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Changes in α-amylase activity in honey during the freeze-drying process

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Abstract. Honey is a natural product, which is appreciated for its sweetness, high nutritional value and health benefits all over the world. Despite all benefits, the usage of honey in food industry is limited due to its high viscosity. The use of dried honey could be an alternative to liquid honey, and would allow to use it as an additive in a range of many different food products such as sauces, beverages, yogurts etc. There are many parameters, which are used to determinate the quality of honey. α-amylase (diastase) activity is one of the most important criteria to determine the quality and freshness of honey. The aim of the present study was to investigate and compare α-amylase activity in liquid honey samples and freeze-dried honey samples. Overall, 18 honey samples were dehydrated using a freeze-drying method. Freeze-drying of the samples was carried out at – 50.6 °C and the pressure was 0.036 mbar for 72 hours. α-amylase activity in the honey samples was tested using Amylazyme test tablets. The obtained results showed variability in α-amylase activity after the freeze-drying process. As hydroxymethylfurfural (HMF) is another important quality parameter of honey, the content of HMF was determined in the samples by high performance liquid chromatography. In some samples the concentration of HMF after freeze-drying increased and was higher than it is allowed according to the International Honey Commission (for example, 55.75 mg kg^{-1}).

Key words: α-amylase activity, freeze-drying, honey, HMF.

INTRODUCTION

Honey is a natural food product, which is well-known due to its sweetness, high viscosity, specific flavour and health improving properties (Ramsay et al., 2019). Honey is composed of approximately 200 substances (Geana & Ciucure, 2020). The main constituents of honey are monosaccharides (mainly fructose and glucose). It also contains a wide variety of minor components such as enzymes, amino acids, organic acids, vitamins, phenolic compounds and proteins (Azeredo et al., 2003). The qualitative and quantitative composition of honey depends on many factors such as their floral and geographical origin and climate, and processing (Da Silva et al., 2016).

Honey, as a supersaturated solution, tends to crystallize (Dettori et al., 2018). The crystallization of honey is a natural process. However, the process of crystallization can negatively affect the quality of honey. During the crystallization process, water activity increases and that can lead to yeast growth and unwanted fermentations (Tappi et al.,

2019). The most common way how to prevent the negative effects of crystallization is thermal processing (Ribeiro et al., 2018). Also, thermal processing can be used to transform liquid honey into powder by drying. Powdered honey provides many advantages: extended shelf-life, ease of packing and transporting, ease of use in food industry (Tong et al., 2010; Kılınç & Demir, 2017). Although honey in powder form is an alternative substitute to liquid honey, the production of pure honey powder is a complicated process. The high concentration of fructose and glucose does not allow to easily transform liquid honey in to powdered honey. Honey as a sugar-rich product tends to form lumps or syrup during the drying process (Umesh Hebbar et al., 2008). This problem has been solved by adding different types of carriers to increase the glass transition temperatures of fructose and glucose (Adhikari et al., 2001; Samborska et al., 2015).

Honey powder can be produced by different drying methods (Cui et al., 2008; Nurhadi & Roos, 2016; Sramek et al., 2016; Samborska et al., 2019). Spray drying is the most widely used method to convert liquid honey into powder (Shi et al., 2013). Freeze-drying also can be used as an alternative drying method to obtain honey-rich powder, but as a slow and expensive drying method it is rarely used for production of honey powder (Subramanian et al., 2007). Despite the methods expensiveness, it allows to produce high quality dry food products. During the freeze-drying process water is removed from a frozen product by sublimation (Prosapio & Norton, 2018).

The quality of honey as a food product is very important. The enzymatic activity of honey is one of the indicators to detect its freshness and quality (Kanar & Mazı, 2019). Honey contains various enzymes such as saccharase (invertase), glucose oxidase, catalase, peroxidase and α-amylase, which is the predominant enzyme in honey (Tosi et al., 2008). α-amylase activity and the concentration of hydroxymethylfurfural (HMF) are used together to evaluate the quality of honey. Usually the values of these parameters are used to indicate the intensity of heating during the processing of honey. According to Council of the European Union Directive (Codex Alimentarius, 2001) should not less than 8, expressed as diastase number DN, and the concentration of HMF should not exceed 40 mg kg^{-1}.

The aim of the present study was to investigate and compare α-amylase activity in liquid honey samples and freeze-dried honey samples.

MATERIALS AND METHODS

Honey samples

In this study, eighteen honey samples were used for freeze-drying. Ten honey samples were derived from Latvian beekeepers in 2018 and 2019 from different districts in Latvia. Four honey samples were purchased from a local supermarket in Jelgava, Latvia in 2018. Another four honey samples were purchased in local markets and supermarkets in Estonia, Italy, Hungary and Tajikistan in 2018 and 2019. The origin of honey samples shown in Table 1.

Table 1. Distribution of studied honey samples

Sample	Type of honey	Production year	Country	District
H1	Multifloral	2018	Latvia	Zemgale
H2	Multifloral	2018	Latvia	Zemgale
H3	Multifloral	2018	Latvia	Latgale
H4	Multifloral	2018	Latvia*	Unknown
H5	Multifloral	2018	Latvia*	Unknown
H6	Multifloral	2018	Latvia*	Unknown
H7	Multifloral	2018	Latvia	Vidzeme
H8	Acacia	2018	Tajikistan	Baljuvor
H9	Multifloral	2018	Latvia	Latgale
H10	Multifloral	2018	Latvia*	Unknown
H11	Forest flower	2019	Latvia	Kurzeme
H12	Buckwheat	2018	Latvia	Kurzeme
H13	Buckwheat	2019	Latvia	Zemgale
H14	Linden flower	2019	Latvia	Kurzeme
H15	Multifloral	2018	Latvia	Vidzeme
H16	Chestnut	2019	Italy	Sicily
H17	Multifloral	2019	Hungary*	Unknown
H18	Multifloral	2019	Estonia	Võrumaa

* – blend of European Union and non-European Union honeys.

Determination of pH and free acidity

Determination of pH and free acidity of liquid honey samples was carried out according to International honey standards (Ohe et al., 2000). 10 grams of honey sample were dissolved in 75 mL of carbon dioxide-free water. pH of prepared honey solutions was measured using pH-meter inoLab® pH7110 (WTW, Germany). Free acidity was determined by titrating the prepared honey solutions with 0.1M NaOH to pH 8.30. Free acidity of honey was expressed as milliequivalents acid kg^{-1} honey. It was calculated using an equation:

$$Free\ acidity = V \times 10 \quad (1)$$

where V – volume of 0.1M NaOH, which was consumed during the analysis, mL.

Determination of fructose and glucose, and hydroxymethylfurfural (HMF)

Sample preparation: 5 grams of liquid honey sample were weighted into a 100 mL beaker and dissolved in 30 mL deionized water. Dissolved material was quantitatively transferred into 50 mL volumetric flask and diluted with deionized water to the mark and inverted multiple times. Prepared sample solutions were centrifuged (Pro-Research, Centurion Scientific Ltd.) for 10 minutes at 10,000 rpm. The content of fructose and glucose was determined by HPLC using an analytical column SUPELCOSIL™ LC-NH_2 (4.6 mm×250 mm I.D., particle size 5 μm). Column and detector temperature were set to 30 °C. The mixture of acetonitrile (HPLC grade, Sigma-Aldrich) and water (HPLC grade) was used as a mobile phase. The ratio of acetonitrile and water was 80:20 (v/v). The analysis of the samples was carried out under isocratic conditions. Flow rate was 1 mL min^{-1}. Injection volume of 10 μL was performed using an autosampler SIL-20A. The retention times of obtained peaks of analysed samples were compared to the retention times of fructose (HPLC grade, Fluka) and glucose (HPLC grade, Fluka)

standard solutions. Chromatographic analysis of the samples was performed on Shimadzu LC-20 Prominence liquid chromatograph (Shimadzu USA Manufacturing Inc, Canby, USA) with a Shimadzu RID 10A Refractive Index detector. The obtained concentrations of fructose and glucose were expressed as g 100 g^{-1} dry matter.

The concentration of hydroxymethylfurfural (HMF) in the samples was determined by HPLC using an analytical column PerkinElmer C18 (4.6 mm × 250 mm I.D., particle size 5 μm). Column and detector temperature were set to 25 °C. The mixture of acetonitrile (HPLC grade, Sigma-Aldrich) and water (HPLC grade) was used as a mobile phase. The ratio of acetonitrile and water was 10:90 (v/v). The analysis of the samples was performed under isocratic conditions. Flow rate was 1.3 mL min^{-1}. Injection volume of 10 μL was performed using an autosampler SIL-20A. Detection of HMF was carried out at wavelength of 280 nm. The retention times of peaks were compared to the retention time of HMF (HPLC grade, Sigma-Aldrich) standard solution. Determination was performed on Shimadzu LC-20 Prominence liquid chromatograph (Shimadzu USA Manufacturing Inc, Canby, USA) with a Shimadzu DAD SPD-M20A detector. The obtained concentration of HMF was expressed as mg kg^{-1} dry matter.

Freeze-drying

Two types of formulations were prepared for freeze-drying experiments: 1) 20% aqueous solutions of honey and 2) 20% aqueous solutions of honey with maltodextrin (STAR-DRI® 10 NG, TATE & LYLE). The ratio of honey and maltodextrin was 1:2. All prepared solutions were poured into plastic freezer containers, and the initial solutions thickness were approximately 10 mm. The containers of solutions were frozen to -20 °C within 2 hours. Afterwards the pre-treatment procedure, the freeze-drying process was performed at an absolute pressure of 0.036 mbar. The temperature of ice condenser was set to -50.6 °C. The duration of the drying process was 72 hours. Freeze-drying was carried out using a freeze-dryer ALPHA 1-2 LDplus (MARTIN CHRIST Gefriertrocknungsanlagen GmbH, Germany).

Determination of moisture content

Moisture content of liquid honey and freeze-dried honey samples was determined using a moisture analyzer AND MX-50 (A&D Company, Limited, Japan). One gram of samples was weighted on glass fibre sheets and placed on the sample pan of moisture analyzer. The samples were heated up at a drying temperature of 140 °C. The time of analysis was set to 20 minutes. The software 'WinCT-Moisture' was used to record moisture data.

Determination of α-amylase activity

α-amylase activity was determined in liquid honey and freeze-dried honey samples using Amylazyme HY tablets (Megazyme, Ireland). Determination of α-amylase activity was carried out according to Amylazyme assay procedure. The absorbance of samples was measured using a spectrophotometer 6405 UV/Vis (JENWAY, the U.K.) at wavelength of 590 nm. The enzyme activity was calculated according to the following Eq. (2) and expressed as diastase number (DN):

$$Schade\ Units = 20.0 \times \Delta Abs \quad (2)$$

where ΔAbs – absorbance of the analysed samples at 590 nm.

Statistical analysis

All experiments were performed in triplicate. The obtained data were expressed as the mean ± standard deviation. The data were processed using MS Office Excel 2016.

RESULTS AND DISCUSSION

Characterization of honey samples

The chemical composition of honey samples was investigated before the drying process. Overall, 18 honey samples were used for freeze-drying experiments. The main characteristic properties of used honey samples represented in Table 2.

Table 2. Chemical composition of honey samples

Sample	α-amylase activity, DN	Moisture, %	Fructose, g 100 g^{-1}	Glucose, g 100 g^{-1}	pH	Free acidity, meq kg^{-1}	HMF, mg kg^{-1}
H1	15.3 ± 0.5	14.8 ± 0.3	34.4 ± 0.3	33.2 ± 0.4	4.12 ± 0.01	23.7 ± 0.3	5.7 ± 0.4
H2	7.6 ± 0.1	12.2 ± 0.4	33.5 ± 0.4	37.7 ± 0.4	4.29 ± 0.01	9.6 ± 0.1	4.7 ± 0.3
H3	18.5 ± 0.3	20.5 ± 0.2	36.4 ± 0.2	31.8 ± 0.2	3.66 ± 0.01	42.0 ± 1.0	41.4 ± 0.5
H4	3.8 ± 0.1	15.2 ± 0.3	39.9 ± 0.4	31.3 ± 0.3	4.59 ± 0.03	10.7 ± 0.3	9.1 ± 0.6
H5	7.2 ± 0.5	18.2 ± 0.6	35.4 ± 0.4	35.1 ± 0.5	3.92 ± 0.01	29.3 ± 0.3	55.8 ± 0.3
H6	3.7 ± 0.1	12.9 ± 0.5	38.7 ± 0.4	30.4 ± 0.5	4.49 ± 0.07	8.8 ± 0.3	11.0 ± 0.2
H7	20.9 ± 0.6	14.1 ± 0.4	34.1 ± 0.4	33.0 ± 0.4	4.34 ± 0.01	22.8 ± 0.3	1.6 ± 0.4
H8	10.6 ± 0.2	14.1 ± 0.5	37.9 ± 0.4	42.5 ± 0.5	3.89 ± 0.01	24.3 ± 0.3	18.7 ± 0.5
H9	25.4 ± 0.8	17.0 ± 0.3	35.8 ± 0.4	37.1 ± 0.5	3.75 ± 0.01	37.7 ± 0.3	21.3 ± 0.4
H10	4.7 ± 0.2	17.2 ± 0.3	40.2 ± 0.5	35.1 ± 0.5	4.53 ± 0.01	5.3 ± 0.3	66.0 ± 0.3
H11	26.1 ± 0.1	15.0 ± 0.3	43.6 ± 0.5	36.5 ± 0.4	4.28 ± 0.01	26.7 ± 0.3	2.4 ± 0.4
H12	27.7 ± 0.2	17.9 ± 0.4	47.7 ± 0.4	37.8 ± 0.4	3.89 ± 0.01	44.6 ± 0.6	8.3 ± 0.3
H13	28.1 ± 0.1	16.5 ± 0.5	47.0 ± 0.4	39.6 ± 0.4	3.88 ± 0.01	41.5 ± 0.5	1.9 ± 0.2
H14	23.7 ± 0.7	15.0 ± 0.3	44.1 ± 0.3	42.7 ± 0.4	3.99 ± 0.04	20.8 ± 0.8	0.8 ± 0.1
H15	28.0 ± 0.1	16.5 ± 0.3	46.3 ± 0.4	37.6 ± 0.3	3.84 ± 0.01	44.5 ± 0.1	8.0 ± 0.1
H16	21.3 ± 0.8	18.3 ± 0.2	45.5 ± 0.4	30.0 ± 0.3	4.62 ± 0.02	36.3 ± 0.3	2.5 ± 0.2
H17	10.5 ± 0.3	14.6 ± 0.4	45.4 ± 0.4	38.4 ± 0.2	3.88 ± 0.01	22.8 ± 0.3	5.3 ± 0.2
H18	22.2 ± 0.6	15.7 ± 0.4	45.2 ± 0.4	40.5 ± 0.3	4.20 ± 0.01	20.2 ± 0.3	0.7 ± 0.1

α-amylase activity and the content of HMF were used to detect the quality of the honey samples. The obtained data of liquid honey samples showed that α-amylase (diastase) activity in 13 of 18 analysed samples were higher than 8. The value of DN in samples H2, H4, H5, H6, H10 was less than 8. These samples, which showed poor α-amylase activity, were blends of European Union and non-European Union honeys, except the sample H2, (Table 1). The differences in α-amylase activity might vary as the enzymatic activity of honey depends on the age of bees, the physical state of the colony, the nectar harvesting period. Also, the profusion of nectar flow can impact the content of enzymes in honey. Large quantity of nectar flow can lead to a lower α-amylase activity in honey (Persano Oddo et al., 1999; Pasias et al., 2017). The content of HMF in the samples ranged from 0.7 to 66.0 mg kg^{-1}. The samples H5 and H10 showed the highest concentrations of HMF (55.8 ± 0.3 mg kg^{-1} and 66.0 ± 0.3 mg kg^{-1}), which were higher than it is allowed in the European Union (Codex Alimentarius, 2001). The

samples H5 and H10 were categorized as low-quality honeys due to their low enzymatic activity and high content of hydroxymethylfurfural.

Freeze-drying of honey

In this study, dehydration of honey was performed by freeze-drying. During the freeze-drying process water was removed from frozen honey solutions by sublimation. This drying technique is gentle and allows to preserve bioactive compounds during the drying process. The dehydration of honey was carried out using two kind of formulations. After 72 hours of freeze-drying the moisture content in the samples decreased (Fig. 1). The highest content of moisture was in the samples, which were prepared as 20 % honey solutions. These samples were not stable and within a few hours rehydrated from the moisture in the atmosphere. Honey is a sugar-rich natural product, which contains low molecular weight sugars such as fructose, glucose and sucrose. The high concentration of these sugars makes it impossible to freeze-dry honey without adding carriers or drying aids (Bhandari et al., 1997). In this case, 20% honey solutions with maltodextrin (MD) were prepared. The ratio of honey and maltodextrin was 1:2. Maltodextrin is a natural polymer with a high molecular weight, increases the glass transition temperature of drying particles and reduces hygroscopicity (Adhikari et al., 2001). The addition of maltodextrin is common practice to obtain dry sugar-rich food products by spry-drying. After freeze-drying the samples, which were prepared as 20 % honey solutions with maltodextrin (MD), did not absorb the moisture from the atmosphere. The results showed that honey in a powder form was obtained by adding maltodextrin (drying aid) to honey. The moisture content in these samples ranged from 4.1 to 6.4 % and did not increase within weeks.

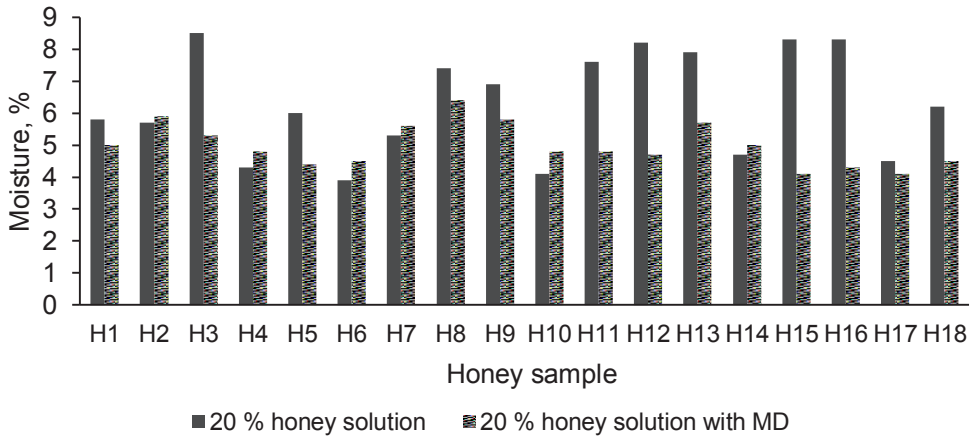

Figure 1. Moisture content of freeze-dried honey samples.

According to study results reported by Sramek and his co-workers (Sramek et al., 2016), they obtained honey powder by the freeze-drying technique. In their study they performed freeze-drying of honey solution in combination with glucose syrup. The final water content of freeze-dried honey powder was 3.1%.

α-amylase activity and the concentration of HMF were determined to detect the quality of obtained freeze-dried honey samples. The obtained results of α-amylase activity were variable (Table 3). There was noted increase of diastase activity in almost all samples after freeze-drying of honey solution. Freeze-drying of honey solutions with maltodextrin (MD) resulted in decrease of α-amylase activity in the most of analysed samples. The obtained results did not clarify the impact of freeze-drying to the enzyme activity. Unfortunately, there is lack of literature data on α-amylase activity changes in honey during the freeze-drying process, which could be used for a comparison of results. The changes of α-amylase (diastase) activity were investigated mainly in studies, where dehydration of honey was performed by spray-drying. In the research, diluted honey solution with Arabic gum was spray-dried. The authors of the research observed reduction of α-amylase activity (Samborska et al., 2017). Sramek and his co-workers stated that diastase (α-amylase) activity as indicator was less suitable and less sensitive to detect the quality of honey during the thermal processing at low temperatures (White Jr. et al., 1964; Sramek et al., 2017).

Table 3. α-amylase activity and HMF concentration in the freeze-dried honey samples

Sample	Freeze-dried honey solution		Freeze-dried honey solution with MD	
	α-amylase activity, DN	HMF, mg kg^{-1}	α-amylase activity, DN	HMF, mg kg^{-1}
H1	16.9 ± 0.2	39.1 ± 0.3	10.7 ± 0.3	48.0 ± 0.4
H2	10.1 ± 0.1	30.0 ± 0.4	8.1 ± 0.4	47.9 ± 0.5
H3	16.6 ± 0.1	32.7 ± 0.2	10.0 ± 0.3	97.0 ± 0.2
H4	4.4 ± 0.4	47.7 ± 0.3	6.3 ± 0.1	54.6 ± 0.1
H5	7.2 ± 0.3	197.2 ± 0.6	6.8 ± 0.7	98.3 ± 0.5
H6	3.9 ± 0.1	58.2 ± 0.5	5.4 ± 0.2	54.1 ± 0.9
H7	21.8 ± 0.4	20.4 ± 0.4	16.9 ± 0.1	40.1 ± 0.2
H8	13.2 ± 0.6	60.0 ± 0.5	10.0 ± 0.4	55.0 ± 0.5
H9	24.2 ± 0.8	55.9 ± 0.3	18.9 ± 0.9	46.0 ± 0.3
H10	5.2 ± 0.2	119.8 ± 0.3	7.7 ± 0.5	75.9 ± 0.5
H11	26.9 ± 0.1	7.3 ± 0.3	22.4 ± 0.4	33.3 ± 0.4
H12	27.6 ± 0.1	53.8 ± 0.4	26.1 ± 0.2	51.1 ± 0.2
H13	28.2 ± 0.1	17.0 ± 0.5	32.7 ± 0.1	36.7 ± 0.1
H14	23.7 ± 0.1	10.0 ± 0.3	16.1 ± 0.9	34.3 ± 0.4
H15	27.7 ± 0.1	66.9 ± 0.3	28.3 ± 0.5	45.8 ± 0.2
H16	22.7 ± 0.4	30.7 ± 0.2	15.1 ± 0.4	40.5 ± 0.3
H17	10.9 ± 0.3	46.8 ± 0.4	8.9 ± 0.3	45.0 ± 0.6
H18	22.7 ± 0.1	9.6 ± 0.4	16.1 ± 0.1	35.1 ± 0.3

The content of HMF is used as an indicator along with α-amylase activity to detected thermal processing of honey. The content of this chemical compound was also evaluated in the freeze-dried samples (Table 3). The highest concentration of HMF was detected in the samples H5 and H10. The formation of hydroxymethylfurfural in honey is unpreventable, as it is mainly composed of fructose and glucose. This heterocyclic organic compound is formed by dehydration of fructose and glucose, which is acid catalysed reaction. Also, pH, low temperature and moisture content are the factors, which catalyses the formation of HMF in honey (Tosi et al., 2004; Stöbener et al., 2019). The content of HMF in the freeze-dried honey samples H1, H2, H3, H7, H13, H14, H16,

H18, which were obtained from diluted honey solutions without maltodextrin, was not higher than it is allowed in the European Union countries. The detected concentration of HMF in the sample after freeze-drying was lower than the initial concentration of HMF. This unusual observation could be caused by high hygroscopicity of the sample. As it was noticed that all samples, which were freeze-dried without adding maltodextrin, were not stable and absorbed the moisture from the atmosphere within a few hours. All other samples showed a very typical increase of HMF after the drying process. The samples H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H12, H15 and H17, which were obtained by drying diluted honey solutions with maltodextrin, contained higher concentration of HMF than it is regulated (Codex Alimentarius, 2001). The observed results showed that concentration of hydroxymethylfurfural in most of the analysed samples after freeze-drying was higher than initial concentration of HMF.

CONCLUSIONS

The present study showed that freeze-drying can be used to obtain dehydrated honey. The samples, which were prepared adding maltodextrin as a drying aid, showed better stability against the moisture in the atmosphere. Unfortunately, α-amylase activity in freeze-dried honey was not suitable tool to detect the enzymatic activity of the samples correctly. The freeze-dried samples, which were obtained without adding maltodextrin, contained HMF in the allowed levels.

Further studies are needed to examine freeze-drying technique as another alternative method of obtaining high quality honey-rich powders. In further studies, there should be improved some freeze-drying conditions to optimise the lyophilization process. The optimisation of freeze-drying would allow to produce high-quality honey-rich powder, that could increase the usage of honey in food industry.

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