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Effect of incorporation of Ashwagandha (*Withania somnifera* L.) in Quarg cheese at different levels for its improved physico-chemical and sensorial properties

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Abstract
India has been recognised as the global leader in terms of both size and rate of growth in the market for milk and milk products. The majority of milk inside the nation is generated by individuals classified as small-scale farmers and landless labourers. The milk output in India has shown a recent growth rate of 6.2% per annum. Hence, it is imperative to enhance the dairy processing infrastructure in order to meet the demands of the expanding dairy industry inside the nation (Anonymous, 2022). Dairy products can be classified into many categories, including heat desiccated, high in fat content, frozen, heat acid coagulated, and fermented dairy products. Among these categories, fermented dairy products are particularly widespread. Research has demonstrated that fermentation has the capacity to enhance the availability of minerals and the digestion of proteins and carbohydrates, while also enhancing the sensory qualities of the final product. This research paper investigates the incorporation of ashwagandha powder at different levels in the preparation of Quarg cheese. Ashwagandha (*Withania somnifera* L.) is a widely recognized medicinal herb with potential health benefits. The study aimed to determine the impact of ashwagandha powder on the sensory characteristics, physicochemical properties, and antioxidant activity of Quarg cheese. Four levels of ashwagandha powder (0.5%, 1%, 2.5% and 5% w/w) were incorporated into the cheese formulation. Sample T_4 was found to be better in terms of fat (10.89%), protein (13.47%), lactose (2.27%), ash (1.46%), acidity (0.62%), antioxidant activity (86.31% inhibition), phenolic content (54.85 mg GAE/g) compared to other trails. Sensory profile of T_4 was found to be more acceptable than other trails.

Keywords: Quarg cheese, Ashwagandha, sensory characteristics, physicochemical properties, antioxidant activity

Introduction
Cheese is among the most widely consumed dairy products, being highly favoured for its production from milk. Cheese has been widely consumed as a component of a nutritionally balanced diet in various regions of the globe. Cheese manufacturing is predominantly concentrated in Western countries, serving as a prominent example of food preservation achieved through the processes of fermentation and ripening (Singh, 2011) [1]. The diversity of cheese types can be attributed to the advancement in cheese-making methods, as well as the increased knowledge in the fields of biochemistry and microbiology pertaining to cheese ripening (Farkye, 2004) [3]. Cheese exhibits a notable presence of casein, a protein known for its high content of bioactive peptides that are enzymatically produced by proteases. According to Gomez-Ruizet *et al.,* (2002) [4], these peptides exhibit several biological features, including anti-carcinogenic, anti-hypertensive, anti-oxidative, and opioid action, among others. In addition to the presence of bio-active peptides, cheese is known for its high calcium content and its potential to contribute to overall health (Kansal and Chaudhary, 1982) [5]. Cheese is known to have higher levels of conjugated linoleic acid (CLA), a type of fatty acid that has been associated with potential benefits such as weight loss, cancer prevention, and modulation of the immune system. The production of processed cheese from natural cheese results in a further increase in the concentration of conjugated linoleic acid (CLA). Quarg, also known as Speisequark in the German language, is a type of unripened, fresh cheese that is produced on a significant scale within Germany and enjoys widespread popularity in the country. The substance in question can be described as a milk protein paste that is produced through the acid coagulation of milk using specific bacterial cultures, such as *Streptococcus cremoris* and *Leuconostoc citrovorum*.

Additionally, a tiny quantity of rennet is added to facilitate the separation of the protein coagulum from the whey, resulting in improved overall yields. The cheese in question is widely consumed in Central European countries such as Germany, Poland, and Austria. According to Gahane (2008) [7], this product is known by several names in different countries, including Kvarg, tvarog, tworog, twarog, Sauermilchquark, and Speisequark.

Ashwagandha (*Withania somnifera* L.) is a popular traditional medicinal herb known for its adaptogenic properties, antioxidant activity, and potential health benefits. Incorporating ashwagandha powder into dairy products, such as cheese, provides an opportunity to develop value-added functional food products (Aini *et al.*, 2017) [6]. According to Ayurveda, ashwagandha is classified as a rasayana herb, which is known for its non-specific effects in promoting overall health and longevity. This particular plant is also recognised as an adaptogen, a type of herb that is non-toxic and operates in a non-specific manner to restore normal physiological function. It achieves this by targeting the Hypothalamic Pituitary Adrenal (HPA) axis and the neuroendocrine system. The utilisation of the plant's roots and berries is observed in the field of herbal medicine. In the practise of Ayurveda, it is common to boil the fresh roots in milk before the drying process, as a means of extracting unwanted elements. The utilisation of berries as a viable alternative to rennet in the process of milk coagulation for cheese production is seen.

Materials and Methods

The acquisition of unpasteurized bovine milk was conducted at the Dairy Farm of the Institute of Agricultural Sciences, Banaras Hindu University (BHU). The cheese culture, specifically identified as NCDC-149, was obtained from the National Collection of Dairy Culture, which is housed within the Dairy Microbiology Division at the National Dairy Research Institute (NDRI) in Karnal, Haryana. The microbial rennet, known as 'Meito', was procured from CHR Hansen Ltd. in Mumbai for the purpose of producing a solid and appealing cheese of the Quarg variety.

Technological aspects of Ashwagandha incorporated Quarg cheese preparation

The quarg cheese variant was produced with the technological advancements pioneered at NDRI, Karnal, as outlined by Gahane (2008) [7], with some adjustments made to the specified methods, shown in Fig 1. Four different levels (0.5%, 1%, 2.5% and 5% w/w) of ashwagandha powder were incorporated into the quarg cheese formulation. The cheese samples were prepared in triplicate for each ashwagandha level.

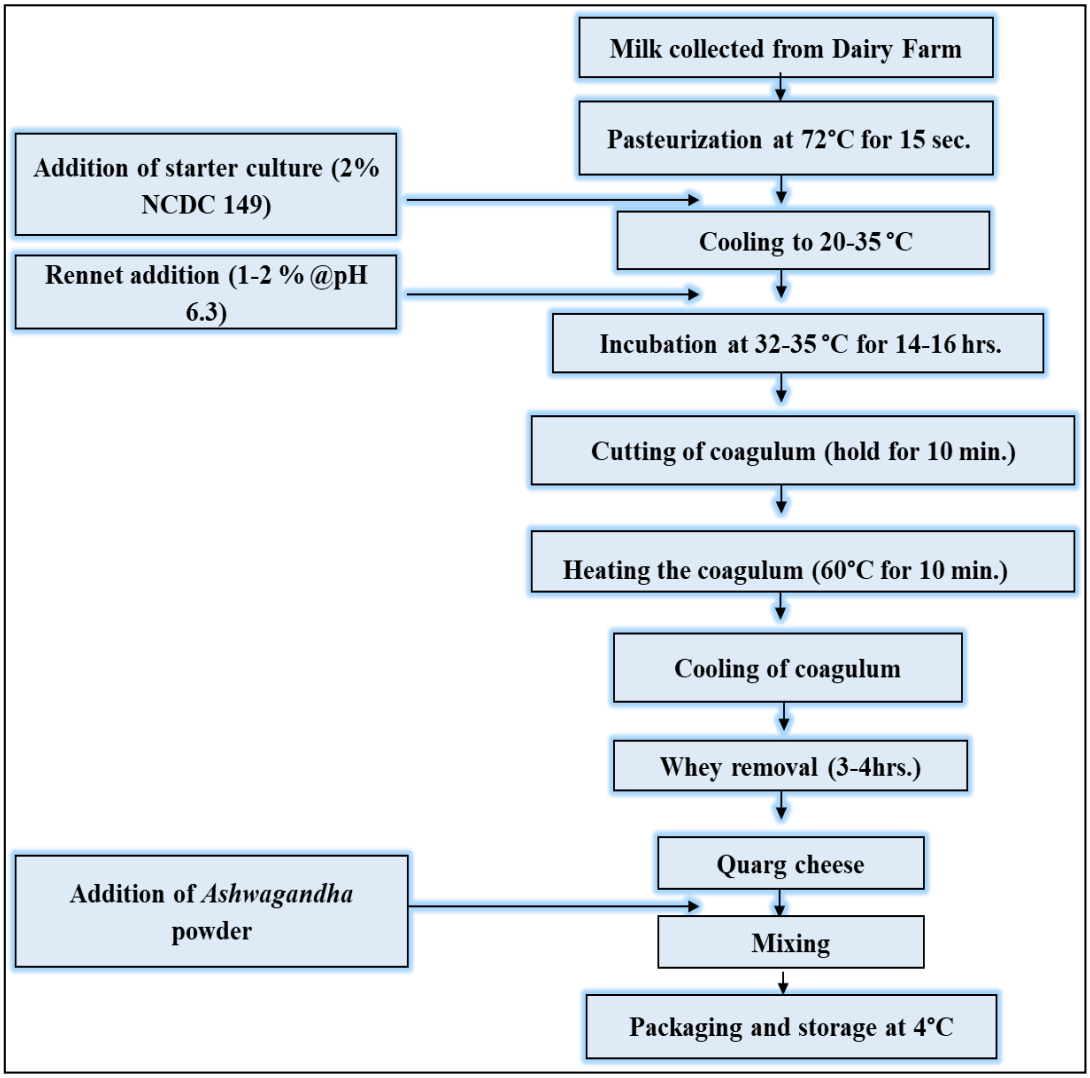

Fig 1: Flowchart for the preparation of Ashwagandha enriched Quarg cheese

Physicochemical analysis
The pH, moisture content, fat content, and protein content of the Quarg cheeses were determined using standard analytical methods.

Antioxidant activity
The antioxidant activity of the cheeses was assessed using a suitable method, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay or ferric reducing antioxidant power (FRAP) assay. Methanolic extract of sample was prepared by centrifuging 1 gram of sample with 10 ml of methanol at 6000 rpm for 10 minutes and the supernatant was collected. 1 ml of the standard DPPH solution prepared (4mg/10ml methanol) was added to 5 ml of sample (supernatant) and was dispersed thoroughly using vortex. The sample was dark incubated for 30 mints. The absorbance was taken in UV-VIS spectrophotometer at 517 nm against blank solution. % DPPH inhibition = Absorbance of blank− Absorbance of sample Absorbance of sample × 100.

Total Phenolic Content
The total phenolic content of the extracts was determined by the Folin Ciocalteau method with some modifications. 0.5 ml of sample (methanolic extract as mentioned in DPPH) was added to 0.5 ml of Folin Ciocalteau reagent (FCR) (1 ml FCR in 9ml distilled water), followed by 1 ml of 7.5% of Sodium Carbonate solution. The above solution was then kept in dark incubation for one hour. Absorbance was measured at 760 nm using UV-1800 spectrophotometer. The standard calibration curve of Gallic acid (0 - 800 mg/L) was produced and phenolic content was expressed in mg of Gallic acid equivalents (GAE)/ g of extract.

Results and Discussion
Following the determination of the optimal level of ashwagandha in quarg type cheese, various treatments of quarg type cheese were created and subsequently subjected to physico-chemical and microbiological analyses. The treatment details are as under:
T_0: Control (Quarg cheese)
T_1: Quarg type cheese added with 0.5% Ashwagandha powder
T_2: Quarg type cheese added with 1.0% Ashwagandha powder
T_3: Quarg type cheese added with 2.5% Ashwagandha powder
T_4: Quarg type cheese added with 5% Ashwagandha powder

Physicochemical properties
The addition of ashwagandha powder affected the physicochemical properties of the Quarg cheese. The pH of the cheeses remained within an acceptable range for cheese products. The moisture content, fat content, and protein content showed slight variations with the addition of ashwagandha powder, suggesting changes in the cheese composition. Sample T_4 was found to be better in terms of fat (10.89%), protein (13.47%), lactose (2.27%), ash (1.46%), acidity (0.62%), antioxidant activity (86.31% inhibition), phenolic content (54.85 mg GAE/g) compared to other trails. The results obtained for fat content are in agreement with Milanovic *et al.,* (2004) [9] where they studied the quality of quarg produced by probiotic application and he found that the fat content of the product was in the range of 12 to 14.5%. Gad and salam (2010) [9] concluded that the use of plant extracts as a source of phenols is preferred as a natural method. The highest protein content was observed in T_0 (13.54%) followed by T_4 (13.47%), T_3 (13.28%) and T_2 (13.14%) whereas lowest was in T_1 (13.09%). This effect may be attributed to protein compositional characteristics of Ashwagandha. The results were in agreement of those reported by Bhoite *et al.,* (2019) [10] reported that the protein content was found ranging from 4.20 to 5.34%.

Bioactive properties of the functional quarg cheese
The Quarg cheeses fortified with ashwagandha powder exhibited enhanced antioxidant activity compared to the control sample (0% ashwagandha). The antioxidant activity increased with increasing levels of ashwagandha powder, indicating a dose-dependent relationship. Control sample (T_0) has the least value i.e., 28.04% whereas the highest value was found in T_4 sample i.e., 86.31%. Similar, results were obtained for the total phenolic content where control sample (T_0) has least value i.e., 30.45 mg GAE/g while the highest value was obtained in T_4 sample i.e.,54.85 mg GAE/g. The bioactive properties were in accordance to the study reported by Aparnna *et al.,* (2023) [11].

Table 1: Effect of Ashwagandha on physio-chemical properties in quarg cheese

Treatment	Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Acidity (%LA)	Moisture (%)
T_0	10.52^{c}	13.54^{a}	2.52^{ac}	0.95^{c}	0.75^{b}	73.65^{bc}
T_1	10.25^{d}	13.09^{b}	2.15^{b}	1.26^{ac}	0.71^{a}	71.28^{acd}
T_2	10.85^{b}	13.14^{bc}	2.02^{b}	1.28^{b}	0.68^{ac}	71.55^{ac}
T_3	10.68^{a}	13.28^{cd}	2.08^{cd}	1.28^{cd}	0.65^{d}	71.84^{ad}
T_4	10.89^{bc}	13.47^{e}	2.27^{c}	1.46^{a}	0.62^{ad}	71.88^{e}
SE	0.274	0.155	0.011	0.048	0.025	0.049
CD @5%	0.578	0.786	0.241	0.136	0.105	0.117

Table 2: Effect of Ashwagandha addition on bioactive profile of quarg cheese

Treatments	DPPH (% inhibition)	TPC (mg GAE/g)
T_0	28.04	30.45
T_1	78.82	46.22
T_2	80.15	48.21
T_3	82.24	51.36
T_4	86.31	54.85
SE	0.015	0.024
CD @5%	0.042	0.075

Sensory characteristics
The sensory evaluation of dairy products exerts a significant impact on customer acceptability, preferences, and market pricing. The sensory evaluation of quarg type cheese, which had undergone various treatments, was conducted by a panel consisting of five judges. The product underwent evaluation for various quality parameters using a 100-point Quarg cheese scorecard. The sensory evaluation results indicated that the addition of ashwagandha powder influenced the sensory characteristics of the Quarg cheese. As the level of

ashwagandha powder increased, there were noticeable changes in flavor, color, and overall acceptability. However, the sensory attributes were generally well-received, and the cheeses with ashwagandha powder exhibited unique flavor profiles. As shown in Table 3, sensory profile of T_4 was found to be more acceptable than other trails.

Table 3: Effect of Ashwagandha addition on the sensory profile of quarg cheese

Treatments	Flavour	Body and Texture	Colour and Appearance	Overall acceptability
T_0	48.62^{c}	38.52^{b}	7.65^{a}	94.79^{ba}
T_1	51.02^{d}	38.55^{a}	8.05^{c}	97.62^{a}
T_2	51.55^{b}	38.62^{bc}	8.18^{ac}	98.43^{b}
T_3	51.65^{a}	38.66^{ad}	8.22^{d}	98.53^{a}
T_4	51.78^{ac}	38.85^{d}	8.26^{b}	98.89^{ac}
SE	0.010	0.007	0.012	0.045
CD @5%	0.035	0.021	0.028	0.152

Conclusion

The incorporation of ashwagandha powder into Quarg cheese influenced its sensory characteristics, physicochemical properties, and antioxidant activity. The addition of ashwagandha powder provided an opportunity to develop value-added functional cheese products with potential health benefits. Sample T_4 was found to be better in terms of fat (10.89%), protein (13.47%), lactose (2.27%), ash (1.46%), acidity (0.62%), antioxidant activity (86.31% inhibition), phenolic content (54.85 mg GAE/g) compared to other trails. Sensory profile of T_4 was found to be more acceptable than other trails. Further research is warranted to optimize the levels of ashwagandha powder for desired sensory attributes and to explore the potential bioactive compounds responsible for the enhanced antioxidant activity observed.

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