

Quality control of home grinded against ready prepared chosen spices from Libyan market

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Abstract: Spices are any pungent, aromatic plant substances used to flavor food or beverages. Plant foods and agricultural commodities including spices are increasingly subjected to adulteration by design or default. This study aimed to compare home grinded spices against ready locally prepared ones in Libyan market. Chosen spices were compared according to their percentage of yield, organoleptic features, and pharmacognostic parameters as macroscopic and microscopic characteristics, pH measurement, microbial contamination (total viable count and microbial identification), and thin layer chromatography (TLC) chromatogram and diphenyl picryl hydrazyl (DPPH) scavenging activity. From the results, the ready prepared samples showed to have higher percentage of yield compared to home prepared ones. There were no difference in organoleptic test results, macroscopic and microscopic characteristics and pH test results. Bacteria in spices samples were too many to count (TMT) in most samples, however thyme, rosemary and cinnamon showed better results. The isolated bacteria were identified as *Salmonella*, *Shigella* and *E. coli* species. TLC chromatogram and DPPH scavenging activity test also showed no difference in both sample groups. All these tests indicates that the samples obtained from market as grinded powder and the same samples of spices that was brought as a raw materials and grinded at home had the same characteristics, which indicated that they are of the same quality which not necessary to be a good one.

Keywords: Spices, adulteration, quality control, Libya.

Introduction

Spices are food products or food supplements, which have been used as flavoring, coloring agents, as food preservatives and herbs in folk medicines for many years in different parts of the world (1). Spices are considered to be good contributors to protein, carbohydrates, fats, vitamins and minerals nutrient intake, leading to enhancement of the nutritional quality of diets (2). Many spices have known to possess digestive stimulant action, carminative effect, anti-microbial activity, antioxidant capacity, anti-inflammatory property, anti-mutagenic ability and anti-carcinogenic potential

(1). In general, the main active constituents in spices are phenolic acids, flavonoids and volatile or essential oils (3). As food supply chains have become increasing global and complex, challenging risks have emerged (4, 5). One of the gaining attentions risks is food ingredient fraud and economically motivated adulteration (EMA) (6). Food fraud may define the intentional or economically motivated adulteration of food ingredients as “the fraudulent addition of no authentic substances or removal or replacement of authentic substances without the purchaser's

knowledge for economic gain of the seller” (7). Commonly used terms to describe food fraud include economically motivated adulteration, economic adulteration and food counterfeiting. Food fraud considered to be foremost an economic issue with less a concern of the traditional food safety or food protection intervention and response infrastructure. Any adulteration results in a change of the identity, purity of the original and purported ingredient by substituting, diluting, or modifying it by physical or chemical means (6).

There is a wide range of food products and risks, food ingredients and additives present a unique risk due to its use in many food products without having unique visual or functional properties that enable easy discrimination from similar ingredients or adulterants (8). Adulteration has been a serious problem for years regarding essential oils. Synthetic flavor blending with natural oil include impurities, these are characteristic of the synthetic route used for preparation (9). The mixing of expensive oils with cheaper ones can be detected by running a GC profile of the oil (10). While food fraud may be economically motivated, there is big risk on the public health. These threats are potentially risky because there are a near infinite number of unconventional adulterants and contaminants (11). Food fraud take place in liquids as well as solids. Grinding of spices into powders makes it easier for adulteration to carry out and less likely to be spotted (12). In most instances of adulteration, spices are adulterated with not highly toxic or carcinogenic material, therefore does not present a significant, immediate public health risk. Economic spices adulteration can have serious implications. In some cases, spices have been adulterated

with toxic materials such as lead-containing pigments, causing serious public health consequences. In Hungary (1994) for example, ground paprika was adulterated with lead oxide, causing the deaths of several people (13).

An incident persisted from 2003 to 2005 in the United Kingdom (UK) involved the fraudulent addition of Sudan red dyes to spices such as chili powder, which led to the largest recall of food in UK to that date involving more than 580 products (14). In reports from Food and Drug Administration (FDA), researchers studied the food safety of spices and found contaminants that range from food borne pathogens such as salmonella, heavy metals, to micro-toxins, which are considered carcinogens.

Spices and herbs are exposed to much microbial contamination during pre- and post-harvest. Such contamination may occur during processing storage, distribution, sale and/or use (15). Spices are commonly heavily contaminated with xerophilic storage moulds and bacteria (16, 17). The most frequent spices fungal contaminants are species from the genera *Aspergillus* and *Penicillium* (18, 19). The dominance of *Aspergillus* and *Penicillium* spp. was reported by Takatori and Ayres (20, 21). Nagy Halim Aziz also stated that *Aspergillus* and *Penicillium* spp. were the main components of cardamom, cinnamon, fennel, coriander, cumin, black cumin, and white pepper, which are all common in the food industry (22). There is big interest in fast, simple and efficient techniques for quality control especially in low funded associations. Pharmacognostic profile of spices was reported helpful in developing standards for quality, purity and sample identification (23,

24). Method of the detection of food adulteration is based on physical, chemical, biochemical, and microscopic technologies. All these methods are still being improved because food adulteration continues in a new form (25).

In this study, some spices were chosen according to their availability and extensive daily use by Libyans in food preparation like thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum zeylanicum*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), black pepper (*Piper nigrum*) and red pepper (*Capsicum annum*) to be qualified, tested and compared according to simple available techniques.

Materials and methods

Plant collection: Plant specimen was mainly obtained from market as 7 different spices in the form of completely grinded powder (thyme, rosemary, ginger, cinnamon, red pepper, black pepper and turmeric), the same seven spices were collected as raw materials and were grinded at home. All spices were exported from East Asia and India to the Libyan market except thyme and rosemary that collected as a fresh material and dried in shadow until weight is stable, then grinded at home to be compared with the ready prepared ones.

Organoleptic tests (OLT): Spices grinded at home were compared to those grinded at market in terms of taste, color and odor.

Microscopic testing's: Dried home and market powdered spices were examined and compared under microscope (under power 40x) to observe the main features.

Macroscopic testing's: Dried home and market powdered spices were examined under microscope (under power 10x) to observe and compare the particle size and to test the presence of sand, filth and strange particles.

pH testing's: Spices were added to aqueous solution and pH was determined using pH meter.

Microbial screening and identification tests: One gm of spices powder was added to 9 ml nutrient broth, incubated for 24 hrs to allow suitable growth of bacteria, then 1 ml of turbid nutrient broth was added to 9ml normal saline, serial dilution was performed 5 times. 100 µl of each dilution 10^{-1} to 10^{-6} was added to normal agar plate and was spreaded by L loop. All agar plates were incubated at 37 C° for 24 hrs. Total count of bacterial was taken after 24 hrs. If the bacterial colonies count was more than 100, it was considered too many to count (TMTC).

Different grown colonies were inoculated in a new agar plate to get pure isolate in order to be identified. Identification was performed using specific media (EMB, XLD, MaC, C).

Extraction and preparation: Spice samples were extracted by cold maceration. Known weight of the spices powder was soaked in methanol in a closed container tank and left for 72 hrs x 3 with stirring from time to time. The methanolic extract was filtered using filter paper. The extract was concentrated by rotary evaporator and collected in glass container which is left uncovered until complete dryness to obtain the crude extract.

Percentage of yield of all extracts was calculated using this formula:

% of yield = (weight of crude extract / weight of original plant powder) x 100

Thin layer chromatography (TLC) test: Methanolic solution of each spice was spotted on TLC plate, left to dry and eluted by different solvent system in TLC chamber to get the best separation for the compounds. Home grinded were compared to those obtained from market. The same spots were investigated under short waves and long waves UV.

Antioxidant (DPPH) scavenging activity: According to the method of Brand-Williams et al. (26). Methanolic solution of each spice was spotted on TLC plate to be compared, left to dry and eluted by suitable solvent system and sprayed with 0.2 % methanolic solution of diphenyl picryl hydrazyl (DPPH) reagent. Change of color from violet to yellow considered positive result. Vitamin C was used as positive control.

Results and discussion

Organoleptic test (OLT) results

Spices grinded at home were compared to those grinded at market in terms of taste, color and odor. The result showed no difference between the market samples and home samples in terms of color, taste and smell except turmeric market sample that was more vibrant in color than the home grinded sample, which may be contributed to addition of some coloring agent that led to this intense color. Which need more specific tests to confirm it?

Microscopic test findings

Dried home and market powdered spices were examined and compared under microscope (under power 40x) to observe the main features and main

characteristics in spices under microscope as shown in figures 1-7.

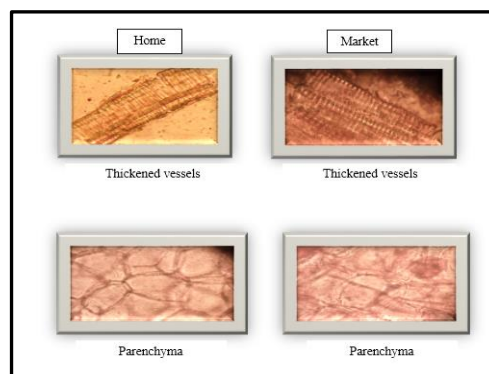


Figure 1: Turmeric main feature of home grinded sample compared to market sample under power x 40 microscope.

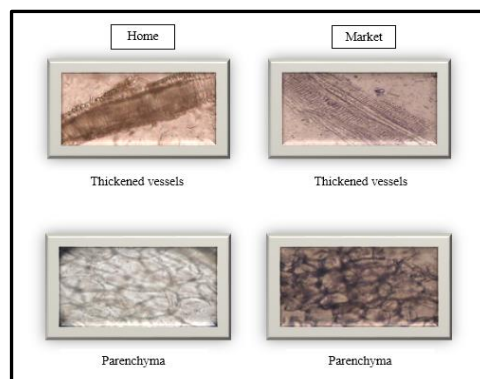


Figure 2: Ginger main feature of home grinded sample compared to market sample under power x 40 microscope.

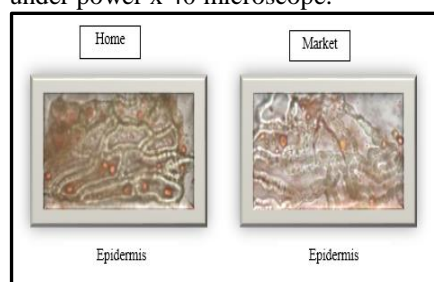


Figure 3: Red pepper main feature of home grinded sample compared to market sample under power x 40 microscope.

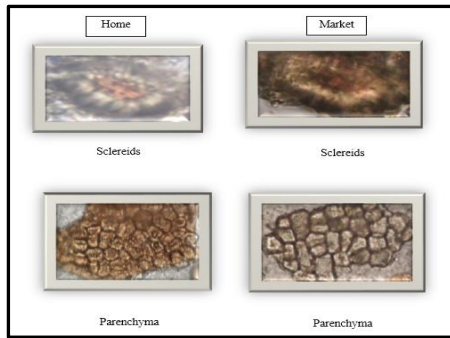


Figure 4: Black pepper main feature of home grinded sample compared to market sample under power x 40 microscope.

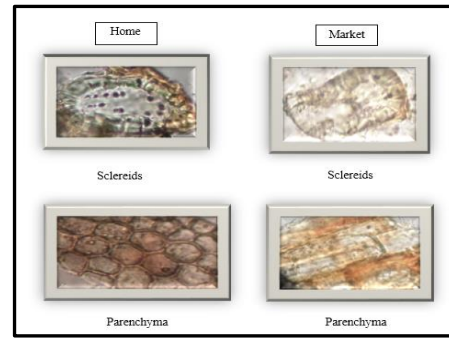


Figure 7: Cinnamon main feature of home grinded sample compared to market sample under power x 40 microscope.

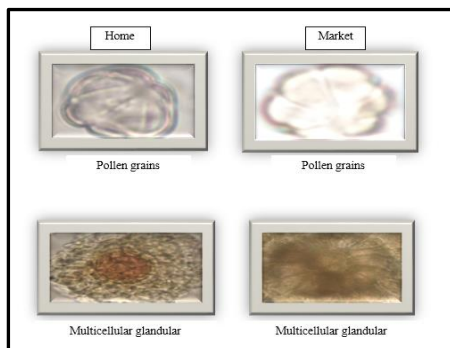


Figure 5: Thyme main feature of home grinded sample compared to market sample under power x 40 microscope.

From all last photographs of microscopic slides of spices samples, it was noted that the market samples under power $\times 40$ were similar to home samples regarding the main features in these plants. In addition to the foreign bodies or filth. This indicates the absence of additives in market samples.

Macroscopic findings

Dried home and market powdered spices were examined under microscope (under power 10x) to observe ampere the particle size and to test the presence of sand, filth and strange particles. Macroscopic results of home and market spices were shown below in figures (8-14).



Figure 6: Rosemary main feature of home grinded sample compared to market sample under power x 40 microscope.

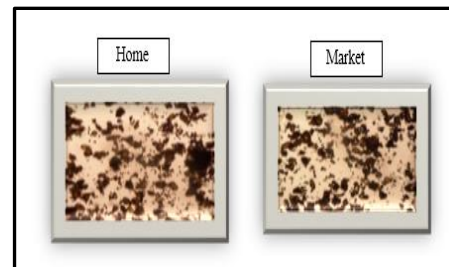


Figure 8: Turmeric sample grinded at home compared to market one under microscope power x 10.

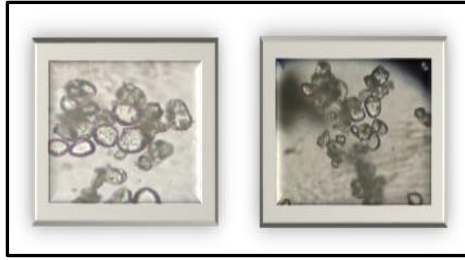


Figure 9: Ginger sample grinded at home compared to market one under microscope power x 10.



Figure 10: Red pepper sample grinded at home compared to market one under microscope power x 10.

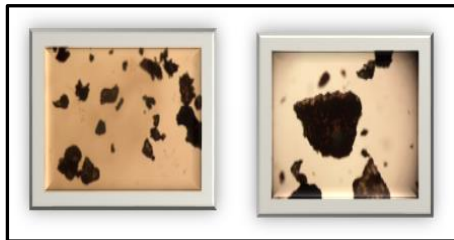


Figure 11: Black pepper sample grinded at home compared to market one under microscope power x 10.



Figure 12: Thyme sample grinded at home compared to market one under microscope power x 10.



Figure 13: Rosemary sample grinded at home compared to market one under microscope power x 10.

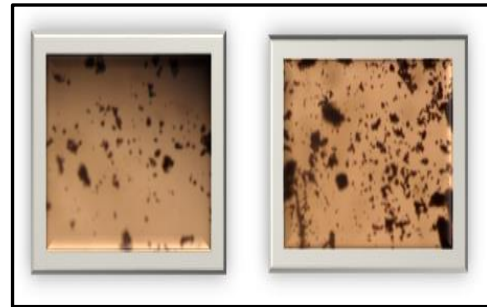


Figure 14: Cinnamon sample grinded at home compared to market one under microscope power x 10.

It was noted from this results that the particle size under the microscope under power of $\times 10$ was similar between the market samples and home samples, which indicated the lack of dust or fine foreign particles in the market samples.

pH test finding

Spices were added to aqueous solution and pH was determined using pH meter as shown in (table 1).

Table 1: pH test results of home grinded spices against market spices

Spices	Home	Market
Black pepper	6.3	6.6
Cinnamon	4.9	4.8
Ginger	4.31	4.35
Red pepper	4.9	5.1
Rosemary	6.24	6.8
Thyme	6.5	6.4
Turmeric	6.9	6.8

From the results, there was no difference in pH values between home samples and market samples; this indicates a lack of additives for market samples which may lead to a change in the pH to a more basic or more acidic conditions. In addition to microbial contamination that may lead to change in pH due to their metabolites and byproducts as reported by (27)

*Microbial contamination test finding**Total count*

Spices powder was added to nutrient broth, incubated for 24 hrs to allow suitable growth of bacteria, and then nutrient broth was added to normal saline, serial dilution was performed. Each dilution was added to normal agar plate and was spreaded by L loop. All agar plates were incubated at 37 C° for 24 hrs. Total count of bacterial was taken after 24 hrs. The result was plot in (table 2). If the bacterial colonies count was more than 100 it was considered too many to count (TMTC).

Table 2: Total count results of home grinded spices against market spices

Spices	(H) Total count	Total count (M)
Black pepper	TMTC	TMTC
Cinnamon	71 ± 1.4	TMTC
Ginger	TMTC	TMTC
Red pepper	TMTC	TMTC
Rosemary	12 ± 0.9	TMTC
Thyme	38 ± 1.2	TMTC
Turmeric	TMTC	TMTC

H= Home, M= Market

The results in the table demonstrate that some of the market samples and home samples of the bacteria were too many to count, however other home samples such as the thyme, rosemary and cinnamon showed better results. This can be due to unsuitable storage condition, poor handling and poor hygiene. In addition to contamination of tools used to grind spices, all these factors led to bacterial contamination or growth of bacteria. On the other hand, Thyme and rosemary had the best results among the group of tested spiced because the process of collection, drying and grinding them was well controlled.

Microbial identification findings

Identification was performed using specific media (EMB, XLD, MaC and C). The result showed that three microbial species were detected which were *Salmonella*, *Shigella* and *E. coli*. The identification of bacteria on specific media by Xylose lysine deoxycholate agar (XLD agar) is a selective growth medium used in the isolation of *salmonella* and *shigella* species. So the result including *salmonella*, can ferment the sugar xylose to produce acid and change the color to yellow and *shigella* colonies cannot do this and therefore remain red. Eosin methylene blue (EMB) is a selective stain for gram negative bacteria on EMB is growing it will give a distinctive metallic green sheen. The existence of these types of

bacterial contamination indicated the lack of hygienic condition in the local shops when handling any kind of spices. In addition, the importance of correct food handling practices and usage of herbs and spices by end users cannot be overemphasized. This result was consistent with a study between April and September 1993, where a nationwide outbreak of salmonellosis occurred in Germany which was traced to contaminated paprika and paprika-powdered potato chips (28). Spices and herbs contaminated with the microbial *Salmonella* were responsible for a variety of food borne outbreaks in Europe and North America (29). *Shigella* also reported to be one of spices contaminant (30) as well *E. coli* (31).

Extraction findings

All spices samples were extracted by cold maceration for 72x3, filtrated and then concentrated. The percentage of yield was calculated (table 3) for all spices samples using this formula:

$$\% \text{ Percentage of yield} = (\text{weight of crude extract} / \text{weight of original powder}) \times 100$$

The results in (table 3) showed that the market samples % of yield were higher than the percentage of yield of home samples, therefore, there is a possibility of presence of bulk additives to the market samples which led to this increment in the % of yield. However this needs to be confirmed by more specific tests.

Table 3: Percentage of yield of home grinded spices against market spices

Spices samples	% of yield of home grinded spices	% of yield of market grinded spices	
Black pepper	9.3	12.1	↑
Cinnamon	14	7.4	
Ginger	6.9	11.8	↑
Red pepper	13.1	24.4	↑
Rosemary	11.7	14.8	↑
Thyme	7.2	6	
Turmeric	5	8.7	↑

Thin layer chromatography (TLC) test
Methanolic solution of each spice was spotted on TLC plate, left to dry and eluted by different solvent system to get the best separation for the compounds. Home grinded were compared to those obtained from market. The same spots were investigated under short waves and long waves UV.

Visual test findings

Thin layer chromatography plate was dried and spots were detected and compared (figure 15). It was observed from the result that the separation of compounds was very similar between the market samples and home samples (The same relative R_F values) which indicated that there was no missing material in the market samples that may caused due to long storage compared to home grinded samples.

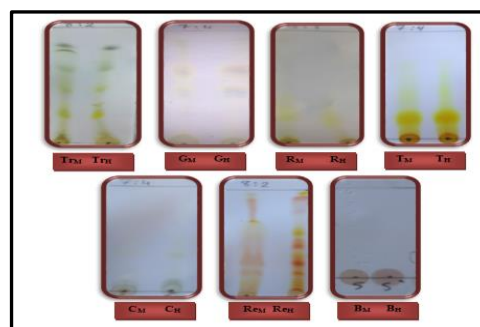


Figure 15: Visual result of TLC test of spices. Solvent= Hexane: Ethyl acetate, H=Home, M=Market, Tr =Turmeric, G=Ginger, R=Rosemary, T=Thyme, C=Cinnamon, Re=Red pepper, B=Black pepper.

Results under UV light (long wave)

The same TLC plate was investigated under long wave UV light as shown in (figure 16). The separation of compounds under long UV wave also showed very tight TLC chromatogram between the market samples and home samples.

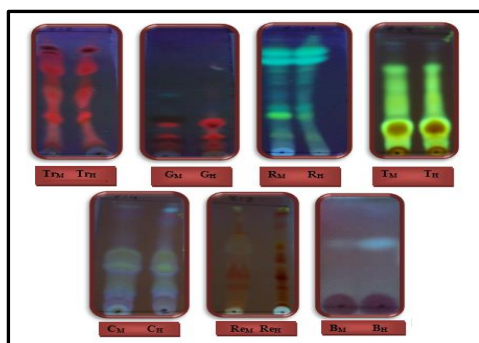


Figure 16: Long wave UV light result of TLC test of spices.

Solvent= Hexane: Ethyl acetate, H=Home, M=Market, Tr =Turmeric, G=Ginger, R=Rosemary, T=Thyme, C=Cinnamon, Re=Red pepper, B=Black pepper

Results under UV light (short wave)

The same TLC plate was investigated under long wave UV light as shown in (figure 17). The results showed that the separation of compounds was very similar between the market samples and home samples. All these results indicated that the market samples still maintaining the same compounds in the raw spices and this was not affected by the storage while grinded.

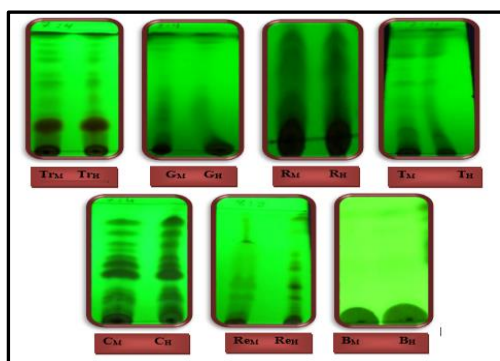


Figure 17: Short wave UV light result of TLC test of spices sample.

Solvent= Hexane: Ethyl acetate, H=Home, M=Market, Tr=Turmeric, G=Ginger, R=Rosemary, T=Thyme, C=Cinnamon, Re=Red pepper, B=Black pepper

Antioxidant (DPPH scavenging activity) findings

Methanolic solution of each spice was spotted on TLC plate to be compared, left to dry and eluted by suitable solvent system and sprayed with 0.2 % methanolic solution of (DPPH) reagent. Change of color from violet to yellow considered positive result. Vitamin C was used as positive control (figure 18).

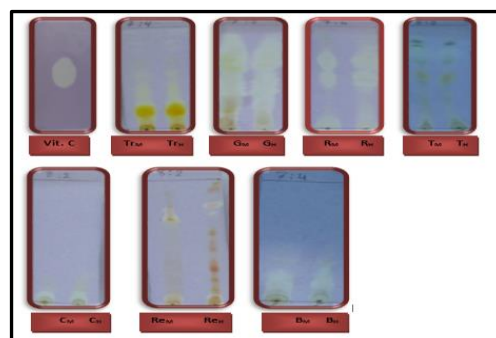


Figure 18: DPPH scavenging test result of TLC spots of spices sample.

Solvent= Hexane: Ethyl acetate, H=Home, M=Market, Tr=Turmeric, G=Ginger, R=Rosemary, T=Thyme, C=Cinnamon, Re=Red pepper, B=Black pepper

The qualitative antioxidant activity (using DPPH assay) revealed that the change in color was similar between the two tested samples of each spice, which can be interpreted that the same compounds responsible for anti-oxidant effect still present in both home and market spices. The result also indicates that those spices still possessing their antioxidant value.

In conclusion: there was no difference in organoleptic, microscopic and macroscopic test among all chosen spices. In addition pH test results of home against market grinded spices also showed no difference. Three bacterial species were isolated from spices (*E. coli*, *Salmonella* and

Shegilla). There was no different in antioxidant test result (DPPH scavenging activity). Furthermore, there was no different in TLC chromatogram. All these tests indicates that the samples obtained from market as grinded powder and the same

samples of spices that was brought as a raw materials and grinded at home had the same characteristics, which indicated that they are of the same quality which not necessary to be a good one.

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