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CHANGES IN HONEY SUGAR AND FREE AMINO ACID CONTENTS AFTER STORAGE

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ABSTRACT

Three Egyptian honeys were stored at 50°C for 44 days. In all the honey samples, amount of sucrose, melezitose and apparent total sugars decreased, whereas that of turanose increased. Changes in amounts of other sugars (maltose, fructosese, glucose) varied with honey type. The total free amino acid content decreased remakably during the storage of all honeys except citrus, but the amounts of some invididual amino acids increased in other honeys. Of the 17 amino acids detected proline was predominent, with phenylalanine second. Changes in the amounts of sugars and free amino acids in the honeys were not clearly related to the exent of darkening.

INTRODUCTION

Other paper by the authors reported a study of changes in colour, total nitrogen content and acidity of the same honeys stored at 50°C, and showed the effects of colour changes of incorporating ascorbic acid or sodium metadisulphite. This article describes changes in individual sugars and free amino acide during hot storage at 50°C for 44 days.

The relationship between the content of amino acids in honey and its colour during storage was studied by many authers: Komamine (1960), Garcia *et al.*, (2000), Shrestha *et al.*, (2000), Azeredo *et al.*, (1999), Bath *et al.*, (1999), Golob and Plestenjak (1999), Perreyra *et al.*, (1999), Sanchez *et al.*, (2001), Baroni *et al.*, (2002) and Gheldof *et al.*, (2002).

The amino acid content of honey has been investigated by various methods. Michelotti and Margheri (1969) used exchange chromatography; Qiu *et al.*, (1999) used N/R spectroscopy, Caceres *et al.*, (2000) used spectrophotometric discrimination, Nozal *et al.*, (2000) applied liquid chromatogrphic method and Garcia *et al.*, (2000) used the near-infrared transflectance spectroscopy.

Changes occurring in levels of individual sugars during the storage of honey are certain to be more complex than changes in amino acids. Changes in carbohydrate content of honey during storage were examined by many authore. (Thrasyvoulou (1997), Singh *et al.*, (1998), Gonzales *et al.*, (1999), Bath *et al.*, (2000) and Marchini *et al.*, (2000). They recorded decreases in the levels of glucose and fructose, increases in the levels of sucrose and maltose and increases in the complexity of the carbohydrates.

The present study describes and discusses results of the deterination of amino acids and carbohydrates in three Egyptian honeys before and after hot storage at 50°C for 44 days.

MATERIALS AND METHODS

The honeys were chosen representative of the main floral sources of honey in Egypt: Citrus *(Citrus Siensi,* Clover *(Trifolium alexandrinium* and Cotton *(Gossypium barbadence)*, the honeys had undergone primary extraction only. Each honey sample (25g) was dissolved in 100 ml 80% ethanol, left to stand for hour and centrifuged at 5000 rpm for 10 minutes. The supernatant was decanted and the residue washed twice with 50 ml 80% ethanol. The washings and supernatant were combined and made up to 200 ml. the residue was discarded.

The amino acids. Sugars and organic acids thus extracted were separated by a procedure based on that of Kliewer (1964). Amino acids and sugars were determined using the procedure of Thomson and Miles (1964).

RESULTS AND DISCUSSION

Table 1 gives the contents of individual sugars before and after storage. The accuracy of determination of mixtures of standard sugars was ± 2%. Therefore, the contents of melezitose, sucrose, and total sugars decreased in all honey samples, and that of turanose increased, for the major sugars, glucose decreased in 2 honeys (Clover and Cotton), and increased in citrus. Fructose content increased in clover and decreased in cotton and citrus. These results go in line with those of Thrasyvoulou (1997), Singh *et al.*, (1998), Gonzales *et al.*, (1999), Bath *et al.*, (2000) and Marchini *et al.*, (2000).

Table(1):Contents of sugars (%) in three honeys before and after storage at 50°C for 44 days, and moisture content (%) before and after storage.

Honey	type	Moisture	Melezitose	Sucrose	Maltose	Turanose	Fructorse	Glucose	Total
Clover	Fresh	19.7	2.4	1.1	4.9	-	35.2	33.0	76.6
	stored		2.1	0.5	4.2	5.1	39.3	25.1	76.3
Cotton	Fresh	15.4	1.5	3.1	4.3	2.3	36.2	33.2	80.6
	Stored		1.1	1.9	4.7	3.9	34.2	32.1	77.9
Citrus	Fresh	24.3	1.7	0.8	2.9	0.7	38.9	28.9	73.9
	Stored		1.6	0.6	3.1	1.1	36.7	29.3	72.6

Table 2 lists the contents of free amino acids before and after storage. The fresh clover and cotton honeys contained 15 amino acids. All amino acids were present in fresh citrus honey except arginine (Table 2). After storage: cotton honey contained 17 amino acids instead of 15, cysteine and methionine were found; citrus honey contained 13 instead of 14, glutamic acid was missed; and clover honey contained 14 amino acids instead of 15 as arginine was missed by storage.

Table (2): Free amino acids in clover, cotton and citrus honeys before and fater storage at 50°C for 44 day (µM/100g.)

Amino acid/100g of each honey type						
Amino acid	Clover		Cotton		Citrus	
	Fresh	Stored	Fresh	Stored	Fresh	Stored
Aspartic	2.1	0.8	8.1	3.4	29.3	4.7
Threonine	5.9	1.9	12.3	2.2	3.8	1.4
Serine	3.3	6.1	16.3	4.1	1.9	2.4
Glutamic	8.6	0.9	8.9	0.7	10.3	-
Proline	478.0	160.4	611.7	161.9	714.0	496.7
Glycine	4.2	4.7	18.7	4.2	8.7	6.3
Alanine	11.0	7.9	51.4	11.1	31.2	17.3
Valine	8.9	5.7	15.7	6.9	16.3	14.5
Cysteine	-	-	-	4.1	-	-
Methionine	-	-	-	3.8	-	-
Isoleucine	5.2	3.9	7.0	2.1	7.8	5.9
Leucine	17.1	3.7	5.3	2.7	4.1	7.2
Tyrosine	4.2	4.3	5.1	2.1	6.3	4.1
Phenylalanine	29.3	16.9	11.2	3.7	27.3	21.2
Lysine	13.2	3.4	18.7	3.6	6.3	9.3
Histidine	4.1	0.8	7.9	1.7	3.9	0.7
Arginine	2.7	-	6.8	2.4	-	-
Total	597.8	221.4	805.1	220.7	871.2	591.7

The total amino acids content of fresh honey was 597.8, 805.1 and 871.2 4 *u*M/100g in clover, cotton and citrus, respectively (Table 2), differences being due principally to variations in the level of proline. Proline was the predominant free amino acid in all honeys, representing approximately: 79.9%, 76.0% and 82.0% of total for clover, cotton and citrus, respectively. Phenylalanine was second to proline in clover and cotton fresh honey (4.8% and 1.4%); aspartic acid was second to proline in citrus honey (3.3%).

The total free amino acids content decreased during storage to 37.0% for clover, to 27.4% for cotton and to 67.9 for citrus. The anomalous behaviour of honey may be explained by its high total nitrogen content, protein hydrolysis and presence of proteolytic enzymes (Garcia *et al.*, 2000; Azeredo *et al.*, 1999; Golob and Plestenjak, 1999; Sanchez *et al.*, 2001 and Gheldof *et al.*, 2002).

A reduction in proline content during storage is responsible for most the decrase in total free amino acids. In spite of the general decrease in free amino acids, the contents of some amino acids increased during storage

especially in citrus honey (Table 2). These results are comparable with those of Shrestha *et al.*, (2000), Bath *et al.*, (1999), Perreyra *et al.*, (1999) and Baroni *et al.*, (2002).

When honeys are stored, certain changes occur in the contents of free amino acids, due to reaction with reducing sugars and the components of the honey (Marchini *et al.*, 2000). The observed increases in som of the amino acids suggest that changes may be due to protein breakdown (Thrasyvoulou, 1997 and Gonzales *et al.*, 1999). The lack of an apparent relation between changes in honey colour during storage and changes in either free amino acids or sugars is thus not surprising.

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التغيرات الحادثة للسكر ومكونات الأحماض الأمنية الحرة فى العسل بعد التخزين

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تم تخزين ثلاثة أنواع عسل نحل مصرية على درجة حرارة ٥٠م ولمدة ٤٤ يوم وقد نقصت كميات السكر والماليسزتوز والسكرات الكلية فى كل العينات المأخوذة فى حين زادت كمية التسيرانوز.

كما اختلفت التغيرات فى كمية السكرات الأخرى مثل المالتوز ، والفراكتوز، والحلوكوز باختلاف أنواع العسل كما نقص محتوى الأحماض الامينية الكلية بوضوح خلال فتره التخزين لكل أنواع العسل المذكورة فيساعد عسل الموالح وكما زادت كميات بعض الأحماض الأمينية الفردية فى أنواع العسل الأخرى وكان البرولين ثانياً من بين الـ١٧ حامض أمينى يليه الفينيل الأنين.

ولم تكن التغيرات فى كميات السكر والأحماض الأمينية الحرة فى أنواع العسل المذكورة مرتبطة بالظلام.