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FUNCTIONAL EFFECTS: WHEY, PROBIOTIC AND PREBIOTIC

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SUMMARY

In 1998 European group of experts declared functional food with benefit effetcts on one or more "targets"on human body. That kind of food showed benefit effects on human health. Functional ingredient of food are probiotic, prebiotic, substitute for fat and vitamin antioxidants. Probiotic is defined as "live microbial ingredient of food with benefit effect on human health". Prebiotic is defined as " nondigestible ingredient of food with benefit effect on a hoste, because it selectively stimulates the growth and activity of one or more bacteria in colon". Day after day whey, as a functional food, gets more and more popular. Traditionaly people believe that whey is healthy and curative and they have no fear to use it. By functional approach it is possibly to enrich whey with probiotic and prebiotic and in that multipurpose way got whey with better benefit characteristics.

Probiotic have widespread usage as a therapeutical supplement in many kinds of products in Europe, USA and Japan. In future it is lakely to be used in a fermeted vegetable or meat. Yogurt and cheese are good environment for probiotics, but natural liquid whey is a field which must be more explored for this purpose. Whey has been subject of many reserches in past 20 years, but in our country, whey is still believed to be raw material with undeveloped technology. Nowdays usage of whey, when the protection of human environment is an imperativ thing, can be treated as a contribution to it.

Worldwide the products based on whey in powder and concentrat of whey protein have reached real expansion, expecialy in USA, France and Germany. Functional and benefit effects of whey, probiotic and prebiotic on human health, were presented in this work. We also did the research of production of whey probiotic beverage named "Probiofit" with characteristics of functional food. We use data from literature and from our own researches.

As a good environment for probiotics we used whey from Trappist cheese supplemented with prebiotic inulin and probiotic starter microorganism Lactobacillus acidophilus and Bifidobacterium bifidum. The probiotic beverage with characteristic sensor features was produced. Beverage has characteristic number of live probiotic (min 10^6 CFU/ml) with prebiotic inulin and its expiring date is 30 days on 4°C.

KEY WORDS:

whey, Probiofit, probiotic, prebiotic

1. INTRODUCTION

Because of its valuble composition of proteins, albumin and globulin, natural lactosis, minerals (Ca, K, P, Mg), vitamines(B1, B2, B3, B6, folic acid and vitamin C) and orotic acid, whey is classify as functional food. Whey can be food and helpful thing in different diets as well as in

cosmetic. There is lot of published articles about fermented whey beverages with different kinds of microorganism, (2, 35,12,13, 37, 34). There are aricles about products based on UF concentrated whey,(36) and articles about beverages with probiotics based on reconstituted whey in powder, (5,33). But we can not find lots of articles about natural whey with probiotics,(14,26). Prebiotics are food or component of food which "nurse and heal" our good bacteria in colon. Actually they are favorite food for our good bacteria, Dixon (39).

For us the most important probiotics are inulin and fructooligosaccharides(FOS).These are carbohydrates or nondegistable oligosaccharides(NOS) with long history of usage in human diet. Inulin has plenty of adventages, but the most important is promotion of bifidobacteria. This clame has been proved in many in vitro and in vivo studies. Inulin and FOS have a significant role in diet of human population worldwide. Daily intake of those oligosaccharides in USA and Europe is about 10g/day, 1-4, average, (38). Many researches declare that intake of inulin and FOS is compilitely safe. That is why they are safe kinds of food and intake of them in normal proportions cannot have some negativ effects. Mullan (40) submits a unique case of scietists Gay-Crosier et al., (2000) from University hospital in Geneve, which clamed that inulin caused allergic reactions. Apart from this researche there is no more information about this issue.

Persons who ingested 15g/day of inulin, extracted from chicory, had significant stimulation on bifidobacteria, (6). Treshold of inulin ingestion is about 4g/day.

Production of products with characteristics of functional food is challenge for food industry. It is important to give new healthy –safe food to consumers because they more and more each day pay attention on safe food, (16).

Aim of this research was presentation of benefit effects of whey, probiotics and prebiotics, production of probiotic beverage with characteristics of functional food and longer expiring day(minimum 30 days) on 4°C.

2. MATHERIAL AND METHOD

Experiment was done in dairy Mlekoprodukt from Zrenjanin. There were 7 experiments for each kind of beverage done on instalment for pasteurising plant for milk. Basic phases of technological process of production of whey beverages with probiotics were:

- separation of milk fat (on cca 0,1%)
- adding of inulin (1%)
- pasteurisation on 70°C
- inoculation and fermentation on 37°C, duration time was 10-12h
- cooling on 4°C and packing in PVC conteiners (0,5L).

Base of orange 72065 (Etol-Celje) and vitamine C were added to beverage. For whey fermentation we used starter culture FD-DVS ABT-5 (CHR Hansen). It is thermophil culture with *Lactobacilus acidophilus* LA-5,

Bifidobacterium bifidum BB-12 and *Streptococcus thermophilus.* We examined the total count of live lactic acid culture according to IDF Standard 149A: 1997 ANNEX A, first, tenth, 20th and 30th day after production. Microbiological proprety was examined according to Act of Standard of microbiological property of food (Sl. list SRJ br. 26/95)(19). Also we examined the main characteristics according to the methodology of Carić, (31).

Content of Mg was examined by atomic absorption spectophotometric method, K and Na were examined by photometar, Ca by permanganometric method, P by spetrophotometric method with developemen of blue color and vitemine B was examined by HCLP. Profesional commity evaluated sensory properties with point-scoring examination of all parameters of our samples, which were kept on 4°C, every 10 days for next 30 days.

3. RESULTS AND DISCUSION

INFLUENCE OF WHEY, PROBIOTICS AND PREBIOTICS ON SOME PHISIOLOGICAL FUNCTIONS IN BODY

Whey, probiotics and prebiotics have significant benefit effects on health of consumers and also can reduce the risk of some diseases. Whey proteins are known to have anticancer effects so they are classified as functional food, (32). It is also known that these proteins stimulate the growth of lactic afcid bacteria. This is important for preparation of probiotic culture, which grows slowly in milk, (33).

There are plenty of data about benefit effects of probiotics in literature, but some researches, as Mullan (40), indicate some oposite facts about those effects on human health. International Dairy Organization published in bilten 380/2003, resultes of Ouweheanda et al, (2003), about effects of starter and probiotic culture on different kinds of diseases: maltigestion of lactosis, diarrhea, necrotic enterocolitis, constipation, infection with Helicobacter pylori, cancer, allergics, cholesterol, blood pressure, coronar disease, infections of urinary tract and other infections. Authors have concluded that there are positive effects on many sicknesses and diseases as a reduction of duration of infection with rotavirus.

Ristić et al. documented improvement of therapeutical treatment of diet on obese persons with probiotic culture *Bifidobacterium longum* BB536 and diet fiber inulin. They also claim that those should be taken every day otherwise it is with temporaly effect.

Whey beverages with probiotics are conventional food (food with nutritive value and benefit effect) and there is no such product on our market. We can find some similar products or pills with probiotics but no such thing. Anyway, consumers use those products because they want to enhence their health. But if we want real functional food we dont need pills, but we need components of food with benefit effects on organism. Whey has plenty of these components.

Since the interest for inulin have grown, examinations were done,(4). But we need more observations to make clear all inulin implications and its benefit effect on human health, (17). Companies from USA accepted fructan type of inulin but they are still trying to do more examinations and explore principles of human intestinal tract better.

There is great number of benefit effects of whey, probiotics and prebiotics on human health and on prevention or treatment of different kinds of diseases. So the whey has potential for making beverages, which will be classify as functional food with benefit effects on human health and reduction of diseases. Table 1.

TABLE 1. POTENTIAL EFFECTS OF WHEY, PROBIOTICS AND PREBIOTICS (17,24,4,29,16,40)

WHEY	Probiotic	Prebiotic
▪ Enhance digestion ▪ option for constipation ▪decrease problems with ▪high acidity and ulcer in stomach ▪helpful in treatment of liver sickness(sindrom of fatty liver) ▪decrease the level of cholesterol ▪decrease blood pressure, enhance circulation, decrease heart problems caused by high level of Ca and K ▪good for rheumatism (arthritis) ▪improve kidneys and speed up excretion of liquor and unhealthy matter, detract edemas ▪decrease inflammatory process ▪good for healthy skin ▪sublimate organizm and reduce the weight (it makes you feel satiate) ▪good for thirstness ▪whey protein has anticancer effect, especially for urogenital cancers ▪lenitiv effect on nervous sistem ▪prevention of diseases: osteoporosis ▪helpful for cellulit reduction	▪offset lactosis intolerance ▪enhance immunity ▪reduce cholesterol ▪reduce risks of diseases: diarrhea caused by rotavirus and reduction of colon cancer ▪change microecology of GI microflora, reduce toksin productoion and reduce sindrom of infants death ▪normalize intestinal permeability ▪increase intestinal immunity response(IgA) ▪prevent diarrhea and reduce allergic reactions ▪enhance digestion of lactosis and reduce intestinal distension ▪insrease immunity and infection resistance ▪cancer protection ▪decrease level of cholesterol and risks of different coronary diseases ▪prevent and heal ulcer ▪reduce allergic reactions ▪helpful for obesity	▪selectable stimulation and growth of bifidobacteria in colone ▪slow down the growth of pathogen bacteria which cause intestinal, vaginal and other infections ▪speed up intestinal mollality ▪reduce risk of diseases: constipation, piles ▪good for diarrhea ▪good for osteoporosis(enhance the availability of Ca and bone mineralization) ▪good for atherosclerosis ▪reduce risk from obesity and diabetes ▪increase production of fatty acid with smaller number of C atoms ▪increase the absorption of Ca ▪decrease serum cholesterol and triglicerydes ▪immunostimulation

Probiotic beverage named Probiofit is a diet product and also functional food with high biological value. Probiofit can be treated as "half food half medicament". In Table 4 we can see the composition of beverage Probiofit.

TABLE 4. COMPOSITION OF PROBIOTIC WHEY BEVERAGE PROBIOFIT

Parameters of quality	Probiofit (natural)	Probifit (orange)
Protein , %	0,64	0,8
Total sugar , %	5,04	9,8
Milk fat , %	0,17	0,10
Dry matter , %	6,67	11,46
Ash ,%	0,51	0,63
Ca mg, %	78	76
P mg, %	66	53
Na mg,%	32	36
K mg,%	120	122
Mg ,mg%	7,23	7,56
Vitamine B_2(laktoflavin)µg/100g	270	290
Energy value kJ/kcal	102,2 /24,28	188/45,00

Probiofit is ideal electrolyte and mineral beverage with low energy value (102,2/24,28 kJ/kcal/100ml), almost without fat and with higly valued proteins, natural lactosis, lots of minerals, vitamines (B complex and vitamine C). Orange Probiofit has a higher energy value because of fruit content and sucrose. Probiofit can bi liquid or a little bit lusterless beverage with specific taste. Color, taste and odour depend on added fruit. Natural kind of beverage had mild acid "vinegard" taste which is related to probiotic. Some producers add water to reduce specific taste but it is not recomedable because in that case valuble ingredients of whey are diluted. The best result are shown in beverage with fruit, without sugar and supplemented with vitamine C. This vitamine also contributes better taste. Results of commity which evaluated beverage are shown in Table 5.

Acidity and pH value have no changes. The quality of product in first 15 days had no changes. After that period we notice slight acidity but the product was stabled. Beverage was evaluated as good for market place. Probiofit with *L. acidophilus, B.bifidum* and *Str. thermophilus,* inulin and without fruit was stable and with good sensory characteristics during 30 days in refrigerate.

TABLE 5. SENSOR ESTIMATION OF PROBIOFIT

BEVERAGE	TASTE	ODOUR	COLOR	SEDIMENT	OUTLOOK	TOTAL
MAXIMUM SCORE	0-12	0-2	0-2	0-3	0-1	0-20
NATURAL	11.50	1,50	2,00	1,50	1,00	17,50
ORANGE	12,00	2,00	2,00	1,50	1,00	18,50

During storage period we noticed small amount of sediment, which disappeared when product was shaked. We can also fix this sediment by adding stabilizer in product. It is better just to shake product. Probiofit with supplement of fruit (orange) had better marks because there was no specific taste of whey in it. Probiofit without fruit had slightly acid taste and odour characteristic for fermented whey.

Science based on researces and developement of products with characteristics of functional food will have to present all benefit effects of these food to its consumers. This science must describe positiv link between components of beverages as a food and diseases. Positiv role of whey beverages are not hard to prove. Evidence about benefit effects of whey beverages on human health is most precise when doctors and nutritionists present them.

4. SURVIVAL OF PROBIOTIC BACTERIA IN WHEY BEVERAGES

The count of live probiotic bacteria is very important for functional properties of whey beverages. According to Association for fermented milk and beverages from Japan, it is recomended that minimum count of live probiotics should be 10^7/ml in fresh beverage.

Consumers buying probiotic products think that count or type of probiotic is correct but it is not always true. In the last few years Mullan(40) exemined survival of Lb. casei, L. acidophilus and bifidobacteria in comercial products of yogurt.

Acidity of product is the most important factor for survival of probiotic. Generally, during storage the higher is pH value of product the survival degree of bifidobacteria and lactobacilus is lower. It means that yogurt culture has to be measured. In practice that means that there is a need to eleminate Lb. delbrueckii ssp. bulgaricus. We need cultures, which produce less acid, or we must low significantly level of inoculation. In some cases presence of bifidobacteria was not determed but they were present in product. Generally, there was low level of live bifidobacteria and lactic acid bacteria in many products.

H.S.Garcia et al.,(5) have examined the production of probiotic product made of reconstitute whey inocukated with L. reuteri (B1471) and B. bifidum (NCFB2715) in concentration of 0,5%, 1%, and 2%. After 30 days of storage in refrigerate product had needed count of live probiotic and characteristic sensory features. Resuls from our research are similar to those results done by Garcia. The only differece was that our product was made from whey left after cheese production. We fermented our whey with probiotic bacteria and S. thermophilus. According to "Regulations of quality and other requarements for milk, dairy products, composite dairy products and starter cultures"(Sl.list SRJ br.26/02)(20), our product must have at least 10^6cfu/ml probiotic bacteria. In our product the total count of live cells were (4,00-5,00)x10^9 cfu/ml and the count of probiotic bacteria were (1,70-2,30)x 10^9cfu/ml. Higher total count of live cells was in Probiofit without supplements. That means that survival of bacteria depends of added fruit base.

It is very important to say that Probiofit after 30 days of storage in refrigerate had total count of live cells higher than 10^7 cfu/ml, and probiotics 10^6-10^7 cfu/ml, depending on sort of Probiofit. We can conclude that Probiofit satisfy criteria of "Regulation about quality" after 30 days of storage. Also all examined semples of Probiofit were microbiological regular so that is why our product had longer expiring date (30 days). It is

very important to combine all the factors of technological process which influence growth of probiotics. These factors are type, genus of culture, size of bacteria, heating tretman, adding vitamine C, temperature and time of fermentation, pH value after the fermentation, pH changes during storage (slow acidity) and cooling.

We follow the changes during 20 days of storage and we concluded that there were no significant changes of pH value and sensory quality. We only notice the small amount of whey proteins as sediment. After the storage period we notice the low level of acidity but it did not spoil the number of probiotics and characteristic sensory features.

TABLE 5. SURVIVAL OF TOTAL NUMBER OF LACTIC ACID BACTERIA AND L. ACIDOPHILUS AND B. BIFIDUM IN PROBIOFIT DURING 30 DAYS OF STORAGE ON 4°C.

Storage period (days)	Probifit	pH value	Lactic acid bacteria , cfu/ml	L.acidophilus i B.ifidum , cfu/ml
1 day	Natural	4,21	$5,0 \times 10^9$	$2,3 \times 10^9$
	Orange	4,24	$4,0 \times 10^9$	$1,7 \times 10^9$
10 days	Natural	4,22	$3,2 \times 10^7$	$1,6 \times 10^7$
	Orange	4,15	3×10^7	$1,5 \times 10^7$
20 days	Natural	4,18	$2,1 \times 10^7$	$1,4 \times 10^7$
	Orange	4,10	$1,9 \times 10^7$	$4,6 \times 10^6$
30 days	Natural	4,02	$1,7 \times 10^7$	$1,5 \times 10^7$
	Orange	4,04	$1,4 \times 10^7$	$4,0 \times 10^6$

During storage level of pH value decreased for 0,19-0,2 units, so the condition for maintaining count of lactic acid bacteria, specialy L. acidophilus and B.bifidum were above the critical level ($>10^6$ /ml). According to the data from literature, (16), research of marketing of fermented beverages took adventages over beverages with pH value higher than 4,01. Our results were likewise. During storage the beverages were hygienicaly regular.

5. CONCLUSION

With the aplication of functional supplements in production of fermented whey beverages we got the products with benefit effect on human health and these products can also reduce risk of diseases. They can be used as a food or helpful ingredient in diets. Results show the folowing conclusions:

- Mixed probiotic cultures in starter ABT-5, in presence of B. bifidum, L. acidophilus and Str. thernophilus can not produce high level of acidity, so the acidification during storage period is not significant.
- Whey beverages need to have enough count of live cells during 30 days of storage(minimum 10^6 cfu/ml)The product needs adequate count of live cells without changes of pH value.

- Inulin-nondigestible component of food has benefit effects on human health and stimulates the growth and activity of good bacteria in colon. At the same time it limits the growth of bad bacteria in colon, improves the sensory characteristics of beverage(taste, viscosity). Vitamin C and fruit also have positiv effect on sensory characteristics.
- Acidity of beverage (pH 4,2-4,4) during storage period (30 days/4°C) had no significant changes. Small amount of sediment did not spoil the quality of product.

The whey beverages with activ probiotic bacteria have great adventage compared to unfermented beverages. Probiofit has no fat and cholesterol. It contains the most important proteins; high level of Ca and K, vitamine of B compex and vitamine C. Probiofit has real characteristics of functional food.

LITERATURE

1. Arsov Arsov A.(1982) : Mogućnost korištenja surutke u proizvodnji fermentisanih napitaka , Mljekarstvo 32(8) 242-246 , 1982
2.Carić M.,Milanović S., Nemet G.,Panić M.(2003) : Proizvodnja funkcionalnog napitka od surutke , Prehr,ind.1-2,69-73.
3.Carić M., Milanović S., Vucelja D.(2000) : Standardne metode i analize mleka i mlečnih proizvoda,Tehnološki fakultet ,Novi Sad.
4.Duncan Crow (2003) Inulin –A Comprehensive Scientific Review
www.members.shaw.ca/duncancrow
5.Garcia H.S.,Hernandez A.,Robles V.,Angulo J.O., J.De La Cruz-Medina (2002):Preparation of a Whey-based probiotic product with Lactobacillus reuteri and Bifidobacterium Bifidum, UNIDA, Instituto Technologico de Veracruz, M.A.de Quevedo#2779, Col.Formando Hogar, Veracruz, Ver., 91897, Mexico, Session 15 A, Dairy Foods, 2002-06-16.
2002 Annual Meeting and Food Expo-Anaheim,California
6. Gibson , G.R.1995.The concept of prebiotics as applied to fluctooligosaccharides and inulin.Proc.Fifth Seminar on inulin, Marseille, France.
7.Gibson , G.R. ,Bear , E.R:,Wang,X.,&Cummings, J.H.1995.Selective stimulation of Bifidobacteria in the human colon by oligofructose and inulin.Gastroenterology 108:975-982.
8.Gibson ,G.R.&Roberfroid , M.R. 1995.Dietary modulation of the human colonic microbial : introducing the concept of prebiotics.J.Nutr.125(6):1401-1412.
9.Niketić ,G.(1993) Dijetetski UHT sterilizovani proizvodi ,II međunarodni simpozijum Savremeni trendovi u ishrani –mleko i mlečni proizvodi, Kopaonik zbornik radova :58-61
10.GRAS Notice No GRN 000118 ,U.S. Food and Drug Administration,May 5,2003
11. IDF Standard 149A:1997 ANNEX A
12.Miočinović Dragica, Stojanka Mitić, Anka Kasalica (1984): Nove mogućnosti korišćenja surutke kao izvora nutritivnih, proteinskih i drugih komponenata u ishrani dece. Savetovanje "Pravilna ishrana – zdravo dete". Sinopsis, 14, Novi Sad.

13.Miočinović Dragica, Stojanka Mitić, Anka Kasalica (1986): Biological transformation of whey for obtaining refreshement drinks. XXII International Dairy congress, Abstracts – posters, 208, Hague.
14.Miočinović Dragica, Anka Kasalica, Rada Marković (1992): Mogućnosti iskorišćenja nutritivnih svojstava surutke iu ishrani ljudi. Savetovanje proizvođača zdravstveno bezbedne hrane, Risan.
15.Milanović S., Carić M., Đurić M., Škrinjar M.,Panić M., Korherc G., I . Prćić , Kocić S.: (2004) Preh.ind.1-2.20-27.
16.Obradović D. , Ristić G. Karić A .(2002) : Indusrijska primena probiotika , Preh.ind.1-2, 4-7.
17.Roberfroid B.M.(2000) : Prebiotics and probiotics : are they fukctional foods ? 1,2,3
American Journal of Clinical Nutrition , Vol .71 , No 6,1682S-1687s,June
18.Roberfroid B.M.(2000) : Concepts and strategy of functional food science : the European perspectiveThe American Journal for clinical nutrition Vol.71.No .6.1660S-1664s, June 2000. 19.Pravilniku o mikrobiološkoj ispravnosti namirnica u prometu ("Sl. list SRJ" br. 26/95).
20. Pravilniku o kvalitetu i drugim zahtevima za mleko, mlečne proizvode, kompozitne mlečne proizvode i starter kutlure , Sl. list SRJ" br. 26/02 .
21.Popović-Vranješ A., Križan K., G.Altabari , F.Stambolić , Oručević S. (1991) :
Kvalitet nekih novih proizvoda na bazi surutke i permeata , XIX Simozij za mljekarsku industriju , Opatija , 1991.
22..Popović-Vranješ Anka , Mirjana Vulić :Mleko i mlečni proizvodi funkcionalna hrana na našem tržištu ,Savremeni trendovi u mlekarstvu , Zbornik radova,138-140, Zlatibor , 07-11.maj 2003
23.Popović-Vranješ, Mirjana Vulić:Probiotic whey beverages
Biotehnology in animal husbandry,2003, Vol 19,5-6,496
24.Popović – Vranješ Anka , M. Vulić, S. Todorović: Nutrition and terapeutic value of new whey productsAnimal Science and biotehnologies, Temišvar,27-28.maja 2004. Scientifical papers, Vol XXXVII, 382-387.
25. Popović-Vranješ Anka**:** Hranljiva i ljekovita vrijednost surutke i surutkinih proizvoda. IV Međunarodni simpozij, Kopaonik 3-7 IV 1995., Zbornik radova, 65-68, 1995.
26. Popović-Vranješ A . , Veselina Radovanović-Pelagić, Verica Jurić, Mirjana Vulić, Anka Kasalica : Probiofit-novi proizvod sa osobinama funkcionalne hrane:.Prehrambena industrija 14 (1-2)74-78 ,2003 Novi Sad
27.Popović-Vranješ A : Proizvodnja bezalkoholnih surutkinih napitaka- Congress Milk situation and perspective, Zbornik radova 148-156, Zlatibor, 2004
28.Popović-Vranješ , Vujičić I. (1997) :Tehnologija surutke , Univerzitet u Novom Sadu, Poljoprivredni fakultet ,Novi Sad.
29.Ristić G., Jorga J., Maksimović M., Karić A., Plećaš D., Makaj A.,Ille T. 2002 : Efekat funkcionalnog mlečnog proizvoda obogaćenog kulturom Bifidobacterium longum BB536 u tretmanu gojaznosti , Preh.ind,1-2 ,
30.Roberfroid B.M.(2000) : Concepts and strategy of functional food science : the European perspectiveThe American Journal for clinical nutrition Vol.71.No .6.1660S-1664s, June 2000.
31..Carić M., Milanović S., Vucelja D.(2000) : Standardne metode analize mleka i mlečnih proizvoda , Novi Sad .

32.Tamime Y.A. , Božanić R. Rogelj I. (2003): Probiotički fermentirani mliječni proizvodi , Mljekarstvo 53(2)11-134.
33..Drgalić,I., Tratnik , Lj., Božanić ,R.(2003): »Growth of probiotic becteria in reconstuted whey» European Dairy Congress.Milk and dairy Products.Portorož,15-18.Novembar 2003.Slovenia
34.Wiolonec R.(1988): Untersuchungen zur entwicklung und Qualitätsbeurteilung von Mölkengetränken, Band 6,Gissen.
35. Đorđević,J., Niketić, G., Karić, A., Bubanja,N., Marković, D.(1982): Mljekarstvo 32(7)214-218
36. Kršev, LJ., Tratnik, LJ., (1984): Mljekarstvo 34(9)268-271
37. Klupsch J.H. 1984: Saure Milcherzeugnisse Milchmischgetranke und Desserts, 142-153, 216
38. Coussement, P. .(1999) Inulin and oligofructose: safe intakes and legal status, Journal of nutrition.129;1412S-1417S;
39. Dixon, Suzanne; Progress Newsletter-Spring 2002; Prebiotics and Probiotics: What are they and why should I eat them.
40. Mullan M.(2004):Dairy science and food technology , starters , probiotics ,cheese and antimicrobial systems , Dairy and food technology,Departmant of Food Science, at Queens University Belfst.