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RESEARCH ARTICLE

Exploring the dynamic dielectric response of yogurt throughout fermentation across frequencies ranging from 10 to 3000 MHz

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Abstract: Our study delves into the intricate dielectric properties of yogurt, navigating its transformation during fermentation at 40°C and post-fermentation modulation across temperatures from 40°C to 10°C. Employing a sophisticated setup comprising a coaxial probe (Speag DAK-12, Schmidt & Partner Engineering AG, Switzerland) and a vector network analyzer (VNA) (Agilent Technologies, E5071C), operating seamlessly within the frequency spectrum of 10-3000 MHz, we meticulously examined yogurt's behavior. Our findings unveil a compelling narrative: as yogurt undergoes fermentation, its pH gradually decreases from 6.5 to 4.3, inducing a remarkable escalation in the loss factor across all frequencies. This intriguing correlation between pH and loss factor underscores the intricate dynamics of yogurt's fermentation process. The rich dataset gleaned from our investigation not only sheds light on the kinetics of yogurt gel formation but also holds promise for optimizing post-fermentation dielectric heat treatments, enhancing yogurt's shelf life and ensuring its quality during storage.

Keywords: Yogurt, VNA, Open ended coaxial probe, Fermentation, dielectric heat treatment

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Introduction

Yogurt, which is produced by the fermentation of pasteurized milk with bacterial cultures consistency of a mixture of Streptococcus subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaris, is one of the common dairy products and is consumed all over the world due to its high nutritional value and organoleptic properties (Tamine & Robinson, 2007; Shah, 2014; Aleman, 2023). Yogurt is also considered as a vehicle to carry the probiotic bacteria, which keeps health promoting properties, such as antimicrobial and antidiarrheal properties, protection against gastrointestinal upsets, improvement in lactose metabolism, lower blood cholesterol (Shah, 2006; Gill, 2023).

Yogurt is one of the perishable food materials, therefore, in order to have a longer shelf life, it is protected from spoilage during its preparation, storage, transportation and distribution. Since acidity of yogurt is one of the main indices for consumers acceptability, so just after producing the yogurt at desired acidity (pH ~ of 4.5), it is to be cooled to refrigerated temperature of less than 10 °C in order to control post acidification (Shah, 2006). Cooling slows down the activities of starter culture and contaminants, if any, such as yeasts and molds. Generally, the shelf life is 8-10 days when stored at temperature less than 10 °C (Entrup, 2005). Maintaining a low temperature while transporting and distributing especially during hot climate in poor countries is very difficult and it results into shelf life less than 8-10 days. Shelf life and quality not only depends upon refrigerated temperature, but also on milk quality, composition, homogenization, heat treatment, starter culture, production equipment, process techniques, packaging, additives, standard of hygiene used, storage conditions and health of dairy personnel (Nelson et al. 2006). In fact, there are three main indicators that limit the shelf life during storage period, which are occurrence of post-acidification; synthesis and oxidation; and growth of contaminants, such as yeasts and molds (Entrup, 2005; Yeboah, 2023). Out of many methods (Ramesh, 2007) for the food safety control and preservation, food items prepared by chemical methods approved by the respective regulatory bodies, are generally not being respected by the consumers due to risk perception of chemicals in food (Spillman et al. 2011). To some extent, this view of consumers appears to be correct in view of some relevant findings (Julhin, 1981; Eigenman & Haenggeli, 2004; McCann et al. 2007; Maier et al. 2010) showing that added chemicals in food items may induce long-term health risk. On the other hand, heat treatment is one of the old, important and physical technique and it is reported that post-fermentation heat treatment (PFHT) in temperature region 60-65 °C has been found (Chandan & O'Rell, 2013) to increase the shelf life of yogurt to 8-12 weeks at 12 °C by destroying the live nature of yogurt, which is due to presence of lactic acid bacteria (LAB). Such destruction of LAB is against the properties of yogurt that are due to its live nature. Also, according to code of FAO/WHO (2013) yogurt must contain 10^6 cfu/g of micro-organisms, but such amount has not been mentioned in the standards of FDA (2015). Heat treatment, however, is allowed by both FAO/WHO and FDA. But such yogurt is to be labelled as either 'heat treated fermented milk' or 'heat treated after culturing'. Recent findings (Siefarth et al. 2014[a]; 2014[b]) made the comparison of conventional heat treatment (CHT) and dielectric heat treatment (DHT) based on radio-frequency showing that yeasts and molds didn't survive on applying these techniques individually even at 58 °C, while LAB were found partially surviving on application of DHT to 58 and 65 °C, whereas these were inactivated by CHT at the same temperatures. This shows that DHT is better than CHT as undesirable micro-organisms in yogurt did not survive, while desirable ones partially survived. These findings motivated the authors to undertake the present work. Now-a-days, food industry is the major user of dielectric heat treatment for various purposes, such as baking, cooking, thawing and tempering, drawing, pasteurization, sterilization and blanching (Ahmed & Ramaswamy, 2004; Awuah et al. 2014). In the present work, co-axial probe Dielectric assessment kit (DAK) connected to vector network analyzer (VNA) has been used, which is based upon reflection method. The primary aim of present work was to acquire the reliable data of dielectric properties of plain set yogurt in support of the development of radio-frequency as well as micro-wave applicators that are expected to be useful to provide post-fermentation dielectric heat treatment (PFDHT) in order to extend its shelf life. The secondary target, which is pre-product of primary aim was to monitor in real time the fermentation process of milk to produce to set yogurt in view of potential benefits of dielectric study (Pethrick & Hayward, 2002) in understanding the microscopic changes occurring in material under investigation in real time. The dielectric study of fermentation process seems to be helpful in understanding the mechanism and kinetic for the formation of yogurt gel, which however is a challenging subject matter despite the availability of number of techniques, such as small amplitude oscillating rheometry, diffusing wave-spectroscopy, ultrasonic spectroscopy, transmission and scanning microscopy and confocal laser scanning microscopy (Mezzenga et al. 2005).

In the present study the Dielectric Heat treatment (DHT) process is used, in which the rise in temperature of the dielectric material by applied electromagnetic field depends upon the complex permittivity ε^* of that material (Metaxas and Meredith, 1983) which is given by

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \qquad (1)$$

where $j = (-1)^{1/2}$ and ε', the real component is called dielectric constant and ε'', the imaginary component is called loss factor. ε' measures the ability of the material to store electromagnetic energy and measure dissipation of electric energy into heat.

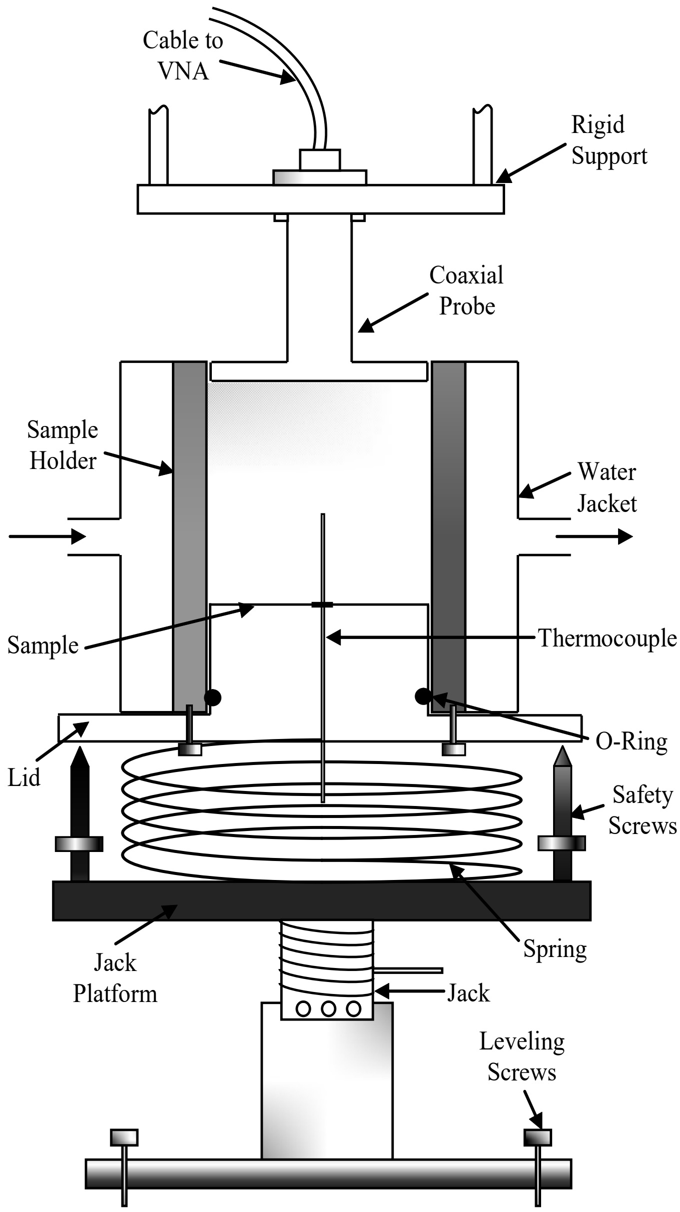

Fig. 1 Experimental setup

Further the penetration depth (d_p) of electromagnetic wave is measured using the values of and . It is defined as the depth where incident wave power density reduced to 1/e (~37%) of its value at the surface of the sample and is determined by the following equation (Von Hippel, 1954)

$$d_p\,(in\ cm) = \frac{c}{2\pi f\sqrt{2\varepsilon'[\sqrt{1+(tan\delta)^2}-1]}} \quad (2)$$

Where is frequency of electromagnetic wave in Hz, is speed of light in free space (3×10^{10} cm/s) and . Measured mean values of and are used to determine. Smaller the values of and higher value of is obtained. During dielectric heating, plays a vital role in selection of the proper thickness of food material for uniform heating (Tang et al. 2002; Wang et al. 2003; Wang et al. 2008; Hu et al. 2024).

The objectives of the present study is to access the (a) pH dependent dielectric properties in the frequency region 10-3000 MHz of acidified milk during fermentation at incubation temperature of 40 °C to produce plain set yogurt at pH ~4.3, (b) temperature dependent dielectric properties so formed yogurt in the temperature region 40-10 °C and frequency region 10-3000 MHz, (c) penetration depth of electromagnetic field so formed yogurt at industrial scientific and medical (ISM) frequencies of 13, 27, 40, 915 and 2450 MHz.

Materials and methods

In this study, we investigate the dielectric properties of Verka Standard Milk, a widely consumed milk variety in Punjab, India. Renowned for its quality and nutritional content, Verka products hold significant prominence in the region. Verka Standard Milk, specifically, is esteemed for its balanced composition, boasting 8.5% solids-not-fat, 4.5% fat, and 3 g/100 ml of protein, rendering it an ideal choice for maintaining health and palatability. Given the increasing demands for advanced analytical insights into dairy products, particularly regarding their dielectric behavior, our research aims to address this need. Verka Standard Milk samples were procured from local markets in Sangrur, Punjab, and subjected to dielectric property analysis within the frequency range of 10-3000 MHz across a spectrum of temperatures ranging from 10 °C to 40 °C. By systematically exploring these parameters, we seek to elucidate the intricate interplay between dielectric properties and the composition and temperature variations of Verka Standard Milk, offering valuable insights into its quality and suitability for various applications.

Dielectric properties measurement apparatus consists of an open-ended coaxial probe (Speag DAK-12, Schmidt & Partner Engineering AG, Switzerland), which works in the broad frequency region of 10-3000 MHz and can be used for liquid, solid as well as semi-solid materials; VNA (Agilent Technologies, E5071C) Agilent. (2005) and a computer. A phase and amplitude stable cable is used to connect the probe to VNA. The calibrated face of the probe is placed in contact with the flat surface of compressed ground sample of rapeseed and the complex reflection coefficient () of electromagnetic field are recorded by VNA. The computer that controls the VNA precisely converts the measured into and with the help of software (DAK, 2014) based on the algorithm developed by Ellison and Moreau (2008).

The arrangement of probe, sample holder, spring and jack is shown in Figure 1. Cylindrical sample holder (50 mm inner diameter with 100 mm height) made of steel is used to hold the yogurt sample. The sample holder containing sample is put under the hydraulic press and pressed in such a way that at top side of sample holder, a depression of ~4 mm is created by inserting a removable brass disc of thickness ~4 mm with diameter just less than 50 mm so as probe can be mounted in this depression. The face of this disc in contact with sample was kept as smooth and plane as possible in order to create minimum aberrations in the surface of sample. Heat is provided to the sample by circulating the hot water at required temperature through jacket fitted with sample holder and temperature of sample is recorded with thermocouple (DTM 3000-Spezial, LKM, Electronics GmbH) that passes through the center of the lid. Sample holder is placed on platform of spring and jack system. To avoid the slipping, ends of spring are fixed in grooves made in lid and jack platform. Jack provides the necessary compressive force to the spring while compressed spring helps in minimizing the air gap between plane of probe, which is rigidly fixed in horizontal direction and face of sample the plane of which can tilt upto certain small angle. In other words, horizontal as well as vertical components of force of compressed spring keep the planes of probe and sample in contact with each other as well as parallel to each other. To avoid the tilting of sample holder beyond a small angle due to any accidental impact, jack platform is provided with three rigidly fixed safety screws (two shown in Figure 1), which can be adjusted in length so that a small gap always remains between the tips of screws and lid of sample holder during measurements so as whole weight of sample holder rests on the spring.

The milk sample was taken in the sample holder. It was heated to 40 °C by circulating the hot water and a starter culture of lactic acid bacteria 0.035 g (1.52×10^{10} cfu/g) was added into it. The initial pH of sample was determined by a standard portable pH meter and calibrated pH probe and was found to be 6.5. After that, the sample was left undisturbed and the constant temperature of (40±1) °C was maintained for next 5 h. The dielectric properties were measured from 10 to 3000 MHz at an interval of 0.5 h and each time the pH was also noted till pH of ~4.3 was obtained. The sample was then cooled from temperature 40 °C to 10 °C by circulating cold water and dielectric properties were again measured at an interval of 10 °C.

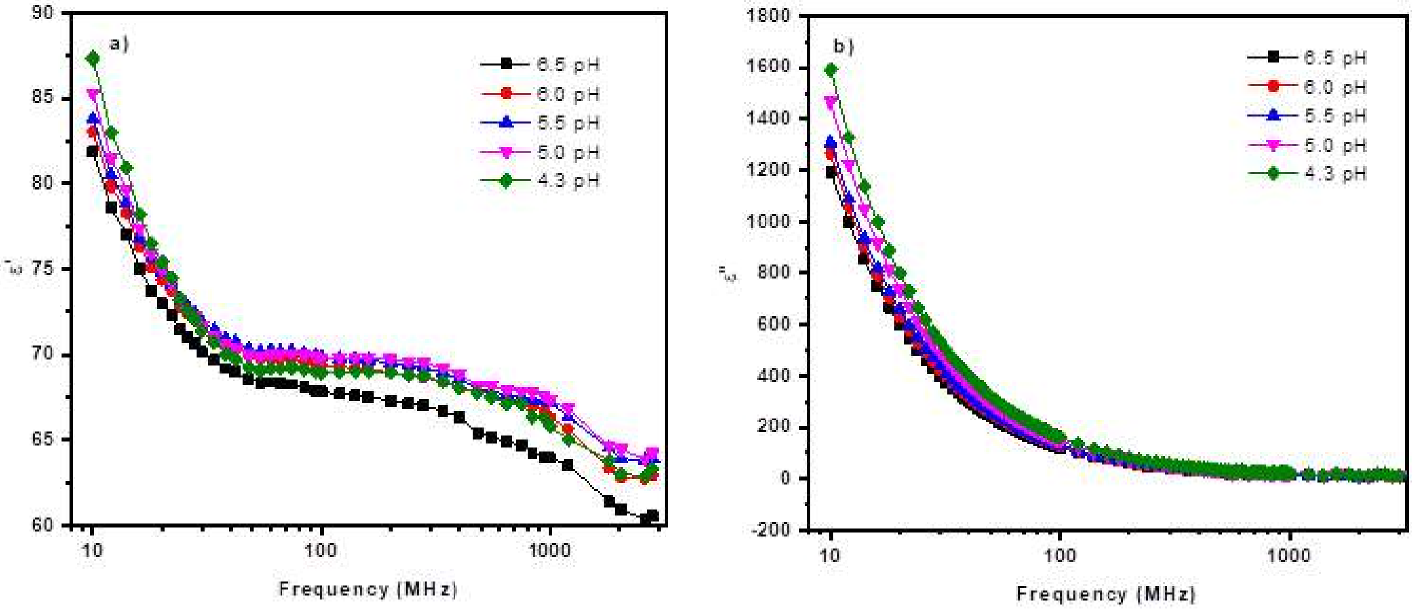

Fig. 2 Variation of a) dielectric constant, and b) dielectric loss factor with frequency at different pH of 4.3, 5, 5.5, 6, and 6.5

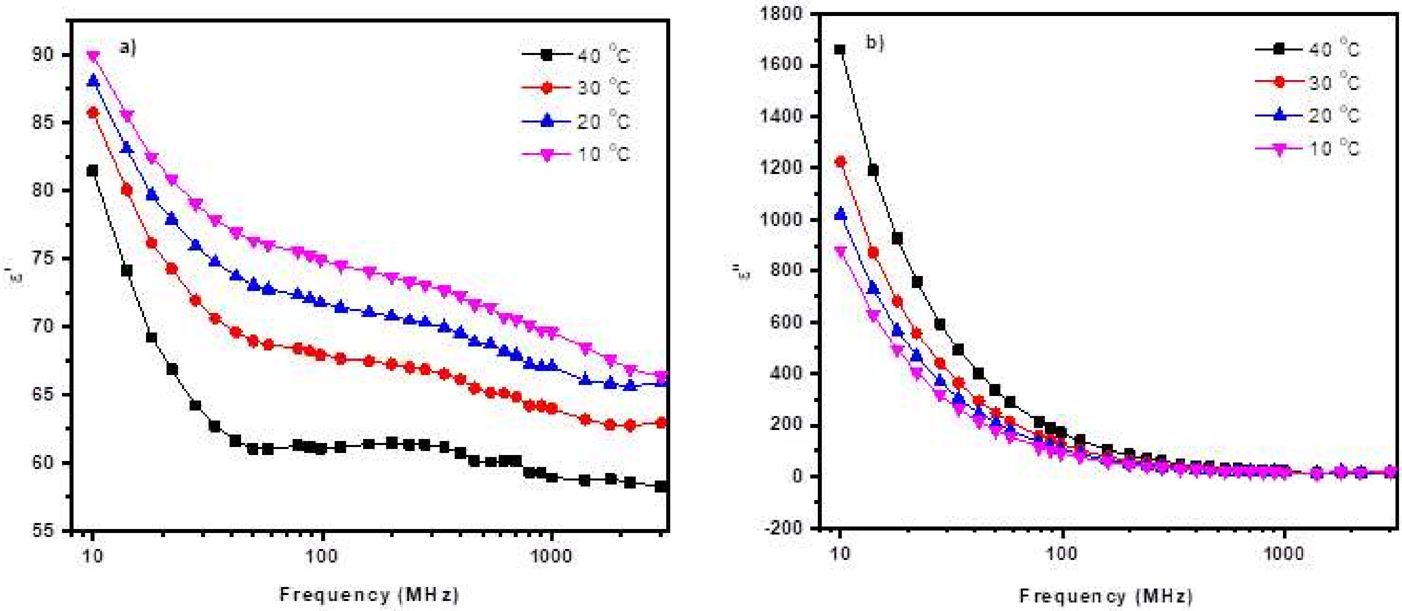

Fig. 3 Variation of a) dielectric constant, and b) dielectric loss factor with frequency at different temperatures of 10 °C, 20 °C, 30 °C, and 40 °C

Results and Discussion

Variation of dielectric constant and loss factor with pH

The obtained values for the dielectric constant (ε×) and loss factor of the milk sample during fermentation at different pH by varying frequencies from 10 MHz to 3 GHz has been shown in Fig. 2a and 2b, respectively. Figures show that both dielectric constant and loss factor decrease with increase in frequency. With increasing pH content, dielectric constant increased with a maximum of around 87 at 4.3 pH and 10 MHz. At each frequency, behavior of increasing dielectric constant was observed with rise in pH, except for pH values of 4.3 and 5. The values of dielectric constant have been found to be decreased at pH 4.3 and 5 as compared to other pH values for the frequency 20 MHz onwards.

Table 1: Calculated penetration depths (in cm) of incident electromagnetic field onto yogurt at five ISM frequencies and at temperatures 10 °C, 20 °C, 30 °C, and 40 °C

Temperature (°C)	Penetration Depth (cm) Frequency (MHz)				
	13	27	40	915	2450
40	7.439	5.297	4.437	1.707	1.028
30	8.793	6.330	5.365	2.070	0.966
20	9.733	7.060	6.015	2.237	0.917
10	10.584	7.744	6.648	2.317	0.851

Dielectric loss factor observed to be increased with increasing pH content as shown in Fig. 2b for the frequency below 600 MHz. There is no influence of pH at higher frequencies of 600-3000 MHz as the loss factor has almost the same value at each pH as well as at each higher frequency. It shows the negative correlation of pH with loss factor during fermentation. With decreasing pH, concentrations of lactic acid increases, but in addition to that, calcium phosphate from the casein micelles would start to solubilize. The acidification of milk causes major physiochemical modifications to both casein micelle and serum. The slow acidification causes a greater rearrangement of casein micelles leading to the formation of a homogenous gel in the entire milk volume. These variations can be explained on the basis of milk composition, which is a mixture of colloidal dispersion and solution consisting of different components like fat, protein, lactose, minerals, vitamins, and water. The milk can be fermented only when lactic acid bacteria convert lactose into lactic acid making milk more acidic through the consumption of orotic and hippuric acids present in yogurt starter culture (Valenzuela et al. 2024). During this process, other contents fat and protein are also disintegrated into several acids declining the pH value of milk (Guo et al. 2018). So, the overall pH value decreased with time increasing the conductivity of yogurt.

Variation of dielectric constant and loss factor with temperature

The yogurt prepared from fermentation of milk is needed to be stored at low temperatures for its safety, shelf life in food industry. In the present work, milk has been fermented at a constant temperature of 40 °C. Therefore, to study the behavior of as prepared yogurt, dielectric properties have been investigated during its cooling at temperatures of 40 °C to 10 °C. The variations of dielectric constant and loss factor have been presented in Fig. 3a and 3b. The values for both dielectric constant and loss factor were observed to be decreasing with increasing frequencies at each temperature. But loss factor has no influence of temperature above 600 MHz due to alike values (Fig. 3b). During cooling, dielectric constant was found to be increased with decreasing temperature at each frequency with a maximum of ~90 at 10 MHz and 10 °C (Fig. 3a). Whereas, loss factor decreases with fall in temperatures having minimum of ~20 after 800 MHz. The maximum value of loss factor was ~1700 at 10 MHz and fermented temperature of 40 °C. The Guo et al. (2018) determine the coefficients of dielectric loss factor with pH and titratable acidity decreased with increasing frequency and present study also show similar trends.

Penetration depth of electromagnetic field in prepared yogurt at ISM frequencies

Penetration depths of incident electromagnetic field in yogurt calculated using Eq. 2 at ISM frequencies of 13, 27, 40, 915 and 2450 MHz in RF and MW region at four temperatures given in Table 1. The values of penetration depth, decreases with rise in frequency and temperature. The results suggest that for dielectric heating of yogurt, thick layered samples can be used in RF system due to larger penetration depth, while thin layered samples are required for MW treatments to overcome the lack of penetration and achieve uniform heating. The results of Dobozi, R. (2023) strengthens our findings on the penetration depth of electromagnetic field.

Conclusions

pH dependent dielectric properties of acidified milk during fermentation at incubation temperature of 40 °C to produce plain set yogurt have been measured and presented by varying frequency from 10 to 3000 MHz. The temperature dependent dielectric properties of so formed yogurt have also been measured with decreasing temperature for storage of yogurt. The results showed that as pH changed from 6.5 to 4.3 during formation of yogurt, the loss factor increased at all frequency showing negative correlation between pH and loss factor during fermentation process. The changes in dielectric properties with reducing pH appear to be useful in monitoring of fermentation process. The measured dielectric properties of formed yogurt by decreasing temperature can be used to design RF/MW applicators for providing the post-fermentation treatment to set yogurt to prolong its shelf life.

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