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Development of Low Cost Nutritional Beverage from Whey

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Abstract: *To establish feasibility and superiority of whey based nutritional beverage using established qualitative and quantitative tests for milk proteins and inclusion of cereal based additives. Additionally ensure that whey produced in processing milk; an important source of nutrition; is utilized to its maximum potential.*
Commercial milk processing recycles whey to extract soluble nutrients, hence whey discarded by commercial milk processing plants is of little use. On the other hand, domestic and low scale milk processing produces whey that has a higher nutritional content and can be utilized locally to produce nutritional drinks that can serve a variety of purposes from refreshment to dietary supplements.
Process Guidelines*: The research includes collection of waste whey from dairies, messes and canteens; Determination of the proteins and other nutrients present in the whey and their quantity, Extraction and concentration of whey protein and addition of additives; nutritional benefit and waste reduction.*
The process described above is to be used on a small scale with limited resources both physical as well as monetary hence the current methods of protein extraction from whey fall short.
Scope*: Whey based nutritional drinks can be produced locally; at low cost; from discarded whey and can play a vital role in combating deficiencies. Whey-Barley beverage contains important nutritional ingredient to address several health problems the common man is facing at the present time. Whey proteins provide excellent nutritional values, in nutrition foods formulated for kids, adults and old aged people as growth tonic for body health maintenance. Additionally, whey protein may help to reduce appetite, improve some diabetes symptoms, increase muscle mass and strength.*
Keywords: *Whey, Whey protein,, nutritional beverage, low cost*

I. Introduction

Commercial milk processing recycles whey to extract soluble nutrients, hence whey discarded by commercial milk processing plants is of little use. On the other hand, domestic and low scale milk processing produces whey that has a higher nutritional content and can be utilized locally to produce nutritional drinks that can serve a variety of purposes from refreshment to dietary supplements. High protein content drinks aimed at increasing muscle mass can also be produced at a significantly lower cost.

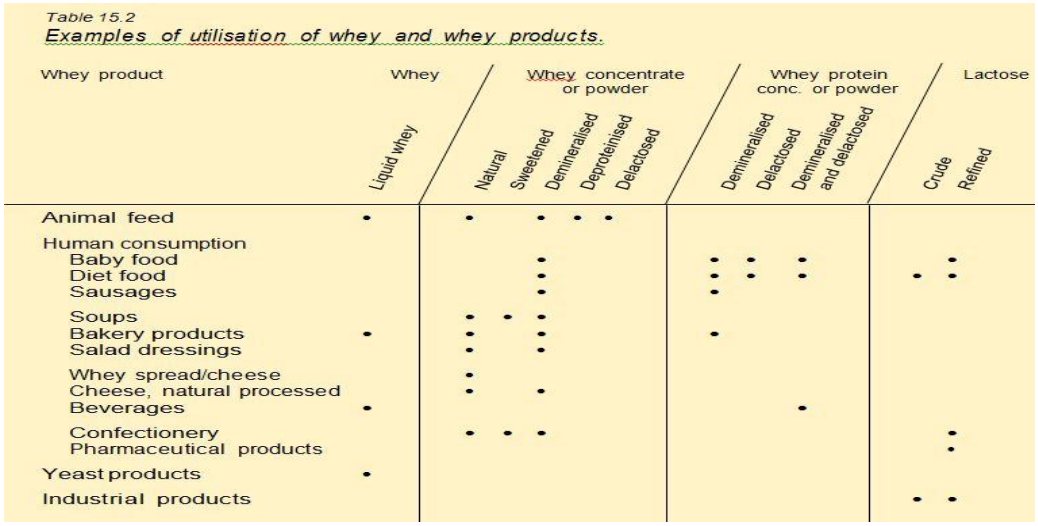
Whey contains a significant amount of beneficial proteins and minerals however it also contains several potential allergens as well as a high amount of low density cholesterol, this prevents direct consumption of whey and requires a scientific process that can eliminate undesirable substances, concentrate the nutrients and serve them in an acceptable manner.

Whey proteins are a combination of various protein fractions

Total Whey Proteins In Milk	:	0.62%
• Lactoglobulin	:	0.30%
• Lactalbumin	:	0.12%
• Serum Albumin	:	0.04%
• Immunoglobulins	:	0.07%
• Proteose Peptones	:	0.06%
• Minor Proteins	:	0.03%

Whey proteins are present in soluble form in the milk. The milk is first coagulated by application of either rennet or acid, casein is precipitated. Rest of the liquid is called WHEY from which Whey proteins are recovered and concentrated.

A widely accepted measure of the general effectiveness of a dietary protein source in providing the metabolic requirements of individual is an index called the **'Biological Value'**. Whey proteins have been known for some time to have biological value superior to that of other naturally occurring proteins (e.g. egg, soya, beef, casein etc.). Biological value is determined by evaluating many aspects but in particular amino acid profile and protein digestibility. Whey proteins have an excellent biological value as they score highly on both counts. It is easily digestible, so that sufficient numbers of these amino acids reach the body's cells to permit them to make the proteins they need.



(Dairy Processing handbook,Bylund, Gösta,Ch-15,Pg-332)

1.1 Whey Protein-Nutritional Value:

The nutritional values of proteins from a physiological perspective are evaluated on the basis of following parameters:

1.1.1 Essential Amino Acid Profile

The details about essential amino acids profile for Whey Proteins, Casein, Soya Proteins, Egg Proteins, Wheat and Rice Protein is given in the table below:

Essential Amino Acid	Protein – Source					
	Wheat	Rice	Soya	Egg	Casein	**Whey Proteins**
Iso-leucine	14	15	21	28	46	**55**
Leucine	27	32	31	34	91	**111**
Lysine	11	15	26	29	77	**88**
Methionine	6	10	5	14	29	**25**
Phenylamine	18	18	19	23	51	**34**
Threonine	12	15	16	21	43	**72**
Tryptophan	5	5	5	5	12	**30**
Valine	18	25	24	29	57	**52**
Histidine	21	21	24	21	30	**22**

(mg/gram of protein)

1.1.2. Biological Value (Bv)

Biological Value measures the amount of protein nitrogen that is retained by the body from a given amount of protein nitrogen that has been consumed.

The comparison chart for **BV** for different sources of proteins is listed below:

Whey Proteins	104
Whole Egg	100
Egg White (Albumin)	88
Casein	77
Rice	74
Soya	59
Wheat	54

1.1.3. Protein Efficiency Ratio (Per)

The Protein Efficiency Ratio is used as a measure of growth expressed in terms of weight gain of an adult by consuming 1gram of food protein.

The table below shows the PER value for various proteins:

Protein Source	PER Value
Wheat	1.00
Rice	1.25
Soya	2.12
Casein	2.50
Lactalbumin	2.86
Whey Proteins	**3**

Based on the above information, it is established by nutritional experts that only 14.5 gram Whey proteins in native form will satisfy the daily requirement of essential amino acids as compared to 17.4 grams Egg proteins; 12 grams Whey proteins are equivalent to 20 grams of Casein proteins in terms of weight gain**.**

1.1.4. Whey Proteins: Standard Composition

Product	Moisture	Protein	Lactose	Fat	Minerals
Whey Protein 80	5	80	04	5	6
Whey Protein 70	5	70	14	5	6
Whey Protein 60	5	60	24	5	6
Whey Protein 45	5	45	40	4	6
Whey Protein 35	5	35	50	4	6

1.2 Whey Proteins-Nutraceuticals Application:

Whey proteins are the wonder proteins for Nutritional, Clinical, Dietetic, Sports, Infant, Protein foods etc. because of:

- The best possible essential amino acids profile
- The best protein efficiency ratio
- The best biological value
- Easy digestibility and assimibility
- Natural taste in native form
- Completely and easily soluble

1.2.1 Nutritional & Dietetic Foods:

Whey proteins provide excellent nutritional values, in nutrition foods formulated for kids, adults and old aged people as growth tonic for body health maintenance. The major products are general health food products as well as protein supplements as under:The USP of whey based products is high protein content, low fat and salt content and are suitable for all age groups. Whey based products have the potential to benefit a large number of people.

Nutrient supplements can be designed :

- For Lactose Intolerance in all age groups.
- As geriatric food formulations – for old aged people with low digestibility.
- For pregnant, lactating and nursing women.
- As Pediatric foods for growth of children, for anemic children, for memory boosting of children.
- For faster post-operative recovery or recovery from illness.
- For general health beverages, stamina building and vitality.

Whey proteins fulfill the need of essential amino acids for growing kids as they require constant supply of high amount of essential as well as non-essential amino acids. The excellent PER value give the regular weight gain as pet their growth requirements.

For adults Whey proteins are highly recommended as these proteins facilitate the weight control management of body.

Lactoferrinpresent in Whey protein improves **iron absorption** in body.

Since Whey proteins are rich in calcium and phosphorous, they provide good bio-availability of these minerals essential for bone formation and better bone strength.

Concentrated protein levels lend Whey proteins powders to use in product categories such as nutritional bars where it leaves maximum space in the formula for addition of other ingredients such as fruits, nuts and honey.

1.2.2 Clinical Foods:

It is established that Whey proteins are digested much faster than less advanced proteins. Increased rate of digestion has been shown – as results in increased plasma amino acid and proteins synthesis attributes, which makes it particularly attractive to the fields of clinical nutrition.

Whey proteins are studied by various nutritionists and it is of great interest that Whey proteins are very useful in operative care of patients as the best digestible proteins. It is highly recommended in body cell repairing and growth as it provides all the essential amino acids in sufficient amount to provide the best possible solution for formulation of clinical foods.

Whey proteins consist of various minor proteins like L-lactoalbumin, Lactoferrin, Immunogloblin, Serum albumin, Lactoperoxidase etc. having high therapeutic values. These constituents have special medical application in dietetic products to treat & prevent a large number of clinical problems such as:

- Special formulations for cancer/tumor patients.
- Special formulations for diabetic patients.
- For cardiac ailments.
- For high cholesterol patients.
- For liver, arthritis patients.
- For burnt patients.
- To deal with situations like renal failure, gout, trauma etc.

1.2.3. Infant Food Formulations:

Formulation of physiologically suitable infant foods necessitate reduction of protein and mineral levels from bovine milk-rich in lactose and Whey proteins and containing appropriately low levels of essential minerals. DemineralisedWhey is an ideal ingredient for infant formula. Demineralisation of Whey permits formulation of infant foods with a gross composition closer to that of mother's milk.

Lactose and its break down products – glucose and galactose have several metabolic and developmental functions in infants. Whey proteins extracted from milk are universally known for their immunological values for infants. That is the most important factor in mother's milk considered best for babies.

In low birth weight baby formulations, the level of Whey proteins is now made mandatory in the ratio 60:40 with Casein; hence fortification with Whey proteins is the only solution to achieve this.

1.2.4. Sports Foods:

The importance of Whey protein supplementation is critical to any individual looking forward to build and maintain muscle mass. Proteins have been called as the **"Building Blocks of Life"** and without proper Whey protein supplementation the task of building and maintaining muscle mass are next to impossible. The reasoning for this is that Whey protein has superior BV.

Every athlete is aware of the importance of protein supplementation. If you are on any strength-training program, building muscles will meet or defeat optimum performance.

Whey proteins have been enriched by nature with Branched Chain Amino Acids (BCAA), namely- L-Isoleucine, L-Leucine, L-Valine. These BCAA must be present in the muscle cell to promote protein synthesis.

These BCAA helps increase the bio-availability of high complex carbohydrate intake and are absorbed by muscle cells for anabolic muscle building activity.

The current theory is that during prolonged exercise, the BCAA's are released from skeletal muscle; the carbon part is used as fuel and the nitrogen part is used to make the amino acid alanine which then goes to the liver where it is turned into glucose for energy. So for athletes who want to protect their existing mass, the idea is to take a BCAA source before and after breakdown exercise. The issues being investigated are whether BCAAs reduce muscle breakdown and act as an energy source during this period. While maintaining exercise performance and delaying exertion, BCAAs are very important for muscle growth.

The use of BCAAs in sports nutrition is especially interesting in making exercise feel easier. Whey proteins also help in speedy repairing of injured and torn muscle during practice and performance.

II. REVIEW OF LITERATURE

Today, the food industries are looking for ingredients, which can provide good functional and nutritional properties for the formulation of various value-added food products. The increasing awareness for nutrition, health and quality food consciousness of consumers and the keen competiton in the market, compel the food industry to search for those ingredients, which can impart specific functionalities to food products, while preserving and enhancing the nutritional quality of foodstuffs, in order to sell their products profitably. In this context, in recent years, much attention has been paid to create a variety of functional properties of milk proteins. The food manufacturing industry has come to realize that milk proteins in general, and whey proteins in particular have potential to improve the quality of food products. This potential has been the subject of many

studies.Due to the unique functional properties, significant market opportunities are expected for WPC that may fall even outside the existing protein market. WPCs are the most valuable ingredients and contribute to a great extent in the development of new food products, as they are capable of fulfilling the diverse functional properties to satisfy different forms of utilization. These are generally recognized as safe (GRAS) for food product applications and not specifically restricted by standards of identity. Owing to their excellent nutritional and functional properties WPCs find numerous applications in food and dairy industries. However, in order to utilize whey protein concentrate as an ingredient in various food product formulations, a thorough knowledge of nutritional and functional behavior of these ingredients in food systems is very essential. Though many reports are available in the literature with regard to nutritional and functional properties of whey protein concentrate, only a few have appeared with respect to application of the specific functional properties in food product formulations. In view of its importance as an ingredient to the food industry, the various aspects of compositional and nutritional importance with respect to food applications and the functional properties such as solubility, viscosity, water binding, whipping, emulsification and gelation and their specific applications needs utilization of whey in developing acceptable nutritional beverage.

Whey was all along been considered as a waste product and looked upon seriously by environmentalists and technologists due to its potent polluting strength. Now-a-days, however, it is no longer considered a waste product but a treasure chest of nutritionally rich whey protein, which has been unlocked by the modern processing technology, enabling them to recover economically in their native form. The advent of this technology has been a great boon to recover these precious solids in the form ofwhey protein concentrate (WPC). The whey proteins are potentially nutritional and functional food ingredients for use in a wide range of food types and can replace expensive ingredients such as egg white and milk proteins. It also contributes to the development of new food products, WPC are generally recognized as safe (GRAS) for food product application and not specifically restricted by standards of identity. This review focuses on functional aspects of WPC in relation to their application in food products formulation.

Whey and whey derived products besides being nutritional ingredients in various foods can also be used as functional ingredients supplying flavor, texture, colour and aeration properties in a variety of foods.

Whey proteins possess very good functional properties, such as solubility, foaming, emulsifying, gelling and water binding. WPCs which contain sufficiently high protein concentrations in a predominantly un-denatured form, with minimal lactose and lipid contents, are highly acceptable as functional protein sources for the food industry. According to studies, the various WPC and WRI will give similar functionality on a protein basis in some instances. This does not occur for low protein (WPC<50% protein). Differences also occur because of differences in the components of whey protein products as a result of manufacturing process.

Two protein ingredients with exactly the same composition can exhibit very different functional properties due to differences in their degree of denaturation because functional food uses normally high protein WPC (75-80%). These products are competitive with other major protein ingredients such as caseinates, soy and egg protein products for numerous applications. Their functional properties have encouraged attempts to use them in a great number of food products replacing other traditional additives such as milk powder and egg albumin.

The functional properties of protein ingredients are ultimately determined by the unique structure and interaction of the protein molecule. WPC may be produced with a wide range of functional properties. It is this characteristic that can increase the attractiveness of WPC. Additionally, these protein products can be specially tailor-made to achieve specific nutritional properties in foods. Many of the desirable attributes of foods may be directly or indirectly related to their functionalities of their protein components. The manner in which food proteins interact with other food components as well as with themselves determine their functionalities . The various functionalities of whey proteins have been reviewed by several authors. The following discussions deal with the possibilities of extrapolating these functional properties in food formulation applications.

Undoubtedly, whey protein concentrates are potential ingredients for food industry, owing to their high nutritional and functional properties. A great potential exists for WPC to use as a specific functional ingredient in various food formulations, besides adding on to the nutritional quality. In this context, research effort has to be focused more on product formulation aspects using WPC as ingredients in each of the product, where it could be used. A systematic protocol has to be given for each product and has to clearly spell out the kind of WPC to be used and the processing conditions, as WPC is a broad term having wide range of proteins and compositional variations. With the introduction of microfiltration, the quality perception of WPC can certainly be improved and thus, WPC will be pivotal ingredients to meet the multi-dimensional requirements of the food industry.

WPC with specific properties have to be developed and in the sense, a tailor-made product has to be developed to suit the individual product requirements.

Whey and buttermilk are the important by products of Dairy Industry. The nutritional and functional properties of these products are of great significance for the formulation of various food products. Technologies have been successfully developed to conserve whey solids and buttermilk solids in dried form. However, limited

reports are available as per the utilization of dried buttermilk and whey protein concentrate in various food formulations. Hence here is the need to develop various food products using whey solids and buttermilk solids to promote the utilization of dried whey protein concentrate (WPC) buttermilk in the market.

In this chapter, the information with respect to the significance of whey solids and buttermilk solids and their utilization in various food products including ice-cream and ready-to-use ice cream which are of relevance to this project have been critically reviewed.

2.1 Significance of Whey

Whey is the liquid left after removal of casein and fat from milk in the manufacture of coagulated products. Its composition varies according to the type of product from which it is derived. Whey containing most of the milk sugar about 20 per cent of milk protein (Whey protein), water soluble vitamins and minerals.(Kennedy, 1985: Marshall and harper, 1988). Though whey constitutes 50 per cent of the milk solids because of the low concentration of the constituents (about 6.4 to 7.0% dry matter), it has not been commonly considered as a byproduct but as a waste product (Sienkiewiez and Riedel. 1990). The growing menace of environmental pollution and the huge loss of nutritional solids resulting due to gross wastage of whey, stress the need to explore the possibilities of utilization for human use.

2.1.1 Nutritional significance of whey

Whey constituents represent a huge quantity of nutritionally rich food. The most valuable whey component is the whey proteins; one of the best proteins known. They consist of about 61.4 per cent beta lactoglobulin, 20.5 per cent alpha-lactalbumin, 6.0 per cent serum albumin and 12.2 per cent immunoglobulin.

The whey proteins are one of the highest quality natural proteins available and their nutritional value is based on the higher concentration of essential amino acids such as tryptophan, leucin, isoleucine, threonine and lysine than casein. They also contain about 2.5g of cysteine and 2.8g of cystine per 100g of proteins. They have all the essential amino acids in excess of FAO standards, of particular importance are isoleucine, lysine, threonine, and tryptophan (Irvine et al, 1984). Whey proteins have shown higher biological value (104) as against whole egg (100) and casein (77). They also have higher PER and NPU than casein. The PER of whey protein is 3.6 as against 3.8 (whole egg) and 2.9 (casein), where as NPU is 94 for whole egg, 76 for casein and 92 for whey proteins (Renner, 1983; Renner and Abd-El-Salam, 1991)

Because of their nutritional significance, whey proteins have long been recognized by the nutritionalists as one of the best proteins available for human needs and is being looked upon seriously as a nutritional ingredient to improve nutrient profile some of foods, especially those popular with children (Jelen, 1978).

Nutritionalists and medical professionals satisfy whey products for specialized dietetic food needs of geriatric and convalescing patients whose digestive function requires special dietary management (Clark, 1979).

2.1.2 Functional properties of whey and whey derivatives

Whey and whey derived products besides being a nutritional ingredient in various foods can also be used as functional ingredient supplying flavor, texture, colour and certain aeration properties in variety of foods.

Whey proteins possess very good functional properties, such as solubility, foaming, emulsifying, gelling and water binding. The solubility of whey proteins is high at all pH values when compared to other proteins like caseins and soy protein which are highly pH dependent (Neilsen, 1988). Whey protein from gels and bind considerable amounts of water when heated and this were found important in baked foods, meat products and processed foods. Whipping and foaming properties of whey proteins was excellent and they were found next only to egg white, whey proteins were also found to be very good emulsifying agents (Neilsen, 1988).

2.1.3 Conservation of whey solids

Many attempts were made in the past to preserve whey solids for longer periods by converting them into a range of products viz. whey powder, dried WPC, granulated high protein whey products and fat filled high protein whey powder. However, it was observed that conservation of whey solids in the form of whey powder and WPC is a better way to redeem the whey solids (Graham et al, 1981). Technologies have been successfully developed by several workers to conserve whey solids in the form of dried whey (Hyde, 1980).

Though dried whey has been used in varieties of food formulation, but because of its high lactose and mineral content it has limited application. In replacing traditional milk constituents to a limited extent, as it results in texture problem in the product formulated. The recent approach is to utilize whey solids in the form of whey protein concentrate.

2.1.4 Composition of whey protein concentrate

Whey protein concentrate is the substance obtained by the removal of sufficient non-protein constituents from whey so the finished dried product contains not less than 25 per cent protein (Renner and Abd-El-Salam, 1991). The average contents of lactose, ash and fat in WPCs with a protein content of 30 to 85 % vary significantly. The extent of variation of protein, fat, lactose, ash and moisture is found to be 30-80, 3-8, 5-55, 4-12 and 2-16 per cent respectively (Engle, 1982). In United States, the requirements for the composition of WPCs are fixed as follows: proteins a minimum of 25 per cent, fat 2-10 percent, ash 2-15 per cent, lactose 60 per cent (maximum) and moisture 1-6 per cent.

2.1.5 Applications of whey protein concentrates in food industry

The WPC as it carries excellent nutritional and functional properties, find great potential in various food formulations.The largest potential use of whey solids as a replacement for non-fat dry milk (NFDM) in the food industry (Mann, 1987). WPC can also be seen competing with casein, egg albumin and soy proteins with the existing markets (Melachouris, 1984).

More product formulation work specially in food industry is needed for WPC. Because of the unique inherent properties of WPC, there lies a strong potential of its utilization in acid foods, beverages, meat products, bakery products, infant foods, humanized milks, cheese, fermented products and dietetic and therapeutic applications (Guptha and Thapa, 1991).

III. MATERIALS AND EQUIPMENT REQUIRED FOR PREPARING FORTIFIED WHEY PROTEIN AT THE LABORATORY

3.1MATERIALS REQUIRED:

- **Buffalo milk**
  Milk and Whey samples were obtained from the dairy of BITS Pilani, Pilani Campus.
- **Coagulation agents**
  Fresh lemon and commercially available good quality citric acid crystals we procured from the local market.
- **Sugar**
  Commercially available good quality cane sugar procured from the local market was used as sweetener.
- **Chemicals**
  All chemicals were obtained from the Laboratory store in BITS Pilani, Pilani Campus.
- **Glass ware**

Conical flask, beakers, volumetric flasks, measuring jars, bacteriological transfer pipettes, test tubes, butyrometers(empty test bottles used for testing fat in whey proteins), petridishes, dilution bottles, acid measures(device to measure sulphuric acid) were used for chemical and microbiological analysis. The glass was thoroughly washed with detergent solution followed by rinse in running water and subsequent draining and drying. The glass wares were sterilized using hot air oven at 180^0C for one hour.

3.2EQUIPMENT REQUIRED:

- **pH meter**

Digital pH meter was used for determination of pH milk, weigh and whey proteins.

- **Incubator**
- **Electronic balance**
- **Electric oven**

All glassware used in experiment, were sterilized in the electric oven at 180C for one and half hours to two hours.

- **Autoclave**

This equipment was used for sterilization of the media prepared in the laboratory.

- **Gerber centrifuge**

Utilized for the estimation of fat.

- **Spectrophotometer**

For quantitative analysis of whey protein

- **Thermometer**

Certified mercury thermometer was used throughout the experiment.

- **Refractometer**

Refractometer was used to determine the carbohydrate percent by Brix's method.

IV. FORMULATION

4.1 FORMULA:

Nutrients	concentration
Whey water	70ml
Barley water	30ml
Sucralose/Sugar	1.4g/10g
Green tea	5ml
Premix	90mg
FOS	2g
Glucosamine sulphate	1 tab
Flax seed powder	5ml
Digene	1/3rd of tab
Sodium citrate	Little

Whey & Whey protein concentrates are very suitable for the formulation of nutritious soft drinks & high protein milk based beverages for school lunches or dietic products. The application of WPC in drinks is, however, governed by high demands on organoleptic & functional qualities, combined with low production costs. This implies that only a bland tasting WPC may be used either as a cheap & nutritious extender for milk, or as a protein supplement for soft drinks with additional functional properties, e.g. cloud stability & viscosity.

Good solubility or colloidal stability of the Whey protein product in the drink is a primary requirement.

4.1.1Additives Used

- **Barley Water**

Barley water is extracted by boiling barley seeds in water. The resultant Barley water is normally assumed to provide diuretic effect and cleanse the kidney to enhance its function. Besides the Barley water contains a compound called β-glucon which is known to reduce the blood cholesterol level which is prevalent in people suffering from – disease and high B.P problem. The beverage was prepared with different levels of barley water to Whey. Fortification of Whey water with 30% Barley water did not alter its physical appearance and flavor of the beverage as assessed for sensory attributes by a panel of judges. Hence addition of 30% Barley water to Whey was found suitable in the development of Whey beverage.

- **Premix**

A mixture of important vitamins i.e. Vit A, C and E along with iron (Ferric ammonium sulphate), Calcium (Calcium glycerate) was added at 0.9% to meet 20% of RDA value. Fortification with premix at 0.9% did not show any change in Whey-Barley water with respect color and flavor.

It is well known that Vit A is known to address problems with vision (cateract), Vit C & E are well known antioxidants to prevent problem due to release of free radicals damaging the functioning of normal cells, Calcium content of Whey is low compared to milk, some portion of Calcium is retained along with casein of milk. Incorporation of premix with important vitamins and minerals would enhance the mixture and therapeutic value of Whey-Barley beverage.

- **Green Tea Extract**

Green tea is unfermented is rich in poly phenol an effective antioxidant and also phyto sterol. Fortification of Whey-Barley water with 10% of green tea extract did not in any way effect its sensory quality particularly color and flavor. On the other hand addition of green tea extract enhanced the flavor of Whey beverage. Most of the diseases and abnormal conditions of health is attributed to the degenerative effect of free radicals damaging the normal cells functioning leading to diseases like cancer, arthritis, etc;

- **Flax Seed Powder**

Flax seed is an ancient food and has become a modern miracle food. It is rich in α-leveolenic acid derived from Omega-3-fatty acids similar to these found in fish such as Salman. Whey-Barley beverage was fortified with flax seed powder oil 1% level to provide heart related specific health benefits. Flax seed is dissolved in Rice bran oil (1g in 3ml) and dispersed in Whey-Barley beverage to facilitate their getting mixed benefits of flax seed as shown by many studies include lowering of cholesterol and LDL cholesterol (bad cholesterol) levels. Other benefits show that flax seed may also help lower blood triglycerides and blood pressure. Apart from this Flax seed is rich in lignan. Lignan in flax seeds shows a lot of promise in fighting disease including a possible role in cancer prevention.

- **Digene:**

Digene is available commercially in the form of tablets which is known to contain digestive enzymes. Addition of 0.5% of this digene powder to Whey-Barley beverage address problems related to indigestion of loss of appetite which is the issue for the Whey beverage gaining acceptability among the consumers.

- **STABILIZERS**

Various functional ingredients are incorporated in Whey-Barley beverage. Some of them are less soluble and result in little sedimentation. To Sodium citrate (0.1%) was added to Whey-Barley beverage as stabilizer. Addition of stabilizer has increased the viscosity and made the beverage drinkable like any other fruit juices.

4.2 Experimental Procedures

These standard procedures adopted for chemical, microbiological sensory quality analysis as well as shelf-life studies of packed whey protein samples are presented below

4.2.1 Chemical analysis:

The analytical methods employed in the chemical analysis of packed whey protein samples prepared from milk are delineated below. A representative sample of whey protein from different containers were allowed to drain for about 5 minutes on a wire gauze then ground in a mortar and pestle till a homogenous mass was obtained. The homogeneous mass so obtained of different whey protein samples was utilized for chemical analysis.

- **Determination of moisture**

Moisture content of the whey protein samples were determined according to IS:4079-1967 by weighing accurately about 2g of the material into a flat bottom dish containing of the stainless steel, having abpoout 8cm diameter and 2.5 cm depth. The dish containing the material was uncovered and heated in the electric oven maintained at 102 +/-1C for about two hours. The dish was cooled in a desicator and weighing at 30 minutes interval until the difference between the two consecutive weighing was less than 1 milligram. The lowest weight was recorded. This procedure was repeated for different samples of packed whey protein selected and the moisture, percent by weight was calculated as follows:

Moisture% by wt $=\frac{100(W1-W2)}{W1-W}$

Where,

W1= weight in g of the dish with the material before drying.

W2= weight in g of the dish with the material after drying and

W=weight in g of the empty dish.

Optimization of a method for small scale production of chhanna based sweets, its fortification with functional ingredients and increasing shelf- life by osmotic dehydration.

- **Determination of fat content**

The fat content in the packed whey protein sample was determined by Gerber method as per manual in diary chemistry (1982 reprint) N.D.R.I.

The fat present in the whey protein samples made from the milk is in the form of an emulsion. This emulsion in broken with sulphuric acid (Gerber sulphuric acid-sp gr.1.820 at 150c) and the fat separation is facilitated by iso-amyl alcohol.

10ml of gerber sulphuric acid was taken into a butyrometer. To this 10.75ml of whey protein and 1ml of iso-amyl alcohol was added. The butyrometer was closed with the stopped, shaken well till all the contents are well mixed. Now the butyrometer was placed in the water bath at 65Cplus or minus 2C for tempering for 3 minutes and shaken periodically until the solution of whey protein sample was centrifuged at 1200 rpm for 5 minutes. The percentage of fat the tested sample is read directly from scale by adjusting the fat column within the scale of the butyrometer.

This procedure of the fat determination was repeated for different brands of the whey protein samples by conducting 5 trials each.

- **Determination of Protein**

The protein content of fortified whey drink was determined by formal titration (pyne's method) as per Manual in the Dairy Chemistry (1982) N.D.R.I.

The percentage of the protein in the fortified whey drink samples was determined by weighing 10g of the samples accurately and mixing the same with 10ml of water in a mortar and pestle. This mixture was ground

well to make a homogenous liquid. 10ml of the homogenous sample was transferred in a 100ml Erlenmeyer flask.

To this 5 drops of phenolphthalein indicator and 0.4ml of saturated potassium oxalate was added and kept aside for 2-4 minutes without disturbing. The whey mixture solution was titrated against 0.1N sodium hydroxide (standard alkali) to its end point. To this titrated solution, 2ml of neutral formalin was added and mixed well. This was titrated against the standard alkali to the same end point as before. The volume of the alkali used in the second titration was recorded.

Volume of N/10 sodium hydroxide required by the 10ml sample treated with formaldehyde was recorded as 'V ml'. the percentage of the protein in the sample is calculated as V x dilution factor x 1.7 (pyne's constant).

The procedure of the protein determination was repaired for different brands of whey samples by conducting 5 trials each.

- **Determination of Acidity**

Titratable acidity of the milk and weight was determined according to IS:1949, Part I (1960).
Acidity of the whey drink was determined by measuring 100ml of the sample in a suitable titration flask. To this one millimeter of phenolphthalein indicator solution (prepared by dissolving 0.5g of phenolphthalein in 100ml of 50 percent ethyl alcohol) was added. After thorough mixing by shaking well, it was titrated against 0.1N sodium hydroxide solution used to neutralize 100ml of the whey drink sample.

4.2.2Microbiological Analysis:

Different whey samples were opened in the laminar flow table sterilized by UV radiations. The contents were emptied individually on separate wire sieves and were collected individually.

- **Sampling**

Eleven g/ml of the whey sample was weighed aseptically in a sterile beaker and transferred to 89ml of sterile normal saline and rendered into a fine paste using a pre-sterilized pestle and mortar thoroughly in a suitable container. Further, 10 fold serial dilutions were prepared using 9ml sterile normal saline dilution banks for the whey samples separately.

Aseptic conditions were maintained during sampling and plating. The procedure for the various viable counts is indicated as follows:

- **Determination of total bacterial count**

The total bacterial count was enumerated according to the procedure described in ISI: 4079- 1967

- **Composition of the media:**

Ingredients	g/liter of water
Peptone	5.0
Yeast extract	2.5
Glucose (dextrose)	1.0
Agar, bacteriological grade	20.0

Medium preparation: The above ingredients were soaked for 3 to 5 minutes in cold water, the mixture was boiled above an asbestos-centered wore gauze over a flame. The completely mixed solution was stirred continuously and efficiently to avoid charring. The pH of the solution was adjusted to 7.0 at 50^{o}C with sodium hydroxide solution. This solution was filtered through a cotton pad till clear filtrate is obtained and sterilized in an autoclave at 121^{o}C for 20 minutes.

- **Composition of dilution water**

Ingredients	Weight
Sodium chloride	9.0g
Potassium chloride	0.42g
Calcium chloride	0.48g
Sodium bicarbonate	0.20g
Water	100ml

Eleven g/ml of the material from each individual sample, using a sterile pipette was weighed and suspended in 89ml of dilution water at 45^{o}C. The contents are mixed properly by agitating the mixture. One millimeter of suitable dilutions is added to the sterile petridishes. The agar medium melted in a conical flask and kept at 45^{o}C to 50^{o}C.

Now the medium was introduced aseptically at 42^{o}C to 44^{o}C, into petridishes and mixed by rotating and tilting the dishes without spreading over the edges. The mixture was spread evenly over the bottom of the plate and allowed to solidify. The plates are now inverted and incubated at 37^{o}C for 48 hours. The colonies were counted with the aid of magnifying lens under the uniform and properly controlled illumination. The bacterial count per gram from the dilutions used in computed and recorded.

- **Determination of coliform count**

Coliform bacteria include all aerobic and facultative anaerobic gram negative, Non-spore forming bacteria which ferment lactose with the production of acid and gas. Development of dark red colonies at least 0.5mm in diameter in a solid medium (violet red bile agar) within 20 to 24 hours at 35^{o} to 37^{o}C may be considered as a positive evidence of the presence of the coliform bacteria. Violet red bile agar is one the standard media used for the determination of general types of coliform organisms including those of fecal origin in water, milk and other materials of sanitary importance.

Coliform counts were enumerated by ALPHA (1978) method using rehydrated violet red bile agar (hi-media) of the following composition.

Ingredients	g/liter of water
Yeast extracts	3.0
Peptone	7.0
Bile salt	1.5
Lactose	10.0
Sodium chloride	5.0
Agar	15.0
Neutral red	0.03
Crystal violet	0.002

- **Determination of yeast and mold count**

The yeast and mold count was determined according to the procedure recommended by American Public Health Association (APHA, 1978) using potato dextrose agar medium (Hi-media). The medium had the following composition:

Ingredients	g/liter of water
Potato infusion	100
Dextrose	20
Agar	15
pH (approx.)	5.6 + or – 0.2

To rehydrate this medium, 39g was suspended in 100ml distilled water and boiled to dissolve the ingredients completely. The medium was sterilized by autoclaving at 121^{o}C for 15 minutes.
Just before using the medium for plating, pH of the medium was adjusted to 3.5 by using sterile 10% tartaric acid.

4.3Sensory evaluation

Sensory evaluation of whey beverage was done by a panel of three judges following 5 Hedonic ratings. Hedonic rating is used to measure the consumer acceptability of food products. Samples are served to the panelist at one session. The acceptability of the product was rated based on a scale of points ranging from "like extremely" to "dislike extremely".

4.3.1Hedonic Rating Test

Taste these samples and check how much do you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for the attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us.

Judges	Color	Consistency	Sedimentation	Flavor	Taste	Average of total
A						
B						
C						

Response	Points
Excellent	5
Very good	4
Good	3
Satisfactory	2
Bad	1

4.4Fortification of nutrients in whey-barley beverage

List of nutrients fortified to enhance its nutritive & therapeutic value & concentrations of each:

Nutrients	**Concentration**
Whey water	70ml
Barley water	30ml
Sugar	10g
Green tea	5ml
Premix	90mg
FOS	2g
Glucosamine sulphate	1 tab
Flax seed powder	5ml
Digene	1/3rd of tab
Sodium citrate	As required

V. EXPERIMENTAL READINGS

TABLE 5.1- CHEMICAL QUALITY OF WHEY WATER:

TRIAL NO.	ACIDITY%	Ph	FAT%	PROTEIN%
1.	0.21	5.15	0.6	0.85
2.	0.22	5.2	0.65	0.78
3.	0.21	5.1	0.6	0.85

Interpretation:

Whey water was examined for its chemical quality. The results are recorded in TABLE 1.

The acidity and pH of raw milk is the normal range of 0.21 to 0.22% & 5.1 to 5.2 respectively. Fat was 0.6 to 0.65%. Protein content was found in the range of 0.78 to 0.85%. All the chemical parameters of whey water meet the standard requirement. Hence whey water is found to be of good quality.

TABLE 5.2- BACTERIOLOGICAL QUALITY OF WHEY WATER:

SAMPLE	TOTAL BACTERIAL COUNT $x10^1$cfu/ml
1.	560
2.	360
3.	410

Interpretation:

Whey water was subjected to bacteriological analysis and the results attached are shown in TABLE 2. The total bacterial count is found in the range of 360 to $560x10^1$cfu/ml. All the bacterial counts of whey water are found to be on the lower side. Hence the quality of whey beverage is considered to be unsatisfactory.

TABLE 5.3 –CHEMICAL QUALITY OF WHEY BEVERAGE FORTIFIED WITH BARLEY AND OTHER ADDITIVES:

%BARLEY	ACIDITY%	pH	FAT%	PROTEIN%	CARBOHYDRATE%
10%	0.19	6.5	0.5	0.1	8.5
20%	0.185	6.55	0.55	0.2	9.0
30%	0.18	6.6	0.35	0.2	10.0

Interpretation:

The Whey beverage was examined for its chemical quality. The results are recorded in TABLE 3. The acidity and pH of raw milk is the normal range of 0.18 to 0.19% & 6.55 to 6.6 respectively. Fat was 0.5 to 0.55%. Protein content was found in the range of 0.1 to 0.2% and the carbohydrate content was ranging from 8.5 to 10.0%. All the chemical parameters of whey beverage meet the standard requirement. Hence whey water is found to be of good quality.

TABLE 5.4 –MICROBIOLOGICAL QUALITY OF WHEY BEVERAGE FORTIFIED WITH BARLEY AND OTHER ADDITIVES:

%BARLEY	TOTAL BACTERIAL COUNT $x10^1$cfu/ml	COLIFORM COUNT cfu/ml	YEAST & MOULD COUNT cfu/ml
10%	450	Nil	Nil
20%	290	Nil	Nil
30%	330	Nil	Nil

Interpretation:

The low calorie whey beverage fortified with Barley was subjected to microbiological analysis and the results obtained are attached are shown in TABLE 4. The total bacterial count is found in the range of 290 to $450x10^1$cfu/ml with nil coliform and yeast mould count. Hence the low calorie whey beverage is found to be good with respect to its bacteriological quality as the bacterial population is less than 80,000 per ml with the complete absence of coliform and yeast &mould count. The beverage therefore is considered safe for consumption.

TABLE 5.5 -SENSORY EVALUATION OF WHEY BARLEY BEVERAGE FORTIFIED WITH FLAX SEED POWDER ALONG WITH SODIUM CITRATE (0.15%):

JUDGES	COLOR	CONSISTENCY	SEDIMENTATION	FLAVOR	TASTE	AVERAGE OF TOTAL
A	Brownish yellow 3	Good 4	Nil 4	No off flavor 4	Acceptable 3	3.6
B	Pale brown 3	Good 4	Nil 4	No off flavor 4	Good 4	3.8
C	Light brown 3	Good 4	Nil 4	No off flavor 4	Good 4	3.8

Interpretation:

Whey water recovered from milk was fortified with barley and flax seed powder. The processed solution was subjected to sensory evaluation by a panel of three judges. The avg. sensory score awarded to the solution was 3.8 out of 5 by 5point hedonic scale. As per the standards the solution was found to be of fair quality.

TABLE 5.6 –EFFECT OF HEAT TREATMENT OF WHEY ON SEDIMENTATION:

HEAT TREATMENT	SEDIMENTATION	CLARITY OF WHEY
90^0C for 0 min	+++	Light turbid
Boil for 1 min	++	Little
Boil for 3 min	+	Clear
AVG. RESULTS OF 3 TRIALS		

Interpretation:

Initial trials were canned out to the effect of different heat treatment of whey on sedimentation. During this stage sedimented whey solids were recorded and presented in the TABLE 24. Results obtained have shown

that on heat treatment of whey by boiling for 3minutes resulted in minimum and most of sediments settling at the bottom. The results obtained will be useful to adopt this heat treatment for all further experimental trials.

TABLE 5.7 -SENSORY EVALUATION OF WHEY BARLEY BEVERAGE FORTIFIED WITH 10ml GREEN TEA IN 90ml WHEY SOLUTION:

JUDGES	COLOR	CONSISTENCY	SEDIMENTATION	FLAVOR	TASTE	AVERAGE OF TOTAL
A	Pale brown 3	Good 4	Nil 4	Faint tea flavor 3	Acceptable 4	3.6
B	Greenish yellow 3	Good 4	Nil 4	Faint tea flavor 3	Good 4	3.6
C	Greenish 3	Good 4	Nil 4	Slight tea flavor 3	Acceptable 4	3.6

Interpretation:

Whey water being the bi product of Channa recovered from milk was fortified with green tea & in the proportion of 90:10. The processed solution was subjected to sensory evaluation by a panel of three judges. The sensory surveys awarded are presented in TABLE 11. The avg. sensory score awarded to the solution was 3.6 out of 5 by 5point hedonic scale. As per the standards the solution was found to be of fair quality.

TABLE 5.8 -SENSORY EVALUATION OF WHEY BARLEY BEVERAGE FORTIFIED WITH 20ml GREEN TEA IN 80ml WHEY SOLUTION:

JUDGES	COLOR	CONSISTENCY	SEDIMENTATION	FLAVOR	TASTE	AVERAGE OF TOTAL
A	Dark brown 2	Good 4	Nil 4	Slight tea flavor 3	Good 4	3.4
B	Dark brown 2	Good 4	Nil 4	Slight tea flavor 3	Good 4	3.4
C	Dark brown 2	Good 4	Nil 4	Tea flavor 3	Good 4	3.4

Interpretation:

Whey water being the bi product of Channa recovered from milk was fortified with green tea & in the proportion of 80:20. The processed solution was subjected to sensory evaluation by a panel of three judges. The sensory surveys awarded are presented in TABLE 12. The avg. sensory score awarded to the solution was 3.4 out of 5 by 5point hedonic scale. As per the standards the solution was found to be of fair quality.

TABLE **5.**9 -SENSORY EVALUATION OF WHEY BARLEY BEVERAGE FORTIFIED WITH 30ml GREEN TEA IN 70ml WHEY SOLUTION:

JUDGES	COLOR	CONSISTENCY	SEDIMENTATION	FLAVOR	TASTE	AVERAGE OF TOTAL
	Deep brown 3	Good 4	Nil 4	Tea flavor 3	Acceptable 3	3.4
	Deep brown 3	Good 4	Nil 4	Tea flavor 3	Acceptable 3	3.4
	deep brown 3	Good 4	Nil 4	Tea flavor 3	Satisfactory 3	3.4

Interpretation:

Whey water being the bi product of Channa recovered from milk was fortified with green tea & in the proportion of 70:30. The processed solution was subjected to sensory evaluation by a panel of three judges. The sensory surveys awarded are presented in TABLE 13. The avg. sensory score awarded to the solution was 3.4 out of 5 by 5point hedonic scale. As per the standards the solution was found to be of fair quality.

VI. DISCUSSION

Whey is a highly nutritious by product of cheese, paneer& casein manufacturing which contains about 0.6% protein. The Whey proteins represent the non-casein proteins as well as the fractions & fragments of the caseins which remain soluble when the casein has been precipitated enzymatically by rennet or iso electrically by acid. They represent even more heterogeneous precipitation mixture than the casein and share very few common characteristics except that of being soluble under conditions that precipitate the caseins. Whey precipitations have been regarded as superior to most other precipitations in nutrition. It has been said that the adult daily requirement of amino acids can be furnished by 14.5g of lac albumin, whereas it requires 17.4g of whole egg precipitation and 28.4g of cow's milk precipitation. Whey is rich in vitamins especially those of B group which are limiting in wheat proteins. Whatever method of scoring is used for assessing the nutritional quality of foods, Whey protein rates extremely high. Some of the Whey proteins seem to have distinct physiological & biochemical roles for e.g. lactoferrin strongly binds iron; α-lac albumin is a constituent of lactose synthetase& lysozyme is an enzyme that destroys the bacterial cell wall with respect to β-lacto globulin, which is the dominant Whey protein in bowine milk, not much information regarding any specific physiological role is available. Inspite of its nutritional significance, most of the Whey product is not utilized properly causing substantial loss of Whey solids. Much more important is its high BOD of 35,000-45,000 mg/l & 100l of Whey has a polluting strength equal to the sewage product by 45 people. Recovery of Whey solids solves the disposal problems thereby providing high quality Whey solids for food use. On average, the manufacturers of one ton of cheese results in the production of 8 tones of Whey, In India, it is estimated that about 1000 million kg of Whey is annually derived as a byproduct.

The recent development in Whey & Whey products utilization is their use in certain food system because of their excellent nutritional and functional properties.

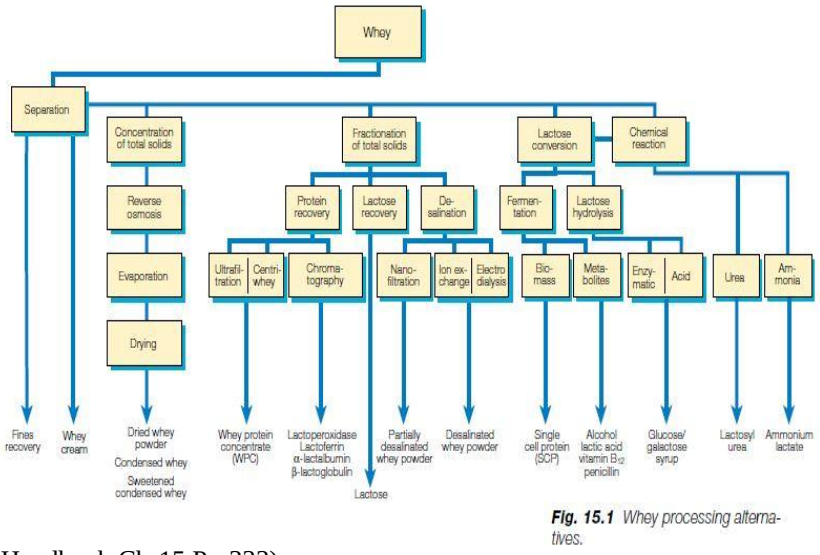


Fig. 15.1 *Whey processing alternatives.*

(Dairy Processing Handbook,Ch-15,Pg-333)

VII. Summary

Health is clearly not the mere absence of disease. Health, care covers not merely medical care but all aspects of preventive care. A significant percentage of our population suffers from chronic deficiencies and is malnourished. Hence food is no longer the only means to fulfill the nutritional needs of the society and thereby solve the myriad health problems. Hence nutraceuticals serve as the main source for instant remedy for good health & counteract the diseases. Hence there is increased demand for tailor made functional foods, be it be a solid food or liquid beverages. Nowadays developed countries have ploughed in lot of finance in the development of health oriented foods. In this context an earnest asset has been made in this investigation to standardize a method for preparation of liquid beverage known as 'Nutrilife' by incorporation of effective functional ingredients. Greater emphasis has been given to fortification of the Whey released as a byproduct in the preparation of Channa based sweets. So far lot of Whey based beverages has been attempted & the same could not find a proper demand in the market. This was mainly attributed to constipational problems faced by the commuters after consuming the Whey beverage. In this investigation care has been taken to address problem of indigestion by incorporation of digestive multienzymes in the form of 0.5% Digene. Sensory evaluators found that the Whey beverage fortified with functional ingredients did not pose any indigestion problem and were comfortable after relishing the taste of this beverage. Whey contains all the important nutrients in the form of Whey protein (high biological value and NPU), lactose (harbors growth of probiotic lactic enzymes in the

intestinal tract), water soluble vitamins and essential minerals. In this investigation Channa Whey was first clarified in the centrifuge to remove all suspended casein particles. The casein free Whey was subjected to 90^0C for 2-3 minutes, cooled to 50^0C followed by incorporation of functional ingredients namely – Premix (Vit A, C & E, Calcium & iron in the form of Ferric ammonium sulphate), Barley water to the extent of 30% (Dieturic effect, purification and cleaning of kidney to enhance its function & above all rich in β-glucon (to lower cholesterol), Flax seed powder (known to contribute to lowering of total cholesterol and LDL), Green tea extract (rich in antioxidant to cap free radicals) & FOS being the powerful fibre (control constipation & ideal for diabetic subjects).

Major emphasis on the development of Whey beverages is laid on by inclusion of 30% Barley water in Whey beverage which has several health benefits to benefit the consumer.

Low calorie Whey-Barley based beverage was developed by replacing sugar by sucralose (1.4%) which carries almost the same sugar intensity & as that of full calorie fortified Whey-Barley beverage. Further there are certain important ingredients which do not mix with the Whey water easily and hence two important stabilizer ingredients in the form of Sodium citrate and Xanthum gum at a concentration 0.1% each has helped in achieving homogenous liquid beverage.

Fortified Whey-Barley beverage is a refreshing drink containing important nutritional ingredient besides functional ingredients to address several health problems the common man is facing at the present time. Since the beverage is prepared from discarded Whey water, it is contributing to lower the cost and thus proved to be economically viable.

The findings of this investigation would benefit the household consumers and related industry to market this overall health oriented Whey-Barley beverage with promising functional ingredients to solve several health problems in the day to day active life.

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