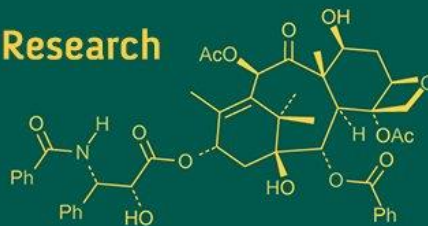
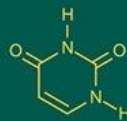
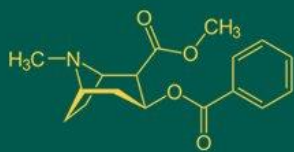


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2025): 5.29

IJABR 2025; 9(11): 421-425

www.biochemjournal.com

Received: 18-09-2025

Accepted: 21-10-2025

Pothale BB

M.Sc. (Agri), Department of Animal Husbandry and Dairy Science, College of Agriculture, Vasant Rao Naik Marathwada Krushi Vidyapeeth, Parbhani, Maharashtra, India

Padghan PV

Associate Professor, Department of Animal Husbandry and Dairy Science, College of Agriculture, Vasant Rao Naik Marathwada Krushi Vidyapeeth, Parbhani, Maharashtra, India

GK Londhe

Head of Department, Department of Animal Husbandry and Dairy Science, College of Agriculture, Vasant Rao Naik Marathwada Krushi Vidyapeeth, Parbhani, Maharashtra, India

Narwade SG

Associate Professor, Department of Animal Husbandry and Dairy Science, College of Agriculture, Vasant Rao Naik Marathwada Krushi Vidyapeeth, Parbhani, Maharashtra, India

Dhawale GR

M.Sc. (Agri), Department of Animal Husbandry and Dairy Science, College of Agriculture, Vasant Rao Naik Marathwada Krushi Vidyapeeth, Parbhani, Maharashtra, India

Corresponding Author:

Pothale BB

M.Sc. (Agri), Department of Animal Husbandry and Dairy Science, College of Agriculture, Vasant Rao Naik Marathwada Krushi Vidyapeeth, Parbhani, Maharashtra, India

Value addition of whey drink by using Ashwagandha (*Withania somnifera* L.)

Pothale BB, Padghan PV, GK Londhe, Narwade SG and Dhawale GR

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i11f.6312>

Abstract

The present investigation was undertaken to study the effect of adding *paneer* whey drink with *Ashwagandha* (*Withania somnifera*) extract at different levels (T₁: 100:0, T₂: 98:02, T₃: 96:04, T₄: 94:06 v/v) on its bioactive and microbiological properties. The drink were analyzed for total phenolic content, total flavonoid content, iron content, crude fibre and shelf life. The inclusion of *Ashwagandha* significantly improved the bioactive composition, with phenolic content ranging from 2.45 to 2.49 mg GAE/100 mL, flavonoids from 1.16 to 1.21 mg QE/100 mL, iron from 0.13 to 0.16 mg/100 mL and crude fibre from 0.15% to 0.40%. Shelf-life analysis showed that higher *Ashwagandha* levels suppressed microbial growth and preserved sensory quality for up to 12 days under refrigerated storage. These results indicate that *Ashwagandha*-enriched whey drink offer both functional and preservative benefits, making them suitable as health-promoting dairy-based drinks.

Keywords: *Paneer* whey, *Ashwagandha* extract, bioactive compounds, crude fibre, shelf life, microbiological quality

Introduction

The growing consumer interest in functional beverages has motivated researchers to explore fortification strategies that enhance both nutritional and therapeutic properties of dairy-based drinks. Whey, a by-product of paneer and cheese manufacture, is a rich source of high-quality proteins, lactose, minerals, and water-soluble vitamins. However, despite its nutritional potential, whey is often underutilized or discarded, causing environmental concerns. Transforming whey into functional beverages not only adds value but also addresses waste management issues in the dairy sector.

Ashwagandha (*Withania somnifera*), a medicinal plant widely recognized in Ayurvedic literature, is valued for its adaptogenic, antioxidant, anti-inflammatory and antimicrobial properties. The root, stem and leaf of *Withania somnifera* revealed the presence of Withaferine A and Withanolide D across all plant parts, though their proportions varied significantly. Withanolide D is most concentrated in the roots (0.193 per cent) and much less prevalent in the leaves. Conversely, Withaferine A was found in higher amounts in the leaves (0.236 per cent). The stem had the lowest overall concentration of Withaferine A and D combined, at just 0.055 per cent (Ganzera, 2003) [3]. Its roots are rich in polyphenols i.e 19.80 mg. Additionally, the free radical scavenging activity (DPPH) was measured at 67.16 Kumari and Gupta (2016) [6].

These compounds are known to scavenge free radicals, boost immunity and enhance overall health. In addition, the antimicrobial properties of *Ashwagandha* may help improve the microbiological stability of food products. *Ashwagandha* roots contain 21.0 to 25.0 per cent crude fiber, 6.09 to 9.46 mg/g starch and 0.39 to 0.82 mg/g tannins. They also include various minerals such as potassium (K), manganese (Mn), sodium (Na), iron (Fe), zinc (Zn), copper (Cu), aluminum (Al), calcium (Ca), cadmium (Cd) and nickel (Ni) (Mali *et al.*, 2024) [7].

Incorporating *Ashwagandha* extract into whey drink can thus simultaneously enhance the bioactive profile and extend shelf life by inhibiting microbial growth. This study aims to evaluate the impact of different inclusion levels of *Ashwagandha* extract on total phenolics, flavonoids, iron content, crude fibre and microbiological stability of whey drink during room and refrigerated storage.

Materials and Methods

Collection of Raw Materials

Buffalo milk with 6% fat and 9% SNF was procured from the local market. Fresh *Ashwagandha* roots were sourced from an Ayurvedic herb supplier. Food-grade crystalline sugar was used for sweetening and rose essence for better flavour. All chemicals and reagents used in analytical determinations were of analytical grade.

Preparation of *Ashwagandha* whey drink

Ashwagandha whey drink was prepared with slight modifications to the method of Yadav *et al.* (2010). Paneer whey was centrifuged at 800 rpm for 30 minutes to obtain clear whey. *Ashwagandha* extract was added according to treatment levels (2%, 4% and 6%), along with 6% sugar. The mixture was heated to 60 °C, stirred to dissolve the sugar and blended uniformly, followed by the addition of 0.5% rose essence for flavour. The drink was bottled, pasteurized at 85 °C for 30 minutes and stored under refrigeration at 5 °C.

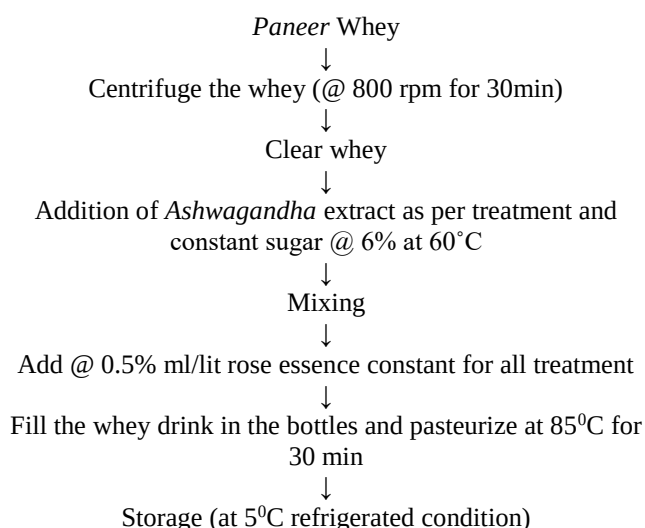


Figure Flow chart for preparation of *Ashwagandha* whey drink.

Formulation of Whey Beverages

Paneer whey added with *Ashwagandha* extract in ratios:

- T₁ = 100 parts whey (control) + 6% sugar + 0.5% rose essence
- T₂ = 98 parts whey + 2 parts *Ashwagandha* extract + 6% sugar + 0.5% rose essence
- T₃ = 96 parts whey + 4 parts extract + 6% sugar + 0.5% rose essence
- T₄ = 94 parts whey + 6 parts extract + 6% sugar + 0.5% rose essence.

Bioactive Compound Analysis

- **Total Phenolic Content:** Determined using Folin-Ciocalteu reagent, expressed as mg gallic acid equivalents (GAE)/100 mL.
- **Total Flavonoid Content:** Estimated by aluminium chloride colorimetric method, expressed as mg quercetin equivalents (QE)/100 mL.
- **Iron Content:** Determined using AOAC method with atomic absorption spectrophotometry.
- **Crude Fibre:** Measured using AOAC gravimetric method.

Microbiological and Shelf-Life Analysis

Samples stored at 5 °C and 27 °C were analyzed at 0, 3, 9 and 12 days for:

- Coliform count (MPN method)
 - Yeast & mould count (pour plate method)
- Sensory evaluation for color, flavor, consistency and overall acceptability was conducted using a 9-point hedonic scale by a semi trained panel of trained judges.

Results and Discussion

Iron Content (mg/100 mL)

From the table it can be clear that the iron absent in control treatment supported by the statement that milk is deficit in iron as the *ashwagandha* extract add the iron content increased in the developed drink from T₂ to T₄ from 0.13 to 0.16. The increased iron content in developed drinks supported by Kumari and Gupta, (2016) [6] mentioned that *ashwagandha* contained 3.3 mg iron, Gulati *et al.*, (2017) [4], (*Withania somnifera* L.) roots content iron varied from 595.83 to 983.33 ppm and Sayyad *et al.*, (2024) [12], investigated that *Ashwagandha* is rich in vital nutrients, providing a good source of key vitamins and minerals such as vitamin C, iron and calcium. In a 100-gram serving, it offers the following nutritional values: Energy: 245 Kcal, carbohydrates: 49.9 grams, Dietary Fiber: 32.3 grams, Protein: 3.9 grams, Iron: 3.3 mg.

Crude Fibre Content (%)

Crude fiber, a component of dietary fiber found in plant-based foods, plays a vital role in digestion and overall health. It adds bulk to food, aiding in bowel movements and promoting healthy gut function. It can be observed that from T₂ to T₄ the level of crude fiber was significantly increased with an increased in the level of *ashwagandha* extract, ranging from 0.15 (T₂) to 0.40 (T₄), due to the abundant amount of crude fiber presence in *ashwagandha* supported by Kumari and Gupta, (2016) [6], analysed the *ashwagandha* powder and recorded 32.3 g crude fiber, Gulati *et al.*, (2017) [4] mentioned crude fiber content ranged between 17.4 per cent and 37.3 per cent and Mali *et al.*, (2024) [7], recorded that *ashwagandha* roots contain 21.0 to 25.0 per cent crude fiber in their works.

Bioactive Properties

Total Flavonoid Content (mg CE/100 mL)

Flavonoids contribute not only to antioxidant activity but also exhibit anti-inflammatory and immune-boosting effects. Flavonoid content showed a similar upward trend with extract addition ranges from 1.16 (T₂) to 1.21 (T₄). This is due to the presence of natural flavonoid in the *ashwagandha* and supported by Baskaran *et al.*, (2012) [2] studied on the phytochemical analysis of *ashwagandha* and identified several bioactive compounds, including alkaloids, flavonoids, carbohydrates, steroids and saponin glycosides.

Total Phenolic Content (mg GAE/100 mL)

The phenolic content increased steadily with the level of *Ashwagandha* extract, reflecting the high concentration of polyphenols in the root. These compounds are potent antioxidants capable of neutralizing free radicals, thus enhancing the functional benefits of the drink. From the table it can be observed that from T₂ to T₄ the level of total phenol content was increased with increased in the level of *ashwagandha* extract ranges from 2.45 (T₁) to 2.49 (T₄), this

is due to the presence of natural phenol in the *ashwagandha* also supported by Baskaran *et al.*, (2012)^[2] and Munir *et al.*, (2022)^[10].

Treatment	Crude fiber	Iron	Total flavonoid content	Total phenol content
T ₁	0.00	0.00	0.00	0.00
T ₂	0.15 ^c	0.13 ^c	1.16 ^b	2.45 ^a
T ₃	0.32 ^b	0.14 ^b	1.19 ^{ab}	2.47 ^a
T ₄	0.40 ^a	0.16 ^a	1.21 ^a	2.49 ^a
SE(M)	±0.008	±0.009	±0.006	±0.006
C.D at 5 percent	0.03	0.02	0.01	0.02

Shelf Life and Microbiological Quality

Sensory shelf life was assessed under room condition (approx. 5⁰ and 27⁰ C using the sterilized bottle). Evaluation were performed freshly and throughout storage using the same semi expert judges of panel.

Sensory shelf life at room temperature

Colour and appearance ranges from 7.98 to 5.20 for all treatments it is clearly notice that colour and appearance score reduced more in control treatments as compared to developed treatments, flavour reduced from ranges from 8.78 to 5.78 which was also more in control treatment as compared to developed treatments. taste/mouthfeel ranges from 5.98 to 5.09 at 3rd days of storage period for progressive treatments, found better score for treatments T₃, overall acceptability ranges from 4.51 to 5.23 at 3rd days of storage period and rejected the product. It indicate that the storage period of developed product increased as the treatment increased from T₁ to T₄. Product remain acceptable up to 3 days at room temperature. Drink containing *ashwagandha* extract showed slightly better score compared to control. It may be due to the antioxidant properties of whey owing to *ashwagandha* extract supported by Mishra (2000), Gupta and Rana (2007)^[5], Alam *et al.*, (2012)^[1], Singh and Ramassamy (2018)^[13], Nile *et al.*, (2022)^[11] and Mohmad *et al.*, (2023) and antimicrobial properties.

Table Sensory shelf life of whey drink at room temperature

Treatment	DAYS			
	0 DAY	1 st DAY	2 nd DAY	3 rd DAY
T ₁	7.98	7.02	5.66	4.48
T ₂	7.02	6.42	5.48	4.78
T ₃	7.98	8.38	6.28	5.09
T ₄	7.64	7.39	6.22	5.20
Flavour				
T ₁	8.78	7.28	6.72	5.41
T ₂	8.61	8.05	7.21	6.05
T ₃	8.48	7.89	6.33	5.48
T ₄	8.19	7.25	6.09	5.78
Taste/Mouthfeel				
T ₁	8.24	7.78	4.64	5.98
T ₂	7.92	7.48	6.22	5.29
T ₃	6.42	6.09	6.57	6.98

T ₄	6.51	6.23	6.08	5.09
Overall acceptability				
T ₁	7.21	6.58	5.64	4.51
T ₂	7.05	6.72	5.58	4.98
T ₃	7.58	6.78	6.82	5.08
T ₄	7.28	7.01	6.99	5.23

Coliform Count (MPN/mL)

Table Coliform count of the developed whey drink at room temperature at (Log 10⁻¹ cfu/ml)

Treatment	Replication				
	0 day	1day	2day	3day	Mean
T ₁	0.00	0.00	0.00	0.00	0.00
T ₂	0.00	0.00	0.00	0.00	0.00
T ₃	0.00	0.00	0.00	0.00	0.00
T ₄	0.00	0.00	0.00	0.00	0.00

Table presents the coliform level (cfu/ml) in the *ashwagandha* whey drink stored at room temperature. The freshly prepared drink exhibited no detectable coliform and these remained consistent even after 3 days of storage. All treatments stayed within the ISS: 9617 (1980) Limit of 100 cfu/g, confirming hygienic production.

Yeast and mould count at room temperature:

Table Yeast and mould count of whey drink at room temperature at (Log10⁻¹ cfu/ml)

Treatment	Day				
	0 day	1day	2day	3day	Mean
T ₁	0.00	0.00	0.50	0.55	0.30 ^a
T ₂	0.00	0.00	0.69	0.58	0.28 ^a
T ₃	0.00	0.00	0.00	0.00	0.00 ^a
T ₄	0.00	0.00	0.00	0.00	0.00 ^a

The values with different small letters superscripts row wise differ significant at 5 per cent level of significance.

According to table yeast and mould count were determined by counting colony-forming unit per ml. Treatment T₃ and T₄ had no detectable count, while T₁ and T₂ showed the minimal growth: 0.30 and 0.28 cfu/ml, respectively on 2nd and 3rd days of storage period. All count remained well below the permissible IS limits of 100 cfu/g, ensuring the product safety.

Sensory shelf life at refrigerated temperature

According to table, the *ashwagandha* extract whey drink remain acceptable upto 5 weeks under refrigeration. Sensory score decrease over time. Colour and appearance ranges from 8.46 to 4.01, Flavour ranges from 8.78 to 4.46. Taste ranges from 8.24 to 5.21 and overall acceptability ranges from 8.49 to 4.56.

The extended shelf life under refrigeration temperature was attributed to lower temperature and controlled humidity. While *ashwagandha* showed a positive influence on shelf life, its effect was not statistically significant, likely due to small quantity used.

Table Sensory score at refrigerated temperature

Treatment	Days				
	0 DAY	3 rd DAY	6 th DAY	9 th DAY	12 th DAY
T ₁	8.46	8.15	7.94	7.48	6.01
T ₂	8.09	8.01	7.73	6.78	5.29
T ₃	7.98	8.38	6.28	6.01	4.71
T ₄	7.64	7.39	6.22	5.20	4.01
Flavour					
T ₁	8.78	8.28	7.49	6.33	5.79
T ₂	8.65	8.05	7.42	5.64	5.21
T ₃	8.48	7.85	6.82	4.99	5.04
T ₄	8.27	7.11	6.09	4.79	4.46
Taste/Mouthfeel					
T ₁	8.24	7.78	6.64	5.79	4.46
T ₂	7.79	7.41	6.36	5.27	5.09
T ₃	6.62	6.12	6.01	5.73	5.49
T ₄	6.74	6.49	6.08	5.09	5.21
Overall acceptability					
T ₁	8.49	8.07	7.35	6.53	5.42
T ₂	8.17	7.82	7.17	5.89	5.19
T ₃	7.69	7.45	6.37	5.57	5.08
T ₄	7.55	6.99	6.13	5.02	4.56

From the above table it can be observed that the as the days increases quality of the product decline. The colour and appearance ranges from 6.01 to 4.01, flavour ranges from 5.79 to 4.46, taste or mouthfeel ranges from 4.46 to 5.21 and the overall acceptability ranges from 5.42 to 4.56. The storage life at refrigerated is good for 12 days than at room temperature. This is because the storage temperature and humidity of April and May season which affect the quality of the product. The storage or shelf life of different developed whey drinks were found large variation as shown below may be due to the processing technic, ingredient used, preservatives and packaging.

Coliform count at refrigerated temperature

Table confirms that coliforms were absent throughout 9 days of refrigerated storage (less than 5 °celcius), meeting IS standards and confirming hygienic handling.

Treatments	Days				
	0 days	3 rd days	6 th days	9 th days	12 th days
T ₁	ND	ND	ND	0.90	1.30
T ₂	ND	ND	ND	0.60	0.90
T ₃	ND	ND	ND	ND	0.60
T ₄	ND	ND	ND	ND	ND

Coliform count at refrigerated temperature

Table shows coliform count in drink showed under refrigeration temperature growth was observed after 9 day of storage T₁ was more affected than T₄ which can possibly due to antimicrobial property of *ashwagandha*.

Table Yeast and mould count at refrigerated temperature at (Log10⁻¹ cfu/ml)

Treatments	Days				
	0 Day	3 rd Day	6 th Day	9 th Day	12 th Day
T ₁	ND	5.59	6.80	7.89	9.02
T ₂	ND	5.96	6.86	7.06	8.48
T ₃	ND	6.82	6.02	6.93	8.20
T ₄	ND	7.90	7.40	6.76	7.10

Table shows yeast and mould count in drink shored under refrigeration temperature growth was observed after 1 day of storage T₁ was more affected than T₄ which can possibly due to antimicrobial property of *ashwagandha*.

Conclusion

Ashwagandha addition enhanced the bioactive composition and microbiological stability of whey drink. Increased phenolic and flavonoid contents contributed to antioxidant potential, while higher iron and fibre improved nutritional value. Microbial growth was significantly reduced in higher inclusion levels, extending shelf life from 3 days to 12 days and preserving sensory quality. Fortification at 4-6% is recommended for optimal bioactivity and storage stability.

References

1. Alam N, Hossain M, Mottalib MA, Sulaiman SA, Gan SH, Khalil MI. Methanolic extracts of *Withania somnifera* leaves, fruits and roots possess antioxidant properties and antibacterial activities. BMC Complementary and Alternative Medicine. 2012;12(1):1-8. <https://doi.org/10.1186/1472-6882-12-175>
2. Baskaran C, Velu S. Phytochemical analysis and in-vitro antimicrobial activity of *Withania somnifera* (Ashwagandha). Journal of Natural Products and Plant Resources. 2012;2(6):711-716.
3. Ganzera M, Choudhary MI, Khan IA. Quantitative HPLC analysis of withanolides in *Withania somnifera*. Fitoterapia. 2003;74(1):68-76. [https://doi.org/10.1016/S0367-326X\(02\)00002-5](https://doi.org/10.1016/S0367-326X(02)00002-5)
4. Gulati S, Madan VK, Singh I, Singh S. Chemical and phytochemical composition of Ashwagandha (*Withania somnifera* L.) roots. Asian Journal of Chemistry. 2017;29(8).
5. Gupta GL, Rana AC. *Withania somnifera* (Ashwagandha): A review. Pharmacognosy Reviews. 2007;1(1):129-136.
6. Kumari S, Gupta A. Nutritional composition of dehydrated ashwagandha, shatavari and ginger root powder. International Journal of Home Science. 2016;2(3):68-70.

7. Mali PD, Adangale SB, Deokar DK, Lawar VS, Lokhande AT, Chougale C, Chawke A. Effect of supplementation of Ashwagandha (*Withania somnifera*) root powder on body weight gain and egg quality in layers. Journal of Experimental Agriculture International. 2024;46(7):1049-1055. <https://doi.org/10.9734/jeai/2024/v46i72313>
8. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. Alternative Medicine Reviews. 2000;5:334-346.
9. Mohamed EH, Mahmoud TA, Hegazy MA. Major biochemical constituents of *Withania somnifera* (Ashwagandha) extract: A review of chemical analysis. 2023.
10. Munir N, Zahed M, Shahid M, Afzal MN, Jahangir M, Shah SMA, et al. *Withania somnifera* chemical constituents' *in vitro* antioxidant potential and their response on spermatozoa parameters. Dose-Response. 2022;20(1):15593258221074936. <https://doi.org/10.1177/15593258221074936>
11. Nile SH, Nile A, Gansukh E, Baskar V, Kai G. Subcritical water extraction of withanosides and withanolides from Ashwagandha (*Withania somnifera* L.) and their biological activities. Food and Chemical Toxicology. 2019;132:110659. <https://doi.org/10.1016/j.fct.2019.110659>
12. Sayyad MR, Yunus A, Gayke AR, Salve MT, Shinde PN. Novel formulation and evaluation of health immunity booster capsule (prevention of various diseases) for Spirulina with Ashwagandha, beetroot and apricot. International Journal of Novel Research and Development. 2024;ISSN:2456-4184.
13. Singh M, Ramassamy C. *In vitro* screening of neuroprotective activity of Indian medicinal plant *Withania somnifera*. Journal of Nutritional Science. 2018;6:e54. <https://doi.org/10.1017/jns.2017.54>