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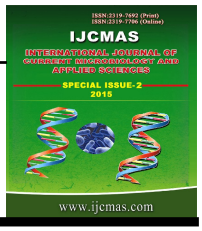

Original Research Article

WHEY: Waste to Health and Wealth

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Dairy Industry, Whey, Culture media, Preservative, Antimicrobial agent, Probiotic Health drink

A B S T R A C T

Whey is a valuable by-product of dairy industry but with no commercial value, therefore it was discarded as waste which resulted in severe environmental pollution. Whey mainly contains lactose and protein. The protein components of the whey include lactoferrin, beta- lacto globulin, alpha- lactalbumin, glycomacropeptide, and immunoglobulin. Whey has the ability to act as an antioxidant, antihypertensive, antitumor, hypolipidemic, antiviral, antibacterial and chelating agent. The aim of this research was to scientifically establish the multifaceted usefulness of this dairy industry effluent. Whey from cow milk was subjected to physicochemical characterization which revealed the presence of 89% protein, 46% carbohydrate, and high concentration of chloride, sodium and potassium ions and negligible fat. Therefore whey is an ideal probiotic health drink. Whey showed antimicrobial potential against Gram positive Staphylococcus aureus ATCC 6358p and Gram negative E. Coli NCIM 2065. Whey along with agar as a solidifying agent was used as basal growth medium for bacteria and fungi. Whey was used to coat peanuts as a food preservative. These properties of whey convert it from waste to health and wealth.

Introduction

Whey is a by-product of the dairy industry, which for years was thought to be in significant and was either used as an animal feed or it was disposed of as waste. Over the past few years several studies were carried out concerning the beneficial properties of whey i.e., its nutritional value and pharmacological properties have antimicrobial, antiviral and anti-oxidant properties; and can offer a kind of protection against cancer and heart diseases and assist at the enhancement of immune defence (Charu Gupta *et al.*, 2012). Whey proteins and amino acid in sports nutrition has a huge market (Ayar and Burucu, 2013). Whey requires a scientific processing that can eliminate undesirable substances, concentrate the nutrients and serve them in an acceptable manner (Nupur Goyal and Gandhi, 2009). Whey that has a higher nutritional content can be utilized locally to produce nutritional drinks that can serve a variety of purposes from refreshment to dietary supplements to probiotics

(Perasiriyan *et al.,* 2013). The use of whey as edible films and coatings in food protection and preservation has recently increased since they offer several advantages over synthetic materials, such as being biodegradable and environmentally friendly (Manab *et al.,* 2010). Antimicrobial substances incorporated into edible films can control microbial contamination of foods by reducing the growth rate and/or inactivating target microorganisms (Badr *et al.,* 2013). The main purpose for edible films from biopolymers is to control mass transfer of multiple compounds including gas, aroma, oil and water vapour into or out of a food, preserving food quality (Regalado *et al.,* 2006). Whey is thought to exert its effects by conversion of the amino acid cysteine to glutathione, a potential intracellular anti-oxidant (Khaled G. Abdel-Wahhab *et al.,* 2013). Whey based tea is reported to be a healthy drink (Perasiriyan *et al.,* 2013).

Material and Methods

Macroscopic and microscopic characterization of whey

Whey sample from cow milk was collected, isolation of Lactic Acid bacteria was done on MRS media, microscopic and biochemical characterization-sucrose, galactose, glucose, maltose, mannitol, and lactose was performed.

Physicochemical properties of whey

Total protein was estimated by Folin Lowry method; -Alkaline solution 5ml

FolinC reagents 0.5ml

Total carbohydrate was estimated by Phenol Sulphuric method-5% of aqueous phenol 1ml, concentrated sulphuric acid 5ml. Reducing Sugars was estimated by DNSA method-DNSA reagent 1ml, distilled water 8ml. Total fat was estimated by Gerber's method. Electrolytes were determined by Flame Ionization method.

Whey media: Whey - Agar- 2.5 gm was autoclaved, and Test organism used- Escherichia coli, Staphylococcus aureus and fungi. The cultures were streaked on whey media plates and the plates were incubated at 37^0C for 24hrs.

Antimicrobial activity: Whey sample, Standard bacterial culture (*Staphylococcus aureus* ATCC 6358p and *Escherichia coli* NCIM 2065), sterile micro tips (0.1 ml and 1ml), Cork borer, Alcohol 70%, Sterile distilled water, Grover and Randal medium. The suspension of *S. aureus* and *E. coli* were prepared. OD of bacterial suspension of 0.1 was adjusted at 620nm. 0.1ml of saline suspension of test organism was inoculated into the sterilized seed agar butt.

The mixture was poured into sterile base agar plate and allows it to solidify. Cork borer was sterilized by using alcohol. The wells were prepared in each plate. Whey sample (100µl) was transferred in each plate. Plates were incubated at 37ºC for 24hrs. After incubation the zone of inhibition was recorded and compared with Standard penicillin concentration.

Whey coating: 50 ml of Whey solution were heated in water bath for denaturation at 90ºC for 30 min (heat treatment promotes water insolubility which may be beneficial to maintain film and food integrity). Resulting solution is rapidly cooled at room temperature. Thus whey forms film on the nut surface. Whey coated nuts were kept for 3 months and uncoated nuts were kept as control. The shelf life of coated and uncoated nuts was checked.

Antifungal testing for whey preservatives

Nuts/peanuts, Whey sample 50 ml, Crucible. Solution of whey sample which was used for coating (denatured and solidified solution) was inoculated with Aspergillus culture kept for 48 hrs as normal whey (Without denatured and solidified) solution was also inoculated with Aspergillus culture as a control.

Whey tea: Fresh whey 50 ml, green tea bags. Whey was heated and green tea bags was used to make whey tea.

Results and Discussion

Isolation of Lactic acid bacteria from Whey sample:

All the tests performed were in triplicates. Results of every test performed are given as an average of the triplicate data.

The whey samples were streaked on MRS plates and incubated at 37°C for 24 hours (Fig. 1). A Gram positive bacillus was observed (Fig. 2).

The physiochemical and chemical properties of whey were studied and tabulated in tables 3 and 4. It was estimated that whey content high protein, high electrolyte and low fat which can therefore be used as a health drink as shown in figure 3 and 4.

Antimicrobial activity (Fig 5 and 6):

Whey showed antimicrobial property against Staphylococcus aureus ATCC 6358p and Escherichia coli NCIM 2065.

Whey media:

Whey used as culture media for isolation of *E. coli* (Fig. 7), *S. aureus* (Fig. 8) and fungi (Fig. 9).

Whey coating (Fig. 10):

Denatured whey was directly applied to nuts and some nuts were kept (without applying denatured whey) as control. Thin whey film on nuts was formed successfully and it was proved that this coating increases the shelf life of nuts and was observed for 3 months (continuing).

Antifungal testing for whey as preservative (Fig. 11 and 12)

It is becoming more and more evident that development of probiotic products largely depends on their quality. It was observed from the characterization, that whey contains high electrolyte high protein, low fat, and low carbohydrate content. Whey is super rich in protein, vitamin, and minerals. Whey can be used as an electrolyte health drink, and it provide a good basal media for isolation of Gram positive and gram negative organism including fungi. Whey can be used as edible preservative in the form of films which can serve as carrier for a wide range of food additives including various antimicrobials, which can extend product shelf-life and reduce the risk of pathogen growth on the surface of food products. “Whey Tea” acts as excellent antioxidant, anticancer and has numerous health benefits.

The value of physiochemical properties like fats, proteins, minerals like sodium, potassium, chloride& lactose were comparable. The amount of electrolyte and protein content is more and the carbohydrate and fat content was negligible as compared to that of the same reference paper (Nupur Goyal and D.N. Gandhi 2009).Whey turned out to be the best electrolyte drink where mineral concentration was high and fat concentration was less.

Table.1 Physiochemical properties of whey sample

Components	Sample
Sodium(mg/l)	34.26 ±1.68
Potassium(mg/l)	98.67 ±4.54
Chloride(mg/l)	100 ±1.2

The electrolytes were determined by Flame Ionization.
The sodium content was34.26±1.68mg/l,Potassium content was98.67±4.54mg/l, and Chloride content was 100±1.2

Table.2 Chemical properties of whey sample

Sr. no.	Protein concentration(mg/ml)	Carbohydrate concentration(mg/ml)	Reducing sugar concentration(mg/ml)
1.	0.89	0.46	0.41

The concentration of protein in whey sample was 0.7 mg/ml, total carbohydrate was 0.46mg/ml, and reducing sugar was 0.41mg/l

Fig.1 Growth of Lactobacilli spp.on MRS plate

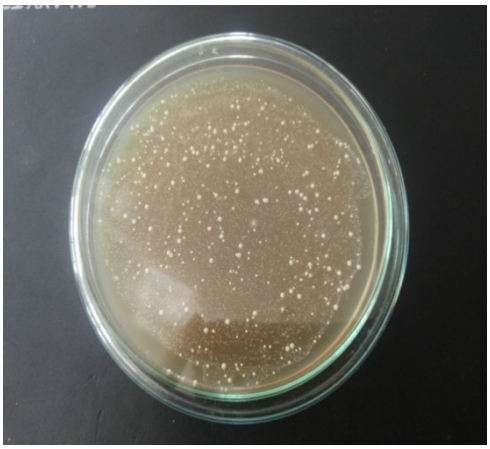

Fig.2 Gram positive rods

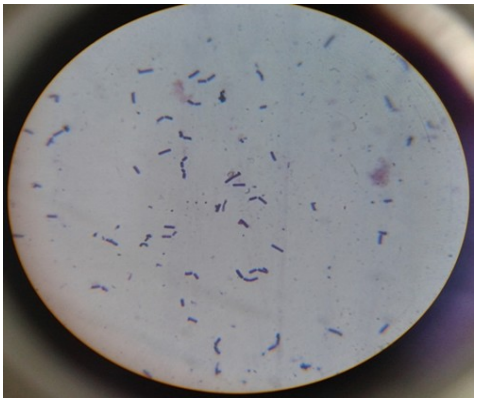

Fig.3 Flavoured whey health drink contains fresh whey sample with flavouring agent- vanilla

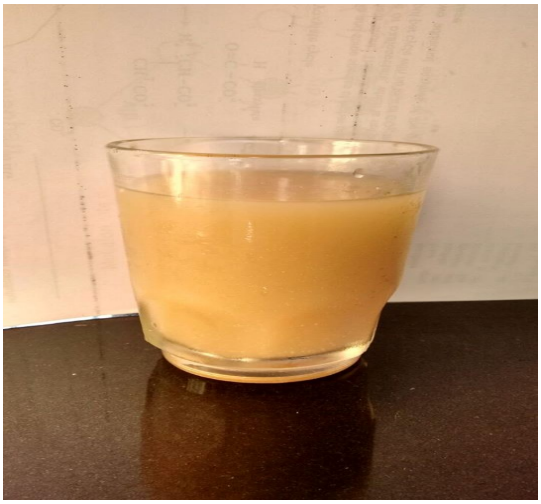

Fig.4 Antioxidant whey tea prepared by heating whey and adding tea bags to it

Fig.5 Zone of inhibition against *S. aureus*

Fig.6 Zone of inhibition against *E. coli*

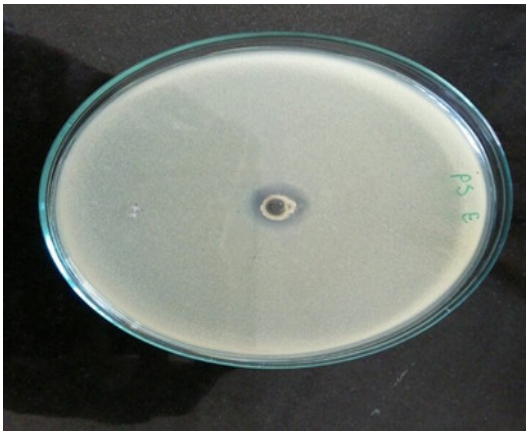

Fig.7 Isolation of *E. coli* in Whey media

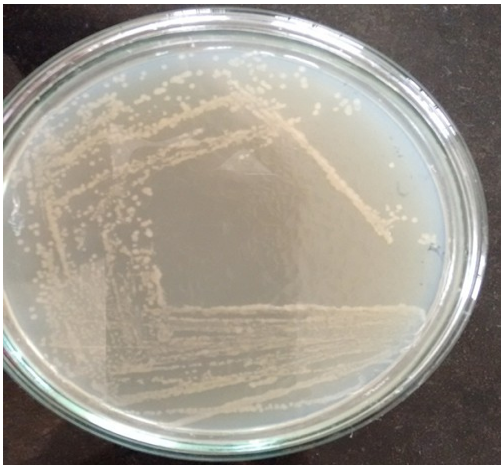

Fig.8 Isolation of *S. aureus* in whey media

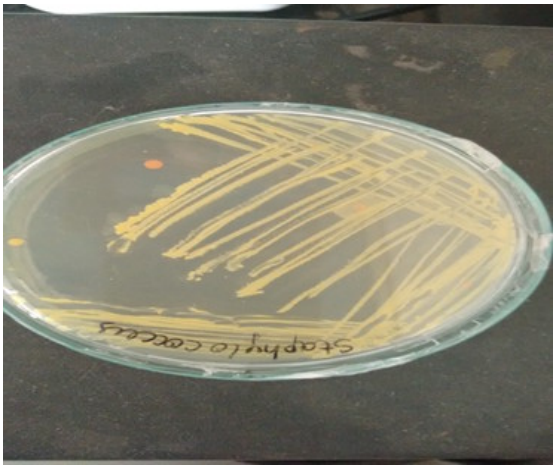

Fig.9 Isolation of fungi in whey media

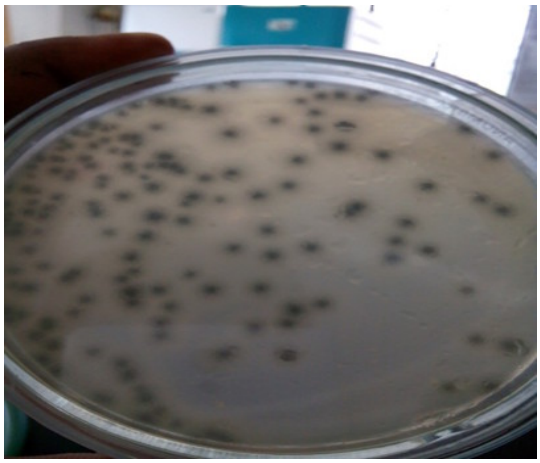

Fig.10 Peanuts coating a. whey coated nuts; b. nuts without whey coating

Fig.11 No growth was seen in Whey (denatured and solidified whey)

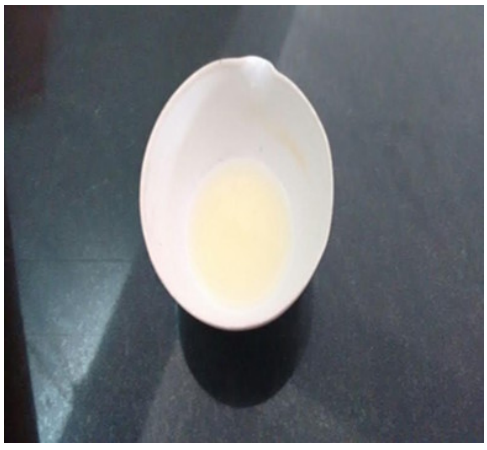

Fig.12 Growth was seen in control whey

Hence whey is more suitable for the preparation of whey based beverages or electrolyte drink for the replenishment of the lost minerals. Probiotics are used in health applications in order to maintain healthy gut micro flora, including a reduction in detrimental bacteria such as, Staphylococcus aureus and *E. coli* and an increase in beneficial bacteria such as Lactobacillus spp. and *Bifidobacterium*. Whey act as excellent antimicrobial agent against gram positive as it affects the cell wall formation in pathogenic Gram positive bacteria and gram negative bacteria. It acts as a complete food for the growth of microorganism (Badr *et al.,* 2013). This waste can be converted a value added product like using whey agar basal medium for isolation of microorganisms (VethaKanraj Helen Shiphrah *et al.,* 2012). Antimicrobial substances incorporated into edible films can control microbial contamination of foods by reducing the growth rate and inactivating target microorganisms. Whey edible films provide mechanical integrity and a selective barrier to oxygen which have potential to increase the shelf life of foods such as peanuts by reducing lipid oxidation rate. Thus whey acts as preservatives. It increases the shelf life of food at the same time retaining its quality (Regalado *et al.,* 2006). The "Whey tea" prepared with green tea bags received wide acceptability in terms of appearance, colour, flavour and taste. Whey contains many essential amino acids like leucine, isoleucine, most of the water soluble vitamins and minerals. It lacks polyphenol which are abundant in tea leaves. Hence combining tea with whey yield a healthy beverage enriched with polyphenol (Perasiriyan *et al.,* 2013). Therefore, whey previously designated as waste could be converted to health to improve quality of life. The dairy industries should take the initiative to convert this effluent into value added products like dehydrated culture media for growing microbes, health drink, food preservative and antimicrobial agent and whey tea which acts as an excellent antioxidant.

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