Short Research Article

'Morphological and anti-microbial study of whey protein concentrate prepared by dehydrating milk serum'

Abstract

The main aim of this study was to dehydrate milk serum and extract whey protein concentrate. Proximate analysis reveals the WPC's protein, fat, ash, and moisture content. WPC contains 42 percent protein, 4.40 percent fat, 10.37 percent ash, and 4.27 percent moisture. The WPC's antimicrobial properties are also particularly effective against S. aureus (gramme positive) and E. coli (gram negative). SEM and X-RD were used to study the morphology, which revealed the microstructure and nature of the compound, as well as the chemical, empirical formula of the molecule.

Comment [u1]: pass the method description on the abstract

Comment [u2]: convey conclusions

KEY WORD: Milk serum, Whey protein, SEM, X-RD, anti-microbial property.

1. Introduction

Whey is the yellow-green-coloured liquid fraction of milk, often known as cheese serum, that is obtained after the curd has been separated and the milk has been coagulated with proteolytic enzymes or acids [1].For decades, it was regarded as a major dairy waste due to disposal challenges associated with its high biological oxygen requirement and high organic matter [2].

Milk whey (also known as Milk Serum) is a complex and diverse protein mixture with several biological, nutritional, and technological applications in the production of modern foods and beverages. Whey proteins are distinct in that they include all of the essential amino acids found in high-quality protein [3]. The most important sources of natural bioactive components, such as particular proteins, peptides, lipids, and carbohydrates, are bovine milk and colostrum [4]. Amino acids are present in protein-rich foods like milk and milk products and are essential for human survival. Humans require eight amino acids, which must be obtained from diets containing animal proteins or an appropriate combination of plant proteins, as the human body is unable to synthesise all of them [5]. The valorisation of whey components, a plentiful dairy by-product, is linked to the recovery and concentration of whey proteins as novel ingredients for the food and non-food sectors, as well as an increase in economic revenue for the dairy industry [6,]. Whey protein improves muscle strength and body composition, as well as preventing cardiovascular disease and osteoporosis when taken as a dietary protein supplement [7].

Furthermore, milk whey proteins are regarded as healthy nutrients due to a number of benefits connected with frequent consumption, including appetite control, workout recovery, and inducing satiety[8].

The principal components of whey proteins are -lactoglobulin, -lactalbumin, B, and immunoglobulin, as well as a variety of other proteins such as lactoferrin, lacto peroxidase, protease peptone, osteopontin, and lysozyme [9]. The features and composition of milk whey vary depending on the milk source (cow, sheep, goat, etc.), the milk-producing animal's nutrition, the lactation stage, the processing method employed, and the time of year when the milk samples were taken [10].

Several membrane filtration applications have recently enabled the use of various whey protein components as food supplements. After the milk is coagulated, the whey protein is isolated in two primary forms using selective membranes: whey protein concentrates (WPCs), which contain 34–89 percent protein, and whey protein isolates (WPIs), which contain at least 90 percent protein [11, 12].

The objective of this research was to evaluate morphological and ant-microbial study of the whey protein concentrate producing through the dehydrating milk whey. The morphological was done by the SEM, X-RD, and microbial study was carried out by Agar well Diffusion method BY using gram positive and gram negative bacteria (S. aureus, E. coli)

2. MATERIAL AND METHOD

2.1 Material

The experiment was carried out at the laboratory of Food Science and Technology, Babasaheb Bhimrao Ambedkar university, Lucknow. In this research 4 liter of milk serum was used as raw material. The milk serum was collected from the 'Lovely Milk Dairy' near Babasaheb Bhimrao Ambedkar University gate no. 1 Shahid path.

2.2 Equipment

Tray, boiler, sieve, gas burner, centrifuge, dehydrator etc.

2.3 Experimental procedure

When cheese is made in a dairy, the whey that forms after the casein coagulates is drained away. Drain whey was collected, and the whey protein concentrate was prepared according to the flow chart below. After draining, it was pasteurised and sieved to remove small casein particles, then evaporated in a dehydrator to obtain crystalline whey protein, which was then transformed into fine powder and kept for packaging or further treatment.

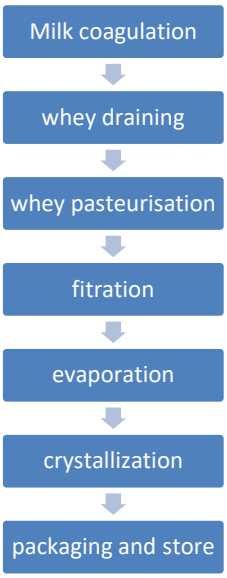

Fig. 1 Processing of WPC

2.4 Nutritional composition of whey protein concentrates

The usual analytical approach was used to determine the approximate content of the whey protein concentrates. The total protein, fat, moisture, and ash of the WPC were measured using the micro-khjeldal and soxhelte method, which is a common process.

2.5 Scanning electron microscopy (SEM)

SEM is method for the imaging the morphology and microstructure of the materials. The microstructure of the WPC was analysed by scanning electron microscopy using a model: JSM6490LV and make JEOL, JAPAN.

Sample was mounted on the aluminium stub using double sided carbon tape and then sample coated with platinum by using sputter coater (JEOL JFC-1600) auto fine coater. The was capture at the 10kV: ,X500:500µm, X1000:10µm, X2000:10µm

2.6 X-RD (X-ray diffraction)

X-ray diffraction analysis (XRD) is a technique for determining a material's crystallographic structure and amorphous structure. Model: D8 Advance Eco and Make: Bruker, Germany are used in the XRD process. Grinding the sample to powder form/tale (0.062mm) is used to prepare it for x-ray diffraction. Then spread the sample to a thin layer in the centre of the sample holder, then place in desiccator and transfer to sample holder for XRD analysis. After that, it's time to load the sample and scan it.

2.7 Anti-microbial properties of WPC

The antimicrobial activity of one sample was evaluated against the microbes S. aureus and E. coil. The activity was evaluated by the method of **Agar Well Diffusion**[13].

For this test, first of all for the bacterial isolates the Mueller Hinton Agar (MHA) Media was prepared as per the standard composition given by Himedia that is 38gms of the media was suspended in 1L water and the media were autoclaved at 121ºC and 15psi for 15minutes using autoclave (Gentek India Pvt. Ltd.). After the sterilization media was poured in sterile glass petri dishes under the Laminar air flow (Toshiba, India) using the aseptic techniques, each plate was poured with 20ml of the culture media. The plates were allowed to solidify properly then the media was inoculated with the respective bacteria isolate the bacterial isolate S.aureus and E. coli on the MHA media by spread plate technique, for which 100µl of the culture broth of each isolated was added over the media and uniformly spread using sterile glass rod. The extract samples were prepared for concentration i.e., 100µg/ml, 200µg/ml, 300µg/ml and 1mg/ml in respective solvent or water. These samples were used in this study to evaluate their antimicrobial activity. Ten minutes after spreading, wells were punched into the media plates using sterile micro tips, and then each well was loaded with 20µl of the respective sample on separate plates. The samples were allowed to diffuse through the well into the media and then the plates were sealed with paraffin and incubated at 34ºC for 24hrs. The plates had two well one of the positive control that is ciprofloxacin of 0.8ppm concentration and the negative control well was loaded with water. Next day after incubation the plates were observed for the clear zone around the well called as the zone of inhibition, and the diameter of these zones was measured in mm and recorded.

3: RESULT AND DISCUSSION

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3.1 Nutritional analysis

Amino acids, protein, fat, vitamin A, phosphorus, and calcium are all good sources of micro and macro nutrients in WPC. The preceding thesis study provided all of the value.

Parameters	WPC% (prepared)	WPI%(market)
energy	340.37kcal	388.8kcal
Protein	42	90
carbohydrates	39.10	00
Ash	10.37	2.30
Fat	4.40	3.20
Moisture	4.27	4.50

Table: 1 proximate analysis of whey protein concentrate

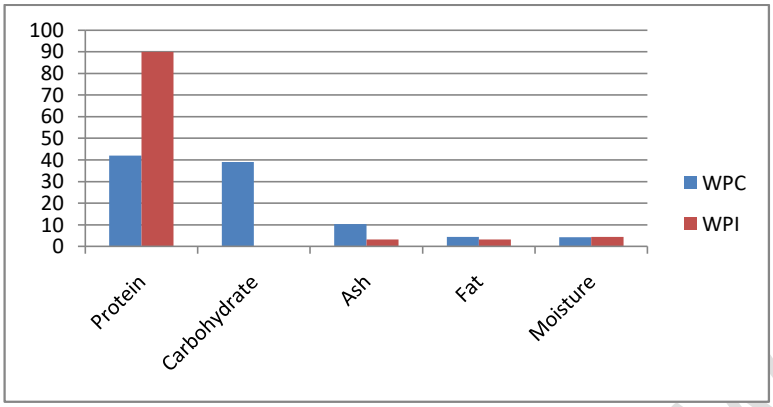

Fig. 2 proximate analysis of whey protein concentrates

Total Energy: the total energy was calculated by according to the **Nielson(1998)**formula from us found that WPC contains about 340.37kcal/100g [14].

Protein: The protein content of milk serum was 20 percent (6.3g/L), [12], however after dehydration, it was enhanced by 22 percent is equal to 42 percent. (Table 1)

Carbohydrates: The carbohydrate content of WPC is about 39.10 % which gives about the 157.6Kcal energy which was calculated according to the **Nielson (1998)**[14].

Ash: Because WPC contains more carbs than WPI, the ash level of the prepared WPC was determined to be around 10.37 percent, which was higher than WPI (2.60 percent).

Fat: By offering a difference of 1%, the fat content of both proteins is determined to be approximately similar.

Moisture: The moisture content of the WPC is 4.27% which was less than that of WPI which has moisture about 5%. (Fig. 2)

3.2: Analysis of Morphology

The image was captured at 10kV by magnifying X500 with a surface area of 50µm in Fig. 3 (A). It depicts a particle with an uneven size and a somewhat smooth surface.The image in fig.3(B) was captured at 10kV and shown at X1000 on a 10µm surface area. It demonstrates the irregular form of the particle, which contains protein, lactose, and a small amount of unevenly distributed fat.Figure 3 (C) On a 10 m surface, the image was recorded at 10kV and magnified at X2000. It depicts a smooth surface with uneven bar-shaped lactose and crystalline, irregularly sized protein.The image in fig. 3 (D) was captured at 10kV and magnified at X2500 on a 10µm surface area. It has a smooth surface with a small quantity of fat and protein in the centre.

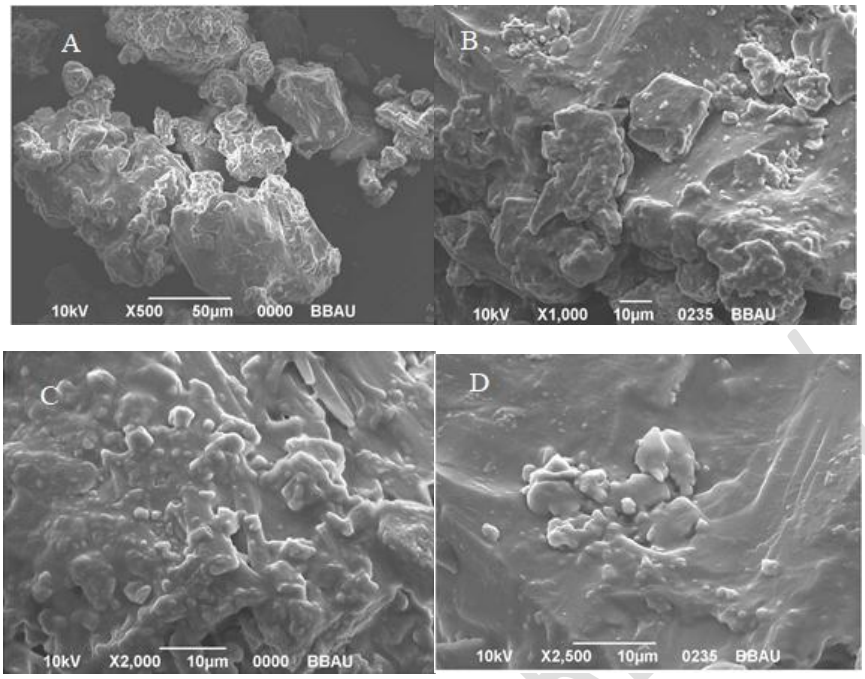

Fig. 3 Image of WPC (A)magnifying at X500 on area of 50µm. (B) At the X1000, (C) at the X2000, and (D) at the X2500 on the surface area of 1µm

3.3 X-RD analysis of WPC

The nature of the prepared WPC utilising X-RD patterns shows a strong peak around (20.115) at 4000, (12.548) at 1700counts fig.4 (A) and many more, indicating that the material has a good quantity of crystalline material. When the X-RD analysis data was compared to the X-RD library data, the pdf No. 00-065-1393 was found.

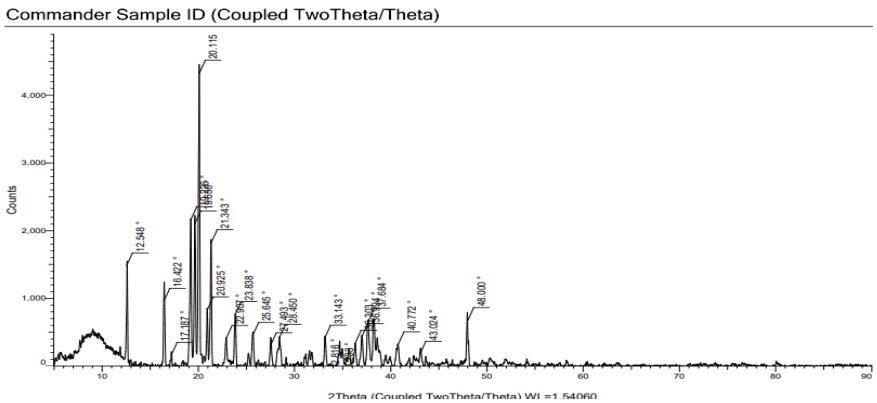

Fig. 4 (A) X-RD analysis of WPC

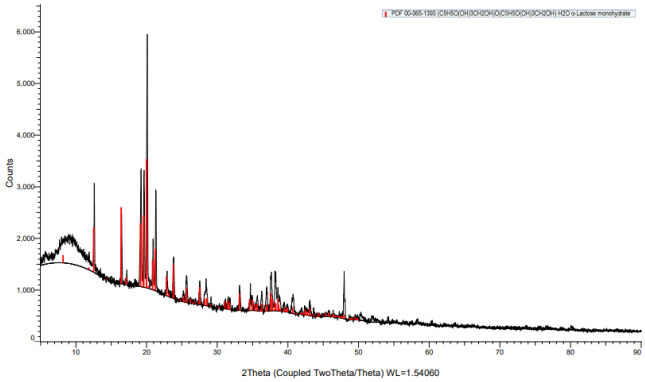

Fig. 4 (B) matching the graph of reference pdf and WPC data

When comparing the WPC graph to the reference pdf data, it shows that they are only partially matched. Because of amorphous part of WPC was not detected in X-RD, from where got the compound name empirical formula molecular weight and chemical formula.

Compound name: alpha-lactose monohydrateEmpirical formula: $C_{12}H_{24}O_{12}$Chemical formula: $(C_5H_5O(OH)CH_2OH)O(C_5H_5O(OH)_3CH_2OH).H_2O$ Molecular weight: 360.31g/mol

3.4 Anti-microbial property

Using the Agar well diffusion method, the antibacterial properties of produced WPC at varied concentrations of 100, 200, and 300µg/ml against S. aureus and E. coli were examined. WPC effectively inhibited the growth of the bacterial strain, according to the findings Table 2

S.NO	Sample	Organism	Zone of Inhibition (mm)
1.	100µg/ml	*Staphylococcus aureus*	Nil
2.	200 µg/ml		Nil
3.	300 µg/ml		25
4.	Positive control		39
5.	Negative control		Nil
1.	100 µg/ml	*E.coli*	Nil
2.	200 µg/ml		17
3.	300 µg/ml		24
4.	Positive control		30
5.	Negative control		Nil

Table 2 Zone inhibition

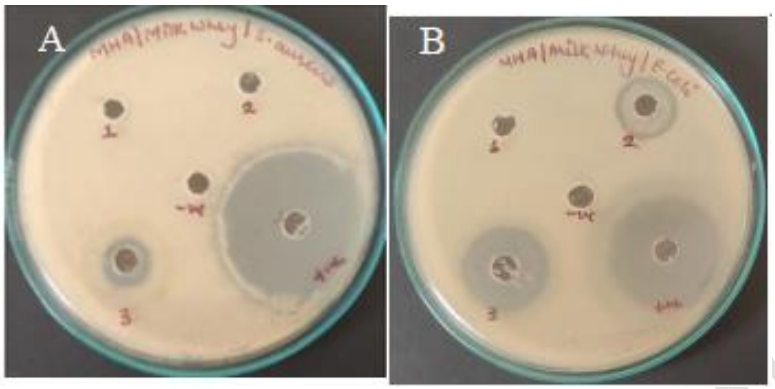

Fig. 5 anti-microbial activity of WPC (A) S. aureus (B) E. coli

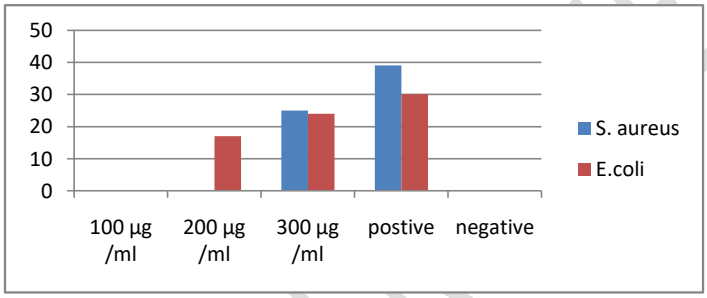

Fig. 6 Anti-microbial activity of WPC

WPC showed the greatest zone of inhibition against S. aureus (25mm) and E. coli (24mm) at a dose of 300µg/ml (Fig.5 and 6), while at 200 µg/ml, S. aureus inhibition was nil and E. coli (17mm). At 100g/ml, however, both strains were found to be completely inhibited.As a result, a concentration of 300µg/ml is effective against both strains, and this concentration can be useful for further research.

4. Conclusion:

A simple and effective approach for preparing whey protein concentrate (WPC) was successful in this investigation. SEM and X-RD were used to investigate morphology, and it was discovered through proximate analysis that the protein content of WPC is around 42 percent, which meets the RDA about 50 percent for athletes.WPC's antimicrobial properties are quite good, as it effectively reduced the growth of both S. aurus (25mm) and E. coli (24mm) at a concentration of 300µg/ml.The findings show that WPC made by dehydrating milk serum is a valuable source of bioactive components that can be used for good nutrition, as well as for therapeutic and palliative purposes in medical disciplines such as obesity and diabetes type 2.[]

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is

absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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