SACCO system

Supporting food culture & life

The various nuances of kefir yesterday and today

Thursday, October 14th 2021

Billund

Denmark

INTRODUCTION

Kefir, is traditionally recognized as acid-alcholic fermented milk with helthy featurs

Nowadays, the word kefir is addressed to a wide range of fermented milk

Cultures as a natural solution to prevent the most known issues

Kefir is a traditional, acidic, slightly alcoholic, fermented milk from the Caucasian regions, which is popular in Eastern European countries.

Kefir grains are used as a starter.

They are gelatinous and irregular particles with a diameter of about 8–10 mm, which are composed of:
a mixture of LAB (mainly lactobacilli),
lactose-fermenting yeasts,
and acetic acid bacteria.

The cells are embedded in kefiran, an HePS composed of glucose and galactose , which is produced by *La. kefiranofaciens*.
Kefiran is the matrix of the kefir grains and plays an important role in maintaining the ecological niche.
It has a protective function when the grains are recovered, dried, and re-used for successive milk inoculations.
Physical contact between both *La. kefiranofaciens* and *Saccaromyces caerevisiae* seems to be responsible for stimulation of the kefiran production by *La. kefiranofaciens* in mixed culture.

Starters

Kefir grains are white or slightly yellow and incorporate a microflora including:

A – lactic acid bacteria , B – acetic acid bacteria

C - yeasts, D - mould *Geotrichum candidum*, and various contaminants.

The indigenous microflora of kefir grains is variable.

The lactic microflora of 'grain starter' consists of:

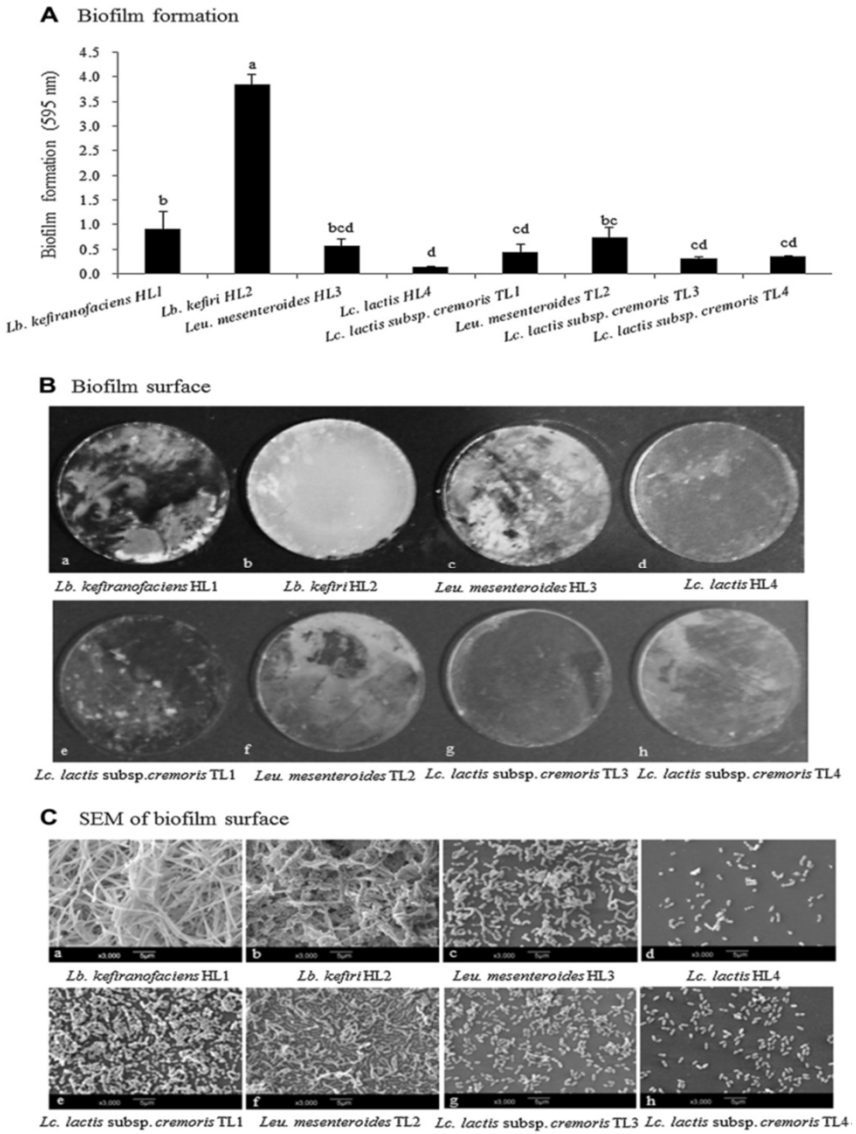
Lactococcus lactis subsp. *lactis* and *cremoris*, homofermentative and heterofermentative lactobacilli (15 species),

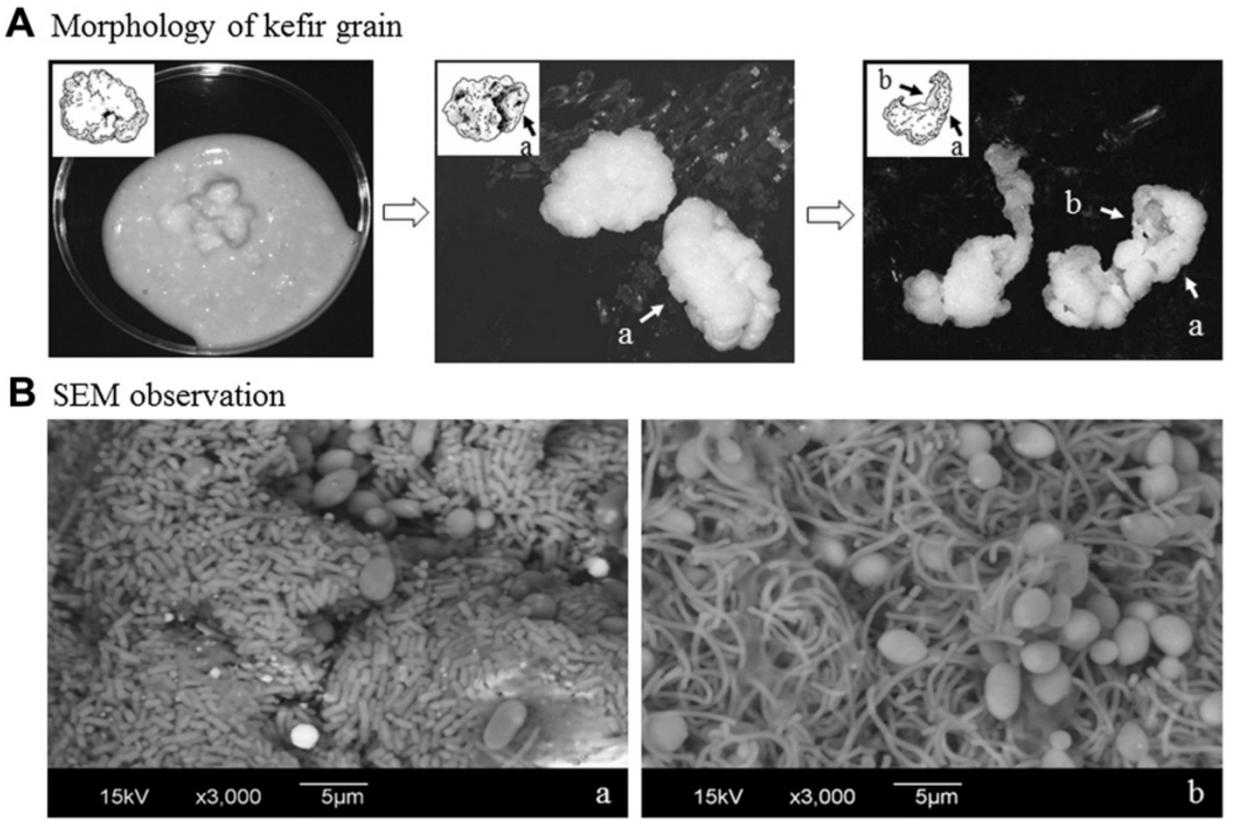
Leuconostoc mesenteroides subsp. *dextranicum*,

Streptococcus thermophilus.

Yeasts of the genera *Saccharomyces*, *Kluyveromyces*, *Candida*, *Mycotorula*, *Torulopsis*, *Cryptococcus*, *Torulaspora*, *Pichia*,

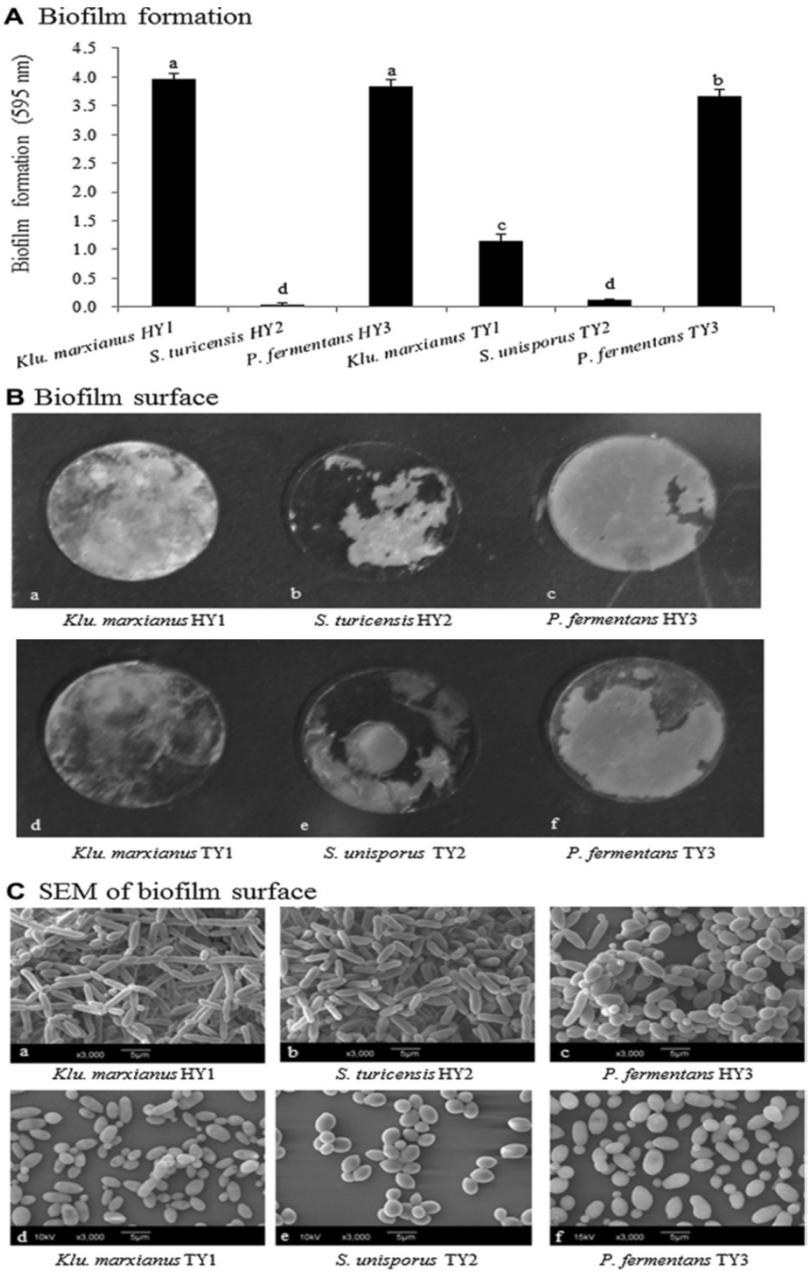
acetic acid bacteria *Acetobacter aceti* and *Acetobacter racens*.





(A) Biofilm formation; **(B)** morphology of lactic acid bacterial biofilm surfaces; and **(C)** SEM observation by LAB isolated from kefir grain and viili starter.

The columns and vertical bars are mean SD of three samples. The columns without a same superscript differ significantly .



Structure of kefir grains

A section of a whole grain examined by microscopy and photographed. The grains are irregular in shape with a diameter of 10 - 15 mm and structurally it could be opened and extended as a thick biofilm.

The whole outer layer of the grain is more densely populated by microorganisms than the inner part.

At a higher magnification, the outer layer of the grain was found to contain lactobacilli, lactococci and yeasts.

The inner layer of grain was similar except that the lactobacilli were much longer, and more yeast cells were found in the inner layer of grain.

(A) Biofilm formation; **(B)** morphology of yeast biofilm surfaces; and **(C)** SEM observation by yeasts isolated from kefir grain and viili starter. The columns and vertical bars are mean SD of three samples. The columns without a same superscript differ significantly ($p < 0.05$).

SACCO system

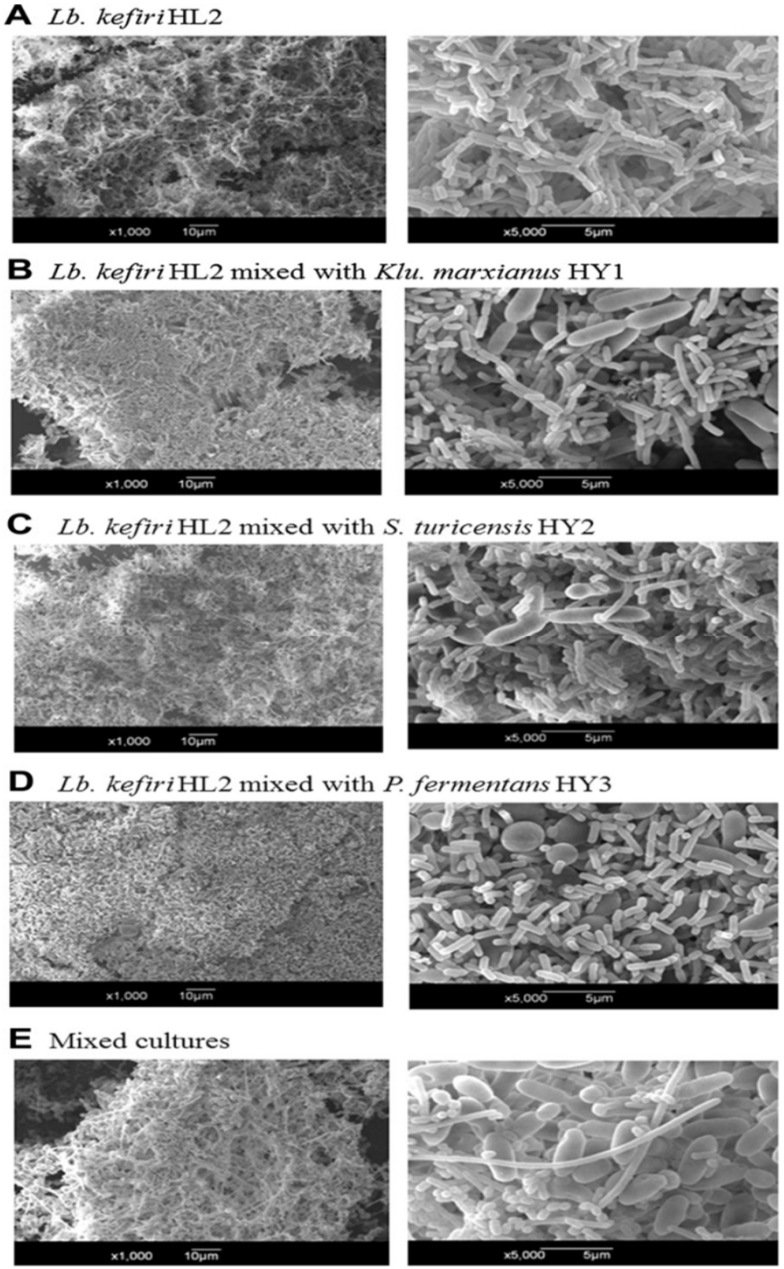
The cell-cell interaction among LAB and yeasts.

The effects of mixed species culture on the co-aggregation and
biofilm formation were investigated.
The pH and strains significantly affected the co-aggregation ability of the mixed kefir LAB and yeast combinations .
Co-cultured kefir LAB and yeast at pH 4.2 showed a faster and better co-aggregation ability than that at pH 6.2.

Mixing Lb. kefiranofaciens with Klu. marxianus or S. turicensis and mixing Lb. kefiri with S. turicensis produced the **highest co-aggregation ability**.

In contrast, mixtures of viili LAB and yeast strains exhibited weak coaggregation ability .
In terms of biofilm formation, a mixture of Klu. marxianus with various individual kefir LAB strains and Lb. kefiri with various individual kefir yeast strains induced higher biofilm formation than the other mixed kefir strains.

Conversely, except for P. fermentans, the mixed viili species displayed lower biofilm formation than the mixed kefir strains .
Biofilm formation by P. fermentans mixed with individual viili LAB was significantly higher than with the other pairs of viili strains .
Overall, Lb. kefiri mixed with Klu marxianus, demonstrated a significantly higher biofilm formation than any other mixed species pair
The structure of the various biofilms was observed by SEM. The results indicated that, in single culture, Lb. kefiri formed a high density and rigid biofilm.
At a higher magnification, the short bacilli were connected together to form network with voids.
In mixed culture, however, yeasts filled the voids and bound together with Lb. kefiri to form a thick biofilm layer.
When a mixture of the four kefir LAB strains and three yeast strains
from kefir were co-cultured, the mixed species biofilm demonstrated a thick bi-layer structure.
The surface layer was covered by long lactobacilli and yeasts.
Short lactobacilli form an inner second layer that was similar to that found in kefir grains .



Scanning electron micrographs of biofilm surface of :
(A) Lb. kefiri HL2;

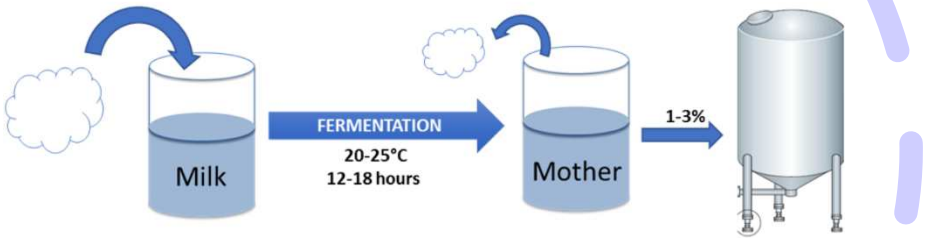
(B) (B) Lb.kefiri HL2 cultured with Klu.marxianus HY1

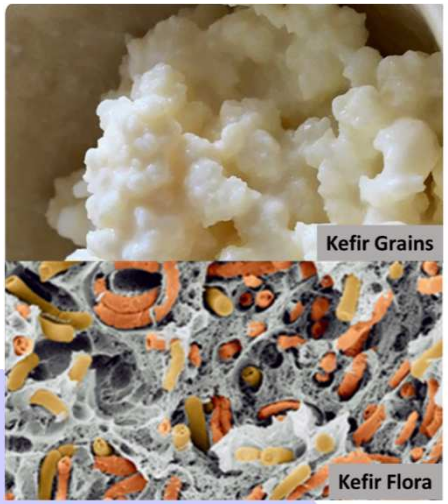
(C) Lb. kefiri HL2 cultured with S. turicensis HY2;

(D) Lb. kefiri HL2 cultured with P. fermentans HY3

(E) 4 LAB cultured with 3 yeasts.

SACCO system





FOCUS ON KEFIR GRAINS - APPLICATION

On a larger scale, kefir production involves a multistep process.

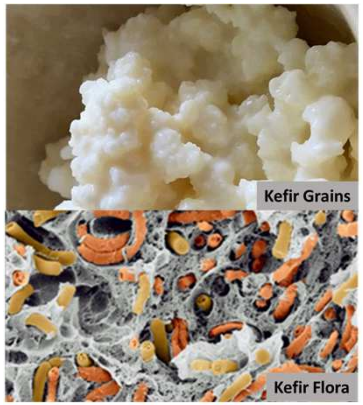
1 - The culture is prepared by incubating milk with kefir grains (2%–3%).

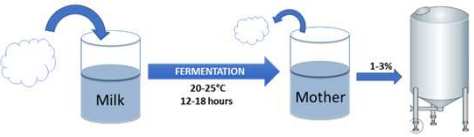
2 - The grains are then removed by filtration and the resulting liquid mother culture is

3 - added to milk (1%–3%), which is fermented for 12 to 18 h at 20°C –25°C to get kefir.

4 - Kefir grains removed by filtration in the first step are used for subsequent fermentations .

FOCUS ON KEFIR GRAINS - APPLICATION





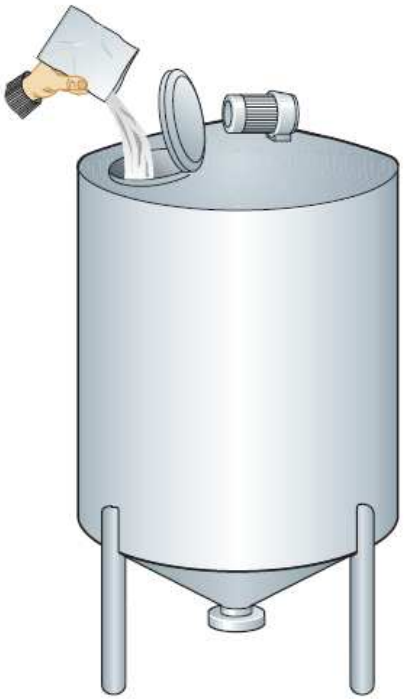
- **<u>The traditional method</u> of kefir preparation involves pouring milk in skin bags on a daily basis,**
- **followed by the addition of kefir grains (2%–10%), which leads to natural fermentation.**
- **The bags, or recipient, are regularly shaken to ensure the milk and kefir grains are well mixed.**

- **The finished product has high acidity and varying amounts of alcohol and carbon dioxide.**
- **Kefir can be produced from milk of various species including cow, ewe, goat, and buffalo,**
- **The use of <u>cow milk</u> is more common.**

GRAINS	MICRORGANISMES	KEFIR
5-25%	*Lactococcus* spp. *Leuconostoc* spp. *Streptococus* spp.	80%
10-15%	*Yeasts (Kluyveromyces* spp. mainly*)*	10-15%
65-80%	*Lactobacilli* spp.	5-10%

FOCUS ON KEFIR TYPE GRAINS - APPLICATION

THE USAGE OF STARTER

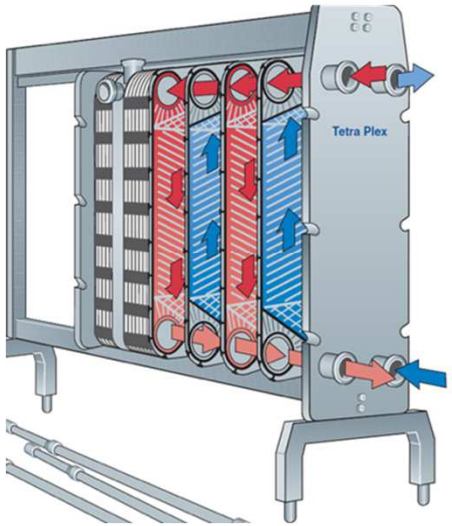


- EASY TO USE
- STEADY MICROBIAL COMPOSITION FROM BATCH TO BATCH
- STABLE STARTER ACTIVITY
- REDUCTION OF QUALITY COMPLAINTS
- POSSIBILITY TO CHOICE A CUSTOMIZED SOLUTION
- DEFINED DOSAGE

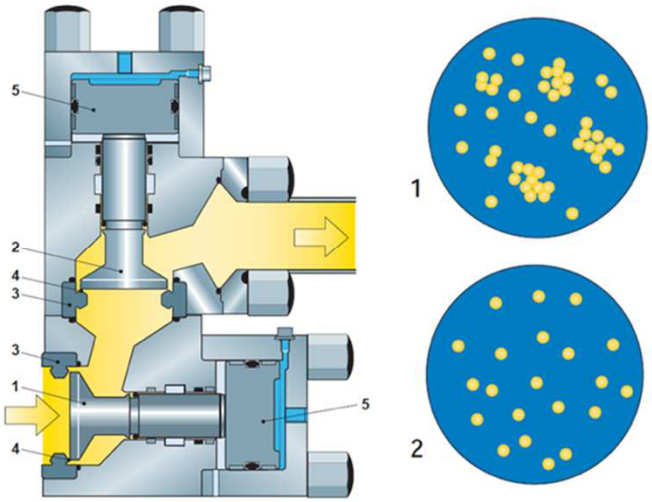
APPLICATION CONDITIONS

- MILK BASE PREPARATION AND STANDARDIZATION
- INOCULATION AT PRODUCTION TEMPERATURE : 28 – 32°C
- FERMENTATION , TILL pH 4,60 +/- 0.05; ACIDITY 80/120°T – 35/ 60°SH/100 ml
- COOLING AND PACKING.
- DISTRIBUTION

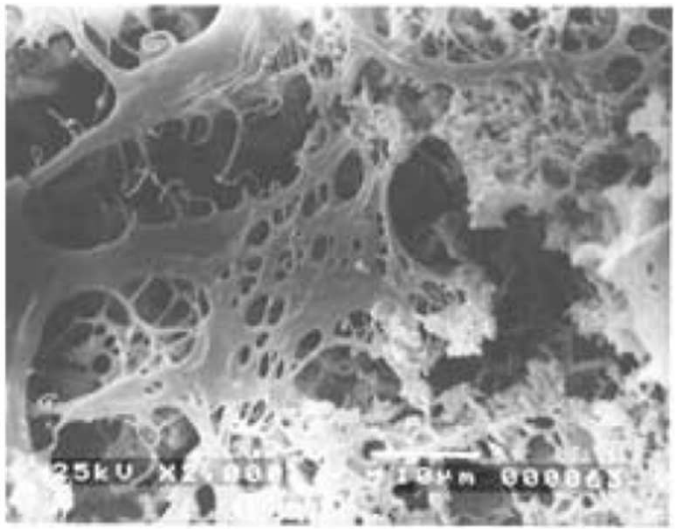
KEFIR and KEFIR DRINK - LACK OF BODY



HEAT TREATMENT
95°C for app. 5 minutes



HOMOGENIZATION
May vary according to Fat and PT content
i.e. full fat min.2 stages >200 Bar



CULTURES EPS PRODUCERS
Act as a natural stabilizer

KEFIR and KEFIR DRINK - LACK OF BODY and the role of HePS

KEFIR GRAINS:

CHECK PRODUCTION OF HePS AND GRAINS COMPOSITION.
CULTURES HePS PRODUCERS

Act as a natural stabilizer

In this case, the HePS producers, has to develop in the natural grains blend, and keep the caracteristics during the regular starter production.

KEFIR DRINK / KEFIR TYPE

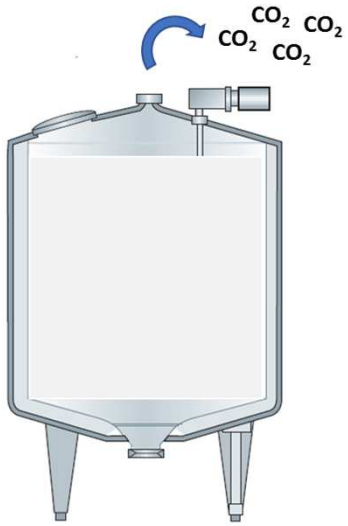
SUBSTITUTION WITH HePS PRODUCED BY LACTIC BACTERIA CULTURES HePS PRODUCERS

Act as a natural stabilizer.

Today are available in the market , culture prepared specifically for kefir drink application carrying bactera HeSP producers.

The application , is in conformity to the regular production parameters.

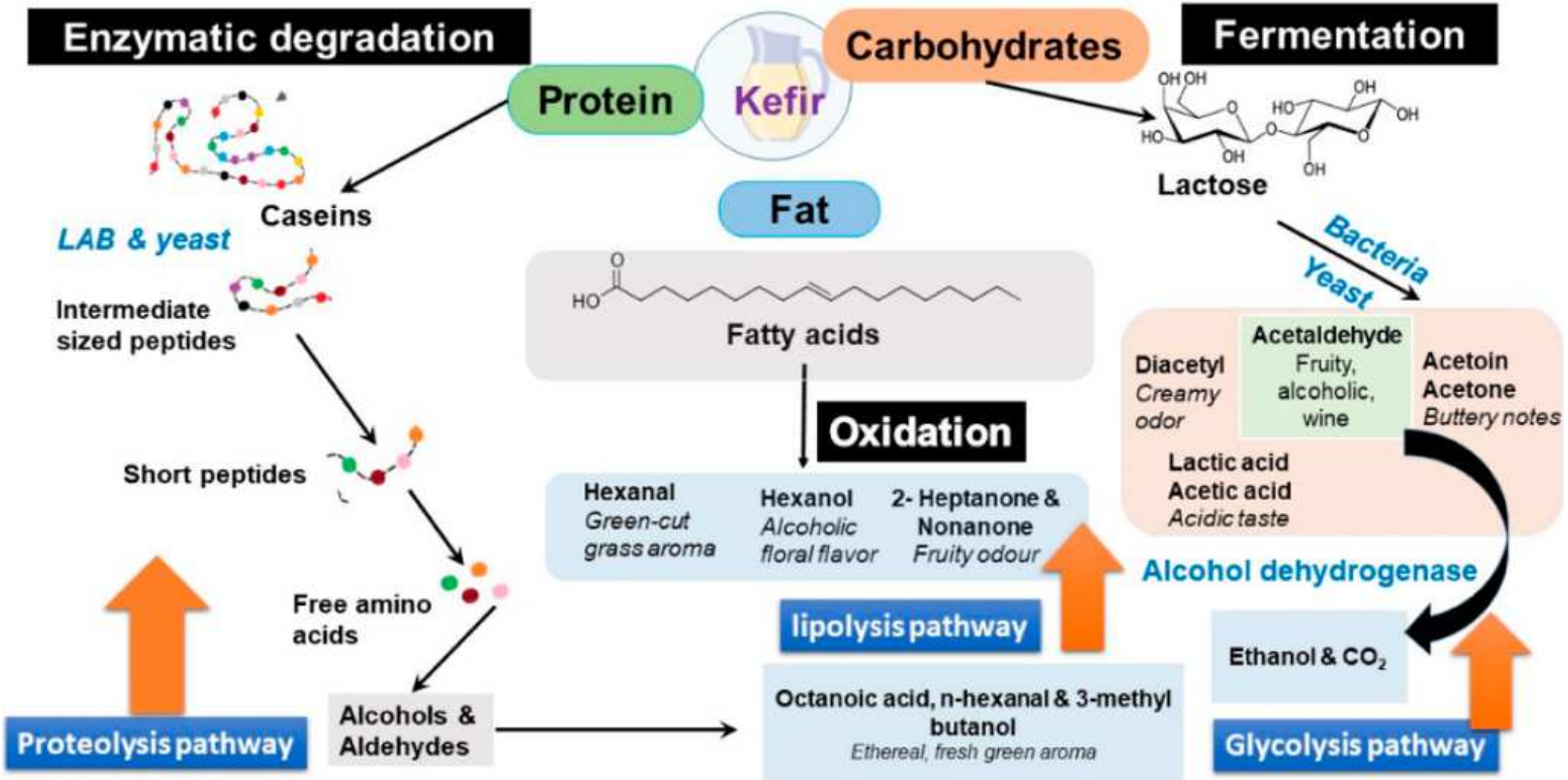
KEFIR and KEFIR DRINK BLOWING ISSUES



1. EXCESS OF YEAST
2.RESIDUAL CITRATE
3.SET PRODUCT WITH WRONG CULTURES

1.KEFIR EXPECTED
2.RIGHT YEAST
3.GOOD CULTURES FORMULATION

FLAVOURS


Enzymatic degradation
Protein
Kefir
Carbohydrates
Fermentation
Caseins
LAB & yeast
Intermediate sized peptides
Short peptides
Free amino acids
Alcohols & Aldehydes
Proteolysis pathway
Fat
Fatty acids
Oxidation
Hexanal
Green-cut grass aroma
Hexanol
Alcoholic floral flavor
2- Heptanone & Nonanone
Fruity odour
lipolysis pathway
Octanoic acid, n-hexanal & 3-methyl butanol
Ethereal, fresh green aroma
Lactose
Bacteria
Yeast
Diacetyl
Creamy odor
Acetaldehyde
Fruity, alcoholic, wine
Acetoin
Acetone
Buttery notes
Lactic acid
Acetic acid
Acidic taste
Alcohol dehydrogenase
Ethanol & CO2
Glycolysis pathway

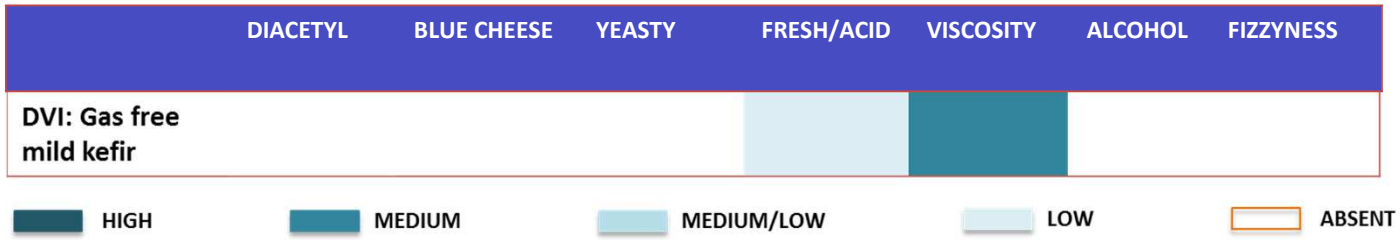
KEFIR DRINK
YOGHURT «KEFIR» SAB - Y - Y+

Faster fermentation through the usage of Yoghurt cultures

Fermentation T° 38-43°C
Time to target pH 6-8 hours

Pleasant and tasty!

A good base to add your favourite topping



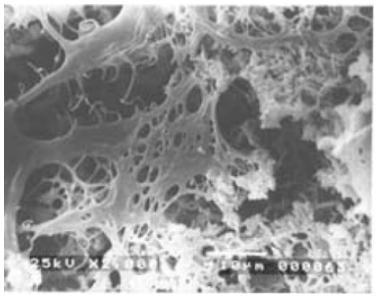
Sacco Lyofast Y as base

Sacco Lyofast ST HePS producers

Sacco Lyofast M - MO HePS producers

KEFIR DRINK
BODY ENHANCER - NO EXTRA GAS

- *Streptococcus thermophilus*
- *Lactococcus lactis* ssp. *cremoris HePS producer*
- *Lactococcus lactis* ssp. *lactis*
- *Lactococcus lactis* spp. *lactis* biovar. *diacetylactis*
- *Lactobacillus brevis*
- *Leuconostoc* spp.



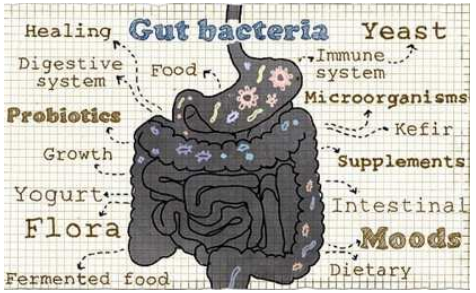
	DIACETYL	BLUE CHEESE	YEASTY	FRESHNESS	VISCOSITY	ALCOHOL	FIZZYNESS
DVI:Yeast free Kefir							

HIGH | MEDIUM | MEDIUM/LOW | LOW | ABSENT

KEFIR DRINK
BODY ENHANCER - WITH PROBIOTICS

- *Streptococcus thermophilus*
- *Lactococcus lactis* ssp. *lactis*
- *Lactococcus lactis* ssp. *Cremoris*
- *Lactococcus lactis* spp. *lactis* biovar. *diacetylactis Leuconostoc mesenteroides*

- *Lactobacillus delbrueckii* ssp. *lactis*
- *Lactobacillus brevis*
- *Bifidobacterium lactis BLC 1*
- *Lactobacillus acidophilus LA 3*
- *Lactobacillus casei BGP 93*
- *Lactobacillus rhamnosus SP1*
- *Lactobacillus plantarum LPLDL*
- *Kluiveromyces marxianus B0399*



	DIACETYL	BLUE CHEESE	YEASTY	FRESH/ACID	VISCOSITY	ALCOHOL	FIZZYNESS
DVI: Yeast free with probiotic							

HIGH | MEDIUM | MEDIUM/LOW | LOW | ABSENT

KEFIR DRINK
TRADITIONAL AND DRINKABLE

Streptococcus thermophilus,
Lactococcus lactis ssp. *cremoris*
Lactococcus lactis ssp. Lactis
Lactococcus lactis spp. *lactis*
biovar.*diacetylactis*
Lactobacillus brevis
Leuconostoc spp
Kluiveromyces marxianus
Saccharomyces bayanus

Streptococcus thermophilus
Lactococcus lactis ssp. *cremoris*
Lactococcus lactis ssp. *lactis*
Lactococcus lactis spp. *lactis biovar. diacetylactis*
Lactobacillus brevis
Leuconostoc spp
Saccharomyces bayanus

EXTRA CULTURES

Kefir could be used as a functional food,

1 - As it suppresses increase of **blood pressure.**

2 - Reduces serum **cholesterol levels.**

3 - And relieves **constipation.**

For instance, *La. kefiranofaciens*, an HePS-producing strain isolated from kefir, displays interesting probiotic properties.

4 - Moreover, the water-soluble HePS from kefir grains **retards tumor** growth *in vivo* when administrated orally.

5 - Furthermore, kefir biofilms and their **polysaccharide compounds** may be good antimicrobial.

6 - Anti-inflammatory, and cicatrizing agents for use in a variety of infections.

EXTRA CULTURES

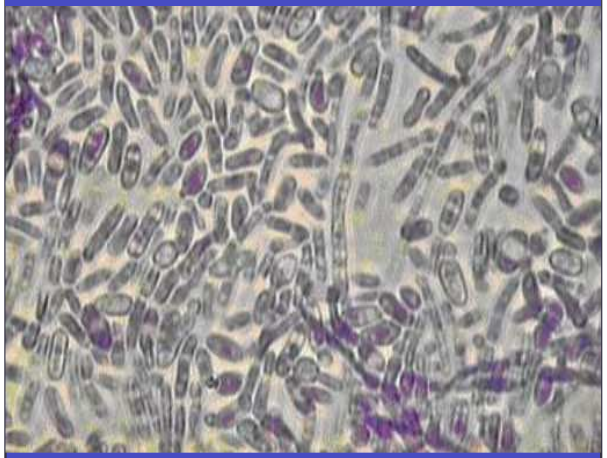
Extra YEAST

KL8: ***Kluyveromyces lactis***

KM7: ***Kluyveromyces marxianus***

DH1: ***Debaryomyces hansenii***

Lyofast MT F 036 LV



PROBIOTIC LACTIC YEAST B0399 ®
Kluyveromyces marxianus ssp fragilis
From Caucasian Kefir Grains
Probiotic

Probiotics

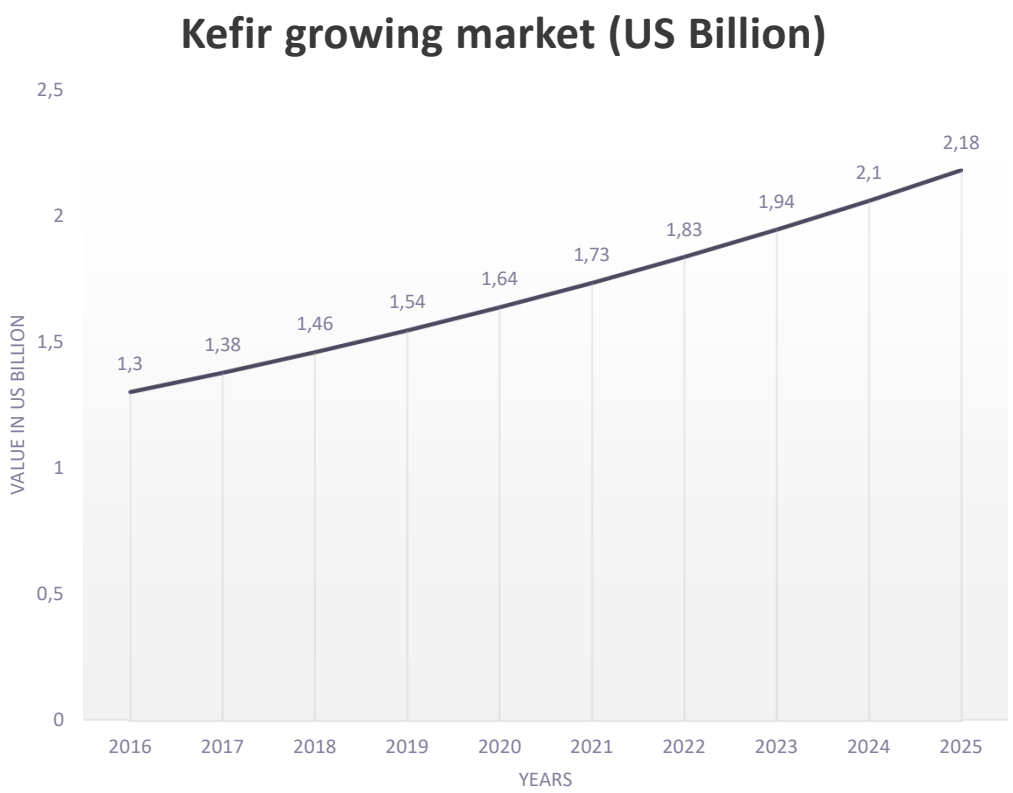
Lyofast SAB series
Streptococcus thermophilus EPS producer
Lactobacillus acidophilus
Bifidumbacterium animalis ssp lactis

Lyofast LP LDL
Lactobacillus plantarum

Lyofast YAB series
Streptococcus thermophilus EPS producer
Lactobacillus bulgaricus
Lactobacillus acidophilus
Bifidumbacterium animalis ssp lactis

Kefir Market 1/3

- Kefir is having a relevant success thanks to the health benefits ensured.
- Kefir consumption is a recent phenomenon derived by the willingness to follow a healthy diet. This because it has essential and good nutrients, useful also for who suffers for lactose intolerance.
- According to Transparency Market Research, the global kefir market is expanding at a **CAGR of 5.9%** in a period from 2017 to 2025



In 2018, the global kefir market was valued USD 1.47 Billion and the forecast for 2025 is that the market will reach the value of USD 2.2 Billion

Kefir Market 2/3

- **Europe** (powered by U.K and Germany) represents the most important market holding more than 50%, in 2016, of the overall kefir market.

- **North America** rests the second most important kefir market. Application of flavoured kefir are increasing in pharmaceutical formulations. In addition, low fat kefir and dietary supplements consume is increasing among obese individuals for its property to enhance metabolic activity.

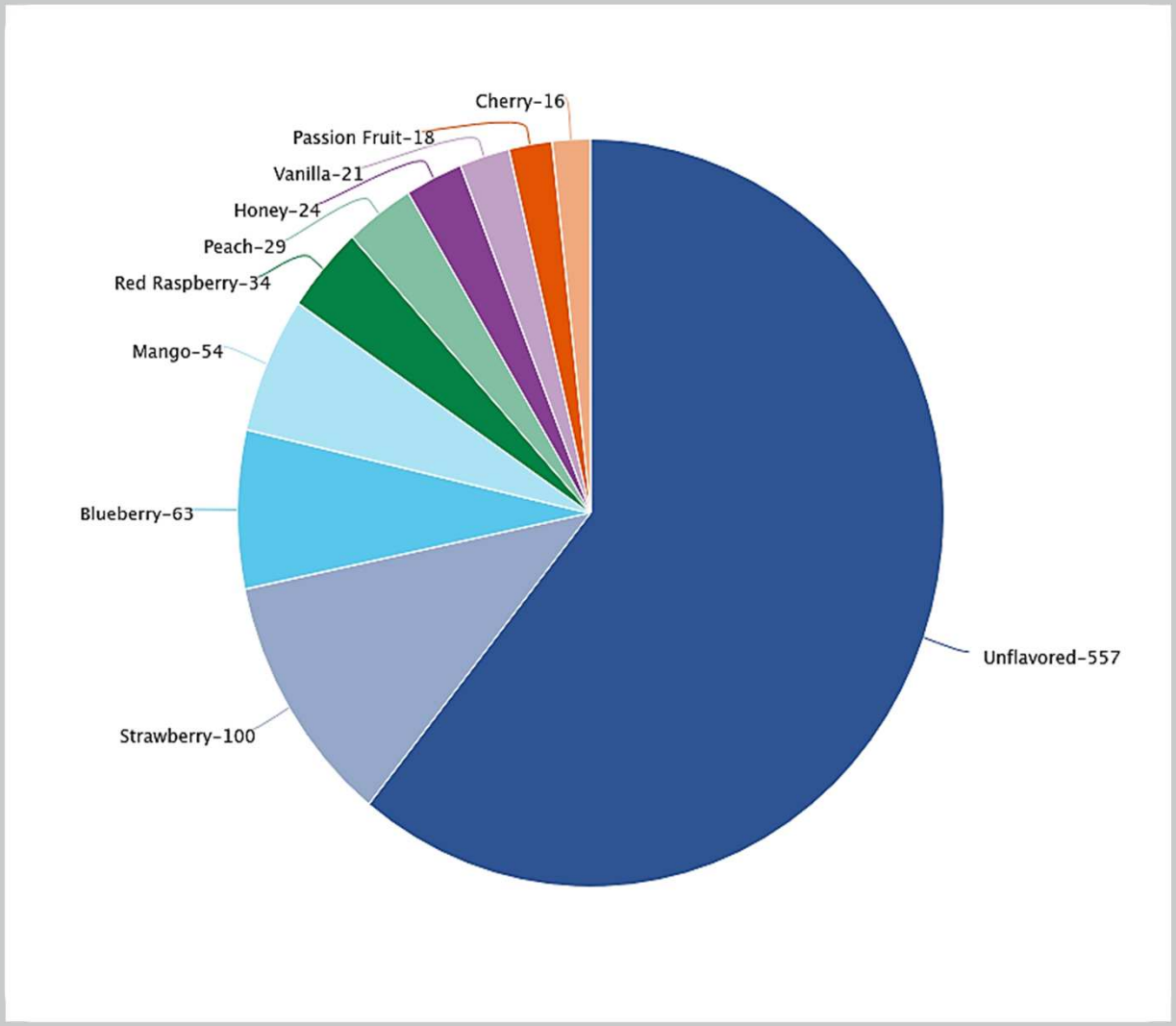
- **Asia Pacific, Latin America, and the Middle East and Africa,** rising health consciousness increased purchases of healthy products, including kefir. **The Asia Pacific kefir market is booming due to increasing spending on health drinks and dietary supplements in India, Japan, and China.**

- **Asia Pacific is projected to be the fastest growing kefir market with CAGR of 9.04% from 2016 to 2025.**

Kefir Market 3/3

- **Kefir best flavors and product**

- The unflavored kefir is the rule despite many other flavored products are launched and going to be launched.
- The chart is a representation of new kefir products launches in the last five years.
- The result is that **unflavored kefir leads the market.**



our network

We are a system of indipendent companies with their autonomy and self identity, grouped in a unique network

Family Company

SACCO system

our market areas

food

probiotics

agrovet

labware

THANKS FOR YOUR ATTENTION

Paolo Cernuschi

p.cernuschi@saccosrl.it

Regional Director - Sacco System

Via A. Manzoni 29/A, 22071

Cadorago, CO Italy

Direct No +39 031 8866 765

Mobile +39 348 60 10 792

www.saccosystem.com