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ADDITION OF WHEY PROTEIN CONCENTRATE IN BISCUITS AND COOKIES

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Abstract

Whey is concentrated to produce different types of whey protein products such as whey protein concentrates (WPC). Whey proteins contain protein contents from 25- 89% and contains 162 amino acid residues consisting of the nine essential amino acids primarily consisting of β - lactoglobulin (BLG), α -lactalbumin (α -LA), bovine serum albumin (BSA) immunoglobulins. Lipid found in WPC is highly enriched in phospholipids. As the concentration of protein in WPC expands, the lactose concentration diminishes. The temperature is inversely proportional to the solubility of whey protein. The sodium caseinate present in it is proficient in absorbing water. Whereas the gel formation occurs after the unfolding of the protein chains. The nutritional effect of addition of whey protein concentrate include the rise in the amount of proteins, carbohydrates and minerals whereas the fats and moisture content is found to be decreased. The total plate count and fungal count was found within the limit during microbiological evaluation. The aim of this paper is to study the effect of addition of WPC on the cookies and its possible application in the bakery industry.

1. Introduction

Whey or milk serum which is obtained from skim milk post casein elimination is the by-product of the dairy industry (Manoi, 2009). An estimated more than 3 Million Tones of whey is produced by Indian dairy industries, while more than 2 lac Tones are dumped into gutter due to costly processing techniques (Khamrui and Rajorhia, 1998). Also, the total worldwide production of whey is estimated at around 180-190 million tons/year. (Yadav et al., 2015; Mollea et al., 2013). Water-soluble residues in whey are 5 to 7% dry matter is majorly comprising by lactose which is around 4-5% and the remaining is protein (1%). It majorly includes water that is 93%. Therefore, it is concentrated to produce different types of whey protein products such as whey protein concentrates (WPC) and whey protein isolates (WPI) and hydrolyzed whey protein (HWP) (Manoi, 2009). UF-diafiltration, evaporation, and spray drying are used in the production process of WPC. Microfiltration is also

utilized to lower the fat content for more protein WPC products (Manoi, 2009). WPC made by the UF process has a protein content from 34 to 80% and spray dried WPC has a bulk density of 0.35 to 0.5 g/cm³. Whey proteins contain protein contents from 25- 89% which are also called serum protein. It contains 162 amino acid remnants consisting of the nine essential amino acids. It primarily consists of β -lacto-globulin, α -lact-albumin, bovine serum albumin, immune-globulins, minor proteins, and enzymes (Fitzsimons et al., 2007). The two most abundant proteins in whey proteins are β -lactoglobulin (BLG) which accounts for 52% and α -lactalbumin (α -LA) which is about 20% and therefore these two are majorly responsible for the functional properties of the globular proteins called whey proteins. The small globular β -lactoglobulin structure is formed with the help of two di-disulphide bonds which is formed in between cysteine amino acid on one side and the other side is of one free sulfhydryl group at the position of 121st cysteine. It majorly consists of α -helical (10-15%), β -sheet (43%), and random coil structures (47%) and is responsible for the process of aggregation and denaturation of β -Lactoglobulin pH dependent (Swaigood, 1986; Boye et al., 1996). β -Lg in its secondary structure comprises of single α -helix and nine anti-parallel β -sheets. β -Lg is bound to association and dissociation. The degree of association is pH-dependent (Farrell and Thompson, 1974; Dalgalarondo et al., 1990; Boye et al., 1996; Sawyer et al., 1999). α -lactalbumin is a small, pH dependent calcium metalloprotein whose molecular weight is 15–16 kDa. According to some authors (Gordon and Kalan, 1974; Whitney et al., 1976; Bernal and Jelen, 1984; Eigel et al., 1984), it exists in two genetic forms but another scientist Fox (1989) added a third form (C) to them. It is a single polypeptide chain consisting of 123 amino acid remnants. Mother molecule of α -La is made up of two domains: a large α -helical domain and a small β -sheet domain, which are connected by a calcium-binding loop (Permyakov and Berliner, 2000). It has around four disulfide bridges and has comparatively high metal binding capacity which as a result forms tough complexes with calcium ions. It has no free sulphhydryl groups. Whey protein ingredients have high contents of Essential Amino Acids and versatile functionalities having many applications in the food industry. Their beneficial functional properties such as turbidity, foaming, viscosity, coagulation, and heat-induced gelation, retention and water binding, dispersability, emulsification, and solubility have been fundamentally explained and applied to the food processing sector (de Wit & Klarenbeek 1984; Morr & Ha, 1993). The structural properties and different applications in the food industry, large-scale availability, and its use in nutraceutical and pharmaceutical industries have made whey a product of great significance. Whey is used as a gelling agent, texturizer, foaming agent, stabilizer, emulsifier, in the manufacturing of edible films, hydrogels, nanoparticles, and coatings (Akhtar & Dickinson, 2007, De Castro et al., 2017). These are also a good source of essential amino acids, which helps in the neural function of body and its metabolism (De Castro et al., 2017). To enhance the physicochemical stability of the WPs different ways can be used as protein encapsulation, enzymatic hydrolysis, molecular chaperones and complexation of carbohydrates (conjugation and electrostatic). Likewise, enzymatic cross-linking, sugar addition, ultrasonication, and mineral chelation can also be used to hamper the protein aggregation process (Kelly, 2019). Transparent beverages are viable products made up of whey protein ingredients. In the production of ready-to-drink beverages, thermal processing is required to enable microbiological safety and quality. For acidic

beverages with pH <4.6, thermal processing conditions are not regulated, instead, hot-fill processes equivalent to heating at about 88°C for 2 min is used by the beverage industry (Etzet,2004). Whey proteins have an immense scope application in liquid meat replacements, sports beverages, processed meats, baked products, artificial coffee creams, soups, salad dressings, ice creams, and other dairy products (Fox and McSweeney,2003; Fitzsimons et al.,2007). From an ecological perspective, Because of the higher values of the Biochemical Oxygen demand (27-60g/l) and chemical oxygen demand (50-102g/l), it is prohibited to dispose whey in civil sewars. On the other hand whey contains high levels of organic and inorganic nutrients, therefor whey has high application potential in the value added products in the food industry. (Svanborg, Johansen, Abrahamsen,& Skeie,2015).The diet of Indians primarily consists of cereals, but these cereals cannot provide some of the major nutrients like protein, minerals, and vitamins. Hence, the lower socioeconomic groups who are living below the poverty line experience a deficiency of nutrients. The food industry is realizing that milk proteins in general and whey proteins, in particular, can enhance the quality of food products (Kumar et al. 2018). The solution to controlling malnutrition is by fortifying the daily used items that belong to the average diet of the poor with high-quality protein from a less expensive source. Therefore, the purpose of this article is to highlight the whey protein properties and their possible use in the bakery industry.

2. Physicochemical Properties of Whey Protein Concentrates

Whey protein exists as compact, globular proteins (Lee et al., 1992) with high solubility because of the large area of surface hydrophilic residues. When whey is put through denaturing agents such as heat, the hydrophobic groups become susceptible and sulfhydryl/disulfide exchange chain reactions take place between the exposed cysteine remnants. As a result, aggregation dissociation and partial unfolding take place (Lee et al., 1992). Environmental factors such as temperature, ionic strength, pH, solvent condition, and protein concentration determine the pathways and rates of these physicochemical reactions (Brandenberg et al., 1992; Marangoni et al., 2000; Iordache and Jelen, 2003). The bioactivity of whey protein peptides is well known and can be liberated through hydrolysis processes catalyzed by chemical or enzymatic agents. These enzymes are used to gain popularity because of their specificity for some active-sites, particularly in terms of biological functionalities which result in ingredients and products with improved functional properties, (Le Maux et al., 2016; Ojha et al., 2016). Chemical, physical and functional properties of WPC depend on the manufacturing processes of the respective. Overall, it contains 4% dampness. Whey proteins concentrate powder recuperated by particle trade adsorption is portrayed by high protein (> 85%) and low (< 0.5%) lactose, low debris, and low lipid substance. The commercial whey protein has protein contents of 34% and 80%. Major Changes in functionality are observed when these are added to food on a solid basis owing to the differences in protein content. Most of the food formulations need some protein content and therefore WPC is used on a constant protein basis. Elimination of differences between protein content should be eliminated. The increase in the protein content might also change the composition of other components in the WPC and therefore can affect the functionality of WPC. Lipid found in WPC has high value of phospholipids and membrane material of milk fat globule. Banishment of remnant lipid from whey increases ultrafiltration flux and improves WPC functionality. When the lipid

content of WPC increases this result as increase in protein content. Some lipids like remnant lipid are known for being harmful for the abilities of WPC, particularly foaming and flavouring qualities of the product. Minor measures of fat in whey protein arrangements cause the fast breakdown of froths that are generally steady. Due to the concentration of Whey to form WPC, the mineral content of whey is changed. Ash content for industrially accessible WPC created by ultrafiltration, electrodialysis, and metaphosphate complex arrangement range from 0.5 to 15%. Calcium content is from 13.9 to 2180 mg/100 g test, whereas the phosphorous substance goes from 0.26 to 3.53%. The samples precipitated contain the largest amount of phosphate. Increased ash content restrains the frothing and emulsion qualities of WPC. WPC has been appeared to work better in various formation, where minerals is taken out or its content adjusted. These applications are utilized for frozen yogurt, new-born child recipes, bread kitchen items, and dietetic nourishments. The mineral that has got the most consideration concerning its impact on usefulness is calcium. The solvency of WPC was enhanced with substitution of calcium, most of the improvement occurred at the isoelectric point. The boundaries in warmth incited gel additionally expanded like sodium is supersede calcium, this did an opportunity which frame a coagulum around 70°C. Overwhelm is diminish by calcium substitution while solution viscosity and foam stability were influenced and affected by non- linearly. Calcium concentrations have a large effect on the heat stability of both β -lactoglobulin and α -lactalbumin. The reliance on heat denaturation on calcium content is presumably answerable for the impacts of heat denaturation on gel quality. At low fixations, the expansion of calcium builds gel quality. when the concentration of calcium increments past a specific maximum level, it further increases which causes decreased quality of gel. On low concentrations, the calcium will increase gel quality by helping the arrangement of cross links that are essential for appropriate gel development. On higher concentration levels of calcium, the precipitation of protein occurs at higher rate than does crosslink development, and gel quality is debilitated due to the weakening of gel strength. As the concentration of protein in WPC expands, the lactose concentration diminishes. Values for the lactose content of economically accessible WPC range from 0.1 to 46%. Values beneath 5% are derived from products produced by an ion exchange procedure, while the estimation of 46% was for a 35% protein WPC. For the most part, lactose is viewed as filler that has little impact on protein usefulness. Lactose is a reducing sugar and can react with proteins using non-enzymatic browning to deliver less nutritious and lower utilitarian products. This must not be an issue for WPC stored at a reasonable moisture level. Lactose can build heat stability and lactose concentration has been identified to be relatable with the dissolvability of whey proteins following heat treatment. This ought not, nonetheless, to be a critical factor in the dissolvability attributes of most WPC. Care is unquestionably justified in the preparation of products that contain extremely low concentrations of remnant lactose and this may elaborate on the perception that WPC created by ion exchange is hard to fabricate with high solubility.

3. Functional properties of whey protein concentrate

Whey protein concentrates are famous for its high nutritious content and its exceptional functional properties when we talk about milk-based products. Whey protein is used in the food industry as whey protein concentrates

basically (Morrand Foegeding,1990). Whey protein concentrates is the powder which is produced by drying of the retentates by ultrafiltration of the whey. Its protein content is of dry matter present in it, which is in the range of 25%-80%. It is important to concentrate the whey 5 times to obtain a whey of 35%, that will be having the solid content of 8%. Properties, which regulate the overall behavior of proteins in foods during manufacturing, processing, storage, and consumption all depend on the property known as the functional properties. There are two types of properties primarily. The first one is nutritional and another one is functional. The nutritional attributes of the whey protein concentrate depend principally on the essential amino acid lysine, tryptophan, cysteine, and methionine. That meets the requirements of its use in the fortification of cereals. Whey proteins are familiar in the industry because of their unique and useful property i.e. solubility to a wide range of pH, and capability to produce high temperature stable gels, and also improved water with fat binding properties.

3.1 Water solubility

Solubility is a very vital property of WPC. As, it is associated with the other functional properties which are emulsifying, gelling, and foaming property directly. The temp. is inversely proportional to the solubility of whey protein. Hence, in other words, we can say it is heat sensitive. Among them, the important one immunoglobulin is the heat sensitive as they become denatured at 70°C afterward α -lactalbumin, β -lactoglobulin, and serum albumin can sustain the temperature of 100°C, which is commonly observed. Denaturation by the heat is basically due to the breaking of Sulphur link within the molecules resulting in unfolding and insolubility. The insolubility is at the best at pH of 4 despite it the solubility is around 60%. The solubility of the proteins at pH 4 and 7 is determined by the method of (Morr et al.(1985).

3.2 Water absorbing capacity

Whey protein absorbs water, naturally after 5-10 minutes. The sodium caseinate present in it is proficient in absorbing the whey protein concentrates. A small change in pH does not affect the absorbing capacity. The concentration of the salt but heating generally affects the natural flavor. Predominantly when the temperature is around 80°C. It is the most accepted method, used in the bakery industry.

3.3 Gel forming ability

The gel formation occurs after the unfolding of the protein chains. The formation of hydrogen along with the ionic bonds in the structure is responsible for this unfolding. When protein chains are attached by the hydrogen bond, hollows get formed in them, which are accountable for absorbing water, and the structure formed by this gets converted to a network of three-dimension encircling pockets of the water molecule.

3.4 Foaming ability

Foaming is the desired property which is accomplished intently by the addition of the air to form the stable structure. Foam development depends on the partial unfolding property of the chains in the protein at the air-liquid interface. Whipping property is relatively less in denatured whey protein concentrates. So, to enhance and to have a healthier whipping capability, higher heat treatment of whey proteins should be avoided. Slight or controlled heat is required to progress the whipping ability of the whey proteins. Foaming ability can be

checked by the whipping time, which is detected by the overrun test and the observation of foam constancy. The good quality whey protein is termed based on this test and it is of the highest soluble nature. There will be mild but not nil denaturation. The whey protein concentrate can replace the use of the egg white; it will be a better option for vegetarian people.

3.5 Emulsifying ability

This is an important property of a whey protein concentrate. It is a surface property of the whey protein which makes it a respectable emulsifying agent. This property is responsible for the foaming and whipping capability. This property is observed in the food, which is having in the fat and water content in high proportion. Emulsifying capacity is based on the quantity of oil and the fats, in the product. On this basis, we can go for the selection of the product to be taken into use. Emulsion stability depends on the capability of the protein to form the emulsion, which remains unchanged till the desirable time. The emulsifying ability in other word is the capacity which depends on the solubility of the whey protein concentrates. Hence, the factors that affect the solubility, controls the emulsion formation. The clarification treatment enhances the emulsifying action of whey protein concentrates at the pH of 7. This result seems that it is associated with the lipid content of the product. In this context, Patel and Kilara (1990) found that the emulsifying capability of the whey protein concentrates with high lipid content (9 to 14%), was suggestively lower than the whey protein concentrates with lower lipid content (4 to 5%). Emulsifying nature gets affected by the lipids, mainly phospholipids, and the monoglycerides. It results in a reduction in the amount of the protein adsorbed in the interface due to the competitive mechanisms and to the direct reaction with proteins. Which results in their uncertainty and promote aggregation (Damodaran, 2005; McClements, 2008). The whey protein concentrates formed by the ultrafiltration process gives higher solubility and also an emulsifying nature. Thermotactic precipitation improved the emulsifying capacity at the pH of 7. Other properties like the solubility, foaming properties, and stability of the emulsion were not enhanced by the clarification. The whey protein concentrates also shows better emulsifying capacity. The mass is used in the food industry as its functional property is an important aspect. That is solubility and emulsifying properties. Nevertheless, the emulsifying activity of whey protein concentrate at pH4 was not up to the mark after the use of the clarification technique, possibly due to the different effects of the pH of the whey protein concentrates. Whey protein concentrate at pH7 showed higher emulsifying activity over pH 4. At pH 7 it gets slightly lower in the emulsifying activity. Over the pH 4, Casper et al. (1999) observed better emulsifying capability than the pH 3 and pH8.

4 Structural Chemistry of Whey Protein

Bovine protein consists of a diverse sequence of the protein. β -Lactoglobulin (β -LG), α -lactalbumin (α -LA), bovine serum albumin (BSA), are the main whey proteins. Immunoglobulins (Ig), lactoferrin (Lf), and some negligible proteins like different proteins.

4.1 β -Lactoglobulin

About 60% of the bovine serum is fulfilled by β -Lactoglobulin, one of the essential contents. The molecule has a 2 nm radius. (Bolder et al.2007). Residues of an amino acid are 162. (Yadav et al.2015). The structure is chiefly β -sheet(Liu et al.2006). Nine strands are there; therefore, the loops form a plane respectively with another β -sheet form a plane with the loop and with the COOH end. The parallel β -sheet unwinds into a cone-shaped barrel protected with α -helix and the gate is formed by a loop into the barrel. In acidic conditions gate is sealed, while the gate remains open in alkaline conditions, therefore within the side chains, some hydrophobic ligands can bind with the residual. Existence of minimum of two hydrophobic restrict the locations in the monomer structure. One is the internal cavity and the second location in the surface between β -sheet and α -helix (Paul et al. 2014). Corrosive side chains of hydrophobic and aromatic amino acids in β -Lactoglobulin have a higher possibility of cleavage sites in protein but are not present for a chemical reaction(Hernandez-Ledesma et al.2006). The structure show variations when β -Lactoglobulin is heated in the range of 90-100°C for the period of 5 to 10 min. (Guo et al.1995). The remaining β -lactoglobulin structure represents the unfolded β -lactoglobulin, disulfide bonds balanced the structure. (kuwajima, Yamaya and Sugai 1996). In whey proteins, 66, 106, 119, 121, and 160 are the sites for cysteine β -lactoglobulin. Cys 66–16 and Cys 106–119 make two disulfide bonds. Cys 121 is remain freed. (Guo et al. 2014). Temperature and pH define the Quaternary structure in β -Lactoglobulin. β -lactoglobulin exists as a dimer when placed at room temperature and in pH of range 5.2 to 7. (Chatterton et al.2006). The dimer forms a long ellipse like structure at impartial conditions, of length 6.95nm and width of 3.6nm, after heating the ellipse got partially unfold and forms monomer. (Schokker et al.1999). In pH range 3.5 and 5.2, β -lactoglobulin forms octamer. At an extreme pH of above 8 to beneath 3, β -lactoglobulin separates to form a monomer (Neg et al.2016).

4.2 α -Lactalbumin

Also known as conservative globular protein. Single peptide includes 123 amino acids (Konrad and Kleinschmidt 2008). di-sulfide balanced the structure of α -lactalbumin, Cys6–Cys120, Cys61–Cys77, Cys73–Cys91, and Cys28–Cys111. free thiol gathering is not present. (Schokker et al. 2000). A cleft separates the α -lactalbumin into two spaces one is the simple helix structure of the α domain and the other contains the crucial content of sheet like the structure of β -domain. (Redfield et al. 1999). The restricting loop of calcium links the two spaces. Three α -helices (pH stable), α -helix of pH subordinate, and 2 short 310 helices together build the space of α domain. On the other hand progression of the loop made the β -sheet, 3 stranded sheet (L-pleated), and a short 310 helix. (Permyakov and Berliner 2000). Carboxylic group of 3 Asp residue, 2 carbonyl club of the peptide spine are the oxygen ligands which shapes the calcium restricting site into the circle, between the helices (Permyakov and Berliner 2000). 1:1 is the ratio in which the calcium is firmly bound with protein. locally unfolded and completely unfolded are the state of transition in a globular protein (Arai and kuwajima, 1996). Some moderate states of α -lactalbumin are collapsed. α -lactalbumin shows exemplarity as liquid globule (Permyakov and Berliner 2000).

4.3 Bovine Serum Albumin

Bovine Serum Albumin is known for its importance in plasma protein. BSA is a globular non-glycoprotein with single chain. (Liu et al. 2004). 64 KDa is the atomic weight of BSA. BSA structures are remarkably like that of humans (HSA). 583 amino-acid residues make the BSA. BSA has 35 cysteines out of which 34 make disulfide bonds and one free sulfhydryl group. 134 and 213 residues are deposited by 2 tryptophan. 67% of BSA structure is α -helix. BSA reassemble heart shaped protein, accompanied by 3 homologous areas. Space is divided into 9 loops which are divided by 17 bonds of disulphate. (Papadopoulou, Green, and Frazier 2005). All the spaces consist of 2 sub-domains. Albumin can bound with ligands. (Carter and Ho 1994).

4.4 Lactoferrin

Lactoferrin atomic weight is 80KDa, it is a glycoprotein with the iron-binding ability of the transferrin group. Consist of two polypeptide side chains of carbohydrates. There are 2 homologous nodes included in bovine milk. In the interdomain part, there is a binding site of iron. The lactoferrin_holo-lactoferrin and apo-lactoferrin have completely identical structure but some areas like C2 and N2 varies. In holo-lactoferrin, the structure is tightly bound and closed. On the other hand, Apo-lactoferrin is an open structure. Apo-lactoferrin is less steady than holo-lactoferrin Bovine. There are 689 amino acid residues of amino acids. (Pierce et al. 1991)

4.5 Immunoglobulin

IgG, IgM, IgE, IgD, and IgA are the immunoglobulin classes. The fundamental structure of Immunoglobulin is defined by 2 heavy chains which are bonded with 2 light chains. Disulfide bonding is the linkage between heavy and light chain, resulting in the Y shape unit. The steady areas of heavy and light chain are grey-toned and variable locales are in blue tone. IgG occurs as a monomeric while others like IgM and IgA occur as polymeric immunoglobulin.

4.6 Minor Proteins

Growth factors, lactoperoxidase, milk fat globule film, and nutrient restricting protein are some of the essential minor proteins in bovine milk.

4.6.1 Growth Factors

Development factor-I like insulin, incorporate as growth factors, and transform the beta-2 development factor. IGF atoms are linked by 3 disulfide linkages. These are the polypeptide solitary chain with the weight of 7.6KDa.

4.6.2 Lacto-peroxidase

There are 612 amino acid residues of the amino acids. The lactoperoxidase structure consists of a solitary polypeptide chain.

4.6.3 Milk Fat Globule Membrane Proteins

The radius of the cross-section of MFGM is about 10-20nm. Act as an emulsifier. Enzymatic deterioration gets shielded by MFGM. The protein combination is not predictable. (Fong et al. 2007).

4.6.4 Nutrient Binding Proteins

There are eight disulfides stretch. The protein is a single polypeptide with a corrosive residue of 222 amino acids (Svendsen et al.1984). D protein and B12 are examples for another nutrient which restrict the protein.

5. Conversion of whey to whey protein concentrate

Whey is focused and dried for a few reasons, for example, to diminish costs for transportation and storage or to prompt crystallization of lactose. It takes much energy to bubble off water from the whey, from a solid's substance percentage of 6.5% to 50 - 60%. This energy is ordinarily is steam under decreased pressure. To decrease the measure of steam required, the dissipation station is ordinarily planned as a different impact evaporator. At least two units work at logically lower weights and subsequently prompting progressively decreased boiling temperatures. There is a club of cylinders in the falling film evaporator in which the stream of whey is pumped in as slight film into the surface. Under vacuum condition, the warming jacket of the stream is kept. The condenser of the machine reduces the H₂O and consolidated fume. For isolation of fume, a rotator is included. To increase the impact of the evaporation one just need to attach more evaporator into the series, up to 7 impacts which will improve the steam economy. These impacts relate to a condenser and a vacuum source. consequently, the temperature contrast (15°C) between the impacts is the equivalent, and the measure of water eliminated in each impact is roughly equivalent. Whey is siphoned from an equilibrium tank to the pasteurizer and shipped consistently in-line with the primary impact of the evaporator. Fume from the primary impact with the boiling temperature of 70°C is used as a warming hotspot for the subsequent one, and so on. The halfway focused whey is isolated from the fume in the twister and siphoned to the subsequent impact. In this impact, the vacuum is maximum, compared to a lower boiling temperature for additional centralization of the whey. The third impact has a boiling temperature of 40°C, bringing about the ideal last focus for additional handling of the whey. A thermo-compressor is frequently used to refine the thermal effectiveness of the evaporator. The structure shows the steam-jet blower, which packs part of the fume from the principal impact. This blower goes about as a warmth siphon which uses a venturi to [the fume pressure and consequently the temperature from 70° to 85°C. The compressed fume is then used to warm the principal impact once more, which expands the thermal proficiency significantly. Higher boiling temperatures, as well as longer habitation times in evaporators, are utilized to deliver "high warmth whey powders". These powders are appropriate for specific application purposes, for example, heating of bread.

5.1 Drying of whey concentrates

The drying cycle might be viewed as a continuation of the grouping of whey to deliver a steady low dampness item for practical and healthful end-use aspects. Modern dryers may be recognized as drum dryers used for specific purposes and splash dryers which are utilized in several drying measures. Drum or roller dryers are used for drying milk as well as protein items, which need surge heat treatment for purposes, for example, caseinates, high warmth milk powder, and some whey items. A roller dryer comprises of two metal drums with a distance across of around 1-meter, the internal surface is warmed with means of steam with a surface temperature of about 100°C. The drums are turning in inverse ways through a box of the concentrate or are taken care of by spraying the material on the drums. Even though drum drying is the least expensive drying

method, but this technique may cause heat damage to the most useful component of whey items. Spray drying is the least difficult structure for the atomizing cycle of milk or whey gathers in hot air inside a drying chamber. The air at inlet temperatures is in between the range of 150°C-250°C and eliminates water from concentrate dropping during drying. Usually, the temperature of vaporization in the water inlet is in the range of 65-75°C. The drying temperature in particle never exceeds this range. In industry the drying using spray dryer can be done using a single stage to a multistage dryer, depending upon the product. The three-stage includes the chamber, internal and external liquid bed. The concentrated whey through the atomizer enters the point of highest drying point. The atomizer provides the drops like structure into chambers. Moisture-free air is pumped into the chamber at a temperature of around 225°C. In the chamber, the moisture content of drops is reduced to 6% and converts into various powder particles. The air takes fine particles in it to the rotator. Then these fine particles are extracted out from the air with the help of a rotator. The uniform stream of fine particle travel from the interior to outer liquid bed. This end has the packaging with a base of perforated pattern for easy passes of hot air, This further reduces the moisture content to 3-4%. After packaging the crystallization in lactose help to avoid moisture during storage

6. Effects of incorporation of whey protein concentrate on biscuits and cookies

6.1 Physiochemical changes occurring due to incorporation of whey protein concentrate in cookies

Biscuits and cookies are consumed on a large scale in our country in the category of products which is produced on an industrial scale using refined wheat flour, hydrogenated fats, sugar, emulsifiers, and some of the food additives. These are manufactured from the non-leavened dough and are produced from a combination of water and refined flour which may include fat and other ingredients combined into the dough which is kept to rest for a period and then passed between rollers to make a sheet (Okaka & Porter, 1997). Among the baked food items, biscuits, and cookies are claimed as the biggest category of snack items in the whole world (Akubor, 2005). In a few countries, cookies are enhanced with incorporated and mixed flour to enhance its nutritional value (Baljeet, Roshan, 2010). Cookies are included in one of the main diet plans followed in routine by all consumer profiles. For obtaining wide varieties of cookies, variable concentrations of basic components, and some minor ingredients is being made in the main mixture (Gallagher & Arendt, 2005). Many products have a longer shelf life and an excellent quality such as cookies, crackers, biscuits, wafers, and many more which are consumed worldwide. To increase the usage of local crops the flour obtained by milling of millets, maize, rice, and sorghum are being added to the wheat flour (Ramacharitar, Matsuo, 2005). India comes in the second position and the first position is occupied by the USA. Many bakery products have wheat flour as their basic fundamental component like white bread, brown bread, cookies, chapattis, rotis (Tortillas), buns, puffs, patties, cakes, pancakes. In India, from the total wheat produced seventy percent of the wheat is consumed as chappatis in both rural and urban areas and it has various types based on the cooking methods like naan, tandoori roti, poories, paranthas, and the other thirty percent is used in the industrial and local production of bakery items like cookies, pastries, cakes, and bread (Butt & Nadeem, 2007). Wheat contains (23.53 to 38.71%) wet gluten, (7.51 to 13.52%) dry gluten, (9.38 to 10.43%) moisture, (10.13 to 14.74%) crude protein, (1.9 to 2.5%) crude fat, (2.3

to 2.9%) crude fibre, (78.7 to 85.1%) nitrogen-free extract, (1.3 to 1.8%) ash content among different varieties of wheat (Kamaljit & Amarjee, 2010). There are many food products which can be fortified by incorporating Whey Protein Concentrates in their food formulations like bread, crackers, confectioneries, beverages, soups, ice creams, gravies, wafers, pastries, pasta, and some special dietic foods which are made for a specific set of individuals (Munaza & Gayas, 2012). Some products like icings, cakes, sponges, biscuits, cakes, and rolls are incorporated with Whey Protein Concentrates for improving the product's flavor, texture, and appearance. Whey protein-fortified baked products have some specific set of properties. Some of the properties which are included in this set are foaming, gelation, water binding capacity, absorption, cohesion, viscosity, emulsification, adhesion, and elasticity. In the processing and manufacturing of these bakery products, these characteristics are considered very important. In India, nutritional deficiencies are still a major concern. Younger age groups are receiving nourishments dependent on root crops and cereals cannot prevent the problem of malnutrition, because the foods they consume are not enough for meeting their daily nutrient requirements, they have insufficient micronutrient content, the energy density of these foods is lower than the minimum requirement and also have a poor protein quality. Protein incorporation in baked products is in focus because awareness is increasing among the consumers toward wellbeing and having a good quality baked product. Composite flour is used in the cookies which are widely utilized as protein fortification vehicles as they have higher acceptability and longer shelf life (Figuerola et al. 2005). Other than acting as a source of quality protein, various other health benefits have been observed in the utilization of cereal products enhanced with whey protein. Thus, the present study was aimed to study the effect of incorporation of WPC into the physicochemical textural and microbial evaluation of wheat-based cookies (Wani et al. 2015). In research conducted by (Wani et al. 2015), whey protein concentrate was incorporated in cookies at different concentrations. Analysis of the cookies was done based on given attributes- physicochemical, texture, microbial, color, and sensory attributes. According to the Physicochemical examination, the cookies which were enhanced with 6 % WPC content were having increased moisture content, ash content, protein content, and fat content as compared to control but a decrease in carbohydrate and crude fiber was observed. With an increase in WPC supplementation level the maximum thickness, diameter, and weight gradually decreased. However, the highest overall acceptability was obtained by the cookie which was enhanced with 4% WPC. In the microbial examination, the total plate count level for the enhanced cookies was under acceptable threshold limits (Wani et al. 2015).

6.2.1 Effect of WPC on Proximate composition of cookies

According to an experiment conducted by (Waniet al.2015), The increasing supplementation of WPC results in increasing the moisture content. This supplementation increased the moisture may be due to an increase in inbound water (O'Brien & Arendt, 2003). An increase in protein content is observed with the gradual increase of supplementation of WPC. The fat content of the cookies also increased with the increasing concentration of WPC incorporation. According to the results increased concentration of WPC incorporation causes a decrease in the overall crude fiber content of the cookies. Increasing the concentration of WPC also affected the carbohydrate content as it decreased in higher concentrations. So according to the obtained results, it was clear

that there was an increase in protein, moisture content, ash content, and fat content but it harmed the levels of crude fiber and carbohydrate. Similar results were obtained by Singh and Mohammed (2007) because they also reported a decrease in carbohydrate content in the incorporation of soy protein in cookies.

6.2.2 Effect of WPC on dimensional properties of cookies

According to the values obtained in the experiment conducted by (Waniet al.2015), the cookies showed a depleted average value for thickness with an increase in WPC fortification levels in the wheat flour. A significant difference in thickness was obtained for samples T2 (4% WPC incorporation) and T3 (6% WPC incorporation). Gallagher et al. (2005) reported a similar decrease in the value of thickness in fortified biscuits in their study.

6.2.3 Effect of WPC incorporation on the calorific value of cookies

Standard values were taken for all the nutrients including carbohydrate (4kcal/g), protein (4kcal/g), and fat (9kcal/g). The term Atwater factor ($4 \times \text{protein}$, $4 \times \text{carbohydrate}$, and $9 \times \text{fats}$) was used in the study of Akubor 2013 for analysis of calorific value. The observations from the tests conducted by (Wani et al.2015) revealed. The highest calorific value was observed in the sample which had a 0% concentration of WPC in them whereas the sample with the highest concentration of WPC showed the lowest calorific value. So we can conclude from the results that incorporation of whey protein concentrates has a negative impact on the calorific value of cookie samples.

6.2.4 Effect of WPC enhancement on sensory perception

According to the results obtained in the sensory analysis conducted by (Wani et al.2015), WPC incorporation resulted in variations in the sensory characteristics like color, texture, flavor, taste, and overall acceptability. The results revealed that the average score for color increased with the increasing concentration of WPC because the highest score was obtained at 6% incorporation. The average score of texture decreased noticeably due to increased incorporation in the sample. An increase in fortification level of WPC also affected the flavor because it imparts bitterness in the taste of cookies hence the lowest average score for flavor was obtained in 6% concentration. The overall acceptability of the samples is analyzed by keeping in mind all the attributes like texture, color, taste, the flavor of the cookies. So according to the sensory analysis, the Overall acceptability of the cookies was highest in the 4% incorporation of Whey Protein Concentrate in cookies. On the other hand cookies with 6% concentration was at the bottom among the four concentrations which were 0%, 2%, 4%, and 6% of WPC incorporation.

6.3 Nutritional Analysis of WPC Incorporated Biscuits and Cookies

6.3.1 Protein quality

In a study of whey protein concentrate fortification in cookie variety biscuits conducted by Parate et al. 2011, it was observed that all biscuits incorporated with whey protein concentrate had greater content of protein than

control biscuits. This indicates that the addition of whey protein concentrate in the production of control biscuits increased the protein content. Hence, the protein content is found to be increasing proportionately with increasing level of whey protein concentrate added in it. Whey protein concentrates include the highest amount of all amino acids and is labeled as high-quality proteins consisting of all the amino acids required for the human body. Cereal grains often contain a low amount of lysine and low amounts of sulfur-containing amino acids which are methionine and cysteine respectively (Zainab et al. 2016). Therefore, the incorporation of whey protein concentrate provides an advantage over conventional wheat flour cookies as it increases the amount of lysine and other amino acids present in the treated sample (Ahmed et al. 2019). In a similar study by Munaza et al. (2012), it was observed that there was an increase in protein content in biscuit samples with an increase in the level of whey protein concentrate from 4% to 10%.

6.3.2 Moisture

Biscuits are most likely a superior vehicle of enrichment with protein because of their popularity, high nutrient density, and long shelf life since they are extremely low in moisture (Narmada et al. 2020). It was reported that the dough enriched with whey protein concentrate, which is used for preparing the biscuits, required excess water than the control dough to have an appropriate consistency. The moisture content of all the whey protein concentrate incorporated biscuits was, in this manner, a bit greater than the control biscuits, but anyways for all degree of whey protein concentrate incorporation, the moisture content was within the specification limit set by the Bureau of Indian Standards (BIS) for protein incorporated biscuits. Hence, if the level of addition of whey protein concentrate is increased in the production, it results in a proportionate expansion in the moisture content of biscuits. So the proportionate expansion in water content of the dough is due to the expanding level of whey protein concentrate in the production of biscuits (Parate et al. 2011). There was a steady increase in the moisture content with an increase in the level of whey protein concentrate from 4% to 10% (Munaza et al. 2012).

6.3.3 Fats

Biscuits incorporated with whey protein concentrate were found to have fat content a bit greater than the control biscuits, but still, the fat content was found to be within the BIS specification limit for any intensity of whey protein concentrate. The amount of fat increased a bit with an expanding level of whey protein concentrate as whey protein concentrate had more fat than wheat flour (Parate et al. 2011). Another study conducted by Munaza et al. 2012 reported that whey protein concentrate added to wheat flour consisted of lower fat content. So, the externally added fat is a major factor in determining the total fat content of biscuits during the preparation. As a result, the amount of fat content decreases as the level of whey protein concentrates incorporation increases from 4% to 10%.

6.3.4 Carbohydrates

Results from statistical analysis found that there was a significant reduction in the amount of carbohydrate content when whey protein concentrate was incorporated into the biscuits (Narmada et al. 2020). This is due to the fact that whey protein concentrate has less amount of carbohydrates than wheat flour, and when it is

incorporated into wheat flour at increasing levels, the overall amount of the resultant carbohydrates decreases. The carbohydrate content of cookies was observed to be significantly decreased from 65.54 to 53.72% with increasing levels of whey protein concentrate from 4% to 10% (Munaza et al. 2012).

6.3.5 Minerals Analysis

Whey protein concentrates are rich in calcium, phosphorus, and water-soluble vitamins. This makes whey protein concentrate biscuits a highly nutritious product (Parate et al. 2011). In a study conducted by Ahmed et al. 2019, it was observed that the number of minerals like Calcium, Magnesium, Sodium, Potassium, Phosphorus in whey protein concentrate incorporated biscuits were found to be the highest (37.37, 19.08, 18.50, 430.00 and 259 mg/100g respectively). The statistical analysis displayed a significant difference in mineral content at increasing levels of whey protein concentrates. Also, the amount of Ca and Mg content in wheat flour were observed to be lower than the results reported by Tang et al. 2008 and Araujo et al. 2008, on the other hand, Ca content was observed to be greater than the value reported by et al. jack 2009 which is 4.86 mg/100g for wheat flour biscuit. It was observed that there was an increment in the value of Ca and Mg in the whey protein incorporated wheat flour with the expanding levels of whey protein concentrate from 5 % to 15 %. Potassium content was within the range shown by Araujo et al. 2008, but it was higher than that reported by Taha 2000. As whey protein concentrates have a low amount of iron, the content of iron in biscuits gets decreased with increasing level of whey protein concentrate in the whey protein concentrate incorporated wheat flour from 5 % to 15 % (Ahmad et al. 2019).

6.4 Microbiological analysis of whey protein concentrate incorporated biscuits and cookies

In a study conducted by Wani et al. 2015, the microbiological analysis of whey protein concentrate enriched biscuits was done by treating the samples with different levels of WPC and checking the total microbial growth on nutrient agar medium as well as fungal count on Potatoes Dextrose Agar. It was reported that the maximum value for the total microbial count, i.e. 4.62×10^2 was found in T3 (6% whey protein concentrate incorporated cookies) on nutrient agar medium and the minimum value was found in T0 (control cookies) which is 3.05×10^2 . Also, the maximum fungal count value was observed in T3 (6% whey protein concentrate incorporated cookies) i.e. 0.13×10^3 while the lowest value was observed in T0 (control cookies) which is 0.5×10^1 . The results showed that T0 and T3 differed significantly while T1 and T2 did not differ significantly in the case of the fungal count. There was an increase in the total microbial count of whey protein concentrate incorporated cookies as compared to control and it was speculated to be because of an increase in moisture content with expanding whey protein concentrate incorporation levels. The total plate count was found to be less than 105 cfu/g when the total microbial count of the whey protein concentrate incorporated cookie samples were compared with microbiological standards of fortified blended product, and the value was still found to be within the acceptable limit.

6.5 Changes during storage period for whey protein concentrate incorporated biscuits and cookies

6.5.1 Changes in moisture content and water activity

A study by Islam et al. 2017 reported that there was an increment in the moisture content of the WPC incorporated biscuit samples with an increment in the level of whey protein concentrate. The sample having 50% WPC was found to have the highest value of moisture content, i.e. 2.89 during 30 days. Similarly, storage in inappropriate conditions often leads to physical changes in dried food products and this may lead to a significant reduction in the shelf life of the products. For instance, there will be a moisture uptake when dehydrated foods are stored at high humidity which leads to the products getting soggy, resulting in reduced shelf life and bad quality (Singh, 2000). In a similar study conducted by Gallagher et al. 2005, as compared to the control, biscuits incorporated with 5 and 10% WPC were higher in moisture. An increment in moisture content was observed for biscuits having 5% and 15% WPC while checking the readings at 8 weeks. Biscuits containing 15% WPC were observed to have the lowest water activity values due to the presence of more bound water in the system. Same observations were reported by O'Brien et al. 2003, where little increment in water activity was observed over time. These increments may have been there due to recrystallization of sucrose leading to the release of water and hence higher water activity. Ash content was found to be increasing in WPC incorporated biscuit samples with expanding levels of whey protein concentrate. The sample having 60% WPC was found to have the highest value of ash content, i.e. 1.26 during 15 days, and the lowest value was found to be in control samples (0% WPC), i.e. 0.23 (Islam et al. 2017). The same type of results was reported by Hahn et al. (1990), where it was found that oats contain 1.56 percent ash. In this regard, Tariqual et al. (2007) observed the ash content in soybean flour to be 2.1 percent. Mebpa et al. 2007 found that wheat flour contains 0.90 percent ash. From this study, we can observe that there was an insignificant difference in levels of ash content at different time intervals when different levels of whey protein concentrate were added (Islam et al. 2017). The control sample was observed to have the highest protein content, i.e. 25.47 at 0 days which was the fresh sample, and the lowest value was observed in the sample containing 60% WPC during 45 days duration, i.e. 09.41. The reason behind the decrease in protein content may be due to the activity of enzymes, storage, and temperature conditions. Thus, this study indicates that the amount of protein content decreases slightly in WPC incorporated biscuits with an increasing period at different levels of whey protein concentrate (Islam et al. 2017). The amount of fat present in WPC incorporated biscuits and cookies tends to decrease with increasing levels of whey protein concentrate as they have less fat content than wheat flour. (Munaza et al. 2012). However, the amount of fat slightly increases during the storage period and this may lead to rancidity or oxidation if proper storage and temperature conditions are not maintained, and that may lead to degradation of quality. The highest amount of fat content was observed in control (WPC 0%) samples in 0 days and the lowest value of fat content was observed in the sample containing 60% whey protein concentrate at a time interval of 30 days. The changes in the fat content of WPC incorporated biscuit samples at different time intervals and different levels of whey protein concentrate are not much significant and are within the acceptable limits according to the standard (Islam et al. 2017).

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