REVIEW ARTICLE

Yogurt: The fermented milk

Nathanon Trachoo

Yogurt has been known to mankind for over 6,000 years. The word "yogurt" is possibly derived from the Turkish word "jugurt" which first appeared in the 8th century (Rasic and Kurmann, 1978) but today various names are used to refer to yogurt or similar products. These names include Dahi or Dahee in India, Roba in Iraq, and Fiili in Finland (Tamime and Deeth, 1980; Tamime and Robinson, 1985) and several others. It is likely that yogurt originated in the Middle East where there was a limited availability of milk due to the desert environment. Ancient Turks who lived as nomads possibly introduced yogurt to village people as a preserved milk product (Akin and Rice, 1994; Rasic and Kurmann, 1978; Tamime and Robinson, 1985). Popularity of yogurt is greatly attributed to Professor Elie Metchnikoff of the Pasteur Institute in Paris, who shared the Nobel Prize in Physiology and Medicine in 1908 and authored the book, "The Prolongation of Life" in which he advocated the health benefits of yogurt.

Yogurt was first introduced to the U.S. in the early 20th century and gained significant consumer popularity during the 1960's and 1970's. Sales in the U.S. increased from 141 million kg in 1974 to 616 million kg in 1995 (Anon, 1996). Yogurt has now become a popular subject for researchers nationwide as it has been claimed to be a healthy food.

During the past decade, full fat yogurt consumption has declined due to changes in dietary habits of consumers, in particular, reduction in milkfat consumption. Many modifications in yogurt manufacturing have therefore been developed to reduce milkfat content in yogurt resulting in the availability of nonfat and lowfat yogurt.

Definition of yogurt

According to the Code of Federal Regulations of the FDA (FDA, 1996c), yogurt is defined

Ph.D. (Food Science and Technology), Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44000 Thailand.
Corresponding e-mail: nathanon.t@msu.ac.th
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as the "food produced by culturing one or more of the optional dairy ingredients (cream, milk, partially skimmed milk, and skim milk) with a characterizing bacteria culture that contains the lactic acid-producing bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*". Other descriptions are included as follows. "To increase the nonfat solids content of the food, other optional ingredients (concentrated skim milk, nonfat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins and modified whey) may also be added and shall be included in the culturing process. Yogurt, before the addition of bulky flavors, contains not less than 3.25% milkfat and not less than 8.25% milk solids not fat, and has a titratable acidity of not less than 0.9%, expressed as lactic acid. Yogurt may be heat treated after fermenting to destroy viable microorganisms for a longer shelf life of the food".

Lowfat yogurt and nonfat yogurt are similar in description to yogurt but contain 0.5 to 2% and less than 0.5% milkfat, respectively (FDA, 1996a; FDA, 1996b).

In many other countries, regulatory agents follow the definition of yogurt defined by Food and Agriculture Organization/World Health Organization (FAO/WHO). The FAO/WHO Codex Alimentarius Commission defines yogurt as "a coagulated milk product obtained by lactic acid fermentation through the action of *Lb. delbrueckii* subsp. bulgaricus and *St. thermophilus* from milk (pasteurized or concentrated milk) with or without additions (milk powder, skim milk powder, etc.). The microorganisms in the final product must be viable and abundant" (Mareschi and Cueff, 1989; Rasic, 1987).

Manufacture of yogurt

Although, yogurt was accidentally discovered in ancient times through natural processes, yogurt manufacturing procedures are now highly developed. Before yogurt bacteria were discovered, no one knew what caused milk to coagulate. Yogurt was traditionally made from boiled milk inoculated with yogurt from the previous day. Inoculated milk was kept overnight at room temperature (Tamime and Deeth, 1980; Tamime and Robinson, 1985). Since the first commercial production of yogurt by Danone in Spain in 1922, yogurt manufacturing has expanded dramatically and quality has improved. Some guidelines for yogurt manufacturing have been described by Norling (1979) and White (1995).

Raw materials. Yogurt was traditionally made from milk with no added ingredients. To improve yogurt texture, milk or skim milk was fortified with other materials such as nonfat dry milk (NDM), whey protein concentrate (WPC) and some other dairy or plant-based ingredients. Milk from at least nine different species is used commercially; cow, mare, ass, goat, buffalo, yak, ewe, reindeer and camel (Kehagias and Dalles, 1984; Kroger *et al*., 1989; Rasic and Kurmann, 1978; Tamime and Robinson, 1985). These milks differ in composition from each other, which considerably affects yogurt qualities. For example, yogurt made from milk of ewe, buffalo, yak or reindeer is high in fat (6.5 to 11.0%) and has better consistency than that made from milk of cow, goat and ass (1.4 to 4.0% fat) (Rasic and Kurmann, 1978).

Preparation of yogurt mix often involves addition to milk of milk powder, sweet buttermilk powder (BMP), whey powder, WPC, casein powder or plant protein, and concentration by evaporation, reverse osmosis or ultrafiltration (Broome *et al*., 1982; Bundgaard *et al*., 1972; Chapman *et al*., 1974; Jepsen, 1979; Kolar *et al*., 1979; Mistry and Hassan, 1992; Norling, 1979; Tamime and Deeth, 1980; Tamime and Robinson, 1985; White, 1995). The addition of 3 to 4% of NDM to yogurt mix is common to increase total solids (TS) for lowfat, nonfat and low calorie yogurt (Morris and Ghaleb, 1995; Tamime *et al*., 1994). Researchers (Harwalkar and Kalab, 1986) found that yogurt with a higher TS content was less susceptible to syneresis and had shorter casein particle chains. Modler *et al*. (1983) compared the physical and sensory properties of yogurt fortified with casein-based proteins (sodium caseinate, ultrafiltered milk and NDM) and whey proteins concentrated by three different methods (ultrafiltration, ion exchange and electrodialysis/lactose crystallization) to increase protein content by 0.5 and 1.0% in yogurt mixes. It was found that yogurts with added casein-based proteins had a coarser texture than those with added WPC. To increase gel strength and reduce syneresis of yogurt, sodium caseinate was the most effective supplement. However, they did not recommend the use of WPC as a replacement for starch and hydrocolloids because of excessive syneresis (Modler and Kalab, 1983; Modler *et al*., 1983).

Nonfat and lowfat yogurt can also be made from plant-based ingredients such as soybean milk. However, such yogurt has a beany flavor and produces flatulence. Soybean yogurt mix with added NDM, evaporated milk or whey-based ingredients can reduce beany flavor and flatulence due to improved fermentation by yogurt cultures (Buono *et al*., 1990; Granata and Morr, 1996; Karleskind *et al*., 1991). Sufficient yogurt flavor intensity cannot be obtained in soy milk-based yogurt (Lee *et al*., 1990). To increase amounts of nutrients such as vitamin A, C and dietary fiber, Collins *et al*. (1991) used milk with added sweet potato as raw material for yogurt production. This yogurt received a mean score of 7.7 out of 10 for flavor. Soy protein isolates have also been investigated to replace NDM in yogurt manufacturing to improve viscosity and reduce syneresis (Kolar *et al*., 1979).

A scientist in Cornell University's Food Science and Technology Department (Kosikowski, 1979) used diluted ultrafiltered skim milk for low-lactose yogurt production. The yogurt flavor was flat, thus BMP and/or sodium citrate were added to improve lactic acid fermentation. Becker and Puhan (1989) reported that using ultrafiltered milk in yogurt manufacturing could increase the nutritional value of yogurt because of higher protein, calcium and phosphorus content in the product.

Homogenization. Homogenization breaks down fat into smaller globules which prevents the formation of a cream line. This improves the consistency and viscosity of yogurt, thus a greater stability to syneresis can be obtained (Rasic and Kurmann, 1978; Tamime and Deeth, 1980; Tamime and Robinson, 1985). Furthermore, homogenization of yogurt mix breaks up powdered ingredients resulting in uniform distribution of the ingredients (Vedamuthu, 1991). According to Schmidt and Bledsoe (1995), homogenization has an adverse impact on yogurt with a lower fat content; it increases syneresis or reduces water-holding capacity due to empty spaces between casein matrices, and lack of native milkfat globule membrane (FGM) (Schmidt and Bledsoe, 1995). In higher fat yogurts clusters of fat globules can fill up these spaces, thus syneresis can be minimized. Darling and Butcher (1978) studied FGM in homogenized cream and reported that caseins and whey proteins were adsorbed on the fat-serum interface. The whey proteins in unpasteurized cream could be easily removed from the interface, but after pasteurization they strongly bonded and were more difficult to remove.

Heat treatment. The objectives of heat treatment of yogurt mix are to kill pathogenic microorganisms, to minimize spoilage microorganisms and to inactivate lipase and hence to prevent lipolysis (Rasic and Kurmann, 1978). Yogurt mix is normally heated at a higher temperature and longer time than normal pasteurization, ranging from 90 to 95°C for 5 to 10 min, to help improve product consistency through whey protein denaturation (Mottar *et al*., 1989; Rasic and Kurmann, 1978; Tamime and Deeth, 1980; Tamime and Robinson, 1985). The degree of denaturation depends on the intensity of heat applied. Low TS yogurt may require more whey protein denaturation than high TS yogurt (Rasic and Kurmann, 1978). Whey proteins which participate in casein aggregation in yogurt are α-lactalbumin (**α-LA**) and β-lactoglobulin (**β-LG**). The former has a denaturation temperature of 62°C and the latter, 78°C (Wong *et al*., 1988). Denaturation of whey proteins is determined not only by heat treatment but also by other conditions. For example, the removal of calcium prevents α-LA redenaturation. The native tertiary structure of heat treated α-LA can be recovered upon cooling because of its high affinity to calcium. Thus calcium aids in reconstruction of the tertiary structure of α-LA (Bernal and Jelen, 1984). β-LG accounts for 50% of whey proteins in skim milk or 9% of total protein, two times more than α-LA, therefore it plays an important role in casein aggregation (Wong *et al*., 1988). When κ-casein is heated in the presence of β-LG, κ-casein - β-LG complex is formed via disulfide bonds (Davies *et al*., 1978; Elfagm and Wheelock, 1978; Long *et al*., 1963; Tessier and Rose, 1964). β-LG could also react with α-LA during heating (Elfagm and Wheelock, 1978; Elfagm and Wheelock, 1978; Tamime and Deeth, 1980; Tamime and Robinson, 1985). Mottar *et al*. (1989) indicated that the ratio of β-LG to α-LA at micellar surface and the level of heat treatment affected yogurt texture. Rasic and Kurmann (1978) indicated that during heat treatment there were two possible events depending on heat intensity; very high heat treatment such as 90°C for 10 min induces both α-LA and β-LG to participate in protein aggregation resulting in less susceptibility to syneresis in yogurt, but a lower heat treatment (vat pasteurization) causes β-LG to participate rather than α-LA because 80 to 90% of denatured α-LA is reversible after heating (Wong *et al*., 1988). Incorporation of β-LG at the micellar surface gives a long chain casein micelle linked by a finely flocculated protein resulting in a loose structure and an increase in syneresis (Modler and Kalab, 1983).

Microbiology of yogurt

Early investigations of yogurt microorganisms were conducted by a number of scientists such as Metchnikoff (1904, 1907), Grigoroff (1905), Maze (1905) and Guerbet (1906). They found rod and coccal-shaped bacteria, yeasts and molds in yogurt (Rasic and Kurmann, 1978; Tamime and Robinson, 1985). Much of the credit for the study of yogurt bacteria was contributed to Orla-Jensen. Yogurt bacteria are now characterized as lactic acid bacteria belong to the *Lactobacillaceae* and *Streptococcaceae* genera. Generally, yogurt cultures are *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, which are thermoduric, homofermentative lactic acid bacteria (Tamime and Deeth, 1980). Some other strains such as *L. helveticus, L. jugurti, L. acidophilus* and *Bifidobacterium* spp. are also sometimes used as adjuncts.

Lactobacillus delbrueckii subsp. *bulgaricus* can ferment glucose, fructose, galactose and lactose to lactic acid. It produces D (-)-lactic acid up to 1.7% in milk and has a growth temperature of 22 to 60°C (Rasic and Kurmann, 1978) with an optimum growth temperature of 40 to 50°C (Mayra-Makinen and Bigret, 1993). The growth temperature for *St. thermophilus* ranges from 20 to 50°C with an optimum of 40 to 45°C. It can ferment glucose, fructose, lactose and saccharose and produces L (+)-lactic acid up to 0.7 to 0.8% in milk. It can grow in the presence of bile salts, but is very sensitive to antibiotics. Both yogurt organisms are non-motile. The average size of *L. delbrueckii* subsp. *bulgaricus* is 0.8 to 1.0 × 4 to 6 µm, and that of *S. thermophilus* is 0.7 to 0.9 µm in diameter (Rasic and Kurmann, 1978).

When a single strain of either *L. delbrueckii* subsp. *bulgaricus* or *S. thermophilus* is used, lactic acid and acetaldehyde production is lower compared with that in a mixed culture (Hamdan *et al*., 1971; Rasic and Kurmann, 1978). There are two stages involved in yogurt fermentation. In the first stage, *L. delbrueckii* subsp. *bulgaricus* stimulates the growth of *S. thermophilus* by liberating essential amino acids from casein by proteolytic activity (Sandine and Elliker, 1970). In this stage, *L. delbrueckii* subsp. bulgaricus grows slowly because it is microaerophilic (Vedamuthu, 1991). At the end of the first stage, the growth of *S. thermophilus* is slowed down because of the high lactic acid concentration. When *S. thermophilus* produces enough formic acid, which stimulates growth of *L. delbrueckii* subsp. *bulgaricus*, the second stage begins. By this symbiotic action, the desirable acidity of the final yogurt can be achieved (Rasic and Kurmann, 1978). *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are proteolytic bacteria. The former has a higher proteolytic activity.

Slocum *et al*. (1988) reported that in yogurt with 10.0 to 17.5% total solid (TS) maximum proteolysis occurred at 14.5% TS.

Although, there is no regulatory requirement in the U.S. regarding the number of viable *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, it has been established with respect to therapeutic properties that yogurt should contain live lactic acid bacteria (Roberts and Maust, 1995). In countries such as Japan, South Korea and Poland, legislation requires viable lactic acid bacteria in the final product ranging from 10^6 to 10^8 cells/g (Hamann and Marth, 1984; Orihara *et al*., 1992).

Media for rods and cocci. Media for differentiation of rods and cocci in yogurt have been studied. Lee *et al*. (1974) differentiated rod and coccus colonies based on the capability for saccharose utilization of *L. delbrueckii* subsp. bulgaricus and *S. thermophilus*. In the presence of saccharose in a basal media with added bromcresol purple indicator, referred to as Lee's media, *S. thermophilus* ferments both saccharose and lactose and lowers pH to change the color of the bromcresol purple indicator, and thus forms yellow colonies. Although *L. delbrueckii* subsp. bulgaricus is a stronger acid producer, it can ferment lactose but not saccharose. Thus, it produces less acid, and forms white colonies in this media. Matalon and Sandine (1986) modified Elliker's lactic agar by addition of 0.1% Tween 80 and 7.0% sterile reconstituted NDM. This media was referred to as Yogurt Lactic Agar. As *L. delbrueckii* subsp. bulgaricus can produce more lactic acid, its larger colonies have cloudy zones, which result from casein precipitation, whereas colonies of *S. thermophilus* in the same media are smaller and lack cloudy zones.

Acetaldehyde production in yogurt

Generally yogurt flavor, other than lactic acid, consists of acetaldehyde, acetone, acetoin and a small amount of diacetyl (Sandine and Elliker, 1970). Among these, acetaldehyde is believed to be responsible for typical yogurt flavor (Bottazzi and Dellaglio, 1967). *Lactobacillus delbrueckii* subsp. bulgaricus is more capable in both acid and acetaldehyde production compared to *S. thermophilus* (Singh and Sharma, 1982). Acetaldehyde is mainly produced from glucose via pyruvate catabolism (Keenan and Bills, 1968; Lees, 1969; Lees and Jago, 1978). Yogurt bacteria also have threonine aldolase, which converts threonine to acetaldehyde and glycine. *Streptococcus thermophilus* may be responsible for increased acetaldehyde production as its threonine aldolase is stimulated by the addition of threonine (Wilkins *et al*., 1986). Marranzini *et al*. (1989) used ultrafiltration to deplete free amino acids in the skim milk in their study. Lactose and standard free amino acids except threonine and glycine were added to skim milk. They found that threonine aldolase activities of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were reduced in the high glycine and low threonine media, but increased in high threonine and low glycine media. Threonine and glycine concentrations did not affect yogurt bacteria growth and acid production. Marshall and El-Bagoury (1986) increased acetaldehyde level in goat's milk yogurt by adding 0.1% threonine to yogurt mix made by ultrafiltered milk.

Textural properties of yogurt

Textural properties for yogurt include viscosity, firmness and syneresis. Measuring viscosity of yogurt is challenging because it is non-Newtonian, i.e. viscosity changes as shear stress changes (Charm, 1971). Therefore, in order to report on the apparent viscosity of yogurt, one has to precisely specify the measurement conditions used.

Viscosity of yogurt is affected by composition, type of starter cultures, heat treatment and stabilizer. As the TS increase, viscosity and firmness increase (Becker and Puhan, 1989; Guirguis *et al*., 1984). Ropy strains of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* have been studied and used to produce smooth and viscous yogurt (Hess *et al*., 1997; Vedamuthu, 1991). These bacteria, often called slime-producing bacteria, produce exopolysaccharides, which help increase viscosity.

Heat treatment affects textural properties of yogurt. Labropoulos *et al.* (1981) indicated that yogurt made from UHT-treated milk (149°C, 3.3 s) had lower viscosity than that made from vat-treated milk (63°C and 82°C, 30 min). This was confirmed by Salji *et al.* (1984). When less intense heat treatment was used (Schmidt *et al.*, 1985), yogurt made from UHT-treated milk (138°C, 3 to 6 s) had lower viscosity and firmness but less syneresis than that made from vat-treated milk (82°C, 20 min). Whey protein denaturation was believed to be responsible for this phenomenon.

Becker and Puhan (1989) noted that yogurt fortified with ultrafiltered milk had higher viscosity and firmness compared to yogurt fortified with NDM and evaporated milk with similar composition. Susceptibility to syneresis of 2.0% fat and 5.0% protein yogurts fortified with various dairy ingredients including NDM, BMP, sodium caseinate and WPC (35 to 75% protein) were studied by Guinee *et al.* (1995). They reported that BMP yogurt yielded the least syneresis.

During cold storage of milk that has been contaminated by psychrotrophic bacteria, κ-casein is subjected to break down by proteolysis. This causes the yogurt made from such milk to be firmer, more viscous and sensitive to syneresis; however, sensory qualities of the yogurt were not reported (Gassem and Frank, 1991).

Microstructure of yogurt

Microstructure of yogurt is usually studied by transmission electron microscopy (TEM) of thin section and scanning electron microscopy (SEM) (Kalab *et al.*, 1983). Sample preparation for TEM is time consuming but it allows for the visualization of casein aggregation, whereas with SEM sample preparation is easier and aids in the study of gel structure (Kalab and Harwalkar, 1973). Skriver *et al.* (1995) used SEM to differentiate the microstructure of yogurt made with ropy and nonropy strains of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. Harwalkar and Kalab (1986) found that an increase in TS increased the density of yogurt matrices which resulted in decreased syneresis. Interestingly, casein particles of 30% TS yogurt were finer than those of 10% TS yogurt. Yogurt made by fermentation to pH 3.85 was more porous and firmer than that made by fermentation to pH 4.5.

Heat treatment of a yogurt mix causes changes in the microstructure of finished product. As previously mentioned, denaturation of whey proteins is responsible for the changes in gel formation. Protein micelles in yogurt mix when heated at 90°C for 10 min have appendages which are complexes of whey proteins and κ-casein, but without casein fusion (Davies *et al.*, 1978). During the fermentation process, these appendages inhibit casein fusion resulting in a loose structure (Davies *et al.*, 1978; Mottar *et al.*, 1989). Davies *et al.* (1978) noted that not only temperature of heat treatment but holding time was also an important factor influencing the appendage formation on the micelle surface. Mistry and Hassan (1992) studied the use of high milk protein powder (18.9% protein) in the production of nonfat yogurts (5.04 to 7.39% protein). They indicated that a high protein yogurt (7.39%) yielded a less porous structure and had lower syneresis than lower protein yogurts.

The existence of FGM in reconstituted BMP and yogurt made from such powder was confirmed by TEM (Kalab, 1980). Tamime *et al.* (1989) noted that FGM of large fat globules (5 μm) in Labneh (6.8 to 8.2% protein and 9.2 to 10.5% fat) were broken by homogenization, whereas FGM of small fat globules were intact but they could not conclude if incorporation of FGM improved yogurt quality (Tamime *et al.*, 1991). Aguilera and Kinsella (1991) reported that recombined fat globules with diameters < 1.26 μm increased the compression stress of a gel consisting of 8.3% NDM, 3% whey protein isolate (92.7% protein) and 4.4% fat.

In a recent development, confocal scanning laser microscopy was used to study the microstructure of yogurt during fermentation in real time without sample preparation (Hassan *et al.*, 1995). It was noted that there were three stages involved in yogurt fermentation: lag phase, aggregation and contraction of milk proteins.

Nonfat and lowfat yogurt

Nonfat and lowfat yogurts were introduced to meet consumer needs. During the past decade, many attempts have been made to produce nonfat and lowfat yogurts which are similar in quality to full fat yogurt. In order to be able to state "nonfat" or "lowfat" on the label, a yogurt manufacturer must produce yogurt conforming to fat content requirements. Roberts and Maust (1995) measured fat content in nonfat, 1% and 1.5% fat yogurts and reported that 50% of these commercial yogurts did not meet the requirements for fat content.

To manufacture reduced fat yogurt, proper ingredient selection is important such as use of skim milk and NDM. However the consequences can be undesirable. Adding NDM increases caloric value of yogurt and acid production as about 50% of NDM is lactose (Kalab *et al*., 1983). A powdery flavor can be detected in yogurt fortified with too much NDM (Tamime and Robinson, 1985). Using fat replacers is another effort to reduce fat content in yogurt. Farooq and Haque (1992) reported that nonfat low calorie yogurt produced by adding a sugar ester as a fat substitute, aspartame as a sweetener and a cream-like substance as a flavor enhancer had a better mouthfeel and texture compared with yogurt without the fat substitute. Microstructure of yogurt containing whey protein-based fat substitute, Simplesse 100™, or anhydrous milkfat were compared by Tamime *et al*. (1995). Both the fat substitute with 0.1 to 3 µm diameter and the anhydrous milkfat interacted with protein matrices, but yogurt with the fat substitute was softer and more susceptible to syneresis. In a study on the effect of seven different starch-based fat substitutes on nonfat yogurt (0.1% fat) firmness of yogurt increased with the substitutes, but flavor and mouthfeel worsened (Tamime *et al*., 1996). Lowfat yogurt (1.5% fat) made with oils from olive, groundnut, sunflower and maize differed in flavor and aroma from yogurt made with anhydrous milkfat but the oils did not affect lactic acid bacteria (Barrantes *et al*., 1996; Barrantes *et al*., 1996).

Nonfat yogurt is normally low in TS (10 to 12%) and consequently suffers from whey separation or syneresis (Harwalkar and Kalab, 1986; Schellhaass and Morris, 1985). To reduce syneresis, thickening agents such as gelatin, starch, cellulose derivatives, alginates and carrageenan can be legally added (FDA, 1996b). Gelatin and carrageenan result in the clustering of casein micelles (Kalab *et al*., 1983), therefore potentially reducing syneresis (Modler *et al*., 1983). As indicated earlier, yogurt texture may also be improved by the utilization of ropy strains of yogurt bacteria (Hess *et al*., 1997; Teggatz and Morris, 1990).

Summary

Yogurt manufacturing has long been known to mankind. Yogurt gained its popularity from health-concerned consumers worldwide. Due to the presence of cholesterol in milkfat, nonfat and lowfat yogurt were invented. Similar to Bulgarian milk, other nutritive and therapeutic properties are of significance.

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