ELSEVIER

Food Chemistry 67 (1999) 389–397

Food Chemistry

www.elsevier.com/locate/foodchem

A comparison between *Helianthus annuus* and *Eucalyptus lanceolatus* honey

Parminder Kaur Bath, Narpinder Singh*

Department of Food Science and Technology, Guru Nanak Dev University, Amritsar-143 005, India

Received 15 March 1999; accepted 25 March 1999

Abstract

A comparison between physico-chemical, enzymatic activities and rheological properties of *Helianthus annuus* and *Eucalyptus lanceolatus* honey was made. *Eucalyptus lanceolatus* honey showed higher protein, diastase and catalase activity than *Helianthus annuus honey*, while proline, conductivity, total acidity, free acids and lactone content were higher in *Helianthus annuus* honey. Consistency coefficient and activation energy for flow were lower for *Helianthus annuus* than *Eucalyptus lanceolatus* honey. Confirmation of monofloral origin of honey types was accomplished through microscopic examination. The effects of convective and microwave heating on hydroxymethylfurfural (HMF) formation in both honey types were also investigated. Total acidity, free acids and lactone content increased in both honey types during heating. HMF formation varied linearly with temperature and time of heating in both honey types. Microwave heating of both honey types also caused an increase in HMF formation. Effect of storage of honey, heated under different conditions, on HMF content was also investigated. Statistical analysis revealed that storage duration had the greatest effect on HMF formation, followed by heating duration and heating temperature, in *Helianthus annuus* honey, while heating duration showed the most pronounced effect, followed by heating temperature and duration, in *Eucalyptus lanceolatus* honey.

Keywords: Honey; Composition; Heating; Microwave heating; Hydroxymethylfurfural; Storage

1. Introduction

The physico-chemical properties of honey produced in different geographical locations have been reported by several researchers (Langridge, 1977; Mateos-Nevado, Saenz De La Maza & Mateos-Nevado, 1994; Siddiqui, 1970; Singh & Bath, 1997). Some of the popular types of honey available in India are of monofloral origin, which include *Trifolium* spp, *Helianthus annuus*, *Eucalyptus lanceolatus*, and *Brassica juncea*. The composition and properties of honey vary with the floral sources utilized by the honey-bees, as well as regional and climatic conditions (Abu-Tarboush, Al-Kahtani & El-Sarrage, 1993; Perez-Arquillue, Conchello, Arino, Juan & Herresa, 1994; Salinas, Montero De Espinosa, Osorio & Lozano, 1994). Heating of honey is an essential step during processing to prevent granulation and fermentation (Singh, Singh, Bawa & Sekhon, 1988). Heating of honey results in hydroxymethylfurfural (HMF) formation. The temperature and duration of heating must be controlled, because any excessive amount of HMF indicates excessive heating and loss of freshness of honey (Isbell, Frush, Wade & Hunter, 1969; Thrasyvoulou, 1986; White, 1978). Temperature and duration of heating during processing must be controlled in relation to the floral source from which the honey has been extracted (Singh & Bath, 1997). Higher values of HMF also suggest the possibility that the honey has been adulterated by invert syrup (Doner, 1977). HMF in honey has also been reported to depend on storage temperature and duration (Gupta, Kaushik & Joshi, 1992; Papoff, Campus, Floris, Prota & Farris, 1995; Sancho, Muniatgeui, Hudiboro & Simal, 1992; White, Kushnir & Subers, 1964). On the other hand, Cherchi, Porcu, Spanedda and Tuberoso (1997) did not observe significant changes in HMF, acidity, moisture content diastase index in three types of honey or even after a storage period of 24 months at referigration and ambient temperature. The objectives of the present study were to: (1) compare physico-chemical properties of *Helianthus annuus* and *Eucalyptus lanceolatus* honey, (2) investigate the effect of convective and microwave heating on HMF formation in honey types and (3) study the effect of storage of honey types, heated under different conditions, on HMF formation.

* Corresponding author.

0308-8146/99/$ - see front matter
PII: S0308-8146(99)00132-6

2. Materials and methods

2.1. Collection and preparation of samples

Samples of honey were collected to represent two different sources, viz. *Helianthus annuus* and *Eucalyptus lanceolatus,* from the 1997 season. Honey used in the present study was ripe honey, harvested from hives, which were located in the areas where *Helianthus annuus* and *Eucalyptus lanceolatus* constituted the major flora, for the collection of nectar. Extraction of honey was carried out by filtration and squeezing the honey through muslin cloth and stored in air-tight plastic jars for further analysis.

2.2. Analysis

°Brix was determined using an Abbemat Digital Automatic Refractometer (Dr. Wolfgang Kernchen GMBH, Seelze, Germany). Refractive indices of honey types were measured using an Abbemat Refractometer at 20°C and corresponding moisture content (%) was calculated using the relationship between refractive index and water content (AOAC, 1990). Colour was determined at 420 nm using a Spectronic-20D (Milton Roy, USA) after diluting 5 g honey to 20 ml with distilled water. Total acidity, pH, free fatty acid, lactone and proline contents were measured in fresh honey samples using AOAC methods (Helrich, 1990). Changes in pH, total acidity, free acids and lactone contents in honey samples heated at 80°C for 1 h were also measured. To determine conductivity, 20 g of each honey sample was dissolved in 100 ml of distilled water and conductivity (mhos) was measured using a digital conductivity meter, model NDC-732 (NAINA Electronics, Chandigarh).

2.3. Pollen morphology

For floral identification of honey samples, 5 g of diluted honey sample was centrifuged at 10,000 rpm for 15 min to separate the pollens. Samples of separated pollen grains were spread with the help of a brush on a slide containing a drop of lactophenol. The slides were examined microscopically at 45× using a bright-field microscope (Olympus, Tokyo).

2.4. Protein, diastase and catalase activity

Protein content of honey was determined by the Lowry method (Lowry, Rosebrough, Farr & Randall, 1951). Diastatic number was determined using the AOAC method (Helrich, 1990). Catalase activity of honey samples was determined following the method of Luck (1965), with minor modifications. Phosphate buffer (110 mM) of pH 7 was made by mixing equal parts of KH_2PO_4 (1.496 g/100 ml) and K_2HPO_4 (1.914 g/100 ml). H_2O_2 (6.5 ml) of 1.25×10^{-2} M was diluted to 25 ml using phosphate buffer. The reference sample contained 1.6 ml H_2O_2 solution plus 400 μl of distilled water. The test samples (2 ml) consisted of 1.6 ml H_2O_2 and distilled water-containing honey (50, 100 and 150 μl). A Shimadzu UV-1601 spectrophotometer with TCC (electronic automatic temperature control) equilibrated at 37°C with an enzyme kinetics software package was used to monitor the decrease in absorbance at 240 nm after 0, 15, 30, 45 and 60 s. Catalase activity (μmol/min) was calculated using the following expression:

$$\frac{\text{Change in absorbance/min} \times \text{total reaction mixture (2 ml)} \times \text{dilution factor}}{\text{Extension coefficient of } H_2O_2 \times \text{amount of sample taken}}$$

2.5. Convective heating

To study the effect of temperature and duration of heating on HMF formation, 5 g of honey was placed in a beaker and heated at 50, 60, 70 and 80°C for 15, 30, 45 and 60 min, immediately cooled to room temperature and analyzed for HMF content.

2.6. Microwave heating

Honey samples (5 g) were poured into Teflon beakers and heated at 70, 140, 210 and 280 W power levels for 30, 90, 150, 210 and 270 s in a domestic microwave oven (Kelvinator, Model-T 23). After each test, the door of the microwave oven was kept open for 5 min to facilitate cooling. The power output of the magnetron of the microwave oven, specified by the manufacturer, was 700 W and the operating frequency was 2450 MHz. The determination of HMF was carried out using AOAC methods (Helrich, 1990).

2.7. Rheological properties

Rheological properties of honey samples were measured using a Brookfield viscometer (Brookfield Engineering Inc., Model DV-II) as described earlier (Singh & Bath, 1997).

2.8. Storage

Honey samples (200 g) were heated at 50, 60, 70, and 80°C for 15, 30, 45 and 60 min in a waterbath, cooled, sealed in air-tight glass containers and stored at room temperature. After 1, 2 and 3 months of storage, samples were analyzed for HMF content.

2.9. Statistical analysis

The data reported are averages of triplicate observations. The data were subjected to statistical analysis using Minitab statistical software (State College, PA).

3. Results and discussion

3.1. Composition

Moisture contents of honey types differed significantly, which mainly depends on the harvest season and degree of maturity in the hive (Perez-Arquille et al., 1994). Honey samples with high moisture contents are more prone to fermentation, so it is an important parameter, when considering the shelf life of honey during storage (Sanz, Gradillas, Jimeno, Perez & Juan, 1995). Moisture contents from 16 to 18% indicate a proper degree of maturity, and stem from the current use of modern hives by beekeepers. *Helianthus annuus* honey had moisture, Brix and proline content of 19.5%, 77.9 and 19.3 mg/100 g, respectively against 16.5%, 81 and 30.4 mg/100g, respectively in *Eucalyptus lanceolatus* honey. Lunigo, Ciurlo, Balletto, Novari and Malerba (1993) reported a proline content from 19.55 to 65.3 mg/100 g (mean value 36.3 mg/100 g) for honey samples imported from Argentina. *Helianthus annuus* honey had a darker colour than *Eucalyptus lanceolatus* honey, as indicated by its higher optical density (OD) measured at 420 nm (Table 1). The colour of honey is an indicator of its mineral content, the higher the mineral content, the darker would be the colour and vice versa (Perez-Arquille et al. 1994). Total acidity, free acids and lactone contents of *Helianthus annuus* honey were higher than *Eucalyptus lanceolatus* honey. Heating of both honey types at 80°C for 1 h resulted in an increase in total acidity, free acids and lactone contents and decrease in pH (Table 2). Honey obtained during the spring flowering season, i.e. February–June, is often more acidic than in the autumn season, when it usually contains more honeydew (Perez-Arquille et al., 1994). Similar effects of heating honey on total acidity, free acids and lactone contents have been reported earlier (Garcia et al., 1997). The presence of organic acids, particularly gluconic acid in equilibrium with lactones or esters, and inorganic ions, such as phosphate and chloride, contribute to the acidity of honey (Echigo & Takenaka, 1974). Conductivity values of *Helianthus annuus* and *Eucalyptus lanceolatus* honey were 1455×10 and 545×10 mhos, respectively. Variations in conductivity values of the honey types may be attributed to the variation in concentration of inorganic salts. Hence, *Eucalyptus lanceolatus* honey is less ionic than *Helianthus annuus* honey. The conductivity of honey depends, in addition to minerals, on organic acids, protein, completed sugars and polyols (Crane, 1975).

3.2. Protein content and enzymatic activities

Protein content, diastatic number and catalase activity of *Eucalyptus lanceolatus* were found to be higher than *Helianthus annuus* honey (Table 3). Protein contents of *Helianthus annuus* and *Eucalyptus lanceolatus* honey were 0.036 and 0.6%, respectively. This variation may be attributed to the type of flora. Protein content of honey from flowers of Tilia, *Ziziphus jujuba*, Robinia, Litchi, *Astragalus sinicus*, Codonopsis, *Sesame*, and cotton plants has been reported to vary between 0.048 and 0.42%, with the highest value in honey from Tilia flowers and the lowest in Robinia flowers (Peng & Pan, 1994). *Eucalyptus lanceolatus* honey showed higher diastase and catalase acitivities than *Helianthus annuus* honey. Schepartz (1966a) described a direct correlation between catalase and diastase activity. Pollens have

Table 1
Physico-chemical properties of honey types[a]

Type	Moisture (%)[b]	°Brix[b]	Colour[b]	Proline (mg/100 g)[b]
Helianthus annus	19.5b ± 0.14	77.9a ± 0.8	0.611a ± 0.008	19.3a ± 0.08
Eucalyptus lanceolatus	16.5a ± 0.25	81.0b ± 1.08	0.683b ± 0.010	30.4b ± 0.32

[a] Values with similar letters do not differ significantly ($p < 0.05$).
[b] Higher ranked letters are significantly different from lower ranked letters in the following form b > a.

Table 2
Chemical characteristics of honey types[a]

Type	PH[c]	Free acidity (meq/kg)[c]	Lactone (meq/kg)[c]	Total acidity (meq/kg)[c]
Helianthus annus	3.67	30c ± 1.6	40c ± 1.50	70c ± 1.6
Helianthus annus[b]	3.24	40d ± 2.4	40d ± 1.0	82d ± 1.6
Eucalyptus lanceolatus	4.48	15a ± 0.8	33a ± 0.4	48a ± 1.2
Eucalyptus lanceolatus[b]	4.13	25b ± 0.85	35b ± 0.5	60b ± 1.6

[a] Values with similar letters do not differ significantly ($p < 0.05$).
[b] Heated at 80°C for 1 h.
[c] Higher ranked letters are significantly different from lower ranked letters in the following form d > c > b > a.

been reported to be the main source of catalase (Schepartz, 1966b), while Vansell and Freeborn (1929) and Lothrop and Paine (1931) assert that diastase in honey mainly comes from the bee, pollens and the nectar. High activity of catalase in *Helianthus annuus* honey may be attributed to the type of flora.

3.3. Pollen morphology

Pollen grains of *Helianthus annuus* honey were characterized by a thick, coarsely granular exine, bearing sharp conical spines (Fig. 1), while pollen from *Eucalyptus lanceolatus* honey (Fig. 2) had a smooth form, exine without spines or flecks or other decorations, triangular in outline and with three prominent germ pores (Wodehouse, 1965).

3.4. Heating

Helianthus annuus honey showed an initial HMF content of 4.45 mg/100 g against 1.23 mg/100 g for *Eucalyptus lanceolatus* honey. These values are higher than those reported previously (Doner, 1977; Lungo et al., 1993). Honey produced in subtropical climates has been reported to have higher HMF content (LaGrange and Sanders, 1988). Heating at 70°C for 60 min resulted

Table 3
Protein and enzyme activity of honey types[a]

Type	Protein (mg/100g)[b]	Diastatic number[b]	Catalase (μmol/min)[b]
Helianthus annuus	36a ± 0.80	31.4a ± 0.32	158a ± 4.0
Eucalyptus lanceolatus	60b ± 1.6	42.9b ± 0.73	366b ± 5.0

[a] Values with similar letters do not differ significantly ($p < 0.05$).
[b] Higher ranked letters are significantly different from lower ranked letters in the following form b > a.

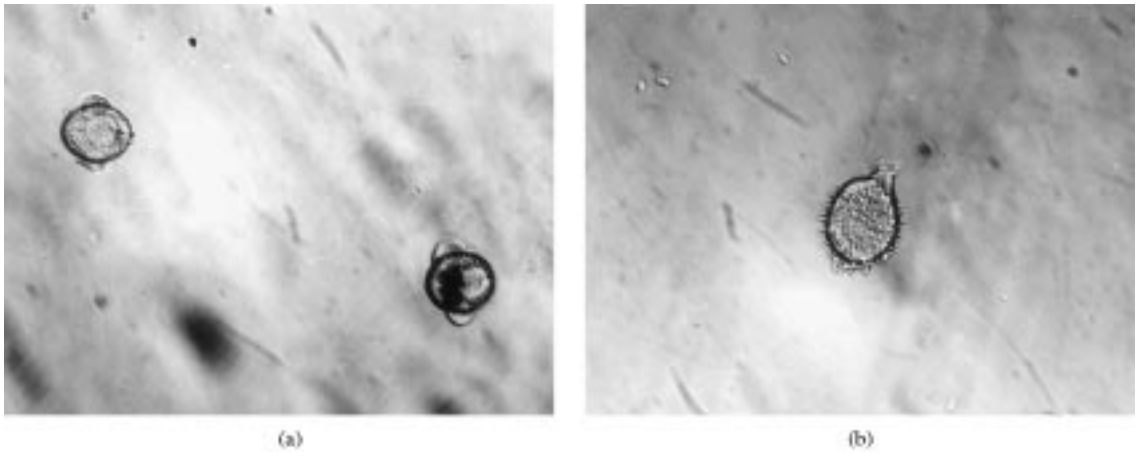

Fig. 1. Pollens separated from *Helianthus annuus* honey at (a) 10× and (b) 45× under light microscope.

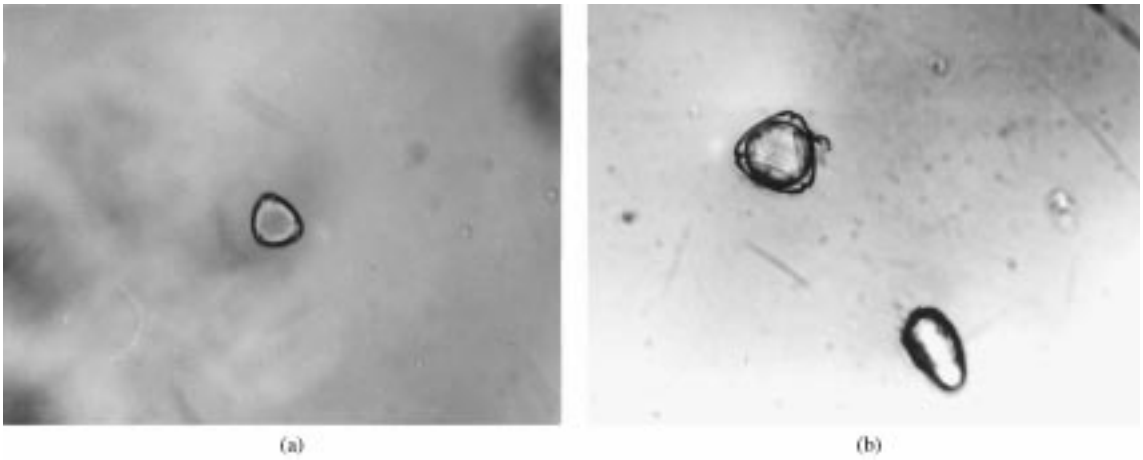

Fig. 2. Pollens separated from *Eucalyptus lanceolatus* honey as seen at (a) 10× and (b) 45× under light microscope.

Table 4
Analysis of variance of data shown in Figs. 3 and 4

Type	Source	DF	MS	*F*-value[a]
Helianthus annuus	Temperature	3	6.24	547.8***
	Heating duration	3	31.28	2743***
	Temperature×heating duration	9	0.142	12.45***
	Error	32	0.0114	
	Total	47		
Eucalyptus lanceolatus	Temperature	3	9.13	3088***
	Heating duration	3	14.30	4839***
	Temperature×heating duration	9	1.62	548***
	Error	32	0.003	
	Total	47		

[a] ***$p < 0.005$.

Table 5
Regression analysis for HMF formation in honey types

Type	Temperature (°C)	Regression equation[a]	R^2
Helianthus annuus	50	$Y = 4.243 \times 0.027X$	0.982
	60	$Y = 4.186 \times 0.044X$	0.929
	70	$Y = 5.420 \times 0.036X$	0.986
	80	$Y = 7.475 \times 0.039X$	0.912
Eucalyptus lanceolatus	50	$Y = 0.996 \times 0.036X$	0.935
	60	$Y = 4.186 \times 0.023X$	0.922
	70	$Y = 5.420 \times 0.026X$	0.870
	80	$Y = 7.45 \times 0.099X$	0.982

[a] Y, HMF (mg/100g); X, heating duration (minutes).

in an increase in HMF formation from 4.45 to 7.66 mg/100 g, in *Helianthus annuus* honey and from 1.23 to 3.1 mg/100 g under similar conditions in *Eucalyptus lanceolatus* honey. The effects of heating temperature and duration on HMF formation in *Helianthus annuus* and *Eucalyptus lanceolatus* honey are shown in Figs. 3 and 4. The higher rate of HMF formation in *Helianthus annuus* honey may be attributed to its lower pH. It has already been reported that honeys with low pH value produce more HMF on heating (Hase, Suzuki, Odate & Suzuki, 1973; Singh & Bath, 1997, 1998). The statistical analysis showed significant effects of temperature and duration of heating on HMF formation in both honey types (Table 4). However, temperature showed a greater effect on HMF formation in both honey types. The interaction effect of temperature and duration of heating, on HMF formation, was also highly significant. The regression models for the formation of HMF as a function of temperature and duration of heating showed a linear relationship with R^2 value in the range of 0.91–0.98 in *Helianthus annuus* and 0.86–0.98 in *Eucalyptus lanceolatus* honey (Table 5).

Ghazali, Tan and Hashim (1989) reported that microwave heating could be used (in small-scale industries) for preventing deterioration of honey. They did not observe any significant changes in pH, total acidity, ash, glucose, fructose and sucrose contents of honey during microwave heating. These authors did not report the effect of microwave heating on HMF formation. The effects of heating the honey types in a microwave oven at different power levels and durations are reported in Table 6. HMF content increased with increase in the microwave power levels and duration of heating in both the honey types (Figs. 5 and 6). The statistical analysis, shown in Table 7, revealed that power levels had a greater effect on HMF formation, in both the honey types, than duration of heating. The variation in HMF formation during microwave heating may be attributed to differences in dielectric constant and loss factors of the honey types. The differences in these properties may be due to differences in composition of honey types. The absorption of microwaves by a material has been reported to depend on its loss factor. The greater the loss factor, the greater the absorption and faster is the rise in temperature (Hudiara, 1998). *Helianthus annuus* honey is more ionic, as indicated by its higher conductivity. The ionic or conductivity contribution to the loss factor, ε''_σ, can be expressed by the following equation (Buffler, 1992):

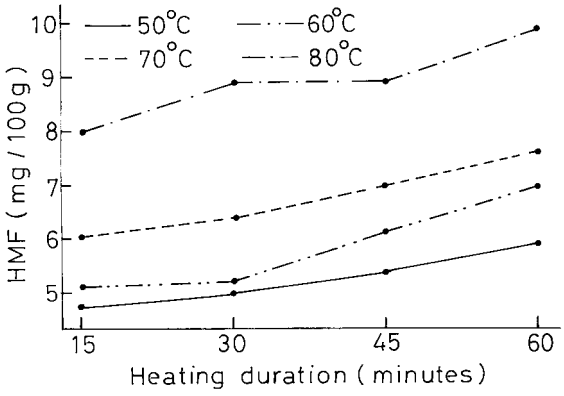

Fig. 3. Effect of heating temperature and duration on HMF formation in *Helianthus annuus* honey.

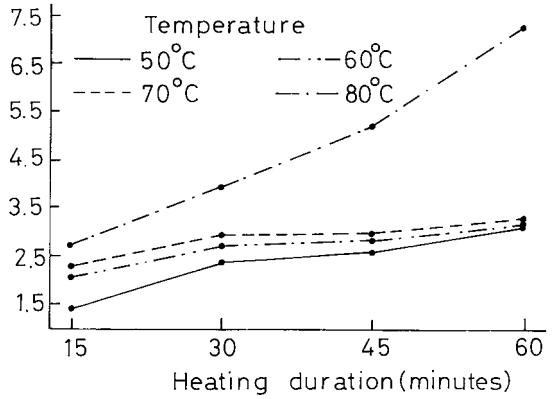

Fig. 4. Effect of heating temperature and duration on HMF formation in *Eucalyptus lanceolatus* honey.

Table 6
Effect of microwave heating on HMF formation

Type	Heating time (s)	Power levels (W)			
		70	140	210	280
Helianthus annuus	30	11.19075	14.00	15.831	17.061
	90	12.938	14.258	17.554	19.452
	150	13.385	17.461	19.456	20.145
	210	16.091	18.038	19.932	22.234
	270	16.850	19.10	20.000	25.789
Eucalyptus lanceolatus	30	4.775	5.4715	4.7775	5.3742
	90	4.903	5.4865	5.9206	6.5119
	150	5.1197	5.6511	6.0927	7.2080
	210	5.6137	5.7559	6.8263	8.4805
	270	6.2448	6.2533	8.5104	8.5478

$$\varepsilon''_{\sigma} = \sigma/2\pi f^{\circ} \quad (1)$$

where σ represents the direct current conductivity (mhos), f is the frequency in Hertz and ε is permittivity of free space (8.85×10^{-12} f/m). Eq. (1) can be written in the following more simple form:

$$\varepsilon''_{\sigma} = 1.80\sigma/f \quad (2)$$

where σ and f are in mmhos/cm and Ghz (more commonly used units), respectively.

Therefore, at 2.45 GHz, a system with greater conductivity contributes more to the loss factor as compared to the system with lower conductivity.

3.5. Flow behaviour

Consistency coefficient, flow behaviour index and activation energy of flow, for both the honey types, are shown in Table 8. *Helianthus annuus* honey had a lower consistency coefficient than *Eucalyptus lanceolatus* honey. These results are in agreement with those reported earlier (Singh & Bath, 1997). Consistency coefficient progressively decreased with increase in temperature in both honey types. The effect of temperature on consistency coefficient can be correlated by an Arrhenius-type equation:

$$\eta = \eta_{\infty} \exp(E_a/RT) \quad (3)$$

Table 7
Analysis of variance of data in Table 6

Type	Source	DF	MS	*F*-value[a]
Eucalyptus lanceolatus	Power levels	3	13.26	678.4***
	Time	4	9.57	490***
	Power levels×time	12	0.678	34.7***
	Error	40	0.0195	
	Total	59		
Helianthus annuus	Power levels	3	123.078	6699.35***
	Time	4	65.267	3552.61***
	Power levels×time	12	2.378	129.45***
	Error	40	0.018	
	Total	59		

[a] ***$p < 0.005$.

Table 8
Flow behaviour and activation energy for flow of honey types

Type	Temperature (°C)	Consistency coefficient (Pa s^n)	Flow behaviour index	E_a (kcal/mol)
Helianthus annuus	15	1.839	0.94	7.90
	25	0.640	0.95	
	35	0.430	0.99	
	45	0.095	1.00	
	55	0.0313	1.30	
Eucalyptus lanceolatus	15	5.370	0.93	22.80
	25	1.981	0.99	
	35	1.164	0.95	
	45	0.394	0.92	
	55	0.185	0.90	

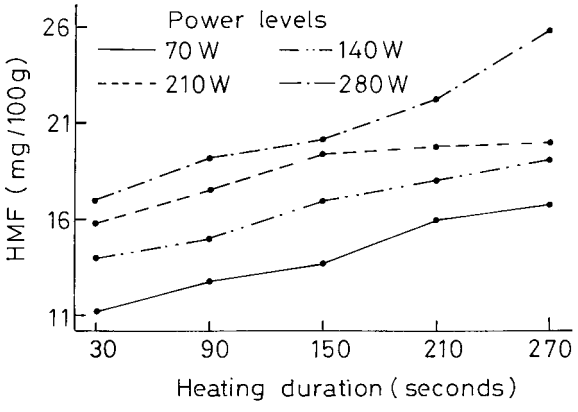

Fig. 5. Effect of power levels and heating duration on HMF formation in *Helianthus annuus* honey during microwave heating.

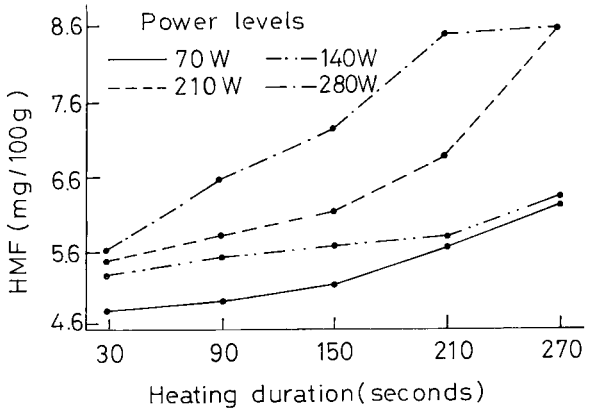

Fig. 6. Effect of power levels and heating duration on HMF formation in *Eucalyptus lanceolatus* honey during microwave heating.

Table 9
Effect of storage on HMF (mg/100 g) content of honey types

Temperature (°C)	Heating duration (min)	Storage time (months)	*Helianthus annuus*	*Eucalyptus lanceolatus*
50	15	1	11.9	5.30
50	15	2	13.1	5.14
50	15	3	16.0	5.05
50	15	1	11.7	6.00
50	30	2	15.0	5.27
50	30	3	15.4	5.16
50	45	1	12.7	6.01
50	45	2	15.5	6.52
50	45	3	15.7	6.84
60	15	1	12.0	5.55
60	15	2	15.5	4.97
60	15	3	15.9	5.23
60	30	1	13.0	6.14
60	30	2	15.7	6.8
60	30	3	16.0	7.16
60	45	1	13.4	6.97
60	45	2	16.7	7.0
60	45	3	16.8	7.39
70	15	1	12.4	4.93
70	15	2	15.5	5.0
70	15	3	16.2	6.0
70	30	1	14.2	6.44
70	30	2	16.1	5.95
70	30	3	16.7	6.13
70	45	1	16.6	6.19
70	45	2	16.5	6.53
70	45	3	16.8	6.74
80	15	1	14.3	5.68
80	15	2	15.2	6.07
80	15	3	15.4	6.18
80	30	1	14.7	6.94
80	30	2	16.6	7.15
80	30	3	16.6	7.65
80	45	1	18.1	7.31
80	45	2	18.0	7.31
80	45	3	18.0	7.75

where η is the viscosity, η_∞ is a pre-exponential factor, E_a is the activation energy for flow, R is the perfect gas constant (1.987 cal g mol^{-1} k^{-1}) and T is the absolute temperature (K). Eq. (1) can be expressed in the logarithmic form in order to estimate the parameter E_a for both honey types. Semi-log plots, of consistency coefficient versus inverse of absolute temperature, gave significant correlation coefficients when plotted using the linear regression analysis. Estimated values for E_a for both honey types are reported in Table 8. It was observed that *Helianthus annuus* honey had a lower activation energy for flow than *Eucalyptus lanceolatus* honey. These differences may be attributed to the differences in soluble solids as indicated by °Brix values. The results are in accordance with those reported previously. Giner, Ibarz, Garza and Xhian-Quan (1996) reported an E_a value of 14.6 kcal/mol for clarified cherry juice at 74°Brix.

Table 10
Analysis of variance of data in Table 9 for *Helianthus annuus*

Source	DF	SS	*F*-value	P
Temperature	3	70.85	1051	0.000
Heating duration	2	56.22	1251	0.000
Storage	2	127.45	2836	0.000
Temperature×heating duration	6	12.42	92.12	0.000
Temperature×storage	6	24.54	182.06	0.000
Duration×storage	4	9.69	107.83	0.000
Temperature×heating duration ×storage	12	14.51	53.85	0.000
Error	72	1.617		
Total	107	317.33		

Table 11
Analysis of variance of data in Table 9 for *Eucalyptus lanceolatus*

Source	DF	SS	*F*-value	P
Temperature	3	21.52	1569	0.000
Heating duration	2	39.50	4318	0.000
Storage	2	2.28	250	0.000
Temperature×heating duration	6	4.22	153	0.000
Temperature×storage	6	1.15	41	0.000
Heating duration×storage	4	0.61	33	0.000
Temperature×heating duration ×storage	12	5.25	95	0.000
Error	72	0.32		
Total	107	74.90		

3.6. Storage

HMF contents in honey types, stored for 1, 2 and 3 months after heating at 50, 60, 70 and 80°C for different durations, were also determined (Table 9). The data reported in Table 9 were subjected to analysis of variance. This significant effect of storage duration, heating temperature and heating duration on HMF formation in both honey types is shown in Tables 10 and 11. Storage duration showed the most pronounced effect, followed by heating duration and heating temperature, on HMF content in *Helianthus annuus* honey, while heating duration showed the greatest effect on HMF formation in *Eucalyptus lanceolatus* honey, followed by heating temperature and storage duration. The interaction effect of all three factors, with each other, on HMF content was also significant in both honey types. Heating temperature interaction effect with storage duration, in *Helianthus annuus*, and with heating duration, in *Eucalyptus lanceolatus* honey, on HMF formation, was highly significant. The effects of storage duration on HMF formation in honey types heated under different conditions are illustrated in the interaction plots plotted using Minitab statistical software (Figs. 7–10). The more pronounced effect of storage duration on HMF formation in *Helianthus annuus* than in *Eucalyptus lanceolatus* honey may be attributed to the compositional

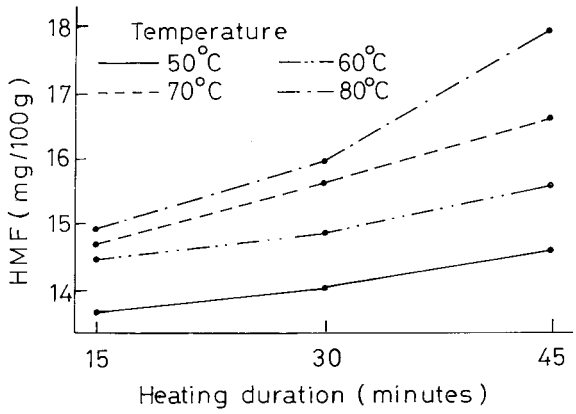

Fig. 7. Interaction plot showing effect of temperature and duration of heating on HMF formation in stored *Helianthus annuus* honey.

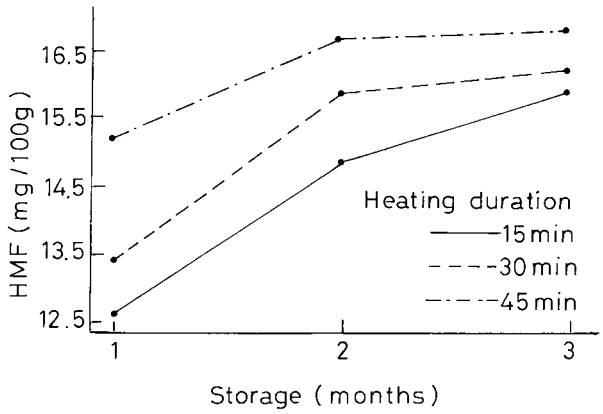

Fig. 9. Interaction plot showing effect of storage and heating duration on HMF formation in *Helianthus annuus* honey.

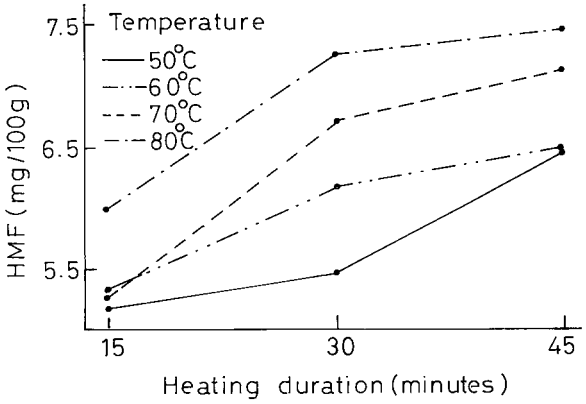

Fig. 8. Interaction plot showing effect of temperature and duration of heating on HMF formation in stored *Eucalyptus lanceolatus* honey.

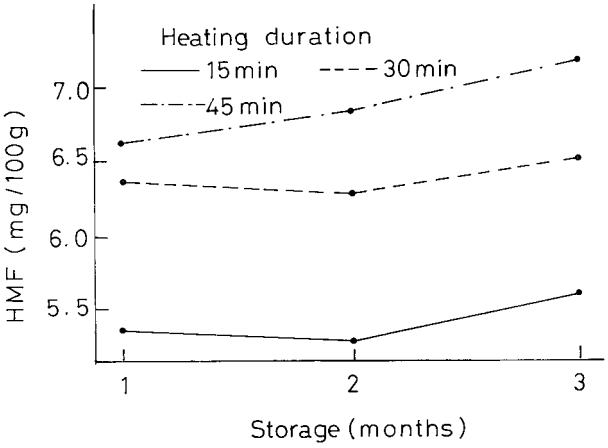

Fig. 10. Interaction plot showing effect of storage and heating duration on HMF formation in *Eucalyptus lanceolatus* honey.

differences in honey types. *Helianthus annuus* had higher moisture, free acids and total acidity than *Eucalyptus lanceolatus* honey. All these constituents have been reported to create favourable conditions for degradation of reducing sugars by promoting enolization (Papoff et al., 1995; Schade & Marsh, 1958; Toribio & Lozano, 1987).

4. Conclusion

Helianthus annuus and *Eucalyptus lanceolatus* honey differ significantly in their physico-chemical, enzymatic and rheological properties. The intensity of HMF formation in both the honey types also varied significantly. HMF formation in honey during storage depends on source and heating conditions under which the honey has been processed. Therefore, heating conditions, storage duration and source of honey must be considered together during processing of honey. *Helianthus annuus* honey showed greater HMF formation than *Eucalyptus lanceolatus* honey under similar heating and storage conditions.

Acknowledgements

P.K.B. acknowledges a CSIR Senior Fellowship from the Council of Scientific and Industrial Research, New Delhi.

References

Abu-Tarboush, H. M., Al-Kahtani, H. A., & El-Sarrage, M. S. (1993). Floral types identification and quality evaluation of some honey types. *Food Chemistry, 46*, 13–17.

Buffler, C. R. (1992). *Microwave cooking and processing*. (pp. 47–68) New York: Van Nostrand Reinhold.

Cherchi, A., Porcu, M., Spariedda, L., & Tuberoso, C. I. G. (1997). Influence of aging on the quality of honey. *Industria Conserve, 72*, 266–271.

Crane, E. (1975). *Honey: a comprehensive survey*. Heinernatin, London: International Bee Research Association.

Doner, L. W. (1977). The sugars of honey: a review. *Journal of the Science of Food and Agriculture, 28*, 443–456.

Echigo, T., & Takenaka, T. (1974). Production of organic acids in honey by honeybees. *J. Agric. Chem, Soc. Japan, 48*, 225–230.

Garcia, M. A., Valcarcel, M., Fernandez, M. I., Herrero, C., Latorre, M. J., Garcia, S., & Pena, R. (1997). Changes in acidity of Galician honeys during packaging. *Industria Conserrve, 72*, 52–56.

Ghazali, H. M., Tan, C. M., & Hashirn, D. M. (1989). Effect of microwave heating on the storage and properties of starfruit honey. *ASEAN Food Journal, 9*, 30–35.

Giner, L., Ibarz, A., Garza, S., & Xhian-Quan, S. (1996). Rheology of clarified cherry juices. *Journal of Food Engineering, 30*, 147–154.

Gupta, J. K., Kaushik, R., & Joshi, V. K. (1992). Influence of different treatments, storage temperature and period on some physico-chemical characteristics and sensory qualities of Indian honey. *Journal of Food Science and Technology, 29*, 84–87.

Hase, S., Suzuki, O., Odate, M., & Suzuki, S. (1973). Changes in quality of honey on heating and storage. I. Changes in hydroxymethylfurfural (HMF) content of honey. *Journal of Food Science and Technology, 20*, 248–256.

Helrich, K. (1990). *Official methods of analysis* (15th ed.). Arlington, VA: Association of Official Analytical Chemists, Inc.

Hudiara, I. S. (1998). Microwave complex permittivity of water at high temperature. *IETE Technical Review, 15*, 221–223.

Isbell, H. S., Frush, H. L., Wade, C. W. R., & Hunter, C. E. (1969). The use of HMF and diastase as criteria of quality of Greek honey. *Journal of Apicultural Research, 25*, 185–195.

LaGrange, V., & Sanders, S. W. (1988). Honey in cereal-based new food products. *Cereal Foods World, 33*, 833–858.

Langridge, D. F. (1977). A study of some quality factors of Australian honey. *Food Technology Australia, 29*, 109–112.

Lothrop, R. E., & Paine, H. S. (1931). Diastatic activity of some American honey. *Ind. Eng. Chem., 23*, 71–74.

Lowry, O. H., Rosebrough, N. L., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin-phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Luck, H. (1965). In H.-U. Bergmeyer, *Catalase. Methods of enzymatic analysis* (pp. 885–894). New York: Academic Press.

Lungo, T. D., Ciurlo, R., Balletto, A., Novari, G., & Malerba, A. (1993). Proline content in honeys imported from Argentine. *Industrie Alimentari, XXXII*, 349–353.

Mateos-Nevado, M. D., Saenz De La Maza, J. A., & Mateos-Nevado, B. (1994). Honey from Seville province II. Chemical and physicochemical characterization of honey from La Campina regin. *Anales-de-Bromatologia, 44*, 101–104.

Papoff, C. M., Campus, R. L., Floris, I., Prota, R., & Farris, G. A. (1995). Influence of temperature storage on the food quality of strawberry-tree honey (*Arbutus unedo* L.). *Industrie Alimentari, 34*, 268–273.

Peng, Z. F., & Pan, J. G. (1994). Protein content in honey and its effects on precipitation of beverage. *Food Science (China), 12*, 6–8.

Perez-Arquille, C., Conchello, P., Arino, A., Juan, T., & Herresa, A. (1994). Quality evaluation of Spanish rosemary (*Rosmarinus officinalis*) honey. *Food Chemistry, 51*, 207–210.

Salinas, F., Montero De Epnosa, V., Osorio, E., & Lozano, M. (1994). Determination of mineral elements in honey from different floral origins by flow injection analysis coupled to atomic spectroscopy. *Revista Espanola de Ciencia y Tecnologia de Alimentos, 34*, 441–449.

Sancho, M. T., Muniateugi, S., Hudiboro, J. F., & Simal, J. F. (1992). Aging of honey. *Journal of Agricuture and Food Chemistry, 40*, 134–138.

Sanz, G., Gradillas, G., Jimeno, F., Perez, C., & Juan, T. (1995). Fermentation problem in Spanish north-coast honey. *Journal of Food Protection, 58*, 515–518.

Schepartz, A. I. (1966a). The glucose oxidase of honey. IV. Some additional observations. *Biocim. Biophys. Acta., 118*, 637–640.

Schepartz, A. I. (1966b). Honey catalase: occurrence and some general properties. *Journal of Apicultural Research, 5*, 167–176.

Siddiqi, I. R. (1970). The sugars of honey. *Advances in Carbohydrate Chemistry and Biochemistry, 25*, 285–309.

Singh, N., & Bath, P. K. (1997). Quality evaluation of different types of Indian honey. *Food Chemistry, 58*, 129–133.

Singh, N., & Bath, P. K. (1998). Relationship between heating and hydroxymethylfurfural formation in different honey types. *Journal of Food Science and Technology, 35*, 154–156.

Singh, N., Singh, S., Bawa, A. S., & Sekhon, K. S. (1988). Honey—its food uses. *Indian Food Packer, 42*, 15–25.

Thrasyvoulou, A. T. (1986). Use of HMF and diastase as quality of Greek honey. *Journal of Apicultural Research, 25*, 186–195.

Toribio, J. L., & Lozano, J. E. (1987). Formation of 5-hydroxymethylfurfural in clarified apple juice during heating at elevated temperatures. *Lebensm. Wiss. Technol., 20*, 59–63.

Vansell, G. H., & Freeborn, S. B. (1929). Preliminary report on the investigations of the source of diastase in honey. *Journal of Economic Entomology, 22*, 922–927.

White, J. W., Kushnir, I., & Subers, M. H. (1964). Effect of storage and processing temperatures on honey quality. *Food Technology, 555*, 153–156.

White Jr., J. W. (1978). Honey. *Advances in Food Research, 24*, 287–374.

Wodehouse, R. P. (1965). *Pollen grains: their structure, identification, and significance in science and medicine*. New York and London: Hafner Publishing Company.