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A novel and holistic functional food

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ABSTRACT

Functional foods are those wholesome fortified, enriched or enhanced foods that provide health benefits beyond the provision of essential nutrients when they are consumed as part of the diet. Scientists, today are identifying physiologically active components of food that can reduce the risks of a variety of diseases and optimize health. The consumer self-care phenomenon has increased and hence there is a rapid growth in the market for health and wellness products.

A functional food containing pink guava, whey and walnut were developed. The formulation was evaluated for its health benefits like antioxidant, probiotic, immune modulatory and antimicrobial activity. The formulation was freeze-dried for stability. Standardization was done using UV spectroscopy and high-performance liquid chromatography methods. The formulation showed distinct probiotic and antimicrobial activity. Immunomodulatory activity was evaluated using animal models. The formulation also showed significant values for protein, total fat content, vitamin C, pectin, and energy value.

The product is a novel, holistic and economical combination and thus it can be used as a rejuvenator for an efficient, effective and healthy lifestyle.

Keywords: *Functional foods, Probiotic, Immunomodulator, Antioxidant.*

1. INTRODUCTION

About 2000 years ago, Hippocrates correctly emphasized: “Let food be your medicine and medicine be your food". The concept of “**Functional food**” was introduced in Japan in the 1980s.[1] since then various efforts have been made to define functional foods. Broadly “**Functional food**” may be defined as *a food which influences specific functions in the body that may provide added health benefits or remedy from some diseased condition following the addition/concentration of a beneficial ingredient, or removal/substitution of an ineffective or harmful ingredient.* Foods might inherently possess these supposedly beneficial qualities, or they may be fortified/modified and/or genetically altered.[2]

Functional foods are those that when consumed regularly exert a specific health-beneficial effect beyond their nutritional properties i.e., a healthier status or a lower risk of disease. Thus, functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates necessary for healthy survival. This type of health-promoting products is getting more popular amongst health-conscious consumers and thus, a large list of functional foods containing phytochemicals from foods is now available in the market. Some of the most common phytochemicals found in the functional food market are polyphenols such as anthocyanins, proanthocyanidins, flavonols, stilbenes, hydroxycinnamates, coumarins, ellagic acid and ellagitannins, isoflavones, lignans, etc.[3]

There is a lot of confusion regarding the terminologies *like “nutraceuticals”, “functional foods”, “dietary supplements” “designer foods”, “medical foods”, “pharmafoods”, “phytochemicals”* etc.[4]

A functional food containing a combination of pink guava, walnut and whey was formulated to provide all the required nutrients to the body and protect the body from the effects of changing lifestyle and modernization.

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2. MATERIALS AND METHODS

Figure 1

Collection and Authentication:

Fresh fruits of *Psidium guajava* and *Juglans regia* were collected from local market and authentication was carried out from Blatter Herbarium, St. Xavier's Institute, Mumbai, and their identity was confirmed to be *Psidium guajava*, Family- Myrtacae (Herbarium accession no. 26280) and *Juglans regia,* Family- Juglandacae. Whey was obtained as a by-product of cheese from the local market.

Extraction:

The *Psidium guajava* fruit (pink-fleshed) was cut into small pieces and was fed to a food processor to obtain a puree, the seeds were then strained and removed. The puree of *Psidium guajava* (guava) fruit thus obtained was then dried in an oven, the resulting lumps using powdered, then extraction with methanol was carried out with soxhlet apparatus (temp 45℃) using methanol(80%) solvent. *Juglans regia* (walnut) fruit was powdered in a food grinder. The resulting powder was then dried in an oven, and was subsequently extracted with methanol (80%) in an ultrasonic bath for 2hrs. After completion of extractions, the solvent methanol was evaporated in an evaporating dish on a water bath to obtain a dry extract. Whey aqueous solution, obtained as a by-product of cheddar-cheese was used.

Evaluation:

Organoleptic evaluation:

Table 1

Characteristic	**Guava fruit**	**Walnut fruit**	**Whey**
Color	Pink-(flesh)	Brown-colour	White
Odour	Characteristic	Characteristic	Sour
Taste	Sweet	Characteristic	No taste

Chemical evaluation (Phytochemical screening, qualitative and quantitative evaluation):

Table 2

Extracts / Test	*Psidium guajava* methnolic extract	*Juglans regia* methanolic extract	*Whey* aqueous extract
ACID	-	-	-
ALKALOIDS	-	-	-
CARBOHYDRATES			
Molisch test	+	+	-
Barfoed's test	+	+	-
FLAVONOIDS			
Shinoda test	+	+	-
Alkaline reagent test	+	+	-

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Zinc hydrochloride test	+	+	–
GLYCOSIDES			
a) Anthraquinone glycosides	-	-	-
Borntrager's test			
b) Cardiac glycosides			
Legal's test	-	-	-
Baljet's test	-	-	-
c) Coumarin glycosides	-	-	-
d) Cyanogenetic glycosides	-	-	-
e) Saponin glycosides			
Froth formation test	+	-	-
FATS AND FIXED OILS			
Saponification test	-	+	-
VOLATILE OILS	**-**	+	**-**
TANNINS			
Ferric chloride test	+	+	**-**
Test for Catechin	**-**	+	**-**
Test for Chlorogenic acid	+	+	**-**
STEROIDS AND TRITERPENOIDS			
Libermann-Burchard test	+	-	-
Salkowski test			
	+	-	-
PROTEINS			
Heat test	-	-	+
Trichloroacetic acid test	-	-	+
Biuret test	-	-	+
Xanthoproteic test	-	-	+
AMINO ACIDS			
Millon's test	-	-	+
Ninhydrine test	-	-	+
NAPHTHOQUINONES			
Juglone test	-	+	-

□ Dam-Karrer test	-	+	-
MUCILAGE	+	-	-
STARCH	+	-	-

Standardization and quantification of *Psidium guajava, Juglans regia,* and whey

For *Juglans regia* extract:

Diluent: Methanol

Reference compound: Catechin

Different concentrations ranging from 50, 100, 200, 300, 400, and 500µg/ml was prepared.

The λmax of Catechin in methanol was found to be 280nm. Hence the absorbance of the above mentioned standard was recorded at 280nm.

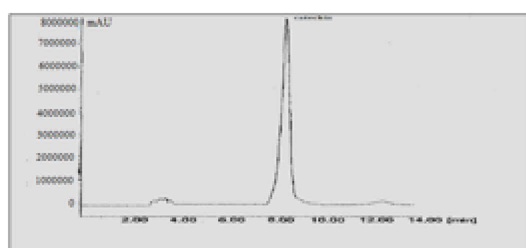
Juglans regia solution was prepared: 300 and 500µg/ml using methanol as solvent

This solution was then filtered using membrane filter before injecting into the HPLC system.

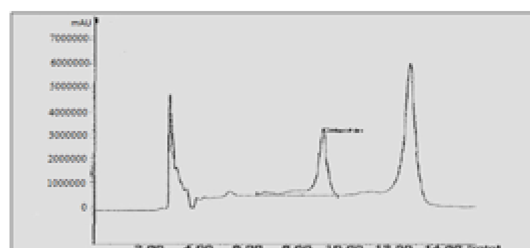
The separation system consisted of a C18 reversed-phase column, an isocratic elution system of acetonitrile /water/ortho- phosphoric acid, and a PDA detector.

Elution conditions for the detection of Catechin were as follows:

- Acetonitrile: water: ortho phosphoric acid 15: 84.9: 0.1
- Flow rate 1 ml/min in isocratic mode
- 20 µL injection volume
- PDA detection at 280 nm
  Before use, the mobile phase was degassed by an ultrasonic bath. Separation was performed at room temperature.



Graph of standard Catechin concentration 500µg/ml



Methanolic extract of *Juglans regia* (conc. 500µg/ml)

Figure 2

Methanolic extract of *Juglans regia* contains 5.0 % of catechin.

Standardisation of *Psidium guajava*:

Diluent: Dichloromethane

Reference compound: Lycopene

Different concentrations ranging from 50, 100, 200, 300, 400, and 500µg/ml was prepared.

The λmax of Lycopene in dichloromethane was found to be 470 nm. Hence the absorbance of the above mentioned standard was recorded at 470 nm.

Psidium guajava was accurately weighed and dissolved in 10 ml HPLC grade dichloromethane to get stock solution of 1mg/ml. This stock solution of 1mg/ml was further diluted with HPLC grade dichloromethane to get different concentrations of 300 and 500µg/ml.[4] This solution was then filtered using whatman filter before injecting into the HPLC system.

The separation system consisted of a C18 reversed-phase column, an isocratic elution system of Acetonitrile: Chloroform (92:8) with 1% TEA (trietanolamine), and a PDA detector.

Elution conditions for the detection of Lycopene were as follows:

- Acetonitrile: Chloroform (92:8) with 1% TEA
- Flow rate 1 mL/min in isocratic mode
- 20µL injection volume

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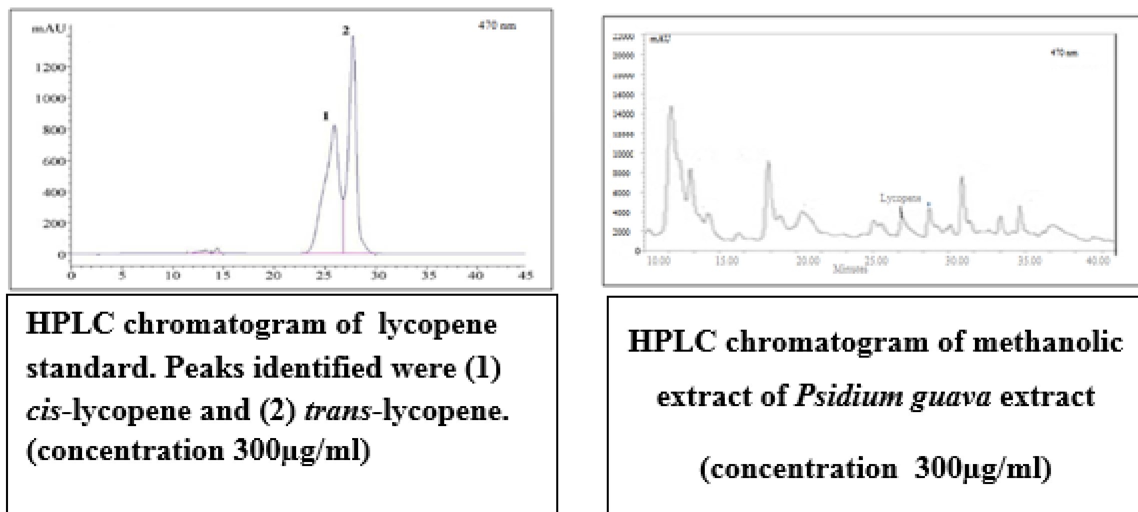
- PDA detection at 470 nm
  Before use, the mobile phase was degassed by an ultrasonic bath. Separation was performed at room temperature.



HPLC chromatogram of lycopene standard. Peaks identified were (1) *cis*-lycopene and (2) *trans*-lycopene. (concentration 300µg/ml)

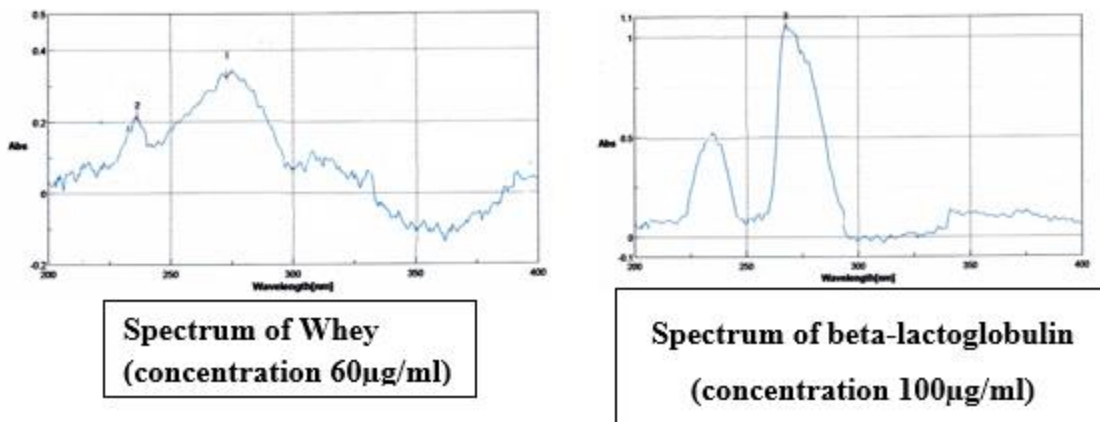
HPLC chromatogram of methanolic extract of *Psidium guava* extract (concentration 300µg/ml)

Figure 3

Dichloromethane extract of *P.guajava* contains 0.5 % of lycopene.

Using UV/vis spectroscopy for quantitation:

Materials and Methods:
All chemicals and solvents used were of the Spectroscopic grade. Beta-lactoglobulin, the standard compound was procured from Sigma Aldrich. UV VIS was recorded on Schimadzu UV-1700 Spectrophotometer.



Spectrum of Whey (concentration 60µg/ml)

Spectrum of beta-lactoglobulin (concentration 100µg/ml)

Standard solution (100µg/ml) was scanned in UV-VIS range (200-800nm) for maximum absorbance after enabling blank correction for water in the above region. The λmax of Beta-lactoglobulin in water was found to be 290 nm.

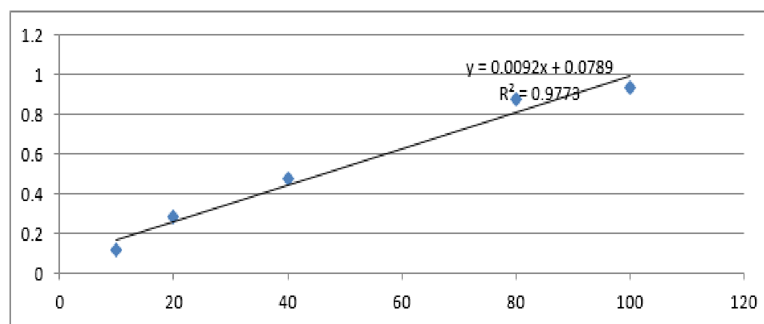


Figure 4

Calibration curve of standard beta-lactoglobulin

Using the data obtained from the linearity curve, the concentration of beta-lactoglobulin present in Whey was calculated. From calculation, Whey contains 2.0 % of beta-lactoglobulin.

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Formulation development:

With this essence in mind, a puree of Guava, Walnut, and Whey was developed in the laboratory.

Ingredients	**Quantity**
Guava	**20g**
Walnut	**5g**
Whey	**100ml**
Honey	**q.s (5 ml)**

Psidium guajava (guava) pulp and peel, *Juglans regia* (walnut) seed and Whey (drain-off cheddar cheese) in the above-mentioned quantities were fed to the food-processor to obtain a puree. Honey was added to the puree sufficient to taste good and mixed well. The formula was designed according to active pharmacological constituents of *P.guajava, J.regia,* and Whey and by sensory analysis. The puree was then subjected to freeze-drying.

Freeze drying procedure:

The puree obtained as described above was freeze-dried in a VIRTIS Model 50 SRC 5 (Piramal, India) freeze dryer. An approximately 1.5 cm thick layer was placed on each tray and frozen overnight at -25°C; then the heating plate temperature was set to 46°C and the vacuum to 55mTorr to initiate drying. After drying for 48 hrs, blocks of guava, walnut, and whey powder were removed from trays and ground in a food grinder and the fine powder was stored in plastic containers at refrigerated temperature (15ºc) and protected from light.

Evaluation of Freeze-Dried Formulation:

1) Organoleptic Evaluation

Table 3

Characteristic	**Observation**
Colour	Pink
Odour	Characteristic odour of guava and whey
Taste	Sweet

2) Physical evaluation

Table 4

Test	**Result**
Moisture Content	Loss on drying: 4.014%
Extractive values	Water soluble: 18.8329% Alcohol soluble: 23.3609%
Total tannins	23.43%
Proteins	Water Extract: 27.33% Methanolic Extract: 40.7%
Fat	12.34%
Titrable acidity	Puree: 12.8% Formulation: 13.90%
Ascorbic acid	Puree: 24.26mg Formulation: 12.13mg
Crude Fiber	28.83%
pH	Puree: 5.42 ± 0.5 Formulation: 4.26 ± 0.5
Energy Value	Formulation: 848.45 cal

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3) Evaluation of flow properties of freeze-dried formulation

Table 5

PARAMETER	FORMULATION
Bulk density	0.46
Tapped density	0.53
Carr’s index	13.21
Hausner’s ratio	1.152
Angle of repose	35.6 °

4) Determination of Total Aerobic Count of a freeze-dried formulation

Table 6

	Plate count in 1gm of the formulation (CFU/g)	IP limits (CFU/g)
For Bacteria	60	300
For Fungi	70	100

Evaluation of probiotic activity:

1) Materials:

Whey-making:

The Cheese (Cheddar-cheese) variety was selected as its technology does not include curd treatments such as steady stirring, high-temperature cooking, direct salt addition, curd washing, etc., that can impair viability and increase the loss of probiotic bacteria in the whey. Cheddar-cheese was prepared by using *L.acidophilus* as the starter culture.

Probiotic culture: (standard)

The lyophilized commercial culture of Lactobacillus species, a strain of *L. acidophilus* (MTCC no.447) was studied. (Chandigarh imtech)

Culture media:

MRS medium: (de Mann Rogosa Sharpe)

2) Methods:

Determination of Cell Viability:

Cell viability of freeze-dried samples was determined by the standard plate count method.[5]

Procedure:

A sterile borer was used to prepare cups of 6mm diameter in MRS medium of each petridish under aseptic conditions. The freeze-dried powder (1g) of GWW was rehydrated with 10 ml of sterile distilled water. The rehydrated samples were kept on a shaker for 30 min to allow complete dissolution. Suitable dilutions of feed solution of GWW sample (before freeze-drying) and rehydrated samples of GWW (after freeze-drying) using sterile distilled water were prepared by serial dilutions (0.1, 0.3, 0.5, 1 mg/ml) and were added to the cups with the help of a micropipette in different petri-plates. The plates were then kept in a refrigerator for pre-incubation diffusion for 15 min in an inverted position (The pre-incubation diffusion ensures the diffusion of the Freeze dried formulation throughout the MRS agar and also ensures the same metabolic stages of the organism). The plates were then removed from the refrigerator and kept at room temperature in a sterile area for 15 min. A control of plain water and a standard, *L.acidophilus* was also run simultaneously. Colony forming unit (cfu) was determined after incubation for 48 hr at 37°c. The percentage viability of the freeze dried sample was calculated according to Reddy *et al.* [14]

% viability = 100 x Nr/Nf,

Where Nr was log cfu/ml of rehydrated sample (freeze-dried) and,

Nf was the log cfu/ml of feed solution.

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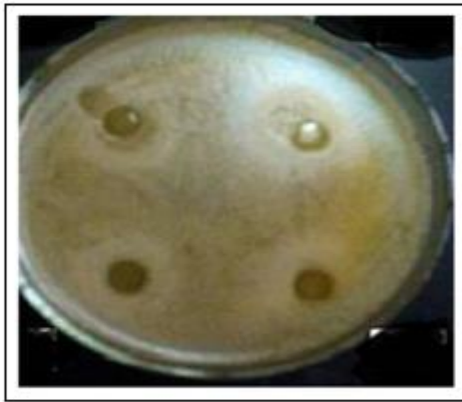
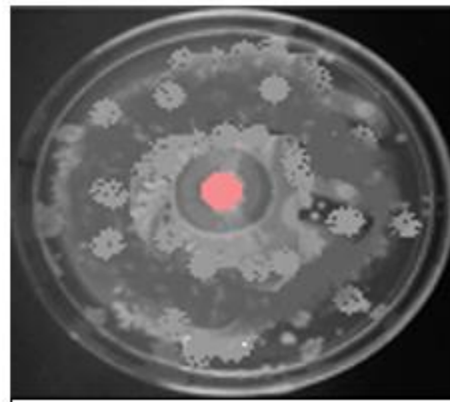
1) Observations:



Zone of diffusion of Whey (30 days trial)

Figure 5

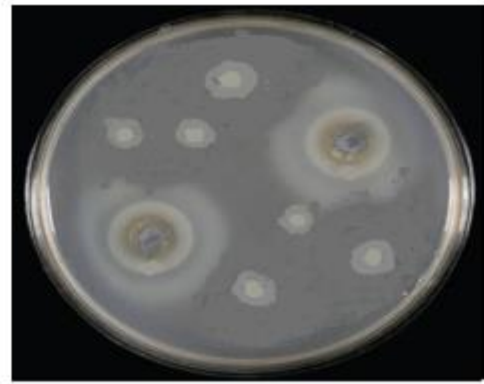


Isolated grey colonies of lactobacilli of freeze-dried formulation

Figure 6

Zone of diffusion of *Lactobacillus acidophilus*

Figure 7



Zone of diffusion of Whey

Figure 8

Evaluation of probiotic characteristics of whey:

- **Determination of Tolerance to simulated gastric juice:**

Procedure:[6]

Simulated gastric juice was a solution of pepsin (0.3% w/v) and NaCl (0.5% w/v) adjusted to pH 2 and 3. An overnight culture of *L.acidophilus* and Whey (30 ml) were centrifuged (6000 rpm, 20 min, 5ºc), washed twice in 50 mM K_2HPO_4 buffer (pH 6.5) and resuspended in 3 ml of the same buffer. One ml of washed cell suspension was harvested by centrifugation (12,000 rpm, 5 min, 5ºc) and resuspended in 10 ml of gastric solution pH 2 and 3. Total viable counts were performed, as it was detailed above, before and after an incubation period of 3 hr at 37ºc. The results were expressed as the difference in these colony counts. (log orders CFU mlÀ1).

- **Determination of Bile resistance:**

Procedure:

L.acidophilus strain and Whey was inoculated (4% v/v) into MRS broth with 0.3, 0.5 or 1% (w/v) of bile (Sigma Chemical Co.) Cultures were incubated at 37ºc and after 24 hrs, A_{560}nm was measured and compared to a control culture (without bile salts). The results were expressed as the percentage of growth (A_{560} nm) in the presence of bile salts compared to the control.

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- **Determination of Hydrophobicity:**

Procedure:

The culture of the strain *L.acidophilus* and Whey were harvested in the stationary phase by centrifugation (12000 rpm, 5 min at 5ºC- refrigerated centrifuge), washed twice in 50mM K_2HPO_4 (pH 6.5) buffer and finally resuspended in the same buffer. The cell suspension was adjusted to an A_{560}nm value of approximately 1.0 with the buffer and 3 ml of the bacterial suspensions and Whey were put in contact with 0.6 ml of n-hexadecane and vortexed for 120 s. The two phases were allowed to separate for 2hr at 37ºc. The aqueous phase was carefully removed and the A_{560}nm was measured. The decrease in the absorbance of the aqueous phase was taken as a measure of the cell surface hydrophobicity (H%), which was calculated with the formula:

$H\% = [(A_0 - A)/A_0] \times 100$

Where, A_0 and A are the absorbances before and after extraction with n-hexadecane, respectively.

3. RESULTS

Probiotic activity:

Trial 1: Whey

Table 7

Time (Days)	pH	Cell counts (log10 CFU $mL^{-1)}$
0	3.50 ± 0.06	5.97 ± 0.31
5	3.42 ± 0.08	5.82 ± 0.10
15	2.35 ± 0.05	5.18 ± 0.23
30	2.00 ± 0.10	3.88 ± 0.33

Fig 7.4.1 pH and cell counts of Whey

Trial 2: *L.acidophilus*

Table 8

Time (Days)	pH	Cell counts (log10 CFU $mL^{-1)}$
0	2.45 ± 0.08	8.19 ± 0.21
5	2.30 ± 0.05	8.04 ± 0.16
15	2.09 ± 0.10	7.87 ± 0.07
30	1.25 ± 0.08	7.70 ± 0.38

Fig.7.4.2 pH and cell counts of *L.acidophilus*

Trial 3: Freeze-dried formulation (GWW)

Table 9

Time (Days)	pH	Cell counts (log10 CFU $mL^{-1)}$
0	4.35 ± 0.04	6.58 ± 0.14[a]
5	4.28 ± 0.07	6.26 ± 0.08[b]
15	4.19 ± 0.05	4.73 ± 0.12
30	4.12 ± 0.10	2.26 ± 0.35

Fig.7.4.3 pH and cell counts of a freeze-dried formulation

Time: total time (incubation + storage)

All counts as CFU ml^{-1} ± SD and the means of four determinations.

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Probiotic characteristics and resistance to biological barriers for probiotic bacteria (mean Æ standard deviation, n=3)

Table 10

Test substance	H%[c]	Resistance to gastric juice at[a]		Growth (%) respect to a control in the presence of bile		
		pH 3	pH 2	0.30%	0.50%	1%
L. acidophilus	47.9 ± 5.7	1.7± 0.3	4.3 ± 0.5	87.4 ± 4.1	83.4 ± 2.1	79.9 ± 1.9
Whey	43.8 ± 3.7	3.7±0.4	3.7 ± 0.4	93.5 ± 3.9	89.5 ± 3.9	79.1 ± 2.3

a: decrease in viable cell counts after exposure to low pH (3 and 2) solutions during 24hr at 37ºC

c: Hydrophobicity percentage

4. DISCUSSION

Authentication and standardization of raw materials were performed and it was found to contain the active constituents. Phytochemical screening of the raw materials was performed. The freeze dried formulation was prepared and evaluated for probiotic activity. About 2.5litres of gastric juice at a pH of approximately 2 is secreted each day in the stomach, which causes the destruction of most microorganisms ingested. In this sense, resistance to human gastric transit is an important selection criterion for probiotic microorganisms. Whey exhibited good resistance to gastric juice at pH 3 compared to pH 2.

The relevant physiological concentrations of human bile range from 0.3% to 0.5%. In this sense, it is generally considered necessary to evaluate the ability of potentially probiotic bacteria to resist the effects of bile acids. Bile tolerance is considered to be an important characteristic of L. acidophilus, as tested in this study, *L.acidophilus* showed, in fact, the highest bile salts tolerance. Whey showed moderate bile resistance.

5. REFERENCES

[1] Functional Foods: Their Role in Disease Prevention and Health Promotion A Publication of the Institute of Food Technologists Expert Panel on Food Safety and Nutrition Claire M. Hasler, Ph.D.
[2] Functional foods in India: Mandeep Ahuja (prm 28)
[3] Review- Nutraceuticals: Facts and fiction Juan Carlos Espin, Maria Teresa Garcia-Conesa, Francisco A. Toma´s-Barbera´n, September 2007
[4] K.W. Konga, A. Ismail food and bioproducts processing, 89 (2011),53–61
[5] Intl. J. Microbiol. Res., 2 (3): 243-251, 2011
[6] C.G. Vinderola, J.A. Reinheimer / Food Research International 36 (2003) 895–904.

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