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Original article

Protein content and amino acids composition of bee-pollens from major floral sources in Al-Ahsa, eastern Saudi Arabia

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A B S T R A C T

Protein content and amino acids composition of bee-pollens from major pollen floral sources in Al-Ahsa, Saudi Arabia were determined to investigate the nutritive value of pollen protein relative to requirements of honeybees and adult humans. The major pollen sources were alfalfa, date palm, rape, summer squash, and sunflower. Bee-pollens from alfalfa and date palm showed high content of crude protein and amino acid concentrations. Bee-pollen from sunflower had low content of those components. Eighteen amino acids were found in bee-pollens from the five major floral sources. The highest concentrations of individual amino acids valine, leucine, isoleucine, phenylalanine and proline were obtained from alfalfa bee-pollen; lysine, arginine, cysteine, tryptophan and tyrosine from date palm; methionine, histidine, glycine and alanine from summer squash; threonine, serine and glutamic acid from sunflower; and aspartic acid from rape bee-pollen. The amino acid composition obtained from sunflower bee-pollen showed the lowest concentrations of the essential amino acids: isoleucine, leucine, methionine, phenylalanine and valine. Apart from methionine, arginine and isoleucine, the essential amino acids of bee-pollen from alfalfa, date palm, summer squash and rape exceeded the honeybees' requirements. Methionine was the limiting amino acid in bee-pollens from the five selected sources. Concentrations of essential amino acids in the tested bee-pollens were variable and significantly correlated to their botanical origin of pollen. Bee-pollens from alfalfa, date palm and summer squash was found to be rich source of protein and amino acids for bees and for humans.

1. Introduction

Bee-pollens are a natural product consisting of nutritional and biologically active substances (Dukhanina et al., 2005). Bee-pollens contain varying amounts of protein, ranging from 2.90% to 33.51%, depending on the botanical origin (Day et al., 1990; Serra Bonvehi and Escolà Jordà, 1997; Rogala and Szymaś, 2004; Somerville and Nicol, 2006; Szczęsna, 2006a; Tasei and Aupinel, 2008; Carpes et al., 2009; Martins et al., 2011; Margaoan et al., 2012; Nicolson and Human, 2013). In particular, more than 16 essential and non-essential amino acids have been found in bee-pollens from different botanical origins (Day et al., 1990; Serra Bonvehi and Escolà Jordà, 1997; Szczęsna and Rybak-Chmielewska, 1998; Szczęsna et al., 1999; Dukhanina et al., 2005; Human and Nicolson, 2006; Somerville and Nicol, 2006; Szczęsna, 2006a,b; Nicolson and Human, 2013).

Eleven amino acids (cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine threonine, tryptophan, tyrosine and valine) cannot be synthesized by the human body (FAO, 2007) and they must be included in the diets. Those are called essential amino acids, and bee-pollens can be one of their important source. The essential amino acids for honeybees are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (De Groot, 1953). The concentrations of essential amino acids in bee-pollens, expressed as the percentage of the total amino acids ranged from 34.59% to 48.49% (Szczęsna, 2006a,b; Nicolson and Human, 2013). Glutamic acid,

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aspartic acid, proline, leucine and lysine have been found to be the most abundant amino acids in pollens protein (Szczęsna and Rybak-Chmielewska, 1998; Szczęsna et al., 1999; Szczęsna, 2006a,b; Nicolson and Human, 2013).

The major floral sources of pollen for honeybees in Al-Ahsa, eastern Saudi Arabia were summer squash (*Cucurbita pepo* Thunb), date palm (*Phoenix dactylifera* L.), alfalfa (*Medicago sativa* L.), sunflower (*Helianthus annuus* L.) and rape (*Brassica napus* L.) (Taha, 2015a,b). They were contributed as 21.10%, 20.25%, 18.15%, 17.42% and 17.05%, respectively of total yearly collected pollen loads (Taha, 2015a).

The objective of this study is to determine protein content and amino acid composition of bee-pollens from the major pollen sources in Al-Ahsa, Saudi Arabia to investigate their nutritional value compared to requirements of honeybees and humans.

2. Materials and methods

Bee-pollen samples were collected from the apiary of the Agricultural and Veterinary Training and Research Station, King Faisal University, Al-Ahsa eastern Saudi Arabia. Ten colonies (each of 10 combs) of Carniolan honeybees (*Apis mellifera carnica*) in the same population size were used for pollen trapping.

Pollen traps with efficiency of 25% were fitted onto the entrances of the hives. From February to June in 2015, the pollen loads were daily collected from the traps. Pollen loads were dried at 40 °C for one hour, then separated and referred to their floral sources according to Taha (2015a,b). Pollen samples were trapped during February-March for summer squash, date palm, rape and sunflower, and during May for alfalfa, summer squash and sunflower. Each pollen species was represented by five samples (each of 50 g). Samples were collected form traps during 4 days and were stored at −21 °C until analysis at the central laboratory of Kafrelsheikh University.

Sample of 0.07 g dried matter was used to determine nitrogen content by micro-Kjeldahl method using the 5.60 factor for conversion into crude protein (Rabie et al., 1983).

Amino acids profile in bee-pollens were determined according to the method of Szczęsna (2006a). The quantitative and qualitative analyses of the amino acids were performed using ion exchange chromatography with utilization of Automatic Amino Acid Analyzer LC3000 (Eppendorf-Biotronik, Germany). The technique was based on amino acid separation using strong cation exchange chromatography, followed by the ninhydrin colour reaction and photometric detection at 570 nm. A 25 mg DM of a bee-pollen sample was transferred to a hydrolysis tube containing 10 ml of 6 N HCL. The samples were treated with performic acid to avoid the decomposition of Sulphur-containing amino acids (AOAC, 1990). The hydrolysis tube was sealed under vacuum, heated in an oven at 110 °C for 24 h, and then cooled to room temperature. After hydrolysis, the solution was evaporated using EYELA Rotary Vacuum Evaporator at 40 °C, then dissolved in 1 ml distilled water and evaporated again to remove the acid traces. The contents were filtered through Whatman No. 1 filter paper to remove visible sediments. The tube and precipitates on filter paper were then washed with deionized water. The combined filtrate and wash were diluted to the volume of 25 ml in a volumetric flask. Five ml of the filtrate was transferred to a 50 ml beaker. Dry residues were dissolved in 1 ml of lithium citrate buffer (pH 2.2). Twenty μl of the solution were loaded onto the cation exchange column, and then four lithium citrate buffer with pH 2.2, 2.8, 3.3 and 3.7 were successively applied to the column at flow rate 0.2 ml/min. The ninhydrin flow rate was 0.2 ml/min and pressure of reagent was 150 bar. The pressure of buffer was from 0 to 50 bar and 130 °C reaction temperature. The qualitative analysis of the amino acids in bee-pollen samples were identified by comparing the retention time of particular amino acids in the examined solution with reference solutions. The quantitative analysis were performed by comparing the corresponded peak area to those of amino acids.

The nutritive value of protein in bee-pollen samples was determined using the chemical measure of protein quality [Chemical Score (CS)] and Essential Amino Acid Index (EAAI). Based on the amino acids composition of bee-pollens and of that of chicken egg, the EAAI was calculated according to the equation of Oser (1951) as follows:

$$\mathrm{EAAI} = \sqrt[N]{\frac{\mathrm{Arg.P} \times \cdots \times \mathrm{Val.P} \times 100}{\mathrm{Arg.E} \times \cdots \times \mathrm{Val.E}}}$$

where N = the number of amino acid which equal 10, P = refers to the tested protein, E = refers to standard protein (egg protein) according to FAO (1973).

The CS was calculated from the chemical analysis of the essential amino acids of bee-pollens according to the formula of Mitchell and Block (1946) as follows:

$$\mathrm{CS} = \frac{\mathrm{AAP} \times 100}{\mathrm{AAR}}$$

where (P) refers to AA in tested protein, (R) refers to AA requirements for honeybees (de Groot, 1953) or AA requirements for adult humans (FAO, 2007).

Data were statistically analyzed by analysis of variance (ANOVA). Treatment means were compared by Duncan's Multiple Range Test (Duncan, 1955). Pearson correlation between values of amino acids was determined using SAS software (SAS Institute, 2003).

3. Results

As shown in Table 1, protein and amino acids composition of the tested bee-pollens showed the highest significant ($p < 0.01$) values (g/100 g DM) of crude protein (20.23 g) and total amino acids (12.51 g) for alfalfa; total essential amino acids (5.35 g) and percentage of total essential amino acids (42.87%) for date palm. Bee-pollen from sunflower showed the lowest values of their contents. The EAAI ranged from 62.03% for alfalfa bee-pollen to 78.00% for summer squash bee-pollen, with 67.97% on average.

Data presented in Table 2 showed that, eighteen (10 essential and 8 non-essential) amino acids were found in bee-pollen samples of the tested sources. The highest values (mg/g DM) of the essential amino acids leucine (12.90 mg), valine (9.95 mg), isoleucine (7.20 mg) and phenylalanine (3.06 mg) were obtained from alfalfa bee-pollen, lysine (8.94 mg), arginine (4.20 mg) and tryptophan (1.21 mg) from date palm, methionine (0.71 mg) and histidine (6.28 mg) from summer squash, and threonine (5.18 mg) from sunflower. The lowest concentrations of isoleucine, leucine, methionine, phenylalanine, tryptophan and valine were obtained from sunflower bee-pollen. The highest concentrations (mg/g DM) of individual non-essential amino acids in bee-pollens were as follows: proline (0.68 mg) from alfalfa, tyrosine (2.93 mg) and cysteine (2.42 mg) from date palm, glycine (17.64 mg) and alanine (13.49 mg) from summer squash, serine (8.86 mg) and glutamic acid (18.40 mg) from sunflower, and aspartic acid (16.46 mg) from rape. Generally, the most abundant amino acid in bee-pollens were found to be alanine, aspartic acid, glutamic acid, glycine and leucine. The content of individual amino acids ranged from 9.65 mg/g DM (leucine) to 18.40 mg/g DM (glutamic acid) in sunflower bee-pollen.

As shown in Table 3, there were high significant ($p < 0.01$) positive correlations between leucine, isoleucine, phenylalanine and

Table 1
Crude protein, total amino acids and essential amino acids composition of bee-pollens.

Botanical origin	Protein (g/100 g DM)	TAA* (g/100 g DM)	TEA** (g/100 g DM)	TEA%	EAAI*** (%)
Alfalfa	20.23^{a}	12.51^{a}	5.25^{b}	41.97^{b}	62.03
Date palm	19.77^{b}	12.48^{a}	5.35^{a}	42.87^{a}	64.00
Summer squash	16.39^{d}	12.27^{b}	5.13^{c}	41.81^{c}	78.00
Sunflower	15.19^{e}	12.20^{c}	4.73^{d}	38.77^{d}	68.07
Rape	18.86^{c}	12.25^{b}	5.12^{c}	41.80^{bc}	67.74
Average	18.09	12.34	5.12	41.44	67.97

Means of each column followed by the different letter are significantly different at the 0.01 level.
* Total Amino Acids.
** Total Essential Amino Acids.
*** Essential Amino Acids Index (calculated for the 10 essential amino acids).

Table 2
Amino acids composition (mg/g DM) of bee-pollens.

Amino acid	Alfalfa	Date palm	Summer squash	Sunflower	Rape	Average
Essential amino acids						
Arginine	3.14^{c}	4.20^{a}	2.87^{d}	3.89^{b}	3.91^{b}	3.60
Histidine	3.46^{d}	3.20^{e}	6.28^{a}	6.07^{b}	3.98^{c}	4.60
Isoleucine	7.20^{a}	6.53^{c}	5.81^{b}	4.62^{e}	6.00^{d}	6.04
Leucine	12.90^{a}	12.14^{b}	11.70^{c}	9.65^{e}	10.87^{d}	11.45
Lysine	6.37^{c}	8.91^{a}	7.67^{b}	7.57^{b}	7.67^{b}	7.64
Methionine	0.44^{c}	0.36^{d}	0.71^{a}	0.36^{d}	0.50^{b}	0.47
Phenylalanine	3.06^{a}	3.02^{b}	2.57^{c}	1.16^{d}	3.02^{a}	2.55
Threonine	4.74^{c}	4.84^{b}	4.00^{e}	5.18^{a}	4.38^{d}	4.63
Tryptophan	1.19^{a}	1.21^{a}	1.03^{b}	0.50^{c}	1.18^{a}	1.02
Valine	9.95^{a}	9.11^{c}	8.58^{d}	8.29^{e}	9.64^{b}	9.11
Non-essential amino acids						
Alanine	12.72^{a}	11.92^{c}	13.49^{b}	11.30^{d}	12.12^{c}	12.31
Aspartic acid	16.44^{a}	14.16^{b}	16.08^{b}	16.00^{c}	16.46^{a}	15.83
Cysteine	1.79^{b}	2.42^{a}	1.20^{c}	1.22^{c}	1.15^{d}	1.56
Glutamic acid	16.18^{d}	17.81^{b}	13.24^{e}	18.40^{a}	16.73^{c}	16.47
Glycine	15.02^{d}	14.70^{e}	17.64^{a}	16.60^{b}	15.84^{c}	15.96
Proline	0.68^{a}	0.31^{e}	0.56^{c}	0.65^{b}	0.49^{d}	0.54
Serine	7.63^{b}	7.01^{d}	7.62^{b}	8.86^{a}	7.10^{c}	7.64
Tyrosine	2.21^{b}	2.93^{a}	1.55^{d}	1.61^{c}	1.34^{e}	1.93

Means of each row followed by the different letter are significantly different at the 0.01 level.

Table 3
Pearson correlation coefficients for amino acids in bee-pollens from major pollen floral sources in Al-Ahsa, KSA.

	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	TRY	VAL	ALA	ASP	CYS	GLU	GLY	PRO	SER
ARG																	
HIS	−0.39																
ILE	−0.21	-0.80^{**}															
LEU	−0.39	-0.61^{**}	0.95^{**}														
LYS	0.61^{**}	−0.27	0.03	0.04													
MET	-0.75^{**}	0.48	0.01	0.17	−0.26												
PHE	−0.15	-0.72^{**}	0.89^{**}	0.81^{**}	0.12	−0.12											
THR	0.61^{**}	−0.20	-0.72^{**}	−0.29	0.13	−0.94	-0.52^{*}										
TRY	−0.21	-0.74^{**}	0.90^{**}	0.79^{**}	0.14	−0.11	0.83^{**}	0.40									
VAL	−0.06	-0.83^{**}	0.85^{**}	0.66^{**}	−0.23	−0.12	0.81^{**}	−0.11	0.40								
ALA	-0.82^{**}	0.14	0.44	0.62^{**}	−0.24	0.85^{**}	0.52^{*}	-0.81^{**}	0.54^{*}	0.20							
ASP	−0.62	0.38	−0.14	−0.15	-0.89^{**}	0.41	−0.12	−0.28	−0.11	0.18	0.31						
CYS	0.39	-0.068^{**}	0.55^{*}	0.57^{*}	0.61^{**}	-0.50^{*}	0.34	0.39	0.37	0.19	−0.11	-0.82^{**}					
GLU	0.86^{**}	−0.36	−0.20	−0.38	0.29	-0.91^{**}	−0.35	0.90^{**}	−0.32	0.01	-0.90^{**}	−0.36	0.36				
GLY	-0.50^{*}	0.95^{**}	-0.66^{**}	−0.47	−0.29	0.72	−0.40	−0.48	−0.44	-0.68^{**}	0.38	0.45	-0.70^{**}	−0.60			
PRO	-0.61^{**}	0.45	−0.21	−0.16	-0.89^{**}	0.13	−0.40	0.12	−0.41	0.01	0.13	0.82^{**}	-0.55^{*}	−0.17	0.40		
SER	−0.10	0.69^{**}	−0.21	-0.63^{**}	−0.41	−0.20	-0.93^{**}	0.50^{*}	-0.90^{**}	-0.62^{**}	−0.35	0.39	−0.38	0.25	0.46	0.70^{**}	
TYR	0.32	-0.65^{**}	0.57^{*}	0.59^{*}	0.59^{*}	-0.51^{*}	0.32	0.40	0.34	0.23	−0.12	-0.79^{**}	0.88^{**}	0.33	-0.73^{**}	-0.53^{*}	−0.36

Explanations: ARG = arginine, HIS = histidine, ILE = iso-leucine, LEU = leucine, LYS = lysine, MET = methionine, PHE = phenylalanine, THR = threonine, TRY = tryptophan, VAL = valine, ALA = = alanine, ASP = aspartic acid, CYS = cysteine, GLU = glutamic acid, GLY = glycine, PRO = proline, SER = serine, and TYR = tyrosine.
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

valine. Serine was found to be significantly positive correlated with histidine ($r = 0.69^{**}$), threonine ($r = 0.50^{*}$) and proline ($r = 0.70^{**}$). Also, alanine was significantly positive correlated with leucine ($r = 0.62^{**}$), methionine ($r = 0.85^{**}$), phenylalanine ($r = 0.52^{*}$), and tryptophan ($r = 0.54^{*}$). On contrary, glycine was significantly ($p < 0.01$) negative correlated with cysteine, isoleucine, methionine, valine, tyrosine and glutamic acid. Similarly, arginine was significantly ($p < 0.01$) negative correlated with methionine, alanine,

Table 4
Amino acids (g/100 g protein) in bee-pollens compared to the minimum requirements of honeybees and humans.

Amino acids	Alfalfa	Date palm	Summer squash	Sunflower	Rape	Minimum requirements	
						A	B
Essential amino acids							
Arginine	1.55	2.12	1.75	2.56	2.07	3.00	-
Histidine	1.71	1.62	3.83	4.00	2.11	1.50	1.50
Isoleucine	3.56	3.30	3.54	3.04	3.18	4.00	3.00
Leucine	6.38	6.14	7.14	6.35	5.76	4.50	5.90
Lysine	3.15	4.51	4.68	4.98	4.07	3.00	4.50
Methionine	0.22	0.18	0.43	0.24	0.27	1.50	1.60
Phenylalanine	1.51	1.53	1.57	0.76	1.60	1.50	-
Threonine	2.34	2.45	2.44	3.41	2.32	1.50	2.30
Tryptophan	0.60	0.61	0.63	0.33	0.63	1.00	0.60
Valine	4.92	4.61	5.23	5.46	5.11	4.00	3.90
Phenylalanine + Tyrosine	2.60	3.01	2.52	1.82	2.31	-	3.80
Non-essential amino acids							
Alanine	6.29	6.03	8.23	7.44	6.43	–	–
Aspartic acid	8.11	7.16	9.81	10.53	8.73	–	–
Cysteine	0.36	0.47	0.20	0.19	0.22	–	–
Glutamic acid	8.00	9.01	8.08	12.11	8.87	–	–
Glycine	7.42	7.44	10.76	10.93	8.40	–	–
Proline	0.34	0.16	0.34	0.43	0.26	–	–
Serine	3.77	3.55	4.65	5.83	3.76	–	–
Tyrosine	1.09	1.48	0.95	1.06	0.71	–	–

A = Minimal levels of essential amino acids required by honeybees (De Groot, 1953).
B = Minimal levels of essential amino acids required by adult humans (FAO, 2007).

Table 5
Chemical score in bee-pollens compared to the minimum requirements of honeybees.

Amino acids	Alfalfa	Date palm	Summer squash	Sunflower	Rape	Average
Arginine	51.67*	70.81	58.37*	85.36	69.11	67.06
Histidine	114.00	107.91	255.44	266.40	140.67	176.88
Isoleucine	89.02	82.57	88.62	76.04	79.53	83.156
Leucine	141.78	136.46	158.63	141.17	128.08	141.22
Lysine	105.01	150.23	155.99	166.12	135.56	142.58
Methionine	14.51**	12.14**	28.88**	15.80**	17.67**	17.80**
Phenylalanine	100.87	101.84	104.53	50.91	106.75	92.98
Threonine	156.28	163.33	162.70	227.34	154.83	172.90
Tryptophan	60.00	61.00*	63.00	33.00*	63.00*	56.00*
Valine	123.02	115.20	130.87	136.44	127.78	126.66
Average	95.62	100.15	120.70	119.86	102.30	107.73

** 1st limiting amino acid.
* 2nd limiting amino acid.

Table 6
Chemical score in bee-pollens compared to the minimum requirements of adult humans.

Amino acids	Alfalfa	Date palm	Summer squash	Sunflower	Rape	Average
Histidine	114.00	108.00	255.33	266.67	140.70	157.20
Isoleucine	118.67	110.00	118.00	101.33	106.00	110.07
Leucine	108.14	104.07	121.02	107.63	97.63	87.73
Lysine	70.00	100.22	104.00	110.67	90.44	108.67
Methionine	13.75*	11.25*	26.88*	15.00*	16.88*	16.75*
Threonine	101.74	106.52	106.09	148.26	100.9	112.70
Tryptophan	100.00	101.67	105.00	55.00	105.00	82.44
Valine	126.15	118.21	134.10	140.00	131.00	129.89
Phenylalanine + Tyrosine	68.42**	79.21**	66.32**	47.90**	60.79**	64.53**

* 1st limiting amino acid.
** 2nd limiting amino acid.

aspartic acid and proline. Also, histidine and serine were found to be significantly ($p < 0.01$) negative correlated with leucine, isoleucine, phenylalanine and valine.

Apart from methionine, arginine and isoleucine, the essential amino acids of bee-pollens from alfalfa, date palm, summer squash and rape were found to be above the honeybee's requirements (Table 4). Compared to the minimum requirements of honeybees and adult humans, the chemical score values clearly showed that, methionine was the first limiting amino acid in all analyzed pollen samples (Tables 5 and 6).

4. Discussion

The protein and amino acids concentrations in bee-pollens were variable and significantly ($p < 0.01$) depending on the botanical origin. These results were endorsed by the findings of Somerville and

Nicol (2006), Szczęsna (2006a, b), Weiner et al. (2010), and Yang et al. (2013). The protein in pollen loads from sunflower was found to be deficient in both quantity and amino acids contents. The results confirmed the findings of Tasei and Aupinel (2008), Nicolson and Human (2013). Meanwhile, Somerville and Nicol (2006) did not indicate deficiencies in amino acids of bee-pollen from sunflower. The values (g/100 g protein) of leucine, valine, alanine, aspartic acid, glutamic acid, glycine and serine were higher than those obtained by Nicolson and Human (2013), while arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, proline and tyrosine were lower than their values. The values of the estimated amino acids in rape bee-pollen were found to be lower than those determined in *Brassica* pollen (Szczęsna, 2006b). Apart from glutamic acid and tryptophan in bee-pollen from date palm, values of the remaining amino acids were found to be lower than those of date palm pollen grains (Hassan, 2011). That may be resulted from adding nectar sugars during pellets forming, which caused carbohydrate increases, while the protein (amino acids), and other contents decreases in bee-pollen (Nicolson and Human, 2013).

The nutritional value of pollen protein for honeybees depends on the concentrations of essential amino acids relative to requirements of honeybees (de Groot, 1953) and for humans it depends on these concentrations relative to requirements of adult humans (FAO, 2007). Except for arginine, isoleucine, methionine, and tryptophan all essential amino acids in the protein of the tested bee-pollens were above the requirements of honeybees (de Groot, 1953). Unfortunately, values of phenylalanine in bee-pollen from sunflower were lower than requirements of honeybees. Based on the requirements of adult humans, histidine, phenylalanine, threonine, and valine in all tested bee-pollens exceeded the values recommended by FAO (2007). The values of arginine, methionine, and phenylalanine + tyrosine in bee-pollens from the tested five sources, as well as leucine in rape bee-pollen, lysine in bee-pollen collected from alfalfa and rape were lower than the values recommended by FAO (2007).

Compared to total quantified amino acids, the essential amino acids in bee-pollens were found to be 42.87% for date palm, 41.97% for alfalfa, 41.80% for rape, 41.81% for summer squash, and 38.77% for sunflower. These percentages were closed to the findings of Hassan (2011) for pollen from date palm, while they were lower than those obtained by Nicolson and Human (2013) for sunflower, and those recorded by Szczęsna (2006a) for rape.

The nutritive value of bee-pollen protein was estimated as CS compared to the minimum requirements of honeybees, and EAAI. The values (%) of CS in bee-pollens protein could be arranged in descending orders as follows: summer squash (120.70) > sunflower (119.86) > rape (102.30) > date palm (100.15) > alfalfa (95.62). The values (%) of EAAI in bee-pollen protein could be arranged in descending orders as follows: summer squash (78.00) > sunflower (68.07) > rape (67.74) > date palm (64.00) > alfalfa (62.03). The value of rape bee-pollen was higher than the value obtained by Szczęsna (2006a) for CS and lower for EAAI. Although the concentrations (mg/g DM) of most amino acids in sunflower and summer squash bee-pollens were low compared to the other three tested pollens, the protein composition showed the highest levels (g/100 g protein) of arginine, histidine, lysine, threonine, and valine, and subsequently the CS and EAAI. These results were related to the low protein content in bee-pollens from sunflower and summer squash compared to the protein level of the other pollens analyzed.

The CS values clearly showed that, for honeybee, methionine was the first limiting amino acid (14.51, 12.14, 28.88, 15.80 and 17.67%) for alfalfa, date palm, summer squash, sunflower and rape, respectively. Arginine was the second limiting amino acid (51.67 and 58.37%) for alfalfa and summer squash, respectively. Tryptophan was the second limiting amino acid (61.00, 33.00 and 63.00%) for date palm, sunflower and rape, respectively. These results are endorsed for methionine by Hassan (2011) and Basuny et al. (2013) for date palm pollen, Nicolson and Human (2013) for sunflower bee-pollen, and Szczęsna (2006a) for bee-pollen from rape. Compared to the minimum requirements of adult humans, methionine was the first limiting amino acid (13.75, 11.25, 26.88, 15.00, and 16.88%), followed by phenylalanine + tyrosine (68.42, 79.21, 66.32, 47.90, and 60.79%) for alfalfa, date palm, summer squash, sunflower, and rape, respectively.

Based on the concentration of the total essential amino acids, leucine was the most prevalent essential amino acid and reached about 24.57% (alfalfa), 22.81% (summer squash), 22.69% (date palm), 21.23% (rape), and 20.40% (sunflower) of the total essential amino acids quantified. Valine was found to be the second predominant essential amino acid and represented about 18.95% for alfalfa, 18.83% for rape, 17.53% for sunflower, 17.03% for date palm, and 16.72% for summer squash bee-pollen. These results are confirmed by the findings of Hassan (2011), Basuny et al. (2013) for date palm pollen, Nicolson and Human (2013) for sunflower bee-pollen, and Szczęsna (2006a) for rape bee-pollen.

The dominant five amino acids in the tested bee-pollens were found to be aspartic acid, glutamic acid, glycine, leucine, and alanine. They constituted about 58.56, 56.67, 58.80, 58.98, and 58.79% of the total quantified amino acids for alfalfa, date palm, summer squash, and rape, respectively. These results are endorsed by those obtained by Szczęsna (2006a) for rape bee-pollen.

5. Conclusion

The concentrations of essential amino acids expressed as total essential amino acids (g/100 g DM) or as percentages of total amount of amino acids were variable and significantly depended on the botanical origin of pollen. Bee-pollens from alfalfa, date palm and summer squash were important sources of protein and amino acids for bees and human.

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