A Comprehensive Review on Horse Gram (*Macrotyloma uniflorum*) Beverage - Technological Advancements

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Abstract: Horse gram (*Macrotyloma uniflorum*) is a poorly exploited but more nutritious legume that is mostly grown in South and South-east Asia. Horse gram is traditionally eaten cooked, but, in recent years, there has been a paradigm shift: it has been technologically transformed into plant-based milk, and it is now a sustainable and functional substitute of conventional dairy. This review is a review of the technological innovations behind the horse *gram beverage* development, including pre-processing strategies, optimization of extraction, enzymatic and physicochemical modifications, fortification strategies and packaging innovations. Nutritional character of horse *gram beverage* (high in proteins (2225%), dietary fiber, polyphenols, and essential minerals) is considered along with its functional characteristics such antioxidant, anti-diabetic, anti-urolithiatic, and anti-inflammatory. The difficulties associated with beany flavor, low colloidal stability, and anti-nutritional issues are overcome based on the state-of-the-art processing interventions that include ultrasound-aided extraction, high-pressure homogenization, fermentation, and enzymatic hydrolysis. The review also explains on quality parameters, regulatory considerations and future research directions. The milk of horses is one of the good alternatives in the global plant-based dairy sector that has to be explored scientifically at large scale and introduced into the market.

Keywords: *Horse Gram Beverage; Macrotyloma Uniflorum; Plant-Based Milk; Legume Processing; Functional Food; Pulse Technology; Non-Dairy Beverages.*

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I. INTRODUCTION

The last decade has seen the global plant-based dairy market grow exponentially due to the growing interest in lactose intolerance, dairy allergies, environmental sustainability, and the growing popularity of vegan and flexitarian diets (12). Lactose malabsorption is observed in approximately 68 percent of the adult population across the globe, which puts in a dire need of non-dairy alternatives that are nutritionally adequate (17). Plant milks that have taken over the market, such as soy, almond, oat, and pea milk, are Legume-based, which has caused unique ecological and ethical issues such as water usage, land degradation, and allergicity (5).

In this changing scenario, *Macrotyloma uniflorum* Lam. The horse gram, (Verdc.) has become an unexploited source of nutritional and functional possibilities. The horse gram is a low-input legume that thrives on droughts and is planted in large quantities in India, Sri Lanka, Ethiopia, Malaysia, and Australia (1). Historically, horse gram has been viewed as an excellent source of nutritional value, especially in terms of protein (2225 percent), which is of a high quality, complex carbohydrates, dietary fiber, iron, calcium, phosphorus, and a variety of polyphenolic compounds, all of which are recognized today as the pulse of the poor man or cattle fodder (2)

The production of horse gram into a plant-based milk beverage is a major technological breakthrough. When optimized, horse *gram beverage* can provide a lower cost protein rich, allergen free and functionally superior substitute to not only dairy but also current plant based milks. Nevertheless, the commercial viability of the horse *gram beverage* product requires addressing multiple scientific issues, such as issues with the reduction of anti-nutritional factors (tannins, phytases, trypsinases), the removal of beany flavors, the improvement of colloidal stability, and the maximization of the extraction yield and sensory properties (16, 19).

This is a systematic review of existing scientific facts and technological progress in the development of horse *gram beverage* providing a combined look of agronomy, food chemistry, processing engineering, and consumer science. It is aimed at guiding researchers, food technologists and industry players on the path to commercialization of horse *gram beverage* as the next generation functional beverage.

II. HORSE GRAM - AGRONOMIC SIGNIFICANCE AND BOTANICAL PROFILE

A. Taxonomy and Morphology

Horse gram is a member of the Family Fabaceae (Leguminosae) and genus Macrotyloma. Formerly it was classified under Dolichos uniflorus and currently reclassified as *Macrotyloma uniflorum* (Lam.) Verdc. according to molecular phylogenetic research (18). It is an annual, twining herb that bears flattened oblong pods that contain 5-7 seeds in each pod. The seeds are 35 mm long and are dark brown to reddish-brown in color (15).

B. Cultivation and Agroclimatic Adaptations

Horse gram is known to be agronomically resilient. It grows in semi arid areas with rainfall of 600-900 mm and can withstand temperatures of 20-35o C. It is cultivated as a rainfed crop on the Deccan Plateau, India especially in Karnataka, Andhra Pradesh, Tamil Nadu and Odisha (1). Its capacity to fix nitrogen through Rhizobium symbiosis makes it ecologically good which enhances soil fertility with few synthetic chemicals (7). Horse gram is one of the most sustainable legumes that can be utilized in the development of plant-based products with water footprint that is much lower than that of soy or almonds (13).

C. Statistical Production and Distribution on the Globe

India produces about 90 percent of the total horse gram production with annual contribution of more than 300,000 metric tonnes. Other producing countries are Myanmar, Malaysia, Philippines and Ethiopia. Although nutritionally strong and ecologically beneficial, horse gram is an extremely underused crop in contemporary food production chains mainly because of the lack of consumer awareness and industrial processing (4).

III. NUTRITIONAL AND PHYTOCHEMICAL CHARACTERISTICS OF HORSE GRAM

A. Macronutrient Composition

Horse gram has a high nutritional value, protein level of horse gram is 22 to 25% dry weight basis, and it is one of the richest plants protein sources among the underutilized legumes (2). A balanced amino acid profile is determined by the protein quality, with the following concentrations of lysine (6.2 g/100 g protein), leucine (8.5 g/100 g protein), and valine. Total carbohydrates make up about 55-60 percent of dry matter with resistant starch making up a good proportion of it, which bears the properties of low glycemic index (8). The amount of crude fat is between 0.5 and 2.1, and it consists of unsaturated fatty acids such as linoleic (C18:2) and oleic (C18:1) acids (11).

B. Micronutrient Composition

Horse gram is highly concentrated in minerals. The iron content (6.77 9.07 mg/100 g) is higher compared to the majority of common legumes and is close to that of red meat on a per-serving basis (14). Calcium (287–350 mg/100 g), phosphorus (311 mg/100 g), and zinc (3.22 mg/100 g) are present in nutritionally significant quantities (2). It is also nutritionally superior to the traditional sources of plant-based milk due to the B-vitamin complex and especially thiamine (0.41 mg/100 g), riboflavin (0.20 mg/100 g), and niacin (1.50 mg/100 g) (11).

C. Phytochemical and Bioactive Components

Horse gram is also an extremely good source of polyphenolic (quercetin, kaempferol), phenolic acids (caffeic acid, p-coumaric acid), and condensed tannins (11). Its total polyphenol content is 3.5 to 7.2mg GAE/g, which is among the highest food legume polyphenol content reported (14). Bioactive components of functional relevance are also phytosterols (beta-sitosterol, campesterol), saponins, and trypsin inhibitors (2). Raw horse gram contains lectins and hemagglutinins, which are successfully killed by heat to ensure milk processing procedures (16).

D. Anti-nutritional Factors

Horse gram contains anti-nutritional factors (ANFs) which are the tannins (2.31 4.30 mg catechin equivalent/g), phytic acid (0.57 0.91% dry weight), trypsin inhibitors (12.8 18.2 TIU/mg), hemagglutinins, and oxalates (19). These substances decrease the bioavailability of the mineral, block digestive enzymes, and disrupt protein digestion. Their decrease is one of the vital technological aims in the production of horse *gram beverage*, which will be discussed in the further parts of this review.

IV. HORSE *GRAM BEVERAGE* PRE-PROCESSING TECHNOLOGY

A pre-processing step that triggers seed hydration, water-soluble ANFs leaching (tannins, phytases), and endogenous enzymatic activity is soaking horse gram seeds in water (616 hrs). It has been reported that phytic acid and tannins decrease by 30-42 and 25-38 percent respectively after soaking at ambient temperature (16). The ANF reduction, i.e. phytate losses of up to 50% without incurring significant nutritional losses, has been found to be better with alkaline soaking (0.1 0.5% $NaHCO_3$) (8). Soaking water should be washed off before the extraction of milk to avoid re-absorption of ANFs.

A. Thermal Processing

Lipoxygenase enzymes that cause beany flavor development in legume milks are effectively inactivated by blanching (95 C 3 -10 minutes). Manthey and Tweten (10) showed that hexanal (beany flavor volatile) was decreased by more than 90 percent in soy analogs by steam blanching at 100 o C in 5 minutes, which can be applied to horse gram. Autoclaving (121 o C, 15 minutes) destroys trypsin inhibitors and hemagglutinins completely and removes tannin content by 40 55 percent (2). Nevertheless, too much thermal treatment may lead to Maillard browning and loss of B-vitamins, which are sensitive to heat, causing the optimization of the process.

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B. Germination (Sprouting)

Germination is a cheap, very efficient biotechnology tool of ANF reduction and nutritional improvement. Horse gram germination of 2472 hours at 2528 C reduces phytic acid (2852) and tannin (3045) as well as flatulence-causing oligosaccharides (raffinose, stachyose) by significant degrees (7). At the same time, germination leads to the activation of phytase and amylase, which raises the availability of the iron by 2.5 times and improves the digestibility of the proteins by 67-82 percent (2). The pool of free amino acids, especially GABA (gamma-aminobutyric acid), rises significantly during germination, which gives derived milk functional value (14). Horse *gram beverage*, which is germinated, has been reported to have a better antioxidant activity than when it is not germinated (11).

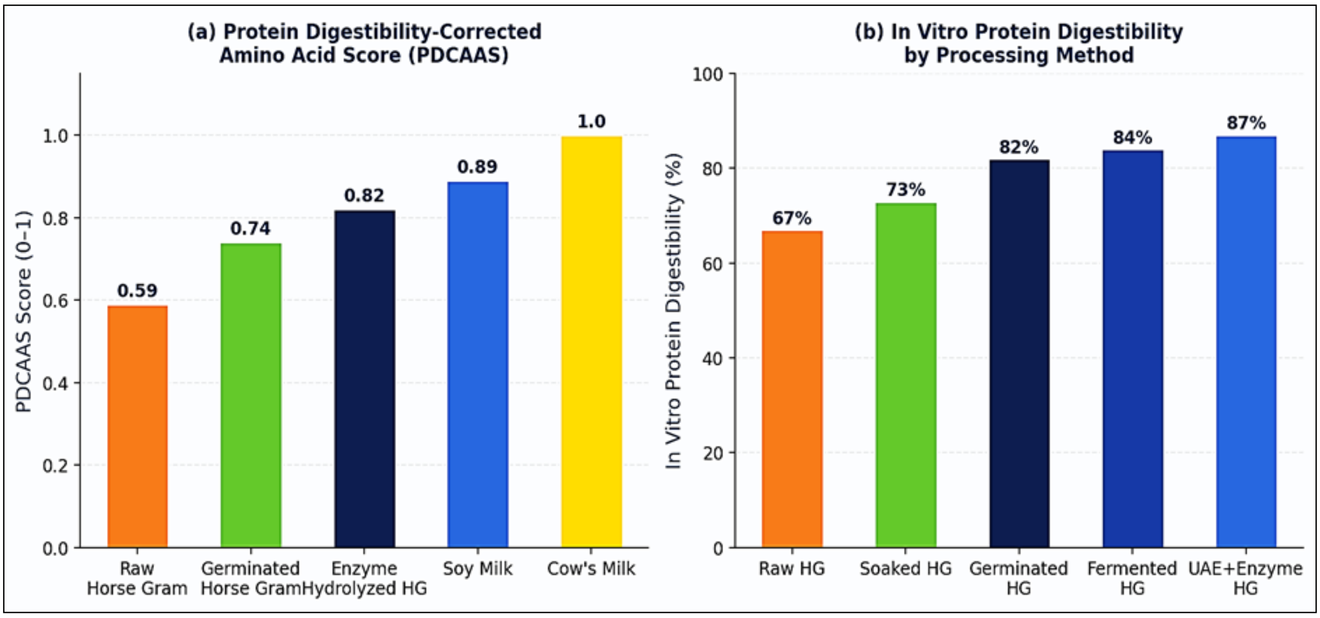

Fig 1. Protein Quality Metrics for Horse Gram Milk Under Various Processing Conditions

(a) PDCAAS = Protein Digestibility-Corrected Amino Acid Score (maximum = 1.0); (b) IVPD = In Vitro Protein Digestibility (%). Sources: (8, 2).

C. Fermentation

The fermentation with lactic acid bacteria (LAB) including Lactobacillus plantarum, L. acidophilus, and Leuconostoc mesenteroides is one of the new pre-processing methods of horse *gram beverage*. LAB fermentation (2448 hours) decreases phytic acid (4060 percent) through microbial fermentation phytase activity and degrades tannin and produces bioactive peptides with ACE-inhibitory and antioxidant functions (19). In studies of animal models, fermented horse *gram beverage* had much better protein digestibility (PDCAAS = 0.78 vs. 0.59 with nonfermented) and a lower glycemic index (11). Probiotic functionality is also provided by fermentation and the volatile flavor compounds are also controlled, eliminating beany notes.

V. OPTIMIZATION OF EXTRACTION TECHNOLOGIES AND PROCESS

A. Conventional Water Extraction

The classic wet-milling process consists of soaking, grinding and aqueous extraction of slurry horse gram followed by filtration and thermal treatment. Ratios of 1:5 to 1:10 (seed to water w/v) are usually used. The conventional extraction yield is between 55 and 65 percent recovery of the protein, and the left-over okara (fiber-protein cake) is a value-added by-product (8). The effects of pH of extraction medium are also a major factor affecting the yield; alkaline extraction (pH 8.59.0) increases protein solubilization by 1520 percent relative to neutral conditions (2).

B. Ultrasound-Assisted Extraction

Ultrasound-assisted extraction (UAE) relies on the acoustic cavitation effect to break cellular matrix, increase in mass transport and increase the extraction efficiency of proteins and bioactive compounds. UAE has been shown to extract proteins with 78-85% yield (300-600 W) applied to horse gram slurries at 2040 kHz over 1030 minutes with a corresponding reduction in extraction time (40 percent) by UAE as opposed to conventional techniques (6). UAE also alters protein secondary structure, enhancing hydrophobicity on the surface and enhancing ability to emulsify, which is essential in milk stability (9). The extraction efficiency of polyphenols increases by 30-45 percent in the ideal UAE conditions.

C. High Pressure Processing (HPP)

High-pressure processing (HPP; 100600 Mpa) is a non-thermal treatment that changes the conformation of proteins, inactivates enzymes and enhances the colloidal stability of bioactive compounds without thermal degradation of bioactive compounds. HHPP (400 MPa, 10 minutes) application raised the protein solubility by 22, beany flavor volatile compounds by 35, and emulation stability index by 68 to 84% in horse gram extract (19). The polyphenol content in HPP treated horse *gram beverage* was 92 percent higher than that of thermally processed counterparts (71 percent), which can be used to show the potential of the technology in producing high-quality functional milk (9).

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D. Enzymatic Extraction

The degradation of the cell wall enzymes (cellulase, hemicellulase, pectinase) are associated with the disruption of the cell matrix in the legumes, which releases the encapsulated proteins and bioactive compounds. Enzyme-aqueous extraction of horse gram with a cellulase-pectinase cocktail (0.5% enzyme concentration, 50 o C, 60 minutes) improved protein recovery by 30-35% and polyphenol recovery by 40% over control aqueous extraction (12). The addition of protease (Alcalase, 0.3% pH 8.0) produces protein hydrolysates, which are more soluble over a broader pH scope, which is essential in the stability of beverage formulations.

E. Microfluidization and nano-emulsification

Microfluidization (10,00020,000 psi) exposes the extracts of horse *gram beverage* to high shear forces and pressure drops to generate submicron droplets (less than 200 nm) of a homogenous distribution. This leads to much higher emulsion stability (>21 days), improved mouthfeel and decreased sedimentation. Microfluidized horse *gram beverage* had a 45 percent smaller D50 particle size, more protein-lipid interactions, and better creaming stability than those homogenized conventionally (6). Bioavailability of lipophilic bioactive compounds such as phytosterols and fat soluble vitamins which are introduced during fortification is also enhanced through nanoemulsification.

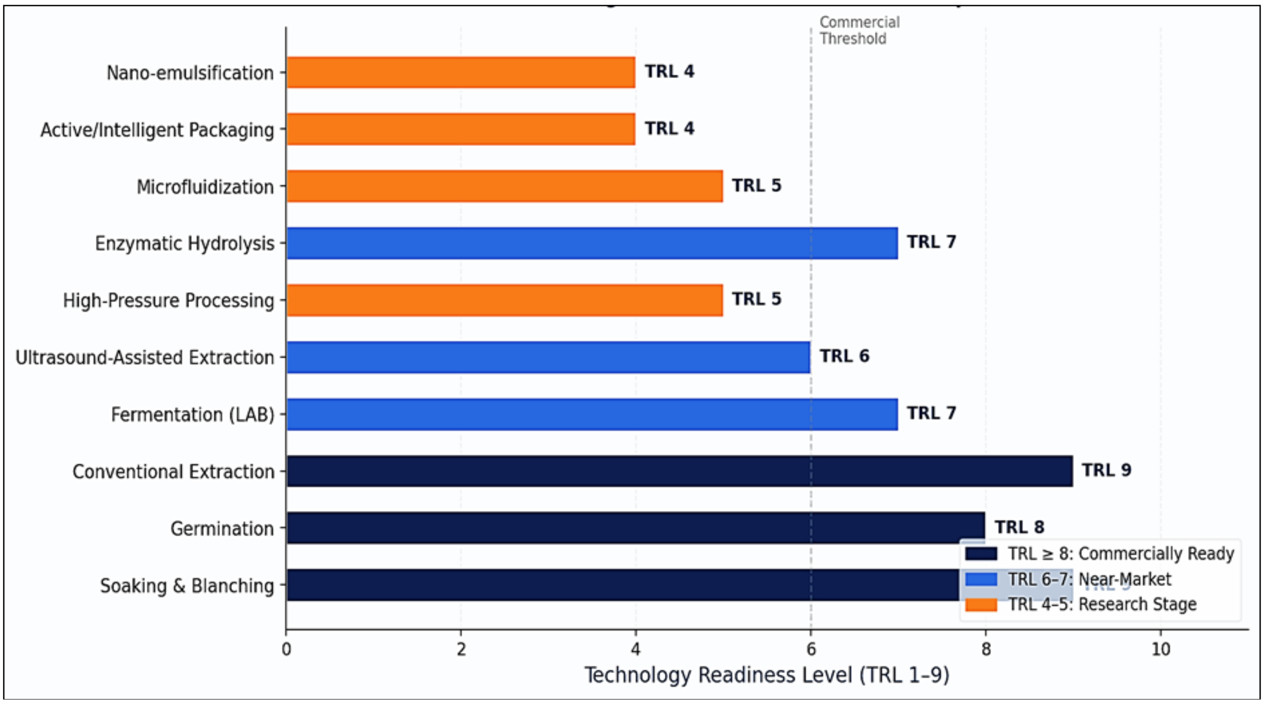

Fig 2. Technology Readiness Level (TRL) Assessment for Processing Technologies Applicable to Horse Gram Beverage Development.

TRL 1–3: basic research; TRL 4–6: development/demonstration; TRL 7–9: deployment/commercial. UAE = Ultrasound-Assisted Extraction; HPP = High-Pressure Processing; LAB = Lactic Acid Bacteria.

VI. STABILITY, SHELF LIFE AND PACKAGING TECHNOLOGIES

A. Physical and Colloidal Stability

Phase separation and sedimentation are the major issues of stability in horse *gram beverage*. Optimal values of horse *gram beverage* are -28 to -42 mV Zeta potential which indicates moderate to good electrostatic colloidal stability. The microfluidization process is applied to decrease the size of particles and the stabilization is performed by adding hydrocolloid stabilizers to improve the stability of creaming. Microbial safety is ensured by thermal processing (UHT: 138 o C, 4 seconds) without negatively affecting the shelf life of aseptically packed products (19).

B. Microbial Safety and Thermal Processing

Ultra-high temperature (UHT) processing is an effective sterilizer of horse *gram beverage* with a 12D inactivation of Clostridium botulinum spores at the cost of 85-90% heat-resistant bioactive compounds. Pasteurization (72 o C, 15 s; 90 o C, 10 s) can be used with refrigerated products that have shelf life of 15-21 days. High-pressure processing (HPP; 400600 MPa) is becoming an improved non-thermal method that has equal microbial lethality, and superior polyphenol (92 to 71% in thermal processing) and sensory retention (9).

Table 1. Comparison of Thermal and Non-Thermal Processing Technologies for Horse Gram Milk Safety, Shelf Life, and Bioactive Retention.

Processing Technology	Conditions	Microbial Reduction	Shelf Life	Polyphenol Retention (%)	Reference
Pasteurization (HTST)	72°C, 15 sec	5D	15–21 days (refrigerated)	85–88	(9)
Pasteurization (Extended)	90°C, 10 sec	6D	21–28 days (refrigerated)	80–85	(9)
UHT Processing	138°C, 4 sec	12D	6–9 months (ambient)	68–74	(19)
High-Pressure Processing (HPP)	400–600 MPa, 10 min	6–8D	45–60 days (refrigerated)	90–95	(9)
UHT + Active Packaging (O_2 scavenger)	138°C, 4 sec	12D	9–12 months (ambient)	70–76	(19)

D = log cycle reduction of Clostridium botulinum spores.

C. Fortification Strategies

To fill the most important nutritional gap of the horse *gram beverage* relative to cow milk, calcium fortification (calcium citrate or calcium carbonate, 120 mg/100 mL) is implemented. Fortification of vitamin D3 (1-2 ug 100mL) improves calcium bioavailability and incorporates immunomodulatory effects. The vegan consumers need vitamin B12 (0.4 µg/100 mL) fortification. Ferric pyrophosphate (2.4 mg/100 mL) as an iron fortification agent encapsulated in lipid matrices helps in avoiding the degradation of colors and flavors and preserving bioavailability. Encapsulation of omega-3 fatty acid (flaxseed oil microcapsules, 0.1%), improves functional profile but with no oxidative rancidity (12).

D. Active and Intelligence Packaging

Antimicrobial agents (nisin, natamycin, silver nanoparticles) and oxygen scavengers in active forms are added to packaging technologies and increase the shelf life of horse gram milk by 30-50. The Oxygen transmission rates of 2.1 cm 3 /m 2/day (comparable to conventional multilayer films) of bio-based packaging materials (PLA/PHBV composites) in line with sustainability goals of the horse gram value chain have been observed. PH-sensitive chromogenic indicators (anthocyanin-based) used in intelligent packaging can give real-time information on the freshness of food, preventing food waste and increasing consumer safety (19).

VII. CHALLENGES AND FUTURE RESEARCH DIRECTIONS

A. Key Technological Challenges

Although there have been tremendous improvements, there are a number of issues in commercializing horse gram milk. Through thermal treatment, protein degradation and browning, remnants of anti-nutritional properties in conventional processed foods, poor shelf stability at ambient temperature without UHT treatment, and high capital expenditure on equipment in the UAE, HPP and microfluidization technologies are challenges to widespread implementation. Processing parameters that are on the laboratory level to pilot and commercial scale bring about extra complications of mass and heat transfer that must be optimized through systematic engineering.

B. Future Research Priority

Future research may prioritizeon (1) Genome-wide association studies (GWAS) - to identify horse gram cultivars with inherently low ANF content and superior protein digestibility for targeted breeding; (2) Structural characterization and bioavailability quantification; (3) Consumer acceptance studies across diverse demographic and cultural units; (4) Randomized controlled clinical trials – to validate health claims (anti-diabetic, anti-urolithiatic, cardioprotective) for regulatory sustainability; (5) Investigation of synergistic blending with complementary plant milks (pea, oat) to achieve nutritional completeness; and (6) Techno-economic analysis of horse gram milk production at commercial scale across global market segments.

VIII. CONCLUSION

Horse gram (*Macrotyloma uniflorum*) is an interesting, underresearched plant-based milk foundation to develop next-generation plant-based milk. This review has revealed that horse gram has a unique nutritional and phytochemical profile with scientifically proven functional properties of antioxidant, anti-diabetic, anti-urolithiastic and cardioprotective actions. The arsenal of emerging technologies such as ultrasound-assisted extraction, high-pressure processing, enzymatic hydrolysis, germination, fermentation and microfluidization can be used to address the critical processing challenges which are; ANF reduction, beany flavor elimination, and colloidal stability improvement.

The integration of technological development, nutritional research, consumer preference to functional beverages based on plant, and regulatory transformation presents an opportunity that has never been seen before in commercializing horse gram milk.

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