The Role of Soaking Process on Mineral HCl- Extractabilities, Phytic Acid Content and Trypsin Inhibitor Activity of Common Bean Bulgur

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Common bean (CB) bulgur, is a variety of new parboiled and dried product, was produced with soaking different soaking time (2, 8 and 12h) and soaking water pH (pH 4, 6 and 8) in this study period. Experiment was conducted according to $(3 \times 3) \times 2$ factorial design. Physical, chemical nutritional, and anti-nutritional components of raw and CB bulgur samples were determined. Total phenolic content and phytic acid reduced at a ratio 41 – 57% and 48 – 57% respectively, while trypsin inhibitor activity was completely eliminated after 12 hour soaking time. The highest in vitro protein digestibility (73.4%) was measured in CB bulgur samples with soaked at pH 8 soaking water. The highest HCl-extractability of ash value (65.57%) of CB bulgur samples obtained by soaking at pH 6 soaking water. HCl-extractability of K, Ca, P, Mg, Zn and Fe increased with levels of 3 – 7%; 17 – 19%; 29 – 34%; 15 – 21%; 15 – 22%; and 28 – 52%, respectively with increasing soaking time. The optimum soaking process condition was found as 12 h soaking time and 8 pH soaking water for obtaining highest nutritional properties of CB bulgur, except an adverse effect on texture and taste score of bulgur.

Keywords: soaking; trypsin inhibitor activity, phytic acid, HCl-extractability of minerals, in vitro protein digestibility, bulgur

Introduction

Dry CB (*Phaseolus vulgaris* L.) is the most widely produced and consumed food legume that have numerous health benefits such as colon cancer prevention, increased satiation; lower glysemic index and decreased cardiovascular disease risks (Anderson *et al.*, 1984; Geil and Anderson, 1994). It is also consumed its high protein content and low price compared to meat products. As reported by Morrow (1991), the utilization of food legumes is limited because of the presence of some anti-nutritional substances. Among these are polyphenols, phytates and trypsin inhibitor. Legumes contain considerable amounts of Fe-binding polyphenols inhibiting Fe absorption (Sandberg, 2002). Phytates can enhance or reduce the bioavailability of proteins and minerals (Nestares *et al.*, 1996). Trypsin inhibitors present in legume seeds can affect the nutritional quality of legumes by reducing protein digestibility (Liener and Kakade, 1980). Therefore legumes need to be nutritionally improve with some processes, such as soaking, boiling, autoclaving or dehulling not only im-

proves flavor and palatability of foods but also increases the bioavailability of nutrients, by destroying the anti-nutritional factors (Chau and Cheung, 1997).

Bulgur, produced from different cereals by soaking, cooking, dehulling, drying and grinding, is a valuable cereal product and a very famous industrially processed ancient wheat product. And also bulgur is a very important pre-cooked wheat product due to its storability, high nutritional value, ease preparation and low cost (Bayram, 2000). Generally, durum wheat is preferred for bulgur production, but some researchers choosed to use oats, corn, triticale, barley, rye and soybean instead of durum wheat (Singh and Dodda, 1979; Elgün *et al.*, 1990; Köksel *et al.*, 1999; Bayram *et al.*, 2004).

Therefore the objectives of this study were to introduce the processing treatments of CB bulgur and to investigate the effects of the soaking time and soaking water pH on nutritional and anti-nutritional properties of CB bulgur.

Material and Methods

Material Seeds of Akman-98 dry CB variety were obtained from Anatolian Research Institute in Eskişehir, Turkey.

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Preparation of bulgurs CB samples free from cracks, dust and other foreign materials were soaked (1:5 w/v) at different soaking time (2, 8 and 12 h) and different soaking water pH (pH 4, 6 and 8) at room temperature. Experiments were conducted according to 3 (different soaking time) $\times$ 3 (different soaking water pH) $\times$ 2 (repetition) factorial design. Soaking water was prepared with 0.01 N NaOH and 0.01 N HCl solutions. After soaking, the seeds removed from the soaking water and then cooked (1:2 w/v) in autoclave under steam pressure at 121°C, 20 min (Hirayama HV-50, Saitama, Japan) which was sufficient to gelatinize the starch entirely. The cooked materials were dried at $50 \pm 5^\circ\text{C}$ to 10% moisture content in the oven (Nüve FN-500, Ankara, Turkey). The dried seeds were conditioned with 2% additional water by mixing for 10 min, milled into course grist on the disk mill (Bastak, Ankara, Turkey) so that all the material passed through a 3.55 mm sieve and over 1.6 mm sieve, and aspirated to remove bran material. Samples were kept in closed glass containers at room temperature until used.

Bulgur yield and color measurements The color of the samples was evaluated by measuring the L^* , a^* and b^* values using a Hunter Lab Color QUEST II Minolta CR-400 (Minolta Camera, Co., Ltd, Osaka, Japan). Bulgur yield was calculated by using the initial CB amount and cooked, dried and sieved bulgur amount, shown as follows:

$$\text{Bulgur yield} = 100 \times (A \times B) / (C \times D)$$

A: Weight of bulgur (g); B: Dry matter of bulgur (%); C: Weight of legume before soaking (g); D: Dry matter of legume before soaking (%).

Nutritional and antinutritional analysis Raw CB and CB bulgur samples were analyzed for their moisture (method 44-19), crude ash (method 08-01), crude protein (method 46-12), crude fat (method 30-25), crude fiber (method 32-10) using standard methods (AACC, 1990). Starch contents were determined by using the polarimeter (Elgün *et al.*, 2005). Gross energy (kcal) was estimated by multiplying the percentages of crude protein, lipid and carbohydrates by the factors 4, 9 and 4 respectively. The mineral element contents of the samples were determined by inductively coupled plasma spectroscopy, ICP-AES (Vista series, Varian International AG, Switzerland) (Bubert and Hagenah, 1987). HCl extractabilities of the minerals were carried out according to Saharan *et al.* (2001). Trypsin inhibitor activity (TIA) was essentially determined according to Kakade *et al.* (1974). Phytic acid was measured by a colorimetric method according to Haugh and Lantzsch (1983). In vitro protein digestibility (IVPD) was determined by the methods given by Bookwalter *et al.* (1987). Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method (Singleton *et al.* 1999) as modified by Beta *et al.* (2005). Total phenolics

were analysed by using Hitachi-U1800 spectrophotometer (Hitachi High-Technologies, Tokyo, Japan) at 725 nm. The results were expressed as mg gallic acid equivalents per g sample.

Sensory analysis Bulgur samples were boiled in water (1:2 w/v) at $100 \pm 2^\circ\text{C}$. After all the boiling water was absorbed, 100 g bulgur sample was served to the panelists at approximately 40°C. Sensory analyses of bulgur samples were conducted by ten panelists. Samples were randomly ordered at the beginning of the panel and each panelist received the samples in the same order. Eight panelists were asked to score the bulgur in terms of hardness, taste, chewiness, color and smell. Bulgur characteristics were rated on a 1-5 scale: 1- dislike extremely; 3- acceptable; and 5- like extremely.

Statistical analysis Commercial software programs (MINITAB and MSTAT) was used to perform statistical analyses. Data were assessed by analysis of variance with two ways ANOVA Duncan's multiple-range test was used to separate means. Significance was accepted at $P < 0.05$ throughout the analysis.

Results and Discussion

Physical properties of CB bulgur ANOVA results showed that soaking time and soaking water pH had a significant ($P < 0.01$) effects on bulgur yield and color L^* values of the CB bulgurs (Table 1). The effect of soaking methods as a variance source on bulgur yield is given Table 2. Higher bulgur yield values were obtained by soaked at 2 h. When alkaline pH (8 pH) used in soaking water of CB samples, the highest bulgur yield values were obtained in CB bulgurs. This might be due to the hardness of the soaked seeds at acidic soaking water and more destruction, therefore obtained lower bulgur yield and higher flour compared the soaked at alkaline water (Bayram *et al.*, 2004). The color of bulgur is the most important parameter for consumer acceptability of the bulgur. The darkening (color loss) of the processed product is a common problem with the processing of bulgur. Generally a light yellow color is acceptable for wheat bulgur. Therefore, L^* and b^* values should be preferred high and, in contrast to low values of a^* by the consumers. In present study, 2 h soaking method gave the highest L^* and the lowest a^* values. Soaking in alkaline water gave higher L^* values of CB bulgur than that of soaking in acidic water.

Nutritional properties of CB bulgur Chemical composition of raw and processed CB samples were showed in Table 2. Crude protein and starch content of CB bulgur samples increased while crude ash, crude fiber and crude fat content reduced compared to raw sample by the bulgur process. As a variance source, soaking time significantly ($P < 0.05$) decreased crude ash and crude protein contents. These de-

Table 1. Anova results of the soaking time and soaking water pH on the composition of CB bulgur samples.

Composition	F values		
	Soaking time (A)	Soaking water pH (B)	A-by-B
Bulgur yield	1112.666**	69.843**	16.365**
Color L*	15.214**	10.653**	12.084**
Color a*	21.800**	—	6.603**
Color b*	—	—	—
Crude protein	7.810*	—	—
Starch	3234.200**	535.400**	1805.000**
Ash	13.901**	—	—
Crude fiber	—	8.223**	—
Crude fat	19.958**	—	—
Energy	22.120**	—	8.178**
IVPD	5.846*	20.962**	7.426**
HCl-extractability of ash	6.979*	13.837**	—
TPC	394.714**	4.959*	18.000**
TIA	—	—	—
Phytic acid	5.728*	60.366**	17.820**
Minerals			
Total P	50.920**	320.982**	14.048**
Total Ca	1083.673**	2567.961**	819.091**
Total Mg	—	—	—
Total K	19359.055**	368.969**	1778.621**
Total Zn	421.289**	42.421**	147.737**
Total Fe	111.647**	179.115**	135.853**
<i>Extractability of minerals</i>			
HCl-extractability of P	—	18.166**	—
HCl-extractability of Ca	—	—	—
HCl-extractability of Mg	40.763**	5.868*	6.019*
HCl-extractability of K	154.401**	200.918**	22.682**
HCl-extractability of Zn	333.820**	213.014**	156.055**
HCl-extractability of Fe	71.441**	9.288**	4.695*

Blank spaces indicate no significance. *Significant at $p < 0.05$ respectively. **Significant at $p < 0.01$, respectively.

A-by-B: Soaking time-by-Soaking water pH.

creases might be attributed to their diffusion into the soaking and cooking water, and due to the leaching of both macro and microelements into the different soaking waters and subsequent autoclaving medium. This is in agreement with data given by Aletor and Ojo (1989) on soya and lima bean. Significant ($P < 0.05$) increases in starch, crude fat contents and energy values were observed with soaking time of CB bulgur samples. The increases in starch and crude fat content in CB bulgur samples were attributed to the loss of soluble solids during soaking and cooking, which would increase the concentration of these contents in cooked seeds. The results agree with those of previous studies by Wang *et al.* (2010) on common bean and chickpea and Siddhuraju and Becker (2005) on mucuna beans. When the Duncan test results were compared according to soaking water pH variance source, crude protein, crude fat, crude ash and energy values of soaked at different soaking water pH and those soaked followed by autoclave were found to be similar (Table 2). CB

bulgur samples soaked at pH 4 soaking water showed the highest starch content, while the seeds soaked at pH 6 soaking water contained the least.

According to ANOVA results soaking time had a significant ($p < 0.05$) effects on IVPD and HCl-extractability of ash, while soaking water pH affected same parameters at $p < 0.01$ significance levels (Table 1). Similar results have been reported by some researchers (El-Adawy 2002; Mubarak 2005) for chickpea and mung bean seeds. The highest HCl-extractability values of ash observed with CB bulgur samples soaked at 12 hour and pH 6 soaking water according to the soaking time and soaking water pH variance source. And CB bulgur samples soaked at pH 8 soaking water gave the highest IVPD values. The combining effect of 8 hour soaking time and pH 8 soaking water gave the superior properties in term of improving the protein digestability (data not shown). Some researchers reported that the alkali-treated sorghum seeds exhibited significant ($P < 0.05$) increase in IVPD as

Table 2. Physical properties, chemical composition and nutritional properties of investigated raw CB and bulgur samples^a.

Composition	Raw CB	Variance Sources					
		Soaking time			Soaking water pH		
		2 h	8 h	12 h	pH 4	pH 6	pH 8
Bulgur yield (g/100g)	—	84.06 ^a	68.29 ^b	67.55 ^b	72.78 ^b	71.37 ^c	75.85 ^a
Color L*	71.18	74.32 ^a	73.12 ^b	74.24 ^a	73.27 ^b	74.36 ^a	74.06 ^a
a*	0.46	1.50 ^b	2.42 ^a	2.11 ^a	1.98	1.99	2.06
b*	7.27	17.16	16.03	16.19	16.02	16.55	16.81
Crude protein ^b (g/100g)	17.92	20.10 ^a	19.98 ^{ab}	19.27 ^b	19.88	19.89	19.58
Starch (g/100g)	39.8	45.03 ^c	45.28 ^b	47.27 ^a	46.25 ^a	45.30 ^c	46.03 ^b
Ash (g/100g)	4.20	2.84 ^a	2.54 ^{ab}	2.50 ^b	2.62	2.65	2.62
Crude fiber (g/100g)	4.22	3.74	3.86	3.85	3.82 ^{ab}	3.91 ^a	3.72 ^b
Crude fat (g/100g)	1.01	0.82 ^b	0.91 ^a	0.95 ^a	0.90	0.91	0.86
Energy (kcal/100g)	239.97	260.64 ^b	269.47 ^a	275.07 ^a	270.51	266.69	267.97
IVPD (g/100g)	68.90	71.06	72.25	72.36	71.55 ^b	70.73 ^b	73.40 ^a
HCl-ext. ash (g/100g)	55.56	60.50 ^{ab}	60.29 ^b	64.85 ^a	58.33 ^b	65.57 ^a	61.73 ^{ab}
TPC (mg GAE/g)	1.66	0.983 ^a	0.883 ^b	0.718 ^c	0.877	0.862	0.847
TIA (TIU/mg)	20.34	nd	nd	nd	nd	nd	nd
Phytic acid (mg/100g)	1152.16	568.23 ^a	561.31 ^{ab}	538.96 ^b	572.91 ^a	595.29 ^a	500.31 ^b

^a Variables were determined by the two- way ANOVA model, means in the same line at the two different variance source, with different letters as superscripts are significantly different. ($n = 3$).

^b Protein = $N \times 6.25$.

^c nd: non determined.

^d IVPD: In-vitro protein digestability, TIA: Trypsin inhibitor activity, TPC: Total phenolic content. HCl-ext.: HCl-extractability.

Fig. 1a. Effect of soaking time and soaking water pH on macroelements (K and P values $\times 10^{-1}$). **Fig. 1b.** Effect of soaking time and soaking water pH on HCl-extractability of macroelements.

compared to untreated seeds (Chavan *et al.*, 1979). Figure 1 and 2 points at macro and microelements of raw CB and its bulgur samples produced by different soaking treatments. At the end of the bulgur process, all macro and micro elements decreased in variable degrees. According to raw CB seed, P,

K, Ca, Fe and Zn decreased 16 – 29%; 20 – 34%; 10 – 31%; 16 – 21%; and 14 – 21% in CB bulgur, respectively. Barampama and Simard (1995) reported that soaking and cooking processes caused considerable losses in soluble solids, especially vitamins and minerals in common bean seeds. As ob-

Fig. 2a. Effect of soaking time and soaking water pH on microelements.

Fig. 2b. Effect of soaking time and soaking water pH on HCl-extractability of microelements.

served by Duhan *et al.* (2004) and Mubarak (2005), Ca, K, P, Mg, Fe and Zn content decreased with soaking treatment in legume (pigeon pea and mung bean) seeds. Change in soluble solids content for different grain products have been reported to be phytic acid, non-protein nitrogenous compounds, sugars, minerals (Fe, Cu, Zn, Mn, P, Ca, Mg), pigments, starch, protein, water-soluble vitamins such as thiamine, riboflavin and niacin (Kon, 1979; Synder and Kwon, 1987; Wang *et al.*, 1979). As stated before during soaking and cooking, minerals leached from the legume seeds into the water at different rates with soaking and cooking treatments. El Maki *et al.* (2007) reported that soaking and cooking treatments decreased the Ca content of CB from 377 mg/100 g to 162 mg/100 g in white common bean seeds. In the present study, the highest K, P and Fe content was observed with CB bulgur samples soaked at pH 8 soaking water. This might be due to the higher disruption and more diffusion into the acidic soaking water. Özkaya and Kahveci (1989) reported that all the investigated minerals in wheat bulgur samples were less than the corresponding wheat samples, and also, Mn, Zn and P contents decreased more than that of the other minerals. Figure 1 and 2 shows the effect of soaking time and soaking water pH on the HCl-extractability of macro and microelements of CB bulgur. As the period of soaking increased from 2 to 12 hour, a significant ($P < 0.05$) increase in the extractability of Mg, K, Fe and Zn was witnessed. Saharan *et al.* (2001), have found similar results with faba bean that on 12 hour soaking. Total P content reduced while HCl-extractability of

P increased because of the reducing the phytic acid content. As expected, the HCl-extractability of P, Ca, Mg, K, Fe and Zn present in CB bulgur samples were significantly ($P < 0.05$) higher than that of the raw whole CB seeds. HCl-extractability of K, Ca, P, Mg, Zn and Fe increased with levels of 3 – 7%; 17 – 19%; 29 – 34%; 15 – 21%; 15 – 22%; and 28 – 52% respectively with increasing soaking time and highest HCl-extractability of P (37% increase) by soaked at acidic soaking water. In contrast to HCl-extractability of P values, highest HCl-extractability of K, Mg, Zn and Fe (19; 20; 21; and 41% increase) values obtained by soaked at 6 pH soaking water. The Recommended Dietary Allowances (RDAs) (Anon., 2011) for adult males are 800 mg of calcium, 10 mg of iron, 1.6 – 2.0 g of potassium, 350 mg of magnesium, 800 mg of phosphorus and 15 mg of zinc. When 100 g (dry matter) CB bulgurs were consumed, 10.9 – 20.2% of RDA for Ca, 45.1 – 52.9% of RDA for Fe, 52.0 – 67.4% of RDA for K, 26.4 – 34.3% RDA for Mg, 37.3 – 46.0 of RDA for P and 16.7 – 19.7% of RDA for Zn were taken by the human body. Especially high Fe contents of CB bulgurs are important to overcome the anemia problem which occurs due to iron deficiency.

Anti-nutritional properties of CB bulgur samples The results of bulgur process included different soaking treatments on various anti-nutritional factors of CB bulgur samples are presented in Table 2. The mean TPC in raw CB seed was 1.66 (mg GAE/g) and during CB bulgur production 41 – 57% reduction in TPC was observed. Significant

differences ($p < 0.01$) in TPC were found in soaking time of CB (Table 1). Xu and Chang (2008) reported that, after soaking about 2 – 12% of TPC in peas and chickpeas were lost with increases in the hydration rate, while about 9 – 38% of TPC in lentil were lost in soaking water. Soaking followed by autoclaving reduced also total phenolics. Siddhuraju and Becker (2005) reported that soaking in bicarbonate solution, followed by autoclaving, brought about significantly ($p < 0.05$) lower TPC in all other processing methods like soaking at water; 0.07% sodium bicarbonate; 0.1% ascorbic acid and 3% free dried moringa leaf powder (due to its antioxidative properties like ascorbic acid). These results agreed with the previous studies of Vijayakumari *et al.* (1998) and El-Hady and Habiba (2003) and Martin-Cabrejas *et al.* (2009) who reported that the soaking, cooking, autoclaving reduced the TPC of legume seeds. Importance of TPC may be discussed from two points of view. First, evaluating the negative effects of consumed phenols and second, estimating their positive contribution to health. Therefore, 2 hour soaking time gave the highest TPC and 4 pH soaking water gave the highest TPC descriptively. The mean TIA value of CB seed was 20.34 TIU/mg and all soaking and followed autoclaving process, completely destroyed the TIA. Similar results reported by Gonzalez de Mejia *et al.* (1988), that soaking and boiling completely eliminated the TIA in tepary beans. According to Frias *et al.* (2000), soaking combined with cooking completely destroyed the trypsin inhibitors. Shimeilis *et al.* (2007), studied with beans and reported that 12 hour soaking reduced the TIA with levels of 9 – 18%, and soaking with sodiumbicarbonate soaking water gave more trypsin inhibitor loss. Khalil and Mansur (1995); Mc Naughton (1981) and Mubarak (2005) reported that boiling and autoclaving of bean seeds completely eliminated TIA. If the cooking process (boiling and autoclaving) of legumes is sufficient, it

is important to not only soften the legumes but also to inactivate or reduce trypsin inhibitors. Two ways ANOVA results showed that soaking time ($P < 0.05$) and soaking water pH ($P < 0.01$) had a significant effect on phytic acid content (Table 1). The interactive effect of soaking time-by-soaking water pH on phytic acid content was also significant ($P < 0.01$). The phytic acid content was 1152.16 mg/100 g in raw CB seeds and during CB bulgur production 49.7 – 59.6% reduction in phytic acid was observed. The combining effect of 2 hour soaking time and pH 8 soaking water gave the superior properties in term of reducing the phytic acid. The loss of phytic acid might be due to leaching of phytate ions into soaking and cooking water. Low phyate phosphorus contents are important in terms of bioavailability of phosphorus from the phytic acid complex. Changeable degrees of reduction in phytic acid content in legume seeds subjected to soaking and the cooking by atmospheric, autoclave and microwave (Duhan *et al.*, 1989; Estévez *et al.*, 1991; Jany *et al.*, 1993; Ummadi *et al.*, 1995; Manez *et al.*, 2002) methods have been reported. Özkaya *et al.* (2004) reported 18.9 – 33.9% phytic acid loss during wheat bulgur production.

Sensory properties of CB bulgur samples Sensory scores of CB bulgur samples produced by different soaking methods are presented in Table 3. Significant ($P < 0.05$) increases in hardness texture score occurred in CB bulgur produced by soaking at higher soaking time and acidic soaking water (pH 4). Low pH might induce hardness of the legume seed due to the acidity effect on the textural properties of legume seed during soaking (Bayram *et al.*, 2004). CB bulgur samples produced by soaking at 2 h resulted in softer texture and more bulgur-like taste. It was found that, soaking time and soaking water pH had not significant effect ($P < 0.05$) on the sensory properties such as color, smell, and chewiness.

Table 3. Effect of soaking time and soaking water pH of bulgur process in common bean bulgur samples sensory properties (1 – 5)^a.

	Variance Sources					
	Soaking time			Soaking water pH		
	2 h	8 h	12 h	pH 4	pH 6	pH 8
Texture	3.000 ^b	4.333 ^a	4.333 ^a	4.333 ^a	4.167 ^{ab}	3.167 ^b
Chewiness	4.333	4.667	4.500	4.667	4.833	4.000
Taste	4.333 ^a	3.333 ^{ab}	2.833 ^b	3.667	3.500	3.333
Color	3.000	3.167	3.667	3.000	3.167	3.667
Smell	3.667	3.500	3.333	3.500	3.167	3.833

^a Variables were determined by the two- way ANOVA model, means in the same line at the two different variance source, with different letters as superscripts an significantly different. ($n = 3$).

Conclusion

The results indicated great influence of soaking process in nutritional and anti-nutritional properties of CB bulgur and soaking in any soaking water pH and at any soaking time may be considered as a potential and cost-effective processing device to reduce and eliminate the anti-nutritional factors. TPC and phytic acid reduced ranged with 57% and 53% by soaked 12 h CB samples, respectively. The highest IVPD values (73.4%) was measured in CB bulgur soaked at pH 8 soaking water. HCl-extractability of K, Mg, Zn and Fe increased with increasing soaking time. TIA was completely eliminated with all soaking treatments by bulgur process. The highest HCl-extractability of ash value (65.57%) of CB bulgur samples obtained by soaking at 6 pH soaking water. HCl-extractability of K, Ca, P, Mg, Zn and Fe increased with levels of 3 – 7%; 17 – 19%; 29 – 34%; 15 – 21%; 15 – 22%; and 28 – 52% respectively with increasing soaking time. As a result, soaking in water at 8 pH for 12 h should be recommended to improve nutritional properties and inactivate/eliminate some anti-nutritional factors of CB bulgur. Thus the CB has high potential to be used bulgur material for the manufacturing of value-added products. When discussed nutritional aspects, CB bulgur is a unique new product especially for child, elder people and pregnant, due to its high protein, high RDA values of mineral, low phytic acid, TIA and TPC values.

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