

Development of Guava Probiotic Dairy Beverages : Application of Mathematical Modelling between Consumer Acceptance Degree and Whey Ratio

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ABSTRACT

Twenty One guava probiotic dairy beverages (*Lactobacillus aciophilus* LA-5 "2% vol/vol" "T₁", *Bifidobacterium bavidium* "2% vol/vol" "T₂" and *Lactobacillus aciophilus* LA-5 "1% vol/vol" and this ratio in *Bifidobacterium bavidium* "T₃") were produced using 0, 20, 35, 50, 65, 80 and 100% (vol/vol) (unsalted whey) which is a product of Ras cheese manufacturing in their formulations and the same percentage interchangeably with cow's milk. The adding of whey had effected on pH values in T₁ treatments. The highest pH values (5.039 to 5.173) recorded in the T₂ treatments, while less values (4.083 to 4.246) recorded in T₁ treatments, but in "T₃ treatments" were a medium values (4.313-4.627±0.012). The effect of adding whey on counts of *B. bavidium* in "T₂" treatments and *L. aciophilus* in "T₂ and "T₃" treatments were highly significant (p≤0.001), while, non-significant effect (p=0.609) of adding whey was showed on *B. bavidium* ("T₃" treatments). In T₁, T₂ and T₃ treatments, the higher values of consumer acceptance degree were 7.267, 7.567 and 7.767 out (9), respectively at 35% whey, while lower values 5 was showed at 0% and 80% whey in T₁ treatments, and (3.233 and 3.867) at 100% whey in T₂ treatments" and T₃ treatments. Mathematical Modelling was created to describe the relationship between consumer acceptance degree (C) and the whey ratio (W) at each type of the previously mentioned starters. Sinusoidal modelling was resulted with a standard error ranging between (0.38 or 0.40). This study is utilized to improve the product, the production of quality products, different consumer degrees and use of dairy wastes "whey", giving an economic value.

Keywords : Guava, mathematical modelling, probiotic, consumer acceptance degree

I. INTRODUCTION

Guava (*Psidium guajava* L.) is widely distributed throughout Egypt. Damietta governorate is considered one of the main districts of guava production in Egypt. The Guava is a soft thin-skinned fruit, sweet in taste and white flesh and many seeds within. It is very susceptible to physical damage. Also, it is highly perishable of ten with potential shelf life of only 2-3 days. Most guava yield are marketed by street vendors. Three days later, the remained fruits have sever decay disorders and become useless. Guava fruit were contains 80% moisture, 20% dry matter, 1% ash, 0.7% fat and 1.5% protein. It is a rich source of Vitamin C and contains other nutraceutical components such as vitamin A, vitamin B₁, B₂, niacin and pantothenic acid. In addition, it also contains a fair amount of phosphorous, calcium, iron, potassium and sodium [1], broad spectrum of

phytochemicals including polysaccharides, essential oils [2 and 3], alkaloids, glycosides, steroids [4], tannins, triterpenes, lectins, fatty acids, dietary fiber, manganese, oxalic and malic acids [5], phenolic compounds [6]. It's contain both major classes of antioxidant pigments such as carotenoids and polyphenols [7].

Its combination with probiotic fermented dairy food like yoghurt, curd and shrikhand will develop high value commodities to increase application of guava in the area of functional foods [8]. Conversion of whey into soft beverages is one of the most attractive avenues for utilization of whey for dairy industry. [9] developed beverage from paneer whey and guava. Here product diversification using whey as a partial replacement of water without much change in quality is quite [10]. Such beverages may be beneficial for the people suffering from gastro-intestinal tract disorders and can be used as

therapeutic soft drinks. The popularity of yogurt products continues to grow; manufacturers are continuously investigating value-added ingredients such as prebiotics and probiotics to entice health-conscious consumers. Probiotics are referred to as “live microorganisms, which when administered in adequate amounts confer a health benefit on the host” [11]. *Lactobacillus* and *Bifidobacteria* species are the most common types of probiotics. Prebiotics are classified as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health” [12]. Currently, the most widely accepted prebiotics include fructooligosaccharides and galactooligosaccharides [13]. When prebiotics are combined with probiotics, their relationship is classified as synbiotic. This combination can improve the survival rate of the probiotics and provide additional health benefits to the host [14]. Whey contains more than half the solids present in the original whole milk including 20% of the proteins and most of the lactose, minerals and water soluble vitamins. There has been increased recognition that the proteins and lactose in whey are valuable nutrients which should not be wasted [15]. In Egypt, most of the whey produced is from Domiati cheese processing (salted whey) little amounts are produced from Ras cheese processing (unsalted whey).

The main objectives of this study were (1) to produce an guava probiotic dairy beverages similar to commercial products that will serve as a basis of comparison for fermented milk with added pulp guava, probiotic cultures and sweet whey (unsalted whey) resulted from Ras cheese manufacturing, (2) Access to the best ingredients between the two types of probiotic bacteria (*Lactobacillus aciophilus* and *Bifidobacterium bavidium*), and (3) Creating a mathematical model describing the relation between consumer acceptance degree and whey ratio at each starter.

II. METHODS AND MATERIAL

Materials:

Guava (*Psidium guajava* L.) pulps were obtained in a Masr Italia for food Industries at a factory of fruit pulp in Damietta El-Jadida city, Damietta Governorate, Egypt. Both fruit pulps were homogenized and repacked in low density polyethylene bags. One part of the samples was

characterized according to moisture content, pH, acidity, ash and soluble solids (Table 1). The other part was stored in a freezer at the temperature of -18°C. until it was used in the experiments.

TABLE 1 PHYSCO-CHEMICAL COMPOSITION OF GUAVA PULP

pH	Acidity	Soluble solids	Black spaces	Color	Y&M	T.C
4.34	0.25	7.7	Non	Good	Nil	Nil

TABLE 2 CHEMICAL COMPOSITION IN COW'S MILK AND UNSALTED WHEY

	F (%)	F/DM (%)	TS (%)	pH value	Acidity
Cow's milk	3.50	29.12	12.02	6.71	0.16
Unsalted whey	0.80	10.66	7.50	6.14	0.14

	SNF (%)	Lactose (%)	Salt (%)	TP (%)
Cow's milk	8.52	4.32	--	3.41
Unsalted whey	6.15	4.22	0.05	1.1

Pasteurized milk (3.5% fat, 12.02% total solid content, 6.71 pH value and 3.41% total protein) obtained from dairy department, Faculty of Agriculture, Damietta University, Damietta, Egypt, and sweet Ras cheese (unsalted) whey (El-Ghazy laboratory, Elsayalem, Damietta), which was obtained during the production of Ras fresh cheese by the enzymatic coagulation process before the salting step (fat 0.80%, pH 6.14, total solids 7.50% and total protein 1.1%), heating to 65°C. to denatured of coagulation enzymes, were used to formulate the probiotic beverages (Table 2).

Starter culture (*Lactobacillus acidophilus* LA-5, *Bifidobacterium bavidium*) were obtained from Ch. Hansen's laboratories, Danmark. Sugar "Elmarwa" "white Sugar", Elmarwa Company for Trading & Distribution, Damietta, Egypt.

Processing of guava probiotic dairy beverage Formulations:

Twenty One beverages were formulated, containing 0 (control), 20, 35, 50, 65, and 80% (vol/vol) whey, with the remaining volume made up with milk, table (3). Preliminary experiments indicated that these whey concentrations were appropriate for sensory tests. Sugar was added to the probiotic beverage at a concentration of 10% (wt/vol) and the mixtures were heat-treated at 83°C for 15 min. After cooling the mixtures to 46°C, the

fruit "Guava pulp" preparation was added at 5% (wt/vol), the probiotic starter culture inoculum at 2% (vol/vol) (Table 3). The mixture was kept at 45°C for the

fermentation process, which was stopped by cooling to 5-8°C. The beverages were stored under refrigeration until the consumer test.

TABLE 3 PROCESSING OF THE PROBIOTIC BEVERAGE FORMULATIONS EACH 1000 ML

Treatments	Milk (L.)	Whey (L.)	Whey (%)	Guava (ml)	Starter (ml)	Sugar (gm)	Total
T ₁	830	----	0	50	20 ml	100	1000
	664	166	20	50	<i>L. aciophilus</i>	100	1000
	539.5	290.5	35	50		100	1000
	415	415	50	50		100	1000
	290.5	539.5	65	50		100	1000
	166	664	80	50		100	1000
	----	830	100	50		100	1000
T ₂	830	----	0	50		20 ml	100
	664	166	20	50	<i>B. bavidium</i>	100	1000
	539.5	290.5	35	50		100	1000
	415	415	50	50		100	1000
	290.5	539.5	65	50		100	1000
	166	664	80	50		100	1000
	----	830	100	50		100	1000
T ₃	830	----	0	50		10 ml	100
	664	166	20	50	<i>L. aciophilus</i> + 10 ml <i>B. bavidium</i>	100	1000
	539.5	290.5	35	50		100	1000
	415	415	50	50		100	1000
	290.5	539.5	65	50		100	1000
	166	664	80	50		100	1000
	----	830	100	50		100	1000

T₁: Probiotic beverage with 2% *L. aciophilus*, T₂: Probiotic beverage with 2% *B. bavidium*, T₃: Probiotic beverage with 1% *L. aciophilus* and 1% *B. bavidium*

Physico-chemical and Microbiological Analyses:

Total solids (TS%), fat, total nitrogen (TN%), lactose content, soluble nitrogen (SN%) and non-protein nitrogen (NPN%) of milk and cheese samples were determined according to [16], the pH was determined with a digital pH meter (Hanna AT 4817). Salt contents of samples were estimated using Volhard method according to [17]. The titrable acidity of guava pulp was estimated by [18]. The Brix percentage of the guava pulp was determined using the refractometer to estimate soluble solids. Total bacterial count (T.C.) of Guava pulp was determined according to [19]. Yeast and Mold (Y&M) were determined according to [19].

The enumeration of *L. acidophilus* LA-5 was carried out in duplicate using de Man, Rogosa, and Sharpe agar supplemented with 0.15% (wt/vol) of bile salts obtained from (El-Gomhoria co., Mansoura, Egypt), incubating anaerobically for 3 days at 37°C [20]. Live strain in *B. bavidium* was inoculated in (MRS) broth supplemented with 0.05% (w/v) cysteine hydrochloride at 37°C under anaerobic conditions for 24 h [19]. Coliform bacteria count according to the method described by [19].

Mathematical Modelling:

Curve expert© 1.3 program was used to model the relationship between the product quality (Independent variable) and whey ratio (dependent variable) for the three types of starter. The average of whey treatments for the three types of starter were used to fit the curve of the consumer acceptance degree which describes product quality. Sinusoidal fit was resulted with a correlation factor 98.59%. Referring to the previous result the sinusoidal fit was chosen to fit the curves which describe the relationship between the product quality and whey ratio at each starter type. Each curve will be fitted to model consumer acceptance degree (C) as a function of whey ratio (W).

Statistical Analysis:

Data were analyzed using [21] computer program, GLM analysis of variance (ANOVA). Differences between means were detected by Duncan's Multiple Range Test [22].

Consumer Test:

Thirty consumers "staff members, graduate and undergraduate students, workers and employees of

Faculty of Agriculture, Damietta University, Egypt" were randomly selected and invited to take part in the test [23]. The samples were presented at $5\pm 3^{\circ}\text{C}$, served in polystyrene cups coded with 3-digit numbers, following the sample presentation design in balanced complete blocks [24] aimed at decreasing the carryover and first-order effects, served 30-50 ml samples to each consumer. Participants were instructed to drink water between samples to cleanse the palate. They evaluated the samples acceptance using a 9-point hybrid hedonic scale [25], where 1 = disliked extremely, and 9 = liked extremely. The consumer test was carried out after samples refrigerated.

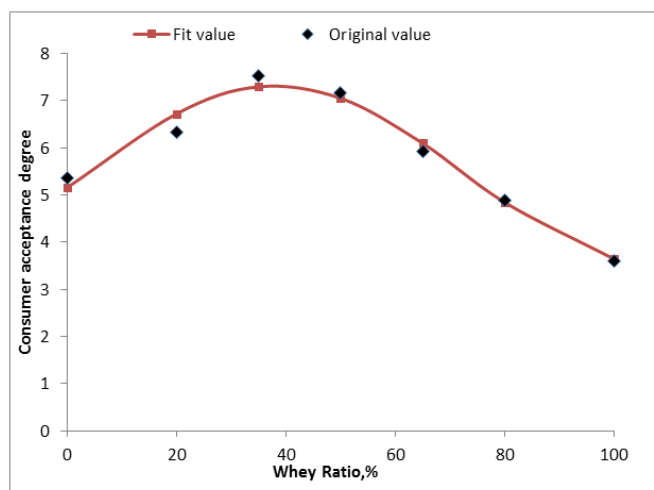


Figure 1 : Fitting of Whey ratio- consumer acceptance degree curve

III. RESULTS AND DISCUSSION

pH and Microbiological Values:

Based on the results, we concluded that the beverages met the standards for human consumption according to the Egyptian legislation, thus allowing their use in sensory tests. Table (4) shows the pH values, *L. acidophilus* and *B. bavidium* counts in whey probiotic beverages. The pH values varied from 4.083 ± 0.009 to 5.173 ± 0.012 , the highest pH values (5.039 ± 0.013 to 5.173 ± 0.012) when the treatments using of *B. bavidium* "T₂ treatments", while the less values (4.083 ± 0.009 to 4.246 ± 0.020) in the using of *L. acidophilus* "T₁ treatments", but in treatments using *L. acidophilus* and *B. bavidium* "T₃ treatments" were a medium values (4.313 ± 0.049 to 4.627 ± 0.012). From the first experiment "T₁ treatments" that included the *L. aciophilus* as a starter, only. It's clear that adding whey during the different stages from 0% up to 100% had highly significant ($p<0.001$) effect on the values of pH and *L. aciophilus* starter. pH values were at range from

4.086 ± 0.009 (20%, whey) to 4.246 ± 0.020 (100%, whey), while the counts of starter were at range from $29.333\pm 0.882 \times 10^5$ (100%, whey) to $57.333\pm 0.333 \times 10^5$ (20%, whey). Also, in the second experiment "T₂ treatments", the effect of adding whey on the pH values and counts of *B. bavidium* $\times 10^6$ starter was found to be highly significant ($p\leq 0.001$). Higher value of pH and counts of *B. bavidium* was recorded as 5.173 ± 0.012 (100%, whey) and $87.000\pm 1.732 \times 10^6$ (0%, whey), respectively, while the lower value was 5.039 ± 0.013 (0%, whey) and $70.333\pm 4.631 \times 10^6$ (100%, whey), respectively. On the other hand, the third experiment "T₃ treatments" included both of *L. aciophilus* and *B. bavidium* as starters. Adding whey during the different stages had highly significant effect ($p<0.001$) on pH values and *L. aciophilus* starter, pH values ranged from 4.313 ± 0.049 (100%, whey) to 4.627 ± 0.012 (65%, whey), while the values of *L. aciophilus* Starter ranged from $5.667\pm 1.333 \times 10^5$ (65%, whey) to $21.667\pm 4.055 \times 10^5$ (0%, whey), in contrast; non-significant effect ($p=0.609$) of adding whey was showed on *B. bavidium* $\times 10^6$ starter. With respect to *L. acidophilus* count, all beverages presented values >8 log cfu/ml, indicating a probiotic level sufficient to provide consumer benefits and to compensate a possible reduction caused by passage through the gastrointestinal tract [26]. In accordance with Egyptian legislation, the whey beverages showed probiotic counts >7 cfu/100 ml of product. The whey content did not interfere in the viability of probiotics in the dairy beverages ($p>0.05$), indicating no limit in the capacity of the probiotic strain to metabolize the peptides present in the whey. These results confirmed the technological application of fresh whey from Minas Frescal cheese as a means to develop probiotic bacteria [27]. Moreover, our findings were comparable to those of other studies involving dairy beverages [28] and to results obtained for other dairy products processed from cheese whey [29 and 30] and other dairy foods, such as yogurts [31], cheese [32 and 33], and ice cream [34 and 35]. All samples were free counts of yeasts and molds and coliforms may be the hygienic or sanitary conditions during the process.

Consumer Test:

Table 5 shows the acceptance of the probiotic whey beverages containing different levels of whey in three experiments "T₁, T₂ and T₃ treatments". The lower value (5.000 ± 0.179) of the consumer acceptance of guava

probiotic dairy beverages with different whey contents in the first experiment "T₁ treatments" was showed at 0% and 80%, whey, while higher value was 7.267±0.117 at 35%, whey. In the second T₂ treatments" and third "T₃ treatments" experiment lower value of consumer acceptance (3.233±0.213 and 3.867±0.150, respectively) was recorded at 100%, whey, while higher value was 7.567±0.092 and 7.767±0.104, respectively at 35%, whey.

Whey content had an effect on consumer acceptance (p<0.05): maximum acceptance was observed for the beverage with 35% whey (mean score of 7.0 on the 9-point hedonic scale). Greater amounts of whey resulted in lower consumer acceptance: samples with 65 and 80% whey presented mean scores of 5.7 and 5.2, respectively [36]. This results was agreement of [37 and 38]. Previous studies reported the use of survival analysis in development of dairy products. [39] used survival analysis to estimate the shelf life of probiotic yogurts. The consumer acceptance indicated values of 35% and 50% whey in the formulations, respectively. Nevertheless, considering the elevated nutritional quality of the whey, the need to reduce the costs of the formulation, and the need to minimize the emission of

polluting substances, the use of cheese whey meets the needs of the food industry.

An analysis of the results presented by the different mathematical modelling allowed for the selection of two probiotic beverage formulations: the first, determined by analysis of the consumer acceptance degree, contained 0 to 100% cheese whey in its formulation, and the second, determined by the best consumer acceptance degree, contained 35 and 50% unsalted whey, respectively.

Figure (2) describes the deviation between sensor degree and resulted values of models. Standard error of the three models varied between 0.38 and 0.40 which means the predicted values is close to the experimental obtained values [40] Cosine function has the property of describing the behavior of a curve that describes a relationship has the trends of both decrease and increase [41].

Thus, we tried to create a mathematical modelling to describe the relation between the consumer's acceptance degree and whey-added ratio for both types of starters, while meeting current demands of the dairy industry.

TABLE 4 PH VALUES AND PROBIOTIC MICROBIAL COUNTS (LOG CFU/ML; MEANS±SD) OF GUAVA PROBIOTIC DAIRY BEVERAGES WITH DIFFERENT WHEY CONTENTS

Whey (%)	pH	L. aciophilus X 10 ⁵	B. bavidium X 10 ⁶
T₁			
0	4.093±0.009 ^{cd}	54.333±1.202 ^a	-
20	4.086±0.009 ^d	57.333±0.333 ^a	-
35	4.160±0.012 ^b	45.333±0.333 ^b	-
50	4.096±0.009 ^{cd}	44.333±1.453 ^{cb}	-
65	4.126±0.012 ^{cb}	39.667±2.906 ^c	-
80	4.083±0.009 ^d	58.333±2.603 ^a	-
100	4.246±0.020 ^a	29.333±0.882 ^d	-
<i>P-value</i>	<0.001	<0.001	-
T₂			
0	5.039±0.013 ^d	-	87.000±1.732 ^a
20	5.080±0.012 ^d	-	85.667±2.963 ^a
35	5.160±0.006 ^{ab}	-	81.333±2.333 ^{ab}
50	5.107±0.009 ^{cd}	-	79.000±1.732 ^{abc}
65	5.133±0.009 ^{cb}	-	74.333±2.963 ^{bc}
80	5.093±0.009 ^d	-	73.667±2.333 ^{bc}
100	5.173±0.012 ^a	-	70.333±4.631 ^c
<i>P-value</i>	<0.001	-	<0.001
T₃			
0	4.337±0.022 ^d	21.667±4.055 ^a	32.333±1.202
20	4.450±0.031 ^c	18.333±2.333 ^{ab}	33.667±1.764
35	4.550±0.021 ^{ab}	15.667±1.333 ^{abc}	29.667±1.202
50	4.530±0.025 ^{cb}	10.333±1.202 ^{cd}	31.333±4.910

65	4.627±0.012 ^a	5.667±1.333 ^d	35.333±3.756
80	4.550±0.025 ^{ab}	18.333±1.202 ^{ab}	30.333±2.603
100	4.313±0.049 ^d	11.667±1.202 ^{bcd}	28.333±1.453
P-value	<0.001	<0.001	0.609

T₁: Probiotic beverage with 2% *L. aciophilus*, T₂: Probiotic beverage with 2% *B. bavidium*, T₃: Probiotic beverage with 1% *L. aciophilus* and 1% *B. bavidium*

TABLE 5 AVERAGE CONSUMER ACCEPTANCE OF GUAVA PROBIOTIC DAIRY BEVERAGES WITH DIFFERENT WHEY CONTENTS

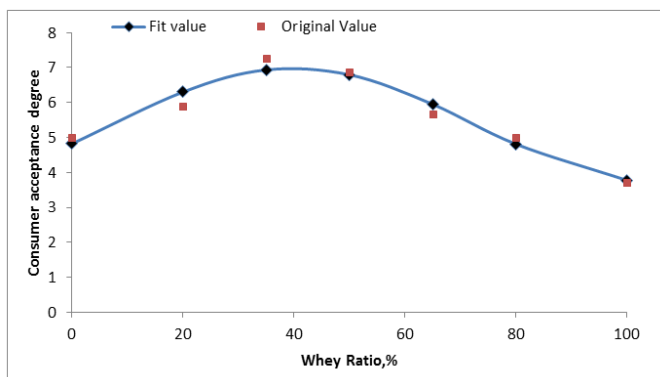
Whey (%)	0	20	35	50	65	80	100
T ₁	5.000±0.179	5.900±0.139	7.267±0.117	6.867±0.115	5.667±0.130	5.000±0.179	3.700±0.204
T ₂	5.367±0.169	6.367±0.122	7.567±0.092	7.033±0.102	5.867±0.115	5.233±0.149	3.233±0.213
T ₃	5.700±0.174	6.700±0.137	7.767±0.104	7.633±0.162	6.233±0.133	4.467±0.124	3.867±0.150

* T₁: Probiotic beverage with 2% *L. aciophilus*, T₂: Probiotic beverage with 2% *B. bavidium*, T₃: Probiotic beverage with 1% *L. aciophilus* and 1% *B. bavidium*
 ** Evaluated on a 9-point hybrid hedonic scale from 1 = disliked extremely to 9 = liked extremely.

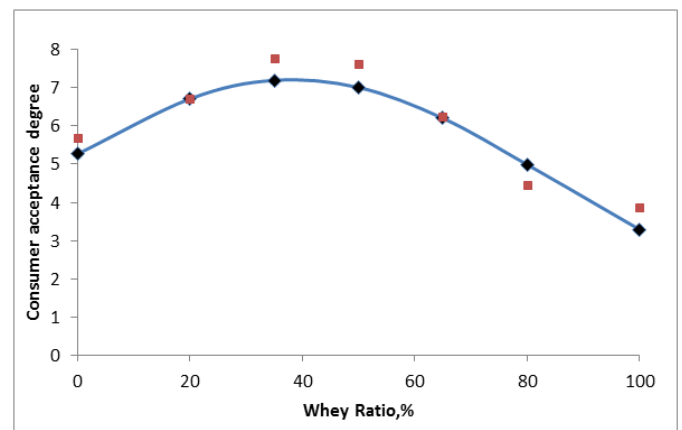
TABLE 6 MATHEMATICAL MODELLING OF CONSUMER ACCEPTANCE DEGREE AS A FUNCTION OF WHEY RATIO

Starter	Formula	Correlation factor, %	Standard error
T ₁	C=5.3444539+1.635956*cos(0.047459805W-1.8955734)	97.53	0.38
T ₂	C=-27.949858+35.123374*cos(0.010003013W-0.49273582)	98.16	0.40
T ₃	C=5.7246037+2.5126344*cos(0.063559216W-2.7262609)	98.14	0.38

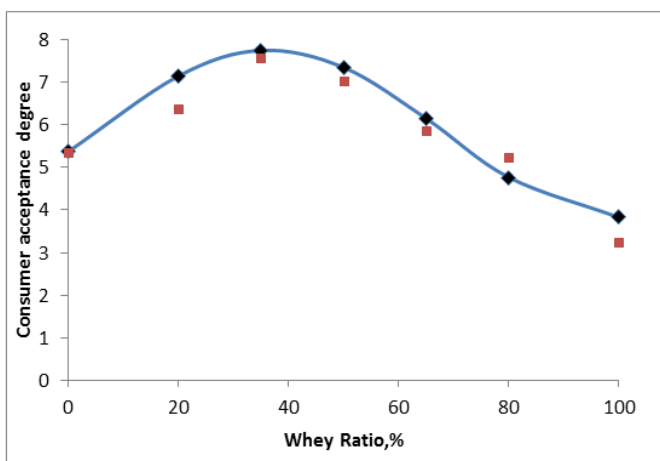
T₁: Probiotic beverage with 2% *L. aciophilus*, T₂: Probiotic beverage with 2% *B. bavidium*, T₃: Probiotic beverage with 1% *L. aciophilus* and 1% *B. bavidium*
 C: The consumer acceptance degree, W: whey ratio percentage interchangeably with cow's milk



A



C



B

Figure 2: Curve fitting of consumer acceptance degree as a function of whey ratio at three types of starters A) T1 B) T2 C) T3

IV.CONCLUSION

pH values in whey probiotic beverages between 4.083±0.009 to 5.173±0.012, the highest pH recorded in T₂ treatments, while the less values in T₁ treatments, but in T₃ treatments were a medium values.

- Higher numbers of *B. bavidium* in beverages were recorded as 87.000±1.732 X10⁶ (0%, whey), while the lower numbers were 70.333±4.631 X10⁶ (100%, whey). On the other hand, the numbers of *L. aciophilus* ranged from 5.667±1.333X10⁵ (65%, whey) to 21.667±4.055 X10⁵ (0%, whey).

- The lower value (5.000 ± 0.179) of the consumer acceptance of beverages in T₁ treatments were showed at 0% and 80%, whey, while higher value was 7.267 ± 0.117 at 35%, whey. In T₂ and T₃ treatments recorded lower value of consumer acceptance (3.233 ± 0.213 and 3.867 ± 0.150 , respectively) at 100%, whey, while higher value were 7.567 ± 0.092 and 7.767 ± 0.104 , respectively at 35%, whey.
- In attempt to create a mathematical modelling to describe the relation between the consumer's acceptance degree and whey-added ratio for both types of starters, while meeting current demands of the dairy industry, we created a mathematical models listed.
- Generally, the whey content had an effect on consumer acceptance ($p < 0.05$): maximum acceptance was observed for the beverage with 35% whey.

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