ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Extending the Shelf-Life of Labneh by Use of Papaya Seeds Extract.

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ABSTRACT

Papaya extracts were prepared from leaves and seeds and their antimicrobial activities were evaluated against different pathogenic strains. Then, the papaya seeds extract (PSE) was used in the manufacture of Labneh. The final product was chemically, microbiologically and sensory evaluated during a storage period of 30 days in cold storage. The PSE exhibited more potent antimicrobial activity than the leave extract against some pathogenic microorganisms. Moreover, addition of the PSE to Labneh during manufactured had no effect on the chemical composition of resultant product. Also, the growth and activity of the bacterial starter cultures were not affected by the added PSE. Coliform bacteria and mold and yeast were not detected in any labneh prepared by the addition of the PSE during storage. The addition of PSE extended the shelf life of labneh up to 30 days compared with 15 days for the control. The organoleptic properties of the different labneh samples slightly decreased and gradually during storage tell 30 days, while, the control lost the acceptability after 14 days only.

Keywords: Papaya, antimicrobial activity, pathogenic microorganisms, labneh.

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ISSN: 0975-8585

INTRODUCTION

Labneh is a popular dairy product. It has a soft, smooth, and spreadable texture with clean acid flavor. Labneh can be made from cow, sheep, goat or buffalo milks. Therefore, Labneh can be differences in the composition and properties of labneh according to the kind of milks used.

Labneh as other milk products is suitable product that can undergo spoilage by the growth and activity of microorganisms [1-2]. Therefore, there is always a need to add preservatives or antimicrobial agents in order to prevent the growth and activity of harmful microorganisms without affecting in the growth and activities of used starter in manufacture.

Mycotoxins are defined as secondary metabolites, with low molecular weight; they are not directly essential for mundane fungal magnification. They are thought to confer a selective advantage to the engenderer strain within involute ecosystems, as they are naturally occurring molecules [3-4]. Indeed, molds are able to grow on a wide range of substrates (including cereals, meats, nuts, cheeses, grapes, coffee beans, apples, and derived products), at any stage of engenderment in the field, during post harvest storage, and under a wide range of climatic conditions. Moreover, early mycotoxin engenderment could additionally sanction molds to rapidly colonize the environment. These metabolites are commonly found in sundry pabulum and victual commodities. Moreover, mycotoxins are kenned to be resistant to industrial processing.

Papaya leaf extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5,7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, chlorogenic acid [5].Papaya leaves extract are usually offered as tea as for treatment of malaria. These leaves have found to contain a substance which kills microorganisms that often interfere with the digestive function called karpain [6]. However, no study has been cited on the possible use of papaya extract as antimicrobial agent in food manufacture.

The aim of this work, study the antimicrobial effect of papaya seeds and leaves extracts on the some species of pathogenic bacteria and fungi to protect labneh from hazardous mycotoxins and contaminated by pathogenic bacteria.

MATERIALS AND METHODS

Fresh buffalo's milk, used in the manufacture of labneh, was obtained from Faculty of Agriculture, Cairo University. Seeds and leaves of Papaya fruits (Carica papaya) were obtained from the Horticultural Research Institute, Agriculture Research Center; Giza, Egypt.

Bacterial starters:

The bacterial strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb-DRI-VAC and *Streptococcus thermophilus* CH-1, were obtained from northern Regional Research Laboratory. Illinois, USA and Chr. Hansens's Lab., Denmark respectively.

Pathogenic strains:

Bacillus cereus B-3711, *Bacillus subtilus*, *Asparagillus flavus* 3357 and *Saccharomyces cerevisiae* Y-2223 were provided by the Northern Regional Research Laboratory Illinois, USA (NRRL). *Listeria monocytogenes* 598 was provided by the Department of Food Science, University of Massashusetts, Amherst MA, USA. *Escherichia coli* 0157: H7 and *Staphylococcus aureus* were isolated and serologically identified by dairy microbiological Lab., National Research Center. *Yersinia enterocolitica* were obtained from Hungarian National Collection of Medical Bacteria, OKI, Gyaliut 2-6, H-1966 Budapest, Hungary. *Aspergillus niger, Pencillium requfortii* J5 were obtained from Department of Microbiology, Swedish University of Agricultural Sciences, *Candidia albicans* were provided by the Institute of Applied Microbiology, University of Tokyo, Japan. The pathogenic strains were routinely transferred into trypton Soya broth, incubated at 37°C for 24 h. Yeast and mold strains were activated in Malt extract broth (Oxoid), incubated at 25°C for 72 h.

Extraction of plant material:

The seed and leaves extract (PSE & PLE) was prepared as described by Junaid et at. [7] using methanol (99%). Briefly, 5 g of each sample (seed or leaves were weighed and mixed with 20 ml of the solvent. the samples and solvent were stirred every 30 min for 3 h and allowed to stand for 24 hours. The extracts were filtered through ordinary cheesecloth and Whatmann (No.1) filter paper. The filtrates were centrifuged at 5000 g for 15 min. The supernatants were Rotary evaporated to half of their original volume, and then are dried and stored at room temperature.

Assessment of the antimicrobial activity of the seed and leaves extracts:

The disc diffusion method according to Alabi et al. [8] with some modification was used to determine the antimicrobial activity of the two extracts powder (leaves and seeds) against pathogenic strains. For pathogenic bacteria, 0.1ml (approximately 10^9 cells /ml) of the tested microorganisms was grown on trypton soya broth media at 37 °C for 24 h. For mold and yeast, the strains were grown in malt extract broth at 25 °C for 72 h, and then spread on the entire surface of the Petri dish using a sterile swab. Each of this powder was diluted 1:1 with dimethyl sulfoxide (DMSO) to facilitate diffusion of the powder through the agar gel during the microbiological assay. Twenty micro liters of each concentration of these DMSO/each extract powder was impregnated on different sterile paper discs (Whatman, No. 1.6 mm) and placed on the surface of agar in Petri dishes. The plates were incubated at 37 °C for 24.0 h. After the incubation period the inhibition zones around the paper discs were measured in millimeters.

Manufacture of labneh using papaya seeds extract:

Labneh was manufactured according to Robinson and Tamime [9]. Fresh buffalo's milk was heated at 90 C for 20 min, cooled to 45^{o}C and then inoculated with 2% of the yoghurt starter culture (*S. thermophilus* + *L. bulgaricus* 1:1) and incubated at 40 ^{o}C for 3 hrs until it was completely coagulated. The coagulum was transferred to cloth bags, which were hung in the refrigerator at 5 ± 1^{o}C for 18 h, to allow drainage of the whey. The resultant coagulant was mixed thoroughly with 1 % NaCl. The resultant Labneh was divided into four portions, the first one was kept as control, and 0.5, 1.0 and 1.5% papaya seeds extract were added to the other three portions respectively, mixed well for 5 min to distribute the added extract uniformly in the curd. The fresh Labneh from each treatment and the control were poured into small plastic containers and stored for 30 days at 5 ± 1 ^{o}C for assessing the shelf life and the keeping quality. The whole experiment was repeated three times. Samples were taken for analysis either fresh (day 0) or during the storage period.

Chemical Analysis:

The samples were chemically analyzed according to AOAC [10] for, total solids, fat, total protein and acidity values. The total volatile fatty acids were determined according to Kosikowiski [11].

Microbiological analysis:

The total starter culture counts were enumerated using Eliker agar medium according to Elliker et al. [12]. The plates were aerobically incubated at 35ºC for 48 h.

Yeasts and Molds count were enumerated using potato dextrose agar acidified to pH 3.5 with sterile lactic acid solution (10 % conc.) [13]. The plates were aerobically incubated at 25 ºC for 4 days.

Coliform bacterial counts were enumerated at 37 º C for 18 h according to Mossel [14] using violet red bile agar medium.

Texture profile analysis:

Force and torque measurements of Labneh treatments were measured using a texurometer model Mecmesin Emperor TM Lite 1.17 (USA) as described by Lobato-Calleros et al. [15].

Organoleptic Properties:

Organoleptic Properties evaluated by scoring panel from (15 members) the member at the NRC according to El Samragy and Zall [16].

RESULTS AND DISCUSSION

Assessment of the antimicrobial activity of the seed and leaves extracts:

Table (1) showed that the different extracts (seeds and leaves) had various degrees of inhibition against the growth of some pathogenic strains. From this table it is clear that both extracts were most effective against *Listeria monocytogenes* while *Bacillus cereus* was the most resistant bacteria species especially when seeds extract was used. Moreover the most sensitive fungi and mold against the extract was *Pencillium requfortii* followed by *Saccharomyces cerevisiae* and in the contrast the more resistant fungus to the extract was *Asparagillus flavus.* Also, from this table it is observed that the seeds extract had more potent antimicrobial properties than the leaves extract. Our results confirmed these reported by Ocloo et al. [17] who found that the seeds of the local variety of papaya contain broad-spectrum antibacterial agents against gram positive and gram-negative bacteria. Also, Peter et al. [18] studied the antibacterial activity through agar well diffusion assay against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* and *Salmonella typhi* aqueous, of chloroform extract of leaves and aqueous, methanolic extract of seeds of papaya This study indicated that the chloroform extract of papaya leaves did not show any inhibition against the bacteria and the aqueous leaf extract was potent to inhibit them. However, aqueous as well as the methanolic extracts of seeds were effective to inhibit the bacterial pathogens.

Table 1: The antimicrobial activity of the seed and leaves extracts against different pathogenic strains

Pathogenic strains	Seed extract	Leaves extract
	Diameter of inhibition zone (mm)	
Bacillus cereus	5	3
Bacillus subtilus	8	5
Staphylococcus aureus	10	7
Listeria monoytogenes	20	15
Salmonella typhamirium	10	8
Escherichia coli	15	14
Yersinia enterocolitica	19	18
Aspargillus niger	12	10
Asparagillus flavus	5	Non detect
Candidia albicans	25	18
Pencillium requfortii	23	20
Saccharomyces erevisiae	22	20

Chemical properties of Labneh manufactured using papaya seeds extract:

From the antimicrobial result we found that seeds extract had more antimicrobial activity than the leaves extract. For these reasons papaya seed extract (PSE) was used in the manufacture of Labneh to extend the shelf life of the final product.

Chemical analysis revealed that fresh plain Labneh (control) contained 35.00±0.2 fat/DM, 32.00±0.01 protein/DM, 29.5±0.2% total solids as in **Fig.(1).** There were no differences between values of chemical analysis in all treatments either in the zero time or during the storage period.

These result means that, the addition of PSE had no effect on the chemical composition of Labneh. There was no paramount difference observed. The chemical composition of all treatments revealed in this study was in acquiescent with the Egyptian specification for Labneh (Egyptian Standards, 2000). Also, during storage, due to moisture loss, both TS and F/DM increased. Ismail et al. [19] mentioned that by addition of six different essential oils there were no observable differences in TS and F/DM of labneh produced. Tamime [20-

21], Tamime and Robinson [22], Mehaia and El Khadragy [23], Aloğlua and Oner [24] also, Desouky et al. [25] reported that the TS of labneh ranged between 22–26% so the present data is different from them. This may be due to difference of milk type and manufacturing process.

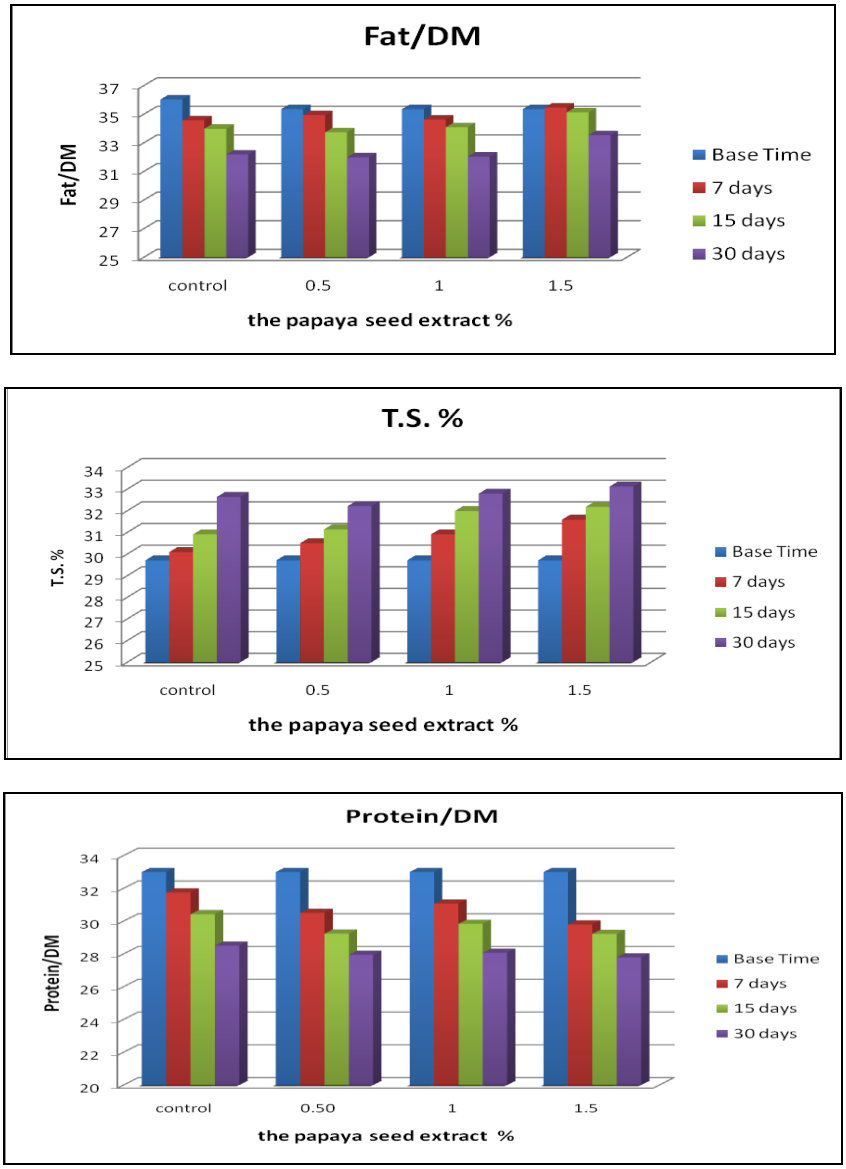

Fig.1: chemical composition in Labneh samples during storage period.

Fig. (2) indicted a very important factor which is the change in titratable acidity (TA), since it affects the shelf life and the acceptability of labneh. Showed that the acidity values of labneh were almost similar for four kinds of fresh labneh and during the storage period. The titratable acidity gradually increased during storing in all samples

Based on these results suggesting that the starter culture and total viable count are not affected by the PSE. The TA increased gradually during storage period in agreement with the results obtained by Abbas and Osman [26]

ISSN: 0975-8585

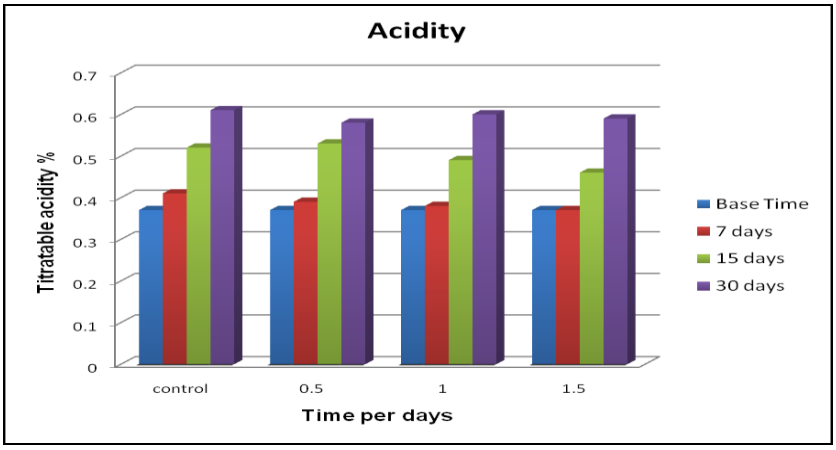

Fig. 2: Titratable acidity (TA) in Labneh samples during storage period.

The percentage of soluble nitrogen of labneh made with the addition of the Seed extract showed a gradual but significant increase in control and all treatments during the storage period **(Fig. 3)**. The results indicate that the addition of Seed extract hadn't any effect on the activity of LAB (starter). This result did not confirm those of Ismail et al. [19] and Al.Otaibi and El.Demerdash [27] who found that the untreated control labneh showed significant decrease SN% compared to labneh made with the addition of the essential oils in all treatments during the storage period and they attributed this to an increase in LAB activity. The same trend was showed in **Fig. (4),** where the addition of Seed extract hadn't any effect on the values of TVFA.

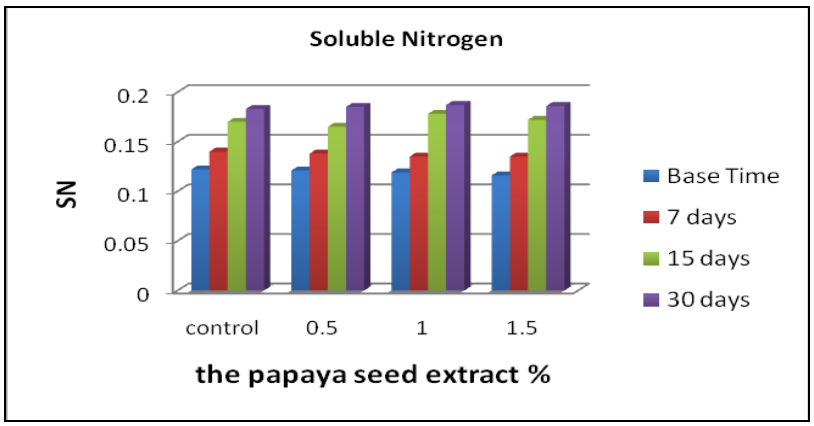

Fig. 3: Soluble Nitrogen content in Labneh samples during storage period.

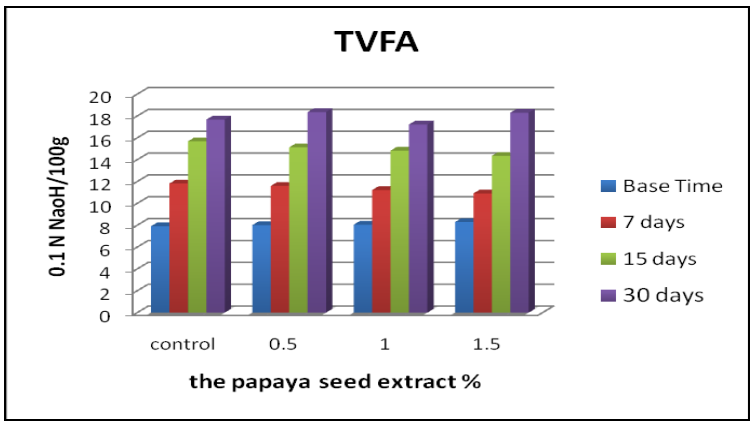

Fig. 4: TVFA content in Labneh samples during storage period.

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Microbiological analysis:

Labneh prepared by adding three different concentrations from Seeds extract to the Labneh was directed to microbiological analysis. Analysis of the results obtained for the total starter cultures counts (Fig.5), indicated that in all cases the respective counts gradually increased up to 15 days of storage and after that decreased but not completely inhibited at the end of storage period. This may be explained by the fact that during the manufacturing process, bacterial starter tend to increase in number and then continue to multiply for approximately 15 days, whilst lactose is available in fermented milk, but after that the bacterial count will be decreased due to increase in acidity [28]. Khaleel [29] and El–Nawawy et al. [30] reported that adding of some essential oils to yoghurt and labneh cheese during its manufacture cause a stimulatory effect on LAB by increasing their growth and acid production. Moreover, El-Nawawy et al. [30] reported that increased the counts of *S. thermophilus* and *L. bulgaricus* compared to untreated controls during storage due to the presence of some herbs in the manufacture of yoghurt.

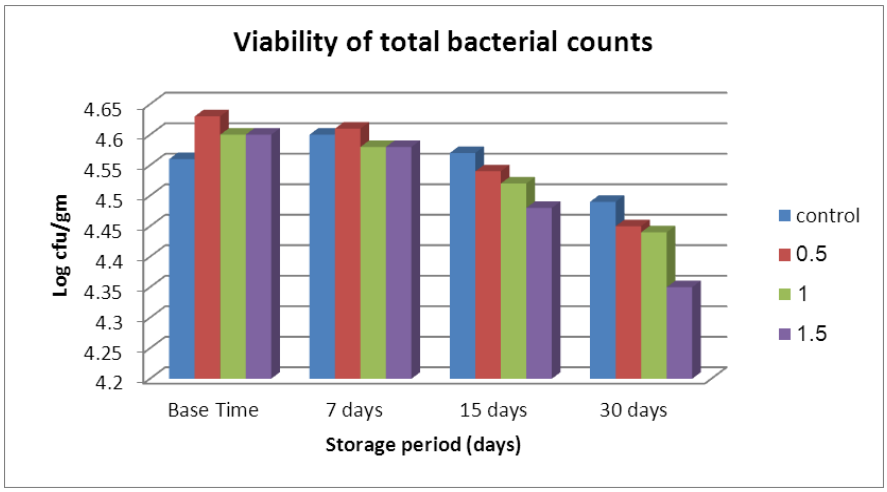

Fig. 5: Total starter cultures counts in Labneh samples during storage period.

Notably, coliform bacteria and *E.coli* were not detected in any of the labneh prepared by addition of the seeds extract. This may not be surprising, as Peter et al. [18] reported that aqueous extract of Carica papaya seeds showed increasing zone of inhibition of *S. aureus*, *P. aeruginosa* and *E. coli* with increasing seed extract concentration as 25, 50, 75 and 100 mg/m.

Moreover, the quality and the shelf life of labneh are indicated by yeast and mould counts. In this regard, yeasts and moulds were detected in the untreated control after 15 days and later of storage (4 and 6 $x10^3$ cfu/g, respectively) but yeasts and moulds were not detected in labneh containing seeds extract throughout the storage period, these results are in agreement with those reported in this study. Generally, the different concentration of seeds extract contributed to increase the shelf-life of Labneh samples up to 30 days compared with control which was contaminated with fungi and yeast after 15 days of storage periods. These results due to the antimicrobial activity of this extract as we mention before in these study.

Textural analysis:

From the data presented in **Table (2)**, it is clear that there is no difference in all textural parameters between control and all other samples that contained different concentration with seeds extract. This observation was expected because there is no change in the chemical composition of the product especially fat and total solids.

ISSN: 0975-8585

Table 2: Textural properties of labneh samples

Labneh samples	Firmness (N)	Cohesiveness (Area B/A)	Gumminess (N)	Chewiness (m/N)	Springiness (mm)
control	3.626	0.669	0.195	1.43	0.676
0.5	3.625	0.667	0.194	1.425	0.673
1.0	3.601	0.659	0.193	1.422	0.67
1.5	3.59	0.652	0.192	1.413	0.667

The organoleptic properties:

The organoleptic properties of the different labneh were investigated as in **Table (3).** There were insignificant differences in the all properties of these treated samples as compared with the untreated control. While, after 14 days only the untreated labneh (control), lost the acceptable. This result may be due to the growth of fungi and the development of undesirable flavors. Other all treated samples the total scores of the sensory evaluation slightly decreased gradually during storage tell 30 days with acceptable flavor and good appearance without any signs of spoilage organisms or fungi. Moreover, the samples that contained different concentration of seeds extract remain acceptable for 45 days of storage. This result means that the addition of PSE was prevented the growth of fungi and spoilage organisms as *E. coli* or coliform group in the samples.

Table 3: organoleptic properties of Labneh samples during storage periods

Samples	Storage periods (days)			
	0	7	15	30
Flavors (50)				
control	45	41	25	0
0.5	45	45	45	40
1.0	45	45	45	40
1.5	45	45	45	40
Body and Texture (40)				
control	35	35	30	25
0.5	35	35	35	35
1.0	35	35	35	35
1.5	35	35	35	35
Appearance (10)				
control	9	9	3	0
0.5	9	9	9	9
1.0	9	9	9	9
1.5	9	9	9	9
Total (100)				
control	89	85	85	25
0.5	89	89	89	84
1.0	89	89	89	84
1.5	89	89	89	84

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