

INFLUENCE OF CONVECTIVE DRYING TYPE ON PHENOLIC COMPOUND COMPONENTS OF THE EGYPTIAN HONEY BEE POLLENS

By

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Abstract

The present work determined the efficacy of three drying methods, 41°C sun drying (SD), 32°C shadow drying (ShD) and 32, 40 & 50°C incubator (ID) on the percentage of moisture losses and phenolic compounds content of pollen grains throughout 72h. SD & ShD drying bee pollens showed the highest number of phenolic compound after 72h. with total peak of 11388228 & 14374500 respectively. The 32°C, 40°C & 50°C ID of bee pollens showed the highest number of phenolic compounds after 24h., with total peak of 12429414, 12078595 & 12578030 respectively.

Key words: Honey bee, Pollens, Drying, Convective, Phenolic compounds.

Introduction

Bee pollen is a beehive product obtained by honey bees (*Apis mellifera* L.) gathering millions of floral pollen grains and mixing it with plant nectar and bee saliva rich in enzymes, thus transforming its composition and improving its therapeutically potential (Cocan *et al*, 2005). The bee pollen drying aimed to remove water to minimize microbial spoilage. There was a significant reduction in weight and volume contributing to reduce the cost of handling, storage and distribution (Sokhansanj and Jayas, 1995). Bee pollen is sold as fresh and dried. Fresh bee pollen needs to be refrigerated between 5°C & 10°C to keep its quality. The drying preserves the bee pollen for longer time at room temperature without refrigeration by preventing rapid fermentation and microbial spoilage, providing ease of marketability for bee pollen and increasing profit of the beekeepers (Barajas *et al*, 2012). Sometimes, the sun drying of bee pollen is used inappropriate because of considerable process time, increased microbial spoilage during drying process and lower with sanitary conditions. The hot-air drying is a suitable and frequently used in a commercial product for reasonable process time, better sanitary and drying control conditions (Crapiste and Rotstein, 1997). Mostly the collected pollen

pellets contain impurities, which should be removed, most efficiently by air specially constructed purifiers. The air should be free of dust and bacteria. In order to have minor losses of nutrients, pollen should be stored in a cool, dry place in well closed glass or plastic containers (Bogdanov, 2004). Nutrient content of pollen changes due to storage. Pollen is best dried in an electric oven, where humidity can continuously escape. The feasible temperature was 40°C and the drying time should be as short as possible to avoid losses of volatile compounds until the humidity was 6% or lower. Such pollen remains stable during storage for 15 months. Pollen containing more than 6% of water easily ferment upon storage. Storage for one year or more reduce the pollen free radical scavenging capacity (Campos *et al*, 2008). Bee pollen with high moisture content (20-30g water/100g product in wet basis or 25-42.9g water/100g dry solid basis) perished after a short period of time from harvest as being highly susceptible to microbial attacks (De-Melo *et al*, 2016). Low temperature may lead to formation of reactive oxygen species (ROS), as superoxide radicals, hydrogen peroxide, hydroxyl-radical and single oxygen. To reduce the oxidation damage, plants use enzymatic and non-enzymatic antioxidant mechanisms to scavenge ROS (Greene,

2002). Among the chemical compounds in plants, secondary metabolites, in particular phenolic compounds (phenols and flavonoids) are of great importance in plant environment relationships (Rezanejad, 2009). These compounds are of a particular interest due to their involvement response of plant to environmental stress as low temperature (Robles *et al*, 2003). Natural antioxidants are found in pollen (LeBlank *et al*, 2009; De Arruda *et al*, 2013). Vitamins A, E, C, B-vitamins, niacin, rutin, polyphenols, and selenium compounds are widely found in pollen (Bonvehi, *et al*, 2001). Pollen grain contains bioactive compounds, which if present even in small amount, play an important role on human health. The bee pollen contains carotenoids, phenolic compounds and in particular flavonoids produced from plants' metabolism possessing numerous phenolic compounds with antioxidant activity (Bogdanov, 2006; Medeiros *et al*, 2008; Feás *et al*, 2012). The polyphenols are antioxidants with redox properties act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Campone *et al*, 2014; Ranneh *et al*, 2018). Fanaliet *al*. (2013) found nine polyphenols: *o*-, *p*-coumaric acid, ferulic acid, myricetin, cinnamic acid, quercetin, naringenin, hesperitin and kaempferol. All analytics, with the exception of *p*-coumaric acid and myricetin partially co-eluted with other pollen components, were also quantified in sample. Lv *et al*. (2015) identified pollen samples from Qinghai-Tibetan Plateau by HPLC-DAD-APCI/MS, quercetin and campherol, without routine and isoramnetin detection.

This work aimed to study the influence of convective type drying on phenolic compound components with optimum drying of bee pollens to produce high quality for this product.

Materials and Methods

The first hybrid of Carniolan bees race *Apis mellifera* L. of honey bee colonies were chosen in the apiary at Dokki, Giza 2018.

The pollen grains used in this experiment were collected by honeybees from multiflora were collected as corbicular pellets removed from the pollen baskets on the hind legs of bees as they passed through pollen traps attached to honeybee hives.

After the pollen grains carefully collection from pollen traps, they were exposed directly to different temperatures as the following: Sun rays dry (SD) 41°C, Shadow dry (ShD) 32°C and incubator dry (ID) 32, 40 & 50°C. All treated materials were collected after 24, 48 & 72h., and then the pollen grains were stored at -20°C until analysis.

Moisture reduction% = (weight pollen post temperature exposure/weight pollen before) - 1 X 100.

Determination of the phenolic compounds: Preparing of 10% pollen solution, one g of pollen was solved in 10ml ethanol 70%, and then kept in closed glass tubes for analysis by HPLC instrument. Identification of the phenolic compounds of bee pollen samples was done by a JASCO, using a hypersil C18 reversed- phase column (250 X 4.66 mm) with 5µm particle size. All chemicals and solvents used were in HPLC spectral grade, as well as the standard phenolic compounds were purchased (Sigma).

Statistical analysis: Data were analyzed in (ANOVA) a randomized complete block design and means were compared ($p \leq 0.05$) by Duncan's multiple rang test. All analyses were performed by using a version 20 of the software SPSS.

Results

The 40°C & 50°C ID had the highest significant differences values of the moisture losses (ML%) of bee pollen compared with others after 24h., but with neither significant variation between all temperatures on moisture reduction at 48 & 72h. nor temperatures using the three times (24, 48& 72h.) except for 41°C SD, which showed significantly the highest value 2.32 ML% after 48h (Tab. 1).

Table 1: Effect of different treatments and temperature degree on reduction of moisture percentage of bee pollens.

Treatment	41°C SD			32°C ShD			32°C ID			40°C ID			50°C ID			F	LSD _{0.05}	P ≤ 0.05
Time	W B	W A	%M	W B	W A	%M	W B	W A	%M	W B	W A	%M	W B	W A	%M			
24 h.	42.70	42.13	1.35	49.72	49.44	0.57	43.82	43.66	0.37	41.47	40.86	1.49	52.29	51.62	1.30	26.8	1.15	0.000 ***
	42.24	41.75	1.20	50.16	49.81	0.70	40.48	40.31	0.42	53.60	52.70	1.71	55.77	54.77	1.82			
	50.38	49.96	0.84	49.50	49.19	0.63	41.07	40.98	0.22	56.84	55.69	2.07	57.09	56.15	1.67			
Mean	45.11	44.61	1.13 ^{Bb}	49.79	49.48	0.63 ^{Ac}	41.79	41.65	0.34 ^{Ac}	50.64	49.84	1.77 ^{ABa}	55.05	54.18	1.60 ^{Aa}			
48 h.	43.45	42.50	2.24	51.44	50.79	1.28	38.53	38.15	1.00	55.54	55.11	0.78	40.69	40.24	1.12	2.57	2.99	0.103 ^{ns}
	43.76	42.81	2.22	55.16	54.58	1.07	53.81	53.61	0.37	53.05	52.73	0.61	41.10	40.62	1.18			
	41.15	40.16	2.47	51.19	51.05	0.28	39.95	38.81	2.93	46.91	46.59	0.69	38.39	37.89	1.32			
Mean	42.79	41.82	2.32 ^{Aa}	52.60	52.14	0.88 ^{Ab}	44.10	43.52	1.43 ^{ABab}	51.83	51.48	0.69 ^{Bb}	40.06	39.58	1.21 ^{Bab}			
72 h.	50.90	50.19	1.41	41.12	40.59	1.31	50.19	49.66	1.07	41.78	41.28	1.21	35.63	35.13	1.43	1.11	1.143	0.41 ^{ns}
	48.35	47.71	1.34	41.27	40.75	1.28	38.88	38.43	1.16	41.72	41.31	0.99	41.21	40.62	1.46			
	48.80	48.21	1.22	49.45	49.03	0.86	43.66	42.91	1.74	56.84	55.99	1.52	49.82	49.03	1.61			
Mean	49.35	48.70	1.32 ^{Ba}	43.96	43.46	1.15 ^{Aa}	44.24	43.67	1.31 ^{Aa}	46.78	45.86	1.24 a	42.22	41.59	1.50 ^{ABa}			
Total	4.77			2.66			3.08			3.70			4.31					
F	25.14			2.28			2.88			4.43			4.56					
LSD _{0.05}	0.99			2.31			3.81			2.90			0.82					
P ≤ 0.05	0.0012 ^{**}			0.1837 ^{ns}			0.1329 ^{ns}			0.0657 ^{ns}			0.0624 ^{ns}					

Means data with different capital letters in column and small letters in row at p ≤ 0.05, WB= Bee pollen weight before treatment, WA= Bee pollen weight after treatment, %M = moisture percentage, SD= Sun drying, ShD=Shadow drying, ID=Incubator drying.

Table 2: Influence of convective drying by different methods on phenolic compounds component in bee pollens (mg/100g)

Standards	Control	Sun rays (41°C)			Shadow(32°C)		
		24h	48h	72h	24h	48h	72h
Pyrogallol acid: *benzene-1,2,3-triol	0.0000	0.0000	0.0000	0.9731	0.0000	0.0000	0.0000
*Phenol	0.0115	0.0000	0.0143	0.0000	0.0011	0.0000	0.0178
Salicylic acid : *2-hydroxybenzoic acid	0.0028	0.3019	0.0000	0.0000	0.0000	0.0000	0.0035
Ferulic acid : *3-(4-hydroxy-3-methoxy-phenyl)prop-2- enoic acid	0.0900	0.0000	0.0000	0.0407	0.0562	0.0323	0.0011
*3,5-dimethoxyphenol	0.0000	0.1350	0.0347	0.0000	0.0000	0.0000	0.1095
p-Coumaric acid: *3-(4-hydroxyphenyl)prop-2-enoic acid	0.0004	0.0000	0.0000	0.0000	0.0257	0.0120	0.0045
Phenolphthalein: *3,3-Bis(4-hydroxyphenyl)-2-benzofuran-1(3H)-one	0.0000	0.0000	0.0000	0.0000	0.0378	0.1756	0.0020
Eugenol: *4-Allyl-2-methoxyphenol	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1853
Cinnamic acid: *(E)-3-phenylprop-2-enoic acid	0.0000	0.0051	0.0008	0.0010	0.0000	0.0000	0.0000
Gallie acid: *3,4,5-Trihydroxy benzoic acid	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Resorcinol: *benzene-1,3-diol	0.0000	0.0000	0.5218	0.7305	0.0000	0.0000	0.0000
Protocatchol	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
p-OH benzoic acid *4-Hydroxybenzoic acid	0.0011	0.0000	0.0012	0.0001	0.0000	0.0000	0.0000
Caffeic acid: *3-(3,4-dihydroxyphenyl)prop-2-enoic acid	0.0000	0.0029	0.0000	0.0000	0.0000	0.0000	0.0000
Vanillin: *4-hydroxy-3-methoxy-benzaldehyde	0.0000	0.0000	0.0058	0.0009	0.0000	0.0000	0.0000
Quercetin: *2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy- chromen-4-one	0.0037	0.0141	0.0026	0.0151	0.0000	0.0040	0.0000
Pinocembrin: *(2S)-5,7-dihydroxy-2-phenyl-2,3 dihydrochromen-4-one	0.0000	0.0235	0.0000	0.0064	0.0047	0.0000	0.0000
Chrysin: *5,7-dihydroxy-2-phenyl-chromen-4-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Galangin: *3,5,7-trihydroxy-2-phenyl-chromen-4-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Acacetin: *5,7-dihydroxy-2-(4- methoxyphenyl)chromen-4-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3,5 dihydroxyisoflavone: *3,5-Dihydroxy-3-(4-hydroxyphenyl)chromen-4-1	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000
Pinostrobin: *5,7-dihydroxy-2-phenyl-chroman-4-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Total Peak Area	13139199	10644580	12148065	11388228	10730331	12112796	14374500

The influence of convective drying by the different methods on phenolic compounds value in bee pollens: In 41°C SD phenolic content after 24h., were seven compounds: caffeic acid, salicylic acid, 3, 5-dimethoxyphenol, cinnamic acid, quercetin, pinocembrin and 3, 5-dihydroxy isoflavon. Also, seven phenolic compounds were found after 48h.: Resorcinol, p-ohbenzoic, phenol, vanillin, 3, 5-dimethoxyphenol, cinnamic acid, quercetin, but increased to nine phenolic

compounds. After 72h the resulting phenolic compounds kinds and percentage were not stability. The more stable phenolic compounds were cinnamic acid and quercetin. In 32°C ShD the number of phenolic compound unstable through 72 h. were five, four & seven respectively, after the first 24h. ferulic acid was the highest compound 0.0562 mg/100g, while phenol phthaleinit (0.1756 mg/100g) was the highest one after 48h. (Table 2 continued):

Standards	Control	Incubator (32°C)			Incubator (40 °C)			Incubator (50 °C)		
		24h	48h	72h	24h	48h	72h	24h	48h	72h
Pyrogalllic acid: *benzene-1,2,3-triol	0.0000	1.2697	0.0000	0.0000	1.3219	1.2079	0.0000	0.7806	0.4341	0.7922
*phenol	0.0115	0.0000	0.0000	0.0122	0.0000	0.0000	0.0000	0.0000	0.0000	5.5771
Salicylic acid : *2-hydroxybenzoic acid	0.0028	2.1413	0.0000	2.5651	0.0020	0.0000	0.9681	0.0036	0.1026	0.0000
Ferulic acid: *3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid	0.0900	0.0169	0.0000	0.0000	0.0036	0.0000	0.0182	0.0963	0.0000	0.2997
*3,5-dimethoxyphenol	0.0000	0.0000	0.5807	0.1258	0.0000	0.0000	0.0000	0.0000	0.0000	1.2907
p-Coumaric acid: *3-(4-hydroxyphenyl)prop-2-enoic acid	0.0004	0.1547	0.0000	0.0000	0.0022	0.07725	0.0000	0.0000	2.9091	0.0000
Phenolphthalein: *3,3-Bis(4-hydroxyphenyl)-2-benzofuran-1(3H)-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Eugenol: *4-Allyl-2-methoxyphenol	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Cinnamic acid: * (E)-3-phenylprop-2-enoic acid	0.0000	0.0009	0.0000	0.0000	0.0014	0.0285	1.8323	0.0000	7.7647	0.0000
Gallic acid: *3,4,5-Trihydroxy benzoic acid	0.0000	0.0000	0.0129	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Resorcinol: *benzene-1,3-diol	0.0000	0.3364	0.0000	0.0000	0.2900	0.0000	0.0000	0.8725	0.0000	0.0000
Protocatechol	0.0000	0.0051	0.0000	0.0000	0.0044	0.0000	0.0000	0.0060	0.0000	0.0000
p-OH benzoic acid *4-Hydroxybenzoic acid	0.0011	0.0011	0.0000	1.0516	0.0011	0.0000	1.1897	0.0009	7.9380	9.8671
Caffeic acid: *3-(3,4-dihydroxyphenyl)prop-2-enoic acid	0.0000	0.0000	0.0000	0.0000	0.0000	1.4652	0.0000	0.0000	0.0000	0.0000
Vanillin: *4-hydroxy-3-methoxy-benzaldehyde	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	6.0731	0.0000
Quercetin: *2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-chromen-4-one	0.0037	0.0234	0.0000	4.2376	0.0218	0.0000	1.0311	0.0247	0.0163	4.0814
Pinocembrin: *(2S)-5,7-dihydroxy-2-phenyl-2,3-dihydrochromen-4-one	0.0000	0.0000	0.0000	0.0000	0.0084	8.9172	0.0000	0.0000	7.3439	0.0000
Chrysin: *5,7-dihydroxy-2-phenyl-chromen-4-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.8292	0.0000	0.0000	0.0000
Galangin: *3,5,7-trihydroxy-2-phenyl-chromen-4-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.7936	0.0000	0.0000	0.0000
Acacetin: *5,7-dihydroxy-2-(4-methoxyphenyl)chromen-4-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	5.0083	0.0000	0.0000	0.0000
3,5-dihydroxyisoflavone: *3,5-Dihydroxy-3-(4-hydroxyphenyl)chromen-4-one	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pinostrobin: *5,7-dihydroxy-2-phenyl-chroman-4-one	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Total Peak Area	13139199	12429414	10377088	11482122	12078595	11204660	11505611	12578030	10515447	12307766

Eugenol (0.185mg/100g) was the highest compound after 72h., while the more stable compounds were ferulic acid, coumaric acid and phenolphthalein. In 32°C ID phenolic compounds were 10 after the first 24h., and decreased to two & five after 48 & 72h., respectively, without stable phenolic ones. In 40°C ID ten phenolic compounds were detected after the first 24h., decreased to five after 48h., and increased to eight after 72h. The cinnamic acid was stable after 72h. In 50°C ID seven compounds were found after 24h., increased to eight and decreased to six compounds after 48 & 72h., respectively, with three phenolic ones were stable in this treatment pyrogalllic acid, p-oh benzoic and quercetin. Stable phenolic compounds at 41°C SD were cinnamic acid and quercetin. Quercetin increased after 72h., to 0.0151mg/100g compared to control 0.0037mg/100g. Pinocembrin at 40°C ID after 48h drying showed the highest 8.9172mg/100g. It has the primary multifunctional flavonoid used in pharmaceutical industry. After 72h drying ferulic acid was detected. Pyrogalllic acid recorded 1.2697mg/100g & 1.3219mg/100g at 32°C ID &40°C ID after 24h. respecti-

vely, but with lowest amount was 0.434mg/100g &0.7806mg/100g at 50°C ID after 48h & 24h., respectively. Most of the phenolic compounds useful properties increased at 40°C ID & 50°C ID. p-oh benzoic for instance reached 1.1897mg/100g & 9.8671mg/100g for 40°C ID and 50°C ID, respectively. At 40°C ID vanillin recorded 6.0731mg/100g as compared to 41°C SD, which recorded 0.0058 mg/100g.

Discussion

Temperature is one of the most critical factors that affect product quality and shelf-life. In general, in literature, the effects of heating on the degradation of biologically active compounds have been assessed in terms of the levels of phenolic compounds (Georgé *et al*, 2011). Barajas *et al*. (2012) found that the drying process of bee pollens at 45°C had the moisture content (7-8%), but at 35°C moisture content increased to reach 9-11%. Dried bee pollens at 35°C & 45°C were not enough to determine parameters in drying kinetics. Hot air drying affected organoleptic properties, & pollens. Midilli *et al*. (2000) dried bee pollens at 45°C in solar and electric assisted dryer and measured weight

changes of pollens during the drying. They were unable to study drying kinetics of bee pollen at one temperature. Many factors contribute to the composition of bee pollen, as the plant source, climatic conditions, soil characters and the beekeeper activities (Morais *et al*, 2011; Estevinho *et al*, 2012). The relative instability of most phenolic compounds from plants showed their sensitivities to drying method (Lim and Murtijaya, 2007). Phenolic constituents concentration depends on various factors, including plant species used by the bees, the plant health, season, environmental factors...etc. (Küçük *et al*, 2007). The commonest phenolic acids were chlorogenic, gallic, ferulic, cinnamic (Almaraz-Abarca *et al*, 2004), caffeic acids (Kędzia, 2008) and hydroxyl cinnamic, ortho-coumaric and paracoumaric acids (Bonvehi *et al*, 2001; Almaraz-Abarca *et al*, 2007). Šarić *et al*. (2009) in phenolic composition of Croatian bee pollens by HPLC analysis detected seven compounds, which were flavonol (pinocembrin), flavanols (quercetin, kaempferol, galangin, & isorhamnetin), flavones (chrysin) and phenylpropanoids (caffeic acid).

In Egyptian bee pollen, quercetin, rutin, catechin, epicatechin, kaempferol, apigenin, naringenin, and luteolin were detected (Mohdaly *et al*, 2015) that agreed with Buchner *et al*. (2006) who found that the thermal treatment of quercetin was the most important flavonoid widely used in human drugs. It has antimicrobial, anti-inflammatory, antioxidant and anticancer activities (Rasul *et al*, 2013). Ewald *et al*. (1999) found that some phenolic compounds, such as ferulic acid and *p*-coumaric acid, existed as free acids, soluble esters and insoluble esters in rice, corn and other pollen grains. Even when free phenolic acids are in low concentration in foods, they may increase in processed food underwent freezing or fermentation, and could affect by food processing as well, especially those involved in thermal treatment. The pyrogallol was used as antioxidant and as a topical anti-psoriatic (Budavari, 1996), also used in conjunction with ultraviolet B

for resistant psoriasis (Siage, 1976). *p*-hydroxy benzoic acid, chemical obtained naturally and synthetically has anti-microbial, anti-algal, anti-mutagenic, anti-estrogenic, hypoglycemic, anti-in-flammatory, anti-platelet aggregating, nematocidal, antiviral, antioxidant...etc. (Manuja *et al*, 2013). Vanillin sensitive to sunlight, on heating gives CO & CO₂ with anti-microbial activity (Kumar *et al*, 2012). Isik *et al*. (2019) found that sun drying was the widely used traditional method for pollen drying, but with some disadvantages such as long drying times, microbial contamination risk, insect infestation susceptibility, large drying area, and high man power costs without control of drying conditions. Hot air drying has shorter time, low microbial contamination risk, more effective sanitary conditions and better control of drying conditions. So hot air dried at 40°C was recommended for the drying of bee pollens.

Conclusion

Determination of the effect of drying temperature and drying time on Phenolic compounds, of bee pollen is important to obtain bee pollen products with high quality characteristics. Sun and shadow drying bee pollen showed highest phenolic compounds after 72h., with a peak area 11388228 & 14374500 respectively. At 32°C, 40°C & 50°C incubator drying of bee pollen showed highest compounds after 24h. with a peak area 12429414, 12078595 & 12578030 respectively. Moisture reduction was of no significant in all drying methods after 48 & 72h. Recommended for of bee pollens, drying was hot air at 32°C ShD, 32, 40 & 50°C incubator 24h., while 41°C SD 48h.

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