

## Effects of different temperature profiles and corn-sago starch ratios on physical properties of extruded tilapia diets

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### Abstract

Sago starch is a locally grown and produced starch resource in Malaysia. In this study, corn starch to sago starch ratios (CS:SS) of 0:20, 5:15, 10:10, 15:5 and 20:0 were included in feed mixture to contain 20% starch and produce five isonitrogenous and isocaloric (30% crude protein and 16.7 kJ/g, respectively) tilapia *Oreochromis* sp. diets. Diets were preconditioned to contain 40% moisture and extruded using a single-screw extruder at screw speed of 120 rpm using three different temperature profiles (I 60-100-140-180°C; II 60-100-120-160°C; and III 60-120-120-180°C). Effects of these factors were evaluated on physical properties of extrudates including expansion ratio, bulk density, water stability, floatability, sinking velocity and durability. From the results, different temperature profiles and CS:SS had significant effects ( $p < 0.05$ ) on expansion ratio and floatability. Sago starch performed as a good binder as it gave higher percentage of water stability and pellet durability. The mixture with 10:10 of corn to sago starch extruded using temperature profile II (60-100-120-160°C) produced the best extrudates with desirable physical properties.

**Keywords:** Aquafeed, Corn starch, Extrudates, Pellet physical properties, Sago starch, Tilapia, Temperature profiles.

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## Introduction

Extrusion technology is a cooking and shaping process widely used in manufacturing aquafeeds. It is a short time process using high temperature to minimize losses in vitamins and amino acids (Harper and Clark, 1979). During extrusion, starch component undergoes gelatinization which is a process of thermal disordering of crystalline structures in native starch granules (Tester and Morrison, 1990). Gelatinization of starch plays an important role in the aquafeed production because starch acts as a binder which improves feed durability, expansion ratio and buoyancy; and also making starch digestible for fish consumption (De Silva and Anderson, 1995; Bureau *et al.*, 2002; Kannadhasan *et al.*, 2011). In addition, adjustment of the extrusion operating parameters such as moisture and temperature helps in obtaining the desired characteristics for the final product (Chinnaswamy and Hanna, 1988; Singh and Muthukumarappan, 2015). The digestibility of starch varies among fish species and omnivorous fish species such as tilapia can derive a high amount of energy from modified starch (Hardy and Barrows, 2002). Inclusion of starch in diet has been proven to directly affect faeces quality as it increases particle size of faeces, thus enhancing the removal efficiency of waste which contributes to a sustainable aquaculture (Amirkolaie, 2013).

Corn starch is used as a source of carbohydrate and as a plant-derived binder in the aquafeed industry. Due to

the USA legislative mandates and proposals which brought biofuel to a heavy demand, corn is now mainly cultivated for ethanol production, resulting in the rising price of corn products (Baker and Zahniser, 2006; Luchansky and Monks, 2009). Consequently, this has raised concern about the cost of manufacturing aquafeed leading to a review on the utilization of sustainable plant products in aquafeeds used in the USA (Gatlin *et al.*, 2007). In 2014, the world's largest fuel ethanol producer was the USA with 14,300 million of gallons produced (RFA, 2015). A local approach using alternative starches such as green banana, sago, tapioca, taro, and broken rice; as binder has also been explored in previous years (Umar *et al.*, 2013, Sarawong *et al.*, 2014, de Cruz *et al.*, 2015).

Sago (*Metroxylon sago*) is an indigenous crop of the South East Asia; and in Malaysia, sago palms are mainly cultivated in the state of Sarawak. Malaysia is reported to be one of the biggest sago exporters in the world with about 48,314 metric tonnes of sago starch valued at RM 87 million, exported mainly to Japan, Taiwan and Singapore (DOA, 2012). Thus, the utilization of sago starch in Malaysia is increasing because of its abundance (Abd-Aziz, 2002). Sago starch has been identified as corn starch replacement in producing industrial sugars using single-screw extrusion (Govindasamy *et al.*, 1995). It is also used as textile, paper and plywood adhesives; and as

stabilizer in the pharmaceutical industry (Singhal *et al.*, 2008).

This study was conducted to investigate (1) the potential of sago starch as corn starch replacement in producing tilapia extruded feed and (2) to determine the effects of temperature and corn-sago starch ratios on the physical properties of the extruded diets.

## Materials and methods

### *Feed formulation and experimental design*

In this 5×3 factorial experimental design, five different ratios of corn to sago starch and three different extruder barrel temperature profiles were used. Five isonitrogenous (30% crude protein) and isocaloric (16.7 kJ/g) tilapia (*Oreochromis sp.*) diets containing 20% starch at different corn: sago starch ratios (CS:SS) were formulated using Winfeed 2.8 software (Cambridge, UK). The tested ratios were 0:20, 5:15, 10:10, 15:5 and 20:0 (Table 1). The proximate composition of each ingredient was predetermined following methods by Association of official analytical chemists, (AOAC, 1990) before formulation. All treatments were in triplicates. Raw ingredients used in the diets were supplied by Nutri-Vet Trading, Malaysia and all ingredients were initially ground to ≈1 mm particle size using a laboratory scale pulveriser (Pulian International Enterprise Co. Ltd., Taiwan). The ingredients were then blended with a mixer (KitchenAid, USA) for 20 minutes, preconditioned to

40 % moisture content and kept in polyethylene bags at room temperature for 24 hours to allow moisture equilibration.

### *Extrusion-cooking process*

The extrusion-cooking process for the feed mixtures was performed randomly using a single-screw extruder (BrabenderKE19, Brabender GmbH and Co. KG, Germany). Screw length with a compression ratio of 3:1 was used. The temperatures at the feeder, mixing, compressing and die zones were set as follows: (1) 60-100-140-180°C, (2) 60-100-120-160°C, and (3) 60-120-120-180°C (Table 2). The extruder screw speed was set at 120 rpm while the X-bladed cutter speed was adjusted to 300 rpm. A die head of 3mm in diameter was used. After extrusion, the extrudates were allowed to cool and dry in an air-blow dryer for 24 hours. The physical properties of dried extrudates were subsequently measured.

### *Measurement of pellet physical properties*

Approximately 1000g of extrudates was produced for each treatment and physical properties for each treatment were measured in triplicates unless stated. Expansion ratio, bulk density and pellet durability were measured according to Kannadhasan and Muthukumarappan, 2010. Diameter of the extrudates were measured using a digital calliper (DigiMax, Switzerland) with  $n=10$ .

**Table 1: Ingredient components in five different feed mixture.**

Feed Ingredient	20CS:0SS	15CS:5SS	10CS:10SS	5CS:15SS	0CS:20SS
Fish meal <sup>a</sup>	19.9	19.9	19.9	19.9	19.9
Corn meal	2.8	2.8	2.8	2.8	2.8
Soybean meal	39.7	39.7	39.7	39.7	39.7
Rice bran	12.2	12.2	12.2	12.2	12.2
Vegetable oil <sup>b</sup>	3.4	3.4	3.4	3.4	3.4
Vitamin premix <sup>c</sup>	1.0	1.0	1.0	1.0	1.0
Mineral premix <sup>d</sup>	1.0	1.0	1.0	1.0	1.0
Sago starch	0	5.0	10.0	15.0	20.0
Corn starch	20.0	15.0	10.0	5.0	0
Total	100.0	100.0	100.0	100.0	100.0

<sup>a</sup>Fish meal with 59% crude protein.

<sup>b</sup>mixture of canola and oil (Naturel™).

<sup>c</sup>Vitamin premix: (g/kg premix); ascorbic acid, 45; myoinositol, 5; choline chloride, 75; niacin, 4.5; riboflavin, 1; pyridoxine, 1; thiamin mononitrate, 0.92; Ca-pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.083; vitamin K menadione, 1.67;  $\alpha$ -tocopheryl acetate (500 IU/g), 8; biotin, 0.02; folic acid, 0.09; vitamin B12, 0.001; cellulose, 845.11.

<sup>d</sup>Mineral premix (g kg<sup>-1</sup> premix): KCL, 90; KI, 0.04; CaHPO<sub>4</sub>.2H<sub>2</sub>O, 500; NaCl, 40; CuSO<sub>4</sub>.5H<sub>2</sub>O, 3; ZnSO<sub>4</sub>.7H<sub>2</sub>O, 4; CoSO<sub>4</sub>, 0.02; FeSO<sub>4</sub> 7H<sub>2</sub>O, 20; MnSO<sub>4</sub>.H<sub>2</sub>O, 3; CaCO<sub>3</sub>, 215; MgOH, 124; Na<sub>2</sub>SeO<sub>3</sub>, 0.03; NaF, 1.

**Table 2: Temperature profiles used during the extrusion cooking (°Celsius).**

Temperature profile	Feeder zone(°C)	Mixing zone(°C)	Compressing zone(°C)	Die zone(°C)
I	60	100	140	180
II	60	100	120	160
III	60	120	120	180

Expansion ratio percentage (%) was calculated as the ratio of the extrudates to the diameter of the die used times 100. For measuring bulk density, each treatment was poured into a 1000 mL of cylinder until it reached the mark. The weight of 1000 mL diet was then determined and the bulk density was recorded as g/L. For measuring pellet durability, 50 g of extrudates was tumbled for 10 minutes at 25 rpm using friabilator (DF-3 Distek, North Brunswick, NJ) and then sieved using a 0.5 mm sieve. The weight before and after tumbling was recorded. The pellet durability index (%) was then calculated as the mass of extrudates

after tumbling to the initial mass of extrudates.

For sinking velocity, extrudates ( $n=10$ ) were dropped into a 1L measuring cylinder filled with distilled water. The distance length of the cylinder was 0.343 m and the sinking rate was timed. The calculation for sinking velocity was determined by the ratio of distance travelled to the time taken for the extrudates to reach the bottom of the cylinder (cm/s). Extrudates that did not sink were given the value 0 (Glencross *et al.*, 2010). Floatability of the extrudates was determined by dropping randomly selected extrudates ( $n=50$ ) in a beaker

filled with 1L distilled water. The percentage of floating extrudates was calculated as the number of remaining extrudates on the water surface after 60 seconds (Schwertner *et al.*, 2003). The water stability was measured through weighing 3.0g of extrudates in a pre-weighed basket with a bottom mesh of 2.0 mm. The basket was then submerged in a tray filled with 5.0 cm depth distilled water for 20 minutes before being carefully removed and left to stand for 10 minutes for excess water to drip off. Extrudates and basket were then oven-dried at 130°C for 2 hrs, cooled in desiccators and weighed. The percentage of water stability (%) was calculated as the weight of dried extrudates remaining in the basket over its initial weight multiplied by 100 (Lim and Cuzon, 1994).

#### *Statistical analysis*

All values were reported as mean ( $\pm$  standard error) in triplicates (unless specified) and subjected to two-way ANOVA while differences between means were tested using Duncan's new Multiple Range Test at  $p < 0.05$ . Percentage data were arcsine transformed before undergoing statistical analysis. The analyses were performed using SPSS 16 for Windows (SPSS Inc., Chicago).

#### **Results**

Table 3 shows the physical properties of extrudates from five different CS:SS cooked with three temperature profiles.

#### *Expansion ratio*

Temperature profile I produced extrudates with a significantly higher ( $p < 0.05$ ) expansion rate in comparison with temperature profiles II and III. The best expansion ratio was  $161.33 \pm 6.49\%$  (10:10) cooked in temperature profile I and the least expanded extrudates was  $97.33 \pm 0.88\%$  (20:0) cooked in temperature profile II.

#### *Bulk density*

Extrudates with the lowest bulk density ( $p < 0.05$ ) was produced by temperature profile III at  $403.36 \pm 3.06$  g/L,  $436.88 \pm 3.60$  g/L and  $458.96 \pm 3.98$  g/L (0:20, 5:15 and 15:5, respectively) and by temperature profile II at  $441.77 \pm 2.98$  g/L (10:10).

#### *Floatability*

There was an increasing floatability percentage with increasing sago starch ratio for temperature profile I. The best floating extrudates ( $p < 0.05$ ) was produced by temperature profile II produced at  $64.00 \pm 11.72\%$ ,  $62.00 \pm 10.26\%$ , and  $57.33 \pm 7.86\%$  (15:5, 10:10 and 5:15, respectively). Mixtures 20:0 in temperature profile II and mixtures 20:0, 10:10 and 5:15 in temperature profile III failed to produce floating extrudates. On the other hand, we observed that floatability percentage for temperature profiles II was significantly improved ( $p < 0.05$ ) when 5% corn starch or more was replaced by sago starch.

**Table 3: Physical properties of extrudates with five different CS:SS ratios cooked with three temperature profiles (I, II, III).**

Main effect		Physical properties					
Temperature	CS:SS (g/kg)	ER (%)	BD (g/l)	F (%)	SV (m/s)	WS (%)	PDI (%)
I	20:0	158.67 ± 2.33 <sup>a</sup>	488.83 ± 7.62 <sup>ab</sup>	2.00 ± 1.15 <sup>ef</sup>	6.67 ± 0.03	89.69 ± 0.75	99.96 ± 0.01
	15:5	157.67 ± 2.96 <sup>a</sup>	469.33 ± 0.50 <sup>ab</sup>	4.00 ± 3.06 <sup>ef</sup>	5.15 ± 0.11	89.20 ± 1.78	99.96 ± 0.01
	10:10	161.33 ± 6.49 <sup>a</sup>	470.71 ± 4.96 <sup>ab</sup>	14.67 ± 6.67 <sup>cde</sup>	3.06 ± 0.08	91.09 ± 0.83	99.97 ± 0.00
	5:15	158.33 ± 2.03 <sup>a</sup>	485.03 ± 13.62 <sup>ab</sup>	17.33 ± 11.10 <sup>de</sup>	3.66 ± 0.05	87.53 ± 0.45	99.98 ± 0.00
	0:20	151.33 ± 1.76 <sup>a</sup>	483.57 ± 9.32 <sup>ab</sup>	40.67 ± 8.51 <sup>abc</sup>	1.68 ± 0.13	85.52 ± 1.38	99.96 ± 0.00
II	20:0	97.33 ± 0.88 <sup>c</sup>	488.21 ± 17.56 <sup>ab</sup>	0.00 ± 0.00 <sup>f</sup>	4.64 ± 0.05	88.32 ± 0.36	99.97 ± 0.00
	15:5	112.67 ± 3.18 <sup>cd</sup>	480.42 ± 26.07 <sup>ab</sup>	64.00 ± 11.72 <sup>a</sup>	1.13 ± 0.28	87.24 ± 1.66	99.96 ± 0.00
	10:10	123.00 ± 1.00 <sup>bcd</sup>	441.77 ± 46.24 <sup>bc</sup>	62.00 ± 10.26 <sup>a</sup>	1.71 ± 0.22	89.02 ± 0.43	99.95 ± 0.01
	5:15	124.00 ± 1.53 <sup>b</sup>	511.21 ± 7.83 <sup>a</sup>	57.33 ± 7.86 <sup>ab</sup>	2.03 ± 0.03	88.75 ± 0.10	99.97 ± 0.00
	0:20	123.33 ± 2.33 <sup>bc</sup>	516.93 ± 17.60 <sup>a</sup>	23.33 ± 15.72 <sup>cde</sup>	3.74 ± 0.02	89.01 ± 0.18	99.93 ± 0.01
III	20:0	99.00 ± 3.22 <sup>c</sup>	469.58 ± 8.70 <sup>ab</sup>	0.00 ± 0.00 <sup>f</sup>	5.32 ± 0.09	90.30 ± 1.35	99.96 ± 0.00
	15:5	101.67 ± 4.33 <sup>e</sup>	458.96 ± 16.24 <sup>abc</sup>	9.33 ± 6.36 <sup>def</sup>	3.07 ± 0.10	87.29 ± 0.22	99.96 ± 0.01
	10:10	99.00 ± 3.18 <sup>c</sup>	467.29 ± 10.29 <sup>ab</sup>	0.00 ± 0.00 <sup>f</sup>	4.03 ± 0.06	88.87 ± 0.33	99.98 ± 0.01
	5:15	112.00 ± 1.00 <sup>d</sup>	436.88 ± 16.93 <sup>bc</sup>	0.00 ± 0.00 <sup>f</sup>	3.62 ± 0.09	88.48 ± 0.27	99.96 ± 0.01
	0:20	120.67 ± 7.88 <sup>bcd</sup>	403.36 ± 15.44 <sup>c</sup>	29.33 ± 8.97 <sup>bcd</sup>	2.09 ± 0.04	88.01 ± 0.87	99.96 ± 0.01
<b>Mean Temperature</b>							
I		157.47 ± 1.61 <sup>a</sup>	479.49 ± 3.97 <sup>a</sup>	15.73 ± 6.90 <sup>b</sup>	4.04 ± 0.86	88.61 ± 0.96	99.97 ± 0.00
II		116.07 ± 2.84 <sup>b</sup>	487.71 ± 13.36 <sup>a</sup>	51.67 ± 12.71 <sup>a</sup>	2.65 ± 0.66	88.47 ± 0.33	99.96 ± 0.01
III		106.60 ± 2.83 <sup>c</sup>	447.21 ± 12.39 <sup>b</sup>	7.73 ± 5.69 <sup>b</sup>	3.63 ± 0.53	88.59 ± 0.50	99.96 ± 0.00
<b>Mean CS:SS ratio</b>							
20CS:0SS		118.33 ± 10.15	482.21 ± 6.32	0.67 ± 0.67 <sup>b</sup>	5.54 ± 0.60 <sup>a</sup>	89.44 ± 0.59 <sup>a</sup>	99.96 ± 0.00
15CS:5SS		124.00 ± 8.75	469.57 ± 6.20	25.78 ± 19.17 <sup>a</sup>	3.12 ± 1.16 <sup>b</sup>	87.91 ± 0.65 <sup>ab</sup>	99.96 ± 0.00
10CS:10SS		128.00 ± 9.23	459.92 ± 9.13	25.56 ± 18.71 <sup>a</sup>	2.93 ± 0.67 <sup>b</sup>	89.66 ± 0.72 <sup>a</sup>	99.97 ± 0.01
5CS:15SS		131.44 ± 6.99	477.71 ± 12.76	24.89 ± 16.98 <sup>a</sup>	3.10 ± 0.54 <sup>b</sup>	88.25 ± 0.37 <sup>ab</sup>	99.97 ± 0.01
0CS:20SS		131.78 ± 5.47	467.95 ± 18.35	31.11 ± 5.08 <sup>a</sup>	2.50 ± 0.63 <sup>b</sup>	87.51 ± 1.04 <sup>b</sup>	99.95 ± 0.01
<b>Statistical analysis</b>							
Temperature		***	**	***	ns	Ns	ns
CS:SS ratio		ns	ns	***	**	*	ns
Temperature x Ratio		***	*	***	*	*	ns

Mean ± SE (n=3); T, Temperature profile; FM, Feed mixture; ns, non-significant; \*, p<0.05; \*\*, p<0.01, \*\*\*, p<0.001.

### Sinking velocity

There was no significant interaction (p>0.05) between tested factors. The slowest sinking velocity was recorded at 1.13±0.28 cm/s (15:5) and 1.71±0.22 cm/s (10:10). However, the fastest sinking velocity was at 6.67±0.03 cm/s (20:0) followed by 5.32±0.09 cm/s (20:0). Generally, the sinking velocity

of the extrudates was decreased with increasing sago starch ratio for temperature profiles I and III. For temperature profile II, the sinking velocity of extrudates was decreased with the inclusion of sago starch in feed mixtures.

### Water stability

Sago-corn starch ratio had a significant effect on the water stability of extrudates ( $p>0.05$ ). The best stability in water was recorded for 10:10 ( $91.09\pm 0.83\%$ ) and it was the least stable in water for 0:20 ( $85.52\pm 1.38\%$ ). Temperature profile and the interaction did not affect ( $p>0.05$ ) the water stability.

### Pellet durability

Generally, extrudates produced by all three temperature profiles with different sago starch ratios had similar durability percentage. The percentage of fines produced was less than 0.1% for all extrudates.

### Discussion

Expansion at higher extrusion temperatures leads to higher gelatinization, producing extrudates with greater expansion ratio, and reducing bulk density (Case *et al.*, 1992). In this study, all the values in temperature profile I were significantly higher compared to other temperature profiles. Both factors and interaction between factors had significant effects which indicated that expansion ratio is influenced by temperature profiles and CS:SS ratio. A steady increase of temperature throughout the cooking process and equal CS:SS ratio produced extrudates with the best expansion ratio in this study. Several studies show that inclusion of another types of starch decreases the expansion ratio in starch-based snacks, e.g. increasing ratio of soy protein concentrate to corn starch

(de Mesa *et al.*, 2009); and starch ratio in cassava starch (Tongdang *et al.*, 2008).

Bulk density is important in determining the storage volume for transportation and storage containers. Specific bulk density range for floating tilapia feed has been established at  $<480$  g/L (Riaz, 2009). Temperature profile III produced extrudates in this range for all CS:SS ratio. There were significant effects for temperature profiles and its interaction between factors but none for CS:SS ratio. This showed that higher temperature at die zone affects the bulk density of the final product. Similar results have been reported where bulk density value decreases with increasing barrel temperature (Case *et al.*, 1992, Kannadhasan and Muthukumarappan, 2010, de Cruz *et al.*, 2015).

Pellet floatability plays a significant role in tilapia feeding. Cruz and Ridha (2001) reported that tilapia juveniles fed with sinking extrudates have significantly better growth performance and feed conversion ratio compared to fish fed with floating extrudates. In contrast, sea bream (*Sparus aurata*) shows best performance when it is fed with floating tilapia extrudates (Sadek *et al.*, 2004). In our study, inclusion of sago starch significantly improved the floatability of extrudates. With just 5% inclusion, the extrudates had better floatability as observed in temperature profile II (15CS:5SS) and temperature profile III (15CS:5SS) compared to no floatability for the 20% corn starch extrudates for both temperature

profiles. Accordingly, all factors and interaction between factors had significant effects indicating that pellet floatability was influenced by temperature profiles and CS:SS ratio. The percentage of moisture content in our study is also similar to another study on sago and tapioca mixture which proves that any sago combination with another starch has better pellet floatability at 40% moisture content (Umar *et al.*, 2013).

There were significant effects for starch ratio and temperature profile for the sinking velocity in this study, although there was no significant effect for interaction between factors. A somewhat similar result has also been reported by Kannadhason and Muthukumarappan (2010). It was observed that all extrudates that contained 20% CS had high sinking velocity. This suggested that inclusion of sago starch lowered the sinking velocity of the extrudates which is a desirable characteristic during fish feeding. Schwertner *et al.* (2003) noted that sinking velocity is an unsatisfactory indicator as some of the extrudates floated until they disintegrated which also occurred in this study. Therefore, both floatability and sinking velocity tests performed for an unbiased evaluation.

Pellet durability is the ability to withstand breakage and disintegration caused by stress during handling and transportation (Thomas and van der Poel, 1996), as well as having high water stability to prevent nutrient leaching and disintegration in water

(Obaldo *et al.*, 2002). Previous studies demonstrate that extrudates with low water stability may cause oil separation in the gut and lead to oil-belching in rainbow trout afflicted by osmoregulatory stress (Baevefjord *et al.*, 2006, Aas *et al.*, 2011). Our results showed that there were no significant effects for individual factors and interaction between factors for pellet durability. Corn-sago ratio had a significant effect on the water stability of extrudates. However, these factors have been observed to have significant effects on water stability and pellet durability when mixtures of DDGS-cassava starch, DDGS-potato starch or DDGS-corn starch are used as the dietary starch source (Kannadhason *et al.*, 2009, Rosentrater *et al.*, 2009a, Rosentrater *et al.*, 2009b). In another study, using sole starch source had better durability in comparison to mixtures of two to three sources (Ah-Hen *et al.*, 2014). This difference in results could be attributed to the different type of starches used in our study. Low percentage of fines (<0.10%) and high percentage of pellet stability in water (>85%) suggested that sago starch is a good binder for producing quality tilapia extrudates.

Based on the results, it can be concluded that inclusion of sago starch significantly improved the expansion rate and floatability percentage for extruded tilapia feed. Temperature manipulation played an important role in reducing bulk density. On the other hand, interaction between temperature and sago starch ratio did not have



significant effects on sinking velocity, water stability and pellet durability ( $p>0.05$ ). Sago starch can be considered as a good binder as it gave the extrudates high percentage of water stability and pellet durability. This study demonstrated that extrusion of a feed mixture containing 10:10 corn to sago starch ratio at a barrel and die setting of 60-100-120-160°C produced the best tilapia feed with desirable physical properties.

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