

Saccharose degradation over time in stored honey: influence of time, temperature, enzyme activity and botanical origin

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Summary

During the ripening process of honey, saccharose is converted in the bee hive to fructose and glucose by the enzyme invertase. The maximum content of saccharose in honey is limited by the European legislation. However, honey samples might show elevated levels of saccharose even though other quality parameters indicate that the ripening process did proceed normally. Honey samples with a saccharose content between 3.5% and 17% were analysed for conditions optimal for degradation of saccharose in stored honey. The honey samples were incubated at 15 °C, 21 °C and 37 °C for at most 9 months. After 9 weeks at 37 °C, degradation of saccharose was completed with a mean degradation rate of 3.42% per week, but invertase activity was reduced by almost 40%. At 21 °C, the average rate of degradation was 1.4% per month. At 15 °C, the process was slowed down to 0.81% saccharose per month. Analysis of saccharose degradation revealed a significant correlation to invertase activity ($p < 0.01$), but also to saccharose content ($p < 0.001$) and pH ($p < 0.01$). The results of this investigation can be used to instruct beekeepers how to handle honey with naturally elevated saccharose content to preserve a natural product conforming to legislation.

Keywords

honey; saccharose; invertase activity

Consumers are more and more conceived with the quality of the food they are consuming. Hence, quality control and quality evaluation of food is of increasing importance. Quality control of honey is performed to determine the degree of ripeness and the botanical origin, as well as to identify heat damage and adulteration. Ripeness is defined as the degree of processing of the honey by the bees [1, 2]. However, nectar quality and source, as well as environmental factors determine the initial conditions. High content of saccharose and moisture, low enzyme activities and low content of the amino acid proline are presumed to be indicators of immature honey. However, high content of saccharose is also one of the criteria for the suspicion of honey adulteration [3–5].

Saccharides are the main components of honey and make up 60–80% of the ingredients. The content of saccharides in the nectar is influenced by many parameters like conditions, age and size of the flowers, as well as environmental factors like air and ground humidity, soil properties and climate [4; 6–8]. The predominant monosaccharides

fructose and glucose derive directly from the nectar or are products of enzymatic cleavage of saccharose during honey ripening process in the hive. The ratio of the three most important saccharides in the nectar, fructose, glucose and saccharose, depends mainly on the floral source [9]. The saccharose proportion varies between 0% and about 75% of the total saccharides. Nectar exhibiting high saccharose levels is preferably collected by foraging bees, compared to nectar containing predominantly fructose and glucose [10].

During the ripening process of honey in the bee hive, most of the saccharose is degraded by the enzyme invertase (alpha-glucosidase, EC 3.2.1.20), usually to fructose and glucose, so that only a very low level of non-converted saccharose remains [11, 12]. Invertase is added to the ripening honey by the bees, being a product of their hypopharyngeal glands [4, 13]. Since invertase is a thermosensitive enzyme, its activity in harvested honey can also be used as an indicator of heat damage or inadequate storage [14]. Many investigations were performed to analyse the kinetics of invertase and

its sensitivity to temperature [15–19]. However, the conditions of saccharose degradation in honey still remain elusive.

Various investigations reported on the antimicrobial activity, antioxidant properties [20–24] and wound-healing effects of honey [25]. The parameters responsible for this special application of honey are also dependent on the processing and storage of honey [26].

According to EU-Council Directive 2001/110/EC, honey containing more than 5% saccharose is not marketable with the exception of some unifloral honeys for which 10% is allowed [27]. The exception reflects the fact that the saccharide spectrum of honey depends on the botanical source [28, 29]. In the area of investigation (Eastern part of Germany), this exception is only valid for robinia honey. However, because the enzyme invertase is still active in the collected honey, proper storage of the honey might help to reduce the level of saccharose even after harvesting.

It is known that the content of saccharose in honey is sometimes inexplicably high. In Eastern Germany, this happens for honey containing nectar predominantly from *Robinia pseudoacacia*, *Tilia* spp. or *Centaurea cyanus* during dry and hot summers. The causes are unknown but climatic factors like temperature and rainfall, or massive honey flow, seem to have an impact on this phenomenon, rather than adulteration of honey with syrup or bee food by beekeepers. In this honey, other quality parameters like moisture and enzyme activity confirmed that, at the time of honey collection, the ripening process of these samples was already completed.

The aim of the study was to analyse conditions optimal for degradation of saccharose in stored honey in order to be able to give advice to beekeepers how to achieve a lower content of saccharose in otherwise unmarketable honey. For this purpose, it was necessary to study the parameters that naturally influence saccharose degradation in honey to avoid artificial processing of honey and preserve the natural character of this bee product.

MATERIALS AND METHODS

Honey samples

All honey samples were from local beekeepers and subjected to quality control and determination of the botanical origin (Tab. 1). Honey samples containing elevated levels of saccharose were collected over three years and stored at $-18\text{ }^{\circ}\text{C}$ to avoid changes of the components.

Analytical methods

The saccharides were determined by Fourier transformation infrared spectroscopy (FTIR) [30]. In case of high content of saccharose, the analysis of saccharides was additionally validated according to Din-Norm-10758 or recommendations by the International Honey commission [31–33] by HPLC.

Determination of invertase activity, contents of 5-hydroxymethylfurfural (HMF), physical properties and melissopalynological analysis were performed according to the German DIN standards [34–38] or recommendations by the International Honey Commission [32; 33].

Production of honey – saccharose mixtures and conditions of storage

Experimentally elevated levels of saccharose were produced by addition of saccharose (D(+)) saccharose for biochemical use, Roth, Karlsruhe, Germany) to honey of different botanical origin (Tab. 1). Saccharose was added (10% w/w) at stirring. In order to dissolve the crystals, the mixture was warmed up carefully, but the honey was not liquefied. Subsequently, content of saccharose and invertase activity were determined. Aliquots of the mixtures were stored in an incubator at $37\text{ }^{\circ}\text{C}$ for 1–9 weeks, on the shelves at room temperature ($21\text{ }^{\circ}\text{C}$ on average) for 27–42 weeks, and at $15\text{ }^{\circ}\text{C}$ (on average) in the basement for 27–42 weeks. The honeys were sampled several times, and content of saccharose as well as invertase activity were analysed again. From each honey, samples of 100g were taken at intervals of 1 week in the first month, then samples were taken monthly.

The experiment was performed twice: First with different honey types, different quantities of saccharose and variable invertase activity (Tab. 1, No-1), and then with more honey types, different quantities of saccharose and similar invertase activity (Tab. 1, No-2; mean $77.3\text{ U}\cdot\text{kg}^{-1}$).

For statistical evaluations, SPSS version 18 (IBM, Ehningen, Germany) and MS Excel 2007 (Microsoft, Redmond, Washington, USA) were used.

RESULTS AND DISCUSSION

Honey samples with naturally occurring elevated levels of saccharose showed a saccharose content between 4.5% and 17%. Palynological analysis of these honeys revealed that they were mainly multifloral honeys with variable parts from *Robinia pseudoacacia*, *Centaurea cyanus* or *Tilia* spp. Additionally, saccharose was added artificially to

Tab. 1. Initial content of saccharose and invertase activity of samples.

No.	Botanical origin of the honey	Saccharose (initial) [%]	Saccharose (6 months, $T = 21\text{ }^{\circ}\text{C}$) [%]	Invertase [$\text{U}\cdot\text{kg}^{-1}$]	HMF (1 week, $T = 37\text{ }^{\circ}\text{C}$) [$\text{mg}\cdot\text{kg}^{-1}$]
R1-1 ^a	Robinia (<i>Robinia pseudoacacia</i>)	12	5.09	44	
R2-1 ^a	Robinia (<i>Robinia pseudoacacia</i>)	13	4.53	66	
R3-1 ^a	Robinia (<i>Robinia pseudoacacia</i>)	8.8	2.38	50	
R4-1 ^a	Robinia (<i>Robinia pseudoacacia</i>)	9	2.81	75	
R5-1 ^a	Robinia (<i>Robinia pseudoacacia</i>)	17	6.31	15	
R6-2 ^b	Robinia (<i>Robinia pseudoacacia</i>)*	9.5	0	76	0
R7-2 ^b	Robinia (<i>Robinia pseudoacacia</i>)	6	1.38	80	0.3
P1-1 ^a	Rape (<i>Brassica napus</i>)*	8	0.05	74	
P2-1 ^a	Rape (<i>Brassica napus</i>)*	9.6	1.02	129	
P3-2 ^b	Rape (<i>Brassica napus</i>)*	9.2	1.08	71	0
P4-2 ^b	Rape (<i>Brassica napus</i>)*	8.3	0	87	3.3
T1-1 ^a	Tilia (<i>Tilia</i> spp.)	10	1.8	56	
T2-1 ^a	Tilia (<i>Tilia</i> spp.)*	10.7	1.85	131	
T3-2 ^b	Tilia (<i>Tilia</i> spp.)*	8.5	0.84	78	2.4
T4-2 ^b	Tilia (<i>Tilia</i> spp.)*	9.4	1.26	71	2.2
T5-2 ^b	Tilia (<i>Tilia</i> spp.)*	13.7	0	58	0
T6-2 ^b	Tilia (<i>Tilia</i> spp.)	7.6	0	64	0
T7-2 ^b	Tilia (<i>Tilia</i> spp.)	10.9	0	66	0
T8-2 ^b	Tilia (<i>Tilia</i> spp.)	14.8	1.91	67	0
T9-2 ^b	Tilia (<i>Tilia</i> spp.)	8.6	0	94	0
T10-2 ^b	Tilia (<i>Tilia</i> spp.)	4.5	0	99	0
C1-2 ^b	Cornflower (<i>Centaurea cyanus</i>)*	9.1	4.62	75	2.6
C2-2 ^b	Cornflower (<i>Centaurea cyanus</i>)	6.3	1.06	77	1.1
C3-2 ^b	Cornflower (<i>Centaurea cyanus</i>)	7.1	0	130	1.6
C4-2 ^b	Cornflower (<i>Centaurea cyanus</i>)	8.6	0	163	1.6
U1-2 ^b	Sunflower (<i>Helianthus annuus</i>)*	9.8	6.02	68	2.8
H1-1 ^a	Heather (<i>Calluna vulgaris</i>)*	7.7	0.05	131	
H2-2 ^b	Heather (<i>Calluna vulgaris</i>)*	8.2	0.88	77	2.8
H3-2 ^b	Heather (<i>Calluna vulgaris</i>)*	8	0	84	1.4
D1-1 ^a	Honeydew*	6.3	0.05	112	
D2-2 ^b	Honeydew*	5.2	3.82	97	2.5
D3-2 ^b	Honeydew*	7.8	0	95	4.7
F1-1 ^a	Multifloral	10.2	2.77	55	
F2-1 ^a	Multifloral	8	0.98	99	
F3-1 ^a	Multifloral*	8.4	4.97	53	
F4-1 ^a	Multifloral*	9.5	0.05	148	
F5-1 ^a	Multifloral*	8.2	0.05	203	
TM1-2 ^b	Tilia/Multifloral	6.5	0	70	0.3
TM2-2 ^b	Tilia/Multifloral	6.5	0.1	70	0.7
PM1-2 ^b	Rape/Multifloral	6.1	0	74	0.7
PM2-2 ^b	Rape/Multifloral	5.4	0	78	0.8
UM1-2 ^b	Sunflower/Multifloral	6.4	1.36	69	2.3
HM1-2 ^b	Heather/Multifloral	5.9	0.77	71	2.5
HM2-2 ^b	Heather/Multifloral	6.1	0	79	1.5
DM1-2 ^b	Honeydew/Multifloral	3.5	0	86	1.2
DM2-2 ^b	Honeydew/Multifloral	5.6	0.45	84	3.1
RM1-1 ^a	Robinia/Multifloral	6.8	0.14	142	
RM2-1 ^a	Robinia/Multifloral	8.1	0.21	123	
PR1-1 ^a	Rape/Robinia	6.8	0.07	104	

* – saccharose added; a – experimental part 1; b – experimental part 2; HMF – 5-hydroxymethylfurfural.

honey or honey samples without saccharose were mixed with such at elevated levels (Tab. 1). The experimentally elevated saccharose content ranged between 3.5% and 13.7%.

The resulting content after adding the saccharose crystals to the samples were lower than 10% (Tab. 1). Since the samples were warmed for dissolving and even distribution of the saccharose crystals, degradation during mixing and stirring might have occurred.

All of the samples were analysed for various physical-chemical parameters (Tab. 2). The pH values ranged from 3.88 to 5.75, the electrical conductivity from 0.11 mS·cm⁻¹ to 0.96 mS·cm⁻¹ and free acidity from 1 meq·kg⁻¹ to 22 meq·kg⁻¹, depending on the floral source. Analysis of the invertase activity and saccharose degradation revealed a significant correlation ($p < 0.01$; Tab. 3). Therefore, we performed a second experimental approach with honey samples having similar invertase activity, to elucidate the effects of other parameters.

After 5 weeks at 37 °C, the saccharose content in samples with an initial level of saccharose of less

than 10% was lower than 5% (legal limit). The degradation was completed after 9 weeks in all samples. The curve of degradation was logarithmic with a correlation coefficient of $R^2 = 0.96$ (Fig. 1). The mean degradation rate was 3.42% per week at a mean enzyme activity of 87 U·kg⁻¹ (Tab. 2). Because of the high temperature, only about 50% of the initial invertase activity was measurable after 9 weeks (Fig. 2). Since other enzymes contained in honey (e.g. α -amylase; EC 1.2.1.1, glucose oxidase, EC 1.1.34) are also sensitive to high temperatures, honey quality would deteriorate at these conditions. The activity rates of all enzymes would fall below the levels prescribed by EU [27], while content of HMF, another indicator for heating of honey, would increase like demonstrated by others [15, 39–42].

In the second experimental approach, the honey samples were probed after one week at 37 °C. Invertase activity was already reduced (14 ± 9.74 U·kg⁻¹; mean \pm standard deviation), but the content of HMF did not increase considerably. Content of HMF was lower than 5 mg·kg⁻¹ in all samples like in fresh, untreated honey,

Tab. 2. Descriptive statistics of the analysed samples.

	<i>n</i>	Minimum	Maximum	Mean	<i>SD</i>
Initial invertase [U·kg ⁻¹]	49	15.0	203.0	86.95	33.76
Initial saccharose [%]	49	3.5	17.0	8.40	2.59
Decrease of saccharose per week ($T = 37$ °C) [%]	46	1.2	6.9	3.42	1.17
Decrease of saccharose per month ($T = 21$ °C) [%]	49	0.5	2.63	1.40	0.48
Decrease of saccharose per month ($T = 15$ °C) [%]	45	0.23	1.46	0.81	0.31
pH	49	3.88	5.75	4.56	0.45
Electrical conductivity [mS·cm ⁻¹]	49	0.11	0.96	0.40	0.22
Free acidity [meq·kg ⁻¹]	49	1.0	22.0	5.82	5.44

SD – standard deviation.

Tab. 3. Pearson's correlation coefficient and significance of the degradation process depending on time and temperature.

Saccharose degradation per month [%]	<i>n</i>	Initial invertase activity [U·kg ⁻¹]		Initial saccharose [%]		pH		Electrical conductivity [mS·cm ⁻¹]	
		<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
All samples, $T = 21$ °C	49	0.364	< 0.01	0.494	< 0.001	0.337	< 0.05	–	–
3. month, $T = 21$ °C	45	0.378	< 0.01	0.349	< 0.05	0.479	< 0.001	0.302	< 0.05
4. month, $T = 21$ °C	42	0.351	< 0.05	0.395	< 0.01	0.419	< 0.01	–	–
6. month, $T = 21$ °C	29	–	–	0.688	< 0.001	0.545	< 0.01	–	–
9. month, $T = 21$ °C	11	–	–	0.873	< 0.001	–	–	–	–
All samples, $T = 15$ °C	49	0.36	< 0.01	–	–	0.49	< 0.05	–	–

r – correlation coefficient, *p* – significance.

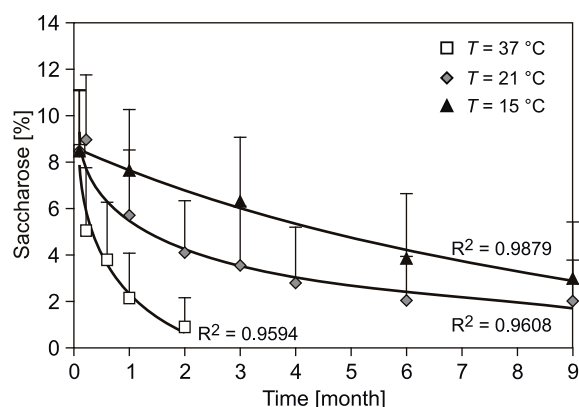


Fig. 1. Saccharose degradation rate at different temperatures and mean invertase activity of $87 \text{ U} \cdot \text{kg}^{-1}$.

Mean, standard deviation and trend lines with coefficient of correlation (R^2), $n = \{46, 49, 45\}$.

showing that this parameter was less sensitive for heat treatment than the enzyme activity (Tab. 1). Therefore, the most important point for all temperature manipulations is to maintain the enzyme activity.

At about 21°C , the saccharose content was still higher than 5% in 20% of samples after three months, depending on starting conditions. All of these samples showed a low invertase activity ($< 70 \text{ U} \cdot \text{kg}^{-1}$). After 6 months, in only 2 of these samples remained the content higher than 5% (legal limit). The curve of degradation was logarithmic with a correlation coefficient of $R^2 = 0.96$ (Fig. 1). The average rate of degradation was 1.4% per month at $87 \text{ U} \cdot \text{kg}^{-1}$ (Tab. 2), which was much lower than at 37°C . At this temperature, the invertase activity was less affected. After 25 weeks, about 80–90% (Fig. 2) and after 41 week about 60–70% of the initial activity was measurable (data not shown).

Even at the lowest temperature (15°C) and low enzyme activity, saccharose was converted. However, the process was slowed down compared to the higher temperatures. The average degradation rate was 0.81% saccharose per month at $87 \text{ U} \cdot \text{kg}^{-1}$ of invertase activity (Tab. 2). In 16% of samples, more than 5% saccharose was measurable after 9 months. These samples were in most cases unifloral robinia honey, which has a higher limit for saccharose (10%, EU [27]). The degradation process behaved exponentially, the correlation coefficient being $R^2 = 0.99$ (Fig. 1). A temperature of 15°C was optimal for conservation of the enzyme activity, 90% of the initial activity being preserved after 6 months (Fig. 2).

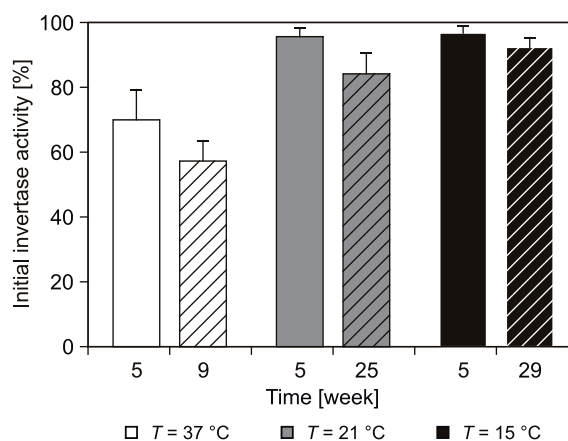


Fig. 2. Remaining invertase activity at different times and temperatures of storage.

Mean and standard deviation ($n = 16$).

We have also taken into account the effect of physical-chemical parameters on saccharose degradation (Tab. 3). At 21°C , the degradation rate of all samples showed a positive correlation to initial invertase activity, saccharose content and pH. However, it changed during the experiments. In the first 4 months, the correlation of the degradation rate to invertase activity was significant ($p < 0.05$). The correlation between the initial saccharose content and the rate of degradation remained significant over the entire duration of the experiment ($p < 0.001$) and was an important factor of the degradation process. The pH value correlated significantly over 6 months ($p < 0.01$), electrical conductivity only over the first 3 months ($p < 0.05$). At 15°C , only the initial invertase activity and pH were relevant for the degradation rate (Tab. 3). It is known that invertase activity is dependent on pH [43]. This could explain the importance of this parameter for saccharose breakdown.

Since the honey samples were not liquefied even after adding the saccharose, it might be possible that incompletely dissolved saccharose crystals were exposed to the enzyme. Therefore, mixing experiments with honey samples having naturally high amounts of saccharose were used to verify our experimental procedure (Tab. 1). The results demonstrated that natural saccharose (mean 1.16% per month, 6 weeks, 21°C) was not degraded at a higher rate than the added saccharose (1.24% per month, 6 weeks, 21°C).

The analysis of the complete set of samples indicated that degradation over all is not dependent on the honey type (Fig. 3). During the first week

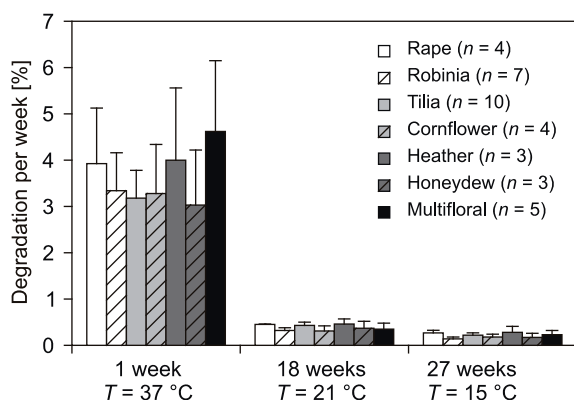


Fig. 3. Degradation rates of saccharose depending on honey type and temperature.

Mean and standard deviation.

at 37 °C, differences were observed between tilia honey and the multifloral samples. At 21 °C and 15 °C, differences could only be seen at robinia honey compared to rape and tilia honey. Since we have shown before that saccharose degradation was dependent on the physico-chemical parameters, these findings could be explained by the variety of the components of the honey samples but not only by invertase activity.

CONCLUSIONS

Based on our results, we obtained a saccharose degradation curve for different temperatures and invertase activities (Fig. 4). If all other quality cri-

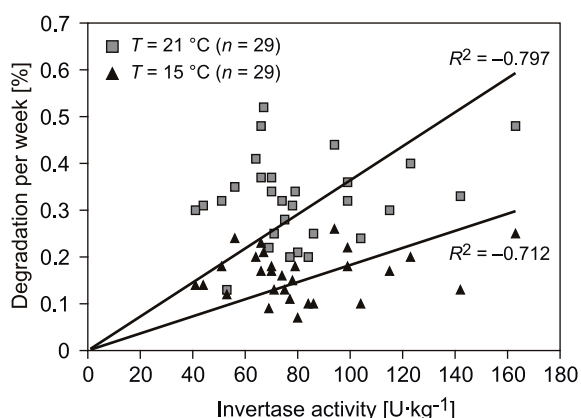


Fig. 4. Degradation rate of saccharose per week at various invertase activities and temperatures.

Trend lines (linear regression) and coefficient of correlation (R^2).

teria conform to the limits as prescribed by EU [27], it is not necessary to generally reject honey samples with an elevated content (more than 5%) of saccharose. Instead, the beekeeper may be advised how to process the honey in order to get a naturally produced honey conforming to legislation by simply adjusting temperature and duration of storage according to the degradation and invertase activity curves given here.

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