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Evaluation of the use of cardoon inulin to make a new type of pasta with a low glycemic index

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1. INTRODUCTION

This work concerns the evaluation of different types of flours obtained by Ancient Whole-grains, and different types of inulin, for the production of a new pasta, with lowering glycaemic index effect on human health. The work was carried out in three years, from November 2014 to October 2017, as PhD research in "Innovation and Management of high healthy value foods", of the university of Foggia. The work was carried out at CNR-ISAFOM in Catania.

The topic, approved from the college, was related with the production of a new type of pasta with a low glycaemic index, based on the use of wholemeal durum wheat flour and the effects of the addition of inulin with different polymerization degree (DP) on the chemical and sensorial properties of spaghetti.

At the beginning, inulin types were extracted, purified and characterized from different plant materials (trial 1); the following trials concerned pasta production and its quality evaluation in terms mainly of consumer's acceptability.

Trial 2 regarded the production and the evaluation of spaghetti of wholemeal durum wheat flour with the addition of inulin, extracted from *Cynara cardunculus*, L. roots and from heads. In this way, the best part of plant to use for the extraction of the polymer was defined.

Trial 3 concerned the evaluation of pasta produced using cv "Russello" flour with the addition of inulin extracted from roots of cardoon plants grown in two different Sicilian environments.

The aim of trial 4 was the evaluation of the characteristics of pasta produced with the use of a type of Sicilian wholemeal flour: "Russello", added with inulin, extracted from tree different sources: *Cynara cardunculus* roots, C*hicorium intybus* roots and *Helianthus tuberosus* tubers. Inulin was added to pasta samples at different percentage: 2, 4 and 8% (w/w).

In Trial 5 the evaluation of characteristics of different types of pasta produced using four different flours, with the addition of inulin with different DP, extracted from roots of

Cynara cardunculus and from roots of C*hicorium intybus*, at different percentage: 2, 4% (w/w), was performed.

Regarding trial 6, experiments using quinoa flour were performed. In particular, different percentages of quinoa were tested to reach the threshold of acceptability of consumers.

The following trials (7) aimed to evaluate the properties of dry spaghetti produced using commercial wholemeal flour with 10% of Quinoa flour and the addition of inulin from two sources (cardoon and Chicorium) at 8% (w/w).

At the end of all trials, the addendum explains the work carried out during the six months of abroad stage.

1.1 FUNCTIONAL FOOD AND NUTRACEUTICALS

In the last decades consumer demand in the field of food production has changed considerably. Consumers more and more believe that foods contribute directly to their health (Young, 2000; Mollet & Rowland, 2002). Among supporting trends it can be mentioned that both experts (such as e.g. medical doctors, nutritional advisers) and consumers have realized and have started to accept that a close connection between nutrition and state of health exists (Hilliam, 1998; Young, 2000).

Scientific evidence, confirming the relationship between food and health, has promoted the rapid development of a new food market in recent years: the functional food market (Siró *et al*., 2008; Viuda-Martos *et al*., 2010).

Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans, but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers (Roberfroid, 2000b; Menrad, 2003).

In this regard, functional foods play an outstanding role. The increasing demand on such foods can be explained by the increasing cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of their later years (Roberfroid, 2000a, 2000b; Kotilainen *et al*., 2006).

Consumers, because of their advancing average age, are becoming increasingly interested in the relations between food and quality of life. 95% of the population believe that food possesses the potential to improve health by doing more than just providing nutrients (Dixon *et al*., 1999).

Functional food can be any food consumed as part of an accustomed diet which, beyond basic nutritional functions, is demonstrated to have physiological benefits and/or reduce the risk of some diseases, improve the general conditions of the body (e.g. pre- and probiotics), decrease the risk of some diseases (e.g. cholesterol-lowering products), and could even be used for curing some illnesses (Menrad, 2003; Mark-Herbert, 2004).

Not only food manufacturers, but also the pharmaceutical industry has become interested in this field. Functional foods have been developed in virtually all food categories (Sirò *et al*., 2008).

It is important to define the difference between functional food and nutraceuticals. Nutraceutical can be defined as a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease. When functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) other than anaemia, it is called a nutraceutical. Functional food can be either an unmodified 'natural food' or a food developed by adding, modifying or removing a component from the food. A functional food for one consumer may act as a nutraceuticals for another consumer (Shanon, 2010).

Both functional foods and nutraceuticals provide an opportunity to improve the health, reduce health care costs and support economic development in rural communities. According to market statistics, the global functional foods and nutraceuticals market is growing at a rate that is outpacing the traditional processed food market (Myeong *et al.*, 2010; El Sohaimy. 2012).

According to numerous studies, consumers are increasingly reflective in matters of health and willing to adopt health-oriented changes in their eating habits (Niva, 2007; Sirò *et al*., 2008).

Consumers are looking for name brand products to assure superior quality, even if the price may be a little higher (Shanon, 2010; El Sohaimy, 2012).

Moreover, it is beyond doubt that persuading people to make healthier food choices would provide substantial (public) health effects (e.g. decreased mortality, and increased quality of life), therefore it is a common economic and public interest (Van Kleef *et al*., 2005; Jones & Jew, 2007; Sirò *et al*., 2008).

This increasing consumer awareness in combination with advances in various scientific domains provides companies with unique opportunities to develop an almost infinite array of new functional food concepts (Bistrom & Nordstrom, 2002; Van Kleef *et al*., 2002; Sirò *et al*., 2008).

It should also be considered, that functional foods are sold at higher prices, thus contain larger profit margins than conventional foods, which obviously make the sector attractive for the players in the supply chain. This development and marketing require significant research efforts. This involves identifying functional compounds and assessing their physiological effects; developing a suitable food matrix, taking into account bio-availability and potential changes during processing and food preparation, consumer education, and clinical trials on product efficacy in order to gain approval for health-enhancing marketing claims (Kotilainen *et al*.,2006;).

It is a multistage process that requires input from commercial, academic and regulatory interests, with a critical need to achieve acceptance by the consumers (Jones & Jew, 2007; Sirò *et al*., 2008). Consumer acceptance of the concept of functional foods, and a better understanding of its determinants, are widely recognized as key success factors for market orientation, consumer-led product development, and successfully negotiating market opportunities (Gilbert, 2000; Grunert *et al*., 2000; Weststrate *et al*., 2002; Verbeke, 2005, 2006; Ares & Gàmbaro, 2007).

The acceptance of a specific functional ingredient is linked to the consumer's knowledge of the health effects of specific ingredients. Therefore, functional ingredients, which have been in the mind of consumers for a relatively long period (e.g. vitamins, fibres, minerals) achieve considerably higher rates of consumer acceptance than ingredients, which have been used since a short period of time (e.g. flavonoids, carotenoids, omega-3 fatty acids, selenium, xylitol, fructans) (Bech-Larsen & Grunert, 2003; Bech-Larsen & Scholderer, 2007; Krygier, 2007; Urala & Lahteenma ki, 2007).

By purchasing functional foods, in general consumers may achieve a modern and positive impression of themselves. These products provide consumers a modern way to follow a healthy lifestyle, which differs from the conventionally healthy diet defined by nutrition experts (Sirò *et al*., 2008).

The beneficial effects of functional foods and nutraceuticals include: reduced risk of cardiovascular diseases, reduced risk of cancer, weight loss/management, reduced osteoporosis, improved memory, quicker reaction time, improved fetal health and reduced risk of many other diseases (El Sohaimy, 2012).

For this reason, governments looking at regulatory issues for functional foods are more aware of the economic potential of these products as part of public health prevention strategies.

1.2 CARBOHYDRATES

Carbohydrates constitute an important class of biomolecules. They occur in nature as simple or complex carbohydrates (Rao, 1998).

Carbohydrates take the form of sugars, oligosaccharides, starches and fibres and are one of the three major macronutrients, which supply the body with energy (fat and protein being the others). Carbohydrates in all shapes and forms are good for our health. They can help to control body weight, especially when combined with exercise, are vital for proper gut function and are an important fuel for the brain and active muscles (Currel & Jeukendrup, 2008).

The most important message for the public recommended from the World Health Organization and the Food and Agriculture Organization of the United Nations on carbohydrates in human is: an optimum diet contains at least 55% of energy from carbohydrates and 20-35 g dietary fibre/day for all those over two years of age, a wide range of carbohydrate-containing foods should be consumed so that the diet is sufficient in essential nutrients and dietary fibre (DF) (Dobbing, 1989).

Moreover, scientific studies suggested that a diet containing an optimum level of carbohydrates may help to prevent body fat accumulation; starch and sugars provide readily accessible fuel for physical performance; DF which is a carbohydrate, helps to keep the bowel functioning correctly (Currel & Jeukendrup, 2008; El Sohaimy. 2012). It is particularly important to consider the carbohydrates intake in people suffering from diabetes.

Most recommendations for the dietary management of diabetes allow a modest amount of ordinary sugar as the inclusion of sugar with a meal has little impact on blood either glucose or insulin concentrations. Apart from the direct benefits of carbohydrates for the body, they are found in a wide range of foods which themselves bring a variety of other important nutrients to the diet. For this reason, it is recommended that carbohydrates be supplied from diverse food sources to ensure that the overall diet contains adequate nutrients. Consuming a wide range of carbohydrate foods is an acceptable part of the diet of all diabetics and the inclusion of low glycaemic index foods is beneficial as they help regulate blood glucose levels (Bolton-Smith & Woodward, 1994; World Health Organization, 1998).

As an important part of a balanced diet, the cereal group provides important amounts of most nutrients (Truswell, 2002).

Cereals can be applied as sources of non-digestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of lactobacilli and bifidobacteria present in the colon and act as prebiotics. Cereals contain water-soluble fibre, such as beta-glucan and arabinoxylan, oligosaccharides, such as galacto and fructo-oligosaccharides and resistant starch, which have been suggested to fulfil the prebiotic concept. Finally, cereal constituents, such as starch, can be used as encapsulation materials for probiotics in order to improve their stability during storage and enhance their viability during their passage through the adverse conditions of the gastrointestinal tract (Charalampopoulos *et al.,* 2002; Brennan & Cleary, 2005; Sirò *et al*., 2008).

1.3 FIBERS

Dietary fibre (DF) was originally defined in 1972 by Trowell as "that portion of food which is derived from cellular walls of plants which are digested very poorly by human beings" (De Vries, 2010; EFSA, 2010; Westenbrink *et al*., 2012). DF is found in plant foods (fruit, vegetables and wholegrains) and consists of portions of plant foods that are edible and non-digestible by humans. It is essential for maintaining a healthy digestive system. Fibre cannot be fully digested and it is often called bulk or roughage (Sivam *et al*., 2011). DFs are as soluble or insoluble, based on whether they form a solution when mixed with water (soluble), or not (insoluble) (Periago, 1993; Ajila & Prasada Rao, 2013).

Soluble fibre, which can dissolve in water, is found in beans, fruits and oat products and can help to lower blood fats and maintain blood sugar. It is abundant in wholegrain, barley and oats, as well as in fruits such as ripe, strawberries and bananas; this type of fibre forms a viscous indigestible mass in the gut and helps trap digestive enzymes, cholesterol, starch, glucose and toxins that are then expelled through the faeces. In this way, soluble fibre can help obese people reduce the amount of calories they absorb from food and help diabetics by reducing the rate of starch digestion and glucose absorption (F.A.C.S., 2006).

Insoluble fibre cannot dissolve in water, so it passes directly through the digestive system. It is found in wholegrain products and vegetables and it increases the rate at which food passes through the gut. Consumption of insoluble fibres such as cellulose and hemicelluloses, as found in bran, leafy vegetables or fruit skins (e.g. apples and pears), serve as roughage and help to reduce the caloric value of diets, which is important in obese and diabetic conditions (Selegean *et al*., 2009).

The insoluble fibre is related to both water absorption and intestinal regulation, whereas the soluble fraction is associated with the reduction of cholesterol in blood and the decrease of glucose absorption by the small intestine. Although soluble fibre is less common in foods than insoluble fibre, it is believed to have important effects in the digestive and absorptive processes (Dreher, 2001; Peressini & Sensidoni, 2009).

High-fibre foods take longer to digest, so keep you feeling fuller for longer. The slow and steady digestion of food through the gut helps to control blood sugar levels and weight maintenance.

DF has been known and investigated for a very long time (Asp, 2004), from being considered as waste to being described as a "universal remedy" that improves any physiological problem within human organism (Rodríguez *et al*., 2006; Yangilar, 2013). Indeed many studies have shown the link between the consume of more fibre (contained in quantity in wholegrains) and the prevention of some disease. The observation of the link between fibres consumption from grains (cereal fibre) or wholegrain foods and the risk of developing ischemic heart disease is consistent, relatively strong (20–50% reduction in risk), and independent of other lifestyle factors and body weight (Morris *et al*., 1977; Kushi *et al*., 1985; Khaw & Barrett-Connor, 1987; Fraser *et al*., 1992; Pietinen *et al*., 1996; Rimm *et al*., 1996; Jacobs *et al*., 1998, 1999; Wolk *et al*., 1999; Liu *et al*., 1999).

Moreover, data from meta-analysis provide some support for a larger effect of soluble than insoluble fibre on blood pressure (BP); furthermore, it is known that insulin may play a role in BP regulation (Landsberg, 2001), and DF has been shown to enhance insulin sensitivity and improve vascular endothelial function (Cleland *et al*., 1998).

Consequently, high intake of fibre, although mainly insoluble types from cereals, has been associated with lower risk of diabetes mellitus type 2 (Salmerón *et al*., 1997a,1997b; Chandalia *et al*., 2000; Meyer *et al*., 2000; Liu *et al*.,2000; Willet *et al*., 2002; Pereira *et al*., 2002).

It is not surprising that fibre promotes bowel regularity and keeping the gastrointestinal tract clean to help reduce the risk of developing diverticular disease and constipation. A high fibre diet may reduce the risk of developing diabetes and colorectal cancer (Dobbing, 1989). Therefore, over the years, DF has received much positive attention with regard to its potential as a pharma food, due to its ability to reduce cholesterol, diabetes and coronary heart disease and ease constipation (Telrandhe *et al*., 2012; O'Shea *et al*., 2012).

Recommended adult intakes for total fibre in countries, which have developed guidelines, range from 21 to 40 g/day, and World Health Organization has recommended that total fibre intake be 25 g/day (WHO/FAO, 2003; Food and Nutrition Board, Institute of

Medicine, 2001). However, estimates of actual total DF consumption range from 14 to 29 g/day, with only a few countries reporting fibre consumption at or above the WHO recommendation, and with most reported values below either national or WHO recommendations (Gray, 2006).

Fibres have long been recognized as the active nutrients responsible for the health benefits of fruits and vegetables to humans. Interest in incorporating bioactive ingredients such as DF and phenolic antioxidants into popular foods like bread has grown rapidly, due to the increased consumer health awareness. They have different functional properties that may affect perceived taste or use indifferent food applications (Fitch & Keim, 2012).

Further researches on DF sources, conducted on DF, conclude that there are so many utilized and under-utilized sources of DF. When incorporated in diet, these may perform various physiological functions related to digestive system, diabetes and heart diseases. Due to their physicochemical properties (such as viscosity, water holding capacity, solubility and gel formation), these carbohydrates improve the textural and rheological properties of food products. These indigestible carbohydrate sources can be used as partial replacement of flour and or fat in various processed food products (Mudgil *et al*., 2013). DF can be used in processed food products not only to improve the fibre content but also to improve the viscosity, texture, sensorial characteristics and shelf life of food products. Many by-products of food industry are rich in fibre and can be used as DF source for incorporation in processed foods. These by-products may include waste from fruits and vegetables industries (such as fruits and vegetables peel or skin), cereals industry (such as wheat bran, rice bran), etc. These fibre-rich by-products can be incorporated in food products as inexpensive, non-caloric bulking agents. However, the maximum level of fibre incorporation in different food products varies because it may cause undesirable changes in colour and texture of foods (Sangnark & Noomhorm, 2004).

The literature contains many reports about additions of DF to food products such as baked goods, beverages, confectionery, dairy, frozen dairy, meat, pasta and soups (Yangilar, 2013).

To be acceptable, a DF added to a food product must perform in a satisfactory manner as a food ingredient (Jaime *et al*., 2002; Figuerola *et al*., 2005).

Consumers may adapt their expectations in a changing world with new technologies. Health and nutrition become relatively more important under conditions of stress and increased environmental pollution and stress. Therefore, it is recently observed that there is an increasing demand for low calory, low fat and low cholesterol (functional) foods. An important development in this regard has been in DF mixed products. The enrichment of foods with DF is an effective way to enhance nutritional and physiological aspects and to promote functionality by influencing rheological and thermal properties of the final product (Yangilar, 2013).

1.4 PASTA

Pasta is a staple food eaten daily or weekly in quantities constituting a dominant moiety of the diet in many countries, it is regularly eaten in such quantities that constitutes a dominant portion of the diet worldwide (International Pasta Organization, 2014). Pasta is favoured by consumers for its versatility, ease of transportation, handling, cooking and storage properties, availability in numerous shapes and sizes, high digestibility, good nutritional qualities and relatively low cost. Therefore, pasta can be used as carrier of specific compounds. It is traditionally manufactured from durum wheat semolina.

Recently, the development of enriched pasta with a high dietary fibre content would be a good way to increase the fibre intake and reduce the glycaemic index of pasta, which would result in a product for specific nutritional purposes (Padalino *et al*., 2015).

1.5 ANCIENT WHOLE GRAINS AND QUINOA

Durum wheat (*Triticum durum*) accounts for approximately 8% of the total world wheat production (Abaye *et al*., 1997).

The habitual and exclusive consumption of flour “00”, the most widespread in both home and industry, is a danger for the health, because the total lack of fibre makes starch easily assimilated and as a result blood glucose rises rapidly. This results in an equally rapid rise in the level of insulin. Constantly high levels of glycaemia and insulin may, over time, predispose to the development of metabolic diseases such as obesity and type II diabetes, cardiovascular disease, and increase the risk of cancer.

Integral or less refined flours (type "0"), however, have the great advantage of keeping intact the nutrients present in the germ, the "soul" of the minerals, amino acids and vitamins, and the bran, which constitutes the more outer part of the grain, which contains the fibres.

The ancient Sicilian grains or local varieties of wholegrains are a series of 52 varieties of native grains of Sicily.

Sicily is one of the few areas of Southern Europe where it is still possible to find landraces of many agricultural species, particularly cereals and durum wheat.

Fortunately, nowadays more and more people are looking for old fashioned and traditional tastes (Gallo *et al*., 2010).

In the human diet, insoluble fibre is mainly derived from wholegrain products and soluble fibre from fruits, vegetables, pulses, and oats (Martinette *et al*., 2005).

Given that insulin resistance increases the risk of type 2 diabetes and cardiovascular disease (Reaven, 1995; Hsueh & Law, 1998; Ruige *et al*., 1998; Pereira *et al*., 2002), insulin sensitivity may be one important mechanism through which wholegrain consumption confers protection.

On the other hand, gluten allergy, or the developed gluten-sensitivity that has been more and more frequently encountered in recent years, is probably due to excessive consumption of modern grain which is rich in gluten. The advantage of using antique grains, wondering or at least distant, the possibility of developing gluten intolerance.

Wheat protein (and the wheat starch) is easily digested by nearly 99% of the human population. However, several screening studies in Europe, South America, Australasia, and USA suggested that approximately 0.5-1% of these population may have undetected celiac disease (Vanga & Kelly, 2014; Di Sabatino & Corazza, 2009). Celiac disease is a condition that is caused by an adverse immune system reaction to gliadin. This causes an inflammatory reaction and interference with nutrient absorption. The only effective treatment is a lifelong gluten-free diet.

While the disease is caused by a reaction to wheat proteins, it is not the same as wheat sensitivity, which require less gluten consume in diet.

Another ancient and overlooked grain is Quinoa (*Chenopodium quinoa*) originated in the Andian region of Equador, Bolivia, Colombia and Perù. It is a pseudo-cereal rather than a true cereal or grain, as it is not a member of the grass family (Kolata, 2009). The nutrient composition is very good compared with common cereals. Quinoa grain contain essential amino acids like lysine and good quantities of calcium, phosphorus and iron (Schlick *et al*., 1993). In contemporary time, this crop has become highly appreciated for its nutritional values, as its protein content is very high (18%). It is a good source of dietary fibres and it is gluten free and easy to digest (Vaughn & Geissler, 2009; Cooper, 2015).

The development of pasta produced using ancient Sicilian wholegrains and Quinoa flour, enriched with a high dietary fibre content would be a good way to increase the fibre intake and reduce the glycaemic index of pasta and the development of gluten sensitivity.

1.6 PROBIOTICS AND PREBIOTICS

Among soluble fibres, we can find prebiotics. Prebiotics are non-digestible dietary ingredients that benefit the host by selectively stimulating the growth and/or activity of beneficial bacteria in the colon (Gibson, 1998; Nazzaro *et al*., 2009). Prebiotics resist digestion in the stomach and small intestine and become available in the colon where they are selectively fermented by specific microbial groups. Dietary fructans that reach the colon may thus select for an enhanced growth and activity of the indigenous bifidobacteria population. The bifidogenic nature of fructans is explained by the nutritional advantage that most strains of bifidobacteria have over other microbial groups in the intestinal tract (Roberfroid *et al*. 1998).

Indeed, we have bacteria living in our gut; some of them are actually beneficial. These "friendly" bacteria help keep bad bacteria and yeast from growing in the intestinal tract. Bacteria also help make vitamin K and keep immune system functioning properly. Normally we have an abundance of friendly bacteria, however antibiotic therapy, stress and poor dietary choices may all cause intestinal dysbiosis, which is a bacterial imbalance that results in overgrowth of bad bacteria and yeast (Kingler, 2007; Lara-Villoslada, 2007; El Sohaimy, 2012).

Beneficial bacteria, which can be found in various foods, are called probiotics. When we eat probiotics, we will add these healthy bacteria to our intestinal tract. Common strains include Lactobacillus and Bifidobacterium families of bacteria. Probiotics bacteria like lactobacilli are naturally found in fermented foods. Some foods will have added probiotics as healthy nutritional ingredients (Keswani & Cohen, 2005; Collins & Gibson, 1999; El Sohaimy, 2012). Bifidobacteria and lactobacilli are associated with a number of beneficial health effects, such as increased colonization resistance against pathogens, stimulation of the host’s immune system and the production of beneficial fermentation products that provide energy to the colonocytes (Clausen & Mortensen 1995) and induce apoptosis of cancer cells (Cherbut *et al*. 1997).

Many studies have demonstrated the potentially extensive impact of prebiotics on the composition of the gut microbiota, stimulating directly or indirectly putative beneficial gut commensal other than lactic acid bacteria. By elucidating the mechanisms of probiosis and prebiosis, scientists can design enhanced functional foods tailored to improve host health (Saulnier *et al*., 2007; Delphine *et al*., 2009).

Prebiotics might enhance the growth and survival of the probiotic cultures by influencing the growth and metabolites of both the probiotic and the starter.

Prebiotics that feed the beneficial bacteria in the gut mostly come from carbohydrate fibres called oligosaccharides. As said above, sources of oligosaccharides include fruits, legumes and wholegrains. Fructo-oligosaccharides may be taken as a supplement or added to foods (Collins & Gibson, 1999; Lamoureux *et al*., 2002; El Sohaimy, 2012).

1.7 INULIN

Prebiotics that are specifically bifidogenic are the well-studied β(2-1)-fructans comprising of inulin, oligofructose and fructo-oligosaccharides (FOS) (Gibson, 2004; Sirò *et al*., 2008). Fructans, or polyfructosylsucroses, are fructose polymers that are derived from sucrose, a disaccharide of glucose and fructose. They are storage carbohydrates, similar to starch and sucrose, which are present in about 15% of flowering plant species. Plants that are able to synthesise fructans are scattered throughout several families, suggesting a polyphyletic origin of the trait. Often, only perennial members of a family store fructans. Specialised organs are often present for the storage of fructans. They are, for example, abundant in the leaf basis of grasses, the taproot of chicory (Cichorium intybus), the tubers of dahlia (Dahlia variabilis) and the bulb of tulip (Tulipa gesneriana) and onion (Allium cepa). Fructans are used by these plants during re-growth after defoliation and for sprouting. They are synthesised and stored in the vacuole. Inulin is a linear fructan consisting of β-(2→1) linked fructofuranosyl units, with a terminal glucose residual. The smallest inulin is the trisaccharide 1- kestose. The degree of polymerisation ranges usually between 3 and 60 and between 2 and 7 for inulin and oligofructose, respectively. The possible benefits on inulin to human health have now been studied for more than a decade. Inulin has been recognised as a beneficial food ingredient. It is a soluble food fiber that can not be digested by humans. It has prebiotic qualities as it is preferably fermented by beneficial bowel bacteria. Inulin is regarded as functional food, that is food (substances) with acknowledged beneficial health effects. (Ritsema & Smeekens, 2003; Apolinario *et al*., 2014; Drabinska *et al*., 2016). Depending on species and age of the plants, inulin presents different DP, from low fructose units (e.g. Cichorium with 20 units of fructose) until 100 units of some Asteracean plants *(Cynara, Echinops, topinambur*, etc.) (Raccuia & Melilli 2004; Raccuia & Melilli, 2010).

Non-digestible oligosaccharides of longer DP are more resistant to saccharolytic fermentation so that the metabolism takes place more distally in the colon (Hughes & Rowland, 2001). This difference in fermentability may also be reflected upon the actual changes that prebiotics have in the microbial community and general metabolism in the colon (le Blay *et al*., 2003).

Chemical degradation or controlled enzymatic hydrolysis of inulin with endoglycosidase enzymes results in oligofructose compounds with a DP between 2 and 20. Several studies have shown that the majority of Bifidobacterium species are capable of utilizing short-chain FOS and inulin (Roberfroid *et al*. 1998; Biedrzycka & Bielecka, 2004; Rossi *et al*. 2005).

Non-digestible oligosaccharides of long chain length are typically less (or more slowly) biodegradable than compounds of shorter chain length (Roberfroid *et al*., 1998).

Inulin needed a longer time of supplementation than oligofructose before positive effects were established. This can be explained by the higher DP.

Inulin is a fructan of longer DP than oligofructose. Therefore, inulin fermentation will take a longer time than oligofructose fermentation, which results in more residual carbohydrate breakdown in the distal colon compartments. These observations make us conclude that inulin as a longer chain fructan has a higher potency than oligofructose of inducing prebiotic effects in the distal region of the colon. It was observed that a combination of short-chain and long-chain fructans is physiologically more active than the individual fractions (van Loo, 2004).

Inulin-type fructans of longer-chain length show more pronounced beneficial effects to an in vitro-cultured colon microbiota than oligofructoses of a shorter-chain length. Additionally, these beneficial changes were noted not only in the proximal colon compartments, but also in the distal colon compartments (Van de Wiele *et al.,* 2007).

It is already known the consume of inulin and oligofructose as bifidogenic agents helps to maintain a good health in several ways. These fructans modulate the hormonal level of insulin and glucagon, thereby regulating carbohydrate and lipid metabolism by lowering the blood glucose levels; Inulin effect on the human organism was assessed in many clinical studies. It is thought it has prebiotic features, lowers plasma glucose and intestinal pH level, which results in higher calcium bioavailability. Additionally, it has a positive impact on the plasma lipid profile, acts as an immunomodulator, affecting digestive systems' lymphatic tissue. Due to its characteristics, it can be used in the diet of obese and type 2 diabetes mellitus patients (Horochowska *et al*; 2017). In recent findings, inulin-type fructans bind as ligands to TLR2 and TLR4 provides a mechanistic explanation for their immunomodulatory properties. Evidence is accumulating that other fructan-types have prebiotic, antioxidant and immunomodulatory properties as well. Fructans and their fermentation products (SCFAs, H_2) may act as signaling compounds and/or cellular redox regulators, differentially affecting different cell types by influencing AMPK and/or NF-κB signaling pathways (Peshev & Van den Ende, 2014). Adding inulin to pasta, made with ancient grain and Quinoa flour, can join the healthy aspect of insoluble fibres with the good effect of the soluble ones. Indeed, inulin is feasible to incorporate dietary fibre ingredients into pasta that may increase its nutritional value to the consumer compared to conventional pasta. Adding inulin to pasta may be a suitable strategy, since it would represent a minimal change in the diet of population and it could be maintained for a long period (Kaur, 2002).

The work is mainly based on the use of inulin with high DP. The effects of inulin with high DP addition on the cooking properties, texture and nutritional characteristics of durum wheat pasta was detected. The aim of the work was the evaluation of the quality of pasta produced using ancient Sicilian wholegrains and a mixture of Sicilian grains and Quinoa flour, both with the addition of inulin (from different sources), in order to obtain a functional food with a low glycaemic index.

The addition of inulin could compromise the sensorial, technological and nutritional aspect of pasta and could not be accepted and purchased by consumers (sensory panel). Aravind (2012) studied that two inulin types with differing DP and crystallinity have different levels of integration with the starch-gluten matrix during pasta preparation.

Carbs and pasta, fibres and inulin (in the right quantity and balance) can sustain the maintenance of a good health if consumed, and mostly if combined, in a healthy diet. For this reason, the purpose of the work was to process and develop a functional food based on the use of antique grains (known for their insoluble fibres, proteins and nutrients content) and inulin, whose effects as soluble and prebiotic fibre have been thoroughly examined. It can aim to contribute, by becoming part of daily diet, to the promotion of a diet, or dietary style, aimed at maintenance of the state of human health and well-being, without excluding that for some individuals, even if unknowingly, may be a nutraceutical food whose consumption is certainly more pleasant than taking supplements in pill form.

2. MATERIAL AND METHODS

GENERAL AIM OF THE WORK

The aim of the work was to evaluate the characteristics of pasta samples produced with the use of alternative flours like the ancient Sicilian wholegrains and Quinoa flour, added with inulins extracted from different sources: *Cynara cardunculus*, *Helianthus tuberosus* and *Chicorium intybus.* In order to reach these results, the work was divided in 7 different trials.

2.1 <u>TRIAL 1</u>: EVALUATION OF THE CHARACTERISTICS OF INULINS FROM DIFFERENT SOURCES

2.1.1 Aim

The aim of the work was to evaluate the quantitative and qualitative characteristics of different types of inulin, extracted from different sources:

- *Cynara cardunculus* L. heads (HI);
- *Cynara cardunculus* L. var. *altilis* DC "Line CDL" roots cultivated in two different environments:
    - Barrafranca (RI-BR);
    - Assoro (RI-AS);
- *Helianthus tuberosus* L*.:* local populations:
    - red "Ramacca" (RRH);
    - red "Santa Tecla" (RSH);
    - red "Pennisi" (RPH);
    - white "Pennisi" (WPH);
- *Chicorium intybus* L. local population "Francofonte" (CIL);
- *Chicorium intybus* L. commercial variety (CIC).

2.1.2 Materials and methods

Inulin extraction and purification from different sources

Cynara cardunculus L. var. *altilis* DC, line CDL, was cropped in the experimental field of Assoro (EN. 37°30'54'' N; 14°16'26'' E, 279 m. a.s.l.). Roots to extract inulin were collected (up to a depth of 40 cm) in May 2014 before plant flowering, when plants maximize yields in long DP inulin. Fresh roots (consisting of both primary and secondary roots) and heads were washed in cold tap water, scraped and ground to a fine powder. 100 grams of the original homogenate was diluted ten-fold with water and put in a boiling water bath for 30 min. After cooling to room temperature the extract was filtered and centrifuged at 3000 rpm for 5 min. The inulin extracted was precipitated (in water) at 0° C overnight. The supernatant was removed and inulin was washed with distilled water and again precipitated at 0° C overnight. The washing process was repeated until inulin was white.

The colour was determinate by colorimeter Minolta CR 400. Inulin was lyophilized in Petri dishes and used for pasta production. On lyophilized inulin the moisture content were determined in a thermo-ventilated oven at 105°C. To test the effect of the DP during the pasta production, commercial inulin from *Chicorium intybus* was purchased from Orafti®. The mean DP of *Chicorium intybus* inulin was 20-25 fructose units.

The other plant sources to extract and purify inulin have been treated as above. In particular, *Helianthus tuberosus* was collected in November 2016 from different areas (Ramacca, Santa Tecla and Pennisi, CT) and *Chicorium intybus* was cropped in the field of Francofonte (SR) in November 2015.

Dry matter

In the laboratory, the moisture content of a representative sample of each inulin source was measured after drying the material to a constant weight in a thermo-ventilated drying oven at 105° C.

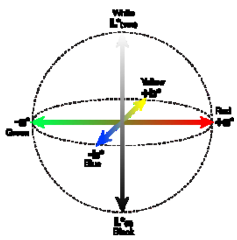
Inulin characterization

A representative amount of fresh samples was washed in cold tap water, scraped and ground to a fine powder with a mortar and pestle under liquid nitrogen. One gram of the original homogenate was diluted fivefold with water and put in a boiling water bath for 30 min. After cooling to room temperature, the extract was centrifuged at 3000 rpm for 5 min. A part of this fraction (TQ) was diluted 10-fold with distilled water to analyze free sugars (glucose, fructose, and sucrose) and another fraction (500 μL) was hydrolyzed (H) at 70° C for 2 hours using 5 μL of 3N HCl to analyze total fructose. Both the fractions were analyzed using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC PAD) (Thermofisher 3000), consisting of a metal-free isocratic pump, a pulsed amperometric detector, a metal-free injection valve with a 20 μL injection loop, and a CarboPac PA10 column (4 × 250 mm) with the guard column. The detection cell contained a gold working electrode (1.0 mm in diameter) and an Ag/AgCl reference electrode; the counter electrode was a titanium cell body across the 25 mm thin-layer channel from the working electrode. The column was regenerated with 1M NaOH for 10 min and equilibrated for 20 min after every run. Pulsed amperometric detection was carried out with the following waveform: t = 0.00 s (E = +0.05 V), t = 0.49 s

(E = +0.60 V), and t = 0.62 s (E = –0.60 V). The integration was at 0.28 s (beginning) and 0.48 s (end). The response time was 1 s, and the electric signal was integrated in nanoCoulomb (nC). All experiments were carried out at 30 °C under the following elution conditions: 90 mM NaOH with 50 mM Na-acetate for 1 min followed by a linear gradient from 50 to 500 mM Na acetate in 90 mM NaOH over a 60-min period with a flow-rate of 1 mL/min. Quantification was performed on the peak areas with the external standards methods for α-glucose, fructose, and sucrose (SIGMA, Steinhem, Germany). The carbohydrate standard solutions to be injected were prepared fresh daily. Inulin content was calculated as suggested by Baert (1997): I = (F + G) − (f + g + s) where F and G are total fructose and glucose after acid-hydrolysis and f, g, and s the reducing free sugars fructose, glucose, and sucrose before acid-hydrolysis. All the analyses were performed in duplicate and are reported on a dry matter (DM) basis. Inulin is reported in g kg^{-1} d.m.. For the qualitative analyses of inulin the TQ fraction was put into the HPAEC following the elution gradient of: 90 mM NaOH with 50 mM Na-acetate for 1 min followed by a linear gradient from 50 to 500 mM Na acetate in 90 mM NaOH over a 138-min period with a flow-rate of 0.8 mL/min. All the other chromatographic conditions remained the same as above.

Inulin colour evaluation

Inulin colour data were collected with the use of a Minolta colorimeter CR, 400. The three parameters L*, a* and b* refers to different sections of the colour spectrum. L* is the luminance, expressed as a percentage (0 for black and 100 for white); a* and b* are two ranges of colors, ranging from green to red and blue to yellow respectively, with values from -120 to +120. Measures were performed against a white plate (CTRL), L*= 88.0; a*= 0.3184; b*= 0.3359. Values of L* upper than 80 has been accepted for purification.



Data analyses

Data were submitted to the Bartlett’s test for the homogeneity of variance and then analyzed using analysis of variance (ANOVA). Means were statistically separated on the basis of Student-Newmann-Kewls test, when the “F” test of ANOVA for treatment was significant at least at the 0.05 probability.

2.1.3 Results

Dry matter

In table 1.1, dry matters of each plant source of inulin were listed.

Tab 1.1: Dry matter of plant materials under study.

SOURCES	DRY MATTER (g 100 g^{-1} f.w.)
HI	39.7±1.98
RI-BR	32.0 ±1.60
RI-AS	74.6 ± 3.73
RRH	69.6 ± 3.48
RSH	73.2 ± 3.66
RPH	72.3 ± 3.61
WPH	79.7 ± 3.98
CIL	18.8 ± 0.94

The highest water content percentage was detected in WPH with 80% and the lowest value was in CIL with 20%.

Inulin characterization

All the samples used for inulin purification presented a content of monosaccharides (glucose and fructose) and disaccharide (sucrose), in a different concentration.

In figure 1.1, a typical chromatogram of the three saccharides is reported.

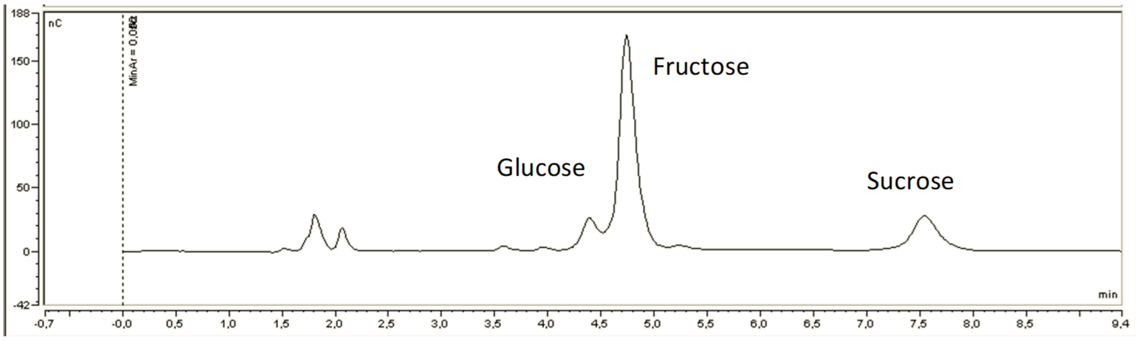


Figure 1.1: Free sugars in inulin extract. Extracts have been 10 fold diluted before injection in HPAEC.

In table 1.2 are reported the amount of each saccharides in all the plant material types, detected using G, F, S as external standards.

Table 1.2: Free sugars content (g kg^{-1} of d.m.) of the studied plant sources. Different letters indicate differences among them at $P< 0.05$.

SOURCES	GLUCOSE	FRUCTOSE	SUCROSE	TOT
	g kg^{-1} of d.m.			
HI	25.00 **c** 10%	115.00 **c** 44%	120.2 **a** 46%	260.2
RI-BR	34.03 **b** 9%	255.64 **b** 70%	75.94 **c** 21%	365.6
RI-AS	21.97 **c** 5%	362.04 **a** 77%	87.62 **b** 19%	471.6
RRH	22.99 **c** 17%	26.65 **d** 20%	84.46 **b** 63%	134.1
RSH	35.54 **b** 29%	38.83 **d** 31%	49.93 **e** 40%	124.3
RPH	56.82 **a** 34%	45.67 **d** 27%	65.31 **d** 39%	167.8
WPH	36.33 **b** 27%	41.13 **d** 31%	56.00 **e** 42%	133.5
CIL	1.60 **d** 9%	4.05 **e** 22%	12.10 **f** 68%	17.7
CIC	max 5 (Analysis carried out by ORAFTI)			
MEANS	29.28 14%	111.12 53%	68.94 33%	209.3

The highest glucose content was shown in *Helianthus tuberosus* (topinambur), population RPH. Fructose was found in a huge amount in RI-AS. The highest sucrose amount was found in CIL (68% on total sugars); it showed the lowest content of sugars (17.7 g kg^{-1} of d.m. in total). The highest fructose amount was found in cardoon roots (255 g kg^{-1} in RI-BR and 362 g kg^{-1} in RI-AS).

Inulin from different sources showed different yields and DP.

Cynara cardunculus

Quantitative analysis showed that it was possible to extract, from one kilogram of dry and peeled AS roots, a total sugar amount, as sum of glucose, fructose and sucrose of 820 g kg^{-1} d.m. and 780 g kg^{-1} in BR roots. 650 g kg^{-1} d.m. was the yield of inulin in heads.

There is a higher content of inulin in roots if compared with heads of *Cynara cardunculus.*

Figures show the qualitative analysis of inulins: HI (figure 1.2), RI-AS and RI-BR (figures 1.3 and 1.4).

The DP of inulin was 23 fructose units (mean) with a max 54 units in HI and 62 fructose units (mean) with a max 124 units in RI (AS).

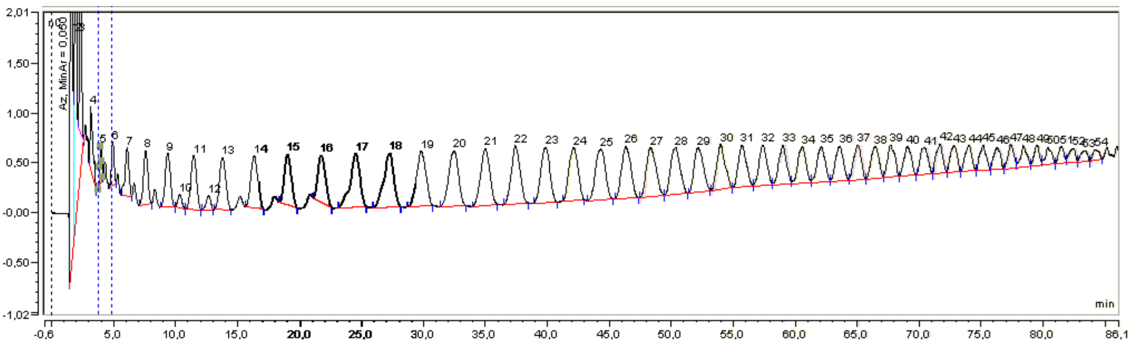


Figure 1.2: Inulin profile from heads of *Cynara cardunculus* (HI).

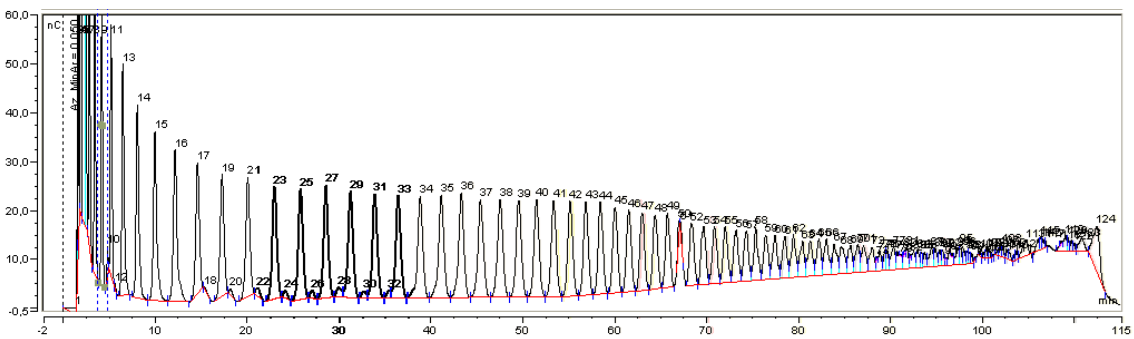


Figure 1.3: Inulin profile from roots of *Cynara cardunculus* (RI-AS).

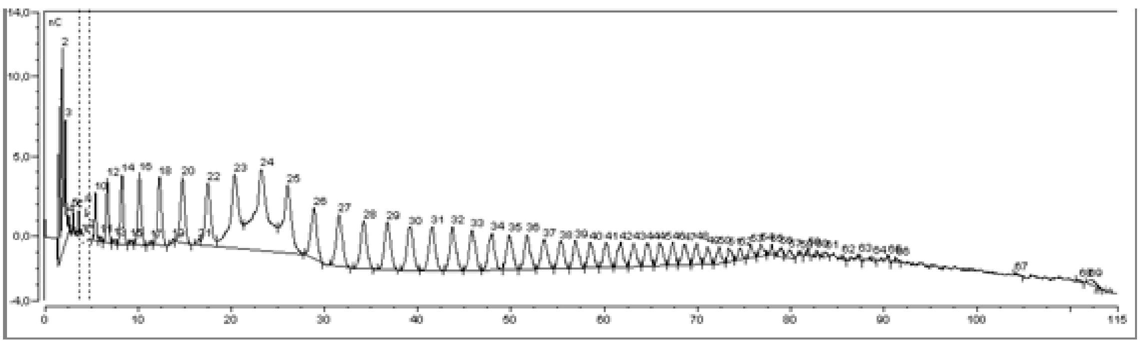


Figure 1.4: Inulin profile from roots of *Cynara cardunculus* (RI-BR).

Helianthus tuberosus

Helianthus tuberosus (HT) crops were collected in 2015 and 2016. The first year the collection of germplasm in the different areas of Eastern Sicily as above mentioned was performed. Collected tubers were grown at the experimental field of Cassibile during the growing season 2016.

Table 1.3 shows the inulin amounts of different population of *Helianthus tuberosus* in the two years of collection.

Table 1.3: Inulin amount in tubers of Topinambur, in the two years of collection. Different letters indicate differences among types of inulin P< 0.05.

POPULATIONS	2015	2016
	(g kg^{-1} d.m.)	
RSH	546.7 **b**	456.6 **b**
WPH	454.8 **d**	392.3 **d**
RRH	479.8 **c**	406.6 **c**
RPH	560.2 **a**	444.0 **a**
means	510.4	424.9

Inulin amount was lower in the second year of cultivation, probably due to the different environmental conditions of growing compared with the first year.

Figures 1.5, 1.6, 1.7 and 1.8 show the different DPs of inulin extracted from different sources of HT on 2015 and on 2016. The dilution before analyses was respectively 100 fold (2015) and 10 fold (2016).

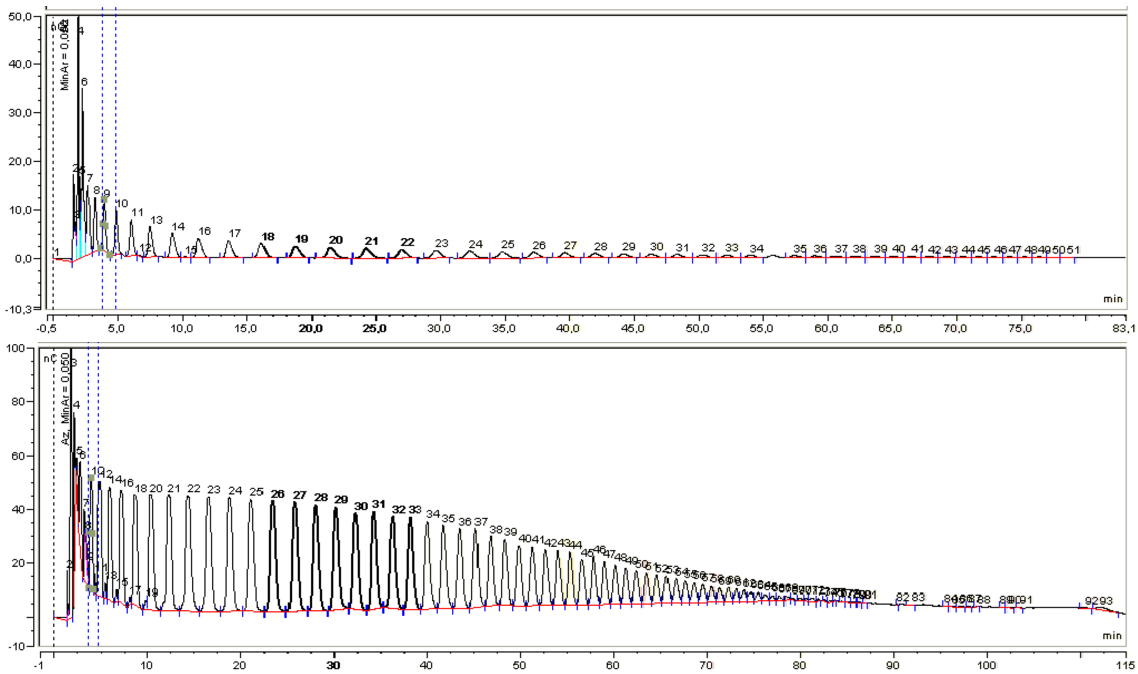


Figure 1.5: Inulin profile from *Helianthus tuberosus* population red “Ramacca” in 2015 (up) and 2016 (down).

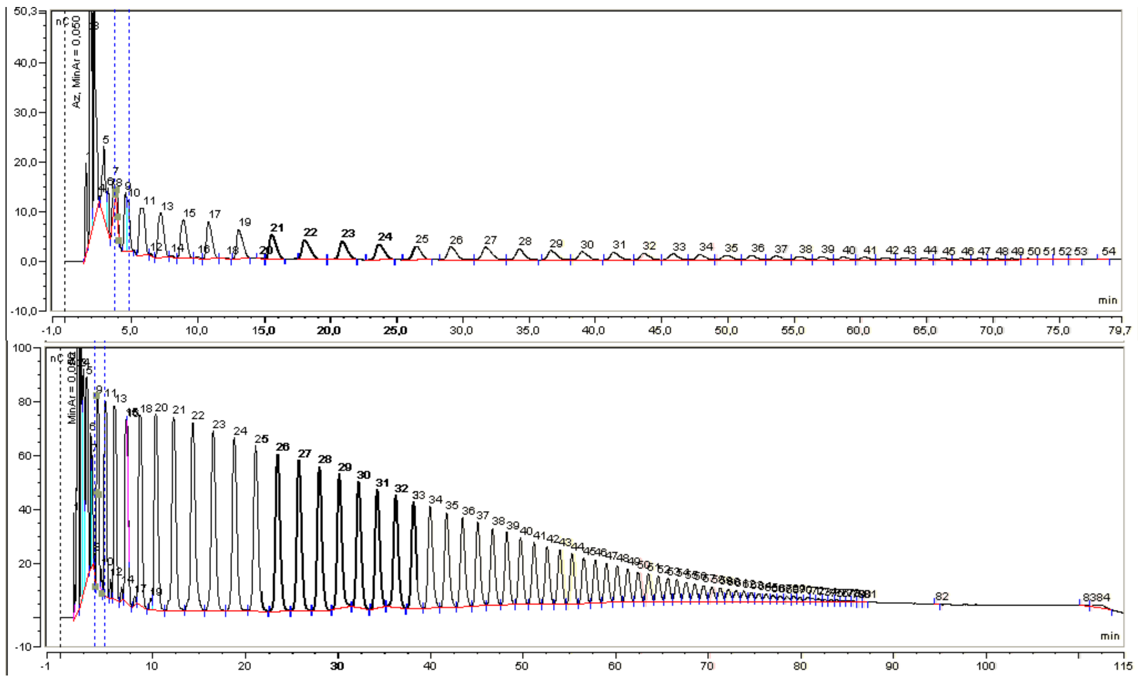


Figure 1.6: Inulin profile from *Helianthus tuberosus* population red “S.Tecla” in 2015 (up) and 2016 (down).

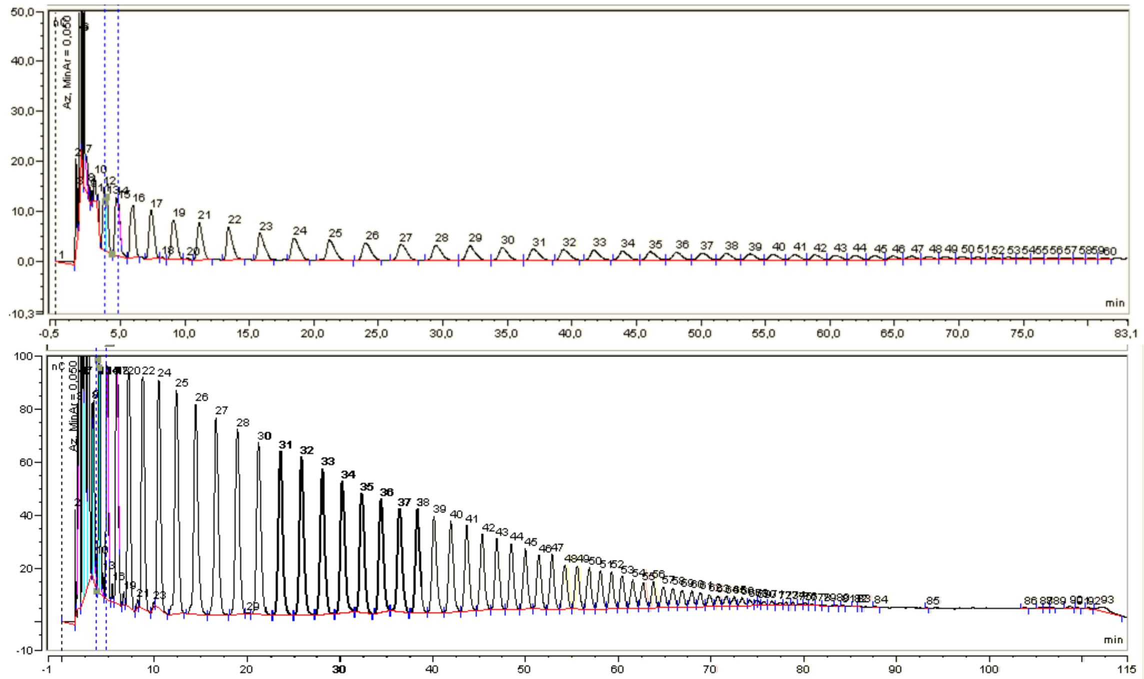


Figure 1.7: Inulin profile from *Helianthus tuberosus* population red “Pennisi” in 2015 (up) and 2016 (down).

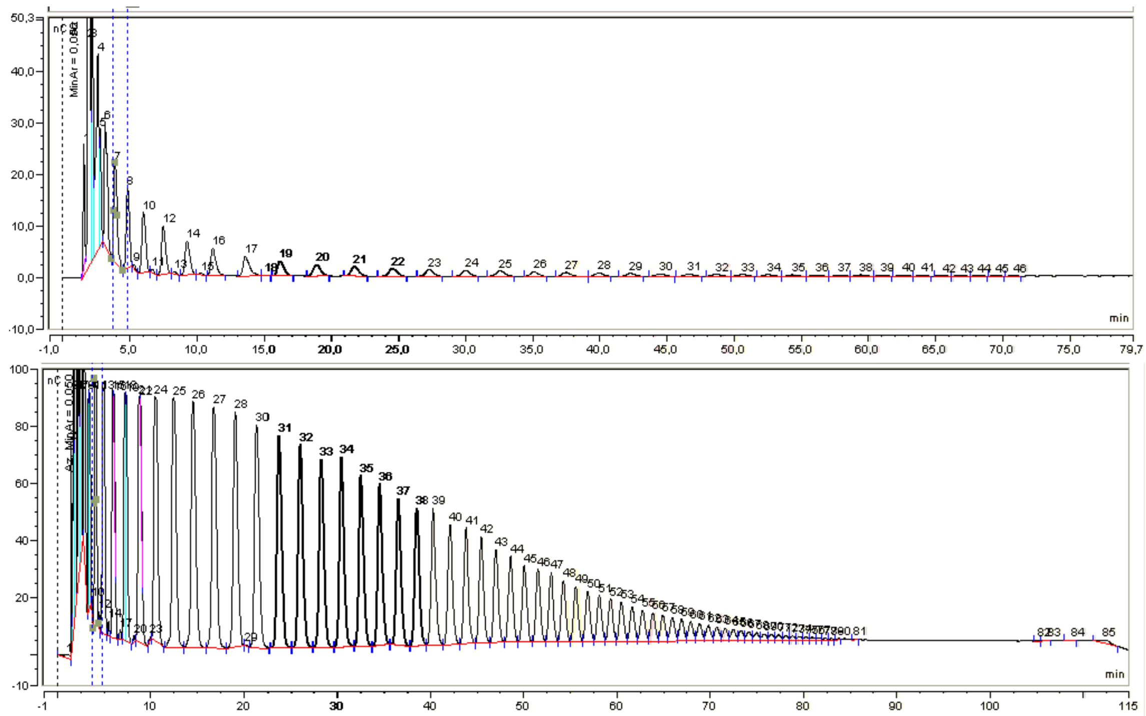


Figure 1.8: Inulin profile from *Helianthus tuberosus* population white “Pennisi” in 2015 (up) and 2016 (down).

Inulin DP in 2015 crop of *Helianthus tuberosus* reached a maximum of about 90 fructose units in red “Ramacca” and red “Pennisi” populations; a maximum of 84-85 fructose units was observed in populations red “S. Tecla” and white “Pennisi”.

The DP showed significant differences among the two years of cultivation. Shorter chain of inulin were detected in the first yield where the maximum DP was 60 fructose Units in pop. red “Pennisi” and the lowest was 46 fructose units in pop. white “Pennisi”.

There was an average of increase in the DP of about 30% probably due to the different environmental condition of cultivation. This could explain the lower sugar content and on the other hand the higher DP.

<u>*Chicorium intybus*</u>

Inulin extracted from *Chicorium intybus* local population had a yield of 144.4 g kg^{-1} d.m. Considering the very low amount, the purification process was not performed.

The DP of *Chicorium intybus* inulin was shown in figure 1.9 for commercial variety and 1.10 for local populations. It was maximum 59 fructose units in CIC (by ORAFTI) and 57 in CIL.

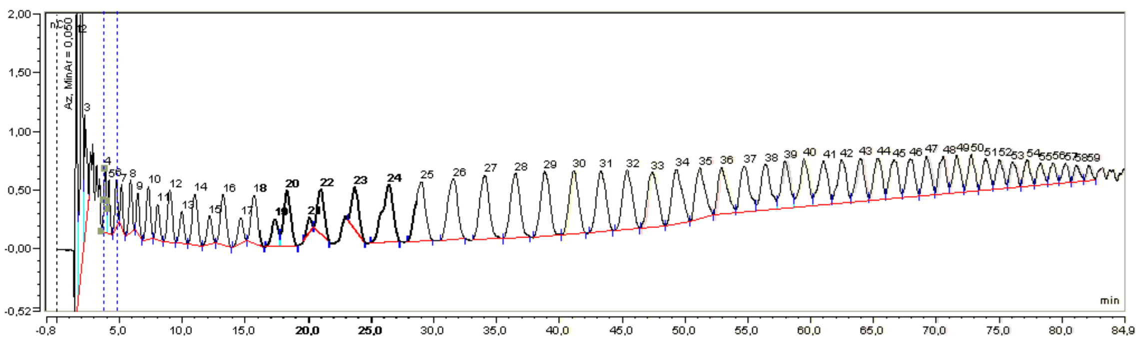


Figure 1.9: Inulin profile of commercial inulin.

Inulin that is manufactured by ORAFTI (Tienen, Belgium) as Raftilose® Synergy 1 is an oligofructose enriched inulin. This is a 1/1 mixture of long chain and short chain fractions of inulin extracted from Chicorium roots (*Cichorium intybus*). Inulin is made by a set of linear chains of fructose molecules, with a DP ranging between 3 and 65. It can be fractionated into a slowly fermentable long-chain fraction (DP ranging from 10 to 65, average 25) or in a rapidly fermentable fraction made of oligofructose (DP ranging from 3 to 8, average 4). Synergy1 is a mixture of both fractions, and has a higher amount of long chains relative to the native product.

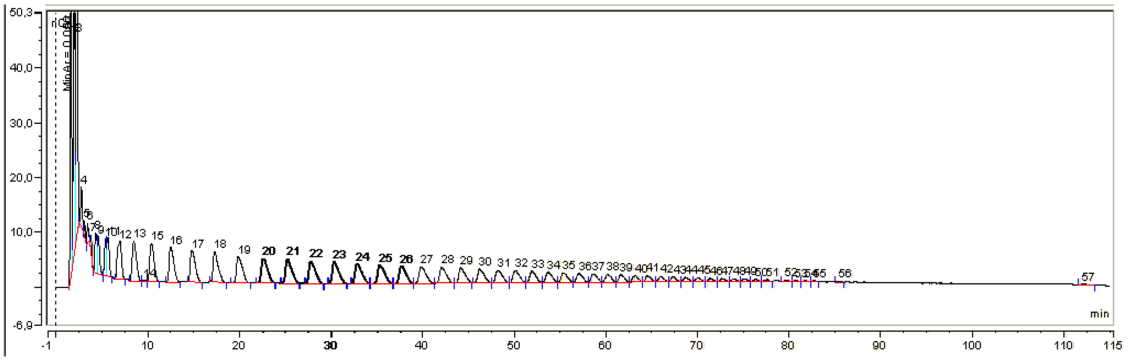


Figure 1.10: Inulin profile of "Francofonte" Chicorium

Inulin extracted from *Cynara cardunculus* roots had a higher yield than inulins extracted from different populations of *Helianthus tuberosus* and *Chicorium intybus*.

Inulin color evaluation

In table 1.4 are shown the L*, a* and b* values reached after inulins purification, from plant different sources. This parameter interferes with the sensorial analyses conducted on pasta added with inulins. Moreover the amount of phenols of *Cynara* roots led to a darker coloration of purified inulins (lower L* values), even if the white coloration reached was acceptable for all the polymers.

Table 1.4: colour scores (L*, a*,b* values) of lyophilised inulin extracted from different sources. Different letters indicate differences among types of inulin $P < 0.05$.

SAMPLE	L*	a*	b*
RI	85.35 **b**	0.17 **d**	8.23 **e**
HI	84.98 **b**	0.15 **d**	8.34 **e**
RRH	83.33 **d**	0.60 **b**	10.08 **b**
RSH	83.29 **d**	0.09 **d**	10.35 **a**
RPH	86.84 **a**	0.29 **c**	8.55 **d**
WPH	84.26 **c**	0.73 **a**	9.71 **c**
CIC	86.81 **a**	-0.75 **e**	4.15 **f**
Means	84.98	0.18	8.49

On the average of L* values (86.41), the highest score was detected in *Chicorium intybus*, the commercial variety, which shown the lowest score of a* value. Among the local germplasm, only RPH gave the same result. On the average of b* values (8.49), the highest score was in RSH inulin.

2.1.4 Conclusions

Concerning *Cynara cardunculus*, different amounts and DPs of inulin from different part of the plant were evaluated. An higher content of inulin was found in cardoon roots as compared with heads and, moreover, inulin DP was higher in RI than in HI.

As regard *Helianthus tuberosus*, even if a decrease in inulin amount was found in the second year of cultivation, an increase in the inulin DP of about 30%: from an average of 53 of fructose units in the first year, to an average of 89 in the second year. These differences could be attributed to the stressful environment of Cassibile, different from the areas of collection, where the plants resulted completely adapted.

In *Chicorium intybus* the content of inulin was the lowest of detected inulins. The DP was of 59 fructose units in the CIC and 57 in CIL.

With the aim to produce a functional food, studying the effect of inulin with a high DP, the extractions and purification were carried out from *Cynara cardunculus* roots and Topinambur tubers.

Concerning the color the highest score of L*was detected, as expected, in *Chicorium intybus*, commercial variety, but all the samples analysed reached the level of white that should not interfere with the sensorial characteristic of the pasta added with inulin.

2.2 TRIAL 2: EVALUATION OF THE PART OF THE PLANT USED FOR THE EXTRACTION OF THE POLYMER.

2.2.1 Aim

The aim of this trial was the evaluation of the characteristics of pasta samples produced using commercial wholemeal flour, with the addition of two types of inulin extracted from flowering receptacles (heads) and from roots of *Cynara cardunculus,* line CDL, selected at the laboratory of ISAFOM CNR, for high biomass production.

2.2.2 Materials and Methods

The inulin has been extracted, purified and characterized from head and roots of *Cynara cardunculus*, L. Line CDL. Plant materials were collected during the spring 2015, before the flowering stage.

After purification process, as described above, inulins from roots (RI) and from heads (HI) were added, to the wholemeal durum wheat flour to obtain fortified pasta.

On the pastas were evaluated: colour (Minolta colorimeter CR, 400), sensorial qualities (panel test), optimal cooking time (OCT) (minutes), cooking quality and loss of inulin (HPAEC-PAD, Thermofisher) after cooking.

Inulin extraction and characterization from Cynara cardunculus L.

The extraction, purification and characterization of *Cynara* inulins, both from roots (RI) and heads (HI), have been carried out as described in trial 1.

Pasta making

Spaghetti were produced with commercial wholemeal durum wheat flour by using the following operating conditions: wholemeal flours was mixed with water with a rotary shaft mixer (Namad, Rome, Italy) at 25 °C for 20 min so as to obtain a dough with 30% moisture content. Then, the different types of inulin were added at 2% (w/w): I) RI and II) HI.

In order to ensure the solubility of the inulins powder, they were previously dissolved in hot water. Pasta without inulin was produced and used as control (CTRL).

Pasta colour evaluation

Pasta colour was evaluated before and after cooking with the use of a Minolta colorimeter CR, 400 in the same way described for inulin colour evaluation in trial 1.

Sensorial Analysis of pasta

Dry spaghetti samples were submitted to a panel of fifteen trained tasters (six men and nine women, aged between 28 and 45) in order to evaluate the sensorial attributes. The panellists were selected on the base of their sensorial skills (ability to accurately determine and communicate the sensorial attributes such as appearance, odor, taste and texture of a product). The panellists were also trained in sensorial vocabulary and identification of particular attributes by evaluating durum wheat commercial spaghetti (ISO 11036, 7304). They were asked to indicate color and resistance to break of uncooked spaghetti. Elasticity, firmness, bulkiness, adhesiveness, fibrous nature, color, odor and taste were evaluated for spaghetti (Padalino, *et al*., 2013). To this aim, a nine-point scale, where 1 corresponded to *extremely unpleasant*, 9 to *extremely pleasant* and 5 to the *threshold acceptability*, was used to quantify each attribute (Petitot, *et al*., 2010). On the base of the above-mentioned attributes, panellists were also asked to score the overall quality of the product using the same scale.

Cooking quality evaluation

These analyses were performed in the laboratories of Foggia University. The optimal cooking time (OCT) was evaluated according to the AACC-approved method 66-50 (2000). The cooking loss and the amount of solid substance lost into the cooking water were determined according to the AACC-approved method 66-50 (2000). The swelling index of cooked pasta was determined according to the procedure desCardoonbed by Cleary and Brennan (2006). For each test, three spaghetti strands (40 mm length) were cooked at the OCT. After cooking, the spaghetti samples were gently blotted and submitted to hardness and adhesiveness analysis by means of 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). The three samples were put side by side on the lower plate, and the superior plate was moved down onto the spaghetti surface. The hardness (mean maximum force, N) and adhesiveness (mean negative area, Nmm) were measured. Six measurements for each spaghetti sample were performed. Trial specifications were as follows: preload of 0.3N; load cell of 1 kN; percentage deformation of 25%; crosshead speed constant of 0.25 mm s^{-1} (Padalino, *et al*., 2013). 2 cycles of pasta production for each type were performed, obtaining about 3 kg of spaghetti.

Inulin cooking losses

On 1 ml of residual water of the cooking test, the amounts of inulin eventually released by pasta was determined in HPAEC PAD, using the same method desCardoonbed for inulin extracted from roots. Only the content of fructose was considered for the calculation, because of the glucose released by starch during acid hydrolysis could interfere with the free glucose of the long inulin chain.

Data analyses

Data were submitted to the Bartlett's test for the homogeneity of variance and then analyzed using analysis of variance (ANOVA). Means were statistically separated on the basis of Student-Newmann-Kewls test, when the 'F' test of ANOVA for treatment was significant at least at the 0.05 probability.

2.2.3 Results

Pasta colour evaluation

Figure 2.1 shows the colour score (L*) of uncooked and cooked dry spaghetti samples.

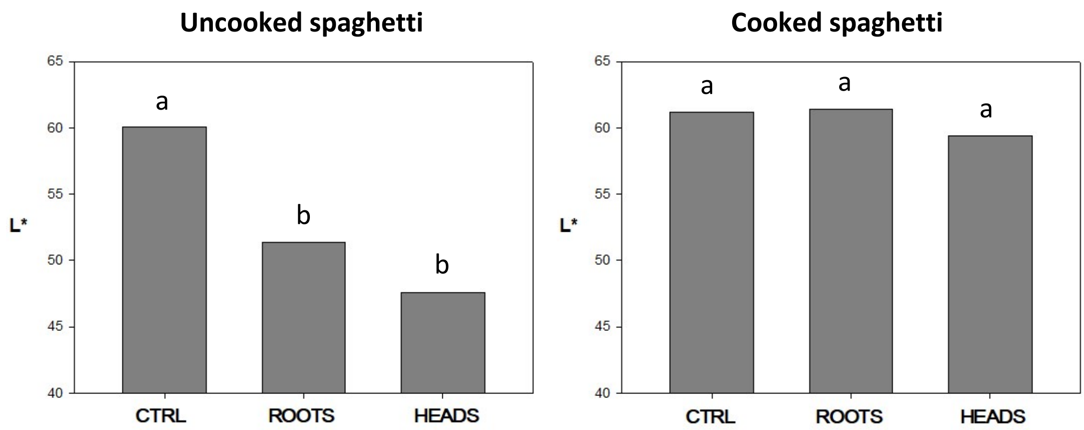


Figure 2.1: Colorimeter data of uncooked (left) and cooked (right) spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

The addition of inulin influenced the L* score in uncooked samples, where particularly the HI decrease the value, but did not give any significant changes in cooked samples. The average of colour score of cooked samples was 60.6 vs 53.0 in uncooked sample, suggesting an improvement of colour score during cooking process.

Sensorial Analysis of pasta

The addition of inulin fibre to pasta could in principle influence the structure of pasta, altering the continuity of the protein network as well as the protein-starch interactions. In other words, the use of inulin fibre in pasta could strongly influence the organoleptic characteristic of this product affecting the consumer acceptability.

The sensorial properties of fresh spaghetti samples investigated in this work were addressed, they are listed in table 2.1 for uncooked spaghetti samples.

Table 2.1: Sensorial analysis of fresh uncooked spaghetti samples. Different letters indicate differences among types of pasta $P < 0.05$.

SAMPLE	COLOR	HOMOGENEITY	ODOR	OVERALL QUALITY
CTRL	7.8 **a**	8.0 **a**	8.0 **a**	7.7 **a**
RI	7.6 **a**	7.5 **b**	7.8 **a**	7.5 **a**
HI	7.7 **a**	7.6 **b**	7.7 **a**	7.5 **a**
Means	7.7	7.7	7.8	7.6

Almost all properties did not show any differences in spaghetti samples with inulin addition, compared with samples control without inulin (CTRL). Homogeneity scores went down but it remains in the range of acceptability with a good value.

Sensorial properties of fresh cooked spaghetti samples are shown in figure 2.2 and listed in table 2.2.

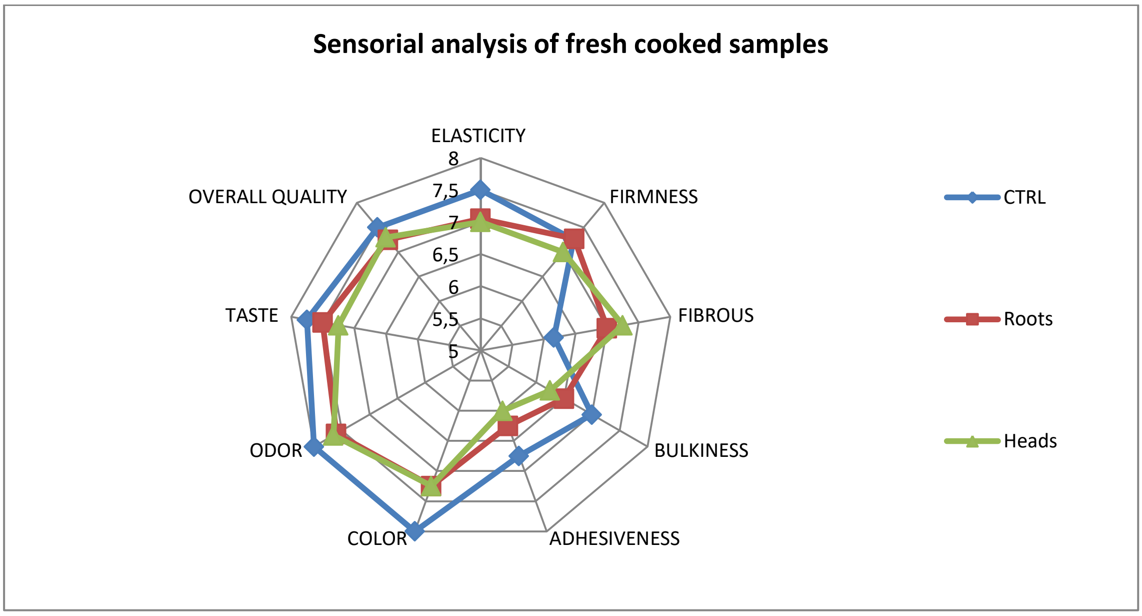


Figure 2.2: Sensorial analysis of fresh cooked spaghetti samples: pasta control (blue), pasta with RI (red) and pasta with HI (green)

No changes were detected in firmness (on average value of 7.2) and, lower compared with control sample, but very good scores, were registered in elasticity, colour, odour and taste.

Bulkiness and adhesiveness had lower scores than CTRL, improving consumer's acceptance. An increase of fibrous score was expected because of the addition of a soluble fibre.

In general, no significant changes were found in fresh cooked spaghetti samples with inulin compared with the CTRL.

Table 2.2: Sensorial analysis of fresh cooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

SAMPLE	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OVERALL QUALITY
CTRL	7.5 **a**	7.3 **a**	6.2 **b**	7.0 **a**	6.8 **a**	8.0 **a**	8.0 **a**	7.8 **a**	7.5 **a**
RI	7.1 **b**	7.3 **a**	7.0 **a**	6.5 **b**	6.3 **b**	7.3 **b**	7.6 **b**	7.5 **b**	7.3 **a**
HI	7.0 **b**	7.0 **a**	7.3 **a**	6.3 **b**	6.0 **b**	7.3 **b**	7.7 **b**	7.3 **b**	7.3 **a**
Means	7.2	7.2	6.8	6.6	6.3	7.5	7.8	7.5	7.4

Table 2.3 lists the sensorial properties of dry uncooked spaghetti samples.

Table 2.3: Sensorial analysis of dry uncooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

SAMPLE	ODOR	HOMOGENEITY	COLOR	BREAK TO RESISTANCE	OVERALL QUALITY
CTRL	8.0 **a**	8.0 **a**	7.2 **a**	6.7 **b**	6.9 **a**
RI	7.8 **a**	7.5 **b**	7.0 **a**	7.0 **a**	7.0 **a**
HI	7.8 **a**	7.7 **b**	7.4 **a**	7.3 **a**	5.3 **a**
Means	7.8	7.7	7.2	7.0	6.4

No differences were found in sensorial analysis of fresh uncooked spaghetti samples in odour and colour values. Homogeneity decreased a bit in samples with inulin added, instead break to resistance had an increase. This resulted in an absence of statistical differences in the overall quality score (OQS) of samples with inulin addition, compared with samples without inulin.

The sensorial properties of dry cooked spaghetti samples investigated in this work are shown in figure 2.3 and listed in table 2.4.

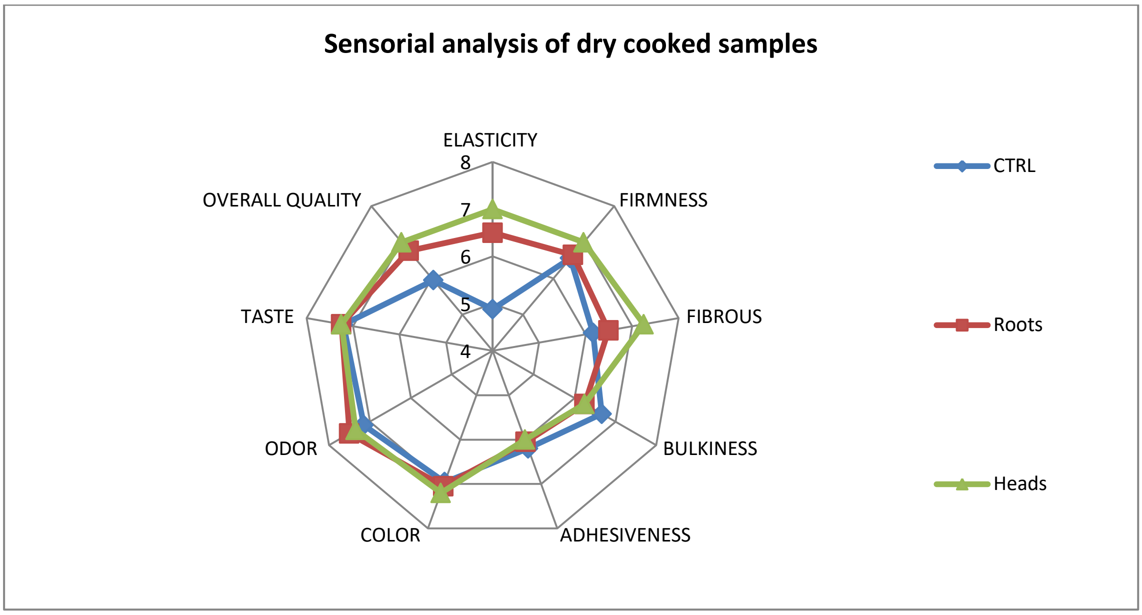


Figure 2.3: Sensorial analysis of dry cooked spaghetti samples: pasta control (blue), pasta with RI (red) and pasta with HI (green).

On the average of elasticity (6.1), the score was improved by addition of inulin, in particular with HI (7.0).

Also on the average score of fibrous (6.7), a higher value was in samples with HI addition (7.2).

Any changes were shown in firmness (average 6.7), bulkiness (6.3), adhesiveness (6.1), colour (7.1), odour (7.3) and taste (7.2) of different samples compared with spaghetti control.

Table 2.4: Sensorial analysis of dry cooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

SAMPLE	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OVERALL QUALITY
CTRL	4.8 **c**	6.5 **a**	6.1 **b**	6.6 **a**	6.2 **a**	6.9 **a**	7.1 **a**	7.2 **a**	5.9 **b**
RI	6.5 **b**	6.6 **a**	6.4 **b**	6.2 **a**	6.0 **a**	7.0 **a**	7.5 **a**	7.2 **a**	6.7 **a**
HI	7.0 **a**	7.0 **a**	7.2 **a**	6.2 **a**	6.0 **a**	7.2 **a**	7.3 **a**	7.2 **a**	7.0 **a**
Means	6.1	6.7	6.6	6.3	6.0	7.1	7.3	7.2	6.6

As shown in figure 2.4, OQS had an average of 6.6 with an improvement with the addition of inulin (on average values of 6.9) compared with the control (5.9). In general, between the two types of inulin, the best score in OQ was in sample with HI, with a score of 7.0.

Cooking quality evaluation

No differences was notice in the OCT (Optimal Cooking Time) that was 10.5 minutes for samples with and without inulin.

Cooking quality characteristics are shown in table 2.5.

Table 2.5: Cooking quality of dry spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

SAMPLE	COOKING LOSS (%)	SWELLING INDEX	WATER ABSORBTION
CTRL	4.9 **b**	6.3 **a**	151 **b**
RI	4.8 **b**	2.0 **b**	161 **a**
HI	5.2 **a**	2.2 **b**	160 **a**
Means	5.0	3.5	157

HI gave a higher cooking loss and water absorption compared with control and pasta with RI. The average of cooking loss was 4.9 with a max of 5.2 in HI samples.

The swelling index expressed as (weight of cooked spaghetti)−(weight of spaghetti after drying)/(weight of spaghetti after drying), decreased with the addition of inulin (average values 3.5). As Tudorica *et al*. (2001) referred, glucose release may be significantly reduced by the addition of soluble dietary fiber and in consequence the swelling index decrease with inulins addition. An increase of water absorption was found in samples with inulin because of its structure that react like a fibre, asking and blocking water in its structure.

Inulin cooking losses

In the pastas with the addition of HI it has been noticed an increase in overall quality in dry cooked samples, compared to the control, but on the contrary there was a greater cooking loss of the polymer (30.8) in samples with HI added, compared with the value recorded in the pasta with RI (27.2) (Figure 2.5).

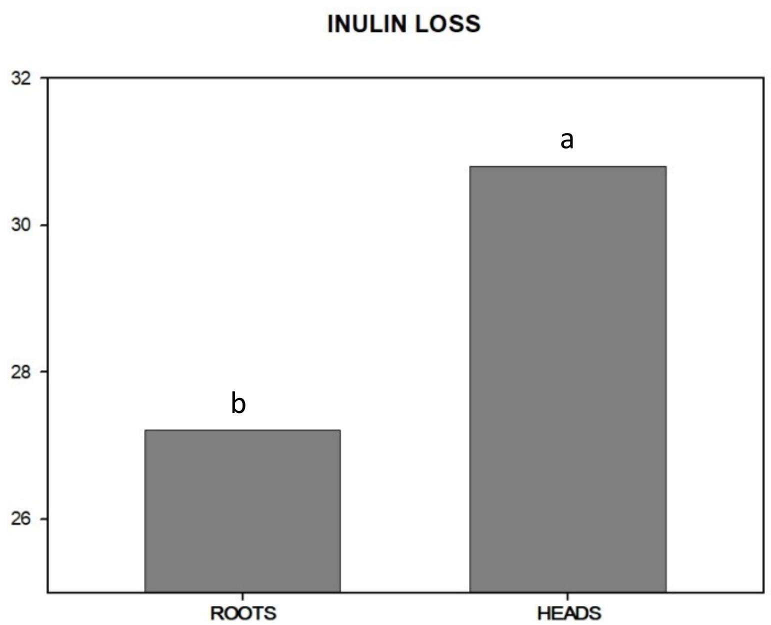


Figure 2.5: Inulin loss during cooking of samples with RI and HI. Different letters indicate differences among types of pasta samples with inulin P< 0.05.

2.2.4 Conclusions

The overall quality (panel analysis) of the pastas obtained with HI was satisfactory but, the high loss of the polymer during cooking, implies an increase of the concentration of inulin to add in the initial phase, making the process non-sustainable on the contrary to what was found by providing the use of the roots for inulin extraction.

2.3 TRIAL 3: EVALUATION OF THE GROWING ENVIRONMENT OF PLANT.

2.3.1 Aim

The aim of this trial was the evaluation of the effect of the growing environment of cultivation of cardoon plants on inulin characteristics. In particular, the effects of its addition on the quality score of pasta was studied.

2.3.2 Materials and Methods

The plants of cardoon were grown in two Sicilian growing environments, Assoro (AS) and Barrafranca (BR). Inulin was extracted from cardoon roots (genotype "CDL") and added to 4% (w/w) to the flour cv "Russello" for obtaining pastas. Pasta without inulin was used as control (CTRL).

On the pastas obtained were evaluated: colour (Minolta colorimeter CR, 400), sensorial qualities (Panel test), optimal cooking time (OCT) (minutes), cooking quality and loss of inulin (HPAEC-PAD, Thermofisher) in cooking.

Location of the trial, plant material and crop management

Cynara cardunculus L. var. *altilis* DC, line CDL, was cropped in two different environments: the experimental field of Assoro (EN.37°30’54”N; 4°16’26”E, 279 m a.s.l.) and Barrafranca (EN. 37°26’39”N; 14°15’26”E, 572 m a.s.l.), both the environments are located in the internal hilly area of Sicily. Cultivated cardoon plants were sown in September 2014, using a density of 6 plant m ^{2}. During the two years of the experiment, the energy inputs for crop management were minimized. Crop water requirements were satisfied by rain and one irrigation per year in May (flowering) with 50 mm of water. In the first year, another irrigation (50 mm of water) was carried during the establishment of the crop. In the second year, the crop regrowth was naturally carried out by rainfall. In both the environments roots were collected in plant two years old.

Inulin extraction and characterization from Cynara cardunculus L.

Inulin from roots of *Cynara cardunculus* cropped in the fields of Assoro (AS) and Barrafranca (BR) was extracted, purified and characterized as trial 1.

Pasta making

Spaghetti were produced with durum wheat flour cv ”Russello” by using the same operating conditions of trial 2. Then, the different types of inulin were added at 4% (w/w): I) inulin extracted from cardoon roots cultivated in Assoro (AS) field and II) inulin extracted from cardoon roots cultivated in Barrafranca (BR) field. In order to ensure the solubility of the inulin powder, they were previously dissolved in water. Pasta without inulin was produced and used as control (CTRL).

Pasta colour evaluation

Pasta colour was recorded before and after cooking with the use of a Minolta colorimeter CR, 400 as seen in trial 1 for inulin colour evaluation.

Sensorial Analysis of pasta

Dry spaghetti samples were submitted to the same panel of fifteen trained tasters as the trial 2, in order to evaluate the sensorial attributes.

Cooking quality evaluation

These analyses were conducted in the laboratories of Foggia University. The optimal cooking time (OCT) was evaluated according to the AACC-approved method 66-50 (2000). The cooking loss and the amount of solid substance lost into the cooking water were determined according to the AACC-approved method 66-50 (2000). The swelling index of cooked pasta was determined according to the procedure desCardoonbed by Cleary and Brennan (2006).

Inulin cooking losses

Inulin cooking losses evaluation was performed as described in trial 2.

Data analyses

Data were submitted to the Bartlett’s test for the homogeneity of variance and then analyzed using analysis of variance (ANOVA). Means were statistically separated on the base of Student-Newmann-Kewls test, when the ‘F’ test of ANOVA for treatment was significant at least at the 0.05 probability.

2.3.3 Results

Inulin extraction and characterization from Cynara cardunculus L.

Qualitative analysis of both inulins extracted from Assoro (AS) and Barrafranca (BR) roots are shown in trial 1. The DP of two types of inulin in AS was >100 fructose units and in BR samples <60 fructose units, both types of inulin extracted from cardoon roots showed an higher DP compared with commercial inulin by Orafti®.

Pasta colour evaluation

The L* index of both cooked and uncooked spaghetti samples are shown in figure 3.1 and listed in table 3.1.

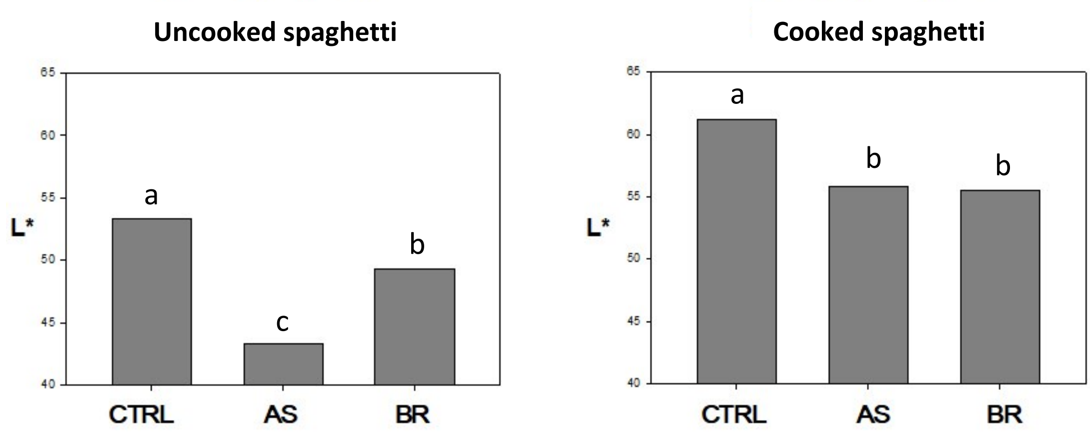


Figure 3.1: Colour of uncooked (left) and cooked (right) spaghetti samples with two different types of inulin. Different letters indicate differences among types of pasta P< 0.05.

In uncooked spaghetti, the CTRL had L* of 53.4. Adding inulin, the brightness decreased, with values of L*49.3 (BR) and 43.4 (AS). These differences resulted less marked after the cooking process, with an increase in L*, on average of AS and BR of 20.3%.

Sensorial Analysis of pasta

Data from sensorial analysis of fresh uncooked spaghetti samples are shown in table 3.2.

SAMPLE	COLOR	HOMOGENEITY	ODOR	OVERALL QUALITY
CTRL	7.5 **a**	6.2 **a**	7.2 **ab**	7.2 **a**
AS	7.0 **a**	6.0 **a**	6.8 **b**	7.0 **a**
BR	7.2 **a**	6.4 **a**	7.7 **a**	7.1 **a**
Means	7.2	6.2	7.3	7.1

Table 3.2: Sensorial analysis of fresh uncooked spaghetti samples. Different letters indicate differences among types of pasta $P< 0.05$.

In fresh uncooked spaghetti samples, there were no changes in almost all characteristics but in odour was a bit influenced by the addition of AS inulin. The best odour score was 7.7 of the sample with BR inulin.

On the average of values, overall quality was not being conditioned by the addition of inulin.

Figure 3.3 shows the sensorial characteristics of fresh cooked spaghetti samples. Values are listed in table 3.2.

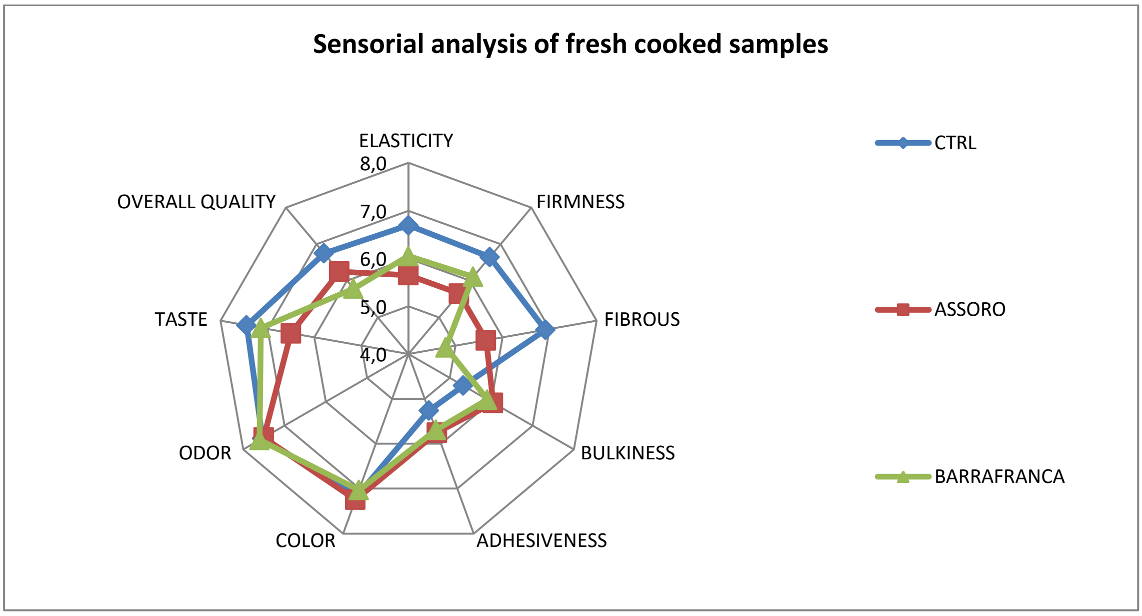


Figure 3.3: Sensorial analysis of fresh cooked pasta samples: CTRL without inulin (blue), pasta with AS inulin (red) and pasta with BR inulin (green).

Average value of both elasticity and firmness was 6.1, with a little but significant decrease with the addition of inulin.

On the average value of fibrous (5.8), the lowest score was detected in AS sample (4.8).

No changes was found in bulkiness, adhesiveness, colour and odour.

On the taste average value of 7.0, AS obtained the lowest score (6.5).

In general, the overall quality was good (6.3) but it went a bit down with the addition of inulin from AS (6.2) and BR (5.8).

Table 3.2: Sensorial analysis of fresh cooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

SAMPLE	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OVERALL QUALITY
CTRL	6.7 **a**	6.6 **a**	6.9 **a**	5.3 **a**	5.3 **a**	7.2 **a**	7.5 **a**	7.4 **a**	6.7 **a**
AS	5.6 **b**	5.6 **b**	5.6 **b**	6.0 **a**	5.7 **a**	7.2 **a**	7.5 **a**	6.5 **b**	6.2 **ab**
BR	6.1 **b**	6.1 **b**	4.8 **c**	5.9 **a**	5.7 **a**	7.0 **a**	7.6 **a**	7.1 **a**	5.8 **b**
Means	6.1	6.1	5.8	5.8	5.6	7.1	7.5	7.0	6.3

Data from sensorial analysis of dry uncooked spaghetti samples are shown in table 3.3.

Table 3.3: Sensorial analysis of dry uncooked pasta samples. Different letters indicate differences among types of pasta P< 0.05.

SAMPLE	COLOR	HOMOGENEITY	ODOR	BREAK TO RESISTANCE	OVERALL QUALITY
CTRL	7.8 **a**	6.7 **a**	7.3 **a**	6.2 **a**	7.1 **a**
AS	6.8 **b**	6.0 **a**	6.7 **a**	5.6 **a**	6.2 **b**
BR	7.7 **a**	6.4 **a**	7.2 **a**	5.7 **a**	7.1 **a**
Means	7.4	6.4	7.1	5.8	6.8

No changes were found in homogeneity, odour and break to resistance.

On the average value of colour (7.4), AS samples obtained the lowest score (6.8). The overall quality was good in all the samples with an average value of 6.8. A decrease of quality was detected in AS samples (6.2).

Data from sensorial analysis of dry cooked samples are shown in figure 3.4 and listed in table 3.4.

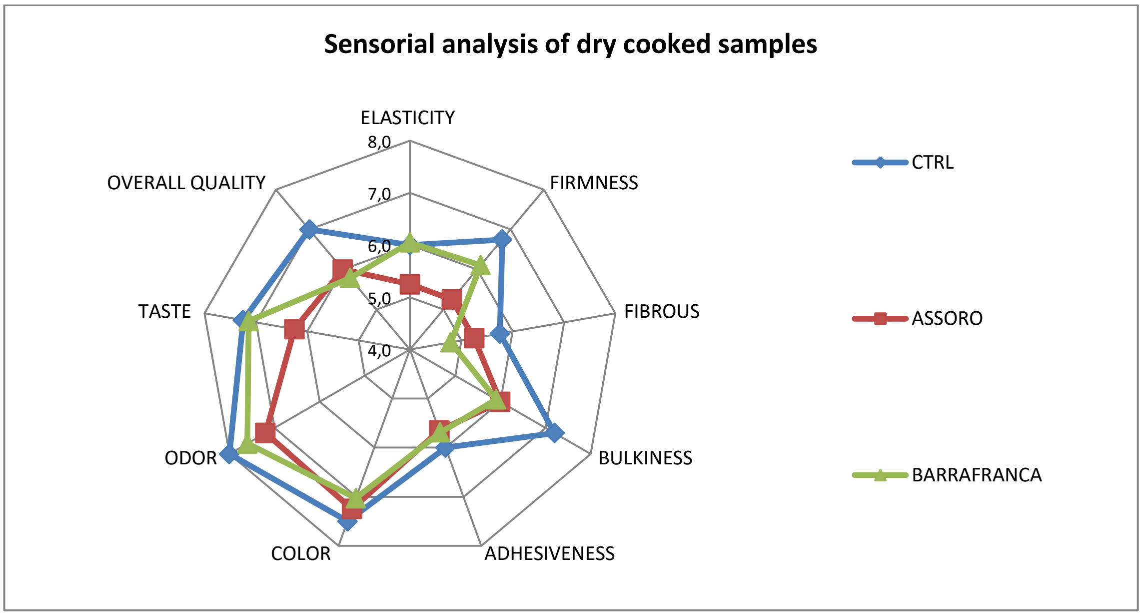


Figure 3.4: Sensorial analysis of dried cooked spaghetti samples

On average of the three types of pastas, the elasticity score was 5.8 with a little decrease in samples with AS inulin (5.2).

Firmness decreased with the addition of inulin, mainly with inulin from AS (5.2).

On the average value of 5.3 for the fibrous, a decrease was found in samples with inulin.

No differences were shown in bulkiness, adhesiveness and colour of pasta samples with inulin, compared with control without inulin.

Adding inulin, the odour score went a little bit down compared to control but it remained good, being respectively 7.2 and 7.6 in AS and BR.

In general it is noted that the overall quality went down with inulin addition (on average 5.9) compared with control (7.0), but still in the range of consumer acceptance.

Table 3.4 lists the sensorial characteristics scores of dry cooked spaghetti samples.

Table 3.4: Sensorial analysis of dry cooked spaghetti samples. Different letters indicate differences among types of pasta $P < 0.05$.

SAMPLE	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OVERALL QUALITY
CTRL	6.0 **a**	6.7 **a**	5.7 **a**	7.2 **a**	6.0 **a**	7.5 **a**	8.0 **a**	7.2 **a**	7.0 **a**
AS	5.2 **b**	5.2 **c**	5.2 **b**	6.0 **a**	5.6 **a**	7.2 **a**	7.2 **b**	6.2 **b**	6.0 **b**
BR	6.0 **a**	6.1 **b**	4.8 **b**	5.9 **a**	5.7 **a**	7.0 **a**	7.6 **ab**	7.1 **a**	5.8 **b**
Means	5.8	6.0	5.3	6.4	5.8	7.3	7.6	6.9	6.3

The overall quality score during cooking process decreased in both samples, fresh and dry. In fresh spaghetti samples it was detected a decrease of 10.4%. In dry samples the decrease was of 15.7%. Even if the decrease found, every score value remains in the range of acceptability (threshold of acceptance >5).

Cooking quality evaluation

The OCT showed a decrease with the addition of inulin (figure 3.5). Particularly spaghetti samples with inulin extracted from roots of cardoon cultivated in BR gave a decrease of 2.5 minutes in cooking time.

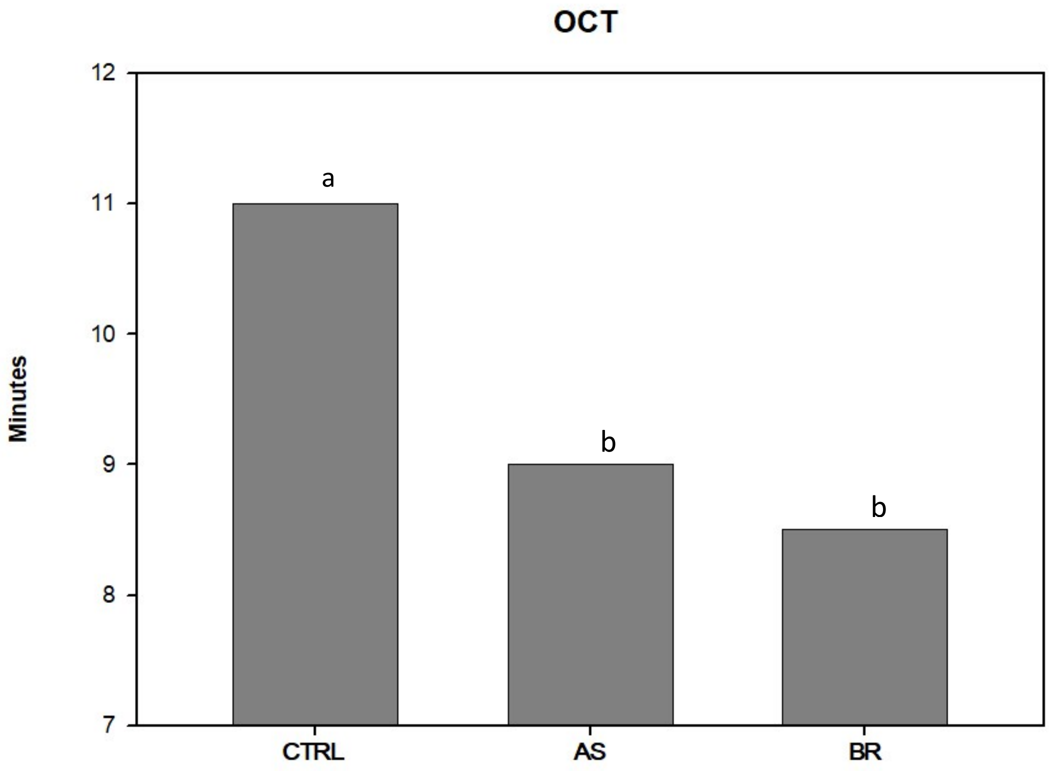


Figure 3.5: OCT of pasta samples with two types of inulin from cardoon roots. Different letters indicate differences among types of pasta P< 0.05.

In table 3.5 are shown data of cooking quality of dry pasta samples.

Table 3.5: Cooking quality of dry spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

SAMPLE	COOKING LOSS (%)	SWELLING INDEX	WATER ABSORBTION	ADHESIVENESS	HARDNESS
CTRL	6.6 **a**	1.9 **a**	149 **a**	0.56 **a**	6.9 **a**
AS	6.4 **a**	1.7 **b**	137 **b**	0.52 **a**	6.5 **a**
BR	6.2 **a**	1.8 **b**	139 **b**	0.52 **a**	6.8 **a**
Means	6.4	1.8	141	0.53	6.7

A statistical difference was noted in the swelling index. It decrease when inulin was added.
Anyway, considering the quality of pastas during cooking, it gave the same values of the control.

Inulin cooking losses

There was not inulin loss in pasta samples produced with the use of “Russello” flour and inulin from both sources.

2.3.4 Conclusions

The quality parameters of the spaghetti added with inulin remain in the range of acceptability for all parameters. The different chain length gave different results after drying the pasta, but the after the cooking process panelist did not reveal these.
Because of the characteristics of quality studied at the end of the drying and cooking process are independent of the environment of cultivation, the cardoon cropping for the production of roots to extract inulin, could be conducted also in marginal areas.

The raw material to produce inulin for pasta used for the development of the work was produced in the field of Assoro, that gave the best results in terms of degree of polymerization.

2.4 TRIAL 4: CHARACTERISTICS OF PASTA WITH INULIN ADDITION

2.4.1 Aim

The aim of this trial was to evaluate the characteristics of pasta produced with the use of "Russello" wholemeal flour added with inulin, extracted from roots of *Cynara cardunculus* and Chicorium *intybus,* and tubers of *Helianthus tuberosus* at different percentage: 2, 4 and 8% (w/w).

2.4.2 Materials and methods

The inulin has been extracted, purified and characterized from roots of *Cynara cardunculus L.*, Chicorium *intybus* and tubers of *Helianthus tuberosus.* The polymer was added, at concentration of 2, 4 and 8% (w/w), to the pasta (spaghetti) produced with cv "Russello" flour. Pasta without inulin was used as control (CTRL).
On the pastas were evaluated the sensorial qualities (panel test) of cooked samples.

Inulin extraction and purification

The extraction, purification and characterization of inulins have been carried out as described in trial 1.

Pasta making and evaluation

Spaghetti were produced with "Russello" durum wheat flour by using the same operating conditions described in trial 2. Different percentage of inulin were added at 2, 4 and 8% (w/w). In order to ensure the solubility of the inulin powder, they were previously dissolved in water. Dry spaghetti samples were submitted to a panel of fifteen trained tasters as described in trial 2. These analyses were conducted in the laboratories of Foggia University as described in trial 2.

Data analyses

Data were submitted to the Bartlett's test for the homogeneity of variance and then analyzed using analysis of variance (ANOVA). Means were statistically separated on the basis of Student-Newmann-Kewls test, when the 'F' test of ANOVA for treatment was significant at least at the 0.05 probability level.

2.3.2 Results

Table 4.1 shows the percentage of variation of each studied factor on sensorial properties of fresh spaghetti samples. In fresh cooked samples, elasticity, firmness, fibrous, bulkiness, colour, taste and consequently overall quality scores were mainly influenced by inulin percentage, followed by interaction between the type of inulin and its percentage. Adhesiveness was influenced at the same way by the type and the percentage of inulin added; the interaction between the two parameters on the values was significant influenced “odour”. Table 4.2 shows the percentage of variation of each studied factor on sensorial properties of dry spaghetti samples. The sensorial properties of fresh and dry cooked spaghetti samples investigated in this trial are shown in table 4.3 and 4.4.

Table 4.1: Mean square (MS) and percentage of variation (%) of sensorial properties of fresh spaghetti samples in relation to the studied factors.

	ELASTICITY		FIRMNESS		FIBROUS		BULKINESS		ADHESIVENESS		COLOR		ODOR		TASTE		OVERALL QUALITY	
	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
INULIN SOURCE (I)	1.1 **	22.9	0.5 *	25.0	2.3 ***	27.7	0.2 ns	22.2	0.3 ns	30.0	0.1 ns	27.0	0.2 ns	64.5	1.2 **	31.6	1.1 **	28.2
INULIN (%)	2.4 ***	50.0	0.9 **	45.0	3.5 ***	42.2	0.4 *	44.4	0.3 ns	30.0	0.2 ns	54.0	0.01 ns	3.3	1.9 **	58.3	2.4 ***	61.5
I*%	1.3 ***	27.1	0.6 ***	30.0	2.5 ***	30.1	0.3 *	33.3	0.4 **	40.0	0.07 ns	18.9	0.1 ns	32.3	0.7 ***	18.4	0.4 **	10.2

Table 4.2: Mean square (MS) and percentage of variation (%) of sensorial properties of dry spaghetti samples in relation to the studied factors.

	ELASTICITY		FIRMNESS		FIBROUS		BULKINESS		ADHESIVENESS		COLOR		ODOR		TASTE		OVERALL QUALITY	
	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
INULIN SOURCE (I)	0.1 ns	0.7	0.6 *	25.7	0.05 ns	2.6	2.1 ***	45.6	0.3 ns	28.8	0.4 ns	44.4	0.3ns	37.5	0.9 **	25.0	1.4 **	30.4
INULIN (%)	2.0 ***	14.1	1.7 ***	73.0	0.8 **	41.0	2.4 ***	52.2	0.7 **	67.3	0.3 ns	33.3	0.3 ns	37.5	2.2 ***	61.1	2.9 ***	63.0
I*%	12.1 ***	85.2	0.03 ns	1.3	1.1 ***	56.4	0.1 ns	2.2	0.04 ns	3.8	0.21 ns	22.2	0.2ns	25.0	0.5 ***	13.9	0.3 **	6.5

In dry cooked samples, elasticity and fibrous were mainly influenced by the interaction between inulin type and its percentage; firmness, bulkiness, adhesiveness, taste and consequently the overall quality score were mainly influenced by the percentage of inulin added. Colour was the only parameter influenced more by the inulin source. Odour scores were influenced in the same way by the type of inulin and its percentage in samples.

Table 4.3: Sensorial properties of fresh cooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

SAMPLE	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OVERALL QUALITY
on average of concentration									
CTRL	6.7 **a**	6.7 **a**	6.9 **a**	5.3 **a**	5.3 **a**	7.2 **a**	7.5 **a**	7.4 **a**	6.8 **a**
Topinambur	5.2 **c**	5.8 **b**	5.7 **b**	5.6 **a**	5.1 **a**	6.9 **a**	7.0 **a**	5.9 **b**	5.5 **b**
Cardoon	6.0 **b**	5.9 **b**	5.5 **b**	5.9 **a**	5.6 **a**	7.2 **a**	7.5 **a**	6.7 **a**	6.1 **b**
Chicorium	5.9 **b**	6.4 **ab**	4.8 **c**	5.8 **a**	5.8 **a**	7.1 **a**	7.7 **a**	7.0 **a**	5.5 **b**
on average of plant source									
CTRL	6.7 **a**	6.7 **a**	6.9 **a**	5.3 **b**	5.3 **a**	7.2 **a**	7.5 **a**	7.4 **a**	6.8 **a**
2	6.5 **a**	6.6 **a**	6.3 **b**	6.1 **a**	5.8 **a**	7.4 **a**	7.4 **a**	7.0 **a**	6.5 **a**
4	5.9 **b**	6.0 **b**	5.2 **c**	5.8 **ab**	5.5 **a**	6.8 **a**	7.4 **a**	7.0 **a**	5.8 **b**
8	4.7 **c**	5.5 **b**	4.5 **d**	5.4 **b**	5.1 **a**	6.9 **a**	7.4 **a**	5.6 **b**	4.8 **c**

In fresh cooked spaghetti samples, on average of concentration, no differences were detected in bulkiness, adhesiveness, colour and odour.

Elasticity (average of 5.7 vs 6.7 of CTRL) went down slowly in samples with inulin from cardoon and Chicorium, but the decrease was more marked in samples with inulin from topinambur.

Firmness went down in samples with inulin (average of 6.0) compared with the CTRL (6.7). Also fibrous was lower in samples with inulin added (average of 5.3 vs 6.9 of CTRL), mainly in samples with Chicorium inulin. Taste was good in all the samples (average of 6.5), but it was lower in samples with inulin from topinambur.

In general the overall quality was good (average of 5.7), but lower compared with the control (6.8).

On the average of plant source, no differences were detected in adhesiveness, colour and odour.

Elasticity and firmness maintained the same score of the CTRL in samples with 2% of inulin, but they went slowly down when the percentage of inulin went up. Fibrous was directly related with the increase of percentage of inulin added. As inulin addition increase, as the values decrease. Bulkiness was good in all samples (average of 5.8), but it was better in samples with 2% of inulin, if compared with the control (5.3).

Taste score decayed at 8% of inulin addition but it was good in the other samples.

In general, scores showed a very good overall quality in samples with 2% of every type of inulin. Quality was in the range of acceptability at 4% but with 8%, the values were out of range (<5).

These results means that until 4% of addition, pasta have good quality and properties. When the addition is higher, most of the pasta characteristics gave lower scores and the quality is out of the range of acceptability.

Table 4.4: Sensorial properties of dry cooked spaghetti samples. Different letters within the same trait indicates differences at P<0.05.

SAMPLE	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OVERALL QUALITY
				on average of concentration					
CTRL	6.0 **a**	6.8 **a**	5.8 **a**	7.2 **a**	6.0 **a**	7.5 **a**	8.0 **a**	7.3 **a**	7.0 **a**
Topinambur	5.6 **a**	5.9 **b**	5.7 **a**	5.5 **b**	5.2 **b**	6.7 **a**	7.3 **a**	6.0 **c**	5.6 **b**
Cardoon	5.8 **a**	5.9 **b**	5.5 **a**	5.8 **b**	5.6 **ab**	7.2 **a**	7.5 **a**	6.5 **bc**	6.0 **b**
Chicorium	6.0 **a**	5.9 **b**	5.7 **a**	5.4 **b**	5.5 **ab**	6.9 **a**	7.4 **a**	6.9 **ab**	5.6 **b**
				on average of plant source					
CTRL	6.0 **b**	6.8 **a**	5.8 **ab**	7.2 **a**	6.0 **a**	7.5 **a**	8.0 **a**	7.3 **a**	7.0 **a**
2	6.8 **a**	6.6 **ab**	6.3 **a**	6.0 **b**	5.8 **a**	7.2 **a**	7.5 **a**	7.0 **a**	6.6 **a**
4	5.9 **b**	6.1 **b**	5.2 **b**	5.8 **b**	5.5 **a**	6.8 **a**	7.4 **a**	7.0 **a**	5.8 **b**
8	4.8 **c**	5.1 **c**	5.3 **b**	5.0 **c**	4.9 **b**	6.8 **a**	7.3 **a**	5.4 **b**	4.8 **c**

In dry cooked samples, on average of concentration, elasticity (average 5.8), fibrous (5.6), colour (6.9) and odour (7.4) did not show any variation respect the control.

A decrease was noticed in firmness (average 5.9 vs 6.8 of the CTRL), bulkiness (5.6 vs 7.2) and adhesiveness (5.4 vs 6.0) for all the types of inulin source, suggesting an increase in consumer's acceptance.

Concerning the taste (average 6.5), the best score, among the inulin sources, was Chicorium (6.9) and the worst was the sample with inulin from topinambur (6.0).

On the average of plant source, the elasticity in sample with inulin in percentage of 2%, was better than the control (6.8 vs 6.0 in CTRL); also the fibrous value was higher in sample with 2% of inulin than the control (6.3 vs 5.8 of the CTRL). Elasticity score, as well as the fibrous value, decreased with the increase of inulin percentage.

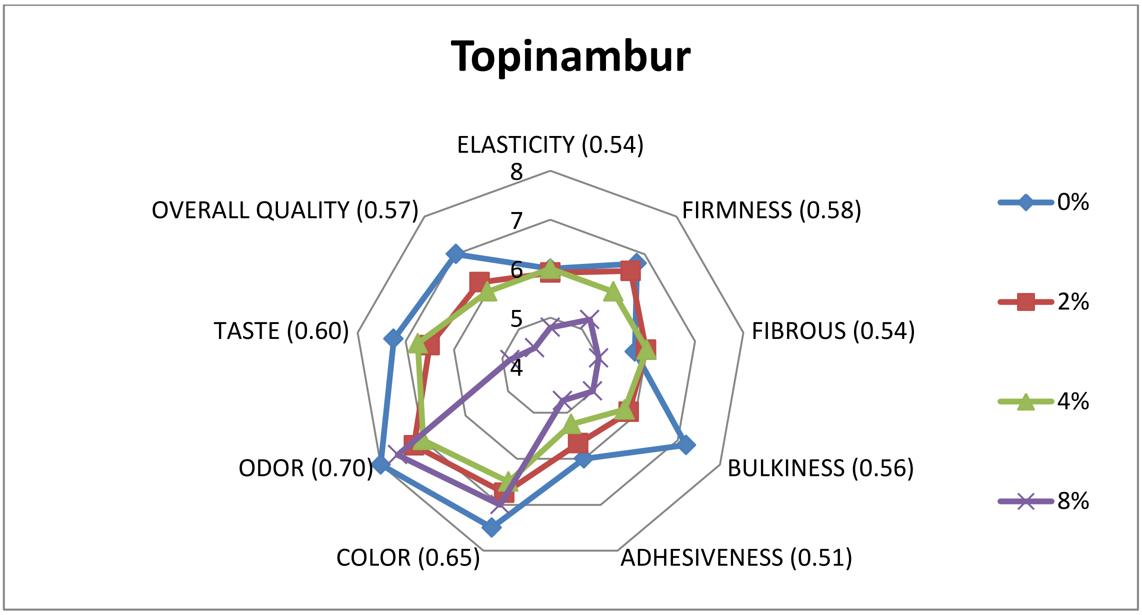
Firmness value was well maintained in samples with inulin at 2% (6.6) but it went down in samples with 4 and 8% of inulin added (respectively 6.1 and 5.1).

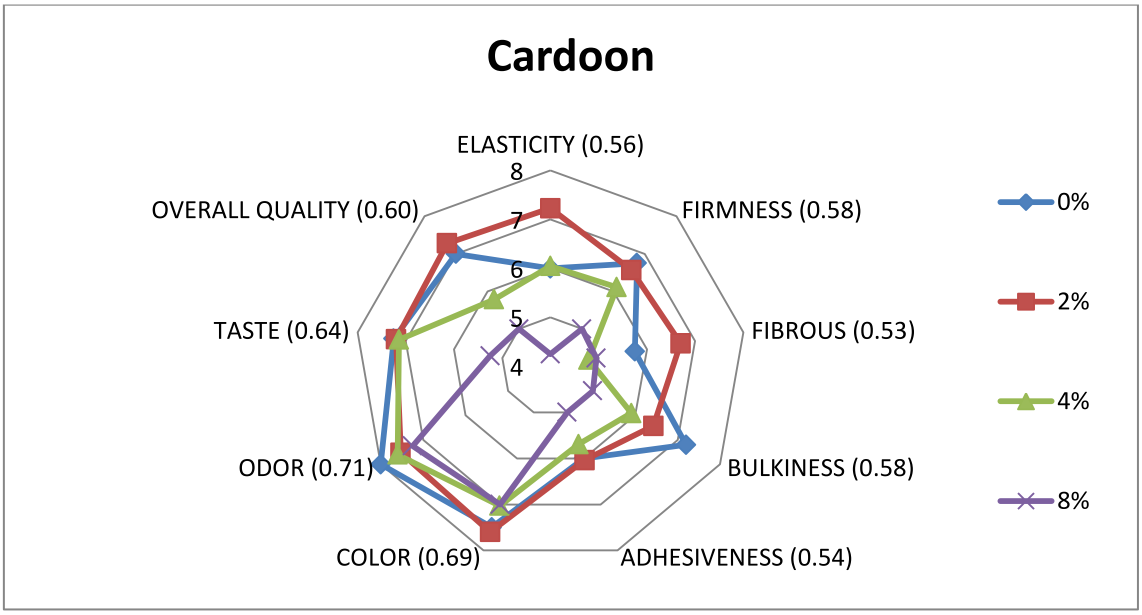
Bulkiness had lower values in samples with a higher inulin percentage. Adhesiveness and taste maintained the same values of the control, a part the samples with 8% of inulin.

No differences were shown in colour and odour of pasta samples.

In general, the overall quality was the same of the control in the samples with the lowest inulin percentage, but it remained in the range of acceptability in sample with 4% of inulin added.

Figure 4.1 shows that, out of the type of inulin used, the concentration of inulin influenced the studied parameters. Within the same source a decay of all parameters were observed passing from 4 to 8%, even if the sensation of fibrous is less evident using long chain inulin.





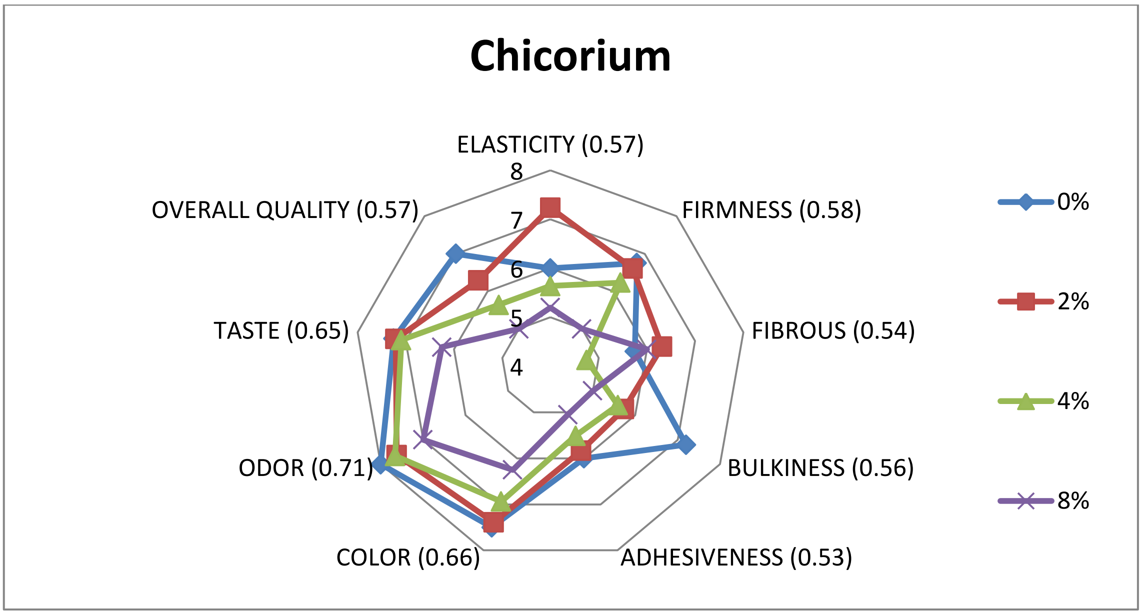


Figure 4.1: Panel analyses of dry spaghetti produced with different types and concentrations of inulin. In each traits, the number between parenthesis indicates LSD at P<0.05.

The global quality score decayed at 8% for all types of inulin studied. Adding Topinambur inulin at 4%, good levels of quality were observed (Figure 4.2).

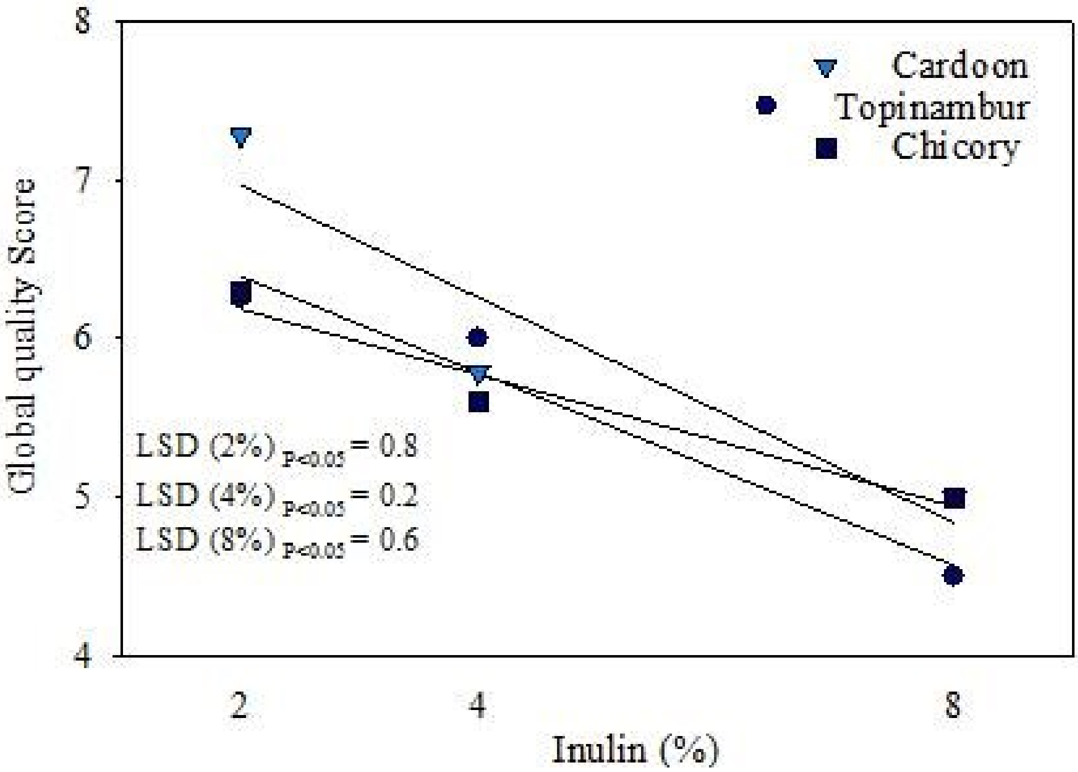


Figure 4.2: Global quality score of dry spaghetti produced with different types and concentrations of inulin.

2.3.2 Conclusions

The linear regression between OQS and inulin percentage, for cooked dry spaghetti, gave the best results at lower concentration in all the inulins used. The best compromise (threshold value > 5) was reached using topinambur at 4%. In both fresh and dry cooked samples, results showed that until 4% of addition, pasta have good quality and properties. When the addition is higher, most of the pasta characteristics give lower scores and the quality is out of the range of acceptability.

In both fresh and dry cooked samples, the overall quality scores was mainly influenced by the percentage of inulin added. In fact, out of the type of inulin used, a decay of all quality parameters were observed passing from 4 to 8%, suggesting that a percentage of inulin higher than 4% is not proper in terms of performance.

Considering these results, other trials were conducted on pasta samples with the addition of no more than 4% of inulins.

2.5 TRIAL 5: EVALUATION OF DIFFERENT TYPES OF PASTA WITH INULIN WITH DIFFERENT DEGREE OF POLYMERIZATION.

2.5.1 Aim

The aim of this trial was to evaluate the characteristics of pasta produced with the use of different wholemeal flours added with inulin with different degree of polymerization (DP).

2.5.2 Materials and methods

The inulin has been extracted, purified and characterized from roots of *Cynara cardunculus* L. (CRI- mean DP 62) and from roots of *Cichorium intybus* (CHI- mean DP 23) and added, to 2 and 4% (w/w), to the pasta produced with 4 different cvs of flours: commercial wholemeal (WMF), Margherito (MAR) Senatore Cappelli (CAP), Russello (RUS), Timilia (TIM).
Pasta without inulin was used as control (CTRL).
All the pastas were evaluated on the sensorial qualities and the overall quality score (OQS) (panel test).

Inulin extraction and purification

The extraction, purification and characterization of inulins have been carried out as described in trial 1.

Pasta making and evaluation

Spaghetti were produced using the same operating conditions described in trial 2. Two different percentage of inulin were added at 2, 4% (w/w). In order to ensure the solubility of the inulins powder, they were previously dissolved in water. Dry spaghetti samples were submitted to a panel of fifteen trained tasters as described in trial 2.
These analyses were conducted in the laboratories of Foggia University as described in trial 2.

Data analyses

Data were submitted to the Bartlett's test for the homogeneity of variance and then analyzed using analysis of variance (ANOVA). Means were statistically separated on the basis of Student-Newmann-Kewls test, when the ‘F’ test of ANOVA for treatment was significant at least at the 0.05 probability level.

2.5.3 Results

Pasta colour evaluation

The color indices detected by Minolta colorimeter, have been influenced mainly by *cv* used, both in uncooked and cooked spaghetti. In particular, as regard uncooked spaghetti, the percentage of total variation resulted: 45% (L*), 91.7% (a*) and 50% (b*). Even if the type of inulin did not influence the yellow index (b*), the concentration of the polymer used was significant relevant (35.6% of total variation). After cooking process, the effect of the source of inulin used began more marked, with values of b* of 21.9% of total variation, while L* and a* values resulted mainly influenced by cultivar of whole-meal flours (Table 5.1).

Table 5.1: Mean square (MS) and percentage of variation (%) of colour scores of uncooked and cooked dry spaghetti samples in relation to the studied factors.

COLOUR

	L*		a*		b*	
	MS	%	MS	%	MS	%
UNCOOKED SPAGHETTI						
Cultivar (Cv)	244 ***	45.7	214.4 ***	91.7	558.5 ***	50.9
Inulin source (I)	96.9 ***	18.1	1.8 ***	0.8	3.9 *	0.4
Inulin (%)	115 ***	21.5	6.5 ***	2.8	390.2 ***	35.6
Cv*I	6.0 ***	1.1	0.5 ***	0.2	29.1 ***	2.7
Cv*%	29.5 ***	5.5	8.6 ***	3.7	81.2 ***	7.4
I*%	38.6 ***	7.2	1.1 ***	0.5	3.7 *	0.3
Cv*I*%	4.8 ***	0.9	1.0 ***	0.4	29.7 ***	2.7
COOKED SPAGHETTI						
Cultivar (Cv)	82.5 ***	61.8	104.6 ***	95.6	16.4 ***	38.0

Inulin source (I)	7.1 ns	5.3	0.6 ***	0.6	9.5 ***	21.9
Inulin (%)	6.2 ns	4.7	2.0 ***	1.8	4.8 ***	11.1
Cv*I	5.4 ns	4.0	0.2 ***	0.2	0.8 ns	1.9
Cv*%	19.2 ***	14.4	1.5 ***	1.4	5.5 ***	12.8
I*%	9.4 *	7.1	0.2 ***	0.2	4.7 ***	11.0
Cv*I*%	3.7 ns	2.8	0.3 ***	0.3	1.4 *	3.3

Out of cultivar used and inulin source, statistical differences have been recorded only for L* index in uncooked spaghetti between 4% and CTRL. The 4% CRI addition improved yellow index in uncooked spaghetti (Table 5.2).

Table 5.2: colour score averages of uncooked and cooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

		UNCOOKED SPAGHETTI			**COOKED SPAGHETTI**		
INULIN SOURCE	**INULIN %**	**L***	**a***	**b***	**L***	**a***	**b***
				Out of cultivars			
CTRL		51.2 **a**	4.0 **a**	21.2 **b**	58.5 **a**	-0.4 **a**	17.1 **a**
CHI	2	51.9 **a**	3.5 **a**	19.6 **b**	57.8 **a**	-0.2 **a**	16.7 **a**
	4	49.3 **ab**	4.2 **a**	27.0 **a**	58.7 **a**	-0.1 **a**	17.0 **a**
CRI	2	49.5 **a**	3.7 **a**	19.6 **b**	58.0 **a**	0.1 **a**	16.4 **a**
	4	44.0 **b**	4.9 **a**	25.8 **a**	56.9 **a**	0.0 **a**	15.6 **a**
				Out of cultivars and inulin source			
	CTRL	51.2 **a**	4.0 **b**	21.2 **b**	58.5 **a**	-0.4 **b**	17.1 **a**
	2	50.7 **a**	3.6 **c**	19.6 **c**	57.9 **a**	0.0 **a**	16.6 **a**
	4	46.7 **b**	4.5 **a**	26.4 **a**	57.8 **a**	0.0 **a**	16.3 **a**

For all the cultivars, averaged, for inulin source and percentage, the cooking process increased the L*, and decrease the yellow index. This phenomenon was much more marked in CTRL than in the other Sicilian cultivars. “Russello” maintained better the yellow index at both 2% and 4% of inulin addition (Table 5.3).

Table 5.3: colour scores of uncooked and cooked spaghetti samples produced with different cvs of wholemeal flours.

INULIN SOURCE			CHI		CRI		
	INULIN %	CTRL	2	4	2	4	Means
COMMERCIAL WHOLEMEAL							
Uncooked spaghetti	L*	60.0	59.5	55.2	51.3	49.6	**53.9**
	a*	-0.5	-0.8	-2.0	-0.6	-2.1	**-1.4**
	b*	24.8	24.7	36.1	32.5	35.2	**32.2**
Cooked spaghetti	L*	61.2	60.3	61.3	61.4	61.5	**61.1**
	a*	-4.1	-4.0	-4.4	-3.3	-4.3	**-4.0**
	b*	18.6	17.6	15.9	17.1	16.0	**16.6**
MARGHERITO							
Uncooked spaghetti	L*	48.0	51.1	48.9	50.8	43.6	**48.6**
	a*	5.6	4.8	4.9	4.6	6.4	**5.2**
	b*	25.8	16.1	29.7	14.4	25.8	**21.5**
Cooked spaghetti	L*	57.0	57.3	59.9	56.9	58.8	**58.2**
	a*	0.2	1.5	0.0	1.5	1.2	**1.1**
	b*	16.7	18.7	17.5	17.9	17.6	**17.9**
RUSSELLO							
Uncooked spaghetti	L*	53.4	52.8	52.6	51.3	43.4	**50.0**
	a*	3.6	3.4	4.9	3.3	7.1	**4.7**
	b*	15.7	16.4	15.9	13.8	24.4	**17.6**
Cooked spaghetti	L*	61.2	57.2	58.4	56.4	55.9	**57.0**
	a*	0.6	0.2	1.4	0.2	1.0	**0.7**
	b*	18.2	16.4	18.0	15.6	16.2	**16.6**
SENATORE CAPPELLI							
Uncooked spaghetti	L*	52.2	53.1	44.7	51.5	40.2	**47.4**
	a*	3.3	3.3	5.8	3.3	6.3	**4.7**
	b*	15.7	16.4	26.5	14.6	20.4	**19.5**
Cooked spaghetti	L*	57.5	57.6	58.1	57.9	52.3	**56.5**
	a*	-0.2	0.2	0.7	0.4	1.0	**0.5**
	b*	15.7	15.9	16.5	16.1	13.4	**15.5**
TIMILIA							
Uncooked spaghetti	L*	42.4	43.2	45.3	42.4	43.1	**43.5**
	a*	8.1	7.0	7.3	7.7	6.6	**7.1**
	b*	24.0	24.6	26.5	22.8	23.3	**24.3**
Cooked spaghetti	L*	55.5	56.3	55.6	57.3	56.1	**56.3**
	a*	1.5	1.3	1.7	1.9	1.3	**1.6**
	b*	16.5	15.1	17.0	15.1	14.8	**15.5**

Sensorial characteristics of fresh uncooked spaghetti

The color detected by panelist reflected the data recorded by Minolta colorimeter, the cv and percentage of inulin influenced mainly this parameter with 23% and 52% of total variation detected by ANOVA (Table 5.4).

Table 5.4: Mean square (MS) and percentage of variation (%) of sensorial properties of fresh uncooked spaghetti samples in relation to the studied factors.

FRESH UNCOOKED

	COLOR		**HOMOGENEITY**		**ODOR**		**OQS**	
	MS	**%**	**MS**	**%**	**MS**	**%**	**MS**	**%**
Cultivar (Cv)	0.4 **	23.8	4.3 ***	83.5	0.4 ***	29.9	0.4 **	36.8
Inulin source (I)	0.1 ns	7.5	0.1 ns	2.1	0.0 ns	3.4	0.0 ns	1.8
Inulin (%)	0.8 ***	51.9	0.1 ns	1.9	0.4 **	32.5	0.4 *	37.7
Cv*I	0.0 ns	1.9	0.0 ns	0.4	0.1 ns	4.3	0.0 ns	2.8
Cv*%	0.1 ns	8.8	0.5 ***	9.7	0.3 ***	24.8	0.1 ns	12.0
I*%	0.1 ns	3.1	0.1 ns	1.6	0.0 ns	0.9	0.1 ns	7.4
Cv*I*%	0.1 ns	3.1	0.0 ns	0.8	0.1 ns	4.3	0.0 ns	1.6

The color perception resulted a little bit lower passing from 2 to 4% of inulin addition both in CHI and CRI, but the scores recorded are quite high in all the samples (<7.9) (Table 5.5).

Table 5.5: Sensory properties score averages of fresh uncooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

FRESH UNCOOKED

INULIN SOURCE	INULIN %	COLOR	HOMOGENEITY	ODOR	OQS
		Out of cultivars			
CTRL		7.4 **a**	6.8 **a**	7.7 **a**	7.4 **a**
CHI	2	7.2 **a**	6.8 **a**	7.8 **a**	7.2 **a**
	4	7.1 **a**	6.7 **a**	7.5 **a**	7.2 **a**
CRI	2	7.4 **a**	6.9 **a**	7.8 **a**	7.3 **a**
	4	7.2 **a**	6.7 **a**	7.6 **a**	7.1 **a**
		Out of cultivars and inulin source			
	CTRL	7.4 **a**	6.8 **a**	7.7 **b**	7.4 **a**
	2	7.3 **a**	6.8 **a**	7.8 **a**	7.2 **ab**
	4	7.1 **b**	6.7 **a**	7.6 **b**	7.1 **b**

In fresh uncooked spaghetti, the homogeneity resulted influenced only by *cv*. In the CTRL the score detected was 8, against 7.4 of the Sicilian whole-meal flours (average value); the odor and the OQS resulted mainly influenced by the percentages of inulin, the panel group in fact, appreciated the use of CRI at 4% in all samples made with Sicilian *cvs*, expecially for "Senatore Cappelli" (OQS 7.5) (Table 5.6).

Table 5.6: Sensory properties of fresh uncooked spaghetti samples produced with different cvs of wholemeal flours.

FRESH UNCOOKED

INULIN SOURCE		CHI		CRI		
%INULIN	0	2	4	2	4	**means**
CV	**COMMERCIAL WHOLEMEAL**					
COLOR	7.8	7.5	7.0	7.7	7.3	**7.4**
HOMOGENEITY	8.0	7.3	7.3	7.5	7.3	**7.3**
ODOR	8.0	7.7	7.7	7.8	7.3	**7.6**
OQS	7.8	7.3	7.2	7.5	7.1	**7.3**
CV	**MARGHERITO**					
COLOR	7.2	7.0	7.4	7.2	6.9	**7.1**
HOMOGENEITY	6.5	6.6	6.9	7.1	6.7	**6.8**
ODOR	7.5	7.8	7.3	7.8	7.6	**7.6**
OQS	7.2	7.1	7.3	7.2	7.0	**7.1**
CV	**RUSSELLO**					
COLOR	7.5	7.3	7.0	7.4	7.2	**7.2**
HOMOGENEITY	6.3	6.7	6.4	6.7	6.4	**6.5**
ODOR	7.3	7.7	7.5	7.8	7.7	**7.7**
OQS	7.3	7.2	7.1	7.2	7.1	**7.1**
CV	**SENATORE CAPPELLI**					
COLOR	7.4	7.2	7.2	7.3	7.3	**7.2**
HOMOGENEITY	6.7	6.6	6.5	6.8	6.7	**6.7**
ODOR	7.9	7.9	7.8	7.9	7.8	**7.8**
OQS	7.4	7.3	7.3	7.5	7.5	**7.4**
CV	**TIMILIA**					
COLOR	7.3	7.3	7.0	7.4	7.2	**7.2**
HOMOGENEITY	6.5	6.7	6.4	6.7	6.4	**6.5**
ODOR	7.8	7.7	7.5	7.8	7.7	**7.7**
OQS	7.2	7.2	7.1	7.3	7.1	**7.2**

Sensorial characteristics of fresh cooked spaghetti

After cooking fresh spaghetti, the ANOVA (table 5.7) showed much more marked differences among cultivars, inulin source and inulin percentages, than that recorded in uncooked spaghetti. In particular, except of firmness, the percentages of inulin used and the plant source influenced the OQS more than cultivar factor. Out of cultivars and inulin sources, the addition of 2% of inulin improved the elasticity and the adhesiveness. The perception of fibrous, due to the wholemeal flours, decreased with the addition of inulin at 4%, but decreased the global score for color, odor and OQS than CTRL. The taste did not show any variation. CRI at 2% and 4% did not result different from CTRL for OQS, while the addition of low DP inulin at 4% decreased the score. However the OQS resulted always higher than 5.0 (threshold of acceptability). Considering the interaction of cv and % of inulin, the elasticity improved mainly at the concentration of 2% in all cvs except in “Margherito”. Within the same cultivars statistical differences have been recorded between the inulin sources, on average of concentrations. In general, CRI inulin gave best results in terms of sensorial characteristics of pasta, especially at 2% (Table 5.8).

Table 5.7: Mean square (MS) and percentage of variation (%) of sensorial properties of fresh cooked spaghetti samples in relation to the studied factors.

FRESH COOKED

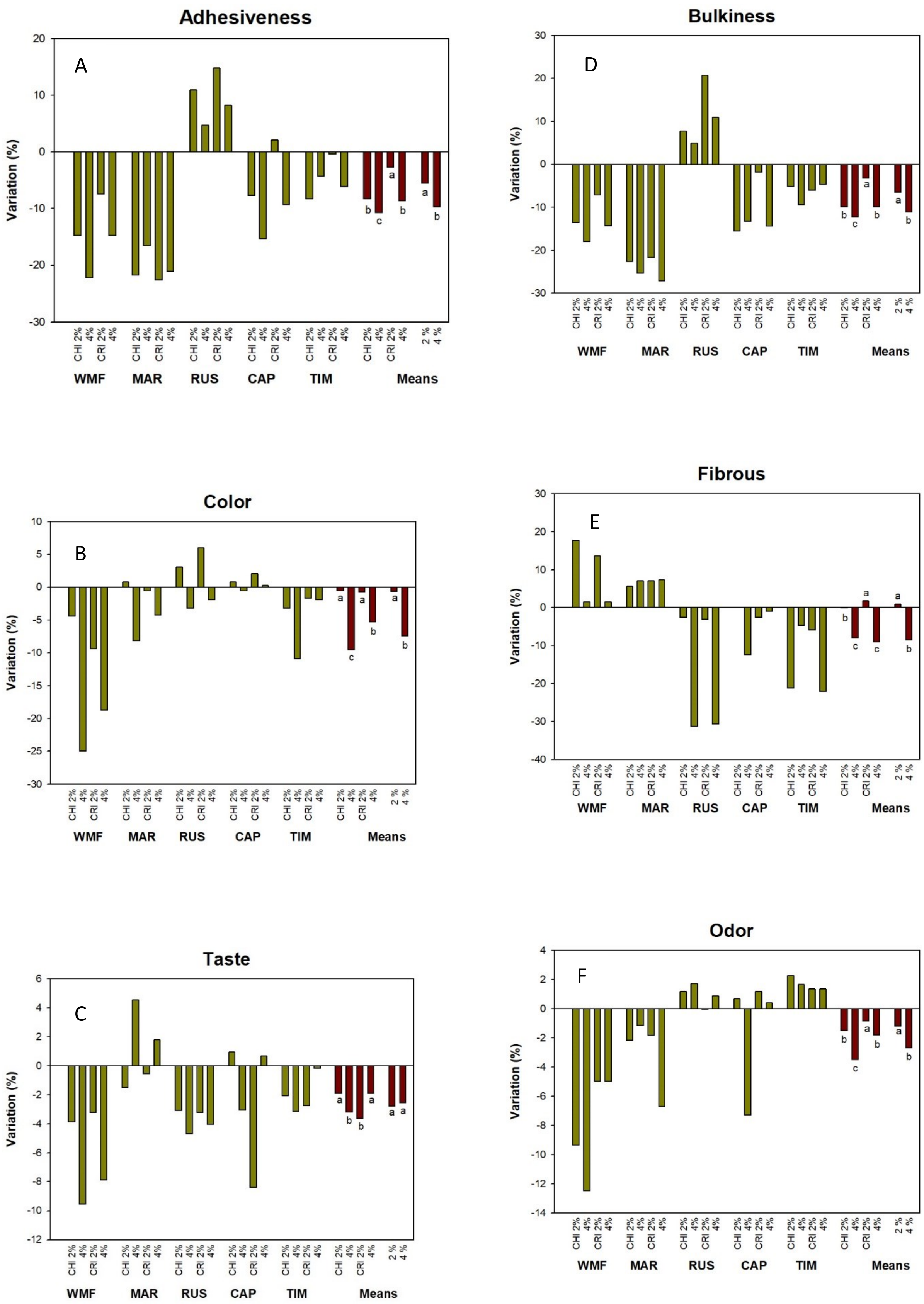
	ELASTICITY		FIRMNESS		FIBROUS		BULKINESS		ADHESIVENESS		COLOR		ODOR		TASTE		OQS	
	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
Cultivar (Cv)	7.8 **	43.8	8.4 ***	74.8	10.2 ***	19.5	1.8 ***	15.5	0.8 ***	9.0	0.4 **	6.4	7.1 ***	14.2	0.3 *	19.7	5.4 ***	27.7
Inulin source (I)	2.3 ***	13.0	0.2 ns	1.5	4.8 ***	9.1	0.9 **	7.6	0.7 **	8.3	0.3 ns	4.7	7.5 ***	15.2	0.0 ns	0.2	3.5 ***	17.7
Inulin (%)	5.7 ***	32.0	2.3 ***	20.2	16.5 ***	31.4	6.4 ***	54.3	4.6 ***	55.2	3.5 ***	63.1	8.8 ***	17.7	0.6 **	46.1	8.7 ***	44.0
Cv*I	0.2 ns	1.0	0.1 ns	1.0	5.4 ***	10.2	0.1 ns	1.0	0.2 *	2.3	0.0 ns	0.7	7.0 ***	14.1	0.0 ns	3.0	0.2 *	1.0
Cv*%	0.8 ***	4.3	0.2 ns	1.3	6.0 ***	11.5	2.0 ***	17.1	1.7 ***	20.6	1.0 ***	18.2	6.2 ***	12.6	0.2 *	17.4	0.3 ***	1.6
I*%	0.7 ***	4.1	0.1 ns	0.4	3.6 ***	6.8	0.4 *	3.5	0.3 *	3.6	0.3 *	5.3	6.5 ***	13.0	0.1 ns	6.8	1.1 ***	5.6
Cv*I*%	0.3 ***	1.8	0.1 ns	0.7	6.1 ***	11.6	0.1 ns	1.0	0.1 ns	1.0	0.1 ns	1.5	6.5 ***	13.1	0.1 ns	6.8	0.5 ***	2.4

Table 5.8: Sensory properties of fresh cooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

FRESH COOKED

INULIN SOURCE	INULIN %	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OQS
					Out of cultivar					
CTRL		6.5 **a**	6.4 **a**	6.1 **a**	6.4 **a**	6.2 **a**	7.4 **a**	7.7 **a**	7.4 **a**	6.8 **ab**
CHI	2	6.4 **a**	6.4 **a**	6.1 **a**	5.7 **ab**	5.6 **a**	7.3 **a**	7.6 **a**	7.3 **a**	6.4 **ab**
	4	5.6 **b**	5.9 **a**	4.3 **c**	5.5 **b**	5.5 **a**	6.6 **a**	6.0 **b**	7.2 **a**	5.6 **c**
CRI	2	6.8 **a**	6.5 **a**	6.2 **a**	6.1 **ab**	6.0 **a**	7.3 **a**	7.6 **a**	7.2 **a**	7.0 **a**
	4	6.1 **ab**	6.0 **a**	5.5 **b**	5.7 **ab**	5.6 **a**	7.0 **a**	7.5 **a**	7.3 **a**	6.1 **bc**
					Out of cultivars and inulin source					
	CTRL	6.5 **b**	6.4 **a**	6.1 **a**	6.4 **a**	6.2 **a**	7.4 **a**	7.7 **a**	7.4 **a**	6.8 **a**
	2	6.6 **a**	6.5 **a**	6.1 **a**	5.9 **b**	5.8 **b**	7.3 **a**	7.6 **a**	7.2 **b**	6.7 **b**
	4	5.8 **c**	6.0 **b**	4.9 **b**	5.6 **c**	5.5 **c**	6.8 **b**	6.8 **b**	7.2 **b**	5.9 **c**

Within the same cultivar, comparing spaghetti added by inulin with the relative control, commercial wholemeal flour gave negative scores for all the studied characteristics when added by inulins in both concentration and inulin source, than pasta obtained without polymer. The pasta of cv “Margherito”, when added by inulin, improved taste (4%), elasticity, firmness and OQS (CRI 2%). Fresh cooked spaghetti obtained by cv “Russello” gave the best results in terms of bulkiness, decay of fibrous, improvement of odor and elasticity (2%). The best performance (OQS), as for “Margherito”, were obtained using CRI 2%. The best results for all the studied parameters, adding inulins, were obtained using the cv of “Senatore Cappelli” to make low glycemic index spaghetti, with improvement of taste, color, elasticity (CHI 2% and CRI 2%), a decay of sensation of adhesiveness , bulkiness and fibrous. The OQS, resulted in a negative variation of about 20%, when the flours were added by CHI 4%, while positive variation than CTRL (“Sen. Cappelli” spaghetti) where recorded using CRI inulin, in both concentrations. The cv “Timilia”, characterized by a low gluten amount is generally used for bread production in some areas of Sicily; the spaghetti obtained with this cultivar added by inulins attained good results only for elasticity (Figure 5.1 A-I).


Adhesiveness
A
Variation (%)
CHI 2% 4% CRI 2% 4%
WMF
MAR
RUS
CAP
TIM
Means
Bulkiness
D
Color
B
Fibrous
E
Taste
C
Odor
F
2 % 4 %
a
b
c

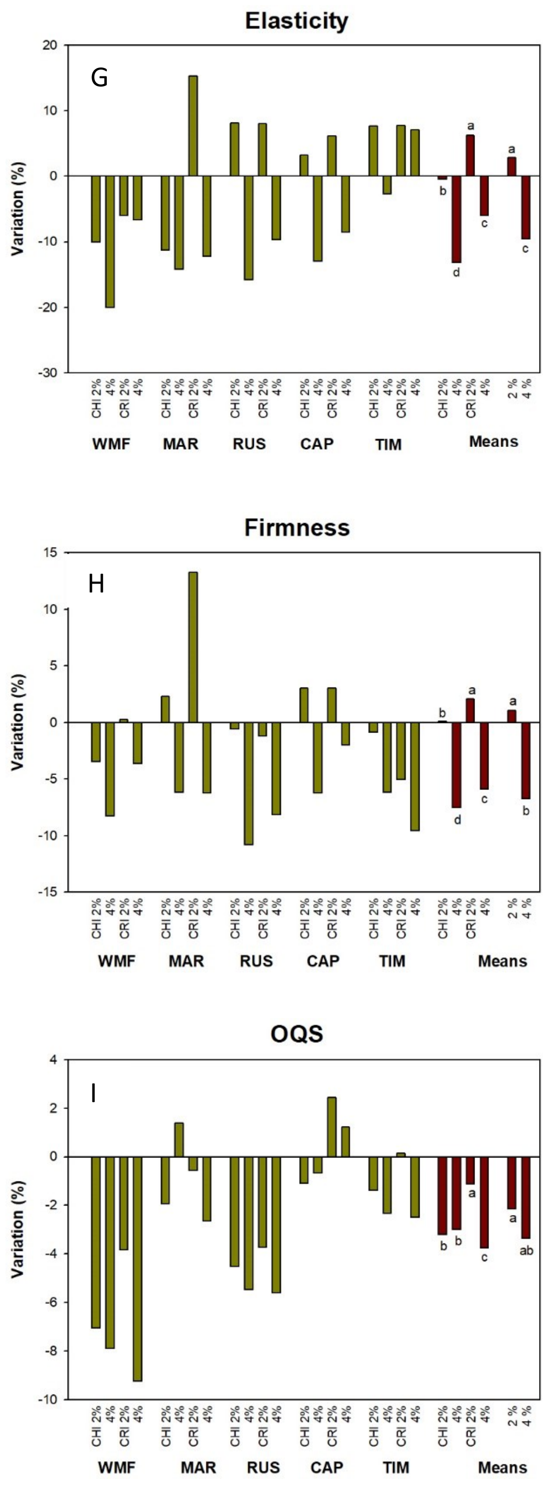


Figure 5.1: Percentage variations of the scores for each parameter, within the same cultivar, vs the relative CTRL.

Sensorial characteristics of dry uncooked spaghetti

After the drying process the characteristics of uncooked spaghetti resulted mainly influenced by the percentage of inulin addition for odor (50.5% of total variation) and color (51.4% of total variation), and by Cultivar for homogeneity (80.7% of total variation) and OQS (70.4% of total variation). The inulin source resulted not significant for all the parameters. Out of cultivars, using inulin at 2%, the characteristics of spaghetti resulted the same of the absolute CTRL, with little decay at 4%. Moreover, at all times the parameters had values much higher than the threshold of acceptability (Table 5.9; Table 5.10).

Table 5.9: Mean square (MS) and percentage of variation (%) of sensorial properties of dry uncooked spaghetti samples in relation to the studied factors.

DRY UNCOOKED

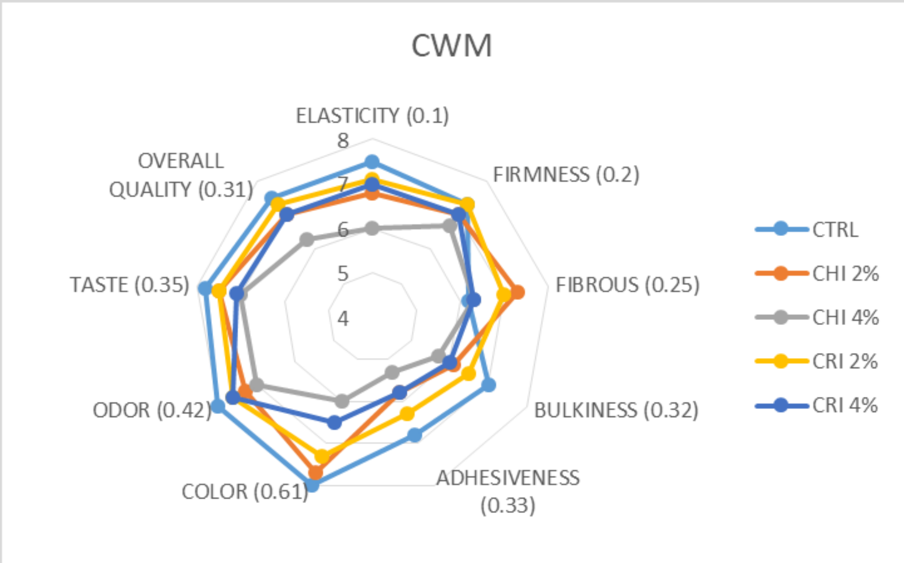
	ODOR		HOMOGENEITY		COLOR		OQS	
	MS	%	MS	%	MS	%	MS	%
Cultivar (Cv)	0.1 ns	15.1	6.3 ***	80.7	0.3 **	17.7	2.6 ***	70.4
Inulin source (I)	0.1 ns	6.5	0.2 ns	2.4	0.1 ns	6.6	0.3 ns	8.7
Inulin (%)	0.5 **	50.5	0.7 ***	8.8	0.9 ***	51.4	0.4 *	10.1
Cv*I	0.1 ns	5.4	0.1 ns	1.4	0.2 *	11.6	0.1 ns	1.6
Cv*%	0.1 ns	15.1	0.4 ***	4.5	0.1 ns	4.4	0.2 *	5.7
I*%	0.0 ns	2.2	0.1 ns	0.9	0.1 ns	3.3	0.1 ns	2.7
Cv*I*%	0.1 ns	5.4	0.1 ns	1.3	0.1 ns	5.0	0.0 ns	0.8

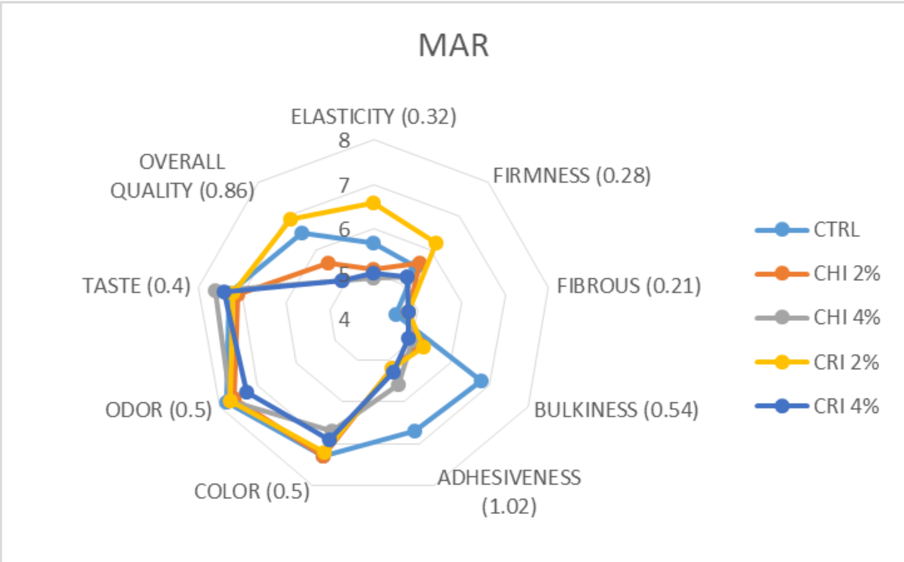
Table 5.10: Sensory properties of dry uncooked spaghetti samples. Different letters indicate differences among types of pasta $P< 0.05$.

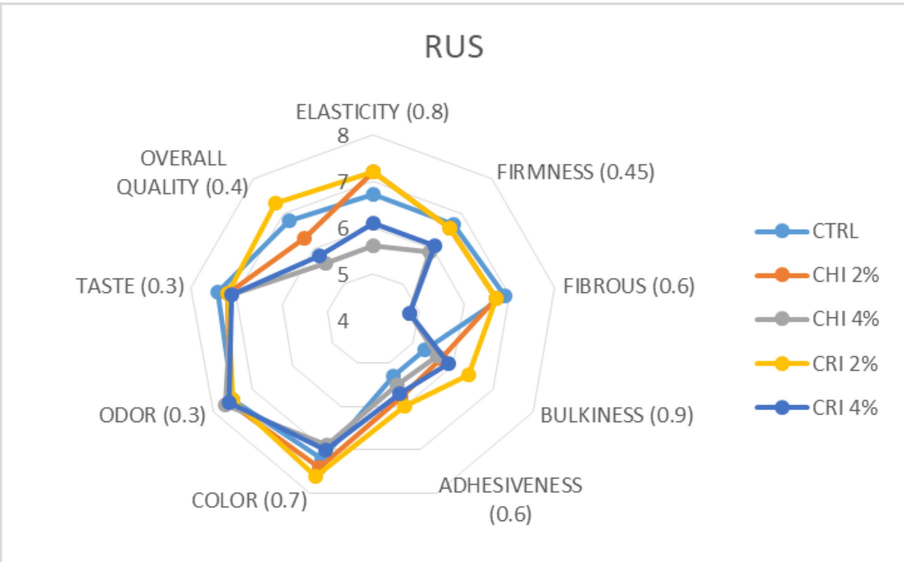
DRY UNCOOKED

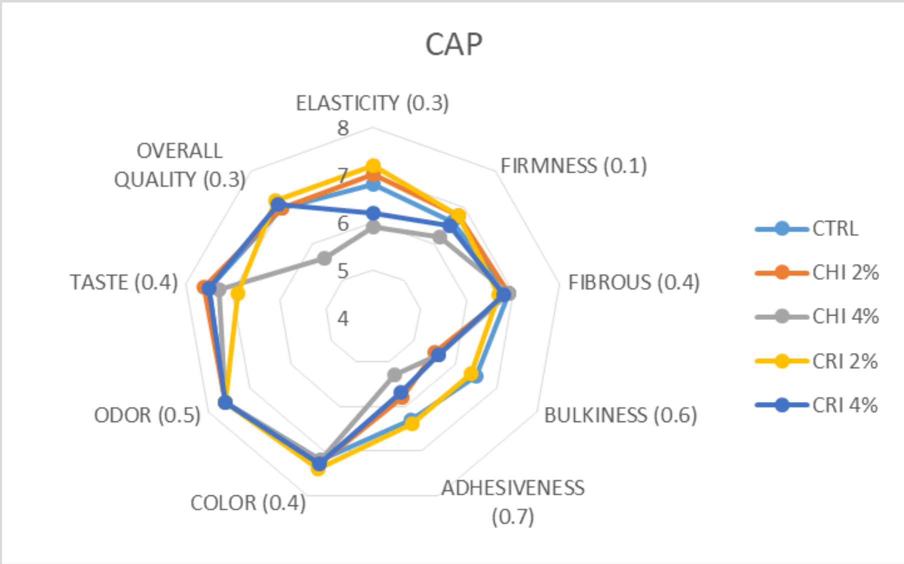
INULIN SOURCE	INULIN %	ODOR	HOMOGENEITY	COLOR	OQS
		Out of cultivar			
CTRL		7.8 **a**	7.0 **a**	7.3 **a**	7.0 **a**
CHI	2	7.8 **a**	6.8 **a**	7.1 **a**	6.8 **a**
	4	7.5 **b**	6.7 **a**	6.9 **b**	6.7 **a**
CRI	2	7.8 **a**	6.9 **a**	7.2 **a**	7.0 **a**
	4	7.6 **b**	6.8 **a**	7.0 **b**	6.9 **a**
		Out of cultivars and inulin source			
	CTRL	7.8 **a**	7.0 **a**	7.3 **a**	7.0 **a**
	2	7.8 **a**	6.8 **b**	7.2 **b**	6.9 **ab**
	4	7.6 **b**	6.7 **b**	7.0 **c**	6.8 **b**

Figure 5.2 showed the variation of each characteristic, within the same cultivar, after the addition of inulin vs the relative CTRL. Even if all the parameter attained values higher than 5.0, it should be noted that all the parameters decreased the scores after the addition of the polymers at the concentration of 4%, independently of the DP. Using CRI at 2% improved OQS in “Russello”, it was not statistically different in the other cvs.


CWM
ELASTICITY (0.1)
FIRMNESS (0.2)
FIBROUS (0.25)
BULKINESS (0.32)
ADHESIVENESS (0.33)
COLOR (0.61)
ODOR (0.42)
TASTE (0.35)
OVERALL QUALITY (0.31)
8
7
6
5
4
CTRL
CHI 2%
CHI 4%
CRI 2%
CRI 4%


MAR
ELASTICITY (0.32)
FIRMNESS (0.28)
FIBROUS (0.21)
BULKINESS (0.54)
ADHESIVENESS (1.02)
COLOR (0.5)
ODOR (0.5)
TASTE (0.4)
OVERALL QUALITY (0.86)
8
7
6
5
4
CTRL
CHI 2%
CHI 4%
CRI 2%
CRI 4%


RUS
ELASTICITY (0.8)
FIRMNESS (0.45)
FIBROUS (0.6)
BULKINESS (0.9)
ADHESIVENESS (0.6)
COLOR (0.7)
ODOR (0.3)
TASTE (0.3)
OVERALL QUALITY (0.4)
8
7
6
5
4
CTRL
CHI 2%
CHI 4%
CRI 2%
CRI 4%



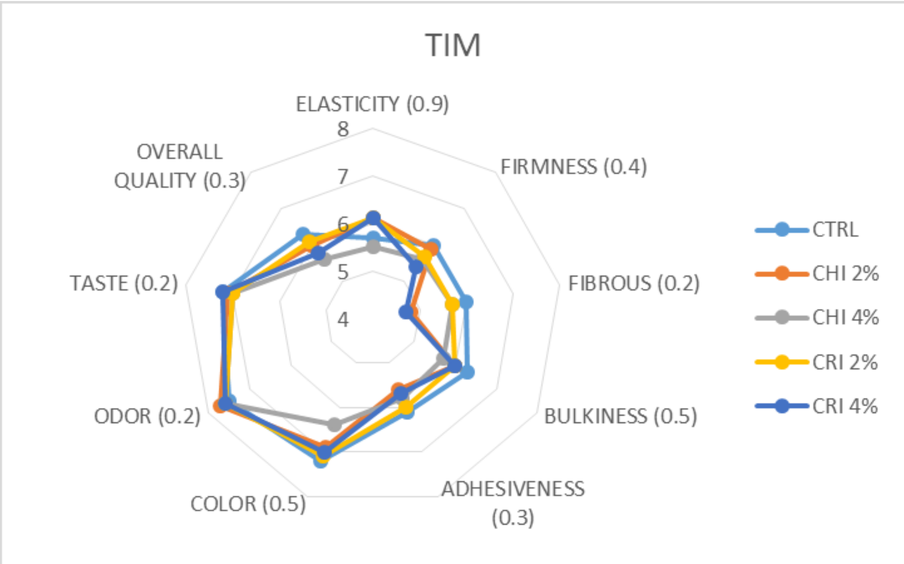


Figure 5.2: Variations of the scores for each parameter in dry uncooked spaghetti, within the same cultivar vs the relative CTRL. In each trait, the number between parenthesis indicates LSD at P<0.05.

Sensorial characteristics of dry cooked spaghetti

The ANOVA (Table 5.11) showed that the studied factor “cv” affected: elasticity (19.5% of total variation), firmness (69.3% of total variation), fibrous (17.1% of total variation), bulkiness (5.3% of total variation), adhesiveness (6.6% of total variation), color (35.0% of total variation), odor (18.7% of total variation), taste (15.7% of total variation) and OQS (15.8% of total variation). The inulin source, e.g. the DP of inulin influenced significantly all the studied traits except firmness, bulkiness and color, while the percentage of inulin influenced all the traits at P<0.001 and the firmness at P< 0.05. Out of cultivar, all the scores resulted higher than the threshold of acceptability, and the best results were obtained using CRI at 2%. On average of cultivars, and inulin source the inulin concentration of 2% improved or maintained elasticity, firmness, bulkiness, adhesiveness, color, odor and the OQS scores. Only the taste resulted more palatable at the concentration of inulin of 4%, while the sensation of fibrous was more marked both in 2 and 4%, than the absolute CTRL (Table 5.12).

Table 5.11: Mean square (MS) and percentage of variation (%) of sensorial properties of dry cooked spaghetti samples in relation to the studied factors.

DRY COOKED

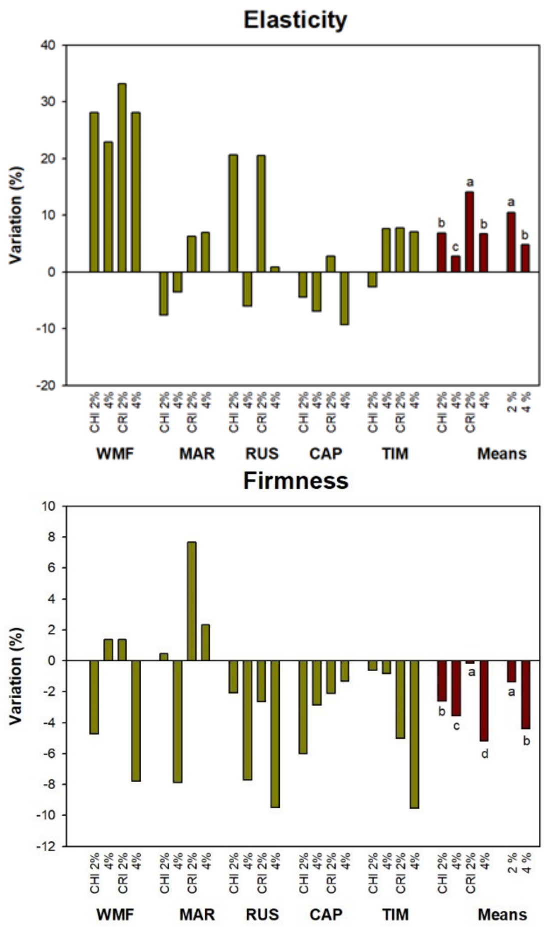
	ELASTICITY		FIRMNESS		FIBROUS		BULKINESS		ADHESIVENESS		COLOR		ODOR		TASTE		OQS	
	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
CULTIVAR (CV)	1.6 ***	19	3.3 ***	69	7.4 ***	17	0.6 ns	5.3	0.3 **	6.6	0.9 ***	35	9.3 ***	19	0.4 ***	16	0.8 ***	16
INULIN SOURCE (I)	1.2 ***	14	0.0 ns	0.1	3.0 ***	7.0	0.8 ns	7.6	0.3 *	6.4	0.2 ns	6.9	8.1 ***	16	0.4 *	15	1.2 ***	22
INULIN (%)	3.1 ***	37	0.8 **	16	14.0 ***	32	8.1 ***	74	3.2 ***	67	0.9 ***	33	6.7 ***	13	0.8 ***	29	1.1 ***	20
CV*I	0.1 ns	1.3	0.3 ns	5.5	5.8 ***	13	0.2 ns	1.8	0.0 ns	0.8	0.1 ns	2.7	7.7 ***	15	0.4 ***	12	0.2 ns	3.4
CV*%	1.8 ***	22	0.1 ns	2.9	5.1 ***	12	0.8 **	7.4	0.7 ***	15	0.4 ***	16	5.8 ***	12	0.3 ***	11	1.3 ***	24
I*%	0.4 **	5.3	0.1 ns	2.8	2.8 ***	6.4	0.3 ns	2.7	0.1 ns	2.1	0.1 ns	2.3	5.9 ***	12	0.2 *	8.2	0.4*	7.5
CV*I*%	0.1 ns	1.3	0.2 ns	3.2	5.0 ***	12	0.1 ns	0.9	0.1 ns	1.9	0.1 ns	3.8	6.1 ***	12	0.2 **	8.2	0.4 ***	6.8

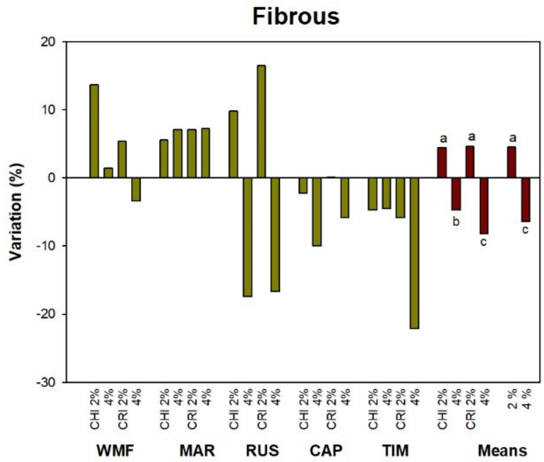
Table 5.12: Sensory properties of dry cooked spaghetti samples. Different letters indicate differences among types of pasta P< 0.05.

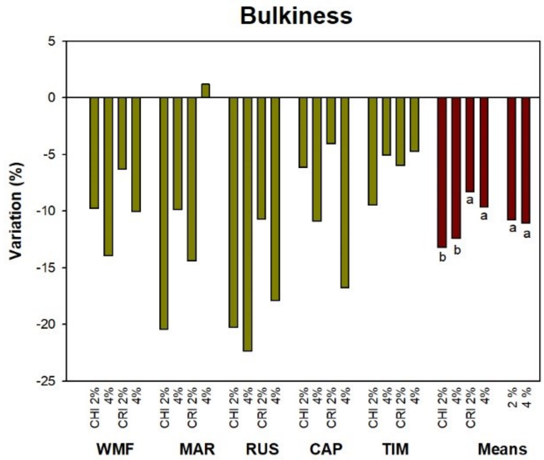
DRY COOKED

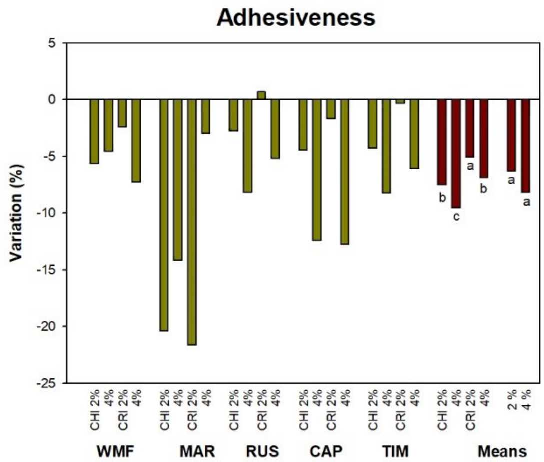
INULIN SOURCE	INULIN %	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OQS
					Out of cultivar					
CTRL		5.7 **b**	6.3 **a**	5.8 **b**	6.6 **a**	6.2 **a**	7.2 **a**	7.5 **a**	7.3 **a**	6.4 **a**
CHI	2	6.1 **b**	6.1 **a**	6.1 **a**	5.7 **c**	5.7 **b**	7.1 **a**	7.4 **a**	6.9 **b**	6.1 **b**
	4	5.8 **b**	6.0 **a**	4.3 **c**	5.8 **c**	5.6 **b**	6.8 **b**	6.0 **b**	7.2 **a**	5.9 **b**
CRI	2	6.5 **a**	6.2 **a**	6.1 **a**	6.1 **b**	5.9 **b**	7.2 **a**	7.6 **a**	7.2 **a**	6.5 **a**
	4	6.0 **b**	5.9 **a**	5.3 **b**	6.0 **b**	5.8 **b**	7.0 **a**	7.5 **a**	7.3 **a**	6.2 **b**
					Out of cultivars and inulin source					
	CTRL	5.7 **c**	6.2 **a**	5.8 **b**	6.6 **a**	6.2 **a**	7.2 **a**	7.5 **a**	7.3 **a**	6.4 **a**
	2	6.3 **a**	6.2 **a**	6.1 **a**	5.9 **b**	5.8 **b**	7.1 **a**	7.5 **a**	7.0 **b**	6.3 **a**
	4	5.9 **b**	6.0 **b**	4.8 **c**	5.9 **b**	5.7 **b**	6.9 **b**	6.8 **b**	7.2 **a**	6.0 **b**

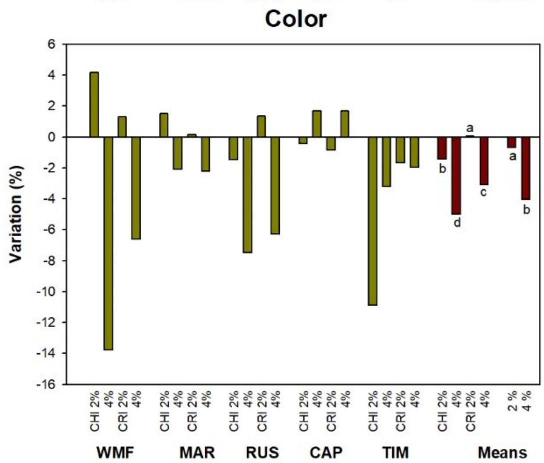
In Figure 5.3 are reported all the variations due to the addition of Chicorium and cardoon inulin, expressed in % vs the relative CTRL, at 2 and 4 %. In general, the results obtained are similar than that recorded in fresh cooked spaghetti, even if some sensorial characteristics resulted improved after the drying process. The sensation of "adhesiveness" recorded in fresh cooked spaghetti made with "Russello" did not result after the drying process, the color remained the same of fresh cooked spaghetti in all the cvs. The taste improved in "Margherito", the bulkiness sensation, as positive variation, was recorded only in "Russello". Odor, elasticity and firmness improved in all cvs, except for WMF. The OQS remained the same than that recorded in fresh cooked spaghetti.




Fibrous
Variation (%)
CHI 2%
4%
CRI 2%
4%
WMF
MAR
RUS
CAP
TIM
Means
2 %
4 %


Bulkiness
Variation (%)
CHI 2%
4%
CRI 2%
4%
WMF
MAR
RUS
CAP
TIM
Means
2 %
4 %


Adhesiveness
Variation (%)
CHI 2%
4%
CRI 2%
4%
WMF
MAR
RUS
CAP
TIM
Means
2 %
4 %


Color
Variation (%)
CHI 2%
4%
CRI 2%
4%
WMF
MAR
RUS
CAP
TIM
Means
2 %
4 %

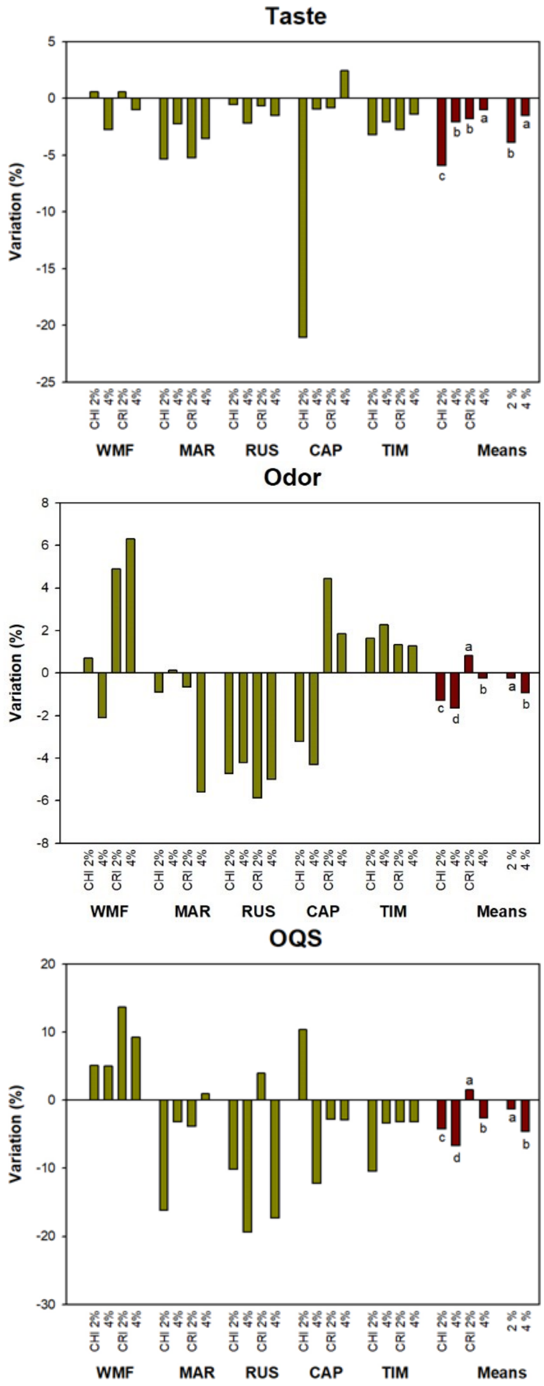


Figure 5.3: Percentage variations of the scores for each parameter, within the same cultivar, vs the relative CTRL.

2.5.4 Conclusions

Concerning the color, the best result was reached by the addition of inulin with an higher DP (CRI) at 4%.

A decrease of the yellow index was detected after cooking process, but only in commercial whole-meal samples than in the other Sicilian cultivars.

In fresh uncooked spaghetti, samples made with "Senatore Cappelli" and CRI at 4% resulted the most appreciated by panellists.

After cooking, CRI at 2% and 4% did not result different from CTRL for OQS, but the addition of low DP inulin at 4% decreased the score. In fact, within the same cultivars, CRI inulin gave best results in terms of sensorial characteristics of pasta, especially at lower percentage.

Commercial whole-meal flour gave negative scores for all the studied characteristics when added by inulins, as compared to pasta obtained without polymer.

The best results for all the studied parameters were obtained using the cv of "Senatore Cappelli", in which the OQS resulted in a negative variation of about 20%, when the flours were added by CHI 4%, while positive variation than CTRL where recorded in the OQS using CRI inulin, in both concentrations.

After the drying process the characteristics of uncooked spaghetti resulted the same of the absolute CTRL using inulin at 2%.

In dry cooked spaghetti the best results were obtained using CRI at 2%.

Concluding, the best cv was "Senatore Cappelli" and the best result was reached adding inulin extracted from roots of *Cynara cardunculus* (higher DP). Considering the scores of sensorial characteristics and the possible positive effect on health, the 4% would be a good percentage to add to pasta samples.

2.6 TRIAL 6: EVALUATION OF QUINOA SPAGHETTI

2.6.1 Aim

The aim of this trial was to evaluate, in a first step, the characteristics of fresh pasta produced with Quinoa flour, and, in a second step, the characteristics of dry spaghetti obtained with durum wheat commercial flour adding 5 (5-QUI) and 10 w/w (10-QUI) of Quinoa flour. All the samples were compared with a CTRL (Commercial wholemeal flour spaghetti).

2.6.2 Materials and methods

Quinoa and commercial wholemeal flours were purchased in “Molino Riggi” (Caltanissetta).

Spaghetti were produced by using the same operating conditions described in trial 2.

Pasta with commercial wholemeal was produced and used as control (CTRL). Fresh spaghetti samples were submitted to a panel of fifteen trained tasters as described in trial 2.

In the second step durum wheat commercial flour were added with 5 (5-QUI) and 10 w/w (10-QUI) of Quinoa flour. Data are presented separately for the different types of spaghetti.

Data analyses

Data were submitted to the Bartlett’s test for the homogeneity of variance and then analyzed using analysis of variance (ANOVA). Means were statistically separated on the basis of Student-Newmann-Kewls test, when the ‘F’ test of ANOVA for treatment was significant at least at the 0.05 probability level.

2.6.3 Results

Sensorial Analysis of Quinoa pasta

Table 6.1 and in Figure 6.1, data concerning the quality of fresh pasta produced with Quinoa flour are reported.

Table 6.1: Sensorial analysis of fresh uncooked spaghetti produced using Quinoa Flour (QF), compared with Commercial Wholemeal flour (CTRL) samples. Different letters indicate differences among types of pasta P< 0.05.

FRESH UNCOOKED

PASTA	ELASTICITY	FIRMNESS	BULKINESS	ADHESIVENESS	ODOR	TASTE	OVERALL QUALITY
CTRL	7.5 **a**	7.2 **a**	7.0 **a**	6.8 **a**	8.0 **a**	7.7 **a**	7.5 **a**
QUI	4.0 **b**	4.0 **b**	4.0 **b**	4.0 **b**	7.0 **b**	5.0 **b**	4.5 **b**
Means	5.7	5.6	5.5	5.4	7.5	6.3	6.0

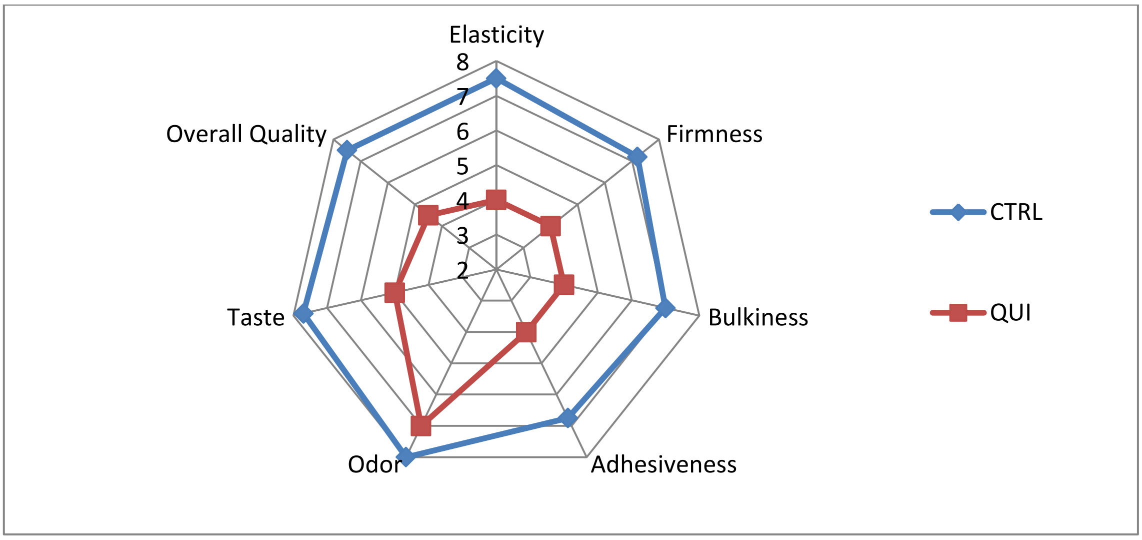


Figure 6.1: Sensorial analysis of fresh uncooked spaghetti produced using Quinoa Flour (QF), compared with Commercial Wholemeal flour (CWF) samples.

In the evaluation of the fresh pasta samples, a big difference with the control of commercial wholemeal flour was noticed, evident in the comparison of the overall quality values that deteriorated in the sample produced with only Quinoa flour. Further evidence was collected during the production of pasta samples in the laboratory, as the difficulty in processing and extrusion of the dough. Moreover, the pasta produced with only Quinoa flour did not pass the drying stage.

Sensorial analysis of pasta added with Quinoa

Table 6.2 lists the sensorial analysis of dry uncooked spaghetti produced using commercial wholemeal flour (CWF) and different percentages of QF.

Table 6.2: Sensorial properties of dry uncooked spaghetti samples based on the CWF enriched with QF. Different letters indicate differences among types of pasta $P < 0.05$.

DRY UNCOOKED

PASTA	**COLOR**	**BREAK TO RESISTANCE**	**OVERALL QUALITY**
CWF	7.2 **a**	6.7 **a**	6.9 **a**
5-QF	6.5 **b**	6.0 **b**	6.8 **a**
10-QF	6.0 **c**	6.0 **b**	6.0 **b**
Means	6.6	6.2	6.6

From the sensorial evaluation of dry uncooked pasta samples produced with CWF and QF, it has emerged that almost all values have lower score with the increase of QF percentage added.

Color went down proportionally with the increase of QF percentage, instead break to resistance showed differences between CTRL and pasta samples with QF addition, but it gave the same score in both percentage of substitution.

Global quality drops slightly in samples with 10% substitution (10-QF), but it was well maintained when we add 5% of QF (5-QF).

Table 6.3 lists the sensorial analysis of dry cooked spaghetti produced using CWF and different percentages of QF. A graphical presentation of these values are showed in figure 6.2.

Table 6.3: Sensorial properties of cooked spaghetti samples based on the CWF enriched with QF. Different letters indicate differences among types of pasta P< 0.05.

COOKED SPAGHETTI

PASTA	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OVERALL QUALITY
CTRL	5.9 **a**	6.6 **a**	6.2 **a**	6.7 **a**	6.2 **a**	7.0 **a**	7.1 **a**	7.2 **a**	5.9 **a**
5-QF	6.0 **a**	6.6 **a**	5.5 **b**	6.0 **b**	5.7 **a**	7.2 **a**	6.6 **ab**	6.0 **b**	6.2 **a**
10-QF	5.2 **b**	5.2 **b**	5.0 **c**	5.0 **c**	5.0 **b**	6.2 **b**	6.2 **b**	5.7 **b**	5.2 **b**
Means	5.7	6.1	5.6	5.9	5.6	6.8	6.6	6.3	5.8

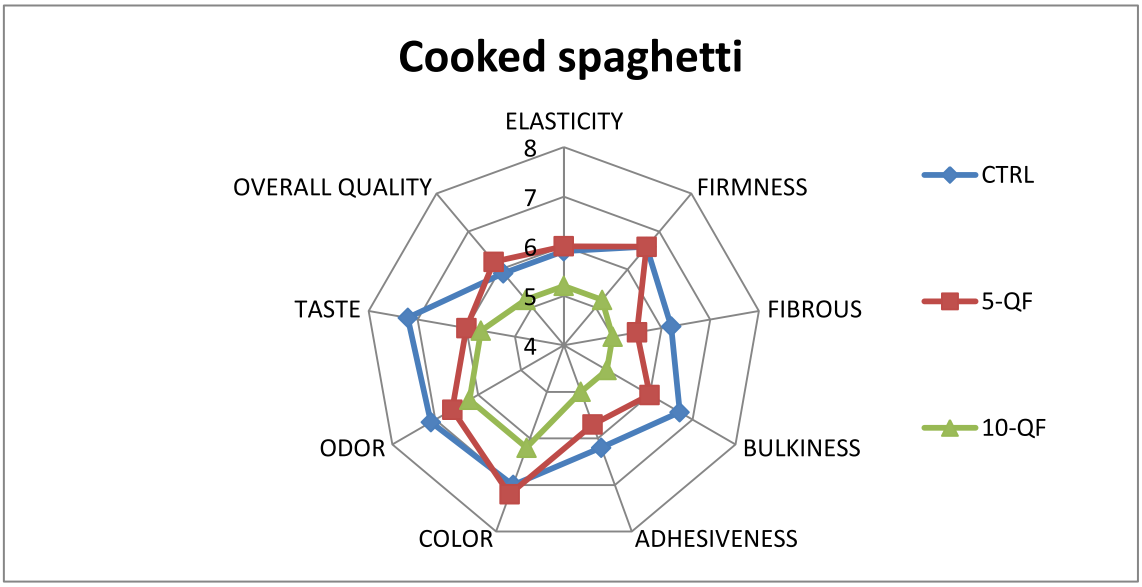


Figure 6.2: Sensorial properties of cooked spaghetti samples based on the CWF enriched with QF.

In dry cooked spaghetti samples, elasticity, firmness, adhesiveness and color did not be statistically different between CTRL and 5-QF but they went statistically down when the percentage of Quinoa flour was higher (10%). Fibrous, bulkiness, odor and taste dropped in 5-QF and over more in 10-QF, compared with CTRL samples.

In general, the quality of each pasta samples was good, with an average value of 5.8. In fact, as seen in uncooked spaghetti, we do not have any statistical differences in overall quality in samples 5-QF, compared with the CTRL. The overall quality scores went down with higher percentage of QF added (10-QF), but they remained in a range of acceptability (>5).

Cooking quality evaluation

In table 6.4 all data concerning the cooking quality are reported.

Table 6.4: Cooking quality evaluation of spaghetti produced using CWF and different percentages of QF. Different letters indicate differences among types of pasta P< 0.05.

FLOUR	COOKING LOSS (%)	SWELLING INDEX	WATER ABSORBTION	ADHESIVENESS	HARDNESS	OCT
CWF	4.8 **b**	1.8 **b**	151 **a**	0.4 **a**	5.8 **b**	10.5 **b**
5-QF	5.0 **b**	1.8 **b**	147 **a**	0.5 **a**	6.0 **ab**	11.0 **a**
10-QF	5.9 **a**	1.9 **a**	149 **a**	0.5 **a**	6.2 **a**	8.5 **c**
Means	5.3	1.8	149	0.5	6.0	10.0

Regarding the cooking quality characteristics, cooking loss, swelling index and hardness increased when the percentage of Quinoa flour added was 10%. Instead, no changes were detected in water absorption and adhesiveness. Optimal cooking time was a bit higher in 5-QF samples and a bit lower than control samples in pasta with 10% of quinoa flour (10-QF). The OCT average was 10.0 minutes.

2.6.4 Conclusions

The score quality of fresh spaghetti samples obtained using QF, except for "odour" did not pass the panel test. Highlighting the difficulty of obtaining pasta with the exclusive use of this flour, and with the aim of obtaining dry pasta samples, pasta with commercial semolina and a percentage of Quinoa flour was produced. In fact, two types of spaghetti samples were produced, 5-QF and 10-QF, and evaluated vs a CTRL. Cooking quality characteristics of all types of pasta were well maintained. If it is true that global quality drops in samples with 10% substitution, both in uncooked and cooked spaghetti samples, it is also true that it is well maintained when we add 5% of Quinoa flour. Moreover, being the inferior value in a minimal way respects the CWF, it will be negligible in the light of the positive effects on human health.

2.7 TRIAL 7: EVALUATION OF DRY SPAGHETTI PRODUCED WITH QUINOA AND INULIN WITH DIFFERENT DEGREE OF POLYMERISATION.

2.7.1 Aim

The aim of this trial was to evaluate the characteristics of dry pasta produced with commercial wholemeal flour plus 10 % (w/w) of Quinoa (10-QUI), with the addition of 8% of inulins from *Cynara cardunculus* (QF/CAR) and *Chicorium intybus* (QF/CHI).

2.7.2 Materials and methods

Quinoa Flour and commercial wholemeal flour were bought in "Molino Riggi" (Caltanissetta).

Inulin from roots of *Cynara cardunculus* was extracted, purified and characterized as trial 1. Spaghetti were produced by using the same operating conditions as described in trial 2. On the pastas, the following parameters were evaluated: colour (Minolta colorimeter CR, 400), sensorial qualities (panel test), optimal cooking time (OCT) (minutes) and cooking quality, as described in trial 2.

Data analyses

Data were submitted to the Bartlett's test for the homogeneity of variance and then analyzed using analysis of variance (ANOVA). Means were statistically separated on the basis of Student-Newmann-Kewls test, when the 'F' test of ANOVA for treatment was significant at least at the 0.05 probability.

2.7.3 Results

Sensorial Analysis of pasta

In table 7.1 and 7.2 are listed the values of sensorial characteristics respectively of dry uncooked and cooked spaghetti, produced using Commercial Wholemeal Flour (CWF) and 10% of Quinoa flour (QF), with the addition of 8% of two types of inulin.

Table 7.1: Sensorial analysis of dry uncooked spaghetti produced using CWF and 10% of QF, with the addition of 8% of two types of inulin. Different letters indicate differences among types of pasta P< 0.05.

DRY UNCOOKED

FLOUR	COLOR	BREAK TO RESISTANCE	OVERALL QUALITY
10-QUI	6.0 **a**	5.0 **b**	6.0 **a**
QF/CHI	6.2 **a**	5.2 **a**	6.2 **a**
QF/CRI	6.3 **a**	5.7 **a**	6.3 **a**
MEANS	6.2	5.3	6.2

Table 7.2: Sensorial analysis of dry cooked spaghetti produced using Commercial Wholemeal Flour (CWF) and 10% of Quinoa Flour (QF), with the addition of 8% of two types of inulin. Different letters indicate differences among types of pasta P< 0.05.

DRY COOKED

FLOUR	ELASTICITY	FIRMNESS	FIBROUS	BULKINESS	ADHESIVENESS	COLOR	ODOR	TASTE	OVERALL QUALITY
10-QUI	5.2 **b**	5.4 **b**	5.0 **b**	5.0 **b**	5.1 **b**	6.2 **a**	6.2 **b**	5.6 **a**	5.2 **b**
QF/CHI	5.6 **a**	6.0 **a**	5.5 **a**	5.4 **a**	5.5 **a**	6.2 **a**	6.5 **a**	5.8 **a**	6.0 **a**
QF/CRI	6.0 **a**	6.2 **a**	5.7 **a**	5.8 **a**	5.7 **a**	6.5 **a**	7.0 **a**	6.0 **a**	6.2 **a**
Means	5.6	5.9	5.4	5.4	5.4	6.3	6.6	5.8	5.8

While in the uncooked spaghetti, we did not have any differences, except for the increase in break to resistance, lower in 10-QUI samples, and this is not reflected in global quality, it is different in cooked samples, where there was a rise in overall quality when inulin is added. An increase was also noted in elasticity (average of 5.8 compared with 5.2 of 10-QUI), firmness (average of 6.1 vs 5.4 of 10-QUI), fibrous, bulkiness, adhesiveness and odor. This was reflected also in the overall quality score.

Cooking quality evaluation

In tab 7.3 are reported all data concerning the cooking quality.

Table 7.3: Cooking quality evaluation of dry cooked spaghetti produced using CWF and 10% of QF, with the addition of 8% of two types of inulin. Different letters indicate differences among types of pasta P< 0.05.

FLOUR	COOKING LOSS (%)	SWELLING INDEX	WATER ABSORBTION	ADHESIVENESS	HARDNESS	OCT
10-QUI	5.9 **a**	1.9 **a**	149 **a**	0.5 **a**	6.2 **a**	8.5 **b**
QF/CHI	5.7 **a**	1.8 **b**	145 **a**	0.5 **a**	6.0 **b**	10.5 **a**
QF/CRI	5.6 **a**	1.8 **b**	147 **a**	0.5 **a**	5.7 **b**	10.5 **a**
MEANS	5.7	1.8	147	0.5	6.0	9.8

No differences were recorded in cooking loss, water absorption and adhesiveness among three samples of spaghetti under studied. OCT was higher in pasta with inulin addition than in the control. Swelling index and hardness went slightly down in samples with inulin.

2.7.4 Conclusions.

Both in uncooked and cooked spaghetti, pasta samples did not have a decrease in sensorial characteristics; on the contrary, it an improvement of many properties in cooked samples when inulin is added is shown. This means a better acceptability from the consumers, moreover a probably good impact on consumer’s health.

3 CONCLUSIONS

The obtained results of the different trials showed:

1) different inulin plant source were considered, to extract inulin, with long degree of polymerization (DP) to use as fiber and fortify spaghetti with nutraceutical effects on human health. The good potential of C*ynara carduncul*us roots and capitula, and *Helianthus tuberosus* tubers, have been taken into account. Concerning *Cynara carduncul*us, different amounts and DPs of inulin from different parts of the plant were evaluated. An higher content of inulin was found in roots, if compared with heads and, moreover, inulin DP was always higher in roots than in capitula. As regard *Helianthus tuberosus*, the inulin DP of about 90 fructose unit was detected in local germplasm. In *Chicorium intybus* local populations the content of inulin was the lowest than the others. The highest score of L*was detected, as expected, in *Chicorium intybus*, commercial variety, but all the samples analysed reached the level of white that should not interfere with the sensorial characteristics of the pasta added with inulin;
2) The evaluation of quality characteristics of spaghetti enriched with inulin purified from roots (RI) and heads (HI) of cardoon, led to choose the roots as part of the plant of cardoon to collect for the polymer purification. In fact, despite the overall quality (panel analysis) of the pastas obtained with HI, was satisfactory, the high loss of the polymer during cooking implies an increase of the concentration at the initial phase, making the process non-sustainable on the contrary to what was found by providing the use of the roots for inulin extraction;
3) the environment of cultivation of cardoon to produce inulin for pasta fortification did not show significant effects on quality characteristics of dry and cooked pasta;
4) the effects of plant material (cardoon roots, Chicorium roots and topinambur tubers) and of different inulin concentrations (2, 4 and 8% w/w) to obtain fortified spaghetti have been investigated in trial 4. In general scores showed a very good overall quality in samples with 2% of every type of inulin. Quality was in the range of acceptability at 4%, but with 8%, the

values were out of range (<5). These results means that until 4% of addition, pasta have good quality and properties. When the addition is higher, most of the pasta characteristics gave lower scores and the quality is out of the range of acceptability. Within all the quality traits, the best compromise in terms of consumer's acceptability has been obtained using inulin extracted from topinambur tubers at the concentration of 4%.

5) Interaction were observed between cultivars of durum wheat, inulin concentration and DP. Among the five wholemeal flours, spaghetti made with the cv "Senatore Cappelli" added with 4% if CRI inulin, gave the best results. On these pasta chemical characterization was performed (Padalino *et al.*, 2017). In general, the increase in inulin amount determined a decline of sensory quality even though the molecular weight of inulin played a key role on pasta acceptability. This effect may be directly ascribed to the probable entanglements created by high molecular weight inulin that generally improved pasta characteristics. Concerning the chemical composition, the sample enriched with 4% of cardoon inulin showed a greater TDF and lower available carbohydrate content with respect to the other samples. Besides, the starch digestibility significantly declined with the increase of inulin. Specifically, the samples supplemented with high DP inulin (CRI) showed a lower starch digestibility than that of samples with low DP inulin (CHI).This result is ascribed to the fact that CHI inulin may have a greater disruptive effect on the starch-protein matrix, thus compromising its cohesive encapsulating layer. In conclusion, the DP of inulin significantly affected its interactions with gluten matrix during pasta formation and as consequence, inulin extracted from cardoon roots allowed realizing a final pasta very interesting from the nutritional point of view and also acceptable for sensory properties and cooking quality (Padalino *et al.*, 2017).
6) In order to produce low GI spaghetti, the Quinoa flour was tested for fresh pasta production. The consumer's acceptance was under 5. Moreover, the pasta produced with only quinoa flour did not pass the drying step. Enriching durum wheat spaghetti with quinoa flour gave good results, the cooking quality characteristics of all types of pasta were well maintained. The scores remained upper than the threshold of acceptability, but the global quality

drops in samples with 10% substitution, both in uncooked and cooked spaghetti, than CTRL. Moreover, being the value lower in a minimal way than CTRL, it will be negligible in the light of the positive effects on human health.

7) dry low GI spaghetti have been produced with commercial wholemeal flour plus 10 % (w/w) of Quinoa (10-QUI), with the addition of 8% of inulins from *Cynara cardunculus* (QF/CAR) and *Chicorium intybus* (QF/CHI), to improve rheological characteristics of the doughs during the pasta production, as well as to fortify spaghetti. Pasta did not have a decrease in sensorial characteristics. This means a better acceptability from the consumers with a good impact on consumer's health.

The good amount of data collected during this research period contributes to improve knowledge about food fortification with fibers, to develop new types of pasta with low glycemic index effect. The reached levels of acceptance of consumer's could be the starting point to produce pasta to be tested in clinical trials for further researches on the effect played on glycemic index after ingestion.

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Appendix

Part of the work of this thesis has been published in the Journal “Carbohydrate Polymers”. Impact Factor (2016): **4.811**

Attached the full paper: “The quality of functional whole-meal durum wheat spaghetti as affected by inulin polymerization degree” by Padalino *et al*., 2017.

Addendum

ABROAD STAGE

Introduction

I completed two research trainings at the University of Plymouth (United Kingdom) during 2016 and 2017 respectively, under the supervision of Dr. Raul Bescos. During these periods I was involved in two different studies investigating:

- The dietary consumption of inorganic nitrate (exogenous source) in vegetarians compared with omnivores and its effect on blood pressure and metabolic rate;
- The effect of acute exercise on endogenous bioavailability of nitrates/nitrites and its effect on the vascular function.

The nitrate/nitrite pathway

Nitrate and nitrite are natural compounds of the nitrogen cycle, and can be found in small amounts in different tissues and fluids of the human body. The level of nitrate and nitrite in the body are modulated by exogenous and endogenous sources. The main exogenous source is the diet, particularly vegetables. Green leafy vegetables have the highest levels of nitrate, with mean values up to 468mg/100g for rocket (European Food Safety Authority, 2008).

Interestingly, dietary nitrate was traditionally associated with harmful effect in humans such as methaemoglobinemia and cancer. In the 1940s Hunter and Comly reported cases of infant methemoglobinemia ('blue baby syndrome') associated with well-water which had a high nitrate content. Subsequent analysis of cases of infantile methemoglobinemia revealed that such cases were rare when the well-water nitrate was below 44 mg/l (Walton, 1951). In 1956 it was reported that N-nitrosamines could cause hepatic tumours in laboratory animals by reacting with nucleic acids (Magee & Barnes, 1956). Then, in 1975 findings from animal research suggested a link between dietary nitrate and development of gastric cancer (Correa *et al.*, 1975). Further concern about dietary nitrate emerged in the 1976, when the research groups of Spiegelhalder in Germany and Tannenbaum in the USA showed that nitrate could be transformed into N-nitrosamines by the reaction of N_2O_3

(derived from the acidification of salivary nitrite) with secondary amines in the diet (Spiegelhalder *et al*., 1976; Tannenbaum *et al,* 1976; Gilchrist *et al*., 2010)(Figure 8.1).

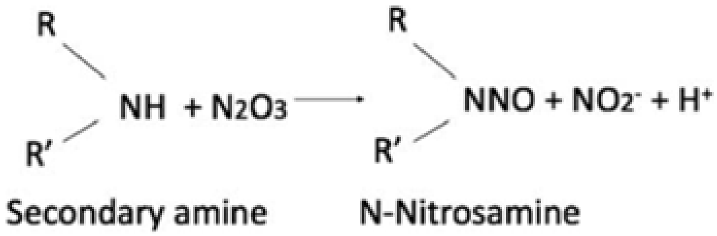

Figure8.1: Nitrosamine production (Gilchrist et al., 2010)

In addition, in 1979, Newberne published a report that dietary nitrite caused lymphomas in rats. Therefore, as a result of all these evidence, an Accettable Daily Intake (ADI) of 3.7 mg/kg/d (EFSA datas) was established in order to reduce the potential harmful effects of nitrate.

However, this view has substantially changed over the last decade. Currently, studies in humans have not found a link between dietary nitrate ingestion and the risk of cancer when the main source are vegetables rather than meats. On the other hand, recent evidence has shown that nitrate and nitrite can be important modulators of the vascular and metabolic response (Hord *et al*., 2017).
Nitrate (NO_3^-) and nitrite (NO_2^-) can be recycled in vivo to form NO, representing an important alternative source of NO to the classical l-arginine–NO-synthase pathway, in particular in hypoxic states (Lundberg *et al*., 2008).

Exogenous pathway (diet)

As indicated above, diet is the main exogenous source of nitrate, particularly vegetables. About 25% of ingested nitrate is reduced by facultative anaerobic bacteria which are found on the surface of the tongue (Doel *et al*., 2005). These bacteria use nitrate in the absence of oxygen to act as a terminal electron acceptor and produce nitrite as a by-product. A small amount of this nitrite is further reduced to NO by bacteria or periodontal acidity (Duncan *et al.*, 1995), but most will be inevitably swallowed and will react with stomach acid to produce a complex mix of nitrogen oxides, including nitrous acid, nitrogen dioxide, dinitrogen trioxide, and nitric oxide (Figure 8.2).

$$NO_2^- + H^+ \underset{pK_a=3.42}{\leftrightarrow} \underset{\text{nitrous acid}}{HNO_2}$$

$$2HNO_2 \rightarrow \underset{\text{dinitrogen trioxide}}{N_2O_3}$$

$$N_2O_3 \rightarrow \underset{\text{nitric oxide}}{NO^{\cdot}} + \underset{\text{nitrogen dioxide}}{NO_2^{\cdot}}$$

Figure 8.2: Nitric oxide formation in stomach (Gilchrist et al., 2010)

Bacterial reduction of nitrate to nitrite (and subsequent systemic reduction to nitric oxide) is necessary in order for it to have biological activity (Lundberg & Govoni, 2004).

In the first study I was involved, we investigated the dietary nitrate intake in UK vegetarians compared with non-vegetarians and its effect on Resting Metabolic Rate (RMR) and blood pressure (BP). The main hypothesis of this study was that dietary nitrate consumption in vegetarian subjects is larger than in omnivores, and this fact, can explain at least partially, the low blood pressure levels and resting metabolic rate found in previous studies. This hypothesis was supported by previous studies showing that dietary nitrate ingestion reduced blood pressure in healthy subjects (Larsen *et al*., 2006; Kapil *et al*., 2010; Siervo *et al*., 2013).

Materials and methods

Dietary nitrate intake was estimated in 23 healthy vegetarian participants and 18 healthy omnivores matched by age, gender, physical activity and body mass index. In addition, blood and salivary levels of nitrate and nitrite were also measured, as well as the Resting Metabolic Rate (RMR) and Blood Pressure (BP) before and after using antibacterial mouthwash for 7 days. This methodological approach disrupts bacterial activity, enabling to isolate the effects of dietary nitrate on metabolic rate and cardiovascular health from other bioactive components of vegetables (Kapil *et al*., 2013). Participants were healthy, not taking any medication and not using mouthwash.

After a week using a placebo mouthwash (distilled water) twice a day, the participants attended the laboratory. Anthropometrical measurements (body weight and height; BMI) were taken at first. Then, the RMR was measured using indirect calorimetry (Jaeger Oxycon Pro, Germany) under fasting conditions (> 7 hours).

The respiratory analyser was warmed up and calibrated one hour before the first test according to the manufacturer instructions. Testing periods lasted 30 minutes with subjects placed on a bed under controlled conditions (23 ± 1°C – 40-50% relative humidity). The first 20 minutes were automatically discarded for the analysis. Oxygen consumption and carbon dioxide data for the remaining 10 minutes were used to analyse the RMR.

After the RMR test, BP was assessed in triplicate in the seated position using an electronic sphygmomanometer according to established guidelines (British).

The mean of the second and third readings was used as the systolic and diastolic BP. Both the operator and the volunteer were blind to these measurements.

Venepunctures were performed on the antecubital vein of the arm by Dr. Raúl Bescós. He is an accredited phlebotomist with experience drawing blood from participants during numerous trials. Overall, 15 mL of blood were taken from each participant on day 1 and day 2. Whole blood glucose (2 mL) was analysed using a glucose analyser (YSI 2300 Stat, Yellow Springs Instrument, Yellow Springs). To analyse the lipid profile, 5 mL of blood were allowed to clot and then centrifuged for serum separation. Serum total cholesterol, high-density lipoproteins, low-density lipoproteins and triglycerides were analysed using enzymatic diagnostic kits on a Smartlab Auto Analyzer (ERBA, Germany). To assess nitrate and nitrite, 5 mL of blood were centrifuged (1000 g for 20 min) within 2 min after extraction. Plasma was separated and stored at -80ºC until analysis by high performance liquid chromatography (HPLC).

Following blood sampling, a saliva sample was taken to analyse salivary nitrate (2-3 mL) and nitrite using the same method as in plasma. Furthermore, The Oral Nitrate Reducing Capacity was also measured by holding a solution of 10 ml of nitrate- and nitrite- free water for 5 min in the oral cavity after which time the mouth rinse was collected into sterile tubes, centrifuged and the supernatants were collected and stored at -80ºC until nitrite and nitrate were determined.

Result and discussion

Only preliminary results are collected. The main results will be part of another thesis.

Endogenous pathway

Nitric oxide is an endogenously modulator of the vascular tone (Lundberg & Weitzberg, 2005, Moncada *et al,* 1988). In the human circulation vasodilatation is thought to be mediated by nitric oxide which is generated by the reduction of circulating L-Arginine. This reaction is regulated by a family of enzymes which are known as Nitrate Oxide Synthases (NOS) (Forstermann & Sessa, 2012).

Nitric Oxide Synthases is a family of enzyme carrying out a complex reaction, which appear to be highly conserved between the mammalian species studied so far. Such conservation between species, together with the diversity of the isoenzymes and the large (and still growing) list of physiological roles that they serve, suggest that synthesis of NO from L-arginine is a regulatory and host defence mechanism of great importance. The combination of the types of role that NO plays, namely intracellular signal, transcellular signal and cytotoxic molecule, is unprecedented in biology. (Knowles *et al*.,1994)

NO Synthases results in the synthesis of NO and Citrulline, requiring L- arginine and NADPH (Leaf *et al*., 1989; Florin *et al*., 1990; McKnight *et al*., 1997; Doel *et al*., 2005; Lundberg& Govoni., 2004; Duncan *et al*., 1995)(Figure 8.3).

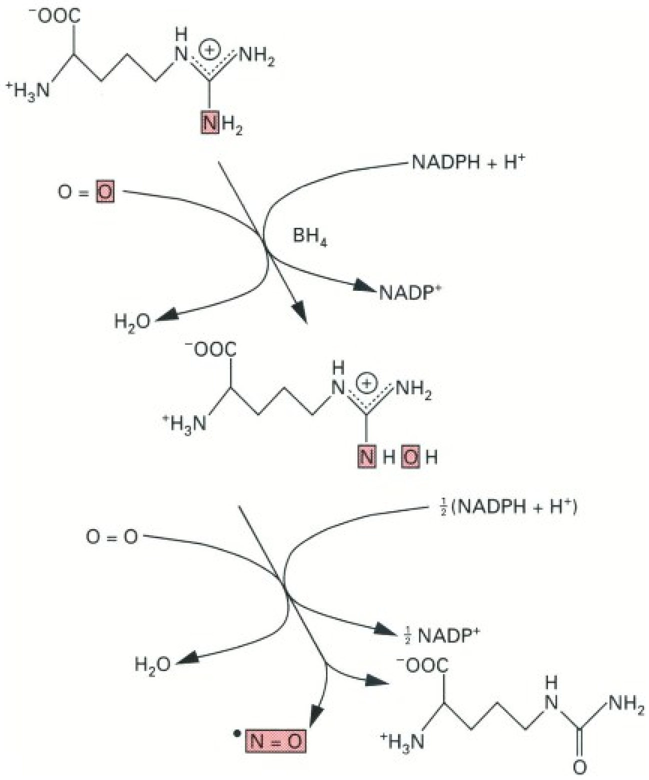

Figure 8.32: NO Sinthases reaction (Knowles et al., 1994)

A fundamentally different pathway for the generation of nitric oxide was recently described in which the anions nitrate (NO_3^-) and nitrite (NO_2^-) are converted into nitric oxide and other bioactive nitrogen oxides (Lundberg & Weitzberg, 2005; Gladwin *et al*., 2005; Lundberg & Weitzberg, 2004; Larsen, 2006). Nitrate and nitrite are the main metabolites of this reaction. Importantly, regular exercise increases endothelial NOS (eNOS) expression and activity, which results in higher circulating levels of nitrate. Then, as discussed above the circulatory nitrate can be oxidised to nitrite in the mouth, which can turn into higher availability of circulatory nitrite and NO . Thus, physical exercise is an effective approach to increase the bioavailability of NO metabolites. This factor has been associated with cardiovascular benefits related to physical exercise. For instance, NO has been suggested to be a key modulator of the physiological phenomenon known as post-exercise hypotension, although the main mechanism behind this intriguing effect is not well understood yet. The study I aimed to investigate the role of endogenous synthesis of nitrate and nitrite on post-exercise hypotension and the vascular function. The main hypothesis of this study was that nitric oxide production during acute exercise increases bioavailability of nitrate and nitrite that can be recycled back to NO during the recovery period. This response is associated with improved post-exercise vasodilation and post-exercise hypotension.

Materials and methods

Participants meeting the inclusion criteria were invited to visit the Nutrition, Exercise and Health Laboratory at Plymouth University in three different occasions following a cross over and double blind design. In the first visit, participants attended the lab under fasting conditions (> 3h) and following baseline anthropometric measurements (body mass, height and body fat) participants undertook a graded treadmill test to assess their maximal oxygen uptake (VOpeak). The test started with a 5-min warm-up running at 5 km/h on a treadmill at 0% of inclination. Following this period, the running speed was kept constant at 7 km/h and the inclination will raise 2% every 2 minutes until volitional exhaustion. Oxygen uptake (VO2), carbon dioxide production (VCO2), and the respiratory exchange ratio (RER) were measured every 15 seconds by a computerized gas analyzer (Jaeger Oxycon Pro, Germany). Heart rate was also measured using a heart rate monitor during the test. The VO2peak was determined as the average of the VO2 over the final 30 seconds of exercise. Using this value, we determined the workload (65% VO2peak) for the next two exercise trials.

In the second and third visit, the same anthropometrical measurements were measured again before the participant lied down for 10 min on a bed in supine position. Then, clinical blood pressure was assessed in triplicate on the left arm. A saliva sample (2-3 mL) was also taken under resting conditions. Finally, the vascular response of the flow mediated dilation of the arm was measured before to start exercise. Participants performed 4 sets of 7 min of running at 65% of VO2peak interspersed with 3 min of passive recovery. After exercise, participants was randomly assigned in a double-blind cross over design to rinse their mouth with antibacterial mouthwash (Corsodyl, 0.2% chlorhexidine, GlaxoSmithKline, UK) or placebo (distilled water with mint flavour) for 1 min following the collection of the first sample, and then at 30, 60 and 90 minutes after exercise. The flow mediated dilation of the brachial artery was measured at 120 min following exercise.

The second and third trial of the study were completed on the same day of the week and at the same time with a resting period of a week between each trial.

Participants were allowed to drink water ad libitum, but food intake was not permitted during the trial. Additionally, a diet with low levels of moderate- or high-nitrate content foods (green leafy vegetables, beetroot, strawberries, grapes, and tea) was followed for 3 days before the trials. In addition, the participants were encouraged to intake the same food and drinks during the 3 days before each trial in order to ensure similar levels of carbohydrate intake. Alcohol, caffeine products, and dietary supplements were excluded 24 hours before each exercise test. A minimum 7-day washout period was included between the two main trials of the study .

Result and discussion

The studies I participated have not been completed yet, and are part of other PhD thesis. Consequently, it is not possible to discuss the main results of them.

However, the main goal of my research training periods in Plymouth was to improve the skills as a researcher. From this viewpoint, by completing these trainings I had the opportunity to learn new techniques that I can apply in future studies. For instance, I developed new skills to measuring the metabolic rate and vascular function. These are important learning outcomes since dietary fiber is known to play a key role in the modulation of these physiological factors.

Conclusions

The participation to these two study was a challenge for me in different way. I went deeper in the processes involved in blood pressure and cardiovascular function regulation, useful if it is considered that my PhD project concerns the production of a new type of pasta with an high fibers content and it is well known the connection between fibers and human health.