



Whey/Broken Chickpea Extract for Manufacture of Probiotic Frozen Yoghurt

FAWZIA ABD RABO¹, M. A. AZZAM¹ and OMAIMA M. DEWIDAR^{*2}

¹Department of Dairy Science, Faculty of Agriculture, Cairo University, Egypt.

²Department of Crops Technology Research, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

Abstract

Probiotic frozen yoghurt is one of the ideal probiotic dairy products for delivery of live probiotic micro-organisms to human diet and thus to human intestinal tract. Probiotic micro-organisms have beneficial effects on human health. These benefits include improvements to the intestinal microflora, anti tumoral activity, reduction of cholesterol in the blood, increased immune response and other health benefits. In this study five probiotic frozen yoghurt mixtures were prepared, Acontrol (mainly from fresh skim milk and skim milk powder), B (mainly from whey/broken chickpea extract and milled chickpea grains), AB₂₅ (75% A + 25% B), AB₅₀ (50% A + 50% B) and AB₇₅ (25% A + 75% B). The resultant frozen yoghurts were sensory evaluated. Panelists arranged their preference as A_{control} > AB₂₅ > AB₅₀ > AB₇₅ > B. Physically, the melting resistance of frozen yoghurt increased with the increase of chickpea ingredients. Nutritionally, chickpea frozen yoghurt contained nutrients not found in Acontrol such as dietary fibers, iron, branched chain amino acids, zinc and vitamins B₃, B₉ and E. Microbiologically, the viable counts of ABT culture strains (*Lactobacillus acidophilus* + *Bifidobacterium bifidum* + *Streptococcus thermophilus*) in all frozen yoghurt samples remained within the limits recommended for the probiotic products. Microbiologically, large numbers (> 10⁷ cfu/g) of probiotic microorganisms present in all stored samples indicated that the resulting frozen yogurt could represent a good source of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and commercially, this product was included in probiotic dairy products.



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CONTACT Omaima M. Dewidar ✉ omaima.dewidar@yahoo.com 📍 Department of Crops Technology Research, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.



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Introduction

The trend towards the use of dairy by-products such as whey, skim milk, butter milk and permeate and some residues of agricultural crops such as broken chickpea seeds, wheat germ, wheat bran, rice bran and etc, as non-dairy by-products in the manufacture of functional dairy foods is increasing worldwide.

Whey is one of the most important dairy by-products. It is a by-product of cheese-making and casein manufacture in the dairy industry. Currently, whey and its components are recognized as value-added ingredients in many food products such as infant formulas, sports nutrition foods and other food products. Among whey components is the protein. Whey protein contains all 20 amino acids and all nine essential amino acids, and it is a rich and balanced source of the sulphur amino acids that serve a critical role as antioxidants.¹ Whey may also include low-molecular weight bioactive peptides formed by the enzymatic degradation of the caseins during the cheese making process.²

The broken chickpea seeds are one of the residues of the process of extracting chickpea seeds from their dry horns. Chickpea (*Cicer arietinum* L.) of legumes are rich in protein, vitamins, minerals and dietary fibers but low in fat^{3,4}. Besides promoting weight loss and preventing obesity and cardiovascular disease, Prebiotics are defined as "selectively fermented ingredients that result in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health."⁵ Prebiotics are also a substrate that is selectively utilized by host microorganisms conferring a health benefit.⁶

Both *in vivo* and *in vitro* studies have confirmed the positive influence of dietary fibers, as prebiotics, on survival of probiotic bacteria such as *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5.^{7,8} showed that supplementing by chickpea flour enhanced the growth of *Lactobacillus debrueckii* ssp. *bulgaricus* in yoghurt. Yoghurt fortified with the watery extract of chickpea had higher *Streptococcus thermophilus* counts than that of the control without the extract.⁹ Thus, dietary fibers and protein contents of chickpea will be a useful ingredient to enhance the quality of any fermented dairy product. In addition to the above, it should be noted that whey and chickpea

are important sources of branched chain amino acids (BCAAs).¹⁰ Branched chain amino acids (valine, leucine and isoleucine) have the effect of promoting protein synthesis and inhibiting protein collapse. Leucine is not only precursor for muscle protein synthesis, but also may play a role as a regulator of intracellular signaling pathways that are involved in the process of protein synthesis.¹¹

Therefore, this study was conducted to investigate the effect of replacing chickpea components instead of milk components on the chemical, physical, nutritional, microbiological and sensory properties of like probiotic frozen yoghurt.

Materials and Methods

Materials

Fresh skim milk (0.1% Fat and 9.43% solids not fat (S.N.F) and fresh cream (45% Fat and 3.48 S.N.F) were obtained by separating raw buffalo's milk using cream separator (Alfa Laval). Low heat skim milk powder made in USA (1.25% Fat and 94.75% S.N.F), cane sugar (sucrose) and raw vanilla were purchased from the local market. Lacta 9060 (combination of selected emulsifiers and stabilizers blend) was obtained from Misr Food Additives (MIFAD). Freeze dried ABT-2 starter culture containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Streptococcus thermophilus* was obtained from Chr. Hansen laboratories (Denmark). Broken chickpea seeds were obtained from Crops Research Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Broken chickpea was milled (using standard electric grinder, Moulinex®, France) and sieved by stainless steel sieves (60 mesh) for obtaining chickpea's powder. The powder product (5.85% fat, 89.04% S.N.F) was stored in glass bottles at °C until use. Broken chickpea extract was prepared according to the following.

Preparation of Broken Chickpea Extract

Preparation of Broken Chickpea Extract steps is shown in Fig.1.

Methods

Experimental Procedure

Preparation of Probiotic Frozen Yoghurt Mixtures

- Two mixtures (A_{control} and B) were prepared from ingredients formed in calculated quantities as shown in Table (1) and standardized to

- contain 14% S.N.F, 8% fat, 13% sugar and 0.4% lacta 9060.¹²
- Equal quantities of calculated skim milk and chickpea extract were heated to 90°C and held for 10 min, then cooled down to the incubation temperature of 35°C. These media
 - inoculated with freeze dried ABT-2 starter culture (0.02%) and incubated at 35°C until the pH value reached 5.5. These fermented media were chilled and stored at 4°C.
 - The process used for manufacture of probiotic frozen yoghurt is shown in (Fig. 2).

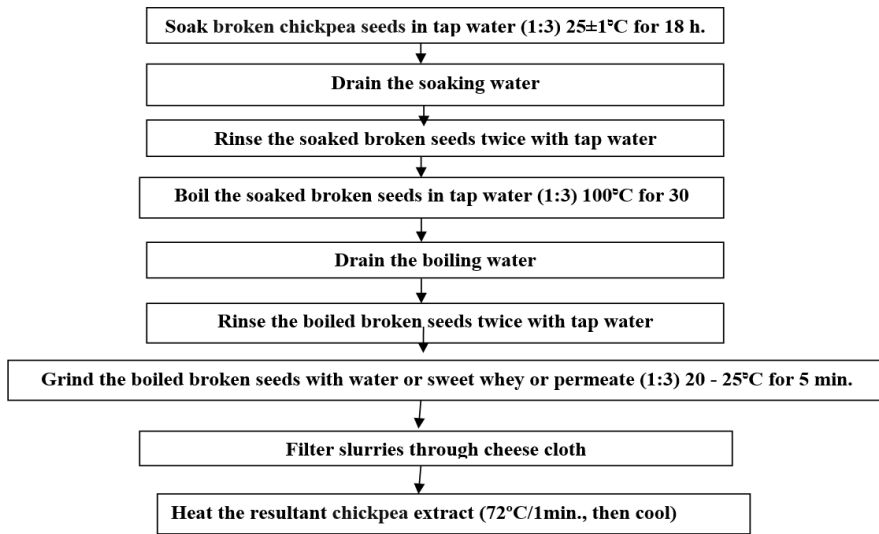


Fig. 1 Flow chart of the preparation of broken chickpea extract

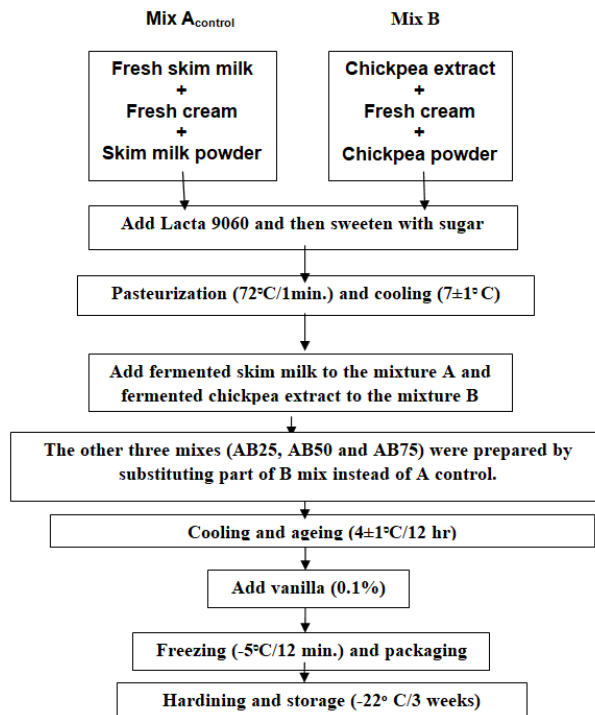


Fig. 2: Flow chart of the process used for experimented frozen yoghurt making

Table 1: Formulation of different probiotic frozen yoghurt mixes

Ingredients	A _{control}	B	AB ₂₅	AB ₅₀	AB ₇₅
Fresh buffalo skim milk	61.13	00.00	45.85	30.57	15.28
Fresh cream	17.42	6.27	14.63	11.85	9.06
Skim milk powder	8.05	00.00	6.04	4.03	2.01
Broken chickpea extract	00.00	72.45	18.11	36.21	54.34
Broken chickpea powder	00.00	7.88	1.97	3.94	5.91
Sugar	13.00	13.00	13.00	13.00	13.00
Stabilizer	0.40	0.400	0.400	0.40	0.40
Total weight (kg)	100	100	100	100	100

Methods of Analysis

Microbiologically

The viable count of ABT cultures (*Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Streptococcus thermophilus*) was enumerated.^{13,14}

Chemically

Total solids, fat, total protein, ash, dietary fibers, minerals (Fe, Ca, P, Na, K, Mg and Zn) and vitamins contents were determined¹⁴ Amino acids content of the product was determined according to the method described in¹⁵ by using High Performance Amino Acid Analyzer. Branched chain amino acids BCAAS (Leucine + Isoleucine + Valine) (g/100g protein) and Fisher ratio of the products were calculated by using the following equation as described by¹⁶

$$\text{Fisher ratio} = \frac{\text{Branched chain amino acids}}{\text{Aromatic amino acids}}$$

Physically

pH value was measured using a digital laboratory pH meter (HI931400 Hanna instruments) with glass electrode. Determination of dynamic viscosity (in Centi-poise, Cp) of aged probiotic frozen yoghurt mixes was carried out using a Brookfield viscometer (Brookfield DV-III, Brookfield Engineering Laboratories, Inc., USA). A Brookfield viscometer equipped with a spindle No.3 to run at 80 rpm in 250 ml cup. All samples were adjusted at 25±1°C before viscosity measurement. The overrun of probiotic frozen yoghurt samples were calculated by using the following equation as described by¹⁷

$$\text{Overrun (\%)} = \left(\frac{\text{Weight per gallon of frozen Yoghurt mix} - \text{Weight per gallon of frozen Yoghurt}}{\text{Weight per gallon of frozen Yoghurt}} \right) \times 100$$

The melting rate of probiotic frozen yoghurt samples was determined 18. The color attributes L (Brightness; 100: white, 0: black), a (+: red, -: green) and b (+: yellow, -: blue) of probiotic frozen yoghurt samples were evaluated using Hunter colorimeter (Hunter ultra-Scan. VIS).

Sensory Evaluation

All the resultant products were sensory rated for flavor (50), body & texture (40) and color & appearance (10) by 10 panelists of experienced staff members at Dairy Science Department, Cairo University and Agriculture Research Center, Ministry of Agriculture, Giza, Egypt according to the ice cream score card of.¹⁷ Total score of flavor, body & texture and color & appearance was defined as total acceptability (100 points).

Statistical Analysis

Statistical analysis was carried out by SPSS Vr. 20 program. Data were expressed as means ± S.D. and the statistical analysis were performed using one-way analysis of variance followed by Duncan's tests. (SPSS, 20).¹⁹

Results and Discussion

Growth Behavior of ABT-2 Culture Strains in Chickpea Extracts

Frozen dairy products such as frozen yoghurt are an ideal carriers for the delivery of beneficial microorganisms to human intestines. In an attempt to increase its functional properties, whey and permeate as dairy by-products and broken chickpea seeds as non-dairy by-products were used in an initial experiment in the manufacture of frozen yoghurt. Therefore, the behavior of ABT-2 culture

(*Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Streptococcus thermophilus*) in three extracts of grounded broken chickpea seeds with water, whey or permeate was investigated to determine the best

extract for making probiotic frozen yoghurt. The mean value of viable counts of ABT-2 culture strains in the three extracts was estimated comparative to that in skim milk as shown in Fig (3).

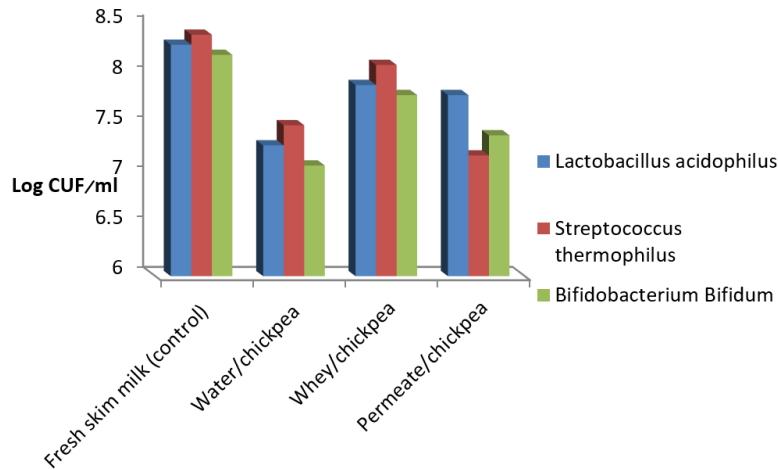


Fig. 3: Flow chart of the process used for experimented frozen yoghurt making

The obtained results showed that the three chickpea extracts were suitable environments for the growth and activity of probiotic bacteria. Whey/chickpea extract was the best extract as the number of viable cells of all ABT-2 culture strains reached a level approximately similar to that of the control skim milk. No microbial activity inhibition due to chickpea ingredients was detected. The dietary fibers and oligosaccharides of chickpea seeds as well as the whey lactoferrin may enhanced the growth and activity of ABT-2 culture strains.^{8,20,21} Therefore, this extract (6.5% fat and 9.34% S.N.F) was used for preparation of experimental probiotic frozen yoghurt mixes Figure 2.

Effect of Chickpea Ingredients on Experimented Frozen Yoghurt Color

Of course, a clean white color is often demanded color of a naturally frozen yoghurt. A_{control}- frozen yoghurt (without chickpea ingredients) exhibited more whiteness as indicated by a high L* value, and low a* and b* values. B-frozen yoghurt (100% chickpea) showed greater yellowish color value (b*) and lesser greenish color value (-a*) than the others (Table 2). Therefore addition of chickpea ingredients decreased the whiteness of the product and this decrease was proportional to replacement ratio of

skim milk and skim milk powder with whey/chickpea extract and milled chickpea seeds in experimented frozen yoghurts (Table 1).

Sensory Evaluation of Experimented Frozen Yoghurt Samples

The resultant probiotic frozen yoghurt products (A_{control}, AB₂₅, AB₅₀, AB₇₅ and B) were evaluated after 2 days of freezing for organoleptic properties (Table 3). The panelists noted that B and AB₇₅ frozen yoghurt melted slowly in the mouth than the others. In other words panelists agreed on the sense of coarseness in sample B, which decreased in AB₇₅ and disappeared in AB₅₀ and AB₂₅. They arranged their preference as A_{control} > AB₂₅ > AB₅₀ > AB₇₅ > B. From the results of the sensory evaluation A, AB₂₅ and AB₅₀ treatments were selected for physical, chemical and microbiological examination.

Physical Properties of the Selected Probiotic Frozen Yoghurt Mixes

Replacement of non-fat milk solids with chickpea ingredients caused a significant decrease in specific gravity (Sp.gr) and weight per gallon and increased viscosity of treated frozen yoghurt mixes as shown in Table (4).

Table 2: Color values of experimented frozen yoghurt

Frozen yoghurts	L*	a*	b*
A _{control}	85.89±0.22 ^a	-3.85±0.04 ^a	10.27±0.07 ^e
AB ₂₅	84.95±0.26 ^b	-3.55±0.08 ^b	12.44±0.17 ^d
AB ₅₀	82.73±0.01 ^c	-3.04±0.13 ^c	15.21±0.22 ^c
AB ₇₅	81.76± 0.38 ^d	-2.44±0.11 ^d	17.94±0.24 ^b
B(100% chickpea)	74.84±0.94 ^e	-2.27± 0.14 ^d	19.88± 0.20 ^a

(L*):The lightness, (b*):The yellowness and (a*): The redness
 Values are means ± SD (n=3), mean number in the same column bearing different superscript letter are significantly different at p < 0.05

Table 3: Sensory evaluation of experimented frozen yoghurt

Mixes	Flavor and Taste(50)	Body and Texture (40)	Color and Appearance (10)	Overall Acceptability (100)
A _{control}	49.00±1.16 ^a	39.25±0.96 ^a	10.00±0.00 ^a	98.25±2.06 ^a
AB ₂₅	47.00±0.82 ^{ab}	38.00±0.82 ^{ab}	9.25±0.50 ^b	94.25±0.96 ^{ab}
AB ₅₀	45.25±0.50 ^b	37.50±1.29 ^{ab}	8.75±0.50 ^b	92.00±2.58 ^{bc}
AB ₇₅	44.50±2.52 ^{bc}	35.25±3.40 ^b	8.00±0.00 ^c	87.75±5.60 ^c
B	42.25±2.06 ^c	30.50 ±1.00 ^c	7.25±0.50 ^d	80.00±2.16 ^d

Values are means ± SD (n=3), mean number in the same column bearing different superscript letter are significantly different at p < 0.05

The high viscosity of treated frozen yoghurt mixes is due to their high content of starch and fibers. Gelatinization of starch is the most prominent change taking place during boiling of chickpea seeds through preparation of its extract. Gelatinized starch has high water holding capacity and it can influence the rheological properties of frozen yoghurt mix.^{22,23}

Physical Properties of the Resultant Probiotic Frozen Yoghurt

With an increase in the level of replacing of non fat milk solids with chickpea ingredients there was a corresponding significant increase in specific gravity and weight per gallon and significant decrease in the overrun of treated frozen yoghurt (Table 5). This lowering in the overrun is due to the high viscosity of mixes which caused a less air incorporation (Table 4). These results are in agreement with the results reported by²⁴ for grape wine lee ice cream,²⁵ for functional ice cream fortified with date pulp and²⁵ for low fat ice cream containing Cactus pear pulp.

Melting resistant of frozen yoghurt samples expressed as a percentage of melted frozen yoghurt during 60 minutes at intervals of 15 minutes is recorded in Table (6).The recorded data indicated that frozen yoghurt samples without chickpea (A_{control}) lost their shape and melted more quickly than those with chickpea (AB₂₅ & AB₅₀). In other words the melting resistance increased significantly as the percentage of whey/ broken chickpea extract increased. A probable cause for this behavior was the impact of fibers and starch of chickpea seeds on the viscosity of the treated frozen yoghurt mixes. These results were in agreement with those reported by.²³ They found that ice creams with the high level of gelatinized malted ragi flour had the highest melting resistance. They attributed that to fibers and starch in ragi flour which may act as stabilizers and thus caused increase in melting resistance of resultant ice cream.

Table 4: Specific gravity (Sp.gr), weight per gallon (Wt/gal) and viscosity of the selected probiotic frozen yoghurt mixes*

Mixes	Specific gravity	weight per gallon (lbs)	Viscosity (Cp)
A _{control}	1.07±0.00 ^a	8.91±0.00 ^a	541.67±1.44 ^c
AB ₂₅	1.04±0.01 ^b	8.66±0.07 ^b	586.70±0.69 ^b
AB ₅₀	1.03±0.01 ^c	8.56±0.05 ^c	657.50±0.00 ^a
weight per = gallon (lbs)	Sp.gr×8.345	(cp) = centipoise.	

Values are means ± SD (n=3), mean number in the same column bearing different superscript letter are significantly different at p < 0.05

Table 5: Specific gravity (Sp.gr), weight per gallon (Wt/gal) and overrun (%) of the resultant probiotic frozen yoghurt.*

Products	Specific gravity	weight per gallon (lbs)	Overrun%
A _{control}	0.75±0.01 ^b	6.29±0.11 ^b	41.73±2.38 ^a
AB ₂₅	0.75±0.01 ^b	6.27±0.42 ^b	38.10±2.01 ^a
AB ₅₀	0.79±0.00 ^a	6.57±0.01 ^a	30.19±0.92 ^b

Values are means ± SD (n=3), mean number in the same column bearing different superscript letter are significantly different at p < 0.05

The Functional Evaluation

Chemical Composition of the Resultant Probiotic Frozen Yoghurt

Total solids and fat contents of all frozen yoghurt were within the limited levels of the standards given by 12 as shown in Table (7). With increased levels of replacement of fresh skim milk and skim milk powder with chickpea ingredients the protein and ash contents of the resulting frozen yoghurt significantly decreased, while the total carbohydrates content significantly increased as compared to A_{control} frozen yoghurt which may be attributed to the high lactose content of whey/chickpea extract.

Nutritionally

The nutritive value of one serving (100 g) of frozen yoghurt samples (A_{control}, AB₂₅ and AB₅₀) was

estimated. The chemical composition, dietary fibers, minerals (Ca, P, K, Fe, Zn and Na) and vitamins (C, B₃, B₂, A, E and B₉) of the of the different samples were determined. Data obtained (Figure 4) showed that AB₂₅- and AB₅₀- frozen yoghurt samples had chemical composition approximately similar to that of A_{control}, but contained nutrients not found in A_{control} such as dietary fibers and iron and contained higher content of zinc (0.94 and 1.00 versus 0.88 mg) and low content of sodium (102.58 and 85.92 versus 119.21 mg), vitamin B₃ (0.20 and 0.22 versus 0.19 mg), vitamin B₉ (19.65 and 28.62 versus 10.66 mg) and vitamin E (0.05 and 0.09 versus 0.04 mg).

Nutritive Values of Frozen Yoghurts Protein

Essential and non-Essential amino acids (EAA) of frozen yoghurt samples ($A_{control}$, AB_{25} and AB_{50}) were also determined Figure 5, 6. The percentage of all amino acids slightly decreased as the percentage of whey/chickpea extract increased in the mixture, but remained within the established limits approved by²⁶ for yoghurt. The branched chain amino acids BCAAS (isoleucine, leucine and valine) percentage

for all amino acids was 46.63 for $A_{control}$, 46.70 for AB_{25} - and 46.85% for AB_{50} -. BCAAS are important for muscle protein synthesis and have a positive effect on mental state.¹⁰ Also, a high fisher ratio was noticed in all frozen yoghurt samples ($A_{control}$ = 2.26, AB_{25} = 2.36 and AB_{50} = 2.39). Fisher ratio is important for maintenance of hepatic function reserve and prevention of progression to liver failure.²⁸

Table 6: Melting resistance of the resultant probiotic frozen yoghurt.*

Products	Melting time (min)			
	15	30	45	60
Weight loss % at 23°C				
$A_{control}$	14.36±0.96 ^a	44.03±0.12 ^a	77.55±1.57 ^a	98.52 ±1.34 ^a
AB_{25}	9.75 ±0.31 ^b	41.96 ±1.16 ^a	74.59 ±0.72 ^b	96.18 ±1.87 ^a
AB_{50}	7.61 ±0.54 ^c	30.55 ±1.52 ^b	59.81±0.91 ^c	81.98 ±1.53 ^b

Values are means ± SD (n=3), mean number in the same column bearing different superscript letter are significantly different at p < 0.05

Table 7: Chemical composition of the selected probiotic frozen yoghurt*

Frozen Yoghurts	Constituents (%)				
	Total solids	Fat	Protein*	Ash	Carbohydrates
$A_{control}$	35.41± 0.10 ^a	8.16 ± 0.10 ^a	4.12 ± 0.12 ^a	1.12 ±0.03 ^a	22.01± 0.13 ^b
AB_{25}	35.43±0.02 ^a	8.00 ± 0.02 ^b	4.08 ±0.10 ^a	0.93± 0.06 ^b	22.38 ± 0.14 ^b
AB_{50}	35.45± 0.02 ^a	8.13 ± 0.01 ^a	3.41 ± 0.29 ^b	0.85 ±0.02 ^c	23.05 ± 0.34 ^a

* Total protein of $A_{control}$ = TN x 6.38, AB_{25} = TN x 6.35, AB_{50} = TN x 6.32

Values are means ± SD (n=3), mean number in the same column bearing different superscript letter are significantly different at p < 0.05

Microbiologically

There was no significant difference between the viable counts of ABT culture strains in all fresh frozen yoghurt samples but they were reduced in $A_{control}$

sample at the end of the storage period compared to AB_{25} - and AB_{50} - samples but still within the limits recommended by²⁹for the probiotic products (Table 8).

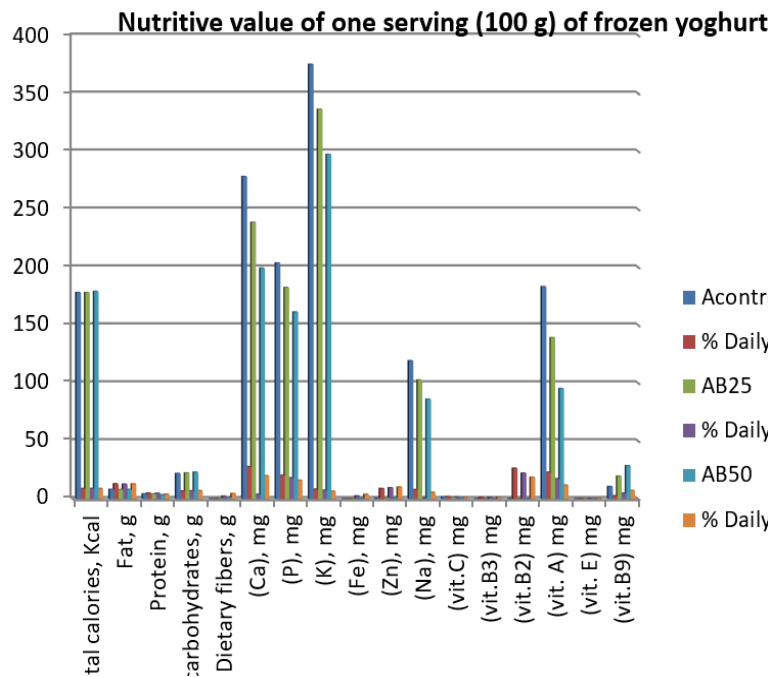


Fig. 4: Nutritive value of one serving (100 g) of frozen yoghurt

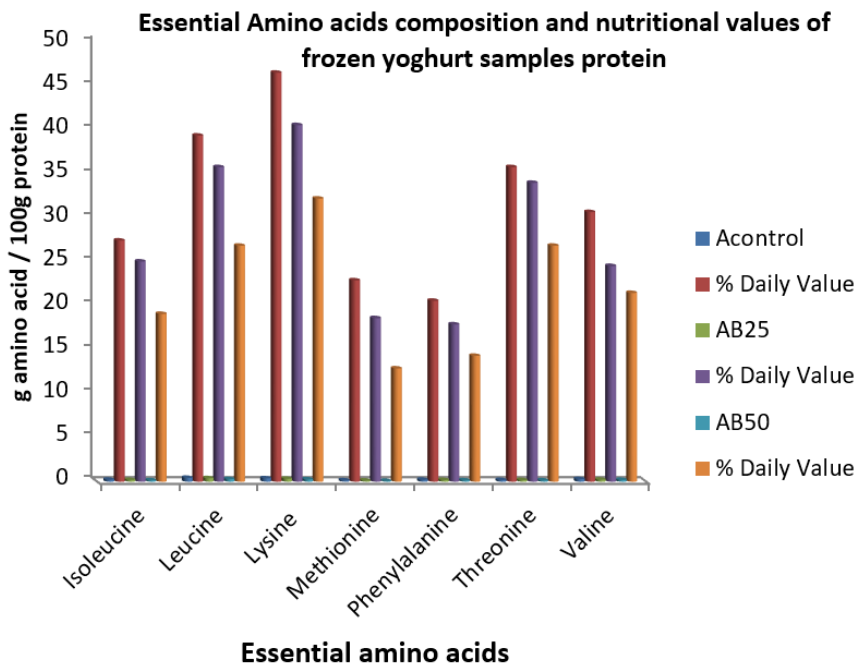


Fig. 5: Essential amino acids composition and nutritional values of frozen yoghurt samples protein

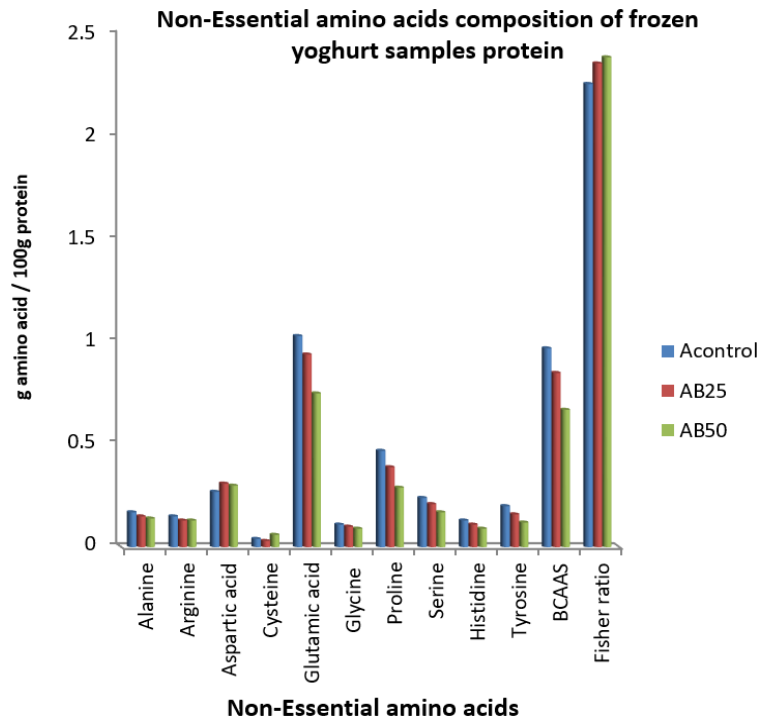


Fig. 6: Non-Essential amino acids composition of frozen yoghurt samples protein

Table 8: Changes in the number of viable count of ABT culture strains during the storage period at -23 ° C for 21 days

Treatments*	Viable count (log cfu/g)	Storage period	
		Zero time	End time
A _{control}	<i>Lactobacillus acidophilus</i>	8.35±0.60 ^a	7.50±0.53 ^b
	<i>Bifidobacterium bifidum</i>	8.20±0.45 ^a	7.20±0.72 ^b
	<i>Streptococcus thermophilus</i>	8.21±0.35 ^a	7.40±0.75 ^b
AB ₂₅	<i>Lactobacillus acidophilus</i>	8.40±0.42 ^a	8.20±0.62 ^a
	<i>Bifidobacterium bifidum</i>	8.25±0.60 ^a	7.65±0.72 ^b
	<i>streptococcus thermophilus</i>	8.30±0.65 ^a	7.80±0.65 ^a
AB ₅₀	<i>Lactobacillus acidophilus</i>	8.30±0.38 ^a	8.10±0.55 ^a
	<i>Bifidobacterium bifidum</i>	8.10±0.58 ^a	7.80±0.72 ^a
	<i>streptococcus thermophilus</i>	8.25±0.71 ^a	7.90±0.70 ^a

* See Table 1

Values are means ± SD (n=3), mean number in the same column bearing different superscript letter are significantly different at p < 0.05

Conclusion

From the above, it could be concluded that using mixture of whey as dairy by product and broken chickpea as non-dairy by product in the manufacture of probiotic frozen yoghurt was succeed. The resulting frozen yoghurt can be successfully used commercially to deliver probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* to consumers from different age groups.

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Conflict of Interest

The authors do not have any conflict of interest.

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