

## INVERTASE ACTIVITY AND CARBOHYDRATE SPECTRUM OF ORGANIC ACACIA AND POLYFLORAL HONEY AFTER ONE-YEAR STORAGE

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### Summary

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The invertase activity and carbohydrate spectrum of organic acacia and polyfloral honeys produced in the South Stara Planina region (Republic of Bulgaria) were investigated. After one year of storage at a temperature up to 25 °C, a mild reduction in invertase activity (3.9–4.79%) has occurred in polyfloral honey. The activity of this enzyme decreased considerably in acacia honey (15.16%). The average content of carbohydrates of acacia honey after one-year storage was: fructose – 42.76±1.24 g/100 g, glucose – 27.5±1.11 g/100 g, fructose/glucose ratio – 1.55±0.05, sucrose – 0.60±0.63 g/100 g, turanose – 1.18±0.21 g/100 g, maltose – 0.54±0.36 g/100 g, trehalose – 0.28±0.04 g/100 g. The respective carbohydrates in polyfloral honey were fructose (41.16±0.81 g/100 g), glucose (27.11±0.85 g/100 g), fructose/glucose ratio (1.51±0.07), sucrose (0.14±0.20 g/100 g), turanose (1.17±0.26 g/100 g), maltose (0.90±0.69 g/100 g), trehalose (0.65±0.19 g/100 g). Neither melibiose nor melezitose were established in both types of honey.

**Key words:** bee honey, carbohydrate spectrum, invertase, organic produce

### INTRODUCTION

The composition of Bulgarian bee honey was described by Ivanov (1978). It contains a variety of enzymes; among them, diastase ( $\alpha$ -amylase), invertase, glucose oxidase, catalase and acid phosphatase are the most important with regard to quality control. Honey enzymes have raised a significant scientific interest for years, because of their essential role for distinguishing natural from adulterated honeys (Von der Ohe & Von der Ohe, 1992). It is shown that their concentrations in honey depend largely on the period of bee development (Huang *et al.*, 1989), the

amount of nectar, and climatic atmospheric conditions influencing the release of nectar by plants (White, 1975).

Invertase is responsible for the conversion of sucrose, maltose, melezitose, raffinose, melibiose and trehalose into glucose and fructose – the predominant sugars in bee honey (Ivanov, 1978). Due to the substantial reduction of invertase activity during storage and after heat processing, this parameter is used for evaluation of the freshness of harvested bee honey (Dustmann *et al.*, 1985). It is proved that invertase is involved in nectar ripening and

production of bee honey (White, 1975). This is one of most thermolabile enzymes of honey, its degradation begins even at 35 °C, a temperature which is common in many countries during the summer. The initial level of invertase activity varies considerably among the types of honey (Karabournioti & Zervalaki, 2001). According to recent literature references, the determination of honey invertase activity is used mostly in the complete evaluation of unifloral honeys in Europe, as well as for determination of features related to the geographical origin of different bee honey varieties (Oddo *et al.*, 1995; 1999; 2004; Bartakova *et al.*, 2007; Serrano *et al.*, 2007).

According to the European Honey Commission (Bogdanov *et al.*, 1997), invertase activity could serve as a criterion to determine whether the honey is "fresh", long-term stored or heated at high temperatures. Although invertase activity is somewhat variable in the different honey types, minimum values of its activity have been proposed (in invertase numbers – IN or Siegenthaler units):  $\geq 50$  IN for highly valuable fresh honeys,  $\geq 20$  IN for honeys with low enzyme activity and  $\geq 10$  IN in unifloral honeys (Bogdanov *et al.*, 1997). In Bulgarian normative documents, the invertase activity is not included as a parameter of honey quality control (Anonymous, 2002a; 2003).

Despite being one of most heat unstable enzymes of honey, due to natural variations of initial activities, some researchers suggest that invertase could not be a reliable indicator of heat processing of the product (Oddo *et al.*, 1999; Karabournioti & Zervalaki, 2001). So far, at a national scale, the invertase activity of honey has been investigated with regard to the integrated characteristics of the types of honey or to provide evidence for heat

processed or adulterated honey (Ivanov, 1978; Dinkov & Vashin, 2001; Dinkov *et al.*, 2002; Zheliazkova *et al.*, 2002). A more recent study in four types Bulgarian honey from two regions of the country aimed at determining the natural variations of honey invertase activity (Dinkov & Valkova, 2009).

According to the cited literature data, invertase activity has been advanced as an additional qualitative parameter to assess the quality of freshly harvested honey and as a secondary marker of honey adulteration through addition of carbohydrates or of honey produced by bee families intensively supplemented with carbohydrate solutions.

During the last years, consumers' interest to organically produced bee honey is continuously increasing at a worldwide scale. An important feature of organic produce is the lack of contaminants, environmental, or physical, chemical and biological pollutants resulting from human activity. The high quality features of organically produced honey should be certified (Anonymous, 1999; 2001; 2008a; 2008b).

For assessment of the influence of unwanted heating, an investigation on the effect of honey processing at relatively low temperatures on invertase activity was conducted. It was demonstrated that honey samples processed at 42 °C for 24 h, exhibited a statistically significantly lower invertase activity ( $P < 0.01$ ) in 80% of cases (Dinkov, 2010).

With regard to the need for harmonisation of quality parameters in standards for organically produced bee honey (Anonymous, 2010), the invertase activity of different types of organic bee honeys should be assayed. That is why, the issue of organic honey storage became particularly important with regard to the preservation

of enzyme activity and carbohydrate composition of the product. No investigations of invertase activity and carbohydrate spectrum of organic bee honeys, stored under proper conditions over a year were found out in available literature.

Therefore, the aim of the study was to determine the changes in invertase activity and carbohydrate spectrum of organic acacia and polyfloral honeys after one year of storage at temperatures up to 25 °C.

## MATERIALS AND METHODS

Representative samples (n=40) were obtained from batches of organic acacia and polyfloral honey, produced in July 2010 from three certified organic apiaries in the South Stara Planina region (Kalofer municipality). The samples were collected after uncapping honeycombs, centrifugation and filtration, without additional heat processing.

The botanical origin of samples was determined by melissopalynological, organoleptic, physical and chemical features (Bogdanov *et al.*, 1997; Anonymous, 2002a; 2003; Oddo *et al.*, 2004; Piana *et al.*, 2004).

The samples were stored at a temperature up to 25°C over a year until the analysis, in compliance with the required conditions for honey storage (Anonymous, 2005).

At the beginning and the end of the storage period, invertase activity of honey was analysed using the method recommended by the European Honey Commission (Bogdanov *et al.*, 1997). The enzyme activity was determined photometrically by measuring the concentrations of p-nitrophenol, obtained by conversion of the substrate p-nitrophenyl  $\alpha$ -D glucopyranoside by invertase at a wavelength of 400 nm. The results were expressed in inver-

tase (sucrase) numbers – IN, representing the micromoles of substrate, converted by honey invertase per 1 min (Bogdanov *et al.*, 1997).

By the end of the storage period, the carbohydrate spectrum of samples was determined by an EC-approved method (Bogdanov *et al.*, 1997).

Invertase activity was analysed at the Laboratory of the Department of Food Hygiene and Control, Veterinary Legislation and Management at the Faculty of Veterinary Medicine – Stara Zagora (Bulgaria). Carbohydrate spectrum analyses were performed at the Laboratory of Apiculture-Sericulture, School of Agriculture, Aristotelian University of Thessaloniki, Thessaloniki (Greece).

The results were statistically processed by the Student's *t*-test.

## RESULTS

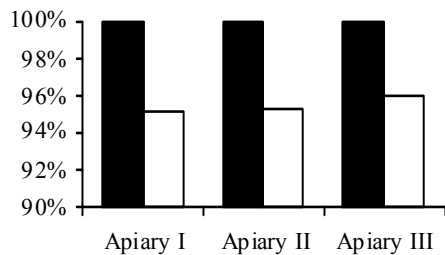
The highest invertase activity was that of polyfloral honey, harvested from the second apiary (33.594±0.017 IN), followed by honeys from the first and third apiaries (28.25±0.01 IN and 24.89±0.94 IN, respectively). After one year of storage, invertase activity was slightly reduced, and the aforementioned order was preserved – highest values of honey from the second apiary (32.03±0.010 IN), followed by the first (26.89±0.04 IN) and lowest activity from the third apiary (23.91±0.02 IN) (Table 1).

Accepting the initial invertase activity of polyfloral honey as 100%, the reduction in activities of honeys after storage over one year at temperatures < 25°C was by 3.9 % in the third apiary; by 4.67% in the second apiary and by 4.79% in the first apiary (Fig. 1).

**Table 1.** Invertase activity (IN) of organic polyfloral and acacia honeys, after one-year storage (from 2010 to 2011). Data are presented as mean±SD (min–max)

	2010	2011
<i>First apiary</i>		
Polyfloral honey (n=10)	28.25±0.01 (28.21–28.26)	26.89±0.04* (26.85–26.96)
<i>Second apiary</i>		
Polyfloral honey (n=10)	33.59±0.02 (33.58–33.62)	32.03±0.10* (31.89–32.15)
<i>Third apiary</i>		
Polyfloral honey (n=10)	24.88±0.94 (24.23–25.96)	23.91±0.02 (23.89–23.93)
Acacia ( <i>Robinia</i> sp.) honey (n=10)	14.29±0.02 (14.26–14.32)	12.12±0.03* (12.07–12.18)

\* statistically significant differences in invertase activities in 2010 vs. 2011 at P<0.001.



**Fig. 1.** Average reduction of invertase activity of polyfloral honey after one-year storage: black bars – initial activity (100%); white bars – activity after one-year storage.

The trend towards decrease of invertase activity was also present in acacia honey samples (Table 1). The one-year storage resulted in reduction of invertase concentrations from  $14.285 \pm 0.018$  IN to  $12.12 \pm 0.03$  IN. In percentage terms, the resulting activity after storage was 84.84% of baseline, equal to 15.16% reduction.

According to results, despite the occurring reduction of invertase after one-year storage, the activities in both polyfloral and acacia organic honeys were compliant to international norms for minimum invertase activity of 10 IN (Dustmann *et al.*, 1985; Bogdanov *et al.*, 1997).

Table 2 presents the carbohydrate composition of stored organic polyfloral and acacia honeys.

The fructose concentrations were the highest in both acacia ( $42.76 \pm 1.24$  g/100 g) and polyfloral honey ( $41.16 \pm 0.81$  g/100 g), followed by glucose ( $27.5 \pm 1.11$  g/100 g and  $27.11 \pm 0.85$  g/100 g in acacia and polyfloral honey samples, respectively). Third came the turanose with  $1.18 \pm 0.21$  g/100 g (in acacia honey) and  $1.17 \pm 0.26$  g/100 g in polyfloral honey.

The other carbohydrates are in negligible amounts. Both studied types of organic honey lacked melibiose and melezitose.

**Table 2.** Carbohydrate spectrum (g/100 g) of organic acacia and polyfloral honey after one-year storage. Data are presented as mean±SD (n=10)

	Acacia ( <i>Robinia</i> sp.) honey (n=10)	Polyfloral honey (n=10)
Fructose	42.76±1.24	41.16±0.81
Glucose	27.50±1.11	27.11±0.85
Fructose+glucose	70.26±2.26	68.28±0.94
Fructose/glucose ratio	1.55±0.05	1.51±0.07
Sucrose	0.60±0.63	0.14±0.20
Turanose	1.18±0.21	1.17±0.26
Maltose	0.54±0.36	0.90±0.69
Trehalose	0.28±0.04	0.65±0.19
Melibiose	ND	ND
Melezitose	ND	ND

ND = not detected.

## DISCUSSION

Average invertase activities obtained in the present study were similar to those reported in honey produced in another Bulgarian region in 2009 (23.762 IN) (Dinkov & Valkova, 2009). This could be attributed to honey production in a similar region of South Bulgaria. The data are supported by data showing a considerable effect of nectar release intensity on honey invertase activity, which could be a prerequisite for close values of honey invertase activities in regions with comparable climatic conditions (White, 1975). The present results about the invertase activity of polyfloral honey as well as results from previous studies of ours – 35.75 IN (Dinkov & Valkova, 2009), were close to results reported from Germany – 34.9 IN (Dustmann *et al.*, 1985) and Italy – 28 IN (Oddo *et al.*, 1999).

The differences between polyfloral and acacia honeys could be explained by the substantial effect of the amount of nectar and atmospheric conditions on honey invertase levels (White, 1975). The differences after storage of polyfloral and acacia organic honeys come in support of

the thesis of Oddo *et al.* (1999), that the period of honey storage could be hardly evaluated only on the basis of this quality trait.

The European Honey Commission has recommended quality indices based on the sum of glucose and fructose, and sucrose content. The glucose+fructose sum in all honeydew honeys should be ≥60 g/100 g and sucrose – ≤5 g/100 g as per the Council Directive (Anonymous, 2002b; Bogdanov *et al.*, 1997). Maltose, melezitose and other sugars are thought important in the identification of unifloral honeys (Oddo *et al.*, 1995). Yet, no regulations about the acceptable changes in honey carbohydrates during storage could be found in available literature sources.

The carbohydrate spectrum of stored acacia and polyfloral honeys in this study could be compared to the fennel (*Foeniculum vulgare* Mill.) bee honey produced in another ecological region of Bulgaria (Parvanov *et al.*, 2011). The fructose content (44.9±0.2 g/100 g), glucose content (25.5±0.3 g/100 g), glucose+fructose sum (70.4±0.3 g/100 g) and the low sucrose content of fennel honey allowed to classify it in the group of honeydew honeys as

per the standards of the European Honey Commission (Bogdanov *et al.*, 1997) and the Council Directive 2001/110/EC (Anonymous, 2002b). The fructose content of acacia and polyfloral honeys in this study were comparable ( $42.76 \pm 1.24$  g/100 g and  $41.16 \pm 0.81$  g/100 g respectively). Glucose concentrations in acacia and polyfloral honeys ( $27.5 \pm 1.11$  g/100 g and  $27.11 \pm 0.85$  g/100 g) were also similar to those in fennel honey type.

It could be also stated that in this study, sucrose concentrations in both honey types were low after one-year storage ( $0.60 \pm 0.63$  g/100 g and  $0.14 \pm 0.20$  g/100 g). This circumstance, along with the glucose+fructose sum ( $70.26 \pm 2.26$  g/100 g and  $68.28 \pm 0.94$  g/100 g in organic acacia and polyfloral honeys, respectively) indicated that they could also be classified as honeydew honeys conforming to the carbohydrate content requirements of the European Honey Commission (Bogdanov *et al.*, 1997) and the Council Directive 2001/110/EC (Anonymous, 2002b).

There were neither melebiose, nor melicitose in acacia and polyfloral honey samples in this research. These carbohydrates were also absent in fennel honey in the study of Parvanov *et al.* (2011). Unlike fennel honey however, which was shown to contain no maltose, the samples of organic acacia and polyfloral honeys contained  $0.54 \pm 0.36$  g/100 g and  $0.90 \pm 0.69$  g/100 g, respectively. The other carbohydrates in acacia honey were compliant to the norms of the European Honey Commission (Bogdanov *et al.*, 1997) and the Council Directive 2001/110/EC (Anonymous, 2002b).

In conclusion, the present study emphasised on the importance of assaying invertase activity and the carbohydrate spectrum of bee honey with regard to the more precise control of the quality of

stored organic honey. After one-year storage at controlled temperature up to 25 °C, studied samples of polyfloral honey exhibited a low extent of invertase activity reduction and a more substantial reduction of invertase activity in unifloral acacia honey. The activity of this enzyme was consistent with the internationally required minimum activity of honey invertase – 10 IN (Dustmann *et al.*, 1985; Bogdanov *et al.*, 1997). The detailed analysis of presented results supported previous findings about gradual equalisation of the levels of some carbohydrates in the different types of honey after storage.

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