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COURSE HANDOUT

BIOCHEMISTRY OF FOODS

Level: Master 1

Agri-food and quality control.

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Preface

Biochemistry of Foods aims to highlight the crucial role of biochemistry in the evolving field of food science and to offer a more in-depth understanding of the chemical changes that occur in foods. This course handout is designed for students in the Master 1 agri-food and quality control program. It encompasses eight chapters focused on the biochemistry of foods: The first chapter covers the biochemistry of plant foods, including the biochemical composition of various human-consumed plant foods. It examines both macro- and micronutrients and provides comparisons among different sources. The second chapter addresses changes associated with food preservation and processing during fabrication. It offers solutions to preserve or minimize alterations in fruits and vegetables during these processes. The third chapter provides an overview of the biochemical composition of animal-origin foods, including eggs, fish, and various meat products. The following chapter explores technological processes affecting food, such as meat oxidation and other alterations that can occur during cooking. Chapter five details the cheese manufacturing process, outlining the steps involved in its production. Chapter six focuses on the fabrication of fermented milk products, discussing different types of fermented milk and the microorganisms involved in these processes. The seventh chapter examines the transformation of animals into carcasses, covering key aspects such as slaughtering techniques, sanitary controls, meat maturation, and the valorization of co-products. Finally, chapter eight provides a summary of the manufacturing process for poultry pâté.

This course is classified as a fundamental teaching unit, carrying 3 coefficients and 6 credits due to its significance in the master's specialization. Studying the biochemistry of foods enhances students' knowledge and scientific understanding of food composition and alterations during the manufacturing process. This knowledge helps minimize food loss by extending the shelf life of products and supports the development of a diverse range of high-quality products.

1. Biochemical composition of plant foods

1.1. Cereal seeds

Cereals stand as the paramount crops globally, boasting an aggregate annual grain production surpassing 2000 million tonnes (mt). While various cereal varieties are cultivated, three main ones – maize (604 mt in 1998), wheat (589 mt in 1998), and rice (563 mt in 1998) – collectively contribute to over 70% of the overall output. Additional cereals in descending order of total production encompass barley, sorghum, millets (comprising several small-seeded tropical species), oats, and rye.

Cereals consist predominantly of carbohydrates, constituting 50–80% of their weight, alongside noteworthy proportions of proteins (5–6%, table 1) and lipids (1–10%). Whole grains serve as significant sources of mineral salts (1.5–2.5%), including phosphorus, calcium, magnesium, potassium, iron, zinc, and copper, as well as various vitamins such as thiamine, riboflavin, niacin, pyridoxine, biotin, folic acid, vitamin E, and vitamin A. These grains comprise four distinct parts: bran, endosperm, germ, and aleurone layer. The endosperm is abundant in starch and reserve proteins (prolamins and glutelin), while the bran, aleurone layer, and germ contain more proteins with essential amino acids, vitamins, minerals, fibers, lipids (with a higher concentration in the germ), and bioactive compounds (e.g., phenolic acids, flavonoids, alkylresorcinols, avenantramides, tannins, carotenoids, lignans, and phytosterols). Whole grains are widely consumed and associated with health benefits, partially due to their dietary fiber content, which varies in type and quantity among different cereals. Typically, whole grains and pseudocereals are rich in both soluble and insoluble fiber, with cellulose, arabinoxylan-glucan, xyloglucan, and fructan being the most prevalent types.

Numerous vitamins can be found in cereals, including thiamine, riboflavin, niacin, pyridoxine, biotin, folic acid, pantothenic acid, vitamins A, E, and K. These vitamins are primarily concentrated in the outer layer and germ of the grain, which can be significantly reduced through refining processes. Furthermore, the overall concentration of vitamins and minerals is influenced by various factors such as the type of cultivar, soil composition, and the extent of refinement.

Table 1: Compositional distribution of proteins found in major cereal grains

Cereal Grain	Albumin	Prolamins	Globulins	Glutelins
Wheat	9-15	33-45	6-7	40-46
Corn	4-8	47-55	3-4	38-45
Rice	5-11	2-7	~10	77-78
Barley	~12	25-52	8-12	52-55
Oats	10-20	12-14	12-55	23-54
Rye	10-44	21-42	10-19	25-40
Zorghum	~4	~48	~9	~37

1.2. Legume seeds

The term "legume" encompasses over 13,000 distinct species belonging to the Leguminosae family. Legumes hold a prominent position in the global human diet. Among the vast array of species, only a select few are commonly cultivated commercially, including soybeans, peanuts, various types of dry beans, peas, broad beans, chickpeas, and lentils. Despite significant differences in their macronutrient makeup, legumes share a consistent seed structure. Each mature seed comprises three primary components: the seed coat (testa), the embryo, and the endosperm. Typically, legume seeds possess minimal endosperm at maturity, as the majority of the seed weight is attributed to the cotyledons of the embryo, which contain the essential reserves for germination and growth. The nutrient composition of legumes varies widely, influenced by factors such as seed type and variety, soil conditions, and environmental variables.

The protein content of legume seeds ranges from 19.30% to 26.12% of the edible portion (Table 2, and 3), although reported crude protein levels vary between 15% and 45%, with certain soybean varieties containing up to 50% protein. Carbohydrate content fluctuates between 24% and 68%, showing an inverse relationship with lipid content. Legume seeds with high carbohydrate levels typically exhibit low lipid content, and vice versa. For instance, peanuts are characterized by a notably high lipid content (49.24%) and relatively low carbohydrate content (16.13%). Potassium stands out as the most prevalent mineral in most legumes, with soybeans containing as much as 1.80 g/100 g of the edible portion. Phosphorus, copper, iron, calcium, and magnesium are among the significant minerals found in legumes.

Niacin and pantothenic acid are the most abundant vitamins in legumes, with many also serving as good sources of folate.

Table 2: Composition and nutritive value of selected legume seeds

Specification	Nutrients (g/kg)			Micro and macro elements (mg/kg)				Vitamins and provitamins					
	protein	**fat**	**Dietary fiber**	**Sodium**	**Potassium**	**Phosphorus**	**iron**	**A (µg/kg)**	**B-carotène (µg/kg)**	**E (mg/kg)**	**B1 (mg/kg)**	**B2 (mg/kg)**	**C (mg/kg)**
White Bean, dry seeds *Phaseolus vulgaris L.*	214	16	157	190	11880	4370	69	0	0	2	6.7	2.3	20
Pea, dry seeds, *Pisum sativum*	238	14	150	300	9370	3880	47	200	1170	3	7.7	2.8	20
Red lentils, dry seeds *Lens culinaris* Medik,	254	30	89	20	8740	3010	58	100	600	2.2.	10.7	4.5	30
Soybeans, dry seeds *Glycine max* (L.) Merr	343	196	157	10	21320	7430	89	20	120	7.8	6.9	1.9	0
Grean Pea, fresh seeds *Pisum sativum* L.	67	4	60	220	3530	1220	19	680	4080	3.9	3.4	1.6	342
Broad Bean, fresh seeds, *Vicia faba* L.	71	4	58	70	2610	570	19	280	1700	4.6	0.9	0.6	320

Eating legume seeds is thought to lower the risk of developing cancer, including colorectal cancer. When these seeds are consumed alongside a meal, they help speed up the movement of food through the digestive system, making it easier to pass waste. Consequently, this reduces the absorption of cholesterol, incompletely digested starch, and decreases the intensity of fermentation processes, all of which are beneficial for one's health. The consumption of fermented yellow lupine (*L. luteus*) seeds promoted the abundance of Bifidobacteria and anaerobic microorganisms in the colon.

Table 3: Chemical composition and content of amino acids in legume seeds

Specification	Chemical composition (g/kg)			Content of amin acids (g/kg)				
	total protein	crude fat	crude fiber	lysine	methionine	cysteine	tryptophan	threonine
White lupine *Lupinus albus* L.	359	100	151	15.9	3.1	5.6	0	16
Yellow lupine *Lupinus luteus* L.	422	70	157	22	3.2	8.4	0	14.5
Blue lupine Lupinus *angustifolius* L.	339	59	168	15.7	2.7	4.6	0	12.9
Pea *Pisum sativum* L.	241	11	71	17.5	2.9	3.9	2.6	9.6
Field bean *Vicia faba* L.	309	10	98	18.1	2.1	3.4	2.5	9.8
Domestic Soybean *Glycine max* (L.) Merr	375	159	82	22.3	5.8	6.4	0	14.7

1.3. Oilseeds

Soybean production is expected to increase by 1.6% annually, compared to the 4.4% annual growth seen over the past decade. Similarly, the growth rate for other oilseeds such as rapeseed, sunflower seed, and groundnuts is anticipated to be slower at 1.4% annually, in contrast to the 3.1% annual growth observed in the previous ten years. The growth in other oilseeds is primarily driven by improvements in yields, which are projected to contribute to 64% of production growth, whereas for soybeans, yield increases will account for 46% of overall production growth.

Globally, the crushing of soybeans and other oilseeds into meal (cake) and oil dominates total usage. The demand for crush will increase faster than other uses, notably direct food consumption of soybeans, groundnuts and sunflower seeds, as well as direct feeding of soybeans. Overall, 90% of world soybean production and 86% of world production of other oilseeds will be crushed in 2028. The crush location depends on many factors, including transport costs, trade policies, acceptance of genetically modified crops, processing costs (e.g. labour and energy), and infrastructure (e.g. ports and roads).

Oilseeds are categorized into major and minor types according to their production levels. Major oilseeds consist of rapeseed, soybean, groundnut, sesame, sunflower, safflower, castor, linseed, and niger, while minor oilseeds include palm and coconut oil. Additionally, there are other oils like cottonseed oil and rice bran oil, though they are available in smaller amounts. Oilseeds are abundant in protein, fat, fiber, vitamins, carbohydrates, minerals, and nutrients (Table 4).

Table 4: Nutritional composition of different types of major and minor oil-seeds

Oil-seed seeds	Major/minor	Moisture (%)	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)
Sunflower	Major	-	18-20	40-45	32-36	3.1
Groundnut	Major	7.4	24.7	46.1	2.83	1.48
Sesame	Major	0.2-3.5	20	52-63	4.2-11.4	1.4-5.93
Mustard	Major	9.5	25	33-45	12-15	9.6-12
Soybean	Major	13-14	36-56	19	5	5
Niger	Major	1-11	10-25	30-40	10-20	-
Rapeseed	Major	12	36-38	1-2	10-12	6-8
Safflower	Major	43-29	12-17	34	20-33	3.45-4.21
Linseed	Major	7-11	20	40	30	4
Castor	Major	6.1-8.4	21-48	1.9-50	2.5-24.5	5.66-6.49
Oil palm	Minor	4.53	24.6-26.7	47.3-49.4	6-24.9	6.44
Coconut palm	Minor	7.51	25-30	30-40	4.27	7.70

Minor oilseed crops contribute to 5-10% of total oil production and find applications in pharmaceuticals, food, and soap industries. Soybean, comprising 18-22% fat content, contains essential polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and other fatty acids like oleic and stearic acid (Table 5). Peanut oil is rich in monounsaturated fatty acids (50%) and contains 14% saturated fatty acids. Sunflower seeds are particularly abundant in polyunsaturated fatty acids (194%). Unsaturated fatty acids play a beneficial role in reducing bad cholesterol levels, thereby lowering the risk of obesity, atherosclerosis, and heart diseases. Additionally, fatty acids aid in the transportation of fat-soluble vitamins and exhibit antioxidant properties, thereby reducing oxidative stress in the body. Oilseed fats are utilized as functional food ingredients and are a significant source of protein, essential for the production of nutritionally balanced products. Soybean, a key oilseed, contains 20-25% protein, while groundnut boasts 22-26% protein content and is notably rich in arginine. Rapeseed contains 34.50% protein, with albumins comprising 39.88% and glutelins 46.25%, while sesame oilseed contains 20% protein, with albumin and glutelin constituting 8.6% and 6.9%, respectively. Linseed contains 21% protein, with albumins ranging from 20-42% and globulins from 26-58%.

Oilseeds are a source of fat-soluble vitamins such as A, E, and K, as well as water-soluble vitamins including B1, B2, B3, B6, and C, though they lack vitamin B12. These vitamins play crucial roles in nerve function, digestion, skin health, and disease prevention. Oilseeds also contain essential minerals like calcium, magnesium, zinc, manganese, phosphorus, potassium, iron, and copper, which serve as cofactors for enzymes. Additionally, oilseeds harbor phytochemicals like polyphenols, carotenoids, phytosterols, and tocopherols, which confer various health benefits to humans.

Table 5: Typical fatty acid composition (%wt)

Oil source	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**
Cocoa butter	26	34	35	-	-
Corn	13	3	31	52	1
Cottonseed	27	2	18	51	<1
Groundnut	13	3	38	41	<1
Linseed	6	3	17	14	60
Olive	10	2	78	7	1
Palm	44	4	39	11	<1
Palm olein	41	4	31	12	<1
Palm stearin	47-74	4-6	16-37	3-10	-
Rape (high erucic)	3	1	16	14	10
Rape (low erucic	4	2	56	26	10
Rice bran oil	20	2	42	32	-
Safflower	7	3	14	75	-
Safflower (high oleic)	6	2	74	16	-
Sesame	9	6	41	43	-

Table 5 (continued)

Oil source	16:0	18:0	18:1	18:2	18:3
Soybean	11	4	22	53	8
Sunflower	6	5	20	60	<1
Sunflower (Sunola)	4	5	81	8	<1
Sunflower (NuSun)	4	5	65	26	-

1.4. Fruits and vegetables

Vegetables

A good source of chemicals with pharmacodynamic activity is vegetables and their byproducts, which include pickled, preserved, frozen, marinated, and dried vegetables, as well as salads, prepared sauces, and pickles, both fermented and nonfermented. Since vegetables are the source of many bioproducts necessary for the life of the animal kingdom, humans and vegetables are inextricably intertwined. From the perspective of the food industry, vegetables are important because of their complex chemical content, which includes organic substances (carbohydrates, proteins, lipids, and organic acids), phytoncides and antimicrobial substances, a high content of minerals (Ca, P, Fe, K, Mg, S, Cl, Zn, and Cu), and a high content of vitamins (A, B complex, C, E, F, C, and Cu). Vegetables' high-water content helps the body stay hydrated, while also stimulating the skeletal and muscular systems, internal glands, and enzymatic activity. All of these benefits are attributed to the complex chemicals found in vegetables, which also have a high nutritional and energy content. Vegetables are an important component of a basic diet because of their high nutritional density, low energy intake, and variety of biological phytonutrients. When veggies are at their most nutritious, it is advised to eat them fresh; nevertheless, some processing and storage techniques might deplete certain nutrients and water-soluble vitamins. Vegetables use solar energy to create a range of fundamental molecules, including proteins, lipids, and carbohydrates, that are essential for their life.

Vegetables are foods derived from plants that have a variety of purposes and are a major source of nutrients. Different plant species have different edible parts, such as the root, stem, bulb, fruit, flowers, seeds, leaves, or the entire plant. The plant organ that is consumed determines how much nutrition is ingested from veggies. While leafy vegetables are very rich in vitamin C, magnesium, chlorophyll, and carotenoids, tubers are rich in starch but low in vitamins; roots and stems, which are rarely eaten as such (e.g., cauliflower), are high in fiber (cellulose and hemicellulose) and contain vitamin C.

Plant species differ in the amount of carbohydrates they contain, or in significant quantities of cellulose. Sucrose, starch, and cellulose are found in lettuce, zucchini, tomato, eggplant, cucumber, greens, onion, okra, carrots, leeks, beets, celery, Brussels sprouts, potatoes, and dry beans. Vegetable carbohydrates, such as cellulose, starch, and pectin, are mostly composed of mono- and disaccharides. It has been found that glucose is a free component in carrots, despite the fact that it is rarely ingested in its monocarbohydrate form. The sweetest monocarbohydrate, fructose, accounts for around 3% of the weight of dried vegetables; typically, veggies contain 1% to 7% fructose.

Because they are not proximally absorbed, inulin-type oligocarbohydrates—which are naturally occurring polymers of fructose, a type of carbohydrate storage—produce 1 kcal/g. Garlic and tomatoes are two other vegetables high in fructo-oligocarbohydrates. Vegetables that have not yet ripened contain high levels of sucrose; however, as fruit ripens, fructose and glucose take the place of sucrose. Certain plants don't have sugar in them.

Starches are complex inorganic compounds with the formula $(C_6H_{10}O_6)_n$. Vegetables like carrots, potatoes, and dry legumes (beans and peas) contain starch as a reserve material. Raw grains and potatoes are difficult to digest because starch granules are encased in stiff cellulose walls in plants, rendering them inaccessible to digestive enzymes. Digestion is made feasible by heat because it causes the granules to enlarge, the starch to gel, and the cell wall to soften and rupture. Large amounts of starch are present in a plant before it develops, which reduces the amount of fruit maturation by changing the fruit's sugar content. Raw carrot starch is safe to consume and is typically advised for young children. Because it has a finer structure than grains, vegetable cellulose is advised for people who suffer from certain digestive disorders.

Vegetables include pectic compounds and cellulose that combine to generate pectin-cellulose complexes, which have the ability to increase secretions and motility. The percentage of vegetable cellulose varies from 3.6% (parsnip) to 0.3% (zucchini). Carrot, beet, and zucchini include hemicelluloses, which help the intestines hold onto water and repair cations. Hemicellulose is an indigestible polycarbohydrate that is linked to cellulose and lignin, with the exception of crops high in fiber like radish, cabbage, and eggplant. Carrots and beets are known for their pectins, which are used to manufacture absorbent gels, antiseptics, and medications for the intestines (such as those used to treat enteritis).

Vegetables have little levels of lipids (excluding oilseeds). Almonds and peanuts contain small levels of saturated fatty acids, while peas, beans, and spinach are good sources of palmitic, myristic, and stearic acids. Of the monounsaturated fatty acids, spinach contains palmitoleic acid (23%) while peas have oleic acid. Soybeans and beans contain omega-3 polyunsaturated fatty acids. Furthermore, soybeans are recognized to be a good source of glycerophospholipids such lecithin and lipids (47 mg/100 g; linoleic to linolenic acid ratio: 7.5:1).

Most vegetables have a protein content of 0.5% to 1.5%; however, dried legumes (20–34% in lentils, dried beans, and soybeans) and green legumes (5%–6% in peas and beans) are exceptions. These legumes contain globulin-type protein, which is found in phaseolin in beans, legumelin in peas and lentils, and glycine in soybeans. Methionine and lysine are present in modest levels, making soybean proteins inadequate from a nutrition perspective even though they contain all of the essential amino acids. A protein is deemed incomplete if it lacks one or more essential amino acids, or if it has all the essential amino acids but not in equal proportions, as in the case of dried beans with low methionine content. Flour is made from soybeans, while the food sector uses protein isolates and concentrates to manufacture soy milk, energy drinks, and other goods.

Minerals found in vegetables include K, Ca, Mg, P, and Fe. They also have trace amounts of oligoelements (Cr, Cu, I, F, Zn, Mg, Mo, and Se), whose proportions vary because they are absorbed from the soil along with water. Vegetables that contain calcium (with an absorption rate >50%) include cabbage, cauliflower, broccoli, parsley, onions, peas, and beans. Beans and soybeans have comparatively high calcium contents. Spinach and tomatoes contain oxalic acid, which forms insoluble calcium oxalate ions and reduces the bioavailability of minerals. Onions, lettuce, and cabbage have the best Ca:P ratios, which facilitates their absorption. Vegetables supply less than 30% of the P that the body requires. Vegetables supply less than 30% of the P that the body requires. Dried legumes have been shown to contain phosphorus in the form of phytic acid, which binds to minerals like calcium, iron, zinc, and magnesium to create insoluble complexes. Potassium is soluble in water and gastric juice and is present in considerable concentrations as carbonate and organic acid salts. Vegetables have a diuretic effect in general due to their high water and K content. Dried beans (1,500 mg/100 g), spinach (700 mg/100 g), and potatoes (500 mg/100 g) are significant sources of K.

Darker green vegetables like lettuce, spinach, green onions, and nettles, as well as dried legumes that contain magnesium as an essential component of chlorophyll, are good sources of magnesium. Vegetables are a rich source of Fe after meat, especially lentils and dried beans. Even with vegetables that have a lower Fe level, a high vitamin C content increases the availability of iron for absorption (carrots, broccoli, potatoes, tomatoes, cauliflower, cabbage, and sweet peppers). Vegetables with elevated levels of phosphate, cellulose, tannin, oxalic acid, and phytate have less Fe that may be absorbed. Boiling and over-refining lead to fragmentation, which increases the loss of iron. Broccoli, cauliflower, and dry beans are the main sources of S. Chlorophyll is typically abundant in vegetables with high zinc content, such as spinach, dried beans, lentils, and peas. Vegetables, with the exception of dried legumes, have less copper than animal products—about 2 mg/day. Cu absorption is decreased when cellulose or thiocyanate is present, which is found in turnips, kale, cauliflower, and cabbage. Vegetables have different quantities of iodine. Vegetables include low levels of selenium (Se); examples include broccoli, soy, garlic, and beans. These items are a low source of sodium and should be included in salt-restricted diets for individuals with edema, renal insufficiency, or hypertension. Vegetables high in sodium include winter radishes, celery, carrots, beets, spinach, and turnips.

A vegetable's vitamin content varies depending on its type, ripening stage, growing medium, and preservation method. Vegetable-based vitamin C has more activity than manufactured vitamin C, and its absorption is improved when combined with vitamin P and other antioxidants. The majority of green vegetables are rich in vitamins and minerals. Leafy greens, some vegetables (tomatoes and peppers), tubers (asparagus and potato), and bulbs (onions) are rich sources of ascorbic acid (vitamin C). The growth and maturation of crops affect the amount of vitamin C present in vegetables. Since the shell contains more vitamin C than the core, vitamin C is lost when the outer layer is removed. Since oxidation destroys vitamin C, vitamin C is lost when sodium bicarbonate is added to cooked vegetables to improve and preserve their color. Foods that are refrigerated or frozen can help retain vitamin C, but heating can cause those foods to lose up to 45% and 52% of their vitamin C content, respectively.

Legumes (dry beans have 0.6 mg%), leafy vegetables (lettuce and spinach), cabbage (0.5–1.5 mg%), and potatoes (0.56 mg%) contain vitamin B1. B2 is present in pulses (lentils at 0.2–0.3 mg%) and green leafy vegetables (broccoli, spinach, and parsley at 0.2–0.3 mg%). Dried legumes (2-4 mg% in lentils) and other vegetables such potatoes, tomatoes, eggplant, spinach, cauliflower, green peas, and beans contain niacin in the form of nicotinic acid. Broccoli, potatoes, and tomatoes are the main plant sources of vitamin B5. Pulses (lentils, beans, and soybeans) and vegetables (carrots, cauliflower, and spinach) are good sources of vitamin B6. Because vitamin B6 is impacted by extended heat treatment, freezing methods, and long-term storage, its bioavailability in plants is lower than that of animal sources. Vegetables like cabbage, spinach, beans, peas, carrots, and tomatoes are good sources of vitamin B7 (H).

Along with potatoes and lentils, leafy green vegetables (spinach, lettuce, and broccoli) are a good source of folic acid (vitamin B9). When vegetables are boiled, reduced B9 is oxidized and destroyed between 50 and 90 percent of the time. The presence or absence of B9-conjugated metal chelates, inhibitors, and chelating agents (B9-linked veggies), the form of which dictates a person's nutritional status, affects the bioavailability of B9. Compared to yeasts or bananas, lettuce has a twice-smaller B9 absorption rate. Vegetables include trace levels of cyanocobalamin (vitamin B12), which is produced by bacteria. If a vegetarian does not take a supplement, after five to six years, their circulation concentrations of vitamin B12 are reduced.

Vegetables are a good source of vitamin D, with spinach and cabbage having the highest concentration. Only plants are able to produce tocopherols and tocotrienols, with plant oils being the most abundant source. 80–95% of a-tocopherol and g-tocopherol are found in soybean oil. Vitamin E is found in seeds, beans, spinach, lettuce, peas, cabbage, and celery. It is well-known for its antioxidant properties. Green leafy vegetables (40–50% of total diet) include parsley, spinach, broccoli, lettuce, turnips, and cabbage. Table 6 displays the stability of vitamins under specific situations.

Table 6: Stability of vitamins when exposed to certain conditions

Phytochemicals	Air	Light	Heat	Maximum loss from cooking (%)
Vitamin A	A	A	U	40
Vitamin C	A	A	A	100
Vitamin D	A	A	U	40
Vitamin K	U	A	U	5
Vitamin B_7	U	U	A	60
Vitamin B_6	U	A	A	40
Vitamin B_2	U	A	A	75
Vitamin B_1	A	A	A	55

U unalterable (no considerable destruction), A alterable (considerable destruction)

Fruits

Fruits provide important minerals, vitamins, and dietary fiber. In addition to these ingredients, they contain carbohydrates and, to a lesser amount, proteins. Fruits help digest meat, cheese, and other high-energy foods by neutralizing acids caused by lipid hydrolysis.

Fruits typically include 10–25% carbs, less than 1% protein, and 0.5% fat. Catabolism involves the breakdown of carbohydrates, sugars, and starches into CO_2, water, and energy. Carbohydrate-rich fruits include banana, plantain, date, raisin, breadfruit, and jackfruit. Dried apricots and figs include proteins and amino acids, while avocados and olives are high in fat. Fruits mostly consist of sugars, which vary in composition. Fruits contain primarily sucrose, glucose, and fructose (see Table 7). Many fruits contain sugar alcohols like sorbitol.

Table 7: Sugar composition of selected fruits.

Fruit	Sugar (g/100 mL of juice)			
	Sucrose	**Glucose**	**Fructose**	**Sorbitol**
Apple	0.82±0.13	2.14±0.43	5.31±0.94	0.2±0.04
Cherry	0.08±0.02	7.5±0.81	6.83±0.74	2.95±0.33
Grape	0.29±0.08	9.59±1.03	10.53±1.04	ND
Nectarine	8.38±0.73	0.85±0.04	0.59±0.02	0.27±0.04
Peach	5.68±0.52	0.67±0.06	0.49±0.01	0.09±0.02
Pear	0.55±0.12	1.68±0.36	8.12±1.56	4.08±0.79
Plum	0.51±0.36	4.28±1.18	4.86±1.3	6.29±1.97
Kiwi fruit	1.81±0.72	6.94±2.85	8.24±3.43	ND
Strawberry	0.17±0.06	1.8±0.16	2.18±0.19	ND

ND: Not detected (less than 0.05 g/ml)

Fruits contain less than 1% protein, but nuts provide 9-20% protein. Plant protein sources often provide dietary protein needs in nations with limited access to animal proteins. Fruits typically have a low lipid content (0.1–0.2%). Avocados, olives, and nuts are exceptions. Despite the fact that fresh fruits contain very few storage lipids, they do contain cell membrane lipids. Lipids form the surface wax and cuticle of fruits, providing a shiny look and protecting against water loss and pathogens.

Fruits and vegetables significantly contribute to our daily vitamin requirements. The nutritional value of a fruit or vegetable depends on its vitamin content and quantity consumed. Fruits and vegetables provide around 50% of daily vitamin A, 60% thiamine, 30% riboflavin, 50% niacin, and 100% vitamin C. Vitamins are affected by many processing circumstances, including heat, cold, oxygen, light, free water, and mineral ions. Trimming, washing, blanching, and canning can reduce the nutritional content of fruits and vegetables.

Minerals in fruits, such as calcium oxalate, may not be entirely nutritionally accessible. Fruit minerals mostly consist of base-forming elements (K, Ca, Mg, Na) and acid-forming elements (P, Cl, S). Minerals commonly found in microquantities include Mn, Zn, Fe, Cu, Co, Mo, and I. Fruits contain the highest levels of potassium, followed by calcium and magnesium.

Dietary fiber includes cellulose, hemicellulose, lignin, and pectic compounds found in fruit cell walls and skins. Fruits typically contain 0.5-1.5% dietary fiber by fresh weight. Water accounts for up to 90% of fruits and vegetables' total weight. Fruits' maximum water content varies depending on their structure. Agricultural circumstances affect plant water content. Water, a fundamental component of fruits, affects their quality and degradation.

Fruit phenolics include chlorogenic acid, catechin, epicatechin, leucoanthocyanidins/anthocyanins, flavonoids, cinnamic acid derivatives, and simple phenols. Fruits typically have a total phenolic content of 1 to 2 g per 100 g fresh weight. Chlorogenic acid is the primary substrate for the enzymatic browning of injured fruit tissue (Oke & Paliyath, 2007). Phytochemicals with anticarcinogenic effects are known as chemopreventive agents. They can reverse or suppress the first phase of carcinogenesis and prevent the progression of cancer cells.

1.5. Other products (coffee, cocoa, etc.)

Coffee

Coffee quality is primarily determined by its chemical ingredients, which contribute to the ultimate flavor and aroma. Coffee bean chemical constituent formation is influenced by various factors, including genetics, environment, nutrition, harvesting techniques, postharvest, drying, processing, and storage, as well as roasting and beverage extraction. Coffee is made from the ripe seeds of *Coffea arabica Linn*., a plant of the *Rubiaceae* family.

Coffee produced from coffee beans, which are also present in crimson fruits. The seeds of the botanical genus Coffea can be raw, roasted, whole, or crushed. The drink made from coffee seeds is also known as coffee. Only 3 out of 70 coffee species are cultivated. *Coffea arabica* accounts for 75% of global coffee production, followed by *Coffea canephora* at 25% and *Coffea liberica* at less than 1%, among others. Coffee is often grown at altitudes ranging from 1000 to 2000 meters. It is native to Ethiopia, Brazil, India, Vietnam, Mexico, Nepal, Guatemala, Indonesia, and Sri Lanka.

Coffee is primarily composed of caffeine, tannin, fixed oil, carbohydrates, and proteins. It has 2-3% caffeine, 3-5% tannins, 13% protein, and 10-15% fixed oils. Caffeine is found in the seeds as a salt of chlorogenic acid (CGA). It also contains oil and wax. Table 8 shows the many ingredients and components of coffee.

Table 8 Constituents along with components of coffee

Constituents	**Components**
Soluble carbohydrates	Monosaccharides: glucose, fructose, galactose, arabinose (traces)
Oligosaccharides	Sucrose, raffinose, stachyose
Polysaccharides	Polymers of galactose, mannose, arabinose, glucose, insoluble polysaccharides
Hemicelluloses	Polymers of galactose, mannose, arabinose
	Cellulose
	Acids and phenols
	Volatiles acids
Nonvolatile aliphatic acids	Citric acid, malic acid, quinic acid
Chlorogenic acids	Mono, dicaffeoyl and feruloylquininic acid
	Lingnin
	Lipids
	Wax
Oil	Main fatty acids: N compounds
Free amino acids	Main amino acids: Glu, Asp, Asp-NH2
	Proteins
Caffeine	Traces of theobromine and theophylline
	Trigonelline
	Minerals

The fragrances of raw and roasted coffee vary significantly. Raw coffee has approximately 250 volatile chemicals, whereas roasted coffee contains over 1000. During roasting, the Maillard Reactions combine reducing sugars with amino acids, resulting in the creation of melanoidins that give coffee its black color. Sensory analysis can be compared to chemical analyses, including acidity (pH, total titratable acidity, greasy acidity), sweetness (total and reducing sugar content), body astringency (caffeine, chlorogenic acids, trigonelline), and aroma/fragrance (volatile constituent analysis, gas chromatography).

Cocoa

Cocoa (Theobroma cacao) is a popular tropical plant grown for its beans, which are the primary ingredient in chocolate manufacture. Theobroma cacao, a member of the *Sterculiaceae* family, originated in Central and South America's tropical regions. The plant is evergreen and grows to an average height of 4-8 meters. It thrives in warm and humid conditions.

Cocoa is naturally rich in minerals and nutrients, providing numerous health benefits. Cocoa powder has numerous health benefits, including brain health, blood pressure stabilization, cholesterol maintenance, antioxidants, diabetes treatment, obesity reduction, mood enhancement, cardiovascular health, cancer prevention, constipation relief, and skin support. Table 9 shows the nutritional content of cocoa.

Cocoa (Theobroma cacao), a tropical plant known for its delicious chocolate beans, contains numerous bioactive chemicals that may provide health advantages. Cocoa contains phenols, which include flavonoids, catechins, and procyanidins. Cocoa's phenolic components contribute to its characteristic flavor and aroma, as well as its antioxidant capabilities. Because of its antioxidant properties, cocoa phenolics assist the body fight off dangerous free radicals, which lessens oxidative stress and may lower the chance of developing a number of chronic illnesses, including cancer and heart problems. Additionally, these phenolic compounds have been linked to anti-inflammatory properties.

Table 9: The nutritional continent of cocoa in percentage per 100g.

Macronutrients	Percentage
Protein	15-21%
Carbohydrates	~ 15%
Lipids	10-15%
Fiber	25-40%
Nutrients	**Per 100g**
Vitamin A (Retinol)	< 0.2 mg
Vitamin E (Tocopherol)	2.5 mg
Vitamin B1 (Thiamine)	0.3 mg
Vitamin B2 (Riboflavin)	0.4 mg
Vitamin B3 (Niacin)	0.7 mg
Sodium (Na)	0.03 g
Potassium (K)	4.3 g
Calcium (Ca)	151 mg
Phosphorus (P)	700 mg
Iron (Fe)	26 mg
Magnesium (Mg)	555 mg
Coopper (Cu)	5 mg

2. Alterations linked to conservation and processing

2.1. Protein denaturation

Vegetables are a valuable source of energy, nutrients, elements, protein, omega-3 fatty acids, and readily available energy for worldwide agricultural production. They are also the most energy-efficient in greenhouses. Vegetables provide for two-thirds of global dietary protein production. Lysine in grains and sulfur amino acids in beans are often identified as restricted amino acid score, which stands for Amino acid score of vegetable protein.

Heat is commonly used to alter the structure and sensory properties of vegetable proteins. Extreme heat stability alters protein structures permanently, causing hydrolysis and aggregation through disulfide, hydrophobic, and electrostatic interactions. This leads to a loss of functional properties.

High hydrostatic pressure (HPP) is a non-thermal technique that uses pressure levels ranging from 100 to 800 MPa for a few seconds. HHP treatment affects the denaturation, aggregation, and interactions of several vegetal proteins. HHP treatment increases muscle hydrophobicity and decreases solubility, leading to aggregation by exposing hidden sulfhydryl groups during unwinding and inhibition. HHP can boost the nutritional value of vegetable proteins, according to studies. Compared to other treatments like improved ultrasonic, microwaving, and raised homogenization, H. HHP was found to be the most effective in reducing allergens in soy isolate for infant formula. The researchers found that although increasing digestion, ultrasonic treatment (25 kHz, 400 W, 1–16 min) reduced soymilk protein ace inhibitory action by 52%. The parts work together to create intense agitation and strong mechanical forces caused by a massive spinning screw that is traveling at peak pressures (1.5–30.0 MPa) and temperatures (90–200 C).

2.2. Browning

Food processing and storage can lead to the common occurrence of browning reactions. These reactions happen when fresh fruits and vegetables are mechanically damaged, as well as when meat, fish, and vegetable products are manufactured. The food products involved experience changes in flavor, appearance, and nutritional value due to browning. Browning, however, is a crucial step in the preparation process for several foods. For instance, it improves the look and flavor of goods made from coffee, tea, beer, and maple syrup, as well as while toasting bread. Some browning is said to be good in potato chips, French fries, and apple juice.

It's critical to comprehend the underlying mechanisms in order to regulate or prevent these reactions.

2.3. Maillard reaction

The Maillard reaction occurs when reducing sugars and amino acids combine with one another, changing the properties of food. It is also known as the nonenzymatic browning reaction.

The Maillard reaction, which occurs after heating or long-term storage, is a deteriorative process in preserved foods. It has also been found in mammalian organisms. The sugar component must have a reactive carbonyl group (reducing sugar), and amino acids can be found in foods as free or as part of protein. Reducing sugars react with amino molecules, resulting in complex colors with varying quantities. The resulting products vary greatly based on the reaction conditions.

The Maillard reaction leads to:

i) The formation of Melanoidins, brown pigments with varying nitrogen content, molecular weight, and water solubility. Their structure remains largely unknown. While most products appear brown, they can exhibit other colors as well. Browning is beneficial in baking and roasting but may be undesirable in foods with distinctive, delicate hues.

ii) The production of volatile compounds, known as premelanoidins, often results in potent aroma and flavor substances during cooking, baking, roasting, or frying. It's crucial for desirable aromas and flavors to develop in these processes. However, during storage, particularly in dehydrated foods, or in heat treatments like pasteurization and sterilization, and in cases of overcooked meat or fish (which can produce bitter substances), as well as in excessively heated tea or coffee, it may also lead to unwanted off-flavors.

iii) The production of reductones and antioxidants, which possess strong reducing properties, plays a role in stabilizing foods against oxidative damage. These compounds are thought to potentially reduce the incidence of degenerative diseases.

iv) Mutagenic compounds are typically generated during extended storage periods.

v) Essential amino acids such as lysine and histidine may be depleted.

vi) Compounds capable of cross-linking proteins are produced, a reaction that also has implications in vivo, particularly in diabetes.

The Maillard reaction is a set of reactions. The reaction is complex, with multiple pathways. Chemical reactions follow three stages (Figure 1):

(i) The early step involves the formation and degradation of N-substituted glycosylamine, leading to rearrangement or fission products.

(ii) The advanced or intermediate stage involves the degradation of the rearrangement product through enolization (C1-C2, C2-C3, etc.) and subsequent secondary reactions. Under acidic conditions, nitrogen is protonated and the positively charged nitrogen acts as an electron sink, facilitating 1,2-enolization. However, alkali and strong basic amines promote 2,3-enolization.

(iii) The last stage involves producing color, aroma, and flavor chemicals, as well as polymers, co-polymers, or melanoidins.

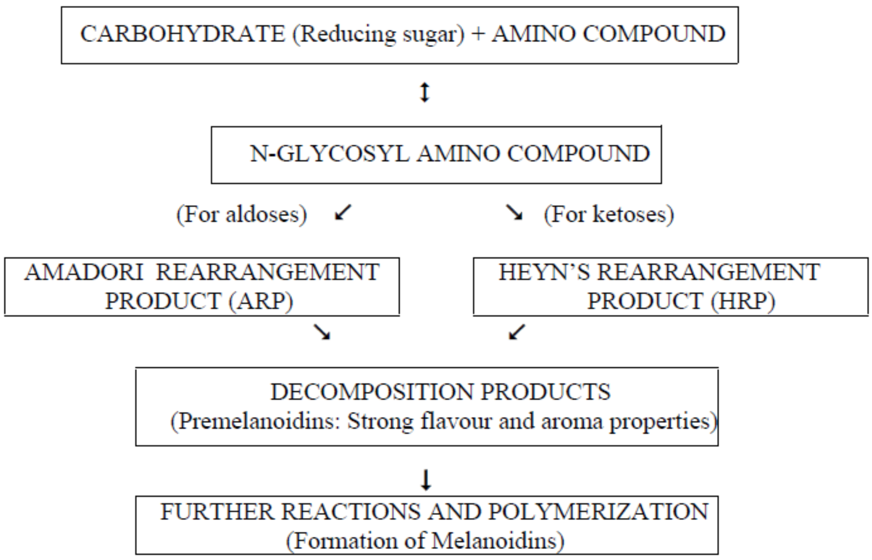

Figure 1: A general outline for the Maillard reaction

Factors effecting the Maillard reaction

Maillard reactions can be influenced by a variety of factors, including pH, temperature, pressure, time, reducing sugars and amino compounds, buffers, water activity, oxygen, and metals.

- ***pH and buffers***: The pH of the medium significantly influences the qualitative and quantitative composition of intermediate and final products. The reaction can take place in acidic or alkaline media, but it is more likely to occur in alkaline conditions where the amino groups of amino compounds are in the basic state. The rate of the reaction rises as the pH increases. Low pH ($\leq$6) promotes furfural production, while high pH (>6) promotes reductones and fission products. Leonard (1996) discovered that phosphate buffer accelerated amino acid loss and brown pigment production at pH 7 and 25^0C, but citrate buffer had no influence on the reaction.
- ***Temperature and duration of heating***: Research on model systems indicates that higher temperatures and longer heating times lead to increased color formation, carbon-to-nitrogen ratio, degree of unsaturation, and chemical aromaticity. Non-enzymatic Maillard reactions occur during food storage and processing at elevated temperatures, as well as at normal or reduced temperatures, including near 0°C. Increasing the temperature by 10°C can accelerate the reaction rate by 2-3 times.
- ***Water activity***: Water is formed during the Maillard reaction. The reaction rate is low for both high and low water activity (aw) values. The reaction peaks at a water activity of around 0.7, which corresponds to a relative humidity of 40-70% in most food systems.
- ***Air and oxygen:*** Melanoidins effectively remove active oxygen species. Oxygen can cause melanoidins to degrade into colorless compounds, resulting in less strong brown coloration.
- ***Metal ions:*** Phosphates and citrates speed up the reaction. Certain metal ions such as Cu^{2+}, Fe^{2+}, and Fe^{3+} accelerate browning, with Fe^{3+} being more effective than Fe^{2+}. Na^+ has no impact on the reaction. Trace metals like Sn^{2+} and Mn^{2+} inhibit the reaction, while some metal ions have a partial influence on browning.

Inhibition and control of the Maillard reaction

Controlling and inhibiting the Maillard reaction is crucial not only because of its impact on flavor, aroma, and color but also due to potential toxicity of its products. In cases where Maillard browning is undesirable, it can be partially controlled or inhibited by creating unfavorable reaction conditions, such as adjusting temperature and modifying water content (using very low or very high levels), managing pH levels, altering the form of reactants, removing one of the reacting substances, or employing chemical inhibitors like SO_2 (sulfur dioxide and/or bisulfites), aspartic acid, glutamic acid, etc. Conversely, in processes where desired traditional products arise, such as during coffee or cocoa bean roasting, the reaction can be effectively promoted under suitable conditions.

2.4. Colored Reactions

Via Amadori Compounds

The processes responsible for the transformation of 1-amino-1-deoxy-1-ketose derivatives into brown pigments or melanoidins are highly intricate and not fully comprehended. Nonetheless, researchers have proposed three distinct pathways, two of which play direct roles in pigment creation (Figure 2). These pathways hinge on various unstable intermediates, specifically the enol forms of Amadori compounds. In one pathway, enolization of 1-amino-1-deoxy-2-ketose occurs at positions 2 and 3, leading irreversibly to the formation of 2,3-enediol. Subsequently, this compound undergoes a sequence of transformations, including the departure of the amine from C1, resulting in a methyl dicarbonyl intermediate.

The second pathway initiates with the formation of 1,2-eneaminol from the Amadori product, wherein a hydroxyl group is lost at C3, followed by deamination at C1 and water addition to generate 3-deoxyhexosulose. Both derivatives, 1-deoxyhexosulose and 3-deoxyhexosulose, are highly reactive and undergo retro-aldolization to yield α-dicarbonyls like glyoxal, methylglyoxal, and 2,3-butanedione. Subsequent reactions involve a complex series of aldol condensation and polymerization, culminating in the formation of nitrogenous compounds that impart dark brown pigmentation. The pH of the environment influences the pathways: a lower pH promotes the 1,2-eneaminol pathway, whereas a higher pH favors the pathway involving the conversion of 2,3-enediol into reductones, subsequently fragmenting into furaneol and pyrones.

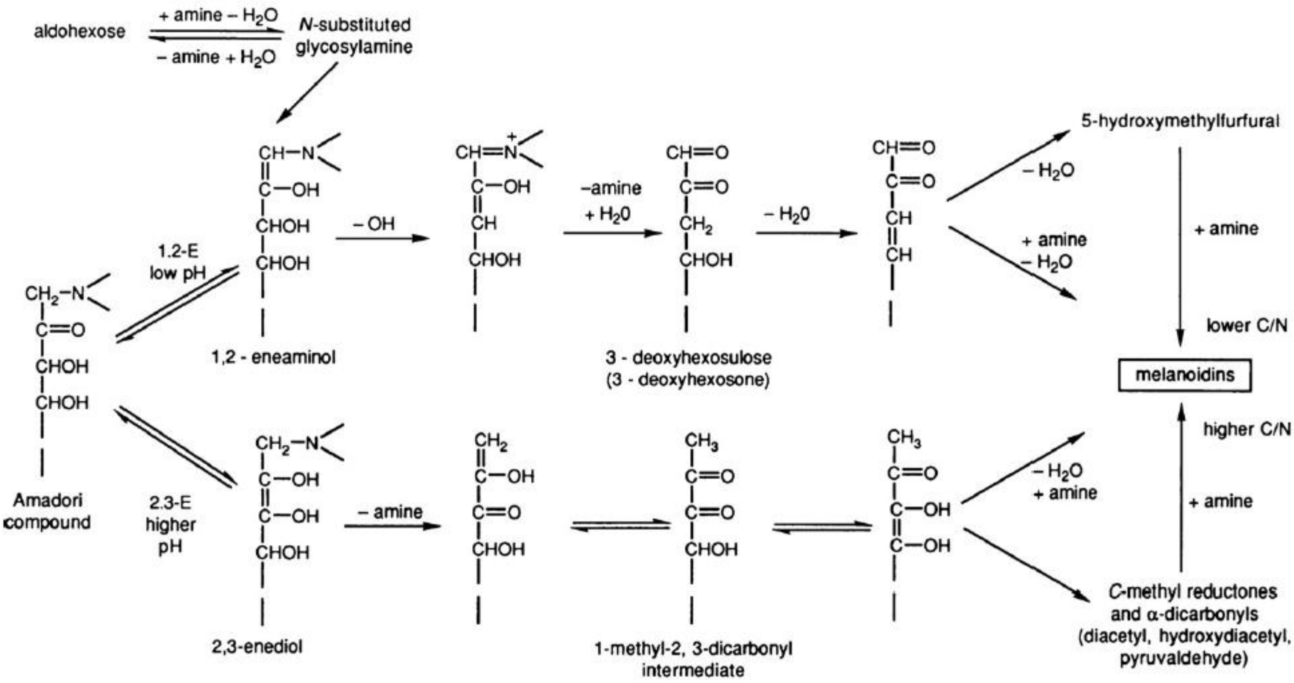

Figure 2: Maillard reaction: two major pathways from Amadori compounds to melanoidins

In the 1970s, Namiki et al. (1973), Namiki and Hayashi (1975), and Hayashi et al. (1977) first reported the occurrence of free radicals in mixtures where carbonyl compounds react with amines or amino acids. Milic et al. (1978, 1979, 1980) subsequently confirmed the generation of free radicals at the outset of the reaction between carbonyl and amino acids for D-glucose and aminobutyric acid isomers. Namiki and Hayashi (1981) observed electron spin resonance spectra with 17 and 23 lines in model systems involving alanine and arabinose. These signals were identified as N,N-dialkyl pyrazine cation radicals, detected before the formation of Amadori compounds. Their findings suggested an alternative pathway where the sugar part of the Schiff base breaks down before Amadori rearrangement, forming glycolaldehyde alkylimine or its corresponding eneaminol (Figure 3).

Further studies by Hayashi and Namiki (1986) confirmed the production of methylglyoxal dialkylimine, a C2 compound, early in the Maillard reaction. They found that glycoaldehyde and methylglyoxal, representing C2 and C3 sugar fragments, exhibited significantly faster browning rates—approximately 2000 and 650 times faster than glucose, fructose, or xylose when heated with β-alanine. Another C3 compound, glyceraldehyde, also showed a nearly 2000-fold increase in browning rate compared to its corresponding sugars. Hayashi and Namiki (1986) summarized that, under acidic conditions, browning typically progresses via osone formation through the Amadori rearrangement. Conversely, under alkaline conditions, they attributed increased browning largely to sugar fragmentation into C2 and C3 fragments (Figure 4). Danehy (1986), however, proposed considering this pathway as occurring concurrently with the established Maillard reaction scheme.

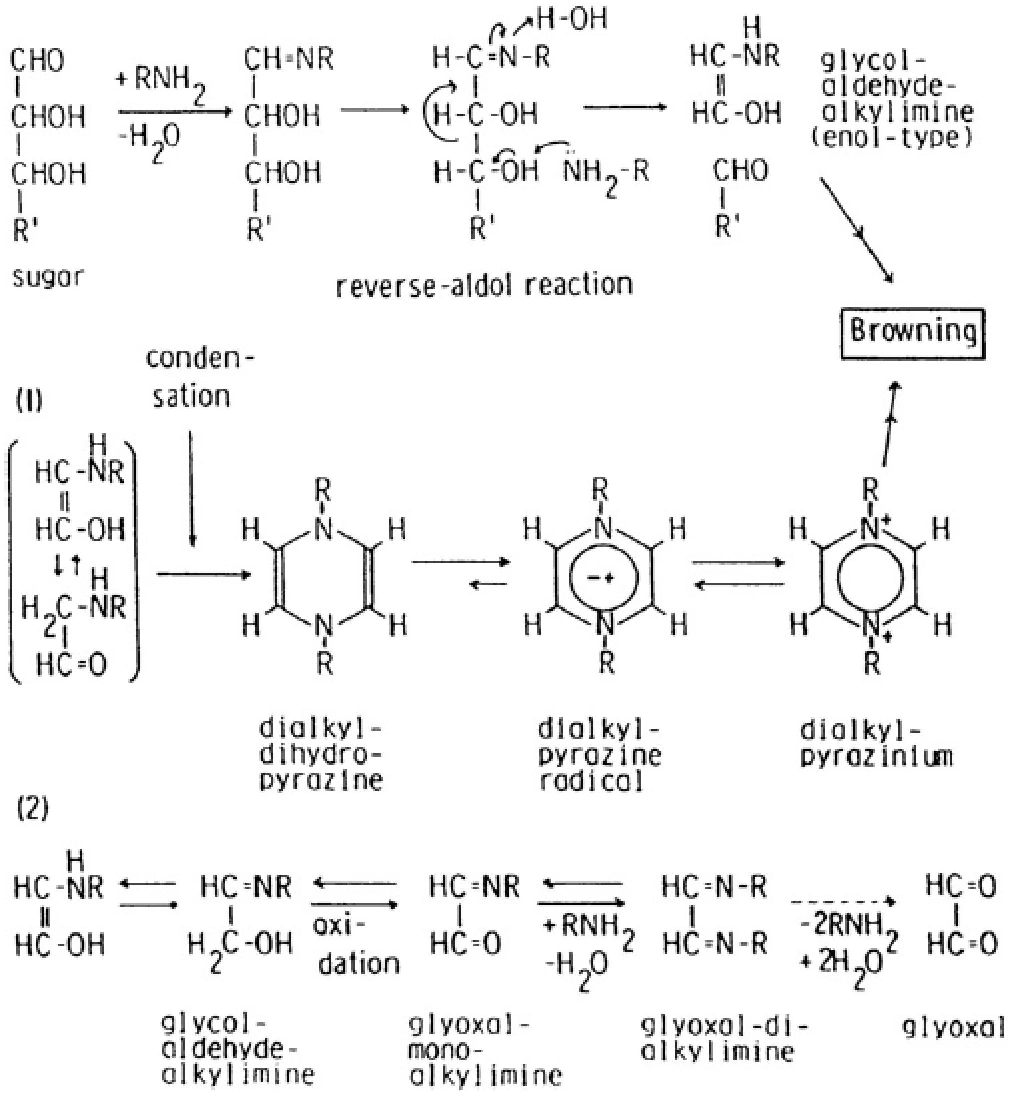

Figure 3: Alternative pathway for browning

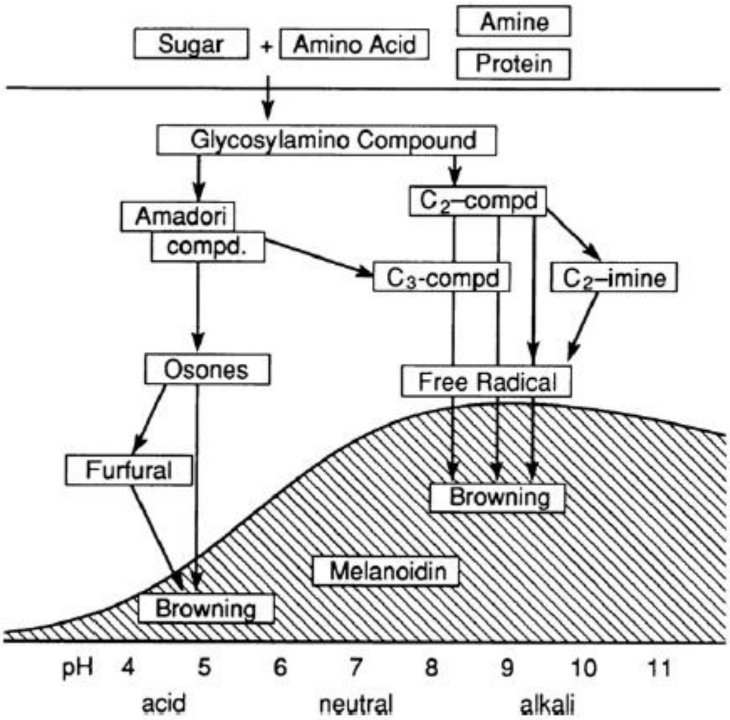

Figure 4: Different pathways for melanoidin formation depending on reaction pH

2.5. Caramelization

Caramelization is a nonenzymatic browning reaction of sugars that produces a caramel-like flavor during high temperature treatment of foods. Sugar degradation is catalyzed by amino acids during the Maillard process, which is distinguished by nitrogen-containing low and high molecular weight molecules. At high temperatures, both processes occur concurrently, with one having an effect on the other. Although the Maillard reaction can occur under gentler settings, sugars are caramelized at temperatures exceeding 120 degrees Celsius. Food products undergo high temperatures during operations such as roasting (180-240°C) and baking (160-240°C). Heating can cause the outer layers of bakery items to dry and reach temperatures exceeding 100°C, leading to browning and flavor development. Caramelization reactions occur in jams, canned fruit goods, juices and concentrates, soft drinks, honey, and sugar syrups during thermal processing or storage. At milder temperatures, pH below three and over nine are frequently necessary to see reactions in moderately faster rates.

Commercially, food caramels come in various forms such as caramel color, burnt sugars, aromatic caramel, and caramelized sugar syrups. Burnt sugars, aromatic caramel, and caramelized sugar syrups are made without additives, whereas caramel color production involves the use of additives. Caramel color serves primarily as a food and beverage colorant and typically does not impart flavor. Figure 5 illustrates the primary reactions involved in sugar degradation.

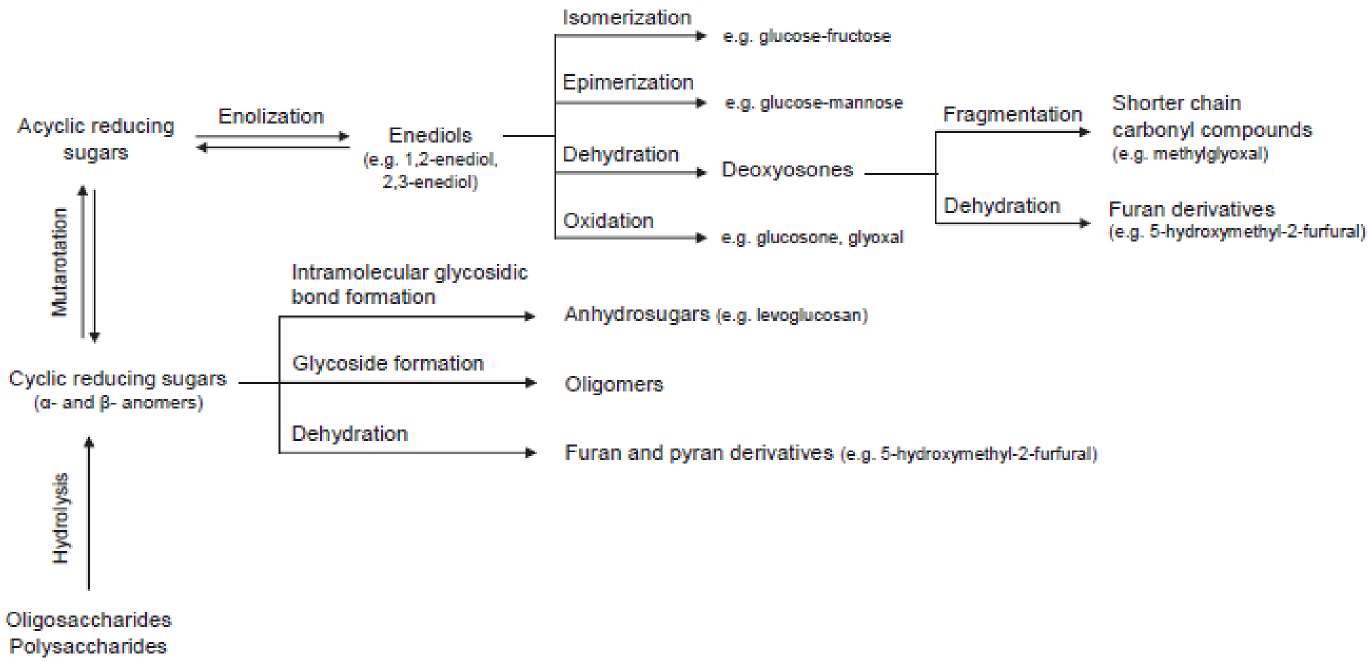

Figure 5: Main reactions of sugar degradation

2.6. Polyphenol peroxidase

Enzymatic browning in fruits and vegetables occurs when they are exposed to air after cutting or slicing, as well as in pulped states, due to mechanical damage during transportation, and during thawing of frozen or cold-stored foods. Polyphenol oxidase (PPO) and peroxidase (POD) are the primary enzymes responsible for this process. PPO is classified as an oxidoreductase enzyme containing four copper atoms as a prosthetic group. It catalyzes the oxidation of hydroxyl groups attached to the carbon atom of the benzene ring in monohydroxy phenols (such as phenol, tyrosine, p-cresol) to o-dihydroxy phenols (like catechol, dopamine, adrenaline), and further dehydrogenates o-dihydroxy phenols into o-quinones. The formation of quinones and subsequent melanin production results in the darkening of foods.

POD, on the other hand, is a thermostable enzyme belonging to the group of oxidases that utilize hydrogen peroxide (H2O2) as a catalyst to oxidize phenolic compounds. POD is associated with undesirable changes in flavor, texture, color, and nutritional quality of foods.

The levels of PPO and POD vary in fruits and vegetables, and their content changes with maturity and senescence, influenced by the ratio of bound and soluble enzymes. The enzymatic reactions leading to changes in color of fruits and vegetables pose significant challenges during harvesting, transportation, storage, and processing. Consumers find the resulting color deterioration, off-flavor, and loss of nutritional value in foods unacceptable.

2.7. Enzymatic browning reaction

Enzymatic browning occurs in a variety of fruits and vegetables, including potatoes, mushrooms, apples and bananas. Tissue that has been wounded, cut, peeled, infected, or exposed to aberrant conditions may quickly darken when exposed to air due to the conversion of phenolic chemicals to brown melanins. Browning reactions can decrease the sensory properties and market value of foods, but they can also add color and flavor to baked and fried foods, coffee, tea, cocoa, and other products. Enzymatic browning results in loss of functional, nutritional, and organoleptic qualities, such as softening, darkening, and off-flavor alterations. The rate of enzymatic browning in fruits and vegetables depends on the concentration of polyphenol oxidases (PPO) and phenolic substances, as well as temperature, pH, and oxygen availability.

Enzymatic browning occurs when the PPO enzyme reacts with oxygen. The substrates involved, primarily polyphenols, reside in plastids, while the enzymes are situated in the cytoplasm. When fruits or vegetables are processed and their tissue is damaged, plastids are ruptured, allowing PPOs to interact with the substrates. PPO then catalyzes the conversion of monophenols to o-diphenols, followed by their oxidation into o-quinones in the presence of oxygen. This reaction is facilitated by PPOs containing two copper atoms at their active sites. Subsequently, the o-quinones undergo nonenzymatic polymerization, forming high-molecular weight pigments known as melanins. These melanins are insoluble, complex dark-colored compounds (Figure 6).

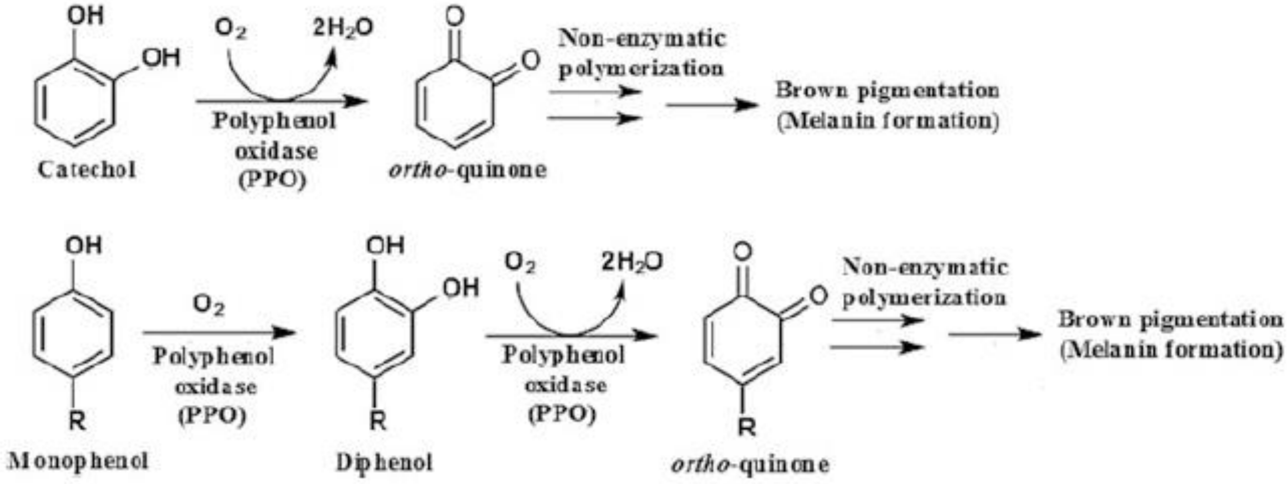

Figure 6: Brown pigment (melanin) formation from phenolic compounds.

2.8. Autoxidation

In recent times, there has been significant interest in food quality, as food components and products undergo numerous chemical reactions during processing, transportation, and storage. Due to the instability of many nutrients, particularly lipids and proteins, it is crucial to study how they change from processing to storage. Since 2003, research on lipid and protein oxidation has notably increased, especially in the past three years, highlighting researchers' focus on nutrient variability in foods. Lipid autoxidation, a continual free-radical chain reaction, can create an unstable and reactive food environment, particularly noticeable in meat.

The presence of free radicals in food systems can lead to protein oxidation, altering protein structures by converting sulfhydryl groups to disulfide bonds. Products of lipid oxidation can accelerate protein oxidation and subsequently induce protein aggregation.

Plenty of research has been conducted on the effects of autoxidation induced by triplet oxygen ($^3O_2$), the most prevalent and stable form of oxygen. However, an alternative oxidation mechanism occurs when food components are exposed to light, even at low temperatures. Various molecules have the ability to absorb light energy, which is then transferred to an oxygen molecule. This results in the transformation of the abundant triplet oxygen into the more reactive singlet oxygen ($^1O_2$).

2.9. Hydrolysis of lipids and proteins

Hydrolysis of lipids

Lipid oxidation significantly contributes to the degradation of food quality and food products. It affects both triglycerides and phospholipids, the two main classes of lipids in food. This oxidation has long been recognized as a major issue in preserving fatty acids during food storage. It occurs through various molecular mechanisms involving the generation of reactive oxygen species and free radicals. Oxidation influences multiple interactions among food components, resulting in both beneficial and undesirable outcomes.

Among food constituents, lipids are particularly prone to oxidation, making oxidation reactions a major factor in the deterioration observed during food manufacturing, storage, distribution, and final preparation. Lipid oxidation products are commonly found in foods, although their types and levels vary widely. Despite generally low levels, lipid oxidation poses significant challenges by compromising the quality of some food products and limiting the shelf-life of others.

Oxidative changes can lead to rancidity, off-flavors, color loss, diminished nutrient content, and potentially the formation of harmful compounds that may affect consumer health. To mitigate these effects, antioxidants and chelating agents are crucial inhibitors of lipid oxidation.

Several factors influence the rate of lipid oxidation. The composition of phases within food impacts lipid oxidation by altering the effectiveness of antioxidants. The oxidation of lipids is influenced by several factors, including: (1) conditions during processing and storage (such as temperature, light, oxygen, metals, and enzymes); (2) the amount of unsaturated fatty acids and how they are distributed in the triglyceride molecule; and (3) the presence of antioxidants (which inhibit oxidation) or prooxidants (which catalyze oxidation). The oxidative reactions of lipids in the presence of air are diverse, with common methods including autoxidation, photooxidation, and enzymatic oxidation.

Hydrolysis of proteins

Protein is a significant nutrient found in diets, along with carbs and fats. Food proteins are subjected to a variety of alterations during the production, processing, and storage stages.

Light, oxidizing enzymes, transition metal ions, and irradiation all cause protein oxidation, whereas the Maillard reaction occurs during thermal treatments when reducing sugars react with amino acids, peptides, or proteins. Both reaction routes result in cascades of reactions with multiple intermediate products that affect food quality, such as flavor, color, protein structure, functional qualities, and nutritional value.

Food protein oxidation causes the loss of thiol groups, as well as the creation of protein carbonyls and particular oxidation products of cysteine, tyrosine, tryptophan, phenylalanine, and methionine residues, such as disulfides, dityrosine, kynurenine, m-tyrosine, and methionine sulfoxide.

Protein oxidation involves a complex series of interactions between oxidizing agents and proteins, leading to the chemical alteration of proteins. Foods contain various oxidative agents, both radical and nonradical, such as superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals ($HO^{\bullet}$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2). These substances are naturally present in small amounts in unprocessed foods but are generated in larger quantities during food processing and storage. Unlike living organisms, processed foods lack active defense mechanisms that regulate redox balance or repair damaged proteins. Nevertheless, inherent enzymes like catalase and glutathione peroxidase, as well as natural or added antioxidants such as tocopherols, polyphenols, and ascorbic acid, can neutralize these oxidizing agents, thereby delaying the oxidation of food proteins. Once the levels of these protective molecules are diminished, proteins, alongside lipids, become vulnerable targets for oxidation.

Dairy products contain significant amounts of transition metal ions and riboflavin. They are subjected to heat and light during processing and storage. Consequently, proteins in dairy may undergo oxidation mediated by reactive oxygen species (ROS), enzymes (such as lactoperoxidase), and light. Muscle meals naturally contain heme (myoglobin or hemoglobin), redox enzymes (glutathione peroxidase and catalase), metal ions (iron and copper), and photosensitizers (riboflavin), all of which can cause or enhance protein oxidation. Numerous studies have demonstrated that muscle proteins undergo ROS-mediated oxidation during processing and storage, which affects textural features mostly due to intra- and intermolecular disulfide cross-linking, but alterations in flavor properties are less noticeable.

The degree of protein oxidation in fruits and fresh vegetables may be less significant from both a chemical and nutritional perspective due to their low protein content (≤5%) and high levels of antioxidants. However, processing methods such as high-pressure treatment, pulsed electric fields, ultrasound, and cold plasma used for sterilizing fruit and vegetable products can introduce or amplify oxidative changes. Plant-based foods rich in proteins, such as legumes and pulses, are utilized to produce protein-rich fractions (e.g., soy and pea protein concentrates/isolates) for applications in meat substitutes and innovative food items. It is crucial to comprehend the oxidative modifications of plant proteins in such cases to develop stable products.

Recent research demonstrated that thermal treatment of iron-enriched pea protein particles led to increased formation of carbonyl groups and insoluble aggregates, indicating significant protein oxidation. Radical-mediated oxidation of rice proteins and soy protein isolates resulted in protein unfolding, accompanied by the formation of protein carbonyls and loss of thiol groups. Likewise, extended storage of rice flour and rice bran in an oxygen-rich environment induced oxidative degradation of thiol groups in rice gliadin and glutelin proteins.

2.10. Crystallization

Solid foods can exist in two primary forms: either as amorphous substances, like hard candies or milk powders, or crystalline structures, such as chocolate or ice cream. The development of crystals in amorphous foods or alterations in the crystal structure, including shape, size, and distribution, frequently lead to shifts in the desired texture and sensory attributes of food, contributing to quality decline. The composition, processing parameters, transportation, and storage conditions play crucial roles in determining the crystalline microstructure of foods and consequently affect the shelf life of these products.

Crystalline phases in foods are primarily formed by key components such as water (ice), sugars, sugar alcohols, lipids, salts, and starches. Additionally, organic acids (like citric acid), proteins, and emulsifiers may also crystallize. These crystals often contribute specific textures found in frozen foods (such as ice cream), grained sugar confections, and fat-based products like butter, margarine, and chocolate.

In confectionery items like fondants and creams, controlled crystallization is crucial to achieve a fine crystal structure that enhances the desired texture. Similarly, in chocolate production, achieving the correct polymorphic form of cocoa butter crystals is essential for producing chocolate with a desirable snap, sheen, and stability during storage. Improper crystallization can lead to chocolate that is dull, soft, and prone to blooming during storage.

In certain foods, the formation of crystals is not desirable but can occur due to thermodynamic factors, as seen in sugar glasses like hard candy. The presence of crystals in such products can lead to various undesirable changes in texture, taste, and other sensory properties, ultimately shortening their shelf life.

For instance, aged cheddar cheese sometimes faces customer rejection because calcium lactate crystals on the surface can be mistaken for mold. Ice cream may develop a coarse texture due to larger ice crystals forming during freeze-thaw cycles in storage, or it may become gritty due to lactose crystallization. Prolonged storage of ice cream, especially in frost-free freezers with heating and cooling cycles, can also cause a characteristic 'freeze burn' on the surface.

3. Biochemical composition of foods of animal origin and derived products

3.1. Muscle biochemistry

Depending on the ratio of fibers to muscle, muscles can range in hue from white to red. Fibers are categorized in several ways. They can be categorized as red, white, or intermediate based on color: White fibers have low levels of myoglobin and demonstrate glycolytic metabolism; intermediate fibers have intermediate characteristics; and red fibers are distinguished by a higher myoglobin content, a greater number of capillaries, and mitochondria. White muscles have a larger percentage of white fibers and are used for support activities, whereas red muscles have a higher proportion of red fibers and are mostly used for locomotion.

The primary constituents of carcasses are muscle, fat, bones, and skin; muscle is the principal component and is also linked to the name "meat." The species, level of fatness, and dressing technique all affect the average percentage of muscle to live weight: 39% for broiler chicken, 36% for pig, 32% for veal, 25% for lamb, 50% for turkey, and 35% for beef. Another significant indicator of muscle mass is the muscle to bone ratio: 2.1 for veal, 4.0 for pork, 2.5 for lamb, 2.9 for turkey, 1.8 for poultry, and 3.5 for beef.

Meat is classified by European legislation as the edible parts of domestic animals, such as caprine, bovine, ovine, and porcine, as well as poultry, farmed, and wild animals (Ahmad et al., 2018). Meat is primarily made up of water, protein, fats, minerals, and carbs. About 72–74% moisture, 20–22% protein, 3–5% fat, 1% ash, and 0.5% carbohydrate are found in lean muscle tissue (Simpson, 2012). High-quality proteins, a range of lipids, including omega-3 polyunsaturated fatty acids, zinc, iron, potassium, magnesium, sodium, vitamin A, B-complex vitamins, and folic acid are all abundant in it. Its composition varies according on the breed, kind of feed consumed, weather, and cut of meat, all of which have a significant impact on the animal's nutritional and sensory qualities.

About 15–22% of the primary chemicals in muscle are proteins, which are crucial to the muscle's integrity, normal function, and structure. Myofibrillar proteins, sarcoplasmic proteins, and connective tissue proteins are the three primary protein categories found in muscle (Simpson, 2012). Lipid content in skeletal muscle varies, ranging from 1% to 13%. The volume of adipose tissue and the degree of fattening are the key determinants of lipid content. Lipids are present in adipose tissue, between muscles (intermuscular), and inside muscles (intramuscular). Triacylglycerols, which are kept in fat cells, and phospholipids, which are found in cell membranes, make up the majority of intramuscular lipids. Lean beef has about 50–70 mg of cholesterol per 100 g. The primary lipids found in intermuscular and adipose tissue are triacylglycerols, with trace quantities of cholesterol (40–60 mg/100 g). Table 10 below lists the nutritional makeup of several types of meat products.

Table 10: Nutritional composition of meat (Ahmad et al., 2018).

Meat Cut	Protein (g)	Sat. fat (g)	Fat (g)	Energy (kcal)	Vit. B12 (µg)	Na (mg)	Zn (mg)	P (mg)	Fe (mg)
Chicken breast, raw	24.2	0.2	8.5	178	0.39	71	0.9	199	1.2
Beef, steak cuts, raw	21	1.9	4.5	123	1.9	59	1.7	167	1.3
Chicken, raw	22.8	0.6	1.9	113	0.7	78	1.4	202	0.7
Beef, calf, loin, raw	20	3.4	7.3	146	1.1	22	3	193	0.1
Beef, loin, raw	20.9	1.5	3.2	115	2	59	3.7	142	1.6
Pork, chop, raw	18.1	10.8	31.7	353	1	60	1.8	190	1.4
Pork, loin, raw	21.9	1.7	4.9	134	1.1	55	1.9	220	0.7
Pork, leg, raw	20.8	2.8	7.8	155	1.2	84	2.6	164	0.8
Turkey, skinless, raw	19.9	1.8	7.1	136	1.9	42	1.5	209	2.1
Duck meat, skinless, raw	19.4	1.8	6.6	130	2.8	90	1.8	201	2.5
Turkey, breast, skinless, raw	23.6	0.5	1.6	106	1	62	0.5	208	0.6
Chicken breast, skinless, raw	23.8	0.4	1.28	109	0.4	59	0.7	218	0.4
Mutton, chop or meat, raw,	20	2.4	4.8	122	2	63	3.6	221	1.9

3.2. Fishes

It is well known that fish is high in macro and micronutrients. Proteins, lipids, and very little carbohydrates make up the macronutrients (Table 11). Micronutrients, such as vitamins and minerals, are also essential components. All of the necessary amino acids, including the sulfur-containing amino acids cysteine and methionine that are missing from plant protein, are found in fish. Compared to other animal protein sources like chicken, beef, etc., fish has a higher satiety impact and is a valuable source of protein. Because fish muscle contains fewer levels of connective tissue than other animal proteins, it is said to be more easily digested. Fish has a high concentration of amino acids (85–95%) and fish proteins are highly digestible.

The composition of fatty acids in fish varies due to factors such as species, diet, and environmental conditions like salinity, temperature, season, geographical location, and whether the fish is wild or farmed. Fish lipids predominantly contain long-chain n-3 polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These PUFA are in a liquid form that flows freely in the bloodstream, distinguishing them from other fats or oils.

The intake of PUFA is widely recognized as important for human nutrition, health, and disease prevention. Omega-3 fatty acids, found abundantly in fish, have various health benefits. They reduce the incidence of myocardial infarction, lower blood pressure, and decrease triglyceride levels in the blood. Fish lipids have demonstrated positive effects in preventing diseases like cardiovascular diseases. Omega-3 fatty acids, particularly DHA, are crucial for optimal brain and neurodevelopment in children, while EPA contributes to improved cardiovascular health.

Table 11: Distribution of Water, Protein, and Lipids in 100 g of Raw Edible Fish including Bones and Cartilage

Fish	Water (g)	Protein (g)	Lipids (g)
Cod	79-81	17-19	0.6-0.8
Hering	78-80	18-20	0.5-0.7
Ling	79-80	17-19	15-17
Mackerel	63-65	17-19	15-17
Monkfish	82-84	14-16	0.3-0.5
Pilchard/sardine	66-68	19-21	8-10
Plaice	78-80	15-17	1.3-1.5
Saithe/coley	79-81	17-19	0.9-1.1
Salmon	66-68	19-21	10-12
Skate	79-81	14-16	0.3-0.5
Sole	80-82	16-18	1.4-1.6
Sprat	68-70	16-18	9-10
Trout (rainbow)	75-77	18-20	5-6
Tuna	69-71	22-24	4-5
Whiting	79-81	17-19	0.5-0.8

Calcium is also necessary for healthy bone density, and calcium ions are involved in most metabolic processes. Minerals like iodine and selenium are abundant in fish. When eaten whole, including the heads and bones, small indigenous species (SIS) can be a great source of several minerals, include ng potassium, calcium, phosphorus, iron, zinc, selenium, and iodine. Fish have a good amount of all the vitamins needed for human health, though the exact amount varies depending on the species. Vitamins A, D, and numerous B-group vitamins are abundant in fish.

3.3. Shellfish

One significant part of the world's seafood production is shellfish. Shrimp, lobster, oysters, mussels, scallops, clams, crabs, krill, crayfish, squid, cuttlefish, snails, abalone, and other seafood are among the particular products (Venugopal & Gopakumar, 2017). High levels of protein (8.1–21 g/100 g), essential amino acids, EPA and DHA (sum of 0.061–4.3 g/100 g), vitamin B12 (0.82–65 µg/100 g), vitamin E (0.75–28 mg/100 g), zinc (0.61–7.9 mg/100 g), iodine (3.1–2100 µg/100 g), and selenium (7.2–590 µg/100 g) can all be found in shellfish. It was discovered that the hepatopancreas of crustaceans had a significantly higher nutritional density than the white flesh. Moreover, it is not possible to regard the shellfish that were part of this study as excellent providers of vitamin D3, niacin, folate, and riboflavin.

3.4. Milks

Breast milk, a white or slightly yellowish liquid, is a biological secretion of animal (including human) breasts that is meant to nourish their young. To make milk, a variety of animal species are employed. The most popular milk is cow's milk. It accounts for 90% of global milk output. Sheep milk has 2%, goat milk has 3%, and buffalo milk has 5% after them.

Many everyday elements, including the animal's age, lactation (stages of milking), reproduction, season, ambient temperature, nutrition, state of health, and animal gestation length, all affect the chemical makeup of milk (Kourkouta Lambrini et al., 2020). A complete diet, cow milk meets nearly all of the requirements of the human body. Specifically, it is made up of calcium, phosphate, lipids, proteins, lactose, and vitamins (mostly D and B2) (Table12). It has a lot of calcium and lysine, which is an amino acid that's frequently lacking in plant proteins. Calcium and phosphorus are the minerals' main constituents, which facilitate the body's absorption of them.

Table 12: The composition of milk from different mammals in g /100 g milk

Species	Water	Proteins	Fat	Lactose	Ash
Cow	87.2	3.5	3.7	4.9	0.72
Sheep	82.7	5.5	6.4	4.7	0.92
Goat	86.5	3.6	4	5.1	0.82
Camel	87.7	3.5	3.4	4.7	0.71

Proteins are made up of either phosphoric acid and amino acids (α and β casein) along with a fraction of carbohydrates (occasionally even k casein) or only amino acids (β-lactoglobulin, α-lactalbumin). The essential polyunsaturated fatty acids for human metabolism are scarce in goat and cow milk. In both circumstances, goat milk has a somewhat higher fat content than cow milk; triglycerides make up more than 95% of total lipids. Cholesterol ranges from 10 to 20 mg per 100 ml, the majority of which is in the free form, while phospholipids make up 30 to 40 mg/100 ml of cow milk, which contains 8 to 10 mg of lipid. Last but not least, the body needs the vitamins A and D found in milk fat. A is vital to the epithelia, which explains its function in reproduction and eyesight, while D is necessary for the binding of calcium and the development of bones.

Le terme « lait traité thermiquement » fait référence au lait qui a été chauffé avant consommation et décrit des produits laitiers créés uniquement à partir de lait cru et sans danger pour la consommation humaine. Le lait pasteurisé et de longue conservation sont deux exemples de lait de consommation traité thermiquement (Table 13).

Table 13: Composition of milk intended for consumption (Kourkouta Lambrini et al., 2020).

Type	Fat (%)	Min. Fat-free solid residue (%)
Whole milk	3.5 (min.)	8.5
Semi-skimmed milk	1.5-1.8	8.5
Partly (partially) skimmed milk	1.8-3.5	8.5
Skimmed milk	0.5 (max.)	8.5

3.5. Eggs

Eggs are particularly noteworthy in the nutritional context since they provide a modest source of calories (about 140 kcal/100 g) along with important fats, proteins, vitamins, minerals, and trace elements. It should be noted that while eggshell membranes are edible, eggshell and the closely related eggshell membranes are often not consumed (Figure 7).

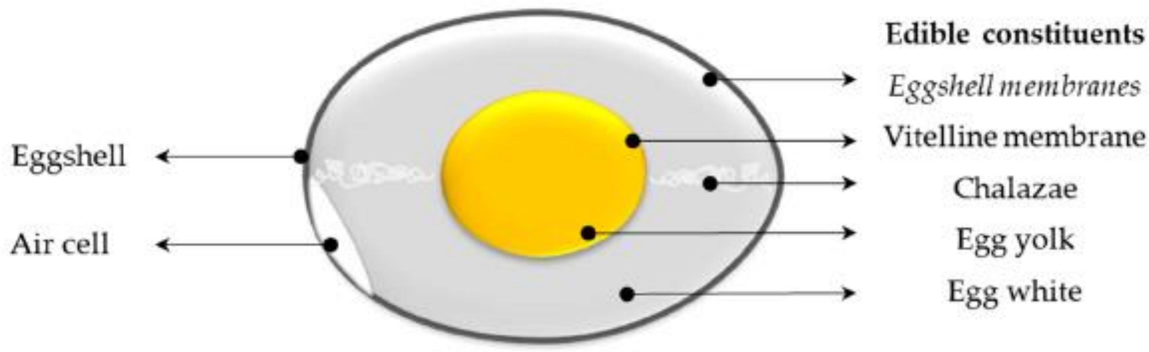

Figure 7: Egg structure

Proteins in eggs are split evenly between the white and yolk sections, while the yolk primarily contains lipids, vitamins, and minerals. The majority of an egg is made of water (Figure 8).

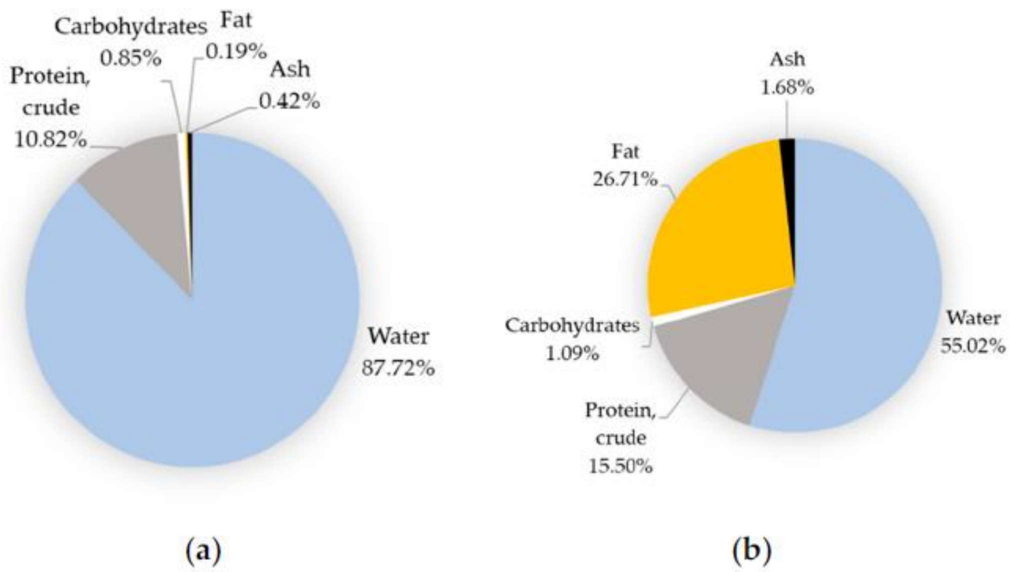

Figure 8: Composition of edible parts of the egg. (a) Egg white; (b) Egg yolk.

Whole raw fresh eggs have an average protein content of 12.5 g per 100 g, but egg yolks with their vitelline membrane and egg white have 15.9 g and 10.90 g of protein per 100 g, respectively. With 68% low-density lipoproteins (LDLs), 16% high-density lipoproteins (HDLs), 10% livetins and other soluble proteins, and 4% phosvitins, yolk is a complex milieu. The most prevalent proteins found in egg yolks are apovitellenin-1, vitellogenins, ovalbumin, serum albumin, immunoglobulins, and ovotransferrin, which together account for about 80% of all egg yolk proteins. The egg white is a gel-like substance that is primarily made up of water (around 80%) and is devoid of lipids. Peptides, glycoproteins (ovalbumin, protease inhibitors), antibacterial proteins (lysozymes), and fibrous structural proteins (ovomucins).

High concentrations of vitamins A, D, E, K, B1, B2, B5, B6, B9, and B12 are found in egg yolks, whereas considerable concentrations of vitamins B1, B6, B8, B9, and B12 are found in egg whites (Table 2). Ten to thirty percent of human vitamin requirements are met by eating two eggs a day.

3.6. Meat pâtés, smoked meats

Pâtés are considered food products with a high animal fat content, ranging from 35% to 50% (Martins et al., 2020). Liver pâté uses various types of poultry liver (such as broiler, ostrich, goose, and duck) and typically contains a higher fat content, ranging from 30% to 50% depending on the specific recipe. The fat plays a crucial role in pâté by enhancing its spreadability. It needs to be evenly distributed within the mixture, encapsulated, and emulsified by the protein structure.

Unlike meat sausages, liver pâté contains less lean meat, which reduces the availability of functional muscle proteins and makes the product less heat-resistant. Excessive heat can release fat or affect the color and flavor, thus liver proteins are primarily responsible for emulsification. However, additional proteins, such as milk protein concentrates, may be incorporated into the recipe to enhance the emulsification process (Totosaus-Sanchez, 2008).

Turkey Pate production technology included the following sequential operations: preparation of raw Turkey meat, fermentation of pork skin, preparation of meat emulsion according to the recipe by successively introducing the recipe's ingredients, placement of the emulsion in the packaging, sterilization, quality control of finished products, and storage (Figure 9).

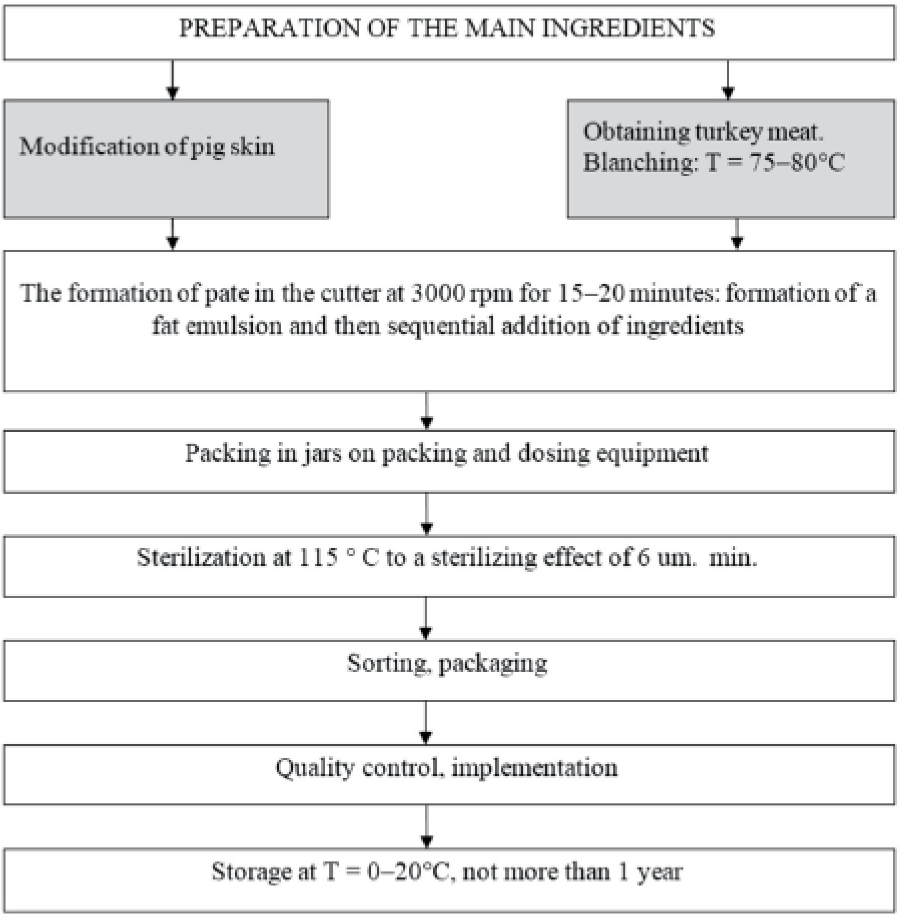

Figure 9: Flow Chart of Turkey Meat Pate Production.

Smoke leads to flavorful meat and poultry. Smokers can add natural smoky flavor to huge pieces of meat, chicken, and turkey breasts. This slow cooking process also keeps them delicate. Smoking is the process of gently cooking food indirectly in the presence of fire. Use a "smoker," an outdoor cooker built for this purpose. To smoke food on a covered grill, place a drip pan with water beneath the meat.

Smoking is a food preservation technique used to extend the shelf life of food by reducing moisture content and microbial load. It also enhances the sensory qualities like taste, aroma, and appearance of smoked meat and fish. There are two main types of smoking processes: "cold" smoking, where product temperature stays below 30°C, and "hot" smoking, which cooks the food such as fish and may raise the center temperature to 60–85°C. Additionally, a third method known as smoke-drying combines hot smoking with a drying step, often used in preserving fish in African countries. This process allows the production of dried products with a water activity of 0.75 or lower, suitable for room temperature storage while controlling bacterial and fungal growth.

During smoking, fish or meat is exposed to smoke generated by partially burning wood, either directly over embers or indirectly in a separate chamber. Traditional methods often use kilns fueled by wood, charcoal, sawdust, chips, bagasse, corn cobs, coconut husks, or shells. Smoke is a complex mixture of approximately 380 compounds, primarily including phenols, aldehydes, ketones, organic acids, alcohols, esters, hydrocarbons, and various heterocyclic compounds. These components influence sensory qualities and can enhance shelf life by inhibiting spoilage bacteria. However, there is a risk of forming carcinogenic compounds such as PAHs, nitrosamines, and heterocyclic amines during smoking, either through the pyrolysis of organic matter or through direct formation within the food due to reactions with heat and food composition.

3.7. Charcuterie

The term "charcuterie," which appeared around the 16th century, comes from "chair cuite," meaning cooked meat. In 1475, in Paris, the guild of charcutiers ("chair cuitiers") became autonomous and distinct from that of butchers.

Charcuteries are products made by processing meats in general (pork, beef, poultry, game, fish), with pork being the primary raw material. They also include products preserved not by cooking but by salt (cured meats), which was the only method available at the time to prevent foodborne infections. Charcuterie and catering gastronomy boast a rich repertoire of over 400 products ranging from traditional to modern. This wide variety encompasses major categories of recipes and specialties.

Cooked hams, cooked shoulders, and dry-cured hams are made from whole parts of limbs. Dry sausages, cooked or to-be-cooked sausages, pâtés, terrines, and rillettes are blends of minced meats prepared in different ways. Blood sausages, andouilles, and andouillettes, tripe dishes, "pieds paquets," and head-based products are made from specific parts of the animal. Pâtisserie charcuteries include items like pâté en croûte, quiches, sausages in brioche, and pastry parcels.

Based on their protein content per 100 g, three groups of products can be defined:

- Have more than 20% protein: cooked and dry-cured hams, dry sausages, dried sausages.

- Have 15 to 20% protein: head pâtés, rillettes, andouilles, and andouillettes.

- Have 10 to 15% protein: pâtés, terrines, sausages, blood sausages, cooked sausages, etc.

Overall, 50% of the charcuterie consumed in France contains less than 20% fat. Common charcuterie items provide minimal amounts: less than 30 mg per slice of cooked ham, less than 80 mg per serving of pâté, and less than 40 mg for four slices of dry sausage. The exception is andouillette, which stands out with 340 mg per serving.

Current cautious recommendations aim for an average daily salt intake of 6 to 8 grams per person to reduce the prevalence of high blood pressure and contribute to cardiovascular disease prevention. Diversity in products and their salt content is a recurring theme in charcuterie. However, since pork meat typically dominates, it provides significant amounts of B-group vitamins, iron (Fe), and zinc (Zn). The iron content is particularly noteworthy because this "heme" iron is well absorbed by the digestive tract, especially when accompanied by vitamin C from liver (in liver pâtés) or vegetables. Black pudding stands out as the food richest in iron.

3.8. Cooked meats

Cooking enhances meat tenderness but also results in weight loss due to heat treatment. The tenderness of cooked meat is influenced by its initial toughness, post-mortem aging, and cooking method. The temperature and duration of cooking are crucial factors affecting meat tenderness during heat treatment, varying depending on the cooking appliance used. Loss of moisture during cooking occurs due to muscle fiber and intramuscular connective tissue contraction, intensified by higher temperatures. Cooking effectively eliminates foodborne pathogens, with recommended temperatures like 70°C for 2 minutes reducing *Listeria monocytogenes* bacteria significantly. However, achieving this temperature uniformly across different cooking methods (e.g., grilling or frying) may vary, potentially allowing survival of pathogens. Cross-contamination risks are also noted, with pathogenic bacteria typically reduced to safe levels or eliminated entirely through adequate cooking, provided environmental conditions do not foster bacterial growth.

There are two categories of cooking techniques:

1. Dry heat cooking method directly exposes the food to heat.

2. Moist heat cooking method indirectly applies heat to the food.

3.9. Yogurts and cheeses

Yogurt

Yogurt is a nutrient-dense food produced through bacterial fermentation of milk. Recent studies highlight its significant health benefits, providing essential nutrients that can enhance the health of vulnerable populations. It is rich in essential amino acids, calcium, vitamin D, riboflavin, vitamin B6, and vitamin B12. Yogurt supports gut health by promoting probiotic bacteria, which can help prevent intestinal infections, lower serum cholesterol levels, alleviate lactose intolerance, and reduce the risk of cancer. It may also contribute to the prevention of osteoporosis, hypertension, and obesity.

Consumption of yogurt is associated with immune-modulatory effects, beneficial in treating conditions like Inflammatory Bowel Disease (IBD), including Crohn’s disease, ulcerative colitis, and pouchitis. Regular consumption of yogurt is linked to a reduced risk of Type 2 diabetes, improved insulin sensitivity, lower blood glucose levels, and reduced triglyceride levels. Yogurt is considered a functional food that serves both preventive and therapeutic purposes in modern health care, underscoring the importance of ongoing public education about its benefits.

Yogurt can be categorized into two main types: standard culture yogurt and bio- or probiotic yogurt. Standard yogurt is produced using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. These bacteria stimulate beneficial microflora in yogurt, which contribute to overall gastrointestinal health. In contrast, bio-yogurts are made with probiotic strains such as bifidobacteria and *Lactobacillus acidophilus*, known for their various health benefits. Bio-yogurts are more popular for their milder, creamier taste and lower acidity. They aid digestion and support overall well-being.

Cheeses

Cheese is defined as a ripened or unripened product produced by the coagulation of proteins in milk, skimmed or partially skimmed milk, cream, whey cream, buttermilk, or a combination of these liquid streams, with a protein concentration derived from the source material. The manufacturing process includes dehydration and, in many cases, fermentation, both of which aim to extend the shelf life of milk. A soft cheese curd is made by coagulating milk proteins using rennet or another suitable coagulating agent, followed by partially draining the whey. The lactose in the cheese curd is fermented to its constituent sugars, galactose and glucose, and eventually to lactic acid by the action of lactic acid bacteria (LAB), which may be added as starter cultures, lowering the pH from 6.7 in the original milk to 4.5 to 6.0 in most cheese varieties.

Cheese comprises the primary milk protein casein, milk fat, the mineral calcium phosphate, and trace amounts of lactose, whey, and other micro components such as salt. The liquid whey contains whey proteins, the remaining lactose, and some soluble minerals. Full fat hard cheeses are composed of approximately 25-30% protein, 30-35% fat, and the balance is primarily water. Softer cheeses include more water and/or fat. Endogenous milk minerals account for around 1% of the cheese weight, the most common of which is calcium phosphate. Cheese also includes sodium chloride, the amount of which varies greatly depending on the type of cheese and may exceed the calcium phosphate concentration.

3.10. Fish products and prepared dishes

Fish products

Fish products have gained popularity as a source of critical nutritional components such as high-quality protein, necessary vitamins, minerals, and beneficial polyunsaturated fatty acids in the human diet. As a result, fresh fish and seafood are the third fastest growing food category globally, trailing only drinkable yogurt (18%) and fresh soup (18%).

A wide range of affordable processing methods for fish products, including live fish, fresh chilled fish, whole cleaned fish, filleted steaks, battered and breaded products, various dried products, smoked fish, fish sausages, and traditional items, are suitable for adoption by small-scale fishers.

To prevent fish and fisheries products from spoiling, proper storage conditions must be maintained. Many developing technologies have the potential to enhance product shelf life. High Pressure Processing, Irradiation, Pulsed Light Technology, Pulsed Electric Field, Microwave Processing, Radio Frequency, Ultrasound, and others are some of the new technologies used in fish processing. Modified atmosphere, active, and intelligent packaging technologies all play essential roles in fish preservation.

Seafood products are classified under heading 03 of the CN code, according to Commission Implementing Regulation (EU) 2017/1925. Specifically:

- 0302 covers fresh or chilled fish (excluding fillets and other fish meat under heading 0304), including various types such as Salmonidae, flatfish, tunas, herrings, cod, tilapias, and fish offal.

- 0303 pertains to frozen fish (excluding fillets and other fish meat under heading 0304), encompassing the same types of fish.

- 0304 is designated for fresh, chilled, or frozen fish fillets and other fish meat, whether minced or not.

- 0305 includes dried, salted, or brined fish; smoked fish, whether cooked before or during smoking; and fish flours, meals, and pellets suitable for human consumption.

- 0306 covers crustaceans, whether live, fresh, chilled, frozen, dried, salted, or in brine; smoked crustaceans, whether cooked before or during smoking; crustaceans cooked by steaming or boiling; and crustacean flours, meals, and pellets suitable for human consumption, such as lobsters, crabs, shrimps, and crayfish.

- 0307 pertains to molluscs, including live, fresh, chilled, frozen, dried, salted, or in brine molluscs; smoked molluscs, whether cooked before or during smoking; and mollusc flours, meals, and pellets fit for human consumption, covering oysters, scallops, mussels, cuttlefish, squid, octopus, snails, abalone, and others.

- 0308 is for aquatic invertebrates other than crustaceans and molluscs, encompassing live, fresh, chilled, frozen, dried, salted, or brined aquatic invertebrates; smoked aquatic invertebrates, whether cooked before or during smoking; and aquatic invertebrate flours, meals, and pellets fit for human consumption, such as sea cucumbers, sea urchins, and jellyfish.

Prepared dishes

Prepared foods are dishes that incorporate multiple ingredients and are ready to eat or heat. To ensure microbiological stability in canned food, the thermal process should use the slowest heating component, which may require overcooking. Freezing separates the components but diminishes eating quality. Refrigeration is the favored preservation technology for maintaining quality and meeting consumer preferences.

3.11. Egg products

Egg products refer to all types of egg presentation, including yolk, albumen, and a combination of the two. The phrase "Egg Products" refers to processed or convenience versions of eggs created by processing shell eggs. Egg products include whole eggs, egg whites, and egg yolks in frozen, pasteurized, and refrigerated liquid and dried forms, which are offered in a variety of product formulations. In particular, the food sector is interested in high quality egg products in a liquefied form, obtained from eggs shelled within 4 days and which have undergone homogenization and pasteurization: their application is primarily related to the creation of egg pasta and bakery items.

4. Technology-related changes

4.1. Oxidation of meat products

Proteins oxidation

The oxidation of dietary proteins is a cutting-edge topic in food chemistry research. Protein oxidation (Pox) refers to the covalent alteration of proteins caused by reactive species or subsequent by-products of oxidative stress. Modifications to muscle food pose both technological and sensory challenges, as well as safety concerns for human consumption. The process involves the general mechanism via free radical basis, leading to the loss of sulfydral groups, formation of cross-linking, formation of carbonyl groups, and lipid-protein interaction.

Oxidation of proteins in meat affects their solubility and functioning, including gelation, emulsifying qualities, and water holding capacity. Oxidation of myofibrillar protein can significantly reduce its nutritional value, including critical amino acid availability and digestibility. The oxidation of amino acids produces carbonyl groups and derivatives. Basic amino acids (lysine, histidine, and arginine) and threonine are necessary for humans. Oxidation of these amino acids depletes their levels in muscle meals. Phenylalanine and tryptophan are important amino acids for humans, and loss in meat due to ROS can significantly reduce their availability.

Lipids oxidation

Storage of meats and other fat-containing meals can lead to quality deterioration due to lipid oxidation. Oxidative degradation of lipids has a direct impact on meat quality, including flavor, color, texture, nutritional value, and safety. Storing fats and oils can cause lipid oxidation and rancidity, a known issue. Food lipids undergo autoxidation processes, followed by secondary reactions (both oxidative and non-oxidative).

Several mechanisms can overcome the spin restriction that prevents groundstate oxygen from interacting with unsaturated fatty acids. These include singlet oxygen, partially reduced or activated oxygen species like hydrogen peroxide, superoxide anion, or hydroxyl radicals, active oxygen-iron complexes (ferryl iron), and iron-mediated homolytic cleavage of hydroperoxides, resulting in organic free radicals.

Ferrous and ferric iron can catalyze lipid oxidation by decomposing lipid hydroperoxides. This produces free radicals that can propagate the oxidative processes. Lipid oxidation produces hydroperoxides, which are colorless, tasteless, and odorless. The breakdown of peroxides produces a complex combination of low-molecular-weight chemicals with different odors and flavors, such as alkanes, alkenes, aldehydes, ketones, alcohols, esters and acids. These chemicals can cause rancid, fatty, aromatic, and other unpleasant flavors in meat.

There are various ways for measuring lipid oxidation in meats. Despite being widely criticized, the TBA test remains the preferred method in most situations. It is easy to use and has high sensitivity and adaptability in detecting lipid oxidation and other radical processes (Gray & Monahan, 1992).

4.2. Cooking-related alterations

Native proteins are denatured during extrusion. The extruder's elevated temperature and shear impair the forces that stabilize proteins' tertiary and quaternary structures. Individual protein molecules unfurl and align with the flow of material to the die. Previously buried amino acid residues are revealed and can react with reducing sugars and other dietary components. The exposure of hydrophobic residues like phenylalanine and tyrosine reduces the solubility of extruded protein in aqueous solutions.

Vitamin loss varies depending on the processing and cooking circumstances. Losses vary based on cooking method and food type. Vitamin degradation is dependent on various factors throughout the culinary process, such as temperature, the presence of oxygen, light, moisture, pH, and, of course, the duration of heat treatment. During culinary operations, the most labile vitamins include retinol (33% retention during vegetable boiling), vitamin C (33% retention due to cooking and oxidation), folate (40%), and thiamine (20-80% retention). Niacin, biotin, and pantothenic acid are relatively stable.

5. Cheese transformation processes

Cheese stands out as the most varied category of dairy products and is arguably the most intellectually intriguing and complex. Unlike many dairy products that remain biologically, biochemically, and chemically stable if made and stored correctly, cheese is biologically and biochemically active. This results in changes to its flavor, texture, and functionality during storage, with the extent of these changes depending on the cheese variety. The process of making cheese involves a precisely coordinated series of biochemical events during manufacture and aging. When these processes are well-timed and balanced, they produce cheeses with highly desirable aromas and flavors. However, if they are not properly managed, the result can be off-flavors and unpleasant odors. Despite using a basic raw material (milk from a few species) and following similar manufacturing protocols, the wide range of cheese varieties produced is truly fascinating.

Cheese production follows a similar technique, with modifications made to achieve desired product characteristics (Figure 10). The main general steps are:

1. Milk is selected, standardized, and, in most cases, pasteurized.

2. Acidification occurs when certain bacteria produce lactic acid on-site.

3. Coagulation of milk through acidification or restricted proteolysis.

4. Various procedures are used to dehydrate the coagulum and produce cheese curd, with some being specific to each species.

5. Forming the curds into distinct shapes.

6. Most cheese kinds require curd maturing to create their distinct flavor and texture.

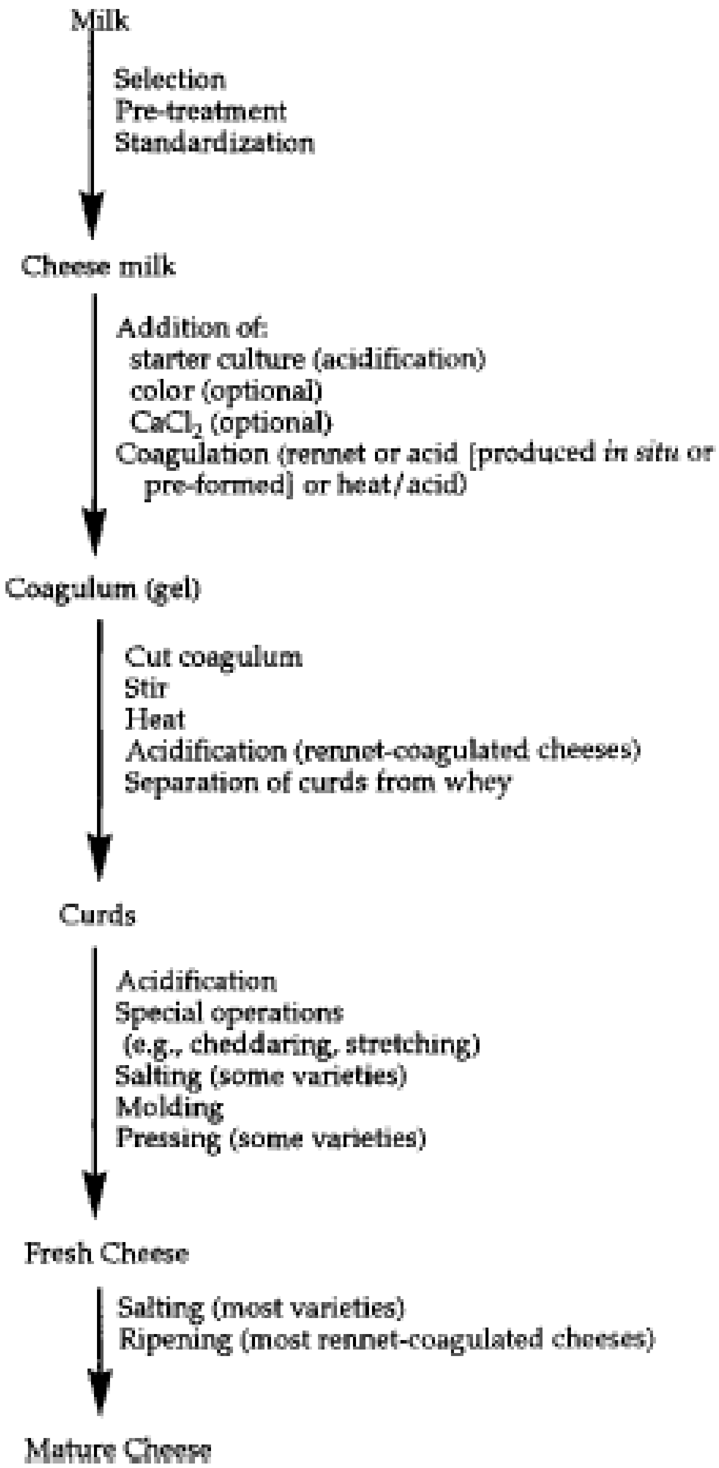

Figure 10: General protocol for cheese manufacture

5.1. Milk preparation

Cheese's composition is heavily influenced by the milk's fat, protein, calcium, and pH levels. Milk composition is influenced by characteristics such as species, breed, individuality, nutritional state, health, and lactation stage of the producing animal. To avoid off-flavors in cheese, milk should be free of chemicals, free fatty acids, and antibiotics that inhibit bacterial growth. Use high-quality milk to avoid bacterial contamination in cheese curds, which can lead to defects and public health issues.

Milk used for cheese undergoes various preparatory steps, each serving specific purposes. Different types of cheese are characterized by their fat-to-dry matter content, which includes a defined fat-to-protein ratio stipulated in the "Standards of Identity" for many cheese types. While the moisture level in cheese, influenced by production methods, primarily determines the combined fat and protein content, the fat-to-protein ratio is primarily determined by the fat-to-casein ratio in the cheese milk. This ratio can be adjusted by several methods, such as natural creaming or centrifugation to reduce fat content, or by adding skim milk, cream, micellar casein, milk powder, evaporated milk, or ultrafiltration retentate to increase it. These additions also increase the total solids in milk, thus enhancing cheese yield.

Calcium is crucial in milk coagulation by rennet and subsequent processing of the coagulum, prompting the common practice of adding $CaCl_2$ (e.g., 0.01%) to cheese milk. The pH of milk is a critical factor in cheesemaking, often adjusted unintentionally by adding 1.5-2% starter culture, which immediately lowers milk pH by approximately 0.1 unit. Starter concentrates, increasingly utilized, do not have an immediate acidifying effect on milk.

To mitigate the depletion of these lactic acid bacteria (LAB), it is increasingly common to introduce a selected LAB culture (lactobacilli) into cheese milk alongside the primary acid-producing culture. Indigenous enzymes like lipase, which can aid in cheese aging, are typically deactivated during pasteurization. Cheese milk is often pasteurized at temperatures below 70 °C to preserve its cheesemaking qualities, as exceeding 72 °C for 15 seconds can detrimentally affect milk's suitability for cheese production.

5.2. Coagulation

To make cheese, the casein component of milk protein is coagulated to form a gel that holds any fat present. Coagulation can be produced through:

- Selective proteolysis by rennets;
- Acidification to ~pH 4.6;
- Acidification to a pH higher than 4.6 (perhaps ~5.2) in combination with heating to ~90°C (Fox et al., 2017).

Rennet is composed of chymosin and pepsin. Chymosin is a proteolytic enzyme found in juvenile ruminants. It curdles the milk, which increases its nutritional value. Chymosin is a highly selective coagulating enzyme, whereas pepsin has widespread proteolytic activity, resulting in lesser cheese output. Chymosin cleaves the peptide link between Phe 105 and Met 106 in κ-casein. It converts κ-casein to insoluble para-κ-casein, resulting in a calcium-based curd. Chymosin is also responsible for texture and flavor changes that occur during ripening. The low pH of the abomasa activates both chymosin and pepsin, which are generated as inactive zymogens.

Aspartic proteases (AP) are the most frequent form of milk coagulating enzymes, found in a wide variety of plants. They are particularly effective in acidic conditions. Serine proteases (SP) with milk coagulating characteristics have been discovered and isolated from a wide range of taxonomic groups and physiological parameters. It is most commonly found in fruits, although it can also be taken from latex, flowers, leaves, roots, stems, and seeds. SP occurs in trees, legumes, crops, and herbs. Cysteine proteases (CP) can be found in a wide range of plants and tissues, but they are most prevalent in fruits. Actinidain, a cysteine protease found in kiwi fruits, destroys undesirable cream milk proteins, resulting in the delicious taste of "Mozzarella" cheeses.

A decreased milk pH during acid coagulation alters casein micelle characteristics. As a result, calcium that is bound becomes detached from the micelles, leading to neutralization of the negative charges on the casein micelles. This promotes aggregation as the casein micelle approaches its isoelectric point (pH 4.6), resulting in the formation of a porous network of loosely interconnected aggregates.

The coagulation of milk is a multifaceted process affected by several factors. pH, calcium levels, and temperature are among the most significant. Lowering pH and raising temperature expedite the coagulation process. As for calcium, higher levels of bound calcium and free calcium ions promote coagulation. Introducing calcium into the milk enhances these levels while also reducing pH.

5.3. Draining

After achieving the desired level of syneresis and pH, the curds are separated from the whey using a variety-specific method. This may include transferring the curds-whey into perforated moulds (e.g., Camembert), allowing the curds to settle in the vat and sucking off the supernatant whey (e.g., Gouda and Emmental), or scooping the curds from the vat and placing them in moulds (e.g., Parmi).

Traditional cheese forms and sizes include small cylinders (Brie and Camembert), taller cylinders (Cherddar and Parmesan), big low cylinders (Emmental), and spheres (Edam). Some cheeses, such as Cheddar and Emmental, are now available in rectangular or square blocks instead of their usual shapes.

5.4. Salting

Except for Domiati cheese, which is salted before rennet coagulation and curd formation, all other cheeses undergo salting afterwards. There are three main methods for salting cheese curds:

- Dry salting: This involves directly adding and mixing dry salt crystals into broken or milled curd pieces towards the end of the cheese-making process. Examples include Cheddar and cottage cheeses.
- Surface dry salting: In this method, dry salt or a salt slurry is rubbed onto the surface of the molded curds. This technique is typical for blue cheeses.
- Brine salting (Brining): The cheese curds are immersed in a brine solution. This method is used for cheeses like Edam, Gouda, Saint Paulin, Provolone, among others.

Sometimes a mix of these strategies is utilized.

5.5. Cheese ripening

Cheese ripening primarily involves the breakdown of proteins, lipids, and carbohydrates, which releases flavor compounds and alters the texture of cheese. Key agents in this process include milk enzymes (such as plasmin and lipoprotein lipase), milk coagulant, starter lactic cultures, secondary cultures, and other ripening agents. The ripening of cheese is a highly intricate process that entails microbiological and biochemical transformations in the curd, ultimately defining the flavor and texture characteristics of each cheese variety.

Microbiological changes during ripening encompass the natural decay and breakdown of starter cultures, non-starter lactic acid bacteria, and secondary microflora, which play crucial roles in many types of cheese. Molds in varieties that ripen with mold and a diverse population of Gram-positive bacteria in smear cheeses significantly influence the flavor and texture profiles of the cheese.

As cheese ripens, its texture typically becomes softer due to the breakdown of casein micelles and alterations in the curd's ability to bind water and its pH. Biochemical changes during ripening can be categorized into primary events, such as the metabolism of residual lactose, lactate, and citrate (glycolysis), as well as lipolysis and proteolysis. Following these primary events, secondary biochemical processes occur, contributing to the development of numerous volatile flavor compounds characteristic of matured cheese varieties.

Rennet-coagulated cheeses typically age for a time ranging from 3 weeks to over 2 years. The duration of ripening is inversely proportional to the cheese's moisture level. Varieties can be consumed at various stages of ripeness, based on consumer preferences and economic circumstances.

6. Manufacturing of fermented milks

6.1. Definition

Fermented milk is a product made by fermenting milk using appropriate microorganisms. The milk used in this process may have been derived from milk products that were modified in composition or not, as allowed. This fermentation process lowers the pH, potentially causing coagulation (iso-electric precipitation).

The starter microorganisms must be viable, active, and present in sufficient quantities in the product until the minimum durability date. If the product undergoes heat treatment after fermentation, the presence of viable microorganisms is no longer necessary. Milk fermentation produces a wide range of products with varying organoleptic properties depending on the fermented microorganisms, recipes, and additives. Yogurts, kefirs, and cheeses are the most often fermented dairy products.

6.2. Microbiology of lactic fermentation

Yoghurt is a popular dairy product that is prepared by fermenting milk. *Lactobacillus bulgaricus*, *Streptococcus thermophilous*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* are the most commonly isolated bacteria from yoghurt. Moderate thermophile LAB are used as starting cultures in yoghurt and yoghurt-like products, including *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. These strains also produce exopolysaccharides, which contribute to reduced syneresis, higher viscosity, and a smoother, creamier texture of the yogurt.

Kefir is a fermented beverage prepared from milk or water and enjoyed in many Asian nations. "The term Kefir is derived from the Turkish word "keyif", which means "good feeling". *Lactobacillus kefiranofaciens*, *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum*, and *Saccharomyces cerevisiae* are among the various bacteria and yeast found in kefir grains (Sionek et al., 2023).

6.3. Biochemistry of lactic fermentation

The vast majority of studies on lactic fermentations have focused and continue to focus on lactic bacteria due to their industrial applications in the dairy sector for the production of cheeses and yogurt. Briefly, lactic bacteria are a group of microorganisms capable of

1 glucose + 2 Pi + 2 ADP $\rightarrow$ 2 lactate + 2 ATP (homofermentation)

1 glucose + 1 Pi +1 ADP $\rightarrow$ 1 lactate + 1 éthanol (acétate) + 1 CO_2 + 1 ATP (hétéro)

transforming simple sugars like lactose or glucose into lactic acid. This transformation yields 1 or 2 molecules of ATP, depending on whether the metabolic pathway is homolactic or heterolactic. These molecules can be utilized for producing the energy necessary for biosynthesis and cellular growth:

Lactose is transformed into lactic acid by lactic acid bacteria during the kefir fermentation process, giving kefir its distinctive flavor. The yeasts in kefir grains also help with the fermentation process by creating ethanol and carbon dioxide, giving kefir its effervescent structure. Kefir fermentation not only improves its flavor, but it also produces a variety of bioactive substances such as vitamins, organic acids, and peptides, all of which add to its nutraceutical benefits.

Many factors can influence the survival of bacteria from the former genus Lactobacillus and *Bifidobacterium spp.* in milk beverages, including the probiotic strains used: pH, presence of hydrogen peroxide and dissolved oxygen, concentration of metabolites such as lactic and acetic acids, buffering capability of the medium, storage temperature, and kind of additives.

6.4. Types of yogurts and fermented milks

Types of yoghurts

Yogurt can be divided into two main types: standard culture yogurt and bio- or probiotic yogurt. Standard yogurt is produced using *L. bulgaricus* and *S. thermophilus* bacteria, which encourage beneficial microorganisms in the gut, supporting overall gastrointestinal health. On the other hand, bio-yogurts are made with probiotic strains like bifidobacteria and *L. acidophilus*, known for their various health advantages. Bio-yogurts are widely preferred due to their smoother, creamier texture, milder taste, and lower acidity, aiding digestion and promoting wellness.

Beyond these classifications, yogurt products available in the market come in a diverse range of flavors, textures, and forms to cater to different tastes and meal occasions. They can be enjoyed as a snack, dessert, or part of a meal. Various types of yogurts can be categorized based on their physical and chemical properties as well as the addition of different flavors.

- ***Based on the chemical composition of the product***: There are three types of yogurts based on fat content: low fat (made from partially skimmed milk), non-fat (made from complete skimmed milk), and regular (made from full fat milk).
- ***Based on the physical nature of the product***: Yogurt has three physical properties: solid, semi-solid, and fluid. Solid yogurts are incubated and cooled for final packaging. Yogurts that are semi-solid and fluid in nature (stirred yogurt and fluid or sipping yogurt) are made by incubating the mixture in a tank, then breaking it down with stirring before chilling and packaging.
- ***Based on the flavor of the product***: The addition of flavors would meet the consumer's needs while producing a range of items. Flavors can be applied before or after homogenization. Yogurt can be classified as plain, fruity, or flavored depending on the taste added. Yogurt is offered in a variety of tastes, including apple, apricot, black cherry, black currant, blueberry, lemon, mandarin, raspberry, strawberry, peach, cereal, vegetables, chocolate, vanilla, caramel, ginger, and more. Flavoring yogurt during manufacture enhances its taste and sweetness (Banerjee et al., 2017).

Types of fermented milks

Cultured buttermilk, a fermented milk low in acidity, is produced by fermenting pasteurized skim milk with a combination of lactic culture and aroma-producing bacteria. High-quality cultured buttermilk features a mild acidic taste with a noticeable diacetyl aroma, and it has a smooth, viscous texture. It appears as a soft white liquid without any gas holes or separation of whey, remaining fresh for at least 10 days when stored at 5°C. Acid production in cultured buttermilk is primarily carried out by *Lactococcus lactis subsp. cremoris* and *Lc. lactis subsp. lactis*, while flavor and aroma are enhanced by citrate-positive strains of *Lc. lactis subsp. lactis biovar diacetylactis* and *Leuconostoc mesenteroides subsp. cremoris*. These bacteria are crucial as they produce diacetyl, a key volatile compound responsible for the distinctive buttery flavor and aroma of cultured buttermilk.

Cultured cream: Sour cream, also known as cultured cream, is a low-acid fermented milk product with a buttermilk-like flavor and scent. The final pH of freshly made sour cream is approximately 4.5. It typically comprises at least 18% butterfat. The starter cultures, primarily *Lc. lactis*, serve the same tasks as in cultured buttermilk. Incubation temperature ranges from 21 to 24 degrees Celsius.

Acidophilus milk is grown with Lactobacillus acidophilus, which produces lactic acid from lactose. Acidophilus milk is fermented at 38 °C with a 2-5% active culture until a curd forms (typically after 18-24 hours). The final product comprises 1.5-2.0% lactic acid and no alcohol. The mixture is cooled to 10°C before being agitated and pumped to a filler for packaging into bottles or cartons.

Probiotic fermented milks include certain strains of bacteria, such as bifidobacteria. Probiotic bacteria often utilized in fermented milk production include *L. acidophilus*, *L. casei*, and *Bifidobacterium spp*. These and other bacteria are believed to provide health and nutritional benefits in the digestive system.

Yakult is well-known in Japan for its purported health benefits. This product contains *Lactobacillus casei subsp. casei* strain Shirota, a probiotic bacterium that thrives in the human gut. Yakult contains 3.6% milk solids and 16% added sugar. Yakult is a fermented milk drink made up of 40% fermented milk and fermented by a single strain culture. The finished result includes 10^8 cfu ml^{-1} of viable *Lb. casei subsp. casei* strain Shirota, with 1.08% protein content.

Kefir is a fermented milk with a characteristic flavor and sour cream consistency. This foamy, effervescent drink with 0.9-1.1% lactic acid and 0.3-1.0% alcohol is popular in eastern Europe but has limited circulation due to packaging and distribution issues.

Kumys, a fermented milk with acid and alcohol, is often created from mare's milk. *Lb. delbrueckii subsp. bulgaricus* and lactose-fermenting yeasts, such as *Saccharomyces lactis* and *K. marxianus*, ferment milk to produce this product. Carbon dioxide gives the product a frothy look and enhances its flavor.

Acidophilus-yeast milk: Only countries in the former Soviet Union produce acidophilus yeast milk. To make 0.8% lactic acid and approximately 0.5% ethanol, heat whole or skimmed milk at 90-95°C for 10-15 minutes, chill to 35°C, then inoculate with a 3-5% mixed starting culture of *Lb. acidophilus* and *Saccharomyces lactis*. The substance is thick, slightly acidic, and has a yeasty taste.

6.5. Manufacturing stages

Generally, the market for fermented milks is dominated by two main types of retail products. One type has a solid, gel-like structure known as set-type, while the other type, called stirred-type, typically has a thick consistency with added fruit flavors, sugars, or other flavors modifying its base taste. There is also a drinking-type variant of the stirred product, which is more liquid and less thick. Despite these differences in final product characteristics, the manufacturing processes for these types of fermented milk products share many similarities. Figure 11, provides an overview of the overall process.

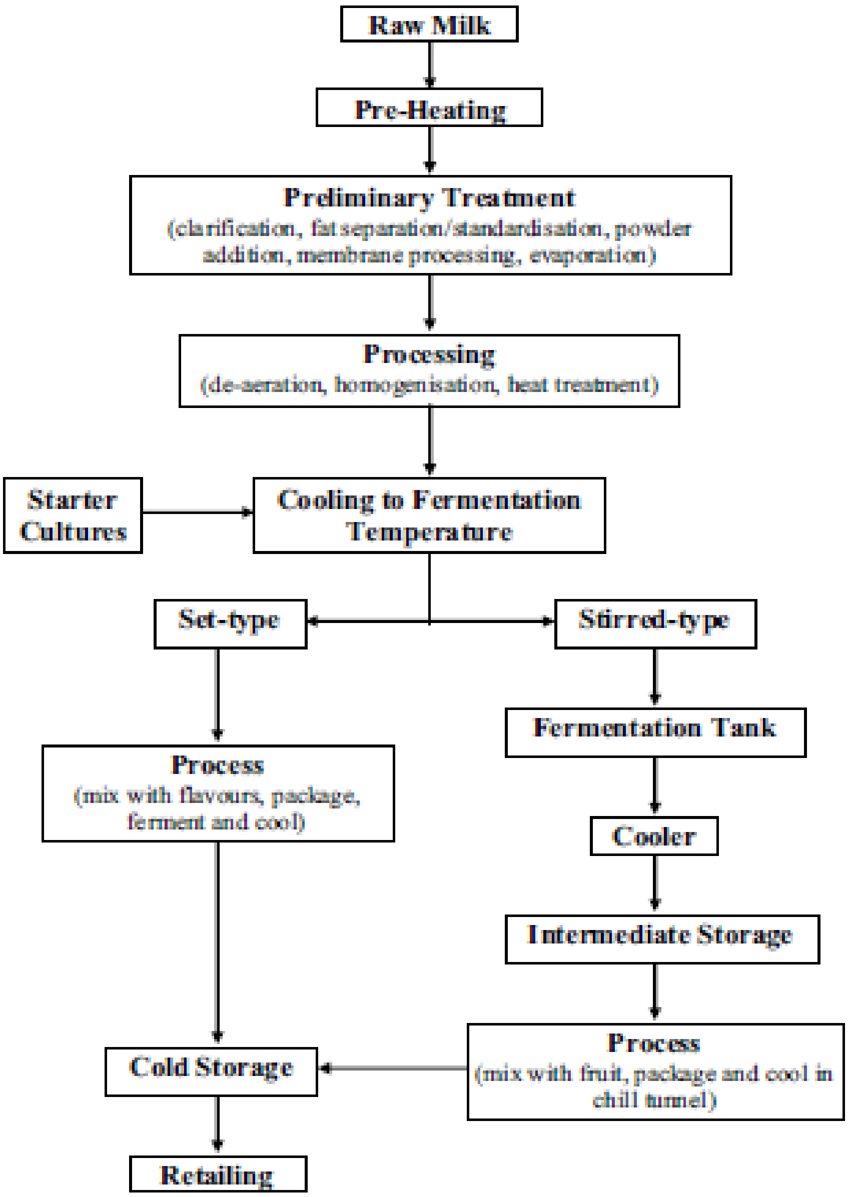

Figure 11: Flow diagram showing the manufacturing stages of fermented milk products.

Although many of these fermentations include specialized bacteria, particularly lactic fermentation. Although manufacturing procedures vary between products and plants, many have commonalities. Typically, commercial milk production begins with fat standardization and SNF fortification at 60-70ºC, followed by homogenization at 10-25 MPa or a pressure based on the product type (e.g., kefir at 10-20 MPa, ymer and ylette at 18-23 MPa, or yoghurt at 20-25 MPa). After homogenization, most types of fermented milks undergo a heat treatment of the milk base to eliminate any microbial contaminants introduced during homogenization. For stirred-types of fermented milks, subsequent processing stages typically include heat treatment, followed by cooling, fermentation, initial cooling of the gel, addition of fruit, packaging, cooling of the packaged product in a chill tunnel, and finally, cold storage. However, variations in plant design and processes are customized to suit the specific type of fermented milk being produced. For instance, in the production of yogurt (whether set or stirred types), kefir, ymer, and ylette, the handling of the milk base follows the process outlined in figure 11. On the other hand, in the production of buttermilk, the skimmed milk is heated to around 95ºC, then cooled to 60ºC, homogenized at 18–20 MPa pressure, cooled further, and fermented at 20ºC. After acidification, the gel undergoes thorough stirring, followed by homogenization at 5–10 MPa pressure at 20ºC, cooling to 4ºC, and packaging.

6.6. Fermented milks and health

Fermented dairy products have a significant impact on human health. They influence health by releasing bioactive compounds through microbial protein digestion and interactions between beneficial microbes and the intestines. Poor diet and unhealthy lifestyles are directly associated with inflammatory bowel disease, irritable bowel syndrome, diarrhea, hypersensitivity, lactose intolerance, and gastroenteritis. Research indicates that fermented dairy products such as fermented milk, yogurt, cheese, koumiss, and kefir enhance immune responses against pathogens by providing antioxidants, antimicrobials, anti-fungals, anti-inflammatory, anti-diabetic, and anti-atherosclerotic agents. Fermented products are also recognized for effectively delivering beneficial probiotic microbes that aid digestion.

Fermented milk offers numerous health benefits. It alleviates symptoms of lactose intolerance and helps prevent colon cancer. Studies have demonstrated that lactic acid bacteria can prevent and inhibit the growth of cancer cells. Additionally, fermented milk acts as a natural laxative, promoting regular waste passage and reducing the risk of constipation and related complications like diverticulosis and colon cancer. Scientific findings highlight that metabolites present in fermented milk and related foods can enhance intestinal immune function by increasing immune proteins like IgA, thereby helping to prevent diarrhea and intestinal infections, particularly in children. Fermented milk is also used to mitigate the severity of winter colds and has historically been used as a potent remedy for treating colds and flu-like illnesses, showing significant efficacy in reducing their severity and duration, although it does not impact influenza treatment. Furthermore, fermented milk helps reduce milk protein allergies, addressing a common issue among individuals sensitive to milk proteins. Fermented dairy products are abundant in essential vitamins and minerals, including vitamins A, B1, B2, B6, B12, niacin, folic acid, pantothenic acid, vitamin D, calcium, phosphorus, zinc, magnesium, iodine, and potassium. Table 14 outlines the bioactive constituents found in fermented dairy items.

Table 14: Biologically active components in fermented dairy products

Product	Lactic acid bacteria	Health effect	active compound
fermented milk	*L. rhamnosus MTCC 5897 L. rhamnosus MTCC 5957*	Anti-oxidant effect High cholesterol Probiotic	Probiotic
fermented milk	*Lactobacillus helveticus*	Stomach and intestinal disorders	Probiotic Peptides
Cheese	*L. chungangensis CAU28*	Immunology	short-chain fatty acids butyrate, acetate, and propionate, gut microbiota
Cheddar cheese	*L. plantarum K25*	Lower cholesterol and heart health	Probiotic peptides
Kefir	*Lactobacillus lactis subs., Leuconostocsubs., Streptococcus thermophilus, Lactobacillus subs., and yeast of kefir*	Colitis	microbial population of the kefir,
Goat's milk	*Pediococcus pentosaceus*	Anti-oxidant	Phenolic compounds and peptides
fermented milk	*Lactobacillus plantarum strain AF1*	Anti-oxidant	Phenolic compounds and peptides

Table 14 (continued)

Product	Lactic acid bacteria	Health effect	active compound
Yoghurt	*Streptococcus salivarius subsp. thermophilus strain fmb5*	Anti-hypertensive	peptides and Aminobutyric Acid
Yogurt-Like Beverages	*Lb. rhamnosus SP1 Lb. plantarum*	Improve protein digestion	Probiotic, peptides and polysacchrids
Yoghurt	*Mixture of L. casei L. bulgaricus S. thermophilus*	Yoghurt Mixture of L. Antibiotics associated with diarrhea in adults	Probiotic
fermented milk	*Bifidobacterium animalis DN-173 010*	Irritable Bowel Syndrome	Probiotic
Yoghurt	*Yoghurt Lactobacillus lactis subs*	Lactose intolerance allergy	polysacchrids
Fermented milk	*Lactobacillus casei subsp Shirota*	Improve protein digestion	
fermented milk	*Lactobacillus lactis subs*	Stomach infections	Metabolites resulting from fermentation
fermented goat's milk	*Lactobacillus lactis subs*	Malnutrition	amino acids short chain
Probiotic Yogurt	*Lactobacillus acidophiius Lactobacillus casei*	Anti-oxidant	Peptides

6.7. Milk fat

Milk and dairy products are considered well-balanced and nutritious components of the human diet. The exceptional nutritional value of dairy products is closely linked to the quality of milk fat, which includes a high concentration of fat-soluble vitamins, n-3 fatty acids, and a significant amount of conjugated linoleic acid (CLA). Milk fat affects raw material processing and imparts flavor and aroma to the product. Cow's milk typically has a fat content of 3.3%-4.4%. Goat's and ewe's milk contain around 3.25%-4.2% and 7.1% fat, respectively. Milk fat content varies based on breed, nutrition, characteristics, and lactation period.

Bovine milk exists as emulsified globules 2 to 4 micrometers in diameter, covered with a membrane produced from the secreting cell. The coat in homogenized milk is largely made up of casein. The globule contains triacylglycerol, which accounts for approximately 98% of the lipid. Phospholipids account for 0.5-1% of total lipids, while sterols make about 0.2-5%. These are typically found in the globule membranes. Cholesterol is the most common sterol, with levels ranging from 10 to 20 mg/dL.

Ruminant milk fat includes up to 400 fatty acids, the majority of which are saturated fatty acids with an even number of C-atoms (C4:0-C18:0 and C18:1). This makes it the most diverse lipid system. Ruminant fats, particularly milk and dairy products, include conjugated dietary linoleic acide, a byproduct of the rumen bacterium's biohydrogenation of linoleic acid.

6.8. Milk skimming

Skimmed milk shall be the milk from which part of the milk fat has been removed and which contains not more than 1 percent of milk fat and not less than 8.5 percent of milk solids (NAFDAC, 2005). This manual operation (skimming) or mechanical operation (by centrifugation) involves separating the cream (fat globules in suspension) from the milk. The cream can then be used for the production of butter or for consumption as cream.

6.9. Butter making

The Codex Alimentarius Commission defines butter as a fatty product obtained only from milk. A 100 g quantity of butter must contain at least 80 g fat, a maximum of 16 g water, and 2 g nonfat milk solids. The EU uses a similar definition in Council Regulation No. 2991/94 for requirements for spreadable fats like butter, mixes, and spreads. Butter is a water-in-oil emulsion that is mostly composed of milk fat. A typical salted butter has 80-82% fat, 16-18% water, about 1% salt, and 1% protein. Most of the milk fat, over 98%, exists as triglycerides, primarily found in milk as fat globules ranging in diameter from 0.1 to 10 µm, stabilized by the milk fat globule membrane (MFGM) (Society of Dairy Technology, 2018). Butter contains fat-soluble vitamins A, D, and E.

Butter is a dairy product produced by churning fresh or fermented cream or milk. Converting milk fat to butter is a traditional method of preserving it. Butter contributes significantly to milk's nutritional value. Butter is commonly used as a spread, condiment, and cooking ingredient for baking, sauce making, and pan frying. Butter is typically created from cow milk, but can also be prepared from the milk of other mammals such as sheep, goats, buffalo, camels, and yaks. Today, butter is primarily produced from bovine milk.

Various types of butter may or may not contain salt. Here are descriptions of several types:

- Pasteurized cream butter: Typically crafted from pasteurized sweet cream, resulting in a gentler flavor compared to butter made from unpasteurized cream.
- Ripened cream butter: Made from cream that develops a pleasant aroma through ripening before churning, often by introducing a lactic culture and maintaining specific temperatures. Well-made ripened cream butter boasts a delicate flavor.
- Unripened cream butter: Produced from cream that hasn't undergone ripening. This type of butter usually has a mild flavor.
- Salted butter: Butter that includes added salt.
- Unsalted butter: Butter made without any added salt.
- Sweet cream butter: Butter where the acidity of the churned cream does not exceed 0.20%.
- Sour cream butter: Derived from cream with more than 0.20% acidity.
- Fresh butter: Butter that hasn't been subjected to cold storage, typically not kept for more than three weeks.
- Cold storage butter: Butter stored at approximately -18°C (0°F) for a period, usually aging from one to six months before being sold.
- Dairy butter (USA): Butter produced on farms, often from unpasteurized sour cream that hasn't been standardized for acidity. This type tends to have a tangy flavor due to its higher acid content.
- Creamery butter: Butter manufactured in a creamery or dairy factory, known for its consistent quality compared to farm-produced dairy butter.

The following diagram provides a summary of the manufacturing process.

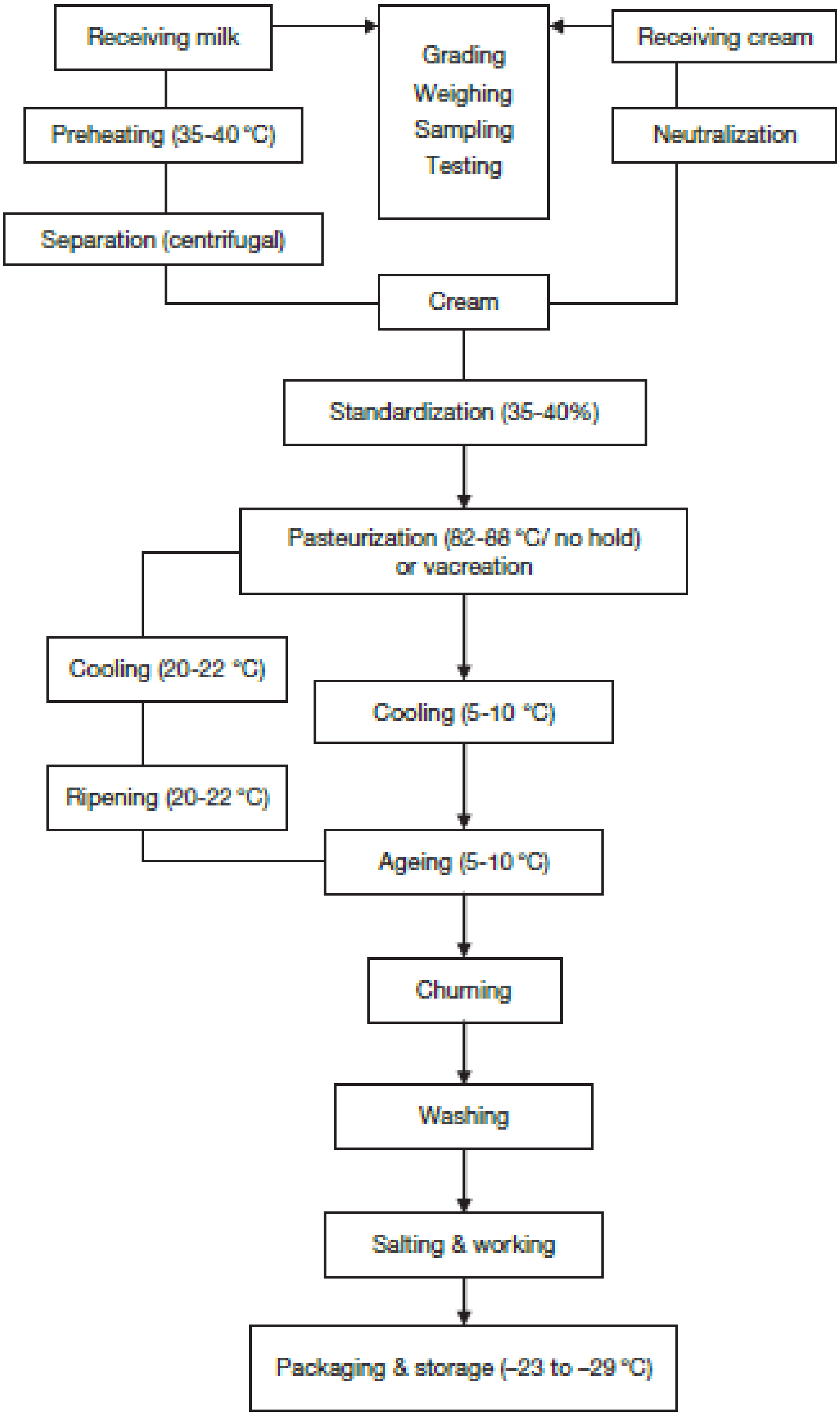

Figure 12: Process flow diagram for butter.

Butter making relies on the ability of milk fat globules in cream (>36% fat) to aggregate when whipped in air, similar to whipped cream. Table 15 summarizes the physical changes that occur during the butter-making process, which are influenced by the milk fat system's natural chemical and physical features.

Table 15: Summary of process for sweet cream salted butter

Key Steps	Physical Changes
Ageing of the cream After pasteurisation, the cream (40% fat) is cooled to 4-5°C and held for several hours (typically 8-15 h).	This allows the higher melting fraction of the milk fat to crystallise and become solid. The time and temperature of the ageing process depends on the relative hardness of the milk fat, which varies mainly according to feed.
Churning the cream Where the aged cream is subjected to a high level of shear and aeration.	Fat globules are most easily disrupted when approximately half of the fat is solid, allowing liquid free fat to escape from the globules. This allows the globules to collect in clusters 'stuck together' by free fat. When these clusters become large enough they appear as small clumps, or butter grains. The remaining liquid is called buttermilk.
Treatment of the butter grains	
Butter grains separated from buttermilk	To reduce the moisture content of the butter
Butter grains physically worked	To reduce the size of the moisture droplets in the butter (ideally to <10 µm to minimise microbial growth)
Salt addition, moisture content adjusted	Salt is added for flavour and as a preservative (lowers aw), moisture to optimise yield.
De-aeration	To remove and standardise the residual air.

6.10. Biological maturation of cream

Cream is among the most significant dairy products. Cream is a rich emulsion of milk fat globules in skimmed milk, separated by gravity or centrifugal force. Creams are classified based on their fat content (g/100 g): double cream (45-50%), cream or full cream (30-40%), single or half cream (15-25%), coffee cream (15-18%), and light coffee cream (< 10%). The United Nations Food and Agriculture Organization (FAO) classifies cream based on fat content:

(a) Cream: 18-26%

(b) Light cream (or coffee cream): more than 10%

(c) Whipping cream: >28%.

(d) Heavy cream: over 35%.

(e) Double cream: More than 45%

Biological maturation is conducted as part of traditional manufacturing practices and for the production of butter with a controlled designation of origin, which requires a minimum duration of 12 hours at temperatures ranging from 9 to 15°C. It entails introducing lactic acid bacteria into the cream at a concentration of 3 to 5%, allowing these bacteria to develop over approximately ten hours to stimulate both lactic and aromatic fermentations.

During lactic fermentation, lactic acid is produced, reducing the pH of the cream to between 4.70 and 5.80, which enhances the butter's shelf life. Moreover, this pH adjustment, nearing the isoelectric point of membrane proteins, facilitates the clustering of fat globules during churning.

Aromatic fermentation primarily arises from the breakdown of citrates by lactic acid bacteria. This process generates a highly aromatic compound, diacetyl or 2-3 butanedione, which imparts the characteristic hazelnut flavor to the butter. While other compounds—whether inherent (such as acids or delta-lactones) or arising from fermentation (like alcohols, aldehydes, ketones, esters, amines, etc.)—contribute to the butter's overall aroma, diacetyl assumes a predominant role.

6.11. Physical maturation of the cream

The consistency of butter is greatly influenced by the thermal and structural characteristics of the triglycerides found in its fat content. Physical maturation, which aims to solidify some of these triglycerides, is essential for consistently producing high-quality butter, even when cream quality varies.

Applying a suitable thermal process directs the crystallization of triglycerides and mitigates seasonal effects. Consequently, the cooling process during physical maturation affects both the quantity of fat solidified by crystallization and the clustering of fat globules. This latter aspect is critical as it impacts the cream's readiness for churning. Fat globules remain in a fragile, metastable state within the cream for approximately ten minutes after cooling. Any mechanical agitation during this period can release liquid fat, causing the globules to cluster. As the cream becomes more viscous, agitation needs to be prolonged and more vigorous.

Agitation can disrupt existing crystals, creating nucleation sites for subsequent nucleation and resulting in more crystals. Minor components including phospholipids (PLs) and diacylglycerides (TAGs) play a key role in structure creation and crystallization. TAGs and PLs delay milk fat crystallization and growth, possibly due to absorption on the crystals.

6.12. Nutritional quality

Yogurt

During fermentation, culture bacteria hydrolyze 20-30% of lactose to glucose and galactose. Yogurt typically has lower lactose levels than milk, but skimmed milk powder or non-fat milk solids may be added during manufacturing. Protein consists of amino acids. Although the overall amino acid concentration of yogurt and milk is equal, yogurt contains a higher proportion of free amino acids due to the proteolytic activity of the bacterial culture, which partially digests the protein during fermentation. There is a suggestion that the presence of bacteria during the fermentation process may enhance the digestibility of fat, although this claim is widely contested. The calorie content of yogurt is influenced by the fat content of the milk used and the inclusion of additional ingredients such as cream or sugar.

Yogurt's vitamin content varies according on milk type, lipid content, bacterial strain, and fermentation circumstances. Lactic acid bacteria require vitamin B12, which can lead to lower levels of some B vitamins. Some cultures can manufacture folk acid, a B vitamin, resulting in higher levels in the final product. The fermentation process has minimal impact on the mineral content of milk. Yogurt is a rich source of many minerals, particularly calcium, zinc, phosphorus, and magnesium.

Cheese

Cheese includes far more fat and protein than milk. Cheese fat and protein contents vary with variety, with values ranging from 3.9 to 35.5% and 13.8 to 39.4%. Cheese retains essential nutrients (Table 16), influenced by factors such as the type of milk (including species, lactation stage, and milk constituents), manufacturing techniques, and ripening methods. While caseins, colloidal minerals, fat, and fat-soluble vitamins remain in the curd, whey proteins, lactose, water-soluble vitamins, and minerals are lost in the whey during production.

Table 16: Chemical composition (in 100g) of some varieties of cheese

Cheeses	Water (g)	Fat (g)	Protein (g)	Cholesterol (mg)	Energy (Kcal)
Beyaz peynir	58.7	19.8	19.7	53.4	250
Cheddar	36	34.4	25.5	100	412
Cottage	79.1	3.9	13.8	13	98
Edam	43.8	25.4	26	80	333
Emmental	35.7	29.7	28.7	90	382
Feta	56.5	20.2	15.6	70	250
Gouda	40.1	31	24	100	375
Gruyere	35	27.2	33.3	100	409
Kashar	44.3	28.3	24.6	73.3	353
Mozzarella	49.8	21	25.1	65	289
Parmesan	18.4	32.7	39.4	100	452
Roquefort	41.3	32.9	19.7	90	375
Stilton	38.6	35.5	22.7	105	411
Tulum	50.5	27.4	19.4	92.6	324

Fat in cheese acts as a carrier for vitamins and dietary fats and significantly influences the texture and microstructure of cheese. Cheese fat composition typically includes approximately 66% saturated fats, 30% monounsaturated fats, and 4% polyunsaturated fats

(PUFA). The cholesterol content in cheese varies widely depending on the variety, ranging from 13 to 105 mg per 100 grams. While fat-soluble vitamins are retained within the cheese fat, water-soluble vitamins (such as B vitamins) are lost in the whey during cheese production. However, certain microorganisms can synthesize B vitamins during the ripening process of cheese.

Cheese is also rich in calcium, phosphorus, and magnesium, with hard cheeses containing high levels of calcium (about 800 mg per 100 grams), which meets the recommended daily intake for adults. Consuming salty cheeses, particularly brine-ripened kinds, can increase sodium intake, leading to hypertension and coronary heart disease. Cheese accounts for only 5-8% of daily salt intake. Other foods in the diet should also be considered for their sodium level.

Butter and Cream

Milk is separated to produce cream. Single cream has approximately 18% fat, double cream has 48% fat, and whipped cream has 40% fat. These creams are mostly utilized in the manufacturing of butter and ice cream. Butter is a water-in-oil emulsion created using the "cream by phase inversion" churning technique. Butter is 80% fat and contains fat-soluble vitamins such as A, D, E, and K. Butter can be made from sweet or acidic creams (culture). Butter may develop off-flavors due to lipolytic and oxidative processes. Butter includes minor amounts of lactose but a substantial amount of cholesterol.

Milk powder

Milk powder, powdered milk, or dried milk is produced by evaporating milk to dryness. The chemical makeup of milk powder from different sources varies, as illustrated in Table 17. Chemical composition and physical qualities (appearance, solubility, particle size, etc.) vary depending on the raw material used in manufacturing.

Table 17: Chemical composition (mean values, %) of some types of powdered milk

Constituents	Whole milk	Skimmed milk	Whey	Buttermilk
Fat	26	1	1	5
Lactose	38	51	73	46
Casein	19.5	27	0.6	26
Other proteins	5.3	6.6	8.5	8
Ash	6.3	8.5	8	8
Water	2.5	3	3	3

Ice Cream

Ice cream is made by freezing and beating water, sugar, flavorings, and other ingredients into a firm foam. Ice cream is classified into four categories based on its ingredients: (a) Dairy-based ice cream, (b) ice cream with vegetable fat, (c) sherbet with fruit juice and milk, and (d) water ice with sugar and fruit concentrate. Ice cream ingredients include fat (12%), milk solids-non-fat (11-11.5%), sugar (10-18%), water, emulsifiers (0.3-0.5%), stabilizers (0.2-0.5%), and flavoring and coloring.

Ice cream is a nutritious frozen dessert with high quantities of milk fat, fat-soluble vitamins, low lactose (ideal for lactose malabsorbers), omega-3 fatty acids, antioxidants, and water-soluble vitamins and minerals (when combined with nuts or fruits).

7. Transformation of animals into carcasses

7.1. Slaughter techniques

Slaughter refers to the killing of animals, particularly for food. When animals reach adulthood, they are sold for slaughter, and their meat is prepared for human consumption. The killing of animals consists of three key steps: pre-slaughter management, stunning, and slaughter.

Pre-slaughter handling: This is the first stage of the slaughter method. In this stage, animals are properly handled before slaughter by giving adequate space and ventilation, as well as ensuring that the animal is stress-free before to slaughter; stress has an impact on meat quality and color. As a result, adequate management and handling are required to produce high-quality meat. Pre-slaughter handling includes animal handling, transportation, resting, fasting, and ante-mortem examination. Pre-slaughter handling should be done appropriately to avoid stress and provide a plenty of food and water, but food and water consumption should be reduced to nothing before to slaughter.

Stunning: After pre-slaughter handling, animals are physically restrained before slaughter and rendered unconscious using mechanical, electrical, or carbon dioxide gas. Mechanical stunning involves firing a bullet through the skull of an animal using a pneumatic instrument. Electrical stunning involves delivering an electric current through an animal's skull to render them unconscious, whereas carbon dioxide gas involves administering carbon dioxide gas to an animal.

- ***Electrical stunning***: Electrical stunning equipment must effectively stun animals of the appropriate species and size. Electrodes must span the brain (Figures 13 and 14) and apply a sufficient voltage (> 200 volts) for at least 3 seconds to induce instantaneous unconsciousness. Applying enough current to the brain can cause an epileptic seizure, leaving the animal unconscious.

Figure 14: Optimum tong position

for head-only electrical stunning of sheep

Figure 13: Optimum tong position

for head-only electrical stunning of pigs

To maintain the current application, head-only stunning tongs for pigs, sheep, goats, and calves should have electrodes with two parallel rows of sharp teeth that can penetrate the skin and prevent sliding after initial contact. To perform the stun operation, the operator must be trained and competent. The electrodes must be correctly placed on the animal's head for the specified duration. The following are indicators of an effective electric stun:

• Tonic phase (lasts 10-12 seconds): Symptoms of a collapsed animal include rigidity, lack of respiratory rhythm, stretched forelegs, and curled hindlegs.

• Clonic phase (duration 20 to 35 seconds): Symptoms may include uncontrolled kicking or paddling, eye rolling or flickering, and salivation.

Electrical waterbaths are commonly used to stun poultry before slaughter. The current passes through the animals to the shackle line, which connects to the earth. Exsaguination of birds involves varying electrical frequencies, waveforms (sine or square wave), minimum current, and blood vessel cuts.

This approach's essential needs include the following: (1) the waterbath should be supplied with a voltage sufficient to ensure that every bird receives the recommended minimum current; (2) the electrode placed in the waterbath must extend the entire length of the bath; (3) there should be good contact between the legs of the birds and the shackle line; (4) the birds' heads should be completely immersed in the bath; and (5) the water should not overflow at the bath's entrance.

➢ ***Mechanical stunning*:** Mechanical stunning involves administering a forceful blow to the animal's skull, causing immediate unconsciousness. The unconsciousness must remain until death. Mechanical stunning devices, commonly known as captive bolt guns (CBGs), fall into two categories: piercing and non-penetrating. Penetrating CBGs (Figures 15 and 16) are commonly used to stun cattle, but can also be used on sheep, goats, pigs, deer, horses, and rabbits.

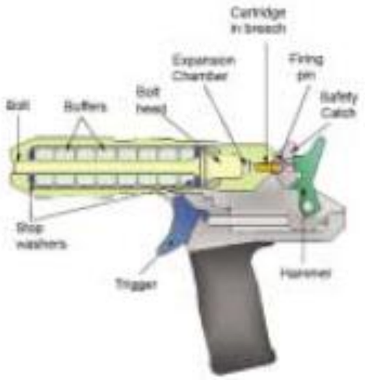

Figure 15: Penetrating CBG with hand trigger

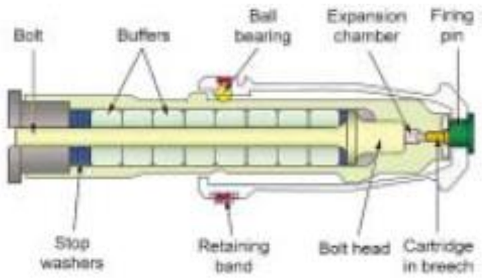

Figure 16: Penetrating CBG with contact trigger

Non-penetrating devices include sledge-hammers, mauls, and mushroom-headed CBG (knockers). The knocker (Figure 17) is the only non-penetrating equipment recommended for practice, as it delivers a regulated blow to the animal's head, unlike hand approaches. Non-penetrating CBGs should only be used on cattle.

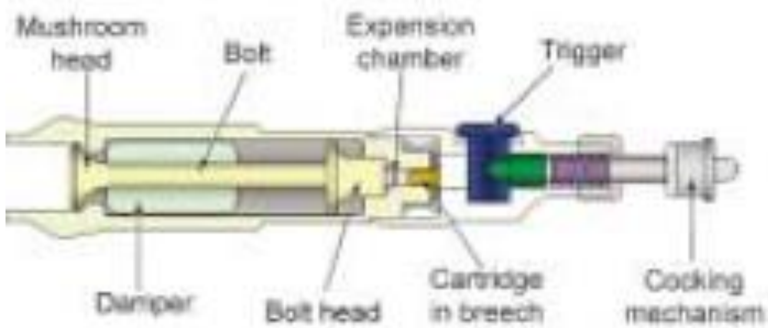

Figure 17: Non-penetrating CBG with hand trigger (knocker)

Shooting positions: To achieve good mechanical stunning, the impact should be directed to a specific location of the skull to cause maximum brain damage. The optimal site for most animals is in the frontal part of the skull, but this might vary according on species, age, and kind of device used (penetrating or non-penetrating). The best shooting location for piercing devices on cattle is the junction of two imaginary lines drawn between the eyes and the center of the base of the opposite horn bud (see Figure 18). Position a non-penetrating device roughly 20 mm above the penetrating instrument.

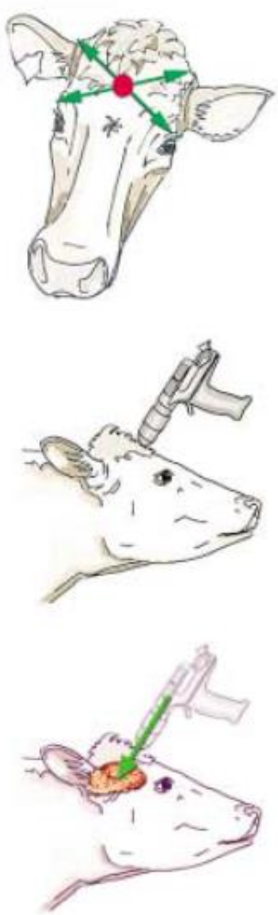

Figure 18: Captive bolt stunning of cattle – gun aimed at right angles to head

For horned sheep, place the captive bolt device beneath the ridge between the horns and aim it towards the base of the tongue (poll position). Animals shot in the poll position must bleed within 15 seconds. For polled sheep, aim the device vertically from the highest point of the head (see Figure 19).

Figure 19: Captive bolt stunning of sheep

For stunning goats (both horned and polled), the proper attitude is similar to that of horned sheep. Position the captive bolt mechanism behind the ridge between the horns, pointing toward the base of the tongue (poll position).

For pigs, position the device on the midline, 20 mm above eye level, and aim towards the tail (Figure 20). To avoid a bony ridge, put older sows and boars 50 mm above eye level and slightly off the midline. CBG stunning can result in severe convulsions in pigs.

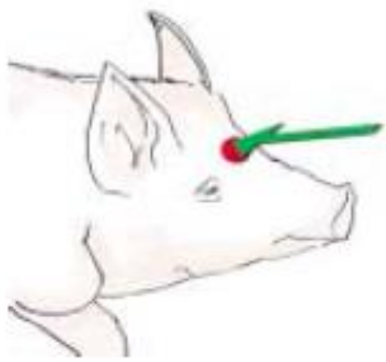

Figure 20: Optimum shooting position for pigs

- ***Carbon dioxide gas stunning:*** Carbon dioxide is disagreeable to animals, and large quantities impair welfare. However, this approach has numerous welfare benefits, including a lower risk of potential human error when compared to, say, electrical stunning, which risks erroneous electrode placement; animals remain in groups; and consistency and efficacy at high throughputs. While a nonaversive gas mixture would be desirable, there are currently no commercially available alternatives.

Newer systems use a succession of automatic gates with pressure sensors to move the pigs forward before loading them into the gas system. This eliminates the necessity for personnel handling as well as the need of handling aids. Figure 21 depicts the gas machine, which is made up of a series of gondolas in a Ferris-wheel design that revolve down into a chamber before rotating up to the top to release the stunned/killed animals.

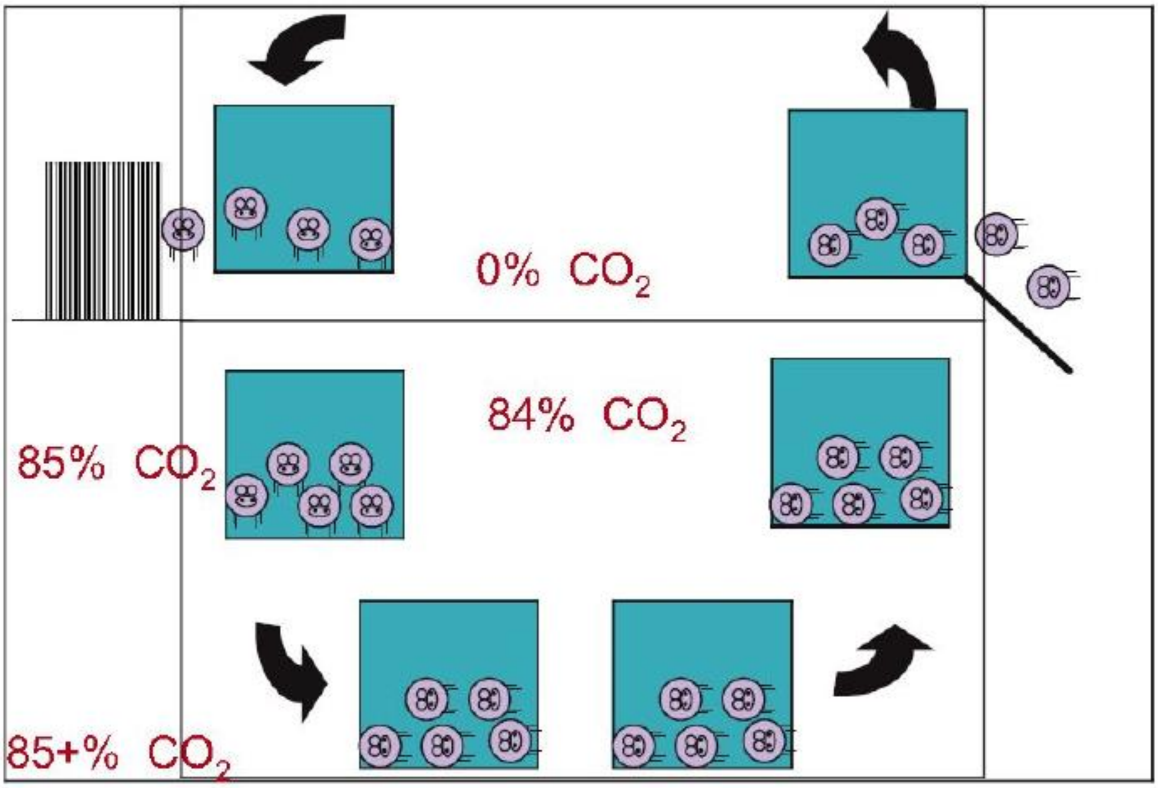

Figure 21: Example of side-entry carbon dioxide stunning machine.

Pigs must not enter the gas stunner if the carbon dioxide concentration is less than 80% by volume. Once inside, they must be transported to the maximum concentration within 30 seconds. Most systems require at least one halt before animals achieve maximum concentration, therefore each cycle should be no longer than 25 seconds.

Gas mixes can be used to stun or kill birds on a conveyor, removing the stress of shackling them. Combining 30% carbon dioxide and 60% argon in air is preferable to employing a high concentration of carbon dioxide.

Slaughtering: After being stunned, the animals are carried to the slaughter house for slaughter. Animals are slaughtered with a knife, cutting the thoracic cavity and severing the carotid artery and jugular.

Animals must be shocked before being stuck. The knife used must be clean and sharp, with a length appropriate for the animal's species and size. Sever both carotid arteries and their originating vessels near the heart. After sticking, allow the animal to bleed to death before proceeding with dressing or electrical stimulation. For pigs, sheep, and goats, the minimum time after sticking is 25 seconds, while cattle and deer require 60 seconds.

<u>Sticking methods</u>:

To use a thoracic stick, first cut the jugular fold at the base of the animal's neck. (b) Insert the knife-point at the base of the breastbone, pointing towards the chest, to disconnect main blood arteries from the heart (Figures 22 and 23).

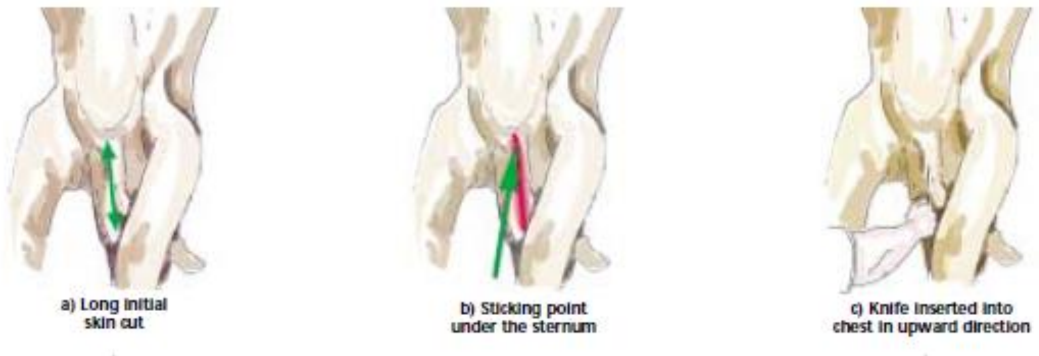

Figure 22: Chest sticking in cattle

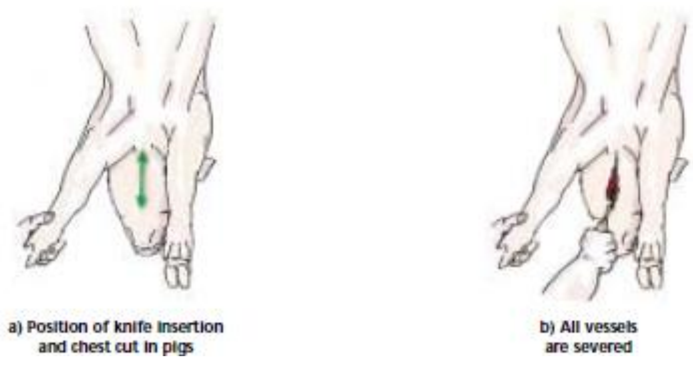

Figure 23: Chest sticking in pigs

To do a neck stick, insert a knife near to the head and cut through the neck, severing the soft tissues between the spine and the front of the neck. Reverse the blade and cut against the spine. This action will sever both carotid arteries and jugular veins (see Figures 24 and 25). These methods can be applied to several species, as follows: • Cattle and calves: (a+b); • Pigs: (b); • Sheep and goats: (b or c).

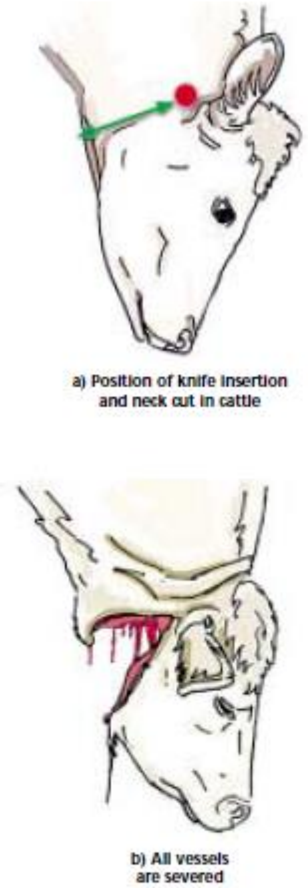

Figure 24: Neck sticking in cattle

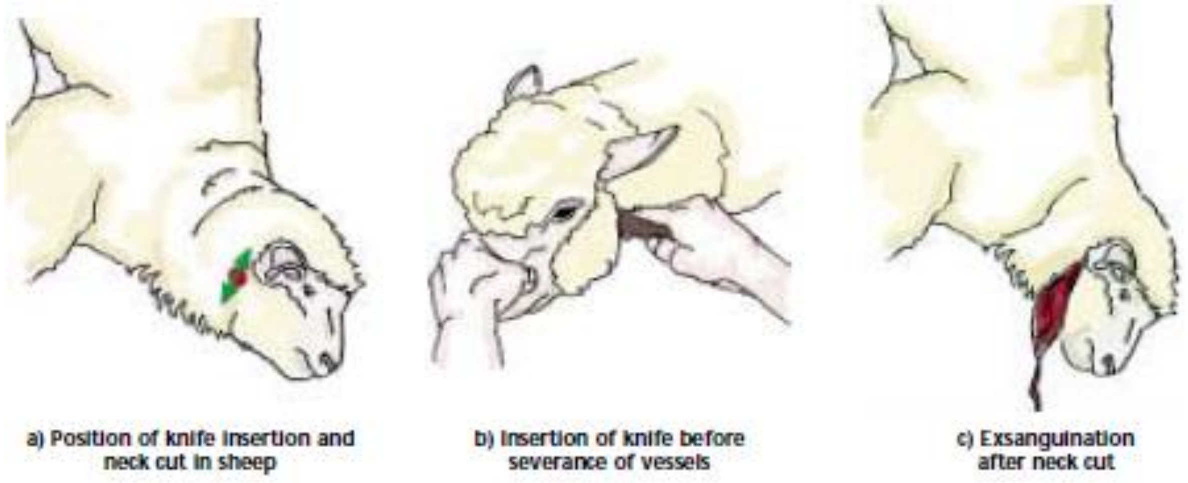

Figure 25: Neck sticking in sheep

Halal Slaughter:

Halal meat is a crucial subject in Islam. To be considered halal, animals and birds must be slaughtered in accordance with Islamic rites. Furthermore, these meat products must be stored separately throughout the supply chain to avoid contamination. Halal slaughtering involves the killing and butchering of non-forbidden animals for consumption. According to

Islamic custom, the conventional way of slaughtering the animal entails cutting the main arteries in the neck, along with the esophagus and trachea, with one swipe of a non-serrated blade (Figure 26).

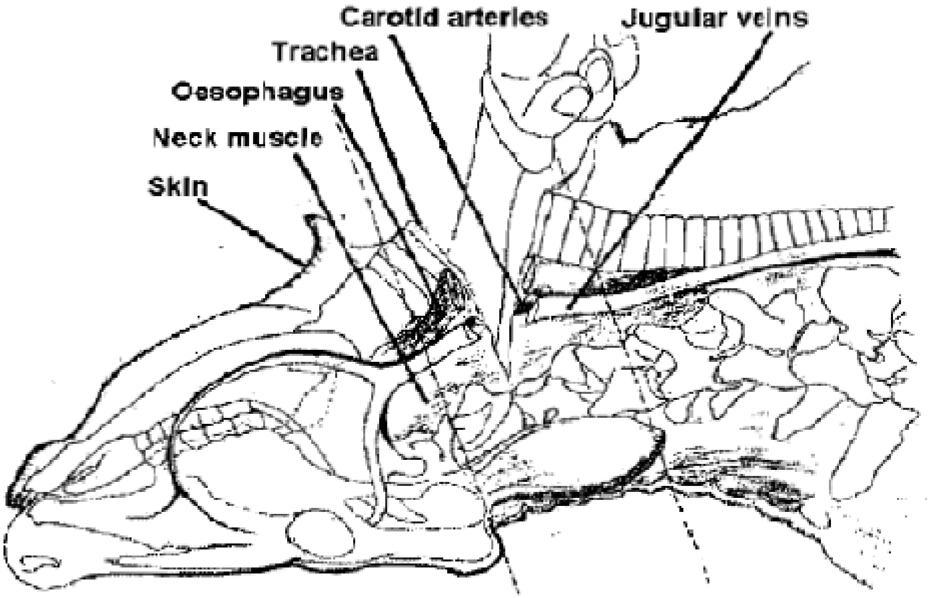

Figure 26: Halal method of slaughtering cattle

Muslims claim that it delivers a somewhat peaceful death while also successfully draining blood from the animal. It is a very particular procedure governed by stringent laws drawn from the holy Quran and Hadith, and it is intended to ensure animal health and welfare as well as food cleanliness. One of the primary requirements of halal slaughter is that the neck be partially cut without decapitation, leaving the spinal cord intact, and the body be released soon after slaughter. When halal animals or birds, such as sheep, cattle, and poultry, are slaughtered using the Islamic way, the flesh is considered lawful to consume.

7.2. Stages of processing cattle and poultry into carcasses

Skinning

Skinning is done after bleeding; skinning in sheep and goats is done by removing the leg skin at the hock joint, following which the entire body's skin is removed to expose the internal organs. Skinning like spectacular is done by hoisting or horizontally.

Hoist skinning: This process involves hanging the animal's body by its hind legs and removing the skin from the hind legs and hock joints. This reveals the tendons and smooth muscles of the rear legs beneath the toes.

Horizontal skinning: In this approach, the animal is placed on a flat surface horizontally, and the skinning process begins with the forelegs and progresses to the belly, sides of the animal's body, and finally the hind legs. The tendons and lax muscles connecting the toes and hind legs are revealed.

Eviscerating

In this procedure, the animal body remains in a hanging position, all of the viscera are exposed, and the intestines are removed before preparing the carcass for human consumption (Faraz & Raza, 2020). For every species, it is crucial during all procedures to avoid puncturing organs like the viscera, urinary bladder, gall bladder, or uterus. In case of accidental puncture, the affected part of the carcass must be removed. All viscera should remain attached to the carcass until veterinary inspection is completed. After inspection, the viscera should be cooled on racks or similar structures to enhance air circulation.

Hands must be cleansed on a frequent basis during evisceration. All knives and saws used in this operation must be sterilized on a regular basis and never placed on the floor. Eviscerators should have access to sanitary facilities so that they can perform their duties properly. For a mechanical conveyor belt, boot-washing, apron-washing, and other washing/sterilizing facilities must be provided. In smaller abattoirs, a hand basin/sterilizer must be available. In all circumstances, there should be sterilization facilities for the evisceration platform and offal containers.

Cattle: The brisket is divided down the center (see figure 27). In the combined horizontal/vertical system, this is performed while the animal rests on the cradle. Subsequently, the carcass is partially hoisted to facilitate hide removal, and then the abdominal cavity is meticulously cut along the midline. The carcass is fully hoisted afterward, suspended clear of the floor to allow the viscera to naturally fall out (Figure 28). These are categorized into thoracic viscera, paunch, and intestines for inspection and cleansing (see figures 29 and 30). If any stomachs or intestines are intended for human consumption, ties are secured at the boundaries between the esophagus/stomach and stomach/duodenum, with the esophagus and rectum having been tied off during hide removal. This precaution prevents cross-contamination between the paunch and intestines.

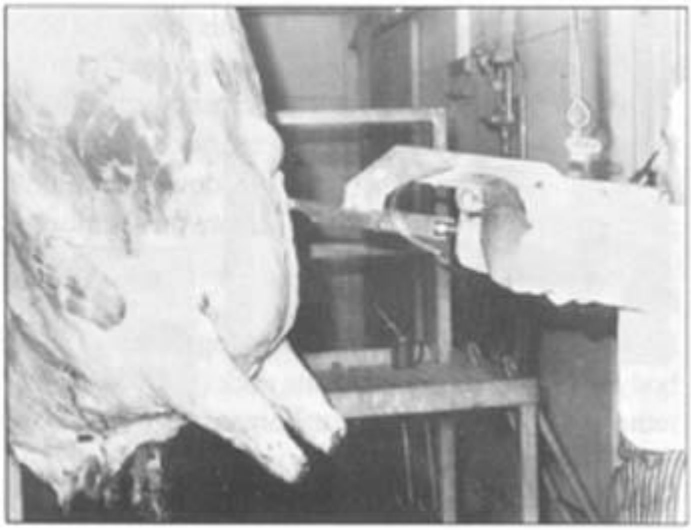

Figure 27: A mechanical saw speeds the splitting of the brisket

but care must be taken not to puncture the viscera

Figure 28: After carefully cutting the abdominal

wall along the midline the viscera fall out under their own weight

Figure 29: A suitable receptacle should catch the viscera so that they are not contaminated by contact with the floor

Figure 30: A portable cart suitable for catching cattle stomachs and intestines with a separate tray for edible offal such as liver, heart and lungs

Small ruminants: A tiny cut is made in the abdominal cavity wall right above the brisket, and the fingers of the opposite hand are inserted to move the body wall away from the viscera while the cut is extended to within about 5 cm of the cod fat or udder. The omentum is withdrawn, the rectum (tied off) is loosened, and the viscera is released and removed. The food pipe (tied off) is dragged up through the diaphragm. The breastbone is broken down the center, being careful not to puncture the thoracic organs, which are then removed.

Pigs: Loosen and knot the rectum. Cut along the middle line through the skin and body wall from the groin to the neck (Figure 31). Cut through the pelvis to remove the bladder and sexual organs. Males' foreskins should not be perforated since the contents are a serious source of contamination. All of these organs are deemed inedible.

Figure 31: The body wall is split down the midline taking care not to puncture the viscera

7.3. Sanitary control

A carefully designed and effectively implemented cleaning and sanitation program for rooms, machinery, and equipment is essential to achieve high hygiene standards. However, solely relying on cleaning and sanitation is insufficient for ensuring hygienic production. Process hygiene and personal hygiene are equally critical factors. Well-planned work routines contribute to maintaining high cleaning standards during processing. This includes cleaning procedures implemented during processing, proper disposal of solid waste, and adequate space in processing areas, all of which facilitate effective cleaning.

Maintaining adequate personal hygiene is crucial for the overall cleaning process. Failure to observe proper personal hygiene, such as washing hands before handling processed materials, can compromise the cleanliness achieved through cleaning efforts. Neither process hygiene, personal hygiene, nor cleaning and sanitation alone can guarantee sufficient hygiene standards. Only when all these aspects are optimally implemented together can a comprehensive hygienic standard be assured.

Defining process hygiene adequately is challenging because the key factors vary depending on:

- The type of processing being conducted

- Characteristics of the processing facilities (location, size, structure)

- Availability of equipment

- Whether personnel are permanent or temporary (including their work routines and training)

- Climatic conditions

- Availability of sanitary facilities

- Supplies of water and energy

- Methods for disposing of liquid and solid waste

A thorough understanding of comparative anatomy of livestock carcasses and organs is essential for conducting proper post-mortem inspection procedures. Identifying animal species and determining gender can be achieved through a detailed knowledge of differential anatomical characteristics, especially within the skeletal system. The inspection anatomy of pigs, ruminants, and poultry is structured into distinct sections for describing their anatomy:

- ➢ Head anatomy
- ➢ Visceral anatomy
- ➢ Carcass anatomy
- ➢ Poultry anatomy

The head can be inspected either after removal or while still connected to the corpse. The inspection involves both an external visual study to describe the muscles and an inside review to capture the skeleton, cranial cavity, and face. Following their retrieval from the carcass, the viscera must be examined. Examining particular organs requires knowledge of their usual properties. Splitting carcasses in half allows for easier evaluation of rib shape, sternum structure, vertebral features, and ischiopubic junction. The examination may reveal information about the varied properties of the various species.

Sampling and laboratory tests conducted at the slaughterhouse serve various purposes, including aiding meat inspection to determine its suitability for human consumption, monitoring and controlling zoonoses and communicable animal diseases, diagnosing transmissible spongiform encephalopathies (TSEs), detecting residues, and monitoring microbial contamination of carcasses and the production environment. Samples and analyses may be part of official controls, surveillance programs, or the food industry operator's own checking system. Always refer to the country's current legislation while doing obligatory analyses.

7.4. Maturation of meats

Following animal harvest, a series of intricate energetic, biochemical, and physical transformations occur in muscle tissues, leading to their conversion into meat. This process initiates soon after harvest, disrupting the animal's homeostatic mechanisms. As the animal undergoes exsanguination and subsequent oxygen deprivation, skeletal muscle continues attempting to maintain cellular balance by synthesizing and using adenosine triphosphate (ATP). However, with oxygen depletion, the muscle begins anaerobically metabolizing glycogen and high-energy phosphates solely for ATP production. Anaerobic metabolism, less efficient than aerobic metabolism, results in ATP hydrolysis rates surpassing ATP generation rates, triggering rigor mortis (Latin for "stiffness of death"). Over time, as postmortem metabolism progresses, ATP production diminishes until all ATP reserves are depleted. In the absence of ATP, myosin irreversibly binds to actin, completing rigor mortis and causing loss of muscle excitability and extensibility. Rigor mortis typically completes within 1 to 12 hours postmortem, influenced by species, muscle fiber type, and pre- and postmortem conditions. During postmortem storage (aging), cytoskeletal protein degradation leads to muscle structural integrity loss, resulting in decreased muscle tension and resolution of rigor mortis.

Another important change that occurs in postmortem muscle under anaerobic conditions is acidification. This occurs due to the accumulation of lactate from postmortem glycolysis and hydrogen ions (H^+) from ATP hydrolysis, as there is no effective mechanism for their removal. Consequently, the pH of the muscle gradually decreases from approximately 7.2 in living tissue to a final pH around 5.6. The speed and extent of postmortem metabolism significantly impact various meat quality characteristics. The rapid, prolonged, or insufficient decline in postmortem pH can negatively affect meat color, texture, and water-holding capacity. Factors such as environmental conditions and pre- and post-slaughter handling can markedly alter the rate of postmortem pH decline. Therefore, understanding these factors that govern postmortem metabolism can enhance the likelihood of producing high-quality meat products.

7.5. Valorization of the 5th quarter

The slaughtering and cutting processes produce a diverse array of by-products, which slaughterers aim to either remove at minimal cost or maximize their value. These materials are known by different names depending on the situation: offal, by-products, co-products, waste, refuse, residues, or scraps. In a broader historical context, all parts of the slaughtered animal that do not qualify as "meat" are encompassed by the term "Fifth Quarter".

The products of the fifth quarter include offal and tripe products, food co-products such as blood, bones, and animal fats, which are processed to re-enter the food supply chain, and skins, a portion of which may be used in gelatin production (Figure 32).

The amount of by-products generated in the meat and poultry industry is substantial. By-products can constitute a significant portion of live animal weight, ranging from approximately 44-56% for cattle, 45-51% for sheep, 22-32% for poultry, and up to 30-48% for pigs. Factors influencing the proportion of by-products include species, sex, age, live weight, and fat content of the animal, as well as the specific definition and collection method of what constitutes the "fifth quarter".

In the field of butchery, "offal" refers to the internal organs of animals located in their head, chest, and abdomen, as well as their glands, cheeks, feet, and tail. These parts constitute the edible portion of the fifth quarter, named in contrast to the four quarters (two forequarters and two hindquarters) of a large cattle carcass.

Referred to as "variety meats," they are typically categorized into two types: red offal and white offal. This distinction does not denote the color of the products but rather their state at sale. Red offal consists of products sold in their raw, minimally trimmed form: examples include liver, kidneys, heart, tongue, snout, tail, cheeks, skirt steak, and flank steak. White offal undergoes varying degrees of preparation at the slaughterhouse and is sold scalded, blanched, or partly cooked, resulting in an ivory-white appearance. Examples include stomach, feet, ears, udders, and veal head.

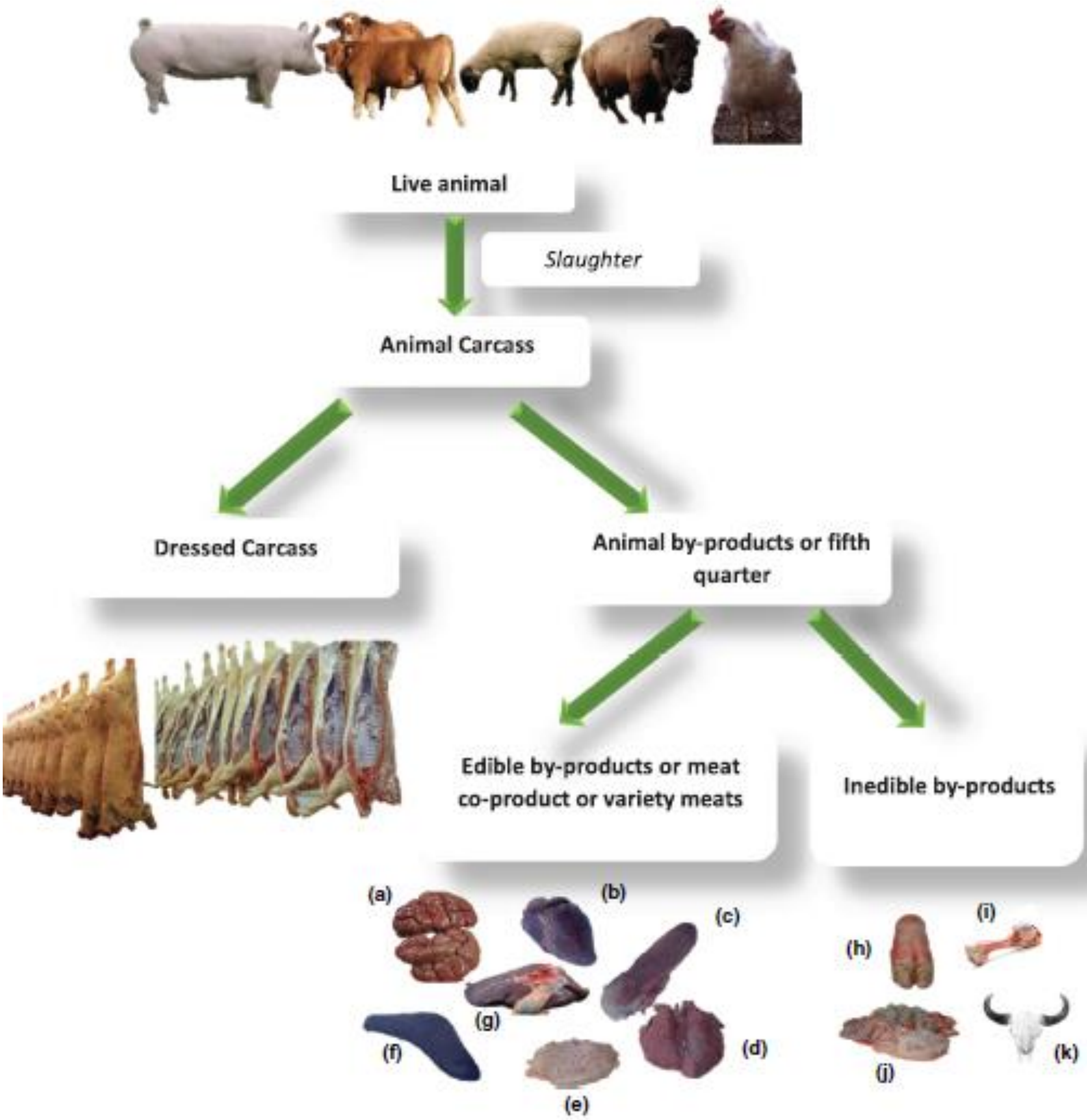

Figure 32: The classification of the fifth quarter with common names used to describe them; Selected fifth quarter labeled as (a) kidney, (b) heart, (c) tongue, (d) lung, (e) stomach, (f) spleen, (g) liver, (h) hoof, (i) bone, (j) digesta in gut, and (k) skull.

The fifth quarter includes both edible and inedible components from butchered livestock. However, markets for edible offal are declining in the Western economy, owing primarily to consumer health and safety concerns. As a result, nonfood uses for the fifth quarter have been identified (Figure 33).

The initial co-product derived from animal slaughter is blood. In Europe and Asia, there is a longstanding tradition of using animal blood in various foods such as blood puddings, sausages, biscuits, blood cakes, and breads. Blood and its proteins are utilized in the food industry for their nutritional value, as binding agents, natural color enhancers, emulsifiers, stabilizers, and agents in meat curing processes.

Organ meats, like kidneys and liver, provide more vitamins and polyunsaturated fatty acids compared to lean meat. Because of its high protein and nutritional content, offal can be used as functional ingredients in novel value-added products with economic potential.

Co-products from slaughterhouses, including hides, skins, tendons, and bones, are significant sources of collagen. Cattle bones, for instance, contain up to 25% collagen, compared to 5% found in cattle hide. Collagen can be hydrolyzed into gelatin, which serves as a highly effective clarifying, stabilizing agent, and emulsifier. It is extensively utilized in the food industry due to its valuable functional properties. The gelling properties of gelatin have made it highly desirable for a wide range of applications, including soups, gravies, dairy products, ice cream, candies, bakery items, and desserts. Its ability to create a melt-in-the-mouth texture has further popularized its use in desserts.

Cattle bones make up approximately 10-15% of the animal's live weight, whereas lamb carcasses contain about 16% bones. Bovine cancellous bone was transformed into a customizable implant material for biomedical purposes through processes involving boiling, solvent treatment, and de-proteinization. Co-products from slaughterhouses, such as bones, horns, and hooves, are frequently utilized as soil conditioners and organic fertilizers.

Animal co-products provide a valuable nitrogen source, and their application to soil enhances microbial biomass, thereby improving soil fertility. Studies suggest that meat-and-bone meal can serve as a cost-effective source of pure phosphate for soil enrichment. It contains high levels of nitrogen, organic carbon, and essential micronutrients like manganese, iron, copper, nickel, and zinc, surpassing those found in chemical fertilizers.

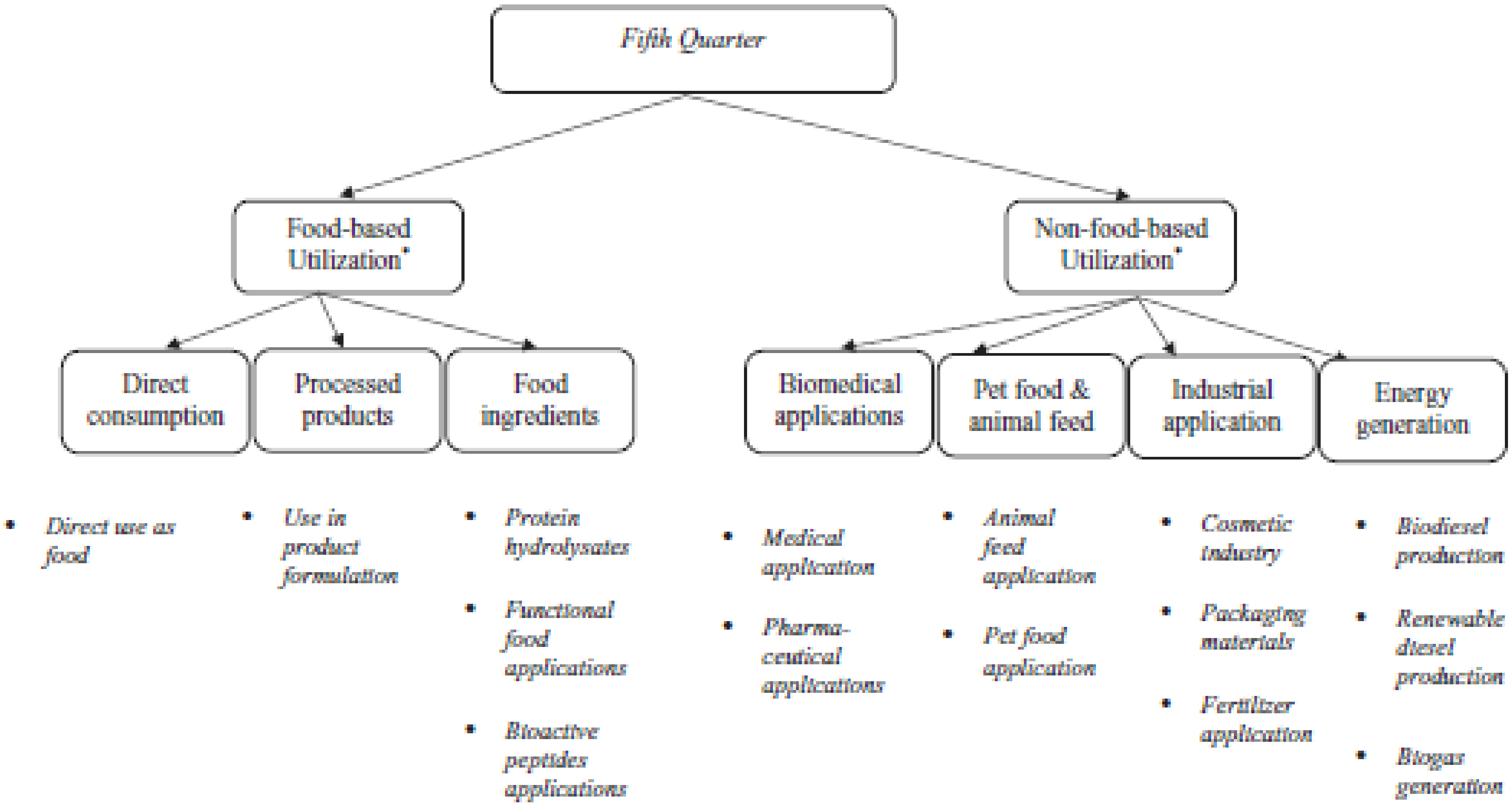

Figure 33: The utilization routes of the fifth quarter across various industries.

8. Poultry pâté manufacturing processes

8.1. Hydration

Water is an essential yet frequently underestimated nutrient in poultry production. It constitutes approximately 65-70% of a bird's lean body mass and plays a crucial role in nutrient transport, temperature regulation, joint lubrication, and various biochemical reactions both inside and outside the cells. It is vital for all metabolic processes in animals. Additionally, water is a fundamental component in two of the most critical life processes for chickens—digestion and respiration—which are integral to maintaining proper body temperature.

Typically, chickens should consume about 1.5 to 2 times more water than their feed intake when environmental temperatures are normal. Water consumption in poultry is influenced by various factors, including:

- **Feed Consumption**: Lower feed intake can lead to decreased water intake, and vice versa.
- **Water Temperature**: Chickens drink less if the water is too hot.
- **Water Quality**: Contaminated water can reduce consumption.
- **Ambient Temperature**: Water intake increases with higher temperatures and decreases when it’s cooler.
- **Type of Drinkers**: The design of the drinkers can affect water consumption.
- **Drinker Height**: The height of the drinkers can influence how much water is consumed.
- **Water Pressure**: Proper water pressure is necessary for optimal consumption.

8.2. Muscle proteins

Meat contains water, proteins (including enzymes and amino acids), mineral salts, lipids, vitamins, bioactive components, and a minor quantity of carbohydrates. Protein accounts for 12-20% of the edible component and 50-80% of the dry weight. The primary ones are myosin, myostroin, and collagen. Myoglobin provides meat its distinctive red color, which turns brown when oxidized. Lipids vary in quantity according on the animal and component. Chicken costs 5p 100, while veal and rabbit cost 5-10p 100 and charcuterie costs 10-20p 100.

Raw poultry meat primarily consists of proteins, lipids, and minerals, with their proportions ranging from 18.4% to 23.4%, 1.3% to 6.0%, and 0.8% to 1.2%, respectively. Breast meat contains less than 3 grams of fat per 100 grams, while dark meat (without skin) has an average fat content of 5-7 grams per 100 grams. Unlike beef fat and dairy products, chicken meat does not contain trans fats, which are associated with coronary heart disease. Instead, about half of the fat in chicken is made up of beneficial monounsaturated fats, while only a third consists of less healthy saturated fats. The World Cancer Research Fund and other health organizations have noted that consuming large amounts of red meat, especially processed meat, can be unhealthy, but this does not apply to chicken meat.

Poultry meat is a significant source of essential polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids. It is generally easier to increase the levels of these valuable long-chain polyunsaturated fatty acids (LC PUFAs) in chicken meat compared to other farmed meats, although this may sometimes affect oxidative stability.

8.3. Mechanism of hydration and swelling power

Water-holding capacity refers to meat's ability to retain or absorb additional water when subjected to an external force, a property dependent on interactions between proteins and water. Water is retained by muscle proteins and is physically trapped within the muscle structure, particularly in the spaces between myofibrils. Various factors, including pH, salt concentration, processing methods, and temperature, affect how proteins bind with water and impact the overall quality of the poultry meat product.

Protein-water interactions are closely linked to the state of meat after death. At the isoelectric point (pH ~5.1), myofibrillar proteins carry a neutral charge and tend to clump together. As pH rises during the resolution of rigor mortis, the proteins acquire a more negative charge, which increases repulsive forces between myofibrils. This causes the muscle to swell, allowing more water to interact with the proteins. Consequently, protein solubility and water-holding capacity improve as proteins become more negatively charged.

Adding salt up to a concentration of 0.6 mol/liter (2–3.5% NaCl) decreases electrostatic interactions between proteins, which enhances protein extractability, solubility, and water binding in both breast and dark muscle. Additionally, the use of alkaline phosphates, along with salt and mechanical action, raises the pH and improves the extraction and solubilization of myofibrillar proteins. Chopping or tumbling the muscle breaks down the muscle fibers, allowing them to absorb water and swell. However, excessive chopping or tumbling can overly disrupt muscle fibers and cause protein denaturation due to increased temperature and shearing forces. Denatured proteins, such as those found in PSE (pale, soft, and exudative) muscle, form aggregates with low water affinity and diminished emulsification and foaming properties.

8.4. Coloration and myoglobin: case of fresh meat

The color of meat is the first quality attribute noticed by consumers when purchasing, often serving as an indicator of freshness. Meat color is influenced by four key components: the first two explain the color of fresh meat, while the latter two account for its changes during storage.

The structural component is related to the muscle's physical structure, particularly its acidity (pH), which affects the product’s brightness, ranging from light red to darker shades. The quantitative component refers to the amount of red pigment in the muscle, which dictates the color’s intensity, from bright red to dull or grayish. Myoglobin, the primary pigment responsible for meat color, is a chromoprotein consisting of a heme group (an iron-containing molecule) and globin (a protein). A higher heme iron content results in more intense red meat and less yellowish hue. During storage, the structural and quantitative components of meat color remain relatively stable.

The qualitative component involves the chemical state of the muscle pigment, which changes over time. Reduced myoglobin (MbO_2, Fe^{++}) is present deep within the muscle or on the surface when stored without oxygen. When exposed to air, this pigment reacts with oxygen to form bright red oxymyoglobin (MbO_2, Fe^{++}), which is associated with freshness and is visually appealing to consumers.

Additionally, meat color tends to appear redder and darker with increasing age, carcass weight, diet, muscle metabolic type, muscle fiber composition, marbling, heme pigment content prior to slaughter, and post-mortem conditions.

8.5. Redox state of the pigment

The color of poultry meat is influenced by the concentration and chemical state of various meat pigments, including myoglobin, hemoglobin, cytochrome C, and their derivatives. Additionally, the presence of ligands that interact with heme pigments also affects the meat's color.

The color of fresh meat is determined by the relative proportions of four redox states of myoglobin: deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb), and metmyoglobin (MetMb) (Figure 34).

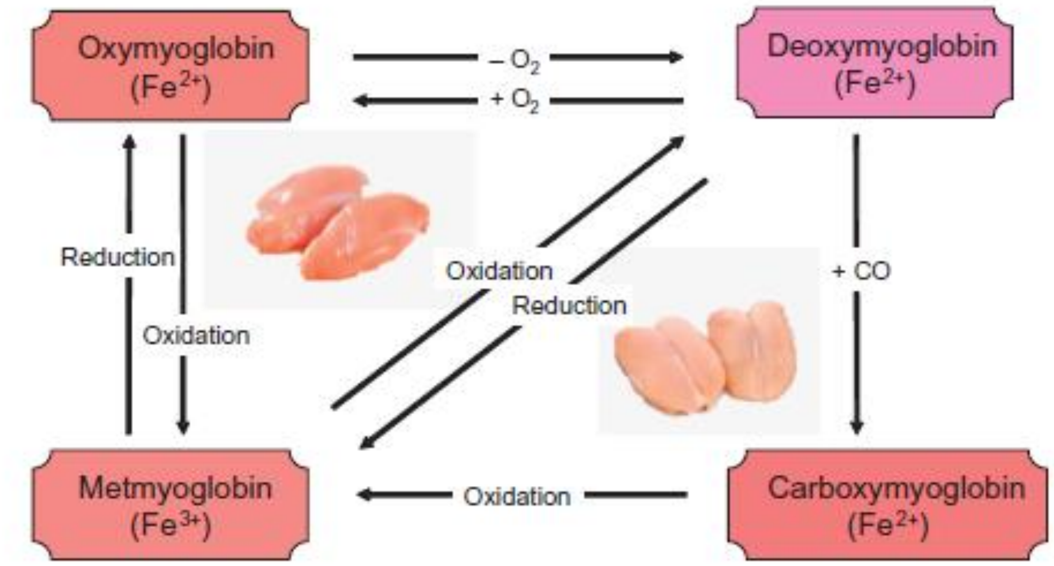

Figure 34: Myoglobin redox forms in poultry meats.

OxyMb and COMb produce cherry-red and red colors, respectively, and these two redox forms are not distinguishable by the human eye. DeoxyMb appears purplish-red. In OxyMb, the sixth coordination site of the heme iron is occupied by oxygen, while in COMb it is occupied by carbon monoxide. In DeoxyMb, the heme iron does not have a bound ligand. Saturating myoglobin with oxygen (O2) results in the cherry-red color of OxyMb. MetMb, which appears brown, forms when the three ferrous (Fe2+) states of myoglobin are oxidized to the ferric (Fe3+) state, leading to discoloration in poultry meat. MetMb has a water molecule bound at the sixth coordination site of the ferric heme and cannot bind oxygen.

8.6. Case of cured products

Curing, or salting, is one of the oldest methods for preserving meat. This process involves immersing or injecting meat pieces—whether small or large, and either boneless or bone-in—with a curing brine or pickle solution. This solution contains salt, phosphate, nitrate/nitrite, ascorbate, and sugar dissolved in potable water, with each ingredient contributing to the distinct flavor and pink color of the cured meat. There are two main curing techniques: wet and dry.

In wet curing, also known as pickle curing, meat cuts are either soaked in the curing solution or injected with it using a multi-needle injector before soaking. This method ensures the even distribution of the curing solution and helps achieve a consistent cured meat color without grey spots. In dry curing, the curing ingredients are rubbed directly onto the meat's surface, which is then stored for an extended period under controlled temperature and humidity conditions.

8.7. Evolution of ions NO_3^- and NO_2^-

Scientific progress enabled the identification of nitrate (NO_3^-) and subsequently nitrite (NO_2^-) as essential components in preserving cured meat products. Sodium and potassium nitrate and nitrite salts are commonly used as food preservatives in meat processing. These compounds effectively suppress microbial growth, delay rancidity, enhance cured meat flavor and aroma, and maintain the meat's characteristic red color.

Nitrites limit the formation of *Clostridium botulinum*, which causes food poisoning in many meats and goods. Nitrates and nitrites are harmful to humans when taken in excess, hence their levels are limited to 150-200 parts per million (ppm, or 150-200 mg/kg finished product). Certain items, such as bacon, have a nitrite content limit of 120 ppm. Nitrites react with secondary amines to generate nitrosamines, which are known to cause cancer.

Nitrates (E251; E252) and nitrites (E249; E250) are approved food additives under Commission Regulation (EU) No. 1129/2011. Nitrates are relatively non-toxic, however nitrites, and nitrites metabolic products such as nitric oxide and N-nitroso compounds, have aroused concerns about potential negative health effects.

8.8. Formation of nitrosated pigment

Nitrite or nitrate interacts with myoglobin in muscle to create a pinkish-red color. To address food safety concerns, many researchers have recently explored alternative ingredients for color formation that contain natural nitrite or nitrate, such as vegetable extracts and bacteria. Researchers discovered that a nitrite concentration of 4×10^{-6} g/g was ideal for marinating chicken meat when combined with ginger paste. However, using more than this amount of nitrite resulted in an undesirable pink color in the drumsticks.

Cooking causes globin to denature, resulting in the formation of nitrosyl hemochrome complex (NHC), a persistent pink color. The minimum sodium nitrite concentration required for noticeable pink color was 14 ppm for beef, 4 ppm for pork, 2 ppm for turkey, and 1 ppm for chicken. Nitrates must be converted to nitrite to produce nitric oxide myoglobin (NOMb), which denatures and converts to NHC when heated.

8.9. Other roles of nitrites

Flavor improvement

Nitrite has the ability to hinder the growth of bacteria such as *Clostridium botulinum*, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, and others. This inhibitory effect of nitrite supports certain fermentative bacteria in cured meat products, enabling them to generate flavor compounds through their own metabolism or through the hydrolysis of proteases and lipases within the meat. Thus, nitrite indirectly influences the development of cured meat flavor by affecting both microbial activity and the action of natural enzymes.

Antioxidant effect

The nitric oxide produced from nitrite can competitively reduce oxygen through self-oxidation, bind to the iron ion in hemoglobin to prevent oxidation, and disrupt radical chain reactions involved in lipid oxidation. These secondary actions of nitrite likely account for its antioxidant properties. It has been demonstrated that sodium nitrite effectively suppresses lipid oxidation during the marination of mutton at a concentration of 1×10^{-4}. Ma et al. (2022) conducted a study comparing the antioxidant properties of phosphorylated nitrosohemoglobin (PNHb) and sodium nitrite in emulsified sausage. They found that the thiobarbituric acid values were 0.62 mg/kg for the PNHb group and 0.67 mg/kg for the $NaNO_2$ group. These results indicate that PNHb exhibits a more potent antioxidant effect compared to $NaNO_2$.

Shelf-life extension

The extended shelf-life results from multiple factors that collectively delay quality deterioration, such as the cleanliness of raw meat, processing techniques, storage conditions (temperature and humidity), packaging methods, and more. Nitrite and nitrate contribute to this by preventing the growth of harmful bacteria and spoilage organisms, thereby enhancing the shelf life of meat and meat products.

8.10. Adverse effects

- ✓ ***Chronic Effect***

Carcinogenic Effect of Nitrates and Nitrites

Nitrites, even when used as food additives, contribute to the production of nitrosamines, some of which are known to be carcinogenic. After considering several cautious assumptions (assuming the worst-case scenario), the panel determined that the formation of nitrosamines in the body from nitrites added within approved limits in meat products poses a low risk to human health. It also highlighted that nitrite inadvertently present in meat due to sources like environmental contamination can also contribute to nitrosamine formation. EFSA's experts concluded that these levels of nitrosamines could potentially raise health concerns, but emphasized the need for further research to address uncertainties and gaps in understanding this complex issue.

Nitrite plays a specific role in human physiology, with some of its physiological effects linked to nitric oxide (NO), impacting arterial blood pressure, immune response, and biofilm formation. However, in acidic environments or under oxidative stress, nitrite can convert into various reactive nitrogen species (RNS). Oxidative and nitrosative stress resulting from an increase in reactive oxygen and nitrogen forms is recognized as a significant factor in many acute and chronic diseases. The levels of nitrosative stress depend largely on the concentration and duration of exposure to RNS, as well as the capacity of cellular antioxidants to neutralize them. These reactive nitrogen species include nitric oxide (NO), nitrogen dioxide (NO2), and peroxynitrite ($ONOO^-$), with NO2 and $ONOO^-$ being highly reactive. In biological systems, their production is tightly regulated because they play crucial roles in various biological functions.

However, unchecked intracellular presence of these species can lead to significant toxicity by targeting a variety of biomolecules such as proteins, DNA, lipids, and carbohydrates. Therefore, elevated levels of nitrite and NO under nitrosative stress conditions may potentially contribute to adverse effects like mutagenesis and carcinogenesis (Figure 35).

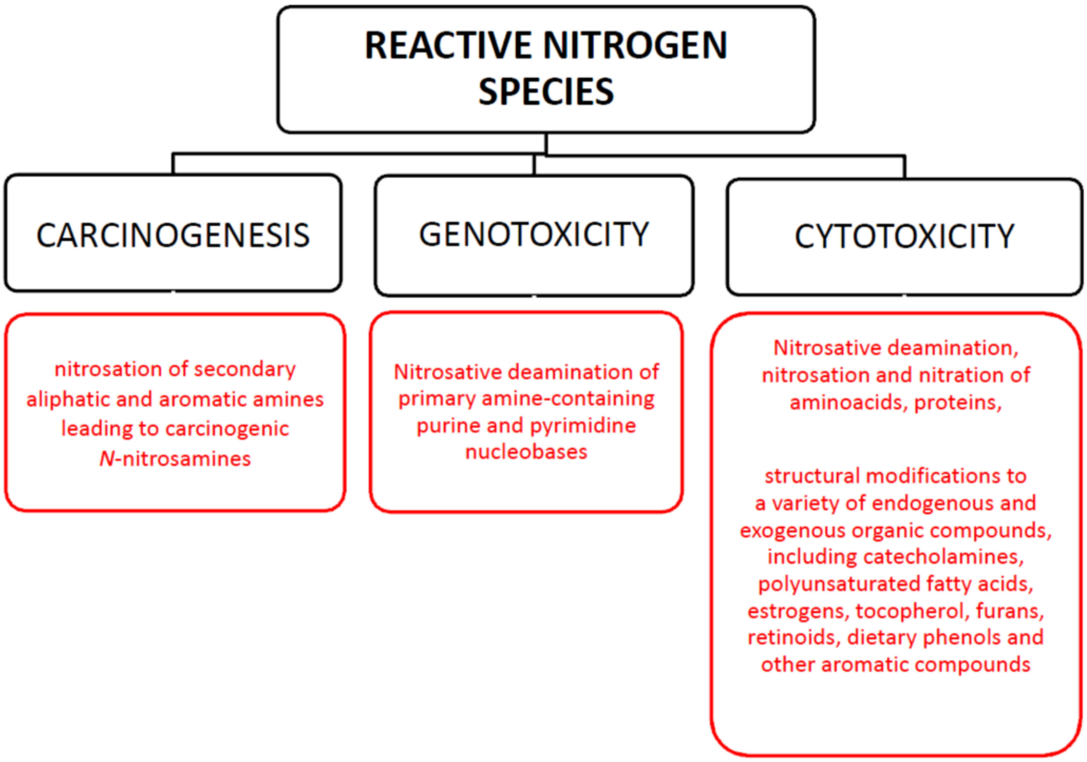

Figure 35: Toxicological effects of reactive nitrogen species

Nitrosative stress-induced oxidation of membrane lipids can be particularly damaging because it alters membrane fluidity and increases tissue permeability by deactivating membrane-bound receptors or enzymes. Furthermore, the process of lipid peroxidation produces a range of relatively stable products that can be detected in plasma and urine, serving as indirect markers of nitrosative stress. These products include unsaturated aldehydes such as malondialdehyde, 4-hydroxy-2-nonenal, and 2-propenal. Some of these compounds are known to react easily with proteins, DNA, and phospholipids, contributing to the development of various diseases.

Proteins are significant targets for reactive nitrogen species (RNS). When proteins are exposed to RNS, it induces substantial changes in their structure, leading to various functional impacts. These include inhibiting enzymatic and binding activities, increasing vulnerability to aggregation and breakdown, and altering their immunogenic properties.

The impact of dietary nitrates and nitrites on cancer risk is depicted in Figure 36. However, the findings from human studies regarding the association between nitrate and nitrite intake and cancer risk vary widely. Some studies indicate a significant link between higher intake of nitrate and nitrite and an elevated relative risk (above 1) of various cancers, including breast cancer, gastric cancer, renal cell carcinoma, adult glioma, colorectal cancer, esophageal cancer, and thyroid cancer.

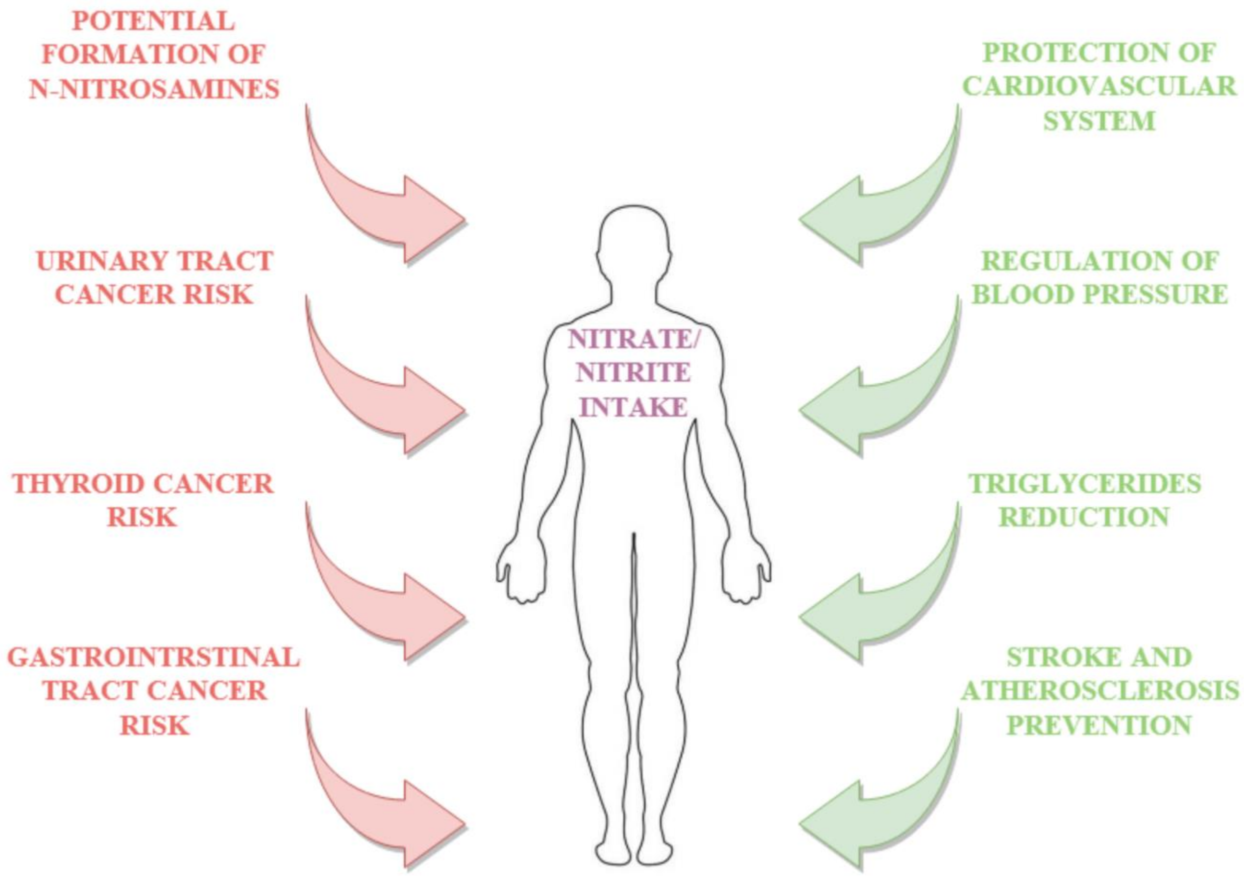

Figure 36: Selected benefits and adverse effects on nitrate nitrite intake

✓ ***Acute Toxicity***

Methemoglobinemia

Methemoglobinemia is a condition affecting the blood where there is an excessive production of methemoglobin (MetHb), which hinders the efficient release of oxygen to the body tissues. This occurs because MetHb represents a modified form of hemoglobin (Hb), where the iron in heme has undergone oxidation from its ferrous to ferric state.

There are two forms of methemoglobinemia: congenital and acquired. Congenital methemoglobinemia results from a deficiency in the enzyme CYB5R3 or a mutation in the CYB5R gene. It can range from a severe condition potentially leading to death to a milder, more treatable disorder. Acquired methemoglobinemia, associated with exposure to nitrates, arises from various agents such as topical anesthetics like Lidocaine and Prilocaine, gastrointestinal infections, and nitrate/nitrite ingestion. Unlike congenital methemoglobinemia, which stems from genetic factors, acquired methemoglobinemia is induced by substances that oxidize the iron in hemoglobin.

Effect on the Thyroid Gland

The thyroid gland is a crucial part of the body's endocrine system, serving as the largest organ specialized for hormonal function in humans. Shaped like a butterfly (Figure 37), it is situated in the front of the throat near the voice box and is richly supplied with blood vessels. This gland plays a vital role in normal body growth during infancy and childhood. It absorbs iodine from the diet and synthesizes thyroid hormones, which are iodine-containing compounds that regulate the body's metabolic rate. Thyroid hormones influence processes such as controlling body temperature and regulating the breakdown of proteins, fats, and carbohydrates in all cells. Additionally, the thyroid gland contributes to growth hormone release, skeletal maturation, and the regulation of heart rate, strength, and output. It supports the growth of the central nervous system and stimulates the production of various enzymes. Muscle tone and overall vitality also depend significantly on thyroid function. The hormone thyroxine, produced by the thyroid gland when sufficient organic iodine is present, plays a critical role in regulating metabolism to a considerable extent.

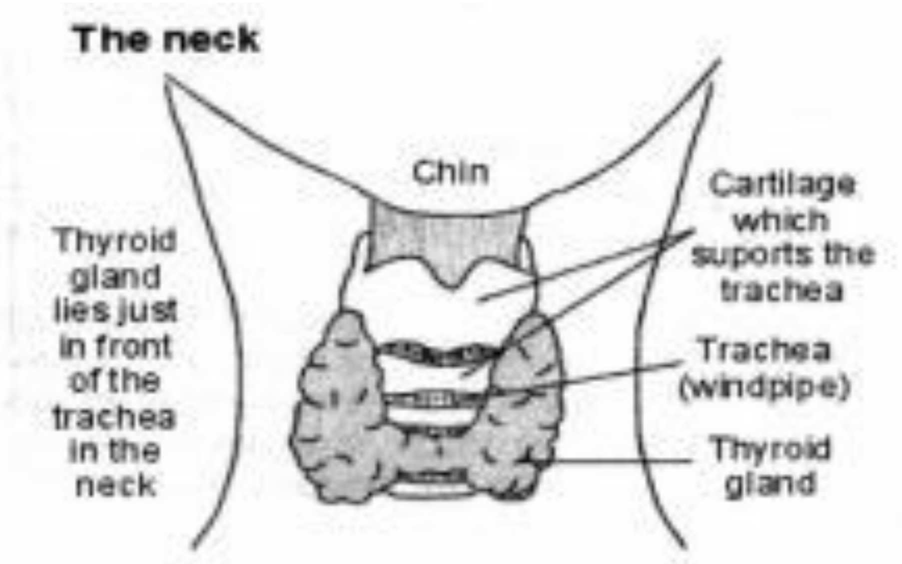

Figure 37: The thyroid gland

Elevated nitrate consumption can impact thyroid function in humans similarly to observed effects in animals. This is because nitrate ions (NO_3^-) can hinder the transport of iodide (I^-) into the thyroid gland, as they utilize the same transport mechanism. This hindrance may result in reduced secretion of thyroid hormones (T4, T3), leading to an increase in thyroid-stimulating hormone (TSH). Ultimately, this sequence of events could potentially cause enlargement of the thyroid gland, known as goiter.

Mellitus Diabetes

Diabetes mellitus is a metabolic disorder of various causes characterized by persistent high blood sugar levels, affecting carbohydrate, fat, and protein metabolism due to deficiencies in insulin secretion, insulin action, or both. This condition can lead to chronic damage, dysfunction, and failure of multiple organs. Symptoms of diabetes mellitus often include thirst, frequent urination, blurred vision, and unintended weight loss. In severe cases, it can progress to ketoacidosis or a hyperosmolar state, potentially resulting in stupor, coma, and, without prompt treatment, death. Symptoms may be mild or absent, allowing elevated blood sugar levels to persist for an extended period before diagnosis. Long-term effects include complications like retinopathy leading to potential blindness, nephropathy increasing the risk of kidney failure, and neuropathy associated with foot ulcers, amputation, Charcot joints, and autonomic dysfunction, including sexual dysfunction. Individuals with diabetes also face higher risks of cardiovascular, peripheral vascular, and cerebrovascular diseases.

Studies conducted in Colorado demonstrated a significant link between the prevalence of insulin-dependent type 1 diabetes mellitus in children aged 1 to 18 years and the concentration of nitrate in drinking water. Similarly, a case-control study in 1990 reported a positive correlation between nitrites, nitrates, and nitrosamines in food and the incidence of this disease, particularly among individuals with diets high in nitrate in Sweden. This association was also noted in Finland, albeit with a weaker exposure to nitrate. In contrast, no significant risk was observed for diabetic children exposed to nitrate in drinking water supplies across Scotland and parts of central England. Studies in the Netherlands and Italy likewise found no connection between different concentrations of nitrate and nitrite in drinking water and insulin-dependent type 1 diabetes mellitus. Overall, these findings indicate limited and conflicting data, emphasizing the need for comprehensive assessments to definitively understand the relationship between nitrate, nitrite, nitrosamine exposure, and diabetes mellitus through various dietary sources.

8.11. Other dyes

Color plays a crucial role in enhancing the appeal of food and beverages, influencing consumer acceptance. Food manufacturers have increasingly favored synthetic colors over natural ones to achieve specific benefits like affordability, enhanced appearance, vibrant color intensity, improved stability, and consistency. However, various foods and drinks on the market may contain unauthorized synthetic colors or excessive use of permitted ones. This misuse can potentially result in serious health issues such as mutations, cancer, reduced hemoglobin levels, and allergic reactions.

The regulation of food dyes in meat products requires ongoing vigilance due to multiple food safety concerns. Certain red colorants like Ponceau 3R (also known as Red Dye No.1), Amaranth (Red Dye No.2), and Scarlet GN (Red Dye No.4) have been banned due to their proven toxic effects in rats. Erythrosine (Red Dye No.3), however, remains permitted in both the USA and Europe, despite studies indicating potential health risks such as thyroid follicular cell hypertrophy, adenomas, and hyperplasia in rats from high intake.

Carmine (E120) and Ponceau 4R (E124) are the most commonly used red dyes in meat products. Along with Allura Red AC (E129), they are the only food dyes currently allowed in meat products under European legislation. However, specific legal limits have been established because these dyes cannot be considered entirely non-toxic. Carmine, for instance, has been associated with adverse reactions in humans.

The regulatory stance on Ponceau 4R in food varies significantly between countries, leading to controversy. In the USA, the Food and Drug Administration has banned this food dye due to studies indicating potential health effects, including heightened hyperactivity in susceptible children with attention deficit/hyperactivity disorder and other behavioral issues (Iammarino et al., 2020).

Industries are actively seeking natural coloring agents and antioxidants that can prevent oxidation in products, address free radicals in human metabolism, and enhance product coloration. Recent exploration focuses on identifying and isolating colored antioxidants from natural sources like herbs, leaves, spices, vegetables, fruits, and even food remnants. In South America, three noteworthy examples include propolis resin, red grape pomace derived from wine and juice production waste, and leaves from the *Moringa oleifera* plant.

8.12. Dispersion and emulsion

An emulsion is a colloidal mixture formed when small droplets of one liquid are dispersed within another liquid in which it is not soluble. The foundation of pâté and related products lies in the emulsification of added fats. A meat emulsion represents a two-phase system where solid particles are dispersed in a liquid phase where they are not soluble. While this definition acknowledges the behavior of insoluble particles in the aqueous phase, it does not fully encompass the characteristics of fats, which form a structure more akin to an emulsion than a simple dispersion. In the realm of processed meats categorized as meat emulsions, fine paste can be defined as a blend primarily comprising lean meat, fat, and water, where the components are so finely mixed that individual grains are no longer discernible to the naked eye. Mortadella, frankfurters, and liver pâté are examples typical of this category.

Emulsion gels are soft solid materials characterized by a gel network structure and stable mechanical properties. Within these gels, emulsified droplets are embedded in a gel matrix, forming a complex colloidal material that can exist in both emulsion and gel states. The unique properties of emulsion gels stem from intricate interactions among their components. In oil-in-water (O/W) emulsion gels, this colloidal structure can arise from the dispersion of emulsion droplets within a continuous gel matrix or from the aggregation of dispersed droplets into particle gels.

Compared to traditional emulsions, emulsion gels demonstrate improved storage stability and have the potential to prolong drug release in the intestines. They also exhibit excellent stability, making them ideal for encapsulating flavor compounds. Moreover, emulsion gels offer a soft solid texture and physical characteristics similar to fat in emulsified meat products, making them particularly suitable for creating healthy, functional foods and serving as substitutes for animal fat to produce low-fat meat products. These gels can effectively replace animal fats while preserving the physical and chemical properties—especially stiffness and water-holding capacity—of the final products. The use of plant oils rich in polyunsaturated fatty acids (PUFAs) to create solid-structured oils and fats as replacements for animal fats has garnered increasing attention. Emulsion gels find widespread application in meat products.

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