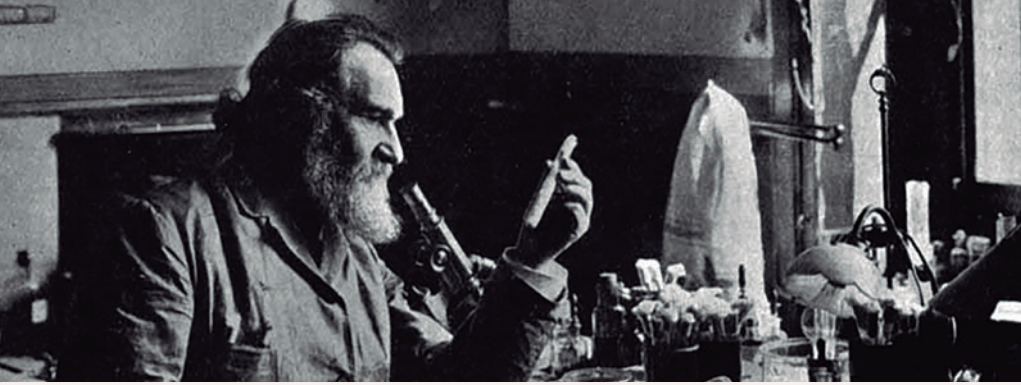
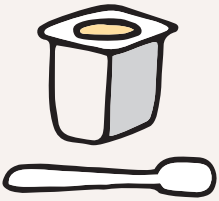


YOGURT

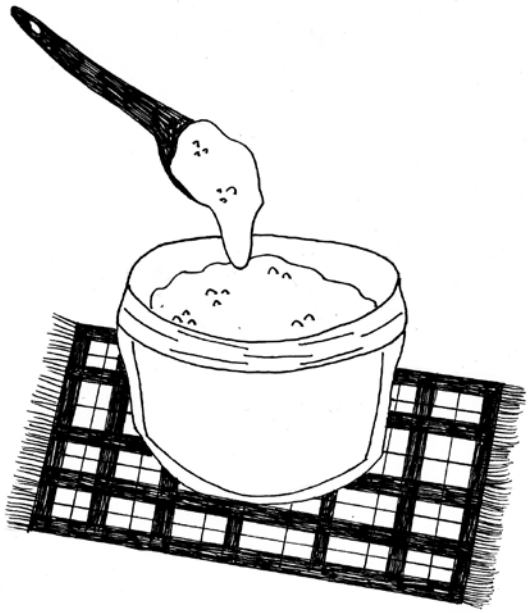
ancient food in the 21st century

Ricardo Weill

This book is the result of extensive research carried out in collaboration with more than ten specialists in general health, nutrition, paediatrics, biochemistry and microbiology, among other disciplines. All specialists consulted offered state-of-the-art scientific and academic information, which was then combined and woven into this single text describing yogurt from its very origin to industrial manufacture.



YOGURT ANCIENT FOOD IN THE 21ST CENTURY



Weill, Ricardo
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FOREWORD

The first draft for this book was drawn up in Berlin in 2015, during the 12th European Nutrition Conference (Federation of European Nutrition Societies), over dinner with my dear friend Esteban Carmuega. On that occasion, Esteban and myself first began carelessly doodling to later give shape to much of the information and life stories woven from the development of yogurt in the Southern Cone and the emerging and increasingly compelling evidence of its contribution to health. This is how, on my return to Buenos Aires, I asked Nora Bär to help me interview scientists in different fields who were considered authorities on the subject.

There comes a moment in life, almost imperceptible, when you begin to look back into the past with a different perspective. Precisely in the light of these reflections the idea was born of collecting some of the stories about yogurt of which, it may be argued, I have been a privileged witness. As a matter of fact, I have not only been professionally linked to milk fermentation in the industrial processing of the most ancient food in our current staple diet, but I have also had multiple opportunities to travel round the world in search of new and better ferments and their uses in cooking, and to learn about the value they acquire in different cultures. I have investigated the effects of ferments in animal experimental

models and in large-scale studies, and I have attended –mostly recently– numerous scientific conferences in which new hard data invariably confirm their epidemiological value in the prevention of disease and the complex mechanisms through which gut microbiota modulation and balance contribute to the prevention of chronic disorders.

These wanderings have rendered many of the comments, opinions, and information shared here, which should not be thought of as a book of a purely scientific nature, but rather as a story, an enjoyable one I hope, whose plot unfolds along the development of yogurt and fermented milks and their benefits, reported through the eyes of researchers, communicators and specialists I have had the opportunity to meet over these years.

I should thank several people, starting with Prof. Roberto Halbinger, who introduced me to the world of good bacteria and became my first mentor. I would also like to thank Pascual Mastellone, who taught me to respect and honour consumers, and Danone, where I have been able to incorporate many of the scientific lessons learnt from cutting-edge research in this field –both locally and internationally– on an industrial scale, paying tribute to state-of-the-art knowledge. I would like to say a word of thanks to Gabriela Perdigón, who introduced me to the world of immunology and probiotics, Nora Bär, who other than being a friend has given me support in carrying out many of the interviews which make up this book, Alejandro Ferrari for his editorial work, Narda Lepes for her many practical suggestions –some of them truly innovative– and a great deal of common sense about the uses of yogurt in staple food, and Florence Abd, whose illustrations make this story much more reader-friendly.

For these reasons, I believe that my role in this book has not been that of an author, but rather of a compiler of stories which randomly come to life at times, leaping from history to basic science, from experimental research to food technology, from epidemiology to nutrition.

Needless to say, it has been a pleasure to compile these stories featuring yogurt as a lead actor, a food which has been there from the beginning of civilisation and of which current science shows benefits –in different cultures over the centuries, many of them not even connected to one another– with real health-associated value.

Ricardo Weill

INTRODUCTION

All it takes is a short walk down any supermarket aisle in the world to find countless yogurt pots of all imaginable types lining up on dairy products shelves. Options include mixed-in cereal or fruit, multiple flavours, regular, low-fat, fat-free, sugar-free, organic, Greek, extra calcium or vitamin D, or even those especially designed to help lower cholesterol levels.

To those not particularly interested in the details of nutrition, it will prove undoubtedly difficult to recognise, in these containers of different sizes and colours, the reformulated version of the food which as far back as five thousand years ago allowed the first peoples who domesticated mammals to survive.

It was back then that fermentation became somewhat popular as a way of preserving milk for extended periods, apparently discovered by chance while the white liquid obtained from cows, goats and sheep was transported in stomachs containing digestive juices and microorganisms which made it acidic.

It was a bit by chance and a little out of necessity in such process that yogurt was quickly adopted in different cultures for its benefits. Throughout history, yogurt has been regarded effective in preventing diarrhoea, as a source of protein for Roman soldiers, and valuable for its alleged cosmetic properties to treat seborrheic lesions.

But it was only at the beginning of the twentieth century that yogurt became the object of scientific studies seeking to verify its presumed health benefits. In 1905, Stamen Grigorov, a Bulgarian medical student, discovered lactobacilli. In the same decade, Russian microbiologist Élie Metchnikoff, who worked at the Pasteur Institute and was awarded the Nobel Prize for his discoveries in phagocytosis and innate immunity, established a link between yogurt intake and the longevity of the Caucasus centenarians, and put forward the hypothesis that the very microorganisms which transformed milk could also balance intestinal flora, thus playing a key role in stretching lifespan.

Although these notions lacked experimental support for decades, interest in the ideas of Metchnikoff was sparked again in the light of studies on the active community of bacteria covering our digestive tract, which finally rendered human microbiota as a newly discovered organ.

In the words of Francisco Guarner from University Hospital Vall d'Hebron, Barcelona, in his introduction to the *Handbook on Gut Microbes* by the World Gastroenterology Organisation, "Our knowledge of the microbial communities that inhabit the human gut has grown exponentially over the last few years and there is a profusion of novel information flowing from basic science laboratories into the clinical scenarios."

Bacteria are prokaryotic cells, that is, unicellular organisms lacking a nucleus, mitochondria or other membrane organelles, which have their DNA in the cytoplasm. As Guarner and his colleague Claudia Herrera from the same hospital explain in this handbook, human beings are associated with a large and diverse population of microorganisms that live on body surfaces and in cavities connected with the external environment. They coexist with the rest of the body in complex but surprisingly beneficial symbiosis.

Even if numbers vary among researchers, Guarner and Herrera estimate that our intestinal tract harbours around two billion of these microbial cells and more than a thousand species. It is well

established now that these cells affect our physiology and pathology in multiple ways, although these ways and the molecular pathways underlying them still need to be specifically characterised.

In this scenario, yogurt gained prominence as a food offering the possibility of balancing or modifying the action of this bustling population weighing around 2 kilos. Medical doctors often suggest incorporating yogurt, more than any other food, in their patients' diets to prevent diseases such as hypercholesterolemia and osteoporosis.

But what is actually true about the promises of this food which has already become a classic? Regardless of nuances, the scientists who answer this question agree on the benefits of this millennial food and make recommendations as to how to improve it, for example, by reducing sugar content. Their experience allows us to peek into the story that brings us here, find out what we certainly know and get ready for what, no doubt, is yet to come.

1

FOOD FERMENTATION: CHANCE AND OPPORTUNITY

We may well wish humanity had developed from a more poetic plot. However, the beginning of humanity was not dominated by magnificent ideals or grand epics, but by simple stories and pressing needs. The first and foremost, the search for food. Long before supermarkets sprang up in almost every block of big cities and had their shelves crammed with all sorts of ready-to-eat products, finding what to eat was a staggeringly time-consuming task. These were times when small groups of human beings might travel as much as dozens of kilometres every day to collect fruit or plants and, from time to time, feed on protein obtained from animals they caught in their traps or hunted with precarious weapons. Our ancient ancestors had no cattle-raising skills, nor were they lucky enough to raise domestic animals. To make matters worse, they still lacked the knowledge required for crop farming.

But then something happened which was totally unexpected. After glaciation, sea levels rose to cover large land areas and modified the habitat of the first humans. TK Derry and TI Williams provide a good explanation in A Short History of Technology (1960): "Neolithic man is a man emerging from savagery, having solved the basic problem of his food-supply by an increasing, though incomplete, reliance on stock-keeping or tillage or a combination of the two.

He could grind and polish, and even drill, his implements of hard stone; ... The immediate result seems to have been a rapid growth of population without earlier parallel "the so-called Neolithic revolution".

Between 10,000 and 4,000 BC, those pioneers moved from a predatory to a productive economy, from crafting weapons to making agricultural tools, producing plants, raising animals and shaping pottery to store crops.

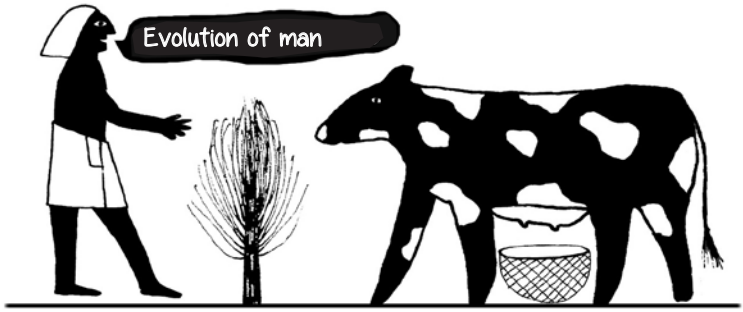
It was during this long transition period that one of the fundamental chapters of the human adventure began to be written. Derry and Williams claim stock-keeping and herding to have come first, as they could be practiced by nomads. But with seas overflowing, ancient peoples were forced to move to the highlands, where they found a natural environment offering crops such as wheat and barley – probably also oats and rye. These first farmers of the Near East then learnt to grow extremely useful crops.

Following Derry and Williams, "Agriculture implied ... at least a temporary settlement. And the very fact of settlement made men conscious of new wants, which the new ease of food production made it possible to satisfy. Time and skill were no longer monopolised by the needs of the stomach, for the agriculturist, unlike the hunter, has seasons of comparative leisure and makes a permanent home."

Moreover, these authors regard the switch from food collection to food production as a consequence of a major technological breakthrough: "Every other use which we have learnt to make of the material universe depends upon our ability to produce food for a given population by means which do not of themselves entirely exhaust the energy and time of that population. Man as a hunter had no such surplus; it was man the keeper of flocks and herd and cultivator of the soil who first accumulated the surplus that has been the basis of all civilisation."

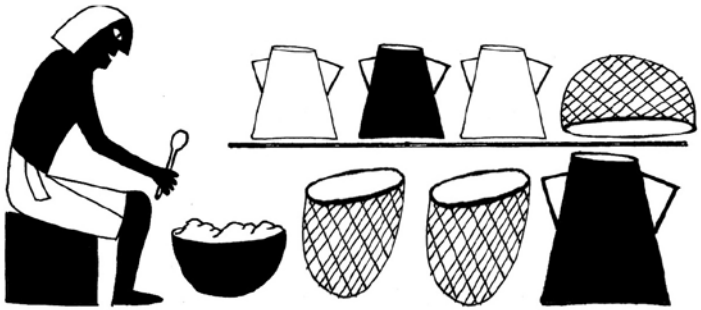
Regarding the domestication of animals, archaeological studies on cave remains have shed light on a theoretically plausible order in which the process may have taken place. Scavengers appear to

CROP FARMING AND CATTLE-RAISING



STORAGE

FERMENTATION



have come first, such as the jackal and the dog. Seasonally migrating animals like the reindeer, the goat and the sheep probably came next, when man was still a nomad himself. Cattle domestication, however, appears to have come last, as it calls for a sedentary life.

In Histoire Naturelle et Morale de la Nourriture (1987), Maguelonne Toussaint-Samat points out that animal domestication and herding were highly developed in what is today the European continent and other areas where sheepherders had gained ground: the Aryans in the Punjab and in the even more humid steppes of the Oxus and Jaxartes rivers of Central Asia, and the Semites on the banks of the Euphrates and the Tigris, whose herds of horses and camels, and their flocks of donkeys, oxen, lambs, goats and pigs expanded and offered abundant food.

Toussaint-Samat agrees that the hunter-gatherers of the Upper Palaeolithic, the men of Cro-Magnon, started raising cattle long before the crop farming technology was developed. This practice began thousands of years before our era, at least in the area which later became the Negev Desert, in the far ends of North Sinai, where archaeological research discovered fences used to enclose gazelles or fallow deer dating back to 20,000 to 10,000 BC.

The origin of agriculture appears to be blurrier. The hunter-gatherers may have either been faced with an unprofitable and overdemanding task, or they may simply have noticed new plants with more food growing from the seeds scattered on the ground.

But be it vegetable or animal food, as soon as production began those men and women were forced to tackle the issue of preservation. Those inquisitive enough must have observed that, for reasons they could not explain, food lifespan could be stretched through processes such as **fermentation by yeast** (a type of unicellular fungi which divide asexually and produce enzymes capable of breaking down sugars or carbohydrates).

Although not completely certain, evidence suggests that yeasts were used as far back as 2,300 BC, and that it may have been the Egyptians who discovered their activity in fermentation by chance.

Two of the first products obtained through this technique were **bread and wine**. Bread in particular is a staple food in much of the Western world and its general consumption spreads worldwide, except in the rice countries. It is as old as civilisation and one of the simplest human manufactures. All it takes is mixing flour with water, salt and yeast. Once this dough is fermented for several hours at around 30°C, it is cut, kneaded and left to rise in the same temperature and humidity conditions for about twenty hours. At this stage yeast breaks sugar, which makes up bubbles of carbon dioxide and gives the dough a soft and fluffy consistency.

Not surprisingly, in those early times yeasts were readily available to humans, as they are frequently found on leaves and flowers and are mainly disseminated by insects. They can also get into fruit by mechanical damage or be found in the soil and in the water of lakes and rivers.

In any case, research indicates that both bread and wine date back thousands of years. In *Un festin en paroles* (1995), French writer Jean-François Revel argues that the first wild cereals must have probably grown on the fertile banks of the Nile. In support of this claim, Revel comments on archaeological findings of tiny mortars used 75,000 years ago, although the first loaf of bread to be recovered was a burnt-out cake, which was cooked 4,000 years BC by someone living in a lake area of what is now Switzerland, and which reached the Landesmuseum in Zurich. Following Revel's argument, the Metropolitan Museum of New York has exhibited another piece, this time round and thin, which had been buried 3,400 years ago with Egyptian princess Meryet-Amun. When referring to Egyptians, Herodotus derogatorily observed that 'they knead dough with their feet and clay with their hands'. Egyptians were the first to discover the secret of fermentation before cooking; they let yeast mix into a semi-solid mixture, producing a spongy mass which served, in turn, as a type of sourdough. This discovery and the skill required to keep this sourdough alive turned bread making into a trade.

In Roman times, yeasts were obtained from the surface of bowls of fermented wine and were later found to be useful in bread making. This process is now known to be carried out by *Saccharomyces cerevisiae*, which is also used to brew beer.

In Revel's views, the phenomenon of fermentation was discovered in the Near East, particularly among Hebrews. The microorganisms responsible for it were either so-called wild yeasts, which are found in pasta bugs, or beer yeast, used in industrial bakery.

Among all the imaginable ways in which the fermentation of wheat flour may have developed, specialists suggest that, in ancient times, a mixture of flour and water may have been accidentally left uncooked and sown by ferments in the air, hence rising due to heat and humidity. This serendipitous event is thought to have inspired the incorporation of alcoholic ferments into dough to obtain yeasted bread, more easily digested than cakes.

Knowledge of bread is already mentioned in the Bible, not only among Hebrews but also among Egyptians. According to Revel, several Egyptian paintings also give testimony of the practice of wheat sowing and harvesting, of wheat grain grinding between two stones, of dough kneading and cooking in pots similar to the current devils, that is two pots of rough clay, one on top of the other, in which the dough could be cooked right on the fire without water. In short, a miniature oven. Referred to by Hecataeus of Miletus in the fifth century BC as 'the bread-eating people', much like Englishmen and Germans spoke of the French of the nineteenth century, Egyptians gave their main food all kinds of shapes, which gave birth to a somewhat artistic kind of bakery.

Though hard to believe, Revel argues that the Greeks of the classical era had up to seventy-two kinds of bread, and that their bakery had gained such good reputation that the master bakers of Rome, even in the times of the Empire and the Early Empire, were practically all Greek. Moreover, baker Teanos features in the writings of poets and philosophers like Aristophanes, Antiphanes and Plato.

Another ancient food in its own right, wine in mentioned in several passages of the Bible which prove its early consumption in Babylon and is also portrayed in Egyptian sculptures of vintage and winemaking scenes. Revel further points out that man has long used all kinds of fruits, berries and fermentable juices in the production of alcoholic beverages. Apples, raspberries, blackberries, pears, strawberries and wild grapes have all been stomped and left to ferment for as long as it took. Absolutely nothing connected with the transformation of sugar into alcohol escaped the footprint of *Homo sapiens*, starting with honey itself, the basis of mead, the oldest of Greek drinks, which is nothing but honey fermented in ten or twelve parts of water.

Both bread and wine have been part of the Western world right from the start. The Greek breakfast consisted of bread dipped in wine, the only time of the day when the Greeks drank pure wine, says Revel, as water would me mixed into wine during and after the rest of the meals. In fact, breakfast was so closely associated to the idea of pure wine that it was even named after it: *akratisma*, which comes from akratos, that is 'unmixed, pure wine'. Akratos was something like a dry drink. From the same origin, *acratidzomai* means both 'drinking pure wine' and 'having breakfast'.

Another fermented food which has been among humans for thousands of years, **cheese** is made from acidified milk of different mammals, such as cattle, goats, sheep, buffalo or camels, through the action of bacteria which transform sugar into lactic acid and gives it a particular texture and flavour. The beginning of cheese making is elusive, although it may be estimated between 8,000 and 3,000 BC, probably at the beginning of sheep herding. The legend goes that it was discovered by an Arab merchant who, while making a long trip through the desert, kept milk in a container made from the stomach of a lamb. And, when he was finally ready to drink it, the milk had curdled and fermented due to the rennet in the stomach of the lamb and the high temperature of the desert. The earliest archaeological

evidence of cheese making was found in murals of Egyptian tombs, roughly dating back to 2300 BC.

Further stories refer to excavations of stilt houses on the shores of Lake Neuchâtel, in current Switzerland, which revealed remains of earthenware bowls with holes in them from about 6,000 years before our era. Archaeologists believe these may have been used as strainers for curdled milk, although it is uncertain whether the populations in this area, already skilled in goat and sheep domestication, had begun breeding and milking practices.

It is well-known that, eighteen centuries ago, the legal code of King Hammurabi II levied taxes on dairy products from the Babylonian market, where the milk of goats and sheep was traded. Cow milk, in contrast, was a luxury good, as it was believed it could only be obtained during cow labour.

As pointed out above, cheese is also thought to have developed from milk stored in the bladder or stomach of sacrificed animals which were used as airtight bags. Revel deems it probable that milk may have curdled quickly, due to heat or neglect, or by the effect of natural enzymes in the stomachs of ruminants. The Jewish people may have obtained their primitive curds in this way, until Moses' laws forbade the mixture of milk and any product of young animals.

Cheese has always been valued as an excellent food for several reasons. It is easier to transport and preserve than milk and it is also high in fat, protein, calcium and phosphorus. The ancient Greeks had hundreds of cheese types and regarded it as a gift from the gods. The discovery of cheese was attributed to a minor god, Aristaeus, the son of Apollo and Cyrene and the patron god of bees. Worth mentioning, *Homer's Odyssey* describes a Cyclops making and storing sheep and goat cheeses.

Skills in cheese making were introduced in Europe from the Middle East. Colder climates meant it could be manufactured with less salt and allowed the use of bacteria and mould, which would give cheese different flavours. Cheese was a staple food in Ancient Rome, consumed daily and manufactured much in the same way as today. From then on, its production and distribution throughout the world have grown with no limit.

France and Italy are now the countries with the widest cheese range, with approximately four hundred types each. An article on *Newsweek* magazine in 1962 quotes an occasional remark by French President Charles de Gaulle, "How can you govern a country which has two hundred and forty-six varieties of cheese?". According to the Food and Agriculture Organization of the United Nations (FAO), worldwide cheese production was about 20 million tons per year by 2010.

But the food playing the starring role in this story also comes to us from ancient times. Even when household meals today no longer feature recipes passed down from generation to generation but are rather riddled with all kinds of mass-manufactured pre-

packaged foods, yogurt is still going strong. The reason for such persistence is still to be unveiled, which will be the task at hand throughout these pages.

2

YOGURT IN THE HISTORY OF MAN

The search for the origin of fermented milk products takes us to human prehistory, and what we know has come to us through countless legends and traditions. The study of these traditions leads us to think that the first types of fermented milk were deer, a species already domesticated by the time of the agropastoral revolution and, above all, of the first development of applicable biotechnologies. Milk fermentation seems to have been born in the Middle East, although its production can be speculated to have spread to Europe and Asia, and later to Africa through the practice dairy animal raising, preferably goats and cows, but also horses and camelids. The analysis of language terms used to refer to milk and its ferments –that is, a paleolinguistic approach– allows us to trace a possible path from their origin to their arrival in Europe and America.

But how did man get to consume animal milk? From a historical and nutritional point of view, according to Mauro Fisberg –paediatrician and nutritionist, Professor at the Universidade Federal de São Paulo and coordinator of the nutrition department at Hospital Infantil Sabará, São Paulo, Brazil– , prehistoric babies were breastfed for long periods of time, usually until the mother's next pregnancy. That is, upon a new pregnancy, breast milk composition

changed, and the baby refused to continue breastfeeding. To Fisberg, one of the leading experts in the introduction dietary fermented milk in Latin America, the explanation is simple: hormonal alterations associated to a new pregnancy modify the flavour of breast milk, which is why infants often perceive a new pregnancy in progress before mothers themselves.

However, this is not the only reason why breastfeeding was interrupted in ancient times. Another probable cause is the very high rate of maternal mortality, both upon and immediately after child birth, which deprived children from breastfeeding right from the start. In these circumstances, communities resorted to the milk of other mothers or, as this was not always possible, alternative food sources. In this context, Fisberg postulates that the use of milk from other animals, especially the goat, the sheep, the mare and the buffalo, may have come up as an option. Over time, this animal milk may have been consumed not only by children in the circumstances described, but also by adults, as it was high in protein and more readily available than meat, which was often scarce.

The introduction of animal milk as a source of nutrients was associated with animal domestication and became a common practice when the conditions were established for small communities to remain in a fixed place. This condition allowed the development of agriculture and animal raising, first non-domestic and then domestic ones. The first types of animals to be herded were rather small, but larger species were soon introduced which allowed greater milk production and the possibility of sharing it with other groups and settlements. In this way, milk became a reliable source of food, available not only to children but also throughout adult life.

The appearance of the first fermented milk products, even yogurt in its contemporary form, can also be traced a long way back in history. Their precise origin may prove hard to identify, as suggested by the multiple names given to fermented milk in oral tradition, some of them still used until not long ago. Such a wide

range of names can be considered proof that fermented milk may have appeared independently in many different places at about the same time. As a matter of fact, its discovery is thought to be completely incidental, occurring several times and in different places and conditions. As we will see below, records have been found which map the origins of fermented milk in the Fertile Crescent, particularly Mesopotamia, but also in the Asian steppes and the Caucasus, with continuous spiral growth to reach India, Scandinavia, the Mediterranean and Egypt.

Fisberg also speculates that the appearance of fermented milk must have been accidental, probably linked to the wide use of containers made up of camel stomach tissue, and places it in the Middle East, a region comprising the current territories of Egypt, Israel, Jordan, Lebanon, Syria, Turkey, Iraq, Iran, Kuwait, Saudi Arabia, Oman, Yemen, Qatar, Kuwait and the United Arab Emirates. Fisberg further explains that the first fermented milk products were most probably obtained from the transport of milk in the camel stomach, for example, in which milk comes into contact with the animal's gastric juices. Although fermentation may have well resulted from milk left in storage, especially in winter, the strongest claim appears to be that of animal stomach transport, which provided evidence of longer-lasting milk preservation. Alternative views place the origins of yogurt in the deserts of Turkey, a territory bordering the Middle East, from fresh milk stored in bags of goat skin saddled onto the camel's flanks, such that the animal's body temperature offered optimal conditions for the multiplication of acid bacteria, which turned milk into yogurt.

In the warm environment of the first huts in the Fertile Crescent, roughly comprising the current Egypt, Israel, Jordan, Lebanon, Syria, Iran and Iraq, milk is likely to have been stored in leather or wooden containers. In these conditions a lactic microbiota most probably developed and made milk curdle, which rendered self-preserved fermented milk in a matter of hours. Some reports indicate that this procedure was discovered about 10,000 years

ago, shortly after the domestication of the small ruminants in the region, and that it proved extremely useful, as keeping the milk in these containers and constantly renewing the curdle as the product was consumed helped preserve it for a whole season.

In turn, excavations by archaeologists V. Gordon Childe and L. Woolley show that the use and organized processing of milk were already in practice in the Sumerian era, between 5,000 and 4,000 years BC. Specialists claim that these Indo-German peoples, native to the highlands of what is now Iran, migrated to the bordering plains of Mesopotamia, between the Euphrates and Tigris, leaving behind beautiful low reliefs found in the remains of temples, faithful signs of a very advanced agriculture. The data obtained by these excavations show that, in addition to milk, these communities processed butter and, most probably, also cheese and fermented milk. Childe and Woolley have inferred that Sumerians and the successive Persians, Semites, Indians and other Asian peoples were able to produce dairy as early as 2,500 BC. The sculptures and old writings in which fermented milk products are frequently mentioned as staple food bear testimony to this. The same applies to the Egypt of the Pharaohs, where well-planned dairy production was already underway.

Truth be told, ancient descriptions of these milk derivates have come from other regions of the globe as well. As Fisberg explains, other than those from the Crescent, simultaneous reports from Africa dating back to 8,000 BC offer proof that both yogurt and other fermented foods were quickly incorporated in the everyday diet, not only as a use for milk, but also as protection or medicine against disease. Likewise, towards the beginning of the Hellenic civilisation and the later Asian and Judeo-Christian civilisations, fermented milk was already used as a product for children and protective factor in armies' diets. It was well known by the Turkish and Roman battle men, who ate it not to get sick.

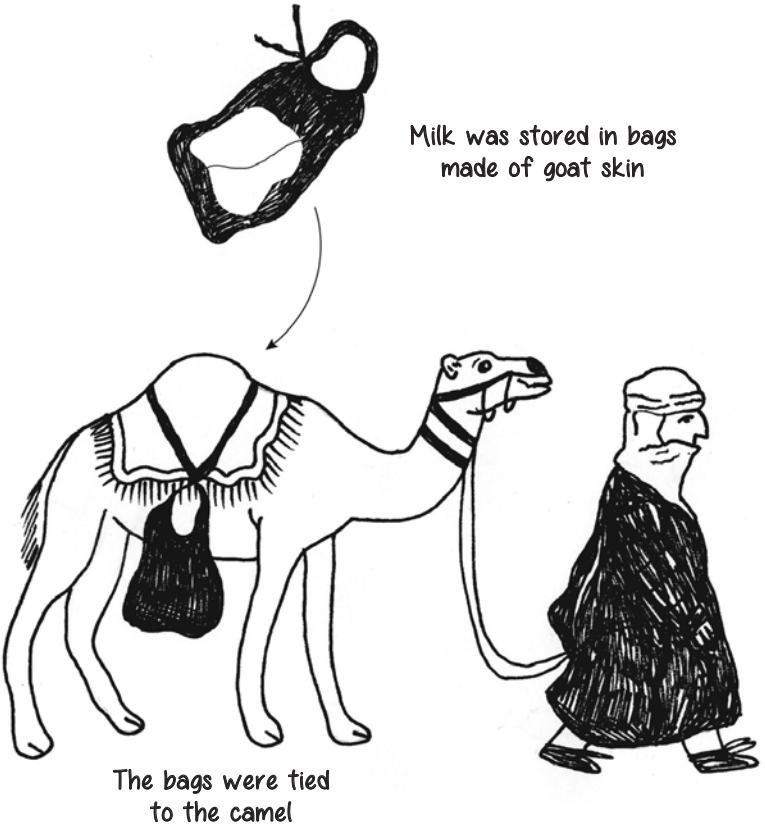
Far more to the east, another historical source of fermented milk features the warriors of thirteenth century Mongolia commanded

by Genghis Khan. According to Giovanni Ballarini, director of Istituto di Clinica Medica Veterinaria at Università degli Studi di Parma, an envoy of the conqueror stopped to replenish water supplies before crossing the steppes of Mongolia but, instead of water, his enemies filled his bottle with milk, in the hope than milk gone sour may eventually be harmful to him. However, the effect was quite the opposite, as the fermented milk gave this messenger more vigour and resistance, to such an extent that Genghis Khan may have imposed the drinking of fermented milk among the Mongols and spread the word of its properties all round Asia.

Biblical texts also contain evidence of the appearance of fermented milk. For example, the Book of Deuteronomy in the Old Testament states that this food appeared with Moses, who considered them vital elements given by God to His people, and the Book of Genesis mentions its mystical origin, as God had the secret of yogurt brought to Abraham by an angel. In turn, the book Judges (5:25) clearly states: "He asked for water, and she gave him milk; in a bowl fit for nobles she brought him curdled milk." In this sense, Dr Gabriel Vinderola –PhD in Chemistry and a researcher at Instituto de Lactología Industrial, Universidad Nacional del Litoral, Argentina– reinforces the idea that fermented milk references are found in several passages of the Old Testament. For instance, angels announce that Abraham's wife Sarah will have a son and, as a sign of hospitality, Abraham offers them curds and milk.

The knowledge acquired by these ancient peoples was later inherited by the Greeks and Romans between 1,550 BD and AD 475. The works of Aristotle, Xenophon, Herodotus, Pliny, Dioscorides and many other ancient authors refer to fermented milk product and their elaboration by the peoples of Asia, Africa and southern Europe, where these products were appreciated not only as nutritious and refreshing foods, but also as a medicine of divine origin. Greek historian Herodotus, for example, reports on a trip north of the Black Sea, about 450 BC, when he heard about women warriors called Amazons who rode the southern Russian steppes

THE DISCOVERY OF YOGURT



on horseback. Around 600 BC, these tribes of Amazons may have started raising sheep, horses and some camels. Archaeological evidence seems to signal their presence from the eastern coast of the lower Don to the end of the southern steppes of the Urals. History and legend concurrently suggest that they may have fed their families with fermented milk.

The polycentric origin of fermented milk can be traced through a paleolinguistic study on the basis of the names given to it in different territories. This approach foinstance supports the claim of two key places of birth for the current habit of preparing and consuming yogurt: one located in Turkey, where the name *yo urt* comes from, and the other one in the Balkans, Bulgaria or Central Asia, where the Bulgarian term *jaurt* was coined. For its part, the *yogurt* form is already found in transcriptions discovered in the first Arabic-Turkish dictionaries of Mahmoud al-Kashgari, published in 1701, in Tsing-Kiang.

But this analysis only reveals a much more complex picture. While the Romans prepared *oxygala*, a type of thick milk considered of great value and seasoned with herbs such as oregano, mint, onion and cilantro, as well as honey, flour and fruit, the Greeks in turn had the *schyston* and the Tartars made *jasma*, a product whose preparation used a kind of fermented milk previously dried called *kaschk*, which also served as a condiment. In Russia and Asian countries, *kumis* and *kefir* were the names given to fermented milk containing a small amount of alcohol, which are regarded as alcoholic milk drinks, as yeasts take part in the fermentation process, and are still consumed today. And the limits are pushed even further away, as *lac concretum* is indeed another example of the spread of curdled milk, this time dried in a round flat shape and mentioned both by Tacitus in his writings on barbarian peoples and by Marco Polo in the accounts of his travels to the East. This suggests the relevance that Tartars had in the dissemination of these products, much like the Arabs are supposed to have had later on in their campaigns.

The persistence of fermented milk throughout history is a remarkably curious fact which responds to several reasons. For example, yogurt is believed to have become the staple food of nomadic peoples for its ease of transport and preservation, whose virtues were already known in ancient times. The spread of yogurt in Asia, Africa, southern and central Europe and the Scandinavian countries was again extraordinary, as can be inferred from its wide variety, each type with somewhat different organoleptic and bacteriological characteristics and all of them still consumed today.

Even without clues to help us travel back in time, the theory goes that –as happened with cheese– the ancient nomadic peoples kept the milk obtained from their herds of cattle, sheep, goats, mares and camels in bags made of animal stomach skin, where the temperature and microorganisms did what those shepherds could not have foreseen.

Heat and milk contact with microorganisms then fostered the multiplication of acid bacteria, which turned milk into a semi-solid curdled mass through fermentation. Once this dairy ferment was consumed, bags were refilled with fresh milk, which was refermented thanks to curdle remains and thus gave a product with the same characteristics. It is easy to imagine this process easily repeated at will.

In Fisberg's somewhat sceptical words, we will probably never know whether it was by mere chance that milk fermentation gave rise to one of the most widely studied foods today, or whether it was a purposeful process. Why not imagine this second possibility? Science has always started from bold minds who observe phenomena and venture hypotheses. And this supports the notion of great minds working hard to come up with a solution for dairy product preservation through winter shortages and even travel, which eventually led to yogurt. And the result was a fast and easily distributed food for the whole community.

From the technological point of view, the main limitation for the production of fermented milk was capacity, in other words, the

type and quantity of livestock. The arrival of nomadic herders and the acquisition of ovine and bovine cattle, as well as new species previously semi-domesticated such as horses, camels and deer in central and northern Europe, all boosted the consumption of fermented milk in the Old Continent.

According to Ballarini, have an animal giving a bit of milk was one thing, and having a dairy animal was quite another. Keeping dairy animals meant fully establishing the domestication process that allowed manual activation of the mother's milk ejection reflex, with no or little offspring participation. The development of new technology posed numerous obstacles: in prolonging the physiological period of milk production, artificial milking brought about problems with grazing and labour control, in addition to those arising from the long preservation of acid fermentation.

The landing of fermented milk in Europe is a special chapter of this story. It was apparently with the arrival of Bulgarians, nomadic herders who came from Asia about AD 679, that yogurt gained most of its fame. However, in the dark Middle Ages, the scarcity of dairy animals and poor social and economic conditions interrupted the spread of fermented milk, which was then only produced in convents and reserved as a luxury food for feudal lords.

Yogurt later conquered France in the sixteenth century, during the Renaissance and amid a renovated zest for healthy food. Indeed, it seems that Francis I, known as the Father and Restorer of Letters and the Knight King, suffered from a bowel infection which was particularly persistent despite the prescriptions of court physicians. In desperate need, Francis then sought help from the Grand Turk, who was thought to rely on expert medical advice from his own court's wise men. The Sultan sent one of his most skilled collaborators, who arrived at the French court with a flock of sheep and a mysterious recipe. Whether by science or mere chance, the sheep milk prepared according to the recipe, apparently fermented with lactic acid bacteria, would succeed in curing the king's resilient infection. Nothing indicates that yogurt actually became popular at that time, perhaps because the recipe was hard to follow, or the ferments were ill-suited to French milk, or because the doctors, too humiliated, refused to believe in this obscure food coming from Ottoman lands.

Yogurt appearance in America dates from much later, as animal milk was not common in the diet of native peoples. According to Fisberg, yogurt consumption appears in the reports of the first European settlers, such as those written by Pêro Vaz de Caminha, an explorer and writer who served as notary public in Pedro Álvares Cabral's fleet during the Portuguese conquest. Although largely undocumented, the introduction of animal milk appears to have produced a major cultural shock, Fisberg explains, as native peoples were physiologically lactose-intolerant and easily fell ill. Fermented milk came up as a partial solution, as it is lower in lactose than regular milk and was then harmless to the health of native Americans. This must have been the case among the peoples of Central and South America, the Aztecs, the Mayans and others.

In the dawn of the Modern Age, foods such as milk, cheese and meat became increasingly available and their use in homemade meals turned into a widespread habit. In contemporary Europe, yogurt first spread out through local producers, and later through

small factories. The first industrial production unit was created by a tireless Thessalonian merchant, Isaac Carasso. The political movements of the time forced him to move to Barcelona, where he continued developing the product he had so much faith in. In 1919, when his son Daniel –affectionately called Danone– turned 10 years old, Carasso officially created the brand that we know today. Danone yogurts were sold in pharmacies at first. However, a decade later and true to his father's philosophy, Daniel Carasso set up his own yogurt factory in Paris. A clear sign of its popularity, the word yogurt and its alternative spelling (*yoghurt, yogourt, yoghourt* and *yaourt*) had its own entry in the Petit Larousse dictionary of 1925.

But Carasso's was hardly the only modern initiative to produce and distribute yogurt. In Argentina, landowner Vicente Lorenzo del Rosario Casares spearheaded the dairy industry with its factory La Martona founded in 1889. Casares' estate was located in a town currently called Vicente Casares in his honour and received milk from dozens of local producers. La Martona, which takes its name from Casares' rather sturdy daughter Marta, was a pioneer in milk industrial processing, with sound health and safety policies and advanced technology. La Martona was the first to produce yogurt with mixed-in fruit and the second largest yogurt manufacturer worldwide by 1908. On a funny note, one of the first advertising brochures for La Martona was written in 1936 by Marta's son and renowned Argentine writer Adolfo Bioy Casares, and Jorge Luis Borges.

From these first developments, yogurt consumption has continuously expanded and is now globally widespread. In Belgium and France, average annual consumption per person went from 1.9 and 4 kilos, respectively, in 1966, to 4.9 and 11.8 kilos in 1983, a substantial increase in less than two decades. In addition to growing, yogurt production has undergone great diversification, with current varieties including multiple flavours, regular, drinkable, low-fat, fat-free, sugar-free and several others.

Average yogurt consumption In Argentina is about 10 kilos per person, the highest figures in the region. France, in turn, is the lead consumer worldwide, with 45 kilos per year per person, followed by Spain, with 25 kilos.

To sum up, tracing back the origins of fermented milk is an exciting challenge which still fuels debate. To a large extent, as Ballarini points out, the biotechnology of fermented milk still lacks specific linguistic historical references, perhaps because, unlike the production of cheese, predominantly a male task, the production of fermented milk has been traditionally mostly in the charge of women.

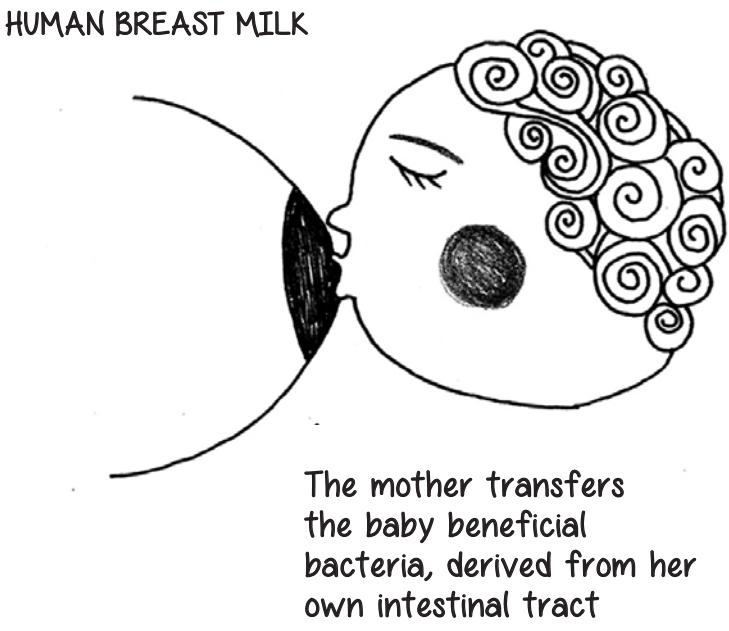
However, yogurt has survived the changes introduced by the great migrations, as well as technological and cultural revolutions, and is now one of the fastest growing foods in in the whole world.

3

THE PIONEERING WORK OF ÉLIE METCHNIKOFF

For thousands of years, the recipe for yogurt was passed by parents on to children by oral tradition, and its alleged healing or beneficial properties remained in the dark. However, in the early twentieth century, yogurt effects on gut flora sparked the interest of Ukrainian zoologist and microbiologist **Élie Metchnikoff**, regarded as one of the fathers of immunology, who then undertook an in-depth analysis. His personal and professional history is closely linked to the development of key concepts such as innate **immunity** and the beneficial functions of **lactic acid bacteria**. For these reasons, it is undoubtedly worth reviewing some of the most important events of his life, starting with his early talent and his great personality, as well as his ability to connect the dots and make amazing discoveries which went unnoticed by his peers.

Born in the village of Ivanovka on 16 May 1845 in what is now Ukraine, Élie was the youngest of five children. His father, Ilya Metchnikoff, was an officer of the Russian Imperial Guard, while his mother, Emilia Lvovna, daughter of a Jewish writer but baptized as a Lutheran, oversaw Élie's education and instilled in him a passion for science. In The Man Who Drank Cholera and Launched the Yogurt Craze, published in Nautilus magazine in April 2015, science journalist Lina Zeldovich describes young Metchnikoff as



a hyperactive boy with a superlative ability to make associations. "When Élie Metchnikoff was 8 and running around on his parents' Panassovka estate in Little Russia, now Ukraine, he was making notes on the local flora like a junior botanist. He gave science lectures to his older brothers and local kids whose attendance he assured by paying them from his pocket money. Metchnikoff earned the nickname "Quicksilver" because he was in constant motion, always wanting to see, taste, and try everything, from studying how his father played card games to learning to sew and embroider with the maids." According to 1920 Vie d'Élie Metchnikoff by Olga Belokopytova, the woman Élie had tutored and later married when she was 16, Metchnikoff asked the 'queerest' questions, often exasperating his caretakers. "He could only be kept quiet when his curiosity was awakened by observation of some natural objects such as an insect or a butterfly", his wife wrote.

In 1856, he entered the Lyceum, where he developed his interest in biology, and later entered the school of science at V. N. Karazin Kharkiv National University, where he completed the four-year course in two, although he missed classes for months to devote himself to the reading of scientific books on subjects he found fascinating. After doing research on the marine fauna of the German island of Heligoland and exploring nematodes, he discovered intracellular digestion in earthworms. In 1867, he returned to Russia to obtain a PhD degree at Saint Petersburg University, where he received the Karl Ernst von Baer prize for his thesis on the development of invertebrate embryos.

At 22 years of age and even younger than many of his students, he was given a Professor position at what is now Odessa University, back then a cultural centre in the Russian Empire, but came into conflict situations and eventually preferred to resign.

And although Metchnikoff's contributions to contemporary science were already remarkable, it was perhaps in those years when his academic life took an almost unexpected turn, abruptly changing his scientific interests. His eyesight problems got in the way of his microscopy studies, so he decided to focus on anthropology. This new field of interest had Metchnikoff travelling through the steppes of Astrakhan and Stavropol, where he found certain communities of **striking longevity**. Metchnikoff soon attributed this longevity to the consumption of **fermented milk**, a notion he would go back to later in life.

Strongly influenced by Charles Darwin's *On the Origin of Species,* the idea that all creatures on Earth share a common origin led him to link the digestive processes in primitive beings with human defence mechanisms. This idea allowed him to combine results from several different lines of research he had taken part in, such as the earthworm digestive system, starfish defence system and human health.

The transparent larvae of starfish would trigger one of his most important discoveries. In lower animals lacking an abdominal

cavity or bowels, digestion is driven by a particular cell type which moves and dissolves food particles, that is mesodermal cells. While analysing mobile mesodermal cells in larvae, Metchnikoff –who was 37 years old at the time– came up with an idea which would later revolutionise the field of health sciences. According to his own account, it dawned on him that similar cells could be used to defend the organism against invaders. To test his hypothesis, he pinned thorns from a tangerine tree into the larvae. If he was right, the larvae would recognize the splinters as foreign and mesodermal cells would gather around to destroy them. And it so happened; the mesodermal cells clustered next to the thorns as if trying to devour them. This led Metchnikoff to call these cells **phagocytes**, a Greek-origin word meaning "devouring cells" which is still in use today, and to think of them as an army purposefully launched against the enemy. In animals which have blood, Metchnikoff further reflected, it had to be white blood cells or leukocytes that rushed to defence, as they populated the site of inflammation where they attacked and destroyed the bacteria. But this idea was faced with scepticism, as his colleagues at the time regarded the presence of leukocytes in areas of infection as proof that they created a favourable environment for bacterial growth and dissemination.

However, experiments carried out by other researchers would finally prove him right and, in 1908, Metchnikoff was awarded the **Nobel Prize in Medicine** for the discovery of phagocytes and his studies on their role in the **human immune system**. From then on, Metchnikoff fully devoted himself to studying the human immune system. He was strongly determined to stretching lifespan, especially after his encounters with the long-lived peoples in the Caucasus. He believed in the power of science to correct the imperfections of nature.

As part of his research into natural immunity, Metchnikoff was rather reckless in experimenting on himself and his collaborators, although, it should be said, this recklessness gained him impressive success in his investigations. As his wife Olga reports in his

biography, Metchnikoff once drank a culture of *Vibrio cholerae*, the bacteria causing cholera which had killed thousands of people in Europe and survived without even getting sick. Not happy with this result, Metchnikoff found a volunteer among his collaborators who repeated the test with a disconcerting result: he did not get sick either. A third volunteer accepted the challenge but, much to Metchnikoff's horror, he did fall ill and nearly died. Although almost disastrous, the experience made Metchnikoff ponder these contrasting results, and ultimately rendered laboratory findings showing that some of the bacteria present in normal gut microbiota hindered the growth of *Vibrio cholerae*, while others stimulated it. This is how he established that human gut bacteria play a key role in the prevention of disease. His claim was that, if the drinking of a bacterial culture could cause disease, the consumption of a different one could be actually healthy and beneficial. This simple but powerful notion paved the way for studies on the modification of gut microbiota composition and its possible beneficial impact on the fight against diseases which had persecuted humans for centuries.

Gut microbiota was a hot topic in the late nineteenth century. A prominent theory held that the large intestine was a reservoir of harmful toxins, some of which were considered the harmful by-products of food digestion. When human digestion was compared to that of birds, it became clear that birds had much more frequent bowel movements. This led some researchers to think of the large intestine as an evolutionary residue from the time when our ancestors needed to flee from predators and found no time for bowel movement. The products of bacterial putrefaction kept in their bowels for too long then became toxic.

Lina Zeldovich goes on to explain that the theory of intestinal putrefaction gained such prestige that British surgeon William Lane proposed the removal of the entire large intestine to remedy digestive disorders. Metchnikoff believed that, while aging was the result of toxic bacteria lodged in the digestive system, it was

possible to achieve microbial balance without appealing to surgery and so set out to find beneficial bacteria. From the experiments to preserve food carried out at the Pasteur Institute, and the anthropological data collected during his travel to the Caucasus, Metchnikoff knew that lactic acid could keep milk from spoiling and thought it could also prolong life. He believed in this so strongly that he himself drank sour milk or yogurt every day. He discussed these ideas in *The Prolongation of Life: Optimistic Studies*, of 1907, in which he exposed the potentially beneficial properties of lactic acid bacteria. His claim was a simple one, if lactic fermentation prevents putrefaction in general, why should it not serve the same purpose in the digestive tract. The idea that our phagocytes 'mutiny' when we grow old and that all the symptoms of old age are the result of their destructive action was already in his mind. He believed this was caused by residual products present in the stomach which, he claimed, could be eliminated by encouraging the proliferation of benign bacteria. All it would take then was eating enough kefir or yogurt, which would guarantee a thriving population of beneficial bacteria in the stomach.

In 1899 he wrote *L'Immunité dans les maladies infectieuses*, which was published in 1901, the same year in which he delivered the Flora of the Human Body conference in Manchester. Metchnikoff advocated for a healthy diet and lifestyle to avoid the intoxication that accelerated aging.

This idea took him in two directions: on the one hand, he studied gut flora and the tissues that age most quickly throughout life; on the other hand, he made progress in anthropological and psychological reflections. He poured his thoughts in *Études sur la nature humaine*: Essai de philosophie optimiste, of 1903, and *Essais optimistes: Etude sur la vieillesse*; *La longévité dans la série animale*, of 1907. Metchnikoff thought that old age could be delayed by scientific procedures and gave bowels a fundamental role. In search of what he called an **orthobiotic bowel state**, he strongly recommended replacing harmful microbiota with a type in which

lactobacilli were predominant. And he advocated for a milk diet, which may acidify bowels and thus reduce toxicity. In this sense, he made two publications in the Annals of the Pasteur Institute, *Études sur la flore intestinale* and *Poisons intestinaux et sclérose*, in 1908 and 1910, respectively.

Following his trail of thought, Metchnikoff came to suggest that the large intestine was altogether harmful. In the preface to the fifth edition of *Études sur la nature humaine. Essai de philosophie optimiste*, he defended himself from the criticisms of his peers by reaffirming that considerable extension of the large intestine was not in harmony with human nature and could even shorten our lives. He further attacked the notion that the microorganisms in our digestive tract are in symbiosis with human health as a misconception, and asserted that, according to his studies, reality showed quite the opposite, that the bacteria nourishing our digestive tract are utterly toxic.

True his theories, Metchnikoff abstained from all raw food and took lactic microbes as part of his diet, which he considered capable of preventing bowel toxicity. Out of strong conviction, Metchnikoff advocated for this lifestyle and encouraged others to follow.

Metchnikoff's vision of the harmony of man in relation to his body was not simply limited to his diet but also extended to certain organs, or parts of them. In this way, chapter four of *Désharmonies dans l'organisation de l'appareil digestif de l'homme* first argues that hair has no useful function and only represents the remains inherited from our ancestors which may become harmful to health, that wisdom teeth are a source of mild disorders in most cases but serious or even fatal in others, and that the appendix is an organ whose absence is harmless as it leaves the body intact when it is removed, but whose normal development may produce serious disorders, to finally assure that the large intestine is also a superfluous organ and that its removal may render positive results. According to Metchnikoff, from the point of view of digestion, the large intestine had no relevant function.

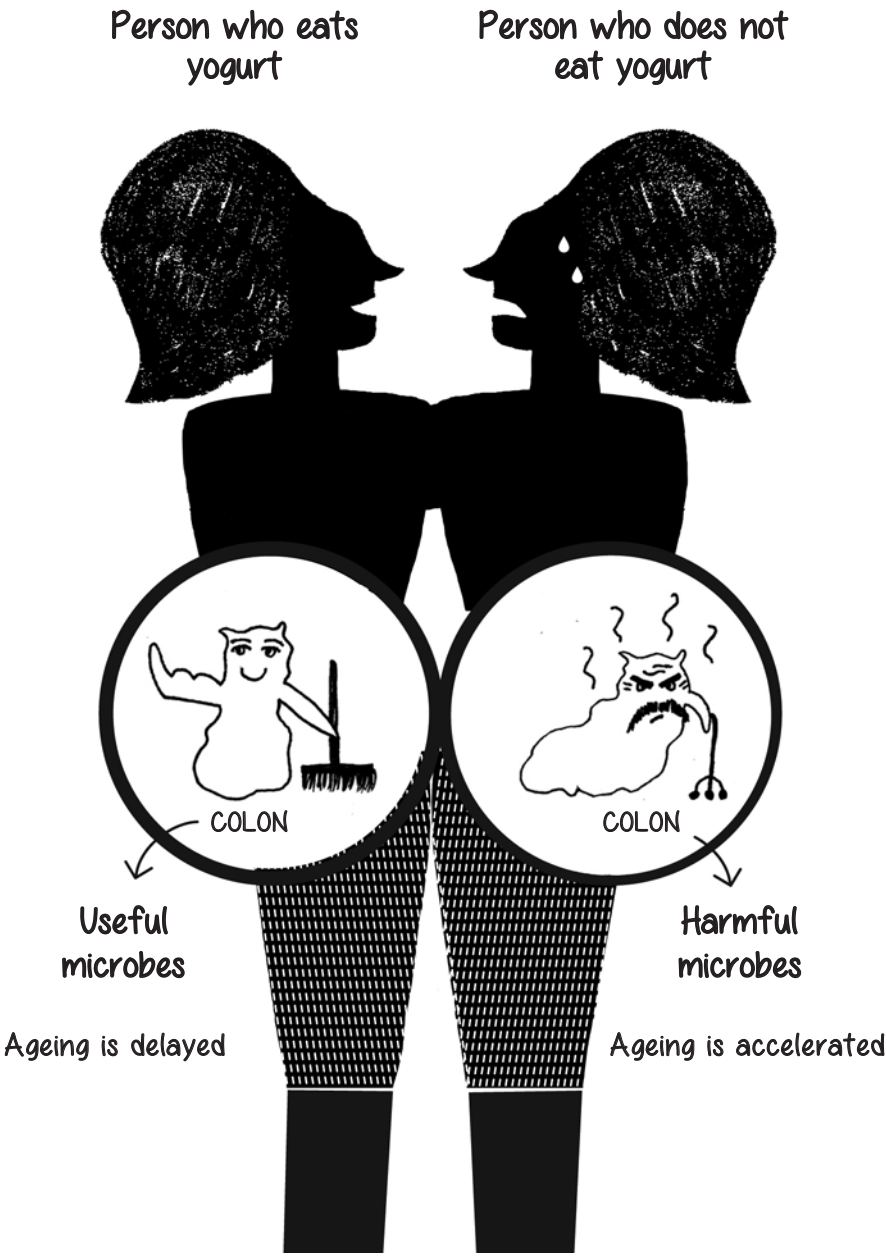
The idea of fighting the microorganisms of the large intestine was appealing. Metchnikoff wondered whether they could be directly attacked using antiseptics, as suggested by Bouchard, who had worked on the disinfection of the digestive tract through of beta-naphthol. However, these studies had shown that this antiseptic substance, like so many others, was not powerful enough against intestinal microbes and could even harm the rest of the body. On the basis of this evidence, Metchnikoff concluded that the idea of destroying gut bacteria with chemical substances was not likely to succeed, as the doses needed to achieve bowel antisepsis were so high that they could only be used in specific conditions and at long intervals. The use of certain purgatives was proposed instead, although far from destroying the gut bacteria they were eliminated mechanically.

During these speculations, Metchnikoff came to think that food has an early influence on the composition of gut microbiota. Indeed, man comes to the world with microorganism-free intestines which upon birth are populated with microbes under the influence of breast milk *Bacillus bifidus*. Given the information available on the relationship between food and the population of bacteria in the newly born, which was studied by analysing the baby' first faeces, Metchnikoff turned to attempts at modifying gut flora and replace harmful microbes with useful ones through diet. After studying several bacterial cultures, he concentrated on *Bulgarian bacilli*, widely used in Eastern Europe to make yogurt. This choice stemmed from his studies in the ancient towns of the Caucasus, where communities consumed a great deal of sour milk.

Unfortunately, Metchnikoff could not bear witness to the benefits the world obtained from his discoveries. He died in 1916 as a consequence of severe heart disease which had afflicted him several times. And, although hard to believe, he kept his curious spirit even on his deathbed. The story goes that, just before dying, he reminded one of his colleagues at the Pasteur Institute, Alexandre Salimbeni, to carefully examine his intestines after death.

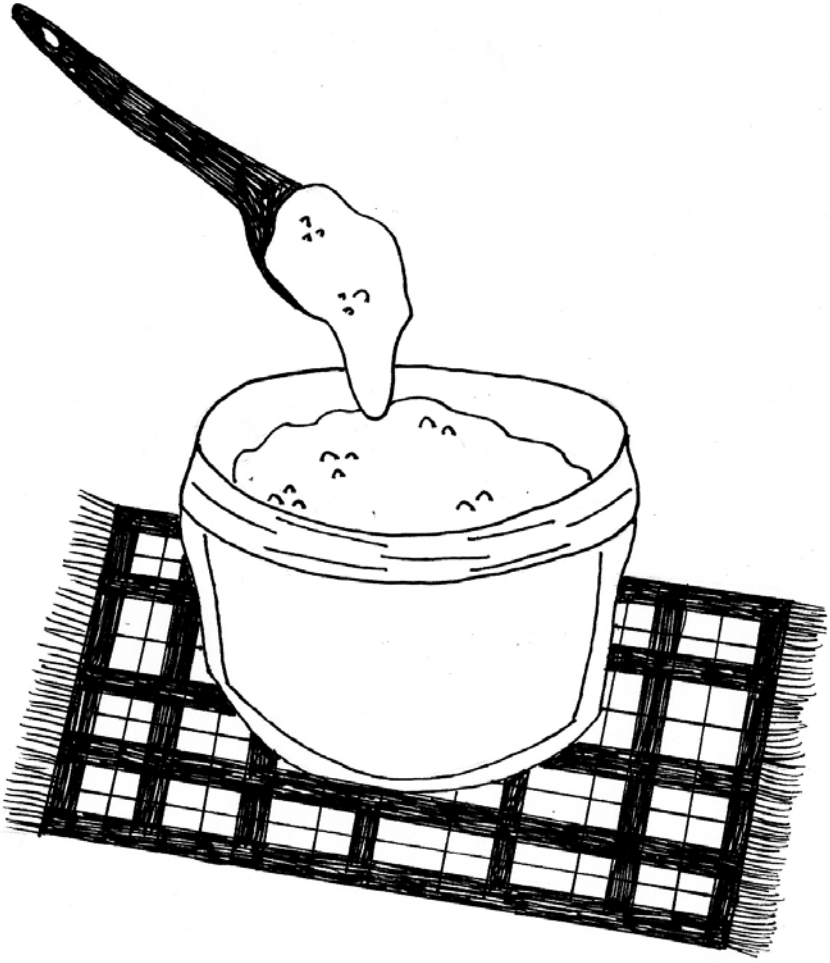
Although initially rejected, the ideas of Metchnikoff gained prestige over time, and some doctors in Europe actually started prescribing sour milk to treat bowel disease. However, an opposite trend emerged in the 1930s with the development of antibiotics, which eradicated pathogens and infections. Scott Podolsky, director of the Centre for the History of Medicine at the Countway Library of Medicine, mentions the Lederle Laboratories in the United States as a clear sign of such transition, as these laboratories switched from acidophilic production to antibiotics production. However, and even if antibiotics were an ominous sign for Metchnikoff's discoveries, their overuse eventually revived his investigations, as scientists soon recognised the potentially negative impact of antibiotics on human health. Superbugs appeared, autoimmune diseases followed, it microbes were found to play a central role in ecosystems such as oceans, forests and soil and to produce vitamins, nutrients and growth factors vital to ecosystems and health. With time and enough scientific evidence, it became clear that we largely rely on microbes for food.

After the Human Genome Project managed to "spell the book of life", sequencing all the genes we are made of, the National Institutes of Health in the United States launched and funded the **Human Microbiome Project**, through which thousands of scientists began to investigate the microorganisms which live in us and their relationship with health and disease. Along the same lines, *Fortune* magazine called 2015 "the year of the microbiome", while the term *probiotic*, derived from the Greek 'in favour of life' and coined in 1989 by Roy Fuller, a researcher in microecology, entered popular language and became the origin of a multi-billion-dollar industry. Such was the scenario in which, during 2016, the prestigious journal *Science* devoted an entire edition to the human microbiome under the title "The Microbes That Make Us". According to editors Elizabeth Pennisi and Kristen Mueller, the invisible microbial world has been in the spotlight over the last 15 years thanks to DNA sequencing methods which allow researchers to detect bacteria and other organisms they


Person who eats yogurt
Person who does not eat yogurt
COLON
COLON
Useful microbes
Harmful microbes
Ageing is delayed
Ageing is accelerated

cannot grow in culture media. First, these techniques revealed vast and diverse communities within our digestive system, in our skin, in our buildings and on every possible surface. Later, studies in germ-free mice and other projects allowed to identify the relationship between microbiota and health, unveiling bacteria with potentially key roles in immunity, obesity and development. Science chose microbiome as a breakthrough item in 2011 and 2013 and published special editions on the subject in 2012 and 2016.

To conclude, Élie Metchnikoff, much ahead of his time, seems to have anticipated in almost a century one of the topics sparking most interest in current science.



4

YOGURT PRODUCTION

A quick look at the world around us and we find proof of just how much technical and scientific progress have changed our everyday lives. Health, technology, transportation. Our whole environment has been transformed by wonderful new knowledge, improving our life standards and stretching our lifespan. But what technical and scientific progress have done with yogurt is rather an exception to the rule, as –in essence– we keep making yogurt much like thousands of years ago. Nothing but high-quality milk left to ferment.

But really nothing else? The fact that the core steps in yogurt production have remained largely unchanged for centuries does not mean science has nothing to say about this magical ancient process. Just as wine is not only grape juice, yogurt is not just milk. And, given proper attention and the right technology, a few key steps can be tuned to improve final quality.

Without a doubt, the starting point is high-quality milk. This means raw material obtained in optimal animal health and safety conditions, but it also implies **milk free of antibiotics or chemicals** which may damage yogurt properties or flavour, and which may even be harmful to consumers' health. Science has come a long way in understanding what medicines can be given to livestock to preserve health while still watching for the quality of the milk

obtained. For instance, one of the most frequent problems in milking is cow mastitis, an inflammation of the mammary glands which usually heals with antibiotics administration. However, as milk containing antibiotics is inedible, scientists have developed methods to determine the time window in which the milk obtained still contains traceable levels of antibiotics after the last administration. In fact, the very presence of beneficial bacteria in yogurt ensures the absence of antibiotics in milk. In other words, milk is still milk, but cow mastitis is a risk factor we have managed to control today, protecting both cattle and consumers.

It is perhaps here, in the handling of the raw material, where we find **the greatest innovation in the production of yogurt**, pasteurisation, originally developed by French scientist Louis Pasteur, who also happened to mentor Élie Metchnikoff during his late scientific career. At the request of Napoleon Bonaparte III, Pasteur set out to investigate the biochemical nature of wine and beer fermentation, which even in the mid-nineteenth century was regarded as a purely chemical process, free of living organisms. Pasteur eventually proved that microorganisms did take part in wine and beer fermentation and, in 1864, demonstrated that heating up wine to 44°C reduced the microbial load and improved beverage preservation without altering its flavour. A few years after this controversial finding, Dr Charles North, an American physician and agricultural scientist working in the promotion of public health, advocated for milk pasteurisation as a form of preservation, together with a series of health and safety rules in milk production including: a) healthy cattle; b) careful milking; c) clean hands and clothing; d) clean and dust-free barns; e) sterilised instruments; and f) quick refrigeration of the milk obtained.

In short, pasteurisation manages to reduce the microbial load that fresh milk inevitably contains even when milked in good health and safety conditions. If not controlled, this initial load could damage milk and be harmful to consumers. Worth highlighting, pasteurisation is not meant to kill all microorganisms –a process

called sterilisation– but to reduce them to levels which are harmless to consumers and help preserve the properties of the original milk as much as possible.

Pasteurisation was originally a slow and somewhat complicated process which consisted in heating milk to 63°C for thirty minutes. This step succeeded in reducing the microbial load but severely slowed down production, as large amounts of milk then required several hours to cool down. This original process was called vat pasteurisation, on account of the large containers it was usually carried out in, but was later replaced by HTST pasteurisation, which stands for high temperature-short time. In this new and better process, milk is heated up to temperatures ranging from 72 to 92°C for fifteen to five seconds, respectively, which provides a much faster result and still keeps the flavour and texture of the original milk. In some particular manufacturing processes seeking a longer shelf life, as is the case with milk cartons, HTST is replaced by UAT pasteurisation, which in turn stands for ultra-high temperature. Under UAT pasteurisation, milk reaches up to 138°C for two seconds, which renders great quality and maximum shelf life.

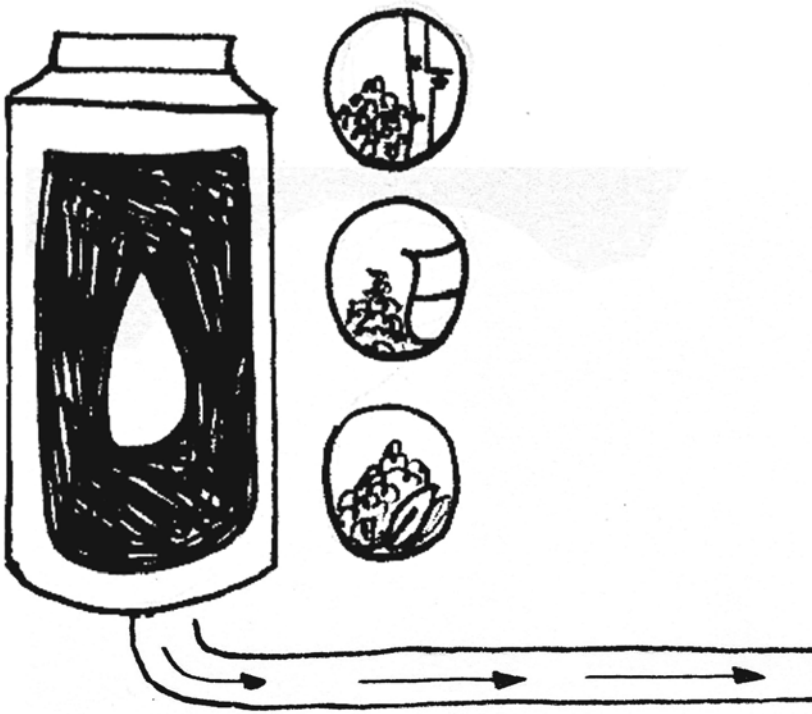
Another example of progress in yogurt production is that the pasteurisation process gives a smoother product texture. During heating, a large proportion of albumin and other globulins present in milk bind to predominant protein casein, which leads to a tastier final product. However, this is an indirect effect, as pasteurised milk still has a long way to go before reaching the consumer in the form of yogurt. Among other things, pasteurised milk is homogenised to obtain a regular mix and better texture and kept cold until the moment of fermentation. This last stage in the process consists in the addition of **ferments** containing specific and controlled amounts of fermenting bacteria which will turn milk into yogurt. This magical moment relies on two types of bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus, both belonging to the microbial group known as **lactic acid bacteria**, because of their ability to produce lactic acid as the main product of

YOGURT PRODUCTION

Every step in the process of yogurt production is carefully controlled to ensure milk microbiological, physical and chemical quality.

① MILK PREPARATION

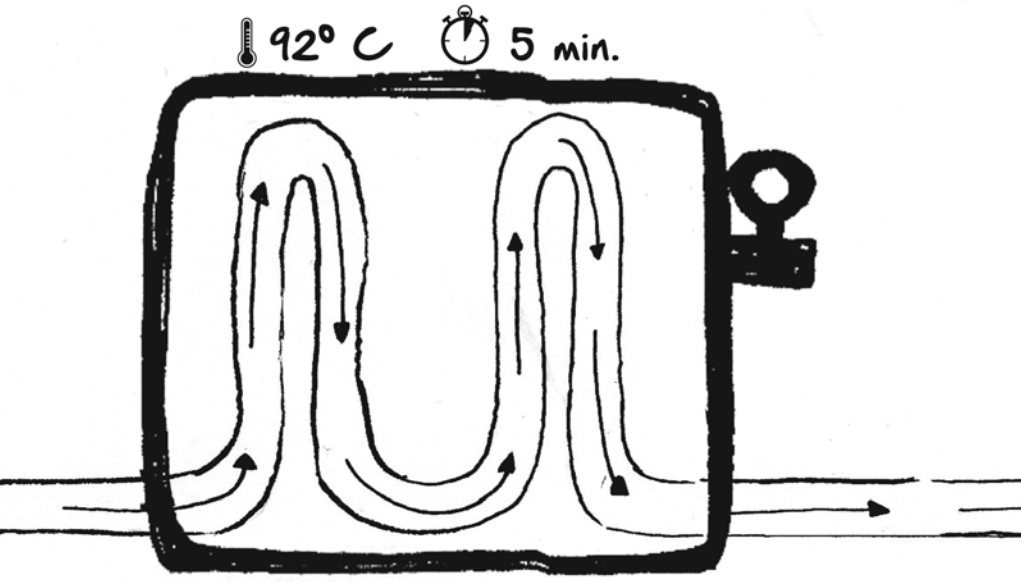
Milk is pumped into tanks and added ingredients according to the different yogurt types: sugar, skimmed milk powder or sweetener.



② PASTEURISATION AND HOMOGENISATION

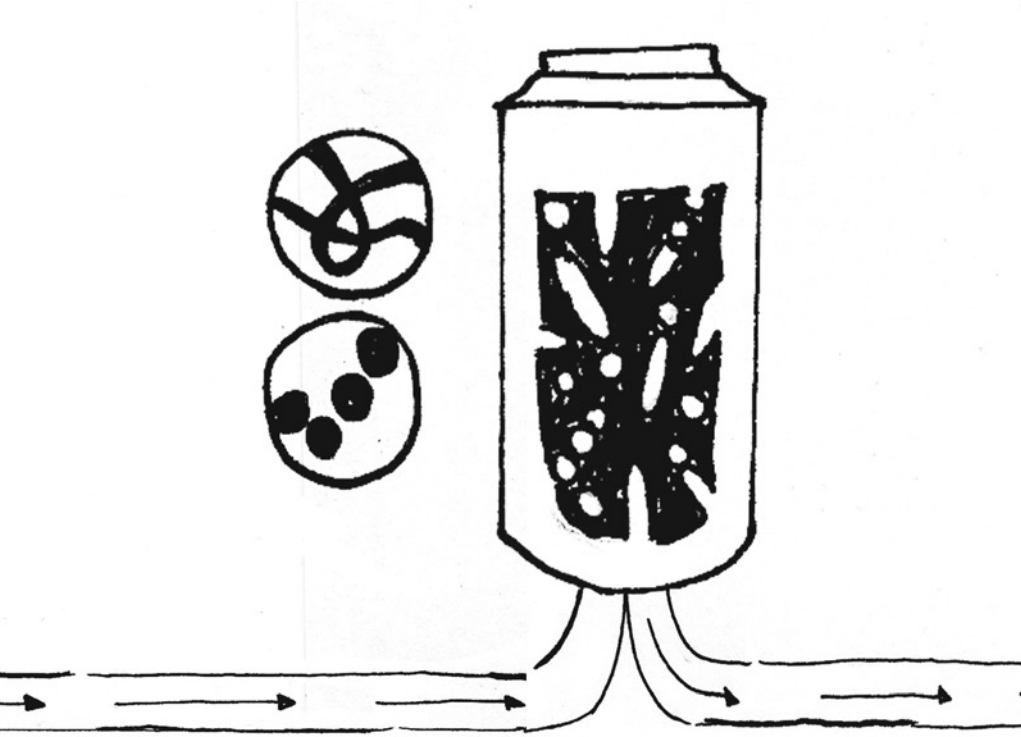
Milk is heated up to remove pathogens and extend shelf life.

Fat bubbles are crushed to prevent milk skin formation.

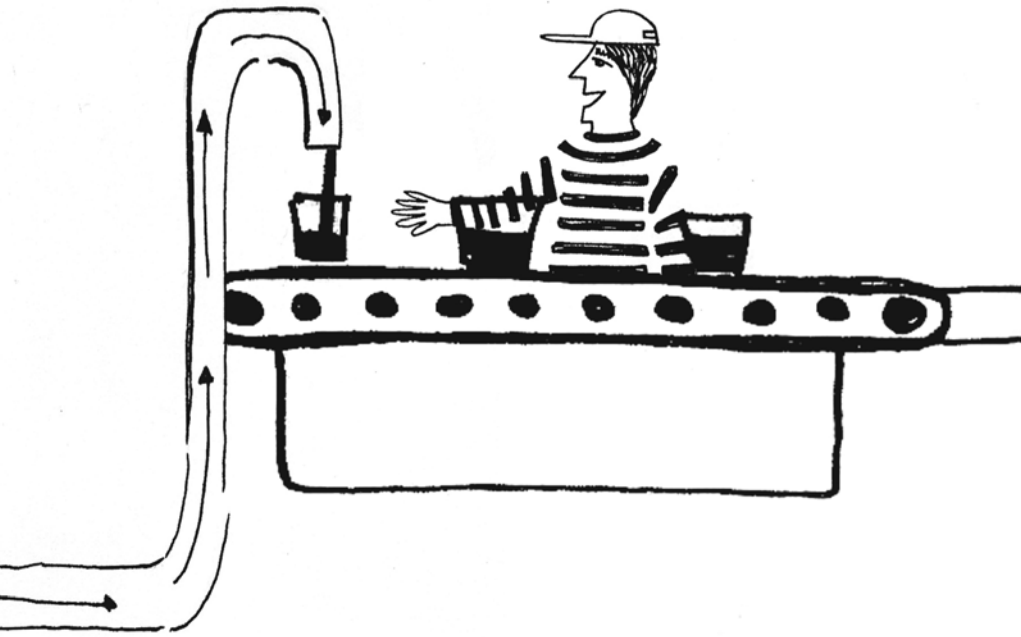


③ FERMENT INCORPORATION

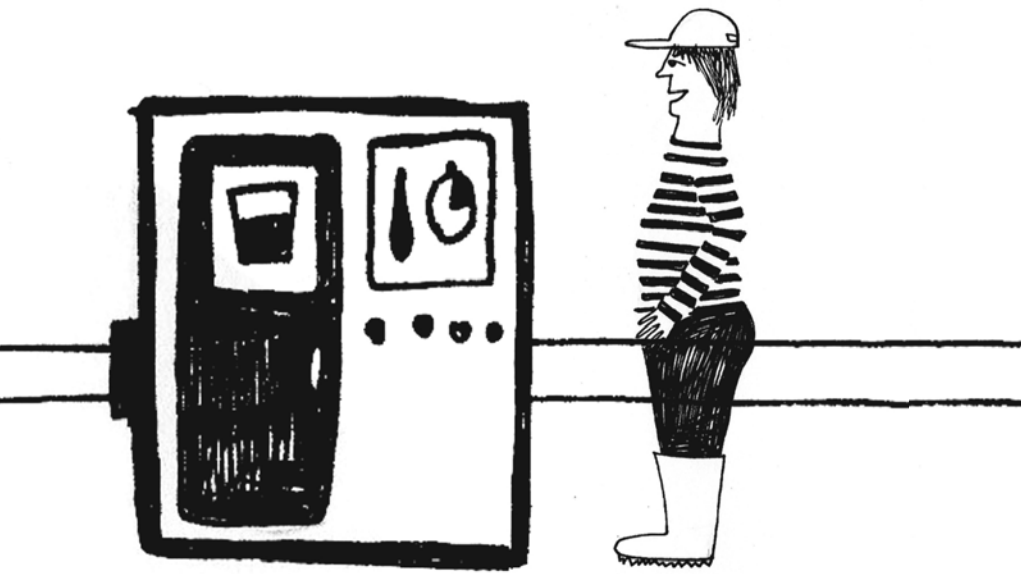
Milk is stored in tanks and added yogurt ferments. The process may vary according to the different yogurt types (thick or drinkable).



④ PACKAGING



⑤ FERMENTATION
Yogurt is left to ferment in pots at warm room



⑥ REFRIGERATION AT 4°C.

their fermentative metabolism. From this point on, ferment-added milk will follow different industrial paths depending on the type of yogurt to be produced, be it thick, drinkable or shake.

In the production of **thick yogurt**, fermented pasteurised milk is cooled down to between 38 and 43°C and straightaway packaged and sealed in the pots which will reach market shelves. All thick yogurt containers are incubated at such temperature for four to six hours, during which lactic bacteria acidify milk by transforming lactose into lactic acid and thus shift acidity levels from a nearly neutral pH of 6.4 to 6.7 to an acidic pH of 4.1 to 4.3. This acidification results in a rigid sugar-protein matrix which characterises thick yogurt, gives it a characteristic flavour and prevents the growth of different microorganisms which could be harmful to consumers' health, such as Escherichia coli or Salmonella. Once fermented, yogurt pots are refrigerated below 10°C until consumed.

On the other hand, the production of **drinkable yogurt** and **shakes** is characterised by milk fermentation and consequent acidification within large tanks. The yogurt obtained, initially thick, is subjected to slight shaking to produce creamy yogurt or shakes, a texture in between drinkable and thick, or more intense shaking to produce drinkable yogurt. Once stirred, the product is finally packaged, sealed and refrigerated below 10°C until consumption, much like thick yogurt.

In this way, the small pots of thick yogurt or the fermentation tanks of drinkable yogurt now replace the old animal stomach skin bags or the wooden pitchers in which ancient yogurt was prepared. In essence, however, magic is still the same.

5

MICROBIOTA: THE INDIGENOUS COMMUNITY

Although we usually imagine ourselves as a collection of cells shaping our organs, the human body can also be understood as a five-star hotel accommodating **billions of microorganisms** essential for our survival, an incredibly complex ecosystem known as **the human microbiome**. The total number of these tiny guests, including bacteria, fungi and other microorganisms which dwell on our mucous membranes and, particularly, in our digestive tract, is roughly estimated as ten times our cells, while the number of microbial genes within us might be one hundred to five hundred times as many as our own genes.

According to t*he Handbook on Gut Microbes* by the **WGO**, the human digestive system harbours around two hundred billion microbial cells of more than a thousand species. In turn, the gastrointestinal tract is a great interface with the external environment and the main site for interaction with the microbial world. Gastrointestinal mucosa exhibits a large surface which, if unfolded, might measure more than three-hundred and seventy square meters, and contains structures and functions adapted for two-way communication with microorganisms.

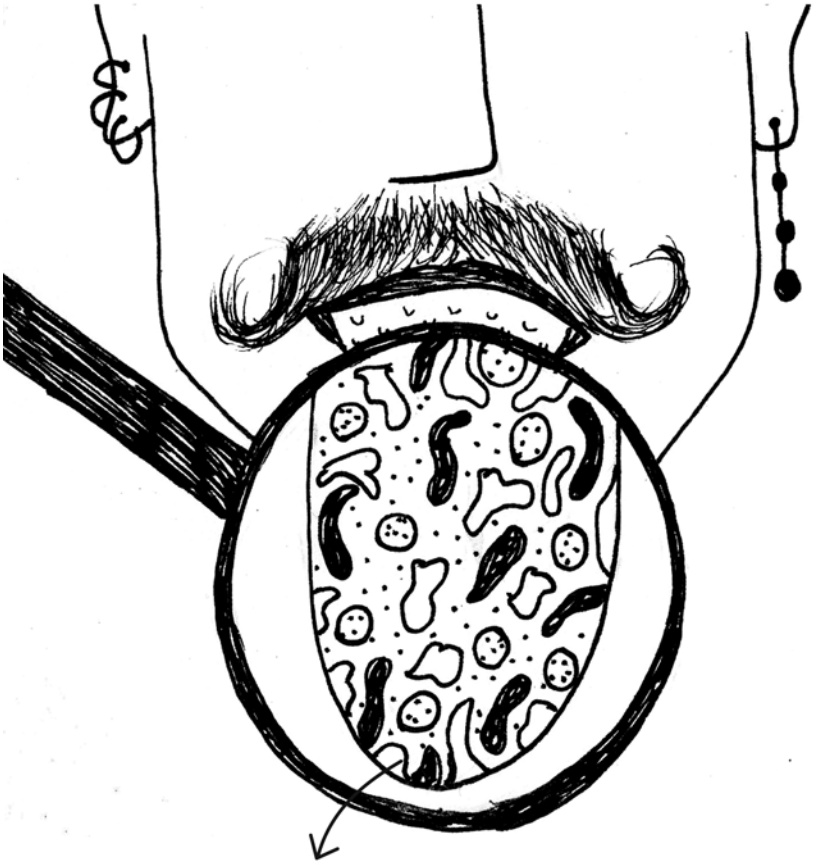
As explained by Claudia Herrera and Francisco Guarner in the introduction to the Handbook, the stomach and duodenum have low

numbers of microorganisms, typically less than a thousand bacterial cells per gram, most of which are **lactobacilli** and **streptococci**. Acids, bile and pancreatic secretions suppress most of the microbes ingested, but the number of bacteria progressively increases to ten million cells per gram in the distal ileum, the final section of the small intestine. According to Herrera and Guarner, these microorganisms usually proliferate in the colon, where transit is slow, fermenting substrates obtained from the diet or endogenous secretions. Countless studies have shown that the bacteria living in the gastrointestinal tract affect host physiology and pathology in multiple ways, as we will see throughout this chapter.

Despite Élie Metchnikoff's predictions reported in **chapter 3**, little was known until recently about this multi-faceted population of microorganisms, other than their key role in vitamin synthesis and defence against pathogens. The way we see bacteria was revolutionised by Dutch merchant Antoni van *Leeuwenhoek*'s design of a single-lensed microscope, which, in the seventeenth century, allowed him to observe microbes for the first time. Born in the city of Delft, Leeuwenhoek eventually managed to design microscopes capable of magnifying up to two hundred and seventy times, which he used to look at animal hairs, wood, seeds and skin scales, and which allowed him to describe red blood cells, plant tissues and lice guts with incomparable levels of detail. In 1675, Leeuwenhoek used his lenses to look at rainwater and spotted a myriad of tiny 'dancing creatures' a thousand times smaller than the mites he had seen on the crust of cheese. However, the notion of good bacteria which did not cause disease was difficult to conceive of. Little by little, though, bacteria were characterised in cats, dogs, wolves, tigers, lions, horses, cattle, elephants, camels and human beings. In 1909, Arthur Isaac Kendall, a pioneer in the study of gut bacteria, described the intestine as a singularly perfect incubator for bacteria whose function was not necessarily harmful to the host. Even Louis Pasteur, while leading the fight against harmful bacteria by developing pasteurisation and paving the way for vaccines and

The digestive tract hosts 70% of the immune cells and 70% of the cells in the autonomic nervous system.

Its good health is closely linked to food intake, physical activity, medicines, stress, etc.



The digestive system harbours around 200 billion microbial cells which give us protection against pathogens and disease.

antibiotics, warned that microorganisms might be useful, perhaps even essential for life. But it was Élie Metchnikoff who took these ideas to the next level.

At present, gut microorganisms are at the core of a vast international effort aimed at unveiling their secrets. In 2008, and with 115-million-dollar funding, the **Human Microbiome Project** was launched on the assumption that the roughly one **thousand species of bacteria** comprised in microbiota play a vital role in health and disease. Throughout the world, this initiative brings scientists, universities, governments and industry together in a joint effort to find out who these numerous tiny dwellers of earth chemistry are and what they do. The reason for such a Herculean task is clear: in the words of Dr Emily P. Balskus, Morris Kahn Associate Professor of Chemistry and Chemical Biology at Harvard University, "Deep in your gut, 40 trillion chemists are hard at work helping you digest your lunch, making essential vitamins and nutrients you can't produce on your own, protecting you from disease, and more." It is undoubtedly essential for us to get to know these 'chemists', studying their characteristics, diversity and capabilities.

The **study of human microbiota** is a bustling field in current international science. Articles published in high-impact journals provide evidence that microbiota might modulate the action of immunotherapeutic drugs, play a role in malnutrition, or even be associated with psychiatric disorders such as depression, autism and schizophrenia, among other examples. However, the alchemy of gut microbiota **varies across individuals and populations**. "For example," Dr Balskus adds, "most people can't digest seaweed. But some residents of Japan are able to do this because their gut microbiomes contain seaweed-eating bacteria." This goes to proving that, within the digestive system, "...Gut microbes carry out chemical transformations that interfere with medications we take and processes that potentially increase the risk of cancer and heart disease." In a way, the microorganisms in our intestinal tract constitute a new organ with its own function, capabilities and limitations.

In an attempt to shed light on the role of this new organ, susceptible to factors such as diet, disease, medication, geographical location and lifestyle, **several scientists have undertaken region-specific studies.** A group of **Argentine scientists** led by Dr Martín P. Vázquez, for instance, conducts research into the local microbiome profile whose first part has already been published in *Frontiers of Microbiology*. According to the researchers, there is growing consensus on a correlation between the structure and composition of this community of microorganisms and numerous diseases. However, given how much this composition may vary, predicting disease-associated alterations requires a thorough characterisation of the healthy **individual's microbiota**, for which American references are not reliable.

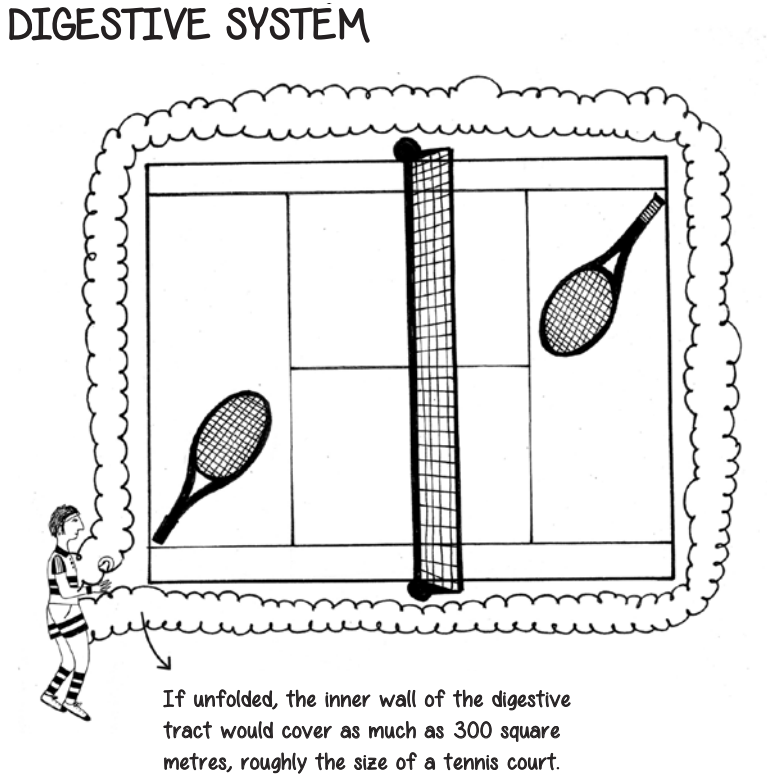
This research project was carried out by scientists from Instituto de Agrobiotecnología and Centro de Diagnóstico Medico de Alta Complejidad, both in Rosario, Argentina, and initially focused on the microbiome composition of skin, mouth, saliva, throat, larynx and bowel, in twenty healthy middle-class individuals between 20 and 50 years of age. Researchers in the group explain that the work yielded thousands of genomic sequences which allowed to identify bacteria. And, despite the wide range of bacteria identified, ten or twelve species were found to prevail, roughly accounting for 80% of the whole range.

Further inroads have been made into regional microbiome profiles. Researchers at Hospital de Clínicas, Universidad de Buenos Aires, have developed a technique through which a patient suffering from an infection with pathogenic *Clostridium difficile* can be **transplanted gut microbiota** obtained from the faeces of a healthy donor. The scientists responsible for this novel strategy indicate that the procedure proved successful in treating the patient's antibiotics-resistant diarrhoea. Moreover, as pointed out by Dr Carlos Waldbaum, head of the gastroenterology endoscopy service at the hospital, the patient recovered as early as two or three days after transplant.

As a large and diverse group of microorganisms, gut microbiota does not take shape quickly but through a rather complex process. Understanding **how our microbiota comes to life** is a challenge which several specialists have risen to, like Dr Gabriel Vinderola, a passionate researcher in the field. "I've been coming to this lab for twenty-one years, and there's not a single day that I didn't want to come." he says. "Sometimes I wake up at three in the morning and start thinking, because my head keeps working." Over the years, this Santa Fé chemist and microbiologist who developed **the first probiotic cheese in Latin America** has been working on yogurt and probiotics, studying how to harness the bacteria in our bodies, among other things.

According to Vinderola, the bowels of newly-born babies are free of bacteria upon birth, but as soon as the next day are colonised by bacteria acquired in the delivery process. Vanderola further explains that the birth canal is populated by **lactobacilli** which come into contact with the baby during natural delivery. In contrast, bacteria colonising the bowels of babies born through C-section come mostly from the hands of the people taking part in the procedure. In 2006, breast milk was shown to contain **bifidobacteria** which is then passed on during breastfeeding and protects the baby against colonisation by other non-beneficial or even harmful bacteria. There is no doubt that the milk of all mammal species is a product improved over 200 million years' evolution. In addition to lactose and bifidobacteria, human breast milk contains fat and oligosaccharides, one of its key components and a vital source of energy for growth which babies, however, cannot digest. At the beginning of the twentieth century, oligosaccharides were found to constitute food for gut bacteria, especially bifidobacteria. As the baby collects new microbial species from their parents and the environment, gut microbiota gradually becomes more diverse, matures and develops the ability to digest, not only milk, but also an adult diet.

On the whole, evidence on the microbial colonisation taking place in the passage of the baby through the birth canal and the



relevance of breastfeeding in the composition of the first microbiota support the notion that premature babies, babies whose mother took antibiotics before childbirth, babies who are not breastfed or those who are raised in aseptic conditions are at a disadvantage in terms of digestive ability and general health.

Scientists cannot be assertive yet as to whether microbiota is the cause, the effect or an accelerator of global health and disease, but they all agree that it is likely to play a starring role. Academic circles are strongly convinced that the digestive system, starting from the mouth, is a highly complex organ which needs microbiota symbiosis with the rest of the tissues. Microbiota may be regarded as single-handedly important, but it is only key in as much as it

interacts with its environment. The space we call digestive tract, from mouth to anus, hosts 70% of the immune cells and 70% of the cells in the autonomic nervous system, and is then thought of as a second brain. And microbiota belongs to this system. These microorganisms are undoubtedly part of an essential barrier made up of epithelial cells which allow the passage of nutrients and prevent the passage of deleterious agents, and which should be kept intact. The digestive tract is then a whole organ in its own right which responds to the environment, food, physical activity, medicines, stress, sleep, fear, joy, in short, our whole lives. It is dynamic and resilient.

Likewise, **microbiota evolves and can be modified** –for better or worse– by several environmental factors, lifestyles, external agents or aging. As discussed above, a baby's microbiota is not the same as that of an older person. In addition, the link between **microbiota and chronic metabolic** and general disease has fuelled extensive debate in the academic field. **Numerous stimuli can modify gut microbiota**, and gut **microbiota in turn regulates what happens in many other tissues.** For example, physical exercise has an impact on microbiota composition, which then has effects on intestinal barrier integrity. However, in addition to the intestine itself, interaction takes place with other tissues such as muscles, as the way our **muscles** become weaker with time is also related to the digestive system. And this is not all. Regarding interaction with the **brain**, records of people's brain activity at the sight of happy or frightening images have revealed a relationship between diet and reaction to stress. Links have also been established between the digestive system and **heart** and, most importantly, between **stress** and microbiota composition.

As mentioned earlier in this chapter, the scientific community was not always aware of this interaction between the microbiome and the rest of the body, and the lesson on the beneficial effects of healthy microbiota had to be learnt the hard way. Half a century ago, mainstream science sought to wipe bacteria off the face of the

Earth because they brought about disease. This vision did have a somewhat bright side, as it allowed to develop asepsis and improve surgical procedures, but we know today that the successful cure of major infection pathologies was dampened by the increasing prevalence of autoimmune and inflammatory disorders. This is how the **hygiene hypothesis emerged**, which stresses the importance of contact with bacteria, as only 0.98% of them are pathogenic. A detailed and careful look at the bacteria colonising our bowels has unveiled links to some of the most worrying health issues round the world such as obesity.

On 24 November 2016, Barcelona magazine *La Vanguardia* featured an article by Roser Reyner Bou under the title 'Gut microbiota makes it clear why it is so difficult to lose weight and not gain it back again'. This striking headline referred to two scientific publications, one about obesity and the other one on diet effects on microbiota and DNA. The article on **obesity** was published in Nature by a group at the Weizmann Institute in Israel and focused on the rebound effect which usually follows dieting. This effect, which authors claim to affect about 80% of those who go on weight loss diets, not only leads to the frustration of weight gain, sometimes even more weight than was lost before, but its cyclic repetition may also increase the risk of certain disorders such as **diabetes**. As part of this work, the authors studied the role of microbiota in the rebound effect and concluded that a diet which may induce obesity also triggers changes in the type of bowel microorganisms which remain despite a weight loss programme. At the end of the diet, these altered microorganisms accelerate the rebound effect, which results in new weight gain, greater with every new attempt.

On the other hand, the work digging into the effects of gut microbiota on host DNA, published in Molecular Cell, indicates that the diet may prove key in the balance of these microorganisms. Dr Francisco Guarner, former president of the WGO, admits that the influence of microbiota on epigenetics beyond the colon is a striking discovery. Guarner further remarks that these two studies

make interesting contributions to the understanding of processes involving gut microorganisms, which contain many more genes than those found in the human body, although he claims we are only beginning to learn about this subject.

In this knowledge framework, it is not surprising that yogurt features in diet recommendations in some regions of the world. Although healthy microbiota cannot be fully and accurately characterised yet, it is thought to be diverse and resilient, especially to the effects of antibiotics. It is also expected to be rich in certain species of bacteria which produce beneficial metabolites such as short-chain fatty acids, some of which are thought to reduce serum lipids and the risk of cardiovascular disease. In addition, healthy microbiota should not include inflammatory species like certain Clostridium. In general terms, exposure to non-pathogenic bacteria and the consequent microbiota diversification make our bodies fitter to fight pathogenic bacteria and remain healthy.

The digestive system is then essential, perhaps much more than we imagine, and should be considered as a whole organ. It is, in some way, the mastermind of well and ill-being. When we suffer slight inflammation out of eating fat or sugar, chronic inflammation starts in the digestive system and then spreads throughout. For these reasons, awareness should be raised on what we eat and the lifestyle we choose. If the inner walls of the digestive tract were unfolded, they might cover an area larger than a tennis court. This tract is the interface with food and the external environment, three-hundred square meters which capture the outer world and take it inside the body.

6

KEFIR, YOGURT'S OLDER BROTHER

"What is that?" This innocent question from a student once puzzled Dr Analía Abraham, today a member of Centro de Investigación y Desarrollo en Criotecnología de Alimentos, at Universidad Nacional de La Plata, Argentina. The question referred to the kefir which Adriana Palacios, then her student and today a biochemist, prepared at home.

We know a great deal now about this older brother of yogurt, but there is still much to learn. Kefir is known to be a milk culture rendering a sour fizzy ferment whose taste is due to the presence of lactic acid bacteria. The production of kefir can be thought of as a variation of yogurt preparation. During fermentation, small whitish jelly-like cauliflower-shaped grains grow in the milk and produce double fermentation, lactic acid and alcoholic. Its characteristic flavour is then partly a consequence of the accumulation of lactic acid and alcohol and the production of carbon dioxide.

From a historical perspective, the beginning of kefir production in somewhat uncertain and largely surrounded by myth. One of the many legends claims that Muhammad offered it to his people, warning them that kefir grains would be of great benefit provided their secret properties remained unknown. As is the case with yogurt, the habit of kefir preparation is thought to have begun

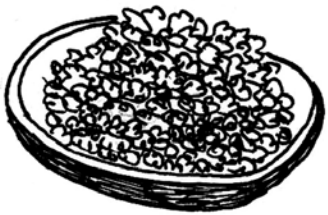
4,000 or 5,000 years ago, when milk was usually stored in bags made up of animal stomach skin and thus harboured a complex association of microorganisms which fermented and gave the first grains. Different versions argue that kefir was first consumed by the ancient Sumerians. As described for the origin of yogurt in Chapter 2, the characteristic kefir grains are likely to have grown on the walls of wooden pitchers where fresh milk was left to curdle in cheese production. These ancient producers then realised that dipping these grains in fresh milk right after milking rendered a drink –kefir milk– which was easier to preserve than fresh milk. And when milk was not at hand, people dipped these grains in water with sugar, which gave a wide range of kefir types in different areas. For example, some studies show that Caucasian kefir, an acidic, moderately alcoholic, fizzy beverage, comes from the manufacture of ayran, a sour frothy drink which also originated in the area. Ayran, in turn, is obtained by fermenting milk in oak containers where bits of calf stomach tissue are added.

The resulting drink is a probiotic food (a concept referring to a better health which will be elaborated on in Chapter 7), as it provides microorganisms which are part of gut microbiota and stimulate bacterial growth and activity in the symbiotic flora, which in turn generates an optimal intestinal microbiological balance. The main virtue of this product is then its ability to regenerate and balance gut microbiota, although folk wisdom gives it all kinds of beneficial properties such as the regulation of bowel function, detoxification, protection against viruses, fungi and bacteria, the stimulation of the immune system, antiseptic and even aphrodisiac effects.

When sharing her first encounters with kefir, Dr Abraham admits she was not quite sure how to approach the study of kefir composition, as she was used to working in sterile conditions and did not feel confident analysing samples riddled with microorganisms. Abraham says she took kefir samples to her workplace, where she was doing research for her thesis on yogurt

KEFIR

Kefir is a ferment of cauliflower-shaped grains which harbours bacteria and yeasts living in perfect harmony.



1- The grains are dipped in milk and left to ferment to obtain a highly acidic milk.

2- This acidified ferment is then strained to remove grains and the resulting milk is consumed.

bacteria and, in particular, on one of the microorganisms generally used to make it, Lactobacillus bulgaricus. The first results on the sample came up as a huge surprise: she found no gram-negative bacteria whatsoever, but rather a world of potentially lactic microorganisms. This finding was a hallmark in her career and eventually led her to the full-time study of kefir, a word which, worth highlighting, is Turkish for blessing.

Scientific studies on the composition of kefir have proven these grains to be complex and long-lasting ecological niches with a highly dynamic microorganism composition and also the ability to keep unwanted microorganisms out. As pointed out above, kefir grains resemble cauliflower in structure but have soft, jelly-like texture, which is why they are sometimes compared to gummy bears. This ferment is made up of a large number of lactic bacteria, acid-acetic bacteria and yeasts, which coexist in symbiosis, a kind of biological harmony. In good conditions, they produce a substance containing polysaccharides and proteins, which constitute the grain matrix, that is everything in it except microorganisms. Within the matrix, microorganisms lead a long life in close association. Acetic bacteria and lactic acid bacteria, or Lactobacilli, produce lactic acid. Yeasts, in turn, produce carbon dioxide and alcohol. In the final mixture, lactic acid is responsible for the sour flavour of kefir (pH 4.2-4.6).

The production of kefir is relatively simple and has remained mostly the same throughout history. The grains are dipped into milk, which is left to ferment and finally gives a very acid, fairly thick or sometimes thinner milk, depending on fermentation conditions. The preparation obtained is then filtered with a cloth strainer, as tradition dictates it cannot get in contact with metals. Grains are then removed, and the fermented milk is consumed. Process repetition gives more and more grains which can be used for more production. This is supposed to be the way kefir production was passed down from generation to generation. Grains dipped in milk can also be frozen and kept for a long time. As a

matter of fact, some grains in Dr Abraham's lab have been stored frozen for as a much as twenty years, when work on kefir began. And, when taken out of the freezer and dipped in milk several times, these grains show they have kept their properties all along.

As with any kind of fermented milk, the beneficial aspects of kefir have been reported by several communities. For example, the longevity of certain ancient villagers has been associated with the consumption of kefir, which was thought to help people keep healthy in times when antibiotics were still to come. Scientific research later corroborated certain properties such as its antimicrobial characteristics and its potential to prevent bowel infection and improve function. Some authors have even suggested anti-tumour effects.

These qualities make kefir a preparation of special interest for scientists, both on account of its composition and properties. As reported by Dr Abraham, a first glance on kefir reveals a highly complex mix of so-called good microorganisms which can inhibit the growth and development of pathogenic ones and produce polysaccharides, which stimulate beneficial bowel microbial populations. Unlike yogurt, the microorganisms in kefir are of multiple types, including different genera and species and also different bacterial strains.

As established in scientific literature, the intake of products rich in microorganisms is followed by microorganism interaction with the individual's own microbiota and their release of metabolism products –usually referred to as metabolites– to the environment. These metabolites in turn take part in an exchange between gut microorganisms and epithelial cells covering the bowel wall. As this is a two-way exchange, scientists regard it as a dialogue in which both microbiota and epithelial cells have something to contribute. The result of this dialogue is a series of products and stimuli which contribute to general health improvement.

This complex framework offers scientists a myriad of aspects to focus on. Experts' views suggest that, when dipped into milk,

kefir grains release part of their microorganisms into the milk later consumed, so that the fermented product contains both grain microbiota and complex microbiota, which brings about changes in milk level of acidity, concentration of lactic acid and acetic acid, and even the appearance of a small proportion of new products. Among these newly generated products, polysaccharide kefiran has been attributed health beneficial properties. For instance, kefiran has been shown to stimulate the proliferation of mouse bifidobacteria, which are part of good gut microbiota.

Several groups of scientists including Dr Abraham's study the composition of kefir grains and fermented milk, and work hard to elucidate the impact of whole kefir on general health, but also the effect of what is known as its non-microbial fraction, made up of the metabolites synthesised by kefir microorganisms during the dialogue with the individual's own microbiota and bowel epithelium. Scientists carry out simple in vitro experiments to analyse the growth of pathogenic or beneficial bacteria, then move on to cell cultures to take a closer look at the molecular systems at play, and finally work on mouse models. In this way, the scientific community has established that the effects of this type of fermented milk, as others, crucially involve not only live bacteria but also the metabolites they produce. In this sense, the most relevant findings of Dr Abraham's group include the isolation of different microbial strains from kefir, and the study of their probiotic properties. This line of work has succeeded in identifying kefir microorganisms capable of inhibiting the growth of Escherichia coli, others which prevent the development of Salmonella, and others which inhibit the proliferation of Bacillus cereus. These studies may have proven utterly unfeasible without the great efforts made to isolate the components of kefir, characterise and analyse them thoroughly.

Kefir is an eminently handmade product, distributed throughout the world. As such, its composition is known to vary across different environments and geographical areas. Some types

do look alike, whereas others have a rather different microbial composition. In Dr Abraham's words, this variability affects some grain and fermented milk properties. Fermentation conditions also considerably modify the characteristics of the fermented product and, although the changes produced by temperature have not been fully elucidated yet, we do know that very high temperatures produce grain destabilisation. For these reasons, kefir stability cannot be fully guaranteed, and this poses an obstacle to product marketing. On the other hand, the fact that yeasts remain viable after bottling makes containers explode as a consequence of gas accumulation. This, in turn, interferes with the addition of sugar or fruit, which would undoubtedly accelerate gas production. In short, although kefir is consumed worldwide, it is only marketed locally.

As the leader of a pioneering group in the study of this preparation which increasingly sparks interest round the world, Abraham reckons that kefir and the lactic bacteria in yogurt may have had a common origin, although she cannot assure it. Nevertheless, the ferment itself has distinctive features, different from all other foods known.

7

YOGURT AND NATURAL IMMUNITY

Although it is hard to pin-point the precise moment when interest was first sparked in gut microbiome and the foods which can modify or balance it, we do know –as discussed in Chapter 3– that the first notions resulted from Élie Metchnikoff's work. We also know that several years ago veterinarians recognised the importance of gut flora in the nutrition, growth and defence of livestock against disease. In Argentina, the first publications on this topic were authored by Dr Luis Bustos Fernández, former head of gastroenterology at Hospital Italiano de Buenos Aires, who acknowledged the key role of bacterial metabolism in the human colon already in the 1960s.

According to Bustos Fernández' disciple and current honorary head of the same service Dr Juan de Paula, **the location of microbiome in the body** is of considerable relevance. These beneficial bacteria live mainly in the colon, where they take part in nutrition and defence functions. On the one hand, they ferment nutrients which cannot be naturally absorbed, allowing the body to incorporate the products of such process as a source of energy. On the other hand, they constitute a bar to pathogenic germs, as they use the nutrients which germs feed on to produce organic lactic and acetic acids, which further inhibit the growth of harmful bacteria. In addition, beneficial bacteria can produce small molecules which eliminate harmful microorganisms.

This is why experimental animals with no gut microbiota –so-called germ-free– exhibit chronic diarrhoea and are prone to infection. This also explains why changes in microbiota modify our natural defences and the level of physiological inflammation. De Paula goes on to highlight the increasing amount of evidence showing that this level of physiological inflammation partly determines the risk of highly frequent diseases such as autoimmune disorders, arteriosclerosis, diabetes and obesity. As a matter of fact, the crucial effect of **prebiotics**, i.e. substances which promote the development of probiotics, and **probiotics** themselves, a term coined by Roy Fuller in the late 1980s to designate beneficial body microorganisms, is reducing the level of unnecessary inflammation. This protective effect is now the focus of major scientific work, with a view to reducing the incidence of endemic disease in modern society.

Beyond scientific efforts and their areas of interest, the relevance of microbiota has captured the attention of popular science and society in general. As proof of this, the book *I Contain Multitudes*, published by popular science writer Ed Yong in 2016, summarises some of the most amazing data about the community of microorganisms that dwell in us. The text explains that microbes help to shape the immune system, even in species as diverse as mice, tsetse flies and zebrafish. These microorganisms promote the creation of whole classes of immune cells and the development of organs which produce and store these cells. They are especially important at the beginning of life, when the machinery of immunity starts working and gets in tune with the world.

The ability of microbiota to regulate the immune system has long dazzled the scientific community. In 2002 and working on immune-deprived mice, Dr Sarkis Mazmanian demonstrated that Bacteroides fragilis re-establishes the normal levels of helper T cells, a crucial type of immune cell which assembles and coordinates the whole system. In his work, Mazmanian showed that this effect is driven by a single molecule of sugar from Bacteroides fragilis membrane, polysaccharide A or PSA. This result constituted first

proof that a single microbe can solve a specific immune problem. Also in mice, Mazmanian's group found that PSA can prevent and cure inflammatory diseases such as colitis, which affects bowels, and multiple sclerosis, which affects nervous system cells.

In a special symposium under the title From Metchnikoff to the Hygiene Hypothesis, held during the 10th Argentine Conference of Nutrition, Esteban Carmuega, director of Centro de Estudios sobre Nutrición Infantil, explained that lack of contact with microorganisms at the beginning of life increases the risk of allergies and autoimmune disease. This '**hygiene hypothesis**' stemmed from a first and simple epidemiological observation in which Dr David Strachan, Professor of Epidemiology and Deputy Director of the Population Health Research Institute at Saint George's University of London, found that certain changes in family size and household hygiene conditions in the districts near London during the twentieth century had helped reduce the incidence of infection among children. However, Strachan pointed out that the appearance of a greater expression of atopic diseases may have also been an associated factor.

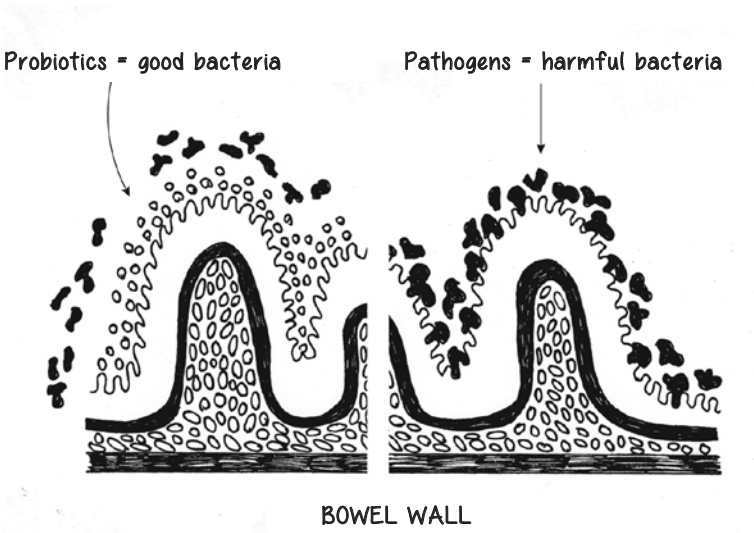
Ten years later, the prestigious journal The Lancet published a paper establishing a relationship between clean environments and atopic diseases, partly because these environments lack certain kinds of stimuli necessary for the early maturation of the immune system. In the New England Journal of Medicine, Jean-François Bach later expanded the theory and argued that the same principle could explain Crohn's disease, multiple sclerosis, type 1 diabetes and other autoimmune diseases. Dr Carmuega remarks that the cleanest environments in urban populations have seen an increase in the incidence of autoimmune diseases along with other chronic diseases.

These observations gave way to **the idea of modulating the immune response through food.** Some experimental and clinical studies showed how changes in gut microbiota can be associated with different levels of mucosal response, and this opened a whole line

of scientific exploration. In this regard, Carmuega ironically points out that clean pigs may get fat too, but they certainly have different gut flora and a different proportion of Firmicutes and Bacteroidetes. And today we know that this proportion is associated with different risk levels of obesity. The possible link between microbiota and the development of obesity is explored in several parts of the world, such as at the University of Turku, Finland, under the direction of Professor of Paediatrics and field expert Dr Erika Isolauri. In 2005, Isolauri published a paper in the International Journal of Obesity establishing the effect of maternal exposure to a probiotics-containing solution on the risk of obesity in children. According to this study, ten years after intervention, a lower incidence of obesity was observed in children born to mothers fed with probiotics.

Although probiotics can be taken in several ways, yogurt is the most wide-spread route round the world. But **yogurt is not only a vehicle for probiotics but also food in its own right.** In Argentina, researcher Nora Slobodianik, chair of Nutrition at Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, studies the **role of yogurt** in food intake. In Slobodianik's view, yogurt provides, among other things, vitamins and zinc. Zinc is closely related to growth, as it participates as a cofactor in numerous enzymes related to both energy and protein metabolism. At the same time, zinc is a key mineral in natural immunity, as it is linked to the development and proliferation of lymphocytes in general and T lymphocytes in particular. Slobodianik further explains that thymulin, a chemical hormone produced in the thymus and involved in the development of T cells, needs appropriate amounts of zinc to perform its function.

Singling out the effects of each nutrient or the consequences of deficiency proves an extremely complex task. Dr Slobodianik claims that animal models of nutrition pose the advantage of focusing on a single variable, for example, to establish the effects of a diet lacking a given protein or mineral, which is virtually impossible in studies involving human beings. This allows to determine the effect of protein deficiency in different tissues and organs, and its



relationship with the thymus and other mechanisms. Slobodianik admits it is difficult to establish, in experimental situations, the true absorption of a nutrient. However, by keeping a close eye on what a group consumes and with a good data collection method, it is possible to estimate what an individual or population consume, while bearing in mind that antinutrients can inhibit nutrient availability for absorption regardless of the amount taken.

Despite all these difficulties, research on **the nutritional effects of yogurt** has shown it **to improve the absorption of other nutrients** and thus help strengthen the immune system. For example, the acidic pH of yogurt has been proven to favour the absorption of numerous common nutrients in the diet, particularly calcium. In addition, one of the functions of lactose –the main carbohydrate in the milk of mammals– is to favour an acidophilic microbiota, which contributes to a decrease in colonisation by other microorganisms,

perhaps pathogenic or harmful to nutrient absorption, and improves the absorption of calcium, iron and proteins.

From a **clinical and social perspective**, Slobodianik has collaborated in several studies linking poverty with malnutrition, such as the one carried out among obese schoolchildren seeking medical attention at Hospital Pedro de Elizalde in the city of Buenos Aires. During this study, a hundred samples were collected among poverty-stricken children and the levels of immunoglobulin A were measured in saliva. This immunoglobulin, usually abbreviated IgA, is an antibody produced by the immune system to identify and neutralise foreign agents such as bacteria, viruses and parasites, especially on the mucous membranes. Results showed that all the children included in the study had low values of IgA, which indicates that their immunological defences were weaker than those of well-fed children of the same age. These findings unveil a strong association between nutrition and general health, particularly regarding the immune system and defences against infections.

But in spite of all the evidence supporting acidophilic microbiota and dating back to Metchnikoff's time, finding cellular and molecular evidence on how **these probiotic bacteria contribute to health** was a pending issue for the global scientific community. Dr Gabriela Perdigón is one of the most widely recognised researchers in the field of immune response mechanisms and their relationship with microbiota. After completing her post-doctoral studies at Laboratoire d'Ecologie Microbienne under the direction of Pierre Raibaud, Perdigón began research into germ-free animals and observed how, as their bowels were colonised by bacteria, their immune cells became more competent. This work was carried out in 1978, when Perdigón met British Professor Roy Fuller. Working with Fuller, Perdigón discovered special non-pathogenic lactobacilli which had the ability to bind directly to the bowel epithelium and, shortly after, Fuller actually coined the term probiotics in 1989. Among lactobacilli observed to bind to the bowel wall, Dr Perdigón focused on *Lactobacillus casei* and, upon returning to Argentina,

devoted full attention to the interaction between these bacteria and the intestinal mucosa. Perdigón was then invited by Dr Aida Pesce de Ruiz Holgado and Dr Guillermo Oliver to join Centro de Referencia para Lactobacilos in Tucumán, where she pursued immunology studies with her group in an experimental phase which would later allow the development of the first probiotic milk. A true breakthrough, her group was the first **to obtain scientific evidence on how these non-pathogenic bacteria interact with bowel epithelial cells.** Her approach was simple and straightforward and led to some of the most significant results in the field. Perdigón explains that evidence was already available on the beneficial effects of lactic acid bacteria in studies of stomach and colon cancer, both in animal models and epidemiological studies conducted by Japanese scientists, but cellular studies had not been undertaken yet on the specific effects of these bacteria on the immune system.

The immune effects of yogurt are amazing, but not magical. A dietary matrix containing probiotics, yogurt is food per se and, as such, not only favours the development of microbiota, balancing it and making it more efficient in keeping immune surveillance, but also exhibits important nutritional properties. Worth highlighting, however, yogurt intake can in no way replace therapy.

Considering that yogurt with probiotics is not a therapeutic food, scientific specialists have drawn attention to the reinforcement of innate immunity, which is the first line of defence coming into play against the entry of pathogenic agents capable of causing disease. In this context, regular lactobacilli consumption has been found to lead to an increase in good bacteria in gut microbiota while still helping to balance it. The goal is not complete replacement, but rather a good balance between gram-positive and gram-negative bacteria. This is crucial because an increase in gram-negative bacteria is known to be among the main causes of inflammatory bowel disease. Indeed, several studies have shown that gram-positive bacteria are associated with good immune system conditions, while gram-negative bacteria are implicated in general inflammatory processes.

YOGURT EFFECTS ON MICE

Mice developing tumours show an increase in the activity of carcinogenic enzymes produced by microbiota.

Therefore, regular consumption of products such as yogurt with probiotics manages to keep a balance between bacterial populations, preventing an increase in the number of microorganisms related to what Metchnikoff called putrefaction.

According to Perdigón, microbiota not only regulates mucous immune functions, key in the maturation of immune cells since birth, but also takes part in blood vessel formation, bowel function regulation and bone homeostasis. In this sense, studies have shown that changes in mouse **microbiota can induce alterations in certain aspects of the central nervous system such as behaviour**, the profile of chemical mediators in the brain and susceptibility to depression. The latest findings indicate that these microorganisms even affect human behaviour in terms of level of anxiety or the perception of pain. Although which members of the ecosystem are responsible for direct action is still to be elucidated, some of the bacteria in the gut microbiota are thought to produce toxic substances which affect the nervous system, so that the regulation of microbiota

Yogurt reduces carcinogenic enzyme activity, modifying microbial populations which produce inflammation.

composition may have seemingly neurological effects. This is a new and largely unexplored field of study, but its results increasingly show the relationship between the different microbial populations and the importance of lactic bacteria in the balance of the intestinal ecosystem.

The interaction between lactic acid bacteria and the bowel epithelium opened the door **to molecular studies on probiotic effects.** Bacteria appear to establish a dialogue through their metabolites, thus contributing to maturation and modulating the population of T lymphocytes to control inflammatory processes. This complex mechanism promotes the maturation of the immune system associated with the intestinal mucosa and triggers the release of cytokines, biologically active molecules which activate other distant cells. The effects of probiotic bacteria are much greater than those of commensal bacteria –which are part of gut microbiota but have no significant beneficial effects– because they are more strongly activated, exert direct stimulation and increase the activation and

maturation of immune cells in the bowel. Among their main findings, Dr Perdigón's group has observed that probiotic bacteria stimulate the activity of anti-inflammatory or M2 macrophages, a class of so-called purifying cells. In other words, the regulation of inflammation is not only mediated by T lymphocytes as part of acquired immunity, but also by macrophages as part of innate immunity. In Perdigón's account, yogurt administration in an animal model of ulcerative colitis has shown potential to improve the inflammatory response. Similarly, yogurt has shown beneficial effects in an animal model of colon cancer through its ability to minimise the inflammatory response and prevent tumour cell expansion. Last but not least, further studies have rendered similar results in diabetes and arthritis.

The beneficial effects of probiotic yogurt are hardly the sum of partial effects but rather a synergic result. For instance, Dr Perdigón's group has shown that lactobacilli consumption in a fermented product exerts stronger anti-inflammatory effects than single-handed lactobacilli **consumption.** Considering the results obtained in an animal model of allergy, Perdigón speculates that lactobacilli may be itself important in immune system activation but enhanced by the fermented product in system modulation. As one of the remaining key questions, this research group attempts to single out the effects of fermentation products obtained with probiotic bacteria **once the bacteria have been removed.** Although preliminary results can be described as amazing, Dr Perdigón still warns that more experiments are necessary to draw conclusions.

Studies by Dr Gabriel Vinderola, a chemist at Instituto de Lactología Industrial, Universidad Nacional del Litoral, Argentina, point in the same direction. Vinderola analysed bowel function parameters associated to immunity in an experimental model of mice fed two, five and seven days with fermented milk. Study results showed an increase in the number of cells producing IgA, pro-inflammatory and regulatory cytokines, and interleukin 6, a molecule produced by the bowel epithelium which helps the development of B lymphocytes, cells specialised in the production

of antibodies. In a nutshell, Dr Vinderola says that, after consuming fermented milk, mice had their bowel system on watch and better prepared, without pathological inflammation.

To test **the clinical effects of products fermented with probiotic bacteria**, Vinderola's group infected the mice which had consumed probiotic milk with *Salmonella typhimurium*, a type of pathogenic bacteria causing gastroenteritis in humans, mice and other mammals. Seven days later, control mice, which had been given placebo milk, were already highly colonised even in the liver, while those who had taken fermented milk had a controlled infection mediated by *anti-Salmonell*a IgA. Moreover, animals given fermented milk had 20% lower mortality rates at the end of the study. Dr Vinderola points out that another major difference was observed when comparing the effects of isolated probiotic bacteria administration to those of probiotic bacteria administration in fermented milk, after both groups of animals had been inoculated *Salmonella*. After fourteen days, animal mortality rates were 20% lower in the group of animals given isolated bacteria as compared to the control group and, astonishingly, null in the group given bacteria in fermented milk

These experiments undoubtedly show the relevance of **the matrix in which probiotic bacteria enter the body to become part of gut microbiota**, which is the focus of Dr Vinderola's research. According to specialists, consuming probiotics as naked cells or as part of a food matrix are two different things. In the From Metchnikoff to the Hygiene Hypothesis symposium mentioned above, Vinderola provided scientific evidence discouraging discussion on probiotics as a class, as effects may be quite different among bacterial strains, or even the same but in different magnitudes. And if the food matrix changes, effects can also change.

According to the specialist, there are multiple reasons to pay attention to the matrix in which probiotic microorganisms are incorporated. First, as happens with yogurt, fermentation produced by lactic acid bacteria induces several **favourable biochemical changes.** Second, yogurt or cheese have a shelf life of one or two

months, a period over which probiotic microorganism viability can be guaranteed, which is an advantage over pharmaceutical drugs kept in a medicine cabinet. Finally, it is essential to consider probiotic food in the context of the general population diet. Fermented milk foods are relatively cheap consumer products, easy to transport and available to the general population. In Vinderola's opinion, there is no point in using probiotics, which should be regularly consumed, in occasional snacks such as ice cream.

In turn, a good food matrix makes sense not only in terms of people's health but also of **probiotic bacteria integrity**. According to Dr Vinderola, the food matrix protects the probiotic bacteria during intestinal transit, as food should remain one or two hours after intake in the bowel in highly acidic conditions. Through artificial digestion experiments carried out in his laboratory, Vinderola has found that the viability, thought of as bacterial health, of three strains of probiotic bacteria greatly benefited from incorporation in cheese as compared to isolated conditions.

Discussion on the dietary matrix of probiotic bacteria further fuels debate on how our microbiota is naturally constituted. Breast milk is the first food we come into contact with during development and the initial route for bowel colonisation. It is a rich matrix containing numerous nutrients which include natural prebiotic substances such as galactooligosaccharides. These substances promote the development of probiotic bacteria such as bifidobacteria, which **breast milk** itself also contains. As pointed out in previous chapters, the mother passes her own bifidobacteria to the baby or, to put it in microbiological terms, transfers the beneficial bacteria colonising her own bowels and somehow reaching the mammary gland. This is one of the key factors which make breast-feeding essential. Somewhat controversial support for this notion has been obtained from studies suggesting that children born by C-section have a higher risk of allergies or immune disorders. According to Dr Vinderola, babies receive bacteria and educate their immune system during the first two years of life, in a way telling this system

what is right and what is wrong. If children cannot educate their immune system during those first years, their susceptibility to allergies will probably be greater in adult life.

Much like Dr Perdigón, Dr Vinderola points out that these experimental results do not mean that probiotic foods provide a final cure for the disorders under study, but that they should be regarded as a complementary or preventive strategy. In turn, not all bacteria have the beneficial effects described, and studying genus and species –for example, *Lactobacillus casei*– may not be enough, as specific strains also play a role. Among thousands of bacterial strains, some have received more scientific attention than others, and some are even regularly used in food industry, although their benefits have not been scientifically verified.

Cancer research is one of the fields in which the distinction between preventive and complementary effects of probiotic foods as part of therapy gains prominence. As already discussed, interaction between microbiota and innate immunity is necessary for healthy development, and the microorganisms incorporated through diet interact with native microbiota in ways which indirectly affect the immune system. As reported by Dr Alejandra Moreno de Leblanc, another researcher at Centro de Referencia para Lactobacilos specialised in this subject, it has been well established that probiotic bacteria can modulate inflammation, and abundant evidence shows how chronic inflammation can be related to tumour growth and progression. It is not surprising then that related beneficial effects of healthy microbiota are increasingly discovered. For example, certain studies suggest that exogenous mutagenic compounds incorporated through the diet cause lesser damage to DNA when they bind to lactic acid bacteria. Along the same line, epidemiological studies have associated the consumption of fermented milk to a decrease in the incidence of certain types of tumours.

Colon cancer is the most widely studied in terms of the beneficial properties of probiotic bacteria, probably because of the heavy influence of diet in this pathology. Dr Moreno de Leblanc's group

has studied certain enzymes regarded as carcinogenic in an animal model of bowel cancer and found a reduction in enzyme activity when animals received yogurt with probiotic bacteria. This means that yogurt induced changes in the profile of bowel microbial populations and, perhaps through this mechanism, decreased mice tendency to develop cancer. These beneficial effects were in turn contrasted with those produced by an anti-inflammatory drug, with results showing that, even if neither group developed tumours, probiotic yogurt administration allowed animals to reach controlled inflammation, characterised by immune system activation. In contrast, anti-inflammatory drug administration only rendered the inhibition of inflammation, while treatment suspension, unlike yogurt suspension, immediately led to rebound effects.

There appears to be no such thing as a unique mechanism of action for probiotic bacteria. Although far from being magical, the regular consumption of yogurt with this kind of bacteria is considered positive from all points of view, all the more so if it is part of a healthy and well-balanced diet.

8

YOGURT AND HEALTH PROMOTION

Medicine advances by leaps and bounds and today offers treatment for a wide range of disorders. However, one of the great merits of the biomedical sciences is seeking prevention even more than cure. In this sense, strategies for health promotion and disease prevention have become crucial tools in world general development. In the context of the concepts and discoveries discussed in the previous chapters of this book, the growing interest in harnessing the ancient properties of yogurt for medical and health purposes is understandable. We will now describe some aspects of general health which can be addressed through diet and, in particular, through yogurt consumption.

TRANSFORMATIONS OF FERMENTED MILK

As we have commented **in chapters 2, 4, 5 and 7**, milk fermentation by lactic bacteria leads to a product which is greater than the sum of its parts. Following Dr Gabriel Vinderola, yogurt production with adequate probiotic properties relies, among other things, on the choice of the correct bacterial strains. In addition, probiotics must be and stay alive, for which fermented milk or yogurt is the best vehicle available today.

As discussed in **chapter 4**, fermentation produces **changes in the biochemical composition of milk.** First, 4.5% of the lactose present in milk as the main **sugar** is reduced by 25% with fermentation, and further reduced by the action of the lactic bacteria used in yogurt production, which stays alive until consumption. Second, certain proteins which may cause food allergies are degraded and, as a result of this degradation, certain **protein** fragments or peptides are produced which are capable of beneficial biological effects, hence called **bioactive peptides**. Finally, a drastic reduction in pH promotes the absorption of nutrients, in particular of certain minerals.

One of the main proteins in milk, casein has the potential to be broken down into 20,000 different peptides. What peptides are produced depends on what enzymes degrade casein and, in turn, this depends on what lactic bacteria are responsible for milk fermentation. In this way, fermented milk becomes a great reservoir of bioactive peptides which have the ability to modulate the immune system, exert antimicrobial effects, promote calcium absorption and even reach the nervous system to induce satiety, good mood, or even act positively on stress.

As Dr Esteban Carmuega says, fermentation appears to impose a kind of alchemy on the components of the original milk. Carmuega already dreamt of being a researcher and change the world at age 14, long before studying medicine. At the age of 15, he received a real microscope as a present, which would later prove a hallmark in his life. He claims to have studied medicine to become a researcher, then specialised in paediatrics and later focused on a career in nutrition, which combined his teenage dreams and his current profession. Dr Carmuega is now the director of Centro de Estudios sobre Nutrición Infantil, named after his mentor, Dr Alejandro O'Donell.

According to Carmuega, the fermentation process triggers a profound transformation in all milk substrates such as **proteins**, **carbohydrates**, **fats and phospholipids**. At the same time, he

says, fermentation is not only at work during production but all along shelf life. For example, 2% or 3% proteins undergo an additional hydrolysis process –additional to that taking place during production– through the metabolism of bacteria which are still alive. Among beneficial transformations other than the production of bioactive peptides, which have a positive impact even on the cardiovascular system, Carmuega highlights macroscopic changes such as **protein coagulation** resulting from their denaturation and a **decrease in pH**. This proves a relevant effect, as milk goes from liquid to semi-solid, which changes the speed of gastric clearance. When we drink liquids, clearance is fast and so is the absorption of nutrients. The gentler these processes, the milder the increase in blood insulin and glucose, factors associated with a better balance and a longer-lasting feeling of satiety.

Other than changes in proteins, **phospholipids** undergo degradation and give rise to other substances such as conjugated linoleic acids, which exhibit antioxidant properties and have shown, at least in experimental studies, to offer protection against certain diseases. In turn, and as already discussed, one of the key effects of fermentation on carbohydrates is probably a reduction in lactose and the consequent decrease in intolerance to this nutrient, along with the development of bacteria which allow sugar digestion through enzymes called galactosidases.

According to Dr Carmuega, **lactose intolerance** is an endemic condition characterised by meteorism and abdominal pain. People are generally born with lactase, the enzyme capable of absorbing breast milk and whose main carbohydrate is lactose, but stop producing this enzyme over the course of 2 to 10 years. Adults can usually easily tolerate a glass of milk if taken slowly, although higher consumption is likely to cause diarrhoea and general intolerance. This is not the case with yogurt though, as living bacteria produce galactosidase, which dissociates lactose and allows its absorption. Then, to some extent, yogurt helps the large proportion of adults suffering lactose intolerance to increase milk consumption.

Dr Ricardo Uauy, faculty and researcher at Pontificia Universidad Católica de Chile and full professor at Universidad de Chile, agrees that, in containing microorganisms, yogurt offers lactose which has already been partially split into galactose and glucose and which can thus be more easily absorbed. The only peoples having enough lactase activity are those who evolved around yogurt as a source of food, claims Uauy. As humans moved away from the tropics, they had less sunlight and more difficulties with calcium absorption and bone function, and people who do not get enough calcium may suffer from osteoporosis at old age. According to Dr Uauy, who also works as a researcher at Instituto de Nutrición y Tecnología de los Alimentos in Chile and is a Professor of Nutrition and Public Health at the London School of Hygiene & Tropical Medicine, University of London, people living in the tropics get enough **exposure to sunlight** and, as a consequence, reach adequate levels of vitamin D which improve calcium metabolism and bone strength. For those living far from the tropics, exposure to sunlight is reduced and the need arises for a diet rich in calcium and vitamin D, precisely the nutrients found in yogurt produced with fermented milk. It is by no means a coincidence, Uauy points out, that the populations which spearheaded the consumption of bovine milk were mainly those of northern Europe and Asia, less tropical, less sunny areas.

Uauy advocates for yogurt intake at all ages, but particularly among the youngest and oldest. Calcium and vitamin D levels should be kept throughout life, and yogurt is a good source of both. Even people who dislike milk tolerate yogurt. The Nordic populations are almost genetically intolerant to lactose but can drink yogurt, as it is fermented or partially digested by lactic acid bacteria. But yogurt is not only a source of calcium and vitamin D, Uauy adds, but also a carrier of beneficial bacteria or probiotics.

According to Dr Carmuega, two lines of research converge and may render a broader picture over the next ten years or so. On the one hand, **controlled epidemiological** studies are showing how certain diets imply a greater or lesser risk of developing chronic

disease. On the other hand, **experimental studies under controlled conditions** are unveiling the mechanisms through which certain foods are associated with a reduction in the risk of obesity, diabetes, osteoporosis or cancer.

Carmuega further explains that the true nature of a **healthy diet** is still to be elucidated, that associated with better health, a longer lifespan and full functional expression. It has been established, however, that populations who consume more and a wider range of vegetables, especially leaf vegetables of different colours, nuts, dairy and yogurt show a better health profile. In this context, Carmuega claims that yogurt appears as an interesting food to be incorporated throughout life. Yogurt has all dairy properties, such as high nutritional density, understood as a high nutrient yield per calorie, but with better tolerance. Also, in controlled conditions, yogurt shows beneficial effects in processes which may reduce the risk of chronic pathologies. Yogurt cannot be held solely responsible for these beneficial effects though, as this may turn it into a so-called drug. Yogurt should be compared to an almond or an apple, a part of a diet whose regular intake is associated with better health conditions. Carmuega concludes this is just the beginning of the road, a road which may help bridging the gap between epidemiological findings and the underlying molecular mechanisms leading to better therapeutic strategies.

YOGURT AND THE PREVENTION OF OBESITY

One of the most inspiring associations is that between **the consumption of dairy products and the lower risk of obesity**. And this risk is actually even lower with yogurt consumption, as suggested by the studies of Jordi Salas-Salvadó in Spain on the effects of the Mediterranean diet. This effect, however, might be attributed to calcium intake, the intrinsic capacity of Mediterranean people to tolerate dairy products, an immunological effect caused by microbiota, or the regulation of inflammatory activity.

It should still be considered that the benefits of yogurt go hand in hand with high fruit consumption and exercise. In this sense, Richard Atkinson, a prestigious researcher at Commonwealth University in Virginia, has conducted several cross-sectional studies proving that people who eat yogurt regularly –at least seven servings per week– have a 20% lower risk of becoming overweight or obese. Atkinson shares a personal anecdote about his 14-year-old granddaughter, who heard all the facts about yogurt and health from her grandfather and remarked that if people could turn to eating yogurt as a healthy food, they might take up other healthy habits as well. Atkinson then has hope that if people who do not usually eat yogurt begin to do so, they may get healthier and lose weight largely because they will lead an overall healthier lifestyle.

A general practitioner and endocrinologist, Atkinson first took interest in obesity when he found an association with hormone alterations. In his own words, the link between yogurt intake and obesity can be summed up in one simple notion: people who eat yogurt simply tend to weigh less than people who do not, and there are about twenty scientific studies to prove it. However, the big question remains whether eating yogurt will make people lose weight. In other words, yogurt intake correlates with lower body weight but cannot be pin-pointed as its cause.

Although largely unverified, hypotheses on how yogurt acts on body weight include the beneficial effect of yogurt bacteria, the role of beneficial milk proteins –such as glucagon or GOP1 peptide, which stimulate the pancreas and increase sensitivity to insulin–, and the role of calcium intake, which is thought to exert positive effects on weight balance.

In any case, Dr Atkinson says, obesity is a highly complex disease. At least sixty genes have been long associated with obesity, some which cause it and others which help prevent it, and the possible combinations for these sixty genes outnumber the world population.

YOGURT AND BONE HEALTH

Beatriz Oliveri is one of the most outstanding Argentine references in bone health. An endocrinologist and researcher in bone metabolic disorders at Instituto de Inmunología, Genética y Metabolismo, Universidad de Buenos Aires, Oliveri highlights three key nutrients found in yogurt, i.e. **calcium, proteins and vitamin D**, in addition to probiotics and prebiotics.

According to this specialist, after the age of 50 one in two women and one in five men suffer bone fractures due to **osteoporosis**, a condition characterised by a reduction in bone mass, deterioration in microarchitecture and bone strength, and consequent increase in fragility. However, several studies show that fractures are not only caused by bone fragility but also by a decrease in muscle mass associated with aging, a disorder called **sarcopenia**. A study published in the *Journal of Osteoporosis and Physical Activity by Oliveri* and her group shows that, despite having normal bone density, women with sarcopenia have a 1.6 times greater risk of suffering fractures. And the combination of osteoporosis and sarcopenia triples the risk among men.

Yogurt enters the equation as a tool for prevention. Even if several drugs are under evaluation to prevent osteoporosis and sarcopenia, no specific medication is available so far to attack a problem which worsens with age. The currently accepted prevention strategy is based on a triad of **nutrition, physical activity and good levels of vitamin D**. According to Oliveri, it is also essential to increase protein intake to roughly three servings per day to avoid sarcopenia and protect muscle as a reservoir for body regeneration. In this context, Olivery singles out yogurt as a good source of protein. It is estimated that by eating about 1.2 grams of protein per kilo of body weight per day an elderly adult can prevent sarcopenia. In addition, yogurt is a good source of calcium, as two daily doses cover the needs of postmenopausal women.

DIABETES AND YOGURT INTAKE

Numerous factors make yogurt a useful food, says Dr Carmen Mazza, former head of nutrition at Hospital de Pediatría Juan P. Garrahan in Buenos Aires and a widely recognised diabetologist. Numerous studies recommend yogurt intake against cardiovascular disease due to the beneficial effect of calcium on insulin resistance, and against type 1 and 2 diabetes on the basis of probiotics ability to act as immunomodulators to regulate inflammation. This is why Dr Mazza incorporates yogurt in weight loss diets and hyperinsulinemia treatment, with a **preventive rather than a curative perspective**. Both in dairy products in general and yogurt in particular, calcium has a positive impact on insulin resistance, she says.

In a study which she replicated and published together with her group, Dr Mazza examined the effect of dissociating food on insulin resistance. The original work, conducted by Swedish scientists, postulated that the strongest stimulus for insulin is the combination of protein and carbohydrates. This study claimed that, upon feeding the same calories and the same nutrients to a group of insulin-resistant and non-resistant women, but separating carbohydrates from proteins, women receiving the dissociated food reduced their insulin resistance more sharply than the others. Dr Mazza's group replicated the Swedish study including two glasses of milk or two glasses of yogurt in the evening. And truth be told, the specialist points out, when children and adolescents have less insulin, they are less hungry.

Yogurt, in addition, is a type of pre-digested milk. According to Dr Mazza, cow's milk protein casein is a macromolecule which can be rather aggressive to young children's bowels. In the first year of life, casein inflames the intestinal mucosa and is one of the mechanisms by which children lose blood and iron. This is the reason for recommendations to sustain breastfeeding, then gradually shift to diluted milk and finally change to full milk at 1 year or so. After 2 years, the adverse effect of casein gets blurred and is outweighed by the positive effect. According to Dr Mazza, milk is

absolutely essential to meet a child's calcium needs, and something of the sort happens with adults, who should drink half a litre of milk or yogurt per day, particularly women. However, 25% adults have little lactase, the enzyme responsible for digesting lactose, and the consumption of such amounts of milk could therefore cause colic and diarrhoea. In this context, the usefulness of yogurt is two-fold, as the lactose in yogurt is more easily tolerated than that of milk.

THE IMPORTANCE OF YOGURT IN METABOLIC SYNDROME PREVENTION

The Predimed and Predimed Plus clinical trials attempt to dig into the seemingly beneficial properties of the hypocaloric Mediterranean diet. In this context, Nancy Babio Sánchez, an Argentine nutritionist teaching and doing research at Universitat Rovira i Virgili in Spain, assesses the effect of yogurt intake on several factors including the incidence of metabolic syndrome. This syndrome is characterised by a combination of hypertension, abdominal obesity, alterations in HDL levels –the so-called good cholesterol– and increased triglycerides. The goal in these trials, Babio Sánchez explains, is to use data from epidemiological studies to assess the habit of yogurt consumption in people with high cardiovascular risk. During the investigation, people who consume more yogurt have shown lower risk of metabolic syndrome factors and diabetes. Some of the most striking data obtained is related to yogurt fat, as results show that those who consume whole yogurt have **lower prevalence of diabetes, hypertension, abdominal obesity, and lesser alterations in lipid levels**. This constitutes, in the views of this researcher, a turning point in widespread nutritional recommendations and tangible proof against the once acclaimed benefits of skimmed dairy. In addition, results showed that people who toe the Mediterranean diet line have 30% lower risk of suffering heart or brain stroke. The amazing fact about this finding is that the level of protection achieved through diet compliance is equivalent

to that obtained from statins, the routine drug prescribed to prevent myocardial infarction via cholesterol reduction.

These results, published in 2013 in *The New England Journal of Medicine*, had great impact on American nutrition and led to modifications in nutrition guidelines. A low-fat diet is no longer recommended, but a selected fat diet is, says Babio Sánchez. As another high-impact and novel factor in these guidelines, talking of nutrients should be replaced by talking of food, as it has been observed that saturated fats are by no means all the same. Fats in yogurt and milk, in particular, seem to be protective against vascular risk disorders, the chronic disease ailing the world these days. Mediterranean diet fats are then beneficial for cardiovascular health, that is, they can be regarded as cardio-healthy along with the fats coming from nuts or olive oil.

Babio Sánchez took interest in yogurt as a member of the dairy food family, one of the food groups most widely and increasingly valued in research for their health benefits, especially in the current study of microbiota. Using consumption data and paying attention to the prevalence of chronic disease, Babio Sánchez' group came up with two major findings: first, that individuals who consume more yogurt have 40% lower risk of developing diabetes and, second, that full yogurt counteracts the negative effects of added sugar, probably due to its protein and nutritional matrix. In the third place, the group has shown that those who consume more yogurt are more likely to reverse abdominal obesity.

Another key recent finding is that calcium and bioactive peptides in yogurt act upon the levels of angiotensin, a hormone regulating hypertension. Recent work has also shown that milk, yogurt and cheese fat **contains certain fatty acids of odd length** –15 and 17 carbon atoms– which are linked to protection against diabetes in favouring tissue sensitivity to insulin, a mechanism which compensates insulin resistance, characteristic of certain stages of diabetes. In this sense, a Harvard University study recently published by Dariush Mozaffarian found that individuals with

higher levels of these fatty acids had better metabolic levels, better sensitivity to insulin and lower risk of diabetes. However, this does not seem to apply to fatty acids in red meat, which further tilts the balance in favour of dairy products.

Dr Atkinson views discussed above speculate that yogurt intake might be accompanied by other healthy habits, and that it is perhaps these other habits that ultimately reduce the risk of chronic disease. Indeed, Babio Sánchez agrees that yogurt consumption is an indicator of overall diet quality, and further comments that people who take yogurt have been also shown to eat more healthily and exercise. However, Babio Sánchez points out that her studies unveil, beyond these healthy habits, an intrinsic protective power of yogurt against chronic disease.

This is exactly the point Andre Marette makes when discussing the consumption of yogurt and the **incidence of cardiovascular disease**. Scientific director of nutrition and functional food at Université Laval, Canada, Marette points out that nothing is certain yet as to whether yogurt is an indicator of a good diet or healthy eating habits associated with less cardiovascular disease, or yogurt itself has a mechanism of action which can explain its cardiac and metabolic benefits. That is the issue which Marette's research aims to tackle, with a view to demonstrating the benefits of yogurt in three animal models and, hopefully, pursuing studies in humans in the future.

Dr Marette's animal studies aim to prevent the development of metabolic disease in obese mice which he feeds diets containing or not containing yogurt. The obesity model includes high levels of sucrose and the general goal is to show that diets comprising yogurt can prevent or ameliorate metabolic syndrome. These studies also compare the effects of yogurt with conventional lactic bacteria to those of yogurt with probiotic bacteria.

Marette also refers to the supposedly beneficial effects of milk fat and points out that the association between cardiometabolic risk and milk fat consumption derives from epidemiological rather than

experimental studies. Although this issue is known to spark great enthusiasm, Marette understands that more science is required to show that yogurt fats are protective and not harmful, and that studies should be further carried out to determine a possible cause-effect relationship, first in animal models and then in humans.

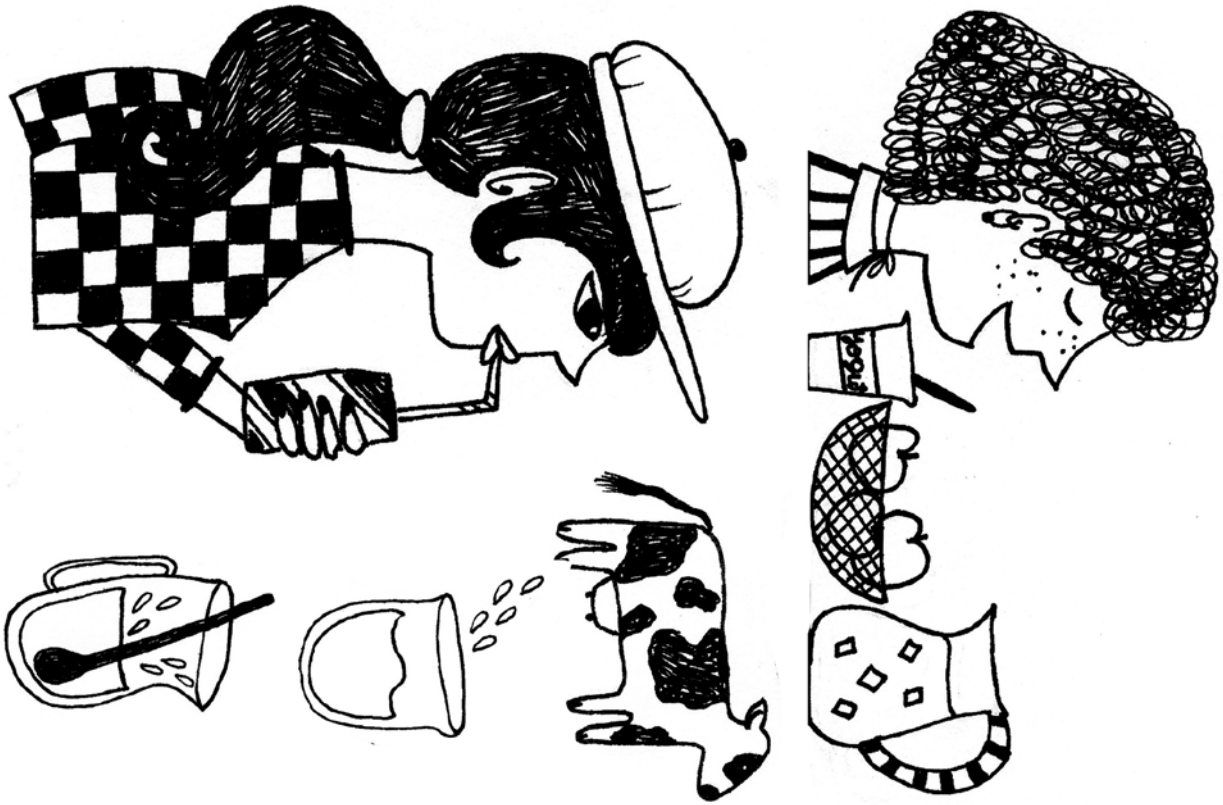
YOGURT IN CHILDREN'S DIET

The implications of yogurt intake among children deserve a separate comment. Paediatrician and nutritionist Mauro Fisberg says that attention to **the role of yogurt in paediatric populations** was first drawn by the increasing consumption of yogurt and cheese –originally intended for adults only– among children who drank relatively little milk. This observation prompted discussion on the possible virtues of these foods, which retrieved historical reports on the benefits of yogurt and, in particular, those describing its effects on the prevention of gastrointestinal problems in children.

According to Dr Fisberg, although yogurt is not indispensable for the whole population, its beneficial properties provide an interesting set of resources which make it attractive in children's diet, for example as a source of calcium, which can be rather low during the first year of life. According to data from studies conducted in **Latin America**, calcium consumption in the region is less than half the recommended amount, and strategies are thus needed to increase supply of this mineral, for example through dairy products. This means that dairy can work as a supplement and help reach the 1000 milligrams of calcium per day required in childhood.

But Fisberg further remarks that yogurt is also recommended for adults, particularly those who have very low dairy intake. The consumption of yogurt may allow this population to supplement dietary calcium and vitamin D, two nutrients which, as mentioned above, help prevent sarcopenia and osteoporosis. The views put forward by Fisberg establish that we should build up our calcium

and vitamin D capital during childhood, as it is between 15 and 25 years of age that we have the greatest possibilities of incorporating dietary calcium and saving up for adult life.



9

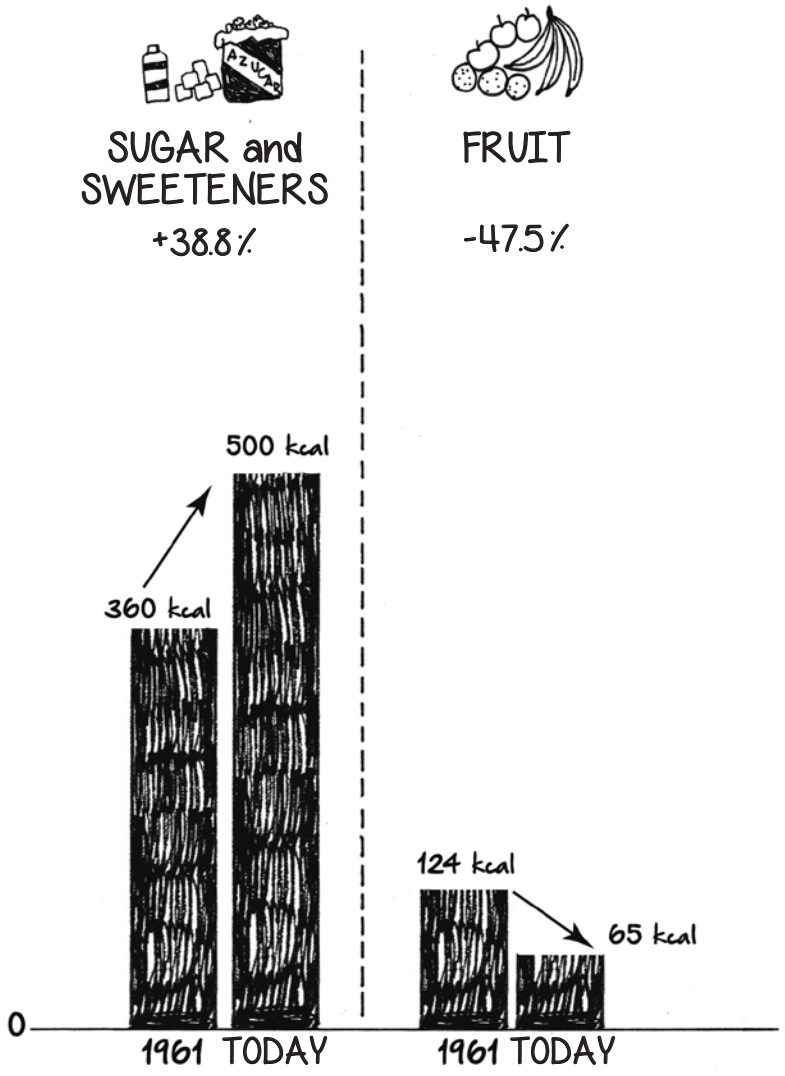
WHAT DO ARGENTINES EAT?

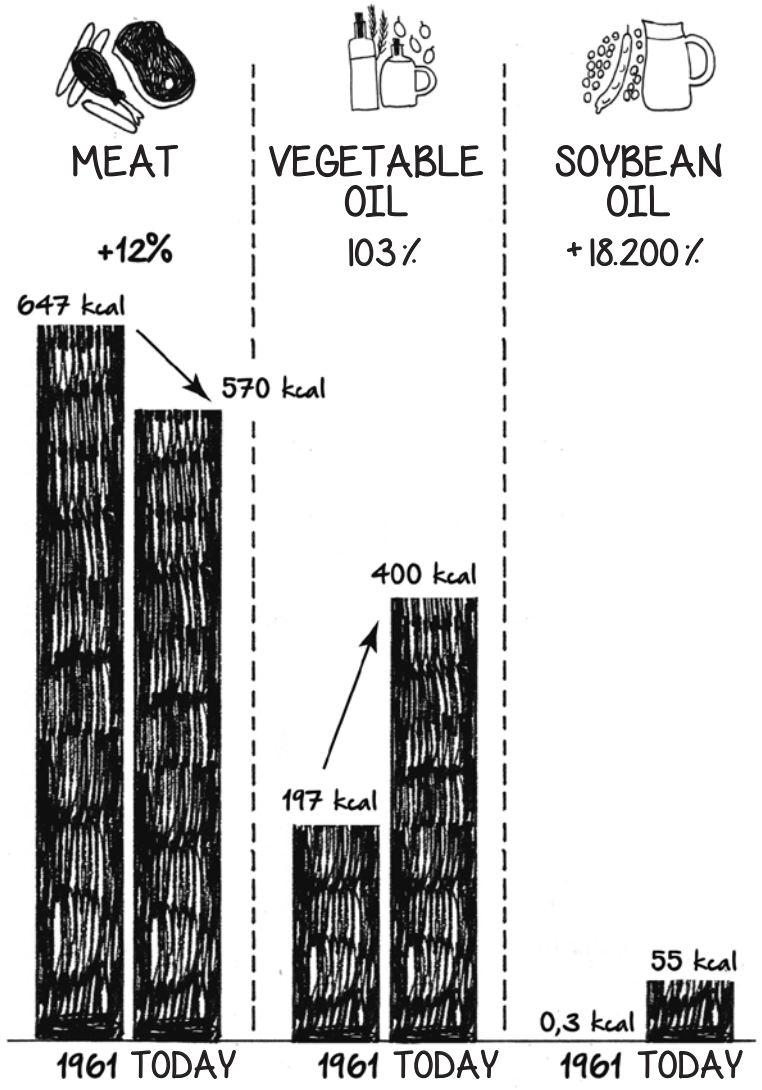
Research on the impact of diet on general health causes increasing concern for adequate nutrition, as the **epidemiological transition** shows a decrease in infectious diseases and an increase in non-infectious ones such as diabetes, heart and neurological disease and dementia.

In particular, the growth in obesity rates and its ubiquity pose a challenge to many of the explanations offered by doctors and researchers. This pandemic disorder in now attributed to the **type of food** we eat rather than the number of calories in it. In fact, a recent study on apparent consumption –which we will define later in this chapter– gathered from food reports by the United Nations Food and Agriculture Organisation (FAO) over the last fifty years surprisingly reveals that Argentines eat today almost the same number of calories as fifty years ago. This work authored by Martín Silberman, who returned to Argentina after working for several years at Universidad Autónoma de Méjico, shows that apparent consumption of daily calories/person/day has remained largely unchanged over the period, going from 3,100 to 3,150. However, Silberman points out, **industrialisation, urbanisation, economic development and globalisation have triggered qualitative changes in food production, processing, distribution and marketing**, with

DAILY INTAKE

Consumption variation since 1961
In kcal per person




MEAT
+12%
647 kcal
570 kcal
1961 TODAY
VEGETABLE OIL
103%
197 kcal
400 kcal
1961 TODAY
SOYBEAN OIL
+18.200%
0,3 kcal
55 kcal
1961 TODAY

a consequent increase in dietary **sugar, fat and oil** intake through mass-manufactured foods.

Sugar, for example, is used in the production of beverages to stabilise the chemical content of salt, to reduce food acidity, to stretch bread shelf life and to inhibit bacterial growth, as high concentrations of sugar help preserve food. Another key ingredient in the elaboration of processed food, vegetable oils have seen apparent consumption more than double, going from 195 kcal/person/day in 1961 to 400 kcal/person/day in 2001. Soybean oil went up from 0.3 to 55 kcal/person/day, while sunflower oil increased by almost 40%. In short, sugar and vegetable oils, alone or in combination, are present in almost all processed foods today.

Dr Julio Montero, former president of Sociedad Argentina de Obesidad y Trastornos Alimentarios, further explains that, although most of the calories we take are of vegetable origin, they do not come from actual vegetable and fruit tissues but from grain-derived products, whose consumption is on the rise. This shift has increased diet energy value at the expense of nutritional value. According to Montero, calorie intake is mostly made up of flours and sugars, which represent more than two fifths of total calories and may reach an overall half when considering the empty calories of starchy vegetables. Vegetables, beans, peas and fruit have lost ground to flours and sugars and, as Dr Montero warns, the human body behaves as a biological unit, and the proportion of body fat/non-fat tissues depends not only on the number of calories taken but also on their quality and their association with additives, which determine their metabolic and addictive effects.

A national survey carried out by Centro de Estudios sobre Nutrición Infantil (CESNI) has rendered utterly devastating results: consumption of fruit and vegetables is going down, while that of rice, cookies, pastry and sodas is going up. When consulted on this issue, CESNI director Esteban Carmuega observes that the last twenty years have witnessed modifications in the structure of food rather than its nutrients. Dr Carmuega, along with fellow

CESNI members María Elisa Zapata and Alicia Rovirosa, conducted a major study on the changes in food and nutrient intake among Argentines over the last two decades and found, much like Dr Silberman, that sugar intake has increased, mainly on account of sodas and beverages, even when calorie intake has remained the same. And this happens mostly among lower-income groups, which consistently show an increase in overweight and obesity rates. The general picture is that of a diet low in critical nutrients and high in caloric value.

This nationwide study was the first of its kind to be conducted in Argentina and, after two years of data processing, unveiled the consumption patterns of different social groups and the nutritional value in their diets. The findings obtained provide in-depth knowledge of household staple diets according to income segments and the changes in food and beverage consumption over the last seventeen years. Public-access data from records of weekly family purchases were obtained from information collected by Instituto Nacional de Estadística y Censos (INDEC) and allowed scientists to estimate adult food intake and then convert food into nutrients, which yielded relevant findings on variations in consumption patterns over time.

Some of the most striking results show, for example, that between 1997 and 2013 **fruit consumption fell by almost 50%**, from 155 to 92 grams per day. Consumption of **soft drinks doubled in general** –from half to a glass per day– and quadrupled in lower-income households, as did the purchase of packaged juice during the same period. Ready-to-eat meals such as pizza, pies, sandwiches and empanadas also quadrupled, while consumption of pre-packaged meats such as burgers and local milanesas tripled. People eat less home-made bread but more cookies and pastry. Intake of free sugars, on the other hand, is higher than the recommended 10% and has increased mainly due to the habit of drinking packaged juice and sodas.

According to specialists, the local diet shows poor variety when studied in the light of public health recommendations. Nutritionist

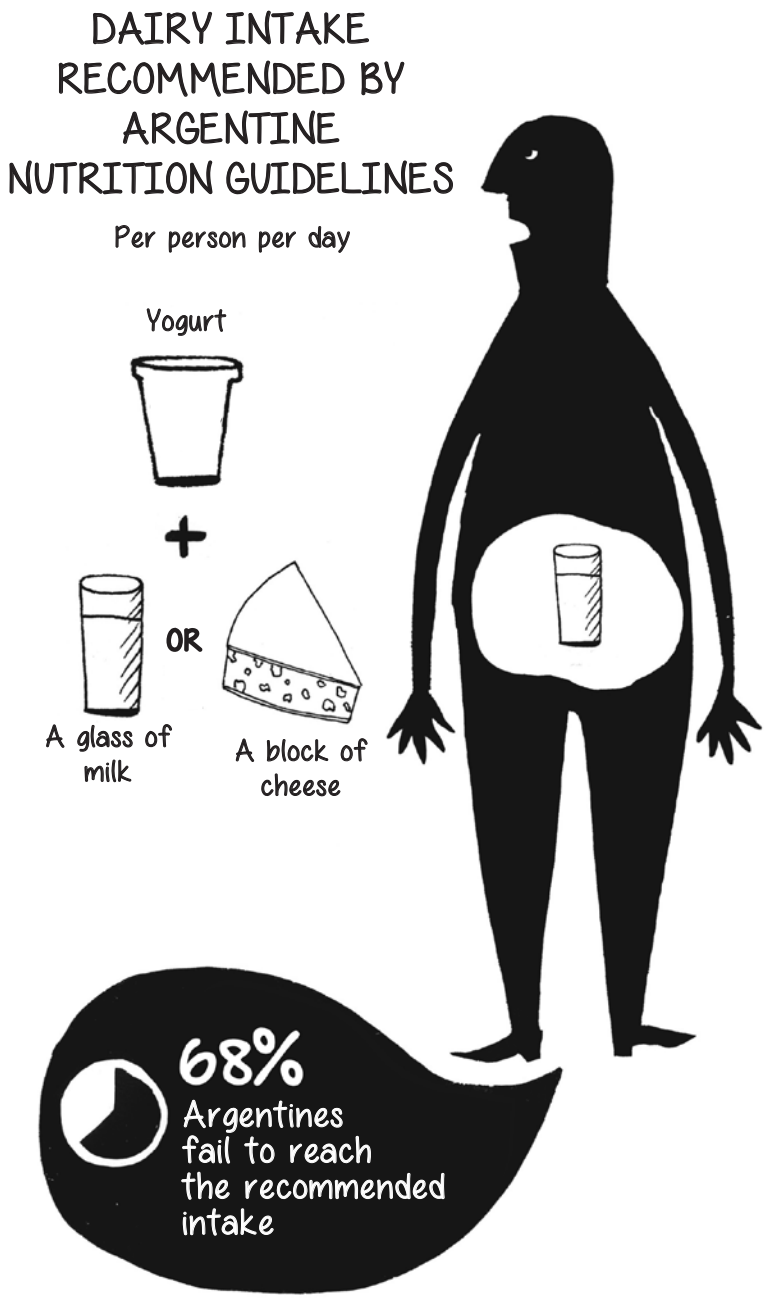
María Elisa Zapata estimates that less than ten food types account for half of the daily calorie intake, i.e. bread, sunflower oil, beef, sugar, dry pasta, rice, wheat flour, cookies and soft drinks. Zapata also remarks that only soft drinks and cookies have been newly added to the diet over the last seventeen years. To make matters worse, Argentines eat only half the 400 grams per day vegetables recommended by the WHO. Most dauntingly, **this poor variety is observed at both ends of the household income range.**

Grain-derived products show similar consumption rates across income levels and over time, but exhibit changes in composition: home-made bread has lost ground to packaged bread, as well as to packaged pie crust and fresh pasta, while the consumption of rice and dry pasta remains stable. In addition, one of the most interesting changes is the increase in consumption of pre-packaged foods, either as take-away or restaurant meals.

YOGURT IN THE ARGENTINE DIET

María Elisa Zapata also conducted a study on the consumption of dairy products in Argentine big cities, which showed consumption to decrease right as from school age. According to Zapata, dairy product intake is adequate among 2 to 4-year-olds, the only group complying with dairy and calcium recommendations for the most part. This consumption begins to fall towards the end of primary school and sharply declines in adolescence, to later partially recover in adulthood, particularly among women of child-bearing age. Men represent the population group with the lowest consumption rates. In general, the study revealed that 90% adolescents, 60% adults and a little lower proportion of children fail to cover calcium intake recommendations.

Data on the population's diet and nutrition may be obtained in different ways. One of them is collection through surveys on individual habits in which subjects are asked to register what they eat. This is how data in the CESNI study were obtained, combining information from a national survey on nutrition and health, a


DAIRY INTAKE RECOMMENDED BY ARGENTINE NUTRITION GUIDELINES
Per person per day
Yogurt
+
OR
A glass of milk
A block of cheese
68%
Argentines fail to reach the recommended intake

nutrition survey in the city of Buenos Aires and the study on dairy consumption patterns.

On the other hand, Zapata explains, part of the data reflects apparent consumption, obtained from purchase records. Researchers estimate that food purchases do not exactly equal food intake but show extremely high correlation. These data are obtained through household expenditure surveys, which in Argentina have been conducted on three occasions: the nationwide survey of 1996/1997 and two previous ones with a narrower scope. In turn, INDEC has carried out another three surveys over the last twenty years, in 1996/1997, 2004/2005 and 2012/2013. These surveys are representative of the whole country and the different income levels. In addition, unlike other countries where only prices are recorded, data in Argentina are collected in terms of grams or kilos of food purchased, for example, how many kilos of potatoes, bread or milk a person has bought in the course of a week, their household size and member ages.

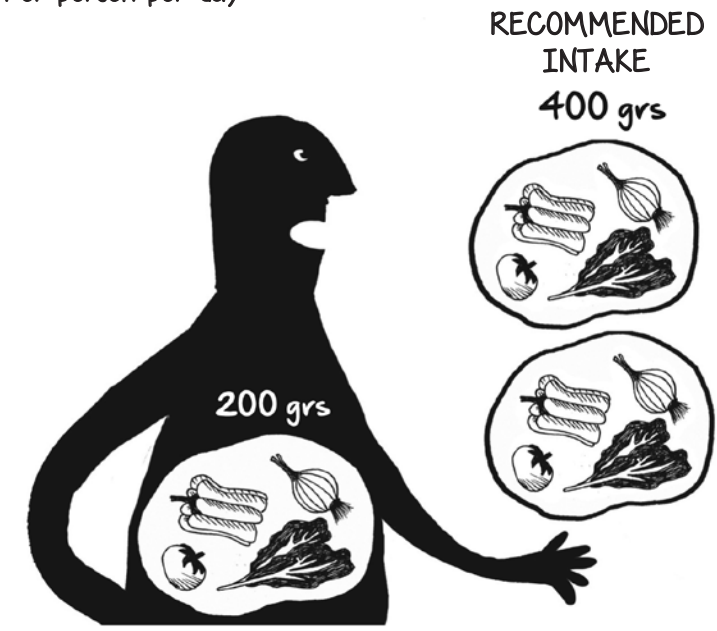
Different methodologies can be applied to these data to infer what people actually eat. In recent years, Zapata has been working on the development of a method to convert this information into grams of net food, which consists of eliminating waste with a correction factor and then determining, quite accurately, what food is available in a given household of a given size for one week. INDEC uses a different method. Lower-income households tend to have more children and fewer adults; as income levels grow, the number of adults goes up and that of children goes down. Then, Zapata explains, to smooth down disparity, INDEC uses a unit referred to as the 'equivalent adult', which is roughly the daily consumption of a 30-year-old man, about 2,700 calories. This method allows to estimate average food intake in grams for an equivalent adult in each income quintile. Regarding yogurt, consumption has gone from 24 to 33 grams per equivalent adult over the last twenty years, which, measured in 125-gram pots, means approximately one and a half pots on average per week.

This study reveals the gap between different income quintiles. In 1996/1997, an adult in the first quintile consumed a quarter of the yogurt consumed by an adult in the last quintile, that is a four-fold difference between the poorest and the richest. Today, that gap is only half, with about 30 grams in quintile 1 and 42 grams in quintile 5, which reflects an increase in consumption over the period.

However, the equivalent adult method raises some controversy, as yogurt, for example, contains nutrients such as calcium whose needs are lower for men than for women or children in comparable situations. Children, teenagers and pregnant women were traditionally advised to eat three servings of dairy per day, while the rest of the population were advised to take two. And, although these days three servings are recommended across the board, no group in Argentina, not even the highest-income ones, can follow these recommendations.

Even when all dairy provides protein and calcium, a closer look quickly reveals differences among products. Milk and yogurt have similar nutritional composition, but yogurt usually contains added sugar, which is not the case with milk. Cheese, on the other hand, only keeps casein after serum protein removal and has high levels of fat and sodium. As a nutritionist, Zapata often listens to patients who do not like milk or yogurt and wonder whether they can substitute calcium through cheese. But the answer to this question depends on the rest of the diet and how much fat and sodium the patient can take. Worth pointing out, yogurt has some extra virtues. Commercial yogurt contains added vitamins and proves a solution for adults suffering lactose intolerance in providing pre-digested lactose, one of the key advantages over milk.

A tool developed in France with CESNI counselling allows to evaluate the actual chances of food substitution for a given population segment. As Zapata explains, and from a practical point of view, it is easier for a person to increase intake of a food type which is already part of their diet than to introduce a new type. And, in this sense, the tool helps identify the possible substitution cases.



Then, substituting yogurt intake for snacks, for example, may allow to estimate the increase in consumption of critical nutrients such as calcium, bearing in mind that this change will only be possible for those who already take at least some yogurt as part of their diet. This instrument then determines the degree of substitution and the proportion of the population which may, for example, increase calcium intake through yogurt. Results in Argentina show that about one fifth of the population might increase yogurt intake, while the rest will probably not, simply because they do not eat yogurt.

As discussed above, calcium intake decreases with age as from childhood, and most adults fail to follow recommendations. Low calcium consumption affects 26% preschool children and 74% older children. A major task for scientists is then to offer expert

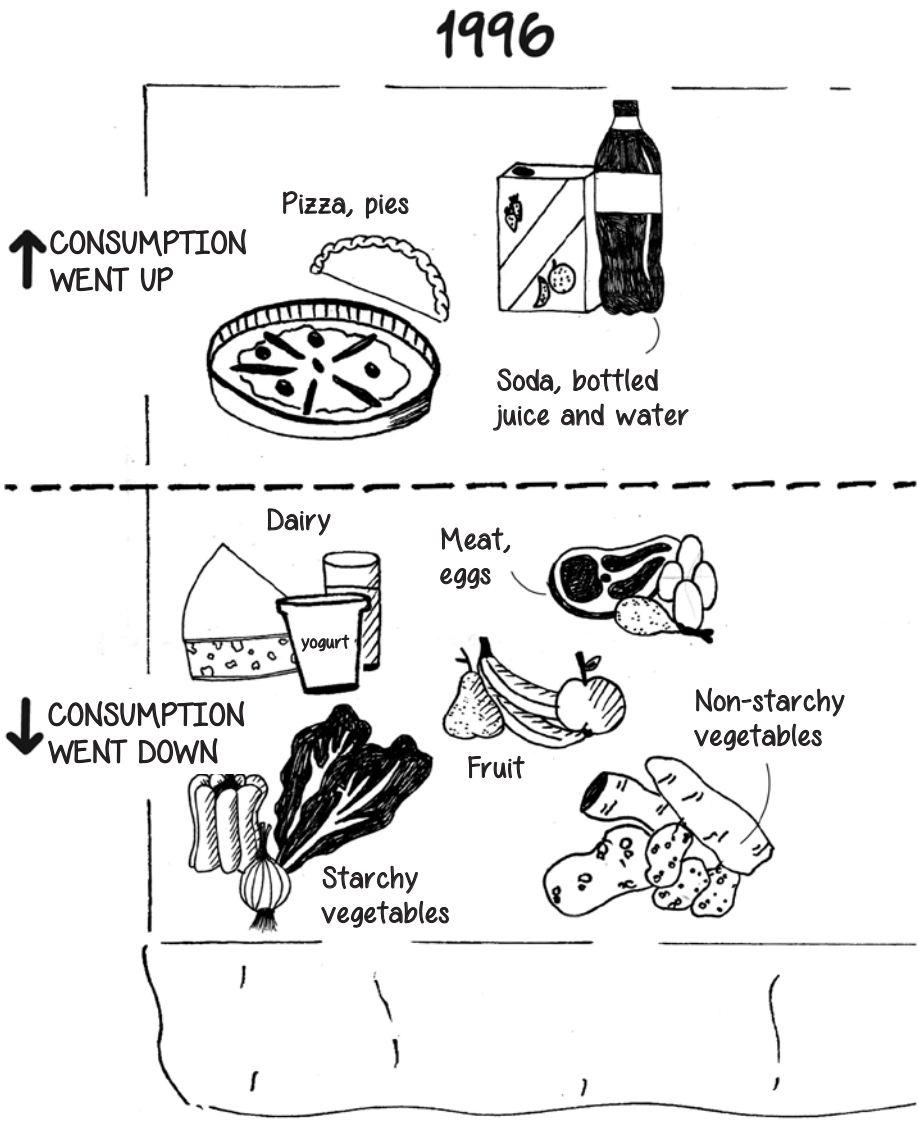
nutritional advice on population habits, which requires the use of complex statistical tools incorporating consumption dynamics, associations and substitutions in real life. In this sense, Zapata's analysis has revealed that the consumption of dairy products by Argentine women in urban groups could be increased by replacing 23.8% infusions, 25.96% cookies and 18.1% breakfast toast, or else replacing 24.4% infusions, 25.3% cakes and pastry, 22.6% toast and 18.15% snacks, with dairy. And, provided these substitutions involve low-sugar low-fat yogurt fortified with calcium, simulations show that free sugar consumption might be reduced by 10%, saturated fat consumption should remain stable and calcium intake might double, which would reduce the calcium intake gap from 64% to 11%.

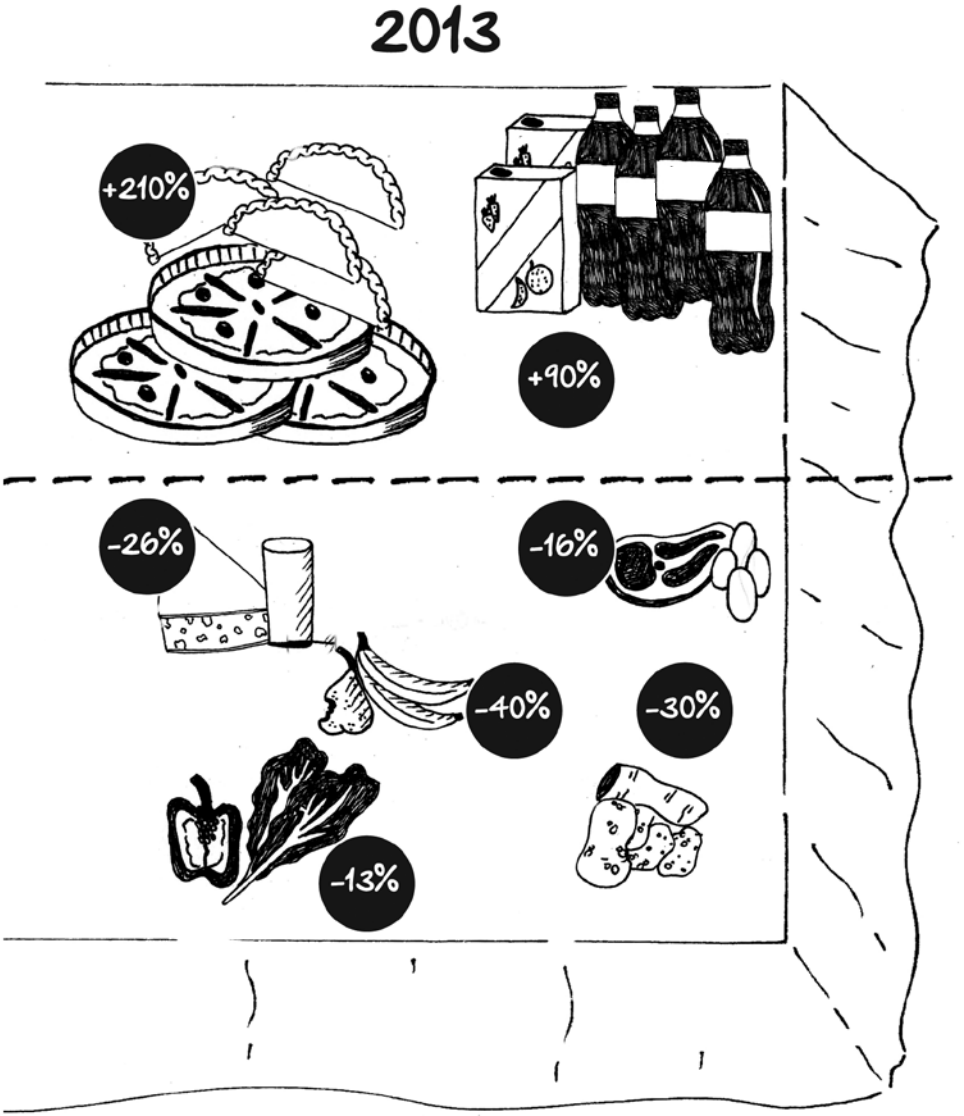
As a matter of fact, the Argentine diet is rather monotonous and there is a population segment which virtually never eats yogurt. For example, on the day the survey was conducted in the city of Buenos Aires, only 10% women of child-bearing age and barely 28% children from 7 to 23 months of age were found to eat yogurt, both groups regarded as of high consumption levels. Similar results were obtained for fruit and vegetables intake.

All children might be expected to eat fruit every day or, at least, three to five times a week, Zapata reflects, but this is not the case. For example, reports show general dairy product consumption, except milk, in 93% children from 7 to 23 months, but regular consumption in only one third of the population. In other words, most people eat dairy sooner or later, but only one third do it regularly.

Although household expenditure surveys have shown that the poor variety of the local diet becomes richer as household income grows, higher-income segments do not necessarily cover recommendations for all nutrients and foods. This is the case with fruit and vegetables, food types whose diet share has shrunk over the last twenty years and is still moving further away from minimum WHO recommendations.

Zapata goes on to explain that the local diet does become a little more diverse as income grows, but this diversity does not


1996
CONSUMPTION WENT UP
Pizza, pies
Soda, bottled juice and water
Dairy
yogurt
Meat, eggs
CONSUMPTION WENT DOWN
Fruit
Non-starchy vegetables
Starchy vegetables


2013
+210%
+90%
-26%
-16%
-40%
-30%
-13%

necessarily mean higher nutritional value. In any case, low dairy consumption is registered in all quintiles, while meat consumption has seen a shift from beef to chicken and pork. But perhaps one the most relevant findings, the survey shows an increase in the consumption of pre-processed meat products such as hamburgers, sausages and nuggets, strikingly not only in high-income segments.

The incorporation of women into the labour market –currently half of those within the working age range– triggered changes in the way families eat, cook and buy. Meals no longer involve families sharing the table, as children eat at school, teenagers eat in their bedrooms watching movies and parents eat at work.

In Zapata's terms, good nutrition is a habit and, like any habit, needs to be first incorporated and then systematised. CESNI research shows that habits which used to change at school age now change before, and 2 or 3-year-old children turn to products which should not be part of their diet such as pre-packaged or processed foods. These days the consumption of candy, soft drinks and ready-to-eat meals begins at a younger age. And in tackling this issue, school and especially the family play a key role. Soft drinks and candy are good weekend or birthday treats, but everyday meals should mean water and everyday breakfast should mean milk or yogurt.

10

CONTROVERSIES IN NUTRITION

Few areas of health have fuelled such fierce debate in recent years as nutrition. With a rampant obesity epidemic getting out of control, the once worshipped nutritional advice issued by medical associations over the last fifty years is now increasingly frowned upon as a global scale experiment. If recent claims prove true, this advice may have led humanity to eating habits which unbalanced natural body circuits and boosted overweight and obesity rates, which in Latin America and the Caribbean are over 50% of the population.

Ancel Keys' famous Seven Countries, *A Multivariate Analysis of Death and Coronary Heart Disease*, which proposed a reduction in fat intake to prevent heart disease and stroke, is a paradigmatic example of the stormy battle waged by nutritionists and epidemiologists seeking the best diet to live longer and better. After decades as a golden standard, the work is now harshly and increasingly criticised partly due to serious methodological flaws, as only seven countries out of a total of twenty-two appear to have been *cherry-picked* to support the author's hypothesis. In addition, some experts argue that, by discouraging fat intake, the work led humanity to replace fat with large amounts of carbohydrates, responsible for metabolic syndrome, diabetes and other chronic diseases.

Another controversy found the Pan-American Health Organisation (PAHO) in the dock. In 2016, this organisation published a novel nutritional profile classifying foods as natural, processed or ultra-processed. In this new profile, which sparked heated debate in the region, PAHO included yogurt among the ultra-processed foods which should be hence avoided.

Dr Chessa Lutter, PAHO's Senior Nutrition and Food Advisor and a researcher at the National Academy of Sciences in the United States, claimed then that this new instrument provided information based on the best scientific evidence available. The document was written by widely renowned Chilean nutritionist Ricardo Uauy, as the head of a team which also included Carlos Monteiro, Juan Rivera, Lorena Rodriguez, Dan Ramdath and Mike Rayner, along with Enrique Jacoby, Chizuru Nishida and Lutter on behalf of PAHO. Most strikingly, Dr Uauy had repeatedly singled out yogurt as a most healthy type of food and admitted that its inclusion in the ultra-processed group had been based on sugar content.

This PAHO tool had immediate and far-reaching repercussions. In an article published in Argentine newspaper *Clarín* in 2017, two specialists of high media exposure, Monica Katz and Alberto Cormillot, criticised the ultra-processed food classification used by PAHO, which included foods containing ingredients not normally used in home cooking, synthetic substances and additives, such as snacks, soft drinks and nuggets, all packaged and ready or almost ready to eat.

Sergio Britos, director at Centro de Estudios sobre Políticas y Economía de la Alimentación (CEPEA), quotes a study by Dariush Mozaffarian discussed above which includes yogurt in a long list of food types associated, in epidemiological non-causal terms, to benefits in weight balance. The study shows yogurt consumption correlation with lower weight gain in all groups. And, although the underlying mechanisms are still unclear, evidence hints at changes in colonising bacteria. Other unmeasured but probably relevant factors include changes towards generally healthier habits by people

who incorporate yogurt. This study focused on data collected by the American Nurses' Health Study (NHS), with a cohort of over 120,000 nurses enrolled in 1976, another of nearly 117,000 younger nurses enrolled in 1989, and a prospective study of 51,000 doctors from across the country enrolled in 1986. Participants were followed up using biennial validated questionnaires including information on diet, exercise and smoking, with exclusion criteria involving obesity, diabetes, cancer, renal, cardiovascular and liver disease. The final analysis included more than 98,000 women and 22,000 men.

Britos argues that the point in question is the frequency, variety and amount of intake for any given ingredient, as no food is harmful per se when consumed in a moderate way. As for ultra-processed food, what matters is the amount of sodium, sugar and, to a lesser extent, saturated fats, rather that it being processed or not. Butter, for example, is not among foods listed as ultra-processed but is still rich in sodium and saturated fat. And using too much sugar in coffee is much more harmful than the worst cookie.

Even if aware that the fewer processed foods we eat, the better, Britos opposes unnecessary food demonisation. Food policies need to deal with prices, ensure that healthier foods have economic incentives to be consumed in greater proportions and regulate industry, which we cannot do without at this point.

Christine M'rini, at Danone, denies the harms of industrial production and points out that yogurt has a very simple manufacturing process. Just milk and bacteria in a large vat, some mixing, some waiting, and yogurt is ready. But, in addition, industrial production can control what and how many bacteria are incorporated and check for unwanted contamination. In other words, no additives, the same as at home, but safer. Also, yogurt bacterial strains Lactobacillus bulgaricus and Streptococcus thermophilus are highly sensitive to antibiotics and thus help ensure that the milk used has no chemical contaminants or antibiotics. These strains also have taste and texture, and specialised staff choose them to comply with certain properties.

From Spain, Nancy Babio Sánchez also disagrees with PAHO's classification of yogurt as an ultra-processed food. Even among dairy, Babio Sánchez claims, toffee pudding is not the same as yogurt or milk. Milk and yogurt have similar characteristics, but yogurt has unique properties through ferments. Cheese is both nutritionally and calorically far from milk and yogurt, and yet features in the same group in PAHO's profile, which calls for thorough revision of nutritional advice given to the population. Sweeteners, for example, are by no means prohibited but rather regulated through tolerable daily intake. The problem lies in overuse, such as drinking two litres of *diet* soda. And although flavour should indeed be taken into account, reducing sweetener consumption should gear people towards less *diet* soda or packaged juice rather than less yogurt. Both in Spain and Argentina, Babio Sánchez remarks, the old habit of drinking water and saving soda for special occasions –she even says her mother used to make juice for her four brothers and sisters– has given way to the widespread consumption of soft drinks, and kids today know nothing else but soda. The soft drink industry has played havoc, she reflects, which is not good.

With sugar and sweeteners now featuring as food villains, criticism of industrial yogurt centres on its content of one or the other. In this regard, Canadian researcher André Marette agrees with Babio Sánchez that adding sugar to yogurt certainly means adding sugar to the diet, but that still falls short of justifying the classification of yogurt as unhealthy food, especially when compared to other sugar sources such as packaged juice, soft or energy drinks. Truth be told, Marette admits, sugar concentration in yogurt should be kept as low as possible, which several companies are aware of now. However, companies also need the product to be appreciated by consumers, which is why a happy medium should be struck in sugar concentrations which are both healthy and appealing to consumers or, even better, have more fresh fruit incorporated in yogurt and thus avoid the need for too much sugar.

Marette also indicates that removing sugar from yogurt may lead people to add it on their own. Research carried out in France shows that people who buy sugar-free yogurt sometimes add more sugar to it than the industry would, which calls for attention to consumer behaviour. The need then arises to make sure consumers like yogurt with sugar contents which are harmless to health, a still largely unexplored field of research.

Richard Atkinson, in turn, is not quite certain that artificial flavours are altogether bad but agrees that sugar intake should be kept low. On the one hand, data on sugar are not solid and its risks are often exaggerated. Just as true, leaving sugar out of the diet may cause no damage whatsoever. Once again, the issue is finding a good balance. Along these lines, Atkinson explains that yogurt is neither perfect nor bad, as it has protein, calcium and bacteria, and that the infelicitous report by PHAO may make people reluctant to buy commercial yogurt and unlikely to take the trouble to make it at home. Discussion with PAHO and WHO officials about paediatric obesity have led Atkinson to describe their views in nutrition and general health as too simplistic, well-meant but not fully supported by evidence.

Atkinson certainly believes that the benefits of yogurt are partly overstated among producers but not in the study published by his group in 2016, as the work presents a review of scientific literature on yogurt and body weight, not even considering probiotics. Other studies have focused on specific types of bacteria and diabetes, while Atkinson's work analysed a significant number of *papers* on classical bacteria yogurt and body weight.

Mauro Fisberg warns yogurt home-making should involve measures to prevent cross contamination and ensure good ferment quality, with little sugar and as few dyes and additives as possible. Still, Fisberg emphasises, almost no qualities are lost in industrialised yogurt and discussion focuses rather on the amount of sugar and dyes. It should be noted that all the scientific evidence available now on yogurt and quality of life and the protection it provides against

diarrhoea, cardiovascular disease and weight gain was obtained through double-blind studies on industry-manufactured yogurt.

Finally, certain naturistic groups object to milk consumption beyond the age of three. However, and except for cases of specific lactose intolerance, in which yogurt may still prove a good alternative, general agreement prevails among the specialists consulted on keeping dairy products as part of the diet. Fisberg explains that we need to rely on dairy as the most readily available source of calcium. In non-dairy-consuming countries, people usually absorb calcium from vegetables and other sources, but this requires consumption in large amounts. Yogurt, in contrast, has very little lactose because fermentation drastically reduces it and is consequently more easily digested even by those with some level of intolerance. Andre Marette also comes up against the views on milk as harmful to adults and points out that milk has been consumed throughout adult life for centuries in regions like the Balkans, the Middle East, and northern Europe, because it is a good source of protein. Elderly people usually take little milk, which is why milk derivates become crucial. Removing milk, cheese or yogurt from the diet can be dangerous then and lead to insufficient protein intake.

Another debate underway is that of the true action of prebiotics and probiotics, which is the subject of a few passages in Ed Yong's *I Contain Multitudes*. Metchnikoff was convinced that beneficial microorganisms could be added to our digestive tract, although microbiologists such as Christian Herter and Arthur Isaac Kendall doubted whether these microorganisms could reach the bowels alive. Japanese microbiologist Minoru Shirota searched for microbes which could reach the intestine safe from stomach acids and found *Lactobacillus casei*, which he used to create the first cultured milk, Yakult. Many food types today include probiotics, some of them grown in live cultures and others lyophilised. These probiotics are bacteria of little presence in the adult intestine, says Yong, as they cannot survive for long.

The WHO defines probiotics as live microorganisms which, when administered in adequate amounts, give health benefits to the host. But, as explained by the scientists interviewed for this study, evidence pointing in this direction is still mostly limited to observational studies in in vitro or animal models. Therefore, Cochrane, a non-profit organisation which reviews medical studies published in a given field, concludes that probiotics can be useful in infectious or antibiotics-induced diarrhoea, or even save lives at risk from necrotising enterocolitis. However, their effect on asthma, allergies, obesity, diabetes and even behavioural problems cannot be assured yet. This is partly why regulators round the world classify them as foods and not as medicines, which prevents companies from advertising them as healing agents.

Active in the nutritional debate in Argentina for several decades, both as a researcher and an industry and public agencies advisor, Esteban Carmuega has got used to discussion and sudden changes in the course of events in the field. Although natural foods are, of course, healthier and better, Carmuega warns that fermentation in uncontrolled conditions in Argentina poses a risk associated to the high prevalence of *Escherichia coli*. Out of all *Escherichia coli*, which are hundreds of thousands, O151 in particular carries a disease in itself which produces haemolytic uremic syndrome, a serious clinical condition in children and the first cause of kidney transplant and dialysis in childhood. In contrast, industrialised yogurt poses no such risk, as all brands involve a double pasteurisation process. Home preparation may be hindered, for example, by dirty hands which contain bacteria and transfer them to milk. Much like meat cross contamination should be avoided among children under six years of age by choosing suitable fridge spots, not using wooden chopping boards and washing forks and knives, yogurt home-making should also be avoided. Carmuega also remarks that comparable yogurt composition should be ensured to validate epidemiological studies underway. This is a research field for the years to come but on

which science is already working today: how to better standardise associations between yogurt consumption and effects observed in epidemiological studies. Whether these associations can be extrapolated to all yogurt types is still uncertain, Carmuega admits, and the yogurt which is best for health, its sugar content and the role of sweeteners need to be fully elucidated.

In Carmuega's opinion, trying to leave industry out of food production is unrealistic. A distinction should be made, however, between producers who merely aim at profits and those who also communicate and educate consumers into healthier habits, consider sustainability and health criteria and modify their products to meet these criteria. Marketing staff should be held responsible for more sensible communication especially for products targeting children, as food needs to meet both hedonistic and health requirements. Two types of intervention may allow humanity to reach 100 or 105 years of active life, probably what our species has genetically determined: physical activity and diet. And both require healthier foods. These are not necessarily those coming from organic orchards, as industrialisation allows food production on a large scale. However, Carmuega remarks, industrialisation processes should be sustainable, developing and advertising products on the basis of scientific evidence. Carmuega's vast experience in nutrition research has persuaded him that it is vital for science and industry to work together, as the chronic diseases currently affecting mostly the developing countries may make healthcare systems collapse in one generation. Leaving the epidemic of obesity and diabetes out of control may force the next generations to allocate their entire health budgets to the treatment of these diseases. In this scenario, and far from the illusion of food miracles or food villains, better food and ethical communication about the benefits of good nutrition prove essential. Carmuega comments on a study carried out by his group on the evolution of Argentine eating habits over the last twenty years in a considerable household sample and sadly observes that Argentines are not

eating better but more lazily: ready-made pies, pre-packaged foods, processed meat, cold cuts and tomato cans. What ongoing household surveys show is that the time devoted to food preparation has been reduced by changes in lifestyle.

Carmuega concludes that food should be tasty, safe, easily available and practical. Recommendations should be made to industry as to what food profiles need to be promoted, communication should be sensible, and consumers should be given clear information and not be misled by *marketing* and advertising strategies. CESNI provides a solid link among basic science, epidemiological research and food manufacturers who

contribute to improvements on the basis of public needs rather than *marketing* figures. Carmuega firmly believes in an ethical dialogue between science and industry to find solutions to an epidemiological puzzle affecting society as a whole: not only industry, not only academia, not only government, not only families. Success in this task is bound to open an interesting future.

11

YOGURT IN EVERYDAY COOKING

by Narda Lepes

I think a lot about what I eat. I think why and whether it makes sense to eat it, and not only if it is tasty. Take tangerine, for instance: tangerine makes a lot of sense. The tree gives shade, the fruit is easily peeled easily and comes in wedges. So, when there is tangerine, let's have a lot. It makes sense. On the other hand, sausage takes hard work. What with slaughtering the pig, removing guts, chopping meat and fat, seasoning, stuffing, ...and waiting. So, let's eat little sausage, savour it, appreciate the work it takes and look after ourselves. Let's eat little sausage.

I have travelled a lot, sometimes to very distant places, far from supermarkets and everything we know. And still some things repeat themselves in different cultures. Chicken soup or broth of some sort is a must everywhere, for example. Another common pattern across cultures is some type of yogurt. As a beverage, in sauce, as dip, in meat marinades, at breakfast, in vinaigrettes, in desserts, pies, dough, bread. Made from cow, sheep, goat, buffalo, camel or yak milk, all of them different and similar at the same time. Some are mild in taste, closer to what we know, and others are sour or strong flavoured, some of them high in fat and others lean. But, basically, all of them are milk and culture. Humans have long learnt how to make milk longer-lasting and more easily digested. And that is the kitchen culture we live on, how we learn and teach what to eat and how to choose and transform what we find in food. It is as mysterious as simple. And by the way, my favourites are Greek yogurt and *labneh*, without a doubt.

Here I share with you some tips on using yogurt in cooking and some useful recipes.

TIPS:

- Before mixing it into preparations, leave yogurt at room temperature.
- If you need to heat it up, always do it slowly and at low heat, or else it will curdle. The best thing to do is add it at the end of cooking (for example, in curries, stews, etc.).
- Yogurt is a good substitute for cream, mayonnaise and cream cheese in several recipes.
- Mind package labels, or you might end up mixing strawberry yogurt into lentils.
- The acidity of yogurt does not get along well with aluminium, so make sure to keep them apart when cooking or wrapping.
- Yogurt will thin down when out of the fridge, so make sure to keep it cool if you need it to be thicker.
- Labneh or Greek yogurt are good options in marinades because they are rather thick and stick well to meats.
- Do not beat or stir yogurt too much and mix it into preparations gently, or else it will get too light.
- Yogurt may be used as a beverage, as in many parts of the world it is drunk either sweet or salty and mixed with herbs, spices, juice, fresh water and, sometimes, sparkling water.

TO USE YOGURT AS A DIET SUPPLEMENT, I RECOMMEND THE FOLLOWING PROPORTIONS:

- To substitute for 1 cup of oil, use 1/2 cup of oil and 3/4 cup of yogurt.
- To substitute for 1 cup of butter, use 1/2 cup of butter and 1/4 cup of yogurt.
- To substitute for 1 egg when baking, use 1/4 cup of yogurt.

12

RECIPES WITH YOGURT, BY NARDA LEPES

Buuz

MONGOLIA

INGREDIENTS

For the dough:

3 1/2 cups of plain flour
2 tbsp of salt
1 1/2 cups of water

For the filling:

700g of chopped lamb
700g of chopped onion
3 spring onions
4 garlic cloves
3 tbsp of coriander
Salt and pepper

For the sauce:

Yogurt

METHOD

- Mix together flour and salt in a bowl. Make a well in the centre and gradually pour in water.

- Pull in flour from the side of the bowl and knead until well mixed in.

- Allow dough to rest for 1 hour in the fridge before using.

- Mix the chopped lamb, chopped onion, spring onions, garlic cloves, coriander, salt and pepper in a bowl, without kneading too much.

- Knead the dough again for 2 minutes and roll it out into a log of 2.5 cm in diameter.

- Cut the roll into 2.5-cm slices. Roll each slice into a ball and lightly dust with flour. Flatten it a bit, then roll it out into a circle about 10 cm in diameter. Make the centre slightly thicker than the edge.

- Place about a teaspoon of filling in the centre.

- Close as small bags, so that there is a small opening in the centre of the top.

- Dip the bottom of each bag into a bit of oil.

- Steam for 20 minutes.

- Use **yogurt** as sauce.

Chusky Burek

BULGARIA

INGREDIENTS

For the peppers:

8 peppers
500g of feta cheese
100g of grated cheese
3-4 crushed garlic cloves
1 tbsp of thyme
50ml of olive oil
5 eggs
Salt and pepper
1 tbsp of baking powder
300g of bread crumbs

For the sauce:

2 crushed garlic cloves
2 tbsp of fresh dill
2 tbsp of parsley
500g of sour **plain yogurt**
1 tbsp of olive oil
1 tbsp of vinegar
1 tbsp of salt

METHOD

- Barbecue the peppers until their skin becomes dark brown. Then peel off the skin and carefully clean the seeds from inside.

- Chop the feta cheese and mix with the grating cheese. If the mixture is too thick, add feta cheese water.

- Add the crushed garlic, thyme, olive oil and 1 egg.

- Season with salt and pepper and mix.

- Fill the peppers with the mixture. Beat the 4 remaining eggs with salt and baking powder. Dip the filled peppers in egg and then in bread crumbs and fry.

- Make the sauce by mixing garlic, herbs, **yogurt**, olive oil, vinegar and salt. Cool in the fridge for 1 hour before serving.

Anatolian Gözleme

TURKEY

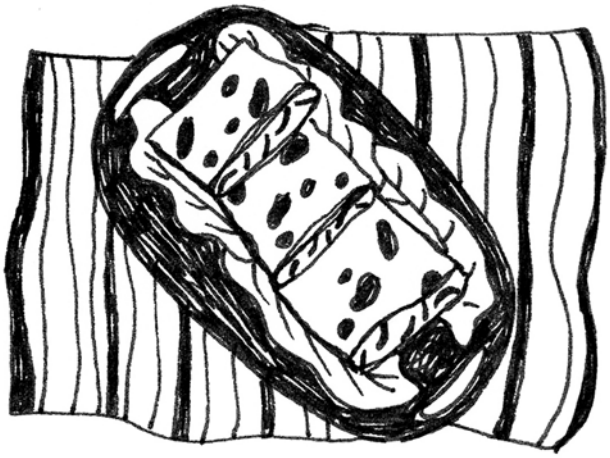
INGREDIENTS

For the dough:

8g of dried yeast
260ml of tepid water
3 cups of strong flour
1 tbsp of salt
45ml of olive oil
30ml of plain yogurt

For the filling:

200g of spinach
1 chopped onion
1 tbsp of red pepper paste
1 grated garlic
15ml olive oil
3 tbsp of **plain yogurt**
230g of feta cheese



METHOD

- Mix the yeast in with a little water and stand for 5 minutes.

- Mix in the flour and the salt. Add the olive oil, the yogurt and the rest of the water.

- Knead into a soft dough and then roll it into 5 balls.

- Put the balls back in the bowl, cover with a tea towel and leave for 30 minutes until they double in size.

- Chop the spinach and mix with the onion, red pepper paste, garlic, olive oil and **yogurt**.
- Add the feta cheese and stir a little more.

- Roll the dough out on a lightly floured surface to make a 40-cm-side square piece.

- Bend the right and left sides inwards until they meet in the centre.

- Place the filling and then fold the upper and lower parts towards the centre, covering the filling well. Press.

- Place on a hot grill, paint with olive oil and cook for 3 minutes.

- Flip and repaint before serving.

Mango Lassi

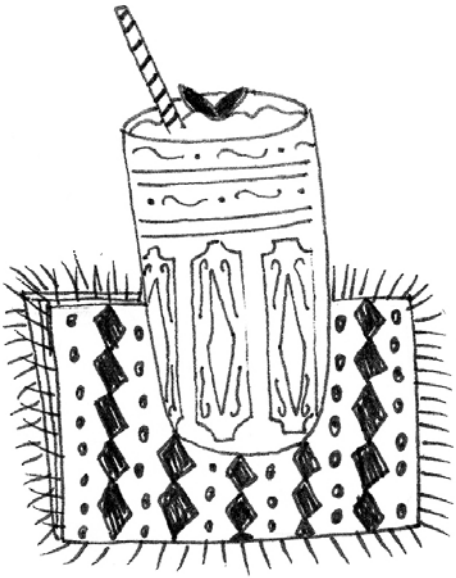
INDIA

INGREDIENTS

1 very ripe honey mango
5 ice cubes
125ml chilled plain whole milk **yoghurt**
Pinch of ground cardamom
Splash of milk or water
1/2 tsp of soft light brown sugar
Squeeze of lime juice

METHOD

- Whisk or blend the ice, mango, cardamom and **yogurt**.
- Add milk or water to thin it down if necessary.
- Add sugar and lime juice.



Portokalopita

GREECE

INGREDIENTS

For the syrup:

400ml of water
400ml of sugar
1 orange zest
1 cinnamon stick

For the cake:

440-450g of phyllo
300ml of corn oil
300g of sugar
200g of **yogurt**
300ml of orange juice
1 1/2 orange zest
1 tbsp of vanilla extract
20g of baking powder

METHOD

- Mix sugar, zest and cinnamon in water, boil for 5 to 10 minutes and let cool.

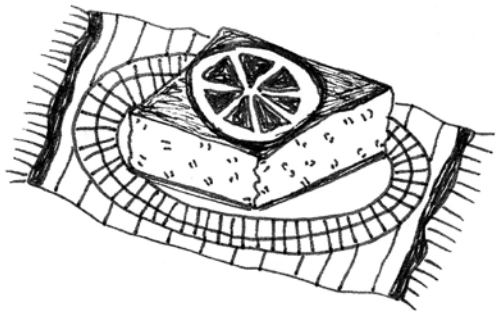
- Remove the phyllo sheets from pack age and shred into pieces.

- Beat the oil and sugar together and add the **yogurt**, juice, zest and vanilla extract.

- Add the baking powder to the mix and gently whisk in the phyllo pieces.

- Pour the mixture into a baking dish with corn oil and bake at 180 °C for 45 to 50 minutes.

- Pour the cold syrup on the hot cake and let cool before serving.



Tarator

BULGARIA, POLAND, UKRAINE, RUSSIA

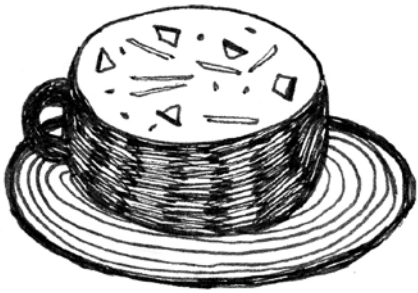
INGREDIENTS

2 or 4 garlic cloves
2 cups of **yogurt**
2 tbsp of olive oil
2/3 cup of chopped walnuts
2 tbsp of lemon juice
1/2 cup of ice
4 cucumbers
Salt and pepper
Optional: spring onion, turnip, boiled potato, hard-boiled eggs (all finely diced)

Toppings: Ice, chopped walnuts, olive oil, fresh dill, mint

METHOD

- Crush the garlic, salt and pepper.
- Whisk the **yogurt** in a bowl and add the olive oil, garlic, walnuts, lemon juice and ice.
- Mix and take to the fridge for 1 hour.
- Dice the cucumbers and add them to the mixture.
- Mix gently and serve with toppings.



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NOTES

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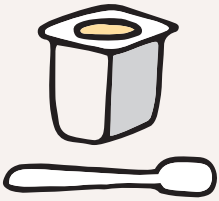
YOGURT



YOGURT

ancient food in the 21st century

The fact that the core steps in yogurt production have remained largely unchanged for centuries does not mean science has nothing to say about this magical ancient process. Just as wine is not only grape juice, yogurt is not just milk. And, given proper attention and the right technology, a few key steps can be tuned to improve final quality.

This book traces, in layman's terms, the historical origins of yogurt preparation and describes its industrial production, as well as the general principles of fermentation. It also discusses the close link between human microbiota "the microorganisms colonising the body" and general health. This is a book written for those with a curious mind, connoisseurs or not, but above all for anyone interested in learning a little more about this ancient product.

Last but not least, widely recognised chef Narda Lepes shares some traditional recipes from round the world including yogurt as an ingredient.