

Inhibitory Effect of Ginger and Turmeric on *Rhizopus stolonifer* Growth on Bread

Ali Muhammad*, Ziaur Rahman, Ayub M, Durrani Y, Ali SA, Abroo Tabassum, Ashbala Shakoore, Majid Khan and Arsalan Khan

Department of Food Science and Technology, The University of Agriculture, Peshawar-Pakistan

*Corresponding author: Ali Muhammad, Department of Food Science and Technology, The University of Agriculture, Peshawar-Pakistan, Tel: 92 91 9216558; E-mail: alizypher@yahoo.com

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Abstract

The inhibitory effect of ginger and turmeric was studied on the *Rhizopus stolonifer* growth on bread. Different ratios of ginger and turmeric were incorporated to bread dough formulation. The treatments were BG₀ (control), BG₁ (3% ginger), BG₂ (4% ginger), BG₃ (3% turmeric), BG₄ (4% turmeric), BG₅ (1.5% ginger + 1.5% turmeric) and BG₆ (2% ginger + 2% turmeric). All the samples were analyzed physicochemically (moisture, ash, pH and water activity), microbiologically (colony count of *Rhizopus stolonifer*) and organoleptically (color, texture, flavor and overall acceptability). The results showed that overall moisture decreased (from 27.46 to 26.41), water activity a_w (from 0.89 to 0.86), color (from 7.4 to 5.58), texture (from 6.94 to 5.34), flavor (from 7.75 to 5.54) and overall acceptability (from 7.38 to 5.48), while percent ash increased (from 0.84 to 0.86), pH (from 5.95 to 6.20) and microbial count of *Rhizopus stolonifer* (3.8×10^1 to 2×10^2). The highest mean value for ash was recorded for treatment BG₄ (0.98), water activity in BG₀ (0.91), moisture in BG₀ (29.69), pH in BG₄ (6.34) Microbial count in BG₀ (1.5×10^2 cfu/g), color in BG₃ (6.98), texture in BG₀ (7.04), flavor in BG₀ (6.90) and overall acceptability in BG₀ and BG₃ (6.96). The overall results showed that BG₃ gave the best inhibitory results against *Rhizopus stolonifer* as well as in physicochemical and sensory evaluation, while BG₆ also presented better inhibitory results in microbial analysis but it was not satisfactory in terms of sensory characteristics.

Keywords: Ginger; Turmeric; *Rhizopus stolonifer*; Bread

Introduction

In terms of both production and utilization, bread is given a special importance in baking industry as compared to all other bakery items. Due to the supportive nature of bread ingredients, bread serves as a media for production, reproduction and multiplication of microorganisms. The common microorganisms responsible for the spoilage of bread are *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* [1]. Among these three, the most destructive and common species in bread is *Rhizopus stolonifer*, generally known as "black bread mold". Most often, the growth of mold initiates with the moisture abundant part of a bread slice, particularly the crease [2].

Bread is basic portion of our routine intake; therefore, studies should be carried out to discover new techniques for extending its storage life. The basic means to increase the shelf life of bread includes sterile environment of baking area, disinfecting mixing premises and guaranteeing sterilized conditions. Numerous chemical antimicrobial agents have been implemented for the improving the shelf life of bread but they are measured liable for toxic and carcinogenic effect in foods. Therefore, consumers hesitate to use foods added with chemical preservatives or improvers and the demand for alternative natural preservatives has increased significantly [3]. Recently, many researchers have attempted the utilization of oils and extracts of many herbs and spices for improving shelf life of foods and to use it as alternative for food preservatives [4-6].

In this era, consumers tend to use safer foods in order to save themselves from being victims of numerous non-communicable illnesses. Therefore, industrialists and food researchers are trying to

improve the quality, standard, safety and variety of food products including bread [7]. For this purpose, they are including natural spices and herbs as optional ingredients in their product formulations. These ingredients are also vital portion of daily diet. For centuries, spices and herbs have been part of food items for the improvement of color, aroma and flavor of food items and as functioning agents for their anti-oxidative, preservative and antimicrobial effect [7].

Turmeric is a member of the ginger family, *Zingiberaceae*. It is a natural spice, obtained from the root *Curcuma longa* [8]. It is bright yellow in color and has been used as a coloring agent in food in USA. It has been used for thousand years as a spice, a food preservative and also for medicinal properties in India (Eigner and Scholz, 1999) [9].

Nowadays, researchers are showing substantial interest in the active compounds of turmeric known as curcuminoids. The main curcuminoid is curcumin (diferuloyl methane), which constitutes about 90% of the turmeric curcuminoid contents, followed by demethoxycurcumin and bisdemethoxycurcumin [10]. Turmeric oil has high antibacterial activity and it was confirmed by testing it against *Bacillus coagulans*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antifungal activity of turmeric oil was examined against *Aspergillus parasiticus*, *Aspergillus flavus*, *Penicillium digitatum* and *Fusarium moniliforme*. After combination with fluconazole or amphotericin B, curcumin showed a greater fungicidal effect in the treatment of systemic fungal infections including candidemia and candidiasis. Even though turmeric has several health improving benefits, still plenty research is needed for the support of powder turmeric being part of our food formulations. Nevertheless, its antimycotic and antioxidative activity has already been reported in butter cakes [11].

Reports revealed that curcuminoids of turmeric have high stability and the level of any cucuminoid is not affected by heating. This quality makes turmeric extremely acclaimed source of functional food constituents like Curcumin [12]. It has been concluded from earlier studies that incorporation of turmeric powder highly improved the antioxidant action of bread. The enhanced antioxidant action of turmeric-bread may probably be due to the integration of phenolic substances like curcumin, which own high antioxidant activity [13].

Ginger (*Zingiber officinale*), belongs to subtropical and tropical family *Zingiberaceae*, which originated from South Eastern parts of Asia and spread to most of the world regions. Ginger comprises naturally active contents which include the key spicy codes, the shogaols and gingerols [14]. Gingerols, which are phenolic in nature, are responsible for the pungency of fresh ginger. Gingerol is present most abundantly and some other gingerols of shorter chains are also there in smaller ratio. The sharp smell of ginger is due to shogaols, which is the dehydrated form of gingerol. Gingerols are converted to corresponding shogaols by heating [15]. Both gingerols and shogaols have high antioxidant activity [16,17], specifically, gingerol is known for its strong antioxidant activity both in vivo and in vitro, as well as for its strong antiapoptotic and antiinflammatory actions [18].

Ginger is available as fresh, dried, powdered, oleoresin, candy (crystallized ginger), preserved in brine or syrup, and in oil form. Ginger is an essential component of food formulations and processing all over the globe due to its biting taste, refreshing pleasant aroma, and carminative characteristics. In western world, it is used broadly for the purpose of cooking in biscuits, ginger breads, cakes, soups, puddings and pickles. In eastern countries, fresh ginger slices, chops, small pieces and ground ginger are extensively used in non-vegetarian and vegetarian foods. Its usage in soft drink preparation, pickling, confectionery and curry powder development is also known [19].

Materials and Methods

The research work was conducted in the laboratory of the Department of Food Science and Technology, The University of Agriculture, Peshawar, Pakistan.

Application of turmeric and ginger to dough

Ginger powder and turmeric powder substitutions were 3% and 4% in wheat flour and were also used in combination at the same levels as described by Lim et al. [20]. 0, 30 and 40 grams of turmeric powder were incorporated in 1000, 970 and 960 grams wheat flour, respectively. Same substitutions were used for ginger powder. In case of combination of both turmeric and ginger, half amount of both were applied, respectively. Amounts of all other ingredients were similar in these different formulations.

Preparation of bread

The bread was prepared from straight grade flour, according to AACCC [21] method 10-09 (12). Wheat flour (1000 g), sugar (60 g), salt (20 g), oil (40 g), yeast (12 g), bread improver (7 g) and water (adjusted accordingly) were mixed together with the help of mixer. This mixed dough was then rested at 30°C for 2 hours. The developed dough was distributed into fragments each having the same mass, molded by hands and kept in baking pans that were already lubricated with oil. These dough fragments were then transferred into a proofing chamber at 70% humidity and 30°C for 50 minutes. The molded loaves were then baked in an oven at 250°C for 20 minutes. Before depanning,

these were rested for some time to become cold. After cooling, the baked breads were packaged in polyethylene bags.

Treatments

- BG₀ = Control (without turmeric and ginger)
- BG₁ = 3% ginger
- BG₂ = 4% ginger
- BG₃ = 3% turmeric
- BG₄ = 4% turmeric
- BG₅ = 1.5% ginger + 1.5% turmeric
- BG₆ = 2% ginger + 2% turmeric

Microbiological analysis

Inoculation of *Rhizopus stolonifer* to bread: Common Bread Mold, *Rhizopus stolonifer*, was grown on PDA medium and the fungal species were morphologically and microscopically identified in the Department of Plant Pathology, The University of Agriculture, Peshawar-Pakistan. After identification, the mold was inoculated in the center of all the bread loaves with the help of inoculation needle to provide a rounded inoculum of about 2mm diameter.

Colony counting: Colony count of *Rhizopus stolonifer* in bread was calculated at 0, 24, 48, 72 and 96 h of storage on yeast and mold agar media by plate count technique as described by Beneke (1962) [22].

$$\text{Cfu/g} = \text{No. of colonies} \times \text{Dilution factor} \times \text{volume factor.}$$

Physicochemical Analysis: The samples were analyzed for moisture, ash, pH, total dissolved solids and water activity by the standard methods of AOAC [23].

Water activity (aw): Water activity (aw) was calculated with the help of Novaseina R.T.D 502 apparatus (Novaseina, Pfafikkion, Switzerland).

Sensory evaluation: The bread samples were assessed organoleptically for color, texture, flavor and overall acceptability with the method of Larmond [24].

Statistical analysis: The results of various treatments were analyzed statistically by using simple CRD and the means were compared by using LSD (Least Significant Difference) test. For all the analyses, the alpha error was set at 0.05% [25].

Results and Discussion

Products prepared from flour are considered as major diet for all human beings. Several studies were carried out on the preservation and shelf life extension of bakery products, mainly bread. Molds and yeasts usually deteriorate these products. Among them, *Rhizopus stolonifer* stands at the top in the list of bread spoilage. In order to inhibit this mold growth, many methods can be employed including the addition of food grade spices that also contain other benefits.

All the bread samples were prepared with total care and were brought to the laboratory of Food Science and Technology, The University of Agriculture, Peshawar, Pakistan for different physicochemical analysis. All the samples were stored at room temperature and were analyzed for % moisture, ash, pH, water activity and microbial count that included *Rhizopus stolonifer* colony count on daily basis. Samples were also evaluated organoleptically at 24 hours interval.

Microbiological analysis

At the start of the experiment, the microbiological values of the samples for *Rhizopus stolonifer* count were recorded as, 0.00 which were progressively increased to 2.9×10^2 , 2.3×10^2 , 2.2×10^2 , 1.8×10^2 , 2.6×10^2 , 1.9×10^2 and 1.9×10^2 cfu/g respectively during 96 hours of storage. The mean microbial values considerably ($P < 0.05$) increased from 0.00 to 2.2×10^2 through storage. For treatments, highest mean values was noted in BG₀ (1.9×10^2), followed by BG₄ (1.5×10^2) and lowest mean value was noted in BG₃ (8.6×10^2) followed by BG₆ (9.0×10^2). Highest percent increase was noted in BG₂ (600%), followed by BG₄ (375%) and BG₅ (320%) while lowest percent reduce was noted in BG₆ (111%), followed by BG₃ (150%).

Storage intervals (hours)							
Treatments	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	% Increase	Means
BG ₀	0	7×10^2	1.6×10^2	2.1×10^2	2.7×10^2	285.71	1.5×10^2 a
BG ₁	0	6×10^2	1.1×10^2	1.8×10^2	2.2×10^2	266.67	1.1×10^2 b
BG ₂	0	3×10^2	1.1×10^2	1.7×10^2	2.1×10^2	600	1.0×10^2 bc
BG ₃	0	0	6×10^2	9×10^2	1.5×10^2	150	6×10^1 d
BG ₄	0	4×10^1	1.2×10^2	1.6×10^2	1.9×10^2	375	1.0×10^2 bc
BG ₅	0	5×10^1	1.3×10^2	1.5×10^2	2.1×10^2	320	1.0×10^2 bc
BG ₆	0	0	9×10^1	1.6×10^2	1.9×10^2	111.11	8.8×10^2 c
Means	0.00e	3.8×10^2 d	1.1×10^2 c	1.6×10^2 b	2×10^2 a		

Table 1: Effect of treatments and storage intervals *Rhizopus stolonifer* growth on bread samples (cfu/g).

Storage intervals (hours)							
Treatments	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	% Increase	Means
BG ₀	31.1	29.73	29.45	29.11	29.06	6.56	29.690 a
BG ₁	26.54	26.36	26.18	25.91	25.79	2.83	26.15d
BG ₂	24.2	24.07	23.92	23.83	23.77	1.78	23.95f
BG ₃	27.87	27.68	27.53	27.41	27.29	2.08	27.55c
BG ₄	28.96	28.62	28.01	27.78	27.45	5.21	28.16b
BG ₅	27.82	27.45	27.23	27.04	26.89	3.34	27.28c
BG ₆	25.76	25.37	25.18	24.93	24.64	4.35	25.17e
Means	27.46 a	27.04b	26.78b c	26.57c d	26.41d		

Table 2: Effect of treatments and storage intervals on moisture percentage of the bread samples.

The statistical analysis showed that the treatments and storage intervals had a considerable ($P < 0.05$) effect on *Rhizopus stolonifer* count of bread sample through storage at ambient temperature. The means were divided by applying LSD test at 5% probability level and standard deviation was applied to the data (Table 1).

The results of microbiological study are in close resemblance to that of the mold growth on bread, reported by Rehman et al., [26] at same storage intervals. Below water activity value of 0.950, yeasts and molds are the cause of most biological alterations of foods [27].

Moisture

At the start of the experiment, the moisture content of the samples were 31.1, 26.54, 24.2, 27.87, 28.96, 27.82 and 25.76% which were gradually decreased to 29.06, 25.79, 23.77, 27.29, 27.45, 26.89 and 24.64% respectively during 96 hours of storage. The mean moisture values considerably ($P < 0.05$) decreased from 27.46 to 26.41 through storage. For treatments, highest mean values was noted in BG₀ (29.69%), followed by BG₄ (28.16%) and lowest mean value was noted in BG₂ (23.95%), followed by BG₆ (25.17%). Highest percent decrease was noted in BG₀ (6.56%) followed by BG₄ (5.21%) while lowest percent reduce was noted in BG₂ (1.78%), followed by BG₃ (2.08%) (Table 1).

The statistical analysis showed that the treatments and storage intervals had a considerable ($P < 0.05$) effect on moisture of bread sample through storage at ambient temperature. The means were divided by applying LSD test at 5% probability level and standard deviation was applied to the data (Table 2).

In a similar study, decrease in the moisture of freshly baked bread was observed by him and Hosney. He reported that bread crumb moisture decreased sharply with time. The crumb of freshly baked bread contained about 47% moisture. However, during 2 hours of cooling the moisture dropped to 41%. Fik et al. [28] in his study observed that the moisture content of calcium enriched bread decreased by 9% in 3 days of initial storage.

Ash

At the start of the experiment, the ash content of the samples were 0.79, 0.78, 0.83 0.90, 0.96, 0.80 and 0.82% which were gradually increased to 0.82, 0.81, 0.86, 0.92, 0.99, 0.81 and 0.84% respectively during 96 hours of storage. The mean ash content increased from 0.84 to 0.86% through storage. For treatments, highest mean value was noted in BG₃ (0.98) followed by BG₆ (0.90%) and lowest mean value was noted in BG₀ (0.79%) followed by BG₂ (0.80%). Highest percent increase was noted in BG₀ (3.70%) followed by BG₂ (3.66%) while lowest percent reduce was noted in BG₁ (1.23%) followed by BG₆ (2.17%) (Table 3).

The statistical analysis showed that the treatments and storage intervals had a considerable ($P < 0.05$) effect on ash content of the bread samples during storage at ambient temperature. The means were separated by applying LSD test at 5% probability level and standard deviation was applied to the data. The ash content in control bread was 0.79% but with the addition of turmeric, this quantity was increased. This anomalous increase of ash content might be due to the fact that ash content in turmeric powder is about four times higher than that of wheat flour, as reported previously by Kang [29]. In another study, Bojnanska et al. [30] observed 0.80% ash in control bread and this aggregate increased with the addition of pulses into the bread that

contain large amount of ash. This finding is closely related to observations of the present study.

Storage intervals (hours)							
Treatments	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	% Increase	Means
BG ₀	0.79	0.8	0.8	0.81	0.82	3.66	0.80d
BG ₁	0.78	0.79	0.79	0.8	0.81	3.7	0.79e
BG ₂	0.83	0.84	0.83	0.85	0.86	3.49	0.84c
BG ₃	0.9	0.91	0.91	0.9	0.92	2.17	0.90b
BG ₄	0.96	0.98	0.98	0.99	0.99	3.03	0.98a
BG ₅	0.8	0.81	0.82	0.82	0.81	1.23	0.81d
BG ₆	0.82	0.84	0.84	0.85	0.84	2.38	0.83c
Means	0.84c	0.85b	0.85b	0.86a	0.86a		

Table 3: Effect of treatments and storage intervals on ash percentage of the bread samples.

Storage intervals (hours)							
Treatments	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	% Increase	Means
BG ₀	5.9	5.9	5.9	6	6	1.69	5.94d
BG ₁	6	6	6.1	6.1	6.1	1.66	6.06c
BG ₂	5.8	5.9	5.9	6	6.1	5.17	5.94d
BG ₃	6.1	6.1	6.2	6.2	6.2	1.64	6.16bc
BG ₄	6.3	6.3	6.3	6.4	6.4	1.59	6.34a
BG ₅	6	6.1	6.2	6.3	6.4	5	6.20b
BG ₆	5.6	5.7	5.9	6	6.2	10.71	5.88d
Means	5.95d	6.00cd	6.07bc	6.14ab	6.20a		

Table 4: Effect of treatments and storage intervals on pH of the bread samples.

pH

At the start of the experiment, the pH values of the samples were 5.6, 5.9, 5.8, 6.3, 6.1, 6, and 6, which gradually increased to 6.2, 6, 6.1, 6.4, 6.2, 6.1 and 6.4 respectively during 96 hours of storage. The mean pH values considerably ($P < 0.05$) increased from 5.95 to 6.20 through storage. For treatments, highest mean values was noted in BG₃ (6.34), followed by BG₆ (6.20) and lowest mean value was noted in BG₀ (5.88), followed by BG₁ and BG₂ (5.94). Highest percent increase was noted in BG₀ (10.71%), followed by BG₂ (5.17%) while lowest percent reduce was noted in BG₃ (1.59%), followed by BG₄ (1.64%) (Table 4).

The statistical analysis showed that the treatments and storage intervals had a considerable ($P < 0.05$) effect on pH of the bread samples through storage at ambient temperature. The means were

divided by applying LSD test at 5% probability level and standard deviation was applied to the data (Table 4).

In a similar study, Shin et al. [31] found pH values of breads from 6.28 to 6.08. They further documented that change in pH of breads was due to the fermentation process. *Rhizopus stolonifer* shows the highest amylase activity (3.9mg/l) at pH 6 [1].

Water activity

At the start of the study, the water activity (a_w) of the samples were 0.92, 0.90, 0.90, 0.89, 0.87, 0.89 and 0.88, which were gradually decreased to 0.91, 0.88, 0.87, 0.86, 0.84, 0.87 and 0.85 respectively during 96 hours of storage. The mean water activity values considerably ($P < 0.05$) decreased from 0.89 to 0.86 through storage. For treatments, highest mean values was noted in BG₀ (0.91), followed by BG₁ (0.89) and lowest mean value was noted in BG₄ (0.85), followed by BG₆ (0.86). Highest percent decrease was noted in BG₄ (3.45%), followed by BG₃ and BG₂ (3.37%) while lowest percent reduce was noted in BG₀ (1.09%), followed by BG₁ (2.22%) (Table 5).

The statistical analysis showed that the treatments and storage intervals had a considerable ($P < 0.05$) effect on water activity of the bread samples through storage at ambient temperature. The means were divided by applying LSD test at 5% probability level and standard deviation was applied to the data (Table 5).

Storage intervals (hours)							
Treatments	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	% Increase	Means
BG ₀	0.92	0.92	0.92	0.91	0.91	1.098	0.91a
BG ₁	0.9	0.9	0.89	0.88	0.88	2.22	0.89b
BG ₂	0.9	0.9	0.89	0.88	0.87	3.33	0.88b
BG ₃	0.89	0.89	0.88	0.87	0.86	3.37	0.87c
BG ₄	0.87	0.86	0.86	0.85	0.84	3.45	0.85e
BG ₅	0.89	0.89	0.88	0.87	0.87	2.25	0.88c
BG ₆	0.88	0.88	0.87	0.86	0.85	3.37	0.86d
Means	0.89a	0.89a	0.88b	0.87c	0.86d		

Table 5: Effect of treatments and storage intervals on water activity of bread samples.

The samples BG₃, BG₄, BG₅ and BG₆ had lower water activity values as compared to the other samples, which might be due to the presence of turmeric powder in these samples. In a similar study, Lim et al. [20] found that increase levels of turmeric powder decrease the water activity of bread samples. He observed that the addition of 2% turmeric powder had a_w of 9.5 while 5% addition of turmeric decreased to 9.4. This finding is closely related to observations of the present study.

Overall acceptability

Initially the mean score of judges for overall acceptability of samples were 7.8, 7.1, 6.9, 7.7, 7.5, 7.4 and 7.3, which progressively decreased to 5.9, 5.4, 5.0, 5.9, 5.5, 5.4 and 5.3 correspondingly throughout storage. The overall mean score of judges for overall acceptability considerably

($P < 0.05$) decreased from 7.38 to 5.48. For treatments, highest value for mean was noted in BG₀ (6.96), followed by BG₃ (6.88), while lowest value for mean was noted in BG₁ and BG₆ (6.36), followed by BG₅ (6.46). Highest percent reduce was noted in BG₂ (27.54%), followed by BG₆ (27.40%), while lowest percent decrease was noted in BG₃ (23.37%), followed by BG₁ (23.94%) (Table 6).

The statistical analysis showed that the treatments and storage intervals had a considerable ($P < 0.05$) effect on the overall acceptability of bread samples throughout storage at ambient temperature. The means were divided by applying LSD test at 5% probability level and standard deviation was applied to the data (Table 6).

Storage intervals (hours)							
Treatments	0 Hours	24 Hours	48 Hours	72 Hours	96 Hours	% Increase	Means
BG ₀	7.8	7.5	7.1	6.5	5.9	24.36	6.96a
BG ₁	7.1	6.9	6.5	5.9	5.4	23.94	6.36d
BG ₂	6.9	6.5	6.2	5.6	5	27.54	6.04e
BG ₃	7.7	7.4	7	6.4	5.9	23.37	6.88a
BG ₄	7.5	7.1	6.7	6.2	5.5	26.67	6.60b
BG ₅	7.4	7.1	6.6	5.8	5.4	27.02	6.46c
BG ₆	7.3	6.9	6.4	5.9	5.3	27.4	6.36d
Means	7.38a	7.05b	6.64c	6.04d	5.48e		

Table 6: Effect of treatments and storage intervals on overall acceptability of bread samples.

Conclusion

From this study, it is concluded that ginger and turmeric does have inhibitory effect on the *Rhizopus stolonifer* growth of bread and these items can be used as natural spices for the improvement of shelf life of bread. The best inhibitory effect against *Rhizopus stolonifer* growth was observed in BG₃, i.e. addition of 3% turmeric to bread flour. This treatment has shown positive effects on all other characteristics of bread including physicochemical and sensory attributes and is considered as the best treatment of this study. BG₆, i.e. combination of both ginger and turmeric 2% each, also showed good results in inhibiting the growth of *Rhizopus stolonifer* but this treatment has affected other physicochemical and sensory characteristics. This study showed that ginger has a detrimental effect on the flavor of bread, while turmeric has no noticeable effect on the flavor or texture of bread.

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