FT-IR determination and Antioxidant activity (DPPH) of Whey protein concentrate prepared by dehydrating Milk serum and its sensory evaluation

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***Abstract*: Whey protein concentrate (WPC) is a food ingredient made from whey (Milk serum). Confectionary, ready-to-eat cereals, nutritious bars, and sports beverages all include them to increase nutritional value.**

***Aim*: The main of this study was to evaluate the study of the prepared whey protein concentrate from milk serum and determination of the functional group, antioxidant activity, and sensory evaluation.**

***Methods*: the functional group was determined by using FT-IR spectroscopy and the antioxidant activity was determined by the scavenging free radical method by using DPPH and observed under a spectrophotometer. The sensory evaluation was done by a well-trained panellist.**

***Result*: FT-IR spectroscopy reveals the functional group by showing the high and sharp peaks. The highest peak was 3524cm^{-1} (O-H stretching i.e., hydroxyl group) and the lowest peak was 466cm-1 (S-S stretching i.e. aryl sulphide group). The antioxidant activity showed the scavenging of free radicals (DPPH). Whey protein scavenges about 50% of free radicals at a 10mg/ml concentration. The sensory evaluation shows that the acceptability of prepared whey protein concentrate is more than that of whey protein isolate.**

***Keywords*: Milk whey, whey protein concentrate, FT-IR, Antioxidant, Sensory evaluation.**

1. INTRODUCTION

Milk has a variety of physiologically active components, including bioactive proteins and peptides, oligosaccharides, immunoglobulins, and fats/lipids, all of which protect against pathogens and illnesses when consumed regularly. Milk comes from a variety of animals, including cows, buffaloes, goats, and sheep. Bovine milk provides about 3.5 percent total protein, lipids, and vital vitamins that help with growth and development [1]. It is a natural and rich source of well-balanced nutrients that show a diverse range of bio functional properties. These properties are because of the presence of milk proteins/peptides, which support infant development, stimulates growth, improves muscle mass, and confers positive health implications beyond basic nutrition [2]. The milk protein system is primarily made up of two types of proteins: roughly 80% (w/w) casein, which is removed from skim milk using either an acid (isoelectric precipitation) or enzymes (rennet coagulation), and 20% whey, which is a residual residue after the casein has been recovered[3]. The whey protein in milk is made up of five fractions that make about 85 percent of the total protein. α-lactalbumin, β-lactoglobulin, glycomacropeptide, immunoglobulins, protease peptone, and serum albumin are among these fractions, whereas the casein component of milk contains -casein,α s1-casein, αs2-casein, and κ-casein [4].

Whey is the yellow-green-colored 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 [5]. For decades, it was regarded as a major dairy waste because to disposal challenges caused by its high biological oxygen requirement and high organic matter [6]. In general, fresh liquid whey from the cheese-making process has 94.2% water and 50% total solids, with 0.8% whey proteins, 0.5% minerals, 0.1% fat, and 4.3% lactose (the primary ingredient)[7]. Whey's content and properties, on the other hand, can change depending on the type of cattle, the animal's diet, the milk used to make it, the processing procedures employed, and other environmental conditions [8]. Whey proteins are globular proteins with a large number of -helix patterns and equally distributed hydrophilic and hydrophobic, acidic and basic amino acids throughout their polypeptide chain [9]. α-lactalbumin (α-LA), β-lactoglobulin (β-LG), bovine serum albumin (BSA), immunoglobulins (IG), bovine lactoferrin (BLF), bovine lactoperoxidase (LP), and modest amounts of glycomacropeptide are the primary elements of whey proteins (GMP). However, the composition of whey protein varies depending on the whey type (sweet or acid), the type of milk (bovine, ovine, or caprine), the type of cattle feed, the lactation stage, and the method of processing. Whey, which is acidic by nature, has a pH of around 5.1 and is created mostly through direct acidification, whereas sweet whey has a pH of around 5.6 and is produced primarily through rennet-coagulation [10].

With the growing popularity of healthy eating, there is a global need for high-protein food products. A sedentary person's daily protein consumption should be 0.8 g per kg of body weight per day (g/kg/day) [11]. This amount of protein is essential to keep the body's nitrogen balance in check and sustain proper metabolic activity. Dairy 2020 comes in a variety of shapes and sizes, with 1 235 being the most common. Egg, soy, hemp, whey, and casein are examples of supplementary proteins. Whey from milk is one of the most common.has the highest percentage of readily accessible and digestible amino acid sensuring that it is properly absorbed by bodily cells [12]. 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% protein, and whey protein isolates (WPIs), which contain at least 90% protein [13,14].

The objective of this research was to prepare whey protein concentrate by utilising the milk serum which is a by-product of cheese and determination of the functional group present in the prepared whey protein concentrate and its antioxidant activity. The functional group determination was carried out by the help of Fourier transform infrared spectroscopy (FT-IR) and the antioxidant activity was carried out using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) by scavenging free radical process.

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 liters 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 pasteurized and sieved to remove small casein particles, then evaporated in a dehydrator to obtain crystalline whey protein, which was then transformed into a fine powder and kept for packaging or further treatment.

Figure .1 preparation of whey protein concentrate

2.4 Functional group determination by FT-IR

The sample was characterized using Fourier transform infrared (FT-IR) spectrometer (Perkin Elmer Frontier Instruments). FT-IR was performed on pressed pellets made with KBr and whey protein concentrate. The FT-IR spectra were recorded between 400 and 4000 cm^{-1} as per the protocol.

2.5 Antioxidant activity by DPPH assay

The antioxidant activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) is calculated via spectrophotometer with small modifications [15,16]. In methanol, the colour of DPPH is dark blue. In its reduced form, the antioxidant compound changes colour from purple to yellow, allowing DPPH to gain electrons. DPPH shows strong absorption at 517 nm, determined by 2, 2-diphenyl-22-pyridyl hydroxylase (DPPH). Briefly, 0.1 ml DPPH solution was mixed with 1g of whey protein concentrate sample prepared in various concentration (20,40,60,80,100 mg/ml). A control sample of 1 ml of methanol was prepared and incubated in darkroom for 30 minutes at ambient temperature. After incubation, the absorbance of the sample was read at 517 nm using a UV Visible spectrophotometer methanol use as a blank. Reduction in the absorbance value shows high activity in scavenging free radicals [17]. It was measured as a percentage of DPPH scavenging activity by using the following formula given below:

$$\textbf{DPPH Scavanging activity} = \frac{\textbf{Acontrol} - \textbf{Asample}}{\textbf{Acontrol}} \textbf{X100}$$

2.6 Sensory evaluation

A panel of trained members was formed. The panel members were selected from the department of 'Food science & technology' Baba Saheb Bhimrao Ambedkar University, Lucknow, India. All the panel member are medically fit to sensory analysis. The formulated treatments were analysed by using 9 points hedonic scale.

Sensory evaluation is a scientific discipline that analyses and measures human responses to the composition of food and drink, e.g., appearance, flavour, colour, odour, texture, and overall acceptability. The sensory evaluation was carried out in between prepare whey protein concentrate and whey protein isolate purchased from the market.

3 Result and discussion

3.1 FT-IR

FTIR investigation of whey protein concentrate yielded infrared spectroscopy data as presented in graph and table and are compared with previously reported data. Infrared spectra were obtained at a frequency region of 4000-500 cm^{-1}. In the infrared spectra, interesting peaks were observed in the range of 3200–3600 cm^{-1}, indicating hydrogen bonding [**18]**. Transmittance peak at 3524 cm^{-1} is due to attributed to O–H stretching of hydrogen bonding and 3379 cm^{-1} secondary amides, which have one N-H bond, generating a band at 3370-3170 cm^{-1}. The small band in this region (ca. 3379 cm^{-1}) is attributed to the N-H stretching of secondary amides **[19]**.

The bands obtained at 2926 cm^{-1} and 2860 cm^{-1} are due to the functional groups -C-H(CH_2) and -C-H(CH_3), respectively representing the asymmetric and symmetric stretching vibrations of the methylene group of fatty acids present in dairy products. Terminal alkyne with C≡C stretch is attributed to transmittance peak at wavenumber 2152 cm^{-1}**[20]**

The lipid peak at 1744 cm^{-1} is characteristic of the ester, C = O stretching from triglycerides **[21]**. 1700-1600 cm^{-1} is attributed to amide I corresponding to C=O stretching vibrations of the peptide bonds and 1600-1500 cm^{-1} is attributed to amide II corresponding to C-N stretching vibrations in combination with N-H bending **[22].** Two major peaks are clearly observed: Amide I in 1658 cm^{-1} and Amide II in 1542cm^{-1} related to peptide bonds. These peaks are closely linked to the concentration of protein **[23]**. Amid I and Amid II bands are considered the backbone of whey protein concentrate, depicting the presence of α-lactalbumin and β-lactoglobulin, which are the main constituents of whey protein concentrate **[20].**
The medium band at 1,436 cm^{-1} was due to H–C–H bending (scissoring) of CH_2 and CH_3 might be due to the presence of alkanes. The transmittance peak at wavenumber 1381 cm^{-1} is due to the H–C–H symmetric bending of CH_2. The C-O stretching of esters was indicated by wavenumber 1259 cm^{-1} **[24].**
The transmittance band at 1085 cm^{-1} is due to the stretching vibrations of C–C links in the hydrocarbon chain **[24].** The stretching vibration of the C-O group of glycerol is due to transmittance at wave number 1032 cm^{-1} **[23].** C=C–H wagging vibration in the plane was denoted by wavenumber 883 cm^{-1}. The 720 cm^{-1} peak represents the overlapping of the methylene (-CH_2) rocking vibration and out-of-plane vibration of *cis*-disubstituted olefins **[24]**
The wavenumber 605 cm^{-1} showing transmittance is due to Disulfides attributed to (S-S stretch). The transmittance peak between 500–430 cm^{-1} due to S-S stretch thus, 552cm^{-1} and 446 cm^{-1} shows the presence of aryl disulfides **[20].**

Figure.2 FT-IR analysis

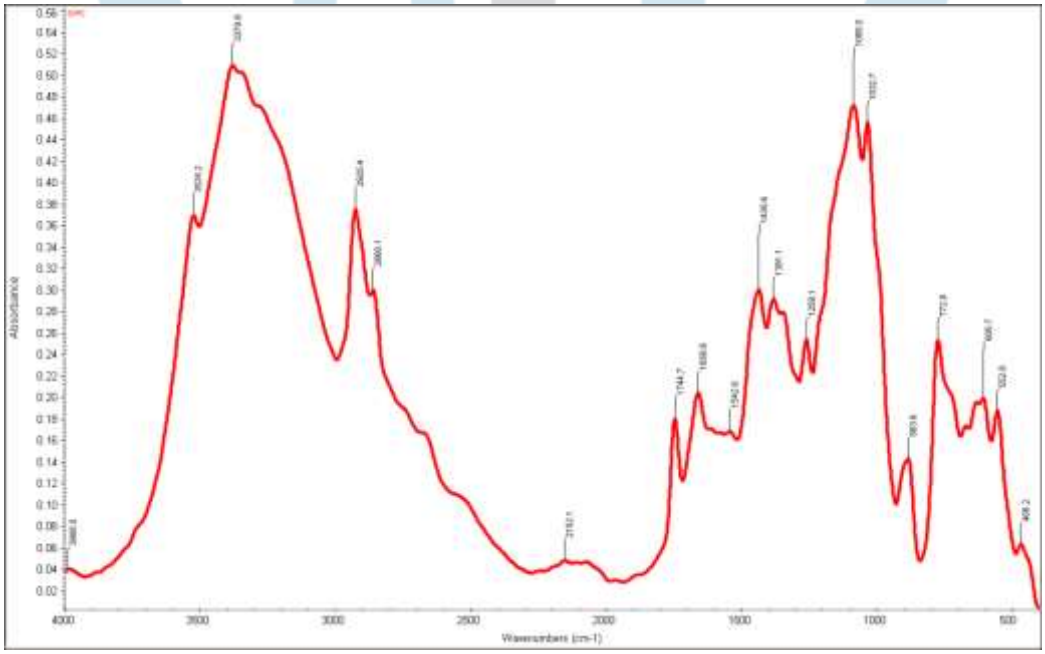


Table 1 Functional group determined

WAVE NUMBER(cm^{-1})	FUNCTIONAL GROUP
3524	O–H stretching
3379	O-H stretching
2925	asymmetric stretching-C-H(CH_2) of lipids
2860	symmetric stretching-C-H(CH_3) of lipids
2152	C≡C, Terminal alkyne
1744	C =O stretching of esters
1658	Amide I (C=O stretching)
1542	Amide II(C-N stretching and N-H bending)
1436	H–C–H bending of CH_2 and CH_3 (scissoring)
1381	H–C–H symmetric bending of CH_2
1259	C-O stretching, aromatic ester
1085	C-C stretch
1032	C-O stretch of alcohol
883	=CH wagging vibration in the plane
772	C–H rocking of CH2 and cis double bond
605	Disulfides (S-S stretch)
552	Aryl disulfides (S-S stretch)
466	Aryl disulfides (S-S stretch)

3.2 Antioxidants DPPH (radical scavenging activity)

DPPH is the most suitable way to determine the antioxidant property of a sample [25]. The colour of the sample changes from purple to yellow as DPPH free radicals are scavenged by antioxidant chemicals. Figure 3 depicts the relationship between whey protein sample concentration (mg) and antioxidant activity (%).

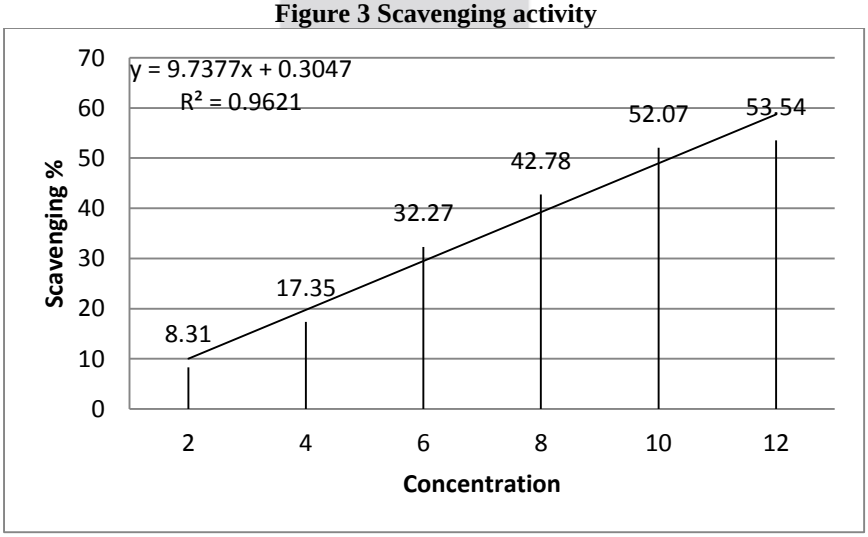
By using a spectrophotometer the optical density of a sample and the optical density of the control can be calculated to determine DPPH behaviour in a sample. According to [26], if DPPH value is below 50 µg/ml it has a very strong antioxidant property, if it lies between 5-10 mg/ml has strong antioxidant property and if it is above 13 mg/ml it has weak antioxidant property. The antioxidant activity of whey protein sample at different concentrations (2,4,6,8,10 and 12mg) was evaluated and the result obtained were illustrated in Fig. 4.5. According to these result, whey protein concentration increases up to 10.29mg. Afterward, the activity of antioxidants was constant.

The equation generated on the above curve was used to determine the IC50 value, where y was replaced with 50 and value of x was calculated which indicated the concentration of extract at which 50% DPPH radicals were scavenge.

Table 2 Scavenging activity

Concentration in mg	Abs at 517nm	Abs. Control	% Scavenging	IC50 value
2	0.375	0.409	8.31	2.022582
4	0.338		17.35	4.076455
6	0.277		32.27	6.130329
8	0.234		42.78	8.184202
10	0.196		52.07	10.23807
12	0.190		53.54	12.29195

Figure 3 Scavenging activity



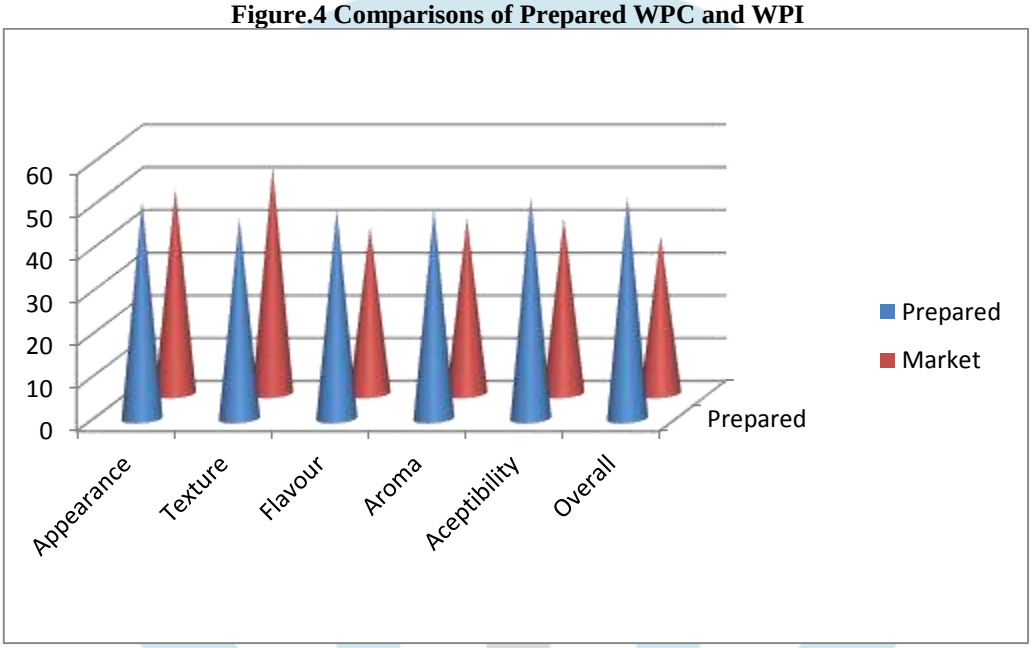
3.3 Sensory evaluation

The assessment by trained judges on samples Whey protein, considering the attributes of look, texture, flavour, aroma and overall acceptance, square measure shown in Fig. 4.6. Fortified cheese sample were assessed by a panel consisted of half dozen panellists (age between 30-55 years old) WHO have expertise and frequently used for assessing these sort analysis. They scored the fifty four out of fifty five in look of Fortified spreadable process cheese. Merchandise and management merchandise were completely different in colour. The flavour could be a vital attribute since it determines client preference [27].

Table 3 Sensory evaluation of prepared WPC and WPI from Market

Protein sample	Appearance	Texture	Flavour	Aroma	Acceptability	Overall
Prepared	51	47	49	49	52	52
Market	48	53	39	41	41	37

Figure.4 Comparisons of Prepared WPC and WPI



From above result we can found that acceptability of prepared Whey Protein have more rating than that of whey protein purchased from the market. And the flavour and aroma also found to be better than Whey protein isolate.

4 Conclusions

A simple and effective approach for preparation of whey protein concentrate (WPC) was successful in this study. Fourier transform infrared (FT-IR) were used to determine the functional group and shows 13 type of functional group in table 1 which have biological function specifically. They act as catalyst and storage of the molecule. They provide mechanical support and strength to the immune system. The antioxidant activity is good enough and it scavenges about 50% of free radical at 10mg/ml concentration, which helps the body to protect against heart disease, cancer and many other diseases. The sensory evaluation was carried out under well trained panellist in the preference to appearance, colour, texture, aroma, and overall acceptability. And the overall acceptability of prepared whey protein was better than that of whey protein isolate purchased from the market. Which help to know for the acceptance in market value of the product.

Competing Interests

Authors have declared that no competing interests exist.

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