

Microbiological and Chemical Properties of Raw Milk Consumed in Burdur

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Abstract: The aim of this study was to determine the microbiological and chemical properties of raw milk consumed in Burdur. A total of 100 samples obtained from different producers were analyzed for microbiological and chemical properties. For this purpose, counts of total aerobic mesophilic bacteria, Enterobacteriaceae, coliforms, *E. coli*, Enterococci, yeast, mold and Micrococcus-Staphylococcus, Coagulase Positive Staphylococcus microorganisms were made. In the raw milk samples, the mean level of total aerobic mesophilic bacteria was 3.95×10^6 cfu mL⁻¹, Enterobacteriaceae 3.0×10^4 cfu mL⁻¹, coliforms 2.0×10^4 cfu mL⁻¹, *E. coli* 1.0×10^2 cfu mL⁻¹, Enterococci 3.2×10^4 cfu mL⁻¹, yeast 7.8×10^3 cfu mL⁻¹, mold 1.0×10^3 cfu mL⁻¹, Micrococcus-Staphylococcus 2.45×10^4 cfu mL⁻¹ and Coagulase Positive Staphylococcus 1.0×10^4 . The mean level of pH, acidity (°SH), non fat dry matter contents (%), density (g mL⁻¹) of raw milks samples were 6.74, 8.41, 8.42 and 1.027 and 6, respectively. In conclusion, raw milk may cause a potential risk to the public and therefore hygienic precautions should be taken by determining critical control points in the phases of production, storage and sale and regular check-ups of milk should be performed at various critical control points according to food regulations.

Key words: Raw milk, microbiological, chemical, properties, phases, Turkey

INTRODUCTION

Food products of animal origin play an important role in sufficient and balanced nutrition of human beings. Milk and milk products are among the most important food products of animal origin. Milk is often described as a complete food because it contains protein, sugar, fat, vitamins and minerals (Komorowski and Early, 1992). Milk is a major component in the human diet all over the world but it also serves as a good medium for the growth of many microorganisms especially pathogenic bacteria (Ruegg, 2003). Traditionally, raw or unpasteurized milk has been a major vehicle for transmission of pathogens (Vasavada, 1988). It is well established that consumers want clean, wholesome and nutritious food that is produced and processed in a sound, sanitary manner and is free from pathogens. To fulfill consumer demands, quality milk production is necessary. Quality milk means that the milk is free from pathogenic bacteria and harmful toxic substances, free from sediment and extraneous substances of good flavor with normal composition, adequate in keeping quality and low in bacterial counts.

In Turkey approximately 12 million tons of milk is produced per year. Turkish milk production consists of 92.35% cow, 5.85% sheep, 1.53% goat and 0.26% buffalo milk. It is reported that in the country, only 54% of the milk is processed in modern plants or small dairies while

35% of the milk produced is consumed at the farm and 11% of milk is sold by street peddlers under unhygienic conditions (Anonymous, 2008). Nearly 243,423,000 tons of milk are produced per year in the city of Burdur making this a key city for Turkish milk production. Of this milk, 80% is transported and processed by other cities. Another percentage of milk is produced and consumed locally in the city by large number of people (Anonymous, 2008).

Milk because it is rich in various nutrients provides a suitable medium for microbial growth. Fresh milk (immediately after milking) has <100 bacteria per mL. Milk contamination resources include the internal and external surfaces of the udder. Other external sources including skin, milking equipment, workers, contaminated water and milk transportation tankers can have more severe effects. Increasing different bacterial populations will also change milk components and can result in unfavorable odor and flavor, increased rate of spoilage and decreases in its maintenance and applications. It also increases the risk of transmission of zoonotic diseases (Chye *et al.*, 2004; Walstra *et al.*, 2006).

The major problem with the fluid milk supply system in Turkey from the consumer's point of view is not only adulteration but also dirty adulteration. The public consumes fluid milk which has been adulterated and diluted to an extent that there is very little nutritive value

left in it, leading to public health concerns and malnutrition. Suppliers of milk appear to have found three ways to increase their margin from the sale of milk: dilution, extraction of valuable components, i.e., milk fat removed as cream and a combination of dilution and extraction of valuable components with the addition of cheap (and sometimes potentially harmful) bulking additives such as low quality flour to bring the total solids to a level that is acceptable to consumers.

The composition and amount of microflora in the raw material has a decisive effect on the quality and safety of dairy products. This study was aimed to determine the microbiological quality and chemical properties of raw milks currently consumed in Burdur.

MATERIALS AND METHODS

Samples: In this study, a total of 100 raw milk samples were obtained from a food bazaar between February and June 2010 in Burdur city. All of the samples were collected aseptically in the sellers usual form (plastic bottles) and brought to the laboratory maintaining the cold state and analyzed immediately.

Microbiological analyses: Traditional microbiological methods and media were used for the isolation and enumeration of Total Aerobic Mesophilic Bacteria (TAMB), Enterobacteriaceae, Coliforms, *E. coli*, Enterococci, yeast, mold, Micrococcus-Staphylococcus and Coagulase Positive Staphylococcus (Table 1). About 10 mL of each milk sample was suspended in 90 mL sterile buffered peptone water (0.85% NaCl+0.1% peptone) and 0.1 mL of 10⁻¹-10⁻⁶ dilutions were spread onto the surface of agar plates.

For the isolation of *E. coli*, presumptive colonies growing on Violet Red Bile (Lactose) agar (Oxoid Ltd., UK) were selected and directly streaked onto Eosin Methylene Blue (EMB) agar and incubated for up to 48 h at 37°C. One suspected *E. coli* colony on the EMB was selected and identified by the indole, methyl red, voges proskauer and simmon’s citrate tests (IMViC tests).

Enterococci were enumerated on Slanetz-Bartley Medium (Oxoid Ltd., UK) after incubation at 37°C for 24-48 h. Typical colonies (pink or dark red with a narrow whitish border) were then counted.

Micrococcus-Staphylococcus and Coagulase Positive Staphylococcus (CPS) were enumerated in Baird-Parker Agar (BD, Becton Dickinson and Company, France) supplemented with egg yolk and tellurite. The plates were incubated at 37°C for 24-48 h. After growth, suspicious colonies were counted. The colonies were classified as typical for *S. aureus* (jet black to dark gray, smooth, convex, entire margins with an opaque zone and a clear halo beyond the opaque zone) and atypical (jet black to dark gray colonies, entire margin without a halo). Ten colonies from each sample were selected and transferred to individual tubes of TSB agar (as stock cultures). A series of tests were then performed on the isolates including Gram stain, catalase, coagulase, anaerobic fermentation of glucose and mannitol, hemolysis in blood agar, production of acetoin, methyl red, voges-proskauer, urease test, DNase and TNase activity (FDA, 1995; Harrigan, 1998).

Physicochemical analyses: The milk samples were analyzed for pH, titratable acidity (^oSH, %Lactic acid), non-fat dry matter content (%) and density (g mL⁻¹). The pH of milk samples was measured electrometrically with a pH meter. The instrument was first calibrated using buffers of pH 7.0 and 4.0 (Metrohm 704 pH meter). Titratable acidity was determined according to the method of Association of Official Analytical Chemists (AOAC, 1990). The samples were titrated with N/10 NaOH solution using a titration kit with phenolphthalein as an indicator. The density (g mL⁻¹) was determined by a lactodensimeter (AOAC, 1990). The non-fat dry matter (%) content was determined by hand refractometry (Atago N-1α, cat.No.2211 Brix0~32%).

Statistical analysis of data: The results were analyzed using Minitab-15 with the descriptive statistics.

Table 1: The media used for the microbiological analyses and incubation conditions

Microorganisms	Media used	Incubation temperature (°C)	Incubation time	Incubation condition
The Total Aerobic Mesophilic Bacteria (TAMB)	Plate Count Agar (Merck, 1.05463.0500)	30	48-72 h	Aerob
Enterobacteriaceae	Violet Red Bile Dextrose Agar (Merck, 110275)	37	24-48 h	Anaerob
Coliforms	Violet Red Bile (Lactose) Agar (Oxoid, CM 0107)	37	24-48 h	Aerob
<i>E. coli</i>	Eosin Methylene Blue Agar (Merck, 1.01347.0500)	37	24-48 h	Aerob
Enterococci	Slanetz-Bartley Medium (Oxoid, CM 377)	37	24-48 h	Aerob
Micrococcus-Staphylococcus	Baird-Parker Agar (Difco, 276840)	37	24-48 h	Aerob
Yeast-Mold	Yeast extract glucose	25	4-5 days	Aerob
	Chloramphenicol Agar (Merck, 1.1600.0500)			

RESULTS AND DISCUSSION

In this study, 100 raw cow milk samples were analyzed for the presence and contamination levels of TAMB, Enterobacteriaceae, Coliforms, *E. coli*, Enterococci, yeast, mold, Micrococcus-Staphylococcus and Coagulase Positive Staphylococcus. For the raw cow milk, the presence of microorganisms is shown in Table 2, the microbial contamination rates are shown in Table 3 and the physicochemical properties are shown in Table 4.

With this study, samples of raw cow milk which were offered for consumption in Burdur were analyzed for the presence of various microorganisms and their counts. The results of the microbiological analysis of the raw milk samples are shown in Table 1. Raw cow milk is considered as having unacceptable hygienic quality when the TAMB exceeds 1.0×10^5 cfu mL⁻¹ according to the Turkish Food Codex (No: 2009/14) and Commission Regulation (EC, No: 1662/2006). In this study, the average TAMB count was 3.95×10^6 which is higher than the limits recommended by either of these agencies. Gran *et al.* (2003), Chye *et al.* (2004), Al-Tahiri (2005), Godic-Torkar and Golc-Teger (2008), Karami *et al.* (2008), Shojaei and Yadollahi (2008), Dan *et al.* (2008), Franciosi *et al.* (2009), Millogo *et al.* (2010) have detected the TAMB counts in raw cow milk as $<10^5$, 12×10^6 , 5.0×10^5 , $4.5 \log_{10}$, 1.36×10^6 , 13×10^6 , 10^6 , $4.18 \log_{10}$ and 10^6 - 10^7 cfu mL⁻¹, respectively. The findings in the present study were consistent with the results of Chye *et al.* (2004), Karami *et al.* (2008), Shojaei and Yadollahi (2008), Dan *et al.* (2008) and Millogo *et al.* (2010) whereas the total aerobic mesophilic microorganisms found in this study were higher than those reported by Gran *et al.* (2003), Al-Tahiri (2005), Franciosi *et al.* (2009). In the present study, the TAMB count was $\geq 10^5$ cfu mL⁻¹ levels in 98% of the raw milk samples. Possible reasons for the high counts could be infected udders of the cows, unhygienic milking procedures or equipment and/or inferior microbiological quality of water used for cleaning utensil and animals as well as the milk storage conditions. Therefore, poor milk quality has often been considered as one of the major reasons for losses and it results in reduced income for the smallholder dairies in Burdur.

Yeast and mold are common contaminants in food. While yeast does not result in food poisoning, it does cause food to spoil (Deak, 2008). A very large number of molds produce toxic substances designated as mycotoxins. Some are mutagenic and carcinogenic, some display specific organ toxicity and some are toxic by other mechanisms (James, 2000). The mean numbers of yeasts

Table 2: Presence of microorganisms in the raw cow's milk samples (cfu mL⁻¹)

Microorganisms	Sample			
	Number (N)	Min.	Max.	Mean
TAMB	100	4.6×10^4	7.1×10^7	3.95×10^6
Yeast	100	$<10^2$	4.6×10^4	7.8×10^3
Mold	100	$<10^2$	1.7×10^4	1.0×10^3
Enterococci	100	$<10^2$	6.6×10^6	3.2×10^4
Enterobacteriaceae	100	$<10^2$	8.0×10^7	3.0×10^4
Coliforms	100	$<10^2$	4.0×10^7	2.0×10^4
<i>E. coli</i>	100	$<10^2$	5.0×10^5	1.0×10^2
Micrococcus-Staphylococcus	100	$<10^2$	1.3×10^6	2.45×10^4
CPS	100	$<10^2$	3.7×10^5	1.0×10^2

Table 3: Microbial contamination rates in raw cow's milk samples (%)

Microbes	$<10^2$	$\geq 10^2$	$\geq 10^3$	$\geq 10^4$	$\geq 10^5$	$\geq 10^6$	$\geq 10^7$	(N)
TAMB	-	-	-	2	10	60	28	100
Yeast	14	2	40	44	-	-	-	100
Mold	28	20	40	12	-	-	-	100
Enterococci	4	-	24	52	10	10	-	100
Enterobacteriaceae	2	8	20	40	20	10	-	100
Coliforms	8	2	12	52	14	4	8	100
<i>E. coli</i>	90	-	2	4	4	-	-	100
Micrococcus-Staphylococcus	14	-	18	54	8	6	-	100
CPS	74	-	6	14	6	-	-	100

Table 4: Physicochemical properties of raw cow's milk

Physicochemical composition	N	Min.	Max.	Mean±SD
pH	100	5.00	7.00	6.7462±0.0361
Titrate acidity (°SH)	100	6.80	12.40	8.416±0.131
Titrate acidity (LA%)	100	0.15	0.27	0.18798±0.0003
Density (g mL ⁻¹)	100	1016.00	1034.00	1027.6±0.333
Non-fat dry matter (%)	100	5.00	11.00	8.420±0.113

and molds found in raw milk samples in this study were 7.8×10^3 and 1.0×10^3 cfu mL⁻¹, respectively which are higher than those reported at 1.5×10^4 and $2.3 \log_{10}$ cfu mL⁻¹ by Al-Tahiri (2005) and Godic-Torkar and Golc-Teger (2008), respectively. We also expected a higher number of yeast and molds in milk when the pasture or the hay was replaced by conserved or ensiled feed. Many researchers have reported a higher number of yeasts, molds and consecutively the higher concentration of mycotoxins in ensiled feed which was used mostly in the winter season. These microorganisms were very often transferred from feed to milk (Blanco *et al.*, 1988; Lopez *et al.*, 2003; Kamkar, 2005).

The bacteria of the genus *Enterococcus* sp., also known as enterococci are considered to be important in foods as indicators of spoilage or potential pathogenic organisms. In dairy products, both *E. faecalis* and *E. faecium* species are relatively heat resistant as well. Most enterococci are also relatively resistant to freezing. In the present study, the average enterococci count was 3.2×10^4 cfu mL⁻¹. A study of the levels of enterococci in

raw cow's milk from 10 New Zealand farms in 1997, revealed an enterococcal minimum count of $<10^1$ cfu mL⁻¹ and a maximum of 1.2×10^4 cfu mL⁻¹, though 95% of the samples from the same study had $<1.9 \times 10^3$ cfu mL⁻¹ (Hill and Smythe, 1997). Other sources report numbers in European raw milk varying from 10^3 - 10^5 cells mL⁻¹ or more without any of the species being markedly represented (Perez *et al.*, 1982). Higher levels of Enterococci in milk are considered to be the result of contamination during the collection or processing of milk (Cogan *et al.*, 1997). In the present study, the Enterobacteriaceae count of microorganisms was between $<10^2$ and 8.0×10^7 cfu mL⁻¹ and the mean of Enterobacteriaceae was 3.0×10^4 cfu mL⁻¹ which is higher than the results obtained at 2.66-5.94 log₁₀ and 1.84 log₁₀ cfu mL⁻¹ by Dan *et al.* (2008) and Franciosi *et al.* (2009), respectively. Therefore, microbiological quality of the samples in this study seems to be low. The Enterobacteriaceae family has earned a reputation as being among the most pathogenic and most often encountered organisms in food.

The Enterobacteriaceae family includes the coliform group (*Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella*) in addition to many other genera (*Salmonella*, *Shigella*, *Morganella*, *Providencia*, *Edwardseilla*, *Proteus*, *Serratia* and *Yersinia*) that are isolated from animal intestines (Hayes *et al.*, 2001). The existence of coliform bacteria may not necessarily indicate a direct fecal contamination of milk but it is a precise indicator of poor sanitary practices during milking and further handling processes. The presence of fecal coliforms, i.e., *E. coli*, implies a risk that other enteric pathogens may be present in the sample (Hayes *et al.*, 2001). In the present study, the average coliform count was 2.0×10^4 cfu mL⁻¹. Chye *et al.* (2004), Al-Tahiri (2005), Shojaei and Yadollahi (2008), Godic-Torkar and Golc-Teger (2008), Franciosi *et al.* (2009), Abd-Elrahman *et al.* (2009) determined coliform counts in raw cow milk samples as 1.7×10^5 , 6.0×10^2 , 1.3×10^3 , $2.0 \log_{10}$, $1.39 \log_{10}$ and $4.157 \log_{10}$ cfu mL⁻¹, respectively.

The detection rate for coliform was in agreement with the results by Abd-Elrahman *et al.* (2009) whereas they were higher than those reported by Al-Tahiri (2005), Shojaei and Yadollahi (2008), Godic-Torkar and Golc-Teger (2008), Franciosi *et al.* (2009) and they were not lower than those reported by Chye *et al.* (2004). The incidence of coliforms in raw milk has received considerable attention, partly due to their association with contamination of fecal origin and the consequent risk of more pathogenic fecal organisms being present, partly because of the spoilage that can result from their growth

in milk at ambient temperatures and not least due to the availability of sensitive and rapid tests for detecting and enumerating coliforms. Coliform counts regularly in excess of 100 cfu mL⁻¹ are considered by some authorities as evidence of unsatisfactory production hygiene. Sporadic high coliform counts may also be a consequence of unrecognized coliform mastitis, mostly caused by *E. coli*. The coliform microorganisms are found also on the surface of the underwashed or moist milking equipment (Bramley and McKinnon, 1990).

In the present study, 10% of the samples collected were contaminated by *E. coli* with a mean count of 1.0×10^2 cfu mL⁻¹ which is lower than the results obtained by Chye *et al.* (2004) but *E. coli* was not isolated by Ekici *et al.* (2004). In spite of generally low *E. coli* counts, their presence indicates the possibility of fecal contamination and implies a risk that other enteric pathogens may be present in the product. The presence of *E. coli* therefore indicates a safety risk and the numbers of *E. coli* should be at the minimum recommended levels in milk products.

In the present study, the average Micrococcus-Staphylococcus count was 2.45×10^4 cfu mL⁻¹ levels in 86% of the samples, the average CPS count was 1.0×10^2 cfu mL⁻¹ and in 26% of the milk samples, CPS counts were above 10^3 cfu mL⁻¹. According to the Turkish Food Codex (No: 2009/14), the *S. aureus* numbers must not exceed a maximum of 5.0×10^2 cfu mL⁻¹. On the other hand, the mean *S. aureus* numbers were 3.0×10^2 , 1.2×10^4 in 60.7% of milk samples and 1.2×10^6 cfu mL⁻¹ by Al-Tahiri (2005), Chye *et al.* (2004) and Mennane *et al.* (2007), respectively. In another study, Godic-Torkar and Golc-Teger (2008) reported that CPS count was $1.97 \log_{10}$ cfu mL⁻¹. One typical pathogen is *S. aureus*, a ubiquitous organism that occurs in the mucous membranes and skin of most warm-blooded animals including human beings. *S. aureus* is widely recognized as a major causative agent of clinical and subclinical mastitis in dairy cattle (James, 2000; Anonymous, 2008). In food, the minimum numbers of *S. aureus* required to produce toxicity in human beings is estimated to be in excess of 10^5 cfu mL⁻¹ (Su and Wong, 1997; James, 2000; Anonymous, 2008). Staphylococcal toxins cannot be destroyed by heating, drying or freezing (James, 2000).

The physicochemical analysis results of raw milk samples are shown in Table 3. It has been explained in the Turkish Food Codex (No: 2006/38) that the acidity of cow's milk is about 0.13-0.20%. The titratable acidity of milk samples ranged from 0.15-0.27%, average $0.18 \pm 0.0003\%$. The value obtained in this study was

almost identical to those in freshly obtained normal cow's milk. However, Turkish dairy milk acidity values have ranged between 4.20 °SH (0.09%) and 12°SH (0.27%) (Sezgin and Kocak, 1982; Isiklar and Kurdal, 1991; Kurt *et al.*, 2003; Ozrenk and Selcuk, 2008). In studies from other countries, the acidity of milk samples was 0.13, 0.15 and 0.17 as reported by Javaid *et al.* (2009), Kanwal *et al.* (2004) and Shojaei and Yadollahi (2008), respectively. The first acidity in milk is due to the amount of casein, phosphate, citrate and carbon dioxide. Then, at the end of the bacterial activity, lactic acid is formed and the acidity of milk increases. The extra acidity value in milk is not desirable (Kurt *et al.*, 2003).

The acidity of milk is usually expressed as pH. The pH of most samples of milk is 6.6-6.8; average 6.7 at 20°C (Walstra *et al.*, 2006). In this study, the pH of milk samples was between 5 and 7 with a mean pH of 6.74. In other studies, various rates of pH readings were reported as between 6.44-6.99 by Gran *et al.* (2003), Kanwal *et al.* (2004), Mennane *et al.* (2007), Shojaei and Yadollahi (2008), Ozrenk and Selcuk (2008), Lingathurai *et al.* (2009) and Milloga *et al.* (2010). Milk pH gives an indication of milk hygiene and milk pH should not be <6.6 or >6.8 when milk temperature is 20°C (Walstra *et al.*, 2006). Cooling milk after milking reduces the risk for the growth of milk bacteria and high milk temperatures must be considered as favourable to the growth of bacteria in the milk (Walstra *et al.*, 2006). The milk would have a high pH value at during mastitis and neutralizer are occasionally used to neutralize the developed acidity of milk (Kurt *et al.*, 2003).

The non-fat solids content of normal cow's milk is 8.9% (Walstra *et al.*, 2006). The mean non-fat dry matter of milk observed in the present study was 8.4% and ranged from 5-11.0%. Results of present study are in line with that of different researchers who have reported that non-fat dry matter content of milk samples was 7.7-9.1% (Sezgin and Kocak, 1982; Kanwal *et al.*, 2004; Ozrenk and Selcuk, 2008; Javaid *et al.*, 2009; Shojaei and Yadollahi, 2008). The non-fat solids content of cow's milk cannot be legally lowered by the addition of water and the resultant product sold as fluid milk. Both titratable acidity and pH are used to measure milk acidity. These tests are used to determine milk quality and to monitor the progress of fermentation in cheese and fermented milks.

The density of milk is rather variable. On average, density of fresh whole milk is about 1029 g mL⁻¹ at 20°C provided that the fat is fully liquid (Walstra *et al.*, 2006). According to the Turkish Food Codex (2006/38), the density of cow's milk is not lower than 1.028 at 20°C. In this study, the density of milk samples was between 1016.0 and 1034.0 g mL⁻¹ and the mean of density was

1027.6±0.332 g mL⁻¹. Likewise in some studies, several rates of density were also reported as average 1.026-1.032 (Sezgin and Kocak, 1982; Kanwal *et al.*, 2004; Ozrenk and Selcuk, 2008; Javaid *et al.*, 2009). Milk that is subjected to malpractices such as skimming and adulteration with water loses its wholesomeness and nutritive value. In the milk samples analyzed in this study, 4% of the samples had additional milk power, 30% of the samples had added water, 6% of the samples had added water and removed fat and 60% of samples were non-adulterated normal milk. The adulteration of milk supplies may be deliberate addition of water, preservatives and neutralizers or it may arise from faulty methods of milk production particularly in the use of sterilizers and in the methods of rinsing milking equipment. Other methods of adulteration likely to be resorted to are the addition of skim milk or the extraction of some fat by skimming. According to Siegentholer and Shulthess (1977), addition of water is the simplest way to increase milk quantity. In addition to the economic part of the problem, watering milk may also cause public health hazards since the available water added may be grossly contaminated. In countries applying a pricing system, milk with a high amount of water receives a low price.

CONCLUSION

According to the microbiological and chemical analysis results, it was determined that the cow's milk consumed and sold in the Burdur bazaar was inappropriate for human consumption due to hygienic and chemical quality and that most of samples were not to the standards of the food regulations. Farmers, milk sellers and collectors need training in milk hygiene and the physical aspects of raw milk.

Routines for minimizing contamination of milk need to be put in place. The cows teats and the milkers hands should be washed carefully before milking starts and all containers used for storing and transporting milk should be cleaned each time milk has been emptied, before being used again. Milking machines should also be cleaned carefully. In order to manage milk containers and cleanliness, the plastic bottles used today should be replaced with milk containers with a large openings and an inside that is easy to clean.

In conclusion, it was concluded that raw milk may pose a potential public health risk and therefore hygienic precautions should be taken by determining critical control points from phases of production, storage and sale. Regular check-ups of milk should be performed at various critical control points according to food regulations.

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