EFFECT OF DIFFERENT TYPES OF HEAT TREATMENT ON INVERTASE ACTIVITY IN HONEY

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Abstract

Honey is important food stuff, which is often consumed fresh or used in other meals. Liquid honey is more demanded in market, so solid honey often is heated in order to melt it. One of the most traditional ways to melt the honey is by heating using thermal treatment – usually in higher temperature than 50 °C. Using microwave ovens are very popular approach to heat food and its popularity is growing, so it is important to understand, how food and its substances are being influenced by microwave heating. One of the most heat-sensitive enzymes is invertase.

The aim of the research is to study and to compare, how invertase activity in honey is affected by heating it in the microwave oven and in the thermostat, while changing microwave oven power, heating duration and heating temperature in thermostat.

p-Nitrophenyl-α-D-glucopyranoside (pNPG) is used as a substrate for the determination of the invertase number in honey. pNPG is split into glucose and p-nitrophenol by α-glucosidase (invertase, sucrase). By adjusting the pH-value to 9.5 the enzymatic reaction is stopped and at the same time nitrophenol is transformed into the nitrophenolate anion, which corresponds to the amount of converted substrate and is determined photometrically at 400 nm.

Analyzing different honey samples, the following results were obtained. When honey samples were heated in 450 W microwave oven, invertase activity significantly decreased after 20 seconds. After 25 seconds invertase activity decreased 5.8 times, after 30 seconds – 12.5 times.

After heating honey samples in thermostat in 60 °C for 120 minutes, invertase activity decreased only twice.

Summarizing research results, we concluded, that invertase activity changes are significantly higher in honey samples, heated in the microwave oven, than honey samples, heated in the thermostat.

Keywords: Honey, invertase activity, thermal treatment, microwave.

Introduction

Food often gets the thermally treated to heat it. Food is heated faster in microwaves than in usual oven. Honey is one of most used foodstuff. Honey in food is used both raw and in confectionery products. In order to better sell honey it's often heated. A small amount of honey can be quickly heated using microwaves.

Microwaves are electromagnetic radiation in the wavelength range between 30 cm to 1 mm (1 GHz–300 GHz), by other data 1 m – 1 mm (300 MHz–300 GHz). Microwaves are essentially short radio waves and in the past they were not separated from radio waves; they are produced by high-frequency power generator, called magnetron (Electromagnetic Energy and Microwaves, 2013).

Microwave radiation intensity depends on the tubes that are used in device. There are separated high power and low power microwave radiation sources. Microwave transmitters and receivers are similar to radio wave transmitters and receivers. Microwave transmitter tubes contain tuned circuit, which is able to perceive or transmit certain frequency bands.

Product heating in microwave oven happens when high-frequency electromagnetic wave energy is transformed in thermal energy; in result products cooking time is two to three times lower than usual. Microwave principle of operation is to broadcast non-ionizing microwave radiation, usually 2.45 GHz, through food. Substances such as water, grease and other receive energy from microwaves in process called dielectric heating.

Many molecules, including water are polar – with a single positive charge on one side and negative charge on the other. Transmitting microwave radiation, water molecules continuously rotates, relative to the prevailing magnetic field that varies 2.45 million times per second. As rotation speed of water molecules increases, product temperature increases as well (Sapunov, 2007; Electromagnetic Energy and Microwaves, 2013).

Affecting food, such as honey, with microwaves denaturates proteins within it, changes vitamin content and also reduces the activity of enzymes (Sapunov, 2007; Hendrickson, 2011).

Honey is losing its biological and antibacterial activity and many of its component volatiles.

Some of the honey quality and biological activity indicators are the enzymes. Enzymes are complex proteins that catalyze reactions within cells. Enzymes have huge impact in human life processes. Honey naturally preserves small amounts of enzymes. Honey mostly contains enzyme amylase, glucose oxidase, catalase and invertase, and their contents can vary depending on the region in which honey is collected (Delaplane, Mayer, 2000).

Enzyme functions and catalyzed reactions determine honey composition and pH level and antibacterial properties.

One of honey quality indicator and thermal treatment indicator is enzyme invertase (Karabournioti, Zervalaki, 2001).

Table 1

Enzyme catalyzed reactions in honey
(Honey enzymes, 2014)

Enzyme in honey	Substratum	Reaction products
Invertase	Sucrose	Glucose and fructose
Glucose oxidase	Glucose	Gluconic acid and hydrogen peroxide
Catalase	Hydrogen peroxide	Water and oxygen
Amylase (Diastase)	Starch	Dextrose, maltose and glucose

Invertase is an enzyme which splits sucrose into glucose and fructose. Invertase activity is expressed as the invertase number or invertase units.

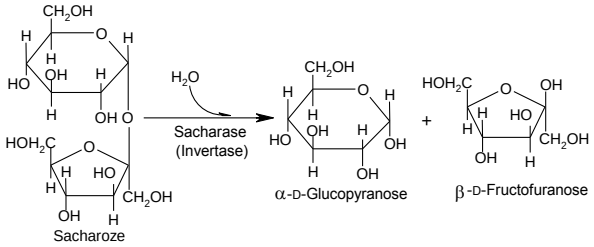

Figure 1. Reaction catalyzed by invertase
(Garret, Grisham, 2005)

Invertase unit is 1 micromole of substrate that is cleaved within 1 minute. Invertase number is the amount in grams of sucrose, which within 1 hour is hydrolyzed by invertase in 100 g honey (1 IN=7.344732 IU kg^{-1}). According to the European Commission's recommendations, invertase number should be higher than 10 (individual honey samples greater than 4) (Bogdanov, 2009; Honey Quality and International Regulatory Standarts, 2014).

The aim was to find out how honey treatment in microwave heating in a thermostat affect invertase activity and hence the quality of honey and its biological properties. To achieve the goal invertase activity were determined in honey samples.

Honey samples were heated in a thermostat, and also treated in microwave oven.

Materials and Methods

In research buckwheat flower honey samples collected in 2013 in Jelgava District Sesavas parish were analyzed. The analysis was performed at the Department of Chemistry of LUA laboratories.

Invertase activity in honey samples were determined spectrophotometrically using Jenway 6300 spectrophotometer.

Research of microwaves influence was analysed, using Samsung microwave oven.

p-Nitrophenyl-α-D-glucopyranoside (pNPG) is used as a substrate for the determination of the invertase number in honey. pNPG is split into glucose and p-nitrophenol by α-glucosidase (invertase, sucrase).

By adjusting the pH-value to 9.5 the enzymatic reaction is stopped and at the same time nitrophenol is transformed into the nitrophenolate anion, which corresponds to the amount of converted substrate and is determined spectrophotometrically at 400 nm (Bogdanov, 2009).

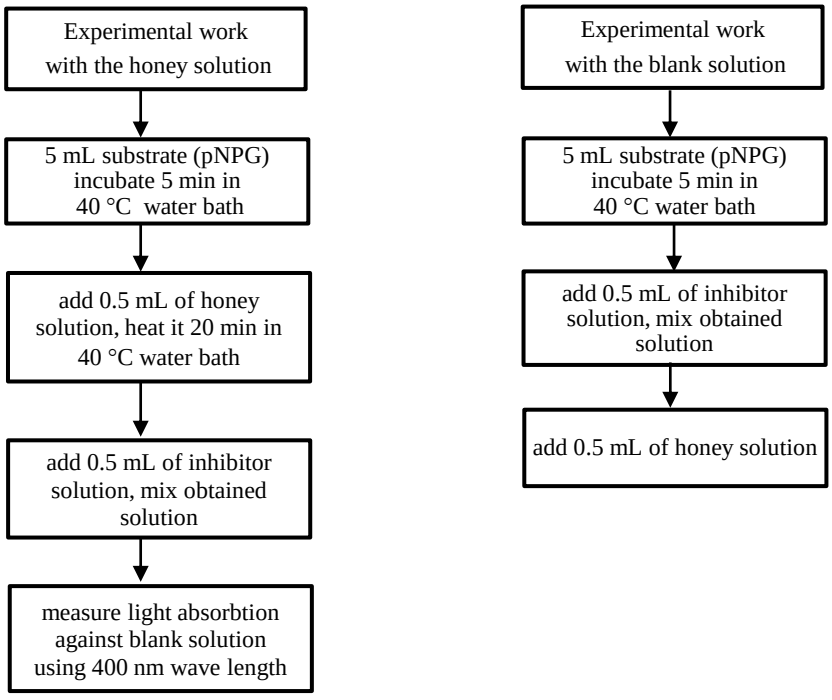

Figure 2. Overall determination scheme of invertase activity

Research on microwave radiation impact were made by treating honey samples in microwave oven 10 s using 180 W, 300 W, 450 W, 600 W in 800 W high power microwave. According from the results, the optimal choice of microwave intensity was chosen.
The thermostat heating temperature was selected by heating the honey samples for 1 h in 50 °C, 60 °C and 70 °C. Then, according from the results the optimal temperature was used in subsequent experiments.
The general methodology for determining invertase activity is shown in Figure 2.

Results and Discussion

Microwave oven power influence on invertase activity in honey is shown in Figure 3.

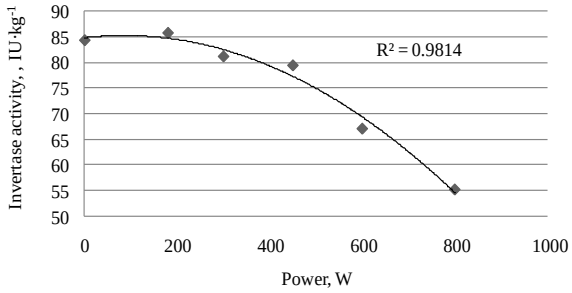

Figure 3. Changes of invertase activity in various microwave oven powers

Honey samples, using 180 W, 300 W, 450 W, 600 W, 800 W microwave oven power, were treated 10 s. As can be seen from the results of Figure 3, increasing microwave power, reduce the invertase activity in honey samples.
Raw honey sample have invertase activity of 85±8 IU, which indicates honey sufficiently high enzyme activity and thus biological - functional properties. This is consistent with the recommendations of the International Honey Commission (IN≥10; IU≥73.45).
To study microwave radiation duration effect on invertase activity, had to opt for microwave power.
It may be more accurate and more appropriate to do this by studying the dynamics of invertase activity decay times by reference to the fresh honey invertase activity.

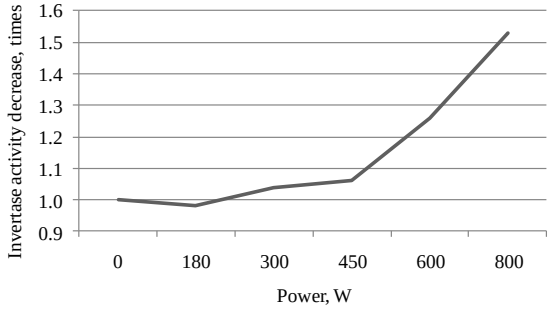

Figure 4. Correlation between decrease of invertase activity and microwave oven powers

As can be seen from the Figure 4 data, a significant decrease in invertase activity occurs from 450 W microwave oven power. Therefore, studies on the effects of microwave radiation on the invertase activity, depending on the microwave radiation exposure time, is carried out using 450 W microwave power.

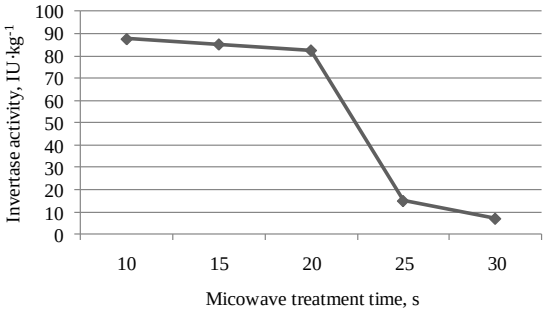

Figure 5. Changes of invertase activity depending on the duration of exposure to microwave radiation

Figure 5 shows that by treating honey samples in a microwave oven power of 450 W, significant changes of invertase activity started to happen after 20 seconds. After 25 seconds of treatment in a given microwave oven power invertase activity decreases 5.8 times, but when treated for 30 seconds – 12.5 times.
It means that significant honey quality changes occur, thus, protein denaturation occurs; it worsens the healing and anti-bacterial properties.
In order to judge the effect of microwave radiation, honey samples were also heated in thermostat using different heating temperatures and heating lengths.
In order to assess the heating effect of temperature on invertase activity in honey were heated for 1 hour at 50 °C, 60 °C and 70 °C.

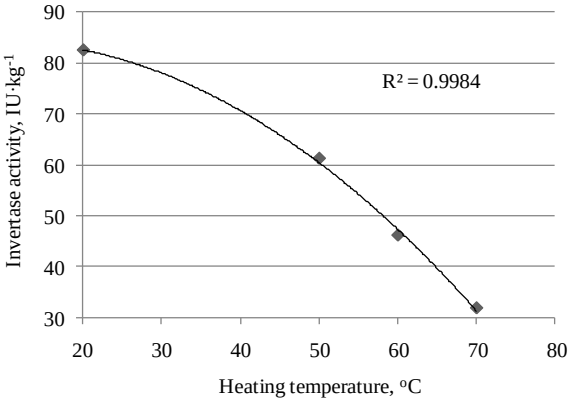

Figure 6. Effect of the heating temperature on invertase activity in honey

As can be seen from Figure 6, increasing the heating temperature, the invertase activity decreases.
In order to characterize the duration of the heating effect of honey on honey invertase activity in honey and correctly select the heating temperature, the

invertase activity change dynamics were characterized by invertase activity decay times relative to the fresh honey (uncooked) invertase activity.

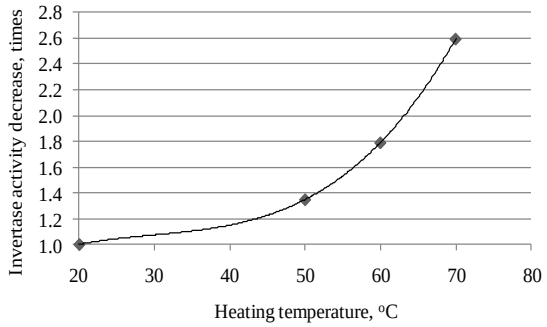

Figure 7. Correlation between decrease of invertase activity and heating temperature

As can be seen from Figure 7, invertase activity starts to decrease significantly from 50 °C temperature. In order to characterize the invertase activity of correlation between the duration of heating, the temperature of 60 °C was chosen because at this temperature for invertase activity has been greatly reduced. Invertase activity of correlation between the duration of the heat treatment is shown in Figure 8.

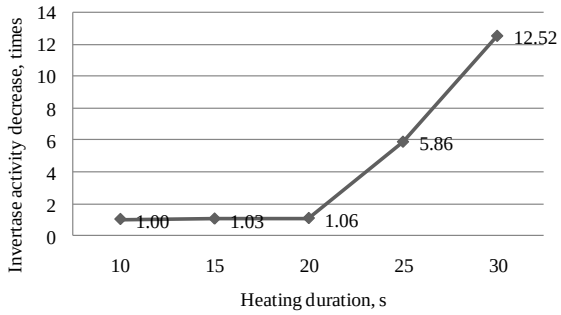

Figure 9. Decrease of invertase activity depending on heating time in microwave oven

As can be seen from the Figure 9 invertase activity changes in the microwave oven heated honey samples are insignificant if honey is heated less than 20 s, on the other hand, from the 20 s invertase activity changes occur very rapidly. On the other hand, if we compare the thermostat heated honey samples, they invertase activity gradually decreased from the beginning of the heating. However the invertase activity decrease is lower than in microwave oven heated honey. For example, at the end of experiment, in thermostat 2 hours heated honey invertase acitivity decreased 2 times (Fig. 10), however in 450 W microwave oven it decreased 5.86 times after 25 s and 12.52 times after 30 s heating.

That means if honey needs to be heated then it should be done using conventional heating. In microwave oven honey can be heated very short time period, and if honey is heated for 30 s or longer, then honey can completely lose its quality.

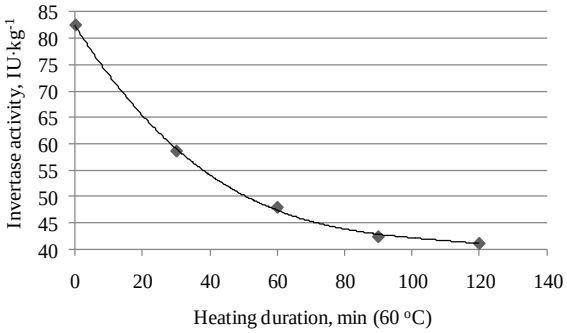

Figure 8. Correlation between invertase activity and the heating time of honey

As you can see from the Figure 8, honey invertase activity changes are not so great when compared to the invertase activity changes in honey samples heated in the microwave.

This is best demonstrated when comparing change in invertase activity decay times in a microwave oven and in a thermostat treated honey samples.

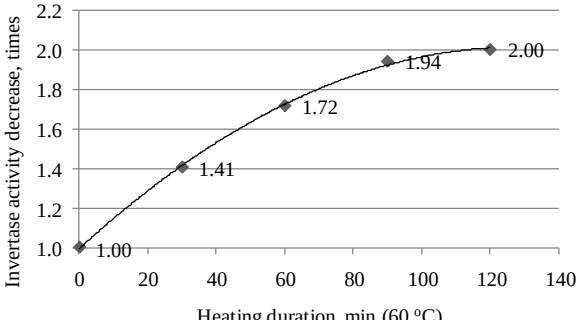

Figure 10. Decrease of invertase activity depending of heat treatment

Conclusions

Treating of the honey samples in a microwave oven, invertase activity changes occur much faster than heating it in an oven.

Treating of the honey samples in Samsung microwave oven significant invertase activity changes occurs if microwave oven power is higher than 450 W.

Treating of the honey samples in a microwave power of 450 W, a significant invertase activity changes occur after 20 s. After 25 s of treatment in a given power invertase activity decreases 5.8 times, while treating the 30 s – 12.5 times.

Heating of the honey samples in a thermostat at 60 °C for 2 h, invertase activity gradually decreases twice.

Treating of the honey samples in microwave oven significantly reduces the activity of biologically active substances.

In thermostat heated honey samples, significant invertase activity changes occurs if heating temperature exceed 50 °C.

Enzymes and other proteins denaturates – lose activity, if they are heated in thermostat or treated in microwave radiation.

The invertase number of analyzed honey samples (11.57) indicates the high quality of honey.

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