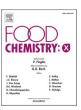
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## Biochemical, dielectric and surface characteristics of freeze-dried bovine colostrum whey powder

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#### ABSTRACT

Colostrum samples of recently registered cow breed "Himachali Pahari" were assorted from high altitude zone (901–2200 m). Prepared bovine colostrum whey powder (BCWP) was analysed for chemical composition, amino acids, minerals, surface morphology (SEM-EDS), FTIR and dielectric properties. Results showed that freeze-dried BCWP contained a considerable amount of nutritional parameters viz IgG (18.55 g/100 g), protein (71.72 g/100 g) and total amino acids (69.64 g/100 g). Additionally, the concentration of essential minerals was found to be adequate, and there was no presence of heavy metals. The BCWP exhibits good dielectric properties (resistance  $\sim\!57$  M- $\Omega$ ). SEM-EDS showed the broken up-wards layer structure with the uniform distribution of minerals on the surface. The FTIR spectra confirmed the presence of a higher proportion of  $\beta$ -sheets and  $\beta$ -turn structures in BCWP. Thus, on account of good functional and nutritive properties, BCWP could be foreseen as the future of functional food.

#### 1. Introduction

The rapid expansion of the world population has escalated the demand for health-promoting dairy products over the past few years. Functional foods are relatively recent developments that played a significant role to meet consumer demand via providing numerous therapeutic applications beyond basic nutrition (Sharma, 2019). Recently, bovine colostrum (BC) has become the most emerging nutraceutical which is also known for centuries for its health benefits (Buttar, Bagwe, Bhullar, & Kaur, 2017). Generically, bovine colostrum (BC) can be defined as immune milk or an alimentative viscous fluid, produced from the mammary glands of mammals right away from calving, that accounts for about 0.5% of the total cow annual yield (Buttar et al., 2017; Mehra, Singh, et al., 2021). This fluid comprises numerous bioactive and nutritional components including, immunoglobin (IgG), lactoferrin,

minerals, amino acids and other minor components in a highly concentrated low volume format (Mehra et al., 2022). These bioactive components were clinically proven in the prevention and pre-treatment of necrotizing enterocolitis (NEC) (Awad, Salama, & Farahat, 2014), upper-respiratory-tract infection (URTI) (Alsayed et al., 2020), enteric dysfunction (Bierut et al., 2021), intestinal permeability, gastrointestinal (Sanctuary et al., 2019) and other chronic diseases.

Bovine colostrum has a limited shelf life due to its high microbial load and low coagulation temperature, which further renders pasteurisation challenging (Mehra et al., 2022). Alternatively, BC can be transformed into liquid whey either by acidification or by rennet coagulation to obtain the product with extended shelf-life as compared to raw (Guo & Wang, 2019). In general, colostrum comprises of elevated level of proteins i.e., whey protein and casein at a ratio of 70: 30 (Christiansen, Guo, & Kjelden, 2010). The rennet acidification of skim

Abbreviations: BCWP, Bovine colostrum whey powder; AAs, Amino acids; ASL, Above sea level; BC, Bovine colostrum; DPs, Dielectric properties; EDS, Energy-dispersive X-ray spectroscopy; Igs, Immunoglobins; SEM, Scanning electron microscopy; WP, Whey powder; FTIR, Fourier transform infrared spectroscopy.

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colostrum results in the non-significant changes in the bioactive components that exist in whey including, IgG<sub>1</sub>, IgG<sub>2</sub>, IGF-1, IgA (Borad et al., 2019). Further, whey and its derivatives exhibit high nutritional value due to the elevated amount of branched-chain amino acids (BCAAs), sulfur-contain AAs and essential amino acids (EAAs) including, valine, isoleucine, leucine and cysteine which are satiating to regulate multiple cellular processes and also represents as pharmaco-nutrient in the prevention of numerous chronic disease (Mehra, Kumar, Kumar, et al., 2021; Naidoo, Naidoo, & Bangalee, 2018).

Whey powder (WP) comprises all the constituents that are present in fresh whey, therefore converting BC whey into powder can be a feasible approach to utilize the BC as a functional food source (Guo & Wang, 2019). Commonly, the milk-based powders were usually prepared by the spray drying method (Zouari et al., 2020), however, freeze-drying or lyophilization is the most favoured dehydration technique for the conversion of BC whey to bovine colostrum whey powder (BCWP) which involve in the expeditious transition of freeze substance from hydrated to dehydrated form i.e., approximately (<4 g /100 g<sup>-1</sup>) of water (Borad et al., 2019; Lapčík et al., 2015). Furthermore, this technique assists in extending shelf-life without altering its native properties and minimum deterioration to thermally fragile components primarily IgG (Borad et al., 2019; Zouari et al., 2020).

The protein, fat, lactose and minerals are the basic compositional elemental of WP. While processing these elements are translocated to the surface of powder showed different concentrations and positions according to the nature of the action (Zouari et al., 2020). Scanning electron microscope (SEM)-energy dispersive spectroscopy (EDS) or (SEM-EDS) is an extensively accepted and performed technique used to determine the surface morphology and distribution of atomic elements on the surface of the powder (Burgain et al., 2017). These surface properties may significantly affect the phenomena linked with adhesion, wicking, wetting and spreading which further played a crucial role in numerous household and industrial applications (Lapčík et al., 2015).

Dairy products exhibit different functional and physical properties, in which dielectric properties (DPs) is one of the main physical parameters. This property assists in predicting the heating rates of the material when exposed or subjected to high frequency in dielectric heating like a microwave (Liu, Guo, & Zhu, 2018). The dielectric property (DP) as a function of temperature and frequency of dairy products including milk or whey-based powders was extensively studied by different researchers across the world (Dag, Singh, & Kong, 2019; Liu et al., 2018). Besides this, another characterization technique is Fourier transform infrared spectroscopy (FTIR) which is widely performed for the estimation of functional groups in food samples (Ostrowska-Ligęza, Górska, Wirkowska, & Koczoń, 2012).

The colostrum samples were assorted from the most recently registered cow breed "Himachali Pahari" of Himachali Pradesh, India. As we reported in our previous study (Mehra, Kumar, Verma, et al., 2021), this rare breed contains an adequate amount of nutritional and bioactive component (IgG) in raw colostrum assorted from the altitude range (901–2200 m). Therefore, the present study was carried out to prepare colostrum whey powder and to analyze its nutritional composition, dielectric properties and surface morphology with a foresight that this study, by utilizing a by-product as functional food ingredient would be significant to the manufacturers and researchers.

#### 2. Materials and methods

#### 2.1. Sample collection

Collection area was selected on the basis of our previous study (Mehra, Kumar, Verma, et al., 2021), all the samples were collected from the same regions, where we observed adequate amount of nutritional composition in raw colostrum. Bovine colostrum (BC) samples (n = 40) were assorted within 72 h. after parturition from the recently registered cow breed "Himachali Pahari" of Himachal Pradesh, India. Assorted

samples were pooled before processing. For the study, all the colostrum samples were collected from healthy multiparous cows, which reared and graze under hilly regions (901–2200 m) without any specific fodder management. Samples were assorted in an HDPE bottle (Tarsons Products Pvt. Ltd., Kolkata) and stored instantly at  $-20\ ^{\circ}\text{C}.$ 

#### 2.2. Processing of bovine colostrum (BC)

Pooled BC samples were defrosted at room temperature ( $26 \pm 1$  °C) and de-fatting was done by refrigerated centrifuge (Sorvall<sup>TM</sup> Legend<sup>TM</sup> XT/XF Centrifuge Series, Thermo FisherScientific, Waltham, MA USA) at 5000 g at 4 °C for 25 min. to obtain skim colostrum as illustrated in Figure ( $S_1$ ). Afterwards, the skim colostrum was pasteurized at 63 °C for 30 min. and maintained at the temperature of  $26 \pm 1$  °C. Furthermore, skim colostrum was treated with 0.01% (w/v) rennet (Meito Microbial Rennet, Meito Sangyo Co. Ltd., Tokyo, Japan; 1,200 IMCU/g). The rennet treated colostrum was allowed to rest for 30 min at 27 °C for curd formation. After the curd formation, the curd was cut into 1 cm<sup>3</sup> sized cubes and liquid whey was separated from casein with the use of muslin cloth (six-fold). Obtained colostrum whey, was dehydrated with lyophilizer (Free Zone, Labconco, Kansas City, MO, USA) to obtain colostrum whey powder.

#### 2.3. Nutritional composition

Nutritional composition of prepared BCWP such as total solids (TS) (Method no. 925.23), protein (Method no. 930.29) and ash content (Method no. 930.30) were analysed by following AOAC International methods (AOAC, 2005). The lactose content was determined through the difference in BCWP composition. The moisture content in BCWP was estimated by the standard method (26:2004) given by International Dairy Federation. The concentration of immunoglobulin (IgG) in BCWP was quantified by a specific Bovine ELISA kit (E11-118, Bethyl Laboratories, Inc. Montgomery) as per manufacture instructions. The absorbance of samples was recorded by an ELISA plate reader (Thermo Scientific, Multiskan Sky Microplate Spectrophotometer) at 450 nm.

#### 2.4. Mineral's estimation

BCWP was analysed for trace (magnesium, iron, sodium and calcium) and heavy elements (arsenic, cadmium, mercury, lead, selenium, tin). Samples were prepared by PerkinElmer's TITAN MPS microwave digestion system and analysed by NexION 2000 ICP-MS (PerkinElmer Inc., Connecticut, USA). BCWP sample (0.25 g) was treated in a microwave digestion vessel by the addition of 7 ml of HNO $_3$  and 0.5 ml of H $_2$ O $_2$ . These samples were kept in a microwave digester and digested as per the above program. After completion of digestion, sample solutions were quantitatively poured into a 50 ml volumetric flask and volume made by using ASTM type 1 water from a Millipore system. For the preparation of standard 1000 ppm stock of standard calibration, 0.1, 0.5, 1, 5, 10, 15, 20 ppb prepared for arsenic, cadmium, mercury, lead, selenium and tin. Another working set of calibration standards was prepared from a 1000 ppm stock of standard calibration standards of 0.1, 0.5, 1, 5, 10 ppm for sodium, magnesium, iron and calcium.

#### 2.5. Amino acids

Total amino acids (EAAs, conditional and non-EAAs) were analysed by the FICCI research and analysis centre (FRAC). The quantification of AAs is done by using a tandem quadrupole mass spectrometer (TQMS) by following EASI-CHE-SOP-25.

#### 2.6. FTIR and SEM-EDS

FT-IR spectrum of bovine colostrum whey powder (BCWP) was determined in the range of 4000–400  ${\rm cm}^{-1}$  by using a Fourier-transform

**Table 1**Nutritional composition of BCWP.

Parameters	BCWP (Mean $\pm$ SD)
Total solids (g 100 g <sup>-1</sup> )	$96.57\pm0.33$
Lactose (g 100 g <sup>-1</sup> )	$17.66\pm0.20$
Protein (g 100 g <sup>-1</sup> )	$71.72\pm0.15$
Fat $(g\ 100\ g^{-1})$	$0.53\pm0.06$
Ash (g 100 g <sup>-1</sup> )	$7.13\pm0.07$
Moisture (%)	$2.96\pm0.06$
Immunoglobin-G (IgG) (g 100 g <sup>-1</sup> )	$18.55\pm0.53$

spectrophotometer (PerkinElmer FTIR-C92035, USA). The potassium bromide (KBr) pelletizing method was followed. The OriginPro, 8E (OriginLab, USA) software was used to deconvolute the FTIR spectra in the region between 1700 and 1600 cm $^{-1}$ . The specific bands at 1640–1600 cm $^{-1}$ , 1650–1640 cm $^{-1}$ , 1660–1650 cm $^{-1}$  and 1700–1660 cm $^{-1}$  are assigned to  $\beta$ -sheets, random coil,  $\alpha$ -helix and  $\beta$ -turns structures, respectively. Scanning electron microscopic–energy-dispersive X-ray spectroscopy (SEM-EDS) (Bruker, Germany) was used for morphological observations of BCWP.

#### 2.7. Dielectric properties

There are multiple techniques widely employed to measure dielectric property of different materials viz transmission line, time domain spectroscopy, lumped circuit, parallel plate and so on (Dag et al., 2019), which significantly depends upon nature of material, accuracy, and frequency range. However, in current study we use parallel plate capacitor to determine the dielectric property of BCWP, due to begin inexpensive coating techniques. Furthermore, the DPs (resistance, dielectric loss and constant) of BCWP were investigated by using an LCR meter (HIOKI, IM3536) at the different temperature ranges from 20 to 100 °C with a frequency range from 40 Hz to 2 MHz in the presence of atmospheric air. The pellets of BCWP (9.8 mm and thickness 3.2 mm), was prepared by using KBR Hydraulic press. The prepared BCWP pellets were coated on both sides with silver paste to make it conductive and to stick with copper wire, which assisted the device to act as a parallel plate capacitor.

#### 2.8. Statistical analysis

All the observations of this work were taken in triplicates and presented as Mean  $\pm$  SD. The coefficient of variation (CV) was evaluated using Microsoft Excel-2019 (Microsoft Corporation, Microsoft Way-Redmond, USA).

#### 3. Result and discussions

It is difficult to relate all the whey powders with the present study due to the different sources of whey was used, milking, pooling, breed, variety of processing methods, environmental conditions and other influencing factors. However, BCWP is being compared in terms of its chemical composition and several properties with equivalent powders prepared from milk and bovine colostrum by other investigators due to limited literature available. Furthermore, each dairy-based powder exhibits its unique composition, therefore the purpose of putting studies of different dairy-based powders is so that we can present in which form this BCWP is inimitable.

#### 3.1. Nutritional composition

Table 1 summarizes the nutritional composition of BCWP. The average total solids (TS), lactose, protein and IgG content in BCWP were 96.57 g  $100~\text{g}^{-1}$ ,  $17.66~\text{g}~100~\text{g}^{-1}$ ,  $71.72~\text{g}~100~\text{g}^{-1}$  and  $18.55~\text{g}~100~\text{g}^{-1}$ , respectively. In general, the protein content in milk-whey powder ranges from 11.0 to 14.5% (Guo & Wang, 2019). This elevated amount of

**Table 2**Amino acid profile in BCWP.

Amino acids	BCWP <sup>a</sup>	CV	SEm±
Essential			
Histidine	$1.28\pm0.04$	0.031	0.02
Isoleucine	$1.84\pm0.04$	0.023	0.02
Leucine	$4.92\pm0.03$	0.006	0.02
Lysine	$4.50\pm0.02$	0.005	0.01
Methionine	$0.75\pm0.08$	0.101	0.04
Phenylalanine	$2.07\pm0.04$	0.019	0.02
Threonine	$1.67\pm0.04$	0.022	0.02
Valine	$4.20\pm0.06$	0.013	0.06
Conditionally			
Tyrosine	$3.11\pm0.04$	0.013	0.02
Cystein + Cystine	$4.07\pm0.09$	0.022	0.05
Non-essential			
Alanine	$2.43\pm0.25$	0.101	0.14
Arginine	$9.03\pm0.07$	0.008	0.04
Aspartic Acid	$5.53\pm0.06$	0.010	0.03
Glutamic acid	$8.53\pm0.25$	0.029	0.14
Glycine	$4.73\pm0.20$	0.042	0.12
Proline	$4.52\pm0.14$	0.031	0.08
Serine	$6.45\pm0.11$	0.017	0.06
Total AA	$69.64\pm0.07$	0.001	0.04
AA nitrogen <sup>b</sup>	10.91		

**Abbreviations:** AAs, amino acids; BCWP, bovine colostrum whey powder; CV, coefficient of variation;  $SEm\pm$ , standard error mean.

protein content in colostrum-based powders was due to the high whey protein; casein ratio in colostrum (70:30) as compare to milk 20:80 (Chatterton et al., 2020). The concentration of IgG % in spray dried-colostrum whey protein isolates and colostrum protein concentrate were reported as  $61.50 \pm 1.04\%$  and  $43.35 \pm 0.84\%$  respectively (Borad, Singh, Meena, & Raghu, 2020). The lactose content in BCWP was found to be 17.66 g  $100 \ g^{-1}$ , which was less than present in the milk-based WP that range from 63.0 to 75.0% and higher than CPC i.e.,  $9.50 \pm 0.02\%$  (Chatterton et al., 2020; Guo & Wang, 2019). This low level of lactose in BCWP and other colostrum powders is due to the concertation of lactose in milk was found to be higher 2.6–4.4% as compared to bovine colostrum 1.2% (Mehra, Singh, et al., 2021). Besides this, the de-fatting of milk results in a significant rise in lactose content (Zouari et al., 2020).

As per American Dairy Products Institute (ADPI Product Standards, 2021), the gross composition of skim (partial removal of fat) colostrum (within 48 h.) powder has a maximum of 35% carbohydrates, 6% fat, 7.5% ash, 6% moisture, 15.0–30.0% IgG and the protein ranged from 50 to 72.5%.

#### 3.1.1. Amino acid composition

Bovine colostrum (BC) is the abundant source of bodybuilding block elements "Amino acids" which served as a precursor for de-novo protein synthesis and pharmaco-nutrient (Naidoo et al., 2018). Obtained results are depicted in the Table 2 revealed that leucine shared the major portion (4.92 g/100 g) of an essential amino acid (EAAs) in BCWP followed by lysine (4.50 g/100 g) and valine (4.20 g/100 g). Methionine was the least EAAs (0.75 g/100 g) found in BCWP. The concentration of leucine, lysine, valine and methionine in low molecular weight fraction of bovine colostrum (CLMWF) (mg 100 g $^{-1}$ ) and whey protein concentrate (WPC) (g/100 g) were 188, 147, 320, 57.6 and 10.4, 9.7, 5.9, 1.9 respectively (Christiansen et al., 2010; Singh & Geetanjali, 2016). Leucine, play a distinct role in to regulate the muscle protein synthesis, and hypertrophy, beside this leucine can be used as a potential source in the prevention of type 2 diabetes and sarcopenia, where isoleucine aids in glycaemic control. Further, a substantial quantity EAAs

 $<sup>^{\</sup>rm a}\,$  All the values of AAs presented in (Mean  $\pm$  SD), expressed in g/100 g.

<sup>&</sup>lt;sup>b</sup> Total AA acid nitrogen calculated as Total AA/6.38.

**Table 3**Concentration of minerals in BCWP.

Minerals	BCWP	CV
Arsenic <sup>a</sup>	$1.55\pm0.25$	0.159
Cadmium <sup>a</sup>	$0.08\pm0.05$	0.643
Mercury <sup>a</sup>	ND	_
Lead <sup>a</sup>	ND	_
Selenium <sup>a</sup>	$34.86\pm2.55$	0.073
Tin <sup>a</sup>	$0.17\pm0.10$	0.577
Sodium <sup>b</sup>	$4058.37 \pm 115.61$	0.028
Magnesium <sup>b</sup>	$141.45 \pm 5.59$	0.039
Calcium <sup>b</sup>	$430.56 \pm 19.01$	0.044
Iron <sup>b</sup>	$1.21\pm0.46$	0.382

Minerals values of BCWP are presented in (Mean  $\pm$  SD), the values are presented in ppb  $^{a}$  and ppm  $^{b}.$ 

Abbreviations: CV, coefficient of variation.

(phenylalanine, isoleucine, threonine and histidine) were also found in BCWP (Table 2), which played an imperative role in numerous body functions. The level of conditional AAs (g/100 g) in BCWP was also found to be in considerable amount i.e., 3.11 tyrosine, 4.07 (cysteine + cystine). The concentration of tyrosine in CLMWF and WPC were 89.8 (mg  $100 \text{ g}^{-1}$ ) and 1.8 g/100 g (Christiansen et al., 2010; Singh & Geetanjali, 2016). Among the non-essential AAs, the concentration of arginine (9.03 g/100 g) and glutamic acid (8.53 g/100 g) was found to be maximum in BCWP. While comparing BCWP with other milk and colostrum-based whey powders, it was observed that the concentration of EAAs (leucine, lysine, valine) and non-EAAs (arginine, glutamic acid) found to be in an amount in all the dairy-based powders, with the exception in arginine. Valine is glycogenic BCAAs, assist in maintaing mental vigor, tissue repairer and involved energy production to perform various body metabolic functions. Phenylalanine is transformed to tyrosine in the body, which is required for certain neural activity, where the histidine assist in to protect nerve cells, and formation of blood cells. Similarly, threonine protect body from different nerve system disorders.

#### 3.1.2. Minerals

The trace and heavy elements in BCWP were analysed due to their essential and noxious nature respectively. Essential elements such as

calcium, magnesium, phosphorus and iron were indispensable in several body functions when present at a normal level. From the results (Table 3), it was found that the concentration of essential trace minerals in BCWP was in the order of sodium > calcium > magnesium > iron > selenium for trace elements. Furthermore, metals are nonbiodegradable, which accumulate in the food chain through biotransformation (Tedesco et al., 2021). Several authors (Rahimi, 2013; Temiz & Soylu, 2012) reported the occurrence of heavy elements (lead, mercury and cadmium) in whole milk or milk-based products. Therefore, BCWP was analysed for heavy metals and the obtained results were tabulated in Table 3. The level of heavy metals, Pb, As and Cd in milkbased whey of cows from the Veneto region, Italy was reported as  $0.0002 \pm 0.0001$ ,  $0.0013 \pm 0.0005$ ,  $0.0062 \pm 0.0010$  mg kg<sup>-1</sup> respectively (Tedesco et al., 2021). The level of heavy metals (Mn, Cr, Ni, Cd, Pb, Hg, As) in sweet/acid concentrated whey was < 0.10 (ppm) (Alsaed et al., 2013).

The differences in the mineral's composition were mainly due to the fodder, grazing, coagulation and processing conditions. For instance, the overall concentration (g/L) of minerals in whey, acidified with acid and rennet ranged from 2.5 to 4.7 and 4.3–7.2 respectively (Guo & Wang, 2019). The recommended daily intake (RDI) of Cu was 0.013 mg Cu/kg/day, where the acceptable limit of lead (Pb) for human consumption given by EU Regulation 1881/2006 is 0.02 mg/kg w/w (Evgenakis, Christophoridis, & Fytianos, 2018). The estimated typical dietary intake of arsenic (As) and cadmium (Cd) for adults (25–30 years) were approximately 9.9  $\mu$ g/day and 2.3  $\mu$ g/kg body weight respectively (Bandara, Towle, & Monnot, 2020).

#### 3.2. Dielectric properties

#### 3.2.1. Resistance

The resistance with varying frequency and temperature ranges from 20 °C to 100 °C was shown in Fig. 1 (a). It was observed from Fig. 1 (a) that the resistance of BCWP is very high  $\sim\!57$  M-ohm  $(\Omega)$  and decreases on increasing frequency and temperature. It was also observed that the material BCWP is highly resistive at room temperature and on increasing temperature, it is going towards semi-conductor while on increasing temperature the BCWP is destroying its property and the colour of the

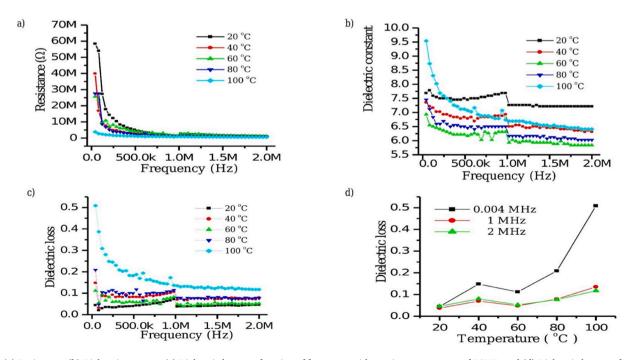
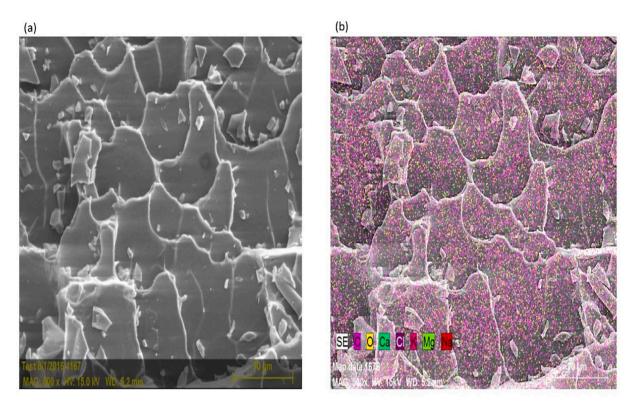


Fig. 1. (a) Resistance, (b) Dielectric constant (c) Dielectric loss as a function of frequency with varying temperature of BCWP, and (d) Dielectric loss as a function of temperature at 0.004, 1 and 2 MHz.

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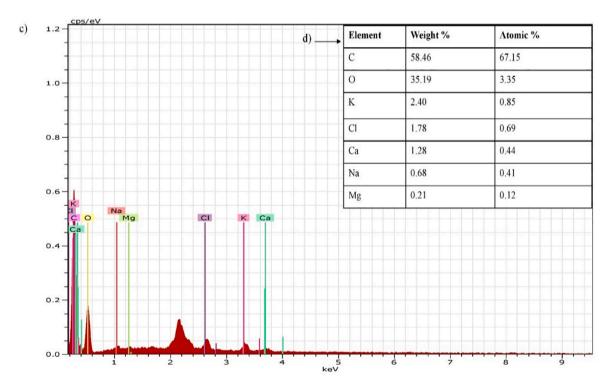


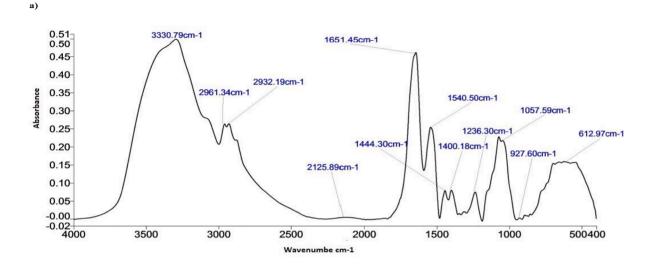
Fig. 2. (a) SEM micrograph of BCWP, (b) Elemental distribution (dot) map, (c and d) EDS spectrum.

device also altering from pale yellow to dark brown. On increasing frequency and temperature, space charge polarization increases in dielectric or insulating materials. Since, whey powder is highly nonconducting, hence due to space charge polarization some conduction of charges occurs, resulting decrement in resistance on increasing frequency and temperature.

#### 3.2.2. Dielectric constant

The dielectric constant as a function of frequency with a varying temperature range from 20 °C to 100 °C was shown in Fig. 1 (b). It was observed from Fig. 1 (b) that the dielectric constant of BCWP is  $\sim$ 7.7 and decreases on increasing frequency and temperature except for 100 °C. For 100 °C the dielectric constant reaches at  $\sim$ 9.5 value, which is naturally very high for BCWP. It can also be seen that the dielectric

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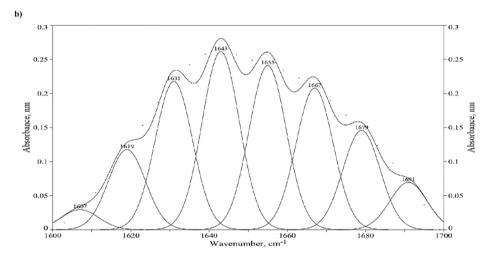


Fig. 3. (a) Fourier transform infrared (FT-IR) spectra (4000–400 cm<sup>-1</sup>), (b) Deconvoluted fingerprint bands (1600–1700 cm<sup>-1</sup>).

values showed very small at all temperatures as compared with other reported work (Muñoz, Gou, Picouet, Barlabé, & Felipe, 2018). Recent studies on whole, skim milk powder (Dag et al., 2019), infants' formula (Lin et al., 2016), and certain other food products (Guo, Liu, Zhu, & Wang, 2011) mentions a number of factors, such as moisture content, variable frequency, temperature, water activity, and overall composition, including fat, lactose, and protein, influence the dielectric properties of any food items.

#### 3.2.3. Dielectric loss

The dielectric loss as a function of frequency with varying temperatures of BCWP is presented in Fig. 1. The dielectric loss is very low at room temperature and almost constant with varying frequency. It was observed from Fig. 1 (c), that as the temperature increases the dielectric loss also increases, which confirms to dissipate the energy at high temperature. The dielectric loss has been shown as a function of temperature in Fig. 1 (d). It can be seen from Fig. 1 (d), that as temperature increases from  $20-40\,^{\circ}\mathrm{C}$  the small increment occurs in dielectric loss and then at  $60\,^{\circ}\mathrm{C}$  there is a small decrement observed, but on increasing temperature, up to  $100\,^{\circ}\mathrm{C}$ , the dielectric loss increases very fast. It happens due to enhancement in electric polarization at a higher temperature. Several authors (Dag et al., 2019; Guo et al., 2011; Lin et al., 2016; Zhu, Guo, Jia, & Kang, 2015) reported changes in dielectric constant and loss when

temperature and frequency increased, which might be attributed to water dipole activity, increased mobility of bound water, stability of compositional elements or decreased ionic conductivity.

#### 4. Surface morphology

The SEM micrograph of BCWP was presented in Fig. 2 (a), showed ambiguous and layered structure with dense brittle texture dispersed with notably sharp edges. Moreover, the particle size of BCWP ranged from 1 to 300 µm to 500 nm as presented in Figure (S<sub>2</sub>). Fig. 2 (b), showed the elemental distribution (dot map) on the surface of BCWP, where the uniform distribution of minerals / compositional elements (Se, C, O, K, Cl, Ca, Na, Mg) on the surface can be seen evidently, Further, the EDS elemental mapping (dot map) of each element is presented in Figure (S<sub>3</sub>). The elemental composition and distribution of BCWP were analysed by energy-dispersive X-ray spectroscopy (EDS). The EDS spectrum Fig. 2 (c) showed the presence of potassium > chlorides > calcium > sodium and > magnesium, as well as the compositional elements carbon and oxygen on the surface of BCWP.

#### 5. FTIR spectral characteristics

FTIR spectra of BCWP is shown in Fig. 3 (a). The IR bands between

1,700 to 1,600 cm $^{-1}$  and 1,500 to 1,590 cm $^{-1}$  were due to C=O stretching vibration and N—H and C—H bending vibration, respectively and attributed to Amide I and Amide II, respectively (Carbonaro & Nucara, 2010). The characteristic peaks at 2,932 cm $^{-1}$  and 2,850 cm $^{-1}$  were due to the presence of lipids in BCWP and were originated antisymmetric CH<sub>2</sub> stretching and symmetric CH<sub>2</sub> stretching bands, respectively (Zhou, Hua, Huang, & Xu, 2019). The IR bands between 1,030 cm $^{-1}$ , 200 cm $^{-1}$ , 930–900 cm $^{-1}$  and 785–755 cm $^{-1}$  were assigned mainly carbohydrate. The characteristic IR band located between 3800 and 3200 cm $^{-1}$  is primarily due to NH stretching vibration and assigned to Amide A band (Carbonaro & Nucara, 2010).

Deconvoluted FTIR spectra were used to characterize the secondary structure of the protein of bovine colostrum whey powder (BCWP) Fig. 3 **(b)**. Amide I (1700–1600 cm $^{-1}$ ) is very sensitive and generally used to determine the secondary structure of proteins. The secondary structure of BCWP consists of a higher number of  $\beta$ -turns (32.8%) followed by  $\beta$ -sheets (28.3%), random coils (20.3%) and  $\alpha$ -helix (18.3%).  $\beta$ -sheet is responsible for the structural stability of protein and is more sensitive to environmental changes and processing conditions than other structures such as  $\beta$ -turns and  $\alpha$ -helix which are responsible for flexibility of protein secondary structure. Results showed that BCWP has a flexible secondary structure due to the presence of a high amount of  $\beta$ -turns (Ye, Zhou, Shi, Chen, & Du, 2017). Meng and Li (2021) also reported the higher proportions of  $\beta$ -sheet and  $\beta$ -turn structures in milk-based powder and whey protein isolate, respectively.

#### 6. Conclusion and future considerations

As a result of enzymatic coagulation of bovine colostrum (de-fatcasein), the nutritional profile of BCWP was found to be higher in protein content, IgG and less in lactose as compared to other dairy powders. Prepared powder comprises a considerable amount of protein, and amino acids, and trace minerals. Moreover, the BCWP exhibits good resistance and dielectric property. The BCWP may represent a novel functional food or ingredient that bears additional research to investigate its whole composition including, proteomics, nucleotide, nucleoside, growth factors and other minor bioactive components. By understanding the dielectric properties of diary-based powders, we may develop a viable pasteurisation strategy. In addition, well-designed clinical trials are needed to determine the long-term safety, efficacy, and optimal dosages of bovine colostrum whey powder. Following the completion of successful clinical trials, bovine colostrum whey powder can be utilised to make infant formula, fortifiers, and various health and nutritional supplements for both infants and adults. Because freezedrying is an efficient technology for preparing powder with minimum loss of bioactive constituents, but it is also fairly expensive and laborious, different standardised time-temperature combinations under vacuuming conditions in spray drying technique are required to cut-off

#### CRediT authorship contribution statement

Rahul Mehra: Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. Shiv Kumar: Formal analysis, Writing – review & editing. Rajat Singh: Writing – review & editing. Naveen Kumar: Writing – review & editing. Deepshikha Rathore: Writing – review & editing, Formal analysis. Gulzar Ahmad Nayik: Writing – review & editing, Formal analysis. Nadiyah M. Alabdallah: Resources, Writing – review & editing. António Monteiro: Validation, Funding acquisition, Writing – review & editing. Raquel F.F. Guiné: Validation, Funding acquisition, Writing – review & editing. Harish Kumar: Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100364.

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