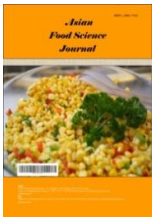

Asian Food Science Journal

14(3): 1-10, 2020; Article no.AFSJ.54331
ISSN: 2581-7752

A Comparative Study of Microbial and Proximate Composition of Hand Shelled and Machine Shelled Melon Seeds (*Colocynthis citrullus L.*) as Sold in an Abeokuta Market

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Authors' contributions

This work was carried out in collaboration among all authors. Author OPS designed the study, performed the statistical analysis, wrote the first draft of the manuscript. Author MOB read the final manuscript. Author SE provided instructions and supervised the laboratory works. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2020/v14i330129
Editor(s):
(1) Dr. Chouitah Ourida, Professor, Department of Biology, University of Mascara, Algeria.
Reviewers:
(1) S. Mahendran, Ayya Nadar Janaki Ammal College, India.
(2) M. M. V. Baig, Yeshwant Mahavidyalaya, India.
(3) Nicoleta Radu, University of Agronomic Sciences and Veterinary Medicine, Romania.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54331

Original Research Article

Received 10 December 2019
Accepted 16 February 2020
Published 26 February 2020

ABSTRACT

Melon (*Colocynthis citrullus* L.) is a widely cultivated and consumed oil-seed in West Africa. Its seeds deteriorate quickly in storage due to microbial attack introduced during the shelling stage of processing.
Aim: This study investigated and compared the microbial and proximate composition of Hand shelled and machined melon seed as sold in the market in Abeokuta, Nigeria.
Study Design: The total bacterial, total fungal, Staphylococcal and coliform counts of the machine shelled and hand shelled melon seeds samples were determined.
Methodology: The mould count was estimated as SFU/g and isolates (*Aspergillus*) were identified through spore formation, production of fruiting bodies, morphological and molecular characteristics.

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The proximate and chemical compositions-Free Fatty Acid (FFA) and Peroxide Value (PV)-were determined by standard analytical methods. Data were analysed using analysis of variance.
Results: There is no significant ($p<0.05$) difference in the proximate composition of both the hand shelled and machine shelled melon seed save the pH. The proximate compositions of hand shelled and machine shelled melon samples were within the recommended limits for *Curcubitaceae*, both FFA (1.88±0.02%, 1.88±0.01%) and PV(2.64±0.01 meq/kg, 2.65±0.02 meq/kg) values respectively were within the Codex Alimentarius range for oily seeds. The pH value of Hand shelled (5.57±0.09) is significantly higher than Machine shelled (6.10±0.06). The fungal count of machine shelled ($92.33x10^8$ SFU/g) was significantly higher than hand shelled ($38.00x10^8$ SFU/g). Staphylococcal count of hand shelled ($59.00x10^8$ CFU/g) was significantly higher than machine shelled ($42.00x10^8$ CFU/g). However, there is no significant difference in total bacterial and coliform counts of both melon samples. The fungal species found in hand shelled and machine shelled melon samples were mainly genus *Aspergillus* with *A. niger* with percentage of occurrence (30%, 22%) and *A. flavus* (8%, 11%) respectively. *Bacillus subtilis* (14%, 40%) and *Staphylococcus aureus* (43%, 20%) were also found in hand shelled and machine shelled melon. These organisms are of spoilage and food poisoning importance.
Conclusion: The study concluded machine shelled melon harbours more spoilage microorganisms and may spoil faster than hand shelled melon seed.

Keywords: Machine shelled; hand shelled; melon; Aspergillus.

1. INTRODUCTION

Microorganisms get into foods from both natural sources and from external sources to which the food comes in contact from the time of production until time of consumption [1]. Major contamination sources are water, air, dust, equipment, sewage, insects, rodents, and employees [2].

Microbiological contamination of food refers to the non-intended or accidental introduction into or inclusion of harmful microorganisms or microbial toxins in food, making it unsafe for consumption. Many opportunities exist for food to get contaminated along the food chain (from production to dining table). Food contaminated by pathogens or chemical substances is a serious issue because it can lead to a wide range of health problems. This is responsible for more than 200 diseases; including typhoid fever, diarrhoea and cancers, among others [3] and can lead to the death of unsuspecting consumers in both developing and the developed countries [4].

Melon (*Citrullus colocynthis lanatus*) popularly referred to as "*egusi*" is one of the important oil seed crops widely grown and consumed in tropical Africa [5]. The proximate composition of melon seed is reported to be 45.95% of fat, 28% of crude protein, and 7.18% of fibre [6] and contains a good amount of sodium, calcium, magnesium, vitamins and irons. Melon is a common fruit in Nigeria because of the edible seeds which are commonly used in the preparation of local soup and snacks such as fried melon. In the eastern part of the country, the seeds are sometimes boiled and eaten as snacks [7].

Processing of melon involves depodding, fermentation, washing, drying, cleaning and shelling [8]. Shelling (manual or mechanical) being the last stage in the processing of melon seeds before consumption is barely the most critical step in the processing of the seeds which determines the microbial safety of melon being offered for sale in the market [9].

The objective of this study is to determine and compare the microbial status and nutritional composition of hand shelled and machine shelled melon to guide and assist consumers in purchasing wholesome products.

2. MATERIALS AND METHODS

One (1) kg each of hand shelled and machine shelled melon seeds (*Colocynthis citrullus* L.) were purchased from Omida market in Abeokuta, Ogun State. Melons were collected and transferred aseptically to the laboratory and stored at 4ºC before microbiological and proximate analyses.

Both melon samples were grinded using a sterile blender and used for microbiological analyses (Total Bacteria counts, Staphylococcal counts, Coliform counts and fungi counts) were carried out using standard microbiological procedures.

Bacterial counts and fungal counts were expressed in CFU/g and SPU/g of samples respectively. Pure bacterial isolates were identified and characterized through colonial and cell morphology and biochemical test [10].

Pure fungi spores were expressed in SPU/g of samples and identified by macroscopic and microscopic characteristics and keys of Barnett and Hunter [11] and molecular identification was carried out using standard procedures for only two moulds isolates; one each from the melon samples.

The proximate composition was determined using the AOAC [12] methods while Peroxide Value (P.V.) and Free Fatty Acid (FFA) were analyzed with the method of Pearson [13].

3. RESULTS AND DISCUSSION

3.1 Proximate and Chemical Composition

The result of the proximate and chemical composition of the hand shelled and machine shelled melon samples revealed that both contain nutrients which are beneficial to human and support microbial growth. There is no significant difference in all the parameters under consideration for both samples save the pH value. The difference in pH values could be a function of biological and biochemical activities occurring in the samples. The pH of food is also important in determining which organisms can survive and thrive on it. All microorganisms have a pH range in which they can grow and an optimum pH at which they grow best [14].

The M.C values obtained in this study were much closed to the value of 5.05% obtained by Olubamiwo et al. for melon samples in Ibadan [15]. The variation in the MC values obtained in this study compare to the values obtained by many researchers at different locations in Nigeria could be due to variation in the humidity of the study location (Abeokuta) compared to others. This view was supported by Bankole et al., who reported that melon seeds from the savannah forest had lower moisture content (4.6%) when compared with the humid south. Moisture content from the humid south ranged from 5.3% to 10.4% [16]. It is noteworthy that the stored product must be of low moisture content possible to keep biodeteriogens at bay.

The FC and CPC obtained for the two samples in this study were relatively high and agreed with the values obtained by Olubanwo et al., fat (48.92%) and proteins (30.16%) [15]; Brisbe et al., 53% fat and 28% protein [5] and Olaniyi et al., with 38% protein and 30-50% of semi-dried oil [17]. The high value obtained is an indication that both melon samples could be a good source of dietary source of protein in food and feeds.

The A.C is a function of micro and macro elements present in the melon samples. The values obtained for mechanically and manually shelled melons; 3.56±0.42, 3.63±0.03 respectively agreed with the value obtained by Ogundele et al. [18] who also reported values of 3.15 to 3.53% for ash; and Achu et al. [19] who reported values of between 3.47 to 4.37% for ash content for melon seeds from different regions of Cameroon.

The CHO and CFC of the melon values were high for both samples. The CHO values obtained for hand shelled and machine shelled melons (11.08 ± 0.23 and 10.90 ± 1.21) agreed with that, 10.88%,obtained by Ogundele et al. [18].

The PV and FFA values for the melon samples were very low. The values P.V for both samples (2.64 ± 0.01 meq/kg , 2.65 ± 0.0 meq/kg) and FFA (1.88 ± 0.02%, 1.88 ± 0.01 %) agreed with the PV and FFA obtained by Bankole et al. [16] for melon dried through various methods, which ranged from 3.13 to 3.67meq/kg and 0.7 to 2.1 % respectively. It is noteworthy that the PV and FFA values obtained for the melon samples were within the Codex Alimentarius standard value of 10 meq/kg and 10% for edible oil [20]. Since the extent of rancidity in fats and oils is determined by measuring Peroxide value and Free Fatty acid [21], it is concluded that the oil from the melon samples is edible.

3.2 Microbiological Composition

The microbial examination of the machine shelled and hand shelled melon seeds as sold in the market revealed that its harbour microorganisms because melon contains nutrients which are beneficial to human and support microbial growth.

3.2.1 Total fungal counts and percentage composition of fungi

The result (Table 2) shows that the fungal count of machine shelled melon, 9.23×10^{9} SFU/g, was significantly ($p < 0.05$) high than $3.80\ x10^{9}$ SFU/g of hand shelled melon seed sample. This could be attributed broadly to the residues of shells in

machine shelled melon during shelling operation; leftover kernel residues in the melon Sheller and cross-contamination from the machine. Most fungi are saprobes; therefore can grow and multiply in the kernel residues left behind in the machine during a previous shelling operation and contaminate fresh batch. Generally, unsanitized milling machines could constitute a high possibility of cross-contamination [22]. The high fungal count agrees with values of between 4.00 $\log_{10}$ CFU/g to 4.38 $\log_{10\ CFU/g}$ in melon oil stored at ordinary room temperature obtained by Kolapo and Oladimeji [10].

Similarly, Table 3 shows the Percentage composition of the fungal species in the melon samples.

From the result, same fungal species were present in both machine shelled and machine shelled melon seed with varying percentages. Moulds of the genus *Aspergillus* dominate the fungi composition in both samples with 61% and 55% in hand shelled and machine shelled melon respectively. This finding is supported by an earlier report of Bankole et al. [23] who found the genus *Aspergillus* as the most frequently associated with 'egusi' melon in Nigeria, which could result from prevalence of their spores in the atmosphere. The presence of *Mucour species, Rhizopus species* and *Saccharomyces cerevisae* which occurred in varying percentage could possibly have resulted from the environment, air, handling and processing of melon seeds. This view is supported with the findings of Bankole et al [24] who found *Rhizopus species*, *Saccharomyces cerevisiae* and *A. niger* among organisms associated with the palm of fast food handlers in Abeokuta.

3.2.2 Characterization of the two selected fungi Isolates

Furthermore, two isolates (S_1 and S_5) of the moulds were selected for further detailed macroscopic and microscopic characteristics and molecular characterization for proper identification (see Tables 6 & 7; Plate 1) i.e. S_1 and S_5 were picked from Hand shelled and machine shelled melon samples respectively.

Plates 1 shows the PCR amplification of the Intragenic transcribed spacers (ITS) genes of isolated fungi with DNA marker 300-10000 bp (Norgen Biotech Corporation, Canada). From the plates, the molecular weight of both isolate was 400 base pairs.

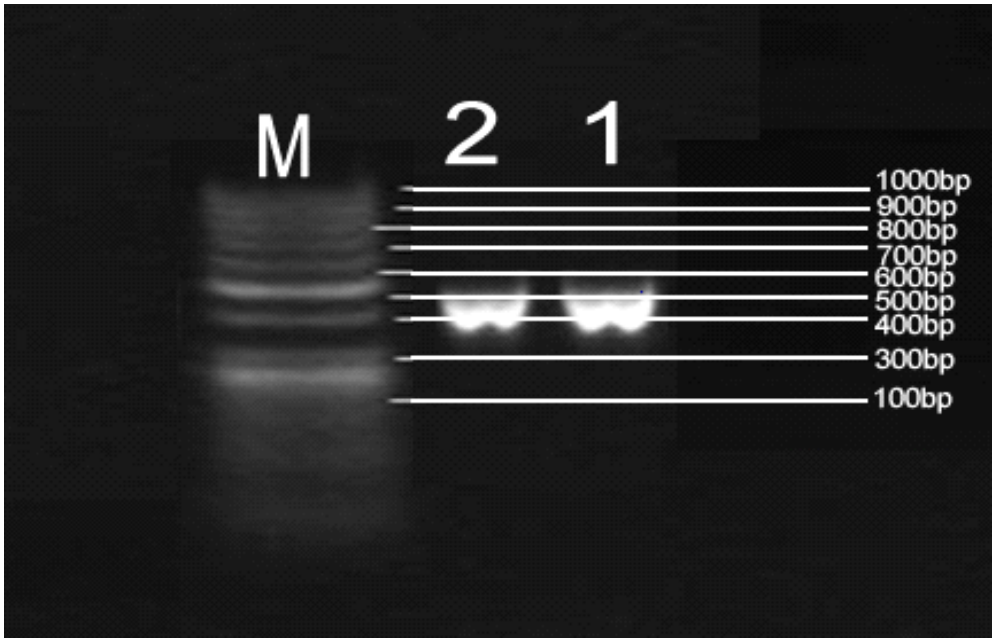

Plate 1. The PCR amplification of the Intragenic transcribed spacers (ITS) genes of the 2 isolates

N.B: At extraction, the molecular weight of the extracted DNA for both isolates was 9000 base pairs

Table 1. The mean proximate and chemical composition of hand shelled and machine shelled melon seeds in (g/100 g dry wt)

Treatments	M.C	F.C	A.C	CFC	CPC	CHO	pH	PV	FFA
Hand shelled melon	5.4±0.4[a]	45.14±0.25[b]	3.56±0.42[b]	11.65±0.85[b]	23.05±0.24[b]	11.08±0.23[a]	5.57±0.09[a]	2.64±0.01[a]	1.88±0.02[a]
Machine shelled melon	5.07±0.[a]	45.46±0.4[b]	3.63±0.03[b]	11.68±0.71[b]	23.25±0.07[b]	10.90±1.21[a]	6.10±0.06[b]	2.65±0.02[a]	1.88±0.01[a]

Key: MC = Moisture content, F.C = Fat content, A.C = Ash content, CFC = Crude fibre content, CPC = Crude protein content, CHO = Carbohydrate, P.V = Peroxide Value in milli equivalent per kilogramme (meq/kg) and FFA = Free fatty acid in %. Values are mean of triplicate samplings; two values in the same column followed by different letters differs significantly (P ≤ 0.05)

Table 2.The mean total microbial counts of hand shelled and machine shelled melon seeds purchased from Omida Market, Abeokuta

Samples	Total fungal counts ($\times 10^8$ SFU/g)/range	Total bacterial counts ($\times 10^8$ CFU/g)/range	Total Staphylococcal Counts ($\times 10^8$ CFU/g)/range	Total coliform counts ($\times 10^8$ CFU/g)/range
Hand shelled melon	38.00 ± 1.00[a]	283.00 ± 3.71[b]	59.00 ± 2.65[a]	1.33 ± 0.67[a]
Machine shelled melon	92.33 ±1.45[d]	282.33± 14.80[b]	42.00 ± 7.57[b]	0.00 ±0.00[a]

Table 3. Percentage composition of the fungal species in the melon samples

Fungal species	Percentage (%) composition of fungal species in melon samples	
	Hand shelled	Machine shelled
*Aspergillus niger**	30	22
*A. flavus**	8	11
A. Fumigatus	23	22
Rhizopus species	23	17
Mucour species	8	17
Saccharomyces cerevisae	8	11

Table 4. Detailed macroscopic and microscopic characteristics of the two mould isolates

Isolate code	Macroscopy Characteristics	Microscopic characteristics	Mould organism
S_1	Mycelia diameter is 3-5. Black filamentous mycelium, produce Black soluble pigment, exudate is present, the reverse colour of mycelia is black.	Black with sulphur-yellow area on the surface single celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bud of the septate hyphae.	*Aspergillus niger*
S_5	Mycelia diameter is 4-6 between. Yellow Filamentous mycelium, produce yellow soluble pigment, exudate is not absent; the reverse colour of mycelia is yellow.	Conidiophores are long and rough just beneath the globolose vesicle. Philades are circumferential and arebiseratial. Conidia are round, smooth or slightly rough and form long chains.	*Aspergillus flavus*

Table 5. Comparative Homology from results of the BLAST for fungal isolates (*Aspergillus niger and A. flavus*)

Isolates Codes	Name of closest identity	Maximum score	Total score	Query cover	E-value	% Identity	Accession number
Isolate 1	*A. niger strain* UBOCCA-A-101076	1002	1002	49%	0	100%	KF225022.1
Isolate 2	*A. flavus* strain TN-432	913	1210	82%	0	98%	JX502763.1

(National Centre for Biotechnological Information, accessed on February 19, 2015 [25]

The amplified segment of the Intragenic transcribed spacers (ITS) genes of *A. niger* and *A. flavus* were sequenced and identified using the Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI) database [25] to establish the identities of the isolates. Genotypic identification results showed that Isolate S_1 from hand shelled melon sample was 100% identified as *Aspergillus niger* strain UBOCC-A-101076 while isolate S_5 from machine shelled sample was 98% identified as *Aspergillus flavus* strain TN-432 .

3.3 Microbiological Analysis

3.3.1 Cultural and biochemical characterization of the bacterial isolates

The results obtained were based on the examination of the colonial characteristics, Gram

staining reactions and biochemical tests performed on the pure bacterial isolates.

3.3.2 Staphylococcal and coliform counts and percentage composition of bacterial in melon samples

Again from Table 2, the total Staphylococcal counts of the hand shelled melon was significantly ($p < 0.05$) high than machine shelled melon seed, both having 5.90 x 10^9 CFU/g and 4.20 x 10^9 CFU/g respectively. However, both Hand shelled and machine shelled samples were not significantly different in Total bacteria and Total coliform counts with 2.83 x 10^{10} CFU/g and 2.82 x 10^{10} CFU/g, and 1.33 x 10^9 CFU/g and 0.00 x 10^9 CFU/g respectively.

In addition to the foregoing, Table 8 indicates that *Staphylococcus aureus* and *Bacillus subtilis* were common composition of hand shelled melon and machine shelled samples with percentage of (43, 20) and (14, 40) respectively.

The higher percentage of *Staphylococcus aureus* in hand shelled melon is expected since *Staphylococcus* is a normal inhabitant of the human skin, nasal passage, throat and hair, and could easily contaminates the hands and fingers being used in manual shelling operation. Some *Staphylococcus* strains are enterotoxigenic; ingestion of food contaminated with the toxin is one of the leading causes of global food poisoning.

Bacillus subtilis is a motile spore forming bacterium present in diverse environmental conditions but mostly in the soil. It is capable of surviving different harsh condition due to production of endospores [26]. The higher percentage composition of *Bacillus subtilis* in machine shelled sample than hand shelled melon could have resulted from transfers from the soil due to unhygienic practice of drying melon on bare concrete floor in addition to cross-contamination from shelling machine being used in processing of other agro-produce without proper and regular sanitization. This finding is supported by Somorin et al. [22] who found *Bacillus species* as the predominant bacteria in market milled yam chips.

Klebsiella pneumonia and *Enterobacter cloaca* are microorganisms which are found in fecal contaminated materials while *Pseudomonas aeruginosa* is ubiquitous with affinity for moist environments and ability to survive at various conditions including the intestinal tract of man and animals [27] is of spoilage importance. The three organisms could have possibly been transferred into the melon samples during the

Table 6. Colonial characteristics of bacterial isolates from hand shelled and machine shelled melon seeds obtained from the market on nutrient agar

Isolate code	Size (mm)	Shape	Colour	Consistency	Edges	Elevation	Opacity
H_1	3-4	Round	White	wet/mucoid	Smooth	Raised	Opaque
H_2	3-4	Irregular	Green	Wet	Rough	Flat	Opaque
H_3	2-3	Round	Creamy white	Wet	Smooth	Slightly raised	Opaque
H_4	3-5	Irregular	Grey	Dry	Rough	Flat	Opaque
H_5	3-4	Round	White	Wet/mucoid	Smooth	Raised	Opaque
H_6	2-3	Round	Creamy white	Wet	Smooth	Slightly raised	Opaque
H_7	2-3	Round	Creamy white	Wet	Smooth	Slightly raised	Opaque
M_1	2-3	Round	Grey	Wet	Smooth	Flat	Opaque
M_2	1-2	Round	Creamy white	Wet	Smooth	Raised	Opaque
M_3	3-5	Irregular	Grey	Dry	Rough	Flat	Opaque
M_4	3-5	Irregular	Grey	Dry	Rough	Flat	Opaque
M_5	2-3	Round	Creamy white	Wet	Smooth	Slightly raised	Opaque

Key: H (Hand shelled melon seed), M (Machine shelled melon seed)

.**Table 7. Biochemical characteristics of bacterial isolates from hand shelled and machine shelled melon seeds obtained from the market**

Isolate code	Shape	GR	SP	CP	CA	CO	MO	IN	OX	CI	UR	MR	VP	G	L	M	Organism
H_1	Rod	-	-	-	+	-	-	-	-	+	+	-	+	A	A	-	*Klebsiella pnuemoniae*
H_2	Rod	-	-	-	+	-	-	-	+	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
H_3	Cocci	+	-	-	+	+	-	-	-	-	-	-	+	A	A	A	*Staphylococcus aureus*
H_4	Rod	+	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*
H_5	Rod	-	-	-	+	-	-	-	-	-	-	-	+	A	A	-	*Klebsiella species*
H_6	Cocci	+	-	-	+	+	-	-	-	-	-	-	+	A	A	A	*Staphylococcus aureus*
H_7	Cocci	+	-	-	+	+	-	-	-	-	-	-	+	A	A	A	*Staphylococcus aureus*
M_1	Rod	-	-	-	+	-	+	+	-	+	+	-	+	A	A	A	*Enterobacter cloaca*
M_2	Cocci	+	-	-	+	-	-	-	-	-	-	+	-	A	-	-	*Staphylococcus Saprophyticus*
M_3	Rod	+	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*
M_4	Rod	+	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*
M_5	Cocci	+	-	-	+	+	-	-	-	-	-	-	+	A	A	A	*Staphylococcus aureus*

Key: H (Hand shelled melon seed), M (Machine shelled melon seed)
Gram reaction (GR), Catalase (CA), Coagulase (CO),Methyl Red (MR), Voges Proskaeur (VP), Glucose (G), Lactose (L), Maltose (M), Indole (IN), Oxidase (OX), Motility (MO) , Urase (UR), Citrate (CI), Spore (SP),Capsule (CP)

Table 8. Percentage composition of the bacterial species in the melon samples

Bacterial species	Percentage (%) composition of Bacterial Species in melon samples	
	Hand shelled	Machine shelled
Staphylococcus saprophyticus	0	20
Staphylococcus aureus	43	20
Pseudomonas aeruginosa	14	-
Klebsiella pneumonia	29	-
Enterobacter cloaca	-	20
Bacillus subtilis	14	40

process of washing of the melon seed pods with water of poor quality. Somorin et al. [22] also found all the three microorganisms in commercial and laboratory milled 'elubo'.

4. CONCLUSION

Both melon seeds (hand shelled and machine shelled) being sold in the market contains acceptable level of nutrients beneficial to man, but was found to harbour microorganisms, with the machine shelled harbours more microorganisms, especially moulds than hand shelled melon seeds and could spoil faster than hand shelled melon.

Melon processors and sellers are advised to cultivate and adhere to good handling practice in the course of their routines. Lastly the melon sellers in the market are advice to keep melon in covered container prior in order to protect the seeds from mould spores and bacterial contaminants in the atmosphere.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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