# UNIVERSITÀ DEGLI STUDI DI FOGGIA


DOTTORATO DI RICERCA IN "INNOVAZIONE E MANAGEMENT DI ALIMENTI AD ELEVATA VALENZA SALUTISTICA"

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TESI DI DOTTORATO

# Pasta with cholesterol-lowering effect on human health

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This work was carried out in three years, from November 2015 to October 2018, as PhD research in "Health food innovation and management", of the University of Foggia. The work was conducted at CNR-ISAFOM in Catania.

The topic, approved from the college, was related with the production of new types of pasta with cholesterol lowering effect, based on the use of commercial durum wheat flour and the addition of typical wild species of purslane, Sicilian populations of lentil, cladodes of prickly pear and oat flour.

Trial 1 regarded the production and the sensorial and chemical evaluation of spaghetti of commercial durum wheat flour with the addition of commercial and Sicilian population of Ragusa lentil; however the use of food additives (CMC and GUAR) was studied to improve sensorial results.

Trial 2 concerned the evaluation of pasta produced using wholemeal flour "Senatore Cappelli" or commercial durum wheat flour with the addition of cladodes of prickly pear (*Opuntia ficus-indica* (L.) Mill) Sicilian populations at different concentrations (5% and 10% w/w). Food additives (Agar and CMC) were used to improve sensorial and nutraceutical characteristics.

The aim of trial 3 was the assessment of chemical composition of three purslane populations under different Mediterranean environmental conditions, for future valorization as novel food sources of omega-3 fatty acids and antioxidant activity. The evaluations were conducted over two years.

In Trial 4 fresh pasta samples with commercial durum wheat and purslane collected in three different areas of Sicily were produced and evaluated. Each of these three populations of purslane was added at three different concentrations (5%, 10% and 15% w/w).

Regarding trial 5, the aims were: the extraction and evaluation of β-glucans in oat (*Avena sativa* L., variety named Genziana) and the production and evaluation of fresh pasta samples produced with the use of commercial durum wheat and oat flour at four different concentrations (5%, 10%, 20% and 40% w/w).

# 1. INTRODUCTION

## 1.1 FUNCTIONAL FOODS AND HYPERCHOLESTEROLEMIA

In the last decades consumer demands in the field of food production has changed considerably. Consumers more and more believe that foods contribute directly to their health (Mollet and Rowland, 2002; Siró et al., 2008). Health is becoming an increasingly important personal and societal value. The prevention of health problems occurring in the first place is becoming very important. A substantial proportion of health complaints are categorized as civilization-related diseases and could be prevented by a healthier lifestyle. Besides physical activity, adequate nutrition is an essential aspect in influencing a person's health status (Goetzke et al., 2014). Consumers are starting to understand that their food choices may influence their health and are paying more attention to the health benefits of food to maintain a healthy lifestyle (Bachl, 2007; Chrysochou, 2010; Goetzke et al., 2014). Therefore, 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 (Menrad, 2003; Roberfroid, 2000). In this regard, functional foods play an outstanding role. The high cost of healthcare, the steady increase in life expectancy, and the desire of older people for improved quality of their later years are causing an increase in demand on such foods (Kotilainen et al., 2006; Roberfroid, 2000; Siró et al., 2008) Previous epidemiologic studies have consistently shown that diet plays a crucial role in the prevention of chronic diseases (Temple, 2000; Willett, 1994). Consumption of fruit and vegetables, as well as grains, has been strongly associated with reduced risk of cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts, and age-related functional decline (Liu, 2003; Temple, 2000; Willett, 1995). The term ''functional food'' was first used in Japan, in the 1980s, for food products fortified (Hardy, 2000; Kwak and Jukes, 2001; Stanton et al., 2005) that may 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 (Mark-herbert, 2004; Menrad, 2003; Side, 2006; Siró et al., 2008). So, in summary, a food marketed as functional contains added, technologically developed ingredients with a specific health benefit (Niva, 2007). Although the term ''functional food'' has already been defined several times (Roberfroid 2000), so far there is no unitary accepted definition for this group of food (Alzamora et al., 2005). In most countries there is no legislative definition of the term (Mark-herbert, 2004; Niva, 2007) and to date, a

number of national authorities, academic bodies and the industry have proposed definitions for functional foods (Bech-Larsen and Grunert, 2003). The European Commission's Concerted Action on Functional Food Science in Europe (FuFoSE), coordinated by International Life Science Institute (ILSI) Europe defined functional food as follows: ''a food product can only be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism thus either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases. The amount of intake and form of the functional food should be as it is normally expected for dietary purposes. Therefore, it could not be in the form of pill or capsule just as normal food form'' (Diplock et al., 1999). On the contrary to this latter statement, since 2001 FOSHU products in Japan can also take the form of capsules and tablets, although a great majority of products are still in more conventional forms (Ohama et al., 2006). European legislation however, does not consider functional foods as specific food categories, but rather a concept (Coppens et al., 2006; Siró et al., 2008; Stanton et al., 2005). 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 nutraceuticals. 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 can act as a nutraceuticals for another consumer (Shanon, 2010).

Use of dietary supplements, functional foods, and nutraceuticals is increasing as industry is responding to consumers' demands (Liu, 2003).

Cardiovascular diseases (CVD) remain the leading cause of morbidity and mortality worldwide, its prevention is quite important. Hyperlipidemia, resulting from the abnormalities of lipid homeostasis, is a common risk factor for the development of CVD (Jain et al., 2007). Reducing serum lipids can reduce the probability of CVD as well as other related metabolic syndromes, such as obesity and diabetes. Healthy diet and exercise are well recognized to have beneficial effects on improving the serum lipid profiles: reducing total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C, the bad cholesterol), while elevating high-density lipoprotein cholesterol (HDL-C, the good cholesterol) (Kelly, 2010). Cholesterol is important constituent of membrane lipid and precursor of steroid hormones, vitamin D and bile

acids. But high level of cholesterol, especially LDL-C is dangerous for human health and the risk of CVD decreases by 2% with a 1% decrease in serum cholesterol (Maxfield and Tabas, 2005; Steinberg, 2006). Human body obtains cholesterol from de novo synthesis mainly in the liver (700–900 mg/d) and absorption from diet (300–500 mg/d). Cholesterol circulates as a component of lipoproteins, such as chylomicron, VLDL (very low-density lipoprotein), LDL (low-density lipoprotein), and HDL (high density lipoprotein). It is eliminated from body via fecal excretion (~600 mg/d), conversion to bile acid (~400 mg/d), and through loss of dead skin cells (~100 mg/d). Cholesterol homeostasis in the body is regulated mainly by intestinal absorption, endogenous synthesis, and hepatic conversion and excretion (Chen et al., 2014).

Some suggested nutraceuticals and functional food against hypercholesterolemia are legume proteins, omega-3 polyunsaturated fatty acid, carbohydrate-dietary fibers and dietary polyphenols.

The consumption of legumes, such as lupin, pea, chickpea, bean, lentil, and butter bean, showed favorable effects in preventing dyslipidemia (Sirtori et al., 2009). The legume proteins are suggested as part of the active ingredients. For example, pea protein isolated from *Pisum sativum* markedly lowered plasma total cholesterol and triglyceride in rats by upregulating LDL-R expression and down regulating fatty acid synthesis genes (Rigamonti et al., 2010). In a human trial, lupin protein compared to casein slightly lowered concentration of LDL-C in hypercholesterolemic subjects, without altering HDL-C (Weisse et al., 2010).

Important dietary ω-3 polyunsatured fatty acids (PUFAs) are DHA (docosahexaenoic acid, 22:6 ω-3), EPA (eicosapentaenoic acid, 20:5 ω-3), and ALA (α linolenic acid, 18:3 ω-3) which is a precursor of the first two. Many epidemiological and interventional studies have demonstrated the beneficial effects of ω-3 PUFAs against CVD, including hypotriglyceridemic, antithrombotic, anti-inflammatory, antiarrhythmic, and antiangiogenic effects (Connor et al., 2007; Micallef and Garg, 2009; Szymczak et al., 2008).

Dietary fibers are resistant to hydrolyzation by the endogenous enzymes in the small intestine of humans and have physiological effects of health benefit as demonstrated by scientific evidence (Phillips and Cui, 2011). Fibers found in plant cell wall are non-starch polysaccharides and consist of a large number of monosaccharide residues joined by glycosidic linkage. This group includes cellulose and hemicelluloses from vegetables, fruits, nuts, legumes, cereals grains, and bran. It also includes pectins (from citrus fruits

and apples), beta-glucans (from cereal grains particularly oats and barley, yeast, bacteria, algae and mushroom), gums and mucilages. Different studies, human trials and meta-analysis showed that the consumption of different types of dietary fiber reduce risk of CVD, diabetes, obesity, and other metabolic syndrome. The beneficial physiological properties of dietary fiber include improved laxation, fermentability by colon microbiota, attenuation of blood total cholesterol, LDL-C, glucose, and insulin and protection against cancers according to specific type of dietary fiber (Chawla and Patil, 2010; Raninen et al., 2011).

Polyphenols are the most abundant antioxidants in diets and important constituents of fruits, vegetables, cereals, and beverages. Some groups among them, such as phenolic acids, flavonoids and lignans, have a lot of beneficial effects against degenerative disease, such as cancer and CVD (Manach et al., 2004). Polyphenols have potent antioxidant activity which may prevent free radical damage to macromolecules. But the precise role played by polyphenols in human health is yet to be elucidated (Chen et al., 2014).

## 1.2 PASTA

Pasta is popular in many countries and is a good source of low glycaemic index carbohydrate (Aravind et al., 2012; Brennan, 2008). It is a traditional cereal-based product accepted worldwide due to the low cost, easy production and sensory attributes (Armellini et al., 2018; Chillo et al., 2008). Therefore, pasta is a staple food because is regularly eaten in such quantities, that constitutes a dominant portion of the diet worldwide (International Pasta Organization, 2014) (Oliviero and Fogliano, 2016). Although pasta is traditionally manufactured using only durum wheat flour, it is possible to use non-durum wheat flour and other ingredients to produce specifically-labelled blended pasta (Bustos et al., 2011). The production of pasta enriched with vegetables is a straightforward strategy to increase the fruits and vegetables intake and it can be a very good carrier of healthy compounds: dried pasta is a very good matrix to stabilize phytochemicals that otherwise, in fresh vegetables, are easily degraded during storage, transportation etc. (Jin et al., 2014; Oliviero and Fogliano, 2016).

But the introduction of other ingredients can dilutes the gluten and weakens the protein network responsible, for example, for leaching of more solids in the cooking water (Torres et al., 2007). To obtain pasta of good quality from raw materials it is often necessary to modify the traditional production process (Kent and Evers, 1994). In particular, balanced formulations and adequate technological production processes have to be adopted to counteract any changes in the rheological properties caused by the incorporation of these new ingredients (Marconi and Carcea, 2001). A strategy can be the use of food additives, such us hydrocolloids that are used in the manufacture of functional foods (Glicksman, 1982). Guar gum, agar and carboxymethylcellulose (CMC) are typical examples of food additives. They are widely used in many fabricated food products in order to impart the required quality in terms of stability, texture and appearance (Padalino et al., 2013b). They are usually controlled by legislation in the form of lists of permitted substances and their presence in food has to be indicated in the manner laid down in regulations governing food-labeling (Yudkin, 1986). Guar gum is a novel agrochemical processed from endosperm of cluster bean. It is largely used in the form of guar gum powder as an additive in food, pharmaceuticals, paper, textile, explosive, oil well drilling and cosmetics industry. Industrial applications of guar gum are possible because of its ability to form hydrogen bonding with water molecule. Thus, it is chiefly used as thickener and stabilizer. It is also beneficial in the control of many health problems like

diabetes, bowel movements, heart disease and colon cancer (Mudgil et al., 2014). Agar is an hydrocolloids that induce stabilization of physical properties of the food product during shelf life and prevention of undesirable changes such as moisture migration, gas cell coalescence or textural profile changes (Aliste et al., 2000). CMC is an anionic polysaccharide obtained from cellulose. The non-toxicity, biodegradability and biocompatibility of CMC make it one of the most important cellulose derivatives. CMC readily dissolves in water to form viscous solutions with a range of thickening, stabilizing and film-forming properties. These properties make CMCs attractive polymers for industrial and consumer applications, including food industry (Arinaitwe and Pawlik, 2014). For example the effect of CMC on the quality of amaranthus spaghetti was evaluated (Chillo et al., 2007) and it emerged that spaghetti samples containing CMC presented better performances especially in cooking with respect to the spaghetti samples with pregelatinized starch (Chillo et al., 2009).

## 1.3 LENTILS

Legume proteins are considered important in vegetarian diets or diets poor in animal proteins, because they have a satisfactory content of lysine and other indispensable amino acids, but have a slightly insufficient content of sulphur amino acids as their main defect. The fractionation by differential solubility separates legume proteins in albumins, globulins, prolamins, and glutelins: globulins are predominant, whereas prolamins and glutelins are very scarce or absent. Legume globulins, which function mostly as storage-proteins, to be mobilized during the course of germination, can be further separated by ultracentrifugation or chromatography into the following two major components: legumins (11S) and vicilins (7S), and some additional other minor fractions. The relative abundance of these legume fractions depends on the species (Arnoldi et al., 2015).

Lentil (*Lens culinaris* Medik.; *Fabaceae*) is one of the most important food legumes worldwide. It contributes to reduce hunger and malnutrition especially with low-income people. Its grains are largely consumed as staple food especially in developing countries and as vegetarian meal and in salads in many parts of the world (Carbonaro et al., 2015; Grusak, 2009). Increased production and consumption of this mineral-rich food could reduce mineral malnutrition affecting more than half of the world's population (Mayer et al., 2008; Shahzad et al., 2014; White et al., 2009). In the Mediterranean region local farmers have repeatedly selected landraces and local cultivars for adaptation to biotic and abiotic stress conditions over a long period of time. A wide diversity of agro-environments (highlands, drylands, more favourable areas) occurs, thanks to the diversity of climatic and edaphic conditions. Lentils collected from these different regions most probably have high molecular diversity and different responses to abiotic and biotic stresses as a result of reproductive isolation and evolutionary differences among populations (Idrissi et al., 2016, 2015). Lentils are also widely cultivated in the Middle East, North Africa, Ethiopia, the Indian subcontinent, North America and Australia (Bhatty, 1988; Coyne and McGee, 2013; Erskine et al., 1990; Ferguson and Erskine, 2001; Idrissi et al., 2018; Sarker et al., 2002; Yadav et al., 2007).

Lentil is high in fibre and low in fat. Brummer et al. (2015) showed that lentil is richer in total soluble fibre than peas and chickpeas. Also, its content of dietary fibre is higher than beans and chickpeas. Most of these are storage proteins located in the cotyledon, containing a low percentage of sulphur-containing amino acids. Lentil proteins are

comprised of around 16% albumins, 70% globulins, 11% glutelins and 3% prolamins (Boye et al., 2010).

Among the Sicilian populations of lentil there is a good variability, due to some nutritional characteristics, such as protein, fiber, total sugars and potassium content; and many populations have a protein content more than 27% (Melilli et al., 2011).

Lentils have cholesterol- and lipid-lowering effects in humans, along with reducing the incidence of colon cancer and type-2 diabetes (Roy et al., 2010). In addition, lentils contain high quantities of of phenolic compounds (6.56 mg gallic acid equivalents $g^{-1}$), flavonoid (1.30 mg catechin equivalents $g^{-1}$) and condensed tannin (5.97 mg catechin equivalents $g^{-1}$). In vitro systems such as DPPH, ABTS, FRAP, ORAC methods showed that pulses with the highest total phenolic content, such as lentil, may exert the highest antioxidant capacity (Campos-Vega et al., 2010; Jamdar et al., 2017; Yeo and Shahidi, 2017). Xu et al. (2007) studied the antioxidant activities of the hydrophilic extracts from nine selected legumes based on copper-induced human LDL oxidation model in vitro and found that the extracts of lentils had significant ($P < 0.05$) longer LDL oxidation lag times (124.2 min) than the LDL control group (94.9 min). Moreover, lentils had higher antioxidant capacities than other pulses such as peas, chickpea and soybeans in both LDL-conjugated dienes assay and LDL TBARS assay. According Garcìa-Mora et al. (2017) the presence of certain type of peptides produced by gastrointestinal digestion of LP (Lentil protein) results in dual antioxidant and angiotensin I-converting enzyme (ACE) inhibitory activities. (Jarpa-Parra, 2018).

Boualga et al. (2009) studied the effects of various dietary purified legumes proteins compared to casein on plasma TG level, VLDL concentration and composition. Moreover, lipoprotein lipase (LPL) activity in epididymal fat, gastrocnemius and heart was investigated to evaluate in these tissues their capacity to release free fatty acids from their TG substrate and the liver capacity to stock the TG. Weaning male Wistar rats were fed ad libitum one of the following diets: 200 g/kg diet of purified proteins of lentil (L), or chickpea (CP) or casein (CAS). At day 28, VLDL were isolated from plasma sample by a single ultracentrifugation flotation. Hepatic lipase and LPL activity in epididymal fat, gastrocnemius and heart were measured by using glycerol tri [9–10(n)-$^{3}$H] oleate emulsion as substrate. Compared with CAS diet, the CP and L protein diets exhibited similar cholesterolemia, but lower triglyceridemia (1.9-fold and 2.5-fold) and VLDL particle number, as measured by their reduced contents of TG and apolipoproteins. CP and L protein diets reduced liver TG and cholesterol by 31 and 45%, respectively

compared to CAS diet. Furthermore, LPL activity in adipose tissue of rats fed CP or L was 1.6-fold lower than that of rats fed CAS. There was no significant difference in heart and gastrocnemius LPL activities with the three proteins. In contrast, hepatic lipase activity was higher in rats fed CP and L diets. So the low food efficiency ratio of purified CP and L proteins related to CAS is associated with decreased plasma VLDL and adipose tissue LPL activity. The low liver TG concomitant with reduced TG and apolipoproteins contents of VLDL confirm that hypotriglyceridemia is essentially due to impaired synthesis, exportation and transport of TG by VLDL which prevent lipid storage in adipose tissue.

## 1.4 CLADODES OF *OPUNTIA FICUS-INDICA*

The cactus *Opuntia* (genus *Opuntia*, subfamily Opuntioideae, family Cactaceae) is a xerophyte with about 200–300 species and is mainly growing in arid (less than 250 mm annual precipitation) and semi-arid (250–450 mm annual precipitation) zones. *Opuntia* plants has a high ecological adaptivity and can therefore be encountered in places of virtually all climatic conditions: North, Central, and South America, the Mediterranean, North, Central, and South Africa, the Middle East, Australia, and also in India (Mohamed-Yasseen et al., 1996; Nobel, 1995). Commercial cultivation is carried out in Italy, Spain, Mexico, Brazil, Chile, Argentina, and California (Inglese et al., 2002). Cactus plants serve as sources for fruits and vegetables, for medicinal and cosmetic purposes, as forage, building material, and as a source for natural colours (Cruse, 1973; Hamdi, 1997; Lòpez, 1995; Mohamed-Yasseen et al., 1996; Vigueras and Portillo, 2001). *Opuntia* spp. is gaining even more importance as an effective food production system including both the vegetative but also the fruit parts, especially in the light of global desertification and declining water resources.

The modified stems, known as cladodes, have a white medullar parenchyma, called core tissue and the chlorophyll containing photosynthetically active parenchyma, called cortex tissue. The latter is covered with spines (modified leaves) and multicellular hairs or trichomes, both forming the so-called areole, which is characteristic of members from the Cactaceae family. .(Stintzing and Carle, 2005) The cladodes are usually consumed after a removing the spines, washing, cutting and decoction (Ramírez-Moreno et al., 2013). They are used in many varieties of salad (after being cut in small cubes and immerged in vinegar), as flour quality enhancers (Kim et al., 2012; Ramírez-Moreno et al., 2015), or consumed as dehydrated foods (Chahdoura et al., 2015; Medina-Torres et al., 2008)

The Opuntia cladodes and fruits are source of a varied number of nutritional compounds. In 100 g dry matter of de-barbed cladodes, (Malainine et al., 2003) found 19.6 g ash, 7.2 g lipids and waxes, 3.6 g lignin, 21.6 g cellulose, and 48 g other polysaccharides, while crude proteins were not assessed. Other authors (Batista et al., 2003; Mizrahi et al., 1997; Mohamed-Yasseen et al., 1996; Pimienta-Barrios, 1993; Retamal et al., 1987a; Rodriguez-Felix and Cantwell, 1988) reported 64–71 g carbohydrates, 18 g fibers, 19–23.5 g ash, 1–4 g lipids, and 4–10 g proteins, the latter consisting. On a fresh weight basis, these values translate into 3–7 g carbohydrates, 1–2 g minerals, 0.5–1 g proteins, 0.2 g lipids, and 1 g fibrous substances per 100 g plant material (Retamal et al., 1987b;

Rodriguez-Felix and Cantwell, 1988; Stintzing and Carle, 2005). Their concentrations being dependent both on the cultivation site, climate and respective fruit variety (Osorio-Esquivel et al., 2012; Osuna-martínez et al., 2014; Park et al., 2010). Cladodes can also be considered a rich source of bioactive and functional compounds, like phenols compounds. In this regard, in recent years, the scientific world has paid particular attention to polyphenols as they have shown antioxidant properties in vitro, together with protective effects against cancer, and the ability to cure and prevent cardiovascular disorders, inflammatory and allergic disease (Del Socorro Santos Diáz et al., 2017; Rocchetti et al., 2018).

The major water soluble polysaccharides extracted from *Opuntia ficus indica* cladodes consists of pectins (Cárdenas et al., 2008) of which the main pectin component is a central linear backbone chain composed of α-D-galacturonic acid units linked by (1→4) glycosidic bonds. Neutral sugars, rhamnose, arabinose, galactose, xylose and glucose are usually present in about 5-10 wt% of galacturonic acid (Lefsih et al., 2016).

The benefits associated with fiber content are well known, especially for the prevention of illnesses such as diabetes, treatment of gastrointestinal disorders, illnesses associated with low dietary fiber intake, reduction of glucose values in the blood, anti-hyperlipidemic and anti-hypercholesterolemic effects (Osuna-martínez et al., 2014).

In general, a prolonged period of satiety was registered after cladode consumption. Fernandez et al. (1994, 1990) highlighted that the reduction of blood lipids triggered by isolated pectin from Opuntia was due to the enhanced binding of bile acid. It was concluded that through reduced bile absorption in the colon the enterohepatic circle was disrupted. In a follow up study, the same authors presented evidence that the low density lipoprotein (LDL)-catabolism was considered to be more important than the modulation and de novo synthesis in the liver. The same pectic-like substances were held responsible for a decreased lipid absorption, lower blood lipid levels, and finally weight reduction (Shapiro and Gong, 2002).

Wolfram et al. (2002) reported a reduction of total cholesterol, LDL, apolipoprotein levels, triglycerides, fibrinogen, blood glucose, insulin and urate, while body weight, high-density lipoprotein (HDL)-cholesterol, apolipoprotein A-1 and lipoprotein A levels were found to remain unchanged. The pulp pectin of cladodes seem to have an anti-hyperlipidemic effects, which both reduced lipid absorption and increased fecal sterol excretion, thus disrupting the enterohepatic circle. Since the level of 3-hydroxy- 3-methyl-glutaryl-coenzyme A, the key enzyme of cholesterol biosynthesis, did not exhibit

any activity changes, the reduced LDL levels and modified LDL composition were ascribed to an enhanced hepatic apo-B/Ereceptor (Palumbo et al., 2003).
The exact mechanisms, however, still need to be elucidated.

## 1.5 PURSLANE (*PORTULACA OLERACEA)*

Common purslane (*Portulaca oleracea* L.), member of the *Portulacaceae*, consists of more than 120 species of often succulent herbs and shrubs. It is fast growing, self-compatible and produces large numbers of seeds that have a long viability. Purslane has a long history of use for human food, animal feed and medicinal purposes (Liu et al., 2000). *P. oleracea* flourishes in numerous biogeographical locations worldwide and is highly adaptable to many drought, saline and nutrient-deficient conditions. These characteristics give purslane competitive advantage over many other cultivated crops. The purslane has already been proved as more salt-tolerant than any other vegetable crop. One important finding is that purslane can produce enough biomass under moderate salinity stress which other vegetable crops cannot (Amirul Alam et al., 2015). Purslane is termed as "Global Panacea" and it is in the list of World Health Organization as one of the most used medicinal plants (Dweck, 2001; Samy et al., 2004). The mixture of phytochemicals present in many of these plants contributes to their protective and health effects.(Shanker and Debnath, 2015) Purslane has an high content of flavonoids, alkaloids, organic acids, vitamins, minerals, sterols, and essential fatty acids (Gong et al., 2009; Jin et al., 2016). Several therapeutic effects have been attributed to purslane, such as antiseptic, antispasmodic, diuretic, antibacterial, wound-healing, analgesic, anti-inflammatory, skeletal muscle relaxant, bronchodilator, antipyretic and antiasthmatic activities (Iranshahy et al., 2017; Masoodi et al., 2011; Zhou et al., 2015). Furthermore, in the past was used for the treatment of various ailments such as fever, diarrhea, and skin, liver, kidney, and spleen diseases (Shanker and Debnath, 2015; Sultana and Rahman, 2013). Purslane has antihyperglycemic and antihyperlipidemic properties (Hadi et al., 2018; Lan and Fu-er, 2003; Movahedian et al., 2007). In particular, purslane has a high percentage of α-linolenic acid (LNA) (Ezekwe et al., 1999; Simopoulos et al., 1992) an omega-3 fatty acid, that is an essential fatty acid because it cannot be synthesised by humans but, has to be ingested. It plays an important role in human growth, development and disease prevention. It is the precursor of the longer-chain omega-3 fatty acids, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). The ability of LNA to convey these health benefits is limited by its poorer conversion to EPA and DHA in humans. The existence of longer-chain omega-3 polyunsaturated fatty acids in purslane have attracted considerable interest in this plant as an alternative source of these nutrients for human consumption (Liu et al., 2000).

Catap et al. (2018) determined the capacity of this plant species to modulate nonspecific immune responses and to confirm its antispasmodic activity in vivo in ICR mice: the ethyl acetate (EA) fraction of *P. oleracea* leaf extract ameliorated the nonspecific immune response of mice which were immunosuppressed through the use of cyclophosphamide. Moreover, the present study showed that the three (EtOH, HEX and EA) solvent fractions of this plant species have potentially effective antispasmodic components. These activities could possibly be attributed to some active compounds present in the *P. oleracea* leaf fractions, which also contributed to its high antioxidant property.

Hadi et al. (2018) in their systematic review and meta-analysis suggested the efficacy of purslane in improving lipid parameters and glucose levels. This result indicated that purslane supplementation has promising implications for improving glycemic status and blood lipid concentrations, especially in diabetic subjects, which have abnormal glucose and lipid metabolism.

Zidan et al. (2014) determined the effects of *Portulaca oleracea* (Po) lyophilized aqueous extract on the serum high-density lipoproteins ($HDL_2$ and $HDL_3$) amounts and composition, as well as on lecithin: cholesterol acyltansferase (LCAT) activity. Wistar rats (n = 12) were fed on 1% cholesterol-enriched diet for 10 days. After this phase, hypercholesterolemic rats (HC) were divided into two groups fed the same diet supplemented or not with *Portulaca oleracea* (Po-HC) (0.5%) for four weeks. Serum total cholesterol (TC) and triacylglycerols (TG), and liver TG values were respectively 1.6-,1.8-, and 1.6-fold lower in Po-HC than in HC group. Cholesterol concentrations in LDL-$HDL_1$, $HDL_2$, and $HDL_3$ were respectively 1.8, 1.4-, and 2.4-fold decreased in Po-HC group. $HDL_2$ and $HDL_3$ amounts, which were the sum of apolipoproteins (apos), TG, cholesteryl esters (CE), unesterified cholesterol (UC), and phospholipids (PL) contents, were respectively 4.5-fold higher and 1.2-fold lower with Po treatment. Indeed, enhanced LCAT activity (1.2-fold), its cofactor-activator apo A-I (2-fold) and its reaction product $HDL_2$-CE (2.1-fold) were observed, whereas $HDL_3$-PL (enzyme substrate) and $HDL_3$-UC (acyl group acceptor) were 1.2- and 2.4-fold lower. Therefore, *Portulaca oleracea* reduces triglyceridemia, cholesterolemia, and improves reverse cholesterol transport in rat fed enriched-cholesterol diet, contributing to anti-atherogenic effects.

## 1.6 OAT AND B-GLUCANS

Oat (*Avena sativa* L.) are an important crop worldwide with a global production of about 21 million tons per year. Oat have many health-promoting components, such as dietary fibres, proteins and minerals (Butt et al., 2008).The health claims approved both by FDA (FDA, 2003, 1997) and by EFSA (EFSA, 2011a, 2011b) and the health benefits linked to oat have increased consumer awareness of this cereal. The European Union allow food producers to market products containing 1 g β-glucan/portion with claims to reduce blood cholesterol concentrations and to attenuate post-prandial glyceamic response (EFSA, 2011a). From the consumer's point of view, it can be challenging to obtain the recommended intake of β-glucan (at least 3 g/day). The hull is the outmost layer of oat kernel and it is mainly composed of cellulose and hemicellulose (Welch, 1995; Welch et al., 1983), with lesser amounts of lignin and phenolic compounds (Emmons and Peterson, 1999). Oat bran is a technical term for a milling fraction containing the outer parts of oat kernel. According to AACCI (Anonymous, 1989), it has a total β-glucan content of at least 5.5% (d.m.) and a total dietary fibre content at least 16.0% (d.m.), such that at least one-third of the total dietary fibre is soluble fibre. The high-quality oat bran should contain at least 18–20% dietary fibre (of which 8–10% soluble) and 6–8% β-glucan, and thus the bran yield is limited to around 30–40% of the starting material (Ganssmann and Vorwerck, 1995). The layers of oat bran (starting from the outer surface) are pericarp, testa (seed coat), nucellum, aleurone, subaleurone and starchy endosperm (Miller and Fulcher, 2011). Starchy endosperm is the largest tissue in oat grains; starch is the major single component in oat endosperm as well as in whole groats The second most abundant component in oats is protein (Bechtel and Pomeranz, 1981). The endosperm cell walls are rich in β-glucan, with smaller amounts of arabinoxylan, cellulose, and glucomannan (Miller et al., 1995; Miller and Fulcher, 1995).

Oat β-glucan, the main soluble fiber in oats, seem to be the main active component responsible for their cholesterol-lowering effect. Health claims regarding the association between cholesterol lowering and soluble fiber from oat products/Oat β-glucan have been approved by food standards agencies in Europe but also worldwide. These approvals are based on a diet containing at least 3 g Oat β-glucan/d (Whitehead et al., 2014). β-glucans are linear polysaccharides which can be viewed as a cellulose chain (≈70% 4-O-linked β-Dglucopyranosyl units) interrupted by 3-O-linked β-Dglucopyranosyl units (≈30%). The

(1-3) linkages occur singly, leading to a structure of predominantly β-(1-3)- linked cellotriosyl and cellotetraosyl units (Wood, 2007).

The United States Food and Drug Administration (FDA) approved a health claim for β-glucan soluble fiber from oats for reducing plasma cholesterol levels and risk of heart disease in 1997. Similarly, in 2004 the United Kingdom Joint Health Claims Initiative (JHCI) allowed a cholesterol-lowering health claim for oat β-glucan. Othman et al. (2011) Investigate if results from more studies are consistent with the original conclusions reached by the FDA and JHCI. Results of this analysis showed that studies support the suggestion that intake of oat β-glucan at daily doses of at least 3 g may reduce plasma total and low-density lipoprotein (LDL) cholesterol levels by 5–10% in normocholesterolemic or hypercholesterolemic subjects. Studies described herein have shown that, on average, oat consumption is associated with 5% and 7% reductions in total and LDL cholesterol levels, respectively. Significant scientific agreement continues to support a relationship between oat β-glucan and blood cholesterol levels, with newer data being consistent with earlier conclusions made by the FDA and JHCI. Whitehead et al. (2014) performed a meta-analysis on 28 published randomized controlled trials (RCTs) comparing ≥3 g of oat β-glucan (OBG) /d with an appropriate control. Was showed that adding ≥3 g OBG/d to the diet reduces LDL and total cholesterol by 0.25 mmol/L and 0.30 mmol/L, respectively, without changing HDL cholesterol or triglycerides.

Different are the proposed mechanisms of action by which oat β-glucan may lower blood cholesterol levels. The main effect of oat soluble fiber on cholesterol metabolism in humans may involve its ability to form a viscous layer at the absorption surface in the small intestine. The increased viscosity decreases intestinal uptake of dietary cholesterol and reabsorption of bile acids (Erkkila and Lichtenstein, 2006; Lund et al., 1989). Inhibition of bile acid reabsorption can therefore increase the synthesis of bile acids from cholesterol and so reduce circulating LDL cholesterol levels (Butt et al., 2008). β-glucan may increase fecal excretion of bile acids (Andersson et al., 2010) and enhance hepatic conversion of cholesterol into bile acids (Bae et al., 2010; Park et al., 2009). Reduced hepatic bile acid levels activate cholesterol 7 a-hydroxylase, the enzyme involved in the synthesis of bile acid from cholesterol (Horton et al., 1994) This will reduce hepatic cell cholesterol content and this upregulates LDL receptor synthesis and enhances plasma LDL cholesterol removal. Decreased intracellular cholesterol content can also upregulate the hepatic synthesis of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol synthesis (Chen and Huang, 2009). Oat β-glucan may also

reduce uptake of jejunal and ileal long-chain fatty acids, and it downregulated the expression of genes involved in fatty acid synthesis and cholesterol metabolism (Drozdowski et al., 2010). However, in overweight subjects, consumption of oat β-glucan (3.8 g) has been shown to increase the release of appetite suppressors, such as cholecystokinin and subsequent decrease in meal intake by more than 400 kJ (Beck et al., 2009). Beverages containing 2.5 and 5 g of oat β-glucan increased satiety-related perceptions after 180 minutes of ingestion in healthy volunteers compared to the beverage without fiber (Lyly et al., 2010). The viscosity of oat β-glucan had a role in its ability to increase satiety (Lyly et al., 2009). Furthermore, the consumption of ready-to-eat oat cereal providing 3 g/day of oat β-glucan by obese adults as part of a dietary program for weight loss (~500 kcal/day) lowered total and LDL cholesterol by 5.4% and 8.7% (Maki et al., 2010; Othman et al., 2011).

# 2. MATERIALS AND METHODS

## GENERAL AIM OF THE WORK

The aim of the research was the development of new types pasta with the potentiality to decrease cholesterol. In this view, the project aimed at solving technological problems to obtain the new types of pasta, evaluating the effect of the molecules substitution on the rheological properties of the doughs, on the organoleptic properties of the obtained fresh and dry pasta and its nutraceutical properties.

To reach the aim of the research pasta samples were produced using durum wheat, added with typical wild species of purslane (*Portulaca oleracea* L.) rich in fatty acids, Sicilian populations of lentil (*Lens culinaris* Medik.) rich in essential amino acids, cladodes of prickly pear (*Opuntia ficus-indica* (L.) Mill) Sicilian populations rich in fiber and with oat flour rich in β-glucans.

## 2.1 TRIAL 1: CHARACTERISTICS OF PASTA WITH LENTIL ADDITION

### 2.1.1 Aim

The aim of this trial was to evaluate the characteristics of pasta samples produced with the use of commercial durum wheat added with commercial lentil and Sicilian lentil population of Ragusa.

In this trial three experiments have been performed:

***Experiment A***

Studied factors:

- Control (durum wheat spaghetti) ("**CTRL"**)
- Durum wheat flours + 40% (w/w) commercial lentil ("**pasta 1"**)
- Durum wheat flours + 40% (w/w) Sicilian population lentil of Ragusa ("**pasta 2"**)

***Experiment B***

Studied factors:

- Control (durum wheat spaghetti) ("**CTRL"**)
- Durum wheat flours + 40% (w/w) commercial lentil ("**pasta 1"**)
- Durum wheat flours + 40% (w/w) commercial lentil + 2% (w/w) of carboxymethylcellulose (CMC) ("**pasta 2"**)
- Durum wheat flours + 40% (w/w) commercial lentil + 2% (w/w) of flour guar seeds (GUAR) ("**pasta 3"**)

***Experiment C***

Studied factors:

- Control (durum wheat spaghetti) ("**CTRL"**)
- Durum wheat flours + 40% (w/w) commercial lentil ("**pasta 1"**)
- Durum wheat flours + 40% (w/w) Sicilian population lentil of Ragusa ("**pasta 2"**)
- 40% (w/w) commercial lentil + 2% (w/w) CMC ("**pasta 3"**)
- 40% (w/w) Sicilian population lentil of Ragusa + 2% (w/w) CMC ("**pasta 4"**)

### 2.1.2 Materials and methods

Commercial lentil and Sicilian population lentil of Ragusa were prepared, as described as follow. For pasta making, commercial lentil and Sicilian lentil of Ragusa were added at different concentration. Pasta without lentil was used as control ("CTRL"). On the obtained pasta samples were evaluated: colour (Minolta colorimeter CR, 400), sensorial qualities (Panel test), optimal cooking time (OCT) (minutes), cooking quality and amino acids content (GC-Ms).

*Preparation of plant material*

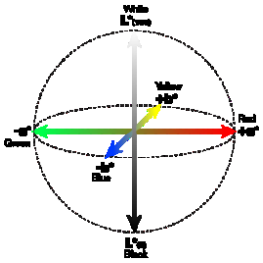
Commercial lentil, CV Easton from Canada was purchased in local market. Sicilian lentil, population Ragusa (RG) is part of germplasm bank of CNR ISAFOM Catania. Lentils, were washed with tap water, dried at low temperature in a termoventilated oven (30°C) and were milled by food processor (mod. IKA) in fine powdered and kept in ermetic bottle until use.

*Pasta making*

Pasta samples were produced using a pilot plant, with an extruder (60VR; Namad, Rome, Italy) for the production of the extruded pasta. Here, 2500 g flour (durum wheat + lentils at different concentrations) were mixed with tap water in a rotary-shaft mixer (Namad, Rome, Italy) at 25°C for 20 min, to obtain dough with 30% moisture content. A screw (length, 30 cm; diameter, 5.5 cm) that ended with a bronze die (hole diameter, 1.70 mm) was used to extrude the dough into a spaghetti shape. The screw speed was 50 rpm. The extrusion pressure was approximately 3.4 bar, and the temperature of the pasta after the extrusion was 27°C to 28°C. A small aliquot of the extruded pasta was pasteurised and used for a first sensory evaluation of fresh cooked pasta (20 g of every kind of fresh pasta was cooked in 400 mL of water for two minutes). The remaining pasta was dried in a dryer (SG600; Namad). In pasta with additives (Experiments B and C), CMC and GUAR were previously dissolved in the water used to hydrate the above formulation, to ensure their solubility.

**Flow sheet production of dry spaghetti**

*Pasta colour evaluation*



Colour was evaluated in dry spaghetti before and after cooking. Pasta 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 colours, 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.

*Sensorial Analysis of pasta*

Fresh and 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, odour, 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 colour and resistance to break of uncooked spaghetti. Elasticity, firmness, bulkiness, adhesiveness, fibrous nature, colour, odour and taste were evaluated for spaghetti (Padalino et al., 2013a). 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 on dry spaghetti in the laboratories of Foggia University. The optimal cooking time (OCT) was evaluated every 30 s during cooking by observing the time of disappearance of the core of the spaghetti by squeezing it between two transparent glass slides. The time at which the core completely disappeared was taken as the OC as described by AACC-approved method 66-50 (AACC, 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 (AACC, 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., 2013a). 2 cycles of pasta production for each type was performed, obtaining about 3 Kg of spaghetti.

***Determination of Cooking quality***

*Qualitative evaluation of amino acids*

A 0.2 g amount of dried samples was mixed with 200 µL of HCl (6 M) and placed in stove at 110°C for 24h. After the aqueous solution was evaporated to dryness under a gentle stream of nitrogen; 200 µL of $H_2O$ and 200 µL of chloroform were added and the aqueous solution was extracted, mixed with a solution ethanol-pyridine (4:1) and well shaked. Subsequently 10 µl of ethyl chloroformate and 50 µL of a solution with ethyl chloroformate at 1% in chloroform were added. In the end the reaction was neutralized with 30 µL of a saturated $NaHCO_3$ solution.

For the separation and analysis of the amino acids, Thermo Scientific DSQ II single quadrupole system in EI (Electron Ionization) mode, working in full scan was used. The capillary column used was a ZB-WAX (30 m x 0.25 mm i.d., film thickness 0.25 µm, (Phenomenex, Italy). The oven temperature was programmed column temperature started at 80°C , increased at 10 °C/min to 230 °C, hold time 3 min, a second gradient was applied to 250 °C at 20°C/min and held for 10 min under isothermal conditions. Helium was used as the carrier gas at a flow rate of 1 mL/min. A sample of 1µl was injected with a split ratio of 1:20. Mass spectroscopy conditions: The ion source temperature was 260°C, the MS transfer line temperature was 265°C and injector temperature was 250°C. Ionization voltage was 70eV and the mass range scanned was 35-550 m/z. Using Thermo Scientific Xcalibur Data system software for Windows peak areas were determined. Triplicate analyses were prepared for each dried sample.

*Data analyses*

Data were submitted to the Bartlett's test for the homogeneity of variance and then analysed 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

*Experiment A*

This first experiment was performed to study how commercial lentil and Sicilian lentil of Ragusa influence the quality of pasta. This pasta samples have been compared:

- Control (durum wheat spaghetti) ("**CTRL"**)
- Durum wheat flours + 40% (w/w) commercial lentils ("**pasta 1"**)
- Durum wheat flours + 40% (w/w) Sicilian lentils of Ragusa ("**pasta 2"**)

*Sensorial analysis*

The panel test results showed that there was no a significant difference between "pasta 1" and "2", so the two types of lentil influenced in the same way the sensorial characteristics of pasta samples. The two samples "pasta 1" and "pasta 2" have been less appreciated than the "CTRL". In figure 1.1 is possible to see the results of panel test in fresh and dry uncooked samples: The results about "pasta 1" (in yellow) and the results about "pasta 2" (in green) were almost coincident. In fresh uncooked samples the overall quality was 7.75 for the "CTRL", 6.33 for "pasta 1" and 6.25 for "pasta 2"; in dry uncooked samples the overall quality was 7.50 for the "CTRL", 5.95 for "pasta 1" and 6.02 for "pasta 2".


**A and B= Uncooked and cooked spaghetti obtained using durum wheat flour and 40% of commercial lentils. C and D= Uncooked and cooked spaghetti obtained using durum wheat flour and 40% of Sicilian lentils of Ragusa.**

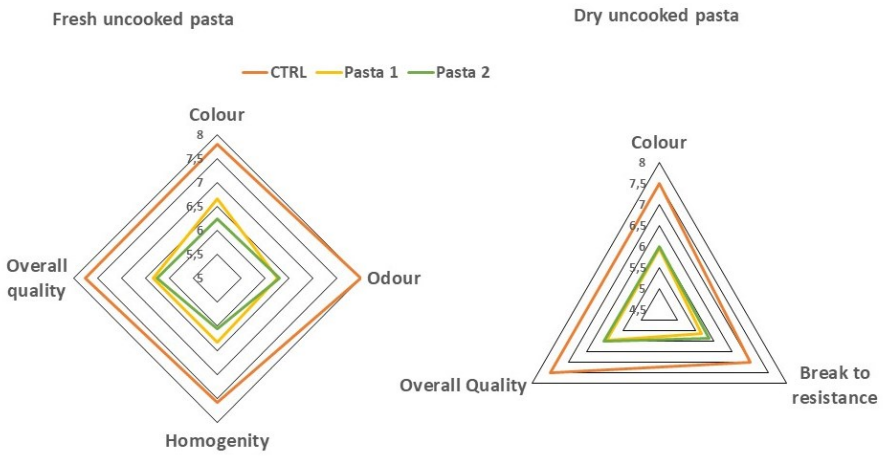



**Figure 1.1: Panel analyses of fresh and dry uncooked pasta obtained with durum wheat flour and lentils.**

The overall quality decreased further in cooked samples. The results of sensorial analysis of fresh cooked samples are reported in table 1.1: both “pasta 1” and “pasta 2” had a score of overall quality of 5 (threshold of acceptability) vs 7.50 of the “CTRL”. The scores of colour, odour and taste in “pasta 1” and “pasta 2” ranged from 6 to 6.75, while the scores of elasticity, firmness, bulkiness and adhesiveness ranged from 5 to 5.65. The table 1.2 shows the results of sensorial analysis in dry cooked samples: also in this case “pasta 1” and “pasta 2” have had the same trend, but the score of overall quality in “pasta 1” (4.95) was lower than that of “pasta 2” (5.25) and slightly below the acceptability threshold.

**Table 1.1: Panel analyses of fresh cooked pasta obtained with durum wheat flours and lentils. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Elasticity | Firmness | Bulkiness | Adhesiveness | Colour | Odour | Taste | Overall Quality |
|---|---|---|---|---|---|---|---|---|
| **CTRL** | 7.50 a | 7.25 a | 7.00 a | 6.75 a | 8.00 a | 8.00 a | 7.25 a | 7.50 a |
| **1** | 5.00 b | 5.25 b | 5.65 b | 5.00 b | 6.00 b | 6.50 b | 6.00 b | 5.00 b |
| **2** | 5.50 b | 5.00 b | 5.25 b | 5.00 b | 6.50 b | 6.75 b | 6.50 b | 5.00 b |
| **Means** | **6.00** | **5.83** | **5.97** | **5.58** | **6.83** | **7.08** | **6.58** | **5.83** |

**Table 1.2: Panel analyses of dry cooked pasta obtained with durum wheat flours and lentils. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Elasticity | Firmness | Bulkiness | Adhesiveness | Colour | Odour | Taste | Overall Quality |
|---|---|---|---|---|---|---|---|---|
| **CTRL** | 7.25 a | 7.00 a | 6.75 a | 6.25 a | 7.50 a | 8.00 a | 7.25 a | 6.87 a |
| **1** | 5.00 b | 5.00 b | 5.25 b | 4.95 b | 6.00 b | 6.50 b | 6.00 b | 4.95 b |
| **2** | 5.25 b | 5.00 b | 5.05 b | 4.95 b | 6.25 b | 6.75 b | 6.50 b | 5.25 b |
| **Means** | **5.83** | **5.67** | **5.68** | **5.38** | **6.58** | **7.08** | **6.58** | **5.69** |

*Pasta colour evaluation*

Unlike what happens for sensory analysis, for the evaluation of colour the pasta samples "1" and "2" did not have the same trend. This because the grain of the commercial lentil presented indices L* lower than the grains of "Ragusa" lentil and so pasta samples enriched with commercial lentil had indices L* lower vs pasta samples enriched with "Ragusa" lentil (table 1.3): L* was 24.75 (uncooked) and 42.38 (cooked) in spaghetti

with commercial lentil, while in spaghetti with Sicilian lentil of Ragusa was 41.24 (uncooked) and 52.30 (cooked).

**Table 1.3: L*, a*, b*in uncooked and cooked pasta obtained with durum wheat flour and lentil. Different letters within the same column indicate statistical differences at P<0.05**

| | Uncooked pasta | | | Cooked pasta | | |
|---|---|---|---|---|---|---|
| **Pasta** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **CTRL** | 60.04 a | -0.52 c | 24.81 b | 61.15 a | -4.06 c | 17.69 a |
| **1** | 24.75 c | 3.73 b | 6.81 c | 42.38 c | 1.85 a | 8.60 c |
| **2** | 41.24 b | 6.52 a | 28.46 a | 52.30 b | 1.55 b | 15.11 b |
| **Means** | **42.01** | **3.24** | **20.03** | **51.94** | **-0,22** | **13,8** |

***Experiment B***

This experiment was performed to improve the characteristics of pasta samples enriched with lentil using additives. In particular, it is needed to understand the best food additives between CMC and Guar in spaghetti enriched in lentil. To reach this aim in this experiment the following pasta samples have been compared:

- Control (durum wheat spaghetti) ("**CTRL"**)
- Durum wheat flours + 40% (w/w) commercial lentil ("**pasta 1"**)
- Durum wheat flours + 40% (w/w) commercial lentil + 2% (w/w) of CMC ("**pasta 2"**)
- Durum wheat flours + 40% (w/w) commercial lentil + 2% (w/w) GUAR ("**pasta 3"**)

*Sensorial Analysis of pasta*

In uncooked pasta samples (fresh and dry) sensory analysis showed a significant difference among "CTRL" and the pasta samples enriched with lentil, while enriched spaghetti did not show difference among them. In fresh uncooked spaghetti the overall quality scores gave satisfactory results with values above 6 for all enriched pasta samples, even if the "CTRL" resulted the most appreciated by panelists (7.75) (table 1.4). In dry uncooked pasta the overall quality scores followed the same behavior of the fresh

uncooked samples (table 1.5). The Overall quality threshold resulted improved vs the data obtained in the first experiment, both for fresh and dry spaghetti.

**Table 1.4: Panel analyses of fresh uncooked pasta obtained with durum wheat flours and lentils. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Colour | Odour | Homogeneity | Overall quality |
|---|---|---|---|---|
| **CTRL** | 7.80 a | 8.00 a | 7.58 a | 7.75 a |
| **1** | 6.67 b | 6.25 b | 6.33 b | 6.33 b |
| **2** | 6.25 b | 6.25 b | 6.00 b | 6.25 b |
| **3** | 6.50 b | 6.25 b | 6.00 b | 6.45 b |
| **Means** | **6.80** | **6.69** | **6.48** | **6.70** |

**Table 1.5: Panel analyses of dry uncooked pasta obtained with durum wheat flours and lentils. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Colour | Break to resistance | Overall Quality |
|---|---|---|---|
| **CTRL** | 7.50 a | 7.00 a | 7.50 a |
| **1** | 5.95 b | 5.65 b | 5.95 b |
| **2** | 6.00 b | 6.00 b | 6.00 b |
| **3** | 6.25 b | 6.00 b | 6.25 b |
| **Means** | **6.43** | **6.16** | **6.43** |

In cooked pasta (fresh and dry) sensory analysis showed that the use of CMC improved significantly all the characteristics of the pasta samples compared with those without additive;

The values of parameters for pasta samples enriched with lentil and CMC, although statistically different from the absolute “CTRL”, were widely acceptable; the overall quality was 6.50 for fresh cooked pasta with CMC (“pasta 2”) vs 5.00 (“pasta 1”) and 5.65 (“pasta 3”), how reported in table 1.6. While in dry cooked “pasta 2” the overall quality was 6.25 vs 4.95 (“pasta 1”) and 5.25 (“pasta 3”) (table 1.7).

In all three types of pasta enriched in lentils, the colour, odour and flavour did not vary, while additives had a positive influence on all the other parameters under study.

**Table 1.6: Panel analyses of fresh cooked pasta obtained with durum wheat flours and lentils. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Elasticity | Firmness | Bulkiness | Adhesiveness | Colour | Odour | Taste | Overall Quality |
|---|---|---|---|---|---|---|---|---|
| **CTRL** | 7.50 a | 7.25 a | 7.00 a | 6.75 a | 8.00 a | 8.00 a | 7.25 a | 7.50 a |
| **1** | 5.00 c | 5.25 d | 5.65 c | 5.00 d | 6.00 b | 6.50 b | 6.00 b | 5.00 d |
| **2** | 6.25 b | 6.59 b | 6.50 b | 6.00 b | 6.25 b | 7.00 b | 6.50 b | 6.50 b |
| **3** | 6.00 b | 6.00 c | 6.00 c | 5.50 c | 6.00 b | 7.00 b | 6.50 b | 5.65 c |
| **Means** | **6.19** | **6.27** | **6.29** | **5.81** | **6.56** | **7.13** | **6.56** | **6.16** |

**Table 1.7: Panel analyses of dry cooked pasta obtained with durum wheat flours and lentils. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Elasticity | Firmness | Bulkiness | Adhesiveness | Colour | Odour | Taste | Overall Quality |
|---|---|---|---|---|---|---|---|---|
| **CTRL** | 7.25 a | 7.00 a | 6.75 a | 6.25 a | 7.50 a | 8.00 a | 7.25 a | 6.87 a |
| **1** | 5.00 c | 5.00 c | 5.25 c | 4.95 b | 6.00 b | 6.50 b | 6.00 b | 4.95 b |
| **2** | 6.00 b | 6.25 b | 6.20 b | 5.95 a | 6.38 b | 7.00 b | 6.50 b | 6.25 a |
| **3** | 6.00 b | 5.95 b | 6.00 b | 5.25 b | 5.95 b | 7.00 b | 6.40 b | 5.25 b |
| **Means** | **6.06** | **6.05** | **6.05** | **5.6** | **6.46** | **7.13** | **6.54** | **5.83** |

*Cooking quality evaluation*

The cooking quality evaluation (table 1.8) showed that the optimal cooking time (OCT) was lower in pasta without additives. The cooking loss increased adding the lentil ("pasta 1") vs "CTRL", but using CMC ("pasta 2") and GUAR ("pasta 3") it decreased; the swelling index did not change significantly, while water absorption was similar in "CTRL", "pasta 1" and "pasta 2" but decreased in pasta adding GUAR (pasta 3).

**Tab. 1.8: Cooking quality pasta obtained with durum wheat flours and lentils. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | OCT (min) | Cooking loss (%) | Swelling Index | Water Absorption (%) |
|---|---|---|---|---|
| **CTRL** | 9:30 a | 6.18 c | 1.80 a | 142 a |
| **1** | 8:30 c | 8.03 a | 1.96 a | 149 a |
| **2** | 9:00 b | 6.97 b | 1.77 a | 138 a |
| **3** | 9:00 b | 7.15 b | 1.74 a | 119 b |
| **Means** | **9:00** | **7.08** | **1.82** | **137** |

*Pasta colour evaluation*

The results of colour parameters were influenced by the addition of lentil flour, leading to the formation of products with low values of L*index, 60.04 ("CTRL") vs 25.87 (average of the three uncooked pasta). a* index increased adding lentil while b* decreased. L* remained constant in "CTRL" after cooking, while increased in "pasta 1", "2" and "3" (table 1.9), indicating the loss of tannins in water, normally present in lentil grains.

**Table 1.9: L*, a*, b*in uncooked and cooked pasta obtained with durum wheat flour and lentils. Different letters within the same column indicate statistical differences at P<0.05**

| | Uncooked pasta | | | Cooked pasta | | |
|---|---|---|---|---|---|---|
| **Pasta** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **CTRL** | 60.04 a | -0.52 c | 24.81 a | 61.15 a | -4.06 b | 17.69 a |
| **1** | 24.75 b | 3.73 a | 6.81 b | 42.38 b | 1.85 a | 8.60 b |
| **2** | 25.73 b | 3.22 b | 7.02 b | 44.98 b | 1.81 a | 9.38 b |
| **3** | 27.14 b | 3.12 b | 7.19 b | 42.64 b | 1.86 a | 8.71 b |
| **Means** | **34.42** | **2.39** | **11.46** | **47.79** | **0.37** | **11.10** |

***Experiment C***

The experiment B highlighted that CMC is a good additive to improve the characteristics of spaghetti enriched in lentil. Therefore, the next step was to study how the two accessions of lentil and the CMC influence the sensorial characteristics of pasta. In this experiment the compared samples are as follows:

- Control (durum wheat spaghetti) (“**CTRL**”)
- Durum wheat flours + 40% (w/w) commercial lentil (“**pasta 1**”)
- Durum wheat flours + 40% (w/w) Sicilian lentils of Ragusa (“**pasta 2**”)
- Durum wheat flours + 40% (w/w) commercial lentil + 2% (w/w) CMC (“**pasta 3**”)
- Durum wheat flours + 40% (w/w) Sicilian lentils of Ragusa + 2% (w/w) CMC (“**pasta 4**”)

*Sensorial Analysis of pasta*

For fresh and dry pasta samples panel analyses showed that there was no significant change among spaghetti enriched with commercial lentil and spaghetti with lentil of Ragusa. Out of concentrations (0%, 40% and 40% + CMC), pasta with commercial lentil and pasta with lentil of Ragusa had a score of overall quality of 6.33 and 6.28 respectively in fresh cooked samples, and a score of 6.02 and 6.04 in dry cooked samples. The figure 1.3 shows that there was no difference in sensorial analysis between spaghetti with commercial lentil and spaghetti with lentil of Ragusa.

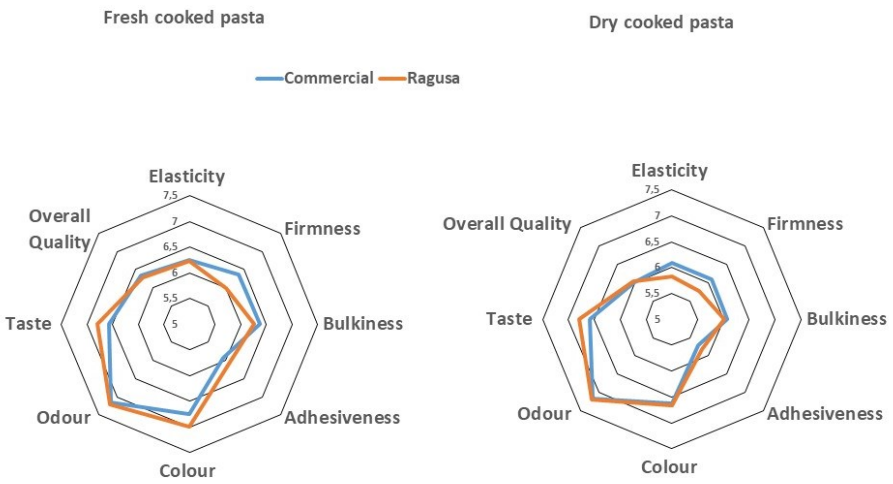



**Figure 1.3: Panel analyses of fresh and dry cooked pasta obtained with durum wheat flour and lentil. Values are averaged for type of lentil and CMC addition.**

The sensorial analysis on average of two accessions of lentil highlighted that the use of CMC did not improve the characteristics of uncooked spaghetti enriched in lentil vs

"CTRL", both in fresh and dry samples, but its positive effect was clear on cooked spaghetti. How reported in table 1.10, in uncooked fresh spaghetti the overall quality was 7.75 ("CTRL"), 6.29 (40% of lentil) and 6.25 (40% of lentil +CMC). The scores of colour, odour and homogeneity decreased with the adding of lentil.

**Table 1.10: Panel analyses of uncooked fresh pasta obtained with durum wheat flour and lentils on average of the two accessions of lentil. Different letters within the same column indicate statistical differences at P<0.05**

| Concentrations | Colour | Odour | Homogeneity | Overall quality |
|---|---|---|---|---|
| **0%** | 7.80 a | 8.00 a | 7.58 a | 7.75 a |
| **40%** | 6.46 b | 6.28 b | 6.19 b | 6.29 b |
| **40% +CMC** | 6.28 b | 6.25 b | 6.04 b | 6.25 b |
| **Means** | **6.84** | **6.84** | **6.61** | **6.76** |

In uncooked dry spaghetti the overall quality score ranged from 5.99 (40% of lentil) to 7.50 ("CTRL"), the colour score from 5.98 (40% of lentil) to 7.50 ("CTRL") and the break to resistance score from 5.75 (40% of lentil) to 7.00 ("CTRL") (table 1.11).

**Table 1.11: Panel analyses of uncooked dry pasta obtained with durum wheat flour and lentils on average of the two accessions of lentil. Different letters within the same column indicate statistical differences at P<0.05**

| Concentrations | Colour | Break to resistance | Overall quality |
|---|---|---|---|
| **0%** | 7.50 a | 7.00 a | 7.50 a |
| **40%** | 5.98 b | 5.75 b | 5.99 b |
| **40% +CMC** | 6.13 b | 6.04 b | 6.13 b |
| **Means** | **6.53** | **6.26** | **6.54** |

In table 1.12 are reported the results of panel test in fresh cooked spaghetti, on average of the two accessions of lentil: the overall quality score was 5.00 in samples with 40% of lentil, but it increased to 6.42 thanks to the use of CMC. CMC did not influence colour,

odour and taste but improved the other parameters (elasticity, firmness, bulkiness and adhesiveness).

**Table 1.12: Panel analyses of cooked fresh pasta obtained with durum wheat flour and lentils on average of the two accessions of lentil. Different letters within the same column indicate statistical differences at P<0.05**

| Concentrations | Elasticity | Firmness | Bulkiness | Adhesiveness | Colour | Odour | Taste | Overall quality |
|---|---|---|---|---|---|---|---|---|
| **0%** | 7.50 a | 7.25 a | 7.00 a | 6.75 a | 8.00 a | 8.00 a | 7.25 a | 7.50 a |
| **40%** | 5.25 c | 5.13 c | 5.45 c | 5.00 c | 6.25 b | 6.63 b | 6.25 b | 5.00 c |
| **40%+CMC** | 5.95 b | 6.17 b | 6.50 b | 6.25 b | 6.38 b | 6.93 b | 6.58 b | 6.42 b |
| **Means** | **6.23** | **6.18** | **6.32** | **6.00** | **6.88** | **7.18** | **6.69** | **6.31** |

The CMC acted in the same way in dry cooked samples: it did not influence colour, odour and taste but it improved elasticity, firmness, bulkiness, adhesiveness and accordingly the overall quality (table 1.13).

**Table 1.13: Panel analyses of cooked dry pasta obtained with durum wheat flour and lentils on average of the two accessions of lentil. Different letters within the same column indicate statistical differences at P<0.05**

| Concentrations | Elasticity | Firmness | Bulkiness | Adhesiveness | Colour | Odour | Taste | Overall quality |
|---|---|---|---|---|---|---|---|---|
| **0%** | 7.25 a | 7.00 a | 6.75 a | 6.25 a | 7.50 a | 8.00 a | 7.25 a | 6.87 a |
| **40%** | 5.12 c | 5.00 c | 5.15 c | 4.95 b | 6.13 b | 6.63 b | 6.25 b | 5.10 c |
| **40%+CMC** | 5.50 b | 5.77 b | 6.23 b | 6.10 a | 6.32 b | 6.93 b | 6.58 b | 6.13 b |
| **Means** | **5.96** | **5.92** | **6.04** | **5.77** | **6.65** | **7.18** | **6.69** | **6.03** |

*Cooking quality evaluation*

ANOVA showed that for all the traits describing the cooking quality evaluation, statistical differences have not been observed between the types of lentil used and the effect of CMC additive used is already reported in table 1.8.

*Pasta colour evaluation*

The grain of the "Ragusa" lentil presented indices L* higher than the grain of commercial lentil and so pasta samples enriched with this Sicilian lentil had indices L* higher vs pasta samples enriched with commercial lentil (tab. 1.14); L* was 36.84 (uncooked) and 49.50 (cooked) in spaghetti with commercial lentil, while in spaghetti with Sicilian lentil of Ragusa was 48.08 (uncooked) and 56.18 (cooked). Therefore, the colour was more appreciable by the consumer in pasta with "Ragusa" lentil.

**Table 1.14: L*, a*, b*in uncooked and cooked dry pasta enriched in lentils. Values are averaged for type of lentil and CMC addition. Different letters within the same column indicate statistical differences at P<0.05.**

| | UNCOOKED PASTA | | | COOKED PASTA | | |
|---|---|---|---|---|---|---|
| **Lentils** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **Commercial** | 36.84 b | 2.14 a | 12.88 a | 49.50 a | -0.13 a | 11.89 a |
| **Ragusa** | 48.08 a | 3.83 b | 27.34 b | 56.18 b | -0.70 b | 15.52 b |
| **Means** | **42.46** | **2.99** | **20.11** | **52.84** | **-0.42** | **13.70** |

On average of the types of lentils, obviously, as discussed above, L* decreased in pasta samples enriched in lentil vs the "CTRL", even if the brightness increased slightly using CMC (L*= 50.03) vs 40% (L*= 47.34) in cooked pasta (Table 1.15).

**Table 1.15: L*, a*, b*in uncooked and cooked dry pasta enriched in lentils on average of the two accessions of lentil. Different letters within the same column indicate statistical differences at P<0.05.**

| | UNCOOKED PASTA | | | COOKED PASTA | | |
|---|---|---|---|---|---|---|
| **Concentrations** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **0** | 60.04 a | -0.52 c | 24.81 a | 61.15 a | -4.06 c | 17.69 a |
| **40** | 33.00 b | 5.12 a | 17.64 b | 47.34 c | 1.70 a | 11.86 b |
| **40+CMC** | 34.34 b | 4.35 b | 17.88 b | 50.03 b | 1.11 b | 11.57 b |
| **Means** | **42.46** | **2.98** | **20.08** | **52.84** | **-0.42** | **13.71** |

*Qualitative evaluation of amino acids*

The total amounts of proteins detected in lentils grains resulted 26.6 g 100 $g^{-1}$ d.m. in commercial lentils cv Easton and 28.2 g 100 $g^{-1}$ d.m. in Sicilian population of Ragusa. In the figure 1.4 is reported the distribution of amino acids in the lentil flours used to enrich spaghetti. The distribution of the aa resulted similar in both the types of grains, expecially for essential amino acids (indicate with *). The grains of Sicilian population of Ragusa resulted more rich in Lys, and Glu, while the cv Easton, genetically improved, resulted more rich in Phe.

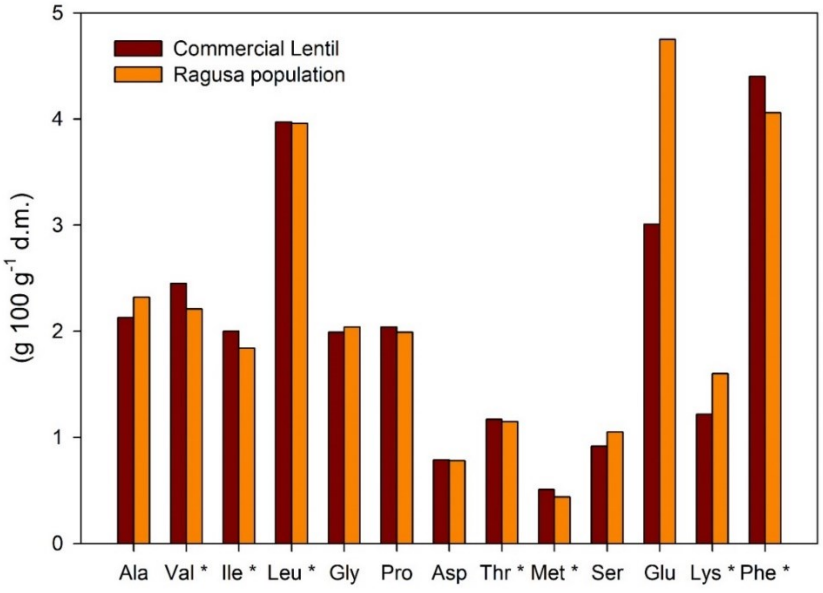



**Figure 1.4: Amino acids content in the two types of lentils. (*) indicates essential amino acids.**

The amino acids content in dry cooked spaghetti is reported in figure 1.5. Although the distribution of the amino acids reflected the type of lentil used, it is worthwhile to note that the amounts of essential amino acid is well maintained after the cooking process. All the amino acids detected in raw material, was recorded in higher concentration in enriched spaghetti, except for Pro, Glu Phe and Met, that are already present in cooked "CTRL". The addition of Ragusa lentil improved the contents in Lys (65%), Val (49%), Ile (60%), Gly (55%), Asp (59%), while the amounts in Leu, Thr, Ser and Lys resulted improved in spaghetti enriched by commercial lentil, whit values of 28%, 61%, 24% and 21%. On average of the types of lentil used, the use of CMC increased mainly the content of Lys (+ 41%) in Ala (+89%) vs the "CTRL"; while Pro, Met, Glu and Phe amino acids did not result affected by the additive (Figure 1.6).

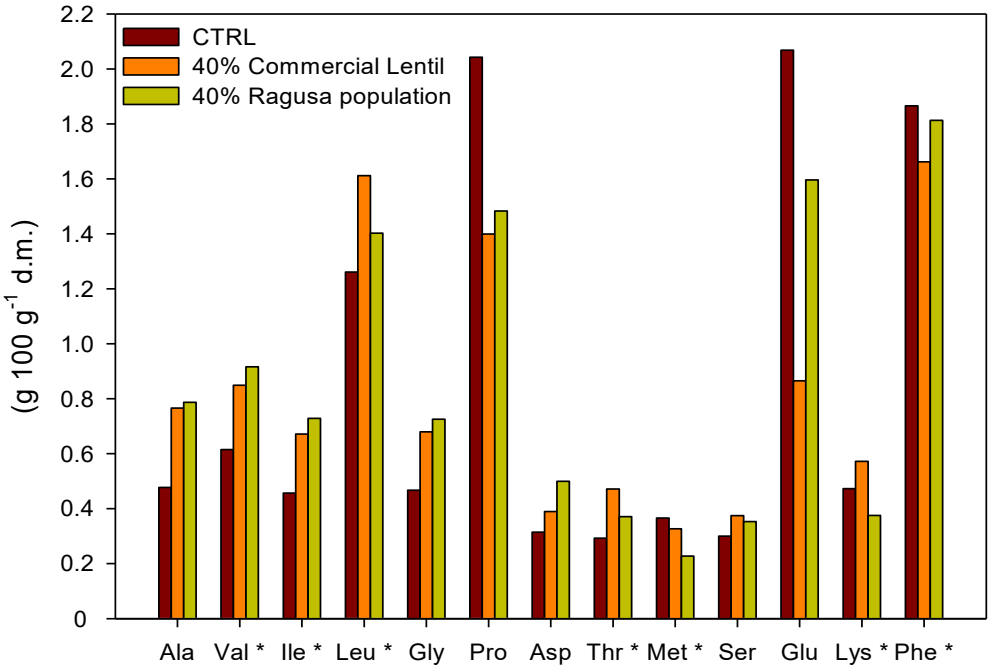



**Figure 1.5: Amino acids content (g 100 $g^{-1}$ d.m.) in the cooked dry spaghetti. (*) indicates essential amino acids.**

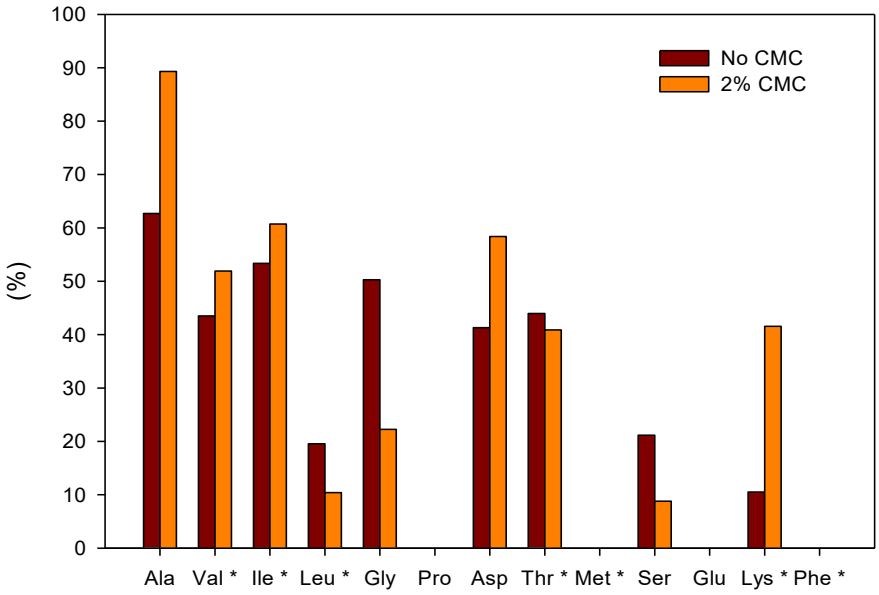



**Figure 1.6: Percentage of variation of amino acid content in the cooked dry spaghetti, on average of the two types of lentils vs the CTRL. The value of CTRL is considered zero. (*) indicates essential amino acids.**

### 2.1.4 Conclusions

Lentil flours modified dough rheological properties and pasta characteristics. The two types of lentil, commercial lentil and Sicilian population of Ragusa, influenced in the same way the sensorial characteristics of pasta samples. The two pasta samples enriched with 40% of lentil were less appreciated than the "CTRL" especially in dry samples and after cooking process: pasta with commercial lentil had a score of overall quality of 4.95 vs 5.25 (in pasta with Ragusa population) and vs 6.87 ("CTRL"). The commercial lentil presented indices L* lower than the grains of "Ragusa" lentil and so pasta samples enriched with commercial lentil had indices L* lower vs pasta samples enriched with "Ragusa" lentil. The experiment B allowed to understand that CMC is a good additive to improve the characteristics of spaghetti enriched in lentil: the values of parameters for pasta samples enriched with commercial lentil and CMC, although statistically different from the absolute "CTRL", were widely acceptable; the overall quality was 6.25 for dry cooked pasta with CMC vs 4.95 (dry cooked pasta enriched in commercial lentil without additive). The optimal cooking time (OCT) was lower in pasta without additives. The cooking loss increased adding the lentil but using CMC and GUAR it decreased; the

swelling index did not change significantly among pasta samples. Subsequently was studied how the two accessions of lentil and the CMC influence the sensorial characteristics of spaghetti: out of concentrations (0%, 40% and 40% + CMC), there was no significant change among spaghetti enriched with commercial lentil and spaghetti with lentil of Ragusa. While the sensorial analysis on average of two accessions of lentil highlighted that the use of CMC did not improve the characteristics of uncooked spaghetti enriched in lentil vs "CTRL", but its positive effect was clear on fresh and dry cooked spaghetti. In particular it did not influence colour, odour and taste but it improved elasticity, firmness, bulkiness, adhesiveness and the overall quality. The distribution of the amino acids resulted similar in both the types of grains, especially for essential amino acids. The amino acids content in dry cooked spaghetti reflected the type of lentil used and the amounts of essential amino acid have been well maintained after the cooking process. The addition of Ragusa lentil improved the contents in Lys, Val, Ile, Gly and Asp, while the amounts in Leu, Thr, Ser and Lys resulted improved in spaghetti enriched by commercial lentil. The use of CMC increased mainly the content of Lys (+41%) in Ala (+89%) vs the "CTRL. The enrichment of wheat flour with legume flours is an effective means to improve the nutritional quality of cereal-based foods: it is well known that the legumes' amino acidic composition is complementary to the one of cereals (Boye et al., 2010). Wójtowicz and Mościcki (2014) obtained similar results in cereal-legume precooked pasta, where the addition of pulses, among which lentil improved nutritional value of the pasta products according to protein, fat, ash and fiber level compare to common wheat precooked pasta. The author concluded that 30% of substitution is the good compromise for precooked pasta. Using CMC it was possible to reach 40% of substitution to obtain dry spaghetti. A recent clinical trial examining the effects of daily consumption of ½ cup of mixed pulses (dried beans, dried peas, chickpeas, lentils) on a cohort of individuals with peripheral artery disease, a form of atherosclerosis that manifests in the limbs, revealed that in only 8 weeks this dietary intervention increased blood flow to the legs (Padhi and Ramdath, 2017). Among all pulses data indicate that inclusion of lentils in the diet can positively alter specific physical and functional vascular parameters leading to increased compliance, even in the presence of established vascular disease. The ability to alter arterial elasticity provides a novel mechanism of action by which lentils exert their beneficial actions. Furthermore, these direct effects on the arterial wall likely explain the enhanced improvement seen with lentils compared to other pulses which only showed LDL-cholesterol lowering. A lentil rich diet could be a useful adjunct

for managing atherosclerotic disease in at risk individuals since pharmaceuticals with similar properties have not yet been developed (Zahradka et al., 2018). Considering the results obtained in this trial, using local populations of lentil until, 40% of substitution to produce fortified dry spaghetti, could increase the intake of this precious legume in an easy way thanks to its addition to regularly eaten products and, at the same time, promote and valorize the rich Sicilian germplasm.

## 2.2 TRIAL 2: CHARACTERISTICS OF PASTA WITH CLADODES OF *OPUNTIA FICUS-INDICA* ADDITION

### 2.2.1 Aim

The aim of this trial was to evaluate the characteristics of pasta samples produced with the use of ancient durum wheat "Senatore Cappelli' and commercial durum wheat added with cladodes flours of prickly pear. The addition of food additives was also tested, to improve the rheological characteristics. Before pasta production, the characteristics of cladodes of three prickly pear populations have been examined; in particular colour, total phenols content (TPC) and antioxidant activity of cladodes were evaluated.

In this trial four experiments have been performed:

***Experiment A***

Studied factors:

3 populations of *Opuntia ficus-indica* cladodes:

- Cladodes collected at Caltagirone, namely "Caltagirone population"
- Cladodes collected at Syracuse, namely "Siracusa population"
- Cladodes collected at Sortino, namely "Sortino population"

***Experiment B***

Studied factors:

- Control (durum wheat spaghetti) ("**CTRL"**)
- wholemeal durum wheat 'Senatore Cappelli'+ 5% (w/w) Sortino cladodes ("**pasta 1"**)
- wholemeal durum wheat 'Senatore Cappelli'+ 10% (w/w) Sortino cladodes ("**pasta 2"**)

***Experiment C***

Studied factors:

- Control (durum wheat spaghetti) ("**CTRL"**)
- Durum wheat flours + 5% (w/w) "Sortino" cladodes ("**pasta 1"**)
- Durum wheat flours + 10% (w/w) "Sortino" cladodes ("**pasta 2"**)

***Experiment D***

Studied factors:

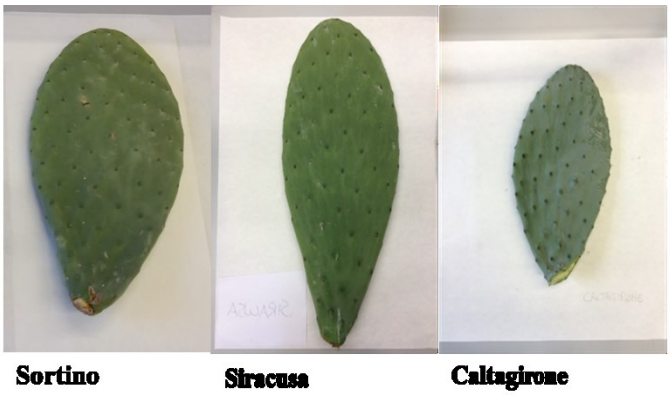
- Control (durum wheat spaghetti) ("**CTRL"**)
- Durum wheat flours + 10% (w/w) "Sortino" cladodes ("**pasta 1"**)
- Durum wheat flours + 10% (w/w) "Sortino" cladodes + 2% (w/w) CMC ("**pasta 2"**)
- Durum wheat flours + 10% (w/w) "Sortino" cladodes + 2% (w/w) AGAR ("**pasta 3"**)

### 2.2.2 Materials and methods

Cladodes were collected and prepared to be added. For pasta making wholemeal durum wheat "Senatore Cappelli" and commercial durum wheat were used and cladodes from Sortino were added at different concentrations. Pasta without cladodes was used as control ("CTRL"). On the pasta samples obtained were evaluated: colour (Minolta colorimeter CR, 400), sensorial qualities (Panel test), optimal cooking time (OCT) (minutes), cooking quality, total fibers content (*Weende* method), total phenols content (*Folin ciocalteu* method) and antioxidant activity (*DPPH* method).

*Preparation of plant material*

Cladodes were collected in three different sites of eastern Sicily: Caltagirone (608 m a.s.l., 37°11'07''N 14°13' 19'' W), Syracuse (17 m a.s.l., 37°3'52'' N 15°17'26'' W) and Sortino (438 m a.s.l, 37°9'24'' N 15°1'39''). Spine of cladodes were removed, after they were washed and dried in a thermoventilated oven at 60° C; the dried material was milled in fine powdered and kept in ermetic bottle until use.




**Cladodes collected in three different sites of Sicily**

*Pasta making*

Spaghetti were produced with wholemeal durum wheat "Senatore Cappelli" or commercial durum wheat by using the same operating conditions of trial 1. Cladodes of Sortino population prickly pear were added at two different concentrations (5% and 10% w/w). Pasta without cladodes was produced and used as control ("CTRL").

*Pasta and cladodes colour evaluation*

Dry pasta colour (before and after cooking) and cladodes colour were recorded with the use of a Minolta colorimeter CR, 400 as described in trial 1.

*Sensorial Analysis of pasta*

Dry spaghetti samples were submitted to the same panel of fifteen trained tasters as the trial 1, 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 (AACC, 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 (AACC, 2000). The swelling index of cooked pasta was determined according to the procedure desCardoonbed by Cleary and Brennan (2006).

*Fiber content*

The fiber were quantified in the "CTRL" and in all dry pasta samples enriched in cladodes, uncooked and cooked. The *Weende* method was used: 3 grams of every sample (dry and powdered) were boiled for 30 minutes in a solution of 225 mL of distilled water and 25 mL of $H_2SO_4$; after boiling the solution was filtered. The residue was washed, boiled in 250 mL of distilled water and filtered. The last washing step was repeated two times. After the residue was boiled in a solution of 200 mL of distilled water and 50 mL of KOH (50 g $L^{-1}$) and filtered using a calibrated filter. The filter with the residue was placed in the thermoventilated stove at 105°C, until the constant weight was reached.

The percentage of raw fiber was defined as:

$$\% \text{ Raw fiber} = \frac{(P1-P2)}{P} \cdot 100$$

P1= weight filter + residue from last filtration

P2= weight empty filter

P= weight sample

For every sample, the protocol was repeated three times.

*Total phenol content*

Total phenol content (TPC) was determinated in the cladodes, in the "CTRL" and in spaghetti samples with 5% and 10% of Cladodes (w/w), uncooked and cooked, using *Folin-Ciocalteu* method as reported by Singleton et al. (1998) with some modifications. One gram of fine powder was extracted with a solution MeOH:H2O (80:20), using a 1:10 dilution factor. Then the solution in ultrasonic bath for 40 minutes; the extracts were filtered and stored in a -20°C freezer. For the determination of TPC 625µL of Folin–Ciocalteu reagent (diluited 5 times) and 1.2 ml of Na2CO3 (7% w/v) solution were added to 125 µL of samples extract. Mixtures were shaken and left to stand at room temperature and in the dark for 1 hour before measuring absorbance at 760 nm using a spectrophotometer (Eppendorf Bio Spectrometer® basic). The TPC was expressed as mg gallic acid equivalent in kg dried samples ($mg_{GAE}$ $kg^{-1}$ d.m.). For every sample, the protocol was repeated three times.

*Antioxidant activity*

The antioxidant activity was determinated in the cladodes, uncooked and cooked pasta samples, using the *DPPH·* (2,2-diphenyl-1-picryhydrazyl) radical scavenging activity (Brand-Williams et al., 1995). One gram of sample was extracted with 4 mL of Methanol. The solutions were centrifuged. The ability of a compound to donate a hydrogen atom was assessed on the basis of the scavenging activity of the stable DPPH• radical. 3 mL of 0.06 mM DPPH• was added to 100 µL of extracts. A control was prepared by adding the same quantity of DPPH• to methanol. The contents of the tubes were mixed and allowed to stand for 20 min at 37°C and absorbance was measured at 515 nm. The results were expressed as $IC_{50}$ (mg $mL^{-1}$), the concentration required to cause 50% DPPH inhibition. For every sample, the protocol was repeated three times.

*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

***Experiment A***

3 populations of *Opuntia ficus-indica* cladodes:

- "Caltagirone population"
- "Siracusa population"
- "Sortino population"

*Colour evaluation*

The results about colour indices are reported in table 2.1. Cladodes of Caltagirone had the highest brightness index ("L*"), 55.32 vs 47.12 (cladodes of Siracusa) and vs 45.96 (cladodes of Sortino). Cladodes of Siracusa resulted greener than those of the other two populations.

**Table 2.1: L*, a*, b* in three different populations of cladodes. Different letters within the same column indicate statistical differences at P<0.05**

| Origin | L* | a* | b* |
|---|---|---|---|
| **Caltagirone** | 55.32 a | -8.05 a | 16.07 c |
| **Siracusa** | 47.12 b | -13.88 c | 25.60 a |
| **Sortino** | 45.96 b | -11.29 b | 23.59 b |
| **Means** | 49.47 | -11.07 | 21.75 |

*Total phenols content and antioxidant activity*

The TPC was measured in the dry powders in order to determine the final amount of total phenols in produced pasta.

Cladodes from Sortino and Siracusa showed the same TPC content while, it decreased in cladodes from Caltagirone. DPPH scavenging activity did not result significantly different among three populations. (Table 2.2).

Therefore, in general the origin of cladodes seems to influence and affect the colour and the content of phenols.

**Table 2.2: TPC ($mg_{GAE}$ $kg^{-1}$ d.m). and IC50, * in three different populations of cladodes. Different letters indicate differences at P< 0.05.**

| Origin | TPC | $IC_{50}$ |
|---|---|---|
| **Caltagirone** | 6360 b | 1.09 a |
| **Siracusa** | 7600 a | 1.08 a |
| **Sortino** | 7610 a | 1.08 a |
| **Means** | **7190** | **1.09** |

Considering the total phenols amounts and the colour indices, the population Sortino was used for the following trials, to obtain pasta samples.

***Experiment B***

In this experiment the studied factors are:

- Control (durum wheat spaghetti) ("**CTRL"**)
- Wholemeal durum wheat "Senatore Cappelli"+ 5% (w/w) cladodes of Sortino (**pasta 1**)
- Wholemeal durum wheat "Senatore Cappelli"+ 10% (w/w) cladodes of Sortino (**pasta 2**).

*Sensorial Analysis of pasta*

In figure 2.1 are reported the results of panel test in fresh and dry uncooked spaghetti. Fresh uncooked pasta samples were appreciated, in fact the overall quality in "pasta 1" was 7.00 and in "pasta 2" was 7.50 vs 7.75 of the "CTRL". The three samples had similar scores for all parameters (colour, odour and homogeneity) except for the presence of black spots; the score of black spots was 7 in "CTRL" and 5.5 in "pasta 1" and "pasta 2". As regard the dry uncooked spaghetti, the two pasta samples enriched in cladodes were less appreciated than the "CTRL": the overall quality score was 6 in "pasta 1", 5.5 in "pasta 2" and 7.5 in "CTRL". There was a decrease of the scores of all the parameters under study, in particular for the presence of the black spots; it was 9 in pasta "CTRL"

and 5 in “pasta 1” and “2”. The results of “pasta 1” and “pasta 2” were not statistically different.

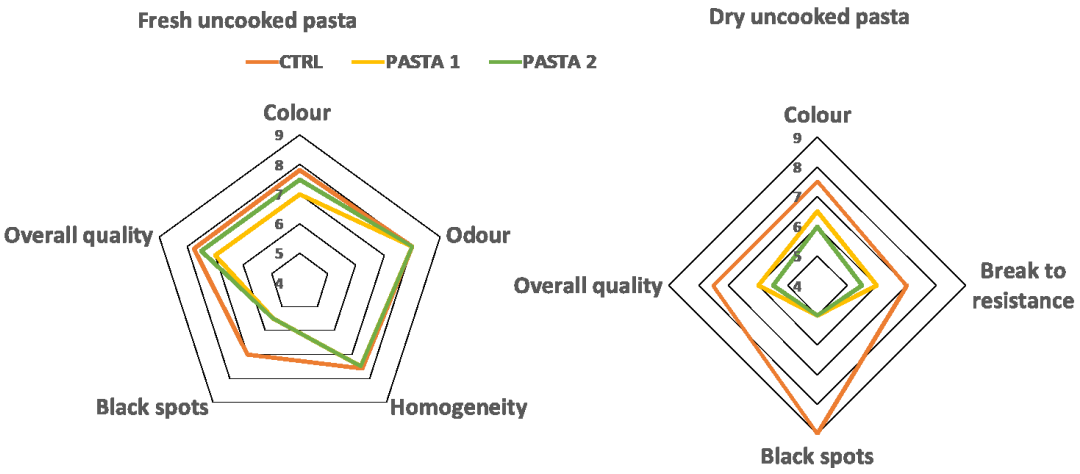



**Figure 2.1: Panel analyses of fresh and dry uncooked pasta obtained with ancient durum wheat “Senatore Cappelli” flour and cladodes of prickly pear**

Also in fresh and dry cooked samples the overall quality decreased in pasta enriched in cladodes: 7.5 in the “CTRL”, 5.5 in “pasta 1” and 6 in “pasta 2”. The poorest results were in dry cooked spaghetti especially in “pasta 2” that had a score of overall quality of 4.50 (below the acceptability threshold). There was no a significant difference between “pasta 1” and “pasta 2” for all parameters, except for the colour (that is 6.5 in pasta 1 and 5 in “pasta 2”); on the other hand “pasta 1” and “pasta 2”were significantly different from the “CTRL”, except for the odour, whose score was 7.5 in all three samples (figure 2.2).

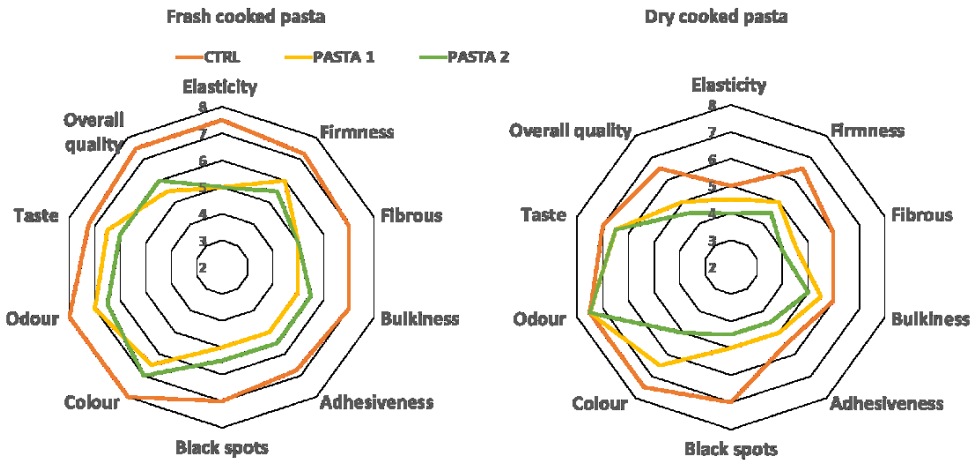



**Figure 2.2: Panel analyses of fresh and dry cooked pasta obtained with ancient durum wheat "Senatore Cappelli" flour and cladodes of prickly pear**

Since the sensorial analyses gave poor results (especially in cooked spaghetti), were not evaluated other characteristics for this pasta samples. The low quality recorded probably was due to the high amount of fiber of wholemeal flour and cladodes. Fibrous is a parameter studied in cooked samples (fresh and dry), and the results effectively showed that this characteristic was not much appreciated in "pasta 1" and "2": in fresh cooked samples it was 5 in both "pasta 1" and "pasta 2" (vs 7 in "CTRL"), while in dry cooked samples it was 4.5 in "pasta 1" and 4 in "pasta 2" (vs 6 in "CTRL").

### *Experiment C*

Considering that the samples with wholemeal durum wheat "Senatore Cappelli" flour did not have good results, this experiment was made using commercial durum wheat flour.

The studied factors are described as follows:

- Control (durum wheat spaghetti) ("**CTRL"**)
- Durum wheat flours + 5% (w/w) cladodes of Sortino ("**pasta 1"**)
- Durum wheat flours + 10% (w/w) cladodes of Sortino ("**pasta 2"**)

*Sensorial Analysis of pasta*

Sensory analysis on fresh cooked pasta samples showed a significant difference between spaghetti enriched with cladodes and "CTRL". Increasing concentration of cladodes the overall quality of the pasta samples decreased, but remaining in an acceptable range

(values> 6.25) (Table 2.3). There was a significant difference also among “pasta 1” and “pasta 2” for elasticity, firmness, bulkiness and adhesiveness and for all these parameters “pasta 2” had the lowest score.


**A and C= Uncooked and cooked spaghetti obtained using durum wheat flour and 5% of cladodes. B and D= Uncooked and cooked spaghetti obtained using durum wheat flour and 10% of cladodes.**

**Table 2.3: Panel analyses of cooked fresh pasta obtained with durum wheat flour and cladodes of prickly pear. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Elasticity | Firmness | Bulkiness | Adhesiveness | Odour | Taste | Overall quality |
|---|---|---|---|---|---|---|---|
| **CTRL** | 7.50 a | 7.25 a | 7.00 a | 6.75 a | 8.00 a | 7.75 a | 7.38 a |
| **1** | 6.50 b | 6.50 b | 6.50 b | 6.00 b | 7.50 b | 7.00 b | 6.67 b |
| **2** | 5.50 c | 6.00 c | 6.00 c | 5.50 c | 7.50 b | 7.00 b | 6.25 c |
| **Means** | **6.50** | **6.58** | **6.50** | **6.08** | **7.67** | **7.25** | **6.77** |

These differences were reduced after drying. In particular, there was no significant differences in values of taste and odour among “CTRL” and the two pasta samples “1” and “2”. However, in “pasta 1” firmness and bulkiness were not statistically different from the “CTRL”. Also in this case “pasta 2” had the lower scores than “pasta 1” and “CTRL” (table 2.4). The overall quality was 6.25 for “pasta 1” and 5.75 for “pasta 2”.

**Table 2.4: Panel analyses of cooked dry pasta obtained with durum wheat flour and cladodes of prickly pear. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Elasticity | Firmness | Bulkiness | Adhesiveness | Odour | Taste | Overall quality |
|---|---|---|---|---|---|---|---|
| **CTRL** | 7.25 a | 7.00 a | 6.75 a | 6.50 a | 7.50 a | 7.65 a | 7.25 a |
| **1** | 6.35 b | 6.95 a | 6.25 a | 6.05 b | 7.65 a | 7.25 a | 6.25 b |
| **2** | 5.75 b | 6.25 b | 5.65 b | 5.25 c | 7.35 a | 7.30 a | 5.75 b |
| **Means** | 6.45 | 6.73 | 6.22 | 5.93 | 7.50 | 7.40 | 6.42 |

*Cooking quality evaluation*

Cooking quality analysis showed that the swelling index did not change in the pasta samples "1" and "2" vs "CTRL", cooking losses and adhesiveness increased by increasing the concentration of the cladodes, while the optimal cooking time (OCT) and water absorption decreased. The hardness decreased only in "pasta 2" (table 2.5).

**Table: 2.5: Cooking quality pasta obtained with durum wheat flours and cladodes of prickly pear. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | OCT (min) | Cooking loss (%) | Swelling Index | Water Absorption (%) | Adhesiveness (Nmm) | Hardness (N) |
|---|---|---|---|---|---|---|
| **CTRL** | 9:30 a | 6.18 c | 1.80 a | 142 a | 0.47 b | 7.25 a |
| **1** | 8:30 . | 6.47 b | 1.84 a | 140 b | 0.54 a | 7.08 a |
| **2** | 8:30 . | 6.76 a | 1.83 a | 139 b | 0.58 a | 6.25 b |
| **Means** | **9:10** | **6.47** | **1.82** | **140.33** | **0.53** | **6.86** |

*Pasta colour evaluation*

The evaluation of the colour (table 2.6) indices L*, a*, b* showed that the yellow index (b*) of pasta samples "1" and "2" increased vs the "CTRL": in uncooked samples it was (on average) 33.51 in "pasta 1" and "2" vs 24.81 in "CTRL", in cooked spaghetti it was (on average) 22.75 in "pasta 1" and "2" vs 17.69 in "CTRL". The brightness (L*) decreased in uncooked "pasta 1" and "2" where it was (on average) 47.12 vs 60.04 in "CTRL"; while in cooked samples L* decreased significantly only in "pasta 2". Regarding a* index, the decreasing values towards negative axes indicated that in uncooked enriched spaghetti "green colour" prevailed vs "CTRL", due to the addition of the cladodes. This difference was not registered in cooked samples, probably due to chlorophylls losses.

**Table 2.6: L*, a*, b*in uncooked and cooked pasta obtained with durum wheat flour and cladodes of prickly pear. Different letters within the same column indicate statistical differences at P<0.05**

| | Uncooked pasta | | | Cooked pasta | | |
|---|---|---|---|---|---|---|
| **Pasta** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **CTRL** | 60.04 a | -0.52 a | 24.81 b | 61.15 a | -4.06 a | 17.69 b |
| **1** | 48.58 b | -1.00 b | 33.83 a | 60.31 a | -4.67 b | 23.02 a |
| **2** | 45.66 b | -1.16 b | 33.20 a | 57.65 b | -4.77 b | 22.48 a |
| **Means** | **51.43** | **-0.89** | **30.61** | **59.70** | **-4.5** | **21.06** |

*TPC and fiber content*

The flour of "Sortino" cladodes added to the pasta samples had 7610 $mg_{GAE}$ $kg^{-1}$ f.w. of total phenols (experiment A); The addition of cladodes at 5% and at 10% enriched "pasta 1" with 693 $mg_{GAE}$ $kg^{-1}$ TPC and "pasta 2" with 1026 $mg_{GAE}$ $kg^{-1}$ TPC. The cooking process did not result in phenol losses, in fact, there was no a significant difference in TPC among uncooked and cooked samples (Figure 2.3).

It is not possible to say the same for the fiber: in fact in the enriched pasta samples the fiber were lost with cooking process. In "pasta 1" the fiber content ranged from 11.6 g $kg^{-1}$ (uncooked) to 4.06 g $kg^{-1}$ (cooked) and in "pasta 2" from 17.6 g $kg^{-1}$ (uncooked) to 11 g $kg^{-1}$ (cooked) (Figure 2.4).

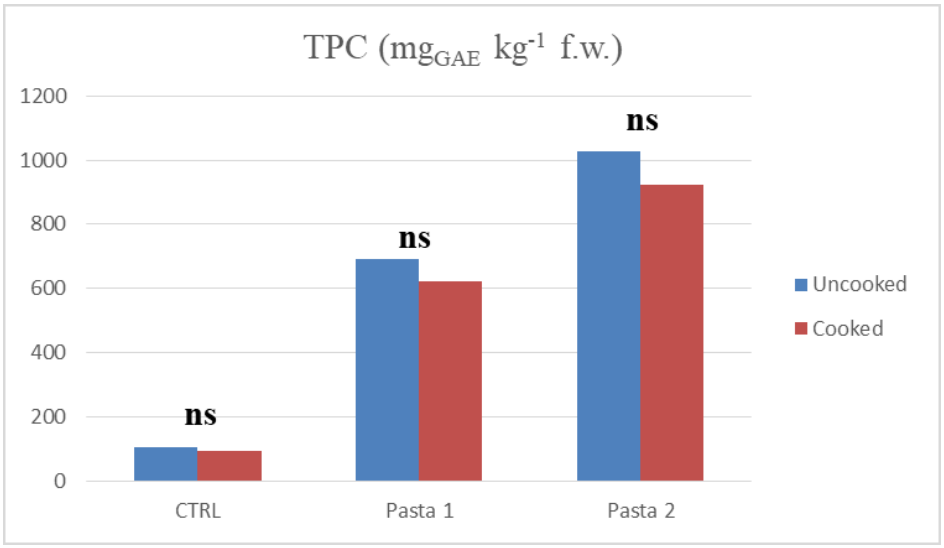



**Figure 2.3: TP content (mg$_{GAE}$ kg$^{-1}$ f.w.) of uncooked and cooked dry pasta obtained with durum wheat flour and cladodes. (ns) indicates not statistically different**

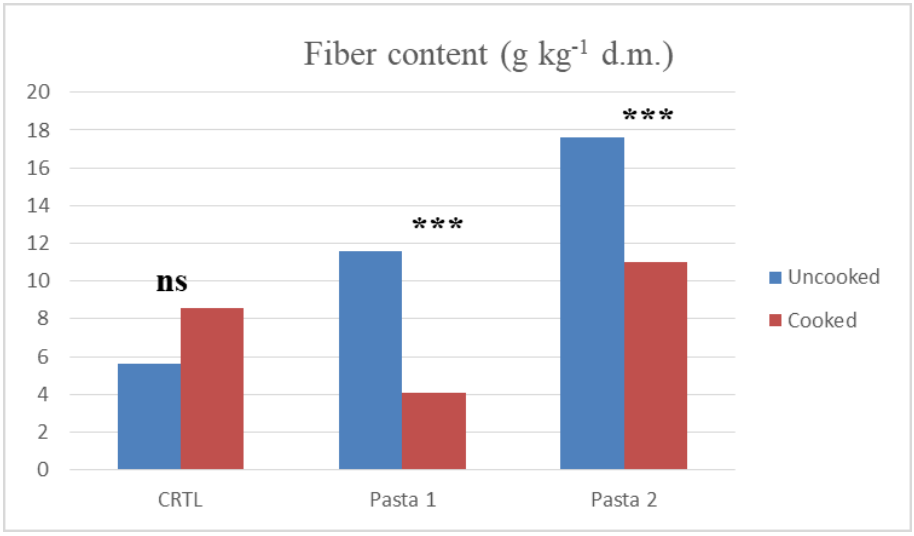



**Figure 2.4: Fiber content (g kg$^{-1}$ d.m.) of uncooked and cooked dry pasta obtained with durum wheat flour and cladodes. "ns" indicates not statistically different. (***) significant at 0.001 probability level**

***Experiment D***

The aim of this experiment was to improve the sensorial and rheological characteristics of spaghetti with 10% of cladodes, maintaining the high fiber content in enriched cooked pasta. Two food additives (Agar and CMC) have been tested to achieve these purposes.

The analyzed samples in this experiment are:

- Control (durum wheat spaghetti) (“**CTRL”**)
- Durum wheat flours + 10% (w/w) cladodes (“**pasta 1”**)
- Durum wheat flours + 10% (w/w) cladodes + 2% (w/w) CMC (“**pasta 2”**)
- Durum wheat flours + 10% (w/w) cladodes + 2% (w/w) AGAR (“**pasta 3”**)

*Sensorial Analysis of pasta*

Uncooked dry pasta with 10% of cladodes (“pasta 1”) obtained a good result in terms of acceptance of consumers also without additives, but the overall quality decreased considerably after cooking, so the role of additives is fundamental during cooking process. In table 2.7 are reported the results of panel test in uncooked samples: there was no a significant difference among all four uncooked dry pasta samples. The overall quality, on average of all samples, was 7.34.

**Table 2.7: Panel analyses of uncooked dry pasta obtained with durum wheat flour and cladodes of prickly pear. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Colour | Odour | Overall Quality |
|---|---|---|---|
| **CTRL** | 7.65±0.25a | 7.65±0.30a | 7.50± 0.30a |
| **1** | 7.05±0.25a | 7.50± 0.30a | 7.20±0.27a |
| **2** | 7.05±0.25a | 7.45±0.25a | 7.25± 0.25a |
| **3** | 6.87± 0.25a | 7.50± 0.27a | 7.40± 0.27a |
| **Means** | **7.16** | **7.53** | **7.34** |

The positive effect of additives is clear in table 2.8, where are reported the results of panel test in dry cooked samples. Both additives improve in the same way the bulkiness, adhesiveness and so the overall quality that ranged from 5.75 (“pasta 1”) to 6.30 (“pasta 3”) and to 6.50 (“pasta 2”) vs 7.25 (CTRL).

**Table 2.8: Panel analyses of cooked dry pasta obtained with durum wheat flour and cladodes of prickly pear. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | Elasticity | Firmness | Bulkiness | Adhesiveness | Colour | Odour | Taste | Overall Quality |
|---|---|---|---|---|---|---|---|---|
| **CTRL** | 7.25±0.25$^{a}$ | 7.00±0.27$^{a}$ | 6.75± 0.30$^{a}$ | 6.50±0.30$^{a}$ | 7.65±0.30$^{a}$ | 7.50±0.25$^{a}$ | 7.65± 0.30$^{a}$ | 7.25± 0.30$^{a}$ |
| **1** | 5.75±0.30$^{b}$ | 6.25±0.27$^{b}$ | 5.65±0.27$^{b}$ | 5.25±0.25$^{b}$ | 7.50±0.27$^{a}$ | 7.35±0.25$^{a}$ | 7.30±0.25$^{a}$ | 5.75±0.25$^{c}$ |
| **2** | 5.85±0.25$^{b}$ | 6.75±0.25$^{ab}$ | 6.35±0.25$^{a}$ | 6.20± 0.25$^{a}$ | 7.50±0.27$^{a}$ | 6.92± 0.27$^{a}$ | 7.25±0.25$^{a}$ | 6.50±0.27$^{b}$ |
| **3** | 5.65± 0.28$^{b}$ | 6.25± 0.25$^{b}$ | 6.20±0.28$^{a}$ | 6.00±0.30$^{a}$ | 7.65±0.27$^{a}$ | 7.20±0.20$^{a}$ | 7.30±0.25$^{a}$ | 6.30±0.26$^{b}$ |
| **Means** | **6.13** | **6.56** | **6.24** | **5.99** | **7.58** | **7.24** | **7.38** | **6.45** |

*Cooking quality evaluation*

The cooking quality results are reported in table 2.9. The optimal cooking time (OCT) decreased adding cladodes, but in particular with additives: it ranged from 7 min ("pasta 2" and "3") to 8:30 min ("pasta 1") and to 9:30 min ("CTRL"). The swelling index and the water absorption decreased in pasta samples with additives. No statistically significant differences among all types of pasta for cooking loss and adhesiveness have been recorded, while hardness was influenced by the adding of cladodes flours, but not by the additives.

**Table 2.9: Cooking quality pasta obtained with durum wheat flours and cladodes of prickly pear. Different letters within the same column indicate statistical differences at P<0.05**

| Pasta | OCT (min) | Cooking Loss (%) | Swelling Index | Water Absorption (%) | Adhesiveness (Nmm) | Hardness (N) |
|---|---|---|---|---|---|---|
| **CTRL** | 9:30$^{a}$ | 6.19±0.04$^{a}$ | 1.80± 0.04$^{a}$ | 142±0.60$^{a}$ | 0.47±0.00$^{a}$ | 7.25±0.30$^{a}$ |
| **1** | 8:30$^{b}$ | 6.76±0.18$^{a}$ | 1.83±0.01$^{a}$ | 139±0.55$^{a}$ | 0.58±0.03$^{a}$ | 6.25±0.22$^{b}$ |
| **2** | 7:00$^{c}$ | 6.26±0.65$^{a}$ | 1.63±0.03$^{b}$ | 126±3.6$^{b}$ | 0.52±0.05$^{a}$ | 6.75±0.23$^{b}$ |
| **3** | 7:00$^{c}$ | 6.64±0.40$^{a}$ | 1.66±0.06$^{b}$ | 122±6.83$^{b}$ | 0.56±0.08$^{a}$ | 6.50±0.27$^{b}$ |
| **Means** | **8:00** | **6.46** | **1.73** | **132.25** | **0.53** | **6.69** |

*Pasta colour evaluation*

ANOVA showed that the additives did not influence the colour of samples. Therefore there was no a significant difference for colour evaluation among "pasta 1", "2" and "3". The results about "pasta 1" are already reported in table 2.6.

*Fiber content*

A purpose of this experiment was to keep the high fiber content also after cooking. The figure 2.5 reports the percentage of fiber improvement vs "CTRL": the amount of fiber increased up to 65-70% adding 10% of cladodes. The best results were obtained using CMC, that maintained the fiber content after cooking; there was no a significant

difference among uncooked and cooked sample of “pasta 2”. In spaghetti with AGAR (“pasta 3”) some fiber were lost after cooking, but anyway less than the fiber that were lost in “pasta 1” (without additive).

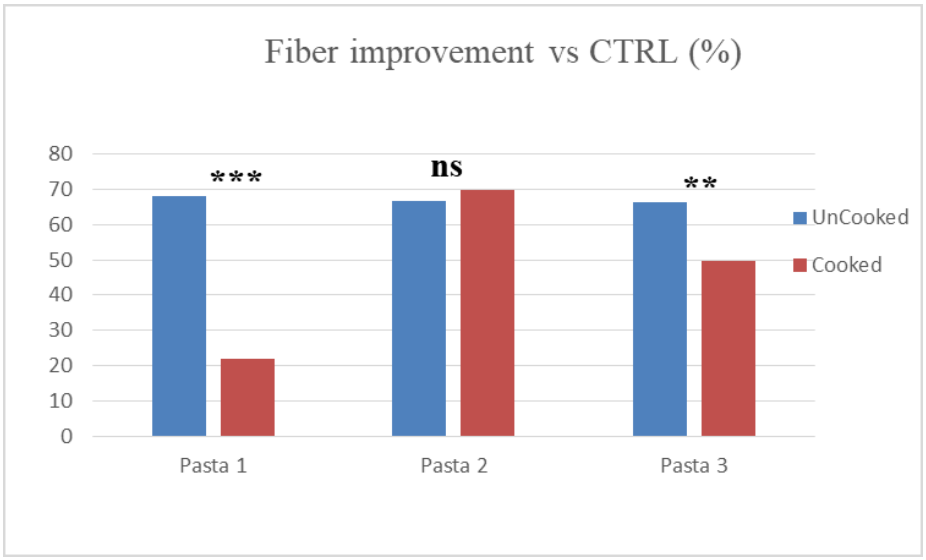



**Figure 2.5: Percentage of fiber improvement vs CTRL of uncooked and cooked dry pasta obtained with durum wheat flour and cladodes. (ns) indicates not statistically different. (***) and (**) significant at 0.001 and 0.01 probability level**

### 2.2.4 Conclusions

Fruits and cladodes have been used to prepare value-added products, such as juice, jam, pickle, cookies, baking bread, nachos, tortillas, because of *Opuntia Ficus Indica* is a good source of natural compounds with functional properties; its fruits and cladodes are used for obesity, arteriosclerosis, diabetes, hepatitis, kidney stones, gastritis, hypercholesterolemia and cardiovascular disorders (Galati et al., 2003). They also have anti-inflammatory, antioxidant, hypoglycemic, antimicrobial and neuroprotective properties (El-Mostafa et al., 2014). Furthermore, they can be used to decrease the circulating levels of triglycerides, cholesterol and serum glucose and to treat burns, asthma and indigestion (Kaur, 2012). Given the widespread use of pasta in the Mediterranean diet and the health benefits related to the *Opuntia*, the production of a new functional pasta may represent an interesting opportunity in the food market. The interest in this topic is very high and in this contest it was possible to obtain dry spaghetti with high TPC and fiber content after cooking, using directly cladodes flours. Other research groups demonstrate the economic feasibility of this new functional pasta (Micale et al.,

2018) and antioxidant, anti-inflammatory hypoglycemic effect properties with putative effect on the aging process and related metabolic diseases are found in pasta enriched with 3% of *Opuntia* extracts (Aiello et al., 2018). Moreover, an estimation of the stability of process in food industry until 38% of *Opuntia* juice in order to assess the final products as "healthy" has been performed by Micale et al. (2017). In this view the valorization of Sicilian germplasm to produce *Opuntia* fortified pasta was taken into account. In the first step the evaluation of cladodes collected in different sites of Sicily showed that the origin influenced the colour and the total phenols content. Among the different germplasm evaluated, cladodes collected in Sortino, resulted more suitable to enrich spaghetti. Dry spaghetti produced with whole-meal durum wheat "Senatore Cappelli" and cladodes dry flours was not appreciated by panelists, probably due to an excess of fiber. The use of commercial durum wheat improved the sensorial quality of cooked spaghetti that had a score of overall quality of 6.25 (5% of cladodes) and 5.75 (10% of cladodes), above the acceptability threshold. The cladodes addition enriched pasta in phenols and fiber. The TPC was 693 mgGAE kg-1 f.w. (5% of cladodes) and 1026 mgGAE kg-1 f.w. (10% of cladodes) vs 100 mgGAE kg-1 f.w. ("CTRL"). While the phenols were not lost with cooking, the same can not be said for the fiber; so the next step was to use food additives (Agar and CMC) to improve further sensorial characteristics of pasta with 10% of cladodes and to maintain fiber after cooking. Actually, the additives improved the overall quality of cooked spaghetti: the score ranged from 5.75 (without additives) to 6.40, and CMC also maintains the amounts of fiber after cooking.

## 2.3 TRIAL 3: ANTIOXIDANT ACTIVITY AND FATTY ACIDS QUANTIFICATION IN SICILIAN PURSLANE GERMPLASM UNDER DIFFERENT MEDITERRANEAN ENVIRONMENTAL CONDITIONS

### 2.3.1 Aim

The aim of this trial was the assessment of chemical composition of three purslane populations under different Mediterranean environmental conditions, for future valorization as novel food sources of omega-3 fatty acids. The evaluations were conducted over two years.

### 2.3.2 Materials and methods

Three different populations of purslane, namely Caltagirone (Cal), Cassibile (Cas) and Santa Venerina (S. Ven), were harvested from native plants found in different areas of eastern Sicily, during the months of July 2016 and 2017. Biomorphological characteristics (colour, dry weight, incidence), total phenols content (*Folin Ciocalteau* method*)*, antioxidant activity (*DPPH* method*)* and fatty acids content *(GC/MS* analysis*)* were evaluated.

*Biomorphological characteristics*

Puslane germoplasm was collected during the months of July 2016 and 2017 in three different sites of eastern Sicily: Caltagirone (Cal), Cassibile (Cas) and Santa Venerina (S. Ven). In the identified sites of collection the plant resulted widespread and cover naturally the degraded soils. The characteristics of the sites of collections are reported in table 3.1. The aboveground biomass was harvested out in all sites of collection the July 2016 and 2017. In each site of collection twenty representative plants were harvested. In laboratory harvested plants were immediately weighed in order to determine the fresh weight (fw). The moisture content of biomass components (stems, leaves and roots) was measured by weighing 100 g of plant material in a precalibrated porcelain capsule and placing it in a thermoventilated oven at 105 °C until constant weight was reached. On fresh leaves the colour indices L *, a *, b * (how reported in trial 1) were measured. All analyses were performed in triplicate for each sampling and are reported on a dry matter (DM) basis. Biomass production per plant was expressed as g plant$^{-1}$ DM. Collected parts of the plant were blended thoroughly for homogeneity and washed with deionized water. After

draining excess water, the biomass (leaves + stalks) was dried at 40°C and ground into powder for further chemical characterization.

Purslane - Caltagirone | Purslane - Cassibile | Purslane - S. Venerina

**Purslane germoplasm collected in different sites of Sicily.**

**Table 3.1: Sites of collection and their characteristics.**

| Location | Coordinates | Altitude (m a.s.l.) | Yearly average temperature [a] | Yearly average Precipitation [a] | De Martonne aridity Index [a] | Soil Characteristics (b) |
|---|---|---|---|---|---|---|
| **Caltagirone (CT)** | 37°11'07'' N 14°13'19''W | 405 | 15-18°C | 400-500 mm | Semiarid | 43 |
| **Cassibile (SR)** | 36°58'33'' N 15°12'18''W | 48 | 18-19°C | 500-600 mm | warm temperate climate | 40 |
| **Santa Venerina (CT)** | 37°40'23''N 15°19'26''W | 201 | 18-19°C | 800-1000 mm | humid temperate climate | 47 |

(Drago, 2005)

(Costantini and L'Abate, 2016)

47 - Haplic e Petric Calcisol; Calcic, Chromic e Skeletic Luvisol; Calcaric e Luvic Phaeozem; Calcaric Fluvisol; Haplic e Calcic Vertisol; Calcic Kastanozem; Eutric, Fluvic, Endogleyic e Calcaric Cambisol; Vitric Andosol; Calcaric Regosol; Calcaric Arenosol.

43- Calcic, Sodic, Gypsic e Haplic Vertisol; Fluvic and Calcaric Cambisol; Calcic Luvisol; Gypsiric Regosol; Calcic e Haplic Gypsisol

40 - Leptic Luvisol; Luvic, Haplic e Calcaric Phaeozem; Calcaric Leptosol; Dystric Andic e Calcaric Cambisol

*Total phenols content*

Total phenol content (TPC) was determinated in purslane germoplasm using *Folin-Ciocalteu method as* reported in trial 2; here the results are expressed as $mg_{GAE}$ 100 $g^{-1}$ D.W.

*Antioxidant activity*

The antioxidant activity in purslane germoplasm was evaluated using the $DPPH^{\cdot}$ radical scavenging activity as reported in trial 2.

*Fatty acids content*

A 0.5-g amount of dried plant tissue was mixed with 2 mL of CHCl3 and 1ml of MeOH; the mixture was sonicated for 45 minutes at 50 °C and then cooled to room temperature; centrifuged at 4000 rpm for 5 minutes and the supernatant was filtered with a 0.45 micron syringe filter and evaporated to dryness under a gentle stream of nitrogen. The obtained residue was dissolved in 100 µL of toluene and 200 µL of KOH in MeOH (10% w/v). The samples was vortexed-for 5 min, and 200 µL of water and 1 ml of hexane were added. The upper phase was collected and 500 µL were added to 200 µL of Internal standard (heneicosanoic acid methyl ester 50 µg/mL) and 300 µL of hexane prior to gas chromatography-mass spectrometry (GC/MS) analysis.

For the separation and analysis of the fatty acid methyl esters (FAMEs) from *Portulaca* DM, Thermo Scientific DSQ II single quadrupole system in EI (Electron Ionization) mode, working in full scan was used. The capillary column used was a ZB-WAX (30 m x 0.25 mm i.d., film thickness 0.25 µm, (Phenomenex, Italy). The oven temperature was programmed column temperature started at 40°C , increased at 15 °C/min to 165 °C, hold time 15 min, a second gradient was applied to 165 °C at 250 °C/min and held for 10 min under isothermal conditions. Helium was used as the carrier gas at a flow rate of 0.8 mL/min. A sample of 1µl was injected with a split ratio of 1:100. Mass spectroscopy conditions: The ion source temperature was 260°C, the MS transfer line temperature was 265°C and injector temperature was 250°C. Ionization voltage was 70eV and the mass range scanned was 35-550 m/z. Using Thermo Scientific Xcalibur Data system software for Windows peak areas were determined and identified by comparison of retention times with those of a FAMEs standard mix (Supelco CRM18918 SUPELCO FAME Mix C8 - C24) separated under the same chromatographic conditions. Triplicate analyses were prepared for each dried plant sample. FAMEs are expressed in mg $g^{-1}$.

*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.

### 2.3.3 Results

*Biomorphological characteristics*

Results of the analysis of variance for most characteristics showed significant differences among populations in both the years of collections (Table 3.2). Significant interaction "P" X "Y" has been observed for stems and leaves incidences on total biomass.

Highly significant sums of squares for populations differences were found in the combined analysis of variance for biomass productions. Plant dry weight (g plant$^{-1}$) and its partitioning (% of total plant weight) are reported in table 3.3. On average of populations, the years of collection did not influenced the plant dry weight, but its partitioning. In 2017 plants produced more stems than leaves. Averaged for year of collection, the population "Cassibile" had the lowest plant biomass, mainly characterized by the presence of leaves (29.9% of total biomass).

**Table 3.2 Analyses of variance of the population characteristics and partitioning of the treatment sum squares (SS expressed in absolute value – AV – and percent of total) into main effects and interactions. (*) Significant at 0.05 probability level; (**) Significant at 0.01 probability level; (***) Significant at 0.001 probability level; (n.s.) not significative.**

| | Population (P) | | | Year of collection (Y) | | | Interaction (P x Y) | | |
|---|---|---|---|---|---|---|---|---|---|
| | **AV** | **%** | | **AV** | **%** | | **AV** | **%** | |
| **Stems (g plant$^{-1}$ d.m.)** | 28.58 | 69.62 | *** | 0.47 | 1.15 | n.s. | 12.00 | 29.23 | * |
| **Leaves (g plant$^{-1}$ d.m.)** | 1.91 | 42.83 | n.s | 1.63 | 36.60 | n.s. | 0.92 | 20.57 | n.s. |
| **Roots (g plant$^{-1}$ d.m.)** | 0.16 | 83.85 | n.s | 0.01 | 2.60 | n.s. | 0.03 | 13.54 | n.s. |
| **Total biomass (g plant$^{-1}$ d.m.)** | 51.32 | 73.30 | ** | 4.65 | 6.64 | n.s. | 14.04 | 20.05 | n.s. |
| **Incidence of stems (% of total biomass)** | 152.65 | 5.99 | n.s | 857.95 | 33.67 | *** | 1537.77 | 60.34 | *** |
| **Incidence leaves(% of total biomass)** | 216.82 | 8.29 | * | 1016.65 | 38.88 | *** | 1381.20 | 52.82 | *** |
| **Incidence roots (% of total biomass)** | 56.63 | 67.48 | n.s | 7.59 | 9.05 | n.s. | 19.69 | 23.47 | n.s. |
| **L*** | 320.24 | 60.78 | *** | 136.84 | 25.97 | ** | 69.84 | 13.25 | n.s. |
| **a*** | 87.16 | 62.79 | *** | 34.89 | 25.14 | ** | 16.76 | 12.07 | * |
| **b*** | 341.07 | 88.20 | *** | 6.82 | 1.76 | n.s. | 38.81 | 10.04 | n.s. |
| **TPC (mg 100$^{-1}$ D.W.)** | 1658 | 91.3 | *** | 21.3 | 1.2 | n.s. | 126 | 7.00 | n.s |
| **$IC_{50}$ (mg/ml)** | 1.12 | 89.6 | *** | 0.11 | 8.8 | *** | 0.02 | 1.6 | ** |
| **Palmitic Acid (mg/g)** | 0.27 | 9.93 | n.s | 2.22 | 80.31 | *** | 0.27 | 9.75 | n.s. |
| **Stearic Acid (mg/g)** | 0.01 | 1.04 | n.s | 0.09 | 7.38 | ** | 1.06 | 91.58 | n.s. |
| **Oleic Acid (mg/g)** | 0.29 | 45.93 | *** | 0.24 | 37.96 | *** | 0.10 | 16.11 | n.s. |
| **Linoleic Acid (mg/g)** | 2.26 | 85.68 | *** | 0.04 | 1.41 | n.s. | 0.34 | 12.91 | n.s. |
| **Linolenic Acid (mg/g)** | 2.70 | 67.58 | *** | 0.03 | 0.83 | n.s. | 1.26 | 31.60 | * |

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**Table 3.3 Plant dry weight (g plant-1) and its partitioning (% of total plant weight), on average of the two years of collection in purslane in relation to site of collection. Different letters indicate differences at P< 0.05**

| Population | Year | Dry weight (g plant$^{-1}$) | | | | Incidence (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | Stems | Leaves | Roots | Plant | Stems | Leaves | Roots |
| **Average of year** | | | | | | | | |
| **Cal** | | 4.01 a | 1.29 a | 0.37 a | 5.67 a | 69.26 a | 21.66 b | 9.08 a |
| **Cas** | | 1.57 b | 0.62 a | 0.13 a | 2.33 b | 62.42 b | 29.90 a | 7.68 a |
| **S. Ven** | | 4.43 a | 1.34 a | 0.26 a | 6.12 a | 67.59 a | 27.60 ab | 4.82 b |
| **Average of populations** | | | | | | | | |
| | **2016** | 3.50 a | 1.38 a | 0.27 a | 5.21 a | 59.52 b | 33.94 a | 6.54 a |
| | **2017** | 3.18 a | 0.78 a | 0.24 a | 4.20 a | 73.33 a | 18.83 b | 7.84 a |

Differences in colour of leaves were observed among genotypes and year of collection (Figure 3.1). In particular, the population influenced for over the 60% of total variation all the indices of color. The brightness (L*) resulted highest in Cassibile for both years, while the population S.Ven resulted more dark with lowest a* and highest b* values, confirming the colour is a parameter genotype dependent.

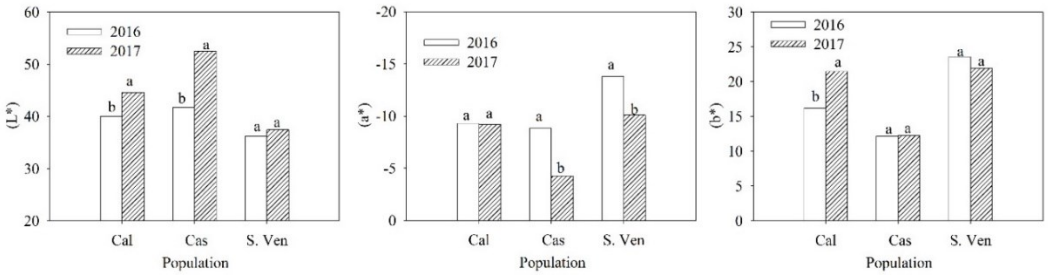



**Figure 3.1: L*, a* and b* variation in relation to population and year of collection. The different letters between years within the same population indicate significant differences at *P*≤ 0.05.**

*TPC and antioxidant activity of Purslane*

Table 3.4 shows the concentration of TPC crude extracts and the antioxidant activities ($IC_{50}$) of *P. oleracea* populations. In this study, TPC were determined compared with standard gallic acid. The Year of collection, did not affect these parameters, the amount of TPC resulted, averaged for the two years 241 mg 100 $g^{-1}$ D.W., with an $IC_{50}$ of 1.28 mg/mL. Out of year of collection, the population "Cas" appears to have the lowest $IC_{50}$ and the highest TPC values among the three populations, indicating its higher antioxidant activity.

**Table 3.4: TPC ($mg_{GAE}$ 100 $g^{-1}$ D.W.) and IC50, on average of the populations and years of collection. Different letters indicate differences at P< 0.05.**

| Population | Year | TPC | $IC_{50}$ |
|---|---|---|---|
| | **Average of populations** | | |
| | **2016** | 240 a | 1.20 a |
| | **2017** | 242 a | 1.36 a |
| | **Average of years** | | |
| **Cal** | | 228 b | 1.38 a |
| **Cas** | | 254 a | 0.93 b |
| **S. Ven** | | 225 b | 1.52 a |

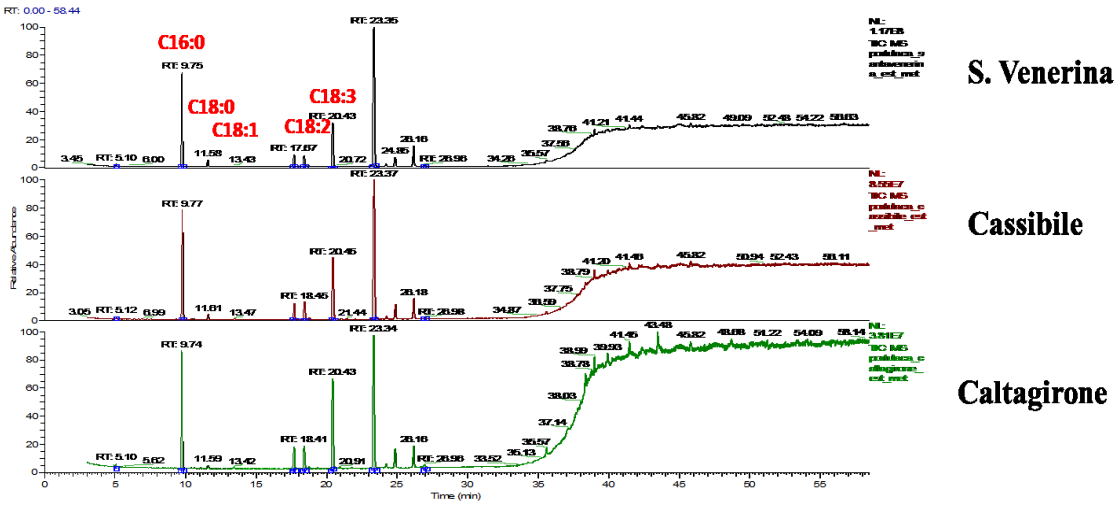
*Fatty acids content*

As regards fatty acids, the results (table 3.5) refer to whole plants since they are both edible. Purslane is considered a rich source of fatty acids, and its content and composition could be considered a key quality factor for genotype evaluation.

ANOVA showed that saturated fatty acid content resulted influenced only by the year of collection, while the polyunsaturated FA resulted influenced by the populations. Only the amount of oleic acid was influenced by both the factors under study. Among FAMEs, on averaged for populations and years of collection, the most abundant fatty acids were linoleic and linolenic acids, with 0.76± 0.26 and 0.83± 0.27 mg/g, respectively. Among the populations, on average of the two years, "Cas" has the highest contents, with 1.01±0.22 mg/g (linoleic acid) and 1.07±0.23 mg/g (linolenic acid). Palmitic acid was detected at significant amounts in all populations. Moreover, linoleic acid was the

prevailing fatty acid for all the genotypes with a ratio of linolenic: linoleic ratio higher than 1.

The correlation analysis of the studied chemical characteristics showed that similar parameter has a highly significant correlation, while among other parameters the correlation is either nonsignificant or less significant or has moderate relation (Table 3.6). In particular, it is worthwhile to note that the antioxidant activity, expressed as $IC_{50}$, resulted significantly correlated not only with TPC content, but also with unsaturated FAMEs content.




**Qualitative analyses of FAMEs in the 3 populations of purslane. C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (linolenic acid).**

**Table 3.5: FAMEs (mg $g^{-1}$ D.W.) content in the different populations of purslane over the two years of collection. Different letters indicate differences at P< 0.05**

| Site of Collection | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid | Linolenic acid | Total | Satured F. A. | Monounsatured F.A. | Polyunsatured F.A. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | **2016** | | | | |
| **Cal** | 0.73± 0.55 a | 0.20± 0.14 a | 0.32± 0.25 ab | 0.91± 0.67 a | 1.07± 0.73 a | 3.22± 2.34 ab | 0.93± 0.69 a | 0.32± 0.24 ab | 1.97± 1.41 a |
| **Cas** | 1.12± 0.27 a | 0.19± 0.05 a | 0.50± 0.11 a | 1.14± 0.29 a | 1.30± 0.30 a | 4.26± 1.01 a | 1.31± 0.31 a | 0.50± 0.11 a | 2.44± 0.59 a |
| **S. Ven** | 0.79± 0.66 a | 0.16± 0.13 a | 0.17± 0.14 b | 0.32± 0.26 b | 0.23± 0.19 b | 1.66± 1.38 b | 0.95± 0.79 a | 0.17± 0.14 b | 0.55± 0.45 b |
| | | | | | **2017** | | | | |
| **Cal** | 0.42± 0.04 a | 0.11± 0.01 a | 0.21± 0.01 a | 0.78± 0.07 a | 0.89 ± 0.08 a | 2.41± 0.22 a | 0.52± 0.05 a | 0.21± 0.01 a | 1.67± 0.15 a |
| **Cas** | 0.39± 0.06 a | 0.09± 0.01 a | 0.20± 0.03 a | 0.88± 0.15 a | 0.84± 0.16 a | 2.41± 0.42 a | 0.48± 0.08 ab | 0.20± 0.03 a | 1.72± 0.31 a |
| **S. Ven** | 0.35± 0.08 a | 0.06± 0.01 b | 0.10± 0.03 b | 0.51± 0.14 b | 0.67± 0.16 b | 1.68± 0.42 b | 0.40± 0.09 b | 0.10± 0.03 b | 1.18± 0.30 b |
| | | | | | **Average of years** | | | | |
| **Site of Collection** | **Palmitic acid** | **Stearic acid** | **Oleic acid** | **Linoleic acid** | **Linolenic acid** | **Total** | **Satured F. A.** | **Monounsatured F.A.** | **Polyunsatured F.A.** |
| **Cal** | 0.58± 0.30 a | 0.15± 0.08 a | 0.27± 0.13 a | 0.84± 0.37 a | 0.98± 0.41 a | 2.82± 1.28 a | 0.73± 0.37 a | 0.27± 0.13 a | 1.82± 0.78 a |
| **Cas** | 0.76± 0.16 a | 0.14± 0.03 a | 0.35± 0.07 a | 1.01± 0.22 a | 1.07± 0.23 a | 3.33± 0.71 a | 0.90± 0.20 a | 0.35± 0.07 a | 2.08± 0.45 a |
| **S. Ven** | 0.57± 0.37 a | 0.11± 0.07 a | 0.13± 0.08 b | 0.42± 0.20 b | 0.45± 0.17 b | 1.67± 0.90 b | 0.68± 0.44 a | 0.13± 0.08 b | 0.86± 0.37 b |
| **Year** | | | | | **Average of populations** | | | | |
| **2016** | 0.88± 0.49 a | 0.18± 0.10 a | 0.33± 0.16 a | 0.79± 0.41 a | 0.86± 0.41 a | 3.05± 1.58 a | 1.06± 0.60 a | 0.33± 0.16 a | 1.65± 0.81 a |
| **2017** | 0.39± 0.06 b | 0.09± 0.01 b | 0.17± 0.02 b | 0.73± 0.12 a | 0.80± 0.13 a | 2.17± 0.35 b | 0.47± 0.07 b | 0.17± 0.02 b | 1.53± 0.25 a |
| | | | | | **General mean** | | | | |
| | 0.64± 0.28 | 0.13± 0.06 | 0.25± 0.09 | 0.76± 0.26 | 0.83± 0.27 | 2.60± 0.96 | 0.77± 0.34 | 0.25± 0.09 | 1.59± 0.53 |

**Table 3.6: Regression coefficients (b1) and r2 among all studied chemical characteristics. ***, ** and * significant at 0.001, 0.01 and 0.05 probability level ns is non-significant. −: negative correlation at 0.05%level.**

| | TPC | $IC_{50}$ | Palmitic Acid | Stearic Acid | Oleic acid | Linoleic acid | Linolenic acid |
|---|---|---|---|---|---|---|---|
| **TPC** | 1 | -0.019 $r^2$=0.777*** | 0.005 $r^2$=0.600* | 0.001 $r^2$=0.651** | 0.007 $r^2$=0.994*** | 0.020 $r^2$=0.997*** | 0.020 $r^2$=0.964*** |
| **$IC_{50}$** | | 1 | -0.337 $r^2$=0.957*** | Ns | -0.342 $r^2$=0.839*** | -0.859 $r^2$=0.731** | -0.850 $r^2$=0.603** |
| **Palmitic Acid** | | | 1 | Ns | 0.836 $r^2$=0.662** | 2.130 $r^2$=0.534* | ns |
| **Stearic Acid** | | | | 1 | 3.500 $r^2$=548* | 11.020 $r^2$=0.676** | 13.050 $r^2$=0.796** |
| **Oleic acid** | | | | | 1 | 2.810 $r^2$=0.983*** | 2.980 $r^2$=0.927*** |
| **Linoleic acid** | | | | | | 1 | 1.080 $r^2$=0.981*** |
| **Linolenic acid** | | | | | | | 1 |

### 2.3.4 Conclusions

The results obtained in this trial indicate that different purslane populations had different chemical composition and nutritional value; in particular, population “Cas” appeared to have higher content of total phenols and higher antioxidant activity than the other two populations (“Cal” and “S.Ven”). Also the polyunsaturated FA, that are important for their beneficial effects, resulted influenced by genotypes. The most abundant unsatured fatty acids are linoleic and linolenic acids and among the populations, also in this case “Cas” population had the highest contents of these fatty acids. The years of collection influenced only the partitioning of the plants and saturated fatty acid content. The data reported by Petropoulos (Petropoulos et al., 2016) on chemical composition of six genotypes of common purslane grown central Greece showed that PUFA/SFA ratio was higher than 0.45 and ranged from 1.31 to 1.92, with great differences among the studied genotypes, and agree with our results. Moreover Uddin et al. (2012) have also reported that mature leaves of wild purslane were rich in PUFAs and palmitic acid. Oliveira et al. (2009) identified twenty seven fatty acids in the leaves samples of purslane germplasm collected in Portugal. Linolenic acid resulted the most abundant, ranging from 27.7 to 39.1%, followed by palmitic (19.3–24.3%) and oleic acids (11.6–19.5%); even if ω-6:ω-3 ratio resulted lower than that recorded in our sample and than that reported by Uddin et al. (2014). These differences are probably due to the time of the germplasm harvest, which occurs in autumn in Portugal, while during summer period in Sicily. The selection of the genotype with the best nutritional and pharmacological spectrum would be important means for the production of functional food with antioxidant, anti-inflammatory, hypocholesterolemic and hypo-glycaemic effects on human health.

## 2.4 TRIAL 4: CHARACTERISTICS OF PASTA WITH *PORTULACA OLERACEA* ADDITION

### 2.4.1 Aim

The aim of this trial was to evaluate the characteristics of fresh pasta samples produced with the use of commercial durum wheat and purslane collected in three different areas (Caltagirone, Cassibile and Santa Venerina). Each of these three population of purslane was added in three different concentrations (5%, 10% and 15% w/w).

### 2.4.2 Materials and methods

Purslane germplasm was prepared to be added. For pasta making, commercial durum wheat was used and three different populations of purslane ("Cal", "Cas", "S. Ven") were added at different concentrations (5%, 10% and 15% w/w). Pasta without purslane was used as control ("CTRL"). On the pasta samples obtained were evaluated: sensorial qualities (Panel test), colour (Minolta colorimeter CR, 400), fatty acids content (*GC/MS* analysis), total phenols content (*Folin-Ciocalteu* method) and antioxidant activity (*FRAP and ABTS* methods).

*Preparation of plant material*

Collected parts of the plant were blended thoroughly for homogeneity and washed with deionized water. After draining excess water, the biomass (leaves + stalks) was dried at 40°C and ground into powder.

*Pasta making*

500 g flour (durum wheat + purslane) were prepared by Pastamatic ARIETE 1591 and mixed for 10.5 min, adding 220 mL of distilled water to obtain a dough with 44% moisture content. The dough was extruded into a spaghetti shape.

*Pasta colour evaluation*

Dry pasta colour was recorded before and after cooking with the use of a Minolta colorimeter CR, 400 as seen in trial 1.

*Sensorial Analysis of pasta*

Fresh-extruded uncooked and cooked spaghetti were submitted to a panel of 10 trained tasters (five men and five women, aged between 27 and 60 years) in order to evaluate the sensory attributes. The panellists were selected on the basis of their sensory skills (ability to accurately determine and communicate the sensory attributes as appearance, odour, flavour and texture of a product). They evaluated colour, the presence of black spots, elasticity, hardness, adhesiveness, stickiness, bulkiness, thickness, global taste and odour (global T & O) and a final global judgment. To this end, a nine-point scale was used: 1 very clear, 9 very dark for colour, 1 absence of black spots, 9 lots of black spots for the presence of black spots and the same principle for elasticity, hardness, adhesiveness, stickiness, bulkiness, thickness while for global T & O and final global judgment 1 correspond to extremely unpleasant, 9 to extremely pleasant.

*Fatty acids content*

The fatty acids content was determinated in "CTRL" and in fresh spaghetti with purslane germoplasm using gas chromatography-mass spectrometry (GC/MS) analysis as reported in trial 3.

*Total phenols content*

Total phenol content (TPC) was determinated in "CTRL" and in fresh spaghetti with 10% of purslane germoplasm, using *Folin-Ciocalteu* method *as* reported in trial 2.

*Antioxidant activity*

Cooked pasta samples with added purslane as well as control were submitted to analysis. Two different aliquots samples were analyzed. 5 g aliquot of each sample was extracted three times with 15 ml of 70% ethanol (v/v) distilled water for three times. After a cleanup step via centrifugation (10 min at 10,000 g, 4°C) and filtration through a Millex HV 0.45 μm filter (Millipore, Billerica, MA), the supernatants of each extraction cycle were recovered, combined, and used for the analysis of antioxidant activity. Antioxidant properties of the extracts from pasta samples were evaluated using two antioxidant assays: the *ABTS radical cation decolorization* assay and the *ferric reducing antioxidant potential* (*FRAP*). *FRAP* assay was performed according to Benzie and Stain (1996). The FRAP reagent was prepared daily by mixing (8:1:1, v/v) 0.3M acetate buffer pH 3.6,

10mM TPTZ and 20mM $FeCl_3$. 160 µl of FRAP reagent was mixed with 30 µl of water and 10 µl of sample or standard. The absorbance at 595 nm was measured after 30-min incubation at 37°C. The calibration curve was constructed using Trolox, an hydrophilic analog of vitamin E. Ferric reducing power was expressed as µmol Trolox equivalent (TE) per 100 g dry weight (DW). All measurements were repeated two times.

*ABTS* assay was performed according to Re et al. (1999). $ABTS^{\bullet+}$ was prepared by reaction of ABTS with potassium persulfate. Samples were analyzed at five different dilutions, within the linearity range of the assay, as described by (Gentile et al., 2016). The calibration curve was constructed using Trolox. The total antioxidant activity (TAA) was expressed as µmol TE 100 $g^{-1}$ DW. All measurements were repeated two times.

*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.

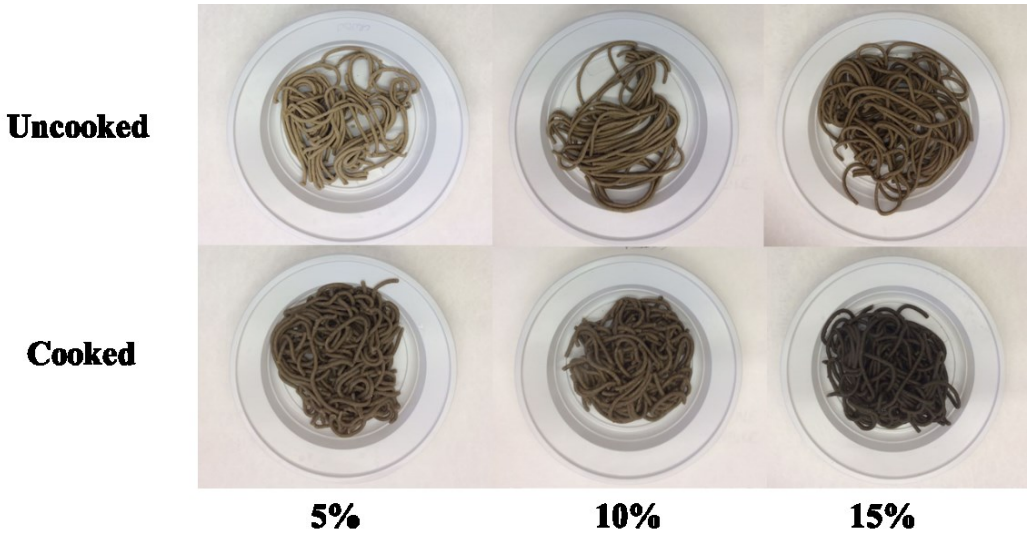
### 2.4.3 Results

In this trial the characteristics of fresh pasta samples produced with the use of commercial durum wheat and purslane collected in three different areas (Caltagirone, Cassibile and Santa Venerina) were evaluated. Each of these three population of purslane was added in three different concentrations (5%, 10% and 15% w/w).

*Sensorial Analysis of pasta*

For fresh pasta enriched in purslane a sensorial analysis have been made to understand how pasta characteristics are influenced by different concentrations and types of purslane. In figure 4.1 results about uncooked and cooked purslane enriched pasta on average of concentrations are reported. All samples had positive scores of Global taste and odour (Global T & O) and of Global judgment. Considering uncooked enriched pasta statistically significant difference among samples have been recorded. Samples enriched with "Caltagirone" purslane (Global judgment =6.35) and "Santa Venerina" purslane (Global judgment=6.39) resulted more appreciated than pasta enriched with "Cassibile" purslane (Global judgment = 5.23). After cooking process these difference were not

appreciable in cooked pasta samples, with values of global T & O > 7 for all the types of pasta, on average of concentrations.




**Uncooked and cooked spaghetti obtained using durum wheat flour and 5%, 10% and 15% of "Cassibile" purslane**

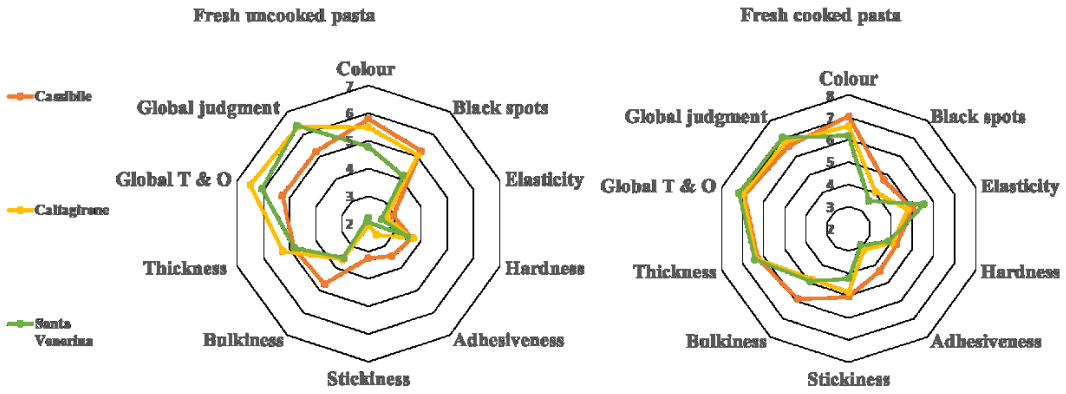



**Figure 4.1: Panel analyses of fresh uncooked and cooked pasta obtained with durum wheat flour and purslane from Caltagirone, Cassibile and S. Venerina on average of different concentrations.**

In tables 4.1 and 4.2 are reported results about uncooked and cooked pasta with different concentrations of purslane on average of origin. It is clear how the presence of black spots

increased considerably in spaghetti with purslane compared to “CTRL”. There was no statistically significant differences in uncooked and cooked pasta samples for bulkiness. Of course increasing concentration of purslane the colour of the dough became darker, while thickness decreased increasing the concentration of purslane. The scores of Global T&O and of the global judgment decreased considerably in pasta samples with 15% of purslane, especially in uncooked pasta, while pastas with 10% of purslane were appreciated, specially cooked: it had 7.30 and 7.03 as scores of Global T&O and of global judgment.

**Table 4.1: Panel analyses of uncooked fresh pasta obtained with durum wheat flour and purslane on average of origin. Different letters within the same column indicate statistical differences at P<0.05**

| Concentration (%) | Colour[a] | Black spots [b] | Elasticity [c] | Hardness [c] | Adhesiveness [c] | Stickiness [c] | Bulkiness [c] | Thickness [c] | Global T & O [d] | Global judgment [d] |
|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 3.00 c | 0.20 c | 4.80 a | 3.60 a | 4.40 a | 3.40 a | 4.00 a | 6.20 a | 6.80 a | 6.60 a |
| **5** | 5.40 b | 5.80 b | 2.87 b | 3.80 a | 2.47 b | 2.20 ab | 4.20 a | 4.93 ab | 6.43ab | 6.87 a |
| **10** | 5.93 b | 6.33ab | 1.73 c | 3.87 a | 1.93 b | 2.13 ab | 4.20 a | 4.87 ab | 5.73 b | 5.93 a |
| **15** | 7.10 a | 6.90 a | 1.40 c | 3.20 a | 1.70 b | 1.70 b | 3.40 a | 3.90 b | 4.80 c | 4.55 b |
| **Means** | **5.36** | **4.50** | **2.70** | **3.62** | **2.63** | **2.36** | **3.95** | **4.98** | **5.94** | **5.99** |

[a]1 very clear - 9 very dark, [b]1 absence of black spots, 9 lots of black spots
[c] 1 low sensation and 9 high sensation; [d] 1 extremely unpleasant, 9 to extremely pleasant

**Table 4.2: Panel analyses of cooked fresh pasta obtained with durum wheat flour and purslane on average of origin. Different letters within the same column indicate statistical differences at P<0.05**

| Concentration (%) | Colour[a] | Black spots [b] | Elasticity [c] | Hardness [c] | Adhesiveness [c] | Stickiness [c] | Bulkiness [c] | Thickness [c] | Global T & O [d] | Global judgment [d] |
|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 3.60 c | 0.40 b | 5.40 a | 4.20 a | 4.40 a | 5.40 a | 5.40 a | 7.20 a | 7.30 a | 7.30 a |
| **5** | 6.33 b | 5.60 a | 5.53 a | 4.00 a | 3.33 a | 4.40 a | 5.60 a | 6.33 b | 7.87 a | 7.90 a |
| **10** | 8.00 a | 5.40 a | 4.93 a | 3.93 a | 2.87 a | 4.53 a | 4.40 a | 6.00 b | 7.30 a | 7.03 a |
| **15** | 8.50 a | 5.00 a | 4.60 a | 4.10 a | 3.30 a | 4.50 a | 5.40 a | 5.90 b | 5.65 b | 5.05 b |
| **Means** | **6.61** | **4.10** | **5.12** | **4.06** | **3.48** | **4.71** | **5.20** | **6.36** | **7.03** | **6.82** |

[a]1 very clear - 9 very dark, [b]1 absence of black spots, 9 lots of black spots
[c] 1 low sensation and 9 high sensation; [d] 1 extremely unpleasant, 9 to extremely pleasant

*Pasta colour evaluation*

In table 4.3 are reported the colour indices of spaghetti on average of the three populations of purslane; how is possible to see, the brightness of pasta samples decreased increasing the concentrations of purslane, both in uncooked and cooked samples and there was a significant differences among all samples. L* index in uncooked samples ranged from 51.00 (15% of purslane) to 79.17 ("CTRL"), while in cooked samples it ranged from 28.78 (15% of purslane) to 65.79 ("CTRL"). It is also evident that the brightness decreased considerably after cooking process. Also yellow index (b*) decreased increasing the concentration of purslane, both in uncooked and cooked samples.

**Table 4.3: L*, a*, b*in uncooked and cooked fresh pasta enriched in purslane on average of the three populations. Different letters within the same column indicate statistical differences at P<0.05.**

| | Uncooked pasta | | | Cooked pasta | | |
|---|---|---|---|---|---|---|
| **Concentration** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **0%** | 79.17 a | -1.23 d | 17.57 a | 65.79 a | -1.42 d | 15.00 a |
| **5%** | 63.17 b | 1.19 c | 16.77 b | 39.69 b | 2.86 a | 14.09 b |
| **10%** | 56.32 c | 1.40 b | 14.43 c | 33.35 c | 1.99 b | 9.79 c |
| **15%** | 51.00 d | 1.89 a | 13.61 d | 28.78 d | 1.52 c | 7.31 d |
| **Means** | **62.41** | **0.81** | **15.59** | **41.90** | **1.24** | **11.55** |

*Fatty acids content*

The fatty acids found in pasta samples were palmitic acid (satured acid), oleic acid (monounsatured acid) and the two essential polyunsatured fatty acids linoleic and linolenic. In figure 4.2 the results were graphed on average of the origins of purslane added in pasta samples, in order to understand how the content of every single acid change according the concentration of purslane and in order to see the quantities of fatty acids lost during the cooking process. The quantity of the palmitic acid ranged from 0.24 ("CTRL") to 0.41 mg $g^{-1}$ (15%) in uncooked samples and from 0.21 to 0.38 mg $g^{-1}$ in cooked samples; on average only the 7% of palmitic acid was degraded by cooking. Uncooked "CTRL" and uncooked pasta samples with 5% of purslane had the same content of oleic acid (0.18 mg $g^{-1}$) vs 0.22 (10%) and 0.26 (15%); on average about 14% of oleic acid was lost after cooking. The content of linoleic acid ranged from 0.63 to 1

mg $g^{-1}$ in uncooked samples and from 0.52 to 0.79 mg $g^{-1}$ in cooked samples; in pasta samples enriched with 5% of purslane a cooking loss of linoleic acid equal to 36%, greater compared to that registered in the other samples (20%) was registered. Of course the "CTRL" did not have the essential polyunsatured linolenic acid, which instead was present in enriched spaghetti: it ranged from 0.05 (5%) to 0.13 mg $g^{-1}$ (15%) in uncooked samples and from 0.03 to 0.11 mg $g^{-1}$ in cooked samples, thus degrading by 22%.

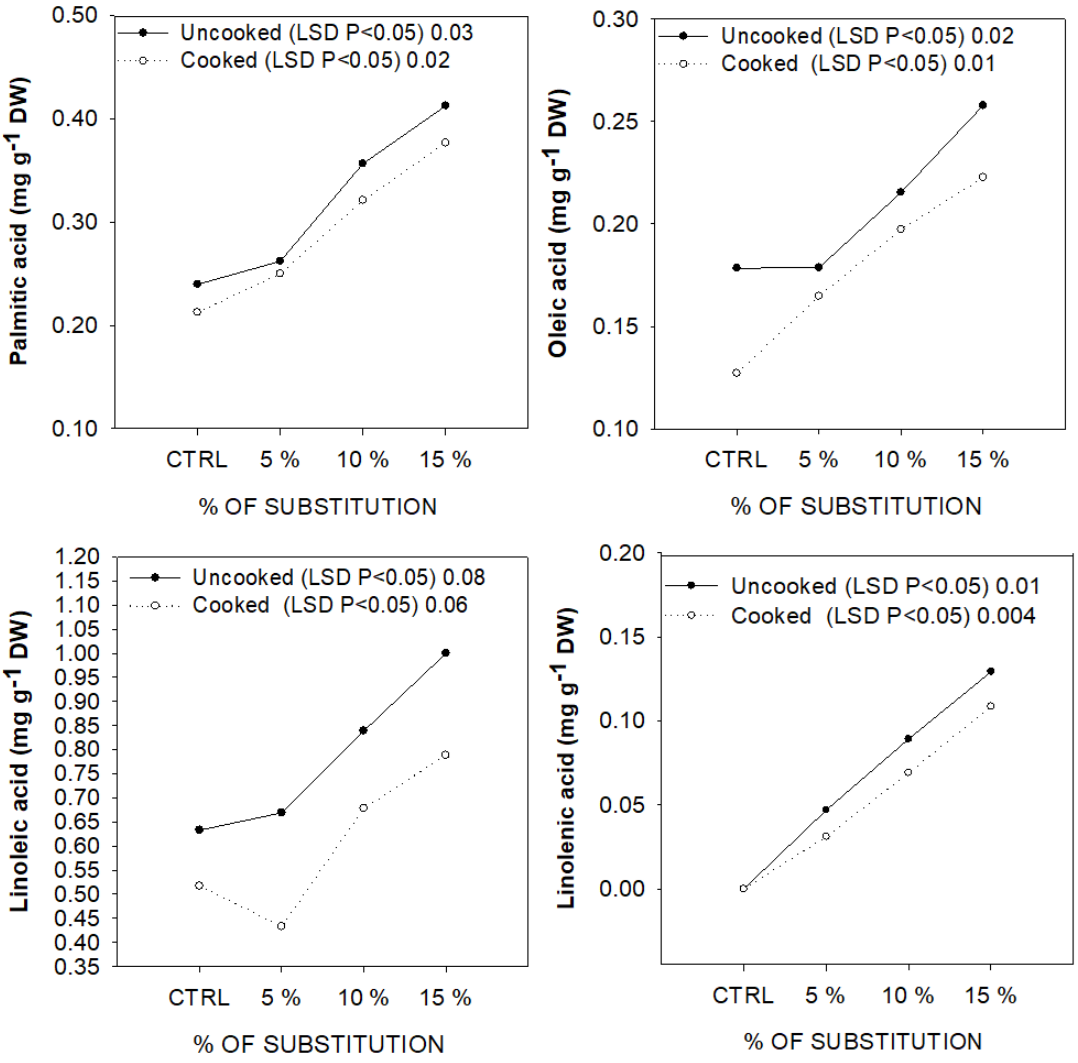



**Figure 4.2: Palmitic, oleic, linoleic and linolenic acids content (mg $g^{-1}$ D.W.) in uncooked and cooked fresh pasta enriched with different concentration of purslane on average of the three populations.**

The content of fatty acids in spaghetti was influenced not only by the % of substitution but also by the origin of purslane used to enrich pasta, how reported in figures 4.3, 4.4, 4.5 and 4.6.

Palmitic acid resulted increased in pasta samples with 10% and 15% of substitution for all the types of purslane added and, except for “S. Venerina” population, the cooking losses were very low at all concentrations. The same trend was observed for oleic and linoleic acids, where they resulted well maintained after cooking process in all samples with 10% of substitutions. All samples at 15% of substitution presented losses in oleic acid, except the “Caltagirone” population. The cooked spaghetti prepared with this purslane maintained also the linoleic acid amount at 15%. The linolenic content recorded in pasta samples is due exclusively to purslane addition. Cooked spaghetti prepared with “Caltagirone” and “Cassibile” purslane presented until 15% of substitution good levels of this important fatty acid. The “S. Venerina” population gave the worst results in terms of concentrations and cooking losses.

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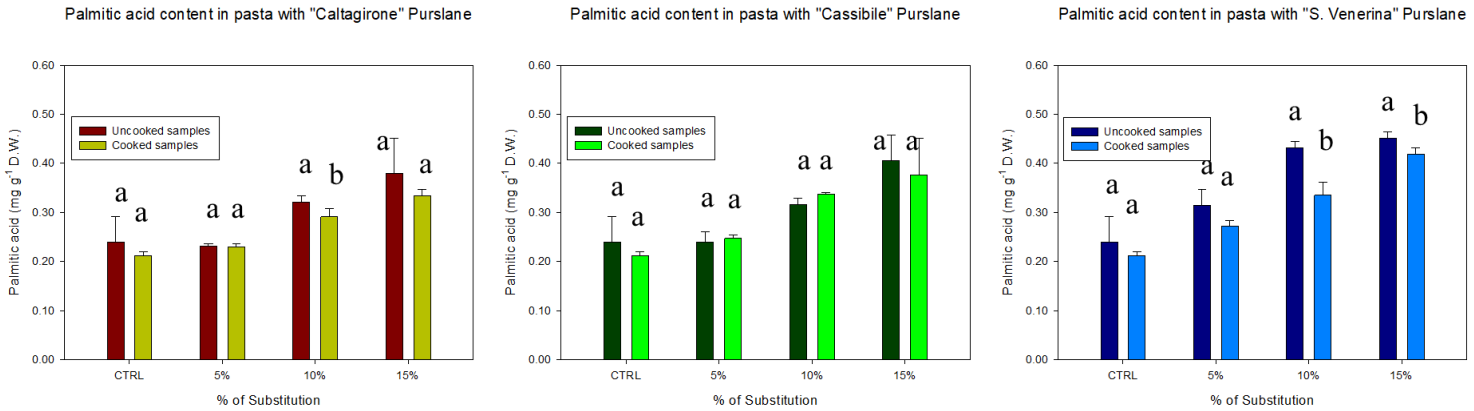



**Figure 4.3: Palmitic acid content (mg $g^{-1}$ D.W.) in uncooked and cooked fresh pasta enriched with different populations of purslane. Different letters within the same concentration indicate statistical differences at P<0.05**

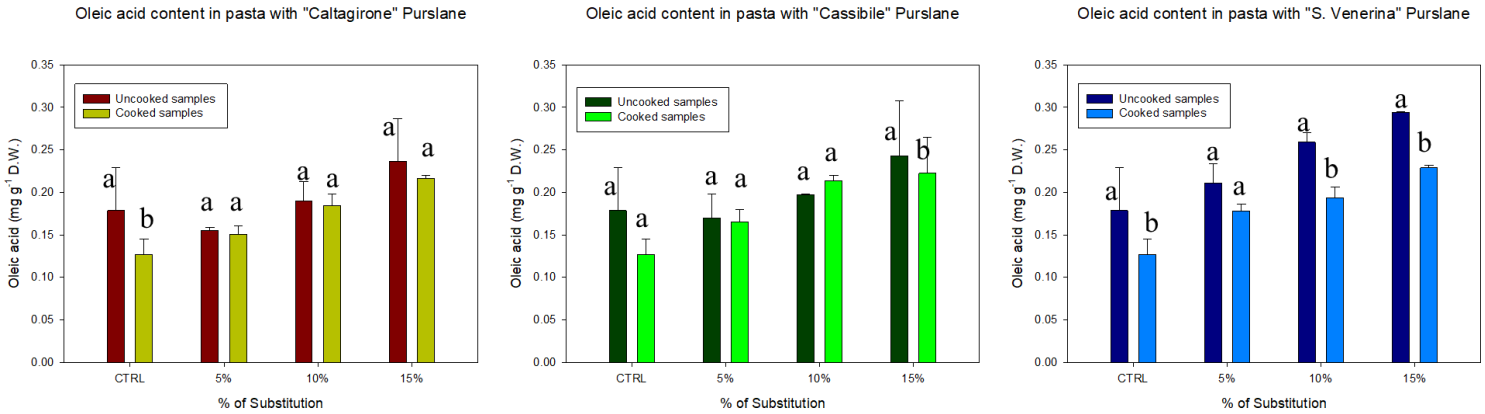



**Figure 4.4: Oleic acid content (mg $g^{-1}$ D.W.) in uncooked and cooked fresh pasta enriched with different populations of purslane. Different letters within the same concentration indicate statistical differences at P<0.05**

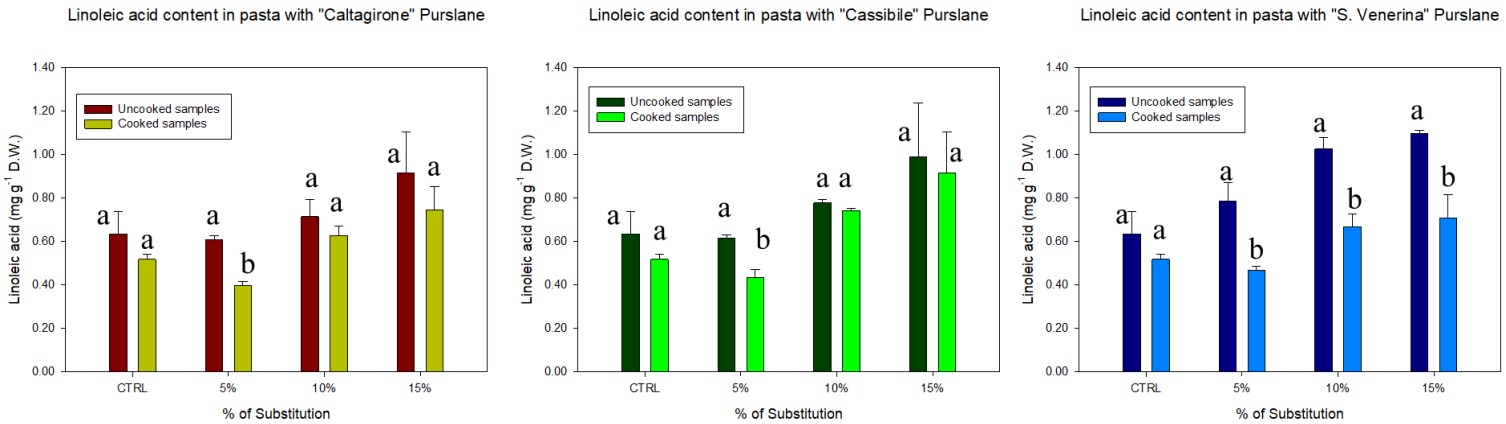



**Figure 4.5: Linoleic acid content (mg $g^{-1}$ D.W.) in uncooked and cooked fresh pasta enriched with different populations of purslane. Different letters within the same concentration indicate statistical differences at P<0.05**

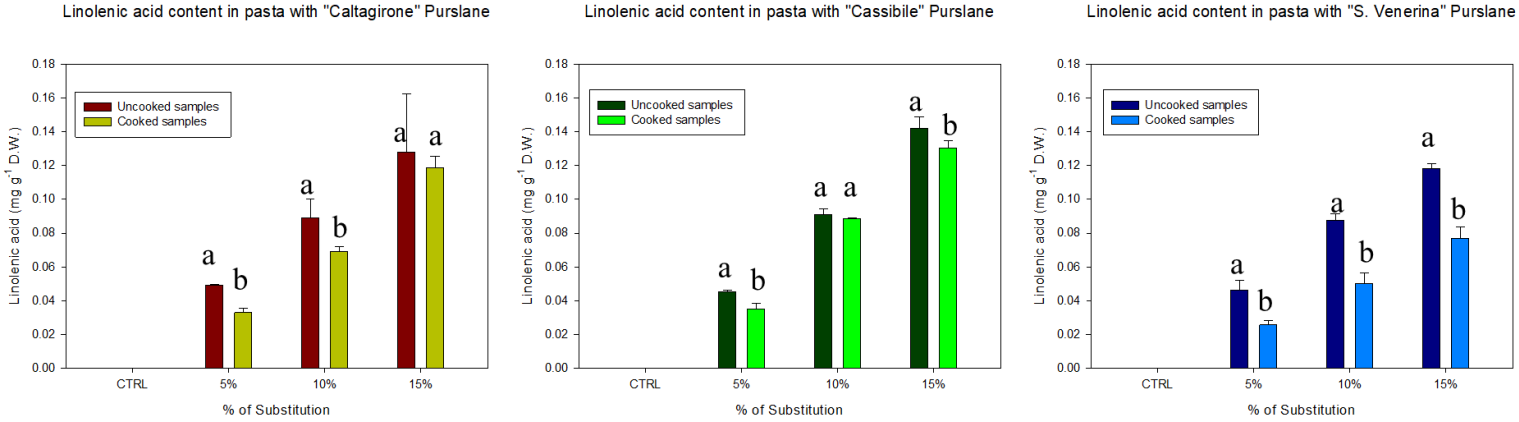



**Figure 4.6: Linolenic acid content (mg $g^{-1}$ D.W.) in uncooked and cooked fresh pasta enriched with different populations of purslane. Different letters within the same concentration indicate statistical differences at P<0.05**

*Antioxidant activity*

Both assays (*FRAP* and *ABTS* assays) highlighted the capacity of purslane to increase the antioxidant potential of enriched pasta samples vs "CTRL". There was no a significant difference among samples enriched with the three populations of purslane at 5%, while among all pasta samples with 10% and 15% of purslane, those with purslane of Cassibile had the higher antioxidant activity. In figure 4.7 are reported the results of *FRAP* assay and in figure 4.8 the results of *ABTS* assay: it is clear that the antioxidant capacity increased increasing the concentration of purslane. Although pasta samples enriched with 15% of purslane had the highest antioxidant capacity, they were not much appreciated during the panel analysis; so it is good to pay attention to the pasta samples with 10% of purslane, which were appreciated and however also had a good antioxidant potential. Regarding the results of *FRAP* assay: pasta with 10% of "Cassibile" purslane had an antioxidant activity of 547.7 μmol TE 100 $g^{-1}$ DW vs 165.7 μmol TE 100 $g^{-1}$ DW ("CTRL"). Pasta samples with 10% of "Caltagirone" and 10% of "S. Venerina" purslane had an antioxidant activity of 438 and 391 μmol TE 100 $g^{-1}$ DW respectively.

The results of *ABTS* assay had the same trend: also in this case pasta with 10% of "S. Venerina" purslane had the lowest antioxidant capacity; it was 132 μmol TE 100 $g^{-1}$ DW vs 152 (pasta with "Caltagirone" purslane) and vs 174 μmol TE 100 $g^{-1}$ DW (pasta with "Cassibile" purslane). Anyway all three enriched samples had of course antioxidant activity higher than the "CTRL" (46 μmol TE 100 $g^{-1}$ DW).

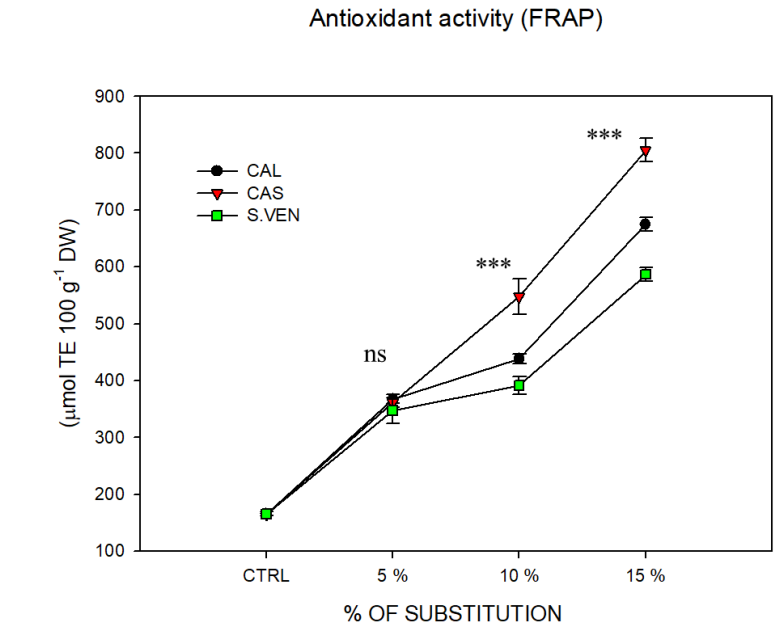



**Figure 4.7: Antioxidant activity (*FRAP* assay) of cooked dry pasta obtained with durum wheat flour and three populations of purslane at three different concentrations. (ns) indicates not statistically different. (***) significant at 0.001 probability level.**

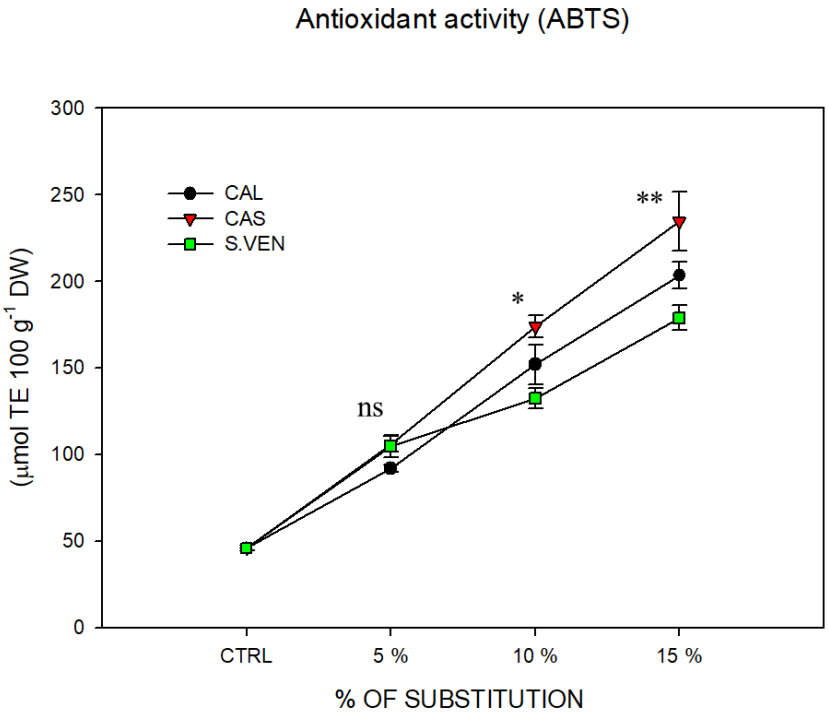



**Figure 4.8: Antioxidant activity (*ABTS* assay) of cooked dry pasta obtained with durum wheat flour and three populations of purslane at three different concentrations . (ns) indicates not statistically different. (**) and (*) significant 0.01 and 0.05 probability level**

*Total phenols content*

It was decided to evaluate the total phenols content only in fresh spaghetti with 10% of purslane substitution, since they were appreciated, while the pasta samples with 15% of substitution did not have a good results in sensorial analysis. The addition of purslane enriched uncooked "Caltagirone" and "S. Venerina" spaghetti with 59 mg $_{GAE}$ 100 $g^{-1}$ and "Cassibile" pasta with 62 mg $_{GAE}$ 100 $g^{-1}$ vs 10 mg $_{GAE}$ 100 $g^{-1}$ of "CTRL". On average of the population, the cooking process caused a phenol loss of 29%. Despite the cooking loss, the enriched cooked spaghetti had an higher TPC than CTRL (Figure 4.9).

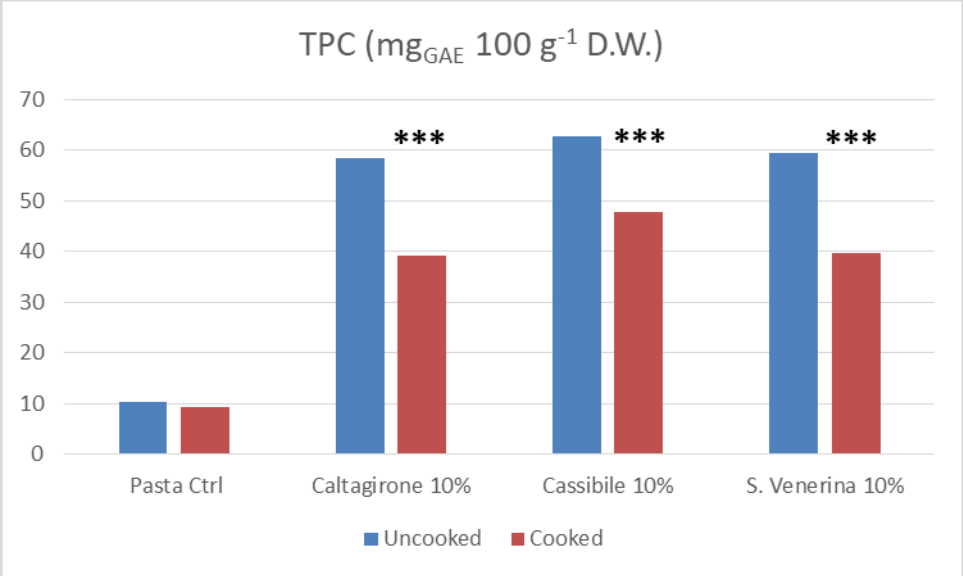



**Figure 4.9: TPC of uncooked and cooked fresh pasta obtained with durum wheat flour and 10% of purslane of Caltagirone, Cassibile and S. Venerina. (ns) indicates not statistically different. (***) significant at 0.001probability level.**

### 2.4.4 Conclusions

The sensorial analysis of uncooked and cooked fresh pasta with purslane from Caltagirone, Cassibile and Santa Venerina on average of concentrations showed that all samples had positive scores of Global judgment: the lower scores were in uncooked pasta enriched with "Cassibile" purslane compared to pastas enriched with "Caltagirone" and "Santa Venerina" purslane. About concentration the analysis showed that the scores of the global judgment decreased considerably in pastas with 15% of purslane, while pasta samples with 10% of purslane were appreciated. The brightness of pasta samples decreased increasing the concentrations of purslane, both in uncooked and cooked

samples. Chemical analysis highlighted that pasta enriched with purslane had the potentiality to be a functional food for polyunsatured fatty acids concentration and antioxidant activity. On average of the origins of purslane added in pasta samples, linoleic acid ranged from 0.63 to 1 mg $g^{-1}$ in uncooked samples and from 0.52 to 0.79 mg $g^{-1}$ in cooked samples (increasing the concentration of purslane). The essential polyunsatured linolenic acid, was detected in enriched spaghetti: it ranged from 0.05 (5%) to 0.13 mg $g^{-1}$ (15%) in uncooked samples and from 0.03 to 0.11 mg $g^{-1}$ in cooked samples. The influence of the origin of purslane used to enrich pasta was clear: "Cassibile" cooked spaghetti had the higher content of linoleic and linolenic acids that in cooked spaghetti with 10% of substitution was 0.75 and 0.09 mg $g^{-1}$ respectively.

The antioxidant capacity increased increasing the concentration of purslane and also in this case pasta samples with purslane of Cassibile resulted the best. Cooked pasta samples with 10% of "Cassibile" purslane had an antioxidant activity of 547.7 vs 165.7 μmol TE 100 $g^{-1}$ DW of the "CTRL" ( according the FRAP Assay) and an activity of 174 vs 46 μmol TE 100 $g^{-1}$ DW of the "CTRL" (according the ABTS assay). Considering that no references are available for the spaghetti enriched in purslane, this trial represent a good point to develop in a larger scale this "functional pasta", having identified the source of FA and the percentage of substitution, related to the chemical and antioxidant properties.

## 2.5 TRIAL 5: EVALUATION OF β-GLUCANS IN OAT AND CHARACTERISTICS OF PASTA WITH OAT ADDITION

### 2.5.1 Aim

The aims of this trial were: the extraction and evaluation of β-glucans in oat (*Avena sativa* L., variety named Genziana) and the production and evaluation of fresh pasta samples produced with the use of commercial durum wheat and oat at four different concentrations (5%, 10%, 20% and 40% w/w).

In this trial two experiments have been performed:

***Experiment A***

- Extraction of β-glucans from oat
- Evaluation and quantification of β-glucans in oat and in extracts.

***Experiment B***

- Production and evaluation of fresh pasta samples produced with the use of commercial durum wheat and oat at four different concentrations ( 5%, 10%, 20% and 40% w/w)
- Evaluation and quantification of β-glucans in produced spaghetti.

### 2.5.2 Materials and methods

For pasta making, commercial durum wheat and oat at four different concentrations (5%, 10%, 20% and 40% w/w) were used. Pasta without oat was used as control ("CTRL"). Two protocols for the extraction of β-glucans were tested (*ethanol* and *enzymatic* extraction). On the pasta samples obtained were evaluated: colour (Minolta colorimeter CR, 400), sensorial qualities (Panel test) and β-glucans content.
The extraction and the evaluation of β-glucans were conducted in the laboratory of Molecular Plant Biology, at the Catholic University of Leuven (KU Leuven), where I have been for six months for my abroad stage.

*Preparation of oat*

Oat (*Avena sativa* L., variety named "Genziana") was purchased in Apsovsementi s.p.a. (Voghera, Pavia). It was washed with tap water, dried at low temperature in a

termoventilated oven (30°C), milled by food processor (mod. IKA) in fine powdered and kept in ermetic bottle until use.

*Pasta making*

Fresh pasta with commercial durum wheat and wholemeal oats (*Avena sativa* L., variety named "Genziana") was produced. Oat was substituted at four different concentrations (5%, 10%, 20% and 40% w/w). 500 g flour (durum wheat + oats) were prepared by Pastamatic ARIETE 1591 and mixed for 10.5 min, adding 220 mL of distilled water to obtain a dough with 44% moisture content. The dough was extruded into a spaghetti shape.

*Pasta colour evaluation*

Dry pasta colour was recorded before and after cooking with the use of a Minolta colorimeter CR, 400 as seen in trial 1.

*Sensorial Analysis of pasta*

Fresh-extruded uncooked and cooked spaghetti were submitted to a panel of 10 trained tasters (five men and five women, aged between 27 and 60 years) in order to evaluate the sensory attributes as reported in trial 4.

*Extraction of β-Glucans*

Two different protocols for the purification of β-glucans were tested: ethanol extraction and enzymatic extraction. The ethanol extraction was made how described by Sayar et al. (2005) : in 15 g of oat flour 175 mL of 82% (v/v) ethanol (EtOH) were added and refluxed for 2 h at 85°C. It was centrifuged at 5000g for 10 min and the residue washed with 50 mL of 95% (v/v) EtOH and centrifuged again. The residue is dry overnight at 40°C and after transferred into bottles, where 150 mL of water were added and the β-glucans were extracted by placing the bottles in shaking water bath at 47°C for 3 h. The solution was centrifuged at 5000g for 10 min, the supernatants were taken and 0.3 mL of 10% of sodium azide was added. Subsequently 300 mL of 95% (v/v) EtOH were added and the oat-gum precipitated; the precipitates were removed by filtration and solubilized in water at 85°C (until complete solubilisation). In the end, to have purified β-glucans, it is freeze-dried.

The enzymatic extraction was performed according the work of Ahmad et al. (2010), with some modifications: 10 g of sieved whole oat flour was refluxed with 80 mL of 80% ethanol for 3 hours, then mixed with NaOH (1M) in 1:50 ratio. After it was stirred for 90 min, centrifuged at 15 000 g for 15 min at 20°C and the pH of supernatant was adjusted at 7 with citric acid. Later, the sample was treated with heat stable α- amylase (375 U), incubated at 70°C for 3 hours and centrifuged at 15 000 g for 20 min at 40°C. The supernatant was treated with protease enzyme, incubated at 60°C for 3 hours and centrifuged at 22 000g for 20 min at 4°C. The supernatant was mixed with ethanol (80%) in ratio 1:2, held for 15 min and centrifuged at 3 500 g at 4°C. At the end, the obtained pellet was dried in oven.

*Evaluation and quantification of β-Glucans*

The evaluation of β-glucans in oats, extracts and produced pastas were made according to the method of De Santis et al. (2018). Flours were treated with enzyme endo 1,3(4) glucanase ("lichenase"). Digestion of β-glucans with lichenase releases two major glucooligosaccharides (GOS) comprising three (G3) and four (G4) glucose units. The sum of these fragments provides a good estimate of total β-glucans while the ratio of G3:G4 GOS provides information on the structure (relative numbers and distributions of β1-3 and β1-4 bonds). 1 ml of 80% (v/v) ethanol was added to 100 mg of flour and heated in a 95 °C water bath for 10 min to inactivate endogenous enzymes present in the samples. After centrifugation (10 000 g for 5 minutes RT), the residue was washed with 80% (v/v) ethanol and then with 95% (v/v) ethanol to remove free sugars, and dried using a Speedvac centrifugal evaporator. The dried powder was resuspended in 1 ml of water containing 10 U of lichenase and incubated at 40 °C for 16 h with continuous rotation in a Thermomixer. Samples were then centrifuged at 13 400 g for 5 min, the supernatants boiled for 30 min to inactivate hydrolases. Finally, oat flour was diluted 1:13 and pastas samples were diluted 1:20 with water and analysed by high performance anion-exchange chromatography coupled with integrated pulsed amperometric detection (Dionex-ICS $5000^{+}$, USA) carried out with a carbohydrate quadruple waveform where Ag/AgCl reference electrode and an Au electrode were used. The column temperature, sampler temperature and run time were set to 32°C, 15°C and 10 °C, respectively. 6 µL sample was injected through a Dionex CarboPac PA-100 guard column (2 x 50 mm) coupled to a CarboPac PA-100 anion exchange column (2 x 250 mm) which had been equilibrated with an isocratic flow pattern (Mobile phase A, 90 mM NaOH, 0.25 ml/min) for 10 min.

A known concentration of both GOS G3 and G4 was used as reference standard to identify and quantify the GOS G3 and G4 originated from the action of the enzyme lichenase on the samples.

### 2.5.3 Results

*Experiment A*

*Extraction, evaluation and quantification of β-glucans*

In this experiment β-glucans were extracted and then evaluated and quantified in oat flour and in obtained extracts.

It was not easy to purify β-Glucans. Both protocols (ethanol and enzymatic) did not give a well purified β-glucans extract and the yield was really low, in particular for ethanol extraction: It had a yield of only 0.84% vs 22% of the enzymatic protocol. But the ethanol extract seemed to have a higher content of β-glucans than the enzymatic one, as showed below.

The sum of G3 and G4 GOS (released by digestion of β-glucans with lichenase) provides a good estimate of total β-glucans while their ratio provides information on the structure (relative numbers and distributions of β1-3 and β1-4 bonds). In figure 5.1 is reported the chromatogram of oat flour treated with lichenase compared with that of the two glucooligosaccharides G3 and G4.

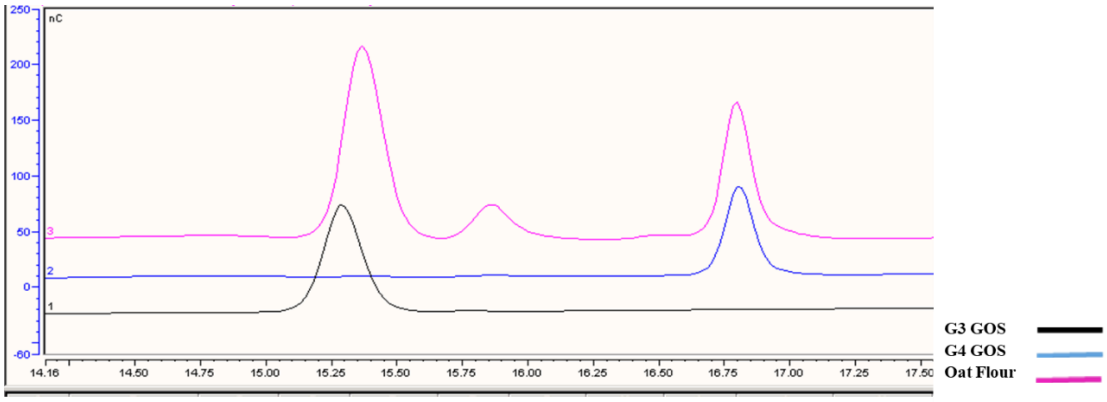



**Figure 5.1: Chromatograms of both GOS G3 and G4 (used as reference standard) and of oat Flour digested by lichenase.**

In table 5.1 it is possible to see that the ratio G3/G4 did not change between the oat flour and ethanol extract (1.82), while it increased in enzymatic extract (2.15).

The content of β-glucans in oats flour was 5.39 mg $g^{-1}$; ethanol and enzymatic extracts had 63.65 and 38.34 mg $g^{-1}$ of β-glucans respectively, so β-glucans in ethanol extract were more purified than in the enzymatic one (figure 5.2).

**Table 5.1: Ratio area's peaks of the G3 to G4 GOS and G3 and G4 GOS quantities released by lichenase digestion of β-glucans in oats flour, ethanol extract and enzymatic extract. Different letters within the same column indicate statistical differences at P<0.05.**

| Samples | Ratio area G3/G4 (nc*min) | G3 (mg $g^{-1}$) | G4 (mg $g^{-1}$) |
|---|---|---|---|
| **Oats flour** | 1.82 b | 3.02 c | 2.37 c |
| **Ethanol extract** | 1.82 b | 35.63 a | 28.01 a |
| **Enzymatic extract** | 2.15 a | 22.18 b | 16.16 b |
| **Means** | **1.93** | **20.28** | **15.51** |

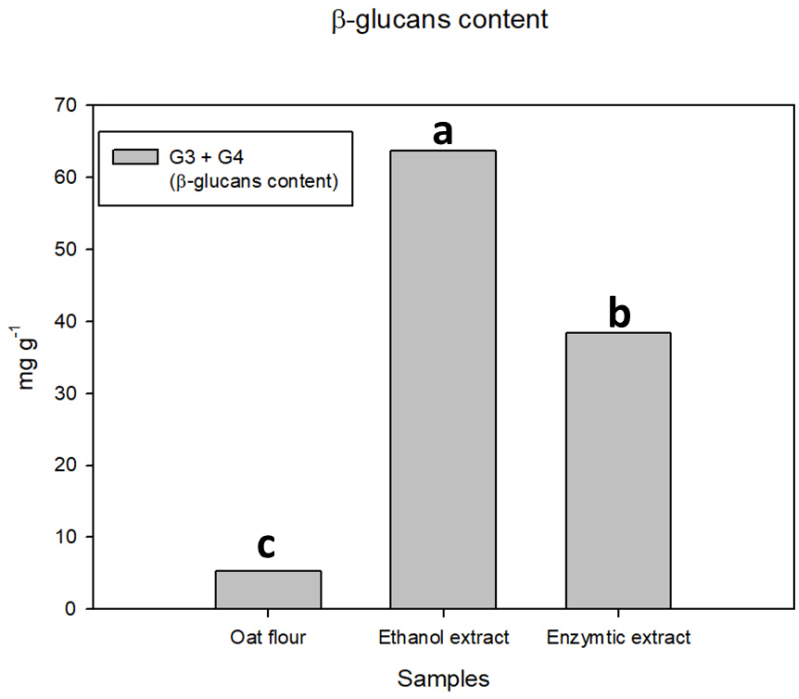


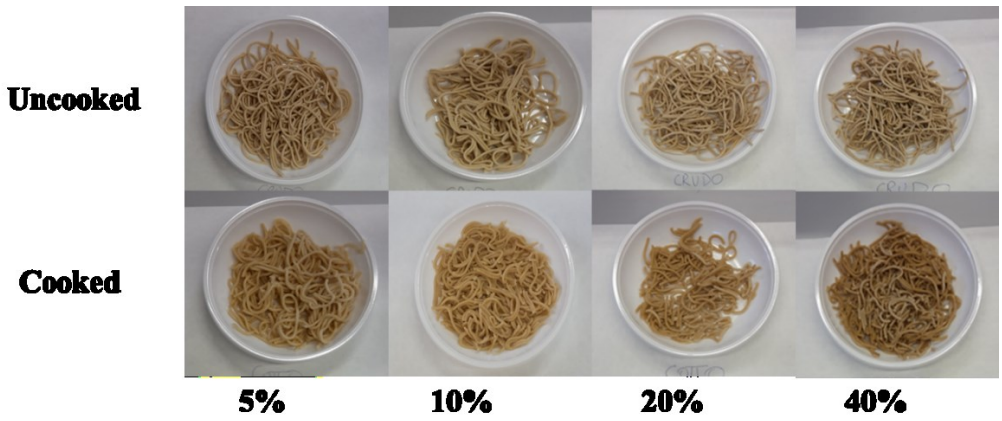

**Figure 5.2: sum of G3 and G4 GOS quantities released by lichenase digestion of β-glucans in oats flour, ethanol extract and enzymatic extract. Different letters indicate statistical differences at P<0.05.**

*Experiment B*

In this experiment fresh pasta samples were produced with the use of commercial durum wheat and oat at three different concentrations (5%, 10%, 20% and 40% w/w). Subsequently in produced spaghetti were evaluated sensorial characteristics and β-glucans content.

*Sensorial Analysis of pasta*

For fresh pasta enriched in oats a sensorial analysis have been made to understand how pasta characteristics are influenced by different concentrations of oat.




**Uncooked and cooked spaghetti obtained using durum wheat flour and 5%, 10%, 20 and 40% of oat.**

In table 5.2 are reported results about uncooked samples: among all pasta samples there was no a significant difference for colour, presence of black spots, stickiness, bulkiness and thickness; elasticity and adhesiveness decreased increasing the concentration of oat, while hardness increased increasing the concentration of oat. The score of Global judgment was 7.4 in all samples except for pasta with the higher content of oat (40%) that had a score of 5.0.

**Table 5.2: Panel analyses of uncooked fresh pasta obtained with durum wheat flour and oat. Different letters within the same column indicate statistical differences at P<0.05**

| Concentration (%) | Colour[a] | Black spots [b] | Elasticity [c] | Hardness [c] | Adhesiveness [c] | Stickiness [c] | Bulkiness [c] | Thickness [c] | Global T & O [d] | Global judgment [d] |
|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 3.4 a | 3.2 a | 3.8 a | 3.6 b | 3.4 a | 1.8 a | 3.4 a | 6.0 a | 8.2 a | 7.4 a |
| **5** | 2.6 a | 3.0 a | 2.4 ab | 3.8 b | 2.8 ab | 2.0 a | 3.0 a | 5.2 a | 8.0 a | 7.4 a |
| **10** | 2.6 a | 2.4 a | 3.6 ab | 4.4 a | 2.8 ab | 2.2 a | 3.8 a | 5.8 a | 7.8 a | 7.4 a |
| **20** | 3.2 a | 4.0 a | 2.8 ab | 5.0 a | 1.8 ab | 1.6 a | 4.0 a | 5.6 a | 7.4 a | 7.4 a |
| **40** | 3.4 a | 3.4 a | 2.0 b | 4.4 a | 1.4 b | 1.8 a | 4.4 a | 4.2 a | 5.4b | 5.0 b |
| **Means** | **3.04** | **3.2** | **2.92** | **4.24** | **2.44** | **1.88** | **3.72** | **5.36** | **7.36** | **6.92** |

[a]1 very clear - 9 very dark, [b]1 absence of black spots, 9 lots of black spots
[c] 1 low sensation and 9 high sensation; [d] 1 extremely unpleasant, 9 to extremely pleasant

Table 5.3 shows the results about cooked spaghetti: the scores of adhesiveness, thickness and stickiness decreased significantly increasing the concentration of oat, while the presence of black spots increased; hardness did not change significantly. Global judgement was 8.15 on average of pasta samples with 0, 5, 10 and 20% of oat and it decreased to 6 in pasta with 40% of oat. Anyway, all samples had positive scores of Global T & O and of Global judgment.

**Table 5.3: Panel analyses of cooked fresh pasta obtained with durum wheat flour and oat. Different letters within the same column indicate statistical differences at P<0.05**

| Concentration (%) | Colour[a] | Black spots [b] | Elasticity [c] | Hardness [c] | Adhesiveness [c] | Stickiness [c] | Bulkiness [c] | Thickness [c] | Global T & O [d] | Global judgment [d] |
|---|---|---|---|---|---|---|---|---|---|---|
| **0** | 2.5 b | 2.6 b | 7.2 a | 4.6 a | 5.0 a | 6.6 a | 4.2 b | 7.8 a | 8.6 a | 8.4 a |
| **5** | 3.0 b | 2.8 b | 5.2 b | 5.4 a | 5.0 a | 6.0 | 3.8 | 6.4 ab | 8.2 a | 8.6 a |
| **10** | 2.8 b | 2.2 b | 4.8 b | 5.4 a | 4.4 | 6.2 | 4.8 | 6.6 ab | 8.4 a | 7.8 a |
| **20** | 4.8 | 4 ab | 5.2 b | 5.2 a | 5.2 a | 4.2 | 5.8 a | 5.6 bc | 7.8 a | 7.8 a |
| **40** | 5.2 a | 5.0 a | 2.8 c | 4.0 a | 3.2 b | 3.2 c | 6.0 a | 4.6 c | 6.0 b | 6.0 b |
| **Means** | **3.66** | **3.32** | **5.04** | **4.92** | **4.56** | **5.24** | **4.92** | **6.2** | **7.8** | **7.72** |

[a]1 very clear - 9 very dark, [b]1 absence of black spots, 9 lots of black spots
[c] 1 low sensation and 9 high sensation; [d] 1 extremely unpleasant, 9 to extremely pleasant

*Pasta colour evaluation*

The brightness decreased with the addition of oat, both in uncooked and cooked spaghetti; but while in uncooked pasta samples enriched in oat L* was the same, regardless of the percentage of substitution (74.78 vs 79.20 “CTRL”), the same did not happen in cooked spaghetti: it was 65.38 (5%), 63.97 (10%), 60 (20% and 40%) vs 68.72 of the “CTRL”. The yellow index (b*) in uncooked pasta decreased increasing the concentration of oat: it was 19.21 in the “CTRL”, 18 in samples with 5% and 10% of substitution and 16 in spaghetti with 20% and 40% of substitution. In cooked spaghetti the opposite happened and b* increased increasing the concentration of oat: it was 18 in pasta with 20% and 40% of oat vs 17 of the other three samples. The adding of oat determined also an increase of the red index (a*) (table 5.4).

**Table 5.4: L*, a*, b*in uncooked and cooked pasta obtained with durum wheat flour and oat. Different letters within the same column indicate statistical differences at P<0.05**

| | Uncooked pasta | | | Cooked pasta | | |
|---|---|---|---|---|---|---|
| **Concentration** | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **0%** | 79.10 a | 0.89 b | 19.21 a | 68.72 a | 0.87 c | 17.15 b |
| **5%** | 75.84 b | 1.60 a | 17.73 b | 65.38 b | 0.87 c | 17.35 b |
| **10%** | 74.16 b | 1.84 a | 18.33 b | 63.97 c | 1.12 b | 17.03 b |
| **20%** | 74.09 b | 1.80 a | 16.02 c | 59.55 d | 2.61 a | 18.92 a |
| **40%** | 75.01 b | 1.76 a | 16.25 c | 60.44 d | 2.67 a | 17.99 a |
| **Means** | **75.64** | **1.58** | **17.51** | **63.61** | **1.63** | **17.89** |

*Evaluation and quantification of β-glucans in produced spaghetti*

In figure 5.3 is reported the analysis of β-glucans in uncooked and cooked pasta samples enriched with different concentrations of oat. The content of G3 and G4 GOS was not statistically different between uncooked and cooked pasta in all the concentrations of oats, so the cooking process did not cause loss of β-glucans. In pasta samples the content of β-glucans increased increasing the content of oats, how it is possible to see in the figure 5.4 that shows the chromatograms of cooked samples: the first peak is G3 GOS, the second is G4 GOS. In the average of uncooked and cooked spaghetti, the content of β-glucans was 2.05 mg $g^{-1}$ in pasta samples without oat, 2.30 mg $g^{-1}$ in spaghetti with 5% of substitution, 2.73 mg $g^{-1}$ in pasta samples with 10% of substitution, 3.80 g $mg^{-1}$ in those with 20% of oat and 5.70 mg $g^{-1}$ in spaghetti with the higher content of oat (40%).

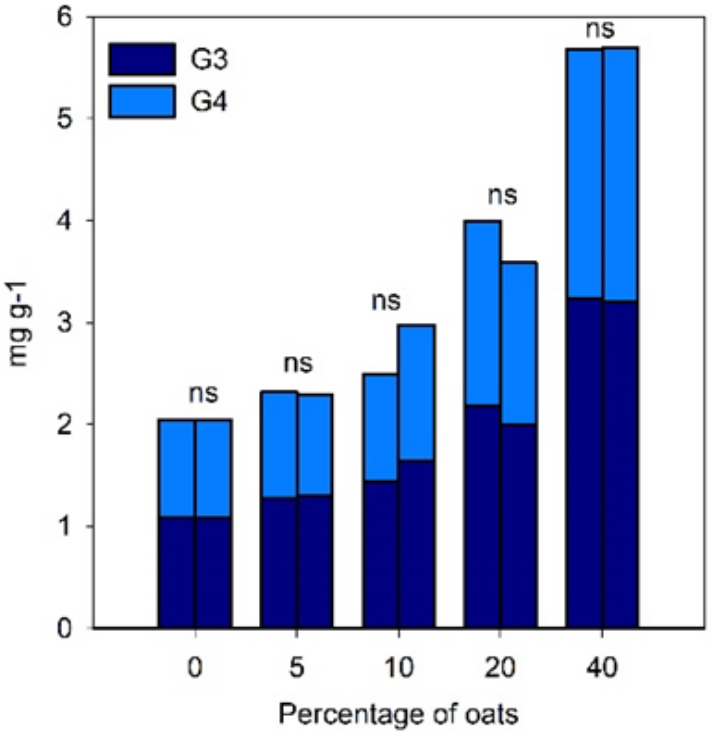



**Figure 5.3: Sum of G3 and G4 GOS quantities released by lichenase digestion of β-glucans in pasta samples enriched with different concentration of oats. Left bars indicates cooked pasta, right bars indicates uncooked pasta. "ns" indicates not statistically different between uncooked and cooked pasta within the same concentration of oats.**

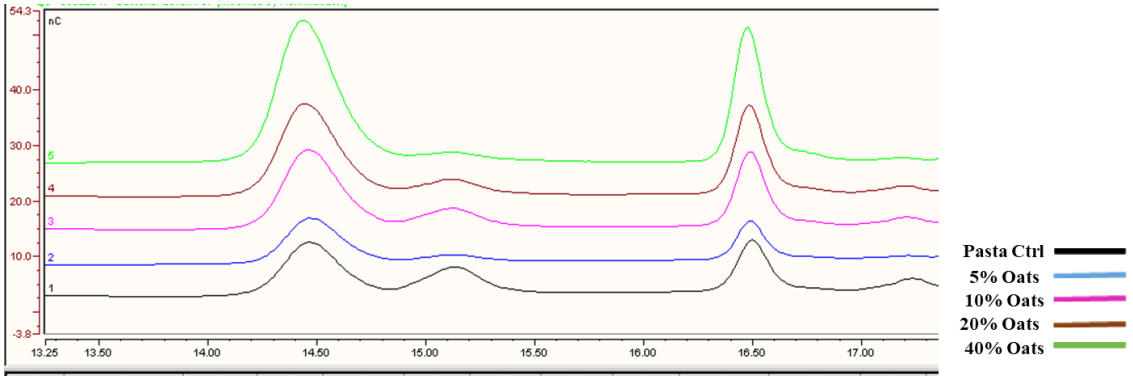



**Figure 5.4: Chromatograms of cooked spaghetti with different concentration of oats. Digestion of β-glucans with lichenase releases two major GOS: G3 (first peak) and G4 (second peak).**

### 2.5.4 Conclusions

The sensorial analysis of fresh pasta enriched with oat showed that the percentage of addition could influence the organoleptic characteristics of this product, influencing the consumer acceptability. The score of Global judgment ranges from 7.4 (0% of oats) to 5.0 (40% of oats) in uncooked pasta and from 8.4 to 6.0 in cooked pasta. Anyway all samples had positive scores of Global T & O and of Global judgment. The substitution of oat influenced also the colour indices of spaghetti: there was a decrease of brightness (L*) and an increase in redness (a*); the same result concerning the colour was obtained by Piwińska *et al.* (2015).

Two different protocols were tested to extract β-glucans: the ethanol trial had a yield of only 0.84%, the enzymatic protocol had an yield of 22%. But in the other hand, β-glucans in ethanol extract were more purified than in the enzymatic one. So probably, it is easier and cheaper to add directly oats to pasta rather than the extracted beta glucans, also because the quantitative analysis of β-glucans in enriched pastas showed that the content of β-glucans increased increasing the content of added oats and there was no a loss of β-glucans with the cooking process. The structural analysis of β-glucans in oat flour recorded a ratio G3:G4 GOS of 1.82, this result is higher than reported by Khan *et al.* (2016)*:* they analysed two different variety of oat, Avon and Sargodha-81 and registered a ratio of 1.44 and 1.49 respectively. Our results are closer to those obtained by Colleoni-Sirghie *et al.* (2003)*,* that observed a ratio ranged from 1.6 to 1.7 according the studied oat line.

A lot of studies showed the capacity of oat to decrease LDL blood levels: in a pilot study, it was considered the effects on health of 30 days intake of pasta enriched with 6% of β-glucan, demonstrating a significant decrease of low density lipoprotein (LDL)-cholesterol, interleukin-6 and advanced glycation end-products, and confirming the capacity of β-glucans intake to lower oxidative stress and inflammatory status (Barera et al., 2016); considering this and also the good sensorial results obtained in this trial, spaghetti enriched in oat have all the potentiality to be a good nutraceutical product.

# 3. CONCLUSIONS

The obtained results of the different trials showed:

1) Spaghetti enriched with 40% of two types of lentil, commercial lentil and Sicilian population of Ragusa, were less appreciated than the "CTRL" especially in dry samples and after cooking process. But thanks to CMC was possible to improve the sensorial characteristics and the cooking quality. Out of concentrations (0%, 40% and 40% + CMC), there was no significant change among spaghetti enriched with commercial lentil and spaghetti with lentil of Ragusa. The sensorial analysis on average of two accessions of lentil highlighted that the use of CMC had a clear positive effect on fresh and dry cooked spaghetti. In particular, it did not influence colour, odour and taste but it improved elasticity, firmness, bulkiness, adhesiveness and the overall quality. The distribution of the amino acids resulted similar in both the types of grains, especially for essential amino acids. The addition of Ragusa lentil improved the contents in Lys, Val, Ile, Gly and Asp, while the amounts in Leu, Thr, Ser and Lys resulted improved in spaghetti enriched by commercial lentil. The use of CMC increased mainly the content of Lys (+41%) in Ala (+89%) vs the "CTRL".

2) The evaluation of cladodes collected in different sites of Sicily showed that the origin influenced the colour and the total phenols content. Among the different germplasm evaluated, cladodes collected in Sortino, resulted more suitable to enrich spaghetti. Dry spaghetti produced with whole-meal durum wheat "Senatore Cappelli" and cladodes dry flours did not obtained good results as regards the sensorial analysis, probably due to an excess of fiber. The use of commercial durum wheat improved the sensorial quality of cooked spaghetti that had scores above the acceptability threshold. The cladodes addition enriched pasta in phenols and fiber. The total phenols content in spaghetti with 10% of cladodes was about ten times higher compared to that of the "CTRL". While the phenols were not lost with cooking, the same can not be said for the fiber; so the next step was to use food additives (Agar and CMC) to improve further sensorial characteristics of pasta with 10% of cladodes and to maintain fiber after cooking. The additives improved the overall quality of cooked spaghetti and CMC maintained the

amounts of fiber after cooking: the amount of fiber increased up to 65-70% adding 10% of cladodes vs “CTRL”.

3) Chemical composition of three purslane populations (“Caltagirone”, “Cassibile” and “S. Venerina”) under different Mediterranean environmental conditions were evaluated over two years. Total phenols content, the antioxidant activity and the polyunsatured fatty acids resulted influenced by genotypes. Population “Cas” had the higher content of total phenols and higher antioxidant activity than the other two populations (“Cal” and “S.Ven”). The most abundant unsatured fatty acids are linoleic and linolenic acids and among the populations, also in this case “Cas” population reported the highest contents of these fatty acids. The years of collection influenced only the partitioning of the plants and saturated fatty acid content.

4) In trial 4 the sensorial and chemical analysis of uncooked and cooked fresh pasta with purslane from Caltagirone, Cassibile and Santa Venerina at three different concentration (5%, 10% and 15%) were performed. On average of concentrations all samples had positive scores of Global judgment; about concentration the analysis showed that the scores of the global judgment decreased considerably in pastas with 15% of purslane, while pasta samples with 10% of purslane were appreciated. The brightness of pasta samples decreased increasing the concentrations of purslane, both in uncooked and cooked samples. Chemical analysis highlighted that the addition of purslane gave to pasta samples the characteristics to be a functional food, thanks to polyunsatured fatty acids concentration, antioxidant activity and total phenols content. The influence of the origin of purslane used to enrich pasta was clear: “Cassibile” cooked spaghetti had the higher content of linoleic and linolenic acids and the higher antioxidant activity.

5) Regarding the extraction of β-glucans from oat, the ethanol trial had a yield of only 0.84%, lower than that of the enzymatic protocol (yield of 22%). But β-glucans in ethanol extract were more purified than in the enzymatic one. The qualitative analysis of β-glucans in oat flour recorded a ratio G3:G4 GOS of 1.82, but this data is linked to the variety used. All fresh pasta samples enriched with

oat (at 5%, 10%, 20% and 40%) had positive scores of Global T & O and of Global judgment, even if increasing the concentration of oat the scores decreased. The substitution of oat caused a decrease of brightness (L*) and an increase in redness (a*). The quantitative analysis of β-glucans in enriched pastas showed that the content of β-glucans increased increasing the content of the added oats and there was no a loss of β-glucans with the cooking process.

The good amount of data collected during this research period contributes to improve knowledge about food fortification with essential amino acids, fiber, polyphenols and fatty acids to develop new types of pasta with the capacity to decrease cholesterol. The reached levels of acceptance of consumer's and the good results from chemical analysis, which have highlighted the nutraceutical potential of the produced spaghetti, could be the starting point to produce pasta to be tested in clinical trials for further researches on the effect played on blood levels cholesterol after ingestion.

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