SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF OLEORESIN FROM MALAGUETA PEPPER (*CAPSICUM FRUTESCENS* L.) ENHANCED BY ULTRASOUND

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Abstract. Supercritical fluid extraction technology came as an alternative to traditional methods of extraction and fractionation of bioactive compounds. The capacity of a supercritical fluid extraction unit is usually changed through the application of combined techniques, such as the use of different co-solvents and ultrasonic waves. The ultrasonic technology is based on the high frequency ultrasonic waves, which are capable of causing cavitations and disrupting the cell walls of vegetable materials. This favors the penetration of solvent and mass transfer, increasing the extraction yield and velocity. The objective of this study was to obtain extracts of malagueta pepper (*Capsicum frutescens* L.) using supercritical fluid extraction (SFE) assisted by ultrasound. The raw material used was malagueta pepper dried at 50°C (5% w. b.) and triturated. The particles with mean diameters between 1.68 to 1.18 mm and 0.342 to 0.177 mm were selected to SFE. To study the influence of ultrasonic waves and particle diameter in the extraction rate and yield, extractions were performed without ultrasound, and with ultrasound at power of 360 W. The extraction unit was constructed with the ultrasonic transducer installed inside the supercritical fluid extractor, which works in a frequency of 20 kHz and a maximum power of 800 W. The SFE conditions were 40 ± 3°C and 15 ± 0.5 MPa. The mass flow rate was fixed at 0.5 ± 0.1 kg/h. The supercritical extraction assisted by ultrasound increased global yield in malagueta pepper extract, when compared to the SFE. The highest increase was obtained in global yield of SFE from particles of 1.68-1.18 mm, while the 0.342-0.177 mm particle diameters achieved low yield increases in SFE+US. The FESEM images reveal morphological changes caused by disturbances on the vegetal matrix due to application of the ultrasound, and also sample characteristics under the electron beam related to the increased global yield in oil/resin of malagueta pepper before the extractions.

Keywords: Ultrasound, Supercritical Fluids, Yield, Pepper and FESEM.

1. Introduction

Red peppers (*Capsicum* sp.) are vegetables rich in capsaicinoids, substances that are responsible for the pungency of the fruits, among which capsaicin is the most representative [1]. Currently, capsaicin is used in the development of new drugs because it has many beneficial properties, such as antioxidant, antimicrobial, anti-inflammatory and antitumor activities, and contributes to the control of diabetes and pain relief [2]. Taking into account the benefits of capsaicin, there is great interest in developing new technologies to obtain concentrated extracts.

The extraction of active compounds from raw materials from plant sources is a promising area in the food industry. On the other side, it is a complex task because, in most cases, the target compounds are oxidative or thermally labile substances. Furthermore, severe legal restrictions are proposing the removal of general use of organic solvents in extraction industrial plants. Therefore, there is considerable interest

in replacing processes such as steam distillation and extraction with organic solvents traditionally used for the recovery of these active compounds [3]

Supercritical fluid extraction (SFE) came forward as an alternative to traditional methods for the extraction and fractionation of active compounds. Among the most commonly used supercritical fluid is carbon dioxide (CO_2), whose advantages in extraction processes are: low cost, nontoxicity, nonflammability, inert, and good extraction capacity [4].

Generally, in a SFE unit one can observe the effects of temperature, pressure, extraction bed size, solvent flow rate, among others, in order to maximize the yield of a specific compound [5]. The morphology of the particle can also influence the extraction yield of a specific compound, since the extraction occurs also through paths where the solvent must pass inside the solid particle, in order to extract specific compounds [6]. Moreover, the SFE unit capacity has changed by using combined extraction techniques, such as the use of different co-solvents and ultrasonic waves [7].

The ultrasound technique is based on the formation of ultrasonic waves of high frequency, which are capable of causing cavitations due to expansion and contraction cycles undergone by the material. Such cycles disrupt the cell walls of the vegetable matrix, favoring the penetration of the solvent and mass transfer, thus increasing the extract yield [8].

The objective of this investigation was to obtain extracts of malagueta pepper by supercritical fluid extraction (SFE) assisted by ultrasound, as well as to adjust the broken and intact cell model to the extraction curves, to verify the influence of particle diameter on the extraction kinetics and to demonstrate the effects of ultrasonic waves on the pepper particles through scanning electron microscopy.

2. Materials and methods

The work was performed in the Laboratory of Supercritical Technology, Extraction, Fractionation e Identification of Vegetal Extracts – LASEFI/DEA-FEA/UNICAMP. The raw material was malagueta pepper (*Capsicum frutescens* L.), a variety of chili pepper acquired at "Central de Abastecimento de Campinas S.A. (CEASA)", a local market in Campinas, southeastern Brazil.

2.1 Sample preparation

The fruits with good physical integrity were selected, sanitized by immersion in a solution of 100 mL/L of sodium hypochlorite for 20 minutes, washed with running water to remove the excess of hygienic solution and finally, conditioned under refrigeration (≈ 4°C) for further utilization. The raw material was subjected to the drying process in a laboratory oven, following the methodology used by Aguiar et al [9]. After drying, the samples were ground in a knife mill (Marconi, model MA 340, Piracicaba) with the objective to homogenize and decrease the resistance to mass transfer during the later stages of extraction.

In order to check the influence of particle diameter on the kinetics of SFE of malagueta pepper assisted by ultrasound, samples of dried and crushed peppers were placed in a vibratory sieve (Tyler series, Wheeling, USA) system (Bertel, model 1868, Caieiras, SP) with sequential openings of 12, 16, 24, 32, 48 and 80 mesh, with the aim of separating two specific particle diameters: diameter A, 16 mesh (1.68 to 1.18 mm), and diameter B, 48 mesh (0.342 to 0.177 mm).

2.2 Supercritical fluid extraction (SFE)

The kinetic experiments were performed in a supercritical fluid extraction assisted by ultrasound (SFE-US) unit consisting of a 0.295 L extraction column; a pneumatic pump (PP 111-VE MBR, Maximator, Nordhausen, Germany); two thermostatic baths to control CO_2 temperature at the pump inlet and SFE temperature; a flow totalizer and manometers to measure pressure. About 20 g of sample were placed inside the column, whose volume was completed with glass spheres, as illustrated in Figure 1.

The conditions of the SFE experiments were pressure and temperature of 15.0 ± 0.5 MPa and 40 ± 3°C respectively, obtained by Aguiar et al. (2012), who optimized carbon dioxide SFE from malagueta pepper. The extraction time was 8 hours, defined after preliminary tests, and the mass flow rate was fixed at 0.5 ± 0.1 kg/h. The extracts were collected in glass flasks, along the time of extraction, and weighed in analytical balance. The solvent used was CO_2 (Gama Gases, Campinas-SP, Brazil) with 99.0 % purity. Figure 2 illustrates SFE-US unit used in experiments.

The ultrasonic system (Unique Group, model DES500) is composed of a transducer unit with frequency of 20 kHZ and a 800 W variable output power controller. The ultrasound probe was installed inside of the SFE column. The ultrasound waves (360 Watts) were applied during the 8 hours of extraction.

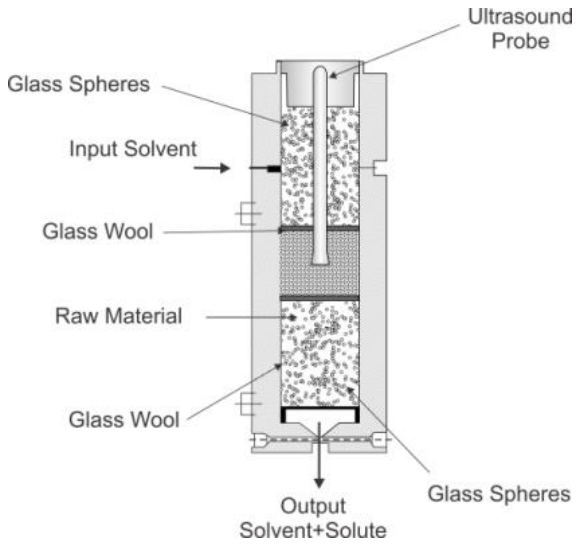

Figure. 1. Settings of the extraction bed for SFE assisted by ultrasound.

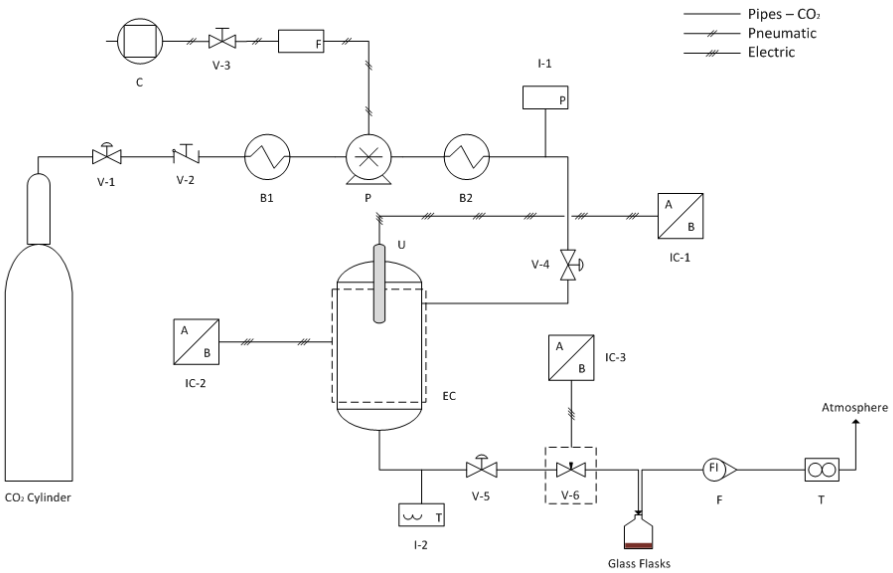

Figure. 2 - Diagram of unit supercritical extraction with carbon dioxide assisted by ultrasound; V-1, V-2, V-3, V-4, V-5 e V-6 – Control valves; V-6 – Micrometer valve; C- Compressor; F- Compressed air filter; B1 –Cooling bath; P - Pump; B2 – Heating bath; I-1 e I-2 – Pressure and temperature indicators, respectively; IC-1, IC-2 e IC-3 – Indicators and controllers of ultrasound power, temperature of extraction column and temperature of micrometer valve, respectively; EC – Extraction column ; U – Ultrasound probe; F – Flow totalizer; T – Flow meter.

2.4 Mathematical modeling

The mathematical model of Sovová [10], for the adjustment of model parameters, was applied through the individual adjustment of each experimental curve. For this purpose, the routine of Powell [11] was used. The used routine is an iterative method of adjustment that works with a range of values of the parameters stipulated by the user in a limited number of iterations. Within this range the routine searches parameter values that minimize the objective function (*f*), which was defined as the sum of squared errors.

Some process data are needed to apply the Sovová [10] model, such as global yield (X_0), extraction bed dimensions (H - height and d - diameter), solid sample mass (F), solvent flow rate (Q), solvent and solid densities (ρ_s and ρ_a, respectively), extract solubility (Y*) and particle diameter (d_p). All those data were directly measured, excepting the following: ρ_s of CO_2 was calculated as function of P and T using an equation of state [12]; Y* was estimated through extrapolation of experimental data from Kwon et al. [13].

2.5 Field emission scanning electron microscopy (FESEM)

Sample microstructures were analyzed before and after the extractions using a scanning electron microscope equipped with a field emission gun (FESEM - FEI Quanta 650). Prior to analysis, the samples were coated with Au in a SCD 050 sputter coater (Oerlikon-Balzers, Balzers, Lichtenstein). Both equipment were available at the National Laboratory of Nanotechnology (LNNano) located in Campinas-SP/Brazil. Analyses of the sample surfaces were performed under vacuum, using a 5 kV acceleration voltage and a large number of images was obtained on different areas of the samples (at least 20 images per sample) to guarantee the reproducibility of the results.

3. Results and discussion

Table 1 shows the values of the input parameters needed to apply the mathematical model of Sovová [10], obtained for the supercritical extraction of pepper variety malagueta (*Capsicum frutescens* L.). It is observed that the extraction bed dimensions were maintained constant for SFE with and without ultrasound, for particle diameters A (1.68 to 1.18 mm), and B (0.342 to 0.177 mm).

Table 1. Process parameters of SFE assisted by ultrasound.

Parameters	Diameter A (1.68 to 1.18 mm)	Diameter B (0.342 to 0.177 mm)
T (K)	313.15 ± 3	313.15 ± 3
P (MPa)	15 ± 0.3	15 ± 0.3
dp (m)	0.00143 ± 0.00035	0.00023 ± 0.00016
X_0 (kg solute/kg solid)	0.0514 ± 0.0051	0.0910 ± 0.0406
ρ_s (kg/m³)	1320.0	1320.0
ρ (kg/m³)	780.23	780.23
Q_{CO2} (kg/s)	0.0001673	0.0001673
H_b (m)	0.02	0.02
d_b (m)	0.05	0.05
F (kg)	0.0204 ± 0.0003	0.0205 ± 0.0007

T - temperature; P - pressure; d – diameter; X_0 – global yield; ρ_s – solid density; ρ – solvent density; Q_{CO2} – solvent mass flow rate; H_b –extraction bed height; d_b –extraction bed diameter ; F- mass of raw material into the extraction bed.

It can be verified in Table 1 and Figures 3 A and B that the global yield values (X_0) vary with particle diameter, from 5.14% to 9.10%, for the diameters A and B, respectively. Such behavior is due to a larger contact area of the particles of smaller diameter, which leads to a higher mass transfer of solute to the solvent. Instead, in the case of particles of larger diameter, the contact area provided with the solvent is smaller, causing a decrease the mass transfer rate, which agrees with results found by other researchers [14, 15, 16]. Another relevant fact is that larger particles contain higher concentration of seeds in the raw material, which causes this significant difference in the value of global yield. These facts are evidenced in Figure 2, which demonstrate the experimental and the predicted data by the model Sovová [10], obtained for the SFE of malagueta pepper assisted by ultrasound, for the largest (A) and the smallest (B) diameter particle.

The curves shown in Figure 3 demonstrate the behavior of supercritical extraction kinetics. The process begins with a period with constant extraction rate (CER), and this stage is characterized by the extraction of compounds readily available to the solvent. When the solute of easy access begins to exhaust, intraparticle diffusion becomes the governing mechanism of mass transfer in SFE. Thus, the extraction curves assume a typical format of a diffusion curve, with reduced extraction rate until the global yield (X_0) is reached.

It is observed in Figures 3 A and B that the influence of ultrasound in the constant extraction rate period is small, because in this period the governing factor of the extraction is the amount of easily accessible solute, which is a characteristic of each raw material. This fact is reflected in the values of the constant extraction rate time (t_{cer}), shown on Table 2. On the other side, during periods of decreasing rate and of diffusion, the application of ultrasonic waves increased yield, and at the end of the extraction increases were approximately from 5% without ultrasound to 6% with ultrasound and from 9% to 9.7% for the largest and smallest particle diameter, respectively. This behavior is explained due to cavitation

near the wall of the cell matrix which causes a disturbance in the vegetable matrix, which leads to the release of the intraparticle material and increase in the global yield of the extraction.

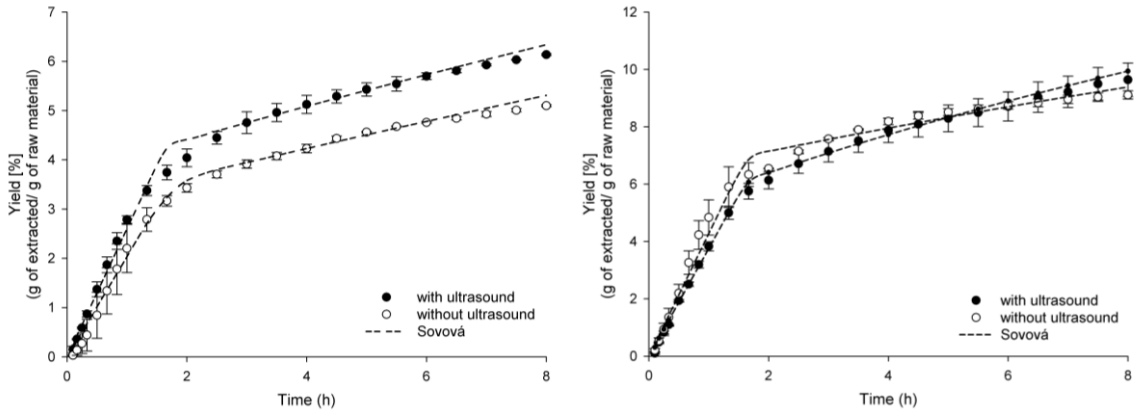

Figure 3. Experimental and modeled SFE curves from malagueta pepper at 15 MPa and 40 °C without and with ultrasound at 360W, for larger (A) and smaller (B) particle diameters, with the respective amplitude of the replicates.

The values of the mass transfer coefficients in the solid phase (k_s), fluid phase (k_f), the concentration of solute inside the particle (X_k), the objective function (*f*) and the constant rate period (t_{cer}) for the extractions performed in this study are shown in Table 2.

Table 2. Adjusted parameters, objective function (*f*) and constant rate period (t_{cer}) adjusted with Sovová model applied to supercritical CO_2 extraction from malagueta pepperat 15 MPa and 40 °C with ultrasound and without ultrasound for larger (A) and smaller (B) particle diameter.

Parameters	Diameter (A)		Diameter (B)	
	Without US	With US	Without US	With US
X_k	0.1462	0.1391	0.1121	0.1207
k_f (s^{-1}) x 10^4	7.1360	9.2965	15.8507	14.0238
k_s (s^{-1}) x 10^6	3.7432	4.6870	6.9302	10.1231
f x 10^8	2.0540	2.3393	6.8162	1.9875
t_{cer} (s)	6141.01	5498.20	5174.11	5241.22

It can be observed in Table 2 that the values of the objective function, which is defined as the sum of squared errors, are below 1.10^{-7}, evidencing a good adjustment of the model, as can be observed in Figures 2 A and B.

The values of the solid phase mass transfer coefficient (k_s) were lower than the fluid phase mass transfer coefficient (k_f) for the evaluated curves. According to Weinhold et al. [17], solute located internally in the particles is more difficult to be dissolved, and thus takes longer to cross the interface between fluid and solid solute, located on the surface of the particles. Therefore, the smaller k_s obtained indicate that the mechanism of diffusion is less representative compared to convection.

It is observed, also in Table 2, that the values of the mass transfer coefficients in both solid and fluid phases were higher for the extraction assisted by ultrasound than without ultrasound, for the SFE with larger particle diameter. On the other hand, the same coefficients at smaller diameter did not have large differences between their values. According to Gao et al. [7], if the particle size too small, the packing density will increase owing to an external mass transfer resistance increase, and the solvent will pass through the way of smaller resistance due to decrease of permeability of the matrix, resulting in the formation of preferential paths.

In order to learn more about the effects of cavitations caused by ultrasonic waves on the matrix, scanning electron microscopy (FESEM) analysis was performed. Figure 4 shows the images obtained by FESEM for malagueta pepper sample before extraction, after SFE and after SFE assisted by ultrasound, for both particle diameters studied in this work. It is clear from the images presented in Figure 4 that the samples that underwent supercritical fluid extraction present a greater amount of particulate material deposited on the surface in comparison to the raw material. In the case of ultrasound assisted process, particle deposition is even more pronounced in both particle sizes. This effect is assigned to a disturbance caused by the supercritical fluid on the cell walls, leading to the displacement of microparticles from the

internal part of the vegetable matrix to its surface. Another observed effect is that the raw material presented a more fragile behavior against the electron beam than the extracted samples. The non-extracted matrix becomes visually degraded under magnifications around 50.000x, which is attributed to the higher amounts of soluble compounds in this material, when compared to the extracted samples.

The increase in extraction yield cannot be explained simply by the abrasive effects or by turbulence created by ultrasonic waves. The experimental observations suggested that intensification of mass transport is due to physical effects on the surface of the particles. The SEM images show evidence of perturbations in the vegetable matrix. The results of mathematical modeling for the samples of larger diameter confirm that the mass transfer coefficient increases when the solid matrix is exposed to such disturbances. According to Balachandran et al. [14], it is possible that these disturbances are caused simply by the rapid changes in density associated with pressure fluctuations induced by ultrasonic waves. However, the authors also consider the possibility of a collapse as a cavitation mechanism. Also according to Balachandran et al. [14], once it is not possible to prove that pressure effects occur, the cavitations near vegetable matrix continue to be the most probable cause of the disruption.

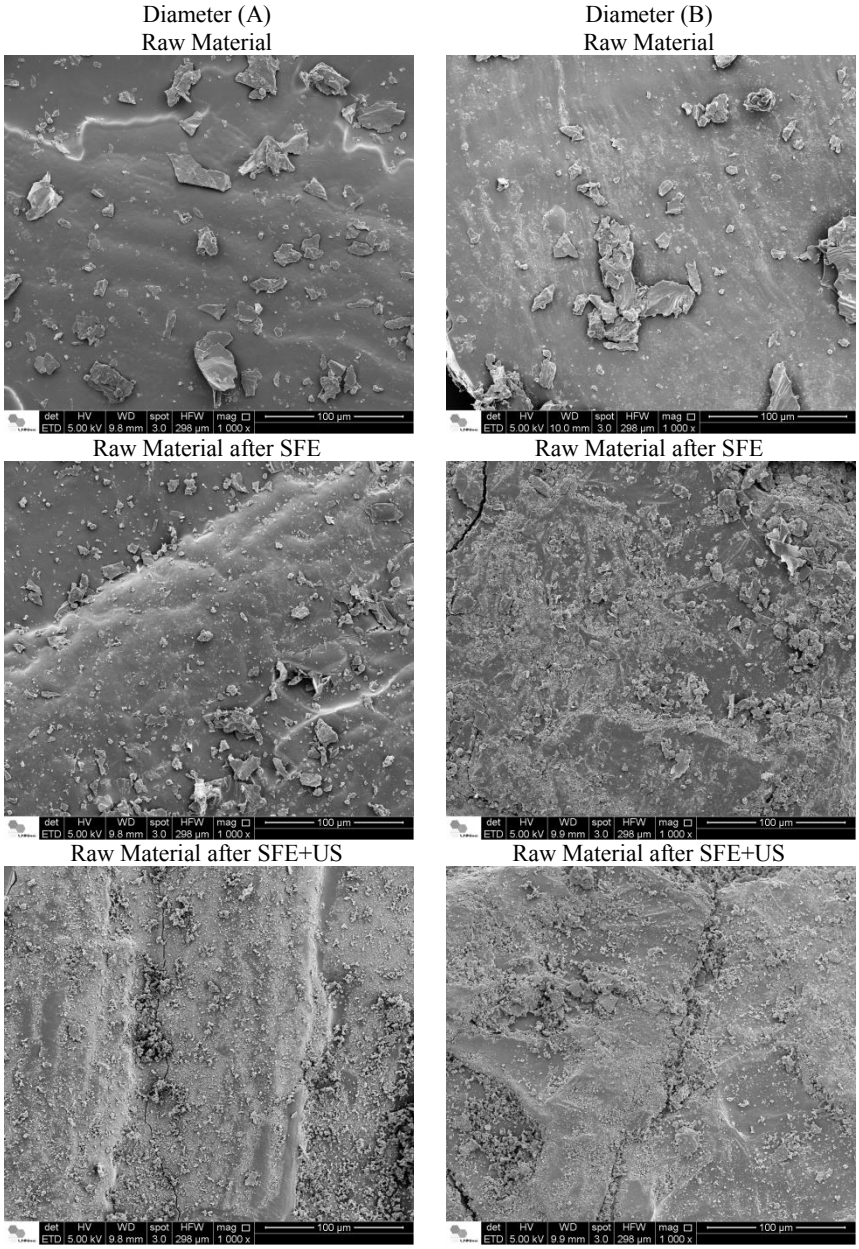

Figure 4. FESEM pictures obtained for the raw material, extracted with supercritical CO_2 and extracted with supercritical CO_2 assisted by ultrasound for two different particle diameters.

4. Conclusions

Supercritical CO_2 extraction assisted by ultrasound increased global yield of malagueta pepper extraction compared to SFE without ultrasound. The highest increase was obtained in global yield of particles of 1.18 to 1.68 mm, while the 0.342 - 0.177 mm particles diameter obtained low yield increases in SFE+US. The model of Sovová [10] proved to be effective in predicting the kinetics of SFE assisted by ultrasound. The FESEM images reveal morphological changes caused by disturbances on the vegetal matrix due to application of the ultrasound, and also sample behaviors related to the increased global yield in oil/resin of malagueta pepper before the extractions.

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