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FINAL DISSERTATION

OPTIMIZATION OF FUNCTIONAL PASTA BASED ON DURUM WHEAT SEMOLINA

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To the loves of my life:
my Mother and my Father

What the father has been silent,
takes the word in the child;
and I have often found that the son
other was not,
if not the naked secret of his father.
Friedrich Nietzsche

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Chapter I

1.1 Origin of pasta

If one could follow the scent of pasta through the millennia, it's no exaggeration to say that the entire history of civilization would be traced. The trail would span cultures and continents ranging from Asia, the Mid-East, Europe, the Americas, and parts of Africa, and reach back at least 3,500 years ago. Yet, in spite of all the myriad of forms it has taken and the countless contexts in which it has appeared, today it is almost universally associated with Italy - though historically, this has not always been the case. In fact, it is only in the last century or so that many regions of northern Italy - including Veneto, Lombardy, Liguria and the Piedmont - have embraced pasta as an essential part of their cuisine. But, there are many aspects of the history of pasta that are surprising.

The early history of pasta does not begin in Italy, but rather in the Shang dynasty in China (1700-1100 BC) where some form of noodles is known to have existed - made with either wheat or rice flour. Pasta also appears to have been a feature in the diet of ancient Greek civilization, flourishing in the first millennium BC. In fact, the word "lasagne" comes from the Greek term "laganon," which consisted of strips of dough made with flour and water.

In the early twelfth century, the Arab geographer Al-Idris reported the existence of the production of dried pasta in Sicily, which was distributed throughout the Mediterranean area, thanks to its long-term preservation properties. Its production was strictly a "family" business, and it was not until the mid-1300s that we have documented evidence of the first artisan pasta shops. Despite its Sicilian origins, the pasta-making industry expanded and flourished in Naples.

Together with the production of dried pasta,the activity was mainly concentrated in southern Italy and Ligurian coasts, thanks to a microclimate that facilitates the drying of the product. We also have documented evidence of small pasta-making businesses that supplied fresh pasta to local demand. These artisans were active in central- northern Italy, where durum wheat semolina was more difficult to find. This explains why the majority of fresh pasta today is still produced with common wheat flour, often with addition of eggs, an important ingredient both from the point of view of nutrition and technology. The vermicelli pasta -makers gave way to factories in the early 1900s, when the first electric motors appeared. Surely the introduction of the continuous press for the kneading process around the 1930s represented a decisive innovation in the development of this product. The production process, which had been discontinuous until this time, became continuous, giving rise to greater productivity and greater cost-savings (Portesi,1957; Serventi e Sabban, 2000).

1.2 The regulatory framework

Pure and simple: wheat and water are what pasta is made of….durum wheat, to be more precise. Durum wheat, deriving from the Latin term Tricutum Durum ("durum" means "hard" in Latin), is grown in many regions of the world, including the Mediterranean countries, North America, the former Soviet Union and Argentina. In Italy, it grows mainly in the southern regions - most notably, in Puglia, which is known for producing some of the finest pasta in the world. One of the most important qualities of durum wheat is that it contains more proteins than common wheat. These proteins, especially gluten, are essential for producing high-quality pasta - that is, pasta that will remain firm, or "al dente," when cooked.

In 1967, a law was passed in Italy requiring that only durum wheat be used in the making of all its dried, store-bought pasta. This guarantees a certain level of quality and nutrition for any industrially produced pasta made in Italy.

According to Italian law, article 6 of Presidential Decree N° 187/ 2001 - Regulation for the revision of laws concerning the production and sale of milling products and pasta- pursuant to Article 50 of Law N° 146/1994, are called “Durum wheat semolina pasta” and “low grade durum wheat semolina pasta” the products obtained by drawing, rolling, and drying a dough prepared respectively and only:

a) with durum wheat semolina and water

b) with low grade durum wheat semolina and water

“Durum wheat whole-meal semolina pasta” is the name to be used for the products obtained by drawing, rolling, and drying dough prepared exclusively with durum whole-meal semolina and water.

The above-mentioned decree also indicates what should be the characteristics of durum wheat semolina and low grade durum wheat semolina used for pasta.

“Durum wheat semolina” or simply “semolina” is the name to be used for the rough, granular product obtained by grinding and sifting durum wheat, which has had any impurities and extraneous bodies removed.

“Durum wheat whole-meal semolina” or simply “whole-meal semolina” is the name to be used for the granular product directly obtained by grinding durum wheat which has had impurities and extraneous bodies removed.

Durum wheat milling products destined for sale must have the following characteristics:

Type and denomination	Maximum humidity (%)	Per hundred parts of dry substance		
		Ash		Minimum protein (nitrogen x 5.7)
		minimum	maximum	
Semolina *	14.50	-	0.90	10.50
Low grade semolina	14.50	0.90	1.35	11.50
Durum wheat wholemeal semolina	14.50	1.40	1.80	11.50
Durum wheat flour	14.50	1.36	1.70	11.50

*Particle size, to be tested using calibrated vibrating sieves: with a mesh of 0.180 mm, no more than 25% may pass through the sieve.

Table 1.1 Characteristics of durum wheat milling products according to Italian Law

Pasta destined for sale must have the following characteristics:

Type and denomination	Maximum humidity (%)	Per hundred parts of dry substance			Maximum Acidity (degrees)*
		Ash		Minimum protein (nitrogen x 5.7)	
		minimum	maximum		
Durum wheat semolina pasta	12.50	-	0.90	10.50	4
Low grade durum wheat semolina pasta	12.50	0.90	1.35	11.50	5
Durum wheat wholemeal semolina pasta	12.50	1.40	1.80	11.50	6

* The level of acidity is the number of cubic centimetres of normal alkaline solution required to neutralise 100 grams of dry substance

Table 1.2 Characteristics of Dried Pasta according to Italian Law.

Therefore the Italian law, Paragraph 4, article of the article mentioned above, does not allow, in any case, the use of soft wheat flour or wheat granites in the production of dried pasta, and not even, as a result, mixtures of semolina and flour, apart from the pasta intended for being marketed and exported to other countries of the European Union or other contracting states on the basis of the Agreement on the European Economic Area .

A very important is article 12 , paragraph 1, which establishes a specific process for the production of pasta with different flours, that is to say " The manufacture of milling products and pasta with requisites different from those set out in this Decree and the relevant Regulatory Body is permitted when the products are destined for exportation to other Member Countries of the European Union or other states which have signed the European Economic Area Agreement, provided that they are not harmful and the manufacturer sends in advance a registered letter to the Agriculture and Forestry Ministry. This letter shall state the type and quantities of goods manufactured, the differences between its characteristics and those set out in this Decree, the type, quantity, and characteristics of the raw materials which are to be used to manufacture it, when work is due to begin and how long the manufacturing process is due to last, and the country to which the goods are to be exported." (https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/6230).

The legislation that we have shown for Italy is similar to that of other European countries (France and Greece). All other countries in the world allow wheat flour, alone or mixed in various proportions with semolina, for the production of dry pasta

1.3 Italian pasta in the world

The total world production of pasta increases annually and from this point of view Italy grabs foreign markets by the throat. Exports of pasta have being growing for nine consecutive years : in 2013it leaped up to5.4 percent. This is a business strategy for Italy if one considers that it is an effective "Multiplier" of Italian exports of all those products that the gastronomic culture associates with pasta, such as tomato paste, olive oil, Italian cheese and those ingredients that contribute to enrich and accompany this dish.

According to Aidepi, the producers' association, in 2013 Italy exported 1.9 million tons of pasta (+ 5.4%) for a value of over 2 billion euro and an increase of about 4 percent. Exports now account for around 60%, in volume and production: a percentage increase for the contraction of the domestic market. The declining consumption in Italy (-1% to -2% in volume and turnover according to Iri) eventually contained the overall growth.

Italy remains the greatest world eater (and producer) of pasta: we consume on average 26 kilograms per capita per year, Venezuela ranks second with 12.7 kilos, followed by Tunisia with 11.7 kilos which is the first African country, Switzerland with 10.1 kilograms and the United States with an annual average of 9 kilos per person stand out as the strongest growing consumer (http://www.ilsole24ore.com/art//2014-04-22/export-pasta-crescita-9-anni-063728.shtml?uuid=ABuzkrCB).

The British prefer spaghetti bolognese, in Spain our lasagne is greatly appreciated, spaghetti in the US, followed by lasagne and macaroni and cheese. And the Italians? Another survey of Pasta Trend says the format that most seduces Italian palates who eat pasta at least once a day and even more in the south are macaroni, preferred by 56% of respondents, used frequently for their versatility, followed by inevitable spaghetti (12.1%), chosen mostly by men, preferably with sauce and al dente, and penne (7.2), loved by young people, combined with pesto sauce and vegetable sauce. Fusilli, butterflies and shells, as well as tortellini, linguini and gnocchi ranked third.

In a few years the market leader Barilla (35%), which also acquired the brand Voiello (3%), followed by companies located in the south as Divella (8.4%) and Garofalo (6%), thanks to communication, widespread distribution and promotion have captured the attention of consumers on durum wheat pasta produced in southern Italy. (http://www.mark-up.it/la-pasta-cresce-grazie-allexport-ed-e-il-pasto-principale-non-solo-degli-italiani/).

1.4 Raw Materials for Pasta

Pasta is a popular wheat-based food worldwide, due to its convenience, cost, palatability and nutritional value. Durum wheat (Triticum turgidum L. var. durum) semolina is the preferred raw material for the production of high quality pasta, due to its unique colour, flavour, and cooking quality. Therefore the quality of the pasta depends on the quality of the semolina, which in turn depends on the quality of durum wheat from the grinding of which pasta was obtained.

The quality of durum wheat includes:

- The yield of semolina (quality milling);
- The quality of the pasta produced (pasta-making quality)

1.4.1 Structure and chemical composition of durum wheat

Wheat grain is a complex structure formed by different tissues (Fig. 1) that have distinct functions and biochemical compositions : bran, endosperm and wheat embryo/germ.

The starchy endosperm (80–85% of grain) is mostly made of starch and proteins, while most of the fibre, vitamins, minerals and antioxidants are concentrated in the outer layers (12–17% of the grain) and the wheat germ (3% of the grain) (McKevith, 2004; Pomeranz, 1988).

Wheat endosperm is surrounded by several adhesive outer layers (including pericarp, testa, and aleurone layer). After milling, a composite material that contains all these different layers is obtained and is commonly called bran.

In wheat, aleurone consists of a mono-layer of cubic cells with cell walls being 6–8 mm thick (Stevens, 1973; Evers & Bechtel, 1988). From a nutritional point of view, aleurone is a source of dietary fibre, minerals, B-vitamins, proteins, phytate and phenolic compounds. In relation to the total amount in the wheat kernel, over 60% of the minerals, 80% of niacin and 60% of pyridoxine are located in the aleurone.

Compared to the endosperm, aleurone proteins are high in lysine, which is the first limitingessent ial amino acid in wheat (79.Betschart, 1988).

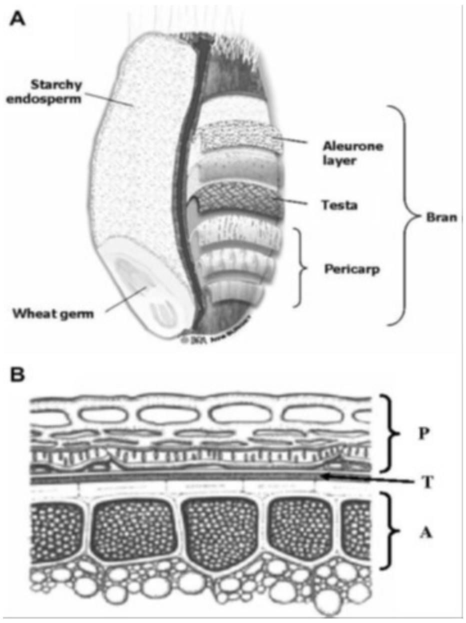

Fig. 1. Structure of wheat grain (1) and of the constitutive layers of wheat bran (2). P: pericarp (fibrous and porous: empty cells); T: testa (hydrophobic layer); A: aleurone (full cells with cellular content rich in proteins, lipids and minerals). Adapted from 6.Surget and Barron (2005).

The main constituents of endosperm cells are starch granules and proteins, but small lipid droplets can be seen associated with the protein. The starch granule in wheat are frequently classified into two size groups: large, elliptically shaped A granules (30-40 µm in dia) and small, round B granules (<10 µm in dia) which are formed later in the grain filling process.

Starch grains are enclosed in the thin layer of adherent protein and located within a protein matrix which fills the individual cells of the endosperm to varying degrees.

Starch in wheat consists of two different molecules of glucose, amylose and amylopectin. Amylose is a linear chain composed by up to 5000 glucose units stuck together by α-1,4-bondings and amylopectin is a highly branched chain composed by up to one million glucose units stuck together with α-1,4-bondings and α-1,6-bondings.

The highest content of protein is observed in the cells of the subaleurone layer of the endosperm. The closer to the center of the grain, the lower the protein content.

The protein content of wheat grains may vary between 10% and 18% of the total dry matter.

Proteins can be fractioned into albumin (water-soluble), globulin (soluble in salt solution), gliadin (extractable in aqueous ethanol solution) and glutenin (soluble in dilute acid), according to the protein classification of Osborne (1907). Albumins and globulins are minor fractions (20%) as compared to monomeric gliadins and polymeric glutenins (80%).

Storage proteins in wheat are unique because they are technologically active in the formation of gluten in dough (11.Hoseney, 1986a).

Gliadin is a monomer protein that is stucked together with peptide bonds and disulphide bridges (Fennema, 1996). When these disulphide bridges come in contact with water, as they do in pasta dough manufacturing, they will break and the gliadin molecule will be unfolded.

The ends of the former disulphide bridges are revealed and can create new bridges with the glutenin, in other words, cross-link with glutenin. These new bridges make the gluten network stronger.

Glutenin is a larger polymer protein that is composed of low-molecular weight and highmolecular weight subunits that are linked together with disulphide bridges and cross bondings (Fennema, 1996). These disulphide bridges will also break in contact with water and reveal their ends and make it possible for new bridges to arise. When both gliadin and glutenin are hydrated with water new disulphide bridges will be found between them and this is the process and interaction that will compose the gluten network that's so important in pasta manufacturing.

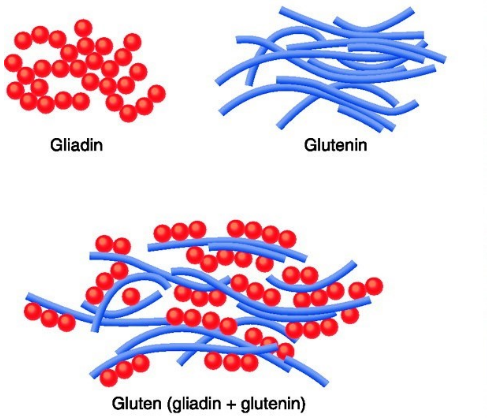

Fig. 2. Formation of gluten in the dough

1.4.2 The evolution of wheat variety

Since the beginning of '900 was made a frantic selection of more productive varieties: in 1915, through a careful genealogical selection Nazzareno Strampelli obtained a wheat seed which he named "Senatore Cappelli" in honour of the promoter of the first agrarian reform in unified Italy. The original intent was to increase productivity. Over time, however, the intent was relegated to a low priority and today, the so-called genetic improvement is subservient to the interests of the food industry, for technological and economic purposes, and requires varieties that produce more and more flour which is rich in gluten without worrying that this leads to the expiration of the organoleptic qualities and health benefits.

In the '70s, those same seeds were bombarded with gamma rays and consequently genetically mutated, and produced wheat with a shorter stem and less risk of "entrapment". These were the years in which the intensive use of chemical fertilizers was beginning, ensuring greater yield but responsible for an increase of the fragility of the stem. The new nano wheat that reduced the risk of entrapment and responded very well to chemical fertilizers, was called "Creso", and still today, together with its "descendants" is one of the dominant varieties on the market.

The small size of the "modern" plants made them less competitive against weeds and the answer was, of course, an increase in the use of herbicides. Over time, the search for and the selection of varieties of hard and soft grains, increasingly resistant to herbicides and pesticides, which were more and more productive especially in terms of protein and starch in expense of trace elements and fibres followed

So conceptually an old wheat cultivar can be defined as a not nano and unregistered genotype, while a modern one can be considered a nano or semi-nano registered genotype. It is noteworthy that modern breeding programs for genetic improvement have been primarily focused on yield improvement, and on the improvement of disease and pest resistance rather than nutritional and functional characteristics.

One of the characteristics that mostly have resulted in the affirmation of modern varieties, besides its production, is the high gluten content, which provides excellent technology feature (resistant to cooking pasta). Unfortunately the best technological qualities of flour and durum wheat flour are not positively correlated with nutritional properties; in recent years, in fact, some epidemiological studies have shown that the presence of high gluten technological quality, produces a sensitization of this protein. The ingestion of gluten causes an immune reaction in people with celiac disease that results in intestinal mucosal lesions able to cause a change in absorption (Vader et al., 2003).

In the semolina obtained from old varieties the amount of gluten is not always lower compared to the new ones (Ghiselli et al., 2010) but differs greatly in quality. Indeed gluten, present in older varieties, contains less toxic epitopes (particular sequences rich in proline and glutamine) namely the amino acid sequences recognized by lymphocytes of people with celiac disease (Van den Broeck. et al, 2010).

Wheat "antique" are rich in a wide variety of biologically active phytochemicals such as polyphenols (flavonoids, lignans, isoflavones) carotenoids, tocopherols and fiber, which play important functions pharmacological activities including anti-tumor, anti-inflammatory, immunosuppressive, cardiovascular, antioxidant and antiviral (Dinelli et al. 2007)

Recent studies on the health benefits of functional wheat products have become increasingly more focused on the importance of introducing phytochemicals through the use of different varieties. As a consequence, there is a renewed interest in the ancient varieties, particularly with regard to potential nutraceutical qualities (Adom et al.,2003; Dinelli et al., 2007; 12 Dinelli et al., 2011; Heimler et al.,2013). In addition to the nutritional and health characteristics sensory details, cannot be neglected.

The pasta, for example, has a more intense and rich aroma. The taste has more character and the nuances conferred by the different flours stand out clearly. A taste that exceeds the standardization imposed by industrial food.

1.4.3 The evolution of the milling technique

Wheat milling is an ancient craft that dates back thousands of years to the dawn of civilization. Milling technology gradually evolved from the mortar and pestle used by primitive cultures 10000 years ago, to the invention of the millstone in Roman times, Millstones remained in vogue until late in the 19th century, when the invention of the purifier, plansifter and horizontal roller mill revolutionized the milling process.

The main purpose of milling is to completely separate bran and germ from the mealy endosperm and to thoroughly pulverize the mealy endosperm into middlings, semolina and flour. This is achieved through the shearing and scraping action of millstones (in windmills and watermills) or mill rollers (in a modern flour-milling factory).

Stone mills are the oldest attrition mills used for making whole grain flours, which simultaneously use compression, shear, and abrasion to grind wheat kernels between two stones and produce a theoretical extraction rate of 100% (Kihlberg et al., 2004).

The lower or bed stone is fixed to the floor of the milling room. The upper stone rotates on a central axis. Wheat is poured from a bin in the upper storey of the mill into a hole (the eye) in the centre of the upper stone. In this way the wheat is positioned centrally between the two millstones. The upper-side of the lower stone and the under-side of the upper stone are fluted The distance between the two stones is in the centre about 1 cm, in the periphery less. During the first few rotations the grains are cut and broken by the edges of the flutings, which work like scissors. Thereafter, the milling good goes in a spiral movement towards the periphery of the stones, where the next step of the process takes place: the inner part of the grains, i.e. the mealy endosperm, is separated from the outer part, i.e. the bran. The large and medium-large endosperm particles that are released during this process are called semolina. The diameter of these particles can vary from about 300 µm to 750 µm (Hoseney RC, 1986).

It have thus, by means of stone mills, semolina darker, more rich in fiber and ash, elements of which are rich in the outer skins and the aleurone layer.

A negative characteristic of the stone mills is that they generate a lot of heat by friction. This can cause considerable damage to the starch, protein, and unsaturated fatty acids in comparison with other milling techniques (Prabhasankar and Rao, 2001).

Around 1850 the idea arose, first in Switzerland and later in Hungary, to use porcelain or steel rollers instead of millstones. The process of roller milling involves separation of the endosperm from the bran and germ followed by gradual size reduction of endosperm (Ziegler and Greer, 1971). Rollermilling comprises several grinding steps, each being followed by a sifting operation. Broadly speaking, the grinding stages may be grouped into two successive sytems: the break system and the reduction system. Primarily, the break system aims to bring about a virtual separation between the bran and the mealy endosperm.

It is carried out with the aid of corrugated or fluted iron rollers, called break rollers. They operate in pairs, revolving in opposite directions and at different speeds. The process includes successive milling steps, in which the corrugations of the rollers become progressively finer and the milling gap (distance between the rollers) smaller. Passage through the rolling mills is alternated with sifting phases carried out by the plansifters and the purifiers. The plansifters, which are made up of superposed oscillating sieves of decreasing mesh size, have the task of separating the ground material according to particle size. The product that comes out of the plansifters is then conveyed, by pneumatic transport devices, to the next phases of the milling process. End products of the break system are mainly coarse offals (bran), germs, coarse endosperm particles (semolina, 300–750 μm and middlings, 125–300 μm) and a certain amount of flour (diameter less than 125 μm).

The reduction system aims to reduce coarse endosperm particles (semolina and middlings) to flour fineness. It involves a series of reduction steps in which, as in the case of breaking, the rollers are set progressively closer at each successive processing step. End products of the reduction system are mainly: fine offals (shorts) and fine endosperm particles (flour) (Russell and Hartley, 1975).

Good purification is essential since bran or other dark particles are readily visible in the semolina. Commercial mills convert 65-75% of the wheat to semolina with 8-12% flour with the remainder being bran and embryo.

There are several noteworthy advantages of making whole grain flour from roller mills as opposed to stone mills. First, the amount of grinding and reduction at each roll can be adjusted to accommodate variations in raw materials, which makes roller milling both economical and flexible (Posner and Hibbs, 2005). Second, the use of selective corrugations and differential speeds subjects the endosperm fraction to minimal shear and compressive forces during the grinding and reduction, which allows less heat to build on reduction rolls and results in less destruction to chemical components in the flour (Prabhasankar and Rao, 2001). A third advantage of making whole grain flours from roller mills is that wheat bran and germ can be separated from the endosperm fraction and subjected to further processing such as heating or fine grinding to affect the storage or functional properties of the flour (Posner and Hibbs, 2005).

1.4.4 Technologies for the use of alternative raw materials

Nowadays, consumers are becoming increasingly health conscious and are demanding natural, wholesome, health-promoting foods. Public concerns about the health effects of dietary fiber have prompted a fast-growing market of high fiber and calorie-reduced products. The term dietary fibre was first coined more than fifty years ago (Hipsley,1953). However it is only recently that an international recognised definition of what constitutes a dietary fibre has been reached (Codex, 2009). The Codex Alimentarius Commission’s Committee on Nutrition and Foods for Special Dietary Uses defined dietary fibre as ‘‘carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans”.

The fibres are conventionally classified in two categories according to their water solubility: IDF, insoluble dietary fibre (cellulose, part of hemicellulose and lignin) and SDF, soluble dietary fibre (pentosans, pectins, gums, mucilage).

The main physiological effect of insoluble fibre is the improvement of gut peristalsis, while the soluble fibre has multiple functions as represents a good substrate for some lactic bacteria and Bifidobacteria strains, which are beneficial for gut health (prebiotic action) (Grizard & Barthomeuf, 1999), it is able to control glycemic index (Tudorica et al., 2002), and it reduces plasmatic cholesterol (Brown et al., 1999).

A very important source of dietary fiber in industrialized countries is wheat bran which is collected as a co-product from the milling process. It represents around 15% (w/w) of the wheat grain and is composed of the grain’s outermost layers: outer and inner pericarp, testa, hyaline and aleurone layers, with remaining adherent starchy endosperm. This milling fraction, due to its high content of dietary fibres and phenolic compounds, has raised nutritional interest. Wheat bran typically contains 36.5–52.4% total dietary fiber, more than 90% of which is water insoluble fiber (Vitaglione et al., 2008).

The recent research demonstrated that lipophilic compounds from bran were cancer preventive agents (L. Liu et al., 2012; L.M. Liu et al., 2012).

Furthermore, wheat bran has higher vitamin and mineral contents than endosperm (Esposito et al., 2005), and high antioxidant content (Li et al., 2007; Liyana et al., 2007; Verma et al., 2008). These characteristics give wheat bran very interesting nutritional properties, and so the addition of bran to different types of foods has been studied extensively (Chillo et al., 2008; Gómez et al., 2011; Robin et al., 2011; Shenoy and Prakash, 2002).

Most recom- mendations (Gray, 2006; National Research Council, 2005) for adults suggest a fibre intake above 25 g/day.

An effective way to promote intakes of dietary fiber is to use dietary fiber supplemented foods. One of the ideal fibre-carrier foodstuffs is pasta. Pasta products are becoming popular in current lifestyle because they are healthy, tasty and convenient for transportation and preparation. Pastas, in general, are considered to have low glycemic indices and, correspondingly, produce low postprandial blood glucose and insulin responses (Jenkins et al. 1983; Granfeldt et al. 1991). The addition of increasing levels of fiber in a long way improves the health status of vast majority of health conscious population who are the main target of this study. However, it is difficult to formulate such pasta to which is added a high level of fiber rich ingredients because they adversely affect color, texture, flavor and taste of the supplemented foods (Onwulata, 2008; Robin et al., 2011).

It has been shown that wheat bran or whole-wheat flour incorporation leads to crucial changes: increased cooking loss (Aravind et al., 2012; Kaur et al., 2011; Kordonowy & Youngs, 1985; Shiau et al., 2012; Sudha et al., 2012), decreased water uptake (Aravind et al., 2012; Kordonowy & Youngs, 1985; Sudha et al., 2012), decreased pasta firmness (Aravind et al., 2012; Chen et al., 2011; Kordonowy & Youngs, 1985; Manthey & Schorno, 2002; West et al., 2013), and reduced extensibility (Shiau et al., 2012) were reported.

Some technological options have been proposed in the scientific literature to solve the above-mentioned problems. The most common solution to improve the quality of high-fibre wheat foods is the addition of hydrocolloids to the dough formulation.

As a definition, 'hydrocolloid' means particles of 10 e1000 nm in diameter dispersing in water as a continuous phase (van Olphen & Mysels, 1975). Alternatively, the term refers to polysaccharides and proteins (Williams & Phillips, 2000) which are used in a variety of industrial sectors, including foods, to control and regulate such a colloidal state.

Food polysaccharides are from various natural sources; agar and carrageenan are from seaweeds, guar gum and locust bean gum from plant seeds, pectin from citrus or apple peels, xanthan gum and gellan gum from microorganisms, and chitin and chitosan from animals. These compounds are generally required at usage levels of less than 2 percent to achieve desired properties in food systems (Anonymous 2001).

The high viscosity and gelling ability at low inclusion levels are of course the main reason they are used in many food formulations. Specifically, hydrocolloids have been widely used in food products to modify texture, improve moisture retention, control water mobility, and maintain overall product quality during storage. Edwards et al. (1995) showed that the addition of xanthan gums improved pasta firmness. Whereas Tudorica et al. (2002) found that the semolina pasta containing guar gum showed reduced or similar cooking loss values when compared to the control sample. This may be due to the fact that the gum forms a network around starch granules, encapsulating them during cooking, and restricting excessive swelling and diffusion of the amylose content. Such encapsulation and integration of the polysaccharide network may strengthen the structural integrity of pasta. Instead studies carried out by Komlenic et al., (2006) showed how the addition of 0.15-0.75% carboxymethyl cellulose to pasta not only did not degrade sensory properties, but improved sensory value over regular pasta The addition of CMC into cereal-based food has shown beneficial affects on blood glucose regulation and fasting plasma cholesterol (Brennan et al., 1996) Thus consumption of dietary fibres, and thereby hydrocolloids would have the potential to improve the feeling of well-being, prevent weight gain by inducing satiety, exercises the digestive system and keep it working effectively, lower cholesterol and keep the cardiovascular system in shape, control blood sugar, and promote the good bacteria in intestine which increase the production of beneficial short chain fatty acids (SCFA).

1.5 Functional pasta

Food and its manufacture are currently attracting significant and public interest due to extensive media coverage of diet-related diseases and their influence on health and wellbeing of communities. This has led to an increased consumer demand for nutritious foods with not only a balanced calorific content, but also with additional health-promoting functions, i.e. functional foods. This trend is promoted and endorsed by governments and consumers worldwide and it is generally accepted that healthy products of high quality and convenience need to be developed through innovative multidisciplinary research programs. To date the primary concern of the food industry has been to produce and provide the consumer with safe food. However, while safety is still of paramount importance, the nutritional and caloric composition of foods is becoming equally important. In addition, if the food is to be considered a ‘‘functional food’’, it should also provide health benefits beyond basic nutrition (American Dietetic Association, 2004; Health Canada, 2004; International Life Sciences Institute, 1999; Thomas & Earl, 1994). ‘‘Functional food’’ is an ambiguous term and its meaning is continually revised by regulatory bodies. The American Dietetic Association (ADA) classifies all food as functional at some physiological level. The commonly accepted definition by several organisations for ‘‘functional foods’’ is: ‘‘foods or ingredients of foods that provide an additional physiological benefit beyond their basic nutrition’’ (International

Food Information Council, 2006; International Life Sciences Institute, 1999). Regardless of the official definition of ‘‘functional food’’ the demand for healthful food has initiated a surge of research and product development in the food industry. In order to adapt to these consumer drivers, the food industry is reformulating food products to enhance the physiological functionality of inherent nutrients or by adding bioactive compounds with largely clinically proven physiological functions.

A food product can be made functional by using any of these approaches:

1) Eliminating a component known to cause or identified as causing a deleterious effect when consumed (eg, an allergenic protein).

2) Increasing the concentration of a component naturally present in food to a point at which it will induce predicted effects (eg, fortification with a micronutrient to reach a daily intake higher than the recommended daily intake but compatible with the dietary guidelines for reducing risk of disease), or increasing the concentration of a nutritive component to a level known to produce a beneficial effect.

3) Adding a component that is not normally present in most foods and is not necessarily a macronutrient or a micronutrient but for which beneficial effects have been shown (eg, vitamin, dietary fibers antioxidant or prebiotic fructans).

4) Replacing a component, usually a macronutrient (eg, fats), whose intake is usually excessive and thus a cause of deleterious effects, by a component for which beneficial effects have been shown (eg, chicory inulin).

5) Increasing bioavailability or stability of a component known to produce a functional effect or to reduce the disease-risk potential of the food (Roberfroid, 2000).

Pasta is the most used food for functional modifications, in fact the World Health Organization (WHO) and Food and Drug Administration (FDA) consider pasta a good vehicle for the addition of nutrients. Pasta was one of the first foods for which the FDA permitted vitamin and iron enrichment in the 1940s. In the last years, grains different from durum wheat have been used (as partial or total substitutes) in production of particular kinds of ‘‘pasta” with healthy characteristics or directed to specific targets, such as people following a coeliac diet (Kasarda, 2001).

The amount of high protein flour (soybean, pea, lupine, bean, chickpea) that can be added or substituted to semolina represents a compromise between nutritional improvement of the pasta and achievement of satisfactory sensory and functional properties (Marconi & Carcea, 2001).

Functional pasta for celiac sufferers is particularly known. To produce this product is necessary to form a network that gives the right consistency in absence of gluten proteins. Lately, there has been significant study on gluten-free products involving diverse approaches. Pectin, carboxymethylcellulose, chitosan, guar flour, alginates, flour of psyllium, xanthans (polysaccharides derived from the culture of Xanthomonas campestris) are the most commonly used ingredients (http://www.europass.parma.it/page.asp?IDCategoria=553&IDSezione=0&ID=313454,2012). Moreover, a recent study observed that the addition of hydrocolloids improved the sensory quality of gluten-free functional spaghetti based on maize flour and oat bran enriched with β-glucan. The best sensory quality for both fresh and dry spaghetti was obtained by the addition of carboxymethylcellulose and chitosan at a concentration of 2% (Padalino et al., 2011). However, the addition of an aliquot of new ingredients (chickpea, amaranth flour) could decrease the quality of pasta. For this reason the incorporation of non-conventional ingredients requires to balance the formulations and to use appropriate processing conditions, for correcting the technological deterioration conferred by the addition of these ingredients.

Design and development of functional foods should not be carried out purely based on the desired nutritional function, without taking into account product properties such as colour, texture, taste and mouth feel because appearance and sensory properties of foods are the most important attributes for consumer and very often they are evaluated prior to the nutritional values.

1.6 Processes and operations in pasta industry

The technology of the pasta includes both processes which operations. The processes can not be separated from operations and vice versa. To process means the chemical and biochemical changes (and consequently also nutritional) that occur in the raw materials during the different technological operations carried out for the production of pasta. For operations means the mechanical, hydro-mechanical and electrical actions affecting all stages of production. In summary they can be summarized as follows:

- Transport of raw materials;
- Mixing;
- Extrusion;
- Pre-drying;
- Drying;
- Conditioning;
- Storage and packaging of pasta.

In the selection of raw materials, the semolina particle size distribution, the protein content and quality, and the starch properties (level of damaged starch and swelling power) are important quality determining parameters (Cubadda et al., 2007; D'Egidio et al., 1990; Delcour et al., 2000a; Delcour et al., 2000b; Dexter et al.,1983; Grant et al., 1993; Oak et al., 2006).

The particle size of the semolina is important since it has an effect on the absorption of water in the dough and therefore also secondary on the drying of the pasta (Pomeranz ,1988; Kulp & Ponte 2000). This influences the quality of the pasta.

In the first stage of the production process following durum wheat milling, semolina is hydrated and transformed into dough. During mixing, the water is distributed as evenly as possible throughout the semolina, thus promoting homogeneous particle hydration. The amount of water added to semolina generally ranges from 25 to 34 kg per 100 kg of semolina. It depends on the initial moisture content of semolina and on the final pasta shape (Dalbon et al., 1996).

The water used should not have off-flavors or smells and needs to be monitored to ensure that it is safe and free from microbiological and chemical contaminants. It should not be too hard, have a low content of sodium, magnesium, and calcium ions as these would give an unpleasant flavor and color.

After mixing (10-20 min), the mixture passes to the vacuum extruder. The extrusion unit is composed of a cylinder fitted with an extrusion screw. The screw rotation pushes the dough towards the head press on which a die is set, where it takes its final shape. The vacuum helps to minimize the oxidation of pigments, reduce enzymatic and oxidative decomposition reactions, and to decrease the likelihood of bubbles being incorporated into the dough, which can cause unsightly appearances in the final pasta.

As a result of both mixing and extruding, starch granules are entrapped in an amorphous protein matrix (Dexter et al., 1978; Resmini & Pagani, 1983). Although the main constituent of pasta by far is granular starch, at this stage of the process, it has no network forming ability (Cornell, 2004).

The feed holes of the die inserts are usually teflon coated to produce pasta with a smooth surface while bronze inserts are used to achieve a rougher pasta surface, which helps sauces to stick better to the cooked pasta. Teflon-coated dies achieve much higher throughput rates. After extrusion the pasta is immediately subject to a blast of hot air to minimize strands sticking together. The strands then enter the predryer. Fresh pasta is then dried in order to reduce its moisture content to about 12% (Dalbon et al., 1996) with low water activity to ensure a long shelf life.

There are three different types of drying temperature used:low temperature (LT) (40-60 °C / 70-80% relative humidity (RH) / 18-28 h), high temperature (HT) (60-84 °C / 74-82% RH / 8-11 h), and very high temperature (VHT) (>84 °C / 74-90% RH / 2-5 h).

This new high temperature process is economically advantageous because of its shorter process time but it requires a more accurate control to obtain an acceptable product. In fact, some undesired effects are emphasised by the high rate of heating and drying, such as Non-Enzymatic Browning (e.g. Maillard reaction) and thermo- mechanical stresses that can break the product and, therefore, the process has to be carefully controlled.

It should be reminded that the onset of stresses and fractures may occur because the water profile inside pasta can cause a different local dough shrinkage (de Cindio et al., 2003). But on the other hand, it is worth to note also that the use of high temperatures implies a rather great improvement of the microbiological safety and the improvement of cooking quality of pasta (Acquistucci, 2000; Dexter et al., 1981) as higher firmness, lower stickiness and lower cooking loss (Aktan & Khan, 1992). This is because the high temperature treatment reinforces the wheat protein network, thus preventing starch leaching during cooking and maintaining satisfactory surface conditions in cooked pasta (Feillet et al., 1989). The final product is then packaged into cellophane or polyethylene bags or cardboard boxes. Packaging is designed to keep the product free from contamination,protect it from damage during shipment and storage, and display the product favorably while differentiating products.

1.7 Assessment of Pasta Quality

The quality of pasta is of great importance to producers and consumers and includes cooking properties, i.e., firmness, stickiness, and overcooking tolerance, as well as water absorption, degree of swelling, and gelatinization rate (Manser 1981).

Good quality pasta is defined as having high degree of firmness and elasticity, which is mainly, termed as ‘‘al dente” (Antognelli, 1980; Pomeranz, 1987).

There are several factors, which affect the characteristics of cooked pasta as firmness and compressibility are primarily affected by protein level, elasticity is related mainly to drying temperature, cooking loss of optimally cooked spaghetti is particularly influenced by protein level and cooking loss of overcooking pasta is affected by both variables.

The structure of cooked pasta is generally described as a compact matrix with starch granules entrapped in a protein network. During cooking, physical competition between starch swelling and properties of polymerised and polymerising proteins determines whether the final cooked pasta is firm and elastic (when a strong protein network is formed and starch particles are entrapped in this network) or rather sticky and soft (in the opposite case of significant starch swelling) (Delcour et al., 2000a, 2000b; Resmini & Pagani, 1983). So texture plays an essential role in determinig the final acceptance by the consumer and it is one of the predominant criteria for assessing pasta quality.

Sensory evaluation is regarded as the ultimate test of pasta cooking quality. The evaluation of pasta products is mostly focused on the sensory tests, examining appearance, colour, shape of dry pasta, breakage susceptibility, and also consistency, shape keeping, stickiness, and appearance of cooked products. A classical definition of sensory evaluation is given by Stone and Sidel (1993) and Dijksterhuis (1997) as follows: “ Sensory evaluation is a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of products or materials as they are perceived by the senses of sight, smell, taste, touch and hearing” . Initiry evaluation or sensory analysis was developed for studying the reactions of consumers to certain characteristics of food products. These reactions are generally in the form of score given to attributes or descriptors perceived in the food stimuli. Next, these sensory data are further analyzed in order to identify consumer’s perception on new food products, predict consumer's future purchase decisions and to provide explicit indices for development and design of new products.

Malcolmson (1991) used a trained texture profile panel to evaluate firmness, elasticity, chewiness, cohesiveness, tooth pack and stickiness of spaghetti using the definitions given in Table 1.3. Unstructured line scales, 15 cm in length were used by panellists to record their rating of each attribute.

Parameter	Definition
Firmness	The force required to bite through the sample
Elasticity	The degree to which the sample returns to its original state after being compressed slightly.
Cohesiveness	The degree to which the sample holds together after chewing
Chewiness	The amount of energy required to chew the sample to the state ready for swallowing.
Stickiness	The degree to which the strands adhere to each other and when lightly touched with a finger.
Tooth pack	The degree to which the sample packs around the teeth during and after chewing.

Table 1.3 Sensory definitions used in the evaluation of cooked spaghetti (Adapted from Malcolmson 1991).

Sensory analysis using highly trained panellists is considered the ultimate tool for measurement of the cooking quality of pasta products but there is no standard method as it is usually based on local preferences and it is difficult to compare results between studies. For this reason, many researchers have sought objective methods for evaluation of pasta cooking quality parameters (Dexter et al., 1985; Feillet et al., 1977; Matsuo and Irvine, 1971). Others have tried to correlate instrumental analysis with sensory evaluation (Voisey et al., 1978a) or chemical tests (Dexter et al., 1985). Generally, instrumental tests are based on measures of compression, adhesion, tension or stretching, shear or cutting and hybrid methods. The use of the Instron Universal Testing Machine is well established for the measurement of pasta firmness (Walsh, 1971). Therefore rapid instrumental methods that consider a number of textural factors have been developed. A very popular one is the texture profile analysis based on the recognition of texture as a multi parameter attribute. The test consists of compressing bite size pieces of food two times in a motion that simulates the action of jaw, and extracting from the resulting force time curve a number of textural parameters. These can be divided into the primary parameters of hardness, cohesiveness, springiness and adhesiveness and the secondary parameters of fracturability, chewiness and gumminess (Bourne,1978).

Aim of the thesis

The aim of the research was the production of functional pasta with a low glycemic index (GI). This was achieved through the following four steps:

- study of the effects of milling techniques on pasta quality;
- study of optimization of product formulation;
- study of the effects of durum wheat bran inclusion on sensory and nutritional pasta quality;
- study of the effects of hydrocolloids inclusion on functional pasta quality.

Under direction of the "Centro della Cerealicultura di Foggia (CRA)", cultivars of wheat were selected and studied in the first step. Subsequently, the selected cultivars of wheat (Anco Marzio, Cappelli, Claudio, Core, Iride and Saragolla) were subjected to two different milling techniques, with stone and by grinding the modern roller. The effects of the different techniques on the cooking quality, the nutritional composition and the GI of spaghetti were analyzed, thus demonstrating that wholemeal spaghetti had a lower GI in comparison to the semolina spaghetti. Specially the variety Cappelli was found a good compromise between sensory and nutritional quality. The second step was aimed to optimize the pasta formulation. Therefore, different cultivars as Timilia, Pr22d89, Pr22d89 enriched in Selenium, in addition to Cappelli were taken into account. Among them, Pr22d89(+Se) variety has been chosen for the great quality and the low glycemic index of related pasta. The third step was aimed to evaluate the effects of substituting semolina with durum wheat bran. The proper amount of bran was optimized (20%) and further hydrocolloids were selected at different concentrations (1%, 2% and 3%) to improve pasta quality. The several analyses carried out on the final funtional pasta assessed that a good formulation was optimized.

Chapter II

2. Materials and Methods

2.1 *Raw materials*: durum wheat seeds (Anco Marzio, Claudio, Core, Iride and Saragolla, Cappelli) were provided from the company of Santacroce Giovanni S.p.a. (Deliceto-Foggia, Italy), whereas durum wheat seeds (Timilia, Cappelli, Pr22d89, and Pr22d89 rich in selenium (Pr22d89 +Se) were provided from the Grain Crowth Research Center (C.R.A.- CER) of Foggia (Italy). The semolina was produced from grinding seeds with a Buhler (Mod MLU 202, Uzwill, Switzerland), whereas wholemeal flour was obtained through with a stone mill (Mod MB250 Partisani). The bran was ground to fine flour on a Tecator Cyclotec 1093 (International PBI, Milano, Italy) laboratory mill (1mm screen – 60 mesh).

2.2 *Spaghetti Preparation*: Spaghetti were made with semolina and wholemeal flour of different durum wheat, using the same operating conditions: semolina or wholemeal flour were mixed with water with a rotary shaft mixer (Namad, Rome, Italy) at 25°C for 20 minutes so as to obtain a dough with 30% moisture content.

For spaghetti enriched with bran there were three steps. In the first experimental phase, the durum wheat bran was added at various concentrations from 5% to 20%. Then, in the second experimental phase the durum wheat semolina and 20% of durum wheat bran was added with CMC (Carboxymethylcellulose sodium salt) at different amounts (1%, 2% and 3%).

The dough based on Pr22d89 (+Se) semolina was used as a reference (CTRL). Furthermore, commercially available spaghetti made up of durum wheat semolina (Barilla, Parma, Italy) named CM was also used as another reference sample.

The dough was extruded with a 60VR extruder (Namad, Rome, Italy). The extrusion pressure was about 3.4 bar, whereas the temperature of the spaghetti after the extrusion was about 27-28°C. The extruder was equipped with a screw (30 cm in length, 5.5 cm in diameter), which ended with a bronze die (diameter hole of 1.70 mm). The screw speed was 50 rpm. Subsequently the pasta was dried in a dryer (SG600, Namad). The process conditions applied were the following: 1st step, time 20 min at 60°C and 65% moisture; 2nd step, time 130 min at 90°C and 79% moisture; 3rd step, time 150 min at 75°C and 78% moisture; 4th step, time 160 min at 45°C and 63% moisture; 5th step, time 1040 min at 50°C and 50% moisture.

2.3 *Sensory analysis*: Dry spaghetti samples were submitted to a panel of ten trained tasters in order to evaluate the sensorial attributes. The panelists were trained in sensory vocabulary and identification of particular attributes, prior to testing, by evaluating commercial spaghetti. The panelists were asked to indicate color, homogeneity, resistance to break and overall quality of non-cooked spaghetti. Elasticity, firmness, bulkiness, adhesiveness, color, odor, taste and overall quality of cooked spaghetti were also evaluated (Padalino et al., 2013). To this aim, a nine-point, where 1 corresponded to extremely unpleasant, 9 to extremely pleasant and 5 to satisfactory was used to quantify each attribute.

2.4 *Nutritional determination*: Dry spaghetti samples were ground to fine flour on a Tecator Cyclotec 1093 (International PBI, Milano, Italy) laboratory mill (1mm screen – 60 mesh). Moisture and ash content (%) were measured according to AACC methods 44-19 and 08-03 (2000). Protein content (%N x 6.25) was analyzed with the micro Kjieldahl method according to AACC method 46-13 (2000). Total dietary fibre (TDF), soluble–water fibre (SDF) and insoluble-water fibre (IDF) contents were determined by means of the Total Dietary Fibre Kit (Megazyme) based on the method of Lee et al., (1992). The available carbohydrates (ACH) were determined according to method of McCleary et al., (2006) as described in the available carbohydrates assay kit (Megazyme). All nutritional analyses of the flour and spaghetti samples were made in triplicate.

2.5 *Spaghetti cooking quality*: The optimal cooking time will be determined according to the approved method 66-50 (American Association of Cereal Chemistry, AACC, 2000). Cooking loss will be determined according to the AACC approved method 66-50 (AACC, 2000). The swelling index and the water absorption of cooked pasta (grams of water per gram of dry pasta) will be determined according to the procedure described by Cleary and Brennan (2006).

2.6 *Hardness and adhesiveness*: After cooking, the spaghetti samples were gently blotted and submitted to hardness and adhesiveness analysis, by means a Zwick/Roell model Z010 Texture Analyzer (Zwick Roell Italia S.r.l., Genova, Italia) equipped with a stainless steel cylinder probe (2 cm-diameter). Trial specifications were described in the work of Padalino et al. (2013).

2.7 *In vitro digestion:* the digestion was carried out as described by Chillo et al., (2011). For the analysis of starch digestion, the samples removed during digestion were added to 2.0 ml of ethanol and mixed. After 1 h, the ethanolic sub-samples were centrifuged (2000 g, 2 min) (Biofuge fresco HERAEUS, Germany) and an aliquot (0.05 ml) of the supernatant was removed. This aliquot was added to 0.25 ml amyloglucosidae (E-AMGDF, Megazyme International Ireland Ltd, 1ml/100 ml in sodium acetate buffer 0,1 mol/L, pH 5.2) for 10 min at 20°C. 0.75 ml DNS solution (10 g 3,5-dinitrosalcylic acid, 16 g NaOH and 300 g Na-K tartarate (Sigma Aldrich, Milan, Italy) made to final volume (1L) was then added to the tubes. The tubes were heated for 15 min in boiling water, then cooled in cold water for 1 h, after which 4 ml of water (15°C) was added. After mixing, the reducing sugar concentration was measured colorimetrically (530nm) using a Shimadzu UV-vis spectrophotometer (model 1700, Shimadzu corporation, Japan). Glucose standards of 10.0 mg/ml were used. The results were then plotted as glucose release (mg) per g of sample v. time.

2.8 *Statistical analysis:* The results of the analysis performed on both flours and spaghetti and were compared by a one-way variance analysis (ANOVA). A Duncan's multiple range test, with the option of homogeneous groups ($p<0.05$), was carried out to determine significant differences between spaghetti samples. STATISTICA 7.1 for Windows (StatSoft, Inc, Tulsa, OK, USA) was used for this aim.

Chapter III

3. Results and discussion

The obtained results of this research will be described separately for each step of the experimental design.

1° Step. Effect of milling techniques on pasta quality

3.1 Effect of milling techniques on nutritional composition

The milling technique had a great impact on the nutritional composition of the samples of the semolina and wholemeal flour made with Anco Marzio, Cappelli, Claudio, Core, Iride, Saragolla variety. In fact, as shown in **Table 1**, the wholemeal flour samples were characterized by a higher content of protein and ash than the semolina samples. In particular, the protein content of the Cappelli sample increased from 13.02% for semolina to 15.26% for whole meal flour. These results are in accordance with Kihlberg et al. (2004) that found a higher protein and ash content in the wholemeal flour (stone-milling) in comparison to the conventional semolina (roller milling). In fact, the ash in wheat is not evenly distributed throughout the kernel, being more concentrated in the bran (6%) than in the endosperm portion (0.4%) of the grain (Pomeranz, 1988). Moreover, ash content indicates how completely and efficiently the endosperm has been separated from the bran. The whole-meal flours also had a higher total dietary fibre content (TDF) content when comparing to the semolina samples. Particulary, there was a significant rise of the insoluble fibre content (IDF) for wholemeal flour in comparison to the semolina sample. In fact, for Iride sample the IDF content value increased significantly from 2.93% for semolina to 9.42% for wholemeal flour. This finding could be explained by the fact that the insoluble dietary fibres are more concentrated in the bran fraction. Besides, the wholemeal flour samples recorded a lowest available carbohydrate (ACH) content in comparison to the semolina samples. In particular, the wholemeal flour Claudio recorded the lower ACH content (62.33) in comparison to the other samples. These results could be due to the fact that the wholemeal samples recorded a high dietary fibre content (non-available carbohydrate) (Mongeau, 2003). **Table 2** reports the nutritional composition of spaghetti samples made with semolina and wholemeal flour. As can be inferred from the Table 2, the wholemeal spaghetti sample showed a higher protein, ash and dietary fibre content and a lower available carbohydrate content in comparison to the semolina spaghetti. In particular, these samples recorded the highest TDF content due to the rise IDF content in comparison to the semolina spaghetti samples. In fact, the wholemeal spaghetti Core recorded a higher IDF content (12.57%) than the semolina spaghetti Core (3.09%). Moreover, it can be seen from the above table that the SDF content of some spaghetti samples increased in comparison to the raw materials. Most probably, these results might be due to the fact that the semolina and pasta have undergone different processing conditions. In particular, the mechanical stress during the extrusion process should be responsible for the breakdown of polysaccharide glycosidic bonds, leading to the release of oligosaccharides and therefore to the increase in the SDF value (Esposito et al., 2005).

Regarding the nutritional composition of the spaghetti samples based on monovarietal durum wheat semolina, also in this case the Cappelli spaghetti sample showed the highest protein and ash contents (12.87% and 0.98%, respectively) and the lowest ACH content (72.00 g/100g) respect to the other spaghetti samples.

3.2 Effect of milling techniques on in vitro starch digestion

Regarding the glucose release during in vitro pancreatic digestion, significant differences were observed between semolina and wholemeal spaghetti. As shown in **Table 3** all wholemeal spaghetti showed a lower glycemic index value than the semolina spaghetti. The wholemeal spaghetti Cappelli and Claudio recorded the lowest glycemic index value (50) whereas the semolina spaghetti Core had the highest GI value (80) among the samples investigated. These results could be due to the fact that the wholemeal samples recorded both a lower available carbohydrate content and a higher fibre content (no-available carbohydrate). In fact, addition of dietary fibers can further reduce the in vitro glycaemic response of pasta and introduce additional health benefits (Yokoyama et al., 1997). Moreover, Brennan et al. (2008) found that bran reduced starch hydrolysis in extruded breakfast cereal. These results also are in agreements with Padalino et al. (2013), which found that the addition of the pepper flour (containing a high level of dietary fibre) caused a lower glycemic index of the pasta of maize flour with respect to the control (100% maize flour).

3.3 Effect of milling techniques on sensory properties

The sensorial properties of the investigated samples were evaluated by means of a group of trained panelists and the results are listed in **Table 4.** The sensory data of spaghetti (uncooked and cooked) showed that the different milling techniques significantly influenced the overall quality of pasta. On the whole, the uncooked wholemeal spaghetti presented low values for color and break resistance and thus a significantly lower overall quality score, when compared to the semolina spaghetti. Specifically, the overall quality of wholemeal sample Core was found to be around the acceptability threshold (i.e., 5.03), due to the low break resistance score (4.73). Conversely, the semolina spaghetti Cappelli showed the highest overall quality score (7.36) due to the high color (7.81) and break resistance (7.68) values. A possible explanation of the observed results is that the wholemeal spaghetti contain bran, with respect to the semolina spaghetti. In fact, bran, by interfering with the continuity of the gluten matrix, causes weakening of the dough and reduces the mechanical strength and the cooking quality of the bran supplemented spaghetti. In addition, several studies found that the spaghetti made from whole wheat or bran-semolina showed a darker brown color in comparison to the light yellow color of the semolina spaghetti. Concerning cooked spaghetti, again the wholemeal spaghetti presented a lower overall quality score, although above the threshold of acceptability, in comparison to the semolina spaghetti, due to the lower values of elasticity, firmness and color. In particular, the wholemeal spaghetti Core and Saragolla showed a lower elasticity (5.16 and 5.50 respectively) and firmness values (6.00 and 6.07 respectively) in comparison to the other samples. Once again, these results could be linked to the highest fibre content of the spaghetti made from wholemeal flour. Hence, the reduction of the elasticity and firmness might be due to the disruptive behavior of fibre on the protein-starch binding during pasta matrix formation (Tudorica et al. 2002). The wholemeal spaghetti, in comparison to the semolina spaghetti, showed a higher bulkiness and adhesiveness score. In particular, the wholemeal spaghetti Core recorded the highest adhesiveness (7.13) and bulkiness (7.07) in comparison to other samples investigated. On the contrary, the semolina spaghetti Core recorded lower bulkiness (5.82) and adhesiveness (5.95) in comparison to other samples. These results could be related to the presence of bran (i.e. fibre), which has a positive effect on the adhesiveness, as found by Cleary and Brennan (2006). In addition, wholemeal spaghetti had an unpleasant taste in comparison to the semolina spaghetti, due to high fibrous (low values). Specifically, the wholemeal spaghetti Saragolla had the lowest taste (6.21) and fibrous (4.71) score. In fact, Aravind et al., (2012) found that the wholemeal spaghetti had floury mouthfeel in comparison to the pasta enriched with bran and control pasta.

3.4 Cooking quality of dry durum wheat spaghetti

As expected, the different milling techniques influenced also the cooking quality of the durum wheat dry spaghetti samples. Data listed in **Table 5** show how the optimum cooking time (OCT) values of the spaghetti samples with wholemeal flour decreased in comparison to those of the semolina spaghetti samples. In particular, the wholemeal spaghetti Saragolla showed the lower OCT value (8.20) whereas semolina spaghetti Cappelli had the highest OCT value (11.15). These results could be due to the physical disruption of the gluten matrix by fibres, providing an easier path for water absorption and a shorter cooking time (Manthey & Schorno, 2002). In fact, the wholemeal spaghetti recorded a higher TDF content (mainly insoluble fibres) with respect to the semolina spaghetti. This is in agreement with the studies of Aravind et al., (2012) which observed a lower OCT value in durum wheat spaghetti enriched with bran (mainly insoluble fibre) in comparison to the durum wheat pasta control.

Cooking loss values (CL) of the wholemeal spaghetti were significantly higher compared with those of the semolina spaghetti. Cooking loss values theoretically reflect the quantity of starch and other biochemical components that are released from the pasta protein matrix and subsequently lost in the cooking medium (Cole, 1991). Specifically, the wholemeal spaghetti Iride and Saragolla recorded greater cooking loss (7.63% and 7.05% respectively) when compared to the other samples. Instead, the semolina spaghetti Ancomarzio and Cappelli recorded a lower cooking loss (4.31% and 4.27% respectively) in comparison to other samples investigated. These results could be due to the high fibre content observed in the spaghetti based on the wholemeal flour. In fact, the samples with greater cooking losses showed a higher fibre content in comparison to the other wholemeal samples. The fibre interfering with the starch-gluten network, allowing more of the gelatinised starch to leach from the pasta during cooking. Specifically, the increased loss of solids encountered in the wholemeal spaghetti samples may be attributed to a loss of continuity of the pasta protein matrix. Also, Manthey and Schorno (2002) found a higher cooking loss value in the pasta sample obtained by whole-wheat. They attributed this in part to disruption of the gluten matrix, as seen by SEM and the presence of soluble water material in the bran. Kunerth and Youngs (1984) reported soluble water non-starch polysaccharides in bran, which might be a component of the measured cooking loss (material leached from overcooked pasta) . Wholemeal spaghetti samples showed a lower swelling index and water absorption values in comparison to the semolina spaghetti.

2° Step – Optimization of product formulation

3.5 Study of the nutritional composition of semolina and spaghetti samples

Analysing nutritional data of semolina of Timilia, Cappelli, Pr22d89, Pr22d89(+Se) variety listed in **Table 6** it could be noted as these monovarietal semolina samples differed both for protein and total fibers content. Cappelli semolina had a higher protein content (16.88%) and a lower ash content (0.91%) in comparison to all the samples, especially Pr22d89 (15.20%). On the other hand, Pr22d89 (+Se) showed a high content of dietary fat (2.15%) and total fibre (8.62%) if compared to all the other samples. These results might be due to the fact that proteins and dietary fibre contents are influenced by durum wheat genotype. In particular, Frank et al., (1999) found that the content and composition of soluble and insoluble dietary fiber varied with genotype. These results were also confirmed by the nutritional analysis of spaghetti samples. In fact, as shown by **Table 7**, the Cappelli variety spaghetti sample always recorded high protein content (16.78%) and lowest ash value (0.98%), while the spaghetti variety Pr22d89(+Se) showed the highest value of dietary fat (2.13%) and total fibre (8,94) in comparison to all samples analysed. The high value of IDF (5.31%) presented in modern variety Pr22d89(+Se) was a data very important because insoluble fibre enriched pasta can also modulate insulin sensitivity by slowing absorption of carbohydrates and possibly reducing glycaemic and insulinemic responses (Jenkins et al., 2002). It is important to note as for some spaghetti samples the soluble fibers content increases compared to the raw materials. Most probably the mechanical stress during the extrusion process should be responsible for the breakdown of polysaccharides glycosidic bonds, leading to the release of oligosaccharides and therefore to the increase in the SDF value (Esposito et al., 2005).

3.6 Sensory analysis of spaghetti

The sensorial properties of the investigated samples were listed in **Table 8**. Sensory data on the spaghetti sample, both uncooked and cooked, showed that the different cultivars significantly influenced the overall quality of pasta. Results on the uncooked spaghetti samples showed that the Cappelli and Pr22d89(+Se) samples had highest overall quality score (7.25 and 7.00, respectively) as opposed to Timilia samples that showed lowest overall quality value (6.01). In particular, Cappelli samples showed a pleasant yellow color when compared to the other spaghetti samples. The colour difference between samples could be due to differences in the ash content among the investigated samples. In fact, a higher amount of this index in semolina leads to an increased brown colour, reducing the semolina and pasta yellowness (Kobrehel et al., 1974; Matsuo et al., 1980; Taha et al., 1987). Regarding to the cooked dry spaghetti, the main discriminating attributes for the spaghetti quality were colour, odour and taste. In particular, Pr22d89(+Se) recorded a higher value of color (7.58), odour (7.15) and taste (7.38). Probably, these results are due to the presence of selenium, that is an essential nutrient of fundamental importance to human biology. Some studies show as selenium may also play a role in the prevention of cardiovascular disease (Rayman, 2000; Stranges et al., 2006; Combs, 2005; Combs et al., 2001; Whanger, 2004). So, analysing the nutritional and sensorial data of the samples, Pr22d89(+Se) variety has been chosen for the production of spaghetti enriched with bran, in order to obtain a pasta with greater quality and a low glycemic index.

3° Step- Effect of durum wheat bran inclusion on sensory quality

3.7 *Sensory quality of spaghetti*

The sensory quality of uncooked and cooked spaghetti determined by a trained panel are reported in **Table 9**. This table shows that there are important differences between the CTRL and all spaghetti enriched with bran. In particular, the spaghetti enriched with 20% of bran showed a lower overall quality score (5.80) in comparison to the semolina spaghetti (7.58), due to the lower values of break to resistance (5.87) for the uncooked spaghetti and for elasticity (5.01), firmness (5.76), fibrous (4.60) and taste (7.08) for cooked spaghetti. Once again, these results could be linked to the highest fibre content of the spaghetti enriched with 20% bran. Hence, the reduction of the elasticity and firmness might be due to the disruptive behaviour of fibre on the protein-starch binding during pasta matrix formation (Tudorica et al. 2002). The bran pasta had no wheat semolina aroma or wheat semolina flavour, but had other odours not found in control which carried over into aftertaste. The colour of spaghetti decreased with addition of increasing amount of bran. In fact, Kordonowy and Youngs (1985) have reported that pasta with 10–30% bran had inferior colour, flavour and texture with 10% having the least effect. Chillo et al. (2008) found differences between bran and control pasta, but similar overall sensory scores up to 20% bran incorporation.

4° Step - Effect of hydrocolloids inclusion on functional pasta quality

3.8 Sensory quality of spaghetti

The sensory data on the spaghetti sample made with 20% of bran plus 2% of different hydrocolloids showed that the type of hydrocolloids significantly influenced the overall quality of pasta. The results are showed in **Table 10.** Bran enriched spaghetti with 2% pectin had lower overall quality score, determined by a low elasticity value (4.08) and odour score (3.44) in comparison to all samples analysed. On the other hand, the spaghetti enriched with 20% of durum wheat bran plus 2% of CMC recorded a good overall quality score. In particular, pasta containing CMC had very similar sensory scores to commercial control pasta (6.50), but had higher value of overall quality (6.60) in comparison to Pr22d89(+Se) plus 20% of bran (5.8), differing significantly in only two measures: firmness and fibrous. The increased of fibrous could be explained by increased of total fibre due to addition of CMC. In fact, CMC is a cellulose derivative with carboxymethyl groups bound to some of the hydroxyl groups of the glucopyranose monomers which make up the cellulose backbone, with food-grade CMC having a degree of substitution in the range 0.65–0.95 (Hoefler, 2000). Komlenic, Ulgarcic-Hardi, and Jukic (2006) also added 0.15–0.75% CMC to pasta without degrading sensory properties. The sensorial data showed in **Table 11** indicate a minimal impact of 1% and 2% CMC on pasta sensorial quality, while the addition of 3% CMC enhanced the firmness and adhesiveness, in line with the impact on technological properties measured. The increase of adhesiveness for the sample enriched with 3% CMC could be explained by the decreased of protein content. In fact, Del Nobile et al., (2005) found that increasing the protein content of pasta has been associated with a decreased in adhesiveness.

3.9 Nutritional quality of spaghetti

The nutritional characteristics of spaghetti samples enriched with 20% of bran plus different percentages of CMC are reported in **Table 12**. From table 12, it emerges that there were important differences among the spaghetti samples with 20% of bran and CTRL. In particular, Pr22d89(+Se) enriched with 20% bran was characterized by a higher value of ash, total fibre and dietary fat content in comparison to CTRL. The increase of the ash content was due to increasing amount of bran added to spaghetti. The ash in wheat is not evenly distributed throughout the kernel, being more concentrated in the bran (6%) than in the endosperm portion (0.4%) of the grain (Pomeranz, 1988). In fact, bran fractionation processes aim at recovering the different layers of the bran separately, to produce rich fractions in the different bran layers, such as pericarp-rich fractions (rich in fiber) or aleuronerich fractions (rich in vitamins, minerals and antioxidant compounds) (Antoine et al., 2004b; Dexter and Wood, 1996). Regarding the TDF content, the Pr22d89(+Se) with 20% of bran plus 3% CMC showed higher values (18.97%) in comparison to all samples. This result is justified by the high value of both insoluble fiber (13.76%) and both soluble fiber (7.21%) in comparison to the commercial control spaghetti and the modern variety Pr22d89(+Se). The increase of IDF could be explained by the fact that the insoluble dietary fibres are more concentrated in the bran fraction. Whereas the increase of SDF is justified by addition of the CMC, that has high cold water solubility (BeMiller, 2008). CTRL showed the highest protein content (15.85%) in comparison to both commercial control (13.63%) and spaghetti samples added with 20% of bran (15.71). This result is probably due to the replacement of semolina with 20% durum wheat bran, thus resulting in a decrease of gliadins and glutenins and an increase of the total fibers for the samples of enriched spaghetti. This result is in agreement with the texural properties, in fact with the increase of protein content adhesiveness decreased.

3.10 In vitro digestion of pasta and estimated glycemic index

Glycemic index has been introduced in order to estimate blood glucose response after food ingestion by humans. It is measured by the postprandial glycemic area of a test meal, expressed as the percentage of the corresponding area of the reference food (fresh white bread). As human subjects would have to be recruited to measure GI, which is also time consuming, invasive, labour-intensive and costly, in vitro testing is commonly used as a faster method to predict in vivo GI (Fardet et al., 1999). The glycemic index values of bran enriched spaghetti samples are listed in **Table 13**. From this table it can be inferred that all spaghetti with 20% of bran showed a low glycemic index value in comparison to the commercial control spaghetti and CTRL. This result can be explained by the fact that the addition of bran, rich in dietary fiber, can further reduce the GI of pasta and introduce additional health benefits (Gatti et al., 1984; Yokoyama et al., 1997). In particular, the sample Pr22d89(+Se) added with 20% durum wheat bran plus 2% and 3% CMC, showed a major positive effect on the reduction of glycemic index value (55) in comparison to the commercial control (61). A specific competitive inhibitory or electrostatic effect of the interaction of the negatively-charged CMC with α-amylase may have been responsible for slower starch digestion rate seen with CMC-containing pasta (Aravind et al., 2012). So the addition of CMC into cereal-based food has shown beneficial effects on blood glucose regulation and fasting plasma cholesterol (Brennan et al., 1996).

3.11 Cooking quality and textural properties of spaghetti

The effect of the addition of bran and CMC on the technological properties of pasta is shown in **Table 14**. The results showed that the addition of bran and CMC had effect on all technological properties of samples in comparison to CTRL spaghetti. In particular, the addition of CMC had effect on pasta cooking time, cooking loss, swelling index and water absorption. The optimal cooking time (OCT) values of the spaghetti samples enriched with

20% bran decreased in comparison to CTRL spaghetti but was higher in comparison to commercial control. These results could be due to the physical disruption of the gluten matrix by fibre, providing an easier path for water absorption and a shorter cooking time (Manthey & Schorno, 2002). In fact, the 20% bran spaghetti recorded a higher TDF content (mainly insoluble fibre) in comparison to CTRL spaghetti. The latter is in agreement with the studies of Aravind et al., (2012) which observed a lower OCT value in durum wheat spaghetti enriched with bran (mainly insoluble fibre) in comparison to the durum wheat pasta control. The addition of increasing percentage of CMC (1%, 2%, 3%) determined further reduction of OCT. This result can be explained by the increase of soluble fibre due to the addition of CMC. Other important technological properties of pasta is cooking loss the values of which theoretically reflect the quantity of starch and other biochemical components that are released from the pasta protein matrix and subsequently lost in the cooking medium (Cole, 1991). As can be seen in **Table 14** the cooking loss values (CL) of the spaghetti made with 20% of bran were higher in comparison to CTRL and commercial control. The values of cooking loss of Pr22d89(+Se) plus 20% bran increased with the addition of increasing percentages of CMC. These results are justified by the high content of fibre, insoluble and soluble, present in all the samples with bran and CMC. The fibre interferes with the starch-gluten network, allowing more of the gelatinized starch to leach from the pasta during cooking. Specifically, the increased loss of solids encountered in the spaghetti samples enriched with bran and CMC may be attributed to a loss of continuity of the pasta protein matrix. Kunerth and Youngs (1984) reported soluble water non-starch polysaccharides in bran, which might be a component of the measured cooking loss (material leached from overcooked pasta). Spaghetti samples made with 20% bran showed a lower swelling index (1.88) and water absorption (147.12%) values in comparison to CTRL (2.10 and 170.25% respectively). These findings could be due to the fact that in food containing bran there is typically less absorption of water by starch (Brennan, et a., 2008) because bran competes for water with starch. There is a correspondence between these results and sensorial analysis. In fact, the sensorial parameters as bulkiness and adhesiveness are connected to the quantity of water absorbed during cooking (Chillo et al. 2008). Pasta water absorption of Pr22d89(+Se) with 20% bran recorded a lower value in comparison to CTRL after having showed a steady increase as more CMC was added and became significantly higher than control pasta at 3% CMC. Komlenic et al. (2006) investigated the effect of adding 0.15–0.75% CMC sodium salt to pasta. They found increased cooking loss and decreased water absorption with CMC addition, which they suggested because CMC absorbs water readily to inhibit starch swelling and absorption of water by the pasta. The data reported by Aravind et al. (2012), by contrast, showed an increase in water absorption and no change in swelling index or cooking loss. Komlenic et al. (2006) obtained improved sensory scores for CMC pasta due to lower stickiness and consistency, otherwise minimal changes were observed, in line with our data on sensory and instrumental stickiness. Olfat, Yaseen, and Aziza (2006) added a CMC–whey protein complex to pasta and found improved cooking quality (increased cooked volume) and improved sensory scores for colour, flavour and appearance using 3% CMC–whey protein compared to 100% semolina pasta.

Regarding the values for the hardness and adhesiveness differences between CTRL spaghetti and spaghetti enriched with 20% bran plus CMC were found. In particular, Pr22d89(+Se) enriched with 20% of bran and 3% of CMC recorded the highest values of adhesiveness and hardness. The increase of adhesiveness was due to the reduction of protein content of samples. This results was in accordance with the study of Del Nobile et al., (2005) that found increasing protein content of pasta, associated with a decrease in adhesiveness.

Conclusions

In this work the manufacturing of spaghetti based on durum wheat semolina (obtained from roll-milling), wholemeal flour (stone-milling) and durum wheat semolina enriched with bran was studied. Initially, Anco Marzio, Cappelli, Claudio, Core, Iride and Saragolla variety were selected and the effects of two milling techniques on cooking and texture properties of spaghetti samples, along with the nutritional and sensory quality of pasta were addressed. The wholemeal spaghetti showed a higher protein, ash and dietary fibre content and a lower ACH content with respect to the semolina spaghetti. In particular, these samples recorded the highest TDF content due to the greatest IDF content. Regarding the rheological properties, the wholemeal spaghetti showed differences in the viscoelastic properties. As regard the cooking quality, the wholemeal spaghetti recorded a greater CL when compared to the semolina spaghetti. Specifically, the wholemeal spaghetti Iride and Saragolla recorded the highest CL values (7.63% and 7.05%, respectively) as opposed to the semolina samples Anco Marzio and Cappelli. Moreover, the wholemeal spaghetti resulted to have lower values of hardness and adhesiveness if compared to the semolina samples. The wholemeal spaghetti showed a lower overall quality due to a lower elasticity, firmness and colour score. However, the wholemeal spaghetti had a lower GI in comparison to the semolina spaghetti. In conclusion, the wholemeal flour improved the nutritional composition of pasta and contributed to a low glycemic response. It is worth noting that wholemeal spaghetti Cappelli was found the optimal compromise between sensory and nutritional quality. Afterwards, the effects of durum wheat bran and soluble fibre inclusion on quality of spaghetti made with semolina of Timilia, Cappelli, Pr22d89, Pr22d89(+Se) cultivars were studied. The spaghetti made up of Pr22d89(+Se) showed a high overall quality score in comparison to all cultivars analyzed. In particular, it differed in comparison to other samples for high odour and taste, and for the high total fibre content (8.94). Pr22d89(+Se) enriched with 20% of durum wheat bran showed a higher ash and dietary fibre content and a lower protein content in comparison to the control semolina spaghetti. In particular, there was an increase of insoluble fibre due to the addition of bran. So, analysing the nutritional and sensory data of all pasta samples, Pr22d89(+Se) variety has been chosen for the production of wholemeal spaghetti enriched with bran, in order to obtain a pasta with a great functional quality and a low glycemic index. The durum wheat bran was added until reaching the pasta unacceptability and then the quality was improved with structuring agents. Among the tested hydrocolloids, CMC exerted the best results. It was tested at different concentrations and 2% seemed to be the optimal amount to improve the technological, sensorial and nutritional value of pasta. Spaghetti enriched with 20% of durum wheat bran had a lower glycemic index (58) in comparison to the Pr22d89(+Se) semolina spaghetti (65) and to the commercial control (61). Adding increasing amounts of CMC, the glycemic index decreased to 55. To sum up, the development of enriched pasta with a higher dietary fibre content represents a good way to increase the fibre intake and reduce the glycemic index of pasta, which would result in a product for specific nutritional purpose. Further clinical experiments are required to ascertain if similar trends can be also observed by *in vivo* test.

Chapter IV

Figures and Tables

Table 1- Nutritional composition of semolina and wholemeal flour samples.

Milling technique	Sample	Protein (%)	Ash (%)	Moisture (%)	IDF (%)	SDF (%)	TDF (%)	ACH (g/100g)
Roller mills	Semolina Anco Marzio	12.47±0.14^{d,f}	0.83±0.01^{g}	13.15±0.24^{d,e}	2.91±0.04^{a}	2.49±0.03^{i}	5.40±0.02^{a}	75.43±0.58^{c,d,e}
	Semolina Cappelli	13.02±0.01^{a}	0.91±0.00^{h}	12.02±0.21^{a,b}	2.15±0.02^{b}	2.12±0.02^{g}	4.27±0.01^{b}	74.27±0.73^{c,d}
	Semolina Claudio	11.67±0.03^{a}	0.63±0.01^{f}	13.36±0.08^{e}	2.38±0.04^{c}	1.03±0.02^{c}	3.41±0.06^{c}	79.67±1.84^{f,g}
	Semolina Core	10.62±0.83^{e}	0.70±0.03^{a}	11.82±0.01^{a}	3.32±0.04^{e}	1.80±0.02^{a}	5.12±0.03^{d}	81.37±2.11^{g}
	Semolina Iride	11.45±0.09^{b}	0.75±0.03^{c}	12.74±0.54^{c,d}	2.93±0.13^{a}	1.46±0.01^{f}	4.38±0.13^{e}	77.50±0.03^{e,f}
	Semolina Saragolla	11.21±0.17^{b,e}	0.73±0.02^{b,c}	12.40±0.27^{b,c}	3.20±0.04^{d}	1.31±0.01^{d}	4.52±0.03^{f}	76.59±0.49^{d,e}
Stone mills	Wholemeal Anco marzio	13.71±0.16^{a}	1.43±0.04^{d}	19.12±0.30^{l}	7.46±0.06^{h}	2.76±0.09^{m}	10.22±0.16^{g}	64.30±0.15^{a}
	Wholemeal Cappelli	15.26±0.12^{g}	1.46±0.01^{d,e}	14.62±0.09^{g}	6.13±0.01^{f}	2.32±0.01^{h}	8.45±0.02^{h}	69.95±0.60^{b}
	Wholemeal Claudio	13.17±0.04^{a}	1.20±0.04^{i}	17.13±0.50^{h}	6.91±0.01^{g}	2.06±0.03^{b}	8.97±0.04^{i}	62.33±0.94^{a}
	Wholemeal Core	12.13±0.64^{c,d}	1.50±0.03^{a,e}	17.70±0.41^{i}	9.11±0.03^{l}	2.57±0.01^{l}	11.68±0.02^{l}	72.65±0.41^{b,c}
	Wholemeal Iride	12.30±0.10^{c,d}	1.54±0.04^{a}	15.70±0.47^{f}	9.42±0.05^{m}	1.80±0.00^{a}	11.22±0.05^{m}	70.00±4.19^{b}
	Wholemeal Saragolla	13.15±0.73^{a}	1.51±0.01^{a}	15.20±0.18^{f}	8.72±0.03^{i}	2.06±0.02^{b}	10.78±0.05^{n}	64.14±1.42^{a}

$^{a-m}$Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 2 – Nutritional composition of the spaghetti samples based on semolina or wholemeal flour.

Milling technique	Sample	Protein (%s.s)	Ash (% s.s)	Moisture (%s.s)	IDF (% s.s)	SDF (%s.s)	TDF (%s.s)	ACH (g/100g)
Roller mills	Semolina Anco Marzio	12.70±0.05^{c}	0.91±0.00^{e}	8.20±0.24^{d}	2.81±0.02^{d}	1.68±0.02^{d,e}	4.49±0.00^{a}	77.93±2.38^{a}
	Semolina Cappelli	12.87±0.03^{c}	0.98±0.02^{f}	7.68±0.26^{a,b}	2.67±0.01^{c}	1.88±0.04^{b}	4.56±0.02^{a}	72.00±1.97^{d}
	Semolina Claudio	11.86±0.01^{b,d}	0.64±0.03^{c}	7.30±0.06^{a,b,c}	2.59±0.01^{b}	1.48±0.03^{a}	4.07±0.02^{d}	78.08±0.01^{a}
	Semolina Core	10.33±0.02^{e}	0.80±0.00^{a}	7.71±0.22^{a,d}	3.09±0.01^{a}	1.78±0.01^{b}	4.87±0.03^{b,c}	87.10±1.56^{e}
	Semolina Iride	11.49±0.04^{a,d}	0.82±0.00^{a}	7.43±0.18^{a,b}	3.16±0.01^{e}	1.60±0.14^{a}	4.77±0.14^{b}	78.52±1.15^{a}
	Semolina Saragolla	11.27±0.11^{a}	0.73±0.02^{d}	7.40±0.14^{a,b}	3.08±0.03^{a}	1.85±0.12^{b}	4.93±0.07^{c}	77.73±0.02^{a}
Stone mills	Wholemeal Anco Marzio	13.69±0.06^{f}	1.66±0.01^{b}	7.14±0.16^{b,c}	5.88±0.06^{g}	1.52±0.09^{a}	7.40±0.16^{e}	70.14±0.36^{c,d}
	Wholemeal Cappelli	15.38±0.01^{g}	1.55±0.00^{h}	7.71±0.38^{a,d}	5.75±0.08^{f}	2.50±0.05^{c}	8.25±0.13^{f}	68.87±0.43^{b,c}
	Wholemeal Claudio	12.98±0.04^{c}	1.39±0.00^{g}	6.51±0.16^{e}	7.38±0.02^{h}	2.12±0.05^{f}	9.49±0.02^{g}	67.67±1.67^{b,c}
	Wholemeal Core	11.24±0.03^{a}	2.03±0.01^{l}	6.84±0.27^{c,e}	12.57±0.00^{m}	2.82±0.09^{g}	15.39±0.10^{l}	75.76±3.21^{a}
	Wholemeal Iride	12.12±0.73^{b}	1.73±0.00^{i}	7.32±0.24^{a,b,c}	9.34±0.01^{l}	2.63±0.03^{c}	11.97±0.02^{i}	66.94±0.02^{b}
	Wholemeal Saragolla	12.14±0.51^{b}	1.67±0.00^{b}	8.50±0.65^{f}	8.66±0.04^{i}	2.58±0.05^{c}	11.24±0.10^{h}	69.71±1.33^{b,c,d}

$^{a-m}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 3 – Glycemic index of the spaghetti samples.

Milling technique	Sample	Glycemic Index
Roller mills	Semolina Anco Marzio	$75{\pm}1.00^{b}$
	Semolina Cappelli	$64{\pm}0.10^{f}$
	Semolina Claudio	$78{\pm}0.30^{g}$
	Semolina Core	$80{\pm}0.20^{h}$
	Semolina Iride	$75{\pm}0.30^{b}$
	Semolina Saragolla	$74{\pm}1.00^{b}$
Stone mills	Wholemeal Anco Marzio	$51{\pm}1.00^{a}$
	Wholemeal Cappelli	$50{\pm}1.00^{a}$
	Wholemeal Claudio	$50{\pm}1.00^{a}$
	Wholemeal Core	$57{\pm}1.00^{d}$
	Wholemeal Iride	$61{\pm}1.00^{e}$
	Wholemeal Saragolla	$55{\pm}1.00^{c}$

$^{a\text{-}h}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 4 – Sensory characteristics of dry, non-cooked and cooked, spaghetti samples.

Sample	Uncooked Spaghetti			Cooked Spaghetti								
	Color	Resistance to break	Overall Quality	Elasticity	Firmness	Fibrous	Bulkiness	Adhesiveness	Color	Odor	Taste	Overall Quality
Semolina Anco Marzio	7.66±0.28^{c,d}	7.24±0.22^{e,f}	7.03±0.24^{a,b}	6.67±0.32^{d,e,f}	7.26±0.27^{c,d}	7.42±0.31^{b}	6.20±0.21^{a,b}	6.21±0.30^{a,b}	7.40±0.21^{b,c}	7.58±0.30^{a}	7.26±0.21^{b}	7.21±0.22^{c}
Semolina Cappelli	7.81±0.26^{d}	7.68±0.37^{f}	7.36±0.23^{b}	7.00±0.32^{f}	7.33±0.21^{d}	7.42±0.31^{b}	6.77±0.21^{b,c}	6.27±0.30^{a,b}	7.20±0.21^{b}	7.56±0.30^{a}	7.23±0.21^{b}	7.34±0.22^{c}
Semolina Claudio	7.26±0.23^{b,c}	6.70±0.34^{b,e}	7.02±0.26^{a,b}	6.85±0.28^{e,f}	6.81±0.24^{b,c}	7.65±0.24^{b}	6.00±0.32^{a}	5.36±0.28^{d}	7.81±0.24^{c}	7.74±0.34^{a}	7.02±0.26^{b}	6.55±0.28^{b,c}
Semolina Core	7.06±0.41^{b}	6.27±0.30^{a,b,d}	6.62±0.35^{a}	6.37±0.35^{d,e,g}	6.52±0.37^{a,b}	7.75±0.32^{b}	5.82±0.31^{a}	5.95±0.31^{a}	7.14±0.24^{b}	7.56±0.37^{a}	7.09±0.30^{b}	6.17±0.23^{a}
Semolina Iride	7.31±0.23^{b,c,d}	6.80±0.32^{b,e}	7.12±0.23^{a,b}	6.77±0.31^{d,e,f}	6.80±0.30^{b,c}	7.42±0.34^{b}	6.02±0.38^{a}	6.10±0.23^{a}	7.40±0.35^{b,c}	7.38±0.34^{a},	7.31±0.23^{b}	6.71±0.40^{b}
Semolina Saragolla	7.23±0.25^{b,c}	6.53±0.33^{b,d}	7.05±0.31^{a,b}	6.23±0.27^{c,d,g}	6.30±0.37^{a,b}	7.78±0.30^{b}	6.07±0.35^{a}	6.05±0.31^{a}	7.21±0.31^{b}	7.58±0.37^{a}	7.50±0.38^{b}	6.28±0.27^{a,b}
WholemealAncoMarzio	5.97±0.26^{a}	5.91±0.30^{a}	7.03±0.32^{a,b}	5.75±0.35^{a,b,c}	6.24±0.24^{a,d}	5.20±0.40^{a}	6.72±0.66^{b,c}	6.71±0.36^{b,c}	6.34±0.33^{a}	7.42±0.30^{a}	6.28±0.26^{a}	6.16±0.28^{a}
Wholemeal Cappelli	5.94±0.22^{a}	5.22±0.31^{c}	5.25±0.28^{d}	5.83±0.28^{b,c,g}	6.80±0.24^{b,c}	5.23±0.30^{a}	6.68±0.37^{b,c}	6.76±0.24^{b,c}	6.32±0.28^{a}	7.41±0.30^{a}	6.31±0.30^{a}	6.37±0.26^{a,b}
Wholemeal Claudio	5.71±0.27^{a}	6.04±0.35^{a,d}	5.93±0.35^{c}	5.70±0.33^{a,b,c}	6.11±0.24^{a}	5.09±0.38^{a}	6.23±0.30^{a,b}	6.20±0.27^{a,b}	6.28±0.27^{a}	7.35±0.33^{a}	6.30±0.22^{a}	6.25±0.26^{a,b}
Wholemeal Core	5.91±0.31^{a}	4.73±0.30^{c}	5.03±0.28^{d}	5.16±0.30^{a}	6.00±0.25^{a}	4.91±0.25^{a}	7.07±0.34^{c}	7.13±0.38^{c}	6.27±0.28^{a}	7.45±0.35^{a}	6.40±0.28^{a}	6.09±0.31^{a}
Wholemeal Iride	5.87±0.35^{a}	5.01±0.21^{c}	5.80±0.34^{c}	5.66±0.34^{a,b,c}	6.30±0.30^{a,b}	4.87±0.24^{a}	6.87±0.21^{c}	6.85±0.38^{c}	6.25±0.32^{a}	7.21±0.26^{a}	6.25±0.25^{a}	6.27±0.26^{a,b}
Wholemeal Saragolla	5.84±0.30^{a}	5.87±0.27^{a}	5.91±0.31^{c}	5.50±0.38^{a,b}	6.07±0.36^{a}	4.71±0.36^{a}	6.68±0.36^{b,c}	6.71±0.27^{b,c}	6.21±0.26^{a}	7.23±0.30^{a}	6.23±0.23^{a}	6.21±0.26^{a,b}

$^{a\text{-}d}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 5 – Cooking quality of dry spaghetti samples investigated.

Milling technique	Sample	OCT (min)	Cooking Loss (%)	Swelling Index	Water Absorption (%)	Adhesiveness (Nmm)	Hardness (N)
Roller mills	Semolina Anco Marzio	9.40	4.31±0.04^{d}	1.76±0.09^{a}	146±4.76^{a}	1.33±0.09^{c,d}	12.34±0.22^{a,d}
	Semolina Cappelli	11.15	4.27±0.28^{d}	1.73±0.00^{a}	145±4.11^{a}	1.28±0.09^{b,c,d}	13.62±0.87^{d}
	Semolina Claudio	9.30	5.00±0.18^{e}	1.92±0.03^{c,f}	156±5.14^{b}	1.40±0.15^{d,f}	11.13±0.88^{a,b,c}
	Semolina Core	9.00	5.98±0.38^{a,b}	2.02±0.06^{c}	157±7.26^{b}	1.68±0.05^{e}	9.94±0.61^{b,c}
	Semolina Iride	9.20	6.53±0.46^{c}	2.01±0.13^{c}	157±6.98^{b}	1.61±0.23^{e}	12.04±0.78^{a,d}
	Semolina Saragolla	9.40	6.20±0.21^{c}	1.90±0.04^{f}	158±4.36^{b}	1.55±0.09^{e,f}	10.93±0.62^{a,b,c}
Stone mills	Wholemeal Anco Marzio	9.00	4.96±0.20^{e}	1.64±0.01^{a,b}	140±3.43^{a}	1.02±0.04^{a}	11.70±0.82^{a,c}
	Wholemeal Cappelli	10.15	5.75±0.26^{a,b}	1.67±0.05^{a,b}	132±2.30^{d}	1.01±0.08^{a}	11.67±0.44^{a,c}
	Wholemeal Claudio	9.20	5.62±0.20^{b}	1.58±0.11^{b,e}	146±2.39^{a}	1.08±0.04^{a,b}	10.67±0.86^{a,b,c}
	Wholemeal Core	8.30	6.20±0.17^{a,c}	1.65±0.03^{a,b}	144±4.38^{a}	0.78±0.12^{g}	9.46±1.78^{b}
	Wholemeal Iride	9.00	7.63±0.23^{g}	1.52±0.02^{d,e}	123±4.16^{c}	1.16±0.05^{a,b,c}	10.64±0.38^{a,b,c}
	Wholemeal Saragolla	8.20	7.05±0.46^{f}	1.44±0.03^{d}	118±5.22^{c}	1.06±0.10^{a}	9.57±0.47^{b}

$^{a\text{-}g}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 6 - Nutritional composition of semolina samples.

Sample	Protein (%s.s.)	IDF (%s.s)	SDF (%s.s)	TDF (%s.s)	Dietary Fat (% s.s)	Ash (%s.s)	ACH (g/100g)
Timilia semolina	16.77±0.07^{c}	3.56±0.11^{a}	3.20±0.14^{b}	6.76±0.25^{a}	1.84±0.01^{b}	0.84±0.03^{a}	76.01±0.32^{c}
Cappelli semolina	16.88±0.06^{d}	5.45±0.01^{d}	2.75±0.05^{a}	8.20±0.06^{c}	1.72±0.04^{a}	0.91±0.00^{b}	74.27±0.73^{b}
Pr22d89 semolina	15.20±0.02^{a}	4.04±0.23^{b}	3.19±0.11^{b}	7.23±0.35^{b}	1.92±0.03^{c}	0.92±0.01^{b}	69.66±0.41^{a}
Pr22d89(+Se) semolina	15.90±0.05^{b}	5.18±0.32^{c}	3.44±0.04^{b}	8.62±0.36^{d}	2.15±0.06^{d}	0.91±0.02^{b}	69.61±0.38^{a}

$^{a-d}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 7- Chemical composition of durum wheat semolina spaghetti samples.

Sample	Protein (%s.s.)	IDF (% s.s.)	SDF (% s.s.)	TDF (%s.s.)	Dietary fat (% s.s.)	Ash (%s.s)	ACH (g/100g)
Timilia spaghetti	16.67±0.07^{c}	2.26±0.06^{a}	5.02±0.03^{b}	7.28±0.03^{a}	1.82±0.02^{a}	0.87±0.01^{a}	77.76±0.10^{c}
Cappelli spaghetti	16.78±0.06^{d}	4.23±0.33^{b}	4.25±0.08^{a}	8.48±0.14^{b}	1.78±0.02^{a}	0.98±0.02^{c}	72.00±1.97^{b}
PR22D89 spaghetti	15.10±0.02^{a}	3.88±0.07^{b}	3.64±0.16^{a}	7.52±0.23^{a}	1.92±0.03^{b}	0.95±0.02^{c}	68.63±0.16^{a}
PR22D89(+Se) spaghetti	15.85±0.05^{b}	5.16±0.38^{c}	3.78±0.08^{a}	8.94±0.30^{c}	2.13±0.04^{c}	0.91±0.02^{b}	68.63±0.16^{a}

$^{a-d}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 8 – Sensory characteristics of dry, non-cooked and cooked, semolina spaghetti samples**.**

Sample	Uncooked Spaghetti			Cooked Spaghetti								
	Color	Resistance to break	Overall Quality	Elasticity	Firmness	Fibrous	Bulkiness	Adhesiveness	Color	Odor	Taste	Overall Quality
Timilia	6.92±0.30^{a}	6.30±0.32^{a}	6.01±0.26^{a}	6.00±0.32^{a}	5.60±0.32^{a}	6.92±0.30 ^{a}	7.03±0.26^{a}	7.10±0.32^{a}	6.73±0.16^{a}	6.35±0.18^{a}	6.38±0.10^{a}	6.08±0.20^{a}
Cappelli	7.50±0.22^{b}	7.05±0.23^{c}	7.25±0.27^{b}	6.52±0.11^{b}	6.20±0.26^{b}	6.83±0.32^{a}	6.82±0.35^{a}	6.83±0.32^{a}	7.08±0.23 ^{a,b}	6.92±0.20^{b}	6.80±0.12^{b}	6.30±0.15^{a}
PR22D89	7.17±0.26 ^{a,b}	6.87±0.34 ^{a,b}	6.72±0.32^{b}	6.58±0.30^{b}	6.32±0.30^{b}	6.85±0.16^{a}	6.87±0.26^{a}	6.90±0.32^{a}	7.20±0.12 ^{a,b}	6.86±0.26^{b}	7.21±0.16^{c}	7.23±0.28^{b}
PR22D89 (+Se)	7.30±0.13^{a,b}	6.90±0.34 ^{a,b}	7.00±0.35^{b}	6.58±0.31^{b}	6.42±0.30^{b}	6.82±0.12^{a}	6.92±0.20^{a}	6.82±0.32^{a}	7.58±0.32^{b}	7.15±0.32^{b}	7.38±0.04^{c}	7.58±0.08^{b}

$^{a-d}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 9– Sensory analysis of uncooked and cooked dry spaghetti samples made with different blends of durum wheat bran.

	Uncooked Spaghetti			Cooked Spaghetti								
	Color	**Break to Resistance**	**Overall Quality**	**Elasticity**	**Firmness**	**Fibrous**	**Bulkiness**	**Adhesiveness**	**Color**	**Odor**	**Taste**	**Overall Quality**
PR22D89 (+Se)	7.30±0.13^{c}	6.90±0.34^{d}	7.00±0.35^{d}	6.58±0.31^{c}	6.42±0.30^{b}	6.82±0.12^{e}	6.92±0.20^{c}	6.82±0.32^{b}	7.58±0.32^{b}	7.15±0.31^{b}	7.38±0.04^{c}	7.58±0.08^{c}
PR22D89 (+Se) +5 %bran	7.20±0.14^{a}	6.75±0.14^{c,d}	7.00±0.14^{c}	6.46±0.09^{c}	6.34±0.09^{b}	6.44±0.14^{d}	6.54±0.09^{b}	6.31±0.17^{a}	6.90±0.18^{a}	6.80±0.05^{b}	7.26±0.08^{b,c}	6.44±0.13^{b}
PR22D89 (+Se) + 10 % bran	7.18±0.14^{a}	6.40±0.22^{b,c}	7.06±0.13^{c}	6.38±0.13^{c}	6.25±0.14^{b}	6.04±0.15^{c}	6.48±0.05^{b}	6.24±0.13^{a}	6.88±0.14^{a}	6.42±0.22^{a}	7.22±0.11 ^{a,b}	6.42±0.09^{b}
PR22D89 (+Se) + 15 % bran	7.00±0.14 ^{a,b}	6.30±0.14^{b}	6.30±0.11^{b}	5.50±0.07^{b}	6.16±0.21^{b}	5.46±0.09^{b}	6.33±0.21^{b}	6.14±0.23^{a}	6.83±0.13^{a}	6.32±0.16^{a}	7.14±0.03 ^{a,b}	6.34±0.21^{b}
PR22D89 (+Se) + 20 % bran	6.86±0.09^{a}	5.87±0.26^{a}	5.96±0.14^{a}	5.01±0.22^{a}	5.76±0.14^{a}	4.60±0.13^{a}	6.00±0.22^{a}	6.00±0.09^{a}	6.78±0.13^{a}	6.06±0.13^{a}	7.08±0.09^{a}	5.80±0.19^{a}

$^{a-e}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 10 – Sensory analysis of uncooked and cooked dry spaghetti samples enriched with durum wheat bran and 2%hydrocolloid.

	Uncooked Spaghetti			Cooked Spaghetti								
	Color	**Resistance to break**	**Overall Quality**	**Elasticity**	**Firmness**	**Fibrous**	**Bulkiness**	**Adhesiveness**	**Color**	**Odor**	**Taste**	**Overall Quality**
CM	7.06±0.09^{a}	6.50±0.07^{d}	6.46±0.05^{e}	5.44±0.09^{e}	5.48±0.08^{a}	5.78±0.19^{e}	5.48±0.08^{a}	5.50±0.07^{a}	6.78±0.19^{b}	6.50±0.07^{d}	7.08±0.08^{c}	6.50±0.07^{e}
PR22D89(+Se)-CTRL	7.50±0.11^{c}	6.90±0.34^{e}	7.50±0.07^{f}	6.58±0.31^{f}	6.42±0.30^{b,c}	6.82±0.12^{f}	6.92±0.20^{d}	6.82±0.32^{d}	7.58±0.32^{d}	7.15±0.31^{e}	7.38±0.04^{d}	7.58±0.08^{f}
PR22D89 (+Se) + 20%bran	6.86±0.09^{b}	5.87±0.26^{c}	5.96±0.14^{d}	5.01±0.12^{d}	5.76±0.14^{a}	4.60±0.13^{d}	6.00±0.22^{b}	6.00±0.09^{b}	6.78±0.13^{b}	6.06±0.13^{c}	7.08±0.09^{c}	5.80±0.19^{c}
PR22D89 (+Se)+ 20%bran+2% AGAR	7.06±0.09^{a}	6.00±0.19^{c}	6.06±0.09^{d}	4.68±0.25^{b,c}	5.78±0.23^{a}	4.50±0.07^{c,d}	6.50±0.07^{c}	6.50±0.07^{c}	7.08±0.08^{c}	7.06±0.05^{e}	7.02±0.04^{c}	6.06±0.09^{d}
PR22D89(+Se)+ 20%bran+ 2%CMC	7.06±0.08^{a}	6.12±0.08^{c}	6.54±0.04^{e}	5.12±0.22^{d}	6.14±0.21^{b}	4.50±0.07^{c,d}	6.50±0.07^{c}	6.46±0.05^{c}	7.08±0.08^{c}	6.16±0.05^{c}	7.34±0.05^{d}	6.60±0.08^{e}
PR22D89(+Se)+ 20%bran +2%GUAR	7.08±0.13^{a}	4.72±0.19^{a}	4.78±0.18^{a}	4.50±0.07^{b}	5.50±0.07^{a}	4.28±0.19^{b,c}	6.50±0.07^{c}	6.50±0.07^{c}	7.08±0.08^{c}	7.06±0.09^{e}	7.08±0.08^{c}	6.04±0.05^{d}
PR22D89(+Se)+ 20%bran +2%PECTIN	7.12±0.13^{a}	5.88±0.09^{c}	6.06±0.09^{d}	4.08±0.08^{a}	5.50±0.07^{a}	3.78±0.19^{a}	6.50±0.07^{c}	6.48±0.08^{c}	7.06±0.09^{c}	3.44±0.09^{a}	3.44±0.09^{a}	3.44±0.09^{a}
PR22D89(+Se)+ 20%bran+ 2%A.TAPIOCA	7.06±0.09^{a}	5.28±0.19^{b}	5.30±0.19^{c}	4.89±0.11^{c,d}	6.14±0.22^{b}	4.50±0.07^{c,d}	6.52±0.08^{c}	6.52±0.08^{c}	6.98±0.09^{b,c}	7.08±0.08^{e}	7.06±0.09^{c}	6.48±0.08^{e}
PR22D89 (+Se)+ 20%bran+ 2%CHITOSANO	7.10±0.10^{a}	5.12±0.13^{b}	5.08±0.08^{b}	4.12±0.13^{a}	6.72±0.19^{c}	4.14±0.11^{b}	7.06±0.09^{d}	7.08±0.08^{e}	6.48±0.08^{a}	4.78±0.19^{b}	4.74±0.21^{b}	4.78±0.19^{b}

$^{a\text{-}f}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 11 – Sensory analysis of uncooked and cooked dry spaghetti samples enriched with durum wheat bran and CMC.

	Uncooked Spaghetti			Cooked Spaghetti								
	Color	**Break to Resistance**	**Overall Quality**	**Elasticity**	**Firmness**	**Fibrous**	**Bulkiness**	**Adhesiveness**	**Color**	**Odor**	**Taste**	**Overall Quality**
CM	7.06 ± 0.09^{b}	6.50 ± 0.07^{b}	6.46 ± 0.05^{c}	5.44 ± 0.09^{b}	5.48 ± 0.08^{a}	5.78 ± 0.19^{b}	5.48 ± 0.08^{a}	5.50 ± 0.07^{a}	6.78 ± 0.19^{a}	6.50 ± 0.07^{b}	$7.08\pm0.08^{a,b}$	6.50 ± 0.07^{c}
CTRL	7.30 ± 0.13^{c}	6.90 ± 0.34^{c}	7.50 ± 0.07^{d}	6.58 ± 0.31^{c}	6.42 ± 0.30^{d}	6.82 ± 0.12^{c}	6.92 ± 0.20^{d}	6.82 ± 0.32^{d}	7.25 ± 0.35^{c}	7.05 ± 0.32^{c}	$7.28\pm0.06^{b,c}$	7.58 ± 0.08^{d}
PR22D89(Se) + 20% bran	6.86 ± 0.09^{a}	5.87 ± 0.26^{a}	5.96 ± 0.14^{a}	5.01 ± 0.12^{a}	$5.76\pm0.14^{a,b}$	4.60 ± 0.13^{a}	6.00 ± 0.22^{b}	6.00 ± 0.09^{b}	6.78 ± 0.13^{a}	6.06 ± 0.13^{a}	$7.08\pm0.09^{a,b}$	5.80 ± 0.19^{a}
PR22D89(Se) + 20% bran+ 1% CMC	$6.97\pm0.07^{a,b}$	6.02 ± 0.08^{a}	6.52 ± 0.04^{c}	5.09 ± 0.09^{a}	$6.14\pm0.21^{c,d}$	4.50 ± 0.07^{a}	6.50 ± 0.07^{c}	6.46 ± 0.05^{c}	$7.08\pm0.08^{a,b}$	6.08 ± 0.11^{a}	$7.16\pm0.15^{a,b,c}$	6.04 ± 0.19^{b}
PR22D89(Se) + 20% bran +2% CMC	7.06 ± 0.08^{b}	6.12 ± 0.08^{a}	6.54 ± 0.04^{c}	$5.12\pm0.22^{a,b}$	$6.14\pm0.21^{c,d}$	4.50 ± 0.07^{a}	6.50 ± 0.07^{c}	6.46 ± 0.05^{c}	$7.08\pm0.08^{a,b}$	6.16 ± 0.05^{a}	7.34 ± 0.05^{c}	6.60 ± 0.08^{c}
PR22D89(Se) + 20% bran +3%CMC	7.04 ± 0.05^{b}	6.12 ± 0.19^{a}	6.10 ± 0.07^{b}	4.98 ± 0.19^{a}	$6.06\pm0.09^{b,c}$	4.46 ± 0.21^{a}	6.38 ± 0.05^{c}	6.34 ± 0.05^{c}	$7.08\pm0.08^{a,b}$	$6.38\pm0.18^{a,b}$	6.96 ± 0.21^{a}	6.48 ± 0.04^{c}

$^{a-c}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$).

Table 12 - Nutritional analysis of dry spaghetti made with different blends of bran and 1%, 2%, 3% CMC.

Sample	Protein (%s.s.)	IDF (% s.s.)	SDF (% s.s.)	TDF (%s.s.)	Dietary fat (% s.s.)	Ash (%s.s.)	Moisture (%)	ACH (g/100g)
CM	13.63±0.06^{a}	4.27±0.25^{a}	1.94±0.06^{a}	6.21±0.31^{a}	2.63±0.20^{b}	1.34±0.04^{a}	9.81±0.04^{c}	72.92±0.99^{e}
CTRL	15.85±0.05^{f}	5.31±0.78^{b}	3,63±0.12^{b}	8,94±0.89^{b}	2,13±0.04^{a}	1.32±0.02^{a}	13.49±0.17^{d}	68.63±0.16^{d}
Pr22d89 (+Se) + 20% Bran	15.71±0.03^{e}	11.19±0.11^{c}	4.49±0.01^{c}	15.68±0.12^{c}	2.88±0.05^{c}	1.45±0.01^{b}	9.72±0.09^{c}	64.00±1.03^{b}
Pr22d89 (+Se) + 20% Bran + 1% CMC	15.11±0.04^{d}	13.70±0.01^{d}	4.84±0.17^{c}	18.54±0.18^{d}	3.00±0.02^{d}	1.49±0.02^{b}	8.25±0.08^{a}	60.12±0.78^{a}
Pr22d89 (+Se) + 20% Bran + 2% CMC	14.98±0.03^{c}	13.22±0.28^{d}	5.63±0.49^{d}	18.85±0.77^{d}	3.10±0.10^{d}	1.98±0.03^{c}	8.31±0.04^{a}	63.79±1.34^{b}
Pr22d89 (+Se) + 20% Bran + 3% CMC	14.43±0.10^{b}	13.76±0.11^{d}	7.21±0.31^{e}	18.97±0.41^{d}	3.16±0.02^{d}	2.16±0.02^{d}	8.64±0.02^{b}	66.65±0.76^{c}

$^{a\text{-}f}$ Mean in the same column followed by different superscript letters differ significantly ($p<0.05$)

Table 13 – Glycemic index of the reconstructed wholemeal spaghetti samples

Sample	Glycemic Index
CM	61±0.75[d]
CTRL	65±0.42[e]
Pr22d89(+Se) + **20% Bran**	58±0.10[c]
Pr22d89(+Se) + **20% Bran + 1% CMC**	57±1.01[b]
Pr22d89(+Se) + **20% Bran + 2% CMC**	55±1.21[a]
Pr22d89(+Se) + **20% Bran + 3% CMC**	55±0.20[a]

[a-f] Mean in the same column followed by different superscript letters differ significantly ($p<0.05$)

Table 14 - Cooking and textural properties of cooked spaghetti made with different blends of bran and CMC.

Sample	OCT (min)	Cooking Loss (%)	Swelling Index (g water/g dry pasta)	Water Absorption (%)	Adhesiveness (Nmm)	Hardness (N)
CM	8'00"	5.58±0.12[b]	1.72±0.01[a]	137.57±0.43[a]	0.59±0.03[a]	7.50 ±0.15[b]
Pr22d89 (+Se)	11'30"	5.00±0.16[a]	2.10±0.02[d]	170.25±0.60[e]	0.65±0.05[a]	6.21±0.25[a]
Pr22d89(+Se) **+ 20 % Bran**	11'15"	5.91±0.30[c]	1.88±0.004[b]	147.12±0.44[b]	0.73±0.02[a]	8.07±0.15[c]
Pr22d89(+Se) **+ 20 % Bran+ 1% CMC**	10'45"	6.22±0.09[c]	1.91±0.01[c]	150.39±2.44[c]	0.77±0.21[a]	10.70±0.33[d]
Pr22d89(+Se) **+ 20 % Bran+ 2% CMC**	10'45"	6.78±0.09[d]	1.92±0.03[c]	155.02±1.70[d]	0.78±0.05[a]	10.96±0.24[d]
Pr22d89(+Se) **+ 20 % Bran+ 3% CMC**	10'40"	7.24±0.23[e]	2.21±0.01[e]	174.66±0.63[f]	1.36±0.37[b]	12.00±0.02[e]

[a-e] Mean in the same column followed by different superscript letters differ significantly ($p<0.05$)

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"Keep your dreams alive. Understand that to achieve anything requires faith and belief in yourself, vision, hard work, determination, and dedication. Remember all things are possible for those who believe"
Gail Devers

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