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VALUE ADDITION OF CURD BY BEETROOT: INFLUENCE ON ASCORBIC ACID, MALONDIALDEHYDE AND LACTIC ACID CONTENTS

Shilpa, J[1]., Athira, A[2].

1. Assistant Professor, Department of Biotechnology Engineering

Sri Shakthi Institute of engineering and Technology, Coimbatore, Tamil Nadu, India.

PIN: 641062. Tel:+91- 9048352365; Email: shilpajoy09@gmail.com

2. School of Health Science,

Kannur University, Thalassery Campus, Kerala, India. PIN: 670661

1*. **Address for correspondence**

Assistant Professor, Department of Biotechnology Engineering

Sri Shakthi Institute of engineering and Technology

Coimbatore, Tamil Nadu, India.

PIN: 641062

Tel:+91- 9048352365

Email: shilpajoy09@gmail.com

ABSTRACT

Curd is the most important fermentative product with high nutritional value. Value addition to the normal curd increases customer attraction, which will have commercial importance. The present study focusses on increasing the nutritional value of curd by adding beetroot juice before fermentation. The change in ascorbic acid, protein and malondialdehyde contents, lactic acid bacteria count, pH and titrable acidity of beetroot flavored curd were evaluated. The result obtained showed that both ascorbic acid and protein contents were increased significantly ($P < 0.001$) in 24h fermentation. Initially the malondialdehyde content was significantly ($P < 0.05$) increased in 24 h fermentation than normal curd. Later malondialdehyde content was significantly ($P < 0.001$) decreased in beetroot flavored curd of 48 h fermentation than the normal curd. So the antioxidant property in the prolonged fermentation of curd gradually increased with the addition of beetroot. The lactic acid bacterial counts in the beetroot flavored curd after fermentation of 24 h were significantly ($P < 0.001$) increased as compared to normal curd. The lactic acid content gradually increased as fermentation proceeds to 48 h in beetroot flavored curd. Thus, pH was decreased gradually in the fermentation product. It was concluded that nutritionally important parameters like protein and ascorbic acid were increased and free radical inducer malondialdehyde was decreased in beetroot flavored curd. Therefore, the beetroot flavored curd will be more nutritious, safe and an acceptable product.

KEY WORDS

Curd; Ascorbic acid; Malondialdehyde; Lactic acid bacteria; beetroot.

I. INTRODUCTION

Curd is a widely accepted and popular fermentation product all over the world. It is produced by a simple procedure called curdling of milk even though the nutritional impact is high. It contains various vitamins, minerals, fatty acids, carbohydrates and nitrogen components [1]. Curd is a rich in wide varieties of bacteria mainly *Lactobacilli* species like *Lactobacillus acidophilus, Lactococcus lactis, Lactococcus lactis cremoris* etc. *Lactobacillus* can convert a sugar into an acid by means of fermentation. These convert lactose into lactic acid, which induce the sour taste to curd [2]. The number of bacteria can be increased by natural fermentation. Prolonged fermentation produces excess amount of acetic or lactic acid that cause the sour taste of product [3]. This study aims to create a high nutritionally valued new curd product. Additives are incorporated with curd that

enhances the content of fibers, starch, minerals and vitamins in curd. Mainly used additives are vegetables, like French bean, beetroot, soybean and sweet potato [4]. Hence, it can be hypothesized that the incorporation of beetroot juice into curd can enhance nutritional value of the curd in addition to the health benefits. Therefore, the aim of the current study was to incorporate beetroot juice into the curd for increasing the nutritional quality, color and flavor of the curd to maximize its commercial importance and to attract the end consumer. The evaluation of biologically important compounds in beetroot flavored curd were done to highlight the relevance of the present study.

The addition of vegetable components into milk prior to curdling enhances the digestibility [5]. Enzymatic activity seen during microbial growth predigest the macronutrients of food in fermentation [6]. Vitamin C is an essential vitamin, supplied through diet. Vitamin C is an antioxidant, along with vitamin E, beta-carotene, and many other plant-based nutrients [7]. Antioxidants block some of the damages caused by free radicals, substances that damage DNA [8]. Acid-fermented vegetables are important sources of vitamins and minerals [9]. Food fermentations increases the protein content or improve the balance of essential amino acids or their availability will have a direct curative effect [10]. The growth of Lactic acid bacteria enhances the pH. This will change the environment of the casein micelles. Thereby, the bacteria can degrade the casein micelles, help the digestion and absorption of casein [11]. The nutritional value of milk protein is preserved during fermentation and curd is a good product for humans with regard to intestinal availability of nitrogen (93%) [12]. Curd is an antioxidant and its various enzymes keep the body cool [13]. Malondialdehyde is a natural product produced by lipid peroxidation. Malondialdehyde causes cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues. Shamberger *et al.* [14] reported that many vegetables and fruits possess a minute amount of MDA. This will reduce the nutritional quality of a product. The present study also aims to decrease the MDA content in fermented product with the help of beetroot juice. Lactic acid bacterial growth will be influenced by the addition of certain additives that contain sugars [15].

This study was done to assess the effect of beetroot juice in regulating ascorbic acid, protein and malondialdehyde contents with the emphasis on enhancing the nutritional quality of curd.

II MATERIALS AND METHODS

Preparation of Curd samples

Preparation of beetroot juice: 250 g of beetroot was cleaned with water to remove sand, adhering dirt and extraneous material. Beetroot was cut into slices, and subjected to extraction using juice extractor. The beetroot pulp was sieved using a sterile cloth to obtain a clear filtered juice. Juice was stored in an air-tight container at -20°c until used.

10 ml of normal curd was taken as control (C+M). Another curd was prepared by mixing 8ml sterile milk, 1ml curd and 1ml beetroot juice (C+M+B).

Ascorbic acid assay

Ascorbic acid content was assayed as described by Omaye et al. [16]. After fermentation, 1 ml of curd samples were centrifuged with 0.5 ml of 10 % TCA solution at 1500 rpm for 15 minutes. To 0.5 ml of the supernatant, 0.1 ml of DTC reagent (2, 4-Dinitrophenyl hydrazine- Thiourea-$CuSO_4$ reagent) was added and mixed thoroughly. The tubes were incubated at 60°C for 1 hour and to this a solution of 0.75 ml of ice cold 65% H_2SO_4 was added. The tubes were then allowed to stand for 30 minutes at room temperature. The absorbance was read at 520 nm in a spectrophotometer. The ascorbic acid level in the curd sample was found from the standard graph with concentration of standard L-ascorbic acid on X axis and absorbance on Y axis. The ascorbic acid content was expressed as mg/10 ml curd.

Determination of protein content

Total protein content in beetroot flavoured curd was determined according to the method of Lowry, et al [17]. BSA was used as the standard. The absorbance was measured at 660nm. Protein concentration in the two curd samples were calculated using the standard graph and expressed as mg protein/10 ml curd.

Estimation of malondialdehyde content

The assay was performed according to procedure of Yagi [18]. Experiment was performed in 3 consecutive time periods (0hr, 24hr and 48hr fermentation). 1 ml of curd sample was incubated at 37°C in a water bath for 2 h. To this, 1ml of 10% TCA was added and mixed thoroughly. Then it was centrifuged at 3000 rpm for

10 minutes. After centrifugation, supernatant was taken and mixed with 2 ml 0.07% TBA. It was kept in boiling water bath for 10 minutes. The tubes were cooled and diluted with 1ml deionized water. The absorbance was measured at 535 nm. The amount of thiobarbituric acid reactive substances (TBARS) was calculated using molar extinction coefficient of malondialdehyde ($1.56\times10^5\ M^{-1}cm^{-1}$).

$$C = \frac{\text{A}}{\text{Ɛ} \times \text{l} \times \text{Dilution factor}}$$

A = Optical density

Ɛ = Molar Extinction coefficient ($1.56\times10^5 M^{-1}cm^{-1}$)

l = Path length of light

Total viable count of Lactic acid bacteria in beetroot flavored curd

One ml of the curd samples was taken and serial dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) were performed in four tubes containing 9 ml deionized water. 100µl sample from 10^{-2} and 10^{-4} dilutions were used for spread plating on MRS agar. After plating, the petridishes were incubated at 37°C for 24 h with 5% CO_2. After incubation, the number of colonies was counted and represented as colony forming unit per 10 ml [19].

$$\text{Total viable count}\left(\frac{\text{Cfu}}{\text{10ml curd}}\right) = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{volume } of curd}$$

Determination of Lactic acid content

pH of the two curd samples were measured. Titrable acidity was determined [20] by titrating the supernatants of curd samples with 0.1 N alkali (NaOH) using 1% phenolphthalein as indicator (3 drops). Titration was continued until the color changed to pink and the titration was repeated to get average result.

% lactic acid is calculated as:

$$\%\ \text{Lactic acid} = \frac{\text{ml of alkali x Normality of alkali x 9}}{\text{ml of solution used}}$$

Statistical analysis

Statistical evaluations used analysis of variance (ANOVA) in GraphPad Instat (version 2.04a; Graph Pad, San Diego, CA). Student Newman-Keuls test was used to compare different groups after ANOVA.

III RESULTS AND DISCUSSION

This study aims to create a high nutritionally valued new curd product. The goodness of curd is universally accepted. Curd was reported to possess several nutritional/dietary advantages over milk [21]. Curd as such is easy to digest. It is used for treating various illness like allergy, urogenital infection, HIV, cancer, *Helicobacter pylori* infection, liver disease, inflammatory bowel disease, irritable bowel syndrome, pancreatitis, diabetes, insomnia and hypercholesterolemia [22]. When it was combined with sugar, pepper, vegetables and/or fruits makes it delicious [23]. Additives enhances the nutritional quality of curd with dietary fibers, starch, minerals and vitamins [24]. The addition of beetroot juice increased the nutritional value, antioxidant property, taste and attraction. The benefits of beetroot flavored curd were evaluated by physical and chemical examinations. Beetroot juice was added with the intention of enhancing the color, antioxidant property and other dietary benefits to the curd. There was a noticeable change in the flavor and taste of the new product. It was due to the masking ability of peculiar flavor of the natural curd by the addition of beetroot juice. The acids formed in curd are involved in the formation of esters, which impart desirable flavors [25].

On first day, the color and taste of normal curd (C+M) was light cream and slightly sour respectively. The color and taste of beetroot flavored curd (C+M+B) was light pink and slightly sour with beetroot taste. On the second day, the color, taste and appearance of C+M was cream color, sour taste and air bubbles with thick appearance respectively. The color, taste and appearance of C+M+B was Light pink, sour with beetroot taste and air bubbles with thick appearance respectively (Fig. 1).

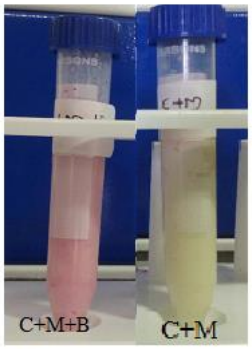

Fig.1

Fig.1. Color, Taste and Appearance of curd after incubation.

C+M+B = 1ml Curd + 8ml Milk + 1ml Beetroot juice, C+M = 1ml Curd + 9ml Milk.

Fermentation reduces the content of non-digestible materials like cellulose, hemicellulose, polygalacturonic and glucuronic acids in vegetables and fruits. Breakdown of these compounds lead to improved bioavailability of mineral and trace elements. Therefore, after fermentation the digestibility and nutritional quality of beetroot flavored curd will be increased [26]. Enzymatic activity seen during microbial growth predigest the macronutrients of food in fermentation [27]. Fermentation increases the content or availability of vitamins such as ascorbic acid, thiamine, riboflavin, niacin or folic acid and can have profound direct effects on the health of the consumers of such food. The ascorbic acid content in C+M and C+M+B on the 1st day showed no significant change. There was a significant ($P < 0.001$) increase in the ascorbic acid content of C+M on 2ndday when compared with C+M of 1st day. The ascorbic acid content in C+M+B on 2nd day was significantly ($P < 0.001$) increased when compared with C+M+B on 1st day. There was a significant ($P < 0.001$) increase in the ascorbic acid content in C+M+B on 2nd day when compared with C+M on 2nd day (Fig. 2).

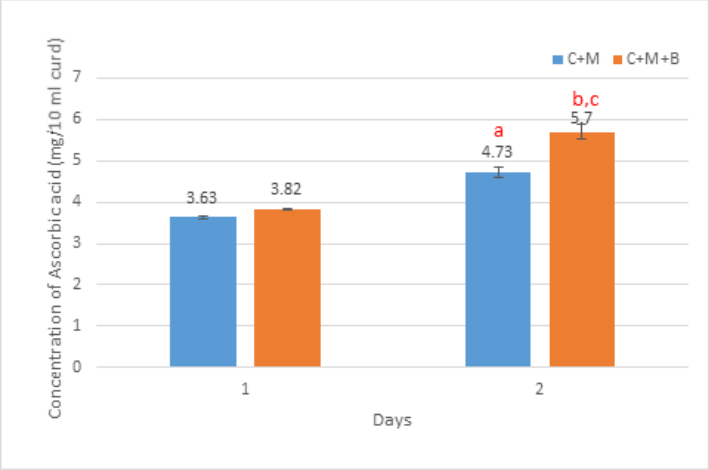

Fig. 2

Fig.2. Ascorbic acid estimation in curd samples.

$^{a}P < 0.001$ when compared with C+M of 1stday. $^{b}P < 0.001$ when compared with C+M+B of 1stday. $^{c}P < 0.001$ when compared with C+M of 2nd day. Values are mean± SEM of four separate experiments.

Most of the vitamins are produced by several species of bacteria, yeasts, fungi and algae may serve to produce essential vitamins [28]. Vitamin C is an essential vitamin, mainly supplied through diet. Vitamin C is an antioxidant [7]. Antioxidants block some of the damages caused by free radicals, substances that damage DNA [8]. The formation of free radicals contributes to the aging process and the development of health conditions such as cancer, heart disease, and arthritis [29]. An important vitamin C deficiency disorder is Scurvy. Acid-fermented vegetables are important sources of vitamins and minerals [9]. The carbon dioxide produced in fermentation, replaces air and provides anaerobic conditions that favor stabilization of ascorbic acid and the natural colors of the vegetables. Vitamin C content will be increased in anaerobic fermentations [30]. Our results also showed an increase in ascorbic acid content due to the presence of beetroot juice in the fermented product. So, this modified curd helps to reduce the dietary deficiency of vitamin C.

In curd, lactic acid fermentation helps to enhance protein solubility and the availability of limiting amino acids [31]. Curd is a very nourishing food and serve as valuable source of protein, essential vitamins, minerals and riboflavin [32]. Food fermentations that increase the protein content or improve the balance of essential amino acids or their availability will have a direct curative effect. The growth of Lactic acid bacteria reduces the pH. This will change the environment of the casein micelles. Thereby, bacteria degrade the casein micelles, help the digestion and absorption of casein [33]. The nutritional value of milk protein is preserved during fermentation

and curd is a good product with high nitrogen content [12]. The protein content in C+M and C+M+B on the 1st day showed no significant change. There was a significant ($P < 0.001$) increase in the protein content of C+M on 2ndday when compared with C+M of 1st day. The protein content in C+M+B on 2nd day was significantly ($P < 0.001$) increased when compared with C+M+B on 1st day. There was a significant ($P < 0.001$) increase in the protein content in C+M+B on 2nd day when compared with C+M on 2nd day (Fig. 3).

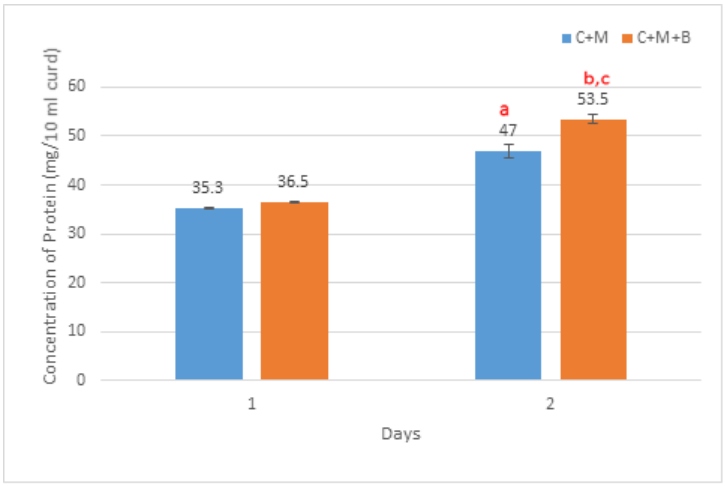

Fig.3

Fig.3. Protein content estimation in curd samples.

$^{a}P < 0.001$ compared with C+M of 1stday. $^{b}P < 0.001$ when compared with C+M+B of 1stday.
$^{c}P < 0.001$ when compared with C+M of 2nd day. Values are mean± SEM of four separate experiments.

The beetroot flavored curd contained high protein content than normal curd. High protein content foods are meat and fish. So, this product helps to reduce the protein deficiency in humans, mainly vegetarians.

Malondialdehyde is a naturally occurring product of lipid peroxidation that enhances the free radicle formation. Naturally many vegetables contain MDA in minute amount [14]. Fermentation reduces these MDA content [34]. So, the addition of beetroot juice on the first day increased MDA when compared to the one with no beetroot juice. There was a significant ($P < 0.001$) increase in the MDA content of C+M+B when compared with C+M on 1st day. On the 2nd day, MDA content in C+M+B was significantly ($P < 0.05$) increased when compared with C+M. There was a significant ($P < 0.01$) decrease in the MDA content of C+M+B when compared with C+M on 3rd day. There was no significant change in MDA content among the C+M of all the three days.

There was a significant ($P < 0.001$) decrease in the MDA content of C+M+B on 2nd day when compared with C+M+B on 1st day. Also there was a significant ($P < 0.001$) decrease in MDA content of C+M+B on 3rd day when compared with C+M+B on both1st day and 2nd day (Fig. 4).

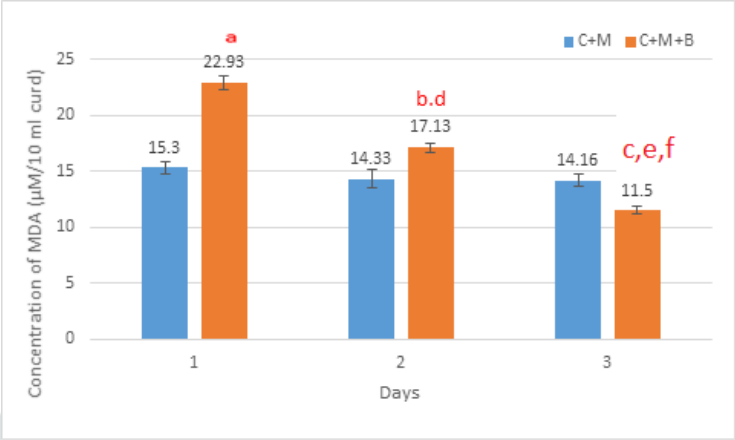

Fig.4

Fig.4. MDA content estimation in curd samples.

$^{a}P < 0.001$ compared with C+M of 1st day. $^{b}P < 0.05$ compared with C+M of 2nd day. $^{c}P < 0.01$ compared with C+M of 3rd day. $^{d}P < 0.001$ compared with C+M+B of 1st day. $^{e}P < 0.001$ compared with C+M+B of 1st day. $^{f}P < 0.01$ compared with C+M+B of 2nd day Values are mean± SEM of four separate experiments.

During the period of fermentation, the amount of saturated fatty acids was significantly lowered in fermentation product. The complex fatty acids are degraded into free fatty acids in fermentation. The increase in free fatty acids content is probably the result of endogenous enzymes activity as well as the activity of enzymes of microorganisms [35]. Intense oxidative changes result in low level fatty acids content [36]. Malondialdehyde is a degradation product formed during lipid oxidation of polyunsaturated fatty acids [37]. Reduction of malondialdehyde values during prolonged fermentation was due to the interaction of malondialdehyde with compounds such as sugars, nitrites, amino acids [38]. Thus, these previous studies supported our findings and highlighted the importance of beetroot flavored curd over normal curd.

Curd contains large amount of Lactic acid bacteria. They have several beneficial physiological effects like antimicrobial property, enhancing the immune potency [39], preventing cancer and lower serum cholesterol levels [40]. The beetroot juice in the curd enhanced the lactic acid bacteria colony count when compared to normal curd. The bacteria utilize beetroot juice for their growth and multiplication. Beetroot juice containing sugars are used

for the growth of Lactic acid bacteria in curd [15]. A difference in bacterial colony morphology was observed in C+M+B. In C+M+B, along with the colonies that were similar to C+M, large colonies were also seen. There was a significant ($P < 0.001$) increase in the Lactic acid bacteria count of C+M+B on the 2nd day when compared with C+M on same day (Table. 1, Fig. 5).

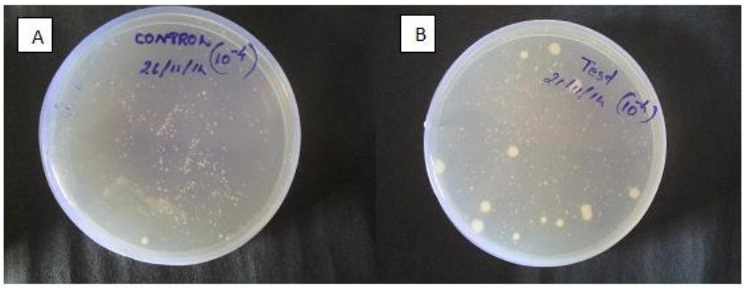

Fig.5

Fig.5. LAB Colony count in MRS media after fermentation.

A – C+M, B – C+M+B. [a]*P* <0.001 when compared to C+M after fermentation. Values are mean ± SEM of four separate experiments.

Table 1, LAB Colony count of curd samples in MRS plates after fermentation.

Control (cfu/10 ml curd)	Test (cfu/10 ml curd)
244000±2081	378000±12767[a]

[a]*P* < 0.001 when compared to C+M after fermentation. Values are mean ± SEM of four separate experiments.

A new strain of lactobacillus species was flourished in the presence of beetroot juice and therefore large and thick colonies were observed for C+M+B when compared to C+M. The initial pH of the C+M and C+M+B was 5.7 and 5.3 respectively. After 2nd day of fermentation, the pH of C+M and C+M+B were reduced to 4.7 and 4.3 respectively. The 3rd day fermentation pH of C+M and C+M+B were again reduced into 4.4 and 4.1 respectively (Table. 2). Maintaining stable pH helps to develop satisfactory flavor and consistency of fermented foods [41]. In our study, the pH of the normal and beetroot flavored curd were reduced after fermentation. The pH of beetroot flavored curd was slightly reduced than the normal. The lowering of pH was most probably due to accumulation of organic acids, mainly lactic acid [42]. Both the milk and beetroot contain large amount of lactose.

The lactase enzyme produced by the lactic acid bacteria convert these simple carbohydrates such as glucose, lactose, sucrose, or galactose to lactic acid [43]. The initial lactic acid content of the C+M and C+M+B was 0.113% and 0.977% respectively. After the 2nd day, the lactic acid content of C+M and C+M+B were increased to 0.803% and 0.846% respectively. At 3rd day of fermentation, lactic acid content of C+M and C+M+B were again increased into 0.977% and 1.026% respectively (Table. 3). Lactic acid content in the beetroot flavored curd was increased gradually compared to normal curd as the fermentation proceeds. Therefore, the pH of product was decreased during fermentation in curd. The rich source of sugar provided by the beetroot juice served as a suitable substrate for the growth of microbes. Thus, beetroot flavored curd tends to be a potent milk product that can enhance the health status.

IV CONCLUSION

Addition of beetroot juice as flavoring agent improved the physical and chemical properties of curd. Beetroot juice in curd improved the acceptability of new product. Ascorbic acid and protein content of the formulated product increased when compared to normal curd. Results suggested that the incorporation of beetroot extract could increase the antioxidant property of curd. The malondialdehyde content was decreased in beetroot flavored curd than normal one, so the prolonged fermentation decreases the lipid peroxidation in beetroot flavored curd than the normal. The lactic acid bacteria count in curd increased with the addition of beetroot juice. Sugar of beetroot juice is utilized by the lactic acid bacteria for energy production. Thus, the count of viable organism is increased in the beetroot flavored curd than the normal curd. Also there was an increase in lactic acid content in beetroot flavored curd. So the pH of new product decreased than normal curd. Based on the results it can be concluded that the inclusion of beetroot juice may improve the nutritional value of curd and increase the consumer preference of new product. This has immense commercial importance.

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