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THE SHELF LIFE OF FRESH PASTA: COMPARISON BETWEEN TRADITIONAL AND NEW TECHNOLOGIES OF CONSERVATION METHODS

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A mia madre e mio padre, che hanno sempre creduto in me
a mia Sorella fedele compagna di viaggio sulle strade della vita
a Luca, che mi ha tenuta per mano con dolcezza in questo percorso
ai miei Nonni ovunque siano…
Un grazie speciale al Professor Osimani per la sua professionalità e disponibilità

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ACRONYMS AND ABBREVIATIONS

a_w	Water activity
CAP	Controlled atmosphere packaging
FT-NIR	Fourier Transform- Near Infrared Spectroscopy Analysis
GAB	Gugghenheim-Anderson-de Boer model
HACCP	Hazard Analysis and Critical Control Points
IAEA	International Atomic Energy Agency
ISO	International Organization for Standardization
LAB	Lactic acid bacteria
MAP	Modified atmosphere packaging
MOCA/FCM	Materials and objects in contact with foods
NIR	Near infrared spectroscopy analysis
NRC	Nuclear Regulatory Commission
SME	Specific Mechanical Energy
TTI	Time–temperature integrator/indicator
VP	Vacuum packaging

INTRODUCTION AND THESIS AIM

Growth in the demand for high-quality food has driven the agribusiness to perfect food conservation techniques (shelf life) to the extent that, in recent years, the scientific literature regarding it has greatly increased. As such, the concept of shelf life (i.e., preservability) has evolved. Initially conceived as 'the retention period of the food product between the date of production and its retail sale', the definition has since extended to the entire 'life period' of the food until consumption. Consequently, Regulation 2073/05 defines shelf life as 'the period preceding the minimum storage term or the expiration date, as defined in Articles 9 and 10 of the Directive respectively 2000/13/EC.' The expire date, is established by the manufacturer, who must provide supporting evidence of that date.

The declaration of shelf life, therefore, is the responsibility of the manufacturer who, in the preservation conditions declared on the label, must guarantee the following:

- the health of the food (microbiological, chemical and physical characteristics); and
- the organoleptic aspect as perceived by the consumer (taste, colour, odour, and texture).

In Italy, filled fresh pasta is regulated by Law no. 580 (04/07/1967), and subsequent amendments. To maintain compliance with Presidential Decree no. 187/2001 (concerning the production and sale of milling products and pasta), the requirements for prepackaged fresh pasta offered for sale are as follows:

- moisture content not less than 24%
- water activity (a_w) not less than 0.920 and no higher than 0.970
- thermal treatment equivalent to at least pasteurisation
- storage at temperatures not exceeding at 4 °C, with a tolerance of 2 °C, from production to sale.

The main factors affecting the shelf life of filled fresh pasta may be classified as intrinsic and extrinsic (Table 0-1).

Table 0-1: Factors affecting the shelf life (Marco Dalla Rosa, 2009).

Intrinsic Factor	Extrinsic Factor
1. Water Activity (a_w)	1. Time-temperature profiles during the process
2. Ph	2. Pressure in the headspace of the packaged food
3. Oxygen (O_2)	3. Relative humidity (RH%) during the process, storage, and distribution
4. Nutrients	4. Exposure to light (UV or IR) during the process, storage, and distribution
5. Natural microflora and surviving microorganisms of partial sanitising treatments	5. Presence of microorganisms in the environment during the process, storage, and distribution
6. Enzymes present in the product or in the formulation	6. Composition of the atmosphere in the packaging and its evolution during storage and distribution
7. Use of stabilising elements (antimicrobials, compositional elements such as salts and humectants) used in the formulation/treatment	7. Heat treatments following the process (such as tempering or cooking before consumption) and profiles, relative time-temperature until consumption.
	8. Consumer manipulation

Intrinsic factors are influenced by the type of raw materials used, the formulation of the product, and its structure. Extrinsic factors depend on the production methods (technological processes), storage, and distribution. Each factor, acting individually or interacting with one or more factors, may affect the shelf life of the product. Hence, each product is a unique system, and to ensure superior quality over time, it is necessary to accurately study the shelf life. The main objective of this thesis is to evaluate the existing scientific data regarding the methods of controlling the intrinsic and extrinsic factors that influence the shelf life of fresh pasta, particularly concerning microbiological parameters.

CHAPTER 1
FRESH PASTA

1.1 Fresh Pasta Definition and Classification

Pasta is one of the main components of the Mediterranean diet as it contains significant amounts of complex carbohydrates, proteins, B-vitamins, and iron. In general, pasta may be made using different kinds of flour (e.g., durum wheat semolina and wheat flour) mixed with water. Fresh pasta comprises more than 24% moisture, and its a_w ranges from 0.92 to 0.99. Hence, it requires refrigeration. Pasta may be prepared with eggs in the dough or by filling a sheeted dough with a spiced mixture of ground meat, cheese, or vegetables (as in the case of tortellini and ravioli). In the past decade, filled fresh pasta has gained national and international popularity. However, distribution continues to represent a significant problem due to rapid microbial proliferation. Such products are highly susceptible to spoilage microorganisms. Therefore, the addition of preservatives or reduced oxygen packaging is necessary to prolong the shelf life that, even under refrigerated temperatures, lasts only two or three days. In particular, without preservatives, the pH value of pasta may drop, thus indicating spoilage and the presence of coliforms.

To reduce the growth of vegetative microbial forms and to improve cooking, Italian law[1] prescribes a pasteurisation treatment before final packaging. The thermal treatment is carried out in an injected steam belt pasteuriser. In addition to reducing a_w, this helps to increase starch gelatinisation resulting in less water absorption during cooking. To prolong the shelf life of fresh pasta, different methods have been applied; the most common approaches are based on chemical preservatives (such as organic acids) and modified atmosphere packaging (MAP), using low oxygen (O_2) (below atmospheric levels) and high carbon dioxide (CO_2) (20% or higher) concentrations coupled with nitrogen (N_2) as an inert gas (Angiolillo et al., 2019).

Pasta products are classified according to the moisture content of dried and fresh pasta and the ingredients in pasta made from durum wheat semolina, special pasta, and filled pasta. Italian

[1] PRESIDENTIAL DECREE N° 187, dated 9 February 2001 (Official Journal n. 117, of May 22, 2001)Regulation for the revision of laws concerning the production and sale of milling products and pasta, pursuant to Article 50 of Law N° 146, dated 22 February 1994. Art.9

dried pasta should have a moisture content no greater than 12.5% whereas the fresh product should have a moisture content of at least 24% and a_w not less than 0.92 and not more than 0.97.

Fresh pasta products are particularly vulnerable from a microbiological perspective according to their water content, some stage in their processing, and the presence of some ingredients in the filling (Ricci, Barone, and Petrella, 2017).

1.2 Fresh Pasta Composition

Egg pasta may only be manufactured using durum wheat semolina and at least four chicken eggs with a total weight of at least 200 grams (without the shells) per kilogram of semolina. Instead of eggs, a corresponding amount of liquid egg product may be used.[2]

Filled fresh pasta is produced from several raw ingredients (as many as 25–30) among which are eggs, which are the preferred media for the growth of several microorganisms. This product has a high moisture content (up to 35%) and a_w values between 0.93 and 0.97 (Zardetto, 2014b). Therefore, fresh-filled pasta is an easily perishable food and for marketing, in addition to heat treatment, a series of technological measures must be implemented to prevent microbiological and sensory decay and guarantee the product's health throughout its shelf life.

1.2.1 Durum Wheat Flour

Durum wheat, also termed pasta wheat or macaroni wheat (*Triticum durum* or *Triticum turgidum* subsp. *durum*), is a tetraploid wheat species. It is the second most cultivated species of wheat after common wheat, although it represents only 5% to 8% of global wheat production. It was developed by the artificial selection of domesticated emmer wheat strains, formerly grown in Central Europe and the Near East around 7,000 BC, which developed a naked, free-threshing form. It is the predominant wheat grown in the Middle East.

Durum in Latin means 'hard', and this species is the hardest of all wheat varieties. Hardness refers to the grain's resistance to milling, in particular the starchy endosperm. This implies that dough made from its flour is weak or 'soft'. Therefore, durum wheat is favourable for semolina and pasta and less practical for flour, since it requires more work than hexaploid wheats such as common bread wheats. Despite its high protein content, durum is not a strong

[2] PRESIDENTIAL DECREE N° 187, dated 9 February 2001 (Official Journal n. 117, of May 22, 2001)Regulation for the revision of laws concerning the production and sale of milling products and pasta, pursuant to Article 50 of Law N° 146, dated 22 February 1994. Art.8

wheat in the sense of strengthening the dough through the formation of a gluten network. Durum contains 27% extractable wet gluten, which is approximately 3% higher than in common wheat.

Table 1-1: Characteristics of durum wheat milling products destined for sale. [3]

	Maximum humidity (%)	**Ash Minimum**	**Ash Maximum**	**Minimum protein (nitrogen x 5.7)**
*Semolina **	14.50	-	0.90	10.50
Low-grade semolina	14.50	0.90	1.35	11.50
Durum wheat whole-meal semolina	14.50	1.40	1.80	11.50
Durum wheat flour	14.50	1.36	1.70	11.50

***Particle size is tested using calibrated vibrating sieves using a mesh of 0.180 mm, so that no more than 25% may pass through the sieve.**

1.2.2 Water

Water is fundamental in pasta production, not only as ingredient but also because it is used to generate steam for pasteurisation. According to current legislation, the use of drinking water is mandatory in food factories. The drinking water supply must be adequate for the needs of the establishment and the scope of production. Under current legislation, ice used in production, or otherwise destined to encounter products, must be obtained from drinking water and manufactured, handled, stored, and used in a way that avoids any possible contamination. The steam that comes into direct contact with food products must be obtained from drinking water and must not contain any substance that presents a health hazard or could cause contamination.

The pipes in the drinking water and steam distribution networks that are intended to come into direct contact with raw ingredients, semi-finished and finished products, must be made of non-toxic and corrosion-resistant material. If the drinking water distribution network includes the use of a chlorination plant, the latter must be equipped with an automatic visual and audible

[3] PRESIDENTIAL DECREE N° 187, dated 9 February 2001 (Official Journal n. 117, of May 22, 2001) Regulation for the revision of laws concerning the production and sale of milling products and pasta, pursuant to Article 50 of Law N° 146, dated 22 February 1994. Art.2

alarm system that signals irregular operation and allows immediate intervention. The reserve tanks, if any, must be kept in perfect maintenance conditions and subjected to regular cleaning according to a specific programme (Magnani, Umberto Randi, M.Giovanna Placuzzi, 1998).

1.2.3 Eggs

Egg pasta may only be manufactured using durum wheat semolina and at least four chicken eggs with a total weight of at least 200 grams (without the shells) per kilogram of semolina. Minimum egg content is verified using the gravimetric dosage of total sterols as digitonides and the fat content of the ethereal extract (Ministerial Decree 23/07/1994). The mandatory characteristics of egg pasta were modified in the Decree of the President of the Republic no. 41 (05/03/2013), which required the ether extract and sterol content to be reduced to 2.50g and 0.130g respectively (referring to one hundred parts of dry matter) (Zardetto, 2014).

An egg's shape resembles a prolate spheroid with one end larger than the other and a cylindrical symmetry along its long axis. An egg is surrounded by a thin, hard shell, inside which there are thin membranes. The egg yolk is suspended in egg white by one or two spiral bands of tissue called chalazae. The larger end of the egg contains an air cell that forms when egg contents cool down and contract after it is laid. The eggshell is lined with a clear film that is visible when peeling a boiled egg. This membrane is primarily composed of fibrous proteins such as collagen type I.

Egg white commonly refers to the clear liquid (also termed the albumen or glair/glaire) within an egg. Initially colourless and transparent, it turns white and opaque upon cooking. In chickens, the albumen is formed of layers of secretions from the anterior section of the hen oviduct during egg passage. The primary purpose of egg white is to protect the yolk and provide additional nutrition during embryo growth. Figure 1 illustrates the composition of a chicken egg.

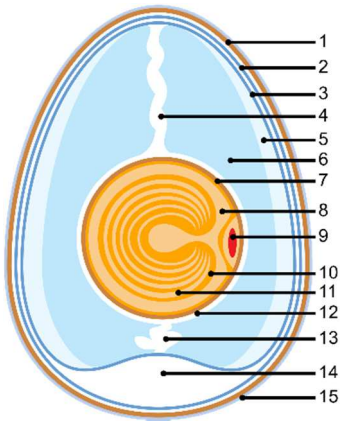

Figure 1-1: A schematic of a chicken egg: 1. eggshell, 2. outer membrane, 3. inner membrane, 4. chalaza, 5. exterior albumen, 6. middle albumen, 7. vitelline membrane, 8. Nucleus of Pander, 9. germinal disc (nucleus), 10. yellow yolk, 11. white yolk, 12. internal albumen, 13. the chalaza, 14. air cell, and 15. Cuticula (www.wikimedia.org).

Eggs are commercially classified at the time of packing, which takes place at the production factory or an authorised packing centre. The eggs are visually inspected (a process known as candling) in special chambers where an operator observes the eggs in semi-darkness. On a conveyor belt, the eggs are positioned against the light so that the inspector may observe the contents in transparency and detect cracks in the shell, blood stains, the presence of inclusions, and the arrangement and size of the yolk and the air chamber. Defective eggs may be downgraded, discarded, or destined for transformation depending on the type of defect. European legislation divides eggs into different quality categories according to freshness and hygiene:

Category A: fresh eggs

Category B: second quality or preserved eggs

Category C: downgraded eggs for the food industry

Category A eggs must possess certain characteristics such as an air chamber height of less than 6 mm and clear, limpid albumen with a gelatinous consistency. Category A eggs may have the wording ‘extra’ added to the labelling when they have freshness characteristics (e.g., an air chamber less than 4mm) guaranteed by more frequent collection and faster marketing. These eggs are delivered to packing centres daily and may retain the wording ‘A extra’ until the seventh day after the packing date or until the ninth day after laying.

Category A eggs are also divided into weight classes:

XL, Extra Large: 73g or more

L, Large: 63g to 73g

M, Medium: 53g to 63g

S, Small: less than 53g

Industrial production of fresh egg pasta typically involves a mixture of whole liquid chicken eggs obtained from hens reared in barns and given feed free of synthetic dyes. This is homogenised, pasteurised, and refrigerated, and 1kg of the product is equivalent to roughly 20 shelled eggs.

1.2.4 Filling

Many ingredients are used to produce pasta filling. Some examples are listed as follows:

- Fine salt
- Cooked mixed bovine-pork meat
- Industrial ricotta
- Double tomato concentrate
- Frozen or dried spinach
- Cured meat
- Seafood products
- Spices
- Breadcrumbs
- Partially skimmed milk powder
- Inulin
- Acidifying additives

The filling has a significant role in the shelf life of filled pasta. Is essential to select microbiologically stable raw ingredients and use technical elements (e.g., vegetable fibers or lactose) that modulate the a_w of the filling.

CHAPTER 2 FRESH PASTA PROCESS

2.1 The Manufacturing Process

The manufacturing process for filled fresh pasta is summarised in Figure 2-1; it includes the following descriptions (Zardetto, 2014):

- filling preparation (weighing, cutting, and mixing the ingredients);
- pasta preparation (automatically or manually measuring and mixing the pasta ingredients);
- laminating or extruding the pasta sheet;
- shaping the product (combining the pasta and filling);
- bulk pasteurisation (first product heat treatment);
- *incartamento* (surface pre-drying of the product);
- cooling;
- modified atmosphere packaging;
- optional pasteurisation (second packaged product heat treatment).

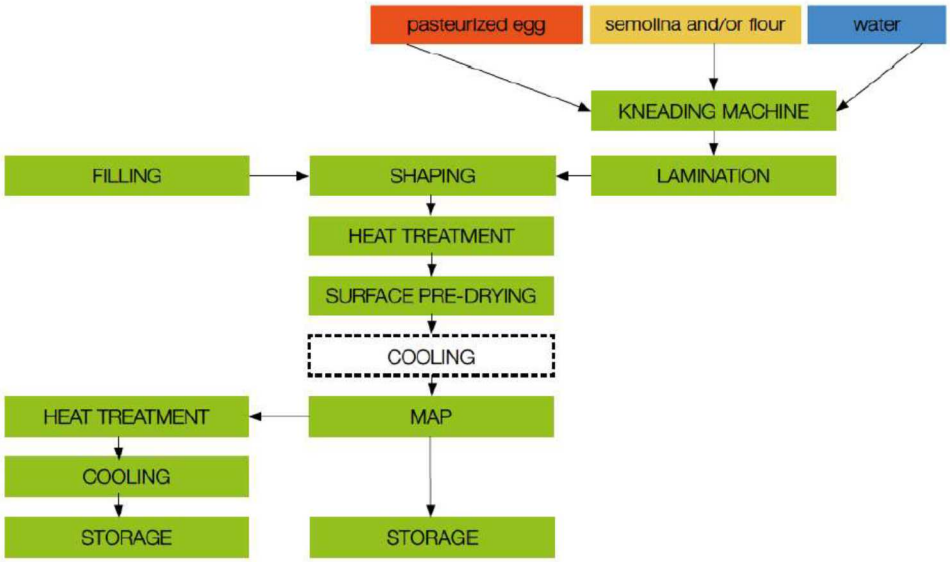

Figure 2-1: Fresh pasta manufacturing process (Zardetto S., 2014a).

2.2 Filling Production

Depending on the type of filling, several types of products are supplied, including cooked meats, ricotta cheese, fresh vegetables, cooked vegetables, matured cold cuts, cooked cold cuts, breadcrumbs, and vegetable fibers. Perishable filling ingredients are stored in cold rooms at a temperature below 4 °C, while non-perishable products are stored at room temperature. It is essential to locally prepare, cook, mix, and store different types of filling in separate containers, which must be stored in a cold room until use. Equipment must be thoroughly washed and stored at the end of production.

2.3 Dough Preparation

Pasta dough is made by mixing durum wheat semolina, and/or wheat flour, with pasteurised eggs and water. This operation uses stainless steel kneading machines. The semolina, eggs, and water are automatically fed into a mixer in precise proportions. The dough is then fed into sheeter and former machines. The formed products (e.g., cappelletti, tortelloni, and ravioli) are transferred to the pasteuriser belt for thermal treatment. Since the environment is a source of contamination, it is essential to implement hygiene interventions for equipment and in rooms. After mixing the ingredients and forming the dough mixture, the dough is laminated. A foil is fed into the forming machine to incorporate the filling (if the pasta is to be filled) or for pasteurisation and cutting (in the case of smooth pasta such as tagliatelle and fettuccine).
The foil may be laminated to the desired thickness (between 0.8 and 1 mm) by either extrusion or rolling (Figure 2-2).

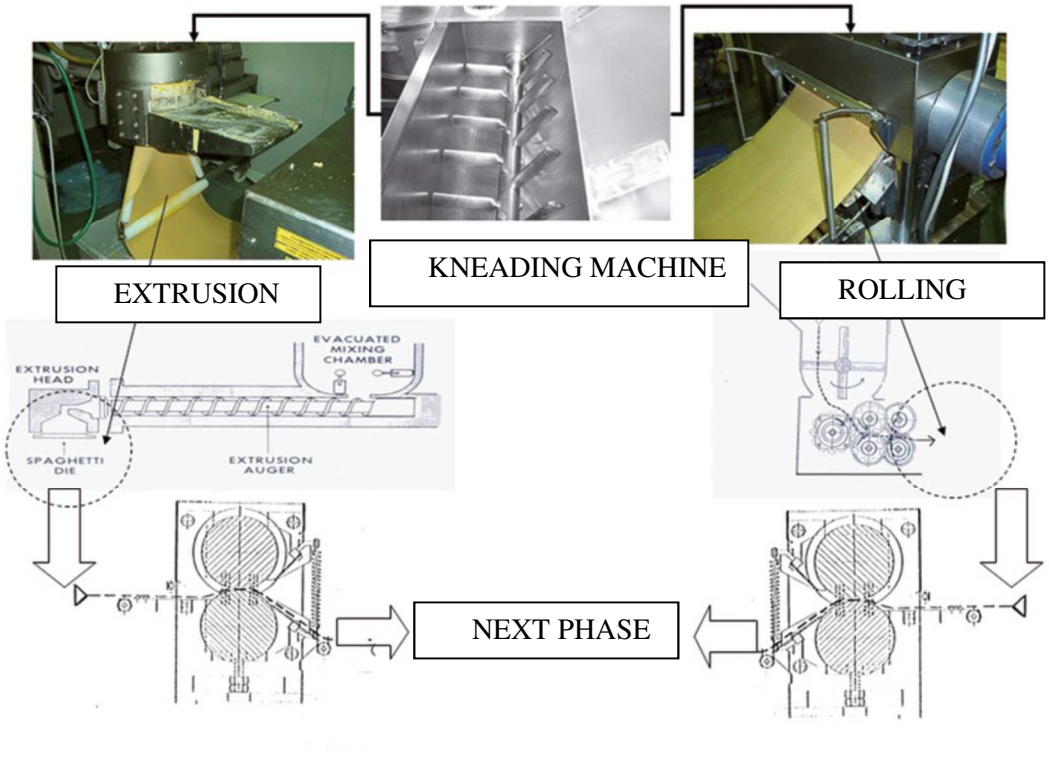

Figure 2-2: The lamination process.

The choice between the two lamination techniques fundamentally determines the quality and commodity value of the finished product. Therefore, it is important to evaluate the effects of the two processing methods on the pasta characteristics, before and after cooking. The choice between the two types of lamination only applies to 'smooth' pasta produced after cutting the pasteurised foil (i.e., long pasta such as tagliatelle, fettuccine, and tagliolini) and filled pasta. In the case of short pasta (e.g., fusilli, penne, and rigatoni) extrusion is the only viable option. Extrusion involves sending the dough into a chamber with a worm screw, at the end of which a diffusion head distributes the dough onto a die. The dough undergoes intense compression (depending on the system characteristics), and the extrusion process affects the final quality of the product by raising the pasta temperature and causing structural changes in the protein network (determined by the implemented operating methods).

In the case of the rolling mill or cylinder, the dough is transferred to a containment tank with a vane kneading shaft. The dough is then sent to a lamination group comprising two or three stainless steel, grooved rollers (malaxers) of different sizes. Afterwards, a pair of smooth surface cylinders compress the dough to form a laminate with a thickness of about 1 cm.

Due to their machine characteristics, the two processes transfer vastly different quantities of mechanical energy to the processed dough. The specific mechanical energy value indicates the amount of energy transferred to the product during the process, i.e., the amount of mechanical energy dissipated as heat inside the product expressed per unit of mass. This parameter, used in all processes, is significant because it determines the final product characteristics. Under usual operating conditions, pasta extrusion transfers approximately 60–70 kJ/kg to the product (Zardetto and Rosa, 2009).

The effects of the two lamination processes on product structure were confirmed in the study of Zardetto and Rosa of 2009 by examining mixtures with the same formulation and obtained under the same mixing conditions. The mixtures were separated into two groups: one was subjected to extrusion and the other to rolling. The samples were then analysed using FT-NIR (fourier transform near-infrared spectroscopic analysis). The results indicated a clear separation between the two groups along with the main component, with noticeable differences depending on the wavelengths. The analysis provided a macroscopic photograph of the changes induced by the two processes, illustrating their effects on several bonds in the gluten-starch-water matrix. In terms of the qualitative attributes perceived by the consumer, the variation results in differences in the level of starch gelation in the pasta, product texture, cooking performance, and colour (Zardetto and Rosa, 2009).

Although both types of products are commercially produced, little scientific data on the differences between them is available. Research into dried pasta suggests that extrusion causes the formation of a protein matrix with several discontinuities (Pagani et al., 1989). The qualities resulting in the best sheet-rolled pasta have also been identified (Pagani et al., 1989). This product has a more developed gluten network (Matsuo et al., 1978; Dexter et al., 1979) and is of better quality than extruded pasta.

2.4 Pasta Formation

Pasta formation is carried out using automatic machines into which the pasta sheet is fed and, in the case of filled pasta, the chosen filling. After rolling, the pasta sheet reaches the forming machine, which has a hopper into which the filling is poured. The hopper has a filling dosing system, and, through a series of high-speed operations, the machine receives the sheet from the rolling mill, cuts it to suitable dimensions, and extrudes the filling through a dispenser, portioning and placing it on the pastry. At this point, the pastry is closed, giving the product its final appearance.

In Italy, filled pasta has shapes that vary according to region and area. In Parma, for example, tortellini are called anolini, and ravioli are called tortelli. In Ferrara, Bolognese tortellini are called cappelletti, and in Mantua, they are known as agnolini. Outside its region, round or square-shaped *Piemontese* pot-bellied agnolotti may be known as ravioli, tortelli, and tortellini. Hence, it is virtually impossible to list all the names given to the various forms of pasta in the different Italian regions (particularly since the same pasta may be identified by distinct names within a few kilometres). The most common pasta varieties are listed below:

- Tortellini have a characteristic shape (considered to be modelled as an imitation of the navel of Venus) and are a specialty disputed between Bologna and Modena. They are the most widely known Italian filled pasta.
- Cappelletti and cappellacci, as the name suggests, are filled pasta shaped like a medieval male headdress. Typical of Emilia-Romagna Region, and of ancient origins, cappelletti are prepared using classic egg pasta dough and are traditionally served in capon or chicken broth.
- Ravioli refers to filled pasta in general, which may be prepared in different shapes such as square or rectangular (e.g., like tortelli and agnolotti), collected (e.g., cappellacci), crescent, and triangular. In some regions, the ravioli are filled with ricotta, with or without vegetables.

2.5 Thermal Treatment

Heat treatment is one of the most significant steps in food processing since the 19th century. In filled pasta manufacture, the product is subjected to two heat treatments. The first treatment is pasteurisation, which is performed immediately after forming and applied to the unpackaged product to destroy pathogenic microorganisms and inactivate degrading enzymes. However, it also has a secondary effect on the product's physical and chemical properties. The second treatment may be performed on the packaged product to prevent potential recontamination, which may occur following initial pasteurisation before packaging. The process may also be applied to increase the shelf life of the product. Single and double thermal pasteurisation have advantages and disadvantages, which are determined by manufacturing criteria and commercial policy (Zardetto and Dalla Rosa, 2015).

Industrial pasta processing comprises one or more heat treatments. Fresh pasta is first steam treated. The pasta is then transferred to another chamber for drying using hot air with a

moisture content of 30–32%. The product is then packaged in MAP. The second treatment, carried out on the packaged product, uses either microwave energy or hot air.

The heat treatment aims to maintain product hygiene and quality. The product is usually pasteurised to kill mould spores and some pathogenic or spoilage microorganisms. However, the thermal process also affects the quality of the pasta on a macromolecular level due to reciprocal interactions between proteins and starch. These interactions are observable in colour changes, decreased a_w, increased starch gelatinisation level, and changes in the quantity of water absorbed during cooking. Heat treatment may also reduce the nutritional value of the food due to the Maillard reaction, which makes amino compounds biologically unavailable.

Heat penetration in food is a physical process that depends on several factors, such as the characteristics of the product (in this case pasta thickness and moisture content) and the pasteurisation conditions, time, and temperature. Managing the process requires a sound knowledge of the key factors that regulate thermal processing conditions. This not only ensures safety but also obtains the desired quality parameters. From microbiological and sanitary perspectives, instability factors that render a product potentially at risk include its composition and relatively high pH and a_w values. The production technology, conditioning, and conservation methods used during processing make a product hygienically safe. Thermal treatment largely contributes to this safety aspect, leading to reduced microbial populations and influencing residual microbiological activity by decreasing a_w and altering the pasta structure. However, thermal treatment also produces several unwanted modifications.

The significance of heat treatment is determined by two contrasting requirements: reducing microbial populations to safe levels and maintaining the sensory and nutritional characteristics of the fresh product.

During thermal treatment, the food is partially cooked. This is particularly the case for pasta, in which thermal damage may cause yellowing and hardening.

Although these alterations are insignificant concerning health and hygiene, they detract from the idea of a fresh product. As such, safeguarding microbiological requirements must be reconciled with initial sensory characteristics.

Electing to use heat treatment depends on shelf life, which represents the time that the product may be displayed for sale and how well it is conserved after packaging.

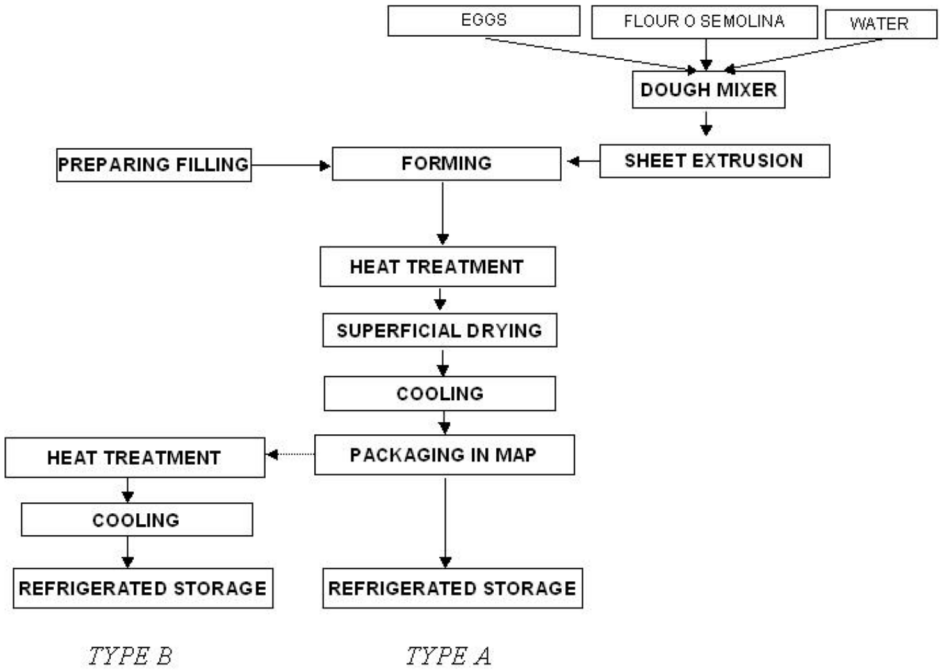

Figure 2-3: Flow chart demonstrating the production of two types of filled pasta subjected to different treatments (Zardetto and Dalla Rosa, 2007).

The adding and combining stages in the basic manufacture process should also be considered due to product diversification. The flow chart in Figure 2-3 demonstrates that a product may be subject to two thermal treatments. The first is always performed on the unpackaged product after sheeting-rolling or extruding the pasta. The second thermal treatment is sometimes carried out on the final product and requires a cooling stage before packing. It aims to eliminate possible recontamination following the first pasteurisation and subsequent packaging. There are several differences between simple and double thermal pasteurisation, both processes having advantages and disadvantages that are determined by manufacturing criteria.

Single thermal treatment (product type A in the flow chart) protects the sensory and nutritional properties of the foodstuff and its image as a fresh product. For this to be effective, however, it requires optimal management at each successive step following pasteurisation, with particular attention to environmental hygiene conditions downstream (high-risk areas). Therefore, only an environment containing filtered and sterilised air may be used. Double pasteurisation (product type B) provides more efficient thermal distribution and permits sterile conditions to be omitted in the downstream stages (although suitable hygienic standards must be observed). A second thermal treatment prolongs the product's shelf life up to 90 days or

more. However, it also results in a loss of sensorial characteristics and significantly increases manufacturing costs.
In both cases, it is critically important to control heat transfer and temperature changes over time. This not only determines the process duration but also temperature distribution. Moreover, changes in the physical and chemical properties of the product are dependent on a time-temperature relationship (Zardetto and Dalla Rosa, 2015).

2.5.1 Thermisation and Pasteurisation

During the initial pasta pasteurisation treatment, two distinct types of equipment are available. In both machines, a belt is used to introduce the loose product into a chamber in which steam is generated from a tank containing boiling water, located either under the belt or outside the plant. The steam is injected directly into the product via tubes placed above and below the belt. In the second pasteurisation treatment, hot air is applied to the packaged product in a spiral oven or static cell, or by using a microwave. Several authors have reported that, in industrial environments, the pasteurisation of loosely filled pasta is sufficient to destroy the vegetative cells of pathogenic microorganisms (Lopez et al., 1998).

2.5.2 Partial Drying

Partial drying is an in-line process conducted on a conveyor-belt dryer. The product leaves the pasteuriser and is collected by a conveyor belt that passes through a machine that allows the superficial drying of the product using air at a temperature of about 70 °C.

2.5.3 Cooling

Cooling takes place in an in-line refrigerated tunnel. The partially dried product is collected on a conveyor belt that passes through the tunnel. The conveyor belt speed is regulated so that the output product has a core temperature of about 9 °C. The belt speed is determined according to the product size.

2.6 Packaging

Packaging is carried out using machines that are fed by the product leaving the cooling tunnel and is usually MAP (Modified Atmosphere Packaging) using a mix of premixed gases.

Most food packaging is manufactured using petroleum-based non-biodegradable polymers, and their disposal is becoming a serious environmental issue. Partially replacing these materials with biodegradable polymers from renewable sources (i.e., biopolymers) can reduce the environmental impact of packaging materials (De Camargo Andrade-Molina et al., 2013).

2.6.1 Modified Atmosphere Packaging

Modified atmosphere packaging substitutes the ambient air in a package with another gas (commonly a mixture of carbon dioxide and nitrogen). It is used in combination with other extrinsic factors to control the growth and toxin production of different moulds. The two major gases used in commercial MAP are nitrogen (N_2) and carbon dioxide (CO_2). Carbon dioxide is the most significant gas in the mixture (Zardetto, 2005). The concentration of CO_2 used in filled fresh pasta varies from 25% to 40% depending on the type of product. An N_2 concentration of over 70% may prevent packages from collapsing due to CO_2 absorption by filled fresh pasta. It is possible to vary the residual oxygen (O_2) concentration in the package by applying this technology, although it is usually less than 2%. A O_2 concentration of 1% or less completely depresses the germination, growth, and sporulation of most moulds. MAP calls for the substitution of air inside the package with other gases (CO_2 and N_2, with O_2 residue < 1-2%) and is used in combination with other environmental factors to inhibit the growth of microorganisms and their production of toxins. Carbon dioxide influences microbial growth by lengthening the lag phase (L) and increasing generation time (μ). Its action is strongly influenced by its concentration level and the product preservation temperature. The inhibitory effect of carbon dioxide on growth parameters increases as the temperature decreases. Figure 2-4 demonstrates the effect of temperature on the speed of the mould growth, causing alteration, found in filled fresh pasta.

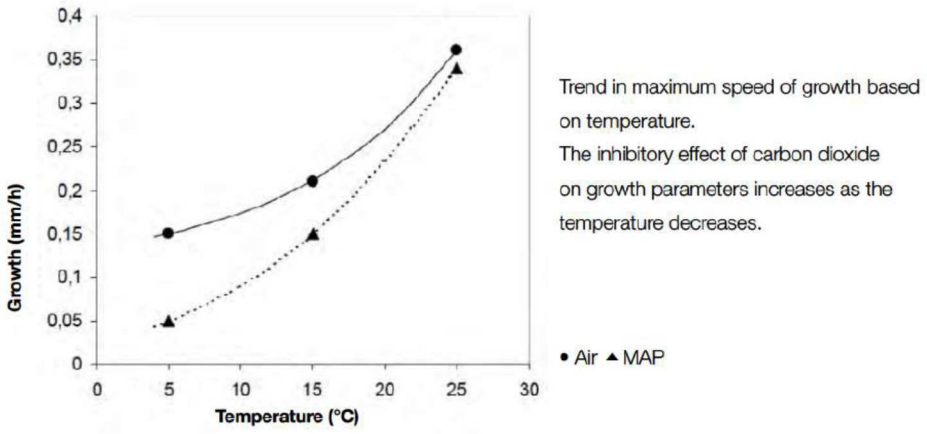

Figure 2-4: The effects of temperature on the inhibitory action of carbon dioxide on mould growth altering packaged filled fresh pasta (Zardetto, 2004).

As previously noted, the inhibitory effect of carbon dioxide increases as the temperature decreases, with the difference in the speed of growth between MAP and open-air samples that increases as the temperature decreases. Therefore, the best results are obtained by employing the synergic effect of MAP with low preservation temperatures (Zardetto, 2005). The carbon dioxide concentration level also influences generation time, particularly under excessive temperatures. Figure 2-5 demonstrates that at a constant temperature (15 °C), the speed of microorganism growth is influenced by the carbon dioxide percentage. As the figure indicates, the growth speed decreases as the carbon dioxide percentage increases, up to a concentration level of 70%, making it possible to completely inhibit microorganisms while preventing product alteration, including under excessive heat conditions (Zardetto, 2005).

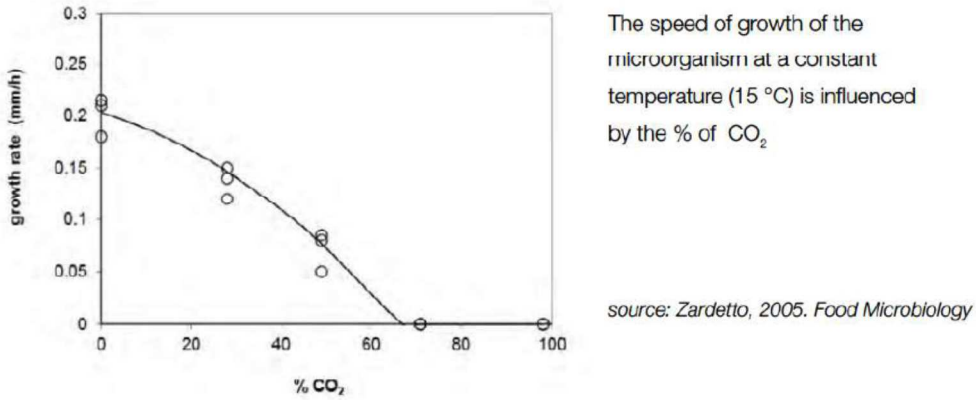

Figure 2-5: The effects of carbon dioxide on the speed of microorganism growth at a constant temperature.

2.6.2 Vacuum Packaging

Although MAP and controlled atmosphere packaging (CAP) largely operate at ambient pressure (101 kPa), storage at reduced atmospheric pressures has been investigated and, in some cases, used for bulk storage (e.g., in the hypobaric storage systems designed by Stanley Burg almost a quarter of a century ago). In the Burg system, produce is stored under atmospheric pressure in the range of 1–10 kPa at refrigerated temperatures. At this low pressure, fresh air saturated with water (RH 80%–100%), is constantly circulated.

Vacuum packaging (VP) is considered a specific type of MAP since part of the usual headspace is removed, leaving an altered initial atmosphere that is not controlled after packaging. VP applies pressure to a product and is only suitable when the product is sufficiently durable. Using a VP system (termed a moderate VP system because it operates at

40 kPa) at 8°C has significantly extended the shelf life of a range of minimally processed fruits and vegetables (Rahman., 2007).

2.6.3 Additional Packaging Techniques

Several studies have shown that antimicrobial agents, such as organic acids, potassium sorbate, bacteriocins, thiosulfinates, enzymes, proteins, antibiotics, fungicides, chelating agents, and metals, may be added to edible films to reduce the growth of microorganisms (Cha and Chinnan, 2004).

The use of that agents must be follow the rules of the Regulation (EC) no. 10/2011 of European Union and the release of substances from food contact materials and articles should not bring about unacceptable changes in the composition of the food. According to good manufacturing practice it is feasible to manufacture plastic materials in such a way that they are not releasing more than 10 mg of substances per 1 dm^2 of surface area of the plastic material.

CHAPTER 3
MICROBIOLOGICAL CHARACTERISTICS OF FRESH PASTA

3.1 The Regulatory Framework

In Italy, fresh pasta is regulated by Presidential Decree no. 187, which sets out a series of requisites for the product to be defined as fresh pasta:

- the product must have an a_w level of less than 0.97;
- it must have undergone heat treatment at least equivalent to pasteurization;
- it must be preserved at a maximum temperature of 6 °C from the point of production to sale.

Although the health and safety objectives of the Decree are clear, the laws regarding fresh egg pasta are less transparent. This type of product is regulated by Law no. 580 (04/07/1967) and subsequent amendments, which establish that pasta containing egg must be sold as *pasta all'uovo* (egg pasta) and produced using at least four chicken eggs with a total weight of at least 200g (without the shell) per kilogram of semolina. Minimum egg content is verified through the gravimetric dosing of sterols, expressed as digitonides, and the fat content of the ethereal extract (Ministerial Decree, 23/07/1994). Egg pasta characteristics were later modified in Presidential Decree no. 41 (05/03/2013) which reduced the ethereal extract and sterol content to 2.50g and 0.130g respectively, expressed as 100 parts of dry matter.

From a microbiological perspective, fresh egg pasta production follows the Hygiene Package requirements and those set by Regulation (EC) no. 178/2002 as part of the EU legislation on food safety. Effective from 1st January 2006, the Hygiene Package comprised four legislative acts:

- Regulation (EC) no. 852/2004

- Regulation (EC) no. 853/2004
- Regulation (EC) no. 854/2004
- Regulation (EC) no. 882/2004

At the present time, Regulations (EC) no. 854/2004 and 882/2004 are no longer in force and have been replaced by Regulation (EU) 2017/625.

Regulation (EC) no. 852/2004 of the European Parliament and the Council of 29th April 2004, on the hygiene of foodstuffs, establishes that food businesses are responsible for food safety, whether produced, processed, and/or distributed. In addition, food business operators must implement and maintain procedures based on Hazard Analysis and Critical Control Points (HACCP) principles. Since microbiological criteria also guide the acceptability of foodstuffs and hygiene procedures during their manufacture and distribution, they should form an integral part of implementing HACCP procedures and other hygiene control measures. This concept was applied in Regulation (EC) no. 2073/2005 which provides microbiological criteria for some food-borne bacteria, microbial toxins, and metabolites in certain foods and food processes.

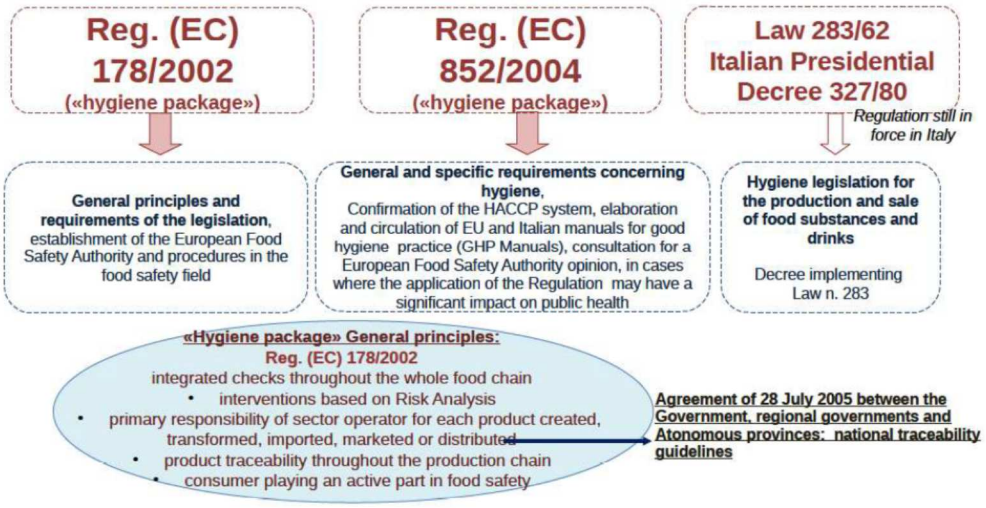

Figure 3-1: European food safety and hygiene regulations (Pastaria International 4/2014).

3.2 Chemical and Microbiological Characteristics

Extensive literature discusses microbiological problems associated with fresh pasta (Zardetto, Stefano Di Fresco, 2000a); 1987; Aureli et al., 1993; Dalla Rosa et al., 1993). The risk associated with pathogenic microorganisms (notably *Staphylococcus aureus, Salmonella spp., Clostridium spp., Bacillus cereus,* and *Listeria monocytogenes*) has been traced to

ingredients used to prepare pasta and fillings and to hygiene conditions in the production environment. According to the literature, *Staphylococcus* and *Salmonella spp*. are the only two microbiological agents to date, which have been associated with food poisoning linked to pasta consumption (Aureli et al., 1993). One case concerned dry pasta contaminated with *Staphylococcus* toxins; the other case involved a preparation based on lasagna (Patano et al., 1994).

From a scientific perspective, the starchy nature of pasta presents problems associated with food or animal products, including eggs (used in dough), meat, and/or milk ingredients. High pH and a_w values are instability factors, rendering a product potentially risky in terms of microbiological and hygiene factors. Production technology, conditioning, and conservation techniques make the product hygienically safe. Thermal treatment contributes to food safety by reducing microbial populations and residual microbiological activity through decreased a_w and altered pasta structure. However, thermal treatment also produces several unwanted modifications (Zardetto and Dalla Rosa, 2007).

Parameter	Limit	Method
Total Mesophilic Aerobic Bacteria	<105 CFU/g	ISO 4833
Enterocacteriaceae	$<10^2$ CFU/g	ISO 21528-2
Molds	$<10^2$ CFU/g	ISO 21527-1
Yeasts	$<10^3$ CFU/g	ISO 21527-2
Escherichia coli	$<10^2$ CFU/g	ISO 16649-2
Staphylococcus aureus	$<10^2$ CFU/g	ISO 6888-1
Salmonella spp	Absent in 25g	ISO 6579
Clostridium perfrigens	$<10^2$ CFU/g	ISO7937
Listeria monocytogenes	$<10^2$ CFU/g	ISO 11290

Figure 3-2: The microbiological limits for fresh pasta and durum wheat flour (Scioscia E. et al, 2016)

3.2.1 Humidity and Water Activity

Pasta products may be classified according to their moisture content (in dried and fresh pasta) and the ingredients in pasta made from durum wheat semolina, special pasta, and filled pasta (Figure 3-3). In Italian dried pasta, the moisture content should not exceed 12.5%. Conversely, fresh pasta should have a moisture content of at least 24% and a_w values of at least 0.92 and no greater than 0.97. Several stabilised pasta have a moisture content of at least 20% and an a_w value no greater than 0.92 (Ricci, Barone and Petrella, 2017).

	DRIED PASTA	FRESH PASTA
INGREDIENTS	ONLY *DURUM WEAT SEMOLINA*	IT'S POSSIBLE ALSO WEAT
UMIDITY	< 12.5%	PRODUCT LOOSE: **THERE ISN'T A RULE** FOR PACKING PRODUCT: **UMIDITY ≥24% aw< 0.97**
CONSERVATION-SHELF LIFE	3 years	PRODUCT LOOSE: Temperature 4°C max 5 days FOR PACKING PRODUCT: Temperature 4°C **THERE ISN'T A RULE** FOR MAXIMUM SHELF LIFE
TECNOLOGY PROCESS	**EXTRUSION** FORMING UNDER PRESSURE (drawing) STABILIZATION: **DRYING**	FORMING: general **LAMINATION** STABILIZATION: **PASTEURIZATION**

Figure 3-3: The differences between fresh and dry pasta according to Italian legislation and from a technological standpoint.

Water is an essential component for living beings and a crucial element in foods. In the middle of the previous century, scientists discovered a relationship between the water content in certain foods and their propensity for deterioration. This led to the discovery that Aw is more significant in food stability than food water content. Between 1953 and 1957, Scott WJ identified a significant correlation between the a_w of a medium and deteriorating food stability caused by microorganism growth (Scott, W. J., 1953). These and many additional studies have made it possible to establish rules for setting limits on food stability using the Aw parameter rather than food water content. Consequently, subsequent research into food degradation has focused on this parameter (Rahman, 2007). Water activity affects not only microorganism growth and metabolism but also determines the chemical and physical characteristics of foods since it supports the biochemical and enzymatic reactions that determine a product's sensory properties and shelf life.

Therefore, a_w must be considered a significant factor in processing food products; the Aw value for fresh foods has been set at around 0.970 and 0.996 by Chirife and Fontan (1982). To regulate a_w, it is vital to consider the ability of different solutes to reduce the amount of free water in foodstuffs, particularly when selecting ingredients and additives for food production.

It is also essential to understand the properties of food packaging materials since these may influence a_w through moisture transfer from the food to the environment and vice versa (Rahman MS, 2007). Combined with additional parameters a_w contributes to a category of intrinsic food factors. Water activity is a thermodynamic parameter, and its values range from 0.000–1.000. It is dimensionless and does not coincide with humidity. However, a correlation between the relative humidity of food and a_w allows these two independent parameters to be placed in a reciprocal relationship using isotherm absorption. Isotherm absorption is specific for each food and calculated at a precise temperature; it may be presented as a graph or equation. Braunauer et al. (1940) classed isotherm absorption into five types (Figure 3-4), demonstrating that the presence of water-soluble crystalline components (such as sugar and salt) in foods resulted in an isotherm with a concave appearance (Figure 3-4 III), while for most foods, the isotherm has a sigmoidal form (Figure 3-4 II).

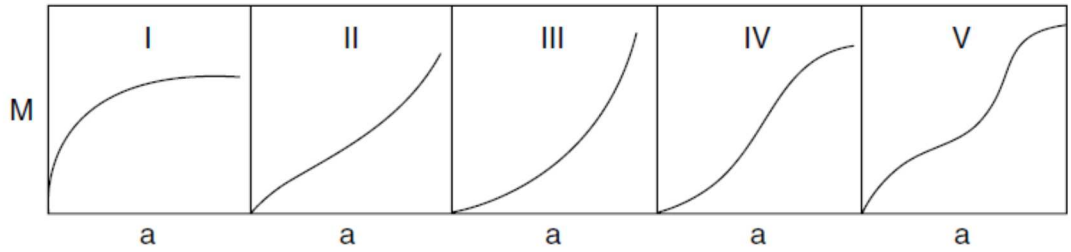

Figure 3-4: The five types of absorption isotherm proposed by Braunauer et al.; in the axes (a) represents water activity while (M) is humidity (Rahman MS, 2007).

The isotherm inflection point is indicated by a change in the ability to bind water or by the relative amounts of free and bound water. For practical purposes, isotherms are presented as equations in empirical or theoretical models. As such, in the existing literature, no isotherm model is valid for the entire range of Aw values in food. The Gugghenheim-Anderson-de Boer (GAB) model is most widely used for its a_w values, which range from 0.10 to 0.90. Another important parameter is hysteresis, which represents the difference in moisture content at the equilibrium between the adsorption and desorption curves (Figure 3-5). In this image, three regions may be identified. The first region represents foods that have a high sugar content or are air dried; here hysteresis largely occurs in the water monolayer below the isotherm's inflection point. In the second region, the water is less tightly held and typically contained in small capillaries. In the third region, the water is dissolved and is contained in large capillaries or free.

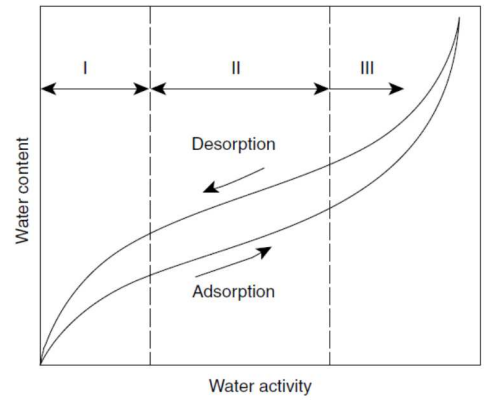

Figure 3-5: A typical isotherm of food product absorption that indicates hysteresis (Rahman MS, 2007).

The practical implications of hysteresis in microbiological and chemical food deterioration are significant in the low and intermediate humidity categories. Consequently, Strasser (1969) and Wolf et al. (1972) have argued that this parameter could be used as an empirical index of food quality deterioration (Rahman MS, 2007).

Another factor to consider is the temperature-dependent nature of a_w, which may be expressed using a modified form of the Clausius-Clapeyron equation:

$$\mathbf{ln} = \frac{\mathbf{aw1}}{\mathbf{aw2}} = \frac{\Delta \mathrm{H}}{\mathrm{R}} \left[\left[\frac{1}{T2} - \frac{1}{T1}\right]\right]$$

Where ΔH is the net isosteric absorption heat corresponding to the food moisture content (J mol $^{-}$1), that is the heat necessary to remove a certain amount of water. As the amount of water in the food decreases, this value increases because it nears the bound water region and therefore requires significant amounts of energy to remove additional water from the mass. The gas constant is R, and T1 and T2 are temperatures expressed in kelvins. There is a linear relationship between a_w and 1/T. The slope of the line is equivalent to ΔH/R and owing to this relationship, it is possible, from the previous formula, to trace a_w values at different temperatures, given the values of a_{w1}, T1, and T2 and the angular coefficient ΔH/R, using the following formula:

$$\mathbf{a_{w2} = a_{w1}\, exp} \frac{\mathbf{\Delta H}}{\mathbf{R}} \left[\left[\frac{\mathbf{1}}{\mathbf{T2}} - \frac{\mathbf{1}}{\mathbf{T1}}\right]\right]$$

Typical examples of variation in a_w caused by temperature, with a constant moisture content, are given as isotherms (Figure 3-6). The a_w change caused by temperature is largely due to the modification of water bonds, the dissociation and physical state of the water, and the increased solubility of solutes. An increase in temperature causes a decrease in the humidity contained

in the equilibrium (Figure 3-6a). The presence of water at the curve intersection, or inversion point, is noted for different temperatures, and this factor depends on the food composition and solubility of the sugars (Figure 3-6b) (Rahman MS, 2007).

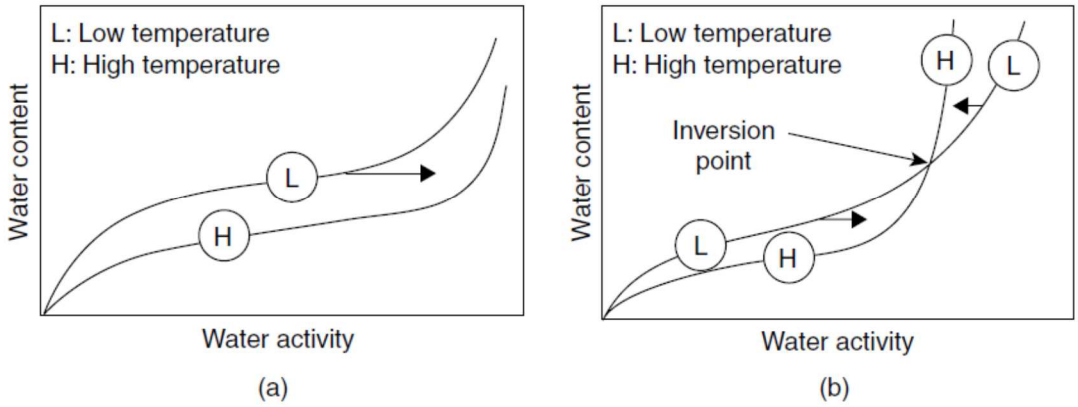

Figure 3-6: The change in water activity in food due to temperature (Rahman MS, 2007).

Therefore, if a_w depends on temperature, humidity absorption will also be dependent upon temperature. Hence, for a given humidity value, the following statements will apply:

a) a_w will increase as temperature increases, according to the Clausius-Clapeyron equation; and

b) for a given a_w value, the quantity of water that can be retained decreases as the temperature increases, according to the Clausius-Clapeyron equation.

One of the main factors affecting the shelf life of fresh pasta is the amount of free water (a_w). The a_w is related to its potential chemical and is defined as pressure relative vapour, that is, as the ratio between the water vapour pressure in the head space over the sample and the water vapour pressure in the headspace above the water at the same temperature.

In the case of a multiphasic food system, characterised by the presence of two or more distinct regions for a different one water concentration (such as fresh stuffed pasta), two o'clock regions (stuffing and pasta) will go meeting, over time, changes in moisture content due of the migration of water from the region to major chemical potential towards the one it presents a lower value until it is reached of balance. The migration of moisture between the different regions is influenced by several thermodynamic factors. The driving force behind water migration is represented by the value of the difference in water assets between the regions involved, once connected to the difference in chemical potential. The driving force of water migration in such complex systems is the potential chemical difference between the two phases.

For fresh-filled pasta, like all food products, a change in humidity produces the following changes:

• a change the product consistency;

• an increase or decrease in microbial growth;

• modified organoleptic levels.

As demonstrated in Figure 3-7, at different asset values, water corresponds to different trigger points related to food degradation processes.

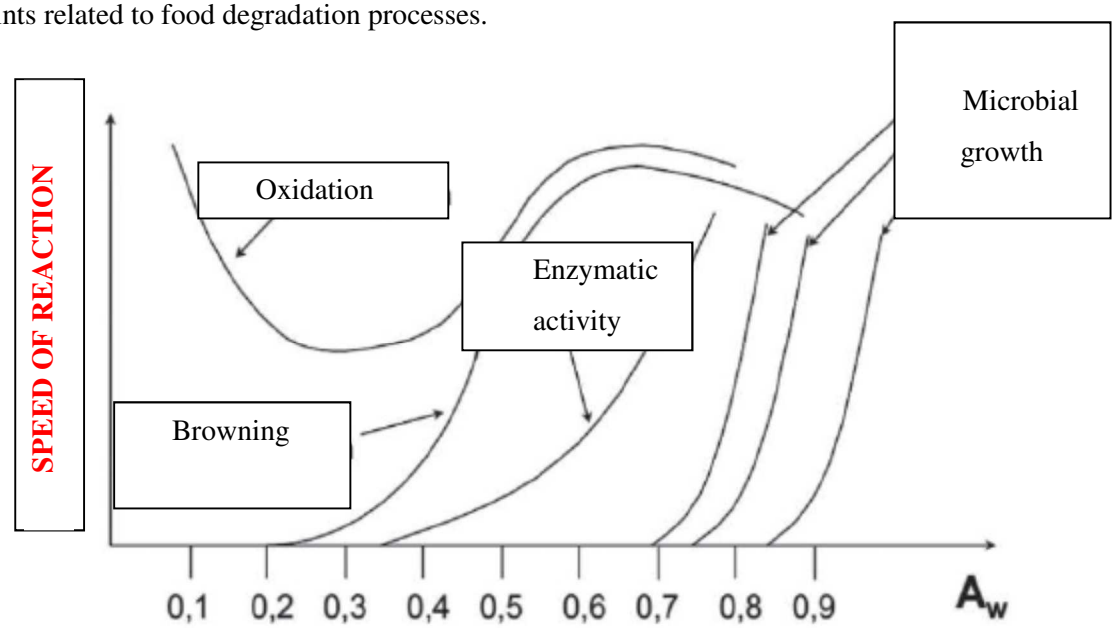

Figure 3-7: The rate of oxidation, browning, enzymatic activity, and microbial growth related to free water content (www.attivitadellacqua.it).

Microorganisms develop at a_w values above 0.80. It is important to consider the following microbial features of each species:

• its optimal a_w range;

• the a_w required for minimum growth.

3.2.2 pH Value

The level of acid in food is a controlling factor in microorganism growth. A pH < 4.5 in a food, stored at temperatures less than or equal to 8°C is sufficient to inhibit the growth of non-proteolytic *C. botulinum*. The pH value of several multicomponent foods may vary within the product due to diffusion and mixing limitations. If the pH level is a factor for controlling safety, a pH of 5.0 or below should be achieved throughout the food and its components. This should be monitored for every production batch, which must be defined by the food business operator. Batch size is a key consideration in risk management. Acidified foods containing meat, fats or oils are notoriously difficult to acidify uniformly and extra care should be taken with these foods (Callaghan, 2008).

3.2.3 Escherichia coli

Escherichia coli is a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the *Escherichia* genus that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Although *E. coli* strains are harmless, several serotypes may cause serious food poisoning in their hosts and are occasionally responsible for food contamination incidents that prompt product recalls. The harmless strains are part of the gut microbiota and can benefit their hosts by producing vitamin K2 (which aids blood clotting) and preventing the colonisation of pathogenic bacteria in the intestine. Hence, they have a symbiotic relationship with their host. *E. coli* is expelled into the environment in faecal matter. The bacterium grows exponentially in fresh faecal matter under aerobic conditions for three days. However, its numbers decline slowly afterwards. Facultative anaerobes such as *E. coli* constitute approximately 0.1% of gut microbiota, and faecal-oral transmission is the major route through which pathogenic strains of the bacterium spread disease. Since the cells may survive outside the body for a limited period, they are potential indicator organisms for testing faecal contamination in environmental samples. A growing body of research, however, has examined environmentally persistent *E. coli* strains that can grow outside a host and survive for many days. *E. coli* is grown and cultured easily and inexpensively in a laboratory and has been intensively investigated for over 60 years. The bacterium is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy. Moreover, *E. coli* is the most widely studied prokaryotic model organism, and a valuable species in biotechnological and microbiological research, as the host organism for the majority of work using recombinant DNA. Under favourable conditions, *E. coli* reproduction takes as little as 20 minutes. Optimum growth occurs at 37 °C (98.6 °F) and may be driven by aerobic or anaerobic respiration, using a large selection of redox pairs. These include the oxidation of pyruvic acid, formic acid, hydrogen, and amino acids, and the reduction of substrates such as oxygen, nitrate, fumarate, dimethyl sulfoxide, and trimethylamine N-oxide. Classified as a facultative anaerobe, *E. coli* uses oxygen when it is present and available. It can, however, continue to grow in the absence of oxygen using fermentation or anaerobic respiration. The ability to continue growing in the absence of oxygen is advantageous for bacteria since their survival increases in environments where water predominates.

It has been previously demonstrated that a temperature of 12 °C is sufficient for significant *E. coli* proliferation inside packaging, even from limited initial contamination (100 UFC per gram) (Awuah, Ramaswamy and Economides, 2007).

3.2.4 Staphylococcus aureus

Staphylococcus aureus is a gram-positive, facultatively aerobic, spherical bacterium. Its cells are not heat resistant and easily destroyed by mild heat treatment. The microorganism does not compete well in mixed populations. However, when other naturally occurring bacteria are destroyed by cooking or inhibited from growth, *S. aureus* introduced into humans survives and grows. It is resistant to low Aw and survives curing solutions that contain salt or sugar. The optimal growth conditions for *S. aureus* are a temperature range of 4–46 °C (for growth and toxin production, with the optimum temperature being 37 °C), a pH range of 4.8–8.0, a lowest reported a_w value for growth of 0.86, salt tolerance of 10–20%, a sugar tolerance of 50–60%, and nitrite tolerance. Since it belongs to a group of facultative anaerobes, it grows in pasta that is both fresh and loose and packaged in a protective atmosphere. *S. aureus* is usually present in local inflammations (e.g., boils and wounds), common in nasal mucus, and even in the hair of healthy individuals. The toxin produced by the bacterium (enterotoxin A) is stable to heat and able to withstand the typical cooking times for dry and fresh pasta. The latter is particularly vulnerable since it has exceptionally thin sheets that require brief cooking times. A temperature of 12 °C is sufficient to reduce *S. aureus* growth. Nevertheless, the law relating to pasta production prescribes a maximum refrigeration temperature of 4 °C, at which the metabolic activity of the bacterium is completely inhibited.

3.2.5 Listeria monocytogenes

Listeria monocytogenes is a gram-positive, rod-shaped bacterium that thrives in anaerobic and microaerophilic (a microaerophile is a microorganism that requires oxygen to survive, but requires environments containing lower levels of oxygen than that are present in the atmosphere) conditions. There are 13 serovars of pathogenic *L. monocytogenes*, which are ubiquitous, can survive for extended periods under adverse conditions, and multiply in foods stored at refrigeration temperatures (the risk may increase during storage). The optimal growth conditions for *L. monocytogenes* are a temperature range of 30–37 °C and a pH range of 4.5–9.6.

L. monocytogenes has been found in raw milk, raw milk cheese, soft-ripened cheeses, raw meats, and seafood. There have also been cases of illness from coleslaw and other raw vegetables fertilised with animal manure or wastewater and inadequately and cleaned before preparation and eating.

The literature concerning *L. monocytogenes* in pasta is scarce and conflicting. Caserio et al. (1989) reported the presence of such microorganisms in fresh-filled, meat-based, pasta samples. While an investigation of 52 filled pasta samples (Foti and Vezzano, 1991) found the microorganism to be absent.

3.2.6 Clostridium botulinum

Clostridium botulinum is a gram-negative, rod-shaped, spore-forming, anaerobic bacterium that forms a neurotoxin. Seven toxin types are known and designated as I, II, III, IV, V, VI, and VII. The toxin is heat-labile and can be inactivated by heating at 80 °C for 10–15 minutes. Heat resistance, measured as D-values for *C. botulinum* type I and proteolytic type II spores, generally ranges from 0.6 to 3.0 minutes at 110 °C. *C. botulinum* type V spores are considerably less resistant than type I or II spores and may be inactivated at or below 100 °C. Growth conditions include a temperature range of 3–48 °C (10–50 °C for types I and II, 3–45 °C for type V), while the optimal temperature for toxin development is 35 °C. The optimal pH range is 4.6–8.9, the lowest reported a_w value for growth is 0.95, and growth is inhibited by NaCl (8%) and nitrite.

C. botulinum strains are divided into four groups based on their physiological characteristics. Of these, groups III and IV have little relevance to cases of food botulism. In terms of group I, which includes proteolytic strains of the microorganism, storage temperature appears to have a significant role in preserving filled fresh pasta. Studies of fresh pasta products have revealed that even under prolonged excessive heat (12°C), toxin proliferation by this microorganism does not occur, instead a temperature of 20°C proved to be efficacious for reducing storage periods and the results were influenced by the type of filling (Glass and Doyle, 1991). Group II includes non-proteolytic strains that are more sensitive to heat and reduced a_w, which (unlike proteolytic strains) multiply and produce toxins at refrigerated temperatures up to 3.3 °C. Risk control for this group of microorganisms includes the elimination of spores in raw ingredients and/or inhibiting germination and growth in those that survive heat treatment.

C. botulinum growth and botulinic toxin generation have been identified by several authors (Del Torre et al., 1998; Glass and Doyle, 1991) as the main risk associated with the production and consumption of fresh-filled pasta. To date, no reported cases have been linked to the consumption of such products. Nevertheless, the possible presence of spores in the filling ingredients renders filled pasta a potential source of risk for such food toxins. In addition to the presence of *C. botulinum*, the main risk occurs during the storage stages when products may be subject to a more or less prolonged rise in temperature (Zardetto and Dalla Rosa, 2007).

3.2.7 Salmonella spp.

Gram-negative, non-spore-forming rods, 2,200 serovars of *Salmonella enterica* subsp. *enterica* exist as natural contaminants of intestinal tracts in animals, birds, and reptiles; *Salmonella* serovar Senftenberg is considered to be the most resistant strain. Growth conditions include a temperature range of 6–49.5 °C (optimum 37 °C), a pH range of 4.1–9.0 (optimum 6.7–7.5), and a lowest reported a_w for growth of 0.93.

Vehicles for the bacterium include inadequate cooking or processing, improper cooling, ingesting raw products, and cross-contamination after heating or cooking: meats and poultry (i.e., beef, pork, turkey, and chicken), raw or improperly pasteurised milk and eggs, homemade ice cream (containing raw eggs), water, fish, shellfish, feeds, fruits, vegetables, and salads.

Salmonellosis symptoms include stomach pain, diarrhoea, nausea, chills, fever, and headache. The infectious dose for this microorganism can be exceptionally low (15–20 cells) depending on the age and health of the host and the *Salmonella* serovars. However, a single cell may cause a person to become ill. Hence, the inactivation of this pathogen through processing and the avoidance of post-processing contamination is critical.

The risk of *Salmonella* contamination is always traceable to raw ingredients, particularly refrigerated fresh eggs, and fresh meat. *Salmonella* spp. are generally absent from marketed fresh pasta (Zardetto and Dalla Rosa, 2007).

Fresh pasta should be consumed after boiling in water for 1 to 10 minutes, depending on the thickness of the pasta. Figure 3-8 provides the survival rates of *S. enteritidis* injected at a concentration of 10^6 UFC/g into fresh pasta with a meat filling.

Time (seconds)	Presence
30	+/+
60	-/-
90	-/-
300	-/-

+ = presence in 25 g of product - initial inoculum 10^6 UFC/g
source: Zardetto et al., 2000, Tecnica Molitoria

Figure 3-8:* Salmonella enteritidis *survival in domestically cooked filled fresh pasta (Zardetto and Di fesco, 2000).

As Figure 3-8 demonstrates, the microorganism was found only when the product was cooked for 30 seconds. The cooking time generally recommended for this type of product (between 2 and 5 minutes) is therefore sufficient to eliminate a *S. enteritidis* concentration of up to 6 log UFC/g (Zardetto and Di fesco, 2000).

3.2.8 Bacillus cereus

Bacillus cereus is a gram-positive, facultatively anaerobic, spore-forming bacterium that is widespread in nature and foods, particularly in its spore state. Its growth conditions include a temperature range of 10–49 °C (optimum 30 °C), a pH range of 4.9– 9.3, and a lowest reported a_w for growth of 0.93.

B. cereus produces two types of illness: diarrheal syndrome, which develops within 20 hours following ingestion, and the emetic (vomiting) response, which occurs 1 to 5 hours after ingestion. The illnesses are caused by toxins associated with the growth of the microorganism in foods (emetic) or the gastrointestinal tract (diarrheal). The diarrheal toxin (enterotoxin) emerges during exponential growth in the gastrointestinal tract, while the emetic toxin (stable under heat and with a pH range of 2–11) is produced by cells growing in the food product. Large numbers (exceeding 10^5 CFU/g) of viable *B. cereus* cells must be consumed for symptoms to develop.

A short-incubation form of *B. cereus* food poisoning is associated with cooked food rich in starch, such as rice, pasta, and potato (Muscolino et al., 2014*).*

3.2.9 Clostridium perfringens

Clostridium perfringens is a spore-forming, anaerobic, pathogenic bacterium. In addition to its invasive factors, which can cause serious trauma infections, it is hazardous because it produces toxins that cause food-borne infections. *C. perfringens* is inhibited at low temperatures. Therefore, the refrigeration of a packaged product in a protective atmosphere is an effective system for inactivating the microorganism and its toxin production. The bacterium largely contaminates meat products, particularly fillings with high a_w, heated for cooking and slowly cooled (Del Torre et al., 2004).

3.2.10 Yeasts and moulds

The deterioration of fresh pasta products is frequently caused by moulds and yeasts. Several types of mould, particularly *Penicillium*, develop slowly at a temperature below 8 °C and alter the product when carbon dioxide levels are insufficient. Since yeasts are microaerophilic microorganisms, they can alter product acidification in the presence of excess heat. The primary process of product decay is observed in organoleptic modifications during storage. Although these modifications do not cause abnormal changes in taste or smell, they do produce

a continuous decrease in the gustative characteristics of the product, up to complete loss of taste.

CHAPTER 4
TRADITIONAL AND EMERGING TECHNOLOGIES FOR THE CONSERVATION OF FRESH PASTA

4.1 The Shelf-Life Determination of Fresh Pasta

Food preservation has been practised since ancient times. The preservation process inhibits the development of microbes such as bacteria and fungi. As such, food preservation has several aims: 1) to maintain food texture, flavour, quality, and nutritional value, 2) to reduce excess food wastage, 3) to maintain product accessibility for an extended period (including in places where it is not produced), 4) to preserve food ingredients during transportation, and 5) to ease the handling of food materials.

Several methods are used to preserve food, including conventional methods and modern preservation technology. Conventional methods such as drying have improved over time to accommodate a vast food industry. Several food preservation methods are discussed in the following sections (Sharif and Mustapha, 2017).

4.2 The Use of Food Preservatives

A food preservative is an additive used to preserve food by lowering its pH value and settling its redox potential. Moreover, preservatives hinder microbial development to prevent food spoilage. Food preservatives are divided into Class I and Class II. Class I refers to natural preservatives while Class II comprises artificial or chemical preservatives. In food products, however, only one type of Class II preservative should be used because their over-consumption can be harmful. Examples include sulphites, benzoates, and sorbates. The classification of both classes of preservatives is presented in Figure 4-1.

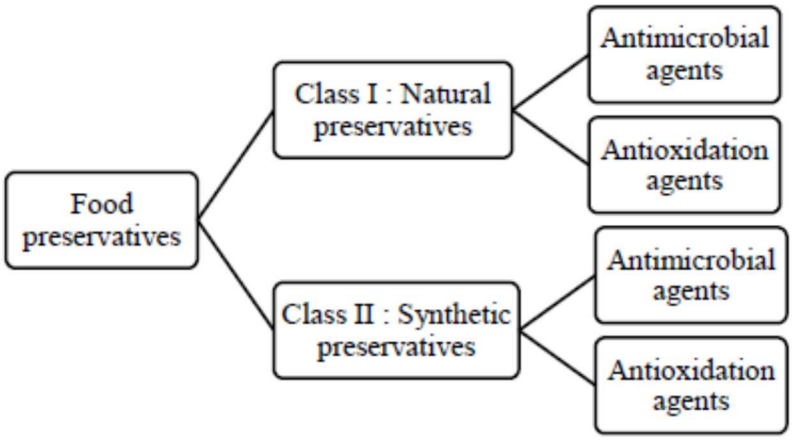

Figure 4-1: Different categories of food preservatives (Rahman MS, 2007).

The use of preservatives has several advantages and disadvantages. The advantages include maintaining food consistency and nutritional value, protecting the food from microbial activity, and enhancing its flavour. Conversely, their over-consumption can cause health problems, such as asthma, kidney failure, and cancer (Sharif and Mustapha, 2017).

According to Italian Ministerial Decree no. 209 (27/02/1996), no type of colouring is permitted in fresh pasta (art. 6, par .2, Appendix IV), although the following additives may be used:

- Acidity regulators: lactic acid (E270), citric acid (E330), tartaric acid (E334), and glucono-delta-lactone (E575) (art. 15, par. 1, Appendix IV, M.D. no. 209, 02/1996). They must be used according to good manufacturing practice, in amounts that do not exceed those necessary to attain the desired objective, and with the provision that they are not used to deceive the consumer.
- Preservatives: sorbic acid (E200), potassium sorbate (E202), and calcium sorbate (E203) are permitted in filled dry and fresh pasta and in packaged gnocchi in amounts of 1g per kg of filling or product (gnocchi) (art. 15, par. 1, Appendix IV, M.D. no. 209, 02/1996).
- Antioxidants: ascorbic (E300) and sodium ascorbate (E301) (art. 15, par. 1, Appendix IV, M.D. no. 209, 02/1996). They must be used according to good manufacturing practice, in amounts that do not exceed those necessary to attain the established purpose, and with the provision that they are not used to deceive the consumer.
- Emulsifiers: mono and diglycerides of fatty acids (E471) and lecithin (E322).

4.2.1 Natural Antimicrobials Agents

As defined by the US Food and Drug Administration (FDA) antimicrobial agents are substances used to preserve food by inhibiting the development of microorganisms and product deterioration. Nature is highly potent against food spoilage microorganism and food-borne pathogens, and the most valuable natural antimicrobials agents are categorised as follows:

- Antimicrobial agents of plant origin: plants are a source of natural antimicrobial agents because they have many valuable bioactive, antimicrobial compounds. In food preservation, they are in high demand by consumers who have become aware of the effects of using synthetic preservatives. Moreover, compounds derived from natural sources, such as phenols, terpenes, and alkaloids, are typically present in many or all parts of the plant. High levels of secondary metabolite protect plants from predators and microbial pathogens by their microbially opposing properties. Among the largest groups in secondary metabolite compounds are the phenolic and polyphenolic groups. Several sub-group compounds inhibit microbial activity. These are flavonoids, quinones, coumarins, phenolic acids, tannins, phenols, flavones, and flavanols. Phenol compounds include the hydroxyl (-OH) group. The sites and numbers of phenol groups in the compound are identified by their relative harmfulness to microorganisms. A higher number of hydroxyl groups represents a higher level of toxicity. Currently, more than 1,340 plants have antimicrobial properties and over 30,000 antimicrobial compounds have been extracted from plants. The US FDA states that numerous essential oils have been recorded as generally recognised as safe (GRAS) and may be used in food preservatives. Essential oils are the most significant phytochemicals used. They have volatile properties and sweet-smelling substances with oily consistencies that are typically formed by plants. Essential oils are extracted from different parts of plants such as flowers, seeds, and leaves. Several methods are used for their extraction, including distillation and supercritical fluid. The oxygenated terpenoids (e.g., alcohol and phenolic terpenes) function as antimicrobial agents in essential oils. However, several hydrocarbons have properties that exhibit microbial activity, including aliphatic, monoterpene, and sesquiterpene hydrocarbons. Herbs and spices have been used for a considerable period for various purposes including as antimicrobial agents.

Extracting essential oils from plants, spices, and herbs requires high vapour pressure and can reach the microbes during the liquid and gas phases. Bassolé et. al (2012) demonstrated the inhibition of *E. coli* and *S. enteritidis* using a standard broth dilution technique with a two-fold dilution of mint essential oil. *E. coli* and *S. enteritidis* development was prevented using the edible coating that contains mint essential oil. A higher concentration of mint essential oil resulted in lower microbial activity. Marin et al. (2016) revealed that anise oil contains several active compounds, such as trans-anethole, α-trans-bergamotene, and limonene, that inhibit bacteria including *S.* Typhimurium, *S. aureus*, and *Vibrio parahaemolyticus*. These microbes are usually found in fish products. Moreover, anise oil may also hinder spore germination. Marin et al. (2016) also reported that cinnamon and clove oils contain several active compounds such as cinnamaldehyde, eugenol, and linalool. Hence, both oils may inhibit microbial development.

- Antimicrobial agents of animal origin: animals are a source of antimicrobial agents that are safe for consumption. Chitosan, for example, has been widely used in food industries and is the polycationic biopolymer commonly found in the exoskeletons of crustaceans such as crabs or lobsters. The use of chitosan in food preservation is limited because it is insoluble in neutral conditions and has a high pH value. Currently, chitosan is incorporated in edible coatings and films, which helps reduce water vapour content, prevent oxygen transmission, and extend the shelf life of fruit. Hence, it prevents food deterioration. Besides chitosan, lysozyme present in eggs and milk is also used as an antimicrobial of animal origin and has been recognised as safe (GRAS). The lysozyme enzyme present in eggs is commonly applied as an antimicrobial agent and preservative for poultry products, meat, and fruits. Lysozyme is widely known for its commercial use in preventing late blowing in semi-hard cheese, caused by *Clostridium tyrobutyricum*. Lysozyme typically inhibits gram-positive bacteria but not gram-negative bacteria. This is due to the lipopolysaccharide layer present on the surface of the cell membrane. Lysozyme's antimicrobial properties are derived from its ability to hydrolyse the ß-1.4 linkage between N-acetylmuramic acids and N-acetyl glucosamine at the microbial cell wall. Murdock et. al (2007) reported that lactoferrin is one of the natural antimicrobial agents found in mammalian secretion such as saliva, milk, and tears. It is also considered to be one of the strongest antimicrobial agents in

milk. Lactoferrin limits the amount of iron in the surrounding environment, Thereby hindering bacteria cell development and freeing liposaccharides from the outer membrane of gram-negative bacteria, causing and membrane distortion. Lactoferrin has also been proved to inhibit microbial activity in *E. coli* and *L. monocytogenes.*

- Antimicrobial agents of microbial origin: several compounds produced by bacteria are effective against other bacteria. These active bacteria hinder and prevent microbial development that causes food spoilage. A protein compound known as bacteriocin acts as an antimicrobial agent, preventing spoilage and pathogenic microbes. Both gram-positive and gram-negative bacteria produce bacteriocins. These proteinaceous compounds permeate the cytoplasm membrane and cause intracellular metabolite leakage. Besides bacteriocins few active bacteria effectively inhibit spoilage microbial growth (examples include reuterin and pediosin). Bacteriocins are produced from lactic acid bacteria (LAB) such as *Lactobacillus acidophilus*, due to their metabolic activity. Moreover, bacteriocins inhibit food-borne pathogens such as *C. botulinum*, *Enterococcus faecalis*, and *L. monocytogenes*. Furthermore, bacteriocins are safe for use as bio-preservatives because they are degraded by protease. Bacteriocins are commonly divided into four classes depending on their chemical and genetic properties. Nisin is the most common bacteriocin listed among European food additives and by the FDA. It is widely used in cheese and sausage production. Nisin is formed from *Lactococcus lactis* and consists of 34 amino acids, including lanthionine, dehydroalanine, and aminobutyric acid. Nisin inhibits several gram-positive bacteria. However, it is ineffective against gram-negative bacteria because of its inability to penetrate the cell wall. Due to the ionic interaction of the C-terminus, nisin attaches to the microbial cell membrane and forms a spore. Consequently, cellular material is released, and the motive force is interrupted. Nisin should be incorporated with chelators such as ethylenediaminetetraacetic (EDTA) and is effective for use in cheese production by inhibiting *S. aureus* contained in raw milk (Sharif and Mustapha, 2017). After packaging, the shelf life of filled pasta depends on the ability of the microorganisms surviving the thermal treatments to grow during storage, overcoming the hurdles determined by a_w, MAP composition, and storage temperature (Sanguinetti et al., 2011).

The use of bioprotective cultures is an interesting strategy proposed with the aim of reducing the risks associated with the growth of undesirable and pathogenic microorganisms and prolonging the shelf life of foods (Oliveira et al., 2018). Bio-preservation uses natural and selected microflora, which control or inhibit spoiling or pathogenic microorganism growth through competition or by producing specific antimicrobial molecules such as bacteriocins, organic acids, diacetyl, and acetoin. (Ghanbari et al., 2013). Lactic acid bacteria (LAB) are ideal candidates for bio-preservation due to their history of safe use in foods and their production of a wide range of antimicrobial compounds (Cifuentes Bachmann and Leroy, 2015). Since a potential drawback in using bioprotective cultures is potentially undesirable effects on the food's organoleptic profile, LAB species choice must consider a low or compatible impact on food flavour.

Tabanelli et al. (2020) evaluated the effects using bioprotective cultures on microbiological patterns in ricotta-based tortelloni during production and storage. Filled pasta was produced in a small factory following a traditional recipe. In addition to traditional microbiological protocols, microbial community profiling was performed through rDNA-targeted pyrosequencing to test the effects of two bioprotective cultures (*Lactobacillus rhamnosus* or a mixture of *L. rhamnosus* and *Lactobacillus paracasei*) on spoilage microbiota during filled pasta storage. Furthermore, the influence of the cultures on the organoleptic profile of the product was studied. Finally, a validation trial was undertaken to optimise the strategy for stabilising the filled pasta. The addition of bioprotective cultures to the fresh pasta filling had a relevant quantitative and qualitative effect on the bacterial microbiota of the product during the storage. Even if the added cultures were never dominant at the end of the shelf life, their presence and/or activity during the overnight incubation of the filling was sufficient to reduce the initial microbiota associated with raw ingredients and could drive microbial community evolution towards a predominantly safer or more organoleptically acceptable species, such as leuconostocs. Although the presence of LAB cultures had several significant effects on the aroma profile of filled pasta, this was attributed to the presence of molecules (such as acetoin and diacetyl) that were compatible with ricotta or cheese-based fillings in tortelloni. The use of such cultures allowed the application of milder thermal treatments to better maintain the traditional textural and flavour

characteristics of fresh-filled pasta combined with a higher a_w needed to preserve the softness of the filling (Tabanelli et al., 2020).

A further study by Schettino et al. (2020) discussed the extension of fresh pasta shelf life using chickpea flour fermented with selected LAB. The use of microorganisms and/or their metabolites to prevent spoilage and extend shelf life is gaining interest as a bio-preservation approach. Lactic acid bacteria are considered a valuable tool due to their ability to synthesise and release several antimicrobial and antifungal compounds from the matrix. Synergistic activities between different compounds synthesised or released during fermentation, such as organic acids and peptides, may influence the overall antifungal effect. Although conventional processes of pasta production do not include a fermentation step, novel recipes including LAB-fermented ingredients, aimed at enhancing the nutritional and functional properties of such products, have recently been proposed, and products exhibiting new sensorial profiles have expanded in the market. In this case, chickpea flour was fermented with selected LAB and used to fortify fresh semolina pasta. The fortification effect on the microbiological shelf life of the experimental pasta was investigated, and a mixture of peptides responsible for antifungal activity was purified and identified. To assess the effect of the fermented chickpea flour on key features of the experimental pasta, an integrated characterisation approach, including technological, nutritional, and sensory investigations, was applied. The study successfully combined a potential antifungal ingredient (chickpea) and fermentation with selected LAB to extend the shelf life of fresh pasta. Besides a high dietary fibre and protein content, nutritional improvements were achieved in fresh pasta. Moreover, the proteolysis operated from both endogenous proteases (activated by the bio-acidification), and the bacterial specific pool of peptidases, led to the release of peptides with high antifungal potential, contributing to an extended shelf life in the fortified fresh pasta compared to pasta containing a chemical preservative (Schettino et al., 2020).

4.2.2 Natural Antioxidation Agents

Antioxidant agents are commonly used to extend food shelf life by preventing oxidative rancidity, degradation, and food colour changes. Natural antioxidants that act as free radical scavengers should consist of phenolic compounds, vitamin C, and vitamin E. Problems usually faced by the seller include the presence of undesirable melanosis on the surface of fresh-cut fruits and vegetables, which change their appearance. This is due to surface reactions, during

which two types of enzymes, polyphenol oxidase (PPO) and peroxidase (POD), act as catalysts. Initially, hydroxylation occurs slowly, changing the monophenol compounds to diphenols. In the second reaction, oxidation of the diphenols to quinines occurs, resulting in browning the fruit or vegetable surface. These reactions are withheld only when the products are surrounded by oxygen. The handling process is one of the factors that lead to the beginning of a melanosis reaction and subsequent food deterioration. Conversely, poultry, meat, and fish face the problem of lipid oxidation, which largely occurs due to the oxidation of myoglobin species and haemoglobin in fish muscle. The antioxidant compounds are divided as:

1. Antioxidation agents of plant origin: plants are a source of antioxidants due to the presence of several active compounds usually found in spices, citrus pulp, peel, and oil seeds. For example, black pepper, turmeric, and garlic hinder the antioxidant properties in distinct food systems. Most spices have antioxidant abilities due to the presence of active compounds such as lignans, flavonoids, polyphenols, and terpenoids. In recent years, essential oils have become a popular choice for antioxidants in food preservation due to the presence of antioxidant compounds, which hindering or postpone the chain reaction in lipid oxidation in poultry, meat, and fish. The presence of phenolic compounds in most essential oils accounts for their antioxidant properties and is the main reason for their use in food preservation. Nugboon and Intarapichet (2015) reported four types of Thai culinary herbs, tested for their antioxidant properties, used in freeze-drying pork meatball batter stored in vacuum packaging at 4 °C. Holy basil and green peppercorn produced a longer shelf life comparable to turmeric and Vietnamese coriander. However, all the meatballs tested with Thai herbs had an extended shelf life compared to the control meat balls which lasted less than 6 days. A study conducted by Supapvanich et al. (2012), revealed that a concentration ratio of 1:1 of pineapple fruit core extract is effective to delay the browning process compared to pulp and peel extract (Sharif and Mustapha, 2017).

   Aromatic herbs are an excellent option due to their high antioxidant power and natural origin. Rosemary extract (*Rosmarinus officinalis L.*) is one of the most valuable, is currently the only herb approved by European legislation. The herb may also exert an antimicrobial effect and is considered a clean label ingredient (i.e., clear, clean, and understandable in food labelling). Research conducted by Ainsa et al. (2021) aimed to enrich pasta using fish by-products to increase nutritional value, evaluate polyunsaturated fatty acid stability during frozen storage, and maintain the necessary

proportions and state throughout its commercial shelf life. Sea bass (*Dicentrarchus labrax*) trimmings are considered a by-product of the filleting process. Regarding cereal source, semolina from durum wheat (*Triticum durum*) and spelt (*Triticum spelta*) were used. The study revealed that the pasta enriched with sea bass by-products included an excellent source of protein, fibre (in the case of spelt pasta, due to the presence of bran), and Ω-3 type polyunsaturated fatty acids. The pasta developed using fish concentrate demonstrated fat stability and effective protection against unsaturated fatty acid oxidation during the commercial shelf life (the study highlighted the ALA conversion in EPA and DHA in 90 days), particularly in samples with additional rosemary extract. Unsaturated fatty acids, notably EPA and DHA, remained in satisfactory quantities during the developed pasta's commercial shelf life and during frozen storage. Finally, the sensory profiles of the sea bass enriched pasta were largely adequate, and improved with the addition of a rosemary extract, regarding a decrease in negative attributes regarded associated with rancidity (Ainsa et al., 2021).

2. Antioxidation agents of animal origin: potential sources of natural food preservative compounds have been discovered in several types of natural materials. Antioxidant agents of animal origin are commonly used for their free radical scavenging, bleaching inhibition and reducing power. These abilities have been tested in honey, which contains active antioxidant compounds such as phenolic acids, vitamins, and enzymes. Most research into the oxidative activities of chitosan indicates that it can delay lipid oxidation and inhibit reactive oxygen species in biological systems and foods. Chitosan acts as an antioxidant by scavenging free radicals from hydrogen donations or lone pairs of electrons. In chitosan, compounds belonging to hydroxyl (-OH) and amino groups (-NH) are fundamental in the antioxidant process. However, these are difficult to break down due to the semi-crystalline structure of chitosan with its strong hydrogen bonds (Sharif and Mustapha, 2017).

Previous literature appears to demonstrate that pasta production and cooking may increase or decrease phenolic acid content and its antioxidant activity (Fares et al., 2010). Processing may affect the levels of free and bound phenolic acids in pasta along with their bioavailability and subsequent physiological effects. Therefore, to achieve the maximum benefits from the food, it is critical to understand its nutrient and bioactive composition as well as the effects of the food formula, processing, and cooking on the composition and bioavailability of these beneficial components. Fares et al. (2010) examined the effects of formulation, pasta making (e.g., extrusion and drying), and cooking on individual phenolic

acids in fresh, dried, and cooked barley spaghetti. The impacts of pasta making and the drying process on phenolic acid composition and the scavenging capacity of free radicals were investigated. Total and individual phenolic acid content was strongly correlated and contributed to the free radical scavenging capacity of pasta. The addition of barley flour into pasta, at all incorporation levels increased the phenolic acid and total phenolic content, demonstrating the antioxidant potential and promoted possible dietary benefits, such as the prevention of chronic diseases related to oxidative stress. Pasta processing did not significantly affect the total phenolic content and free radical scavenging capacity. However, it did modify the phenolic acid content of pasta. A significant reduction in total phenolic acid content after extrusion may have been due to oxidising reactions trigged by water, oxygen, and heat. Dried pasta revealed a phenolic acid content higher than that of the corresponding fresh pasta. Cooking did not affect total phenolic acid content, leading to the conservation of free and bound phenolic compounds. Based on this, different processing technologies have been found to produce varying effects. Therefore, the choice of cereal formula and the technological process is crucial in preserving phenolic acids and their anticipated health-promoting properties.

4.2.3 The Use of Sterile Ohmic Fillings

Ohmic heating, also known as Joule heating, electric resistance heating, direct electric resistance heating, electroheating, and electroconductive heating, passes an alternating electric current through food material. Heat is generated internally due to resistance to the applied electrical current. In conventional heating, heat is transferred from a heated surface to the product interior by convection and conduction and is time-consuming, particularly in longer conduction or convection paths used in the heating process. Electroresistive, or ohmic heating, is volumetric in nature and therefore has the potential to reduce overprocessing by its inside-outside heat transfer pattern. Ohmic heating is not an innovative technology; it was used as a commercial process in the early twentieth century for milk pasteurisation. However, the electropure process was discontinued between the late 1930s and 1960s, ostensibly because of the prohibitive cost of electricity and a lack of suitable electrode materials. Interest in ohmic heating was rekindled in the 1980s when investigators were searching for viable methods of sterilising liquid-large particle mixtures, a scenario for which aseptic processing alone was unsatisfactory.

Ohmic heating is applied to a wide range of foods, including liquids, solids, and fluid-solid mixtures. Commercially, ohmic heating is used to produce liquid egg products in the United

States. It is also used in the United Kingdom and Japan to process whole fruits such as strawberries. Additionally, ohmic heating has been successfully applied in laboratories to a wide variety of foods, including fruits and vegetables, juices, sauces, stews, meats, seafood, pasta, and soups. In 1997, 19 plants using ohmic heating technology operated worldwide. Widespread commercial adoption of ohmic heating in the United States is dependent on regulatory approval by the FDA, a scenario that requires a full understanding of the ohmic heating process regarding heat transfer (temperature distributions), mass transfer (concentration distributions, which are influenced by electricity), momentum transfer (fluid flow), and kinetic phenomena (thermal and possibly electrothermal death kinetics, and nutrient degradation).

Ohmic heating devices consist of electrodes, a power source, and a means of confining the food sample (e.g., a tube or vessel). Appropriate instrumentation, safety features, and connections to other process operation units (e.g., pumps, heat exchangers, and holding tubes) are also crucial. Ohmic heaters may be static (batched) or continuous. Figure 4-2 is a schematic of a static ohmic heating process. Important design considerations include electrode configuration (current flows across product flow path or parallel to product flow path), the distance between electrodes, electrolysis (metal dissolution of electrodes, particularly at low frequencies), heater geometry, alternating current frequency, power requirements, current density, applied voltage, and product velocity and velocity profile. Additional food system factors used in an ohmic heater include the product type and its properties, particularly electrical conductivity and heating rate; other properties include solid percentage, acidity, product viscosity, specific heat, solid density, and solid particle size, shape, and orientation to the electric field.

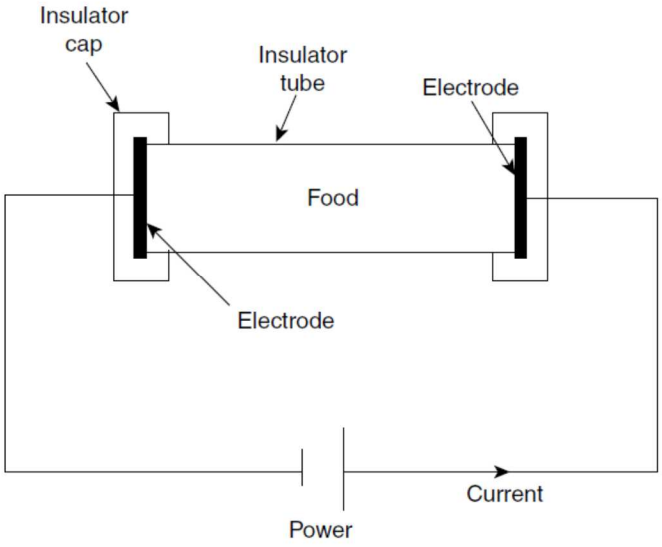

Figure 4-2: A schematic of the ohmic heating process (Rahman MS, 2007).

Ohmic treatment is one of the main innovations in continuous-flow sterilisation of food products. This technology uses the electrical conductivity of food to generate heat, resulting in a rapid rise in product temperature. In addition to preserving food characteristics, through the rapid increase in temperature and the lack of a temperature gradient within the product, ohmic treatment is an inexpensive technology and therefore does not significantly affect production costs. The Italian project named 'Use of a sterile and long-life filling in the production of fresh-filled pasta' made by *Porto Conte Ricerche* Scientific and technological park in Sardinia, aims to apply sterile fillings to traditional Sardinian pasta made with potato and sheep's milk ricotta fillings. The research has demonstrated that the cost of implementing an ohmic plant for the sterilising fillings is particularly onerous, even though the final effect on the product may significantly benefit the shelf life of the product.

In terms of microbial death kinetics, the question concerning whether electricity results in microbial death or if microbial death is solely caused by heat treatment, has received considerable attention. The challenge in modelling microbial death kinetics is the precise matching of time-temperature histories between ohmic and conventional processes. The FDA has published a comprehensive review of microbial death kinetic data regarding ohmic heating. Initial studies in this area have produced mixed results, judging the experimental details to be insufficient to draw meaningful conclusions. Research comparing death kinetics in yeast cells under almost identical time-temperature histories has revealed no difference between conventional and ohmic heating. More recent work in this area has indicated that decimal reduction times in *Bacillus subtilis* spores were decreased significantly reduced when using ohmic heating at identical temperatures (Cho et al., 1999). The researchers also applied

a two-step treatment process using ohmic heating, followed by holding and heat treatment, which accelerated microbial death kinetics; they hypothesised that electroporation may positively influence microbial death kinetics. The inactivation of yeast cells in phosphate, buffered by low-amperage direct current electrical treatment and conventional heating at isothermal temperatures, was also examined. The researchers observed a synergistic effect between temperature and electrolysis when the temperature became lethal for yeast. Further research into microbial death kinetics, survivor counts after treatment, and the influence of electricity on cell death kinetics is necessary to address regulatory issues. At present, the assumption that microbial death is only a function of temperature (heat) results in an appropriately conservative design (Rahman., 2007).

More studies are needed to validate the application of ohmic heating to fresh pasta production. In particular, quantifying the effects of the electrical field on mass transfer properties could optimise promising ohmic heating applications, including drying, extraction, blanching, fermentation, evaporation, and starch gelatinisation.

4.2.4 The Use of Natural By-Products

A study by Lemes et al. (2012) analysed *Spirulina platensis* in wheat flour used to prepared fresh pasta to evaluate the green colour and nutritional enrichment in addition to functional properties due to the presence of the bioactive compounds found in the cyanobacterium. The pasta was evaluated for centesimal composition, microbiological contamination, sensorial acceptance, and technological characteristics such as cooking time, water absorption, volume displacement, and the loss of solids. The superior protein content and the satisfactory technological and sensorial attributes, compared with the control which contained no cyanobacterium, showed the benefits of incorporating *S. platensis biomass* in the fresh pasta. Conventional sources of chlorophyll are spinach and alfalfa, which contain 0.5% (w/v) chlorophyll. However, *S. platensis* may be an alternative source. In this study, an examination of the application of *S. platensis* biomass was undertaken by preparing fresh pasta with integral and special wheat flour, eggs, salts, and different percentages of *S. platensis* biomass, aiming for nutritional enrichment, green colour, and technological properties. The microbiological quality complied with the legislation in force. The sensorial quality was considered satisfactory (Lemes et al., 2012).

Cankurtaran and Bilgiçli (2019) investigated several quality properties in filled and unfilled fresh pasta prepared with wheat milling by-products. The use of wheat bran and wheat germ in fresh pasta enhanced the chemical and nutritional properties. Moreover, using a filling

material provided a significant increase in ash, protein, fat, and more specifically calcium and phosphorus content. Conversely, phytic acid content increased up to fivefold in the presence of wheat milling by-products. The results of this study showed that the highest level of wheat bran and wheat germ revealed a maximum improvement in the nutritional properties of fresh pasta. However, high ratios of wheat bran and wheat germ adversely affected the sensory quality, cooking loss, and colour values of pasta formulations (Cankurtaran and Bilgiçli, 2019).

4.3 Thermal Treatment Methods

The industrial processing of pasta comprises one or more heat treatments. The fresh pasta is first treated with steam. The product is then transferred to another chamber, in which the pasta is dried with hot air to a moisture content of 30–32%. The product is then packaged in MAP. The second treatment, which uses microwave energy or hot air, is carried out on the packaged product. The heat treatment aims to maintain product hygiene and quality. As a part of the processing procedure, the product is usually pasteurised to kill mould spores and spoilage microorganisms. This thermal process, however, affects the quality of the pasta on a macromolecular level due to reciprocal interactions between proteins and starch. These interactions can be detected by colour changes, a decrease in a_w, an increase in the starch gelatinisation level, and a change in the quantity of water absorbed in the cooking phase. The heat treatment can also lead to a reduction in the nutritional value of the food due to the Maillard reaction, which makes amino compounds biologically unavailable. The penetration of heat into food is a physical process that depends on several factors, such as the characteristics of the product (in this case the thickness and the moisture of the pasta), and the pasteurisation conditions (i.e., time and temperature). The control of the process requires a sound knowledge of the key factors. These factors assure the control of the thermal processing conditions, which is necessary not only to ensure safety but also to obtain the desired quality parameters.

From a scientific perspective, the starchy quality of pasta presents problems due to products associated with foods of animal origin, including eggs, as used in dough, meat and/or milk ingredients. The instability factors that render the product potentially at risk, in terms of microbiological activity and hygiene, include the composition, relatively high pH, and high a_w values. The production technology, conditioning, and conservation are used to make the

product safe. The thermal treatment phase of production contributes to this safety aspect. This leads to a reduction in microbial populations and influences residual microbiological activity due to reduced a_w and the structure conferred to the pasta. However, thermal treatment also leads to some unwanted modifications.

The importance of heat treatment is determined by the needs of two contrasting requirements: reducing the microbial population to levels of safety while maintaining sensorial and nutritional characteristics of the fresh product. During the thermal treatment, a partial cooking process of the food occurs, especially in the case of pasta where yellowing and hardening take place due to thermal damage. Although these alterations are not significant concerning safety and hygiene, they detract from the idea of a pure or fresh product and must be reconciled with the microbiological requirements as well as safeguarding the initial sensorial characteristics. The selection of heat treatment depends on the shelf life, which represents the presumed time that the product can be maintained on display and then for sale, and on how well it is conserved after packaging.

4.3.1 Pasteurisation

Pasteurisers used for fresh pasta treatment should be able to reach temperatures high enough for the desired reduction of bacterial load at the centre (core) of the product (in the case of filled pasta, it is the core of the filling) and to maintain such temperatures for the stipulated time. This operation does not present a particularly complex problem but requires adequate devices, supported by a knowledge of how to effectively manage the significant variables. The pasteurisation of unpacked product uses overheated vapour, since wet heat is more efficient than dry heat for killing microbes. The final temperature varies between 85 and 99 ºC. The effective treatment time at this thermal level varies. However, it is normally no longer than 3–8 minutes. These times do not account for the thermal inertia of the system, so the total duration of the treatment would be greater than the indicated value. Heat propagation within the piece occurs by conduction, while the saturated vapour triggers an external mechanism of forced convection, and the relationship between the temperature increase with time is strongly influenced by the chemical and physico-chemical characteristics of the product (such as initial temperature, moisture, piece dimensions and stuffing elements). The product (at room temperature) is introduced after the forming phase by a conveyor belt, the velocity of which can be regulated, allowing for different treatment times inside the vapour pasteurisation tunnel. Vapour emission is regulated by vents located over the product and under the net. The

product leaving the moist heat treatment area is subjected to forced ventilation with hot air to partially dry it.

The pasteurisation of the packed product is terminated by heating the final product (without exceeding temperatures of 95–97 ºC to prevent water boiling inside the product) for a variable time depending on the technology employed. In this case, the process can be discontinuous (in the autoclave or pasteurisation area) or continuous (in a hot area tunnel or microwave combined with forced ventilation). The primary objective of a correct pasteurisation process is to obtain a temperature-time curve displaying a rapid rise to minimise the time spent at low temperatures, which would be inefficient from a sanitary position. The assessment of the thermal treatment performed with different times and temperatures is expressed by the value $F_{70/10}$ for each treatment. This parameter indicates the treatment time (in minutes) at a reference temperature (70 ºC) and allows a comparison of treatments performed in different establishments. For the calculation values of $F_{70/10}$ (pasteurisation effect) the Bigelow method is used:

$$F_{i=\int_{t1}^{t2} 10^{\frac{T-70^\circ C}{Z}} dt} \quad [1]$$

The experimental determination of the time-temperature profile at the centre of the product is performed using a data logger with a penetration probe and involves assessing the heat penetration curve of the temperature at each time (t) interval. From this value F_i the values defined by formula [1] are calculated.

Figure 4-3 gives an example of temperature evolution at the centre of a product and in the pasteurisation chamber. Data is assessed during the pasteurisation of a product with the agnolotto shape. The temperature of the product evolves to reach the value of the vapour in the pasteurisation chamber.

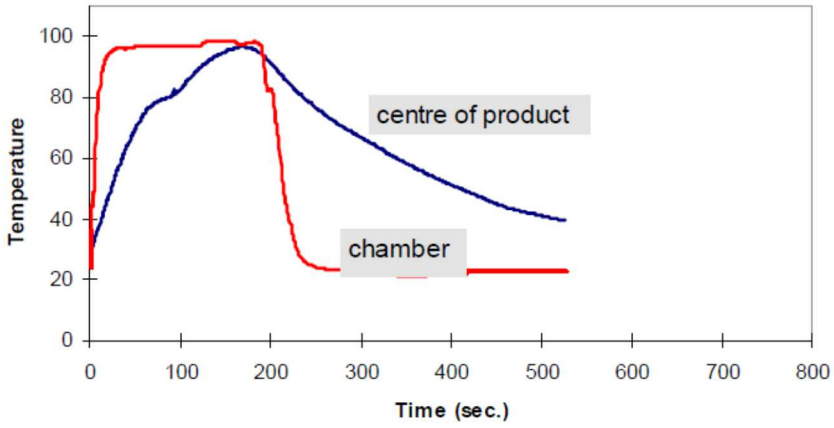

Figure 4-3: Temperature profiles derived from the centre of the product and in the chamber during pasteurization (Zardetto and Dalla Rosa, 2007).

The assessed parameters within the product are, as expected, diverse concerning those in the pasteurisation chamber. The complete treatment duration influences the maximum temperature that can be reached at the centre of the product. Only prolonged thermal treatment allows the temperature at the centre of the product to reach the temperature set on the pasteurisation chamber. This factor is significant for assessing the thermal treatment since it is the temperature achieved at the most unfavourable thermal point that is important for correctly setting the process. Figure 4-4 shows the value of $F_{70/10}$ at the centre of the product concerning the chamber $F_{70/10}$ values, for two different treatment temperatures. The correlation between the two values, after a lag time that varies as a function of the technological characteristics of the heating system, stabilises and becomes linear.

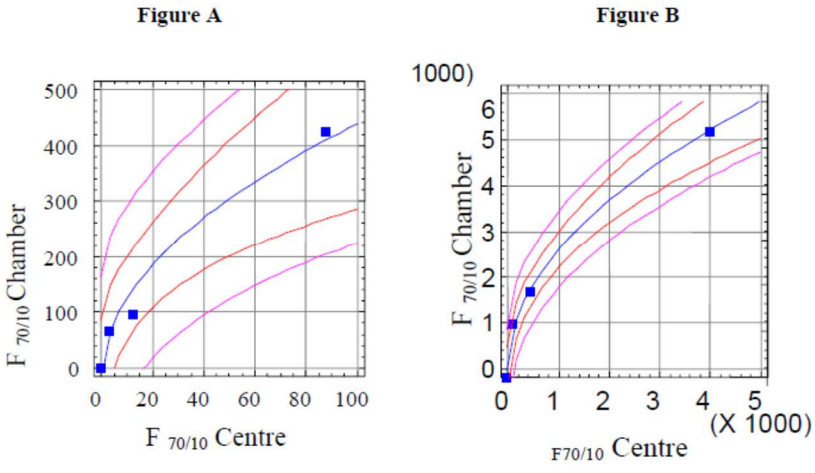

Figure 4-4: Experimental chamber $F_{70/10}$ values versus $F_{70/10}$ values calculated in the centre of the product during two different pasteurisation processes (Zardetto and Dalla Rosa, 2007).

Knowledge of the temperature achieved at the centre of the product, along with the minimum temperature to obtain sufficient pasteurisation, allows for efficient management of treatment time and temperature. These two parameters should be chosen in line with the following considerations: a) the nature and composition of the food to be treated, b) the type and number of microorganisms present in the food, c) the shape and dimensions of the food to be treated, and d) the characteristics of the pasteurisation establishment. With the thermal and physical data, and from experimental and systematic studies of time-temperature changes and related quality modifications, it is possible to develop mathematical models that depict the pasteurisation process of fresh pasta. The combined effect of several process variables, such as thickness, starting temperature, treatment temperature, and the heating media, can be studied and optimised through computer simulation of the temperature distribution and the related changes in quality, depending on the time-temperature relationship.

De Cindio et al. (2000) have developed a mathematical model that can predict the temporal evolution of the temperature profile that occurs in the centre of the product during treatment. In this case, the product is represented by the transverse section of a cylinder through its large axis in a plane containing the same axis. In the case of non-filled fresh pasta, assessment of the product's internal temperature results in a very difficult measurement, due to its thickness (1 mm maximum). In this case, the thin pasta lamina can be assimilated to a rectangular lamina of defined dimensions, as long as the thickness is uniform. Using this model it is possible to estimate the time needed for the temperature at the geometric centre of the product to be equal to the temperature of the chamber (Zardetto, 2005). Hence, the pasta thickness has a significant role since it is appropriate to use the temperature of the product surface for the $F_{70/10}$ calculation only for pasta of a thickness less than 1.1 mm.

From the kinetics of microorganism elimination by heat (the Bigelow law), the destruction at a certain temperature is directly proportional to the number of microorganisms at the outset. The lethality of thermal treatment, expressed through the F value, is linked to the decrease in the microbiological population, expressed by the relationship:

$$F_z^T = D_T(\log N_0 - \log N)$$

Where F expresses the time needed for a certain microbial population, for a given treatment at a given temperature, to be reduced to the desired level, D is the time to reduce the microbial load of a log cycle at a given temperature T. To obtain sanitary safety, the pasteurisation process should assure the reduction of 4D of *Listeria monocytogenes*. This microorganism is used as a reference since it is the most thermo-resistant among non spore-forming pathogens.

The thermal treatment efficacy concerning the total viable count reduction in the stuffing is linked to the treatment time. This is because it influences the maximum temperature achieved at the centre of the product. The obtained reduction in the stuffing is lower (1-3 logarithmic units) and largely linked to the applied $F_{70/10}$ value. Regarding the second thermal treatment, performed after packing under a modified atmosphere, Verratti (2000) reports that it is inefficient for improving the product microbiological quality as the microbial load of the whole product before and after treatment remains constant at around $2 \cdot 10^2$ cfu/g. The a_w value can be correlated with different time-temperature processing combinations. Thermal treatment results in the gelatinisation of starch. The term 'starch gelatinisation' indicates the process through which the starch goes from a natural crystalline structure to an amorphous structure in the presence of heat and water. This transformation implies a series of modifications in starch properties and consequently, variations in the functional and nutritional characteristics of the pasta. Among gelatinised starch properties, the rapid and high capacity for hydration when mixed with cold water and a higher susceptibility to enzymatic digestion leads to a consequent increase in digestibility. The increase in the gelatinisation degree after thermal treatment is influenced by the type of processes that the dough receives before treatment. For the same thermal treatment, sheet-rolled pasta undergoes a significant increase in the degree of gelatinization, whereas in extruded pasta a lower final degree of gelatinisation is obtained (Zardetto and Dalla Rosa, 2007). Thermal treatment thus determines structural modifications in the pasta that influence the quality characteristics of the product. Thermal treatment determines the colour modification in pasta, with diverse variations, as a function of the parameter or lamination modality used during production (i.e., extrusion or sheeting-roll). For extruded pasta, the luminosity is lower in pasteurised samples. Cencic et al. (1995) hypothesised that this characteristic could be dependent on the fact that the Tristimulus colorimeter, being unable to correctly read the reflected light on translucid surfaces, identified the sample as less luminous, whereas upon visible examination it appeared more translucent. The surface of the extruded pasta, immediately after thermal treatment, is visibly more translucent in roll-sheeted samples and this may be associated with starch gelatinisation following different treatments of samples.

The colour changes in pasta, due to thermal treatment, can be interpreted by calculating the h* (hue angle) value. This value describes the sample hue and is obtained according to the following equation:

$$h_{a,b} = \text{arctn}({}^{b^*}/_{a^*})$$

Thermal treatment results in an increase in the colorimetric h* parameter, with values around 90° (yellow). Although pasta samples subjected to thermal treatment always present higher h* values, in non-pasteurised pasta, thermal treatment intensity influences the final value of h*. The damaging effects of thermal treatment on pasta is revealed by the Maillard reaction (the primary reaction between proteins and reducing sugars) or 'non- enzymatic browning' reaction (Pagani et al., 1995). Such reactions cause a nutritional decrease because the amino acids are no longer biologically available. Furosine is produced by acid hydrolysis from Amadori compounds attributed to lysil-ketosis. This molecule is a valid indicator of the initial stages of the Maillard reaction in pasta (Resmini et al., 1990). The intensity of the reaction, for similar reagent concentrations, is controlled by several process parameters, temperature, treatment time, and sample moisture during processing. Pagani et al. (1995) showed that for temperatures higher than 75 °C the key parameter is pasta moisture, or rather its Aw value at the time of treatment. Humidity lower than 16% and a_w values of 0.75-0.80 induced an intense primary stage of the Maillard reaction, with furosine levels between 400mg and 700 mg per 100g of protein. The furosine levels assessed in fresh pasta samples varied widely depending on treatments used. Therefore, for analysis between different time-temperature processing combinations a parameter named "furosine increase" has been proposed, which can be calculated as a function of the furosine content present in the initial sample (Zardetto, Rosab and Frescoa, 2003). The selection of an optimal combination treatment is also linked to a given establishment and product. The combined use of elevated temperatures and short durations (99 °C, 2 minutes) is usually possible for non-filled fresh pasta (e.g., tagliatelle, fettuccine) in which the product shape allows the centre to achieve the desired pasteurisation effect. The same results are harder to achieve in filled fresh pasta (e.g., cappelletti, ravioli). In this case, a combination of lower temperatures and longer durations (95 °C, 6 minutes) is more appropriate.

4.3.2 Sterilisation

Sterilisation is the destruction or elimination of all viable organisms and spores in or on the food product being sterilised. Sterilisation kills yeasts, moulds, vegetative bacteria, and inactivates spore. It allows products to be stored and distributed the at ambient temperatures, with an extended shelf life. Sterilisation procedures involve the use of heat, radiation, chemicals, or physical removal of cells. The sterilisation process consists of four distinct stages. First, the product must be heated to a temperature of 110–125 °C to ensure sterilisation. Second, the product requires a few minutes to equilibrate, since the surface will be hotter than the central portion of the container, causing a temperature gradient. The equilibration stage

allows a reduction in the temperature gradient. Third, the product must be held at this temperature for a given period to ensure a predetermined sterilisation value expressed by the F_0 value. Finally, the product must be cooled to arrest further heat treatment and avoid over cooking. Complete sterilisation will lead to a deterioration in product quality and nutrients. Hence, in practice, commercial sterility is targeted. Commercial sterility refers to a product that has been optimally processed so that, under normal conditions, the product will neither spoil nor endanger the health of the consumer and retain its organoleptic properties and nutrients. The pH of the product is an important factor in determining the severity of the sterilisation process (Rahman., 2007).

For fresh pasta, the shelf-life extension of the processed products depends not only on the surviving cell number, as a consequence of the thermal treatment adopted, and the Aw value, but also the textural or microstructural changes and protein gelation induced by the thermal treatment. The significance of the changes induced by thermal processes suggests that, due to the physico-chemical characteristics of fresh pasta and the need to cook it before consumption, sterilisation heat treatment is not recommended (Lopez et al., 1998).

4.3.3 Microwave Pasteurisation

Microwave heating of foods is attractive due to its volumetric origin, rapid increase in temperature, controllable heat deposition, and easy cleaning potential. It is currently used for a variety of domestic and industrial food preparations and processing applications. Moreover, it has been used successfully for finish drying in potato chips, precooking in chicken and bacon, proofing and frying doughnuts, tempering frozen foods, and drying pasta products. Microwave processing of fresh-filled pasta has become common in Italy since the 1990s, and the technology has been applied to ready-to-eat meals, pasta-based products, and a variety of foods throughout Europe, Japan, and South America (Rahman, Ahmed and Ramaswamy, 2020).

Microwave pasteurisation may be used to prepare ready-to-eat and heat-and-eat pasta dishes, providing consumers with a convenience product with a lower risk of pathogen contamination. This technology may be adapted to similar products, providing the lethality step for pathogens that is critical for the safety of convenience products.

In pasteurisation and sterilization, microwave heating is preferred over conventional heating because the process is fast and requires minimal time to reach the desired process temperature. In microorganisms, the study of destruction kinetics by microwave heating has received considerable interest since the 1940s when initial work by Fleming was reported.

Microwaves are electromagnetic radio waves that exist within a frequency band of 300 MHz to 300 GHz. Microwave heating refers to dielectric heating due to polarisation effects at a selected frequency band in a nonconductor. It differs from capacitive heating by the sample placement. In capacitive heating, the sample is placed between the electrodes, while in microwave heating, the food is commonly housed inside a closed cavity. Microwave heating involves the coupling of electrical energy from an electromagnetic field in a microwave cavity and its subsequent dissipation within the food product. This results in a sharp temperature increase within the product. Microwave energy is delivered at a molecular level through molecular interaction with the electromagnetic field. This occurs through molecular friction resulting from the dipole rotation of polar solvents and the conductive migration of dissolved ions. The principal mechanisms involved in microwave heating are therefore dipole rotation and ionic polarisation. Water in the food is the primary dipolar component responsible for dielectric heating. In an alternating current electric field, the polarity of the field is varied at the rate of microwave frequency and molecules attempt to align themselves with the changing field. Heat is generated rapidly due to internal molecular friction. The second key mechanism of microwave heating is through the polarisation of ions due to the back-and-forth movement of the ionic molecules trying to align themselves with the oscillating electric field. Microwave heating is also affected by the state of the constituents (i.e., whether they are bound or free). Bound ions, for example, have much lower microwave absorption. The volumetric heating rate (Q) of a microwave at a given location is related to the electric field strength, expressed in the following equation:

$$Q = 2\pi f \varepsilon_0 \varepsilon^{"} E^2$$

Where *f* is the frequency of the microwaves, E is the strength of the electric field of the wave at that location, ε_0 is the availability of free space (a physical constant), and $\varepsilon^{"}$ is the dielectric loss factor (a material property called dielectric property) representing the material's ability to absorb the wave. In addition to $\varepsilon^{"}$, there is another dielectric property parameter called the dielectric constant (ε'), which affects the strength of the electric field inside the food.

To produce high-quality microwave-pasteurised pasta, chemical interactions occurring in pasta during cooking and factors defining the pasta quality must be understood. Pasta, made from semolina and water, is a mixed polymer with starch and protein as the primary structuring agents. The rheological properties pasta dough are dominated by the gluten proteins in

semolina flour. Hence, high protein content and gluten strength are key indicators of pasta quality.

In a study by Joyner Melito, Jones, and Rasco (2016) the Microwave-Assisted Pasteurisation System was applied to uncooked pasta, fully cooked pasta, and three incremental stages of parboiled pasta to yield fully cooked pasta with the goal of producing pasta for incorporation into ready-to-eat and heat-and-eat meals that had similar characteristics to conventionally cooked pasta. Microscopy and chemical analyses showed evidence of structural and chemical differences in starch and gluten due to the different microwave treatments, although trends conclusively linking increased starch gelatinisation and gluten polymerisation to increased heat treatment were not observed. Significant differences were observed between the microwave-treated pasta and the control (conventionally cooked) pasta in terms of structure, chemical composition, and mechanical behaviour. Extensibility results showed that the microwave process had a weakening effect on the pasta strands, suggesting that microwave processing may intensify the reactions occurring during product ageing (for example, retrogradation) and the resulting impact on texture. Significant differences were observed between the microwave-treated pasta for many attributes, indicating that the amount of parboiling time given to the pasta prior to microwave pasteurisation impacted the final product properties. However, these results show that microwave pasteurisation of pasta is a feasible processing method for pasta, capable of creating refrigerated, fully cooked pasta for consumers (Joyner Melito, Jones and Rasco, 2016).

Microwave sterilisation has been studied extensively in academic and industrial sectors. However, the commercialisation of the process has had limited success. The major drawback in microwave sterilisation is the lack of availability concerning temperature profiles. Measurements at a few locations does not provide the actual temperature distribution in the product during microwave heating, as the heating pattern may be uneven, subject to change and difficult to predict. Therefore, researchers have obtained inconsistent outcomes. In addition, it is not always true that the microwave-assisted processes result in improved quality retention in food products. The degradation kinetics of quality, sensory, or nutritional qualities depends upon many factors, such as the nature of the food product, food geometry, dielectric properties, and oven design as compared to conventional thermal processing. The dielectric properties of the food product significantly vary during heat processing, particularly above 80 °C in protein and starches, and the heat absorption process. These changes in dielectric properties may affect the heating pattern qualitatively, although such factors are not serious in

conventional thermal processing. The coupling of heat transfer and electromagnetics could account for changes in dielectric properties during thermal treatment.

A study conducted by Zardetto and Dalla Rosa (2015) indicated that the two pasteurizing methods (steam and microwave pasteurisations) used for the heat treatment of filled pasta produced significant differences in colour, gelatinisation, and furosine content. These parameters correlated strongly with the thermal treatment intensity. The microwave heat treatment applied to the packaged product further increased the furosine levels in the pasta and filling. Under the experimental conditions applied in this study, the "nascent steam pasteurizer" produced reduced thermal damage, with lower furosine content in the pasta and the filling, although it also reduced certain favourable parameters, such as starch gelatinisation and colour. This decrease in starch gelatinisation is not desirable in industrially produced pasta, which is subjected to an additional step after heat treatment (i.e., weighing, packaging). Moreover, the degree of starch gelatinisation determines the elasticity of pasta, for which high values reduce product damage in the following heat thermal treatments (shelf-life stability of the product) or during the cooking of the product by the consumer due to increased microstructural stability.

The utility of the microwave sterilisation process depends on the proper selection of equipment and packaging, which could assure its success in food processing industries. Laboratory processing equipment is also essential for process refinement and to study the effect of process and storage time on product quality attributes and microbiological safety factors. It is recognised that microwave sterilisation can produce high-quality shelf-stable food products.

The application of microwave energy for pasteurisation and sterilisation has been studied for half a century with some commercial success. Some researchers have observed non-thermal or enhanced thermal effects associated with microwave heating on the destruction of microorganisms and enzyme inactivation. However, the issue remains controversial. Continuous-flow microwaveable pasteurisers could be used for milk and juice processing.

Microwave pasteurisation of ready-to-eat meals has also been commercially successful in the European countries although US industries are still reluctant to adopt the technology. Commercial sized microwave equipment is now readily available for pasteurisation and sterilisation applications. Some reasons cited for the lack of commercial success in the operation are complexity, high expenditure, nonuniformity of heating, inability to the ensure sterilisation of the entire package, the lack of suitable packaging materials, and unfavourable economics when compared to prepared frozen foods in developed countries (Rahman, Ahmed and Ramaswamy, 2020).

4.3.4 Gamma Irradiation

The irradiation process involves exposing the food, either prepackaged or in bulk, to a predetermined level of ionisation radiation. In this process, it is important to determine the sources of the radiation, how energy is quantified, and its scope, including its advantages and limitations.

Irradiation has been approved by many countries. For example, in the U.S. and Canada, food irradiation has existed for decades (source Canadian Food Inspection Agency. October 31, 2016. Retrieved October 5, 2020). Food irradiation is used commercially and volumes are in general increasing at a slow rate, even in the European Union where all member countries allow the irradiation of dried herbs spices and vegetable seasonings, but only a few allow other foods to be sold as irradiated (source "Annual Reports - Food Safety - European Commission". October 17, 2016). Although there are some consumers who choose not to purchase irradiated food, a sufficient market has existed for retailers to have continuously stocked irradiated products for years. When labeled irradiated food is offered for retail sale, consumers buy it and re-purchase it, indicating a market for irradiated foods, although there is a continuing need for consumer education (Roberts, P. B.; Hénon, Y. M., 2015). Food scientists have concluded that any fresh or frozen food undergoing irradiation at specified doses is safe to consume, with some 60 countries using irradiation to maintain quality in their food supply (Maherani, *et al*. 2016). The Codex Alimentarius represents the global standard for irradiation of food, in particular under the World Trade Organization-agreement. Regardless of treatment source, all processing facilities must adhere to safety standards set by the International Atomic Energy Agency (IAEA), Codex Code of Practice for the Radiation Processing of Food, Nuclear Regulatory Commission (NRC), and the International Organization for Standardization (ISO). More specifically, ISO 14470 and ISO 9001 provide in-depth information regarding safety in irradiation facilities (Roberts, P. B.; Hénon, Y. M., 2015). All commercial irradiation facilities contain safety systems which are designed to prevent exposure of personnel to radiation. The radiation source is constantly shielded by water, concrete, or metal. Irradiation facilities are designed with overlapping layers of protection, interlocks, and safeguards to prevent accidental radiation exposure.

Ionisation radiation interacts with an irradiated material and ionises molecules by creating positive and negative ions by transferring energy in the electrons. The radiation effects on biological materials are direct and indirect. In direct action, the chemical events occur because of energy deposition by the radiation on the target molecule, and the indirect effects occur due to reactive diffusible free radicals formed from the radiolysis of water, such as hydroxyl

radical, hydrated electron, H atom, hydrogen peroxide (H_2O_2), and hydrogen. Hydrogen peroxide is a strong oxidising agent and poisonous to biological systems, while hydroxyl radical is a strong oxidising agent and hydrogen radical is a strong reducing agent. These two radicals can cause several changes in the molecular structure of organic matter.

There are two classes of ionising radiation: electromagnetic and particulate. These are X-rays from radionuclides ^{60}Co or ^{137}Cs, X-rays generated from machines operated at or below 5 MeV, and electrons generated from machine sources operated at or below an energy level of 10MeV. The characteristics of different irradiation sources are summarised in Figure 4-5.

Characteristics of Irradiation Sources

Radiation Source	Characteristics
Cobalt-60	1. High penetrating power 2. Permanent radioactive source 3. High efficiency 4. Source replenishment needed 5. Low throughput
Electron beams	1. Low penetrating power 2. Switch on–switch off capability 3. High efficiency 4. High throughput 5. Power and cooling needed 6. Technically complex
X-rays	1. High penetrating power 2. Switch on–switch off capability 3. Low efficiency 4. High throughput 5. Power and cooling needed 6. Technically complex

Source: D. Kilcast, *Int. Biodeter. Biodegra*. 36:279 (1995).

Figure 4-5: The characteristics of different irradiation sources.

Similar to other preservation methods, irradiation affects microbial growth and changes the food components. Ionisation irradiation affects microorganisms, such as bacteria, yeasts, and moulds, by causing lesions in the genetic material of the cell, effectively preventing it from carrying out the biological processes necessary for its continued existence. The stability of food components also needs to be known for determining its functionality and safety.

The principal targets of irradiation are nucleic acids and membrane lipids. Alteration in membrane lipids, particularly polyunsaturated lipids, leads to the perturbation of membranes and deleterious effects on various membrane functions, such as permeability. The activity of membrane enzymes may be affected as a secondary effect of membrane lipid degradation

(Willemoti et al., 1996). Ionisation radiation acts through changes induced in the DNA structure of the irradiated cells, which result in the prevention of replication or other functions. The energy levels used are sufficient to disrupt certain bonds in the molecules of DNA, thereby making cell reproduction impossible. Nucleic acids, because of their generous size, are the main targets of free radicals generated by irradiation (Willemoti et al., 1996). Chromosomes of bacteria are intrinsically sensitive and lethal damage occurs as a result of exposure to irradiation. The ability of bacteria to repair a limited amount of such damage gives them considerable resistance to such radiation. The efficiency with which different bacteria repair the radiation-induced damage to their DNA varies considerably. The most sensitive vegetative bacteria are *Pseudomona*s, and the most resistant is *Deinococcus*, by a factor of about one hundred (B.E.B. Moseley, 1989).

Food irradiation has been used to increase the shelf life and safety of several products, reducing losses and the occurrence of food-borne diseases (FDA, 2016). Radiation causes DNA and/or protein damage and the generation of free radicals due to the radiolysis of water, which are toxic to cells, leading to bacterial death (Daly, 2009; Trudeau et al., 2012). Free radicals can also interact with macronutrients (proteins, lipids, and carbohydrates) and with minerals, modifying the physical-chemical and sensory characteristics of the food (Bashir and Aggarwal, 2016; Ben Mustapha et al., 2014; Damodaran et al., 2008; Najafabadi et al., 2017; Ocloo et al., 2014; Shi et al., 2015). Dry products such as spices and pasta are irradiated without considerable damage. However, foods with a high moisture content can suffer significant losses in organoleptic properties (e.g., development of off-flavours, discoloration, and a loss of firmness), making it impossible to use gamma radiation to increase its shelf life (Silvestre et al., 2017).

The work of Cassares *et al* (2020) aimed to evaluate the use of gamma radiation to improve the microbiological quality of fresh pasta (gnocchi) during storage at refrigerated and room temperatures and the effect on physical, chemical, and sensory characteristics. Despite the high water content of fresh pasta (gnocchi), the use of gamma irradiation at 13 kGy was effective in maintaining the shelf stability of gnocchi (without chemical preservatives and modified atmospheres) at room temperature (25 °C) for 90 days with no loss of sensorial quality and cooking quality (i.e., the colour and texture of cooked gnocchi), guaranteeing a suitable microbiological quality. When refrigeration was combined as an additional method of preservation, the dose of 10 kGy was enough to obtain the same result (Cassares et al., 2020).

4.3.5 Partial Drying

The preservation of foods by drying is the most common method used by humans and the food processing industry. The dehydration of food is one of the most important achievements in human history, making humans less dependent upon a daily food supply even under adverse environmental conditions.

Drying reduces a_w, thus preserving foods by preventing microbial growth and deteriorative chemical reactions. The effects of heat on microorganisms and the enzyme activity are also important in the drying of foods. In the case of foods to be preserved by drying, it is important to maximise microorganism and enzyme inactivation to prevent spoilage and enhance safety and reduce the components responsible for the deterioration of the dried foods. Also, in the case of drying bacterial cultures, enzymes, or vitamins, minimum inactivation of the microorganism and enzyme is required. Thus, the effects of drying may be desirable or undesirable, depending on the purpose of the drying process.

During the production of fresh pasta, at the end of the pasteurisation phase, the product is partially dried. The dryer is composed of a conveyor belt that advances the product into the drying tunnel where hot air is forced inside it. The speed and conditions of the process can vary depending on the product (e.g., size and type of filling). For example, the air can have a temperature of about 70 ° C, and the treatment time can be set around 20 minutes.

The drying effect is obtained from the circulation of hot air which allows the removal of water that passes from the internal portions of the product to its surface by capillarity. When carrying out this operation, care must be taken not to over-dry the surface part of the product to avoid the formation of the surface crust, and at the end of this phase, the pasta must maintain the mandatory characteristic of fresh pasta (i.e., more than 24% moisture and a_w ranges from 0.92 to 0.97[4]).

This treatment allows the following processes:

a) the reduction of a_w, allowing an increase in the shelf life of the product;

b) surface drying of the product, avoiding adhesion between the pieces and favouring subsequent weighing operations; and

c) the inactivation of some enzymatic complexes, such as oxidase, allowing the maintenance of the colour of the product.

[4] PRESIDENTIAL DECREE N° 187, dated 9 February 2001 (Official Journal n. 117, of May 22, 2001) Regulation for the revision of laws concerning the production and sale of milling products and pasta, pursuant to Article 50 of Law N° 146, dated 22 February 1994. Art.9

4.3.6 Cooling

In the process of fresh pasta after the partial drying step, the product is collected by a conveyor belt that passes through a refrigerated tunnel. The speed of the conveyor belt is regulated in such a way that the output product has a temperature of about 9 °C at the core of the product. The speed of the belt is regulated according to the size of the product treated.
This process is significant as lowering the temperature of the product avoids the subsequent formation of condensation during packaged using MAP. The use of gas mixtures containing nitrogen (cold gas) would in fact lead to the possibility of thermal shock if the product, were still hot, favouring the formation of water droplets in the tray with a possible restart of the microbial activity in the product.

4.4 Packaging Techniques

The first type of packaging material to be discussed is plastic, which is technologically a complex class of material. Packaging materials may be grouped into rigid and flexible structures. Plastic film, foil, paper, and textiles are flexible materials, whereas wood, glass, metals, and hard plastics are examples of rigid materials. The volume of plastics produced each year now exceeds the amount of steel consumed, and practically none of it is recycled. Plastics now account for about 25% of household waste, although less than 20% of the containers we use are plastic. Half of these are used for milk and various carbonated drinks. However, using other forms of packaging would double packaging costs, quadruple the number of waste products, would take more energy to produce, and reduce the number of new jobs per year created by the growing packaging industry. There is increasing recognition of the need to recycle. The main difficulties are the separation of plastics (manual labour) and purity of the final product (energy problems). Because of their lower unit cost and lower energy consumption during manufacture, plastics have tended to replace the traditional packaging materials, glass, paper, and metals, in situations where high barrier properties are not required by the product.
Polymers are the fastest-growing group of materials in food packaging. The first plastic materials used for flexible packaging entered commercial production in 1939, just as the war started, but the main development took place in the mid-1950s. Their foremost advantage is their wide diversity and an extremely broad spectrum of properties. Plastics are cheap, light, easily processed and shaped, and easy to seal.

Polymers (or as they are commonly called plastics) are compounds of extremely high molecular weight. They are constructed of many repeating units or building blocks, combined via a chemical reaction. These building blocks, called mers or monomers, are gases or liquids at room temperature and pressure, whereas polymers are normally solids under these conditions. Polymers can be either natural (familiar examples are starch, proteins, and rubber) or synthetic, the latter being those used in packaging. Other components in plastics are residual monomer and oligomers, additives such as heat and light stabilisers, antioxidants, plasticisers, and UV absorbers, as well as processing aids such as lubricants, slip agents, and antistatic agents. Since no single film can satisfy all packaging requirements, plastic films may be combined by lamination or coextrusion. Lamination is a technique for bonding films together to give a film with the properties of both constituents. By combining the qualities of choice from the raw material films, a laminate can be tailor-made for its application. Each layer in the resulting laminate may exhibit different properties from its free state, such as mutual layer reinforcement in which cracks in a brittle layer are prevented from propagating by high elongation (elastic) layers. For package sterilisation, the material of choice is polypropylene (PP), which is used as the outer and inner plies of the laminate with polyvinylidene chloride (PVDC) as the middle layer to provide an oxygen barrier. Intermediate between these main functional layers will be other plies to contribute appropriate bulk and strength (Rahman., 2007).

Many plastic containers are formed by thermoforming. This involves heating a plastic sheet until it softens and then shaping it by stamping it between two cooled moulds. A growing requirement of moulded plastics is that they be microwaveable, i.e., have a softening point well above 100°C. They must also be shelf-stable. Plastics are slow to degrade under ambient conditions, but they may discolour or become brittle.

Since no single film can satisfy all packaging requirements, plastic films may be combined by lamination or coextrusion. Lamination is a technique for bonding films together to give a film with the properties of both constituents. By combining the qualities of choice from the raw material films, a laminate can be tailor-made for its application. Each layer in the resulting laminate may exhibit different properties from its free state, such as mutual layer reinforcement in which cracks in a brittle layer are prevented from propagating by a high elongation (elastic) layer. This effect depends on good adhesion between the layers. Three factors affect the adhesion between layers:

1. the viscosity or shear rate should match during the melding of layers; to be coextruded, the melt flow viscosities should be similar (a ratio of within 3:1), otherwise one of the plastics will flow over the other, preventing bonding;

2. the temperature, pressure, and period of contact, required to build the bond;

3. the functionality of adjacent resin layers (i.e., they should be sufficiently similar in structure to mix at the contact surfaces).

If all these factors are not present, then an adhesive layer is necessary, and the plastics may be cold bonded with a tie layer of resin adhesive. Adhesives are discussed below. A typical triple-layer film would be composed as follows. Properties of outside layer: high gloss, printable, good lamination, possibly metallised, high slip. Properties of middle layer: strength, stiffness, barrier properties, possibly opaque. Properties of inner layer: easy to seal (hot seal, good hot-tack properties, or good cold-seal properties), low migration rates, barrier properties.

'Materials and objects in contact with foods' (MOCA/FCM) are materials and objects destined to encounter foods (kitchen tools and table flatware and containers, machinery for the transformation of foods, packaging materials, etc.). These terms are also used to indicate the materials and objects that come into contact with water, with the exclusion of fixed public or private water supply systems. Always in Italy, the Lgs. Decree 29/2017 governs the sanctions for the violations of provisions imposed by the regulations concerning materials and objects destined to encounter food products and foods.

In Italy all companies to which the MOCA/FCM legislation applies must be registered with the SUAP (*Sportello Unico Attività Produttive*, or Unified Office for Production Activities) of their relative City and conform with European regulations:

- 1935/2004/EC: concerning the materials and objects destined to encounter food products;
- 2023/2006/EC: concerning the good manufacturing practices for materials and objects destined to encounter food products;
- 282/2008/EC: relative to recycled plastic materials and objects destined to come into contact with foods;
- 450/2009/EC: concerning the active and intelligent materials destined to encounter foods;
- 10/2011/EC: concerning plastic materials and objects destined to encounter food products;
- 1895/2005/EC: relative to the restrictions regarding the use of some epoxies in materials and objects destined to come into contact with food products.

4.4.1 Modified Atmosphere Packaging Techniques

The MAP of respiring food products, such as fresh and minimally processed produce, requires a different approach from the MAP of non-respiring foods. In non-respiring foods, modified atmospheres (MAs) without oxygen are used to minimise oxidative deterioration reactions (such as the brown discoloration of meat or the rancidity of peanuts) or reduce microbial proliferation, e.g., the growth of moulds in cheese and baked goods. High gas barrier films or laminates are used to exclude the exchange of gases (particularly O_2) through the package, which would result in a suboptimal in-package atmosphere. In contrast, respiring products stay metabolically active after harvest, and this activity is essential for preserving their quality. Aimed at extending the shelf life of respiring products, a prerequisite for a MAP system is that the gas composition of the atmosphere allows for a basic level of metabolism, which means that a certain amount of O_2 should be available. The required basic level of metabolism is highly variable among commodities (e.g., type and maturity) and greatly depends on the storage temperature and degree of processing (e.g., trimming, cutting, and slicing). Due to the significant respiratory activity of the product, the gas atmosphere inside the package changes during the storage period. Therefore, expert knowledge concerning these changes is necessary to tailor the package design of an individual product to optimise its quality shelf life.

A packaging strategy that uses MAP is designed to slow the metabolic activity of the product as well as the growth of microorganisms (both spoilage and pathogenic) by limiting O_2 supply and applying an elevated level of CO_2. Since the same strategy underlies refrigerated storage, the MAP of respiring produce is usually combined with this technique.

In MAP, the gas composition within the package is not monitored or adjusted. Therefore, the term *passive* atmosphere packaging is sometimes used in this respect. Depending on the oxygen sensitivity and metabolic activity of the product, air or a predetermined gas mixture is used to flush packages before closing. The use of ambient air as the packaging gas is most economical. However, it is largely an option when respiration activity under prevailing storage conditions is sufficient to reduce the in-pack O_2 level fast enough to levels that do not cause physiological or microbial deterioration. For produce that is extremely sensitive to O_2 (e.g., many minimally processed fruits) or has a low level of respiratory activity, flushing with a gas mixture composed of low O_2 and moderately high CO_2 is often used to shorten the time needed to reach the desired in-pack gas composition. After closing the package, product respiration will cause a decrease in the O_2 content and an increase in CO_2. The altered gas concentrations, however, cause a decrease in the respiration rate. Finally, an equilibrium is reached inside the

package, which is the result of a balance between the metabolic rate of the packed product and the diffusion characteristics of the package materials. This explains the use of another term, equilibrium-modified atmosphere packaging. The package is often designed in such a way that the equilibrium concentration resembles the optimal gas concentrations found in experiments in which products are stored under a range of stable gas conditions.

The course of the atmosphere modification is determined by three interacting processes: commodity respiration, gas diffusion through the commodity, and gas permeation through the film. Each of these processes is strongly influenced by several commodity- and environmentally-generated factors. The respiration rate of a commodity depends on its physiological stage and temperature, O_2 and CO_2 partial pressures, relative humidity, and ethylene concentration. Gas diffusion is affected by temperature, the gas gradient across the limiting barrier, the gas diffusion path, membrane permeability, and the commodity's mass, volume, and respiration rate. Several of these variables may vary according to the maturity stage of the product or even the degree of illumination. Several variables affecting gas permeation through the film are temperature, the gas gradient across the film, the film structure, water vapour gradient, thickness of the package, and surface area. A change in the product amount, free volume, or any of the variables listed above will affect the equilibrium-modified atmosphere and the time in which steady-state conditions are established. Flushing a package with a premixed gas will influence the time needed to attain the equilibrium-modified atmosphere. Strict temperature control in the distribution chain would be a prerequisite for the optimal use of MAP in practice. In most countries, however, the cooling chain between production, distribution, retail, and the consumer has many uncontrolled links. The changes in the permeability of most packaging films to gases in response to changes in temperature are generally lower than changes in product respiration. Currently, most existing plastic films lack the appropriate O_2:CO_2 permeability ratio to provide the ideal MA for many commodities at a given temperature (Rahman., 2007).

Modified Atmosphere Packaging is an established technique in which the gas composition surrounding a product is altered, resulting in an atmosphere different from air. The two major gases used in commercial MAP are nitrogen (N_2) and carbon dioxide (CO_2). The optimal CO_2 concentration for fresh-filled pasta varies from 25% to 40%, while N_2 is used as filling gas to prevent packages from collapsing due to absorption of the CO_2 from the product (Zardetto et al., 2021).

As reported by Zardetto (2005), the reliability of MAP in fresh pasta depends on rigorous temperature control. Temperature is often not constant during the distribution chain,

characterised by different processes such as transport, warehouse storage, picking, and house storage, each with its typical conditions (Zardetto et al., 2021).

Northolt and Bullerman (1982) reported that the MAP combination with a low Aw and low temperature constitutes a prevention measure against mould growth. Moulds are aerobic and are sensitive to CO_2 concentrations. A concentration of more than 40% CO_2 depresses fungal growth and toxin production to different degrees in different species (Northolt and Bullerman, 1982). Concentrations of CO_2 up to 15% stimulate most species of Penicillium, but as the CO_2 concentration increases, fungal growth decreases greatly (Croci et al., 2001). The concentration of CO_2 used in filled fresh pasta varies from 25% to 40% depending on the type of product. Castelvetri (1991) showed that when the concentration of CO_2 used to package fresh-filled pasta is above 30%, the shelf life of the product was over 30 days. An N_2 concentration of over 70% can prevent packages from collapsing due to the absorption of CO_2 by filled fresh pasta. It is possible to vary the residual oxygen concentration in the package in the application of this technology, but it is usually less than 2%. The concentration of 1% or less completely depresses the germination, growth, and sporulation of most moulds (Zardetto, 2005).

4.4.2 Active Biodegradable Packaging

In several cases, a package cannot be designed in such a way that optimal conditions will be reached passively. Active packaging provides a solution by adding materials that absorb or release a specific compound in the gas phase. Compounds that can be absorbed include carbon dioxide, oxygen, water vapour, ethylene, and volatiles that influence taste and aroma. In some leafy vegetables, carbon dioxide levels can induce tissue browning, while for most fruits, increased ethylene levels accelerate ripening (Bugatti, Viscusi, and Gorrasi 2020). Depending on the type of produce, ethylene can induce senescence and maturation processes that reduce the fresh product quality, even at low levels. Including ethylene scrubbers such as potassium permanganate counteracts the effect of ethylene, although the capacity of such scavengers is limited. In transport packaging used for grapes, pouches are often added that slowly release sulfur-containing chemicals to reduce fungal growth. Research has recently been directed towards replacing chemicals with compounds retrieved from plant tissues (green chemicals). The quantity of the required active compounds will depend on a range of interacting factors, including production rates (carbon dioxide, ethylene), the concentrations to be reached, and how long the package should be functional. Several possibilities exist, although it is not possible to precisely control O_2 levels in such packaging (De Camargo *et al*.,2013).

Several intelligent concepts that involve more than scrubbing or emitting compounds have been introduced. These types of packages will only become active when a specific prerequisite has been met. Most of these packages focus on the prevention of problems associated with anaerobic conditions. In one such system, holes are introduced into the package upon exposure to elevated temperatures for a certain time. Originally, the holes were closed by solid hydrocarbons with melting points between 10 and 30 °C. Because product respiration typically increases at a faster rate than gas diffusion following a temperature rise, the holes in the package will prevent O_2 depletion (Stollman, Johansson, and Leufven, 1994).

Another idea is an ethanol sensor that is mounted on a package and informs buyers of its history in terms of possible mechanical damage or temperature abuse. A further concept, which has been used in France and the United States, is the (or time-temperature integrator) (TTI).

In most cases, TTIs are small devices that are attached to the package and indicate the combined time and temperature history of the product by a gradual colour change. TTIs integrate time and temperature using specific enzymatic or chemical reactions that ideally have an identical rate constant to the quality or safety feature of the packed product. The consumer can compare the colour at the time of purchase with the indicated sell-by limit colour. A TTI is an elegant and user-friendly improvement that informs consumers of the expected shelf life at the point of sale. The concept could be extended to the home situation (Stollman, Johansson, and Leufven, 1994).

For fresh pasta, most food packaging material is manufactured using petroleum-based non-biodegradable polymers, and their disposal is becoming a serious environmental issue. The partial replacement of these materials with biodegradable polymers from renewable sources (i.e., biopolymers) may reduce the environmental impacts.

In a study by De Camargo *et al.* (2013), the biodegradable film was produced by blown extrusion using thermoplastic starch, Poly-butylene adipate-co-terephthalate, and potassium sorbate as an antimicrobial agent. Fresh pasta sheets were intercalated with biodegradable films (i.e., film-pasta-film-pasta-film), sealed in low-density polyethylene bags, and stored at 10° C. Microbiological analysis and films characteristic observations were performed before and during storage. Antimicrobial migration to the pasta was also evaluated. The films had mechanical properties suitable for use as active packaging for fresh pasta. The active films controlled the microbial growth, thereby increasing the shelf life of the fresh pasta. The active packaging was formulated using cassava starch and the study results indicated that the biodegradable films generated from a blend of starch, Poly-butylene adipate-co-terephthalate,

glycerol, and potassium sorbate had mechanical properties and a water vapour permeability suitable for the active packaging of fresh pasta. The biodegradable films increased the product shelf life, and the amount of potassium sorbate that migrated to the product was lower than the maximum concentration allowed by Brazilian legislation regarding fresh pasta (De Camargo Andrade-Molina et al., 2013).

A further study by Sousa *et al.* (2016) evaluated the shelf life of fresh lasagna pasta intercalated with extruded films made from blends of rice flour, Poly-butylene adipate-co-terephthalate, glycerol, and potassium sorbate, and quantified the concentration of sorbic acid that migrated from the films into the product. Poly-butylene adipate-co-terephthalate is a biodegradable aliphatic, aromatic copolyester obtained by chemical synthesis, commercialised under the name Ecoflex® (BASF, Germany). It also has the potential to combine with starch and other biodegradable polymers to form polymeric blends (Shi, Ito, and Kikutani, 2005). Rice flour obtained from the broken grains may help lower the cost of films made from biodegradable polyester blends since the cost of Poly-butylene adipate-co-terphthalate is almost 15 times higher than that of rice flour. From a food safety perspective, antimicrobial substances used to develop active films should be approved due to migration. Substances that are recognised as safe (GRAS), such as sorbic and propionic acids and their salts, have been incorporated into polymers used in the production of antimicrobial films. In this study, the use of biodegradable films containing potassium sorbate was demonstrated to be an alternative that guaranteed the microbiological safety in fresh lasagna pasta and avoided the excessive consumption of food additives. Potassium sorbate added to biodegradable films was effective against yeasts and moulds, coliforms at 45 °C, *S. aureus* and *B. cereus*, as well as reducing the growth rate of the psychrophiles. The shelf-life limit of the studied pasta was determined by the appearance of surface slime produced by the psychrophilic microorganisms from a count of $2.0x10^3$ CFU g^{-1}. There was a reduction in moisture content in the pasta containing sorbic acid, proportional to the increase in water migration to the films. The incorporation of 3 g 100 g^{-1} potassium sorbate was recommended to produce a biodegradable film from Poly-butylene adipate-co-terephthalate, rice flour, and glycerol, as a substitute for a synthetic plastic film to package fresh lasagna using intercalation, since this concentration was sufficient to adequately extend the shelf life. Figure 4-6 presents the regression graphs for the microbiological evaluations of psychrophiles (a), yeasts and moulds (b), coliforms at 45 °C (c), and *S. aureus* (d), in fresh lasagna pasta intercalated with polyethylene films and with biodegradable films containing 0 g, 1 g, 3 g and 5 g 100 g^{-1} potassium sorbate (C, MA0, MA1, MA3, and MA5, respectively).

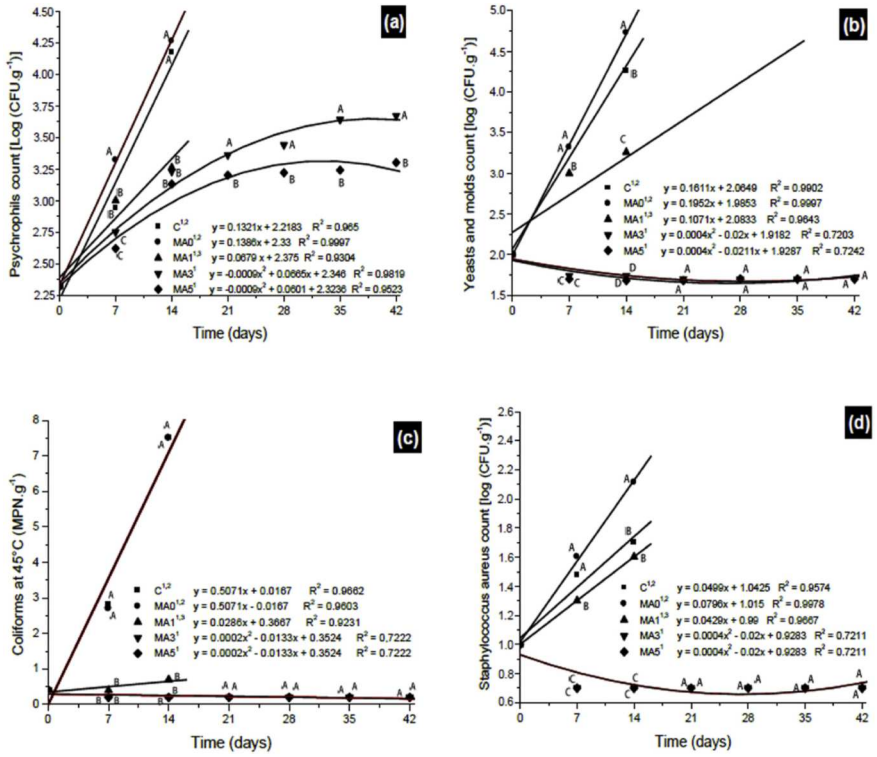

Figure 4-6: Regression graphs giving the microbiological evaluations of (a) psychrophiles, (b) yeasts and moulds, (c) coliforms at 45°, and (d)* Staphylococcus aureus*, in fresh lasagna intercalated with polyethylene films and with biodegradable films (Sousa et al., 2016).

A study by (Kõrge *et al.*, 2020)) used a tannin-rich chestnut extract to enhance the antioxidant and antibacterial properties of chitosan-based film materials used for packing and storing filled fresh pasta. Tannin extracts obtained from chestnut wood (*Castanea sativa*) are prominent antioxidants and are therefore potentially promising as active components in chitosan-based film materials. In this study, chitosan-based active film materials, incorporated with chestnut extract, were used to prepare sachets for packaging fresh pasta. Chitosan is a biocompatible, biodegradable, non-toxic, and partly deacetylated derivative of chitin. Its characteristics largely depend on its degree of deacetylation and molecular weight (Boric et al., 2018). Chitosan also has a film-forming capacity that makes it one of the most favourable biopolymers for the preparation of environmentally friendly films intended for food packaging and protection. The inherent antioxidant and antimicrobial properties of chitosan-based films may be significantly improved by incorporating plant-based active components, helping extend the shelf life of perishable foods. The antimicrobial activity of the film and its interactions within the active chitosan-chestnut extract in the sachets used to package the pasta

during its shelf life was evaluated. The results indicated a retrogradation process within the pasta, producing a hardened texture after nine days of preservation in the chitosan-chestnut extract sachets. Changes in a_w revealed the total phenolic content concentration or dilution, respectively, in the pasta and the chitosan-chestnut extract sachets. Nevertheless, the pasta remained free of microbial spoilage in the chitosan-chestnut extract sachets throughout the 60-day shelf life. The active packaging ingredients did not affect the food surface or its microstructure. Despite the antimicrobial properties, the fresh pasta shelf-life was not achieved during the 60 days due to its unacceptable texture. Therefore, further research is required to improve the chitosan-based film permeability properties and/or to investigate alternative food products with a higher natural moisture barrier (Kõrge et al., 2020).

4.4.3 Bio-Packaging Based on Pure Cellulose Coupled with Cellulose Acetate

A study by Bugatti, Viscusi, and Gorrasi (2020) applied active packaging based on pure cellulose, coupled with cellulose acetate coated with layered double hydroxide, to host 4-hydroxybenzoate (listed in EC-Directive 10/2011) as an antimicrobial agent. The active packaging's capacity to inhibit *Pseudomonas* spp*., E. coli*, *Salmonella* spp. and lactic acid bacteria was evaluated, as well as global migration using three different food simulants (acetic acid [3% v/v], ethanol [50% v/v], and vegetable oil), demonstrating, in compliance with EU regulations concerning migration limits, the suitability of the prepared packaging to be employed as food contact material. Ready-to-eat, cooked, tomato pasta was packaged in active trays and in uncoated (control) packaging for up to 30 days at 4°C. Organoleptic characteristics, mould evolution, total mesophilic aerobic counts, *Enterobacteriaceae*, lactic acid bacteria*,* and *Pseudomonas* spp., expressed as colony-forming units per gram (CFU/g), indicated significant 4-hydroxybenzoate activity in increasing the shelf life of the ready-to-eat pasta. The active coating was based on a food-grade resin filled with layered double hydroxide nanofiller hosting 4-hydroxybenzoate as an antimicrobial, listed in EC-Directive 10/2011/EC (14/01/2011). In the active trays, in-vitro inhibition of *Pseudomonas* spp., *E. coli*, *Salmonella* spp. and lactic acid bacteria was analysed, and significant antibacterial activity was detected, in particular concerning *Pseudomonas* spp. and *Salmonella* spp. (Bugatti, Viscusi and Gorrasi, 2020).

4.5 The Optimal Storage of Chilled Pasta

According to the Decree of the President of the Republic no. 187 (09/02/2001) (Italian regulation for the production and marketing of flour and pasta), pre-packed fresh pasta must 'be stored, from production to sale, at a temperature not exceeding + 4 °C, with a tolerance of 2 °C'.

According to Italian law, the denomination of fresh pasta is given to products that have an a_w level below 0.97, have been submitted to one heat treatment equivalent to pasteurisation, and stored at a maximum temperature of 6°C in all its commercial steps (from initial storage to the distribution chain, until its final destination). For fresh pasta, refrigerated storage is compulsory; temperature abuse at about 10°C has been found under real conditions (Zardetto, 2017).

Zardetto (2005) reported that the reliability of MAP in fresh pasta depends on rigorous temperature control. Temperature is rarely constant during the distribution chain, which includes several processes such as transport, warehouse storage, picking, and house storage, each having unique conditions. The temperature has been established as a critical factor affecting the food shelf life. Most degradation is Arrhenius-like, i.e., the higher the temperature, the faster the rate of degradation (Pedro and Ferreira, 2006). Nevertheless, several food reactions involved in matrix phase changing may significantly cause deviations from Arrhenius behaviour concerning temperature (Labuza and Riboh, 1982).

The influence of slight temperature variations in refrigerated products is frequently underestimated. Moreover, there is a tendency to assume that small variations (e.g., 1–2 °C) have an insignificant effect on product preservation. Figure 4-7 provides the values (in hours) required for a colony of *Penicillium aurantiogriseum* mould to reach a diameter of 3mm, a size that is visible to the human eye and therefore results in the product being rejected. Under optimum storage conditions (4 °C) and in MAP, the colony is visible after 60 days, reducing to 22 days with a constant storage temperature of 6 °C with a reduction in the product shelf-life of over 50% (Zardetto,2005).

	Temperature °C				
Testing conditions	**4**	**5**	**6**	**15**	**25**
w/o MAP (hrs)	164	134	115	55	31
MAP (hrs)	1452	800	384	106	52
fonte: Zardetto 2004, Tecnica Molitoria					

Figure 4-7: Product rejection time (Zardetto, 2004).

Fresh egg pasta packaged in MAP has a long shelf life, from 30 to 70 days (Pagani et al.,2007), but very few published data are available on this important aspect. Several studies have shown that microbiological stability of the industrial pasteurised product packed in MAP is not the most critical factor limiting its durability (Costa et al., 2010; Lucera et al., 2014; Sanguinetti et al., 2011). Determine food structure and properties, both for fundamental research and as an online sensor for process monitoring. NIR (Near Infrared Spectroscopy Analysis) has been applied to egg pasta, indicating its high potentiality for discriminating fresh pasta submitted to different processing steps. Zardetto (2005) reported that FT-NIR (Fourier Transform- Near Infrared Spectroscopy Analysis) could be used to rapidly determine the $F_{70/10}$ value in fresh egg pasta submitted to different pasteurisation processes. Zardetto and Dalla Rosa (2006) also reported NIR spectroscopy's potential application in the discrimination of fresh egg pasta obtained using two different production methodologies, extrusion, and lamination. NIR analysis was also used to determine egg content and moisture in dried pasta (Fodor et al., 2011; Temmerman et al., 2007) and to the prediction of the starch gelatinisation index in fresh egg pasta (Zardetto, 2004). Nevertheless, only a few studies have addressed the NIR spectroscopy application to monitor chemical and physical indices evolution during food storage.

The aim of the study conducted by Zardetto et al., (2021) was two-fold: 1) to assess the main modifications occurring in physical and chemical characteristics monitored with traditional analytical methods during storage at different temperatures and 2) to evaluate the suitability of FT-NIR spectroscopy as a rapid non-invasive approach for monitoring the main changes occurring during storage in fresh egg pasta.

The study demonstrated that several physico-chemical characteristics in fresh egg pasta, such as starch gelatinisation, a_w, freezable water, and hardness, were influenced by different storage temperatures. The lowest temperature (0°C) resulted in the formation of a less compact protein-starch network, impacting several pasta characteristics. Water activity and freezable water decreased significantly during storage time, producing a reduction in water mobility in the system and suggesting an increase in physico-chemical interactions between water and hydrophilic compounds, represented by egg, wheat grain proteins, and starch. This result is significant concerning fresh egg pasta stability, indicating that it depends not only on water content and its thermodynamic status but also on water compartmentation in the matrix and its mobility decrease. Analysis using FT-NIR confirmed several modifications involving the starch-water-protein matrix, with consequent changes in several macroscopic pasta properties (e.g., hardness, water absorption during cooking). The approach used in this study may be applied, combined with sensorial assessment, to evaluate which of the studied parameters

affects sensory acceptability (such as texture and/or cooking behaviour). Since consumer experience often fails to correlate adequately with physico-chemical determinations, further research is needed to carefully evaluate sensory aspects to understand which of the studied parameters is most influent in determining acceptability limits related to the shelf-life of fresh pasta, based on consumer dissatisfaction (Zardetto et al., 2021).

CONCLUSIONS

This thesis has produced the following conclusions:

1. The quality and value of pasta are derived from its raw ingredients. Innovative raw ingredients could function as components that increase the shelf life of pasta. For example, bioprotective cultures or ingredients such as *S. platensis* biomass and turmeric, are not only beneficial from a microbiological perspective but also increase the organoleptic and functional characteristics of the product.
2. In Italy, the manufacturing process of fresh pasta has evolved by altering and optimising several production phases to improve product characteristics. In particular, a study of the existing literature has confirmed that the standard processes of heat treatment using pasteurisation, partial drying, and cooling are the best methods of ensuring product shelf-life. Research into fresh pasta products has consistently verified that using heat treatment and controlled packaging and preservation processes at temperatures below 6 °C guarantees the safety of a product.
3. Currently, the use of several synthetic preservatives is largely unjustified.
4. The environmental problems associated with non-recyclable packaging means that the process where research should hasten the times is the packaging of fresh pasta. Food packaging materials account for almost 26% of plastics produced worldwide, about 90% of which becomes waste after only one use. Fresh pasta must be packaged in single-use plastic, and since this is a non-recyclable material, it represents a genuine environmental dilemma. As the demand for packaging rises, the development of improved food packaging options that are decoupled from limited resource use becomes increasingly important. Based on circular economy principles, food packaging materials should be reusable, recyclable, or compostable by design and meet the microbiological requirements for preserving the shelf life of assorted products.
5. Fresh pasta is an Italian product that has become increasingly popular in the European market, with a strong export trend. However, Italian laws regarding pasta manufacture and sales have not always kept pace with changes in technology and the market.

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