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ORIGINAL RESEARCH PAPER

DEVELOPMENT OF THE METHOD OF PEANUTS DETOXIFICATION AND IMPROVEMENT OF ITS DIGESTION

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Heat treatment, namely hydrothermal treatment, followed by roasting was chosen in order to reduce the content of toxicants and antinutritional substances. Rational duration of hydrothermal processing is the temperature of 100º C at which the content of oxalic acid and its salts is reduced by 67.2...76.0%, and copper salts – by 28.8...38.0%, duration of 30...40 min being chosen. The duration for peanut kernels roasting (t = 120º C) was determined by the change of its color. It was determined that rational colour-parametric characteristics of peanut kernels (dominant wavelength 581.3 - 582.5 nm, color purity 35.9 - 36.0%, brightness 37.1 - 38.1%) are achieved during roasting for 30 - 35 min. The digestibility of peanut protein increased by 20 mg of tyrosine per 1 g of protein indicating the improvement of enzymatic hydrolysis of protein.

Keywords: peanuts, oxalic acid, copper salts, hydrothermal treatment, roasting, digestibility

Introduction

Nowadays nuts market is saturated enough, but peanut among them is a leader according to the frequency of consumption. World production of peanuts is over 35 million tons per year (Office of Global Analysis, FAS, USDA, 2016). Its main supplier is China (more than 45% of the world harvest). In Ukraine, peanuts share of all imported nuts is 80%. It has a high nutritional value and its price is the lowest compared to other nut crops. Rich chemical composition and high nutritional value of peanuts is associated primarily with high content of easily digestible fats (about 50%) and more than 20% of proteins. Fatty acid composition of peanut oil is characterized by a high content of unsaturated fatty acids and high quality amino acid content of protein is closer to the animal one. In addition, peanuts are a source of vitamins, minerals, polyphenols, phytosterols, resveratrol, and other biologically

active substances that makes it an essential product for an adequate and healthy diet (Arya *et al*., 2016; Salesa *et al*., 2014; Hasan *et al*., 2012; Talcott *et al*., 2005).

However, peanuts also contain harmful substances that can have antinutrient and toxic effects. These are such substances as oxalic acid and its salts (Chai *et al*., 2005; Judprasong *et al*., 2006; Chicago Dietetic Association, 2000; Sasaki *et al*., 2008), allergens (Burks *et al*., 1997; Shreffler *et al*., 2004; Barre *et al*., 2005), proteolytic enzyme inhibitors (Shherbakov *et al*., 2003), heavy metals salts (Rahbar *et al*., 2004; Radjabi *et al*., 2010; Tinggi, 1998; Angelova *et al*., 2006; Ching, 2008; Ching *et al*., 2008), radionuclides (Akkurt *et al*., 2012), mycotoxins (Park, 2006; Oliveira *et al*., 2009; Bakhiet *et al*., 2010; Kamika *et al*., 2011; Abbas *et al*., 2012; Stojanovska-Dimzoska *et al*., 2013).

The high content of oxalic acid and its salts, copper salts as well as poor digestibility and allergenicity does not allow safe use of domestic peanut in healthy diet products and requires searching the ways of its detoxification.

Scientists from many countries of the world are involved in the study of toxic and antinutrient substances in peanuts and the ways of reducing them.

The emergence of new peanut varieties and their genotypes as well as of new technologies that are actively implemented in agriculture requires determining peculiarities of the chemical composition of precisely those varieties that are adapted to the specific soil and climatic conditions of Ukraine. The results of our research showed that peanuts adapted to growing in Ukraine contain a rather high amount of oxalic acid and copper salts in its composition (Dubinina *et al*., 2013a; Dubinina *et al*., 2013b). To create peanut-based products of high quality it is necessary to minimize the content of these toxic substances and antinutrients in peanuts.

For some types of natural toxicants and pollutants, post-harvest processing and some technological methods of cooking can contribute to the destruction of endogenous and exogenous toxic substances or to the reduction of their toxicity. To reduce toxicity in the products of plant origin, a lot of ways are applied including washing, cleaning, soaking, blanching, boiling, canning, etc. Due to these operations, it is possible to reduce the content of toxicants up to 90% (Chernenok, 1993; Seljutina, 2001; Shaporova, 2002; Frolova, 2007; Olhovska, 2009; Lenert, 2010; Dubinina, 2014).

The best way to reduce the level of heavy metals in plant raw material is hydrothermal processing (boiling, blanching, poaching). As a result, the loss of these substances can reach 60% (Díaz *et al*., 2004; Lisiewska *et al*., 2007; Perello *et al*., 2008; Dubinina, 2014; Hajeb *et al*., 2014).

It is known that the reduction of oxalic acid in plant raw materials is influenced by the previous wet-heat processing such as soaking, boiling, and blanching. It was determined that after blanching, the total amount of oxalate is decreased by 9...19%, while by boiling by up to 50% (Kmiecik *et al*., 2004; Jaworska, 2005; Kmiecik *et al*., 2005; Dubinina *et al*., 2008). This occurs due to the fact that oxalic acid has high solubility in water and diffuses into solution.

Judprasong *et al*. (2006) studied the effect of roasting on the change of oxalic acid content in peanuts. It is proved that this type of processing reduces insignificantly its content (up to 7%). This is due to the partial removal of the shell that contains a small amount of this toxicant.

Peanut is one of the most common food allergens. It is included in the list of products listed in the Supplement III of the European Commission legislation as an allergenic ingredient the presence of which must be indicated on the label (Pele, 2010). At present, 13 allergens of peanut (Ara h 1 – Ara h 13) originating from 7 families of proteins are identified. It is determined that the peanut allergens Ara h 1, Ara h 2 and Ara h 3 are the greatest threat (Burks *et al*., 1997; Shreffler *et al*., 2004; Barre *et al*., 2005).

Scientists work successfully at development of the ways of reducing allergenicity of peanuts. It was determined that it is possible reduce its allergenic effects by using heat processing (boiling, frying). Increasing allergenic properties of peanuts during high-temperature heat processing due to the flowing of Mayer reaction was found in the works of Kopper *et al*. (2005), Maleki *et al*. (2001). In vivo research on mice proved that peanuts of dry roasting are probably more allergenic than raw ones (dry roasting causes chemical modification of peanuts proteins that activate the immune system) (Moghaddam *et al*., 2014). However, Vissers *et al*. (2011a) determined that, after heating for 20 min at 145°C, Ara h allergen 2/6 degranulated.

Oil legumes to which peanuts belong are characterized by high content of trypsin and chymotrypsin inhibitors. In general, inhibitors of enzymes account for about 6% of the proteins content. Peanuts contain both high molecular (Kunits inhibitor with the weight of 21.5 kDa) and low molecular inhibitors (Bauman-Birk inhibitors, S-P, D-P, E-1 with the molecular weight of 12...14 kDa) (Shherbakov *et al*., 2003). Bauman-Birk inhibitors and D-P form triple complex with trypsin and chymotrypsin: chymotrypsin-inhibitor-trypsin. Kunits inhibitor forms a double complex with trypsin: inhibitor-trypsin. Proteolytic enzymes being part of these complexes are completely devoid of catalytic activity because peanut proteins digestion by the body is significantly reduced. Inactivation is usually carried out by thermal denaturation of protein inhibitors.

Carrying out various types of peanuts kernels processing (boiling, microwave cooking, autoclaving and roasting) is described and their effectiveness is compared in the research of Embaby (2011). It was determined that all types of heat processing allow reducing phytic acid content by 3.8...24.7%, tannins by 6.7...68.5%, lectins by 75...100% and improving protein digestibility by 21.4...100%. Autoclaving, boiling, roasting-salting and oil-roasting proved to be the most effective.

Thus, scientists suggested methods of removing toxicants from peanuts, but they are flawed for many reasons. First, most methods cannot be used in the production technologies of peanuts products because they change significantly the properties of raw material and thereby make it impossible to obtain a quality product. Second, many ways do not present processing modes that make them difficult to use. Third, most methods can reduce the levels of certain toxicants and they are not suitable for the detoxification of others.

In this regard, it was necessary to develop a more universal method of processing peanuts that would be suitable for detoxification of many toxicants and appropriate for use in the existing technologies of its processing.

The objective of this work is to develop a method of processing peanut kernels that will allow reducing the content of toxic and antinutrient substances and increasing digestibility.

Materials and methods

Raw material

Peanuts sort of Pale Pink-2 Collection of the Institute of oilseed of the National Academy of Agrarian Sciences of Ukraine adapted to growing in Ukraine were selected for conducting this research. The specimen is grown in the South Ukraine steppe arid zone on the neutral soils.

Research of the oxalic acid content

The method of quantitative determination is based on the removal and sedimentation of oxalic acid in the form of calcium oxalate that is almost insoluble in cold water.

An extract derived from raw material was used to determine free and bound oxalic acid in the form of calcium salt. Sample material in the amount of 5 g was crushed and transferred to a volumetric flask and topped up with water to 250-500 ml, shaken and left for several hours or overnight. Free oxalic acid was determined in aqueous extracts without acidification.

To sediment oxalic acid, a volume of 50 ml extract was taken in the flask, added to the alkaline reaction ammonia and 1-2 g of boric acid (the latter was added for making difficult the sedimentation of tartaric acid salts and its optical isomers). Then a volume of 10 ml of reagent for the sedimentation of oxalic acid was added and left for 48 hours at a temperature below 7ºC. To prepare the reagent for sedimentation, 25 g of calcium chloride were dissolved in a small amount of water and 50% acetic acid was then added up to 500 ml; 330 g of crystalline sodium acetate were dissolved in 300 ml of water; both solutions were mixed well. The reagent was then allowed to stand for 48 hours at the temperature of 3-7ºC and was finally filtered. Calcium oxalate sediment together with filter is transferred into the flask, dissolved in a precise volume of volumetric solution of H_2SO_4 and the excess of acid is titrated with 0.1 N alkaline solution (1 ml 0.1 N H_2SO_4 equals 4.5 mg of oxalic acid) (Kovalchuk *et al*., 2004).

Copper salts content

Copper salts content was determined by atomic-absorption method according to GOST 30178-96 (1997) that was based on the product mineralization by the method of dry or wet ashing, while the element concentration in the mineralizing solution was determined by method of flame atomic absorption on the atomic absorption spectrophotometer of Perkin Elmer 2380.

Nitric acid in the amount of 10 cm^3 per every 5 g of the product was placed into a flask with product sample, was allowed standing for 15 min, and then was covered

with glass cork and heated on the electric hot plate, thus boiling down the contents of the flask up to 3-5 cm^3 of volume. Afterwards it was cooled. This procedure was repeated 2-4 times. Nitric acid in the amount of 10 cm^3, 5 cm^3 of sulfuric acid, 4 cm^3 of perchloric acid per every 5 g of the product were placed into a flask. The contents of the flask was boiled down to a volume of about 5 cm^3. The flask was cooled to room temperature, 5 cm^3 of nitric acid and 2 cm^3 of perchloric acid were added and then heated until white fumes of sulfur trioxide appeared.

Aliquot of tested solution (different volumes volumes ranging from 10 to 50 cm^3, depending on the requirements for the degree of concentration) was placed into one cup, while the second cup was filled with the same volume of control solution. The content of both cups was brought to a final volume of 50 cm^3 using zero standard.

Standard solutions were prepared for a device calibration. Settings, standardization and procedure of determination were performed according to the instruction manual of the atomic absorption spectrophotometer for analytical methods.

Determining color characteristics

The method of the International Commission on Illumination (ICI) based on determining integral coordinates of color X, Y, Z by which chromaticity coordinates x, y are calculated with the help of the diffuse reflection coefficients was used. They allow determining the chromatic parameters such as color purity, brightness, dominant wavelength (dominant tone).

Spectral characteristics were obtained in the range of 380-780 nm, with a pitch of 10 nm and the number of accumulation cycles being 20 (Kozlov *et al*., 2004).

Determining protein digestibility

Analysis of the protein ability to be digested using proteolytic enzymes *in vitro* system was conducted by the method of gradual action on the protein substances of the studied object by the proteinases system consisting of crystalline pepsin and trypsin (Antipova *et al*., 2001).

Fermentation was conducted in two stages, the duration of each was 60 min. The first stage of fermentation by pepsin was conducted at pH environment of 2.0, the second by trypsin at pH environment of 8.0.

pH level was checked with the help of Laboratory pH meter inoLab pH Level 1.

General chemical composition of the peanuts test sample was determined in the previous works of the authors (Dubinina *et al*., 2012).

The product sample containing 150 mg of protein was placed in two test tubes, and 15 cm^3 of 0.02 N solution of HCl was then added. Afterwards, 15 mg of pepsin were added to the obtained mixture into the experimental test tube. The control sample did not contain any enzyme. Samples were exposed at a temperature of 38°C for 60 min with constant stirring of the mixture. To determine soluble products of pepsinolysis, a volume of 0.2 cm^3 hydrolysate was collected, was mixed with 2 cm^3 of 10% solution trichloracetic acid, exposed for 20 min at room temperature and then centrifuged for 10 min at 6000 rpm/min. In the supernatant the amount of soluble

products of protein pepsinolysis was determined by Lowry method (Lowry *et al.*, 1951) and the degree of protein cleavage under the action of pepsin is calculated.

To conduct trypsinolysis, the content of the experimental and control tubes was neutralized with alkali (pH=7.0), namely 15 cm^3 of 0.08 N of $NaHCO_3$ solution was added. In particular, 30 mg of trypsin were added to the sample into the experimental test tube. Trypsinolysis was conducted for 60 min at a temperature of 38°C with constant stirring of the tubes contents.

To determine the total digestibility (the amount of soluble products of protein hydrolysis based on sequential action of pepsin and trypsin), a volume of 0.2 cm^3 hydrolysate was collected from the test tubes, and was mixed with 2 cm^3 of 10% solution of trichloracetic acid, exposed for 20 min at room temperature, and finally centrifuged for 10 min at 6000 rpm/min. In the supernatant the amount of soluble products of protein pepsinolysis was determined by Lowry method and the total digestibility of protein and the degree of protein cleavage under the action of trypsin were calculated.

Statistical analysis

The statistical analysis of the results was performed by means of Excel Program, Microsoft Office 2007. Differences were considered significant at $P < 0.05$. All results were presented as mean value ± standard error.

Results and discussion

Determining optimal parameters of hydrothermal treatment for peanut kernels detoxification

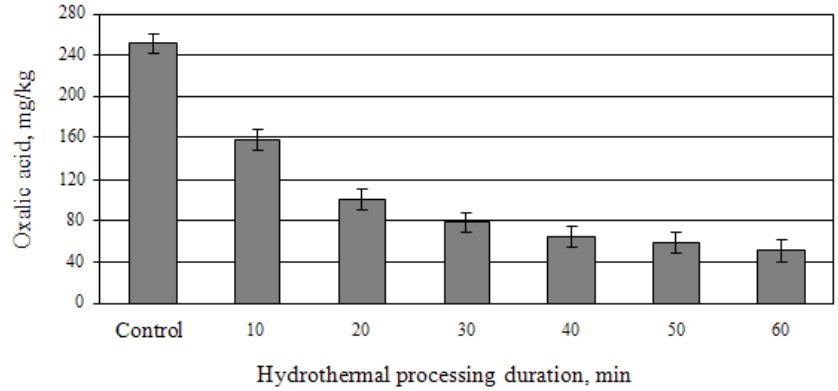
In order to reduce the content of toxicants and antinutrient substances we have chosen the physical method – hydrothermal processing that involves boiling followed by roasting. Hydrothermal treatment simultaneously affects the reduction of oxalic acid and its salts, heavy metals salts due to the diffusion into the solution. In addition, hydrothermal treatment leads to inactivation of trypsin and chymotrypsin inhibitors, thereby peanut protein is easier to digest. At the same time, roasting affects the improvement of organoleptic indicators of peanut quality.

To establish the optimal duration of hydrothermal processing, the interval duration of processing of 10...60 min was selected by us. The content of toxic substances was recorded every 10 min.

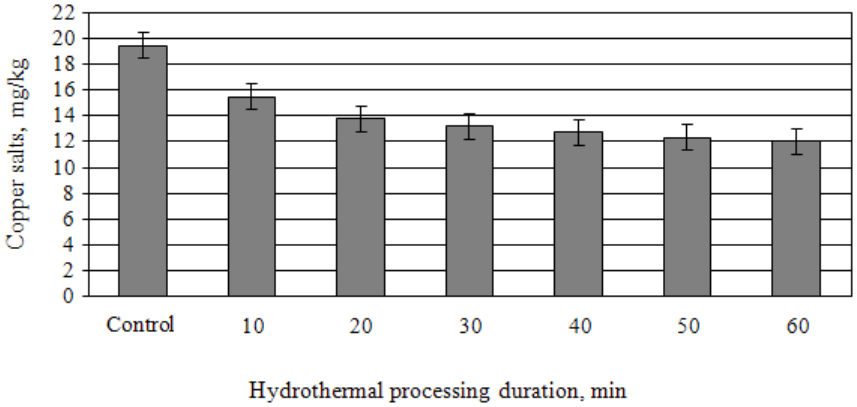
Peanut kernels together with seed coat were boiled in distilled water (ratio of peanuts:distilled water was 1:3). To evaluate the effectiveness of the proposed methods, changes in the contents of oxalic acid and copper salts after hydrothermal processing of samples were studied. Peanuts without hydrothermal processing were selected as a control sample. The results of the conducted experiment are presented in Figure 1.

Oxalic acid can manifest both toxic and antinutrient impact on the human body. It creates deficiency of substances essential for the body, thereby reducing nutritional value of the product. Antinutrient impact of oxalates can be identified taking into

consideration the ratio of oxalate / calcium (oxalate index). If it is greater than the unity, the product has antinutrient properties (Evtjugin *et al.*, 2007).



a)



b)

Figure 1. Evolution of oxalic acid and oxalates (a) and of copper salts (b) during the hydrothermal processing

The results of the research show that the process of boiling affects significantly the reduction of oxalic acid and copper salts contents in peanuts. During the hydrothermal processing within the first 10 minutes the contents of oxalic acid and its salts is reduced by 34.0...40.3%, and copper salts by 16.4...24.4%. The duration of hydrothermal treatment (20 min) makes it possible to reduce the content of toxicants by 58.1...62.1% and 25.6%….32.7%, respectively, in 30 min by 67.2…70.3% and 28.8...35.6%, and in 40 min by 73.5…76.0% and 31.5...38.0%. The amount of oxalic acid and copper salts during further hydrothermal processing also tends to reduce but at a slightly slower rate. Achieving concentration of copper salts lower than Maximum Residue Limits (MRL) (15 mg/kg) in the test sample was observed after 20 min of processing and reduction of oxalic acid to the level of

oxalate index of ≤1 was achieved in 30 min. But this duration of processing may not be enough to eliminate antinutrient impact for peanut varieties with high contents of oxalic acid and low contents of calcium. Therefore, rational duration of hydrothermal processing is 30...40 min, during which the content of oxalic acid and its salts is reduced by 67.2...76.0% and copper salts by 28.8...38.0%. Duration of hydrothermal processing over more than 40 min is inappropriate as it is energy-intensive and contributes to the loss of biologically active substances contained in peanuts.

One of the most important technological operations during peanuts products cooking is roasting. During this operation peanut seed coat is removed, favoring the improvement of the organoleptic indices and the decrease of the threat of microbial spoilage development.

We have selected roasting in the oven with air convection at a temperature of 120ºC. Such temperature of roasting is conditioned by the previous studies of the authors who determined that roasting at temperature of 120...145ºC contributes to the improvement of the hypoallergenic properties of peanuts, and, on the contrary, roasting at higher temperatures (150...170°C) increases the allergenicity of peanuts due to the course of Maillard reaction (Beyer *et al*., 2001; Vissers *et al*., 2011).

Duration of peanuts roasting was controlled visually and according to the quantitative characteristics of samples color.

Color characteristics of test samples in CIE XYZ systems were determined with the help of firmware SFScan. The spectral tristimulus values x and y were obtained with the help of chromaticity diagram in the form of a unit area (x+y+z=1). The 3D color space allowed determining the following indicators: dominant tone (dominant wavelength λ); color purity P, %; brightness T, % (Table 1).

During investigation of the impact of roasting duration on color parametric characteristics of peanut (Table 1) it was determined that "dominant wavelength", "color purity" and "brightness" parameters of the test sample, the roasting period of which is 25 min, does not affect significantly the change of color compared to the control sample (without roasting).

Increase in the period of processing up to 30...35 min is characterized by a shift of the parameter (λ, nm) to the red spectrum region of 573.0 nm for control to 581.3 nm and 582.5 nm for samples №2 and №3 respectively, the color of which is visually characterized as yellow-orange with brown shade.

Further increase in the period of processing leads to significant darkening of the sample №4 that will adversely affect the color formation of the finished product. However, reducing "color purity" parameter for the sample №4 up to 25.4% and "brightness" parameter up to 23.3% makes possible to conclude that color darkening of the mentioned sample takes place and it turns to achromatic color due to introduction of black color. Visual evaluation characterizes the color of the mentioned sample as dark brown. According to flavor profiles, sample №1 had a slightly beany flavor that characterizes insufficient duration of roasting. Sample №4 had a bitter flavor due to excessive roasting and burning. The best flavor profiles

were identified in samples №2 and №3 (roasting during 30 and 35 min): a pleasant flavor of roasted nuts was obtained.

Table 1. Effect of peanuts roasting on color parametric characteristics of peanuts

Test samples	Dominant wavelength λ, nm	Color purity P, %	Brightness T, %	Visual evaluation of samples color
Control (without roasting)	573.0	19.9	40.4	Light gray with yellow shade
№1 (roasting during 25 min)	573.5	21.3	38.9	Gray with yellow shade
№2 (roasting during 30 min)	581.3	36.0	38.1	Yellow-orange with brown shade
№3 (roasting during 35 min)	582.5	35.9	37.1	Yellow-orange with brown shade
№4 (roasting during 40 min)	590.9	25.4	23.3	Dark brown

Thus, the data obtained allowed determining a rational mode of peanuts roasting – at a temperature of 120ºC during 30...35 min. This color parametric characteristics of roasted peanut kernels had the following meanings: dominant wavelength – 581.3...582.5 nm, color purity – 35.9...36.0%, brightness – 37.1...38.1%.

The impact of peanuts processing on protein digestibility

Thermal processing also improves significantly digestibility of peanut proteins by digestive enzymes due to inactivation of trypsin and chymotrypsin inhibitors. That is why we studied the impact of hydrothermal processing on digestibility of peanut proteins. The results of the research are presented in Table 2.

Digestibility of peanut protein that was subjected to hydrothermal processing during 30...40 min, increased by 12.4 mg of tyrosine, and during extra roasting for 30...35 min, increased by 20 mg of tyrosine per 1 g of protein (Table 2). All this proves the improvement of enzymatic hydrolysis of proteins and digestibility of nutrients.

Since it is known that boiling peanuts in water (100...110°C) and roasting (120...145°C) also results in the loss of allergenic proteins of peanuts and reduction of the reactivity of immunoglobulin (lgE) to peanut (Beyer *et al*., 2001; Blanc *et al*., 2011; Vissers *et al*., 2011b; Sayers *et al*., 2014; Turner *et al*., 2014), it can be presumed that the type of peanut kernels processing proposed by us will allow not only increasing digestibility of proteins, reducing the content of oxalic acid and its salts as well as copper salts to a level lower than MRL, but also reducing allergenicity of this nut.

Table 2. Digestibility of proteins *in vitro* of the control and test samples of peanuts

Sample	Amount of soluble products of protein hydrolysis, mg of tyrosine per 1 g of protein in the product		
	pepsinolysis	trypsinolysis	pepsinolysis + trypsinolysis
Peanuts before hydrothermal processing (control)	0.0	39.6 ± 1.9	39.6 ± 1.9
Peanuts after hydrothermal processing during 30…40 min	3.3 ± 0.1	48.7 ± 2.3	52.0 ± 2.6
Peanuts after hydrothermal processing during 30…40 min and roasting (30…35 min)	6.6 ± 0.3	53.0 ± 2.6	59.6 ± 2.9

Conclusions

In order to reduce the content of toxic and antinutrient substances, the method of thermal processing of peanuts with further roasting (hydrothermal processing during 30...40 min followed by roasting at a temperature of 120ºC during 30...35 min) was used. It was determined that this process favors the reduction of the content of oxalic acid and its salts by 67.2...76.0%, and copper salts by 28.8...38.0%, and the increase of protein digestibility by 20.0 mg of tyrosine per 1 g of protein. This results on peanuts processing encourage their use for obtaining high quality traditional products suitable to be used in healthy diets.

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