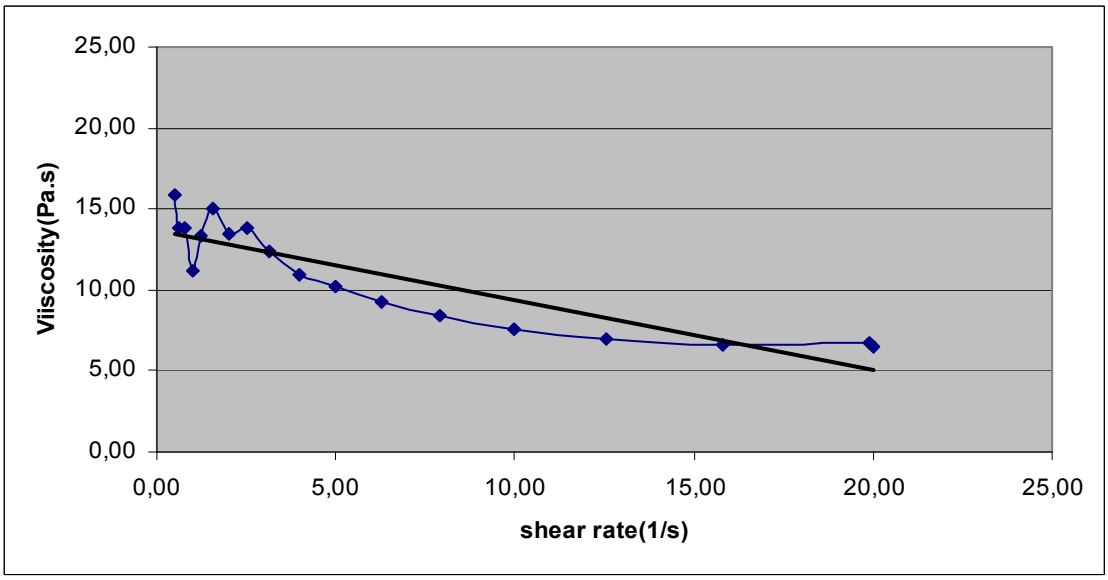

Figure 3.27 Viscosity profile of thermal treated cotton honey (60°C) in Pa.s versus shear rate (1/s).

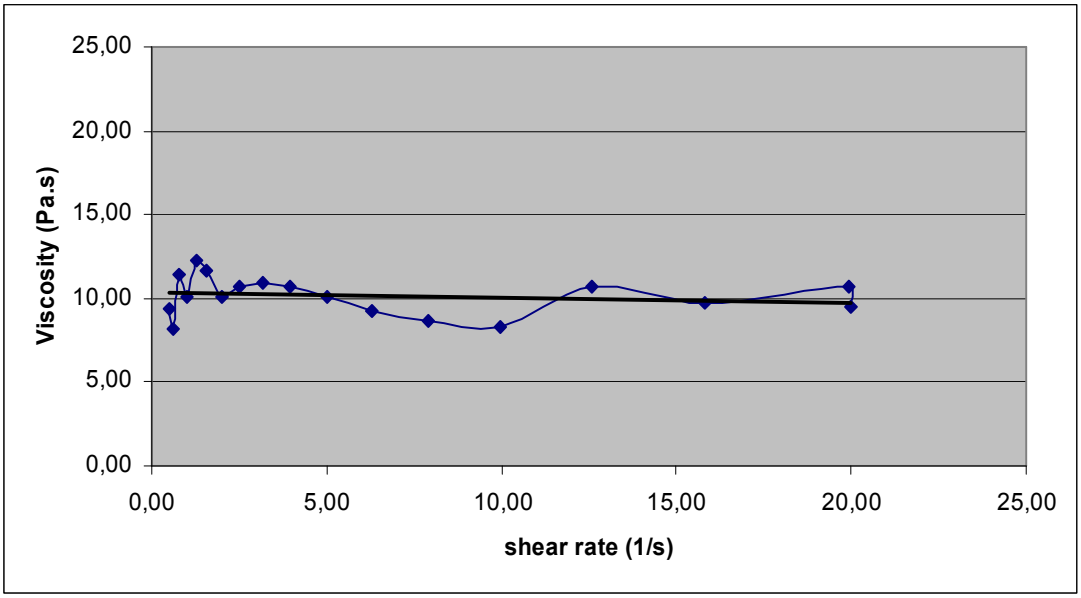

Figure 3.28 Viscosity profile of thermal treated canola honey (60°C) in Pa.s versus shear rate (1/s).

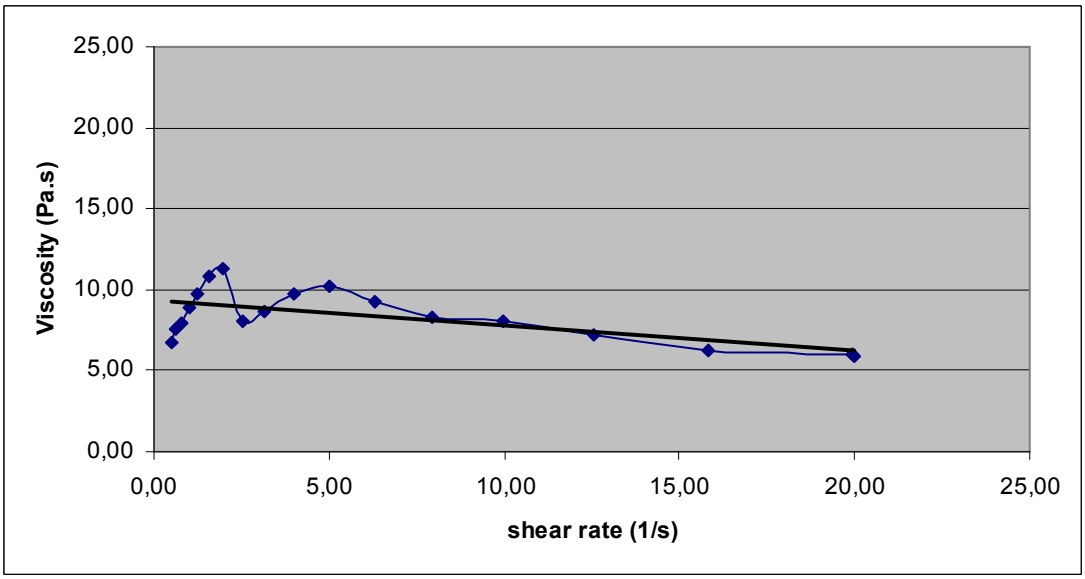

Figure 3.29 Viscosity profile of 100% amplitude- 7 mm probe- cycle 0,5 ultrasound treated cotton honey in Pa.s versus shear rate (1/s).

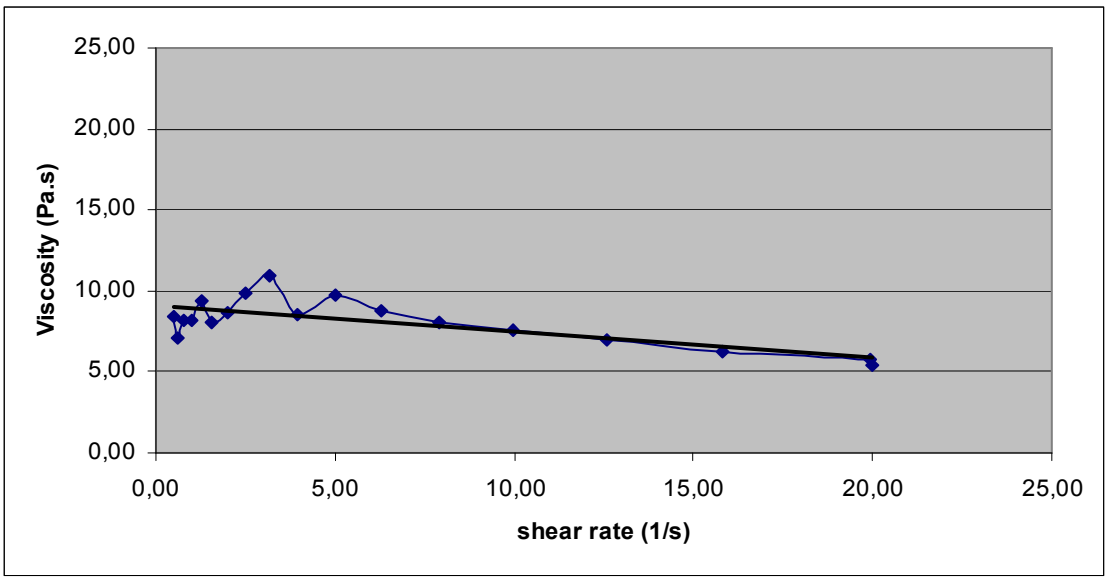

Figure 3.30 Viscosity profile of 100% amplitude- 7 mm probe- cycle 0,5 ultrasound treated canola honey in Pa.s versus shear rate (1/s).

As shown in figure 3.25 and figure 3.26, crystal cotton and canola honeys showed non-Newtonian behaviour like crystal sunflower honey. They had shear-thinning structure. Viscosity values decreased as shear rate increased.

The ultrasound and thermal treated honey samples had no or slightly low crystals. That's why, they showed Newtonian behavior but as shown in figure 3.29 and figure 3.30 the viscosity values decreased slightly as shear rate increased. The reason could be the presence of remaining crystals after ultrasound treatment. This could be eliminated with increasing the processing time of ultrasound application to dissolve the crystals completely and control crystal presence with a microscope, not only visual observation.

3.3.6 Microbiological Analysis in Ultrasound Treated Sunflower, Cotton and Canola Honey

Total aerobic bacteria, *E. coli*, coliform and yeast and mould analysis were performed to three honey types to understand the effect of best ultrasound combination on microorganisms in honey. No coliform and yeast counts were observed for three honey samples. Total aerobic bacteria results before and after ultrasound treatment for selected conditions (100% amplitude – 7 mm probe – Cycle 0,5) are represented in Table 3.3.

Table 3.3 Total aerobic bacteria in honey samples

Sample	Total aerobic bacteria (cfu/g)
Untreated sunflower honey	170
100% amplitude - 7 mm probe - Cycle 0,5 (sunflower honey)	80
Untreated cotton honey	1020
100% amplitude - 7 mm probe - Cycle 0,5 (cotton honey)	990
Untreated canola honey	80
100% amplitude - 7 mm probe - Cycle 0,5 (canola honey)	40

The results showed that, ultrasound have a destructive effect on microorganisms. The total aerobic bacteria counts were decreased around 50% for cotton and canola, 3% for sunflower honey after ultrasound treatment.

According to D'Arcy (2007), the microbial inactivation by power ultrasound was considered to occur due to cavitation, localised heating and free radical formation. During transient cavitation, the bubble size increased quickly and these bubbles collapsed producing temperatures up to 5000 °K and pressure up

to 100 MPa. Such a pressure was sufficient to disrupt the cell wall structures leading to cell disruption. However, these temperatures occur instantly, and the immediate vicinity of the cells is likely to be affected. In addition, microstreaming created by stable cavitation produced a shear force, which rub against the surface cells causing the microbial cells to shear and breakdown.

Leadly and Williams (2002) suggested that free radicals were formed during the application of ultrasound to liquids due to sonolysis of water, and these free radicals had a bactericidal effect.

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

This study was conducted in two parts. In the first part, HHP treatments with the selected pressure and temperature combinations with required time for complete liquefaction of sunflower honey crystals were conducted. Among the selected parameters, it was found that increasing pressure level has no significant effect on liquefaction time ($p>0.05$). For 220 and 330 MPa applications, the liquefaction times were the same. Temperature and pressurization time were the main parameters that affected the HMF level ($p<0.05$). Lower application temperatures are generally selected, to maintain minimum destruction in honey quality parameters. Pressure level has no significant effect on HMF level ($p>0.05$). According to the results obtained, the evaluation of the data collected with cost considerations, the best treatment combinations were determined as 220 MPa pressure - 50°C HHP treatment.

Hence HHP application has three parameters (pressure-temperature-time), when we discuss all of them, we could see that HHP applications enabled shorter liquefaction times and lower HMF values than conventional thermal treatments (50°C and 60°C). HHP suppressed the formation of HMF as a result of Maillard reaction. On this basis the study pointed out that HHP can be offered as an alternative method to traditional thermal treatment for the liquefaction of sunflower honey crystals. Shorter application times and lower HMF values would be an advantage for honey quality but HHP is proposed

with much higher operational and investment costs when compared with the current thermal treatments; HHP could not be cost-efficient and advantageous.

In the second part of the study, Ultrasound treatments with the selected combinations with 7 and 40 mm sonotrodes for complete liquefaction of crystals was conducted. Among the selected parameters, the best combination was determined, based on the chemical and physical analysis. The temperature profiles and application times were analysed. With ultrasound application, shorter application times than thermal thermal treatment were achieved. According to the results obtained, the best combinations were determined as 7 mm probe- 0,5 cycle (batch) applications. Amplitude has a significant effect on application time ($p<0,05$), with no significant effect on chemical and physical parameters of honey ($p>0,05$). That's why, amplitude level can be determined according to the application time preferred and power could be spent. Probe and application type (continuous or batch) have statistically significant effect on HMF and diastase number of sunflower honey ($p<0,05$).

Different honey types (cotton and canola) were also analyzed with the selected parameters. The results obtained were very close to those of sunflower honey. The effect of ultrasound did not vary among selected honey types.

In the light of the results obtained, 7 mm probe – 0,5 cycle combinations favored lower HMF values, with no decrease in diastase number, no significant change in color and viscosity profile. These results are all desirable for honey processing and quality.

On this basis the study pointed out that Ultrasound can be offered as an alternative method to traditional thermal treatment for the liquefaction of honey crystals. When compared to thermal treatment shorter application times, desirable quality characteristics obtained are advantageous and less thermal

energy is needed, the application of ultrasound helps to save processing costs when compared to conventional heating. The conduct of trials using a bench-top size sonication system was recommended. Preliminary tests should be conducted in batch mode, while further processing trials require a flow cell for pressurized recirculation or in-line testing (www.hielscher.com).

As a recommendation, the research can be sustained by covering more honey types and with more HHP combinations. Microwave heating can be studied. The change in nutritional value of honey types by HHP and Ultrasound can be analyzed. As indications of the amount of crystals disintegrated, by taking photographs before and after the treatments by using a microscopy and viscosity, density (by picnometer) analysis can be performed. Calculation of the energy requirement for ultrasound liquefaction of crystal honey can be analyzed. Also, for the determination of the shelf-life the results of the physical and chemical analysis can be supported and verified by sensory evaluations during the storage period of treated honey. As liquefaction of Turkish honey by ultrasound waves has not been studied upto now, the continuation of efforts for the search for an effective and efficient method to liquefy honey, using ultrasonic waves is highly recommended.

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APPENDIX A

RESULTS AND CALCULATIONS

Table A.1 HMF content in ppm for sunflower honey treated at 220 and 330 MPa pressures at 50°C temperature for 106 minutes and 50°C thermal treatment for 120 minutes.

Sample	Measurements		Mean	St. Dev.
	1	2		
Untreated honey	2,31	2,29	2,30	0,01
50°C-120 min (thermal)	2,55	2,63	2,59	0,06
220MPa-50°C-106 min	2,45	2,47	2,46	0,01
330MPa-50°C-106 min	2,40	2,44	2,42	0,03

Table A.2 HMF content in ppm for sunflower honey treated at 220 and 330 MPa pressures at 60°C temperature for 23 minutes and 60°C thermal treatment for 48 minutes.

Sample	Measurements		Mean	St. Dev.
	1	2		
Untreated honey	2,31	2,29	2,30	0,01
60°C-48 min (thermal)	2,75	2,85	2,80	0,07
220 MPa-60°C-23 min	2,66	2,72	2,69	0,04
330MPa-60°C-23 min	2,72	2,70	2,71	0,01

Table A.3 HMF content in ppm for sunflower honey after thermal treatment with the same application times with pressure treated honey samples.

Sample	Measurements		Mean	St. Dev.
	1	2		
Untreated honey	2,31	2,29	2,30	0,01
50°C-106 min (thermal)	2,54	2,56	2,55	0,01
60°C-23 min (thermal)	2,71	2,77	2,74	0,03

Table A.4 HMF levels in ppm of ultrasound and thermal treated sunflower honey samples.

Sample	Measurements		Mean	St.dev
	1	2		
Untreated honey	2,40	2,34	2,37	0,042
100% amp.-7 mm probe-Cycle 1	3,09	3,11	3,10	0,014
80% amp.-7 mm probe-Cycle 1	2,35	2,31	2,33	0,028
60% amp.-7 mm probe-Cycle 1	2,21	2,19	2,20	0,014
40% amp.-7 mm probe-Cycle 1	1,97	1,87	1,92	0,071
20% amp.-7 mm probe-Cycle 1	1,92	1,88	1,90	0,028
100% amp.-7 mm probe-Cycle 0,5	1,59	1,60	1,60	0,007
80% amp.-7 mm probe-Cycle 0,5	1,69	1,40	1,55	0,205
60% amp.-7 mm probe-Cycle 0,5	1,50	1,30	1,40	0,144
40% amp.-7 mm probe-Cycle 0,5	1,35	1,30	1,32	0,033
20% amp.-7 mm probe-Cycle 0,5	1,52	1,43	1,48	0,060
100% amp.-40 mm probe-Cycle 1	4,37	4,36	4,37	0,005
80% amp.-40 mm probe-Cycle 1	4,05	4,07	4,06	0,010
60% amp.-40 mm probe-Cycle 1	4,11	3,95	4,03	0,111
40% amp.-40 mm probe-Cycle 1	4,54	3,89	4,22	0,461
20% amp.-40 mm probe-Cycle 1	3,44	2,53	2,99	0,640
100% amp.-40 mm probe-Cycle 0,5	4,41	4,17	2,36	0,174
80% amp.-40 mm probe-Cycle 0,5	2,16	1,96	2,06	0,143
60% amp.-40 mm probe-Cycle 0,5	2,71	2,01	2,36	0,494
40% amp.-40mm probe-Cycle 0,5	2,44	2,12	2,28	0,220

Table A.4 (continued)				
20% amp.-40 mm probe-Cycle 0,5	2,39	1,90	2,14	0,352
60°C thermal treatment	2,68	3,00	2,84	0,227

Table A.5 Diastase numbers of ultrasound and thermal treated sunflower honey samples.

Sample	Measurements		Mean	St. Dev.
	1	2		
Untreated honey	13,9	13,9	13,9	0
100% amp.-7 mm probe-Cycle 1	1	1	1	0
80% amp.-7 mm probe-Cycle 1	2,5	2,5	2,5	0
60% amp.-7 mm probe-Cycle 1	5	5	5	0
40% amp.-7 mm probe-Cycle 1	6,5	6,5	6,5	0
20% amp.-7 mm probe-Cycle 1	6,5	6,5	6,5	0
100% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0
80% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0
60% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0
40% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0
20% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0
100% amp.-40 mm probe-Cycle 1	13,9	13,9	13,9	0
80% amp.-40 mm probe-Cycle 1	13,9	13,9	13,9	0
60% amp.-40 mm probe-Cycle 1	13,9	13,9	13,9	0
40% amp.-40 mm probe-Cycle 1	13,9	13,9	13,9	0
20% amp.-40 mm probe-Cycle 1	13,9	13,9	13,9	0
100% amp.-40 mm probe-Cycle 0,5	13,9	13,9	13,9	0
80% amp.-40 mm probe-Cycle 0,5	13,9	13,9	13,9	0
60% amp.-40 mm probe-Cycle 0,5	13,9	13,9	13,9	0
40% amp.-40mm probe-Cycle 0,5	13,9	13,9	13,9	0
20% amp.-40 mm probe-Cycle 0,5	13,9	13,9	13,9	0
60°C thermal treatment	10,9	10,9	10,9	0

Table A.6 L values of ultrasound and thermal treated sunflower honey samples.

Sample	Measurements		Mean	St.dev.
	1	2		
Untreated honey	7,01	7,12	7,07	0,078
100% amp.-7 mm probe-Cycle 1	6,88	7,09	6,99	0,148
80% amp.-7 mm probe-Cycle 1	8,11	8,16	8,14	0,035
60% amp.-7 mm probe-Cycle 1	7,47	7,56	7,52	0,064
40% amp.-7 mm probe-Cycle 1	7,6	7,98	7,79	0,269
20% amp.-7 mm probe-Cycle 1	7,12	7,93	7,53	0,573
100% amp.-7 mm probe-Cycle 0,5	9,05	9,73	9,39	0,481
80% amp.-7 mm probe-Cycle 0,5	8,05	8,27	8,16	0,156
60% amp.-7 mm probe-Cycle 0,5	7,97	8,26	8,12	0,205
40% amp.-7 mm probe-Cycle 0,5	8,44	8,47	8,46	0,021
20% amp.-7 mm probe-Cycle 0,5	8,26	8,33	8,30	0,049
100% amp.-40 mm probe-Cycle 1	6,15	6,29	6,22	0,099
80% amp.-40 mm probe-Cycle 1	6,24	6,4	6,32	0,113
60% amp.-40 mm probe-Cycle 1	6,3	6,38	6,34	0,057
40% amp.-40 mm probe-Cycle 1	5,44	6,02	5,73	0,410
20% amp.-40 mm probe-Cycle 1	8,28	8,33	8,31	0,035
100% amp.-40 mm probe-Cycle 0,5	6,87	7,23	7,05	0,255
80% amp.-40 mm probe-Cycle 0,5	6,25	6,62	6,44	0,262
60% amp.-40 mm probe-Cycle 0,5	7,66	7,3	7,48	0,255
40% amp.-40mm probe-Cycle 0,5	7,55	7,31	7,43	0,170
20% amp.-40 mm probe-Cycle 0,5	8,2	7,76	7,98	0,311
60°C thermal treatment	20,01	19,93	19,97	0,057

Table A.7 a values of ultrasound and thermal treated sunflower honey samples.

Sample	Measurements		Mean	St.dev.
	1	2		
Untreated honey	-0,55	-0,17	-0,36	0,267
100% amp.-7 mm probe-Cycle 1	0,89	0,55	0,72	0,240
80% amp.-7 mm probe-Cycle 1	0,05	0,11	0,08	0,042
60% amp.-7 mm probe-Cycle 1	0,44	0,52	0,48	0,057
40% amp.-7 mm probe-Cycle 1	0,26	0,03	0,15	0,163
20% amp.-7 mm probe-Cycle 1	0,09	0,03	0,06	0,042
100% amp.-7 mm probe-Cycle 0,5	0,18	0,26	0,22	0,057
80% amp.-7 mm probe-Cycle 0,5	0,12	0,34	0,23	0,156
60% amp.-7 mm probe-Cycle 0,5	0,12	-0,01	0,06	0,092
40% amp.-7 mm probe-Cycle 0,5	0,01	0,03	0,02	0,014
20% amp.-7 mm probe-Cycle 0,5	0,25	0,30	0,28	0,035
100% amp.-40 mm probe-Cycle 1	1,74	1,82	1,78	0,057
80% amp.-40 mm probe-Cycle 1	1,16	0,99	1,08	0,120
60% amp.-40 mm probe-Cycle 1	1,20	0,88	1,04	0,226
40% amp.-40 mm probe-Cycle 1	0,19	0,35	0,27	0,113
20% amp.-40 mm probe-Cycle 1	0,50	0,44	0,47	0,042
100% amp.-40 mm probe-Cycle 0,5	0,75	0,50	0,63	0,177
80% amp.-40 mm probe-Cycle 0,5	1,98	1,92	1,95	0,042
60% amp.-40 mm probe-Cycle 0,5	2,17	1,99	2,08	0,127
40% amp.-40mm probe-Cycle 0,5	1,79	1,93	1,86	0,099
20% amp.-40 mm probe-Cycle 0,5	1,85	2,03	1,94	0,127
60°C thermal treated	1,74	1,86	1,80	0,085

Table A.8 b values of ultrasound and thermal treated sunflower honey samples.

Sample	Measurements		Mean	St.dev.
	1	2		
Untreated honey	5,11	5,14	5,13	0,021
100% amp.-7 mm probe-Cycle 1	5,48	5,26	5,37	0,156
80% amp.-7 mm probe-Cycle 1	5,49	5,53	5,51	0,028
60% amp.-7 mm probe-Cycle 1	7,11	7,29	7,20	0,127
40% amp.-7 mm probe-Cycle 1	5,70	5,64	5,67	0,042
20% amp.-7 mm probe-Cycle 1	6,75	6,91	6,83	0,113
100% amp.-7 mm probe-Cycle 0,5	6,58	6,27	6,43	0,219
80% amp.-7 mm probe-Cycle 0,5	7,05	7,12	7,09	0,049
60% amp.-7 mm probe-Cycle 0,5	6,74	6,79	6,77	0,035
40% amp.-7 mm probe-Cycle 0,5	6,85	6,83	6,84	0,014
20% amp.-7 mm probe-Cycle 0,5	6,26	6,22	6,24	0,028
100% amp.-40 mm probe-Cycle 1	5,64	5,84	5,74	0,141
80% amp.-40 mm probe-Cycle 1	4,72	4,87	4,80	0,106
60% amp.-40 mm probe-Cycle 1	5,31	5,61	5,46	0,212
40% amp.-40 mm probe-Cycle 1	6,25	6,19	6,22	0,042
20% amp.-40 mm probe-Cycle 1	5,60	5,37	5,49	0,163
100% amp.-40 mm probe-Cycle 0,5	7,36	7,29	7,33	0,049
80% amp.-40 mm probe-Cycle 0,5	7,80	7,76	7,78	0,028
60% amp.-40 mm probe-Cycle 0,5	7,50	7,37	7,44	0,092
40% amp.-40mm probe-Cycle 0,5	8,51	8,24	8,38	0,191
20% amp.-40 mm probe-Cycle 0,5	7,92	7,73	7,83	0,134
60°C thermal treatment	29,93	29,54	29,74	0,276

Table A.9 Viscosity profile of 100% amplitude- 7 mm probe- cycle 1 ultrasound treated sunflower honey in Pa.s.

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
7,21	0,50	14,41
8,89	0,63	14,12
11,25	0,79	14,20
14,19	1,00	14,23
18,20	1,26	14,49
23,02	1,58	14,56
29,30	1,99	14,72
37,65	2,51	15,02
47,73	3,16	15,13
61,11	3,97	15,39
76,97	5,00	15,39
96,96	6,30	15,40
119,90	7,92	15,13
149,60	9,98	15,00
185,50	12,56	14,77
228,40	15,81	14,44
282,20	19,91	14,18
283,10	20,00	14,16
	St.Dev.	0,460

Table A.10 Viscosity profile of 100% amplitude- 7 mm probe- cycle 0,5 ultrasound treated sunflower honey in Pa.s.

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
8,33	0,50	16,65
10,13	0,63	16,10
13,11	0,79	16,55
17,13	1,00	17,17
20,76	1,26	16,53
26,41	1,58	16,70
33,70	1,99	16,93
42,92	2,51	17,13
53,83	3,16	17,06
68,28	3,97	17,19
84,42	5,00	16,88
104,70	6,30	16,64
129,90	7,92	16,39
162,00	9,98	16,23
202,90	12,56	16,15
254,90	15,81	16,12
318,50	19,91	16,00
323,00	20,00	16,15
	St.Dev.	0,406

Table A.11 Viscosity profile of 100% amplitude- 40 mm probe- cycle 1 ultrasound treated sunflower honey in Pa.s.

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
3,07	0,50	6,14
3,91	0,63	6,21
5,33	0,79	6,73
6,54	1,00	6,55
8,29	1,26	6,60
10,87	1,58	6,88
14,31	1,99	7,19
18,39	2,51	7,34
24,91	3,16	7,90
30,88	3,97	7,78
38,28	5,00	7,66
46,46	6,30	7,38
55,31	7,92	6,98
68,15	9,98	6,83
82,55	12,56	6,57
101,90	15,81	6,45
122,00	19,91	6,13
122,90	20,00	6,15
	St.Dev.	0,574

Table A.12 Viscosity profile of 100% amplitude- 40 mm probe- cycle 0,5 ultrasound treated sunflower honey in Pa.s

shear stress(Pa)	**shear rate (1/s)**	**viscosity (Pa.s)**
2,55	0,50	5,11
3,40	0,63	5,40
4,34	0,79	5,47
5,72	1,00	5,74
7,57	1,26	6,03
9,20	1,58	5,82
13,05	1,99	6,56
14,82	2,51	5,91
19,44	3,16	6,16
24,81	3,97	6,25
30,74	5,00	6,15
39,62	6,29	6,30
49,23	7,92	6,21
57,02	9,98	5,72
69,03	12,56	5,50
86,99	15,81	5,50
105,70	19,90	5,31
107,00	20,00	5,35
	St.Dev.	0,412

Table A.13 Viscosity profile of 60°C thermal treated sunflower honey in Pa.s.

shear stress(Pa)	**shear rate (1/s)**	**viscosity (Pa.s)**
6,09	0,50	12,18
7,56	0,63	12,02
9,77	0,79	12,32
12,30	1,00	12,33
15,41	1,26	12,27
19,52	1,58	12,35
24,91	1,99	12,51
30,69	2,51	12,25
38,65	3,16	12,25
49,51	3,97	12,47
61,82	5,00	12,36
79,53	6,30	12,64
94,55	7,92	11,93
123,10	9,98	12,34
155,20	12,56	12,35
191,50	15,81	12,11
237,00	19,90	11,91
238,40	20,00	11,92
	St.Dev.	0,206

Table A.14 Viscosity profile of untreated crystal sunflower honey in Pa.s.

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
93,59	0,50	187,30
100,50	0,63	159,70
112,60	0,79	142,20
131,90	1,00	132,30
158,70	1,26	126,40
195,20	1,58	123,40
230,00	1,99	115,50
274,50	2,51	109,50
338,20	3,16	107,20
361,80	3,97	91,10
420,20	5,00	84,04
413,50	6,29	65,69
444,20	7,93	56,05
495,90	9,98	49,71
443,80	12,56	35,34
412,10	15,82	26,05
	St.Dev.	42,513

Table A.15 Liquefaction times and final temperatures of ultrasound and thermal treated cotton honey samples.

Sample	Final T(°C)	Liquefaction time(min)
100% amp.-7 mm probe-cycle 1	86	5
80% amp.-7 mm probe-cycle 1	84	6
100% amp.-7 mm probe-cycle 0,5	75	11
80% amp.-7 mm probe-cycle 0,5	79	13
60°C thermal treatment	58	60

Table A.16 Liquefaction times and final temperatures of ultrasound and thermal treated canola honey samples.

Sample	Final T(°C)	Liquefaction time(min)
100% amp.-7 mm probe-cycle 1	82	4
80% amp.-7 mm probe-cycle 1	80	6
100% amp.-7 mm probe-cycle 0,5	73	10
80% amp.-7 mm probe-cycle 0,5	72	13
60°C thermal treatment	58	55

Table A.17 HMF levels in ppm of ultrasound and thermal treated cotton honey samples.

Sample	Measurements		Mean	St.dev
	1	2		
Untreated honey	0,90	1,20	1,10	0,212
60°C thermal treatment	2,16	2,04	2,10	0,085
100% amp.-7 mm probe-Cycle 0,5	1,05	1,35	1,20	0,212
80% amp.-7 mm probe-Cycle 0,5	0,95	1,25	1,10	0,212

Table A.18 HMF levels in ppm of ultrasound and thermal treated canola honey samples.

Sample	Measurements		Mean	St.dev
	1	2		
Untreated honey	0,96	0,84	0,90	0,085
60°C thermal treatment	1,74	1,66	1,70	0,057
100% amp.-7 mm probe-Cycle 0,5	1,00	1,20	1,10	0,141
80% amp.-7 mm probe-Cycle 0,5	0,97	1,03	1,00	0,042

Table A.19 Diastase numbers of ultrasound and thermal treated cotton honey samples.

Sample	Measurements		Mean	St.dev.
	1	2		
Untreated honey	13,9	13,9	13,9	0
60°C thermal treatment	10,9	10,9	10,9	0
100% amp.-7 mm probe-Cycle 1	1	1	1	0
80% amp.-7 mm probe-Cycle 1	2,5	2,5	2,5	0
100% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0
80% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0

Table A.20 Diastase numbers of ultrasound and thermal treated canola honey samples.

Sample	Measurements		Mean	St.dev.
	1	2		
Untreated honey	13,9	13,9	13,9	0
60°C thermal treatment	10,9	10,9	10,9	0
100% amp.-7 mm probe-Cycle 1	1	1	1	0
80% amp.-7 mm probe-Cycle 1	2,5	2,5	2,5	0
100% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0
80% amp.-7 mm probe-Cycle 0,5	13,9	13,9	13,9	0

Table A.21 L values of ultrasound and thermal treated cotton honey samples.

Sample	Measurements		Mean	St.dev.
	1	2		
Untreated honey	6,54	6,68	6,61	0,099
60°C thermal treatment	25,83	25,69	25,76	0,099
100% amp.-7 mm probe-Cycle 0,5	5,63	5,59	5,61	0,028
80% amp.-7 mm probe-Cycle 0,5	6,06	6,12	6,09	0,042

Table A.22 a values of ultrasound and thermal treated cotton honey samples.

Sample	Measurements		Mean	St.dev.
	1	2		
Untreated honey	-0,58	-0,40	-0,49	0,127
60°C thermal treatment	-0,20	-0,08	-0,14	0,085
100% amp.-7 mm probe-Cycle 0,5	0,51	0,45	0,48	0,042
80% amp.-7 mm probe-Cycle 0,5	0,72	0,66	0,69	0,042

Table A.23 b values of ultrasound and thermal treated cotton honey samples.

Sample	Measurements		Mean	St.dev
	1	2		
Untreated honey	2,74	2,86	2,80	0,085
60°C thermal treatment	18,99	19,15	19,07	0,113
100% amp.-7 mm probe-Cycle 0,5	2,72	2,88	2,80	0,113
80% amp.-7 mm probe-Cycle 0,5	3,68	3,78	3,73	0,071

Table A.24 L values of ultrasound and thermal treated canola honey samples.

Sample	Measurements		Mean	St.dev
	1	2		
Untreated honey	6,36	6,44	6,40	0,057
60°C thermal treatment	21,89	21,73	21,81	0,113
100% amp.-7 mm probe-Cycle 0,5	4,87	4,93	4,90	0,042
80% amp.-7 mm probe-Cycle 0,5	7,43	7,47	7,45	0,028

Table A.25 a values of ultrasound and thermal treated canola honey samples.

Sample	Measurements		Mean	St.dev
	1	2		
Untreated honey	-0,42	-0,36	-0,39	0,042
60°C thermal treatment	-0,48	-0,48	-0,48	0,000
100% amp.-7 mm probe-Cycle 0,5	0,43	0,41	0,42	0,014
80% amp.-7 mm probe-Cycle 0,5	-0,13	-0,09	-0,11	0,028

Table A.26 b values of ultrasound and thermal treated canola honey samples.

Sample	Measurements		Mean	St.dev
	1	2		
Untreated honey	3,40	3,54	3,47	0,099
60°C thermal treatment	9,01	9,07	9,04	0,042
100% amp.-7 mm probe-Cycle 0,5	3,25	3,19	3,22	0,042
80% amp.-7 mm probe-Cycle 0,5	4,81	4,71	4,76	0,071

Table A.27 Viscosity profile of untreated cotton honey in Pa.s

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
1449,00	0,50	2898,00
874,50	0,63	1390,00
841,30	0,79	1062,00
881,70	1,00	883,70
1073,00	1,26	854,90
1324,00	1,58	837,30
1131,00	1,99	568,20
1346,00	2,51	537,00
1349,00	3,16	427,60
1525,00	3,97	383,90
1723,00	5,00	344,70
1925,00	6,30	305,90
2169,00	7,93	273,70
2606,00	9,98	261,10
3149,00	12,56	250,70
3860,00	15,81	244,20
4676,00	19,91	234,90
4622,00	20,00	231,10
	St.Dev.	651,423

Table A.28 Viscosity profile of untreated canola honey in Pa.s.

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
352,00	0,50	703,90
386,00	0,63	613,20
467,40	0,79	589,90
518,80	1,00	520,10
595,40	1,26	474,00
713,00	1,58	451,00
795,30	1,99	399,50
928,50	2,51	370,50
1073,00	3,16	340,30
1244,00	3,97	313,10
1451,00	5,00	290,20
1702,00	6,30	270,30
2031,00	7,92	256,40
2390,00	9,98	239,60
2876,00	12,56	229,00
3365,00	15,81	212,80
3764,00	19,90	189,10
3725,00	20,00	186,20
	St.Dev.	157,405

Table A.29 Viscosity profile of 60°C thermal treated cotton honey in Pa.s.

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
7,95	0,50	15,89
5,54	0,63	13,80
7,03	0,79	13,88
11,18	1,00	11,21
16,79	1,26	13,37
23,77	1,58	15,03
26,91	1,99	13,52
34,57	2,51	13,79
38,91	3,16	12,33
43,47	3,97	10,94
51,04	5,00	10,21
58,52	6,30	9,30
67,04	7,93	8,46
75,65	9,98	7,58
87,08	12,56	6,93
104,00	15,81	6,58
133,00	19,91	6,68
130,90	20,00	6,54
	St.Dev.	3,198

Table A.30 Viscosity profile of 60°C thermal treated canola honey in Pa.s.

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
4,66	0,50	9,32
5,16	0,63	8,19
9,00	0,79	11,36
10,11	1,00	10,13
15,34	1,26	12,21
18,43	1,58	11,65
20,04	1,99	10,07
26,94	2,51	10,75
34,47	3,16	10,93
42,68	3,97	10,75
50,39	5,00	10,08
58,40	6,30	9,28
68,44	7,92	8,64
83,33	9,98	8,35
94,92	12,56	10,75
109,90	15,81	9,79
130,70	19,91	10,71
128,70	20,00	9,55
	St.Dev.	1,119

Table A.31 Viscosity profile of 100% amplitude- 7 mm probe- cycle 0,5 ultrasound treated cotton honey in Pa.s.

shear stress(Pa)	**shear rate (1/s)**	**viscosity (Pa.s)**
3,35	0,50	6,70
4,79	0,63	7,61
6,30	0,79	7,95
8,84	1,00	8,86
12,24	1,26	9,75
17,07	1,58	10,79
22,47	1,99	11,29
20,26	2,51	8,09
27,28	3,16	8,65
38,59	3,97	9,72
51,19	5,00	10,24
58,23	6,30	9,25
66,17	7,93	8,35
79,99	9,98	8,02
90,34	12,56	7,19
98,85	15,81	6,25
119,70	19,91	6,02
117,80	20,00	5,89
	St.Dev.	1,608

Table A.32 Viscosity profile of 100% amplitude- 7 mm probe- cycle 0,5 ultrasound treated canola honey in Pa.s.

shear stress(Pa)	shear rate (1/s)	viscosity (Pa.s)
3,23	0,50	8,47
4,43	0,63	7,03
6,46	0,79	8,16
8,13	1,00	8,15
11,81	1,26	9,40
15,95	1,58	8,09
23,15	1,99	8,63
32,32	2,51	9,90
34,41	3,16	10,91
42,01	3,97	8,58
48,70	5,00	9,74
54,96	6,30	8,73
63,72	7,92	8,04
75,69	9,98	7,59
87,00	12,56	6,93
99,58	15,81	6,30
114,50	19,91	5,75
107,90	20,00	5,39
	St.Dev.	1,444

Table A.33 ANOVA table for the effect of pressure on the HMF level of HHP treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,000E-04	1	1,000E-04	,003	,962
Within Groups	6,850E-02	2	3,425E-02		
Total	6,860E-02	3			

Table A.34 ANOVA table for the effect of temperature on the HMF level of HHP treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6,760E-02	1	6,760E-02	135,200	,007
Within Groups	1,000E-03	2	5,000E-04		
Total	6,860E-02	3			

Table A.35 ANOVA table for the effect of time on the HMF level of HHP treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6,760E-02	1	6,760E-02	135,200	,007
Within Groups	1,000E-03	2	5,000E-04		
Total	6,860E-02	3			

Table A.36 ANOVA table for the effect of pressure on the liquefaction time of HHP treated honey crystals.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,000	1	,000	,000	1,000
Within Groups	6889,000	2	3444,500		
Total	6889,000	3			

Table A.37 ANOVA table for the effect of amplitude on the HMF level of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,078	4	,269	,233	,915
Within Groups	17,328	15	1,155		
Total	18,406	19			

Table A.38 ANOVA table for the effect of probe on the HMF level of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7,284	1	7,284	11,789	,003
Within Groups	11,122	18	,618		
Total	18,406	19			

Table A.39 ANOVA table for the effect of cycle on the HMF level of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7,900	1	7,900	13,536	,002
Within Groups	10,506	18	,584		
Total	18,406	19			

Table A.40 ANOVA table for the effect of amplitude on the diastase number of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6,075	4	1,519	,063	,992
Within Groups	363,825	15	24,255		
Total	369,900	19			

Table A.41 ANOVA table for the effect of probe on the diastase number of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	115,200	1	115,200	8,141	,011
Within Groups	254,700	18	14,150		
Total	369,900	19			

Table A.42 ANOVA table for the effect of cycle on the diastase number of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	115,200	1	115,200	8,141	,011
Within Groups	254,700	18	14,150		
Total	369,900	19			

Table A.43 ANOVA table for the effect of amplitude on the L value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,531	4	,383	,385	,816
Within Groups	14,901	15	,993		
Total	16,432	19			

Table A.44 ANOVA table for the effect of amplitude on the a value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,299	4	7,464E-02	,109	,977
Within Groups	10,268	15	,685		
Total	10,566	19			

Table A.45 ANOVA table for the effect of amplitude on the b value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1,014	4	,253	,220	,923
Within Groups	17,293	15	1,153		
Total	18,307	19			

Table A.46 ANOVA table for the effect of probe on the L value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6,160	1	6,160	10,796	,004
Within Groups	10,271	18	,571		
Total	16,432	19			

Table A.47 ANOVA table for the effect of probe on the a value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5,832	1	5,832	22,174	,000
Within Groups	4,734	18	,263		
Total	10,566	19			

Table A.48 ANOVA table for the effect of probe on the b value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,318	1	,318	,318	,580
Within Groups	17,989	18	,999		
Total	18,307	19			

Table A.49 ANOVA table for the effect of cycle on the L value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3,136	1	3,136	4,246	,054
Within Groups	13,295	18	,739		
Total	16,432	19			

Table A.50 ANOVA table for the effect of cycle on the a value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,493	1	,493	,881	,360
Within Groups	10,073	18	,560		
Total	10,566	19			

Table A.51 ANOVA table for the effect of cycle on the b value of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9,577	1	9,577	19,748	,000
Within Groups	8,730	18	,485		
Total	18,307	19			

Table A.52 ANOVA table for the effect of amplitude on the final temperature of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	283,000	4	70,750	,589	,676
Within Groups	1802,750	15	120,183		
Total	2085,750	19			

Table A.53 ANOVA table for the effect of probe on the final temperature of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1674,450	1	1674,450	73,280	,000
Within Groups	411,300	18	22,850		
Total	2085,750	19			

Table A.54 ANOVA table for the effect of cycle on the final temperature of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	42,050	1	42,050	,370	,550
Within Groups	2043,700	18	113,539		
Total	2085,750	19			

Table A.55 ANOVA table for the effect of amplitude on the liquefaction time of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3852,700	4	963,175	1,440	,269
Within Groups	10034,250	15	668,950		
Total	13886,950	19			

Table A.56 ANOVA table for the effect of probe on the liquefaction time of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3200,450	1	3200,450	5,391	,032
Within Groups	10686,500	18	593,694		
Total	13886,950	19			

Table A.57 ANOVA table for the effect of cycle on the liquefaction time of ultrasound treated honey.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4176,050	1	4176,050	7,741	,012
Within Groups	9710,900	18	539,494		
Total	13886,950	19			