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Legumes in low input agricultural systems: agronomical traits, sustainability aspects and nutritional features

Presentata da:
Dott.ssa Camilla Tibaldi

Coordinatore Dottorato
Prof. Massimiliano Petracci

Supervisore
Prof.ssa Ilaria Marotti

Co-supervisori
Prof. Giovanni Dinelli
Dott.ssa Francesca Truzzi

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Abstract

The general trend towards on-farm mechanization of modern agricultural production systems over time negatively impacted on the environmental features. This situation, exacerbated by climate-driven issues, brought to a gradual reduction in biodiversity of ecosystem, soil erosion, reduced drinking water and carbon stocks. In parallel, the growing global population is driving towards the need to increase food production, although limited availability of arable land, water and fossil fuels. In this perspective, sustainable agriculture may be a potential solution to enable agricultural systems to feed a growing population within the changing environmental conditions. Furthermore, the recent greater focus on consuming plant-protein sources and functional foods is revitalizing legume production and consumption, leading to policies and initiatives that aim to place legumes again at the base of a more sustainable agri-food system.

Besides being important component of diversified farming systems, grain legumes deliver the unique combination of being high-protein source for both human and animal feed, nitrogen fixing, they also improve cropping systems in terms of reduced pests, diseases and weeds, enhance soil quality and support positive environmental impacts, such as reduction in greenhouse gas (GHGs) emissions and increased biodiversity. The high nutritional value composition of legumes is given by the high content of proteins, fiber, starch, minerals, vitamins and bioactive compounds.

In the present study, grain legumes (*Pisum sativum* L. var. Turris) were cultivated in organic farming system and under low input conditions with the aim to evaluate the agronomic performance among two times of sowing (autumn and spring) in two growing environments (mountainous and hilly) of Emilia-Romagna region (Italy) over two consecutive cropping seasons (2021 and 2022). Additionally, the harvested organic pea grain was totally substituted to soy-based meal in dairy cattle feeding and the examined diets were studied in terms of nutritional composition and their relative effect on milk yield and quality. Finally, the nutritional features of harvested organic peas among the growing environments were investigated and deepen with the evaluation of functional characteristics of wheat-based baked snacks (crackers) enriched with 6% of the respective organic pea flour.

The results gave interesting insights showing that, despite mountainous (autumn sowing) environment appear to be more promising in terms of growth parameters (plant height, internodes, number of flowers and pods), the grain yield of hilly (spring sowing) environment displayed comparable results to the autumn sowing. This might indicate that a key-strategy to improve the pea production within sustainable agronomic systems may be to evaluate the best agronomic strategy according to the pedo-climate conditions of the chosen growing environment. In terms of milk yield and nutritional quality of dairy cows feed, peas substitution showed an overall positive effect on main parameters and thus may be considered as a valuable and sustainable alternative to soybean in the nutrition of dairy cattle. As regards human consumption, weather conditions during the vegetative and reproductive crop and seed development demonstrated to display a strong impact on the nutritional quality of the pea grain. From technological perspective, it was found that the incorporation of the pea flour into wheat-based crackers improves the dough and final food product characteristics, in terms of functional (rheology and texture) and nutritional quality parameters, as well for sensory analysis; thus may be considered as a valuable and sustainable alternative vehicle to deliver a plant-based meat alternatives protein source.

Chapter 1 – General Introduction

1.1 Climate change and sustainability of agronomic productions

Since ancient times, humans have developed techniques to satisfy their basic needs, related to the production of food for the sustenance of society. During centuries, agriculture has undergone to huge expansion to guarantee world population growth, and consequently agricultural systems were developed and adapted to the geographical context, in order to find balance between the necessity to suit the consumers' needs and the sustainability of natural resources. In an anthropocentric cultural context, humans became able to change and shape the surrounding land for their sustenance (Tarolli et al., 2019), leading to a profound modification of the landscape and accelerating the soil erosion (Montgomery, 2007; Tarolli and Sofia, 2016) through the spread of new agricultural practices. In fact, from 2001 and 2012, the potential soil loss increased by 2.5% globally, due to change in land use of about 4 km^2, caused by forest decline and expansion of semi-vegetable areas and cultivated areas (Borrelli et al., 2017). As regards soil erosion, it negatively impacts on the ecosystem services, agricultural production, drinking water and carbon stocks (Evans et al., 2016). This issue can be related to the abandonment of the land (and thus the lack of maintenance), due to depopulation of agricultural areas in favor to cities. Moreover, the evolution of cultivation techniques by heavy mechanization caused considerable pressure on the terrain, leading to soil compaction and degradation at global level (Brunoni et al., 2018; Batey 2009; Bogunovic et al. 2017; Schreck et al. 2012). In the study of Renard et al. (1997), using the Revised Universal Soil Loss Equation (RUSLE) it was observed that the potential soil erosion is 37% higher in mechanized fields compared to non-mechanized fields, mainly due to the reduction of soil permeability (Pijl et al., 2019).

A further critical factor, to be added to previous, is climate change. The excess of carbon dioxide, methane, and other heat-trapping gases are accumulating in the earth's lower atmosphere (troposphere), and are causing warming earth’s surface (Houghton et al., 1996). The main noteworthy and potentially critical aspects are the temporal evolution of average precipitation and rainfalls. Flooding and droughts will be more common, and food productivity is expected to decrease in certain parts of the world. It has been estimated that during the 21st Century the frequency and intensity of global precipitation will tend to increase with high probability (IPCC, 2019). With a particular focus on Northern Italy, it is expected an increase of rainfall events by the end of this century (Gao et al., 2006; Zollo et al., 2016), as already observed for the past century (Sofia et al., 2017). In this context, although agricultural practices and processes can result in release of significant amounts of methane and nitrous oxide (greenhouse gases), agriculture is also severely affected by climate change. Rainfall is an important factor which has the capacity of inducing soil erosion both through the erosive effect of raindrops and runoff (Zuazo et al., 2005), and therefore it is important to understand the evolution of intensity and duration of extreme events. In addition, air pollution may also damage crops, plants, and forests (Reidmiller et al., 2017), due to the fact that plants absorb large amounts of ground-level ozone, consequently reducing photosynthesis, lowering growth, and increasing sensitivity to diseases (EPA, 2022).

One of the best ways to mitigate climate change issues is to create sustainable food systems based on sustainable agriculture. Sustainable agriculture provides a potential solution to enable agricultural systems to feed a growing population within the changing environmental conditions (Rockström et al., 2017). The sustainability of agricultural practices has the aim to develop an integrated system of technologies and management approaches, environmentally and economically sustainable and this must be assessed in terms of economic, social and environmental issues. It has to combine productivity, profitability, resilience, land/water management, decent work and well-being, in order to capture its multidimensional nature (FAO, 2020). On this complex effort governments, non-governmental organizations (NGO), scientists and stakeholders should focus to ensure that sustainable agriculture spreads throughout the World (Tarolli et al., 2020). Already in 2007, the United Nations reported on "Organic Agriculture and Food Security in Africa", where stated that using sustainable agriculture could be a tool in reaching global food security without expanding land usage and reducing environmental impacts (Stanislaus et al., 2009). Moreover, in the perspective to obtain high-quality food, in 2016 the Food and Agricultural Organization (FAO) designated the *International Year of Pulses*, to promote the sustainable cultivation of grain legumes, which represent the main contributors for dietary protein sources (Calles et al., 2019). Starting from this initiative, both political parties and food companies have globally tried to spread and raise awareness about the need to increase production, as well as consumption of legumes instead of relying on animal products for dietary protein sources (Ferreira et al., 2021).

1.2 Role of grain legumes in cropping system

Given the considerable growing importance that sustainable production is gaining in agriculture and food systems, legume crops could play an important role in this context by delivering multiple services in line with the environmental sustainability principles. Considering their environmental and socioeconomic benefits, legumes could be introduced in modern cropping systems to increase crop diversity and reduce use of external outputs. They also perform well in conservation systems, intercropping systems, thanks to their multiple functions, as fixing the atmospheric nitrogen, releasing in the soil high-quality organic matter and facilitating soil nutrients' circulation and water retention (Stagnari et al., 2017).

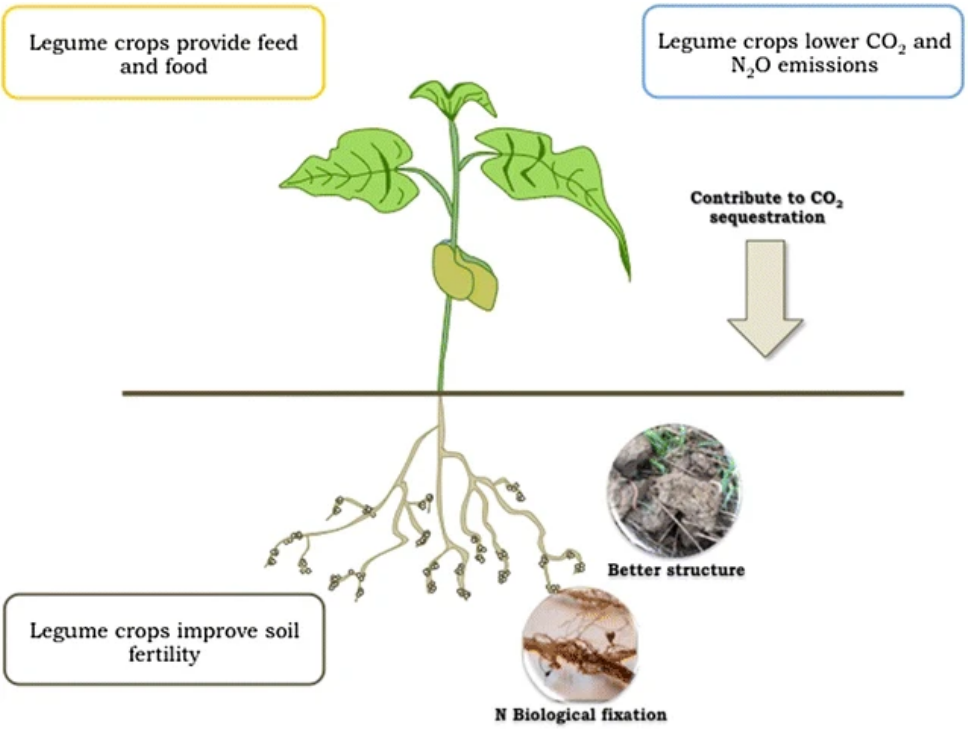

Figure 1.1. Legume crops multiple beneficial functions above and below ground. Source: Stagnari et al. (2017).

Recently, legume crops have been studied for their positive effects on yield and quality characteristics on the subsequent crops within crop rotations (Kirkegaard et al., 2008; Preisseel et al., 2015; Luce et al., 2015). The agronomic benefits provided by grain legumes in crop rotation are related to N supply from biological nitrogen fixation (BNF) (Peoples et al., 2009), which was observed to be higher in soils with low N fertilization (Preisseel et al., 2015). In addition, although is not specific for legume crops, it was demonstrated that legumes may improve the soil organic matter and structure (Hernanz et al., 2009), phosphorous mobilization (Shen et al., 2011), soil water retention and availability (Angus et al., 2015), and reduced pressure from diseases and weeds (Robson et al., 2002). In this perspective, several studies showed the yield benefits of legumes for subsequent cereal crops. In Australia, it was reported that wheat cultivation after legumes increased +30% of wheat yield, compared to pure cereal crop sequences (Angus et al., 1991; Angus et al., 2015). In Europe as well were reported the yield benefits of grain legumes in cropping rotations, and it was observed that they strongly depend on climatic factors (Reckling et al., 2014). The yield advantage to subsequent cereal crops provided by legumes depends also on the species and amounts of fixed N (Walley et al., 2007; Zander et al., 2016). In particular, it was observed that field pea and faba bean accumulate about 130 and 153 kg N/ha in their aboveground biomass, respectively, and significant quantities may also be stored in belowground biomass (Peoples et al., 2009).

In parallel, intercropping system is recognized for its several beneficial aspects against pest control (Chevalier et al., 2016), and in favor of competitive yields with reduced inputs (Monti et al., 2016; Tosti et al., 2010), pollution mitigation (Luo et al., 2016), more stable forage yields per unit area (Smith et al., 2013). Recent studies have focalized on the potential of intercropping in sustainable productions, with a particular focus on grain legumes, considered their capacity to fix N_2 through biological mechanisms (BNF). Indeed, legumes are pivotal in many intercropping systems, and most frequently used as intercrop species (Hauggaard-Nielsen et al., 2005). One of the basic spatial arrangements used in intercropping is strip cropping, in which two or more crops grow together in strips sufficiently wide to allow the production of separate crops to be produced, but close enough for the crops to interact. In this system, non-legumes crops obtain additional N released by legumes into the soil (Li et al., 2013; White et al., 2013) or via mycorrhizal fungi (Wahbi et al., 2016). It was demonstrated that legumes may contribute up to 15% of the N in an intercropped cereal (Li et al., 2009), thus increasing biomass production (Pappa et al., 2012) and reducing synthetic mineral N-fertilizer use (Beaudette et al., 2010).

1.3 Grain legumes as possible solution for a more sustainable agricultural system

Considering that world population is expected to reach 10 billion in the next decades (Until, 2013), humanity has to face both shortages (hunger) and excesses (obesity) of calorie and nutrient intakes (Ulian et al., 2020). Moreover, limited availability of arable land, water and fossil fuels represent an important issue that have to be considered in this future perspective (ISF, 2011). Biodiversity is fundamental to addressing this double challenge, which involves a better understanding of the global state of food resources. Facing higher food demand may involve larger crop cultivation areas and yield increases, as well for higher livestock production. Indeed, recent predictions suggest that global meat intake will increase by about 76% by mid-century (Godfray et al., 2018). Considering that livestock production requires significant land areas and freshwater supplies, if this food consumption patterns do not change, consumers' demand pressure will push upon earth's limited resources (Ferreira et al., 2021). Currently, both grazing land and animal feed crops cover 80% of total agricultural land (Giovannucci et al., 2012). In addition, around 29% of the water footprint of the global agricultural sector is related to the production of animal products (Mekonnen and Hoekstra, 2012). At European level, in 2016 livestock production systems represented 28% of land use (European Environment Agency, 2019a). It was also calculated that feed and animal production require around 25% of total water extraction within the agriculture sector in the EU (European Commission, 2019b). Moreover, it was widely demonstrated that livestock production produces important amounts of the three main greenhouse gases responsible of global warming: carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Godfray et al., 2018). Meat production is the main source of CH_4, generating approximately 80% million tons of per CH_4 year, representing around one-third of all CH_4 anthropogenic emissions (Ferreira et al., 2021) and almost 80% of agriculture emissions (Peoples et al., 2019).

In this perspective, lower livestock production in favor of protein-rich plant crops cultivation could help reduce the huge environmental disadvantages related to animal-based production (Stagnari et al., 2017). Consequently, it can be hypothesized that feed crops may be converted into human food and thereby not compromise long term food security (Giovannucci et al., 2012). According to the literature, a dietary shift toward more plant-based protein food sources, like grain legumes, could help to mitigate global warming and therefore lighten climate changes (Willett et al., 2019). Legumes are nitrogen fixing, require less or no nitrogen fertilizers (Burgess et al. 2012) and reduce the carbon footprint of other crops grown in rotation with them (Gan et al. 2011). More in particular, to face the increasing food market demand, the vast use of N-rich artificial fertilizers over time affected global ecosystem, considering that loss of labile reactive forms of N threatens the quality of air, soil, and water resources (Sutton et al., 2011). The ability of legumes to biologically fix the atmospheric N_2, in symbiotic association with soil bacteria rhizobia, provides continuous N supply within agroecosystems without using additional artificial fertilizers (Clúa et al., 2018). In this perspective, using legumes in mixed cropfields could stimulate soil fertility and enhance yields, contributing to reduce environmental impact, suggesting that including legumes in crop rotation system may increase overall crops' yield and profitability and reduce total production costs (Preissel et al., 2015; Mahmood et al., 2018). Moreover, using legumes in cropping rotations supports the associated diversity of wild flora, fauna and soil microbes (Peoples et al. 2009b; Köpke and Nemecek, 2010). It was estimated that synthetic N fertilizers account approximately 12% of the annual average 5180 million tons of CO_2 equivalent GHG emissions, associated with agriculture activities between the year of 2010 and 2014 (Peoples et al., 2019) and it was found that nitrogen pollution can cost the EU up to €485 billion per year (Sutton et al., 2017). Moreover, if compared to ruminant's meat production, the production of plant-based foods was estimated that can produce 25–150 times less GHGs emissions (Clark et al., 2019). Hence, using legume crops that require no or less synthetic nitrogen fertilizers, thus saving fossil energy resources and substituting meat production with grain legumes cropping system, could indirectly lead to a reduction up to 74% in GHGs emissions, leading to a reduction of 6% (22 million t CO_2 eq) of the carbon footprint of the EU agricultural sector by 2030 (Zander et al., 2016; European Commission, 2019b). A focus of interest can be spent on the type of grain that can be used. In particular, some LCA studies compared soybean-based feed with European-grown grain legumes, and it was concluded that including EU-produced pea, faba bean or lupin in feed rations has showed to significantly reduce energy demand, GHG emissions and acidification potential (Cederberg and Flysiö 2004; Eriksson et al. 2004; van der Werf et al. 2005; Baumgartner et al. 2008; Topp et al. 2012).

In parallel, population growth can be a factor pushing the rising interest in plant-based meat to respond to the increasing demand to proteins and to limit the sustainability issues associating animal proteins to increasing feed supplies and higher levels of greenhouse gases production (Palanisamy et al., 2019). Until the last 50 years, modern diets were characterized by high intake in calories and heavily processed and animal source foods. At European level, the intake of animal proteins, composed by meat and dairy products, has doubled and currently remains twice the global average (64 kg/year) (European Environment Agency, 2019b). In the new impetus for a more sustainable diet and consumption by 2050, addressed by sustainability and healthy reasons, the transformation to healthier diets requires important dietary changes, which may include a consistent reduction in global consumption of unhealthy foods, such as red meat (Ferreira et al., 2021).

The increased awareness of the need to shift to a more sustainable food systems is revitalizing legume production and consumption. Recently, health-conscious consumer preferences shifted towards the consumption of plant-based products in the perspective of seeking more safe and healthy products (Xazela et al., 2017). Despite the benefits of meat consumption, a meat-based diet could lead to human health issues related to high content of cholesterol and saturated fatty acids (Vang et al., 2008; Wang et al., 2009). Moreover, plant-based diets are more cost-effective and present lower risk of cardiovascular disease, blood pressure, diabetes, and mortality (Farmer et al., 2011; Springmann et al., 2018; Mohamed et al., 2017). On the other hand, the development of plant-based meat analogues to replace animal products generated significant breaks for food industries against the above-mentioned health, environmental and ethical concerns (Palanisamy et al., 2019; Hartmann et al., 2017; Malek et al., 2019). In the last decade, legumes have re-emerged as an interesting and balanced source of nutrients. They are considered as high-quality foods, characterized by considerable content of proteins, fiber, and several minerals, such as iron, zinc, and potassium (Grela and Samoli, 2017) and vitamins, such as thiamine, niacin, folate, riboflavin, pyridoxine, vitamin E, and A (Mudryj et al., 2014).

Finally, grain legumes may have an important role in protecting environmental biodiversity (Ferreira et al., 2021). Until last decades, the intensification of modern agriculture production has favored the cultivation and spread of the most agronomically profitable crops (Everwand et al., 2017). This evidence affected the diversity of landscapes and natural habitats of different species. The ecosystems are globally losing the ability to ensure crop pollination, clean air and water, and control of floods and soil erosion (European Commission, 2011). Moreover, the excessive use of N inputs by artificial fertilizers caused acidification and direct toxicity of soil, over other environmental negative consequences (European Commission, 2018b). In this perspective, it was demonstrated that the presence of legumes within cropping agronomic system may promote the conservation heterogeneity and continuity of multispecies habitats (Peoples et al., 2019). The study of Marzinzig et al. (2018) highlighted that leguminous plants offer vital floral resources that guarantee the survival of population of pollinators, which, in turn, benefit from food production and plant reproduction. Therefore, the beneficial effects of legumes in increasing biodiversity should be more widely used as an incentive to promote their production.

1.4 Historical cultivation of grain legumes

Legumes have a long history associated with the development of agricultural practices, as they are one of the earliest domesticated plants (Ahmed and Hasan, 2014), indeed they are considered to have marked the transition from a hunting-life to agricultural practices (Phillips, 1993). These food crops have different origins of domestication. It seems that lentils were already present within cropping systems of ancient Egyptian civilizations and carbonized seeds already 7,000 to 8,000 years B.C. in Turkey (Ahmed and Hasan, 2014). Peas and dwarf field beans seem to have been cultivated in Switzerland between 4000 and 5000 B.C. (Ahmed and Hasan, 2014). Moreover, in China it was found that soybean was cultivated between 3000 and 2000 B.C. (Ahmed and Hasan, 2014). Archaeological sites revealed signs of domestication of bean crops as early as 10,000 years ago in Mexico and Peru (Gomes and Vasconcelos, 2014). Hence, over 3000 years ago, beans, soybean, and staple crops started being domesticated in America and Asia (Ahmed and Hasan, 2014).

According to these findings, pulses (beans, peas, and lentils) have been extensively consumed for at least 10,000 years among the world, thus they can be considered important both economically as well as nutritionally for human being (Mudryj et al., 2014). Indeed, ancient Romans in 37 B.C. were found to use legumes in pastures and for soil improvement purposes, supposing that they already understood the nitrogen-fixing abilities of legumes (Gomes and Vasconcelos, 2014). However, the recognition of the value of these crops seems to have faded over the centuries (Ferreira et al., 2021).

Legume crops have distinctive sizes, shapes, colors, and flavors that contribute to consumer appeal. Thus, these products may be prepared and cooked according to many geographically specific traditions (Amin & Borchgrevink, 2022). These crops are agronomically well suited to cultivation also in tropical and humid climates, whereas pulses are more adapted to semiarid areas (Affrifah et al., 2023).

1.5 Global and European production of grain legumes

Recently, global food industry has increasingly oriented its activities and products reflecting the current dietary trends (i.e. vegetarians, vegans, gluten-free, etc). It was estimated that animal protein substitutes expressed an annual growth rate of 14% (European Commission, 2018c). To reflect these habits, the incorporation of legumes and legume-based ingredients in the food products has increased, thus contributing to a more sustainable food system (Lascialfari et al., 2019). Indeed, in 2010 the development of new food products enriched with pulses, such as chickpea, pea, bean or lentil has increased (European Commission, 2018a). Such products have been mostly promoted based on nutrition-related claims, namely the nutrient-dense high-protein quality of legumes (European Commission, 2018a). In this perspective, since 2014, the European market demand for lentils and chickpeas for human consumption has increased by 24% and 20% respectively (European Commission, 2018a).

At European level, around 1960s the grain legume production (composed by chickpea, cowpea, groundnut, lentil, and common bean production) for human consumption occupied 67% of total production area, and in 2013 it decreased to 27% (Watson et al., 2017). This trend was driven by rising competition from cheaper imports, especially from Canada, and the substitution of legumes intake by meat in the Mediterranean countries (European Commission, 2018a). Afterwards, in the 1980s, field pea and soybean became the two most widely cultivated protein crops for animal feed in Europe (Watson et al., 2017). Peak production areas of these crops exceeded 1.3 and 1 M ha, respectively, but both have declined since the 1990s (Zander et al., 2016). As regards field peas and broad beans, their combined production reached 4.4 million tons in 2018 (European Commission, 2018b). It was calculated that around two-thirds of grain legumes production is directed to animal feed, whereas only 20% is destinated for human consumption (European Commission, 2018b). Indeed, grain legumes cultivation in 2018 covered only 1.4% of the total crop area in Europe (European Commission, 2018b), which corresponds around 10% of their average role in cropping systems at global level (Watson et al., 2017). Moreover, only 43% of the food legumes consumed in Europe are produced on European farmland (Watson et al., 2017). Europe's domestic production expresses a deficit of about 70% of high-protein materials, 87% of which rely on imported soybean and soymeal (Watson et al., 2017). These findings suggest that legume production will continue in declining in Europe (Stagnari et al., 2017), as already explained, probably due to relative economic un-competitiveness compared to more profitable crops, such as cereals, which account for 31% of the total utilized agriculture area in Europe (European Commission, 2018b).

However, recently consumers' interest has shifted toward a more sustainable agri-food production system, driven by the environmental and health impact of producing and consuming meat and animal-based foods. Therefore, consumers are notably reducing or foregoing meat use and opting for sustainably produced plant foods and non-meat protein alternates (Hill, 2022; Uebersax et al., 2023).

1.6 Nutritional quality of grain legumes

Legumes can be produced in an environmentally sustainable system, thereby they could represent a sustainable and economical resource of proteins compared with animal sources (Uebersax et al., 2023). It was demonstrated that regular consumption of legumes offers a variety of widely studied health benefits (Didinger & Thompson, 2021). However, pulses are characterized by a very rich nutritional profile, as they are consistent sources of proteins, dietary fibers (DF), carbohydrates (digestible and resistant starch), selected minerals, vitamins, and bioactive phytochemicals (Sreerama et al., 2012). Legume proteins are higher in essential amino acids, especially lysine, as compared with animal proteins (Kumar et al., 2022). Considering their high nutritional value, legumes are characterized by an average containing 60.76 g carbohydrates, 23.10 g proteins, and 15.55 g DF per 100 g (**Table 1.1**). Nevertheless, legumes contain several anti-nutritional factors (ANFs) including α-galactosides, trypsin and chymotrypsin inhibitors, phytates and lectins (Srivastava & Srivastava, 2003), although it was demonstrated that processing cooking methods reduce ANFs and improve legumes' digestibility (Wiesinger et al., 2022).

Table 1.1. Proximate composition of pulses (per 100g) Source: USDA (2022).

Pulses	**Water (g)**	**Energy (kcal/kJ)**	**Protein (g)**	**Total lipid/fat (g)**	**Ash (g)**	**Carbohydrate (g)**	**Dietary fiber (g)**
Black bean	11.02	341/1427	21.60	1.42	3.60	62.36	15.50
Adzuki bean	13.40	329/1377	19.90	0.53	3.26	62.90	12.70
Chickpeas	7.68	378/1582	20.47	6.04	2.85	62.95	12.20
Cowpeas	11.05	343/1435	28.85	2.07	3.39	59.64	10.70
Faba bean	10.98	341/1427	26.12	1.53	3.08	58.29	25.00
Lentils	8.26	352/1473	24.63	1.06	2.71	63.35	10.70
Lupin	10.44	371/1552	36.17	9.74	3.28	40.37	18.90
Pigeon peas	10.59	343/1435	21.70	0.38	3.45	62.78	15.00
Red kidney bean	11.75	337/1410	22.53	1.06	3.37	61.29	15.20
Average	*10.74*	*344/1438*	*23.10*	*1.86*	*3.50*	*60.76*	*15.55*

The protein content of pulses (per 100 g) ranges from 19.90 g in adzuki beans to 36.17 g in lupin (**Table 1.1**), and it is typically twice the amount of dietary protein levels found in cereals. Legume proteins are divided into macrogroups of globulins, which constitute the major (72%) storage proteins, and albumins, that are the minor (25%) protein fraction. However, legumes contain relatively low amounts of some essential sulfur-containing amino acids (cystine and methionine) that are present in larger concentration in cereal grains (Affrifah et al., 2023). On the other hand, cereal grains contain lower content of lysine and tryptophan, as compared with legumes. Hence, legumes can be successfully used in complementation with cereals in order to improve dietary aminoacid profile of prepared foods. Besides the nutritional value given by the aminoacid composition, legume proteins are also an important source of bioactive peptides. Previous studies reported that both albumin or globulin fractions contained in legumes display a significant inhibitory capacity among inflammatory markers (Duranti, 2006). In addition, in the study of Jeonget et al. (2009) it was found that 43-amino acid peptide named lunasin exerted a specific capacity to inactivate the division of cancerous cells.

Furthermore, legumes are excellent source of complex carbohydrates, containing 40.37 g/100 g in lupin and up to 62.36 g/100 g in beans (**Table 1.1**), consequently representing considerable contributors to diets rich in fibers and low glycemic index (GI) (Collins, 2020). Legumes are a valuable source of DF ranging from 5% to 37%, with significant content of soluble and insoluble fiber. As rich source of fibers, legumes are digested much slower as compared with starchy cereals and tubers, thus stimulating satiety. Moreover, fiber content allows to better control blood glucose levels by reducing spikes after meal intake (Conti et al. 2021). It can be considered that DF in legumes plays a key role in gut functioning and is believed to lower the risk of several chronic diseases including some cancers, heart disease, and diabetes (Affrifah et al., 2023). Starch, composed by amylose and amylopectin, is the main storage carbohydrate in legume grains (Punia et al., 2020). However, legumes showed to have high content of resistant starch (RS) and raffinose-family oligosaccharides (RFOs), all of which are widely studied and demonstrated to exhibit prebiotic activities (Maphosa & Jideani, 2017). More in particular, it was demonstrated that these compounds (RS and RFOs) while passing through the stomach and the small intestine remain undigested, due to lack of enzymes responsible to break them into simpler sugars. In the colon, they can act as a source of prebiotics for resident probiotics, which by fermentation results in the formation of short-chain fatty acids, thus gaining the potential to improve colon health (Bird et al., 2010). However, the fermentation is also associated with the production of gases causing bloating, cramping, and flatulence, which is the most important factor in deterring people from including more legumes in their diet. Considering that most of legumes require heat treatment to a safe consumption, it was demonstrated that thermal treatment, such as boiling, microwave, and autoclaving have a significant effect on increase anti-nutritional factors (ANFs) losses (Samtiya et al., 2020; Popova & Mihaylova, 2019)

As regards crude fats, legume seeds are characterized by varied content of total lipid, based on the variety, origin, location, field production conditions, and soil type (Tiwari & Singh, 2012). According to Maphosa and Jideani, (2017), legumes are largely low in fat and contain no cholesterol, in fact the fat content per 100 g ranges from 0.38 g in pigeon peas to 9.74 g in lupin (**Table 1.1**). The low-fat content of legumes makes them an attractive food to the consumers, as they can be seen as a healthy choice, beyond representing an economic advantage on the current market.

Finally, legumes are rich also in micronutrients, including zinc, calcium, copper, magnesium, etc. (Affrifah et al., 2023). For this reason, they can be considered as highly promoting healthy foods, as these elements play a fundamental role in several cellular metabolic activities (Höhn et al., 2017). Moreover, legumes provide considerable amounts of B-group vitamins (folate, thiamin, and riboflavin), although are relatively poor of fat-soluble vitamins and vitamin C (Maphosa & Jideani, 2017).

Literature demonstrated that legumes promote weight reduction due to their satiety value (Li et al., 2014) and help to moderate blood sugar levels after meals, thereby improving insulin sensitivity (Didinger & Thompson, 2021; Mollard et al., 2011). Given this evidence, legumes act in favor to the control of body weight and obesity, probably because they give greater satiety (Ferreira et al., 2020).

In the perspective that global consumers' market is increasingly demanding for a more sustainable food system and healthier plant-based protein sources, the incorporation of legumes in different food products can potentially expand the utilization of legumes beyond traditional uses and consumption patterns. In this regard, there is a heightened potential in the food industry for using legume ingredients in various food systems (Carbonaro et al., 2015; Dhull et al., 2022). Legume-based ingredients are highly suitable for developing diverse food products, including (1) composite mixes and doughs, (2) meat alternatives and extenders, (3) gluten-free products, (4) baked products, (5) snack foods, (6) dairy products, and (7) regional or ethnic products (Hill, 2022). Additionally, legume proteins have appreciable techno-functional properties (e.g., emulsification, foaming, water absorption) (Neji et al., 2022). It was found that legumes proteins may be used in food applications for the nutritional content as well as the organoleptic and functional properties of plant-based meat alternatives (PBMAs). In combination with starches and fibers, legumes proteins are fundamental components for food functional properties and interact together to help to imitate meat analogues. These functional properties are water-holding capacity, gelation, emulsification and fat-absorption capacity and may contribute to juiciness as well as to the mouthfeel including chewiness and supports the creation of a meat-like texture (Möller, 2021). In recent studies it was seen that legumes' protein isolates exhibit good foaming capacity, emulsion capacity, solubility, and emulsifying activity index even at extreme pH (Gundogan & Karaca, 2020; Lafarga et al., 2020). These characteristics depend also on starch presence, which is a predominant component in legumes and strongly influence gel formation and rigidity. In recent years consumers' interest has increased in isolating protein-rich fractions from legumes, such as lentils, beans, cowpeas, pigeon pea (Ladjal-Ettoumi et al., 2016), although pea and soy protein isolates and concentrates were already commercially available since decades. However, when incorporating protein fractions in nutritional food for protein fortification purposes, the digestibility and bioavailability of the proteins should be evaluated, as well for the regulatory guidelines (Hill, 2022).

1.7 Functional properties of grain legumes

Legumes, besides providing the adequate nutritional intake, can be considered as functional foods. They are characterized by the presence of compounds that can exert beneficial effects on one or more of the organism's functions either for the improvement of health or for a reduction in the risk of diseases. Functional food, therefore, contributes to general well-being and their positive effect is attributable to the components intrinsically present in the food itself. Many of the functional compounds present in species of agricultural interest, and particularly in cultivated species, are secondary metabolites. Given their considerable high-quality nutritional components, legumes display a health-promoting role, thanks to their different constituent bioactive compounds (Murphy et al., 2018), e.g., phenolic acids, tannins, and flavonoids. Polyphenols represent a large and varied group of at least 10,000 known different compounds, that could be unified by the presence aromatic ring and several attached hydroxyl groups. These compounds offer protection to the plant from pathogens, free oxygen radicals, UV rays, and parasites (Naczk et al., 2004). The most common polyphenols could be classified according to the number of phenolic rings they contain in their chemical structure, including phenolic acids, flavonoids, lignans and stilbenes (Truzzi et al., 2021). Among polyphenols, flavonoids represent the major group and the most abundant bioactive constituent of legumes, particularly ferulic acid (Affrifah et al., 2023). The polyphenol classification present in food legumes is represented in **Figure 1.2**.

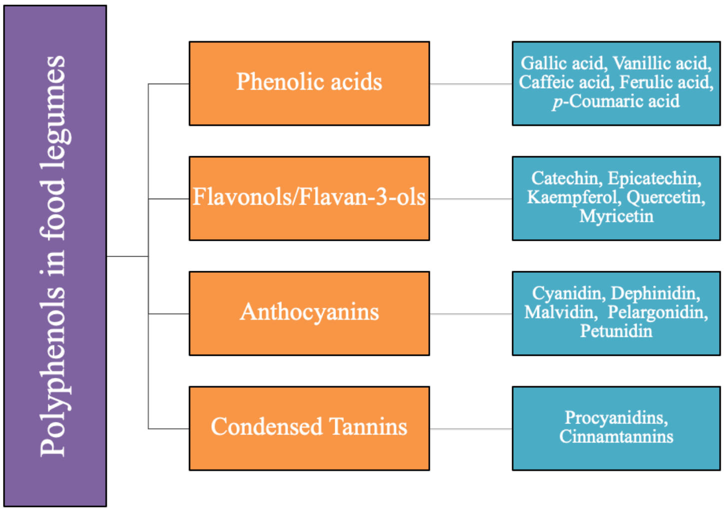

Figure 1.2. Representative examples of phenolic compounds in food legumes. Source: Adapted from Wiesinger et al. (2022).

The most common phenolic acids in legumes include caffeic, *p*-coumaric, sinapic, ferulic, gallic, and *p*-hydroxybenzoic acids. Phenolic acids are physiologically contained in the cotyledon and especially in the seed coat, and their content in legumes may widely vary (Lin et al., 2008). It was studied by Lin et al. (2008) that legumes' phenolic acids largely influence the color and pattern of the seedcoat of some bean cultivars. More in particular, high anthocyanin content is associated with dark colored beans, consisting in typical red, black, pink, etc. seed coat. Whereas the presence of condensed tannins confers light yellow or pink spotter seed coat. The content of phenolic acid in dry beans averages around 31.2 mg/100 g, ranging between 19.1 and 48.3 mg/100 g. (Luthria and Pastor-Corrales, 2006).

These compounds are known for their antioxidant potential, and amongst other health-protective effects, such as hypertension, heart disease and cancer (Singh et al., 2017; Hoffmann and Sonenshein, 2003). In addition, literature evidenced that legume consumption is associated with lower risk of developing noncommunicable diseases, as showed to display positive outcomes on cardiovascular risk factors, such as, blood lipid profile, glycemic control, inflammatory status, oxidative stress, as well as gut microbiota composition, and activity (Ferreira et al., 2021). In addition to these beneficial properties, it was observed that these bioactive compounds exert a role in the modulation of cell proliferation, metabolism, and homeostasis. Furthermore, they possess antioxidant properties that are critical for the prevention of oxidative stress and related diseases (Zhao et al., 2014).

In this perspective, legumes may be considered as functional and also nutraceutical foods, due to the presence of these secondary metabolite phytochemicals (polyphenols and flavonoids), which highlighted their antioxidant, antimutagenic, and anticarcinogenic biological activities (Chávez-Mendosa et al., 2017; Yang et al., 2018). Hence, the increased consumption of grain legumes as well as consumption of targeted functional foods and dietary supplements (or nutraceuticals) incorporated or based on legumes could help in exert their bioactive compound positive health effects (Sirtori et al. 2009).

However, it was widely demonstrated that the phytochemical synthesis within crops is induced by adaptive responses to environmental factors, which are triggered by abiotic inductors (drought, temperature, UV irradiation, and salinity) and chemical/biochemical elicitors, both abiotic (mineral element nutrition) and biotic (Cabrera-De la Fuente et al., 2018).

1.8 Aim of the research

In the current context the worsening of climate climate-driven changes (rising temperatures and altered precipitations) is affecting and pressing structurally and functionally the ecosystem and biodiversity and are expected to continue to impact the environment. This situation is exacerbated by global increasing population, which is leading to a growing food demand on the market, although availability of arable land, water and fossil fuels is limited. These current concerns drive to the need for a more sustainable food system that can support human and natural resources health. In this perspective, the organic cultivation of legumes may give a considerable contribution to a sustainable production of safe and healthy food.

It was already widely demonstrated that legume production can be considered one of the possible solutions to face these issues, since it showed to be more environmentally sustainable in terms of pollution, biodiversity pressure, soil erosion and energy use, generating less greenhouse gas (GHG) emissions and requiring less energy, water, and land use compared with livestock production. From agronomic point of view, legumes, in particular peas (*Pisum sativum* L.), are characterized by wide adaptability to weather conditions, as they can be cultivated in cold and wet climatic regions, thanks to their capacity to withstand both cold and drought conditions. Thus, they may represent a key-point to strengthen the current agronomic system to a more sustainable perspective.

In addition, recently considerable consumers interest in plant-based diet has increased worldwide, consequently leading to the growth demand for vegetable protein sources in the market. Given the agronomic and sustainable potential of legumes, they cover a considerable importance also from nutritional point of view. Indeed, pulses are characterized by a high-quality nutritional profile, as they are consistent sources of proteins, dietary fibers (DF), carbohydrates (digestible and resistant starch), selected minerals, vitamins, and bioactive phytochemicals. It was largely showed that both nutritional and phytochemical compounds within crops is induced by adaptive responses to environmental factors, which are triggered by abiotic inductors (drought, temperature, UV irradiation, and salinity) and chemical/biochemical elicitors, both abiotic (mineral element nutrition) and biotic. Therefore, the requisite to study the impact of environmental inductors and agronomic management practices on nutritional and phytochemical content, with the objective of identifying which factors can be used as suitable strategies for inducing physiological increases in phytochemicals, has become an increasingly important research focus.

Moreover, among grain legumes used for livestock products, it was demonstrated that totally or partially replacing soybean-based feed with field pea, faba bean or lupin in feed rations has showed to significantly reduce energy demand, GHG emissions and acidification potential, by reducing transport and facilitates nutrient cycling between crops, animals, manure and soil.

Considering global consumers' market is increasingly demanding for a more sustainable food system and plant-based protein sources, the incorporation of legumes in different food products can potentially expand the utilization of legumes beyond traditional uses and consumption patterns. In this regard, there is a heightened potential in the food industry for using legume ingredients in various food products.

Combining the need to increase local production together with improving food quality for human and feed use, against GMO food, the research carried out during my PhD touched different themes and included the cultivation of organic peas (*Pisum sativum* L. var. Turris) in organic farming system and under low input conditions over two consecutive cropping seasons in contrasting two Italian environments of the Emilia-Romagna region (Italy).

The objectives of the following chapters were:

- Evaluate the possible impact of agronomic and environmental conditions on the agronomic performance (growth and yield parameters) of peas (*Pisum sativum* L. var. Turris) cultivated in two different Italian environments over two cropping seasons, in order to identify the best agronomic management strategy in response to the local pedo-climate conditions (Chapter 2).

- Study and characterize the nutritional composition of the organic pea (*Pisum sativum* L.) grain, as total replacement of soybean meal (*Glycine max* L.) in dairy cows feeding, and investigate the relative effect on milk yield and composition obtained from the examined cattle (Chapter 2).
- Assess the nutritional and health potential of organic peas (*Pisum sativum* L.) cultivated in two different Italian environments over two cropping seasons, in order to investigate how the environmental and meteorological conditions affected the nutritional composition of the pea grain (Chapter 3).
- Study the incorporation of organic pea flour (*Pisum sativum* L.) on wheat-based savory snacks (crackers) through the investigation of the appearance, physical properties (rheology and texture), nutritional composition and sensory analysis of the related food products (Chapter 3).

The above listed research points are summarized in a flowchart in **Figure 1.3**.

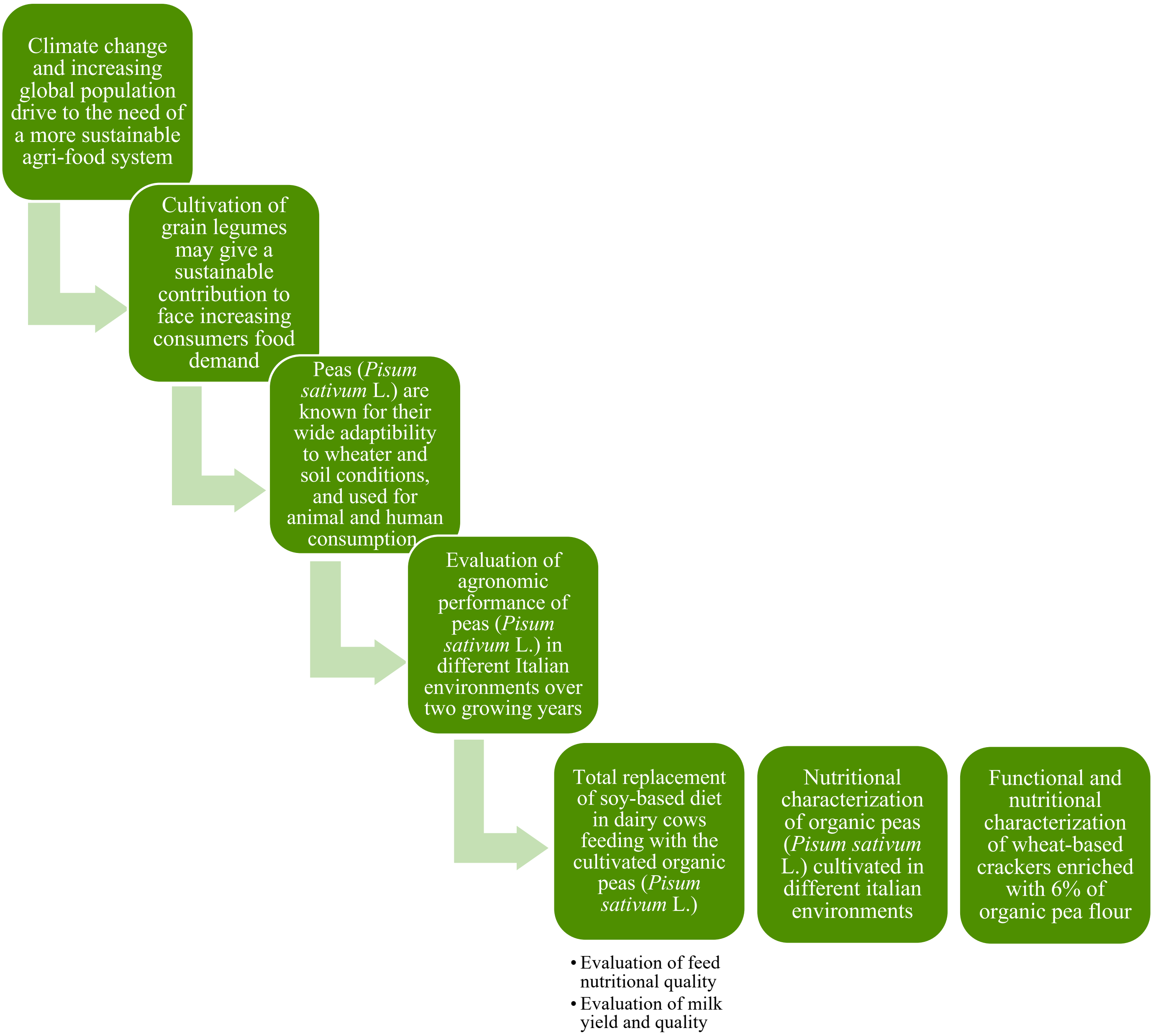

Figure 1.3. Flowchart of the main research topics touched during my PhD research activity.

References

Affrifah, N. S., Uebersax, M. A., & Amin, S. (2023). Nutritional significance, value-added applications, and consumer perceptions of food legumes: A review. Legume Science, e192.

Ahmed, S., & Hasan, M. M. (2014). Legumes: an overview. RADS Journal of Pharmacy and Pharmaceutical Sciences, 2(1), 34-38.

Amin, S., & Borchgrevink, C. P. (2022). A Culinology® perspective of dry beans and other pulses. Dry beans and pulses: Production, processing, and nutrition, 453-480.

Angus, J. F., Herwaarden, A., Howe, G. N., & Van, H. A. (1991). Productivity and break crop effects of winter-growing oilseeds. Australian Journal of Experimental Agriculture, 31(5), 669-677.

Angus, J. F., Kirkegaard, J. A., Hunt, J. R., Ryan, M. H., Ohlander, L., & Peoples, M. B. (2015). Break crops and rotations for wheat. Crop and pasture science, 66(6), 523-552.

Batey, T. (2009). Soil compaction and soil management–a review. Soil use and management, 25(4), 335-345.

Baumgartner, D. U., de Baan, L., & Nemecek, T. (2008). European grain legumes–Environment-friendly animal feed? life cycle assessement of pork, chicken meat, egg, and milk production. Grain Legumes Integrated Project (GLIP). New strategies to improve grain legumes for food and feed. Food quality and safety. WP2.2, Environmental Analysis of the Feed Chain, Final Report. Agroscope Reckenholz-Tänikon Research Station (ART), Zürich. http://kurzlink.de/Baumgartner-et-al-08.

Beaudette, C., Bradley, R. L., Whalen, J. K., McVetty, P. B. E., Vessey, K., & Smith, D. L. (2010). Tree-based intercropping does not compromise canola (Brassica napus L.) seed oil yield and reduces soil nitrous oxide emissions. Agriculture, ecosystems & environment, 139(1-2), 33-39.

Bird, A. R., Conlon, M. A., Christopherson, C. T., & Topping, D. L. (2010). Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics. Beneficial Microbes,1, 423–431.

Bogunovic, I., Bilandzija, D., Andabaka, Z., Stupic, D., Rodrigo Comino, J., Cacic, M., ... & Pereira, P. (2017). Soil compaction under different management practices in a Croatian vineyard. Arabian Journal of Geosciences, 10, 1-9.

Borrelli, P., Robinson, D. A., Fleischer, L. R., Lugato, E., Ballabio, C., Alewell, C., ... & Panagos, P. (2017). An assessment of the global impact of 21st century land use change on soil erosion. Nature communications, 8(1), 2013.

Brunori, E., Salvati, L., Antogiovanni, A., & Biasi, R. (2018). Worrying about 'vertical landscapes': Terraced olive groves and ecosystem services in marginal land in central Italy. Sustainability, 10(4), 1164.

Burgess, M. H., Miller, P. R., & Jones, C. A. (2012). Pulse crops improve energy intensity and productivity of cereal production in Montana, USA. Journal of sustainable agriculture, 36(6), 699-718.

Cabrera-De la Fuente, M., González-Morales, S., Juárez-Maldonado, A., Leija-Martínez, P., & Benavides-Mendoza, A. (2018). Plant nutrition and agronomic management to obtain crops with better nutritional and nutraceutical quality. In Therapeutic Foods (pp. 99-140). Academic Press.

Calles, T., Xipsiti, M., & del Castello, R. (2019). Legacy of the international year of pulses. Environmental Earth Sciences, 78, 1-8.

Carbonaro, M., Maselli, P., & Nucara, A. (2015). Structural aspects of legume proteins and nutraceutical properties. Food Research International, 76, 19-30.

Cederberg, C. (2004). Environmental assessment of future pig farming systems: quantifications of three scenarios from the FOOD 21 synthesis work. SIK Institutet för livsmedel och bioteknik.

Chávez-Mendoza, C., & Sánchez, E. (2017). Bioactive compounds from Mexican varieties of the common bean (Phaseolus vulgaris): Implications for health. Molecules, 22(8), 1360.

Chevalier Mendes Lopes, T., Hatt, S., Xu, Q., Chen, J., Liu, Y., & Francis, F. (2016). Wheat (Triticum aestivum L.)-based intercropping systems for biological pest control: a review. Pest Management Science, 72.

Clark, M. (2019). Changing Dietary Patterns as Drivers of Changing Environmental Impacts, 172-177.

Clúa, J., Roda, C., Zanetti, M. E., & Blanco, F. A. (2018). Compatibility between legumes and rhizobia for the establishment of a successful nitrogen-fixing symbiosis. Genes, 9(3), 125.

Collins, K. (2020). Carbohydrate quality part 2: Pulses and healthy eating. Nutrition Research in Perspective. Retrieved fromhttps://karencollinsnutrition.com/pulses-healthy-eating/(September 18, 2022).

Conti, M. V., Guzzetti, L., Panzeri, D., De Giuseppe, R., Coccetti, P., Labra, M., & Cena, H. (2021). Bioactive compounds in legumes: Implications for sustainable nutrition and health in the elderly population. Trends in Food Science & Technology, 117, 139-147.

Dhull, S. B., Bamal, P., Kumar, M., Bangar, S. P., Chawla, P., Singh, A., ... & Sihag, S. (2022). Fenugreek (Trigonella foenum graecum) gum: A functional ingredient with promising properties and applications in food and pharmaceuticals—A review. Legume Science, e176.

Didinger, C., & Thompson, H. J. (2021). Defining nutritional and functional niches of legumes: A call for clarity to distinguish a future role for pulses in the dietary guidelines for Americans.Nutrients,13, 1100.

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. Fitoterapia, 77(2), 67-82.

EPA. (2022). Ecosystem effects of ozone pollution. Retrieved 3/18/2022.

Eriksson IS, Elmquist H, Stern S, Nybrant T (2004) Environmental systems analysis of pig production—the impact of feed choice. Int J Life Cycle Assess 10:143–154. doi:10.1065/lca2004.06.160

European Commission (2011). Communication From the Commission to the European Parliament, the Council, the Economic and Social Committee and the Committee of the Regions—Our Life Insurance, our Natural Capital: an EU Biodiversity Strategy to 2020. Brussels: European Comision.

European Commission (2018a). Market Developments and Policy Evaluation Aspects of the Plant Protein Sector in the EU—Final Report. Brussels: European Commision.

European Commission (2018b). EU Agricultural Outlook for Markets and Income, 2018–2030. Brussels: European Commision.

European Commission (2018c). Report from the Commision to the Council and the European Parliament on the development of plant proteins in the European Union. Brussels: European Commission.

European Environment Agency (2019a). Climate Change Adaptation in the Agriculture Sector in Europe.

European Environment Agency (2019b). Land and Soil in Europe. Why we Need to Use These Vital and Finite Resources Sustainably. Copenhagen: European Environment Agency.

Evans, R., & Boardman, J. (2016). The new assessment of soil loss by water erosion in Europe. Panagos P. et al., 2015 Environmental Science & Policy 54, 438–447—A response. Environmental Science & Policy, 58, 11-15.

Everwand, G., Dauber, J., Cass, S., and Williams, M. (2017). “Legume Crops and Biodiversity,” in Legumes in Cropping Systems, eds D. Murphy-Bokern, F. L. Stoddard, and C. A. Watson (Wallingford: CAB International) 55–69.

Farmer B, Larson BT, Fulgoni VL et al (2011) A vegetarian dietary pattern as a nutrient-dense approach to weight management: an analysis of the national health and nutrition examination survey 1999–2004. J Am Diet Assoc 111:819–827. https://doi.org/10.1016/j.jada.2011.03.012

Ferreira, H., Pinto, E., & Vasconcelos, M. W. (2021). Legumes as a cornerstone of the transition toward more sustainable agri-food systems and diets in Europe. Frontiers in Sustainable Food Systems, 5, 694121.

Ferreira, H., Vasconcelos, M. W., Gil, A. M., and Pinto, E. (2020). Benefits of pulse consumption on metabolism and health: a systematic review of randomized controlled trials. Crit. Rev. Food Sci. Nutr. 85. doi: 10.1080/10408398.2020.1716680.

Food and Agricultural Organization (FAO) of the United Nations, Indicator 2.4.1 (2020) - Proportion of agricultural area under productive and sustainable agriculture - Key results. http://www.fao.org/sustainable-development-goals/indicators/241/en/, 2020c (accessed 16 January 2020).

Gan, Y., Liang, C., Campbell, C. A., Zentner, R. P., Lemke, R. L., Wang, H., & Yang, C. (2012). Carbon footprint of spring wheat in response to fallow frequency and soil carbon changes over 25 years on the semiarid Canadian prairie. European Journal of Agronomy, 43, 175-184.

Gao, X., Pal, J. S., & Giorgi, F. (2006). Projected changes in mean and extreme precipitation over the Mediterranean region from a high resolution double nested RCM simulation. Geophysical Research Letters, 33(3).

Giovannucci, D., Scherr, S. J., Nierenberg, D., Hebebrand, C., Shapiro, J., Milder, J., & Wheeler, K. (2012). Food and Agriculture: the future of sustainability. The sustainable development in the 21st century (SD21) Report for Rio, 20.

Godfray, H. C. J., Aveyard, P., Garnett, T., Hall, J. W., Key, T. J., Lorimer, J., ... & Jebb, S. A. (2018). Meat consumption, health, and the environment. Science, 361(6399), doi: 10.1126/science.aam5324.

Gomes, A. M., & Vasconcelos, M. W. (2014). The legume grains: when tradition goes hand in hand with nutrition. ISEKI Food Series, 10.

Grela, E. R., and Samoli, W. (2017). Content of minerals and fatty acids and their correlation with phytochemical compounds and antioxidant activity of leguminous seeds. Biol. Trace Elem. Res. 180, 338–348. doi: 10.1007/s12011-017-1005-3

Gundogan, R., & Karaca, A. C. (2020). Physicochemical and functional properties of proteins isolated from local beans of Turkey. Lwt, 130, 109609.

Hartmann C, Siegrist M (2017) Consumer perception and behaviour regarding sustainable protein consumption: a systematic review. Trends Food Sci Technol 61:11–25

Hauggaard-Nielsen, H., & Jensen, E. S. (2005). Facilitative root interactions in intercrops. Root physiology: From gene to function, 237-250.

Hernanz, J. L., Sánchez-Girón, V., & Navarrete, L. (2009). Soil carbon sequestration and stratification in a cereal/leguminous crop rotation with three tillage systems in semiarid conditions. Agriculture, Ecosystems & Environment, 133(1-2), 114-122.

Hill, H. (2022). Utilization of dry beans and other pulses as ingredients in diverse food products. Dry beans and pulses: Production, processing, and nutrition, 307-329.ù

Hofmann, C. S., & Sonenshein, G. E. (2003). Green tea polyphenol epigallocatechin-3 gallate induces apoptosis of proliferating vascular smooth muscle cells via activation of p53. The FASEB journal, 17(6), 702-704.

Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., Van Der Linden, P. J., Dai, X., ... & Johnson, C. A. (2001). Climate change 1995: Contribution of working group I to the third assessment report of the intergovernmental panel on climate change. Climate change 2001: The scientific basis, 388.

IPCC, Shukla, P. R., Skea, J., Calvo Buendia, E., Masson-Delmotte, V., Pörtner, H. O., Roberts, D. C., ... & Malley, J. (2019). IPCC, 2019: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.

ISF (International Seed Federation) (2011) Agriculture under pressure. http://www.worldseed.org/isf/home.html

Jeong, H. J., Lee, J. R., Jeong, J. B., Park, J. H., Cheong, Y. K., & de Lumen, B. O. (2009). The cancer preventive seed peptide lunasin from rye is bioavailable and bioactive. Nutrition and cancer, 61(5), 680-686.

Keskin, S. O., Ali, T. M., Ahmed, J., Shaikh, M., Siddiq, M., & Uebersax, M. A. (2022). Physico-chemical and functional properties of legume protein, starch, and dietary fiber—A review. Legume Science, 4(1), e117.

Kirkegaard, J., Christen, O., Krupinsky, J., & Layzell, D. (2008). Break crop benefits in temperate wheat production. Field crops research, 107(3), 185-195.

Köpke, U., & Nemecek, T. (2010). Ecological services of faba bean. Field crops research, 115(3), 217-233.

Kumar, Y., Basu, S., Goswami, D., Devi, M., Shivhare, U. S., & Vishwakarma, R. K. (2022). Anti-nutritional compounds in pulses: Implications and alleviation methods. Legume Science, 4(2), e111.

Ladjal-Ettoumi, Y., Boudries, H., Chibane, M., & Romero, A. (2016). Pea, chickpea and lentil protein isolates: Physicochemical characterization and emulsifying properties. Food Biophysics, 11, 43–51.

Lafarga, T., Álvarez, C., Villaró, S., Bobo, G., & Aguiló-Aguayo, I. (2020). Potential of pulse-derived proteins for developing novel vegan edible foams and emulsions. International Journal of Food Science & Technology, 55(2), 475-481.

Lascialfari, M., Magrini, M. B., & Triboulet, P. (2019). The drivers of product innovations in pulse-based foods: insights from case studies in France, Italy and USA 1. Journal of Innovation Economics & Management, (1), 111-143.

Li, L., Zhang, L., & Zhang, F. (2013). Crop mixtures and the mechanisms of overyielding.

Li, S. S., Kendall, C. W. C., de Souza, R. J., Jayalath, V. H., Cozma, A. I., Ha, V., Mirrahimi, A., Chiavaroli, L., Augustin, L. S. A., Blanco Mejia, S., Leiter, L. A., Beyene, J., Jenkins, D. J. A., & Sievenpiper, J. L. (2014). Dietary pulses, satiety and food intake: A systematic review and meta-analysis of acute feeding trials. Obesity,22, 1773–1780.

Li, Y., Ran, W., Zhang, R., Sun, S., & Xu, G. (2009). Facilitated legume nodulation, phosphate uptake and nitrogen transfer by arbuscular inoculation in an upland rice and mung bean intercropping system. Plant and Soil, 315, 285-296.

Lin, L. Z., Harnly, J. M., Pastor-Corrales, M. S., & Luthria, D. L. (2008). The polyphenolic profiles of common beans (Phaseolus vulgaris L.). Food Chemistry,107, 399–410.

Luce, M. S., Grant, C. A., Zebarth, B. J., Ziadi, N., O'Donovan, J. T., Blackshaw, R. E., ... & Smith, E. G. (2015). Legumes can reduce economic optimum nitrogen rates and increase yields in a wheat–canola cropping sequence in western Canada. Field Crops Research, 179, 12-25.

Luo, S., Yu, L., Liu, Y., Zhang, Y., Yang, W., Li, Z., & Wang, J. (2016). Effects of reduced nitrogen input on productivity and N2O emissions in a sugarcane/soybean intercropping system. European Journal of Agronomy, 81, 78-85.

Luthria, D. L., & Pastor-Corrales, M. A. (2006). Phenolic acids content of fifteen dry edible bean (Phaseolus vulgaris L.) varieties. Journal of Food Composition and Analysis, 19, 205–211

Mahmood, F., Shahzad, T., Hussain, S., Shahid, M., Azeem, M., & Wery, J. (2018). Grain legumes for the sustainability of european farming systems. Sustainable Agriculture Reviews 32: Waste Recycling and Fertilisation, 105-133.

Malek L, Umberger WJ, Goddard E (2019) Committed vs. uncommitted meat eaters: understanding willingness to change protein consumption. Appetite 138:115–126.

Maphosa, Y., & Jideani, V. A. (2017). The role of legumes in human nutrition. In M. Chávarri Hueda (Ed.), Functional food–Improve health through adequate food (pp. 103–121).

Marzinzig, B., Brünjes, L., Biagioni, S., Behling, H., Link, W., & Westphal, C. (2018). Bee pollinators of faba bean (Vicia faba L.) differ in their foraging behaviour and pollination efficiency. Agriculture, ecosystems & environment, 264, 24-33.

Mekonnen, M. M., & Hoekstra, A. Y. (2012). A global assessment of the water footprint of farm animal products. Ecosystems, 15(3), 401-415.

Mohamed Z, Terano R, Yeoh SJ, Iliyasu A (2017) Opinions of non-vegetarian consumers among the chinese community in malaysia toward vegetarian food and diets. J Food Prod Mark 23:80–98. https://doi.org/10.1080/10454446.2017.1244795

Mollard, R. C., Wong, C. L., Luhovyy, B. L., & Anderson, G. H. (2011). First and second meal effects of pulses on blood glucose, appetite, and food intake at a later meal. Applied Physiology, Nutrition, and Metabolism,36,634–642

Montgomery, D. R. (2007). Soil erosion and agricultural sustainability. Proceedings of the National Academy of Sciences, 104(33), 13268-13272.

Monti, M., Pellicanò, A., Santonoceto, C., Preiti, G., & Pristeri, A. (2016). Yield components and nitrogen use in cereal-pea intercrops in Mediterranean environment. Field Crops Research, 196, 379-388.

Möller, N. (2021). Functional properties of legumes important for food applications.

Mudryj, A. N., Yu, N., and Aukema, H. M. (2014). Nutritional and health benefits of pulses. Appl. Physiol. Nutr. Metab. 39, 1197–1204. doi: 10.1139/apnm-2013-0557

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. Journal of chromatography A, 1054(1-2), 95-111.

Neji, C., Semwal, J., Kamani, M. H., Máthé, E., & Sipos, P. (2022). Legume protein extracts: The relevance of physical processing in the context of structural, techno-functional and nutritional aspects of food development. Processes, 10(12), 2586.

Palanisamy, M., Töpfl, S., Berger, R. G., & Hertel, C. (2019). Physico-chemical and nutritional properties of meat analogues based on Spirulina/lupin protein mixtures. European Food Research and Technology, 245, 1889-1898.

Pappa, V. A., Rees, R. M., Walker, R. L., Baddeley, J. A., & Watson, C. A. (2012). Legumes intercropped with spring barley contribute to increased biomass production and carry-over effects. The Journal of Agricultural Science, 150(5), 584-594.

Peoples, M. B., Brockwell, J., Herridge, D. F., Rochester, I. J., Alves, B. J. R., Urquiaga, S., Boddey RM, Dakora FD, Bhattarai S, Maskey SL, Sampet C, Rerkasem B, Khan DF, Hauggaard-Nielsen H. & Jensen, E. S. (2009). The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis, 48, 1-17.

Peoples, M. B., Hauggaard-Nielsen, H., Huguenin-Elie, O., Jensen, E. S., Justes, E., & Williams, M. (2019). The contributions of legumes to reducing the environmental risk of agricultural production. In Agroecosystem diversity (pp. 123-143). Academic Press.

Phillips, R. D. (1993). Starchy legumes in human nutrition, health and culture. Plant Foods for Human Nutrition, 44, 195-211.

Pijl, A., Barneveld, P., Mauri, L., Borsato, E., Grigolato, S., & Tarolli, P. (2019). Impact of mechanisation on soil loss in terraced vineyard landscapes. Cuadernos de investigación geográfica: Geographical Research Letters, (45), 287-308.

Popova, A., & Mihaylova, D. (2019). Antinutrients in plant-based foods: A review. The Open Biotechnology Journal, 13(1).

Preissel, S., Reckling, M., Schläfke, N., & Zander, P. (2015). Magnitude and farm-economic value of grain legume pre-crop benefits in Europe: A review. Field Crops Research, 175, 64-79.

Punia, S., Dhull, S. B., Sandhu, K. S., Kaur, M., & Purewal, S. S. (2020). Kidney bean (Phaseolus vulgaris) starch: A review. Legume Science,2, e52.

Reckling, M., Preissel, S., Zander, P., Topp, C. F. E., Watson, C. A., Murphy-Bokern, D., & Stoddard, F. L. (2014). Effects of legume cropping on farming and food systems. Legume Futures Report 1.6.

Reidmiller, D. R., Avery, C. W., Easterling, D. R., Kunkel, K. E., Lewis, K. L., Maycock, T. K., & Stewart, B. C. (2017). Impacts, risks, and adaptation in the United States: Fourth national climate assessment, volume II.

Renard, K. G. (1997). Predicting soil erosion by water: a guide to conservation planning with the Revised Universal Soil Loss Equation (RUSLE). US Department of Agriculture, Agricultural Research Service No. 703, 404 pp.

Robson, M. C., Fowler, S. M., Lampkin, N. H., Leifert, C., Leitch, M., Robinson, D., ... & Litterick, A. M. (2002). The agronomic and economic potential of break crops for ley/arable rotations in temperate organic agriculture. Advances in agronomy, 77, 369-427.

Rockström, J., Williams, J., Daily, G., Noble, A., Matthews, N., Gordon, L., ... & Smith, J. (2017). Sustainable intensification of agriculture for human prosperity and global sustainability. Ambio, 46, 4-17.

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: An overview. Food Production, Processing and Nutrition, 2, 1–14.

Schreck, E., Gontier, L., Dumat, C., & Geret, F. (2012). Ecological and physiological effects of soil management practices on earthworm communities in French vineyards. European Journal of Soil Biology, 52, 8-15.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., ... & Zhang, F. (2011). Phosphorus dynamics: from soil to plant. Plant physiology, 156(3), 997-1005.

Singh, B., Singh, J. P., Shevkani, K., Singh, N., and Kaur, A. (2017). Bioactive constituents in pulses and their health benefits. J. Food Sci. Technol. 54, 858–870. doi: 10.1007/s13197-016-2391-9.

Sirtori CR, Galli C, Anderson JW, Arnoldi A (2009) Nutritional and nutraceutical approaches to dyslipidemia and atherosclerosis prevention: focus on dietary proteins. Atherosclerosis 203:8–17. doi:10.1016/j.atherosclerosis.2008.06.019

Smith, J., Pearce, B. D., & Wolfe, M. S. (2013). Reconciling productivity with protection of the environment: Is temperate agroforestry the answer?. Renewable Agriculture and Food Systems, 28(1), 80-92.

Sofia, G., Roder, G., Dalla Fontana, G., & Tarolli, P. (2017). Flood dynamics in urbanised landscapes: 100 years of climate and humans' interaction. Scientific reports, 7(1), 40527.

Springmann, M., Wiebe, K., Mason-D'Croz, D., Sulser, T. B., Rayner, M., & Scarborough, P. (2018). Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modelling analysis with country-level detail. The Lancet Planetary Health, 2(10), e451-e461.

Sreerama, Y. N., Sashikala, V. B., Pratape, V. M., & Singh, V. (2012). Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. Food Chemistry,131, 462–468

Srivastava, R. P., & Srivastava, G. K. (2003). Nutritional value of pulses. Indian Journal of Agricultural Biochemistry,16,57–65.

Stagnari, F., Maggio, A., Galieni, A., & Pisante, M. (2017). Multiple benefits of legumes for agriculture sustainability: an overview. Chemical and Biological Technologies in Agriculture, 4(1), 1-13.

Stanislaus, Dundon (2009). "Sustainable Agriculture". Gale Virtual Reference Library.

Sutton, M. A., Oenema, O., Erisman, J. W., Leip, A., van Grinsven, H., & Winiwarter, W. (2011). Too much of a good thing. Nature, 472(7342), 159-161.

Tarolli, P., & Sofia, G. (2016). Human topographic signatures and derived geomorphic processes across landscapes. Geomorphology, 255, 140-161.

Tarolli, P., & Straffelini, E. (2020). Agriculture in hilly and mountainous landscapes: threats, monitoring and sustainable management. Geography and sustainability, 1(1), 70-76.

Tarolli, P., Cao, W., Sofia, G., Evans, D., & Ellis, E. C. (2019). From features to fingerprints: A general diagnostic framework for anthropogenic geomorphology. Progress in Physical Geography: Earth and Environment, 43(1), 95-128.

Tiwari, B. K., & Singh, N. Höhn, A., Weber, D., Jung, T., Ott, C., Hugo, M., Kochlik, B., Kehm, R., König, J., Grune, T., & Castro, J. P. (2017). Happily (n)ever after: Aging in the context of oxidative stress, proteostasis loss and cellular senescence. Redox Biology,11, 482–501.12). Pulse chemistry and technology. Royal Society of Chemistry.

Topp, C. F. E., Houdijk, J. G. M., Tarsitano, D., Tolkamp, B. J., & Kyriazakis, I. (2012). Quantifying the environmental benefits of using home grown protein sources as alternatives to soyabean meal in pig production through life cycle assessment. Adv. Anim. Biosci, 3, 15.

Tosti, G., & Guiducci, M. (2010). Durum wheat–faba bean temporary intercropping: Effects on nitrogen supply and wheat quality. European Journal of Agronomy, 33(3), 157-165.

Truzzi, F., Tibaldi, C., Zhang, Y., Dinelli, G., & D′ Amen, E. (2021). An overview on dietary polyphenols and their biopharmaceutical classification system (BCS). International Journal of Molecular Sciences, 22(11), 5514.

Uebersax, M. A. & Siddiq, M., (Eds.). (2022). Dry Beans and Pulses: Production, Processing, and Nutrition. John Wiley & Sons, 307–329.

Uebersax, M. A., Cichy, K. A., Gomez, F. E., Porch, T. G., Heitholt, J., Osorno, J. M., ... & Bales, S. (2023). Dry beans (Phaseolus vulgaris L.) as a vital component of sustainable agriculture and food security—A review. Legume Science, 5(1), e155.

Ulian, T., Diazgranados, M., Pironon, S., Padulosi, S., Liu, U., Davies, L., ... & Mattana, E. (2020). Unlocking plant resources to support food security and promote sustainable agriculture. Plants, People, Planet, 2(5), 421-445.

Until, E. (2013). World Population Prospects: 2012 Revision. United Nations Department of Economic and Social Affairs: New York, NY, USA.

USDA (US Dept of Agriculture). (2022). Food Data Central. Retrieved fromhttps://fdc.nal.usda.gov/.

Van der Werf, H. M., Petit, J., & Sanders, J. (2005). The environmental impacts of the production of concentrated feed: the case of pig feed in Bretagne. Agricultural Systems, 83(2), 153-177.

Vang, A., Singh, P. N., Lee, J. W., Haddad, E. H., & Brinegar, C. H. (2008). Meats, processed meats, obesity, weight gain and occurrence of diabetes among adults: findings from Adventist Health Studies. Annals of Nutrition and Metabolism, 52(2), 96-104.

Wahbi, S., Maghraoui, T., Hafidi, M., Sanguin, H., Oufdou, K., Prin, Y., ... & Galiana, A. (2016). Enhanced transfer of biologically fixed N from faba bean to intercropped wheat through mycorrhizal symbiosis. Applied Soil Ecology, 107, 91-98.

Walley, F. L., Clayton, G. W., Miller, P. R., Carr, P. M., & Lafond, G. P. (2007). Nitrogen economy of pulse crop production in the Northern Great Plains. Agronomy Journal, 99(6), 1710-1718.

Watson, C. A., Reckling, M., Preissel, S., Bachinger, J., Bergkvist, G., Kuhlman, T., ... & Stoddard, F. L. (2017). Grain legume production and use in European agricultural systems. Advances in Agronomy, 144, 235-303.

White, P. J., George, T. S., Gregory, P. J., Bengough, A. G., Hallett, P. D., & McKenzie, B. M. (2013). Matching roots to their environment. Annals of botany, 112(2), 207-222.

Wiesinger, J. A., Marsolais, F., & Glahn, R. P. (2022). Health implications and nutrient bioavailability of bioactive compounds in dry beans and other pulses. Dry beans and pulses: Production, processing, and nutrition, 505-529.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., ... & Murray, C. J. (2019). Food in the Anthropocene: the EAT–Lancet Commission on healthy diets from sustainable food systems. The lancet, 393(10170), 447-492.

Xazela, N. M., Hugo, A., Marume, U., & Muchenje, V. (2017). Perceptions of rural consumers on the aspects of meat quality and health implications associated with meat consumption. Sustainability, 9(5), 830.

Zander, P., Amjath-Babu, T. S., Preissel, S., Reckling, M., Bues, A., Schläfke, N., ... & Watson, C. (2016). Grain legume decline and potential recovery in European agriculture: a review. Agronomy for sustainable development, 36, 1-20.

Chapter 2 – Cultivation of organic pea (*Pisum sativum* L.) as substitute to soybean meal (*Glycine max* L.) for dairy cows feeding in Italian environments

Camilla Tibaldi[1], Mattia Alpi[1], Elettra Frassineti[1], Marcella Avondo[2], Massimiliano Lanza[2], Serena Tumino[2], Giorgio Borreani[3], Giovanni Dinelli[1], Ilaria Marotti[1*]

[1] Department of Agricultural and Food Sciences, Alma Mater Studiorum – University of Bologna, viale Giuseppe Fanin, 44 – 40127, Bologna, Italy

[2] Department of Agriculture, Food and Environment, University of Catania, via Santa Sofia, 100 – 95123, Catania, Italy

[3] Department of Agricultural, Forest and Food Sciences – University of Turin, Largo Paolo Braccini, 2 – 10095, Grugliasco (TO), Italy

[*]Corresponding author: ilaria.marotti@unibo.it

Abstract

In the context of the expanding global food demand and the climate-driven issues affecting the environment and biodiversity, organic cultivation of grain legumes can play a crucial role in making agri-food systems more sustainable. Among legumes, pea crop (*Pisum sativum* L.) gains considerable interest, thanks to its wide adaptability to soil characteristics and climatic conditions, while shows several nutritional and health benefits for both human and animal feed. In this study, the agronomic performance of peas cultivated in organic farming system in different Italian (Emilia-Romagna) environments (mountainous and hilly) and at different sowing times (Autumn and Spring) over two cropping seasons (2021 and 2022) was evaluated. In parallel, the harvested pea grain was administered to a group of dairy cows selected for experimental purposes, in substitution of soy-based diet, with the purpose to assess the nutritional value of the feed and the milk yield and quality of the examined animals was evaluated as well.

The results showed that environmental conditions (altitude, environment, sowing time, etc.) overall displayed a strong impact on both growth traits and yield parameters of the crop, showing significantly higher results ($P < 0.05$) in 2021 and mountainous (autumn sowed) samples. While, the incorporation of organic pea in dairy feed diet compared to the Control diet (soy-based) improved the nutritional composition of the feed and increased the milk yield produced by the cattle.

Keywords: legumes, peas, sustainable agronomic food system, dairy feed, milk quality.

2.1 Introduction

The world population is expected to reach 10 billion within the next decades, and to keep pace with it, food production is required to grow significantly to satisfy the expanding food demand (Ulian et al., 2020), despite the limited availability of arable land, water and fossil fuels (ISF, 2011). Global yield of main stable crops (wheat, rice and maize) reflected this trend, but after a worldwide increase (FAO, 2023; Brandão et al., 2010) actually it seems to reach the "peak" of the possible maximum rate in the near future. This situation is exacerbated by climate-driven changes (rising temperatures and altered precipitations), which are affecting and pressing structurally and functionally the ecosystem and biodiversity (Bélanger et al., 2019, Alae-Carew et al; 2020), and are expected to continue to impact the environment (Weiskopf et al., 2020). These current concerns drive to the need for a more sustainable food system that can support human and natural resources health. In this perspective, organic farming can be considered one of the possible solutions to face these issues, since it showed to be more environmentally sustainable in terms of pollution, biodiversity pressure, soil erosion and energy use (Brandão et al., 2010; Tuomisto et al., 2012). Recently, organic farming is significantly growing at global level, and it has been reported that organic farming is covering an average area of approximately 15.6 million hectares (22% globally) in Europe, displaying an increase of 1.25 million hectares compared to recent years (Willer et al., 2020).

In the perspective to ensure a sustainable production of safe and healthy food, the organic cultivation of legumes may give a considerable contribution to the revitalization of a sustainable agronomic system. Legumes are known and used for food and animal feed, and some of these crops, such as peas and lentils, are nitrogen fixing, require less or no nitrogen fertilizers (Burgess et al. 2012) and reduce the carbon footprint of other crops grown in rotation with them (Gan et al. 2011).

Peas (*Pisum sativum* L.) can be cultivated in cold and wet climatic regions, thanks to their capacity to withstand cold and drought conditions (Saha et al., 2018). Moreover, the health benefits of pea seed derive primarily from the qualities of proteins, starch, vitamins, fiber, protein, phytochemicals and minerals, as well as healthy promoting antioxidants. Peas are known and can be also used as animal feed (Hagenblad et al. 2014) thanks to their rich nutritional value and healing properties. Hence, they could represent a valid option to support the protein requirements of livestock in replacement of soybean, largely imported with high environmental challenges (deforestation, GMO, excessive water consumption, etc..). In particular, the ban of GMO soybean, as protein source in organic farming systems, improved the utilization of grain legumes together as in low input farming systems (Bonanno et al., 2011). In terms of dairy cattle feeding, although peas show lower protein content than soybean meal (SBM), they are rich in starch, being comparable to barley for starch rumen fermentability (Masoero, 2006). Furthermore, the pea proteins are richer in lysine, although lower in methionine, but combined with corn provides a more balanced aminoacid supply (Masoero et al., 2006). Hence, pea protein content provides higher rumen degradability if compared to SBM and a presence of some antinutritional factors is reported although often with no detrimental effect on animal performance when supplied as crude peas (Formigoni et al., 2007).

Given the significant importance of grain legumes in the perspective of a more sustainable agronomic system, a focus towards the effect of agronomic factors on growth and health conditions of the plant, may give a contribute to the revitalization of the production of sustainable and healthy food. Combining the need to increase local pulses production for feed nutrition, to substitute GMO feeds, the aim of the present study is to evaluate the agronomic performances of pea (*Pisum sativum* L.) for grain cultivated in organic farming system, under low input conditions over two consecutive cropping seasons in two contrasting environments of the Emilia-Romagna region (Italy). Additionally, the pea grains were characterized in terms of nutritional composition for dairy cows feeding and the relative effect on milk yield and composition in comparison with soybean-based diet was studied to evaluate the health status of the cows.

2.2 Materials and Methods

2.2.1 Experimental locations

The study was divided in two parts: the grain production in two contrasting environments and the feeding trial of dairy cows in an organic dairy farm. The field studies were conducted over two consecutive years (2021 and 2022) at two experimental locations: experimental farm of the University of Bologna in Ozzano dell'Emilia (BO, Italy) and an organic farm "Solaria bio" located in Loiano (BO, Italy). Geographical coordinates and soil types of the experimentation sites are provided in **Table 2.1**. The soil composition at the experimental fields was classified as loamy (12%, 28% and 60% of sand, silt, and clay, respectively) and fine and mixed (36%, 28%, and 36% of sand, silt, and clay) for Loiano and Ozzano location, respectively. The meteorological data (temperature and precipitation), for the entire duration of the trials, comprising each location, was obtained from the Arpae weather station, located in Emilia Romagna (https://simc.arpae.it/dext3r/) and are showed in the **Figure 2.1**. As regards the experimental field located in Ozzano, the trend in average temperatures appear to be very similar between the two cropping years (**Figure 2.1, A and B**), although temperatures are higher in 2022. However, the two growing years differed in terms of rainfalls, whereby higher precipitations were observed in the month consecutive to sowing in 2021 (April), while in 2022 rainfalls were significantly higher in the current month of the sowing. Same temperatures and rainfalls trend was observed for the experimental field of Loiano (**Figure 2.1, C and D**). In addition, in 2022 total rainfalls were more abundant during the vegetative and reproductive development of the crop (months from Feb-May 2022) if compared to 2021 cropping year.

In parallel, the experimental study on dairy cows was carried out at the organic dairy farm "Solaria Bio", located about 800 m above the sea level in Loiano (BO, Italy).

Table 2.1. Geographical coordinates, altitude and soil type at the experimental sites.

Location	Geographic coordinates	Altitude	Environment	Soil Type
Ozzano dell'Emilia (BO, Italy)	44°24'49.7"N 11°28'24.5"E	200 m	Hilly	Fine, mixed
Loiano (BO, Italy)	44°17'55.3"N 11°21'13.2"E	800 m	Mountainous	Loamy

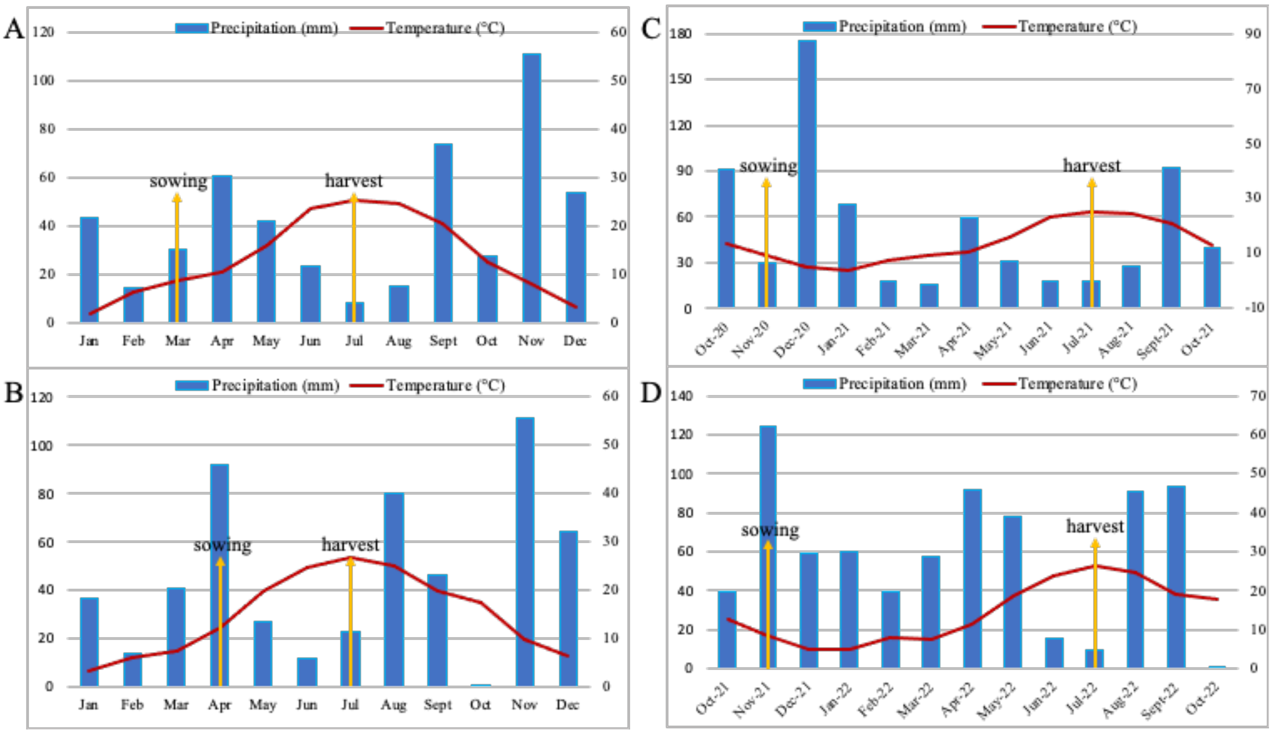

Figure 2.1. Monthly precipitation and average temperature at each location for all cultivation cycles between 2021 and 2022. A= Ozzano 2021, B = Ozzano 2022, C = Loiano 2021, D = Loiano 2022. Meteorological data supplied by the Arpae weather station, located in Emilia Romagna (https://simc.arpae.it/dext3r/).

2.2.2 Field trials

Organic *Pisum sativum* L. var. Turris grain was purchased from Arcoiris S.R.L (Modena, Italy). In both cultivation years (2021 and 2022) the seeds were sown at 200 kg/ha (80 seeds/m^2) of seed density. Within each location and season, the total cultivation area was 5000 m^2 for both mountainous and hilly environment. Planting distances within the designated area was 22 cm inter-row in both fields. The sowing time differed depending on the growing environment and on the elevation of the sites: for the mountainous environment was chosen an Autumn Sowing (AS), in November, while for the hilly environment a Spring Sowing (SS) in March/April. At both locations, organic farming system was followed: no pesticide or herbicide treatments were performed on the crop. Weeding at both environments was performed manually during the crop cycle.

During the growing cycle, phenological and agronomical variables (plant heigh, number of branches, flowers and pods) and weed surveys were periodically (weekly) recorded on a statistically significant number of pea plants on 1 m^2 randomly located plots in the field until the harvest.

The harvest was performed at two phenological stages: green ripening (BBCH-79) and dry ripening (BBCH-89) in June and July, respectively, at each environment and year of cultivation. Moreover, the agronomic yield, in terms of number of plants, grain biomass, weight of 1000 seeds and harvested yield, was estimated by randomized sampling on 1 m^2 randomly located plots for each environment in the two cultivation growing seasons and it was reported in Mg dry matter (DM)/ha.

2.2.3 Cows feeding trials

The feeding trials were carried out at the organic dairy farm "Solaria Bio", located about 800 m above the sea level in Loiano (BO, Italy).

The study had a duration of three years (2020, 2021, 2022) and the pea grain harvested during the open-field trial was used to carry out the 2021 and 2022 feeding trial. The cows (Holstein Friesian), housed in a free environment in a natural ventilation barn, were fed ad libitum once a day at 0700h at 110% expected intake. Among all the dairy cows, twenty multiparous (3rd-4th lactation) Holstein Friesian cows at late lactation stage (milk yield, 22.4±7.3 kg peer d; days of lactation, 247±134 d, body weight of 635 kg±55kg) were selected. Subsequently, the twenty cows were divided in two homogeneous blocks for milk yield and days of lactation and fed 21 kg of the Total Mixed Ration (TMR) reported in **Table 2.2.** The two treatments considered were: the control diet, based on soybean meal, and a reformulated diet, using the pea meal too replace soybean meal. The diets were formulated (NDS Professional, RUM&N Sas, Reggio Emilia, Italy) to be isonitrogenous and isoenergetic, and to meet the nutrient requirements of a 650 kg cow producing 23 kg/d milk with 3.5% fat and 3.1% protein. Briefly, the cows group named Pea diet was fed with a diet including peas in total replacement of soybean meal and partial replacement of corn and barley, while the Control group received a diet including soybean meal and a higher proportion of the cereal grains.

Table 2.2. Feed composition of the two experimental organic diets (% as fed).

Ingredient	Control diet	Pea diet
Alfalfa hay	33.4	33.4
Mixed hay	33.4	33.4
Corn	13.6	10.4
Barley	13.6	10.4
Peas	-	11.3
Soybean meal	5.0	-
Mineral and vitamin mix	1.0	1.0

2.2.4 Feed quality analysis

A two-period cross-over design was adopted. Each period lasted 21 days, with a 21-day washout between periods, during which the cows were fed with a mix of the two experimental diets at equal proportions (50%).

At the beginning of each feeding trial, samples for pea grain and total mixed ration (TMR) were collected. Feed samples for analyses were obtained by mixing an equal amount of the collected subsamples and analyzed for dry matter (DM), crude proteins, fats, ash, and structural carbohydrates (ADL, NDF and ADL) according to AOAC procedures (2005). Moreover, the two experimental feeding diets were analysed for total polyphenols (TP) and flavonoid (TF) content, as previously described by Di Silvestro et al. (2012), and TP quantification was carried out according to the Folin-Ciocalteau spectrophotometric (765 nm) method using gallic acid (GA) as a reference standard (Singleton et al., 1999). TF were measured by spectrophotometric (510 nm) colorimetric assay with catechin (CA) as a reference standard (Adom et al., 2003). The DPPH assay was performed by measuring the reduction (515 nm) of DPPH• to 1,1-diphenyl-2-picryl hydrazine (Floegel et al., 2011) and tannin content as described by Adegbus (2022).

During each feeding trial and during each 21-day experimental period, every 7 days, individual milk yield was measured twice daily at 05.00 and 17.00, and samples were collected from the morning and afternoon milking. Milk samples were analysed for fat, lactose, protein, casein, and urea content by chemical laboratory of ARAER (Funo, Italy), by means of a MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark), according to the International Dairy Federation standards.

2.2.5 Statistical analysis

Statistical analyses were conducted using the Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA). Two-way analysis of variance (ANOVA) in conjunction with Tukey’s honest significant difference was performed to compare the growing environments with the cultivation years. Significant differences between means were determined by least significant difference values for $P < 0.05$. Pearson's correlation coefficient (r) was calculated at significance level of $P < 0.01$.

Milk yield and composition data were analysed using the repeated measures GLM procedure (SPSS for Windows, Inc., Chicago, IL, USA). The statistical model included the following factors: diet, block and period. Days of lactation was used as covariate for milk yield and gross composition. Covariate with no significant effects was excluded from the model.

2.3 Results and Discussion

With the focus on a more sustainable agronomic food system, driven by the expanding global food demand and the environment and biodiversity climate-driven issues, the revitalization of organic production of legumes may support human and natural resources health, and thus it may become a topic of great interest for both consumers and food industry (McClements et al. 2009; Benítez et al., 2013). In the framework of the local production, *Pisum sativum* L. is an important crop characterized by wide adaptability to different soil types and high production potential, with several health benefits for both human and animal feed.

In this perspective, a multidisciplinary approach was used to evaluate whether and how environmental conditions (i.e. altitude, temperature, precipitations, sowing time) influence the agronomic performance of organic pea crop cultivated in different growing locations in Emilia Romagna (Italy) over two consecutive years (2021 and 2022) (**Figure 2.2**, **A** and **B**).

The parameters measured were growth profile (height, internodes, flowers and pods) and agronomic yield, over two phenological stages (BBCH-79 and BBCH-89), in terms of number of plants, grain biomass, weight of 1000 seeds and harvested yield.

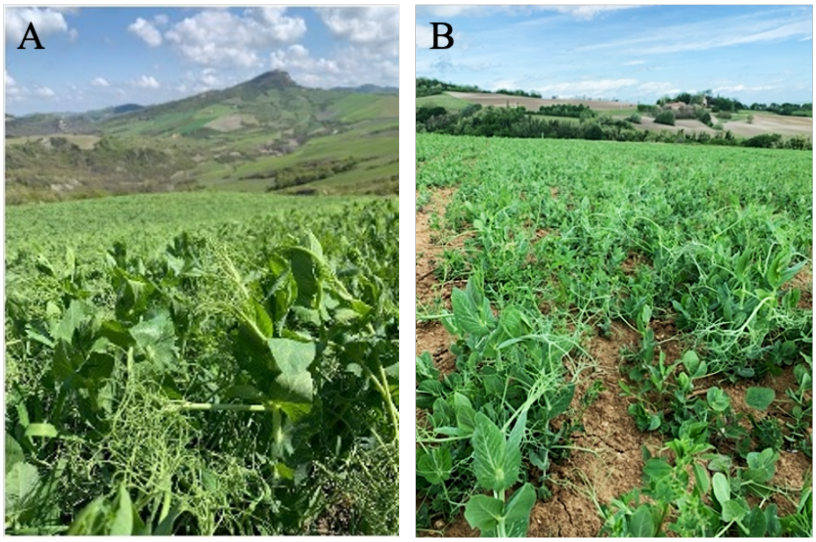

Figure 2.2. Organic cultivation of pea (*Pisum sativum* L.) in the experimental location of Loiano (A) and Ozzano (B) in the cropping year 2021.

2.3.1 Growth traits of peas (Pisum sativum L.) for location and year

Growing parameters (height, internodes, flowers, pods) on a statistically significant number of plants were recorded during the vegetative and productive development of the crop and analysed in terms of sowing time (differed by growing environment) and year of cultivation (**Table 2.3**).

Table 2.3. Mean values of agronomic traits of pea plants in the growing environments with different sowing times over two years of cultivation. Different letters within each column: significant values ($p \leq 0.05$, Tukey's least significance difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. ns= not significant. S x Y = interaction sowing time x year of cultivation.

		height	**internodes**	**flowers**	**pods**
	u.m.	(cm/plant)	(#/plant)	(#/plant)	(#/plant)
Sowing time	autumn	60.21 ***a***	14.55 ***a***	2.39 ***a***	4.07 ***a***
	spring	27.36 ***b***	11.45 ***b***	0.87 ***b***	1.42 ***b***
Year	2021	46.76 ***a***	14.03 ***a***	2.05 ***a***	2.98 ***a***
	2022	40.81 ***b***	11.98 ***b***	1.21 ***b***	2.51 ***b***
	S x Y	***	*ns*	*ns*	***

Sowing time and environment showed a positive impact in the growth of the pea plants, as reported also by Reguera et al. (2018), observing the height of the crop significantly higher in the autumn sowed plants (60.2 cm/plant), as well for all the other growing parameters. In this perspective, it can be demonstrated that autumn sowing, used as agronomic strategy for the cultivation of peas in mountainous environment, considering the longer growing period compared to the spring sowed grain, allows to a longer period of accumulating biomass, and a more effective utilization of post-winter water (Prusiński et al. 2016). As also showed by Urbatzka et al. (2012), cropping winter peas may have several agronomic advantages if compared with spring peas, in terms of higher N_2 fixation, higher N preceding crop effect, more efficient suppression of weeds, higher yield potential and stability (Urbatzka et al. 2011a; Urbatzka et al. 2009; Urbatzka et al., 2011b; Stoddard et al., 2006). A further explanation may be the fact that in the mountainous environment, a significant presence of weeds (estimated average coverage of 30%), in particular of *Sinapis arvensis*, was found in the post-flowering phase of the plant. It is therefore presumable that the pea plants may have invested in height growth to compete with weed species.

In addition, the cultivation year displayed a significant influence on the growth traits, which showed to be significantly higher in 2021. More in particular, strong differences on meteorological parameters were observed between the two cultivation years, with a cumulative precipitation of 299.8 mm in 2021 over 344.7 mm in 2022, while a milder differential in average annual temperatures was observed of 14.6 and 16.9°C for 2021 and 2022, respectively. Hence, given the large climatic differences, occurred mostly in rainfalls, over the two growing years, it can be supposed that excessively wet and rainy weather conditions may limit the agronomic development of the plant.

Among the growing traits, plant height and number of pods displayed a strong significant interaction between sowing time and cultivation year (**Table 2.3**) and thus, the respective interaction was studied and represented in **Figure 2.3**. To the respect of both parameters (height and pods), it can be observed that a synergic effect exists between the examined factors, year of cultivation and time of sowing (**Figure 2.3**). This can be due to spring environmental conditions in 2021 and 2022 exerted on the pea crop were quite similar. Whereas the autumn environmental conditions proved to be very different over the two growing seasons. In particular, cumulative rainfalls occurred during spring sowing environment were 134 and 131 mm for 2021 and 2022, respectively, while in autumn sowing environment were 292 and 340 mm for 2021 and 2022, respectively. Similar trend may be observed among mean temperatures, displaying 11.8 and 13.3 °C respectively for 2021 and 2022 in the spring hilly environment, while slight differences were observed in mountainous autumn sowed environment (6.25 and 6.86 °C). Overall, these observations imply that the environmental conditions have a strong impact on the analysed parameters.

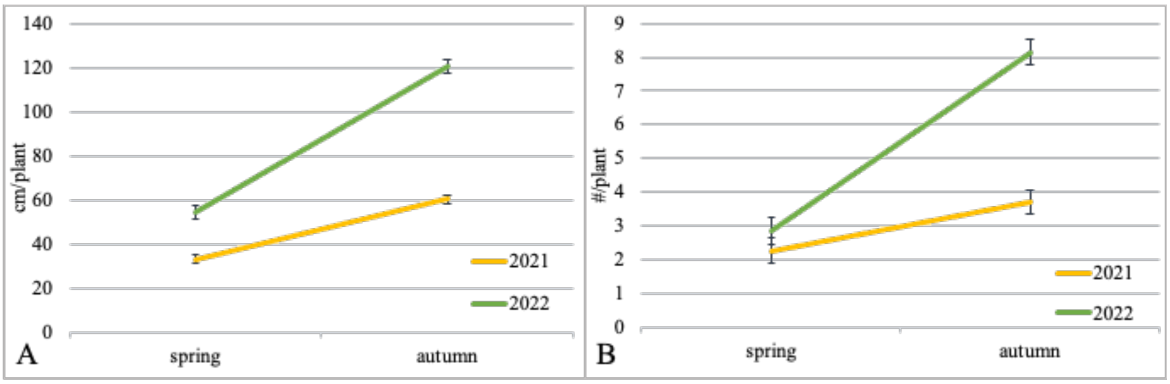

Figure 2.3. Interaction model of agronomic growth features of pea plants with different sowing times (different environments) over two years of cultivation. A = height in cm/plant, B = number of pods/plant.

2.3.2 Yield parameters of peas (Pisum sativum L.) for location and year

Yield is one important determinant parameter influencing the potential success of pea crops in the context of recently increased consumers' demand. In the present study, the number of plants for each m^2 ranged between 55.6 and 84.8 for each m^2, and were significantly higher in 2021 growing season (84.8), in terms of green ripening plants. The same trend was observed in the dry ripening plants (72 and 37.25 for 2021 and 2022, respectively.) This result is due to seed dehiscence detected at harvesting and related to delayed sowing timing, as occurred in the hilly environment in 2022 season (April 2022, **Figure 2.1**), which negatively impacted pea seed germination in the growing environment, leading to lower growth of the plants in the field.

As regards grain biomass (g/m^2), in green ripening peas ranged between 370 and 500 g/m^2, while for dry ripening peas it ranged between 126 and 180 g/m^2, displaying in both phenological stages no significative differences among sowing time and year of cultivation.

However, autumn sowed grain reported significantly higher weight of 1000 seeds over spring environment, displaying 342 and 309 g/m^2 and 184 and 145 g/m^2 in both green and dry ripening grain, respectively. It was highlighted by Tao et al. (2017) that seed size and weight can be linked to better seedling survival, which could ultimately produce higher yields, compared to smaller seed plants. Indirectly also influencing the number of pods in the plants.

The mean grain yield harvested in the two Italian environments (mountainous and hilly) over the two growing seasons (2021 and 2022) ranged from 4.11 and 4.17 Mg/ha and 3.70 and 4.58 Mg/ha, respectively. The showed data are in line with organic pea grain yield analysed in literature (Annicchiarico, 2006). However, no significant differences were observed between the sowing times (different environments) and growing seasons, in terms of green ripening biomass. Given this evidence, despite mountainous environments usually have lower yields (Stelling et al.,1997), autumn sowing could be a considerable strategy that gives good results comparable to the hilly environment yields, thanks to greater crop biomass and better ability to control weeds in the early stages of the crop cycle. In addition, spring sowing method in hilly environment do not penalize the grain effective final yield, although the plant growth values displayed were lower (**Table 2.3**). Thus, both agronomic methods may be worth of consideration, according to the pedo-climate conditions of the examined field, in order to optimize the productivity of the crop. As regards dry biomass, the most abundant harvested grain was obtained in spring sowed samples to the respect to autumn sowing (1.79 and 1.26 Mg/ha, respectively), although no significant differences were observed between 2021 and 2022 growing seasons (1.40 and 1.65 Mg/ha, respectively). In this case, the lower autumn yield was related to the excessive presence of weeds (30%) and high incidence of wild animals in the late phenological stages of the crop in the mountainous growing environment in 2021 during the vegetative development of the plant, that severely damaged the amount of biomass in the mountainous field.

Table 2.4. Mean values of agronomic yield parameters (number of plants, grain biomass, 1000 seeds and yield) of peas (*Pisum sativum* L.) cultivated in two growing environments with different sowing times over two years of cultivation. Different letters within each column: significant values ($p \leq 0.05$, Tukey's least significance difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. ns = not significant. S x Y = interaction sowing time x year of cultivation.

		Green ripening (BBCH-79)				**Dry ripening (BBCH-89)**			
	Parameter	Plants	Grain biomass	1000 seeds	Yield	Plants	Grain biomass	1000 seeds	Yield
	u.m.	#/m^2	g/m^2	g	Mg/ha	#/m^2	g/m^2	g	Mg/ha
Sowing time	autumn	64.91 ***a***	417.06 ***a***	342.29 ***a***	4.17 ***a***	61.08 ***a***	126.17 ***b***	183.66 ***a***	1.26 ***b***
	spring	75.5 ***a***	453.23 ***a***	309.39 ***b***	4.11 ***a***	48.16 ***b***	179.84 ***a***	144.73 ***b***	1.79 ***a***
Year	2021	84.83 ***a***	370.17 ***a***	342.50 ***a***	3.70 ***a***	72 ***a***	140.79 ***a***	187.87 ***a***	1.40 ***a***
	2022	55.58 ***b***	500.12 ***a***	309.18 ***b***	4.58 ***a***	37.25 ***b***	165.22 ***a***	140.52 ***b***	1.65 ***a***
	S x Y	**	*ns*	*ns*	*ns*	**	*ns*	*ns*	*ns*

As reported in **Table 2.4**, the significative interaction between environment conditions (sowing time) and cultivation years is deepen, in terms of number of plants/m^2 of both in green and dry ripening plants and it is reported in **Figure 2.4**. In the case of the dry ripening peas, a similar trend of growing traits interaction (**Figure 2.3**) can be observed. This means that the climatic conditions observed in the spring sowed environment were very similar between the two cultivation years under investigation (2021 and 2022), whereas the environmental conditions of the autumn sowing location proved to be quite different between 2021 and 2022. Thus, it is plausible that a synergic effect of these factors was exerted on the number of plants in the dry ripening peas. As regards the number of plants in green ripening crops, a synergic trend between the two examined factors (sowing time and cultivation year) was observed. In this case, it can be due to increased seed germination that occurred in the spring (hilly) environment during the vegetative development of the plants. This observation was also supported by Stelling et al. (1997), which assessed that higher yield proportions of peas were found under dry and warm weather conditions.

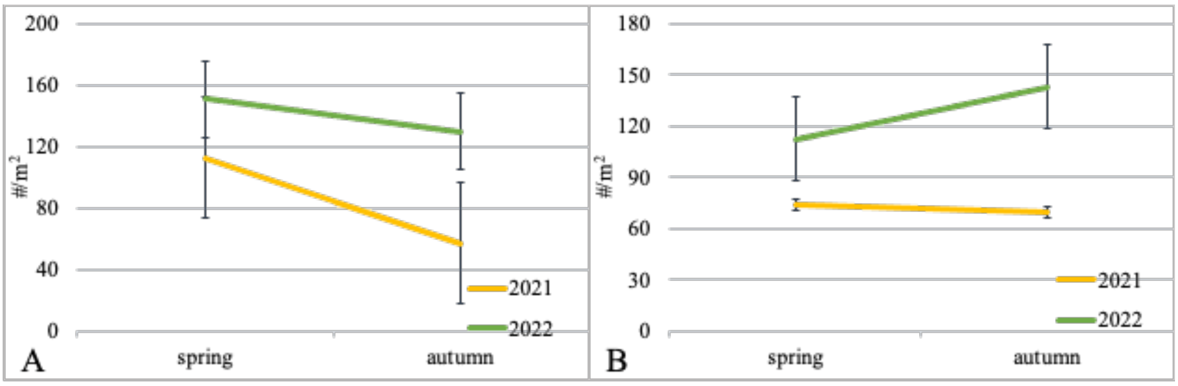

Figure 2.4. Interaction model of yield parameters of peas (*Pisum sativum* L.) cultivated with different sowing times (different environments) over two years of cultivation. A = green ripening number of plants/m^2, B = dry ripening number of plants/m^2.

2.3.3 Pea-Based Diet for Dairy Cattle Feeding

In the framework of livestock production and the supplementation of local forages production aimed to a more sustainable agronomic system, the healthy and nutritional potential of pea-based diet for dairy cattle feed was evaluated, with the respect to typical soybean-based feeding. The nutritional parameters measured were: nutritional characterization of the two diets (soy-based and pea-based), in terms of dry matter, crude proteins, fats, ash, NDF, ADF, Lignin, NFC, Starch and Total Sugars, Total Polyphenols (TP), Total Flavonoid (TF), DPPH and tannin content, and dairy milk yield and composition (Fat, Protein, Lactose, Casein, Urea).

Figure 2.5. Groups of dairy cows (Holstein Friesian) feed with the pea-based diet in the organic farm "Solaria Bio" during the experimental trial.

2.3.3.1 Dairy cattle diets

In **Table 2.5** is reported the chemical composition of the diets. The forage:concentrate ratio was 68:32 (on a DM basis) for both the diets thus respecting the minimum dietary threshold (60 %) of roughage according to EU organic rule 848/2018 (EU, 2018). The two diets were formulated to have similar content of crude protein and fiber, although a certain difference was found for both parameters. Protein content was different between dietary treatments across the three years of study being higher in Control diet compared to Pea diet, particularly in the 3rd year of the experimental trial (12.1 vs. 10.7 % DM, respectively). Apart from the 1st year, NDF content resulted higher in Pea diet in the 2nd and 3rd year (42.8 vs. 41.3 % DM and 45.7 vs. 41.9 % DM, respectively in Pea diet and Control diet), thus denoting a moderately lower quality of this diet compared to control diet. Hence, it could probably be inferred that the difference in these chemical parameters lies in the pea grains cultivated and used in the different years. In fact, Wang and Daun et al., 2004 reported that environmental conditions have significant effects on starch, fiber fractions, fat contents, and on some sugars, minerals and aminoacids. Moreover, starch content was hugely higher (+ 26%) in Pea diet compared to Control diet (20.2 vs. 16 %

DM, respectively) in the 1st year of the study while quite similar between dietary treatments in the 2nd and 3rd years.

Finally, bioactive compounds, such as polyphenols, flavonoids and tannins, recently gained considerable interest because were identified as promising reducers of methane emissions thanks to their capacity to lowering degradability effects on plant material (Morgavi et al., 2012; Formato et al., 2022) and to their modulatory effect that inhibit methanogenic microbes accessibility during rumen fermentation (produces methane emissions) (Chen et al., 2020; Formato et al., 2022). In this perspective, total polyphenol and flavonoid content, and the respective antioxidant activity (DPPH assay) was measured on both examined feeding diets. The results showed that, although TP concentration was higher in Control diet, the pea-based feed displayed higher content of TF to the respect of the soy-based diet, among the three examined years and this is possibly due to flavonoid structure and in the variable degree of substitution, especially in terms of phenolic functions (Formato et al., 2022). While no differences were observed in terms of DPPH assay (antioxidant activity) between the two diets. As regards tannins, these compounds content were lower in the pea diet during the 1st and the 3rd year and slightly higher during the 2nd year. In general, the presence of tannins in the diet can determine, among other effects, a decrease in dry matter intake as reported in cattle and sheep (McNabb et al., 1996; Priolo et al., 2000; Barry and McNabb, 1999) due to astringency effect together with a decreased palatability. Nevertheless, the lack of orts in manger demonstrated that animals fed all the offered rations with no refusals.

Nonetheless, the overall effects of tannins would seem dependent on tannins type (condensed, hydrolizable) and dosage and on basal diets used together with different tolerance among animal species and this account for some controversial effects reported in literature (Frutos et al., 2020). According to dose level and chemical structure, tannins could enhance protein utilization, control internal parasites and improve animal performance, product quality and welfare. Moreover, they can play an important role in improving antioxidant and immunity status of animals (Besharati et al., 2022). The antioxidant power of diets measured through DPPH assay was lower along the three years in peas diet compared to soybean included diet (Control) and consistent with total polyphenols and tannins findings in the two diets. Literature reported a great variation in antioxidant activity expressed as DPPH among different cultivars of Pisum sativum which ranges from 0.44 μmol to over 10 μmol TE/g (Devi et al., 2019). The average DPPH values of peas used in the three year resulted equal to 0.51 μmol TE/g thus more close to the lowest level reported by previous cited authors.

Table 2.5. Chemical composition of the experimental organic feeds. All the samples were analysed for DM. DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, NFC = nonstructural carbohydrate, TP = total polyphenols, TF = total flavonoids, FRAP = ferric reducing antioxidant potential, DPPH = 1,1-diphenyl-2-picrylhydrazyl anti-radical activity.
[1]: average chemical composition of peas (*Pisum sativum* L.) used along the three experimental years. [2]:mg Gallic acid equivalent (GAE)/100 g; [3]: mg Catechin equivalent (CE)/ 100 g; [4]: μmol Trolox Equivalent (TE)/g; [5]: Tannin acid equivalent, %. [6]: average level 3 yrs.

		2020		**2021**		**2022**	
Parameters	Pea grain[1]	Control diet	Pea diet	Control diet	Pea diet	Control diet	Pea diet
Dry matter %	88.3	95.8	96.2	96.0	96.0	96.2	96.2
Crude protein % DM	20.5	13.5	12.8	13.9	12.7	12.1	10.7
Fat % DM	1.2	3.8	2.6	3.8	2.9	2.7	1.8
Ash % DM	3.5	9.4	7.8	8.5	8.3	8.1	8.5
NDF % DM	14.2	43.8	41.3	41.3	42.8	41.9	45.7
ADF % DM	7	30.8	29.7	29.4	30.3	29.0	31.9
Lignin % DM	0.4	5.9	5.4	5.8	5.7	5.5	5.3
NFC % DM	60.6	29.5	35.4	32.5	33.3	35.2	33.3
Starch % DM	51.3	16.0	20.2	17.9	18.4	19.6	18.6
Total sugars % DM	8.3	4.5	4.9	4.9	5.1	5.7	5.6
Total polyphenols[2]	42.23[6]	281.8	271.3	326.6	206.6	297.9	211.5
Total Flavonoids[3]	94.28[6]	136.7	159	133.9	193.9	107.2	167.1
Total DPPH[4]	0.51[6]	2.5	2.2	2.6	1.9	2.2	1.9

Tannins[5]	0.93[6]	9.4	2.5	6.3	7.4	11.6	6.2

2.3.3.2 Dairy cattle milk yield

In **Table 2.6**, milk yield and composition are reported for the three years. Considering that in the late lactation stage the protein requirements of dairy cattle drop down with milk production, thus diets with low crude protein concentration could be fed to animals also to reduce feeding costs and waste nitrogen excretion while maintaining milk yield (Kalsheur et al., 1999). Milk yield was significantly ($P < 0.05$) higher in cows fed diets supplemented with pea grain during the 1st and the 2nd year, as also demonstrated by Masoero et al. (2006), which reported a significant increase of milk yield by replacing soybean meal with peas in dairy cattle feed. However, milk yield was not affected ($P=0.66$) by diet during the 3rd year. However, it can be supposed that higher levels of tannins and polyphenols in the soybean-based diet of the control group may have contributed to the lower milk production as a possible effect of anti-nutritional factors. Tannins can have negative effects on digestibility and performance in ruminants when their levels (in the chemical form of condensed tannins) are higher than 5% of the diet (Frutos et al., 2004). This would be consistent with milk yield differences recorded in the first year (associated with tannin levels of 9.44 and 2.53 in the control and pea diets respectively) and of the third year (lower production in the pea group associated with tannins level above 5% threshold).

As regards milk composition, no dietary effect differences were found between groups, except for a significant ($P < 0.05$) reduction in milk urea during the 1st year, in pea group. This lower content of milk urea showed, at the ruminal level, a better synchrony between the availability of nitrogen and energy. Hristov et al. (2005) tested the effect of the type of carbohydrate on rumen ammonia utilization and observed that starch- and glucose-rich diets resulted in lower concentration of rumen ammonia, plasma urea nitrogen (PUN) and milk urea nitrogen (MUN) compared with a neutral detergent fiber (NDF)-rich diet. Moreover, the higher starch and sugars content and the lower NDF content in Pea diet compared to Control feed in the 1st year could have supported the difference in milk urea between groups. Moreover, the higher starch content in pea diet in the 1st year compared to control (+ 26%) probably provided more energy to animals while in the 2nd and 3rd years the difference in starch content among diets were more limited, although in the 2nd year Pea diet group confirmed a higher milk yield compared to Control meal. Di Grigoli et al. (2018) in a similar trial carried out in Bruna dairy cattle reared according to organic system reported a higher milk production in cows fed on diet including pea in total replacement of soybean meal while no variation occurred in the main milk components. Other studies supported our findings summarizing no negative effect of SBM replacement with peas in dairy cow feeding in terms of milk yield and quality (Van der Poel et al., 2008). In the 3rd year, urea milk level increased in both groups if compared to the levels measured in 1st and, in particular, in the 2nd year without any statistical difference between dietary treatments. This finding was not expected looking at the decreased dietary protein levels in both diets supplied to animals but much more in pea diet compared to Control feed. Several factors affect milk urea level in dairy cattle but related to nutritional factors there are many evidences that over changes in dietary CP content it is also associated to the ratio of dietary CP to energy intake, efficiency of N utilization, or rumen ammonia concentration (Nousiainen et al., 2004).

Table 2.6. Milk yield and composition of dairy cows fed with the two experimental diets among 3 years of experimentation.

	1st Year			**2nd Year**			**3rd Year**		
	Control diet	Pea diet	P	Control diet	Pea diet	P	Control diet	Pea diet	P
Milk yield kg/d	17.70	22.70	0.017	17.03	22.07	0.030	18.04	18.67	0.661
Fat %	3.67	3.43	0.140	3.47	3.31	0.508	4.03	3.88	0.253
Protein %	3.29	3.21	0.437	3.65	3.62	0.911	3.56	3.50	0.589
Lactose %	4.69	4.60	0.093	4.56	4.67	0.231	4.53	4.59	0.530
Casein %	2.57	2.51	0.422	2.84	2.84	0.994	2.80	2.76	0.664
Urea mg/dl	20.2	17.9	0.013	12.73	13.23	0.552	21.19	22.26	0.286

Overall, the pea-included diets in the 1st and 2nd years led to a higher milk production compared to Control feed, thus making the above nutritional differences related to crude protein and NDF of little significance. In the 3rd year, the larger difference in protein and NDF content between diets probably justified the drop of milk yield in Pea group compared to the previous experimental years in comparison with less variable trend in milk yield across the three years in Control group.

2.4 Conclusions

The results obtained in the present study highlighted that the cultivation in different environmental conditions (altitude, environment, sowing time, cultivation year, etc.) of organic pea (*Pisum sativum* L. var. Turris) could significantly impact on the agronomic performance of the crop, in terms of growth traits and yield parameter. In particular, although the growth parameters were promising in the mountainous environment, the hilly location showed comparable yield results with the mountainous environment. Thus, in the perspective of the increased importance of the sustainable agri-food production, to be performed at local level, the evaluation of the pedo-climate conditions of the cultivation field may be the key-strategy to choose the best agronomic methods to apply on the field, in order to optimize the productivity of the crop.

In addition, the inclusion of the harvested organic pea grain in dairy cows feed, as substitute of soy-based diet, showed a positive productive effect on the milk yield of the examined animals and among the nutritional composition of the feed (particularly urea content), compared to the control diet. Overall, peas incorporation showed to be a valuable and sustainable alternative to soybean in the nutrition of dairy cattle in organic farms located in Italian environments.

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References

Adegbusi, H. S., Ismail, A., Mohd Esa, N., & Azuan Mat Daud, Z. (2022). Application of Folin-Ciocalteau colorimetric method in the determination of total tannin in maize and soybean food products. International Food Research Journal, 29(5).

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. Journal of agricultural and food chemistry, 51(26), 7825-7834.

Alae-Carew, C., Nicoleau, S., Bird, F. A., Hawkins, P., Tuomisto, H. L., Haines, A., ... & Scheelbeek, P. F. (2020). The impact of environmental changes on the yield and nutritional quality of fruits, nuts and seeds: a systematic review. Environmental research letters: ERL [Web site], 15(2), 023002.

Annicchiarico, P. (2006). Pisello da granella in agricoltura biologica. L'INFORMATORE AGRARIO, 62(39), 73-75.

AOAC (1990). Official methods of analysis. 15th. ed. Association of Official Analytical Chemists, Washington, DC, USA

Baselice, A., Colantuoni, F., Lass, D. A., Nardone, G., & Stasi, A. (2017). Trends in EU consumers’ attitude towards fresh-cut fruit and vegetables. Food Quality and Preference, 59, 87-96.

Bélanger, J., & Pilling, D. (2019). The state of the world's biodiversity for food and agriculture. Food and Agriculture Organization of the United Nations (FAO).

Benítez, V., Cantera, S., Aguilera, Y., Mollá, E., Esteban, R. M., Díaz, M. F., & Martín-Cabrejas, M. A. (2013). Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes. Food Research International, 50(1), 64-69.

Besharati, M., Maggiolino, A., Palangi, V., Kaya, A., Jabbar, M., Eseceli, H., ... & Lorenzo, J. M. (2022). Tannin in Ruminant Nutrition. Molecules, 27(23), 8273.

Brandão, M., Clift, R., Milà i Canals, L., & Basson, L. (2010). A life-cycle approach to characterising environmental and economic impacts of multifunctional land-use systems: An integrated assessment in the UK. Sustainability, 2(12), 3747-3776.

Burgess, M. H., Miller, P. R., & Jones, C. A. (2012). Pulse crops improve energy intensity and productivity of cereal production in Montana, USA. Journal of sustainable agriculture, 36(6), 699-718.

Business, New Nutrition. (2020). "Key Trends in Food, Nutrition Health 2020." New Nutrition Business: London, UK.

Chen, J., Harstad, O. M., McAllister, T., Dörsch, P., & Holo, H. (2020). Propionic acid bacteria enhance ruminal feed degradation and reduce methane production in vitro. Acta Agriculturae Scandinavica, Section A—Animal Science, 69(3), 169-175.

Devi, J., Sanwal, S. K., Koley, T. K., Mishra, G. P., Karmakar, P., Singh, P. M., & Singh, B. (2019). Variations in the total phenolics and antioxidant activities among garden pea (Pisum sativum L.) genotypes differing for maturity duration, seed and flower traits and their association with the yield. Scientia Horticulturae, 244, 141-150.

Di Grigoli, A., Bonanno, A., Vargetto, D., Tornambè, G., & Marchetta, P. F. (2008). Utilizzazione del pisello proteico in alternativa alla farina di estrazione DI soia in un allevamento biologico di bovine da latte. Proceedings of the VI Convegno Nazionale Associazione Italiana di Zootecnia Biologica e Biodinamica. Arezzo, Italy, 19-24 May.

Di Silvestro, R., Marotti, I., Bosi, S., Bregola, V., Carretero, A. S., Sedej, I., ... & Dinelli, G. (2012). Health-promoting phytochemicals of Italian common wheat varieties grown under low-input agricultural management. Journal of the Science of Food and Agriculture, 92(14), 2800-2810.

E.U. (2018). REGULATION (EU) 2018/848 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007. Official Journal of the European Union, L150: 1-92, 14.06.2018.

Faisant, N., Planchot, V., Kozlowski, F., Pacouret, M. P., Colonna, P., & Champ, M. (1995). Resistant starch determination adapted to products containing high level of resistant starch. Sciences des aliments= Food science: an international journal of food science and technology, 15(1), 83-89.

Fan, X. (2022). Chemical inhibition of polyphenol oxidase and cut surface browning of fresh-cut apples. Critical reviews in food science and nutrition, 1-15.

Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. Journal of food composition and analysis, 24(7), 1043-1048.

Forgrieve J. (2018) The Growing Acceptance Of Veganism Forbes https://www.forbes.com/sites/janetforgrieve/2018/11/02/picturing-a-kindler-gentler-world-vegan-month/

Formato, M., Cimmino, G., Brahmi-Chendouh, N., Piccolella, S., & Pacifico, S. (2022). Polyphenols for Livestock Feed: Sustainable Perspectives for Animal Husbandry?. Molecules, 27(22), 7752.

Formigoni, A., Fustini, M., Mordenti, A., Nocetti, M., & Vecchia, P. (2007). Favino e pisello nella razione, buoni sostituti della soia. L'INFORMATORE AGRARIO, 17, 40-44.

Frutos, P., Hervas, G., Giráldez, F. J., & Mantecón, A. R. (2004). Tannins and ruminant nutrition. Spanish journal of agricultural research, 2(2), 191-202.

Gan, Y., Liang, C., Campbell, C. A., Zentner, R. P., Lemke, R. L., Wang, H., & Yang, C. (2012). Carbon footprint of spring wheat in response to fallow frequency and soil carbon changes over 25 years on the semiarid Canadian prairie. European Journal of Agronomy, 43, 175-184.

Gomes, M. H., Fundo, J. F., Poças, M. F., & Almeida, D. P. (2012). Quality changes in fresh-cut 'Rocha'pear as affected by oxygen levels in modified atmosphere packaging and the pH of antibrowning additive. Postharvest Biology and Technology, 74, 62-70.

Tuomisto, H. L., Hodge, I. D., Riordan, P., & Macdonald, D. W. (2012). Does organic farming reduce environmental impacts?–A meta-analysis of European research. Journal of environmental management, 112, 309-320.

Hagenblad, J., Boström, E., Nygårds, L., & Leino, M. W. (2014). Genetic diversity in local cultivars of garden pea (Pisum sativum L.) conserved 'on farm'and in historical collections. Genetic Resources and Crop Evolution, 61, 413-422.

Halmemies-Beauchet-Filleau, A., Rinne, M., Lamminen, M., Mapato, C., Ampapon, T., Wanapat, M., & Vanhatalo, A. (2018). Alternative and novel feeds for ruminants: Nutritive value, product quality and environmental aspects. Animal, 12(s2), s295-s309.

Hristov, A. N., Ropp, J. K., Grandeen, K. L., Abedi, S., Etter, R. P., Melgar, A., & Foley, A. E. (2005). Effect of carbohydrate source on ammonia utilization in lactating dairy cows. Journal of Animal Science, 83(2), 408-421.

Hykkerud, A. L., Uleberg, E., Hansen, E., Vervoort, M., Mølmann, J., & Martinussen, I. (2018). Seasonal and yearly variation of total polyphenols, total anthocyanins and ellagic acid in different clones of cloudberries (Rubus chamaemorus L.).

Ioannou, I. (2013). Prevention of enzymatic browning in fruit and vegetables. European Scientific Journal, 9(30).

ISF (International Seed Federation) (2011) Agriculture under pressure. http://www.worldseed.org/isf/home.html

Kalscheur, K. F., Vandersall, J. H., Erdman, R. A., Kohn, R. A., & Russek-Cohen, E. (1999). Effects of dietary crude protein concentration and degradability on milk production responses of early, mid, and late lactation dairy cows. Journal of Dairy Science, 82(3), 545-554.

Masoero, F., Moschini, M., Fusconi, G., & Piva, G. (2006). Raw, extruded and expanded pea (Pisum sativum) in dairy cows diets. Italian Journal of Animal Science, 5(3), 237-247.

McClements, D. J., Decker, E. A., Park, Y., & Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Critical reviews in food science and nutrition, 49(6), 577-606.

McNabb, W. C., Waghorn, G. C., Peters, J. S., & Barry, T. N. (1996). The effect of condensed tannins in Lotus pedunculatus on the solubilization and degradation of ribulose-1, 5-bisphosphate carboxylase (EC 4.1. 1.39; Rubisco) protein in the rumen and the sites of Rubisco digestion. British journal of nutrition, 76(4), 535-549.

Morgavi, D. P., Martin, C., Jouany, J. P., & Ranilla, M. J. (2012). Rumen protozoa and methanogenesis: not a simple cause–effect relationship. British Journal of Nutrition, 107(3), 388-397.

Nousiainen, J., Shingfield, K. J., & Huhtanen, P. (2004). Evaluation of milk urea nitrogen as a diagnostic of protein feeding. Journal of dairy science, 87(2), 386-398.

Pinto, M., Coelho, E., Nunes, A., Brandao, T., & Coimbra, M. A. (2015). Valuation of brewers spent yeast polysaccharides: A structural characterization approach. Carbohydrate Polymers, 116, 215-222.

Priolo, A., Waghorn, G. C., Lanza, M., Biondi, L., & Pennisi, P. (2000). Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: effects on lamb growth performance and meat quality. Journal of Animal Science, 78(4), 810-816.

Prusiński, J. (2016). Overwintering and yield of winter cultivars of field pea Assas and white lupine orus. Electronic Journal of Polish Agricultural Universities, 19(4).

Reguera, M., Conesa, C. M., Gil-Gómez, A., Haros, C. M., Pérez-Casas, M. Á., Briones-Labarca, V., ... & Bascuñán-Godoy, L. (2018). The impact of different agroecological conditions on the nutritional composition of quinoa seeds. PeerJ, 6, e4442.

Saha, U., Vann, R. A., Chris Reberg-Horton, S., Castillo, M. S., Mirsky, S. B., McGee, R. J., & Sonon, L. (2018). Near-infrared spectroscopic models for analysis of winter pea (Pisum sativum L.) quality constituents. Journal of the Science of Food and Agriculture, 98(11), 4253-4267.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology (Vol. 299, pp. 152-178). Academic press.

Stelling, D. (1997). Dry peas (Pisum sativum L.) grown in mixtures with faba beans (Vicia faba L.)—a rewarding cultivation alternative. Journal of Agronomy and Crop Science, 179(2), 65-74.

Stoddard, F. L., Balko, C., Erskine, W., Khan, H. R., Link, W., & Sarker, A. (2006). Screening techniques and sources of resistance to abiotic stresses in cool-season food legumes. Euphytica, 147, 167-186.

Tao, A., Afshar, R. K., Huang, J., Mohammed, Y. A., Espe, M., & Chen, C. (2017). Variation in yield, starch, and protein of dry pea grown across Montana. Agronomy Journal, 109(4), 1491-1501.

Ulian, T., Diazgranados, M., Pironon, S., Padulosi, S., Liu, U., Davies, L., ... & Mattana, E. (2020). Unlocking plant resources to support food security and promote sustainable agriculture. Plants, People, Planet, 2(5), 421-445.

Urbatzka, P., Graß, R., Haase, T., Schüler, C., & Heß, J. (2009). Fate of legume-derived nitrogen in monocultures and mixtures with cereals. Agriculture, ecosystems & environment, 132(1-2), 116-125.

Urbatzka, P., Graß, R., Haase, T., Schüler, C., Trautz, D., & Heß, J. (2011). Grain yield and quality characteristics of different genotypes of winter pea in comparison to spring pea for organic farming in pure and mixed stands. Organic Agriculture, 1, 187-202.

Vander Pol, M., Hristov, A. N., Zaman, S., & Delano, N. (2008). Peas can replace soybean meal and corn grain in dairy cow diets. Journal of Dairy Science, 91(2), 698-703.

Wang, N., & Daun, J. K. (2004). Effect of variety and crude protein content on nutrients and certain antinutrients in field peas (Pisum sativum). Journal of the Science of Food and Agriculture, 84(9), 1021-1029.

Weiskopf, S. R., Rubenstein, M. A., Crozier, L. G., Gaichas, S., Griffis, R., Halofsky, J. E., ... & Whyte, K. P. (2020). Climate change effects on biodiversity, ecosystems, ecosystem services, and natural resource management in the United States. Science of the Total Environment, 733, 137782.

Willer, H., & Sahota, A. (2020). The world of organic agriculture, statistics and emerging trends 2020 at BIOFACH 2020.

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Chapter 3 – Nutritional features of organic peas (*Pisum sativum* L.) cultivated in different Italian environments and rheology profile of the respective pea-enriched baked snacks

Camilla Tibaldi[1*], Sónia Oliveira[2], Giovanni Dinelli[1], Ilaria Marotti[1], Anabela Raymundo[2]

[1] Department of Agricultural and Food Sciences, Alma Mater Studiorum – University of Bologna, viale Giuseppe Fanin, 44 – 40127, Bologna, Italia

[2] LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

[*]Corresponding author: camilla.tibaldi2@unibo.it

Abstract

Legumes are one of the most important components of human diet and the main plant-based protein sources. Considering their wide characteristics supporting human health, they have gained considerable interest globally, as they can be suggested as Plant-Based Meat Alternatives (PBMAs), in the perspective of a more sustainable and healthy food system. Among pulses, peas (*Pisum sativum* L.) are considered a good source not only of proteins, but also of fibers, starch, minerals and vitamins. In this study, the effect of environmental conditions on nutritional profile of peas cultivated in organic farming system in different Italian environments (mountainous and hilly), over different cultivation years (2021 and 2022). Moreover, the respective pea grain was used to prepare pea-based baking products. Appearance, physical properties (rheology and texture), nutritional profile and sensory analysis of the crackers were evaluated. The results showed that environment exert a strong impact on most of the nutritional components of peas, related to climatic conditions during the vegetative and reproductive stage of the crop and during the seed development. Moreover, the incorporation of cultivated peas in wheat-based crackers enhanced the functional and nutritional quality of these baked products, thus may be suggested as a more sustainable and healthy consumers choice.

Keywords: legumes, peas, PBMAs, nutritional composition, legume-based crackers, rheology, texture

3.1 Introduction

Recently, health and nutritional benefits of pulses and their by-products have gained significant attention from researchers and consumers, consequently promoting their cultivation and production, to both meet the global increasing demand and obtain high-quality food. Pulses have a positive impact on human health, contributing to reduce the risk of cancer and coronary hearth diseases or diabetes (Geil et al. 1994; Leterme et al., 2002). Among pulses, peas (*Pisum sativum* L.) are an important nutritional source of proteins, carbohydrates, resistant starch, dietary fibers, minerals, and vitamins (Hall et al., 2017). In addition to their nutritional value, peas have increasingly gained attention as a functional or nutraceutical food, due to the presence of secondary metabolite phytochemicals, that present antioxidant, antimutagenic and anticarcinogenic biological activities (Hall et al., 2017; Singh et al., 2017).

However, the global increasing awareness of environmental, human health and food safety issues has generated considerable focus and recommendation for alternative protein sources to meat (McClements et al., 2009; Mohammed et al., 2018). Indeed, recently, a new generation of Plant-Based Meat Alternatives (PBMAs) attracted considerable consumers interest, as they are considered as a valid and sustainable substitute to meat-based diet (Hu et al., 2019). Environmental studies reported that PMBAs production generates less greenhouse gas (GHG) emissions and require less energy, water, and land use compared with livestock production

(Heller et al., 2019). Legumes are a good source of plant-based proteins and may represent one of the largest sources of PBMAs; based on this trend, the production of legumes and pulses has recently increased at European level (European Commission, 2018; Notz et al., 2022).

With the new impetus focused on a more sustainable food system, the integration of legumes into the human diet may have significant health benefits, by enhancing the nutritional and health properties of food products, and thus it may become a topic of great interest to both consumers and food industry (McClements et al., 2009; Benítez et al., 2013). Baked snacks, such as crackers and biscuits, are usually well accepted and consumed throughout the world, and can be excellent vehicles for nutraceutical and protein enrichment because of their wide consumption and long shelf-life (Cookies and Crackers, accessed 2023). Previous studies have incorporated pea flour into wheat crackers and observed an increased nutrition potential of the biscuits (Kohajdovà et al., 2013). Furthermore, legume proteins are rich in lysine but deficient in sulphur-containing amino acids, whereas cereal proteins are deficient in lysine but have adequate levels of sulphur-containing amino acids. Hence, the combination of cereal and legume proteins can provide a better overall balance of essential amino acids (De la Hera et al., 2012) and may improve beneficial diet effects by controlling and preventing various metabolic diseases, such as diabetes, cardiovascular disease, and some forms of cancer (Arab et al., 2010; Siddiq et al., 2010; Angioloni and Collar, 2012; De la Hera et al., 2012).

Given the significant importance of nutritional quality of legumes, a focus directed towards the effect of crop growth factors on the nutritional and functional properties of peas and pea-based products, may contribute to study which environmental factors and agronomic management practices can be used as suitable strategies for improving nutritional and physiological qualities of these legumes and their respective incorporated food products. Combining the need to increase local production together with improving food quality, the aim of the present work is to evaluate the nutritional composition of peas (*Pisum sativum* L. var. Turris) cultivated in an organic farming system and under low input conditions over two consecutive years of cultivation, in different Italian environments within the Emilia-Romagna region (Italy). Moreover, the incorporation of organic pea flour on wheat savory snacks (crackers) was studied in terms of appearance, physical properties (rheology and texture) and nutritional composition of the crackers, in order to obtain a final plant-based product with high-quality nutritional content.

3.2 Material and Methods

3.2.1 Field trials

Open-field experimental trials were conducted over two consecutive years (2021 and 2022) at two experimental locations within Emilia-Romagna region (Italy), in mountainous and hilly environment. The sowing time differed depending on the growing environment and on the elevation of the sites: for the mountainous environment an Autumn Sowing was chosen (from November to July), while for the hilly environment a Spring Sowing (from March/April to July) for both years. At all locations, organic farming system was followed: no pesticide or herbicide treatments were performed on the plants and the sites were all rainfed. Meteorological data (temperature and precipitation), for the entire duration of the experimental trial, comprising the multiple cycles in each location, was obtained from the Arpae weather station, located in Emilia Romagna (https://simc.arpae.it/dext3r/) and are showed in the **Table 3.1**. Using the temperature data, the mean growing degree days (GDD) was calculated (Hykkerud et al., 2018).

Table 3.1. Monthly precipitation, average temperature and growing degree days, at each growing environment for all cultivation cycles, between 2021 and 2022. Meteorological data supplied by the Arpae weather station, located in Emilia Romagna (https://simc.arpae.it/dext3r/).

		Rainfalls (mm)		**Mean Temperature (°C)**		**Growing Degree Days (GDD)**	
		hilly	*mountainous*	*hilly*	*mountainous*	*hilly*	*mountainous*
2020	**Oct**		91.50		13.4		9.4
	Nov		30.1		9.1		5.1
	Dec		175.4		5.0		1.0

2021	**Jan**	43.8	68.4	2.1	3.6	-1.9	-0.4
	Feb	14.6	17.8	6.6	7.3	2.6	3.3
	Mar	30.8	15.6	8.8	9.2	4.8	5.2
	Apr	61	59.9	10.6	10.8	6.6	6.8
	May	42.2	30.5	16.3	15.7	12.3	11.7
	Jun	23.4	18.2	23.8	23.5	19.8	19.5
	Jul	8.6	17.7	25.5	25.1	21.5	21.1
	Aug	15.6	27.9	24.7	24.3	20.7	20.3
	Sept	73.8	91.9	20.6	20.7	16.6	16.7
	Oct	28.2	39.8	12.9	12.8	8.9	8.8
	Nov	111.2	123.9	8.3	8.6	4.3	4.6
	Dec	54	59.3	3.4	5.0	-0.6	1.0
2022	**Jan**	36.6	60	3.4	5.1	-0.6	1.1
	Feb	14	39.3	6.2	7.8	2.2	3.8
	Mar	41	57.9	7.5	7.7	3.4	3.7
	Apr	91.8	92	12.3	11.3	8.3	7.3
	May	27.2	78	20.1	18.9	15.9	14.9
	Jun	11.8	16	24.9	24.0	20.8	20.0
	Jul	23	9.2	26.9	26.4	22.9	22.4

3.2.2 Plant material

For each location, green ripening pea grain was harvested from 1 m^2 of surface area within each randomized block. The pea grain was stored at 4°C and dried in air oven at 50°C overnight, as described by Liu et al. (2020), the respective dry grain was milled (Billy 200 Hawos, Deutschland) to produce fine pea flour and stored at 4°C until analysis.

3.2.3 Peas nutritional characterization

3.2.3.1 Polyphenol and flavonoid content and antioxidant activity analyses

Polyphenols, comprising both free and bound constituents, were extracted as described previously (Dinelli et al., 2011). Free (FP) and bound polyphenols (BP) were then measured according to the Folin-Ciocalteau spectrophotometric (765 nm) method using gallic acid (GA) as a reference standard (Singleton et al., 1999). Likewise, the free (FF) and bound flavonoids (BF) were individually measured using a spectrophotometric (510 nm) colorimetric assay with catechin (CA) as a reference standard (Adom et al., 2003). From the free and bound polyphenol and flavonoids, the respective totals (TP and FT) were calculated. The DPPH assay was performed by measuring the reduction (515 nm) of DPPH• to 1,1-diphenyl-2-picryl hydrazine (Floegel et al., 2011) and FRAP (reduction of Fe^{2+}) was determined using a spectrophotometric (593 nm) method reported previously (Benzie and Strain, 1996). The antioxidant activity in the free and bound fraction were summed and expressed as total DPPH and FRAP, respectively. All analysis were performed in triplicates.

3.2.3.2 Dietary fiber content

Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were extracted and measured according to the instruction protocols provided by the Megazyme Total Dietary Fibre Assay Procedure kit CAT. NO. K-TDFR (Megazyme International, Ireland). This. Protocol was followed based on previously reported methods (Lee et al., 1992; Prosky et al., 1988). All analysis were performed in triplicates.

3.2.3.3 Lipid content

For the determination of lipid content, the Folch method was used (AOAC 1990). Briefly, 500 mg of pea flour was diluted with 1:20 in chloroform:methanol (2:1; *w/v*) and shaked for 20 min. Tubes were centrifuged at 10,000 ×g (10 min). The upper aqueous phase was transferred into a Büchner funnel and vacuum filtered onto already tared bechers. After overnight hood drying, the bechers were weighted and results were calculated as % of lipids. All analysis were performed in triplicates.

3.2.3.4 Protein content

The nutritional composition of the organic pea flour and crackers was performed accordingly to the AOAC official procedures for baked products (AOC, 2006). The protein content was determined using DUMAS protein/nitrogen analyzer (VELP Scientific NDA 702 DUMAS Nitrogen Analyzer—TCD detector), according to the Dumas method. The total nitrogen content was determined, and the resulting value was multiplied by a conversion factor of 5.7 to obtain the crude protein content of the sample (Batista et al., 2019; VELP et al., 2020). All analysis were performed in triplicates.

3.2.3.5 Digestible and Resistant Starch

Total digestible starch (TDS) and Resistant starch (RS) were extracted and measured through the enzymatic kit Megazyme Digestible and Resistant Starch kit CAT. NO. K-DSTRS (Megazyme International, Ireland). All analysis were performed in triplicates.

3.2.3.6 Sucrose, Fructose, D-Glucose and Raffinose Family Oligosaccharides (RFO) content

The presence of Carbohydrates, such as Sucrose, Fructose and D-Glucose, was determined following the instructions on the protocol provided with the Megazyme Sucrose/Fructose/D-Glucose kit CAT. NO. K-SUFRG (Megazyme International, Ireland), as well for the Raffinose Family Oligosaccharides, following the Megazyme Raffinose/D-Galactose kit CAT. NO. K-RAFGA (Megazyme International, Ireland). All analysis were performed in triplicates.

3.2.3.7 Mineral composition

Organic pea flour as well for respective pea-enriched crackers was analysed for its mineral elements by atomic absorption spectrophotometry and ICP-OES (iCAP 7000 series, Thermo Scientific, Waltham, MA, USA) (Leitão et al., 2021). All analysis were repeated 3 times.

3.2.4 Pea-Based Baked Snacks

3.2.4.1 Crackers Preparation

Savory snacks were prepared according to a previously developed and optimized model formulation using 65.5% wheat flour, 1% salt, 1.5% baking powder, 7.5% sunflower oil and 24.5% water (Batista et al., 2019). In the pea-enriched samples, the ingredients were added in similar quantities, except for wheat flour, which was substituted with 6% of organic pea (*Pisum sativum* L.) flour, derived from the field experimentation. Batch sizes of 100 g were made, corresponding to approximately 30 crackers. All the ingredients were mixed by hand, using an optimized procedure, and then rolled out with a manual dough machine, reproducing the extrusion process (Atlas 150, Marcato, Italy) to a thickness of 1.8 mm. The crackers were then molded into jagged 38 mm squares and baked at 180 °C for 5 min in a convection oven Johnson A60 (Johnson & Johnson, New Brunswick, NJ, USA). After cooling, some crackers (N = 10) were powdered for nutritional composition and other chemical analysis.

3.2.4.2 Dough Rheology

Rheological measurements of the dough were performed according to Mota et al. (2020), using a controlled stress rheometer (Haake MarsIII—Thermo Scientific, Karlsruhe, Germany) equipped with a UTC–Peltier system. Frequency sweep tests were performed within the viscoelastic linear region, which was previously defined through a stress sweep test, at 1 Hz, using a serrated parallel-plate geometry with a 20 mm diameter (PP20). Dough pieces were compacted to a 1.5 mm gap and the edge parts were coated with liquid paraffin to prevent moisture losses during tests. Stress and frequency sweeps were performed at 20°C.

3.2.4.3 Color Analysis

The color of the dough and the final products was measured using a Minolta CR-400 (Japan) colorimeter. The method used was previously described by Mota et al. (2020). The measurements were performed under the same light conditions using a white standard ($L^* = 94.61$, $a^* = -0.53$ and $b^* = 3.62$) at control temperature, replicated at least ten times for each sample (CTRL and pea-enriched snacks) 24h after baking. Total color differences (ΔE^*) between CTRL and the pea-enriched snacks were assessed using the following Equation:

$$\Delta E^* = (\Delta L^* \cdot 2 + \Delta a^* \cdot 2 + \Delta b^* \cdot 2) \cdot 1/2$$

3.2.4.4 Texture Analysis

Texture analysis was performed in a TA.Xtplus (StableMicro Systems, Godalming, UK) texturometer. The measurements were performed at 20°C. Each snack's texture was evaluated with a penetration test, using a cylindrical probe of 2 mm in diameter, plunged 10 mm at 1 mm/s, as described earlier by Mota et al. (2020). Hardness was calculated as the peak force (N) in the force versus time texturogram. This peak corresponds to the maximum force required to break the cracker. Crispiness was also determined, and it is considered as the time needed to reach the maximum peak(s). The shorter the time in which the break occurs, the crispier the material. So, crispiness can be obtained from the time needed to break the cracker, which is inversely related to crispiness; the faster the breakage occurs, the crispier the cracker will be (Bourne et al., 2002).

These tests were reproduced at least eight times for each cracker (CTRL and pea-enriched snacks) 24h after baking.

3.2.4.5 Water activity determination

The water activity (a_w) was analysed using a thermos-hygrometer (HygroPalm HP23-AW, Rotronic AG, Bassersdorf, Switzerland) at 20°C. After 24h of baking, the tests were performed by crushing the crackers and each snack (wheat-based CTRL and pea-enriched snacks) measured in triplicate.

3.2.4.6 Crackers nutritional composition

The nutritional compositions of the snacks were evaluated based on the powdered samples. According to the AOAC 950.36 official method for baked products, the protein content was evaluated using the Dumas method, as described above. Crude fat was measured using ether extraction according to AOAC 2003.05. A minimum of 1.5 g of each snack (with and without pea flour) was weighed into a 26 mm x 60 mm cellulose extraction thimble. The content of petroleum ether lipids was evaluated by Soxtec extraction (Soxtec System HT 1043/1046 extraction unit (Tecator AB, Höganäs, Sweden), with 15 min of boiling and 60 min of rinsing, followed by 15 min of drying. Finally, the lipid content was determined gravimetrically. Ash content, representing the inorganic fraction of the snacks, was measured by incineration at

550°C in a muffle (AACC 08-01.01). Moisture content was determined according to Mota et al. (2020). Total carbohydrates were calculated by difference.

3.2.4.7 Sensory analysis

Crackers were evaluated by un untrained sensory panel (n = 48, age = 18–55, male = 12, female = 36) to assess which snacks (wheat-based CTRL or pea-enriched crackers) were most appreciated. To do so, the crackers containing 6% of pea flour from two different years of cultivation were presented randomly, together with a control cracker (wheat-based).

The cracker samples were evaluated in terms of color, smell, taste, texture, and overall liking (using a nine-levels hedonic scale, ranging from 1 to 9, as “very pleasant” to “very unpleasant”). Purchase intention was also assessed, with nine levels ranging from “I would definitely buy” to “I would definitely not buy”. The tests were carried out in a standardized sensory analysis room, according to EN ISO 8589 (EN ISO 8589, 2017).

3.2.5 Statistical analysis

Statistical analyses were conducted using the Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA). A two-way analysis of variance (ANOVA) along with Tukey’s honest significant difference was performed to compare the growing environments with the cultivation years. Significant differences between means were determined by least significant difference values for $p < 0.05$. Pearson’s correlation coefficient (r) was calculated at significance level of $p < 0.01$.

Results and Discussion

Peas (*Pisum sativum* L.) are a rich source of various bioactive compounds. Previous studies, although scarce, have shown that environment condition may affect nutritional composition of legumes, in particular peas (Ali-Khan et al., 1973). To expand earlier studies by including multiple locations, more recent studies have also showed variation in health promoting compounds based on location (Arif et al., 2020; Maharjan et al., 2019; Tao et al., 2017).

Therefore, a multidisciplinary approach was used to study how different crop growing locations in Emilia Romagna (Italy), as well as different agronomic practices, such as sowing time (Autumn and Spring), may affect the nutritional composition of the harvested grain over two consecutive cultivation seasons (2021 and 2022).

The nutritional parameters measured were the contents of phenolic compounds (polyphenols and flavonoids), antioxidant activities (DPPH and FRAP), fibers, lipids, proteins, digestible and resistant starch, carbohydrates and raffinose family oligosaccharides (RFOs) carbohydrates. In addition, in order to give a commercial impetus for the future to the cultivation of leguminous crops, also in response to the gradually increasing market demand for plant-based proteins to substitute meat (PBMAs), savory snacks enriched with organic pea flour (6%) were produced. Functional (rheology and taste) and nutritional analyses were also carried out on these products, aimed at the revalorization of legume cultivation.

3.3.1 Content of nutritional and bioactive compounds for location and year in peas (Pisum sativum L.) grain

Leguminous plants play a major role in human nutrition and are a good source of saccharides, proteins, micronutrients and bioactive compounds (Balasundram et al., 2006). Phenolic acids are the principal polyphenols found in grains and pulses, which primarily exist as bound derivatives, more in particular as conjugates with polysaccharides and proteins (Chon et al., 2009). Previous studies showed that the content of some phenolics may increase when certain stress condition are applied such as UV radiation, infection by pathogens and parasites, wounding, air pollution and exposure to extreme temperatures (Naczk, 2006; Zobel, 1997). Hence, location and year of cultivation (weather and environment conditions) may actively impact on these bioactive compounds' concentration and relative antioxidant activity in the plant fruit. In **Table 3.2**, significantly higher levels of total polyphenols (TP) and total flavonoid (TF), 128.66 mg GAE/100g, and 54.81 mg CE/100g, respectively, were evident in pea grain cultivated in 2021 over 2022 (94.05 mg GAE/100g and 35.26 mg CE/100g, respectively). Moreover, secondary metabolites responsible for FRAP antioxidant activity showed the same trend of TP and TF, displaying significantly higher levels in 2021 than 2022 (0.98 mmol and 0.78 mmol Fe^{2+}/100g, respectively); while for DPPH no significant difference was observed between the factors. This trend may reflect plant response to specific abiotic stresses on both growing environments during 2021 year of cultivation (Klepacka et al., 2011), in particular average annual rainfall and temperatures conditions displayed lower levels in 2021 (299.8 mm and 14.56°C, respectively) than 2022 (344.7 mm and 16.91°C, respectively), showing that drought and cold conditions evidently stimulated the biosynthesis of phenolic compounds and relative antioxidant activity in the resulting plant and/or grain.

In 2022 samples, SDF content resulted in significantly higher (10.05%) depending on the year of cultivation, and it was observed that a correlation effect exists ($p < 0.01$) between the environmental parameters. Although, no significant differences were found between the two environments, the interaction (E x Y) was significant; it can be supposed that the year of cultivation was strongly influenced by the growing environment, but this should be further investigated in future studies.

As regards pea proteins (PRO), a previous early study (Ali-Khan et al., 1973) based on 19 pea cultivars cultivated in 4 different locations over 3 years found a significant correlation between protein content and location, emphasizing the importance of environment and growing conditions and the cultivation year over the grain nutritional composition. Also, more recent studies confirmed the importance of environment over the protein content in peas (Tzitzikas et al., 2006; Nikolopoulou et al., 2007; Bourgeois et al., 2011; Maharjan et al., 2019). In this case (**Table 3.2**), PRO results were in line with previous literature (Harmankaya et al., 2010), and the highest values of PRO were observed in hilly samples (23.90 g/100), suggesting that spring environment conditions support the accumulation of proteins during the vegetative growth of the plant. Moreover, in the study of Tao et al. (2017), it was found that extreme weather (rainfalls and temperatures) conditions during sowing and vegetative growth of the plant have a strong impact on pea protein content, thus supposing a better overall plant health condition in hilly environment.

Contrary to PRO, starch content (TDS) showed higher results in mountainous cultivated grain (25.99 g/100g), showing an inverse relationship with the protein content (PRO) within peas cultivated in the same environment, as demonstrated by Daba et al. (2020). Indeed, a study conducted by Mohammed et al. 2018 stated that drier and warmer environmental conditions (65.6 and 48.7 mm of cumulative rainfalls for hilly and mountainous environment, respectively) during the crop's reproductive phase until seed development promote the accumulation of starch in pea grain, due to rapid conversion of sugars into starch within the grain. Same trend was observed for RS (Tao et al., 2017), which recently gained attention (Haenen et al., 2013; Sun et al., 2015). It is a portion of starch that during the process of digestion keeps fermented in the colon (Sun et al., 2015) and may potentially help in controlling diabetes and energy balance, and the short-chain fatty acids produced by fermenting colonic bacteria provide direct health benefits to the colon (Birt et al., 2013).

No significant differences of LIP content, as well for IDF and RS, were observed between the two growing environments in the two years of cultivation.

As regards the SUC content, significantly higher levels were observed in mountainous harvested grain (6.37 g/100g), in line with findings of Mohammed et al. (2018). Moreover, a significantly lower content was evident in 2022 pea grain compared to 2021 (4.45 and 6.66 g/100g, respectively). It can be noticed an inverse relationship with RFO content in the examined samples. RFO are considered as antinutritional compounds because they are believed to be responsible for causing flatulence in humans, which is the most important factor in deterring people from including more legumes in their diet. However, **Table 3.2** shows that RAF and GLCT (RFO compounds and precursors) concentrations were significantly higher in 2022 pea grain (0.55 and 0.039 g/100g, respectively). It was found that RFO biosynthesis occurs in the latter stages of seed development and is strictly dependent on sucrose content (Gawłowska et al., 2017). This correlation can be due to sucrose active role in the first steps of Raffinose synthesis (Peterbauer et al., 2002) and also because sucrose is a source of UDP-glucose, which is involved in the Galactose (RFOs precursor) biosynthetic pathway (Peterbauer and Richter, 2001), thus assuming that elevated concentration of sucrose can stimulate the RFOs pathway by increasing the effectiveness of metabolic activity of specific enzymes.

Table 3.2. Mean values of nutritional, anti-nutritional and health-promoting compounds in examined peas (*Pisum sativum* L.). Different letters withing each column show significant different values ($p \leq 0.05$, Tukey's least significant difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. ns = not significant, TP = total polyphenols, TF = total flavonoids, FRAP = ferric reducing antioxidant potential, DPPH = 1,1-diphenyl-2-picrylhydrazyl anti-radical activity, IDF = insoluble dietary fiber, SDF = soluble dietary fiber, LIP = lipids, PRO = proteins, TDS = total digestible starch, RS = resistant starch, GLU = glucose content, SUC = sucrose content, FRU = fructose content, RAF = raffinose content, GLCT = galactose content, GAE = gallic acid equivalent, CE = catechin equivalent, TE = Trolox equivalent and E x Y = Environment x Year.

		TP	**TF**	**FRAP**	**DPPH**	**IDF**	**SDF**	**LIP**	**PRO**	**TDS**	**RS**	**GLU**	**SUC**	**FRU**	**RAF**	**GLCT**
	u.m.	mg GAE/100g	mg CE/100g	mmol Fe^{2+}/100g	µmol TE/g	%		g/100g								
Environment	montainous	117.75 **a**	46.55 **a**	0.93 **a**	2.55 **a**	27.32 **a**	8.84 **a**	1.97 **a**	20.69 **b**	25.99 **a**	7.75 **a**	0.09 **b**	6.37 **a**	0.12 **a**	0.86 **a**	0.059 **a**
	hilly	104.96 **a**	43.52 **a**	0.82 **a**	3.07 **a**	28.95 **a**	9.02 **a**	2.23 **a**	23.90 **a**	23.37 **b**	6.74 **a**	0.17 **a**	4.73 **b**	0.22 **a**	1.14 **a**	0.079 **a**
Year	2021	128.66 **a**	54.81 **a**	0.98 **a**	2.91 **a**	27.11 **a**	7.81 **b**	2.04 **a**	22.79 **a**	25.08 **a**	6.79 **a**	0.16 **a**	6.66 **a**	0.20 **a**	0.55 **b**	0.039 **b**
	2022	94.05 **b**	35.26 **b**	0.78 **b**	2.70 **a**	29.15 **a**	10.05 **a**	2.15 **a**	21.79 **a**	24.28 **a**	7.70 **a**	0.10 **a**	4.45 **b**	0.14 **a**	1.46 **a**	0.099 **a**
	E x Y	*ns*	*ns*	*ns*	*ns*	*ns*	**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

3.3.2 Effects of Weather Parameters on Nutritional profile of pea (Pisum sativum L.) grain

To further investigate the impact that growing environment and cultivation year exert on the nutritional profile of legumes, more in particular in pea (*Pisum sativum* L.) grain, the relationship between nutritional compounds and weather conditions was correlated (**Table 3.3**). An inverse relationship between temperature and the expression of TP, TF and FRAP was evident and in line with already discussed findings about these compounds (Klepacka et al., 2011). Whereas secondary metabolites (not belonging to phenolics compounds) responsible for DPPH antioxidant activity, involved in plant protection from abiotic stresses, exert a positive significant correlation with temperatures and negative with rainfalls during the vegetative growth of the crop.

Moreover, LIP and PRO content showed to be positively correlated to environment temperatures and negatively to rainfalls. It was studied by Sattari Vayghan et al. (2020) that lipid content is strictly dependent on seasonal temperatures, which may affect structural modifications at high exerted temperatures. However, in both the growing environments the pea grain was harvested before this thermal threshold, thus confirming the positive correlation with temperatures. Referring to proteins, the early study of Karjalainen and Kortet et al. (1987) and the more recent study of Tao et al. (2017) already showed that too wet and too dry conditions during seed development may limit protein accumulation in peas, corroborating the direct relationship between proteins and rainfalls, found on this study.

Furthermore, an inverse significant relationship was observed for TDS, RS and SUC over temperatures, while a significative positive correlation with rainfalls was found. Mohammed et al. (2018) and Tao et al. (2017) studied this behavior of TDS, RS, and consequently SUC, demonstrating to be strictly dependent to temperature conditions during the vegetative and reproductive stage of the plant and the seed development.

As regards RFOs (RAFF e GLCT), a significant and positive correlation with temperature conditions was found, it may be supposed that this relationship depends on the content of SUC (inversely correlated with RFOs), which was found to play an active role in the biosynthesis of these anti-nutritional compounds (Peterbauer and Richter, 2001; Peterbauer et al., 2002).

Table 3.3. Pearson correlation between weather parameters (cumulative rainfalls, mean temperatures) and the nutritional profile (TP, TF, FRAP, DPPH, IDF, SDF, LIP, PRO, TDS, RS, GLU, SUC, FRU, RAFF, GLCT) in pea (*Pisum sativum* L.) grain. The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level.

	Rainfall (mm)	**Temperatures (°C)**
TP	0.12	-0.47
TF	-0.27	-0.20
FRAP	0.11	-0.33
DPPH	-0.55*	0.52*
IDF	-0.37	0.62*
SDF	-0.09	0.44
LIP	-0.85**	0.99***
PRO	-0.98**	0.81**
TDS	0.74*	-0.79**
RS	0.83**	-0.50*
GLU	-0.69*	0.37
SUC	0.50*	-0.84**
FRU	-0.46	0.22
RAFF	-0.20	0.62*
GLCT	-0.22	0.64*

Overall, in terms of nutritional profile of organic peas cultivated in different locations of Emilia-Romagna region, it can be assessed that all the nutritional and bioactive compounds are strictly interconnected between each other and, also, an interesting relationship with weather parameters was found. Hence, it can be confirmed that growing environment conditions may impact among nutritional composition and thus improve the quality of the grain, in order to face and encourage the recent global consumers interest through high-quality food products in a more sustainable food system.

3.3.3 Content of micronutrients for location and year in peas (Pisum sativum L.) grain

From the perspective of micronutrient composition (**Table 3.4**) within the peas harvested at the two growing environments (mountainous and hilly), in the two cultivation years (2021 and 2022). It was observed that the environment has no impact on most of the micronutrients components, except for Potassium (K), Calcium (Ca) and Phosphorous (P) concentration. More in particular, K was the most abundant element, presenting range of values between 905.46 and 939.59 mg/100g. P was found to range from 440.58 to 500.77 mg/100g. All pea samples contained a higher amount of potassium and phosphorus than other minerals present. Furthermore, peas cultivated in hilly (spring sowed) environment showed a significantly higher content of K, Ca and P (939.59, 93.71 and 500.77 mg/100g, respectively) over mountainous samples, assuming that spring weather parameters during vegetative growth of the crop evidently supported better health conditions of the plant and relative grain. Whereas peas cultivated in 2021 showed higher content of Na and Ca (5.56 and 89.10 mg/100g, respectively), and 2022 samples presented higher K content (933.75 mg/100g).

Table 3.4. Mineral composition (mg/100g) in peas (*Pisum sativum* L.) cultivated in the two growing environments over two cultivation years. Different letters withing each column show significant different values ($p \leq 0.05$, Tukey's least significant difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. ns = not significant, E x Y = Environment x Year.

		Na	**K**	**Ca**	**Mg**	**P**	**S**	**Fe**	**Cu**	**Zn**
Environment	mountainous	5.46 **a**	905.46 **b**	71.94 **b**	136.94 **a**	440.49 **b**	186.58 **a**	6.02 **a**	1.24 **a**	4.79 **a**
	hilly	5.24 **a**	939.59 **a**	93.71 **a**	139.07 **a**	500.77 **a**	197.54 **a**	6.51 **a**	1.20 **a**	5.20 **a**
Year	2021	5.56 **a**	911.30 **b**	89.10 **a**	132.85 **b**	468.76 **a**	191.34 **a**	6.60 **a**	1.18 **a**	5.01 **a**
	2022	5.14 **b**	933.75 **a**	76.55 **b**	143.15 **a**	472.50 **a**	192.78 **a**	5.92 **a**	1.26 **a**	4.98 **a**
	E x Y	*ns*	*ns*	*ns*	**	*ns*	*ns*	*ns*	*	*ns*

Moreover, the study of Hacisalihoglu et al. (2021), the interconnection between Ca and Mg was found, and as well as a positive correlation with Cu, P, K and S was observed as well, suggesting that qualitative content in peas could be improved by targeting either Ca or Mg. However, several studies reported environmental factors displayed a strong impact on mineral content in peas (Wang et al., 2004, Wang et al., 2008; Gawalko et al., 2009), and in particular Wang et al. 2004, found that Cu and Zn were negatively correlated to Mn or P between field environments; nevertheless, main researched results suggest that genetics is the most driving factor for the accumulation of minerals in grain legumes (Hacisalihoglu et al., 2021).

3.3.4 Incorporation of organic pea (Pisum sativum L.) in wheat-based baked snacks (Crackers)

In the perspective of encouraging legumes production for a more sustainable agronomic system and to respond to the growing consumers demand for more plant-based protein alternatives to meat, the preparation of savory baked products (crackers) enriched with organic pea flour was set up. To assess the impact of pea incorporation on the technological properties of the dough and the final product, a wheat-based control (CTRL) was prepared. In parallel, to investigate the impact of different locations and year of cultivation on the preparation of the savory baked snacks, four pea-enriched crackers were prepared: two different growing environments (mountainous and hilly) over two cultivation years (2021 and 2022).

The dough and crackers were analyzed in terms of rheology, color, texture, nutritional composition and microelements concentration in both dough and final products.

3.3.4.1 Physical Characteristics of Dough

In **Figure 3.1**, the viscoelastic behaviors of the four alternative flours in cookie doughs are represented. For all doughs, elastic modulus (G′) was greater than viscous modulus (G″), throughout the selected frequency range (0.1 to 100 Hz), which shows a predominance of the elastic behavior of doughs. Greater elastic behavior indicates greater mechanical strength and shape retention ability of doughs (Oliveira et al., 2022). The G’ (storage modulus) and G” (loss modulus) increased with increasing frequency range and revealed a weak gel-like rheological behavior, which is characteristic of crackers doughs and in agreement with the results demonstrated by Mota et al. (2020) and Fradinho et al. (2015). Moreover, the mechanical spectra also showed that pea-enriched samples have higher G’ and G’’ modulus than the wheat-based CTRL. Thus, pea flour should help strengthening the dough structure, due to building-up a stronger network between pea protein and polysaccharides with wheat flours compounds, as previously reported by Mohammed et al. (2012). More in particular, previous studies showed that legumes incorporation in wheat dough led to higher protein water absorption, thus limiting water availability for the development of the gluten network when in competition with wheat proteins (Des Marchais et al., 2011). Zucco et al. (2011) highlighted that legumes flours contain an increasing number of hydrophilic sites available, due to increased protein content, that compete for the limited free water in dough. Moreover, other components such as sugars and fibers may also affect the formation of structured matrix of the dough (Kamaljit et al., 2010; Atef et al., 2011).

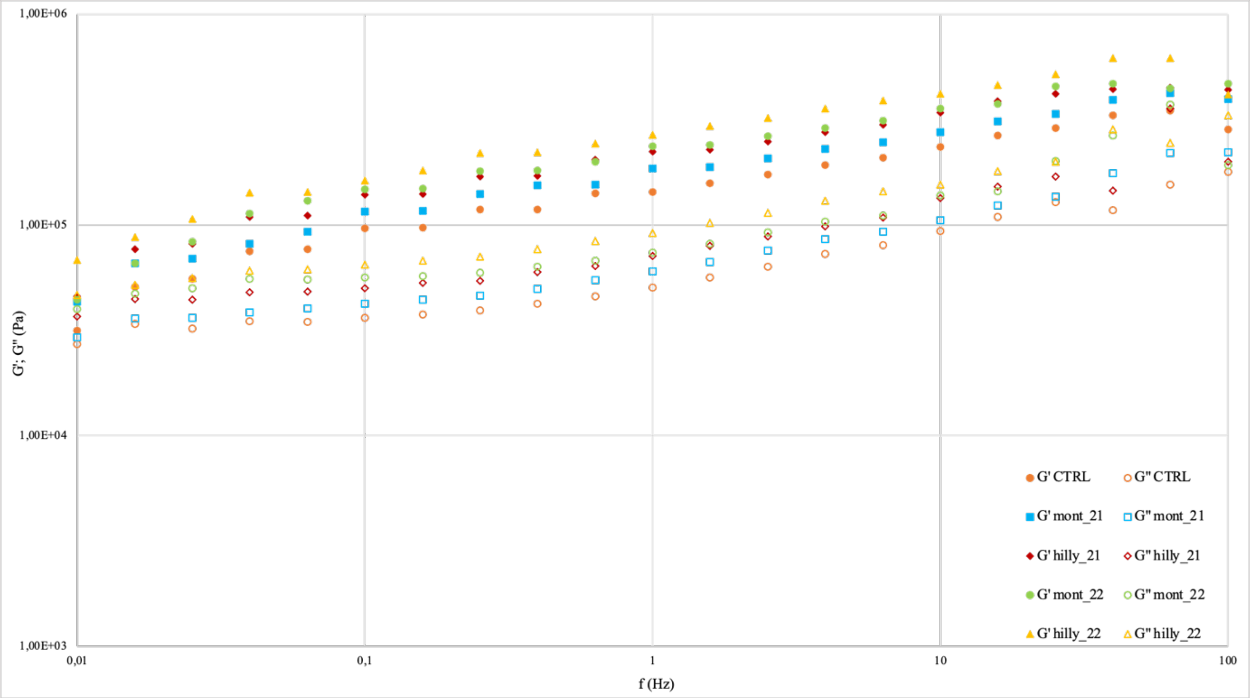

Figure 3.1. Mechanical spectra of CTRL and crackers doughs prepared with 6% of organic pea flour derived from two different environments (“mont” and “hilly”) in two cultivation years (2021 and 2022). Closed symbols represent G’ (elastic modulus) and open symbols represent G” (viscous modulus).

The mechanical spectra, presented in **Figure 3.1**, provides the values for G′ and G″ at 1 Hz (**Table 3.5**). The G′ and G″, throughout the selected frequency (1 Hz), of pea-enriched samples were significantly higher than the CTRL, indicating that the elastic behavior of the dough was enhanced by the pea incorporation. This trend highlights the evidence that pea (legume) proteins play an important role in dough formation and stabilization due to their interaction with polysaccharides (free sugars) present in the flour and their ability to absorb more water.

Table 3.5. Viscoelastic properties (G′ [Pa], G″ [Pa] at 1 Hz) of CTRL and pea-enriched doughs. Different letters withing each column mean significant different values ($p \leq 0.05$, Tukey's least significant difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. Ns = not significant, E x Y = Environment x Year.

		G' 1 Hz (Pa)	**G'' 1 Hz (Pa)**
Environment	CTRL	1.51E+05 **b**	5.25E+04 **b**
	mountainous	2.05E+05 **a**	6.77E+04 **a**
	hilly	2.37E+05 **a**	7.84E+04 **a**
Year	CTRL	1.51E+05 **b**	5.25E+04 **b**
	2021	2.07E+05 **a**	6.86E+04 **a**
	2022	1.25E+05 **a**	7.75E+04 **a**
	E x Y	*ns*	*ns*

3.3.4.2 Color Analysis of Dough

Table 3.6 shows the color parameters of snacks dough. The color variations between the CTRL and pea-enriched doughs, expressed in terms of ΔE* values, were greater than 3 for all doughs, indicating that the color differences amongst the pea-enriched crackers and the CTRL were visually distinguishable by the human eye (Moradi et al., 2019). Regarding the lightness parameter (L*), increasing additions of pea flour to doughs let to a significant reduction in lightness (Mohammed et al., 2012; Atef et al., 2011; Zucco et al., 2011). As regards the color parameter a*, which measures the range between green (− 60) and red (60), wheat-based CTRL snack showed a slight positive low value (0.14), while at all the pea-enriched samples the color changed into a significantly darker green (between -3.85 and -4.80), due to high levels of plant pigment's (chlorophyll) that leads to the formation of green-colored doughs. Regarding the b* parameter, which indicates the range between blue and yellow, comparing to CTRL a significant ($p < 0.05$) increase was also observed, with the addition of organic pea to the dough.

Table 3.6. The ΔE*, L*, a*, b* values for CTRL and pea-enriched doughs. Different letters withing each column mean significant different values ($p \leq 0.05$, Tukey's least significant difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. Ns = not significant, E x Y = Environment x Year.

		L*	**a***	**b***	**ΔE***
Environment	CTRL	74.89 **a**	0.14 **a**	17.80 **c**	
	mountainous	66.99 **c**	-4.48 **c**	23.97 **a**	11.04
	hilly	68.38 **b**	-4.17 **b**	23.15 **b**	9.46
Year	CTRL	74.89 **a**	0.14 **a**	17.80 **c**	
	2021	68.25 **b**	-3.85 **b**	23.36 **a**	9.54
	2022	67.12 **c**	-4.80 **c**	23.76 **a**	10.97
	E x Y	*ns*	*ns*	*ns*	

3.3.4.3 Physical properties of Crackers

The physical properties of baked snacks frequently determine their attractiveness and desirability (or undesirability). As such, color and texture parameters (**Table 3.7**) of all crackers with and without pea flour were evaluated.

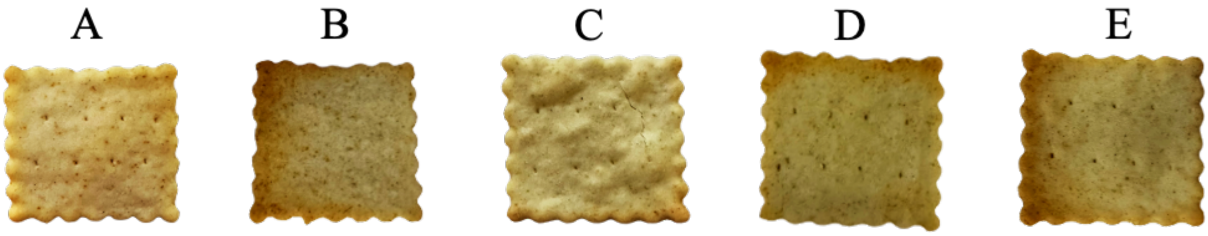

Figure 3.2. Representative images of wheat-based CTRL and pea-enriched crackers prepared with 6% of organic pea flour derived from two different environments over two cropping seasons (2021 and 2022). A = wheat-based CTRL, B = peas from mountainous 2021, C = peas from hilly 2021, D = peas from mountainous 2022, E = peas from hilly 2022.

Regarding the color parameters, pea-enriched crackers showed significantly lower a* levels (ranging between -2.17 to -3.10) compared to CTRL (0.96) and the color intensity resulted in a slightly green compared to the previously discussed doughs. Similarly to doughs, increasing addition of pea flour to crackers in visually different crackers (ΔE* > 3), as it can be seen in **Figure 3.2**. This behavior is possibly due to Maillard reactions between reducing sugars and amino acids, but also possibly to starch dextrination and sugar caramelisation (Gómez et al., 2008; Zucco et al., 2011). Moreover, it was found that higher levels of pea flour (up to 20%) caused also significant reduction in taste, odor and overall acceptance of final products due to higher intensity of leguminous taste and odor.

The texture results (**Table 3.7**) showed that the variations observed for several parameters studied on the dough are reflected also in the final products. Crackers prepared with pea flour revealed higher levels of hardness (ranged between 10.51 and 11.78 N) in comparison to the wheat-based CTRL (9.13 N). The impact of pea incorporation in the product is related to macromolecules structure within the pea flour, due to the absence of gluten. Indeed, Dhinda et al. (2012) described that pea flour promoted a structural rearrangement leading to diverse interactions amongst starch and proteins (protein-starch complex) and altered by the heat treatment that occurs during cooking. Moreover, the water content was kept the same when replacing wheat flour (in the CTRL) by pea flour. Increased protein-starch interactions promote a structuring effect, enhanced by pea flour ability to absorb water. Same trend is also observed on the brittleness parameter. Brittleness is the distance (in mm) to reach the maximum breaking peak and is considered as an indicator of crispness. The pea-enriched crackers show an increase of the distance necessary to reach the maximum peak (Brittleness), indicating a higher degree of crispness compared to the CTRL, although no significant differences were observed. Hence, thanks to all these findings, it can be concluded that pea-enriched baked products may appear more attractive to the consumers.

Table 3.7. The ΔE*, L*, a*, b* values and Texture properties for CTRL and pea-enriched crackers. Different letters withing each column mean significant different values ($p \leq 0.05$, Tukey's least significant difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. Ns = not significant, E x Y = Environment x Year.

		Crackers Color				Crackers Texture	
		L*	**a***	**b***	**ΔE***	**Hardness**	**Brittleness**
	u.m.					N	mm
Environment	CTRL	74.14 **a**	0.96 **a**	19.17 **b**		9.13 **b**	0.68 **a**
	mountainous	72.76 **a**	-2.71 **b**	21.83 **a**	4.74	11.63 **a**	0.70 **a**
	hilly	73.25 **a**	-2.56 **b**	21.37 **a**	4.25	10.74 **ab**	0.92 **a**
Year	CTRL	74.14 **a**	0.96 **a**	19.17 **b**		9.13 **b**	0.68 **a**
	2021	72.45 **a**	-2.17 **b**	21.92 **a**	4.50	11.78 **a**	0.83 **a**
	2022	73.55 **a**	-3.10 **c**	21.27 **a**	4.62	10.51 **a**	0.81 **a**
	E x Y	*ns*	***	***		*ns*	*

3.3.4.4 Nutritional properties of Crackers

The proximate analysis of foods involves the determination of the principal components, such as water activity (a_w), moisture, ash (total minerals), lipids, proteins and carbohydrates, as well as bioactive compounds (phenolics) and relative antioxidant activity. **Table 3.8** presents the approximate chemical compositions of the crackers prepared with pea flour compared with the wheat-based CTRL.

Pea-enriched crackers exhibited significantly higher protein content than the CTRL, as expected. However, no significant difference between the environments and the cultivation years, in terms of protein content, was observed.

As regards the ash, mountainous and 2021 crackers samples displayed significantly higher content (2.39 and 2.30 %, respectively). On the other hand, the highest a_w levels were observed in hilly and 2022 samples (0.28 and 0.29, respectively), with the lowest result for the wheat-based CTRL (0.12). Same trend was observed for moisture content, due to pea protein capacity to absorb more water.

Several studies observed increases in a_w values with the addition of apple fibers to cookies (Uysal et al., 2007) and with the addition of microalgae with a high protein content (Bastista et al., 2017). A similar behavior was also found for the pea-enriched crackers, the increases in the protein content led to lower water-holding capacity values compared to the wheat-based CTRL. However, the a_w values remained at low levels and the addition of pea did not modify the preservation characteristics of the food products.

Finally, total polyphenols (TP), total flavonoid (TF) and antioxidant activity assays, showed overall higher levels in pea-enriched samples compared to CTRL, both from environment and cultivation year perspectives, thus demonstrating that organic pea incorporation improves nutritional quality and bioactive compounds profile in the final food product. These results suggest that promoting legume-based crackers may be a sustainable and healthy consumers choice more or equally attractive and preferable over wheat-based snacks.

Regarding the mineral profile, the results are listed in **Table 3.9**. It was observed that pure pea flour is a source of Potassium (K), Phosphorus (P), Magnesium (Mg) and Copper (Cu) (Reichert et al., 1982; Dahl et al., 2012). This induces similar results in crackers containing it, although cooking heating treatment reduced levels of minerals (Poblaciones et al., 2018). However, in terms of K, P, Mg, and Zn significant higher content in pea-enriched crackers was observed compared to wheat-based CTRL. In this perspective, it can be concluded that incorporation of pea flour in wheat-based baked snacks may guarantee higher nutritional quality of the food products.

Table 3.8. Functional and nutritional composition of CTRL and pea-enriched crackers. Different letters withing each column mean significant different values ($p \leq 0.05$, Tukey's least significant difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. TP = total polyphenols, TF = total flavonoids, FRAP = ferric reducing antioxidant potential, DPPH = 1,1-diphenyl-2-picrylhydrazyl anti-radical activity, GAE = gallic acid equivalent, CE = catechin equivalent, TE = Trolox equivalent, ns = not significant and E x Y = Environment x Year.

		a_w	**Moisture**	**Ash**	**Fats**	**Proteins**	**TP**	**TF**	**FRAP**	**DPPH**
	u.m.			%		g/100g	mg GAE/100g	mg CE/100g	mmol Fe^{2+}/100g	µmol TE/g
Environment	CTRL	0.12 **c**	0.72 **c**	1.79 **b**	10.5 **ab**	9.65 **b**	60.93 **b**	233.04 **b**	0.44 **b**	0.83 **b**
	mountainous	0.25 **b**	3.21 **b**	2.39 **a**	12.47 **a**	10.33 **a**	80.13 **a**	291.31 **a**	0.51 **a**	1.03 **a**
	hilly	0.28 **a**	4.24 **a**	2.02 **b**	9.85 **b**	10.38 **a**	64.21 **b**	288.63 **a**	0.43 **b**	0.68 **b**
Year	CTRL	0.12 **c**	0.72 **b**	1.79 **b**	10.5 **b**	9.65 **b**	60.93 **b**	233.04 **b**	0.44 **b**	0.83 **ab**
	2021	0.23 **b**	3.40 **a**	2.30 **a**	13.85 **a**	10.41 **a**	69.33 **a**	293.39 **a**	0.48 **a**	1.01 **a**
	2022	0.29 **a**	4.04 **a**	2.10 **ab**	8.47 **b**	10.30 **a**	75.01 **a**	286.55 **a**	0.46 **ab**	0.70 **b**
	E x Y	***	*ns*	***	*ns*	*ns*	*	***	*ns*	**

Table 3.9. Mineral composition (mg/100g) of CTRL and pea-enriched crackers. Different letters withing each column mean significant different values ($p \leq 0.05$, Tukey's least significant difference test). The number of stars represent significant differences at the 0.05 (*), 0.01 (**), and 0.001 (***) probability level, respectively. ns = not significant and E x Y = Environment x Year.

		Na	**K**	**Ca**	**Mg**	**P**	**S**	**Fe**	**Cu**	**Zn**
						mg/100g				
Environment	CTRL	949.85 **a**	174.40 **b**	19.22 **a**	22.87 **b**	299.10 **c**	3.21 **a**	99.57 **a**	0.32 **a**	0.67 **b**
	mountainous	929.73 **a**	248.60 **a**	23.42 **a**	32.82 **a**	318.30 **b**	3.47 **a**	102.44 **a**	0.35 **a**	1.04 **a**
	hilly	960.50 **a**	243.67 **a**	22.17 **a**	32.49 **a**	327.47 **a**	3.21 **a**	102.31 **a**	0.34 **a**	1.02 **a**
Year	CTRL	949.85 **ab**	174.40 **b**	19.22 **a**	22.87 **b**	299.10 **b**	3.21 **a**	99.57 **a**	0.32 **a**	0.67 **b**
	2021	920.72 **b**	242.67 **a**	22.79 **a**	32.33 **a**	322.96 **a**	3.21 **a**	102.19 **a**	0.33 **a**	1.04 **a**
	2022	969.51 **a**	249.61 **a**	22.80 **a**	32.98 **a**	322.80 **a**	3.47 **a**	102.55 **a**	0.36 **a**	1.02 **a**
	E x Y	*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

3.3.4.5 Sensory analysis of Crackers

Sensory analysis was carried out on one wheat-based CTRL and two pea-enriched snacks, both from mountainous environment in two different cultivation years (2021 and 2022). Evaluation of the sensory analysis attributes of snacks (**Figure 3.3**) revealed that color parameter was significantly ($p < 0.05$) differently perceived by the sensory panel, between the pea-enriched crackers and the CTRL, similar effects was observed by Hallén et al. (2004), Mohammed et al. (2012), Atef et al. (2011), Zucco et al. (2011) and Tiwari et al. (20119, showing to be the major criterion that affects the appreciation and the quality of the food product (Atef et al., 2011). In general, both pea-enriched and wheat-based (CTRL) snacks presented the most desirable sensory attributes (color, odor, flavor, texture and global appreciation) with no significant difference.

Regarding purchase intentions (data not shown), between all the three samples (wheat-based CTRL and the two pea flour from mountainous environment over 2021 and 2022) no significant difference was observed, demonstrating that pea incorporation may not affect the overall presentation and attractiveness of the final food product. Indeed, it was found that higher levels of pea flour (up to 20%) caused also significant reduction in taste, odor and overall acceptance of final products due to higher intensity of leguminous taste and odor.

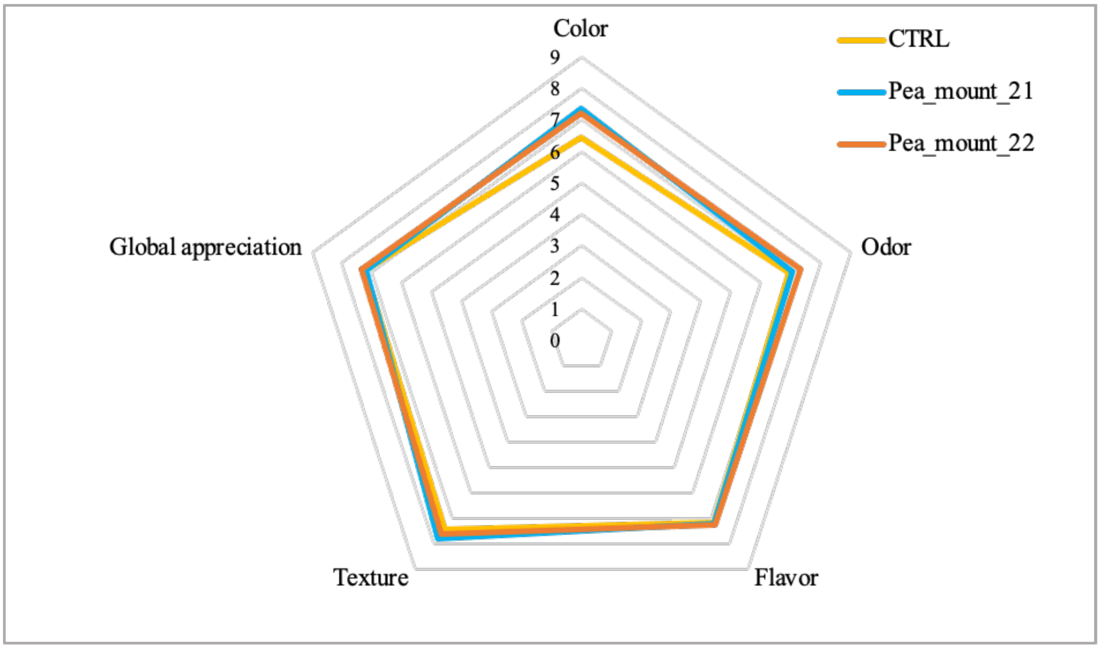

Figure 3.3. Sensory profile of baked snacks: evaluation of sensory attributes (color, appearance, aroma, texture and flavor) and global appreciation. Values are presented as mean.

Overall, the increases in the amounts of protein and in several physicochemical parameters corroborate that the addition of pea flour can increase the quality of the savory snacks produced. Therefore, the use of pea may provide more advantages to the final food product, thanks to its high quantities of digestible plant protein, starch and dietary fiber and a particular low quantity of antinutritional compounds.

3.5 Conclusion

Leguminous plants represent a sustainable, nutritious and convenient source of protein that offers a promising solution to the challenges of meat production. Their nutritional value, low environmental impact and human health benefits make them a key choice in promoting sustainable diets and mitigating the negative impacts of the meat industry. In the current market demand, legume-based products are becoming increasingly popular as functional foods with the use of alternative flours, providing both added nutritional value and bioactive healthy compounds. The present study was aimed to the investigation of agronomical and environmental conditions on the nutritional and bioactive composition of peas cultivated in different Italian environments over different cultivation seasons. This was considered important towards prioritizing this crop for future research, development, and innovation for plant-based protein products. Moreover, an efficient delivery method for incorporating high nutritional quality organic pea flour into baking products was presented. Nutritionally, the environment displays a strong impact on the nutritional quality of the grain, mostly due to weather conditions during the vegetative and reproductive stage of the crop and seed development. Technologically, it was found that the incorporation of the pea flour (6%) into wheat-based crackers has improved the dough and final product, in terms of functional and nutritional quality parameters. Furthermore, pea-enriched crackers showed improved color, rendering their appearance more attractive to the consumers; hence, demonstrating to be a good vehicle to deliver a plant-based meat alternatives protein source.

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References

Atef, A. M. A. Z., Mostafa, T. R., & Al-Asklany, S. A. (2011). Utilization of faba bean and cowpea flours in gluten free cake production. Australian Journal of Basic and Applied Sciences, 5(12), 2665-2672.

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. Journal of agricultural and food chemistry, 51(26), 7825-7834.

Ali-Khan, S. T., & Youngs, C. G. (1973). Variation in protein content of field peas. Canadian Journal of Plant Science, 53(1), 37-41.

Angioloni, A., & Collar, C. (2012). High legume-wheat matrices: an alternative to promote bread nutritional value meeting dough viscoelastic restrictions. European Food Research and Technology, 234, 273-284.

AOAC (2006). Method 950.36, Protein in Bread. In Official Method of Analysis, 18th ed.; Association of the Official Analytical Chemists: Gaithersburgs, MD, USA.

Arab, E.A.A., Helmy, I.M.F., Barch, G. F. (2010): Nutritional evaluation of functional properties of chickpea (Cicer arietinum L.) flour and the improvement of spaghetti produced from its. J. American Sci. 6 (1), 1055-1072

Arif, U., Ahmed, M. J., Rabbani, M. A., & Arif, A. A. (2020). Assessment of genetic diversity in pea (Pisum sativum L.) landraces based on physic-chemical and nutritive quality using cluster and principal component analysis. Pak. J. Bot, 52(2), 575-580.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food chemistry, 99(1), 191-203.

Batista, A. P., Niccolai, A., Bursic, I., Sousa, I., Raymundo, A., Rodolfi, L., ... & Tredici, M. R.

(2019). Microalgae as functional ingredients in savory food products: Application to wheat crackers. Foods, 8(12), 611.

Batista, A. P., Niccolai, A., Fradinho, P., Fragoso, S., Bursic, I., Rodolfi, L., ... & Raymundo, A. (2017). Microalgae biomass as an alternative ingredient in cookies: Sensory, physical and chemical properties, antioxidant activity and in vitro digestibility. Algal research, 26, 161-171.

Benítez, V., Cantera, S., Aguilera, Y., Mollá, E., Esteban, R. M., Díaz, M. F., & Martín-Cabrejas, M. A. (2013). Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes. Food Research International, 50(1), 64-69.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry, 239(1), 70-76.

Birt, D. F., Boylston, T., Hendrich, S., Jane, J. L., Hollis, J., Li, L., ... & Whitley, E. M. (2013). Resistant starch: promise for improving human health. Advances in nutrition, 4(6), 587-601.

Bourgeois, M., Jacquin, F., Cassecuelle, F., Savois, V., Belghazi, M., Aubert, G., ... & Burstin, J. (2011). A PQL (protein quantity loci) analysis of mature pea seed proteins identifies loci determining seed protein composition. Proteomics, 11(9), 1581-1594.

Bourne, M. (2002). Sensory Methods of Texture and Viscosity Measurement. Food texture and viscosity. Elsevier: Amsterdam, The Netherlands; pp. 259–266, ISBN 978-0-12-119062-0

Chon, S. U., Heo, B. G., Park, Y. S., Kim, D. K., & Gorinstein, S. (2009). Total phenolics level, antioxidant activities and cytotoxicity of young sprouts of some traditional Korean salad plants. Plant Foods for Human Nutrition, 64, 25-31.

Cookies and Crackers Europe Statista Market Forecast. Available online: https://www.statista.com/outlook/40100300/102/co okies-crackers/europe (accessed on 20 June 2021)

Daba, S. D., & Morris, C. F. (2022). Pea proteins: Variation, composition, genetics, and functional properties. Cereal chemistry, 99(1), 8-20.

Dahl, W. J., Foster, L. M., & Tyler, R. T. (2012). Review of the health benefits of peas (Pisum sativum L.). British Journal of Nutrition, 108(S1), S3-S10.

De la Hera, E., Ruiz-Paris, E., Oliete B., Gómez, M. (2012): Studies on the quality of cakes made with wheat-lentil composite flours. LWT-Food Sci. Technol. (in press) DOI: 10.1016/j.lwt.2012.05.009

Des-Marchais, L.P., Foisy, M., Mercier, S., Villeneuve, S., Mondor, M. (2011): Bread-making potential of pea protein isolate produced by a novel ultrafiltration/diafiltration process. Procedia Food Sci. 1, 1425-1430

Dhinda, F., Lakshmi, J.A., Prakash, J., Dasappa, I. (2011): Effect of ingredients on rheological, nutritional and quality characteristics of high protein, high fibre and low carbohydrate bread. Food Bioprocess Technol. in press DOI: 10.1007/s11947-011-0752-y

Dinelli, G., Segura-Carretero, A., Di Silvestro, R., Marotti, I., Arráez-Román, D., Benedettelli, S., ...

& Fernadez-Gutierrez, A. (2011). Profiles of phenolic compounds in modern and old common wheat varieties determined by liquid chromatography coupled with time-of-flight mass spectrometry. Journal of Chromatography A, 1218(42), 7670-7681.

EN ISO 8589. Sensory Analysis—General Guidance for the Design of Test Rooms. 2017. Available online: https://www.iso.org/standard/36385.html (accessed on 10 January 2022)

European Commission (2018a). EU Agricultural Outlook for Markets and Income, 2018–2030. Brussels: European Commision

Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. Journal of food composition and analysis, 24(7), 1043-1048.

Gawalko, E., Garrett, R. G., Warkentin, T., Wang, N., & Richter, A. (2009). Trace elements in Canadian field peas: a grain safety assurance perspective. Food Additives and Contaminants, 26(7), 1002-1012.

Gawłowska, M., Święcicki, W., Lahuta, L., & Kaczmarek, Z. (2017). Raffinose family oligosaccharides in seeds of Pisum wild taxa, type lines for seed genes, domesticated and advanced breeding materials. Genetic Resources and Crop Evolution, 64, 569-578.

Geil, P. B., & Anderson, J. W. (1994). Nutrition and health implications of dry beans: a review. Journal of the American College of Nutrition, 13(6), 549-558.

Gómez, M., Oliete, B., Rosell, C. M., Pando, V., & Fernández, E. (2008). Studies on cake quality made of wheat–chickpea flour blends. LWT-Food Science and Technology, 41(9), 1701-1709.

Hacisalihoglu, G., Beisel, N. S., & Settles, A. M. (2021). Characterization of pea seed nutritional value within a diverse population of Pisum sativum. Plos one, 16(11), e0259565.

Haenen, D., Zhang, J., Souza da Silva, C., Bosch, G., van der Meer, I. M., van Arkel, J., ... & Hooiveld, G. J. (2013). A diet high in resistant starch modulates microbiota composition, SCFA concentrations, and gene expression in pig intestine. The Journal of nutrition, 143(3), 274-283.

Hall, C., Hillen, C., & Garden Robinson, J. (2017). Composition, nutritional value, and health benefits of pulses. Cereal Chemistry, 94(1), 11-31.

Hallén, E., İbanoğlu, Ş., & Ainsworth, P. (2004). Effect of fermented/germinated cowpea flour addition on the rheological and baking properties of wheat flour. Journal of food engineering, 63(2), 177-184.

Harmankaya, M., Özcan, M. M., Karadaş, S., & Ceyhan, E. (2010). Protein and mineral contents of pea (Pisum sativum L.) genotypes grown in central Anatolian region of Turkey. South West. J. Hortic. Biol. Environ, 1, 159-165.

Heller, M. C., & Keoleian, G. A. (2018). Beyond meat's beyond burger life cycle assessment: a detailed comparison between a plant-based and an animal-based protein source. Center for Sustainable Systems, University of Michigan.

Hu, F. B., Otis, B. O., & McCarthy, G. (2019). Can plant-based meat alternatives be part of a healthy and sustainable diet?. Jama, 322(16), 1547-1548.

Jones, D. A., DuPont, M. S., Ambrose, M. J., Frías, J., & Hedley, C. L. (1999). The discovery of compositional variation for the raffinose family of oligosaccharides in pea seeds. Seed Science Research, 9(4), 305-310.

Kamaljit, K., Baljeet, S., & Amarjeet, K. (2010). Preparation of bakery products by incorporating pea flour as a functional ingredient. American Journal of Food Technology, 5(2), 130-135.

Karjalainen, R., & Kortet, S. (1987). Environmental and genetic variation in protein content of peas under northern growing conditions and breeding implications. Agricultural and Food Science, 59(1), 1-9.

Klepacka, J., Gujska, E., & Michalak, J. (2011). Phenolic compounds as cultivar-and variety-distinguishing factors in some plant products. Plant foods for human nutrition, 66, 64-69.

Kohajdová, Z., Karovičová, J., & Magala, M. (2013). Rheological and qualitative characteristics of pea flour incorporated cracker biscuits. Croatian journal of food science and technology, 5(1), 11-17.

Lee, S. C., Prosky, L., & Vries, J. W. D. (1992). Determination of total, soluble, and insoluble dietary fiber in foods—Enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. Journal of AOAC international, 75(3), 395-416.

Leitão, I., Sales, J., Martins, L. L., & Mourato, M. P. (2021). Response to stress induced by different potentially toxic elements (As, Cd, Cu and Na) in rapeseed leaves. Plant Physiology Reports, 26(3), 478-490.

Leterme, P. (2002). Recommendations by health organizations for pulse consumption. British Journal of nutrition, 88(S3), 239-242.

Liu, Y., Ragaee, S., Marcone, M. F., & Abdel-Aal, E. S. M. (2020). Composition of phenolic acids and antioxidant properties of selected pulses cooked with different heating conditions. Foods, 9(7), 908.

Maharjan, P., Penny, J., Partington, D. L., & Panozzo, J. F. (2019). Genotype and environment effects on the chemical composition and rheological properties of field peas. Journal of the Science of Food and Agriculture, 99(12), 5409-5416.

McClements, D. J., Decker, E. A., Park, Y., & Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Critical reviews in food science and nutrition, 49(6), 577-606.

Mohammed, I., Ahmed, A. R., & Senge, B. (2012). Dough rheology and bread quality of wheat–chickpea flour blends. Industrial Crops and Products, 36(1), 196-202.

Mohammed, Y. A., Chen, C., Walia, M. K., Torrion, J. A., McVay, K., Lamb, P., ... & Khan, Q. (2018). Dry pea (Pisum sativum L.) protein, starch, and ash concentrations as affected by cultivar and environment. Canadian Journal of Plant Science, 98(5), 1188-1198.

Moradi, M., Tajik, H., Almasi, H., Forough, M., & Ezati, P. (2019). A novel pH-sensing indicator based on bacterial cellulose nanofibers and black carrot anthocyanins for monitoring fish freshness. Carbohydrate Polymers, 222, 115030.

Mota, J., Lima, A., B. Ferreira, R., & Raymundo, A. (2020). Lupin seed protein extract can efficiently enrich the physical properties of cookies prepared with alternative flours. Foods, 9(8), 1064.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of pharmaceutical and biomedical analysis, 41(5), 1523-1542.

Nikolopoulou, D., Grigorakis, K., Stasini, M., Alexis, M. N., & Iliadis, K. (2007). Differences in chemical composition of field pea (Pisum sativum) cultivars: Effects of cultivation area and year. Food chemistry, 103(3), 847-852.

Notz I., Reckling M. and Schuler J. (2022). The role of legume production and use in European agri-food systems. Legumes Translated Practice Guide 2.

Oliveira, S., Sousa, I., & Raymundo, A. (2022). Printability evaluation of Chlorella vulgaris snacks. Algal Research, 68, 102879.

Peterbauer, T., Mucha, J., Mach, L., & Richter, A. (2002). Chain Elongation of Raffinose in Pea Seeds: ISOLATION, CHARCTERIZATION, AND MOLECULAR CLONING OF A MULTIFUNCTIONAL ENZYME CATALYZING THE SYNTHESIS OF STACHYOSE AND VERBASCOSE* 210. Journal of Biological Chemistry, 277(1), 194-200.

Peterbauer, T., & Richter, A. (2001). Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Science Research, 11(3), 185-197.

Poblaciones, M. J., & Rengel, Z. (2018). The effect of processing on Pisum sativum L. biofortified with sodium selenate. Journal of Plant Nutrition and Soil Science, 181(6), 932-937.

Prosky, L., Asp, N. G., Schweizer, T. F., Devries, J. W., & Furda, I. (1988). Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. Journal of the Association of Official Analytical Chemists, 71(5), 1017-1023.

Reichert, R. D., & MacKenzie, S. L. (1982). Composition of peas (Pisum sativum) varying widely in protein content. Journal of Agricultural and Food Chemistry, 30(2), 312-317.

Sattari Vayghan, H., Tavalaei, S., Grillon, A., Meyer, L., Ballabani, G., Glauser, G., & Longoni, P. (2020). Growth temperature influence on lipids and photosynthesis in Lepidium sativum. Frontiers in plant science, 11, 745.

Siddiq, M., Ravi, R., Harte, J.B., Dolan, K.D. (2010): Physical and functional characterisation of selected dry bean (Phaseolus vulgaris L.) flours. LWT – Food Sci. Technol. 43 (2), 232-237

Singh, B., Singh, J. P., Shevkani, K., Singh, N., & Kaur, A. (2017). Bioactive constituents in pulses and their health benefits. Journal of food science and technology, 54, 858-870.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology (Vol. 299, pp. 152-178). Academic press.

Sun, J., Wu, D., Xu, J., Rasmussen, S. K., & Shu, X. (2015). Characterisation of starch during germination and seedling development of a rice mutant with a high content of resistant starch. Journal of Cereal Science, 62, 94-101.

Tao, A., Afshar, R. K., Huang, J., Mohammed, Y. A., Espe, M., & Chen, C. (2017). Variation in yield, starch, and protein of dry pea grown across Montana. Agronomy Journal, 109(4), 1491-1501.

Tzitzikas, E. N., Vincken, J. P., de Groot, J., Gruppen, H., & Visser, R. G. (2006). Genetic variation in pea seed globulin composition. Journal of Agricultural and Food Chemistry, 54(2), 425-433.

Uysal, H., Bilgiçli, N., Elgün, A., İbanoğlu, Ş., Herken, E. N., & Demir, M. K. (2007). Effect of dietary fibre and xylanase enzyme addition on the selected properties of wire-cut cookies. Journal of Food Engineering, 78(3), 1074-1078.

VELP (2020). Elemental Analysis and the Dumas Method. VELP Scientifica. Available online: https://www.velp.com/en-ww/du- mas-method-1.aspx (accessed on 27 August 2021).

Wang, N., & Daun, J. K. (2004). Effect of variety and crude protein content on nutrients and certain antinutrients in field peas (Pisum sativum). Journal of the Science of Food and Agriculture, 84(9), 1021-1029.

Wang, N., Hatcher, D. W., & Gawalko, E. J. (2008). Effect of variety and processing on nutrients and certain anti-nutrients in field peas (Pisum sativum). Food chemistry, 111(1), 132-138.

Zobel, A. M., F. A. Tomas-Barberan, and R. J. Robbins. (1997). "Phytochemistry of Fruit and Vegetables." Clanderon Press, Oxford, UK: 173-204

Zucco, F., Borsuk, Y., & Arntfield, S. D. (2011). Physical and nutritional evaluation of wheat cookies supplemented with pulse flours of different particle sizes. LWT-Food Science and Technology, 44(10), 2070-2076.

General conclusions

Currently, the modern agricultural techniques had negatively impacted on ecosystem patterns and the growing global population is increasingly requiring for higher food production, despite the limited availability of natural resources. In parallel, climate change and associated biotic and abiotic environmental stresses are impacting on farming systems and will increasingly pose serious implications for global food production. This current context is driving toward the need for a more sustainable agri-food system, that may preserve and protect ecological and natural sources. The increase in legume production in low-input agricultural systems could be an important contribution that can help mitigate current health and environmental-related global crises.

Legumes are ecosystem service providers and environmental "guardians", as they reduce the need for synthetic N fertilization, promote soil conservation, and create more diversified and biodiverse agricultural systems. These, as nutrient and energy-dense food sources, could be produced utilizing diverse agricultural production systems that may be both economically and environmentally sustainable, based on the multiple functional benefits that legume cultivation may provide to the agronomic system. Additionally, grain legumes are excellent sources of protein/amino acids, fatty acids, fibers, carbohydrates, and phytochemicals, also characterized by a low glycemic index. The demonstrated nutrient content and health benefits of food legumes align well with the changing consumer trends for healthy food choices. Moreover, consumers are increasingly opting for a reduction or complete elimination of meat-derived proteins. The acknowledgment of these facts may be one important step to the return of legumes within agri-food systems.

The aim of the present study was to comparatively evaluate the agronomical and physiological performance of pea grain (*Pisum sativum* L.) cultivated in an organic agronomic system under low input growing conditions among two different growing environments of Emilia-Romagna region (Italy) over two consecutive cropping seasons. A complete description of the involved pea grain was given, in terms of growth and agronomical parameters, nutrient content and phytochemical composition. In parallel, considering that grain legumes (i.e. pea grain) are known and used also for animal feed, the total replacement of soybean meal (imported with high environmental challenges) with pea-based diet for dairy cows' nutrition was evaluated in terms of nutritional quality of the feed and milk yield and quality of the cattle. Moreover, the physical and functional properties of baked snacks (crackers) enriched with pea flour in comparison with wheat-based product were evaluated.

The research firstly highlighted that the cultivation in different environmental conditions (altitude, environment, sowing time, cultivation year, etc.) of organic pea (*Pisum sativum* L. var. Turris) could significantly impact on the agronomic performance of the crop, in terms of growth traits and yield parameters. Considering that autumn sowing appears to be more promising for the growth traits, spring sowing gave good results in terms of agronomic yield, thus it can be preferably chosen for a more efficient water use in the vegetative and reproductive stage of the plants, in order to optimize the crop production within sustainable and low input agronomic systems. In addition, the inclusion of the harvested organic pea grain in dairy cows feed, as substitute of soy-based diet, showed a positive productive effect on the milk yield of the examined animals and among the nutritional composition of the feed (in terms of milk yield and urea content), showing to be a valuable and sustainable alternative to soybean in the nutrition of dairy cattle in organic farming context.

Moreover, regarding the evaluation of nutritional and phytochemical composition of the pea grain, it was observed that all the nutritional and bioactive compounds are strictly interconnected between each other and, also, an interesting relationship with weather parameters was found. Hence, given the strong impact that the growing environment may exert among the nutritional composition, it can be considered as an agronomic strategy to improve the quality of the grain, in order to face the recent global consumers interest through high-quality food products in a more sustainable food system. Finally, the results showed that the incorporation (6%) of organic pea flour in wheat-based crackers enhanced the functional and nutritional quality of the baking products, suggesting that it can be purposed as a more sustainable and healthy consumers choice.