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PhD thesis

Development of barley based couscous with improved nutritional and functional properties

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CONTENTS

ABSTRACT

Among food products, those from cereals may constitute an important means to introduce bioactive compounds in the diet since it has been shown that they play a fundamental role in the promotion of healthy eating, thanks to their overall composition. In this regard, barley (*Hordeum vulgare* L.) is well suited to develop functional foods because of the presence of bioactive compounds (dietary fibre, ß-glucans, phenolic compounds etc.), which are known for their important physiological properties related to soluble dietary fibre ingestion and to the effects related to the reduction of blood glucose and cholesterol levels.

Among cereal products, couscous could become a good means to introduce bioactive compounds in the diet. In fact, couscous, which is an ancient cereal food typical of North African countries, is now losing its connotation as a typical product of the Arab culture, thanks to globalization and is highly appreciated on an international level, especially in Europe.

In light of these considerations, this research aims to explore unconventional raw materials, such as barley flour, for the production of couscous with high nutritional value and functional properties, developing in three main activities:

1. Evaluation of suitable physical techniques for the enrichment of barley flour in bioactive compounds and the study of balanced formulations, containing semolina and enriched barley flour, for the production of innovative couscous that meet nutritional and health requirements and good technological quality.
2. Production of functional couscous by traditional process in order to verify interactions and/or the changes induced by the addition of non-conventional raw materials.
3. Complete physico-chemical, nutritional and sensorial characterization of innovative products.

The results obtained in the first part of the experimentation show that air classification process is particularly suitable for the enrichment of barley flour in bioactive compounds, in fact, when barley flour is subjected to this process, its final ß-glucans content is increased by almost 90% compared to untreated flour. This allows, in the formulation of functional cereal foods, to reach high ß-glucans amount with a minimum replacement of wheat flour with barley flour thus preserving the technological and processing aptitude of formulas. Further results show that the addition of enriched barley flour to semolina allows for the production of a couscous characterized by quantities of fibre (at least 6g/100g) and ß-glucans (1.0g of ß-glucans per quantified portion) fulfilling the nutritional and health claims identified by FDA and EFSA and implemented through (EC) Regulation No. 1924/2006 and (EU) Regulation n. 432/2012. Moreover, the final product also proved to be enriched with flavan-3-ol compounds (not contained in wheat) and alkylresorcinols, whose content is double in the functional couscous, permitting to introduce different compounds useful for human well-being in their diet. In conclusion innovative couscous, can be considered a functional product with improved nutritional value and good cooking qualities and thus preserves the typical characteristics of classical couscous resulting in a product that can be successful on the market.

This research project was conducted within the PRIN Project 2015 - Prot. 2015MFP4RC supported by MIUR (Italian Ministry of Instruction, University and Scientific Research).

RIASSUNTO

Tra i prodotti alimentari, quelli a base di cereali rappresentano un importante veicolo per introdurre composti bioattivi nella dieta; è stato infatti dimostrato che, i cereali, contengono sostanze con comprovata azione protettiva sulla salute, promuovendo una sana alimentazione. A tal proposito, l'orzo (*Hordeum vulgare* L.) ben si presta allo sviluppo di alimenti funzionali per la presenza di composti bioattivi (fibra alimentare, ß-glucani, composti fenolici ecc.), riconosciuti per le importanti proprietà fisiologiche legate all'ingestione di fibra solubile, nonché per gli effetti ipocolesterolemico e ipoglicemico.

Tra i prodotti a base di cereali, il couscous risulta particolarmente idoneo per introdurre composti bioattivi nella dieta, infatti, pur essendo originario dei Paesi del Nord Africa, oggi, grazie ai fenomeni di globalizzazione e migrazione, sta perdendo la connotazione di prodotto tipico della cultura araba risultando molto apprezzato a livello internazionale e diffondendosi soprattutto in Europa.

Alla luce di queste considerazioni, il presente lavoro di ricerca, ha mirato a valutare l'utilizzo di materie prime non convenzionali, come la farina d'orzo, per la produzione di couscous funzionale ad alto valore nutrizionale, attraverso lo sviluppo di tre attività principali:

1. Impiego di idonee tecniche fisiche per l'arricchimento della farina d'orzo in composti bioattivi e studio di formulazioni bilanciate, contenenti semola e farina d'orzo arricchita, per la produzione di couscous innovativo che soddisfi i requisiti nutrizionali e salutistici, mantenendo una buona qualità tecnologica.
2. Produzione di couscous funzionale mediante processo tradizionale, al fine di verificare le interazioni e/o le modifiche indotte dall'aggiunta di materie prime non convenzionali.
3. Completa caratterizzazione chimico-fisica, nutrizionale e sensoriale del prodotto innovativo.

I risultati ottenuti nella prima parte della sperimentazione mostrano che, il processo di classificazione ad aria risulta essere particolarmente adatto per l'arricchimento della farina d'orzo in composti bioattivi, infatti, quando sottoposta a tale processo, la farina d'orzo presenta un contenuto in ß-glucani più alto di quasi il 90% rispetto allo sfarinato non trattato. Ciò consente, nella formulazione di alimenti funzionali a base di cereali, di raggiungere elevate quantità di ß-glucani con una minima sostituzione della farina di grano con la farina d'orzo preservando l'attitudine tecnologica e di lavorazione delle materie prime.

Ulteriori risultati rilevano inoltre, che l'aggiunta di farina d'orzo arricchita alla semola, consente di produrre un couscous caratterizzato da quantità di fibra (almeno 6,0 g/100g) e ß-glucani (1,0 g di ß-glucani per porzione quantificata) tali soddisfare le indicazioni nutrizionali e salutistiche individuate dalla FDA e dall'EFSA e recepite attraverso il Regolamento (CE) N. 1924/2006 e il Regolamento (UE) n. 432/2012. Inoltre, il prodotto finito risulta essere arricchito anche in flavan 3-oli (non presenti nella semola) e alchilresorcinoli, il cui contenuto è doppio nel couscous funzionale, permettendo di introdurre nella dieta composti utili per il benessere dell'uomo. In conclusione, il couscous innovativo realizzato può essere considerato un prodotto funzionale con migliorato valore nutrizionale e buona qualità di cottura che preserva le caratteristiche tipiche del couscous classico, risultando, perciò, un possibile prodotto di successo sul mercato.

Questo progetto di ricerca è stato condotto nell'ambito del Progetto PRIN 2015 - Prot. 2015MFP4RC supportato dal MIUR (Ministero dell'Istruzione, Università e Ricerca Scientifica).

Chapter 1: COUSCOUS

1.1 Introduction

Couscous is a cereal product which is about 2000 years old. Couscous is also known by different names in different countries: ‘‘Kuskus” in Turkey, ‘‘Couscous” in Morocco, ‘‘Maftoul, moghrabieh” in Lebanon, ‘‘Seksu” in Berber, ‘‘Kusksi” in Libya, ‘‘Keskesu” in Tuareg and ‘‘Kouskousaki” in Greece (Coskun, 2013). The European diffusion of couscous occurred in the seventeenth century by the Arabs, and subsequently moved to the Americas with the Portuguese cargoes from Morocco. Moreover, North African immigration during the 1960s and 1980s to Europe and North America definitely contributed to the international spread of this product (Benkerroum, 2012).

Today couscous is widely known around the world and it can be consumed in various recipes such as salad (tabulleh) or traditional couscous dishes with chicken and meat, fish or vegetables as an alternative to pilaf rice (Yüksel et al., 2017) but it is often consumed also as a sweet dish flavored with dried and candied fruit, chocolate, sugar, honey and cinnamon. Because of its simplicity, affordability and versatility in preparing and cooking, couscous is losing its connotation as a typical product of the Arab culture and is increasing in importance in Europe and on an international level. It has become a trendy and modern food (Abecassis et al., 2012). This trend is surely related to the growing interest in so-called “ethnic foods” and the increase in immigration of people of Arabic origin (D’Egidio and Pagani, 2010).

The *Codex Alimentarius* (Codex Standard, 202-1995) defines couscous as *“the product prepared from durum wheat semolina (Triticum durum) the elements of which are bound by adding potable water and which has undergone physical treatment such as cooking and drying. Couscous is prepared from a mixture of coarse and fine semolina. It can be prepared from coarse medium semolina”*. The high quality of couscous is defined as a product with regular and homogeneous particle size, which must be amber yellow (Chemache, 2018).

Although durum wheat semolina is the main raw material used for couscous preparation, other cereals, like sorghum, millet, corn and fonio (Galiba et al., 1987; Aboubacar and Hamaker, 1999) are used, especially in West and Sub-Saharan Africa but it can also be made from a mixture of durum and soft wheat (Zinedine et al., 2017).

Depending on the formulation, the production process and use, there are three different types of couscous that are widely known; Turkish and short-cut pasta-like couscous and the classic Arabic/African couscous. Turkish couscous is generally produced by coating bulgur granules with semolina, wheat flour and water or milk (Demir et al., 2010). Bulgur is a cereal product obtained from *Triticum durum* through a series of operations such as cleaning, cooking, drying, tempering, peeling, milling, polishing and size classification (Bayram and Öner, 2005, 2007; Hayta et al., 2003; Yıldırım et al., 2008a, 2008b). Short-cut pasta-like couscous, instead, is produced mechanically through extrusion technology (Celik et al., 2004) generally using a machine similar to that used for pasta production. Finally, the classic Arabic/African couscous, which is the most commonly known and used couscous type. It is traditionally produced by hand, but for both the traditional and industrial production of couscous, the basic steps are the same: i) mixing and agglomeration of flour and water, ii) steaming and iii) drying (Aboubacar et al., 2006; Debbouz and Donelly, 1996).

In this study, reference is always made to the classic Arabic/African couscous.

1.2 The worldwide market for couscous

Today, there is not much information on couscous production in the world, however available data show that the worldwide industrial production of couscous is about 420,000 tons/year and about 20% is produced in Morocco (80,000-90,000 tons/year). Europe imports 85% to 95% of couscous from Morocco and the main destination market in the past, were France, the

Netherlands, Germany, Italy, Spain, Belgium, and the UK. In recent years, European countries such as France, Italy and Spain, started to produce couscous autonomously allowing the European couscous production to reach 145.000 tons/year. USA and Canada have a production capacity of 20.000 tons/year of couscous (Zinedine et al., 2017).

More recent data shows that Turkey, where Turkish-like couscous is mostly produced and consumed, is in the nineteenth place for the worldwide quantity of couscous imported between the years 2012 to 2016, while it is ranks forty-second in the list of exporters. The export flow of couscous, together with pasta, occurs mainly from Turkey to Iraq, Japan and United Arab Emirates but, in 2016, the quantity of exported couscous decreased from 268 to 231 tons (Yüksel et al., 2018a).

Extending the observation to other countries of the world, we obtain the data shown in figures 1.1 and 1.2 that report the imported and exported quantities of couscous, by the first fifteen countries in the world, in the three-year period of 2014-2016 (http://trademap.org/Index.aspx).

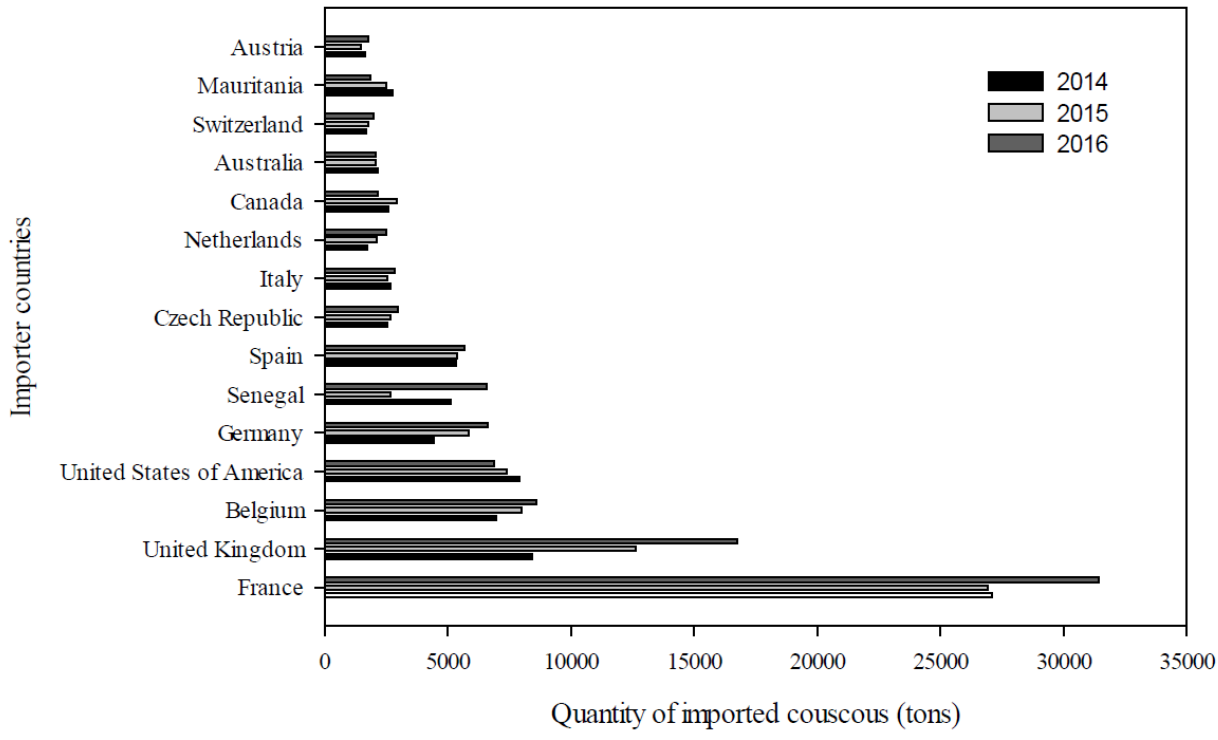

Figure 1.1. Quantity of imported couscous by the first fifteen countries in the world (data from Trade Map)

Worldwide, the total exportation and importation quantity of couscous was 124,481 and 126,799 tons, respectively. In regards to imports, France was the main importer of couscous with 31,436 tons purchased in 2016 followed by United Kingdom (16,763 tons), Belgium (8,597 tons) and United States of America (6,870 tons). Italy ranks only in ninth place (figure 1.1). The situation changes if we consider exports. In 2016, Italy and France were in first and second place with 37,281 and 34,809 tons of exported couscous respectively, followed by ethnic countries such as Morocco, Tunisia and Israel (figure 1.2).

Additional data available, shows that in the first quarter of 2017, Turkey dropped to third place for the quantity of couscous exported, only preceded by France and United States of America (http://trademap.org/Index.aspx).

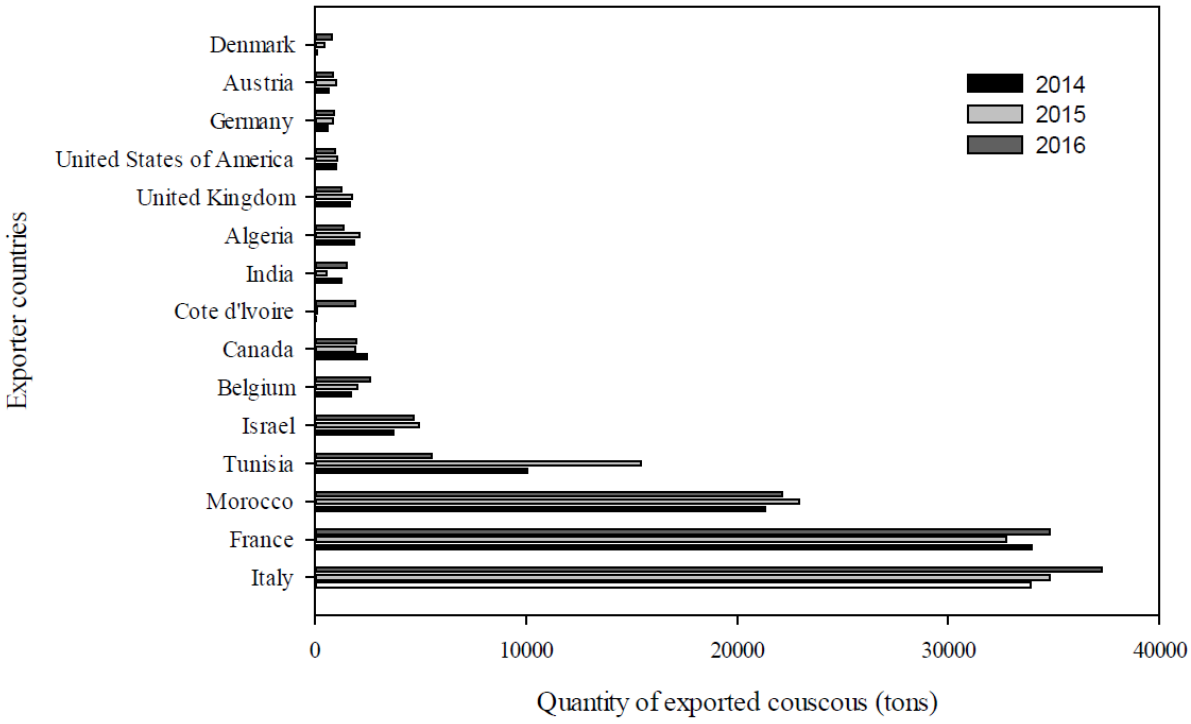

Figure 1.2. Quantity of exported couscous by the first fifteen countries in the world (data from Trade Map)

Couscous and ethnic food in Italy

Italy has always boasted a culinary culture of great value, thanks to the well-known benefits of the Mediterranean diet. However, the desire to try and experiment, surely influenced by the

attention and curiosity raised by Expo 2015, favoured the meeting between the tradition of the "Beautiful Country", with culinary cultures of every latitude.

Nowadays it is estimated that almost 85% of Italians have tried ethnic foods and almost a third eat them regularly in their monthly diet. This experimentation with new flavors takes place through ethnic restaurants, but Italian consumers are gradually introducing these foreign foods into their homes, encouraged by the increasing availability in larger supermarket chains.

In this way the ethnic cuisine fuses with the national one favouring an ever increasing success of these products: in particular, along with couscous, the consumption of Japanese sushi, soy sauce, Chinese rolls, paella, kebab, Greek feta, and Mexican tortillas is growing (Coop Report, 2016).

Table 1.1 shows the results of a survey given to a sample of consumers to understand the trends regarding consumption, purchase and preparation of ethnic foods. Reported data highlighted that about 30% of Italian people consume ethnic food monthly, Arabic preferably, which is easily purchased in a local supermarket, in fact over 48% of those interviewed buy ethnic food in a supermarket, about 30% of interviewed, instead, consume it in restaurants a few times per month, while the 62% of those interviewed prepare ethnic meals at home. In particular, it emerges that among foreign foods, couscous is the most prepared meal at home (Coop Report, 2016). This confirms what has been reported in literary research, where many authors attribute the success of couscous to its availability and easy preparation.

The growing consumption of couscous in 2016, along with that of other ethnic foods, was also confirmed in 2017. In fact, in the first six months of 2017 the ethnic shopping cart, as well as the ready-made one, grew by 6.9% - especially ethnic food like couscous and kebab - thanks to immigration and globalization (Coop Report, 2017).

Table 1.1 Ethnic foods: buy, prepare, eat (Coop Report, 2016)

CONSUMPTION	Do you eat in ethnic restaurants or take away ethnic food sometimes?	Yes, few times per month 29.5% Yes, few times a year 45.1%
	Which ethnic restaurants or take away shop do you frequent most?	Arabic, Chinese, Japanese
PURCHASE	Do you ever buy ethnic food?	Yes, 75%
	Where do you buy ethnic food?	Supermarket 48.3% Food stores managed by foreigners 17.2%
	Which ethnic food do you buy more frequently?	Chinese or Japanese food 38.8% Mexican/Latin American food 27.7%
PREPARATION	Do you prepare ethnic meals?	Yes 61.9%
	Which ethnic meals do you prepare more frequently?	Couscous, Cantonese rice, sushi
	How did you learn to prepare ethnic meals?	Reading recipes on the web 52.1%

Source: Research on data from Osservatorio dell'Istituto Zooprofilattico Sperimentale delle Venezie

As shown in figure 1.3, in 2018 the ethnic shopping cart continued to grow doubling in sales compared to 2010 and this trend is confirmed by Italians' choices in food consumption. In fact, about 56% of Italians appreciate ethnic food (Coop Report, 2018).

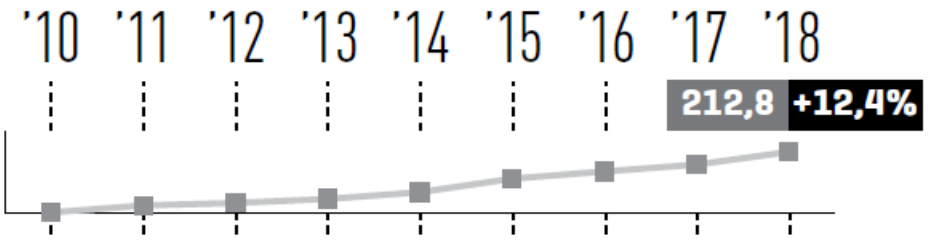

Figure 1.3. Ethnic shopping cart; Index value and percentage change of first half of 2018 /first half of 2017; 2010 = 100 (Source: REF, research on Nielsen data)

Regarding outside consumption, in the first three months of 2018, about 42% of Italians (14 million) chose a different cuisine than Italian food, when eating out at a restaurant, and this percentage rises to 49% in Northern Italy and to 64% among Millennials. The Italian passion for ethnic food it is not new, but it is only in recent years that the phenomenon has become widespread. From sushi to tacos to couscous, about 7.6 million of Italians chose ethnic

restaurants more often compared to 5 years ago, while about 3.6 million Italians eat ethnic meals outside their homes, on average 1.6 times per week (Coop Report, 2018).

Altough the recent "ethnic boom" can be interpreted as a temporary novelty, 2 out of 3 Italians believe that ethnic cuisine is destined to become an integral part of the Italian diet.

1.3 Production process of couscous

Couscous has always been produced at home through a manual process but recently, also thanks to the increase in consumption of this product, an industrial process has been developed and nowadays both processes are widely used.

Couscous artisanal production

Couscous is traditionally prepared by a lengthy, manual mixing of durum wheat semolina and salted water in a large dish, called *gissa*, and rubbing the obtained bulk between the palm of the hand and the container until agglomeration is attained (D'Egidio and Pagani, 2010). The formed granules are then hand-worked, for about 15 minutes, by successive addition of other durum wheat semolina and water, on the basis of personal experience (figure 1.4).

Subsequently, wet granules are forced through a metal sieve in order to have well-shaped and uniformly sized couscous granules. At this stage, based on the desired size, three types of couscous can be produced: coarse, medium and fine couscous (figure 1.5) according to size criteria (Quaglia, 1988).

Figure 1.4. Hand-working of couscous (https://www.alimentipedia.it/cous-cous.html)

After the sieving phase, raw couscous is eventually steamed, in order to have starch gelatinization and a first product structuring, and then sun-dried on the flat roof of the houses (Abecassis et al., 2012) and stored. Finally, before being eaten, couscous is cooked with vapor using a special pot, called couscousière (figure 1.6a), formed by two superimposed pots: a lower pot containing boiling water and an upper pot with a perforated bottom where the couscous is placed to form a layer of about 10-20 cm (Debbouz and Donnelly, 1996; Abecassis et al., 2012).

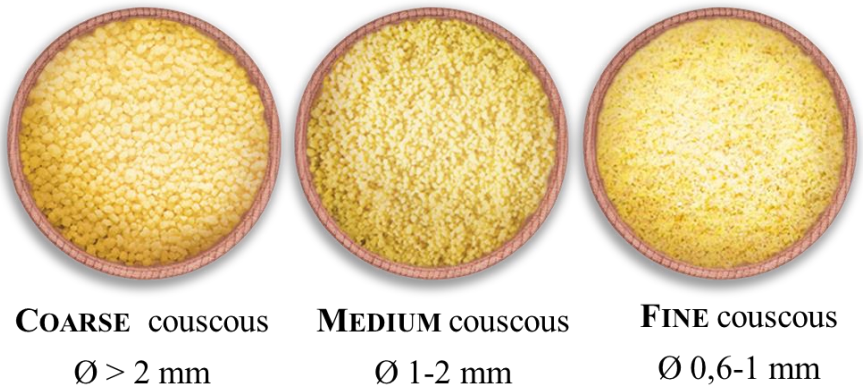

Figure 1.5. Commercial couscous of different sizes (www.storci.com)

The junction between the two sections is usually sealed with a damp cloth (figure 1.6b) to force the steam through the couscous in the upper section and steaming continues for 30-40 minutes

until cooking occurs (D'Egidio and Pagani, 2010). A major limitation of handmade couscous production is the length of time required for final cooking (Pagani et al., 2009).

Figure 1.6. **a)** different type of couscousiére **b)** couscousiére sections sealed with a damp cloth

The artisanal production process of couscous is summarized in figure 1.7.

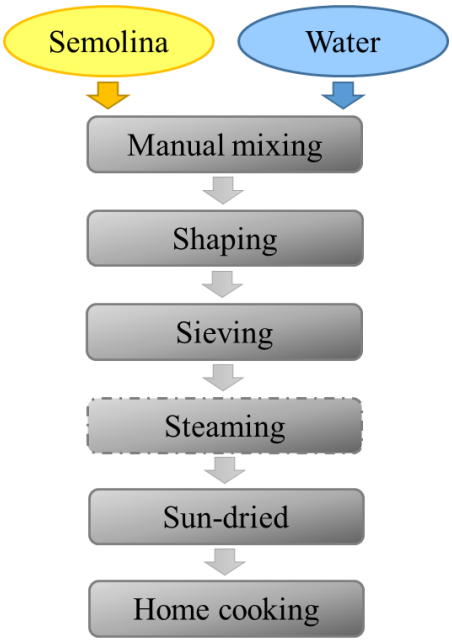

Figure 1.7. Flow sheet of artisanal production of couscous (D'Egidio and Pagani, 2010)

Couscous industrial production

The first information on the process of industrial couscous production dates back to the 1960s in North Africa when the transfer from the pasta making industry to the production of couscous was made. But, it was not until the mid 1970's that fully automated couscous production lines

started in North Africa, and later in other parts of the world, like France, Italy, Greece and more recently in the United States (Abecassis et al., 2012).

The first industrial production lines for couscous processing have working rates ranging from 500 to 1,200 kg/h (Abecassis et al., 2012), with some lines that allow a production of up to 2,000-3,000 kg/h (D'Egidio and Pagani, 2010). In any case, the design of industrial equipment for couscous production should imitate the technique of and reach the final qualities of homemade couscous (Kaulp and Walker, 1986; Quaglia, 1988; Mèot, 2006), in fact, the main steps of the current couscous industrial production process, are the same as the artisanal one and they can be described as three successive stages (figure 1.8):

- *Stage 1:* includes the wetting, mixing, rolling and sieving operation at the end of which an agglomeration of particles occurs and the formation of wet calibrated couscous grains take place.
- *Stage 2:* wet couscous is subjected to the steam cooking heat treatment in order to strengthen the structure and gelatinize the starch.
- *Stage 3:* cooked couscous grains are stabilized by a drying process to ensure a good shelf life of the dried product.

During couscous production, in order to have size classification, two sieving phases are planned after the rolling stage and drying stage (figure 1.8) generating a flow of out-of-scope products (too fine or too large) which must be recycled (Hébrard, 2002).

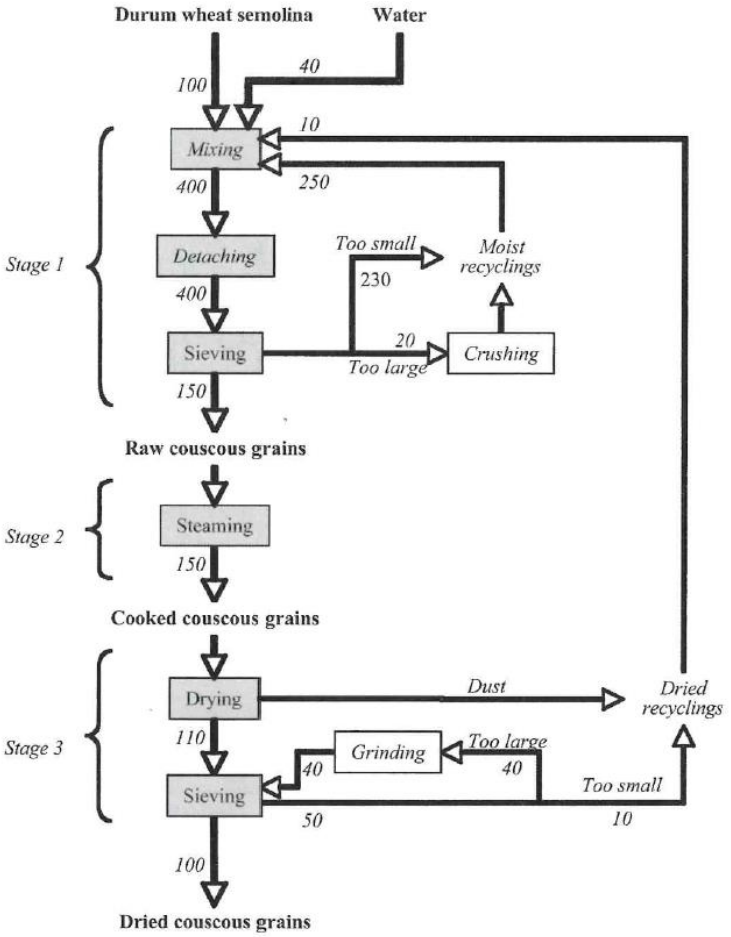

Figure 1.8. Process diagram for couscous production (values are apparent product flow rates, assuming a theoretical durum wheat semolina flow rate of 100) (Abecassis et al., 2012)

The individual phases, of couscous production, are analyzed in detail below.

STAGE 1: particle agglomeration

During this phase, as a result of water addition, durum wheat semolina particles (d_{50} = 300 μm) are transformed into large wet agglomerates (d_{50} = 1,150 μm) which constitute the "raw couscous grains". The characteristics of the wet agglomerates such as size distribution, density and shape greatly contribute to the quality of the final product. Moreover, the agglomeration process directly controls the performance of the couscous production line due to the large amount of material to recycle (about three times the initial semolina flow rate (figure 1.8). For these reasons, agglomeration is a very critical phase.

So that semolina particles agglomeration occurs, two elements are necessary: water and mechanical energy. Water, in a specific proportion, is required to form stable bonds between the semolina particles, while mechanical energy is useful both to have powder-water mass agitation and to promote homogeneous water dispersion and agglomerate growth. By examining the agglomeration process more closely, it is possible to identify three successive phases: i) semolina wetting and agglomerate formation, ii) agglomerate strengthening and iii) agglomerate size classification (Abecassis et al., 2012).

i) Semolina wetting and agglomerate formation

This is a complex process which can occur with two different mechanisms: nucleation and coalescence.

- Nucleation

This process is based on the fall of large water drops directly onto the bed of semolina particles, which generates the formation of a nucleus. This phenomenon is observed inside classical horizontal mixers (equipped with wetting system and rotating blades) and nucleation occurs directly inside the tank of the mixer, at the level of the water addition zone. When water falls on semolina at the beginning of the mixing zone (figure 1.9), one observes the instantaneous formation of agglomerates of very heterogeneous sizes, called nuclei, which are a spontaneous assembly of particles of semolina stabilized by liquid (water) bridges. During this phase, inside water-spry zone, one drop of water tends to form one wet agglomerate and therefore nuclei size distribution is strongly correlated to water droplet size distribution. For this to happen, however, it is necessary that the powder flow through the water-spray zone must be fast enough so that the water droplet that hist the powder surface does not overlap, and the drop penetration time must be fast so that the drop of water must wet into the bed completely before mixing brings it into contact with another partially absorbed droplet on the bed surface.

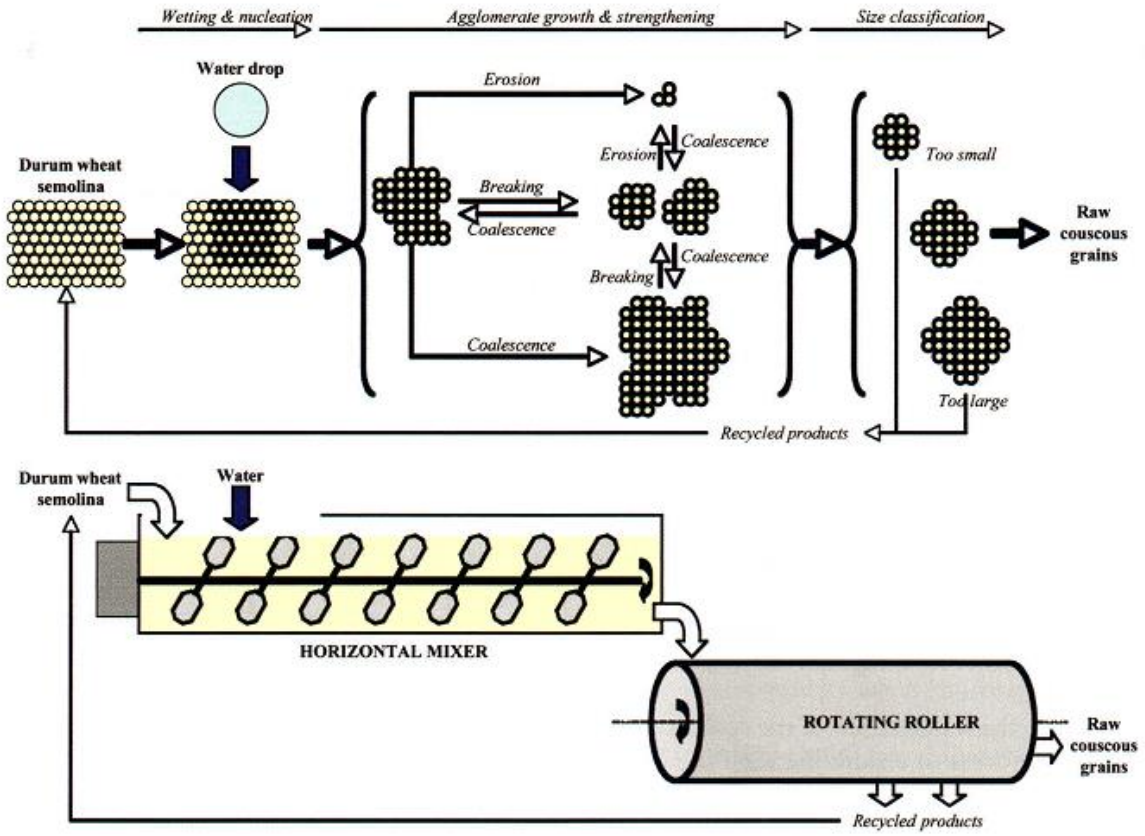

Figure 1.9. Schematic representation of the classical wetting-mixing equipment used to agglomerate semolina (Abecassis et al., 2012)

Immediately after water-drop addition and nuclei formation, the application of mechanical energy forces the water distribution over the powder bed promoting homogeneous nuclei formation. Inside the semolina particles forming the nuclei, one can suppose that water diffusion occurs from the surface to the center of the semolina particles. The kinetic phenomenon of water diffusion allows the hydration of wheat components within the semolina particles. During this phase, in the drop-controlled regime, you can control wet agglomerates formation by modifying mixing condition like water-drop size, mixing temperature, number of revolutions of the mixer blade and mixer filling level (Hébrard, 2002). In particular, it was found that the average size of the wet agglomerates is strongly correlated on the size of the drops of water. In fact, it has been observed that the use of pulverized water rather than the shape of the water drops makes it possible to decrease the fraction of large agglomerates and

increase the fraction of raw couscous grains (yield) (Hébrard, 2002). Other parameters that can be adjusted are: mixer blade geometry, blade rotation speed, and water addition condition.

➢ Coalescence

Coalescence consists of a process during which semolina particles are first wet individually and then subject to mixing actions to form agglomerates (figure 1.10). Unlike nucleation, the coalescence process is observed when centrifugal wetting equipment is used (Hébrard, 2002) and it requires both a good amount of mechanical energy to disperse and individualize the semolina particles before water addition and the size of the water droplet must be sufficiently small to allow the liquid to coat each semolina particle (Iveson et al., 2001).

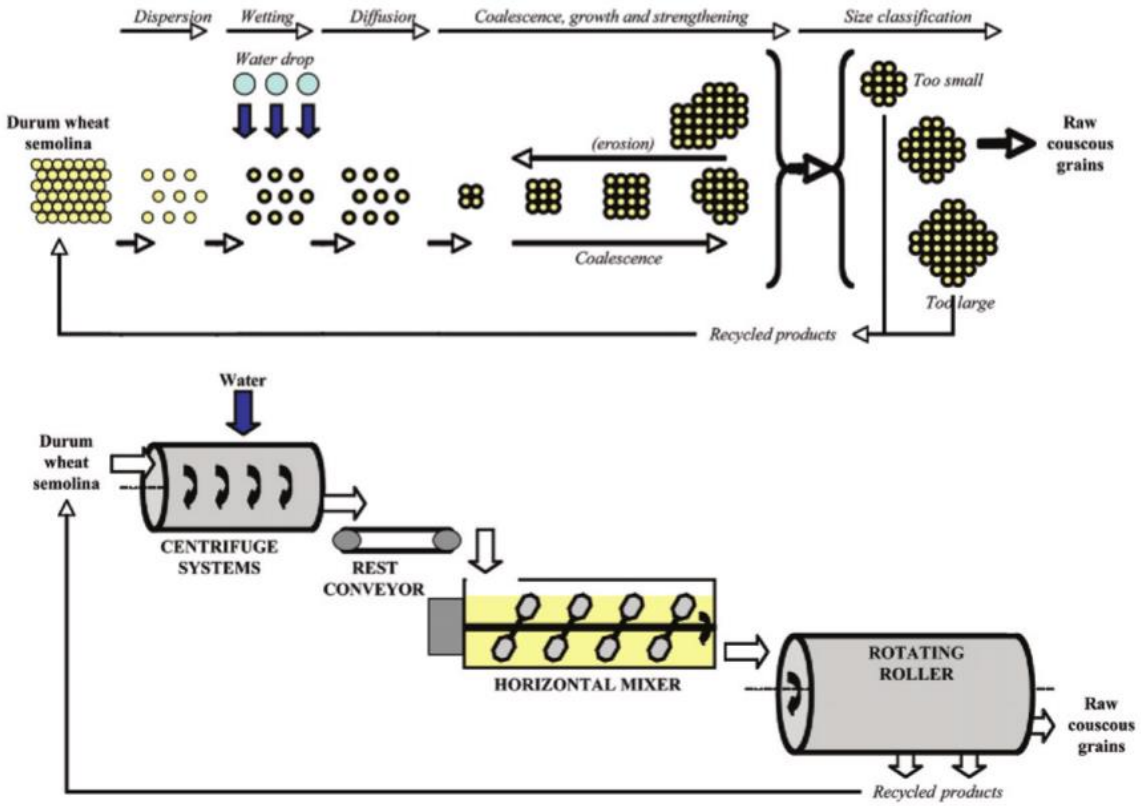

Figure 1.10. Schematic representation of the centrifugal wetting system equipment used to agglomerate semolina (Abecassis et al., 2012)

The particle wetting occurs after there is a collision between the small water droplet and the particle, followed by the spreading of the liquid over the particle surface. The water-delivery

method (water drop size, nozzle height, etc.) has a minimal effect on the nuclei properties while the use of centrifugal wetting equipment is crucial as it allows a homogeneous distribution of water on the surface of the particles in a very short time (less than 1 s).

After a resting period (on a rest conveyor), to allow the diffusion of water inside the semolina particles, the formation of agglomerates by coalescence occurs, through mechanical agitation in the mixer. During this phase, the hydrated semolina particles are subject to impact and contact with each other, which cause plastic deformation and, when the pressure between two particles is high enough, agglomerates are formed thanks to interaction between surfaces of the particles.

An important study (Hébrard, 2002) compared classical wetting-mixing equipment with the centrifugal one coupled with the rest conveyor, and it emerged that the latter leads to better control of the agglomeration yield, a more uniform size distribution of the wet agglomerates and a reduced recycling flow.

However, in both processes, in order to control agglomeration process, the right moisture rate and a good water diffusion inside semolina particles are necessary. For couscous processing the optimum water content ranges from 30 to 40 % depending on the quality of the semolina (Quaglia, 1988; Hébrard, 2002; Ounane et al., 2006). Particularly, the right amount of water must be used to avoid dough formation but, at the same time, must be sufficiently high to allow starch gelatinization during steam treatment.

ii) Agglomerate strengthening

This phase is crucial to obtain agglomerates with right size and uniform size distribution together with consequent stabilized grains formation.

The agglomeration strengthening is conducted through mechanical energy input, during the mixing and rolling stage (figure 1.10), which induce compression phenomena so as to determine the structure consolidation of the wet agglomerates and an increase of density by packing.

The mixing phase lasts about 10-20 minutes and significantly influence the agglomerates size. In fact, through the correct setting of both the rotating mixing blades and the number of rotations, it is possible to reduce the number of fine agglomerates (and single semolina particles). However, following coalescence mechanisms, very large agglomerates can be generated inside the mixer, but they are made smaller due to surface erosion or breaking in several parts.

As for the rolling phase, two technologies are available: rolling over a vibrating sifter and rolling in a drum roller. For the latter, the first section is a non-perforated metal sheet in which compression, rolling, and a slight compaction of wet agglomerates occurs.

During this phase, it is possible to intervene modifying some roller/sifter parameters such as screen sizes, roller length, and rotation speed. Another parameter that can be modified is the hydration level at mixing. With regards to this, Guezlane (1993) indicated that the rotating roller system requires slightly higher water content compared to the sifter system.

Hébrard (2002) has declared that, for the vibrating sifter, agglomerates characteristics such as size, density, form and shape, are not significantly influenced by the rolling condition while, inside the roller, the rotating effect contributes to the spherical shape of the wet agglomerates resulting in more spherical and dense couscous grains than those formed in a sifter, which display a more irregular form.

iii) Agglomerate size classification

Inside the vibrating sifter or the rotating roller, the screening stage also occurs, in order to remove the oversized agglomerates and the overly fine particles. Further, the rotating roller is constituted of three sections: a first not perforated metal sheet, a second perforated sheet with small holes that remove too small agglomerates and a third perforated sheet with holes that sallow the agglomerates with the required diameter to be selected. Large agglomerates are then

discarded at the end of the roller. In a vibrating sifter, a lower portion of the too large agglomerates is favored, for these reasons the choice of rolling techniques could have an influence on the screening yield (Guezlane, 1993). In both systems, discarded agglomerates are recycled in the mixer and mixed with durum wheat semolina after crushing (too large agglomerates) or rehydration (too small agglomerates).

The size calibration of the wet agglomerates is a crucial phase because the excessively large quantities of recycling strongly impairs the efficiency of the process. After this stage, the obtained calibrated wet agglomerates are called "raw couscous grains".

STAGE 2: steam cooking

After calibration, phase 2 follows (figure 1.8) during which couscous is subjected to a continuous steam treatment in a conveyor belt cooker. Raw couscous grains are distributed in an 8-12 cm thick layer and steaming is conducted at a temperature of 100°C at atmospheric pressure (Quaglia, 1988; Guezlane et al., 1998; Méot, 2006; Ounane et al., 2006). In order to guarantee the heat transfer inside the thick product layer, several parameters can be adjusted: residence time inside steam cooker (about 12-18 minutes), thickness of the layer and steam flow. However, during this phase, low quantities of steam are absorbed by raw couscous grains (8-10 % by weight) and, at the end of the process, cooked couscous shows a water content of about 32% wet basis.

Through steam cooking, there are different physicochemical changes in wheat components of couscous. First of all, starch granules lose their initial semi-crystalline configuration and starch gelatinization occurs (55-60 %) (Guezlane et al., 1998; Debbouz and Donelly, 1996). This phenomenon strongly influences some characteristics of final couscous like water absorption, swelling capacity and caking properties (Guezlane, 1993). Also, during starch gelatinization a partial solubilization of the amylose chains extending out of the granules is thought to occur,

which seems to contribute to the formation of very solid links between the particles inside the grains. For these reasons, steam cooking is fundamental for the solidification of the couscous grains. In fact, if uncooked, they would break down during the next steps (Méot, 2006).

As a result of steaming, another important physicochemical change in couscous components is the formation of amylose-lipid complexes which are responsible for some cooked couscous grains properties, such as low stickiness and the lack of starch retrogradation mechanisms during storage. This allows to avoid couscous grains agglomeration, when hydrated before consumption, with consequent increase in their firmness and resistance to overcooking.

Finally, during steam cooking, wheat proteins insolubilization also occurs with formation of large aggregates of gluten proteins and the consequent decrease in the cooked couscous grains stickiness.

STAGE 3: drying

After steaming, cooked couscous grains are dried in order to reduce moisture content of the final product (max 13.5%) and prolong its shelf life.

Couscous drying is conducted using a hot-air rotating dryer in which rotary movement causes a slight mixing of the product promoting the contact between couscous grains and airflow (Quaglia, 1988). During this stage some dust is produced by superficial erosion of grains under rotation, the particles are considered to be too fine and so they are recycled at the beginning of the production line.

Couscous is considered easy to dry for its spherical shape and relative porosity that encourage a rapid water loss and for these reasons many time-temperature combinations can be used: 48h at 25°C, 17h at 55°C and 3h at 95°C (Guezlane et al., 1998; Ounane et al., 2006). After the dryer phase, the hot product layer is cooled to room temperature using a cold air flow.

1.4 Agglomeration and drying: two critical phases

As seen previously, granulation is an important step because it determines the production yield and the final characteristics of the agglomerated couscous grains of durum wheat. For these reasons, Deng and Manthey (2019) have studied the grinding degree influence on the semolina wet agglomerates characteristics. Obtained results show that fine grinded raw materials give rise to more dense agglomerates than those made from coarse flours impacting directly on the wet agglomerate formation and shape. Particularly, coarse flour particles seem to reduce the formation of large agglomerates while fine flour particles have large solid-liquid contact area, which favours water movement into the capillary pores and the agglomerates formation.

During mixing, granules collide with other particles and water migrates outwards, promoting adhesion interaction for agglomerate development. Thus, a greater number of large agglomerates are formed between fine flour particles than from coarse particles. Moreover, fine particles, which have undergone a more severe grinding, show higher level of starch damage, compared to coarse particles and so they can absorb large amount of water during hydration (Farrand, 1964) promoting large agglomerates formation. On the contrary, the use of coarse flours, favours the presence of small agglomerates.

Regarding the drying stage, for some authors, drying conditions do not appear to affect couscous quality, however Guezlane (1993) said that higher drying temperatures cause a reduction in swelling capacity of couscous grains when immersed in cold water while Yüksel et al. (2018b) asserts that in hot-air drying, thermal damage to quality attributes such as color, flavor, nutrients and reduction in bulk density and rehydration capacity of the dried product occur due to high temperatures and long drying time. Therefore, to overcome these problems, Yüksel et al. (2018b) have tested the use of microwave drying for couscous, in order to achieve fast and effective drying while preserving the high quality of products. So, two types of couscous (enriched with different percentages of bulgur flour, milk and eggs) were dried to

12% (w.b.) of moisture content by using packed bed and microwave dryers. The results showed that microwave intensity was more effective on drying rate and drying time but the drying method did not significantly affect the yield of couscous and the sensory attributes of color, smell and general appearance, and had almost the same scores for both drying techniques. It can be concluded that microwave dried couscous is slightly more preferable than packed bed dried ones thanks to better drying rates and lower drying times. In addition, another advantage of microwave drying is that couscous is not mobile in a microwave dryer; therefore, fragile couscous particles will not break during drying.

1.5 Quality attributes of couscous

Couscous quality depends on both the raw material characteristics (durum wheat grain characteristics and milling condition) and the manufacturing process (agglomeration, cooking and drying stages). Both factors can influence not only the physical-chemical mechanisms involved during the production of couscous but also the quality attributes of the finished product. A good quality couscous shows an amber-yellow color, high water-absorption capacity and the ability to hold together, stay distinct, be firm, and have good taste when hydrated (Abecassis et al., 2012). Quality parameters of couscous can be classified in three different categories: characteristics of dry couscous grains, quality parameters related to rehydration behavior (before consumption), quality attributes related to consumption (figure 1.11).

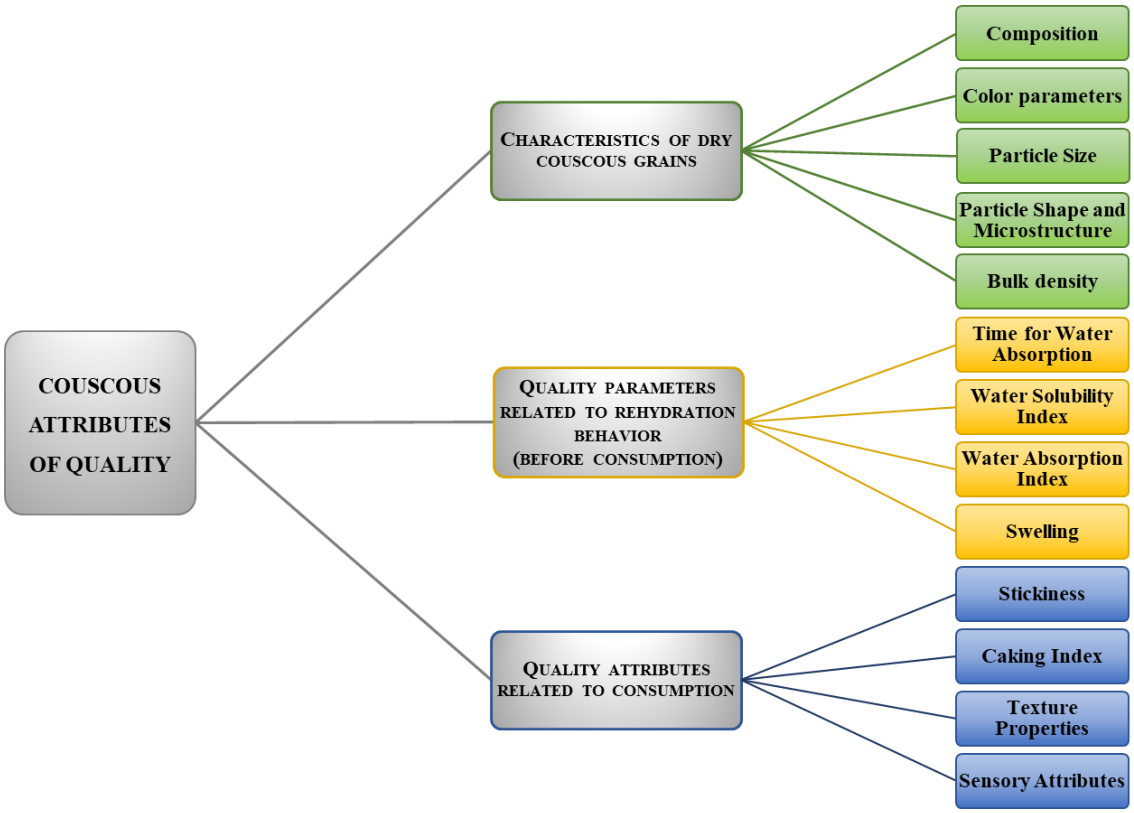

Figure 1.11 Quality attributes of couscous

CHARACTERISTICS OF DRY GRAINS

Composition

The maximum content of moisture and ash for couscous is set to 13.5% and 1.1% respectively as established by *Codex Alimentarius* (Codex Standard, 202-1995) and no food additives may be used during the industrial processing. The indicated moisture value is a legal limit and is essential to obtain a final water activity that allows a good product shelf life.

Table 1.2 shows the biochemical composition of couscous compared to the durum wheat semolina used as raw material and, as expected, there are no differences in the starch (85-86 %), protein (13.5%) and pentosan (1.4-1.7 %) contents. However, if compared with durum wheat semolina (12.7% of soluble protein and 5.9% of gelatinized starch), couscous shows low content of soluble protein (2.2%) and a higher value of gelatinized starch (71.8%), and it is due to physicochemical changes involved in the manufacturing process.

Table 1.2 Physico-chemical characteristics of durum wheat semolina and industrial couscous (Abecassis et al., 2012)

Composition	Durum wheat semolina	Medium couscous
Water content (g/100g product)	14.5 ± 0.4	9.8 ± 0.3
Starch content (g/100g dry matter)	86.2 ± 6.0	85.6 ± 6.0
Gelatinized starch content (g/100g dry matter)	5.9 ± 0.3	71.8 ± 3.6
Total protein content (g/100g dry matter)	13.5 ± 0.5	13.5 ± 0.5
Soluble protein content (g/100g dry matter)	12.7 ± 0.6	2.2 ± 0.1
Total pentosan content (g/100g dry matter)	1.7 ± 0.2	1.4 ± 0.1
Soluble pentosane content (g/100g dry matter)	0.1 ± 0.0	0 ± 0.0

Color parameters

The color of couscous is related mainly to the characteristic of the durum wheat semolina, so couscous grains shows a light yellow color and are normally characterized by a yellow hue (b^*) and a red hue (a^*) ranging between 27-45 and 0-4 respectively and a range of brightness value (L^*) of 21-72 (Guezlane, 1993; Debbouz et al., 1994; Debbouz and Donnelly, 1996). The production system has little influence on this parameter, however, homemade couscous process allows a lower loss of carotenoid pigment, therefore homemade couscous shows slightly higher values of L^* (71.3) and b^* (30.7) compared to industrial couscous (Guezlane et al., 1986; Debbouz and Donnelly, 1996).

Particle size

Couscous particle size is quite variable (tolerance of 6%) and the granularity ranges between 630 and 2000 μm (Codex Standard 202-1995). According to grain size, industrial couscous could be found on the market in three different types: fine, medium and coarse (Quaglia, 1988).

Figure 1.12 shows the particles size distribution of industrial and homemade couscous studying the correlation between the sieve meshes used for the particle size determination and the cumulated weight.

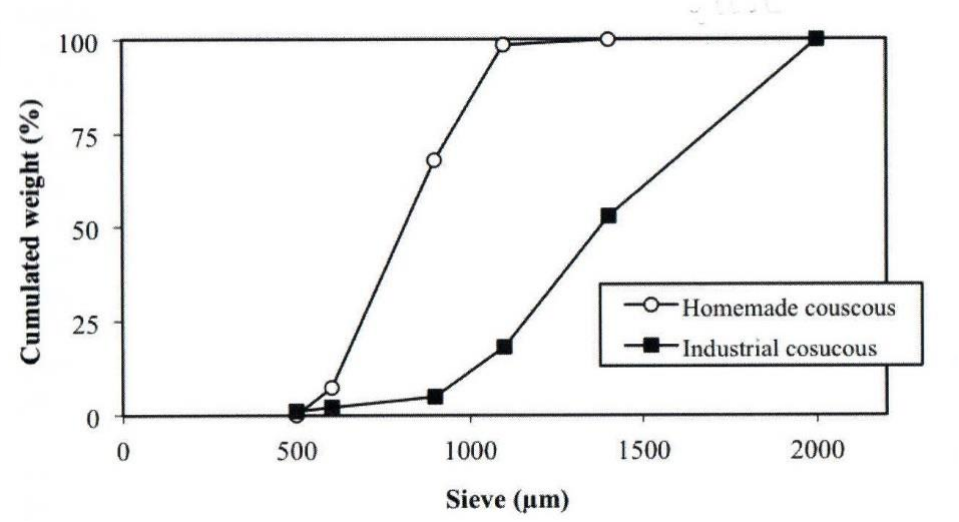

Figure 1.12 Typical particle size distribution for medium-sized industrial couscous and homemade couscous (Abecassis et al., 2012)

Particle shape and microstructure

Through scanning electron micrographs, it has been shown that couscous granules have an almost spherical shape. Differences were found between homemade and industrial couscous, the first is characterized by grains with irregular particles, more or less spherical shape and a rough surface while industrial couscous grains are more regular particles and they show a more homogeneous and spherical shape and smooth surface.

Scanning electron micrographs was also used to evaluate the microstructure of couscous grains. Dry couscous grains can be described as rigid agglomerated large particles formed by more or less molten semolina particles (300-500) among which there is a residual vacuum that is responsible for the porosity of couscous grains (Abecassis et al., 2012).

Bulk Density

According to Guezlane (1993) and Debbouz and Donnelly (1996) the couscous bulk density is measured by filling a 500 ml graduated cylinder with 250 g of couscous. Measurements were expressed as the ratio of couscous weight per unit volume. This parameter is related to both the grains compactness (true density) and the vacuum ratio between the grains (due to their size

and shape distribution) and for these reasons values of bulk density are usually different between homemade couscous (0.60 g/cm^3) and industrial couscous (0.79 g/cm^3).

QUALITY PARAMETERS RELATED TO REHYDRATION BEHAVIOR (BEFORE CONSUMPTION)

The extent to which couscous grain absorb water affects the taste as well as the mouthfeel so, good quality couscous grains must be characterized by a high ability to absorb water or sauce. If it is does not, couscous feels hard and lacks the desired smoothness. This important quality attribute can be evaluated with the following parameters.

Time for water absorption

This parameter measures the time required by the grains to have total water absorption when dry couscous is mixed with water (Debbouz et al., 1994; Debbouz and Donnelly, 1996). Optimum time for water absorption is covered in the range of 10-16 minutes and different values can be related to differences in grain compactness.

Water solubility index

When couscous is immersed in an excess of water some solids are dispersed by solubilization and the amount of these solids expresses the extent of couscous disintegration (Debbouz et al., 1994; Ounane et al., 2006).

The amount of soluble materials lost in water has been related to couscous stickiness so, a high-quality couscous is characterized by low values of water solubility index (typical value 4-16%).

Water absorption index

This index is measured as the change in weight of a sample of couscous when immersed in an excess of water at 30°C (Debbouz et al., 1994) and it is strongly correlated (r = 0.90) with the starch gelatinization extension in couscous grains (Debbouz, 1992). Classical values of water absorption index are in the range 460-490 g of water per 100g of couscous.

Swelling

Swelling is another important parameter to understand couscous rehydration behavior. As reported by Guezlane and Abecassis (1991), Guezlane (1993) and Ounane et al., (2006), it represents the change in apparent volume of a sample of couscous when immersed in cold (25°C) or hot (100°C) water. This characteristic is related to both the water absorption index and the ability of the couscous particles not to compact when hydrated. Moreover, Ounane et al., (2006), correlates couscous swelling at 100°C with water solubility index as well, but this correlation is only partial ($r = 0.55$). Either way, high values of couscous swelling are indicative of a high quality product and typical values of swelling for couscous samples are 280-320 mL/100g of couscous at 25°C and 380-410 mL/100g of couscous at 100°C.

QUALITY ATTRIBUTES RELATED TO CONSUMPTION

Stickiness

During couscous steam cooking, due to starch gelatinization, some exudates can migrate to the surface of couscous grain and generate stickiness. For this reason semolina starch damage and couscous stickiness are strongly correlated ($r = 0.90$). According to the method used for pasta by Debbouz et al. (1994) and Debbouz and Donnelly (1996), couscous stickiness is measured using a texture analyzer and it is evident that a good quality product is characterized by a low values of stickiness.

Caking index

This index measures the extent of the agglomeration phenomena of couscous grains after rehydration (Guezlane, 1993; Ounane et al., 2006) and it is evaluated through the sieving of hydrated and dried couscous grains. A good quality product shows low value of this index, however Ounane et al. (2006) found, caking index value in the range 8.2-32.36 % and they have

also seen that the caking index is negatively correlated (r = -0.59) to the initial particle size of dry couscous.

Texture properties

Couscous texture is normally evaluated using a compression method. In particular, during couscous texture assessment, characteristics like firmness (5.79-7.53 mm), elastic recovery (0.3-0.8 mm) and viscoelasticity (1.3-1.9) are studied (Yettou et al.,1997; Ounane et al., 2006). A weak correlation between texture properties and sensory attributes was found (Guezlane, 1993), and in general low values of firmness and viscoelasticity are indicative of a high-quality product.

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Chapter 2: BARLEY

2.1 Introduction

First archaeological evidence on barley existence date back to 17,000 years ago in Egypt along the Nile River. It is therefore one of the founder crops of Old World agriculture (Badr et al., 2000), or one of the most ancient cereal crops. Also, considerable historical evidence which highlight the importance of barley as a food source in the evolution of humankind were found, and demonstrate that it represented one of the main food grains until the beginning of the twentieth century. There were a lot of opposing arguments about barley domestication before coming to a single accepted theory: barley was first domesticated in the Fertile Crescent in the Near East, which spans present-day Israel, Northern Syria, Southern Turkey, Eastern Iraq, and Western Iran (Newman and Newman, 2008).

Many studies have been conducted to understand the transition from barley wild plants to cultivated/domesticated barley crops. Wild barley was characterized by fragile ears that favoured kernel crushing during the harvest but it is possible that plants which naturally had less fragile ears and larger and more abundant seeds were born accidentally and they were logically preferred and selected for food by hunter-gatherers. Moreover, it has been hypothesized that seeds from these plants were accidentally or intentionally planted giving rise to barley cultivation (Newman and Newman, 2008). This transformation required a lot of millennia (Zohary and Hopf, 1988).

During the first harvests it is probable that kernels were eaten raw and only later did the ancient populations accidentally come to understand that the grain acquired a better texture and flavour if hulls were removed (for hulled types) and kernels were soaked and/or cooked in some way. Simultaneously, early humans learned about fermentation and the use of cereals for the production of alcoholic beverages (Newman and Newman, 2008).

On an environmental level, barley is an undemanding plant, it grows in temperate climates and in periodic drought areas, but it is not suitable to be cultivated in hot-humid regions. However because it is winter-hardy, drought-resistant and because of its early maturing nature, it bears well through various environmental stresses and it is also able to grow in infertile, dry and non-acid soils. It has a short vegetative cycle and requires a reduced amount of nitrogen added during growth and for these reasons it is a low environmental impact plant: it has a good competitive ability against weeds, which are superfluous, or at least reduced, with herbicide treatments. For these reasons it is also more economical to cultivate (Cook, 2013).

Nowadays, barley represents 12% of the total cereal cultivated, ranking in fourth place among the most cultivated crops globally, preceded only by wheat, rice, and maize (Schulte et al., 2009). Of this percentage, only 2% is used directly for human consumption while 33% is intended for malting and the most (65%) is used for animal feed (Sullivan et al., 2013). More recent data shows that global barley production in the cropping year 2016/2017 averaged to 146.20 million metric tons. Australia was the second largest contributor followed by Russia, and exports 65% of its barley production (Statista, 2018). However, barley (*Hordeum vulgare* L.) is primarily cultivated for its starch-rich seeds, but, its utilisation as malt has taken precedence over its value as a food grain.

Also Martínez et al. (2018) confirm these data stating that barley is used mainly as animal fodder but increasingly represents a fermentable material for the production of beer and distilled beverages, and recently the use of barley as an ingredient in healthy food formulations is growing.

2.2 Taxonomy, morphology and anatomy

Hordeum vulgare L., commonly known as barley, is a grass belonging to the family of *Graminaceae* (*or Poaceae*), the tribe *Triticeae*, and the genus *Hordeum*. There are 32 species, for a total of 45 taxa in the genus *Hordeum*, that are separated in four sections (Bothmer, 1992) that differ in the morphological characteristics of the plants, life forms, ecology and geographical area of origin. The great morphological variability observed among cultivated barley plants is due to changes that occurred in cultivation during the years, in the different geographical areas and intense breeding (Wiebe and Reid, 1961).

The taxonomic classification of barley, which includes it in the *Graminaceae* family, reveals the link with other Triticeae's family plants like rice and wheat, but also allows to identify the different barley types and varieties analysing the morphological characteristics of the plant and grain (Chalupska et al., 2008).

One of the main *Hordeum* characteristics which allows a taxonomic distinction is the one flower spikelet. In detail, the barley inflorescence is formed by a central zig-zag rachis that is made from different sections/nodes on each of which, in an alternate position, are inserted three uniflore spikelets, one median and two lateral. If only the central spikelet of each node of the rachis is fertile and the two lateral ones are sterile, the ear has only two rows, so it appears strongly flatted: this is called distichum barley. If instead, all the three spikelets present on each node of the rachis are fertile, we have polistic barley, with six rows (figure 2.1). This latter can be further distinguished as follows:

- caryopses arranged in a regular radial pattern: exastic barley;
- lateral caryopses are very divaricate and almost overlapping between them so as to appear to have 4 rows and quadrangular in section: improperly called tetrastic barley (Newman and Newman, 2008).

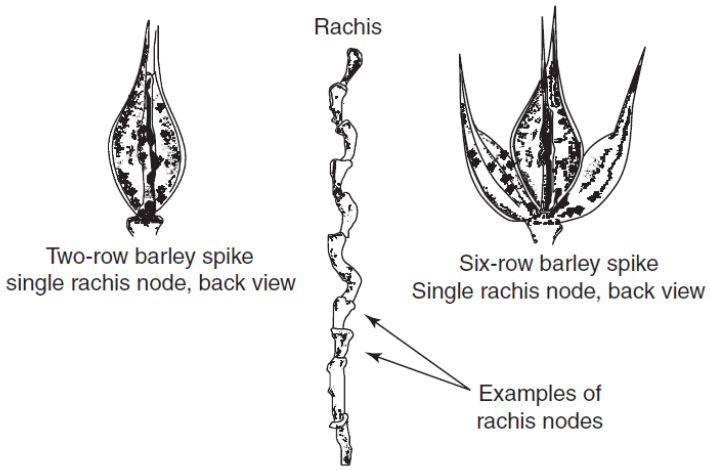

Figure 2.1 Spikes and rachis of barley. (www.brewingtechniques.com)

Initially, it was believed that distiched and polistic barley belonged to two different species: *Hordeum disticum* and *Hordeum hexastichon*, it was however shown that the cultivated barley derives from a common ancestor *Hordeum spontaneum* that, through some mutations, gave rise to the different distiched and polistic species (figure 2.2) (Mozzoni et al., 1994).

Barley grown varieties can be classified not only for the different ear shape but also based on the time of sowing and therefore winter and spring varieties are differentiated.

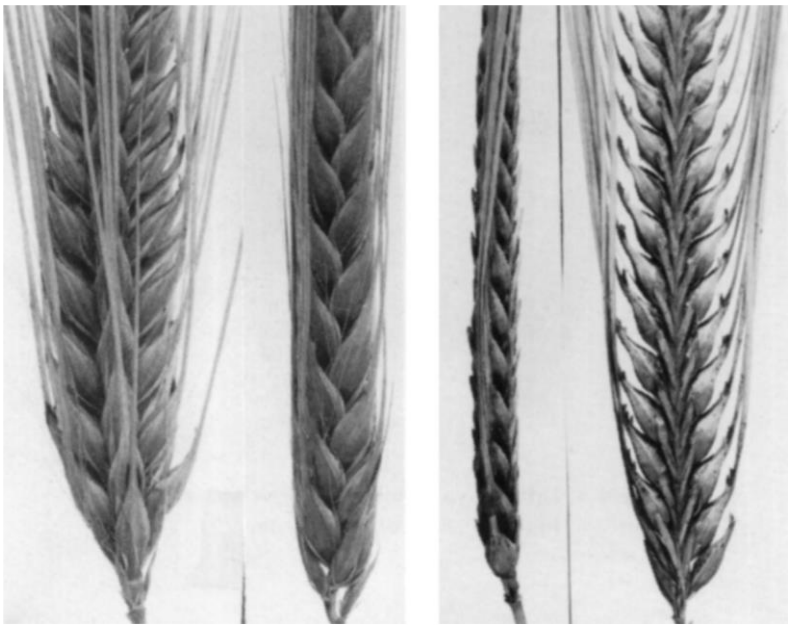

Figure 2.2 Barley heads. *Left:* front and side view of six-rowed barley. *Right:* front and side view of two-rowed barley. (source:Newman and Newman, 2008)

Structure of spikelet and kernel of barley

Barley spike or flower head represent the inflorescence of the plant, it is located at the tip of the stem neck and it consists of varying numbers of spikelets attached at a central stem which is called rachis.

Figure 2.3 shows the barley spikelet structure. Each spikelet is a complete single flower and it consists of two equal lanceolate outer glumes that enclose the floret in which kernel development occurs after fertilization. Similarly, the ovary and stigma, present in each floret, are also enclosed in two flowering glumes, the lemma and palea (Nilan and Ullrich, 1993; Hoad et al., 2016), which in the mature kernel become the hulls. Anatomically, lemma and palea are similar, although palea is the thinnest of the two; additionally, in cultivated barley varieties, lemma presents a thin appendix, called awn or arista, which can be used to make a further classification, in fact there are the aristate, half aristate and mutic varieties of barley. Spikelet, thanks to the presence of both the ovule and the anthers, is a complete flower and so it often self-pollinates (Newman and Newman, 2008).

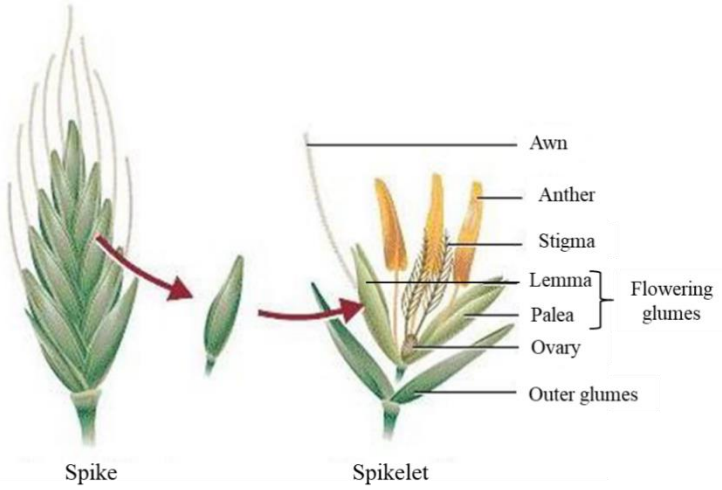

Figure 2.3 Barley spikelet components

About two months following sowing, anthesis occurs in the barley flower within the spikelets and, during this phase, two important phenomena follow one another: pollination and egg cell

fertilization with consequent formation of the embryonic seed or kernel (Briggs, 1978; Reid, 1985).

Barley kernels consist of several layers, starting from the outside (figure 2.4) it shows:

- glume (about 13% of the caryopsis);
- pericarp and head/seed coat (about 3% of the caryopsis);
- aleurone and sub-aleurone layer (about 5% of the caryopsis);
- starchy endosperm (about 76% of the caryopsis);
- germ or embryo (about 3% of the caryopsis).

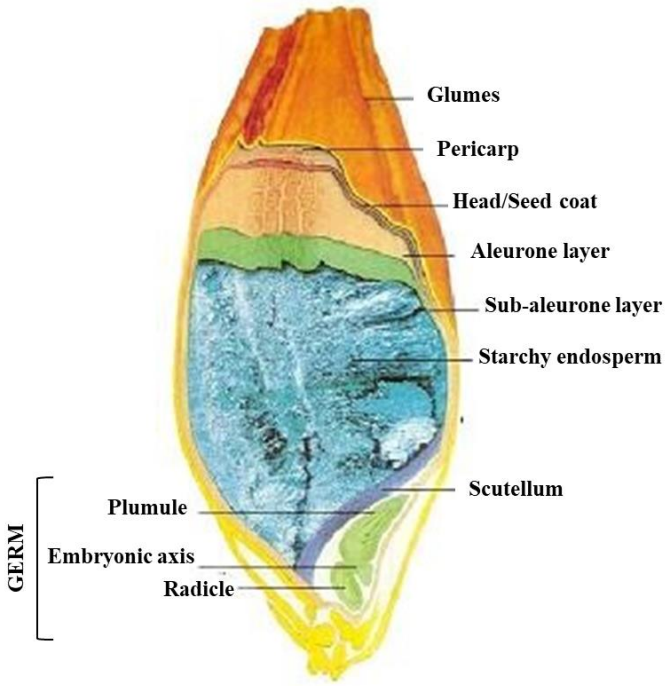

Figure 2.4 Longitudinal section of barley kernel (Crosatti et al., 1993)

Glumes are represented by lemma and palea which, in mature barley kernel, form the hulls and they are constituted mainly of cellulose, lignin, and silica. The formed hulls can be both “glued” to the caryopsis, so they adhere to the surface of hulled genotypes and can be not attached in hulless types (Harlan, 1920).

Below the hulls there is the caryopsis which consists of the pericarp, head (seed coat), endosperm and germ. *Pericarp* represent an outer protective tissue layer, but it is not lignified as are the hulls. The *head*, instead, is a tough membrane composed of cellulose that almost completely cover the kernel, separating the inside from the outside.

The largest portion of the kernel is represented by endosperm that is formed from aleurone layer, subaleurone layer, and starchy endosperm.

The *aleurone layer* is a structure rich in protein which contains, in the own cell walls, two large-molecular-weight polysaccharides, arabinoxylan and ß-glucan, that are important structural components. Immediately below aleurone there is the *sub-aleurone layer*, whose cells are smaller than those of the starchy endosperm and contain more protein and less starch. Finally, endosperm is completed by *starchy endosperm* that represents the largest portion of the kernel and it is formed by cells that are packed with starch grains that are embedded in a protein matrix. Moreover, inside starchy endosperm cell walls, the same two polysaccharides present in the cell walls of the aleurone are found, but ß-glucans are present in higher percentage compared to arabinoxylans (Newman and Newman, 2008).

The last component of the kernel is the *germ* (or embryo). It is located on the dorsal side of the caryopsis at the end, attached to the rachis and it is a very important structure because it contains the material necessary for the initialization of growth of a new plant.

The main constituent parts of the germ are the plumule, which is formed by the vegetative apex and the sketch of the first leaves brought by the small stem and the scutellum which is a protective layer positioned between the endosperm and the embryo, in the form of a concave shield towards the endosperm; the non-germinated grain contains lipid bodies which are replaced during maturation by starch granules.

The subcellular constituents of the embryo include mitochondria, protein bodies, spherosomes that contain lipid, Golgi bodies, and rough endoplasmic reticulum, large nuclei, and thin cell walls transversed by plasmodesmata (Briggs, 1978).

The grain is said to be physiologically mature when dry matter accumulation ceases, but in practical terms, only when kernel moisture is lower than 15% does the grain reaches harvest ripeness.

2.3 Barley composition

From a nutritional point of view, barley has only recently been appreciated for its interesting healthy nutrient profile, however it shows a great variability in the concentration of various components depending on genotype, environment and interaction between the two.

The major nutrients in barley are represented by starch, dietary fibre and proteins while lipids, ash, vitamins and low molecular weight sugars are present in lower concentrations (Andersson and Åman, 2008).

Barley ***starch*** is present in the form of a mixture of large and small granules with a diameter of 15-20 μm and less than 10 μm respectively.

Chemically, starch is a soluble polysaccharide that is very important because in barley, it represents the main source of energy for the nutrition and the growth of a new plant after germination. Moreover, starch is one of the most variable components in barley. In fact, it ranges from 45% and 65% of the kernel depending on environmental conditions, but in some cases, an incomplete filling of the endosperm can determine a much lower than average starch content. As is well known, starch is composed by amylopectin that is a branched-chain polysaccharide in which α-(1,4)-D-glucose units are branched through α-(1-6)-D-glucose linkage, and amylose which is primarily a straight chain of α-(1,4)-D-glucose units (Jane, 2009).

Generally, the two polymer are present in 3:1 ratio with a percentage of 72-78 % for amylopectin and 22-28 % for amylose. However, some cultivars are characterized by starch that is 95-100 % amylopectin. This particularity is due to the presence of the wax gene, that derives from a mutation of the gene that codes for the starch synthase enzyme which is associated with starch granules, and it essential for the synthesis of amylose; these cultivars are called waxy barley.

Ullrich et al. (1986) and Xue et al. (1997) have found that waxy barleys contain a lower starch concentration (5-8 % less total starch) compared to non-waxy cultivar, but this is compensated by a small increase both in simple sugars like fructose, glucose and sucrose and fibre component such as ß-glucans (Newman and Newman, 2008).

Carbohydrates constitute 80% of the weight of barley kernels, in particular 63-65 % is made up of the above mentioned starch, while the remaining 37-35 % is represented by cellulose (4-5 %), soluble carbohydrates in alkaline solutions (8%), water-soluble sugars (1-2 %), and simple sugars (1.5%). Cellulose is a polymer formed by glucose molecule linked by ß-(1,4) bonds that form long linear chains and it is present in variable concentration from 4.1-4.8 % to 2.0-2.9 % in hulled and hulless barley varieties respectively (Xue et al, 1997). Of these percentages more than 96%, is present in the external coating (MacLeod, 1959) while small quantities were found in the aleurone and endosperm and absent in the embryo. Cellulose, together with starch, are large-molecular-weight compounds and they are also known as complex carbohydrates, in opposition to glucose, fructose and sucrose which are simple sugars. Complex carbohydrates are in turn distinguished in starch and non-starch polysaccharides; in barley the main non-starch polysaccharides are represented by a group of compounds which together make up the ***Total Dietary Fibre*** which we will discuss later.

As for ***protein***, their concentration in barley varies from 7 to 25 % (Ullrich, 2002), but on average the quantity fluctuates between 9% and 13% (Newman and Newman, 2005); this great

variability is due to genetic factors but primarily to environmental growing conditions. Inside barley kernels, proteins are responsible for metabolic activity, have structural function, and provide nitrogen for the development of the embryo during germination. Osborn (1924) classified cereal protein (including those of barley) into four classes depending on the solubility in different solvents: albumins soluble in water, globulins soluble in dilute salt solutions, prolamins soluble in aqueous alcohol solutions and the glutelins which are extracted using dilute acid or alkali. A further classification was made by Shewry (1993) which distinguishes proteins in two groups based on biological functions: storage and nonstorage proteins. The reserve ones are the prolamines together with the glutelins and in smaller quantities the globulins; prolamines are the major reserve proteins of barley confined in the starchy endosperm, while glutelins include most of the ribosomal structural proteins of the endoplasmic reticulum and cell wall glycoproteins (Kirkman et al., 1982). Albumin and globulin, on the other hand, constitute the non-reserve proteins that are located in the germ and in the aleurone layer and include proteins that have important biological functions such as enzymes, nucleoproteins, membrane proteins and glycoproteins (Bhatty and Whintaker, 1987).

Lipids are present in concentrations of about 77% and 18% in the endosperm and in the embryo, respectively, the remaining 5% are found in external coatings (Price and Parson, 1975, 1979; Briggs, 1978); those present in the endosperm are found mainly in the aleuronic layer which contains about 67% of the total lipids (Briggs, 1978).

Lipids are classified into two categories: "non-starch lipid" and "starch lipid". The first include all lipids other than those present in starch granules and they are stored inside small oil droplets, called spherosomes, which are surrounded by a membrane containing polar lipids such as phospholipids (Morrison, 1993b), while non polar lipids are represented mainly by triacylglycerol which are located inside the drops together with diglycerides, monoglycerides and free fatty acids. The main fatty acids present in barley triglycerides are linoleic acid (56%),

palmitic acid (23%), oleic acid (13%) and linolenic acid (8%); stearic acid represents less than 1% of total lipids (Morrison, 1993a).

Starch lipids, instead, are represented mainly by phospholipids (Morrison, 1993a) and a small amount (4.4%) of free fatty acids as reported by Acker and Becker (1971) which found palmitic acid as the predominant acid followed by linoleic and oleic acids.

Minerals represent the fraction of a food that is referred to as "inorganic" nutrients. Inside barley, they range from 2.0 to 3.0 % with hulless types that are characterized by lower amount compared to hulled types. About 14 minerals have been identified in whole-grain barley and they were divided into two groups: macroelements and microelements. The first, such as calcium, phosphorus, potassium, magnesium, sodium, chlorine and sulfur, are present in higher concentrations compared to the second such as cobalt, copper, iron, iodine, manganese, selenium and zinc. The greatest concentrations of these elements have been found in the embryo, pericarp and in the aleurone layer (Marconi et al., 2000) although minerals are present throughout the caryopsis. Calcium is the main mineral and it is found mostly in the aleuronic layer and in small quantities in the lemma and in the cell wall of endosperm cells; followed by potassium which is most concentrated in the aleurone, magnesium and phosphorus which are more concentrated in the aleurone, with modest quantities in the endosperm; sulfur and chlorine which are confined to the cell walls of the aleurone and the endosperm (Newman and Newman, 2008).

Vitamins are organic compounds required in small amounts for the maintenance of normal biochemical and physiological functions of a human system and they are considered essential nutrients because we are not able to synthesize them in sufficient amounts to meet our needs and requirements. Based on their solubility, vitamins are divided in lipid-soluble vitamins such as A, D, E and K, and water-soluble vitamins such as B group vitamins (B1, B2, B3, B6, B12),

biotin, pantothenic acid, folic acid, vitamin C, choline and myoinositol. Barley caryopsis contains all of the vitamins and choline with the exception of vitamins A, D, K, B12, and C.

An important group of vitamins associated with barley lipids, thanks to their solubility in fats, is represented by tocopherols and tocotrienols, which are collectively called tocols or vitamin E; they are found in the aleurone, in the endosperm and in the embryo, moreover, most of the tocopherols are concentrated in the germ, while the tocotrienols are more uniformly dispersed in the caryopsis (Panfili et al., 2008). The total content of tocols in the caryopsis depends not only on the variety, but also on the environmental conditions, such as climate and soil fertilization (Cavallero et al., 2004).

2.4 Bioactive compounds of barley kernels

As previously mentioned, a part of the non-starch polysaccharides present in barley is made up of total dietary fibre whose components are classified according to their solubility in water, distinguishing two fractions: soluble and insoluble. The insoluble fibre consists of cellulose and lignin that have mechanical action, favouring intestinal peristalsis, the soluble fraction, instead, consists of arabinoxylans, pectic substances and ß-glucans (Newman and Newman, 2008).

The main feature of these compounds is that they are not digested by man, but arrive undigested in the large intestine where they are fermented by intestinal microorganisms with consequent production of different compounds such as short chain fatty acids: acetic, propionic and butyric acid which are reused as a source of energy from the microbial community (Newman and Newman, 2008). So, these compounds provide little or no energy but are valuable diet constituent for other reasons: they have prebiotic characteristics, in fact they are able to selectively stimulate the growth and/or metabolic activity of microorganisms that are important for the regular functioning of the organism; they reduce the absorption of glucose, cholesterol

and bile salts; they reduce the glycaemic index of carbohydrate foods; they determine an increase in the viscosity of the intestinal contents; they contribute to the increase in faecal mass by slowing down intestinal transit and promoting a greater sense of satiety and more.

Among bioactive compounds, arabinoxylans and ß-glucans are particularly interesting.

Arabinoxylans are polymers of the pentose arabinose and xylose and they range from 4 to 7 % of weight in barley grains with a major concentration in the outer layer of the caryopsis and the hull (Hashimoto et al., 1987), while, only 1.5% of weight is present in the endosperm. Inside the endosperm, arabinoxylans are found primarily in the aleurone and cell walls where they represent 86% and 20-25 % respectively, but they were also found in the hull (Aspinall and Ross, 1963).

A particular characteristic of arabinoxylans is the great variability in the xylose/arabinose ratio. In fact, Aspinall and Ferrier (1957) reports a xylose/arabinose ratio in the hulls of 1:9 while MacGregor and Fincher (1993) have found ratios of 1:3 in starchy endosperm and/or aleurone tissue. Other data (Henry, 1986) reports that the total arabinoxylans amount and xylose/arabinose ratio are related to genotype and are strongly influenced by environmental factors (Messia et al., 2017).

Of all the compounds which constitute barley, the major health effects have been primarily attributed to the dietary fibre fraction, particularly ß-glucans. ß-Glucans are high molecular weight polysaccharides formed by linear chains of glucose linked by ß-(1-3) and ß-(1-4) type glyosidic bonds (figure 2.5).

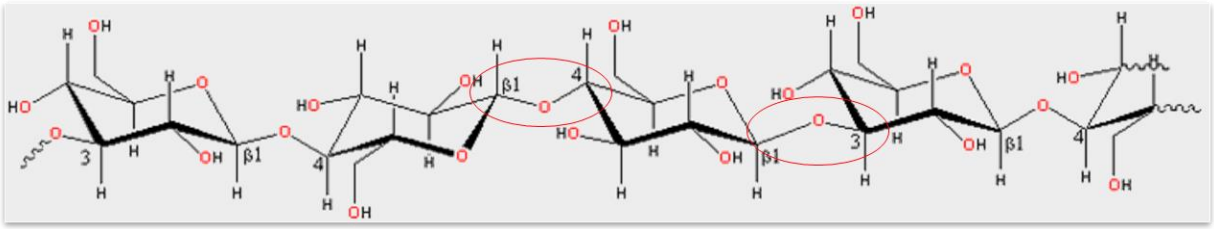

Figure 2.5 ß-glucans structure

The presence of interspersed ß-(1-3) bonds creates a molecule with a particular spatial arrangement, which interrupt the rigid ribbon-like conformation typical of a linear chain made with only ß-(1-4) bonds, conferring flexibility and irregular shape to barley ß-(1-3)(1-4)-glucans which thus results to be soluble in water (Woodward et al. 1983).

The amount of ß-glucans in barley range from 2.0 to 11.0 % also reaching percentages of 15% (Gómez-Caravaca et al., 2015; Messia et al., 2017), but average values are usually fall between 4.0 and 7.0 %. Inside barley kernels these compounds are distributed differently: a smaller percentage (25%) is concentrated in the cell walls of the aleurone layer, while higher concentrations are in cell walls of the starchy endosperm (75%).

In table 2.1 the content of barley fibre components is reported (cellulose, arabinoxylans and ß-glucans) compared with those of other cereals, and their distribution inside caryopsis. The large variation range observed in barley ß-glucans concentration is due to the fact that the barley amount is influenced by environmental factors like hot, dry conditions during kernel maturation, moist and excessive rain during ripening.

Table 2.1. Proportions (%) of cellulose, ß-glucan and arabinoxylan (AX) in aleurone and starch endosperm cells of various cereals

	Cellulose	β-Glucan	AX
Barley			
Aleurone	2	26	67
Starchy endosperm	2	75	20
Wheat			
Aleurone	2	29	65
Starchy endosperm	4	20	70
Maize			
Starchy endosperm	10	65	15
Rye			
Starchy endosperm	8	12	65
Oats			
Starchy endosperm	?	72	28
Rice			
Starchy endosperm	28	20	27

Source: Shewry (2008)

However, it was found that the most influential factor on ß-glucans levels in barley is the genetic composition (Greenberg, 1977; Ullrich, 2002). In fact, it was found that changes in the amylose/amylopectin ratio in barley starch, caused by the presence of the wax gene or the amo1 gene (responsible for high amylopectin and high amylose concentration respectively), has a significant effect on increasing ß-glucans content. Stahal (1996) concludes by stating that the link between ß-glucans content on the presence of wax gene or amo1 gene could not be broken. The great and recent interest in ß-glucans is due to the fact that these compounds have numerous beneficial effects on human health, first of all, the reduction of the risk of cardiovascular disease (Huang et al., 2017; Zielke et al., 2019). Moreover, they contribute in reducing postprandial blood glucose (Singhal and Kaushik, 2016; Tosh, 2013) and serum cholesterol, but also improve glycaemic index and insulin sensitivity (Casiraghi et al., 2006; Cavallero et al., 2002), and favour the production of beneficial short-chain fatty acids (SCFA) (Bindelle et al., 2011; Havenaar, 2011; Mikkelsen et al., 2017; Wood, 2007) as well as promoting intestinal health (Miyamoto et al., 2018), colon cancer control and an increase mineral and vitamin bioavailability (Khalon and Chow, 1997; Klopfenstein, 1988). Furthermore, the solubility of these compounds also makes them highly viscous and this is considered an advantage in dietary and health application (Bhatty, 1992) because, if ingested, ß-glucans can form viscous solutions, thereby decreasing the intestinal transit and delaying gastric emptying enhancing the sense of satiety, decreasing energy intake, reducing the post-prandial glycaemic responses and improving digestive functions. In this regard, many studies have been conducted on the ß-glucans solubility characteristics, also to better evaluate their incorporation in food. Obtained results indicate that the high-level structure and aggregation state of barley ß-glucans is one of the noticeable reasons why the extraction and physical treatment that affect their solution behaviour (Zhao et al., 2020) and consequently both their beneficial effects and their negative implications in some industrial processes.

The importance of dietary fibre, and in particular of compounds such as ß-glucans and arabinolxylans, is more emphasized by the existence of specific legislation that provides for the use of nutritional and health claims related to the presence of these compounds in food, in particular, the Commission Regulation (EU) No 432/2012 stands out, which establishes a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. In table 2.2 the main claims, reported in the aforementioned regulation, are described; it is clear that, in order to have the indicated beneficial effects and therefore use the related claims, the various compounds must be present in specific quantities.

Table 2.2 Annex to Commission Regulation (EU) No 432/2012: list of permitted health claims

Nutrient, substance, food or food category	Claim	Conditions of use of the claim	Conditions and/or restrictions of use of the food and/or additional statement or warning	EFSA Journal number	Relevant entry number in the Consolidated List submitted to EFSA for its assessment
Barley grain fibre	Barley grain fibre contributes to an increase in faecal bulk	The claim may be used only for food which is high in that fibre as referred to in the claim HIGH FIBRE as listed in the Annex to Regulation (EC) No 1924/2006.		2011;9(6):2249	819
Beta-glucans	Beta-glucans contribute to the maintenance of normal blood cholesterol levels	The claim may be used only for food which contains at least 1 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these sources per quantified portion. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these beta-glucans.		2009; 7(9):1254 2011;9(6):2207	754, 755, 757, 801, 1465, 2934 1236, 1299
Beta-glucans from oats and barley	Consumption of beta-glucans from oats or barley as part of a meal contributes to the reduction of the blood glucose rise after that meal	The claim may be used only for food which contains at least 4 g of beta-glucans from oats or barley for each 30 g of available carbohydrates in a quantified portion as part of the meal. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained by consuming the beta-glucans from oats or barley as part of the meal.		2011;9(6):2207	821, 824

Another important class of bioactive compounds in barley is represented by phenolic compounds (Groupy et al., 1999) which are secondary metabolites resulting from metabolic processes, which are not directly involved in normal cell development (Delgoda and Murray, 2017).

Chemically, phenolic compounds consist of at least one hydroxyl-substituted aromatic ring (figure 2.6) and are generally linked to other molecules, mostly sugars and proteins, for example

flavonoids, anthocyanins, and condensed tannins (Morton et al., 2000). Scalbert and Williamson (2000), define phenolic compounds as the most widely distributed and abundant secondary metabolites in plants and they are known for different beneficial properties (Asensi et al., 2011; Santhakumar et al., 2014).

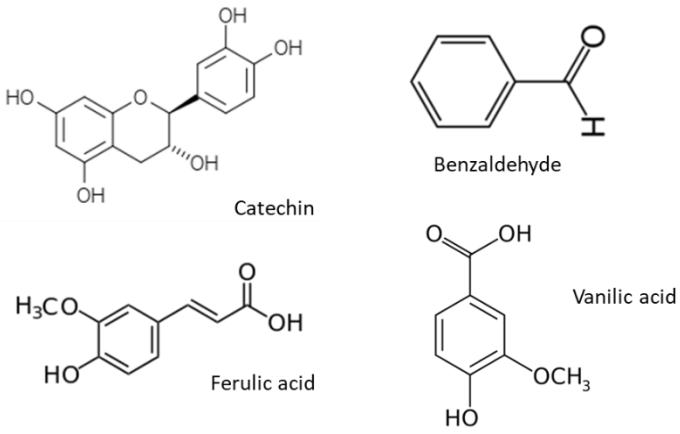

Figure 2.6 Common structure of some phenolic compounds

Phenolic compounds are the primary bioactive components in plants and they are recognized for their wide range of health benefits due to their antioxidant properties, such as reactive oxygen species scavenging and inhibition, electrophile scavenging and metal chelation (Randhir et al., 2004). In this regard, Challacombe et al. (2012) and Ragaee et al. (2011) define phenolic compounds, especially phenolic acids, as the main antioxidants in cereals. In particular, in order to stabilise free radicals, the hydroxyl groups present in the polyphenol structures readily donate hydrogen ions (Pereira et al., 2009). Moreover, in biological systems, polyphenols can inhibit enzymes associated with free radical production, acting as antioxidants. The mechanism by which polyphenols inhibit these enzymes is by either forming hydrogen bonds with the hydroxyl groups or with the benzenoid rings (Parr and Bolwell, 2000).

Moreover, phenolic compounds are considerate interesting especially because they also exhibit pharmacological properties, such as anti-carcinogenic, anti-inflammatory, and anti-mutagenic effects, and anti-proliferative potential (Kaliora et al., 2014) as well as other important

properties such as anti-apoptosis, anti-aging, anti-atherosclerosis, cardiovascular protection and inhibition of angiogenesis (Abozed et al., 2014).

Barley is a source of phenolic compounds, such as phenolic acids and flavan-3-ols (Gangopadhyay et al., 2016) and they can be found free (easily extractable) or covalently bound to macromolecules such as cell wall components (less extractable fractions). Bound phenolic acids are however bioavailable because they can be released by bacterial enzymes in the large intestine and, once freed, they can function as antioxidants and provide an important health protection in the human body (Guo and Beta, 2013).

On the basis of their diverse structure, phenolic compounds are classified in sixteen major classes: benzoquinones, phenolic acids, acetophenones, phenylacetic acids, hydroxycinnamic acids, phenylpropanes, coumarins/isocoumarins, chromones, naphthoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans, and neolignans (Shao and Bao, 2015) of which flavonoids are the most abundant (Giada, 2013).

Phenolic acids and their derivatives, are in turn divided into two further major groups: hydroxybenzoic and hydroxycinnamic acids. The first group includes p-hydroxybenzoic, protocatechuic, vanillic, syringic, and gallic acids, whereas compounds like p-coumaric, caffeic, ferulic, and sinapic acids belong to the group of hydroxycinnamic acid (Rao et al., 2018a). Among phenolic compounds, flavonoids and their derivatives, such as the flavones, flavonols, flavanones, flavanols, anthocyanins, and isoflavones, are highly abundant and have some of the highest antioxidant activity (Waterman and Mole, 1994).

Inside barley kernels the majority of the phenolic compounds are located in the bran layer of whole grain barley (Rao et al., 2018b). Some of the phenolic compounds found in barley include cinnamic and benzoic acid derivatives, proanthocyanidins, flavonols, chalcones, flavones, flavanones, and a range of amino compounds including phenolic acids such as ferulic, p-

coumaric, gallic, vanillic, caffeic, syringic, protocatechuic, chlorogenic, and p-hydroxybenzoic acids (Gamel and Abdel-Aal, 2012).

Depending on the phenolic compound considered, the concentration in barley ranges from 0.21 to 115 mg/100g and this large range is due to the fact that it is strongly correlated to barley variety, and to hulled or hulless barley (Rao et al., 2018a).

Alkylresorcinols also belong to the group of phenolic compounds. Alkylresorcinols are amphiphilic 1,3-dihydroxybenzene derivatives with an odd numbered alk(en)yl chain at position 5 of the benzene ring in the range of 17-25 carbon atoms, giving a mixture of alkylresorcinol homologues C17:0-C25:0 in specific proportions for different cereals. They are located in the intermediate layers between pericarp and head in the grain (Landberg et al., 2008) and are therefore found in large amounts only in wholegrain and bran products, and in very small amounts in refined flour or products (Ross and Kochhar, 2009). Alkylresorcinols are major phenolic compounds in wheat and rye (Bondia-Pons et al., 2009) but modest quantities, are also found in barley, but not in oats (Andersson et al., 2008; Shewry et al., 2008).

Since alkylresorcinols are found only in the outer layers of cereal kernels, it has been suggested that they can be indicated as potential markers of whole grain intake. In this regard, whole grain has shown to have a protective role against Type 2 diabetes in different epidemiological studies. However, underlying mechanisms are poorly understood, but de-creased energy intake, higher insulin sensitivity and improved glucose tolerance have been suggested and partly confirmed in studies (Nordin, 2015). In addition to this feature, alkylresorcinols have been studied mainly for their antioxidant, antimicrobial and antifungal activities, and their effects on biological membranes due to their amphiphilic nature (Ross et al., 2004), properties which make them, together with other phenolic compounds, bioactive compounds of considerable interest.

2.5 Physical methods for the production of ß-glucans enriched barley flour

As mentioned before, in the past barley has been used mainly as animal feed or for the production of malt for beer. Only recently, because of its nutritional potential, has this cereal been revalued and therefore introduced into human nutrition both as whole grain and in the form of barley flour as ingredients of consumer products (bread, pasta, baked products, etc..). In the latter case, the main limitation is related to the difficulties in mixing the barley flour with other conventional flours, negatively influencing the rheological properties of the obtained dough; other problems are related to the alterations of texture, flavour, colour and volume of the finished product which depend on the different barley chemical composition compared to other cereals (Sica, 2009). This problem is partially solved through the use of barley flours enriched in β-glucans that contain a major concentration of this bioactive compound and so they allow to obtain functional foods with the same ß-glucans content (and the resulting beneficial effects) but with a lower percentage of barley flour. β-glucans enriched barley flours represent, therefore, the better compromise between the benefits related to these compounds and the technological limits to the processability of barley in the production of functional foods (Vasanthan and Temelli, 2008). For these reasons, in the last years, industry interest has been growing to produce ß-glucan concentrates. This paragraph aims to reviews the main technologies (figure 2.7) available for the concentration of cereal ß-glucans with a focus on air classification process.

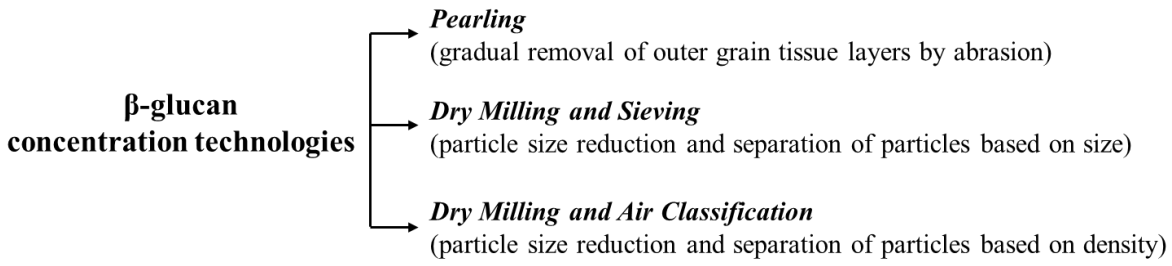

Figure 2.7 Dry technologies for concentration of cereal ß-glucan (Vasanthan and Temelli, 2008)

Sieving

Vasanthan and Temelli (2008) described barley flour as a complex particulate material, where each particle, depending on the extent of size reduction, varies in its chemical composition (i.e. starch, protein, beta-glucan, hemi-cellulose, cellulose, lipids, minerals, etc.); flour particles also vary in terms of their physical characteristics such as size, shape, and density. For these features, flour particles can be separated, according to their size, using simple vibratory sieving and it is possible to obtain a flour fraction where ß-glucans are concentrated, up to 9% (Bhatty, 1997).

Sieving is a physical separation method by which flour particles are separated according to their size. Using sieves with different sizes of mesh lights and applying various sieving steps, in which the coarse fraction (the one that does not pass through the sieve), obtained from the previous step is again ground and sieved, it is possible to obtain, relatively quickly, barley flours with different enrichment degrees in bioactive compounds. The only drawback of this technique is due to the presence of very fine flour particles which, generally, assemble together and then clog the mesh lights of the sieve. By using sieves with nylon cloths and suitable cleaning systems (chains, plastic balls, brushes), this inconvenience can be avoided.

Pearling

Pearling is a physical separation technique on a location basis and is a process widely used for the production of pearl barley to be destined for human consumption. In practical terms, pearling is a dry separation process where the outer layers of grain tissue and the germ are gradually removed by the abrasive action of rollers, through subsequent steps, without cracking the grain (Vasanthan and Temelli, 2008).

With the first pearl passage, the glume is removed with a loss of 7-14 % of the whole caryopsis weight. Further steps, lead to the removal of the outer layers of the caryopsis (head and

pericarp), aleurone, sub-aleurone layer and germ (11-25 % of the initial product), leaving the starchy endosperm rich in carbohydrates (starch and ß-glucans) and proteins intact (figure 2.8).

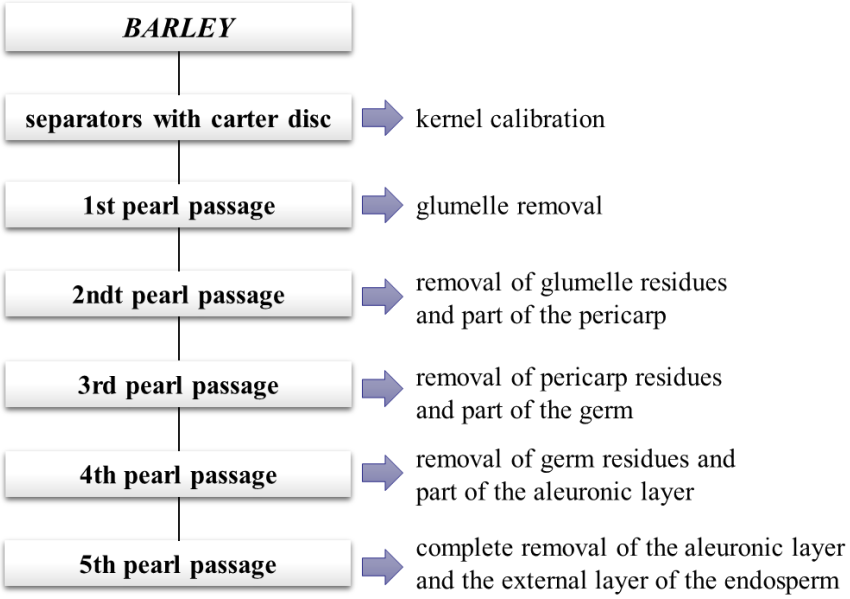

Figure 2.8 Pearling process scheme

Generally, the pearling process leads to a yield of 60-70 % in pearl barley which is used in human nutrition, especially as ingredient in soups and broths, while pearly waste, which represents about 30-40 % of the initial product, are used mainly for animal feed (Sica, 2009). However, in recent years, it has been shown that the pearling waste products are particularly rich in phytosterols, tocopherols and tocotrienols and have a fair content in soluble dietary fibre and β-glucans. For these reasons, pearling is no longer considered simply a technique for the removal of the outer layers of whole grain to obtain pearl barley, but, nowadays, it is seen as a physical technology to produce products which have a high nutritional value and they can be used as ingredients for the production of functional foods (Marconi et al., 2000).

Air classification

Generally, before air classification, barley flour is ground with a hammer mill and micronized, in order to reduce it to a very minute particle size (Kiryluk et al., 2000).

The micronizer is a device whose operating principle is based on the effects of peripheral speed, that is, on the consequences of the kinetic energy that increases with increasing in speed and/or diameter of the area in which one operates. The pulverization of flour particles takes place by impact of the same particles on the surface of blades and on micro-contrasts located inside the device.

At the end of the grinding and micronization phases, it is possible to proceed with the air classification process. This is a physical separation technique which is based on the density differences between particles. An air classification device is usually described as a fan in a closed cylindrical system in which the air flow is directed towards the centre of the cylinder and not peripherally (figure 2.8/C). Inside the device, the flour is subjected to the action of two opposite forces (figure 2.8/A): a traction force, tends to drag the smaller particles towards the centre and a centrifugal force, tends to push the particles towards the external surface of the classifying cylinder. The fine flour particles, therefore, tend to be transported together with the air stream, while the larger particles tend to collect along the cylinder walls (figure 2.8/B); This system is called turboseparation.

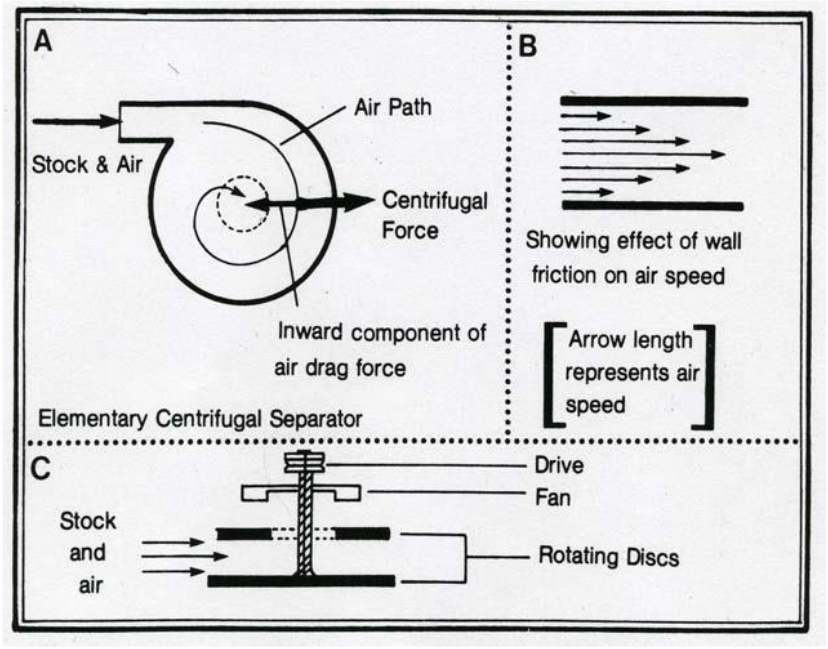

Figure 2.8 Air classification principles; opposite forces; B mass separation; C circulating means (Bass, 1988)

At the end of the process two fractions with different grain size are obtained: a coarse fraction (CF) and a fine fraction (FF). Figure 2.9 shows the air classification scheme;

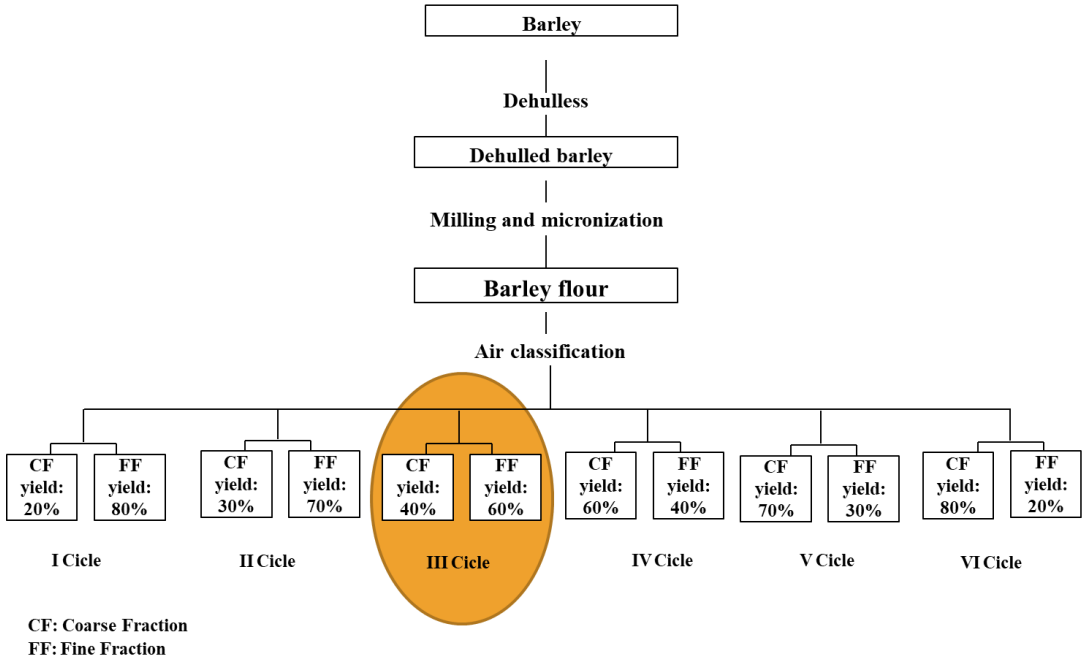

Figure 2.9 Air classification process

Through optimization of air-classification parameters such as feed rate, air flow rate, and classifier wheel speed, a fibre concentrate containing up to 30% ß-glucans can be obtained.

Moreover, by subjecting barley flour to several air-classification steps, it is possible to obtain a good ratio of fine fraction/coarse fraction and a fair compromise between yield and ß-glucans content.

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Chapter 3: FUNCTIONAL FOODS

3.1 Introduction

Recently, the increasing interest in the relationship between health and nutrition has encouraged scientific research towards studying the role of specific food components/ingredients, which are naturally included in many foods, in the possible prevention of diseases such as diabetes and hypertension (Cubadda and Marconi, 2008). It followed that, in the last years, a new category of "enhanced" foods, known as "functional foods", with healthy-pharmaceutical characteristics, have appeared on supermarket shelves and health food stores (Karelakis et al., 2019). These foods, besides satisfying hunger and providing nutrients that are necessary for the organism, also prevent nutrition related diseases and improve the body on both a physical and mental level (Menrad, 2003; Roberfroid, 2000).

Nowadays, no universally accepted definition of functional foods exists. The concept of functional food proposed by the European Commission Concerted Action on Functional Food Science in Europe (FUFOSE), coordinated by the International Life Sciences Institute (ILSI) considered specific features as follows:

- it is beneficial for human health beyond its basic nutritional value, enhancing well-being or reducing the risk of disease;
- its effects are recognized/ensured by the scientific community;
- being a food or food-ingredient which is conventional or consumed daily in the diet;
- the food should be natural and not synthetic, it is not a pill, drug or dietary supplement.

On the basis of these specified characteristics, the Consensus Document on Scientific Concept of Functional Food in Europe published in 1999, proposed the following working definition *"A food can be considered as functional if it beneficially affects one or more target functions in*

the body, beyond its nutritional properties, in a way that can reduce the risk of disease or improve the health status" (EU-ILSI, 1999).

Functional foods are foods that contain specific minerals, vitamins, polyphenols, fatty acids or dietary fibre but also foods where biologically active substances have been added. These molecules can be classified according to their functional role in the prevention of different diseases and /or promoting individual well-being (Polito et al., 2013).

However, in recent years, due to the rapid spread of these products, an atmosphere of confusion and mistrust has emerged between stakeholders and the food sector entities (consumers, health experts, companies) so what followed was the need to regulate production and marketing of functional foods through the definition of standards and guidelines (Benassi et al., 2017). Many countries have therefore adopted regulations on health and nutrition claims for food, in order to ensure their safety and the validity of their healthy properties in a legal way (Steinhauser and Hamm, 2018). For this purpose, Regulation (EC) No. 1924/2006 and Regulation (EU) No. 432/2012 were drafted to regulate the use of health and nutritional claims on labels, approved, upon scientific evidences, by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). EFSA is asked to express its opinion in the authorization of health claims for the different bioactive molecules, the opinions of EFSA are contained in the European Register (http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=search) published online in May 2012. This register is constantly evolving, and it is updated whenever the Authority expresses new favourable or contrary opinions (Benassi et al., 2017).

In particular, the EU Regulation No. 1924/2006, Art. 2, par. 2.4-2.6, reports that the health claims describe the relationship between a food or its ingredient and health and it can be a "general claim" which refers to the nutrient content of food (generic claims) and comparative claims, or a "product-specific claim" which is related to what the product does in terms of health

(specific health claims), well-being and performance, i.e. health claims (EU Commission, 2006). In addition to these, there is also the "risk reduction claim" that refers to the reduction of the risk to develop diseases upon consumption of the food. These claims represent the means by which a consumer can be informed about the health benefits of foods providing specific information that would have been otherwise unknown (Kaur et al., 2017). Moreover, according to the Codex Alimentarius Commission (2013), a claim means *"any representation which states, suggests or implies that a food has particular characteristics relating to its origin, nutritional properties, nature, production, processing, composition or any other quality"*.

An alimentary product can be defined as a functional food if it has components with functions that are beneficial, healthy or that prevent a disease and if said food accomplishes any of the following descriptions: a) is a natural food ; b) is a food to which a component has been added; c) is a food from which a component has been removed; d) is a food in which the nature of one or more of its components has been modified; e) is a food in which the bioavailability of one or more of its components has been modified or, f) any combination of the aforementioned possibilities (Roberfroid, 2011).

Cereals play a fundamental role in the promotion of healthy eating, thanks to their overall composition, although recent advances in research have highlighted the specific protective action of individual components (Cubadda and Marconi, 2008). In fact, they not only contain numerous bioactive compounds located mainly in the outer layer of the caryopsis (table 3.1), but are foods largely and regularly consumed in our daily diet in the form of a wide range of conventional products, from bread to extrudates, pasta and cereal-based foods which are often characterized by optimum content of fibre and polyphenols, as well as by antioxidant activity (Biernacka et al., 2017; Bouasla et al., 2016; Oniszczuk et al., 2015; Wójtowicz et al., 2018).

Table 3.1 Bioactive compounds (Phytochemicals) in the Cereal kernel

BIOACTIVE COMPOUND	KERNEL LOCATION	BIOLOGICAL EFFECTS
ß-glucans (barley and oats)	Starchy endosperm, aleurone layer	Hypocholesterolemic, Hypoglycemic
Tocols (Vitamin E) • tocopherols • tocotrienols	Germ, aleurone layer	Antioxidant, Hypocholesterolemic
Folate	Germ, aleurone layer	Prevention of neural tube defects, reduction of cardiovascular diseases and colon cancer
Fructooligosaccharides	Immature grain at the milky phase stage	Prebiotic
Phytosterols	Germ, aleurone layer	Hypocholesterolemic
(Poly)phenols	Pericarp, aleurone layer	Antioxidant
Phytate	Pericarp	Prevention of colon cancer
Policosanol	Pericarp	Hypocholesterolemic
Pentosans Arabinoxylans	Pericarp	Hypocholesterolemic
Lignan	Pericarp, aleurone layer	Reduction of cardiovascular diseases, reduction in cancer occurrence
Alkylresorcinols	Pericarp	Antioxidant, anticancer

Source: Marconi and Messia (2012)

3.2 Functional foods consumption trends

Functional foods characterise a constantly evolving market and an opportunity for the agri-food sector, but for greater competitiveness in the food industry, financial support for basic research, process and product innovation are necessary. The link between the concepts "health, technology and nutrition" for the development of foods with high nutraceuticals value, in dedicated supply chains, are possible if continuous dialogue and interaction between sector specialists, consumers and industry is promoted (Benassi et al., 2017).

In the last ten years, the increase in costs of health care, life expectancy, and desire for a higher quality of life has led to an increase in the demand for foods and beverages that improve health;

this phenomenon has affected many parts of the world (Diplock et al., 1999). In light of this, a new kind of health tool is represented by functional foods which promise specific effects related to particular food components (Doyon and Labrecque, 2008). For these reasons, the food industry has recently focused research and innovation activities on functional foods that represent a rather interesting sector (Pappalardo and Lusk, 2016). These are foods that are characterized by some particular additional physiological function, beyond their primary nutritional value, and therefore have the potential to have success on the market and become popular among consumers (Karelakis et al., 2019).

On the basis of what has been said so far, it is expected that in the next year, the European functional food market will continue to grow at different rates and the demand of functional foods will be driven by a growing public understanding of the relationship between diet and disease, rising healthcare costs and developments in food technology and nutritional science (Mollet and Rowland, 2002; Young, 2000). This growing trend is also favored by the introduction of foods which report functions in health, for example: for fatigue, stress and sleep (theanine), vision (lutein), memory (Ginkgo biloba), joints (hydrolyzed collagen and glucosamine), blood flow and body temperature (Ginkgo biloba, heseretin) (Iwatani et al., 2019).

A global focus on conscious consumerism, environmental concerns and a move away from chemicals towards more ‘natural’ products has favoured a greater awareness in people about what and how they consume (Angus and Westbrook, 2019) especially in regards to food. Figure 3.2 reports the Compounded Average Growth Rate (CAGR) of health and wellness foods and vitamins/dietary supplements in the time interval between 2012-2017 and it is evident that the consumption of health and wellness foods has increased worldwide with a greater percentage of growth in Asia Pacific, Latin America, and Western Europe compared to that of all foods. As for vitamins and dietary supplements instead, the largest percentage growth was in Asia

pacific and North America followed by Australasia and Middle East and Africa, while a negative average growth rate was found for Eastern Europe and Latin America. Overall the worldwide compounded average growth rate was about 3% in the years 2012-2017.

Figure 3.2 Health and Wellness Foods and Vitamins and Dietary Supplements Outpreform 2012–2017 (Euromonitor International 2018)

Globally, the largest markets worldwide are considered Japan, the US, and Europe while developing markets such as Brazil, Peru, Kenya, and China have emerged as significant exporters of functional components to the developed countries. Specifically, Japan's market is the most dynamic (38.4% of the global market of $24.22 billion), followed by US (31.1%), Europe (28.9%) and Australia (1.6%). Both Japan and the US together are estimated to cover 3/4 of the current global market for functional foods. Among European countries, the United Kingdom, Germany, France, and the Netherlands show the largest functional foods markets while emerging markets are those of Hungary, Russia and Poland (Hilton, 2017).

Upon closer examination of the European market, it emerges that it is a complex market. In fact, there are significant differences in consumption and acceptance of functional foods between various countries, with a major consumption in Central and Northern European countries compared to Mediterranean countries (Van Trijp, 2007). In Greece, for example, protein-fortified products attract new consumers more and more, with a consequent increase in sales in this category. This trend started in 2016, and today, protein-heavy diets are very popular among consumers. Again, the sales of functional foods have seen an overall decrease due to the high prices of these foods compared to conventional alternatives. This situation will deteriorate further as companies are predicted to limit investments in this food category in the future (Euromonitor 2018).

For the same reasons, over the past 2 years in some parts of the world, the growth rates of the functional foods market have slowed down although the global market for functional foods continues to expand. One of the main causes is the global economic downturn that plagues countries worldwide (Karelakis et al., 2019). One way to further increase the success of functional foods is certainly to make producers understand that, perhaps, more important than the financial benefit of companies, is the possibility that these foods can strongly contribute to lowering health care costs and improving health. In fact, the consumer is always more careful about what he eats considering diet as a means of improving health, opting for food or food ingredients that provide health benefits traditionally associated with medicine. However, further research is required to reach generalized conclusions about consumer behavior towards functional foods (Küster-Boluda and Vidal-Capilla, 2017). Generally, we can say that the consumers recognize different kinds of functional foods, resulting in a willingness to pay more for their purchase. They view functional foods as a way to easily follow a healthy and balanced diet and reduce the risk of health problems but they do not always fully understand the health benefits of these foods, based on what is reported on the labeling (Karelakis et al., 2019).

Further studies have been done to assess the differences in worldwide consumption of functional foods, including information on the country, gender and age and taking into account different types of potential health foods such as low-fat and skim milk, probiotic milk products, coffee and tea, cholesterol-lowering products, fermented drinks (red wine), cereals, soy products, fruit juice, fatty fish and other (Ozen et al., 2012).

Generally, results gathered have shown that functional foods like low-fat and skim milk, fruit juice, cereals, and yogurt were consumed by all age groups; however, tea, cholesterol-lowering products, and soy products were more commonly consumed by adults and the elderly; overall the greatest interest in functional foods is from females (Landström et al., 2007; Saher et al., 2004; Bogue and Ryan 2000), although some studies have shown a different attraction of males and females towards different products (De Jong et al., 2003; Ares and Gámbaro, 2007). Education has been proven to be one of the main factors that contribute to healthy eating, in fact, functional food consumers were reported to be more educated than non-consumers (Anttolainen et al., 2001). Other evidence suggests that age might also influence the consumption of some functional foods, as confirmed by a higher interest of older people in the health/beneficial effects of particular foods, than young people. In conclusion, we can say that it is not possible to generalize about consumer choice regarding different functional foods; gender, age, level of education and personal health status can strongly influence the choice of one or more functional foods. At the same time, an essential role in helping to guide food choice is played by the consumer's understanding of health claims of functional foods (Ozen et al., 2012). In this regard, Zafar and Ping (2020) have studied consumers' attitude and preferences of functional food and the analysis of qualitative data demonstrates that the subjects interviewed had the concept and understanding of functional foods and their attributes. The analysis identified the importance of sensory characteristics like taste and texture for the acceptance of this product, while, with reference to non-sensory attribute, the packaging, brand

and price have an important effect in influencing consumers' attitude, behavior and preferences about functional foods. The health claim and benefit attribute emerged as the most important attribute in this qualitative study in shaping people's attitudes and preferences and willingness to buy this type of product.

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Chapter 4: OUTLINE OF THESIS

The recent growing interest in healthy eating and the consequent "boom of functional foods" has encouraged scientific research towards exploring unconventional raw materials for the production of innovative healthy foods. There is a wealth of information about the positive effects of barley and oat ß-glucans on the human body.

ß-glucans content in barley is higher than those of other cereals, and that makes barley a useful ingredient for the production of functional foods.

In order to use barley flour in the formulation of innovative cereal food, and to preserve the technological and processing aptitude of new formulas and the sensorial acceptability of the finished product, the minimum replacement of wheat flour is need. To solve this problem, food technologists have developed different flour fractionation physical techniques for the separation/enrichment of barley flour in ß-glucans.

The research project aims 1) to evaluate the use of waxy barley flour enriched in ß-glucans through the air classification process, as an alternative ingredient for the production of functional couscous; 2) to develop barley based couscous using different percentages of ß-glucans enriched barley flour, in a mixture with semolina; 3) to evaluate the impact of barley flour addition on the chemical-nutritional composition and physical characteristics of the final product, as well as cooking and the sensorial quality of the developed couscous; 4) to analyze phenolic compounds composition of the developed innovative products.

Couscous, thanks to its simplicity, versatility and economic convenience, finds a great success among consumers, resulting in a very trendy product. Consequently, if the proposed innovative product does not lose its characteristic of "ease of use", it would acquire added value determined by the health value brought about by the addition of ß-glucans.

Chapter 5: DEVELOPMENT OF FUNCTIONAL COUSCOUS ENRICHED IN BARLEY ß-GLUCANS

Messia, M.C., Oriente, M., Angelicola, M., De Arcangelis, E., Marconi, E. (2019). Development of functional couscous enriched in barley ß-glucans. Journal of Cereal Science 85, 137-142

ABSTRACT

A flour made from waxy, hulless barley, CDC Alamo, was subjected to air classification to obtain a coarse fraction enriched in ß-glucans. Different percentages of semolina (20 and 30 %) were replaced with this ß-glucan enriched barley flour to prepare functional couscous in a traditional manner (hand rolling). The couscous produced was evaluated for particle size distribution, chemical composition, gelatinized starch, furosine content and cooking quality. The incorporation of ß-glucan-enriched barley flours in couscous production did not cause significant changes in the characteristics or properties of the final product, which had high total dietary fibre content (9.8 and 7.42 % for mixtures 70/30 and 80/20, respectively) and ß-glucans (4.09 and 2.79 % for mixtures 70/30 and 80/20, respectively) compared to that of semolina control couscous (3.61 % fibre and 0.18 % ß-glucans), fully meeting the FDA and Reg. UE 432/2012 requirements for health claims made on foodstuffs.

Keywords:

couscous, barley β-glucans, air classification, functional food

5.1 Introduction

Couscous is an agglomerated and steamed product native to North Africa that is usually handmade from durum wheat semolina. It is known around the world because of its simplicity in production, versatility, and convenience and because of the growing popularity of healthier eating and the “Mediterranean diet”. Consumers appreciate couscous because, similar to pasta, it is a product well-suited to contemporary life with properties like low fat and low glycaemic index (D'Egidio and Pagani, 2010). Sorghum, pearl millet, maize (Aboubacar et al., 2006; Galiba et al., 1988; Taylor et al., 2010) and mixing of semolina with other raw materials such as chickpea flour or buckwheat (Demir et al., 2010; Demir and Demir, 2016) were also used to prepare couscous.

Consumer interest in functional foods in recent years has increased very steeply due to the growing awareness of the link between diet and health and the changes in food regulations. Cereal grains contain phytochemicals that showed positive effect on health and disease prevention (Sidhu et al., 2007).

Barley, one of the earliest cultivated cereals in the world, is now gaining renewed interest in particular for the production of functional foods, mainly because of its high concentration of bioactive compounds such as mixed linkage (1→3), (1→4)-ß-D-glucan hereafter referred to as ß-glucans (De Paula et al., 2017; Sharma and Kothari, 2017). ß-Glucans are a type of soluble dietary fibre located in the cell walls of the endosperm of barley grains. Barley ß-glucans content varies from 2 to 11 % and is influenced by genetic and environmental factors (high amylose and waxy barley varieties have the highest content of ß-glucans).

Over the last two decades, ß-glucans have been considered as bioactive ingredients due to their capacity to lower plasma cholesterol, improve lipid metabolism, and reduce the glycaemic index (Wood, 2007). These positive effects increased the popularity and consumption of cereal-

based foods as well as of many other foods fortified with ß-glucans concentrates and isolates (Lazaridou and Biliaderis, 2007).

Interest in the fractionation of cereal grains has grown in recent years, due to an increase in the demand for functional and healthy foods. Air classification is one physical separation technique used for grain fractionation. It is capable of concentrating components present in fractions of finely ground flour based on particle size, density and mass (Vasanthan and Bhatty, 1995; Wu and Doehlert, 2002). Through optimization of air classification parameters (feed rate, air flow rate, classifier wheel speed) it is possible to obtain barley flours with high bioactive compounds levels (Ferrari et al., 2009). Other processes use non-edible extractants like NaOH or organic solvents, residues of which are prohibited in human foods (Ferrari et al., 2009; Gómez-Caravaca et al., 2015). Moreover, a slight pearling process can be applied on barley de-hulled kernels, before the air-classification, removing 1-2 % of the seed coat, obtaining grains with also a reduced amount of mycotoxins.

In the past, semolina, egg, milk and bulgur were used together to produce nutritionally enriched couscous (Yükşel et al., 2018a), while rice, field beans and chickpea flour were used to produce gluten-free couscous (Benatallah et al., 2008). No information are available on the use of barley flour fractions enriched in ß-glucans to produce functional couscous.

The aim of this study was to produce an innovative functional couscous (enriched in ß-glucans) using a mix of semolina and ß-glucans enriched barley flour with improved technological, nutritional and cooking qualities.

5.2 Materials and methods

5.2.1 Samples

Fourteen samples of commercial couscous manufactured using different cereal species (durum wheat, kamut®, emmer, maize, rice, and barley) were purchased from local supermarkets.

The waxy, hulless barley grain cultivar, CDC Alamo, was grown in experimental fields of the University of Molise.

5.2.2 Chemical analysis

Couscous samples were milled using a refrigerated laboratory mill model IKA A10 (IKA-WERKE GmbH & CO KG, Staufen, Germany).

Moisture, ash and protein content were determined according to ICC methods 110/1, 104/1 and 105/2 respectively (ICC, 1995). Total fat was determined by acidic hydrolysis (AACC, 2000).

Total, soluble and insoluble dietary fibre were determined using the AOAC Method (Prosky et al., 1988). Total ß-glucans were determined using a K-BGLU assay kit (Megazyme, Ireland).

Total and gelatinized starch were quantified enzymatically using the K-TSTA and K-SDAM spectrophotometric assay kits (Megazyme, Ireland).

Furosine content was determined on hydrolysate by HPLC, according to Resmini et al. (1990). A sample amount, corresponding to about 30-70 mg of protein, was hydrolyzed under nitrogen with 8 mL of 8 N HCl at 105 °C for 23 h. The hydrolysate was filtered on filter paper whatmann n. 4 and 0.5 mL of it was purified on a Sep-Pak C18 cartridge (Waters Corp., Milford, MA) and analysed by HPLC (Dionex, Sunnyvale, CA, USA) equipped with an Alltech furosine-dedicated column (250×4.6 mm) (Alltech, Derfield, IL, USA). The eluted compounds were monitored by a photodiode array detector (Dionex, Sunnyvale) set at 280 nm wavelength. The furosine standard was purchased from Neosystem Laboratoire (Strasbourg, France).

5.2.3 Physical characteristics

Sieving analysis: One hundred grams of couscous was sieved using circular, stainless steel, certified test sieves of the ASTM series (Giuliani, Torino, Italy) with mesh sizes ranging from 2.36 mm to 0.85 mm. Colour analysis: The colour of dry, uncooked couscous was evaluated by measuring L* (brightness, 100 = white; 0 = black), a* (+ red; - green) and b* (+ yellow; - blue) parameters by means of a reflectance colorimeter (CR200 Chromameter, Minolta, Japan). Bulk density: bulk density of the couscous samples was determined by filling a 500 mL graduated cylinder with 250 g of couscous. Values were expressed as the ratio of couscous weight per unit volume.

5.2.4 Barley flour ß-glucans enrichment

The waxy barley variety, CDC Alamo (ß-glucan content= 7.6 % d.m.), was utilized for production of barley flour enriched in ß-glucan. Hulless grain of the barley cultivar was milled by a model 8/B (Beccaria S.r.L. Italy) and pin-milled by a model TMX-500 (100–200 kg/h, Separ Micro System, Brescia, Italy). Subsequently, the whole meal (WM) was fractionated by a turbo-separating system (Separ Micro System s.a.s. BS, Italy) in two fractions corresponding to 40 % and 60 % of the total weight of whole flour, respectively. The 40 % and 60 % fractions were defined as coarse and fine fractions, respectively, with regard to the dimension of the flour particles. The fractions and the whole flours were stored at −18 °C before use.

5.2.5 Couscous preparation

Couscous was produced using the handmade traditional procedure. Durum wheat semolina or mixtures of semolina and enriched barley flour (70/30 and 80/20 respectively) were moistened with water (about 40 %) in a dish and stirred by hand. The moistened bulk was rubbed with the palm of the hand to form granules which were compressed between the hand and the container,

and the bulk was compacted by successive throws and drops for approximately 15 min. The granules were compressed through a metal sieve to obtain calibrated granules.

The moistened granules were then placed into the upper section of a couscoussiere, while the lower section contained approximately 1.5 L of boiling water. The junction between the two sections was sealed with a damp cloth to force the steam through the couscous in the upper section. Steaming continued for 10 min. The couscous was then placed into a dish, and again, it was handled (rubbed with the palm of the hand, compressed through a metal sieve and then steamed). This procedure was carried out three times. After cooking, the couscous grains were dried using a static dryer (Namad Impianti, Roma, Italy) set at low temperature (30 °C for 24h).

5.2.6 Cooking quality assessment

Cooking loss (CL): the extent of couscous disintegration during cooking was measured according to a procedure reported by Ounane et al. (2006). Ten grams of cooked couscous was placed in 200 mL of distilled water at 25 °C. After 4 min of agitation, 10 mL of supernatant was recovered and incubated in an oven for 15 h at 105 °C.

Swelling: couscous swelling was determined according to the methodology of Ounane et al. (2006). 20 g of uncooked couscous were placed in a graduated test tube containing 50 mL of distilled water at 100 °C. The test tube was sealed and rotated top-to bottom 10 times successively to ensure that all particles were well moistened. Another 50 mL of water at 100 °C was added to wash down particles sticking to the side of the test tube. The test tube was then placed in a water bath at a controlled temperature (25 °C) and the couscous volume was recorded after 30 min.

Water Absorption Index (WAI): 2.5 g of couscous and 30 mL of ultrapure water were placed in a centrifuge tube. The tube was shaken for 30 min and then centrifuged at 2200 G for 10 min. The supernatant liquid was drained carefully into a container and subsequently placed in an

oven at 105 °C for 4 h and then it was weighed on an analytical balance. The material remaining in the centrifuge tube was weighed and the WAI was calculated (Oliveira et al., 2015).

Water solubility index (WSI): the WSI was calculated as the ratio between the weight of the evaporation residue and the dry weight of the sample (Oliveira et al., 2015).

5.2.7 Statistical analysis

Statistical analysis was carried out using R, Version 3.5.0. The data reported for all parameters are the average values of three results obtained from analysis of three different aliquots of each sample. Analysis of variance was performed to determine significant differences (Tukey's HSD test $*P \leq 0.05$) between means.

5.3 Results and discussion

5.3.1 Chemical characterization of commercial couscous samples

Commercial samples of couscous were at first analysed in order to evaluate the influence of raw material and industrial processes on products' characteristics and to produce an innovative couscous close to the traditional one.

Commercial couscous samples showed varying chemical compositions depending on the types of cereals used (table 5.1). In general, the samples had an average moisture, protein and fat contents of 11.7 %, 12.1 % d.m. and 2.4 % d.m., respectively. The moisture level is typical of this type of product and is a consequence of the drying process to which the product is subjected in the last phase of preparation.

The values found, except for CC10 corn sample, are in accordance with the indications of the Codex Alimentarius (Codex standard, 1995), which defines 13.5 % as the maximum moisture content. For the protein content, the greatest differences were found between couscous from

materials other than durum wheat semolina. Among the samples, maize-based couscous had a lower protein content (average= 6.5 % d.m.) than that of couscous produced by rice, barley, emmer, kamut® and a mix of 4 cereals whose values ranged from 9.8 to 16.1 % d.m. The fat content, as well as ash, is also closely linked to the type of raw material used and the different degrees of refining achieved during the milling process. The highest fat content was found in couscous made from emmer, maize, barley and kamut®. Couscous, unlike pasta or other products (Sharma et al., 2013), does not require kneading and extrusion, but its production involves hydration and subsequent steaming before drying and hence generates a high degree of gelatinized starch and a higher capacity for water absorption when rehydrated during cooking.

The cooking treatment induces significant physicochemical changes of the wheat component of the couscous grains. The gelatinization makes starch more easily hydrolysable by enzymes, such as α- and ß-amylase, due to the marked structural changes determined by this phenomenon. The starches from different cereals behave differently during the gelatinization process, such that it can occur in a wide range of temperatures. Gelatinization is influenced by the relative percentages of amylose and amylopectin (Singh et al., 2003).

Table 5.1 Chemical characterization of commercial couscous samples

Sample	Moisture (%)	Protein (% d.m.)	Fat (% d.m.)	Ash (% d.m.)	Total starch (% d.m.)	Total Fiber (% d.m.)	Gelatinized starch (% d.m.)	Furosine (mg/100g protein)
Durum wheat semolina couscous								
CC1	10.7±0.08	13.0±0.05	1.9±0.02	1.03±0.016	77.9±2.04	4.5±0.72	64.9±2.55	66.4±0.76
CC2	10.6±0.06	13.6±0.08	1.9±0.05	1.15±0.016	74.5±1.35	4.5±0.67	63.6±0.13	48.5±2.92
CC3	10.6±0.01	13.1±0.05	1.4±0.12	1.16±0.000	75.9±2.71	4.8±0.72	72.8±1.01	46.0±0.27
CC4	12.6±0.18	13.8±0.01	2.0±0.11	1.05±0.008	73.4±1.95	4.8±0.79	67.4±0.42	46.8±1.29
CC5	12.1±0.01	13.1±0.00	1.3±0.10	0.90±0.000	75.3±0.80	3.4±0.51	64.4±1.51	53.6±0.60
Durum wheat whole meal couscous								
CC6	10.9±0.02	13.1±0.00	1.8±0.10	1.32±0.000	66.2±0.14	9.9±0.85	62.3±0.61	47.6±0.62
CC7	11.6±0.09	12.2±0.00	3.3±0.04	1.73±0.016	66.2±1.91	10.0±1.00	63.0±2.12	125.7±2.22
Cereals couscous								
CC8 rice	10.3±0.08	9.8±0.11	1.9±0.10	0.58±0.016	83.3±0.86	2.0±0.30	69.2±0.20	32.6±1.78
CC9 barley	10.9±0.06	11.1±0.09	3.4±0.01	1.97±0.008	64.1±0.39	12.4±0.95	58.8±1.48	110.9±0.30
CC10 maize	14.1±0.15	6.2±0.01	3.3±0.22	0.69±0.008	79.2±2.62	4.1±0.54	70.7±0.93	94.2±1.91
CC11 maize	12.9±0.08	7.0±0.02	2.2±0.03	0.44±0.000	79.9±1.96	3.1±0.47	70.6±1.53	44.7±1.07
CC12 emmer	12.2±0.17	13.9±0.02	3.6±0.01	1.71±0.008	66.7±0.22	7.7±0.67	57.3±1.34	110.4±1.73
CC13 kamut®	11.9±0.03	16.1±0.07	3.0±0.01	1.87±0.008	70.2±1.13	8.4±0.95	60.5±0.91	104.3±5.59
CC14 4cereals	11.9±0.09	14.0±0.03	2.2±0.03	1.57±0.096	67.0±1.53	9.1±0.84	62.8±1.14	176.9±3.42
Min-Max	***11.9-14.1***	***6.2-16.1***	***1.3-3.6***	***0.44-1.87***	***64.1-79.9***	***2.0-12.4***	***57.3-70.7***	***44.7-176.9***
Average	***11.7***	***12.1***	***2.4***	***1.22***	***72.8***	***6.3***	***65.0***	***79.2***
RSD%	***9.1***	***22.7***	***40.5***	***8.4***	***32.2***	***50.0***	***7.0***	***52.9***

The lower values of gelatinization were found in barley and emmer couscous (approximately 58 % d.m.) compared to the average of 67 % d.m. in semolina couscous and 65 % d.m. in couscous based on other cereals. As indicated by Debbouz and Donnelly (1996), the gelatinization value considered optimal for this type of product is approximately 69 %. The authors have suggested that a higher content of gelatinized starch is related to a higher Water Absorption Index (WAI), the latter being used as an index to assess the ability of couscous to absorb sauces, and is therefore considered to be a qualitative attribute.

Depending on drying conditions and the relative humidity during drying, reactions between sugars, amino groups and imino groups of free amino acids, peptides and proteins can take place; these reactions are collectively called the Maillard reaction (Hellwig et al., 2018; Sharma et al., 2012). In the first step of this reaction, Amadori rearrangement products are formed and can be quantified as furosine (ε-fructosil-lysine) after acid hydrolysis (Hellwig et al., 2018). Furosine was used with success to identify the non-enzymatic browning in various carbohydrate-protein systems such as pasta, parboiled emmer (Verardo et al., 2017; Messia et al., 2012) and others when undergoing heat treatment. In couscous, furosine may be used to gain insight into the effects of the steam cooking process and subsequent drying on the early stage of Maillard reaction. From the nutritional point of view, the furosine presence is linked to a reduction of the intake of the essential amino acid lysine, thus limiting the nutritional value of a food (Erbersdobler and Somoza, 2007), while, advanced glycation end products (AGEs) in the late stage of the Maillard reaction, are often referred to a risk for human health (Hellwig et al., 2018). In general, the furosine levels found in couscous samples were quite variable and ranged from a minimum of 32.6 mg/100 g protein in rice couscous to a maximum of 176.9 mg/100 g protein in couscous from four cereals. The resulting differences can be mainly attributed to the different conditions used during the cooking and drying processes.

5.3.2 Enriched barley flour production and characterization of couscous ingredients

An air classification system was used to enrich the CDC Alamo barley flour in ß-glucans as previously done also by other researchers (Ferrari et al., 2009). The process diagram of air classification is reported in figure 5.1. The micronization products and the coarse and fine fractions obtained from the process were characterized. As shown in table 5.2, ß-glucans were concentrated in the coarse fraction, which, at the end of processing, had a ß-glucan content of 14.4% d.m. with a yield of 40%.

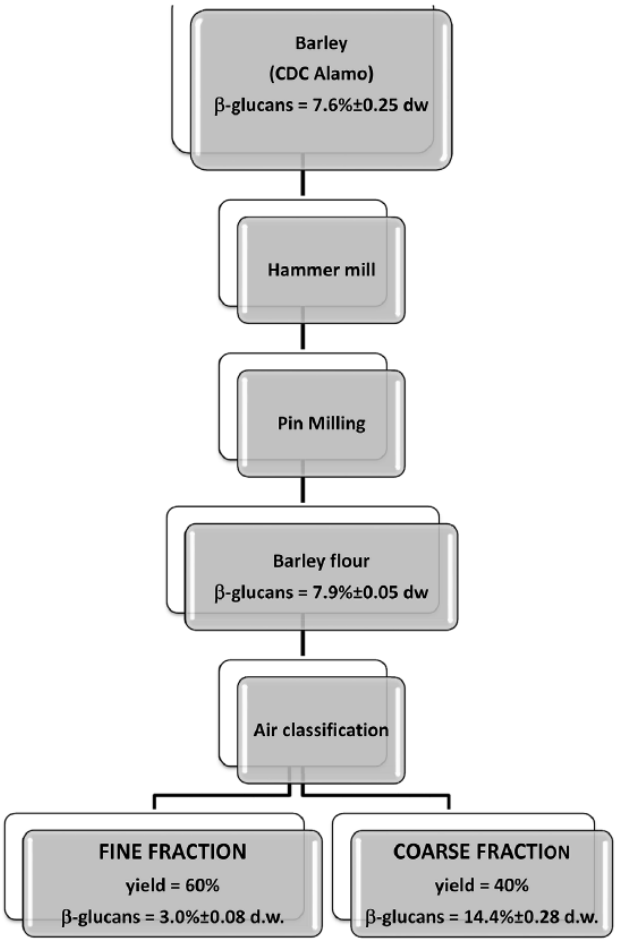

Figure 5.1 Air classification flow-sheet to obtain ß-glucan-enriched barley flour.

The chemical characterization of durum wheat semolina and enriched barley flour, used for the production of functional couscous, are shown in table 5.2. The coarse fraction of CDC Alamo barley, deriving from air classification, showed high total fibre (25.5% d.m.) and ß-glucans

(14.4% d.m.) contents, while total starch content was lower (51.8% d.m.) than the semolina samples. The high content of dietary fibre and ß-glucans and the lowest starch content of the coarse fraction (compared to semolina) make this fraction a functional ingredient because of the presence of high amounts of bioactive compounds together with a reduced caloric intake.

Table 5.2 Proximate composition and β-glucans content of micronized, coarse and fine fraction obtained by air classification of barley flour and durum wheat semolina.

Sample	Protein (Nx6.25) (% d.m.)	Fat (% d.m.)	Ash (% d.m.)	Total starch (% d.m.)	Total Fibre (% d.m.)	β-glucans (% d.m.)
Micronized barley flour	12.1±0.01	2.9±0.02	2.18±0.057	61.8±3.04	14.7±0.14	7.9±0.05
Coarse fraction	13.9±0.59	3.3±0.01	2.35±0.042	51.8±0.85	25.5±1.94	14.4±0.28
Fine fraction	10.1±0.18	2.8±0.10	1.69±0.007	70.2±1.02	9.3±0.09	3.0±0.08
Durum wheat semolina	12.6±0.16	1.7±0.04	0.85±0.033	73.2±1.01	3.5±0.52	0.2±0.00

5.3.3 Functional couscous characterization

Chemical composition

Functional couscous was made by replacing 20 or 30 % of durum wheat semolina with barley flour enriched in ß-glucans. In fact, the chosen ratios (semolina/enriched barley flour 70/30 and 80/20) facilitated product functionality with a minimum replacement of semolina.

The replacement of durum wheat semolina with other flours, such as enriched barley flour, can significantly modify the rheological properties of the mixtures with a consequent decline in its technological capabilities and the sensory properties of the finished product. The presence of the barley-enriched fraction resulted in an increase in ash content (1.32 and 1.26 % d.m. of the couscous mixtures of 70/30 and 80/20, respectively) and fat (1.9 and 1.7% d.m. of the couscous mixtures of 70/30 and 80/20, respectively), compared to 100% semolina couscous, while no significant differences were found in the protein content of both functional couscous and 100% semolina couscous (table 5.3). The produced couscous had high levels of total dietary fibre (9.8

and 7.42 % of the mixtures of 70/30 and 80/20, respectively) and ß-glucans content (4.09 and 2.79 % of the mixtures of 70/30 and 80/20, respectively) compared to couscous made from 100% semolina flour (3.61% fibre and 0.18% ß-glucans).

Both couscous samples (couscous 70/30 and couscous 80/20) fully meet the requirements of Reg. UE 432/2012. According to the abovementioned regulation, the amount of ß-glucans found in functional couscous permits the assertion of the health claim, "beta-glucan contributes to the maintenance of normal cholesterol levels in the blood", as the innovative products contain at least 1 g of beta-glucan from barley per quantified portion (50 g) (table 5.3).

The ß-glucans content of both types of couscous samples (70/30 and 80/20) did not change after cooking. Moreover, during couscous preparation, it was possible to verify that the presence of the coarse fraction did not cause problems with agglomeration and the formation of couscous grains.

Treatment parameters

Gelatinized starch values for functional couscous (60% d.w) (table 5.3) could be a good compromise to have couscous with good water absorption, high absorption capacity and good consistency, as reported by Debbouz and Donnelly (1996). Drying treatment using a static system and low temperatures (30 °C for 24 h) restrains the development of Maillard's reaction. The furosine values of functional couscous and control couscous (24.2, 25.1 and 29.0 mg/100 g protein for 70/30, 80/20 mixtures and control couscous, respectively) were significantly lower than the average value found in commercial couscous (79.2 mg/100 g protein), indicating that the treatment used was milder than those used in industrial plants (55 °C for 17 h or 95 °C for 3 h) (Guezlane et al., 1998); the values were also considerably lower than those found in pasta, a product traditionally prepared from the same two ingredients (water and durum wheat semolina) even when dried at low temperatures (below 60 °C).

Table 5.3 Proximate composition (g/100g as is), β-glucans content, gelatinized starch, furosine, colour, particle size distribution and bulk density of couscous.

Sample	Moisture	Protein	Fat	Ash	Total starch	Fibre			β-glucans	Gelatinized starch	Furosine (mg/100g protein)
						Soluble	Insoluble	Total			
70/30 Couscous	8.2±0.16	12.1±0.05	1.9±0.03	1.32±0.02	59.8±0.04	4.4±0.25	5.4±0.01	9.8±0.24	4.09±0.071	51.2±0.75	24.2±0.88
80/20 Couscous	8.9±0.03	12.0±0.04	1.7±0.03	1.26±0.03	61.2±3.14	3.5±0.16	3.9±0.14	7.4±0.81	2.79±0.078	53.7±0.72	25.1±0.24
Control Couscous	9.4±0.05	11.8±0.07	1.4±0.05	0.84±0.00	67.6±0.06	1.8±0.12	1.8±0.01	3.6±0.11	0.18±0.071	59.9±0.01	29.0±0.41

Sample	Colour			Distribution % of particle size				Bulk density (g/cm^3)
	L*	a*	b*	Ø > 2.360 mm	Ø > 1.400 mm	Ø > 0.850 mm	Ø < 0.850 mm	
70/30 Couscous	+44.09±0.18	+0.99±0.12	+17.13±0.27	1.0	50.4	46.9	1.5	0.56±0.03
80/20 Couscous	+47.38±0.41	+0.38±0.03	+19.99±0.14	1.1	57.0	40.5	1.4	0.60±0.02
Control Couscous	+49.01±0.35	-2.00±0.01	+22.50±0.06	1.3	44.1	51.2	2.9	0.69±0.02

70/30 Couscous: 70% semolina and 30% barley coarse fraction
80/20 Couscous: 80% semolina and 20% barley coarse fraction
Control couscous: 100% semolina

Physical parameters

The presence of barley flour enriched in ß-glucans in the formulation of functional couscous has led to variations in colour compared to control couscous (table 5.3). As reported by Abecassis et al. (2012), homemade control couscous (100% semolina flour) were generally characterized by slightly higher values of L* than commercial/industrial couscous due to the lower loss of carotenoid pigment during processing. Obtained values of L*, a* and b* of control couscous were in the range reported by Guezlane et al. (1998) and Debouz and Donnelly (1996). Functional couscous (70/30 and 80/20 mixes) was darker than semolina couscous. This outcome does not penalize this type of product since the consumer is already accustomed to purchasing fibre-rich products, identifying this feature by darker coloured products.

Sieving tests were carried out on the produced couscous to evaluate particle size, and the obtained values (table 5.3) were compared to those of a commercial semolina couscous sample. The couscous produced experimentally had a granulometry of 1400µm – 850µm, which is, according to the classification of the Codex Alimentarius (Codex Standard, 1995), considered to be medium grain-type couscous.

The bulk density values for the functional couscous (0.56 and 0.60 g/cm^3 for 70/30 and 80/20 mixtures, respectively) were lower than the values obtained for control couscous made with 100% semolina (0.69 g/cm^3).

Cooking quality

Regarding physicochemical-cooking properties, the results obtained on innovative couscous were comparable with values found for control couscous highlighting that incorporating the coarse fraction in semolina only slightly affects the quality parameters related to couscous rehydration behaviour, preserving the quality of the cooked product.

The capacity of couscous to rapidly absorb sauces and maintain its firmness is considered by Debbouz and Donnely (1996) to be a good quality attribute. Both of the functional couscous types showed a WAI index and swelling values higher than control couscous (table 5.4), denoting the good hydration capacity of cooked functional couscous. This feature is due to the presence of ß-glucans in the enriched barley flour, which has a well-established capacity for water absorption (Ahmed and Thomas, 2015). The highest swelling values were recorded for functional couscous, while the lowest ones were obtained for control couscous. As reported by Ounane et al. (2006), the extent of couscous swelling can vary according to the raw materials used.

Table 5.4 Cooking quality of functional and control couscous.

Sample	Cooking Loss (CL) (%)	Water Absorption Index (WAI) (g/100g)	Water Solubility Index (WSI) (%)	Swelling (mL/100g)
70/30 Couscous	17.2±1.14	489.0±4.11	5.0±0.08	478.0±5.35
80/20 Couscous	16.8±0.56	497.9±3.01	4.3±0.02	489.7±11.13
Control couscous	11.5±0.40	415.3±6.48	3.0±0.16	419.8±10.00

70/30 Couscous: 70% semolina and 30% barley coarse fraction
80/20 Couscous: 80% semolina and 20% barley coarse fraction
Control couscous: 100% semolina

Linear correlation coefficients (r) for couscous cooking quality parameters

	CL	WAI	WSI	Swelling
CL	1			
WAI	0.99*	1		
WSI	0.96*	0.90▪	1	
Swelling	0.97*	0.99**	0.87	1

Significant at ** p≤0.01; * p≤0.05;▪ p≤0.1

Values of couscous cooking loss can be related to the cooking behaviour of dry couscous. Generally, low values of couscous cooking loss are indicative of a high quality product.

Functional couscous (70/30 and 80/20 mixes) showed cooking loss values that were slightly higher than those of the control couscous but were in line with what was indicated by Demir et al. (2010) to obtain good quality couscous.

The WSI index, which expresses the extent of couscous disintegration during water absorption, was in the range of 4–16 %, which has been indicated by Abecassis et al. (2012) as classical values for a semolina couscous.

5.4 Sensorial evaluation

In order to verify if the produced functional couscnous are appreciable by the consumer, sensorial evaluation on cooked couscous was carried out. Debbouz and Donelly (1996) procedure was followed, with modifications.

Briefly, a 10-member panel was selected among students and staff of the University of Molise (which usually consume couscous at least 2-3 times a month) to conduct the sensorial evaluation of cooked couscous. The panel was composed by six females and 4 males with an age between 22 and 50.

Commercial couscous, together with produced functional couscous, were evaluated for their cooked sensorial attributes (appearance, mouthfeel, taste, smell and overall acceptability), using a nine-point hedonic scale (1= dislike extremely; 9= like extremely). Before evaluation, optimum sensory attributes were described and explained to the panellist. Sensory evaluation sessions were conducted in the morning, and performed in separate workstations. Therefore, three tablespoons of each cooked couscous sample, coded with three digit random numbers, were presented with a scorecard to each panellist. Finally, the data of each panellist was collected together and processed, obtaining the graph shown in figure 5.2.

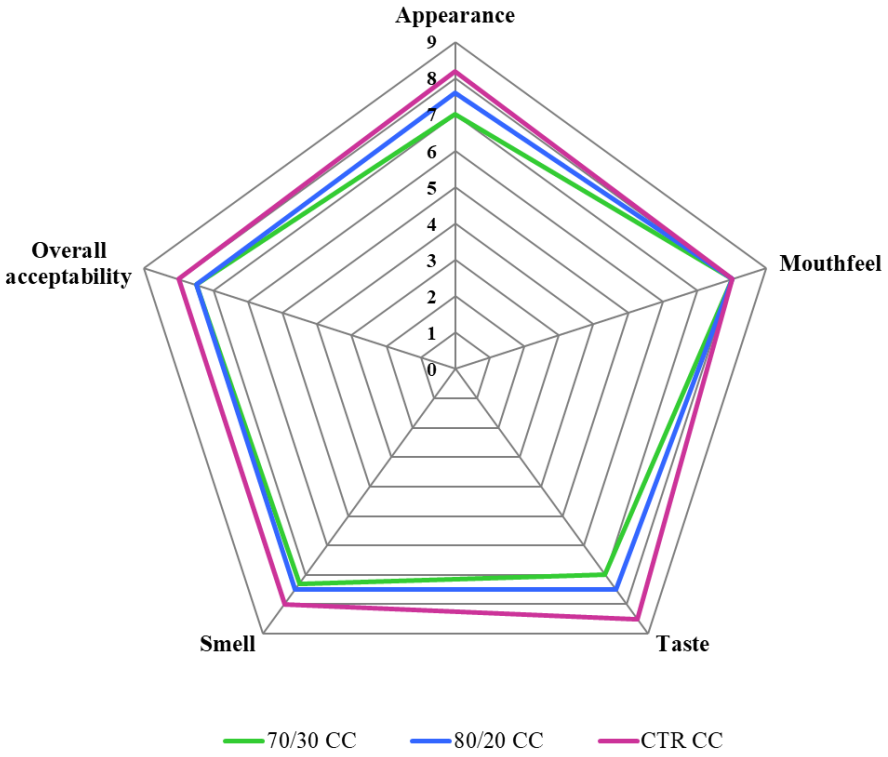

Figure 5.2 Sensorial profile of couscous samples

From obtained results it emerges that all three products have an overall acceptability score between 7 and 8 and, although CTR couscous was more appreciated than the functional couscous, both the products added with barley coarse fraction nevertheless proved to have a very good mouthfeel and a consistency comparable to that of semolina couscous resulting very pleasant products. In detail the 70/30 couscous presented a lower score for appearance and taste, certainly due to the barley fiber which gives to the product a darker color and a slightly bitter taste. However, this outcome does not penalize this type of product since the consumer is already accustomed to purchasing fibre-rich products, identifying this feature by darker colour and a different flavor.

Overall, all the tasters appreciated the innovative products reporting, in the margin notes of the scorecard, that they would have been willing to buy the product enriched in ß-glucans.

5.5 Economic evaluation of functional couscous

From a market investigation, lead on a sample of 20 couscous, it emerged that semolina couscous available on the market, have a variable cost from 1.99 to 5.02 €/kg with an average value of 3.50 €/kg. Moreover, in order to know the wholesale price of ß-glucans enriched barley flour, a further survey at local and neighbouring retailers was carried out, resulting an average value of 1.30 €/Kg. Finally, considering an average wholesale cost of semolina of 0.40 €/Kg (www.ismeamercati.it), a semolina replacement with 30% of enriched flour would lead to an increase in the raw material cost of about 0.27 €/kg.

The information obtained by the companies in the sector in question reveals that, as regards the costs of the commercial semolina couscous, the raw material affects the final cost of the product for a percentage equal to about 18%. Furthermore, the price of the finished product is also influenced by production costs and therefore increases. Assuming, for the functional couscous, both the same price increases and the same production costs of the classic couscous, the cost of the new product proposed is influenced only by the costs of the raw materials and therefore it could have a final price for the consumer of about 5.80 €/kg.

Actually, commercial couscous produced with other cereals (rice, corn, kamut and spelt) and/or legumes (chickpeas and lentils), which are claimed to have beneficial properties related to the absence of gluten or the presence of fibre and proteins, have an average cost of about 7.90 €/kg. With these assumptions, the couscous made in this experimentation, that shows a clear nutritional and functional value, could lead to a higher profit than the budgeted € 5.80/kg, with significant benefits for the company that would produce and market it.

5.6 Conclusion

The functional couscous boasts healthy attributes imparted by the presence of ß-glucans and fibre, while maintaining simplicity and versatility typical of traditional couscous. The quantities of fibre (at least 6 g/100 g) and ß-glucans (at least 1 g per served portion) are compatible with the provisions of the EC Reg. 1924/2006 and the EU Reg. 432/2012, fulfilling the nutritional and health claims related to bioactive compounds. Moreover, the innovative products exhibit good cooking qualities, and therefore, their position in the market of functional products is conceivable, with economic advantages for the companies that would produce and commercialize functional couscous enriched in barley ß-glucans.

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Chapter 6: ASSESSMENT OF PHYTOCHEMICAL COMPOUNDS IN FUNCTIONAL COUSCOUS: DETERMINATION OF FREE AND BOUND PHENOLS AND ALKYLRESORCINOLS

Oriente, M., Díaz-de-Cerio, E., Verardo, V., Messia, M.C., Gómez-Caravaca, A.M., Marconi, E. (2020)
Assessment of phytochemical compounds in functional couscous: determination of free and bound phenols and alkylresorcinols
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ABSTRACT

The aim of this study was to evaluate the content of free and bound phenols and alkylresorcinols in functional couscous enriched with barley coarse fraction obtained by air classification. Two different levels of enriched barley flour (20 and 30 %) were used for the formulation of couscous and they were compared with a control couscous made with 100% of semolina. HPLC-ESI-TOF-MS was used to determine the phenolic composition in couscous samples. Total free phenolic compounds content in control couscous was 182 μg/100g d.m.. This amount increases significantly when coarse barley flour is added reaching value of 2273 μg/100g d.m. and 2978 μg/100g d.m. when 20 and 30 % of barley coarse fraction was used, respectively. Moreover, the main free phenolic compounds in enriched barley samples are represented by flavan-3-ols. Bound phenols ranged from 5242 μg/100g d.m. for control couscous to 27092 μg/100g d.m. for couscous with 30% of barley coarse fraction with a strong prevalence of phenolic acids. Finally, regarding alkylresorcinol compounds, they ranged from 1.01 mg/g d.m. for control couscous to 2.46 mg/g d.m. for couscous with 30% of barley coarse fraction. In conclusion, on the basis of obtained results, barley coarse fraction can be considered a good ingredient to develop functional couscous naturally enriched of phenolic compounds and alkylresorcinols.

Keywords:

waxy barley, enriched barley flour, air classification, phenolic compounds, bioactive, compounds, cereal based food

6.1 Introduction

Couscous is one of the most ancient traditional food developed by the native inhabitants of North Africa. It has traditionally been prepared by mixing semolina and water in a large wooden dish and rolling the two ingredients by hand until agglomeration occurs and couscous granules are formed. Successively, in order to have granules uniformed both in the shape and size, raw couscous is sieved and finally steamed and sun-dried (Debbouz and Donnelly, 1996). It was not until the mid 1970's that fully automated couscous production lines started in North Africa, and later in other parts of the world, like France, Italy, Greece and more recently in the United States.

Nowadays couscous, thanks to globalization and migration, is losing its connotation as typical product of the Arab culture and it is increasingly appreciated in Europe and on an international level (Abecassis et al., 2012), for its simplicity in production, versatility and convenience and because of the growing popularity of healthier eating and the "Mediterranean diet" (Messia et al., 2019). Couscous, in fact, is characterized by a reduced fat content and a low glycemic index (D'Egidio and Pagani, 2010), moreover it is a based cereal food and it is know that cereals contain a wide range of phenolic compounds including benzoic and cinnammic acids, anthocyanidins, quinones, flavonols, chalcones, flavanones and amino phenolics, (Andreasen et al., 2000; Lloyd et al., 2000), which have positive effects on human health. However, the amount of polyphenols in cereals is highly variable in whole grain and refined grain and it also depends on the cereal variety and milling procedure (Adom et al., 2005).

In the last years, industrialized countries, have increasingly focused the attention on healthier eating (AgrifoodMonitor, 2017) and this, together the continuous consumer request for new foods such as novel (Lezaun and Schneider, 2012), functional (Ozen et al., 2012), gluten-free (Saturni et al., 2010) and whole grain food (Slavin, 2004), has encouraged the couscous industry to produce and market products where durum wheat has been partially or totally replaced by

other cereals, pseudocereals or legume flours but preserving traditional shapes (Marconi and Carcea, 2001; Schoenlechner, 2016).

Among cereals, barley (*Hordeum vulgare* L.) is well suited to development of functional foods thanks to the presence of bioactive compounds, such as dietary fiber and phenolic compounds (Newman and Newman, 2008), which are recognized for the important role they play in the reduction of the risk of cardiovascular disease. Phenolic compounds are characterized from one or more aromatic rings with one or more hydroxyl groups (Adom and Liu, 2002) and they have a wide range of health benefits, mainly due to their antioxidant properties, such as reactive oxygen species scavenging and inhibition, electrophile scavenging and metal chelation (Randhir et al., 2004). However, phenolic compounds are known especially for the capacity to contrast formation of free radicals (Abdel-Aal et al., 2012) and for their involvement in the inhibition of human LDL cholesterol oxidation (Abdel-Aal and Gamel, 2008), but also for their anti-inflammatory antioxidant, anticarcinogenic effects and anti-proliferative potential (Kaliora et al., 2014; Sies et al., 2005). Phenolic compounds can be found free or bound to fibre. Free phenolic fraction of barley is constituted especially by flavanols that are found in their monomeric form as catechin and epicatechin or in their polymeric chain as proanthocyanidins (Gangopadhyay et al., 2016), while phenolic acids, like ferulic, coumaric and vanillic acids, are the main compounds of the bound phenolic fraction (Abdel-Aal et al., 2012; Holtekjølen et al., 2006). Phenolic acids are the main phenolic compounds in cereal grains, such as barley and wheat (Abdel-Aal et al., 2012; Adom and Liu, 2002) and they are present primarily in the outer layers of the cereal kernels. Ferulic acid and its dehydrodimer derivatives are the major phenolic compounds in cereals present mainly in bound form, which is ester linked to the cell wall in the outer layers of the grain kernels (Hernanz et al., 2001) constituting the bran. Following the pearl process, these layers are removed and this significantly reduce the antioxidant capacity of whole grains (Baik and Ullrich, 2008; Blandino et al., 2015).

A particular class of phenolic compounds is represented by alkylresorcinols which are one of the major groups of phenolic compounds in wheat, rye and barley grains where they are mainly located in the bran part of the caryopses (Gunenc et al., 2013; Giambanelli et al., 2018) and have been indicated as potential markers of whole grain intake. However whole-grain foods (wheat, rye, and barley) that are rich in dietary fiber contain also good amounts of alkylresorcinols. Therefore, alkylresorcinols and their metabolites may also be used as biomarkers of cereal fiber intake (Landberg et al., 2014).

The interest towards these compounds is due to their antioxidant, antimicrobial and antifungal properties, and their effects on biological membranes due to their amphiphilic nature as well as anticancer activity and the inhibition of different enzymes (Kozubek and Demel, 1981; Ross et al., 2004). These phytochemicals are resorcinolic lipids including very simple homologues of the orcinol-type (1,3-dihydroxy-5-methylbenzene) phenols and a variety of homologues with a dual character, aromatic and acylic. They are synthesised by the addition of an alk(en)yl chain of different length (i.e., C15:0, C17:0, C19:0, C21:0, C23:0, C25:0) to position 5 of 1,3-dihydroxybenzene (Andersson et al., 2008).

Recently, Messia et al. (2019), produced an innovative functional couscous using a mix of semolina and ß-glucans enriched barley flour obtaining a product that shows higher concentration of total dietary fibre and ß-glucans together a lowest starch content, compared to couscous made from 100% semolina flour. Moreover, during couscous preparation, it was possible to verify that the presence of the barley fraction did not cause problems with agglomeration and the formation of couscous grains and the innovative products exhibit improved nutritional value beyond that good cooking quality.

However, information about the effect of barley addition on couscous composition is scarce; because of that, the aim of this work was to study the influence of the incorporation of coarse

barley flour obtained by air classification on the concentration of free and bound phenolic compounds and alkylresorcinols in couscous.

6.2 Materials and methods

6.2.1 Reagents and chemicals

HPLC-grade acetonitrile, ethanol and methanol were furnished by Labscan (Dublin, Ireland). Acetic acid analytical grade (assay>99.5%) was purchased from Fluka (Switzerland). Water was obtained by a Milli-Q system (Millipore, Bedford, USA). Vanillic and ferulic acid, (+)-catechin, procyanidin B3 and vitexin from Sigma-Aldrich (St. Louis, MO) were used for the calibration curves (table 6.1).

Table 6.1 Analytical parameters of the method

Standard	Equation	R^2	Linear range (mg/L)	L.O.D (mg/L)	L.O.Q. (mg/L)
Vanillic acid	y = 1230.2 X + 12426	0.9985	LOQ -100	0.028	0.09
Ferulic acid	y = 8823 X + 3884.5	0.9994	LOQ -100	0.036	0.12
Catechin	y = 54901 X + 5119.2	0.9993	LOQ -100	0.030	0.10
Procyanidin B_3	y = 15962 X + 60507	0.9974	LOQ -100	0.042	0.14
Vitexin	y = 24149.3 X + 1760.1	0.9991	LOQ -100	0.025	0.08

The limits of detection (L.O.D.) and quantification (L.O.Q.) were calculated as the concentration corresponding to 3 and 10 times, respectively, the standard deviation of the background noise.

6.2.2 Samples

Control couscous (100% semolina) and two enriched couscous samples obtained with semolina and different percentage of barley flour enriched in ß-glucan (20 and 30 %) were developed at the Department of Agricultural, Environmental and Food Sciences at the University of Molise, Campobasso, Italy according to Messia et al. (2019). Durum wheat semolina was used to

formulate the control couscous (CTR CC). To formulate functional couscous, 20 and 30 % w/w of semolina was replaced with air classified barley coarse fraction obtaining 80/20 couscous (80/20 CC) and 70/30 couscous (70/30 CC).

Development of barley coarse fraction by air classification

To produce the barley coarse fraction, a waxy barley (variety CDC Alamo) was subjected to air classification process as reported by Messia et al. (2019).

Air classification is a physical system which separates flour components by a combination of particles size, mass and density using an air flow. At the end of the process, the flour is separate into two portions: a coarse fraction (CF) and a fine fraction (FF).

Couscous preparation

Couscous was produced by Messia et al. (2019) using the handmade traditional procedure.

6.2.3 Extraction of free and bound phenolic compounds from couscous samples

Free and bound phenolic fraction from couscous samples was determined according to Verardo et al. (2011a) and Verardo et al., (2011b) method. Briefly, for free phenolic fraction, 3 g of samples was extracted with 30 mL of ethanol/water (4:1 v/v) for 15 minutes in an ultrasonic bath. The extraction was repeated twice and the organic fraction were pooled, evaporated and reconstituted with 2 mL of methanol/water (1:1 v/v). The extracts were stored at -18°C until use.

To extract the bound phenolic fraction, residues of free phenolic extraction were digested with 100 mL of 1 mol/L NaOH at room temperature overnight by shaking under nitrogen gas. The hydrolysed solution was acidified to pH 2 using hydrochloric acid in a cooling ice bath and was extracted twice with ethyl acetate. The organic fractions were pooled and evaporated to dryness. The phenolic compounds were reconstituted in 2 mL of methanol/water (1:1 v/v).

The extracts were filtered through nylon filters with 0.20 µm before HPLC injection.

6.2.4 HPLC-ESI –TOF-MS analysis of phenolic compounds

A liquid chromatography apparatus ACQUITY UPLC M-Class System from Waters (Waters Corp., Milford, MA, USA), including a degasser, a binary pump delivery system and an automatic liquid sampler, was used and coupled to time of flight-mass spectrometer detector (SYNAP G2 from Waters). The HPLC column was a fused-core Poroshell 120, SB-C18 (3.0×100 mm, 2.7 μm) from Agilent Technologies (Agilent Technologies, Palo Alto, CA, USA). The mobile phase and gradient program were used as previously described by Gomez-Caravaca et al., (2014). All solvents were filtered with a 0.45 mm filter disk. Parameters for MS analysis were set using negative ion mode with spectra acquired over a mass range from m/z 50-1100. Phenolic compounds have been quantified according to the relative standards or other with similar structure when not available. Briefly, hydroxybenzoic derivatives were quantified as vanillic acid equivalent, hydroxycinnamic derivatives were quantified as ferulic acid equivalent, catechin and it glucosides were quantified using catechin standard, procyanidins were quantified as procyanidin B_3 equivalent, flavones derivatives were quantified as vitexin equivalent derivatives.

6.2.5 Determination of alkylresorcinols in couscous samples

Alkylresorcinols extract was obtained as reported by Ross (2012). Briefly, 3 g of couscous were extracted in an ultrasonic bath with 30 mL of ethyl acetate for 30 minutes. After centrifugation at 3500 rpm for 10 minutes the supernatants were collected, evaporated and reconstituted with 1 mL of methanol. The extracts were stored at -18°C until use. All samples were centrifuged at 3.500 rpm for 10 minutes before HPLC injection. The analyses were performed using an ACQUITY UPLC M-Class System from Waters (Waters Corp., Milford, MA, USA) coupled to a FLD. The excitation wavelength was 276 nm and the emission wavelength was 306 nm.

The column (Poroshell 120, SB-C18 (3.0×100 mm, 2.7 μm) from Agilent Technologies) was maintained at 60 °C during the analysis.

6.2.6 Statistical analysis

The results reported in this study are the averages of three repetitions. Significance differences by Tukey test were evaluated using Statistica 6.0 (2001, StatSoft, Tulsa, OK).

6.3. Results and discussion

6.3.1 Determination of free phenolic compounds in couscous samples

Table 6.2 shows the MS data of free phenolic compounds identified by HPLC-ESI-TOF-MS in couscous samples. This table includes compound, retention time (min), molecular formula, m/z experimental, m/z calculated and error (ppm).

Thirty-one phenolic compounds have been identified. About phenolic acids, a compound (compound 9) with m/z 167 and molecular formula $C_8H_8O_4$ was identified, according to Dinelli et al. (2009), as vanillic acid; its identity was confirmed by co-elution with an analytical standard. Moreover, *trans* and *cis* ferulic acid (compounds 16 and 17) were identified according to several authors (Gomez-Caravaca et al., 2014; Dinelli et al., 2009; Verardo et l., 2011c) and by co-elution with analytical standard. Nine flavan-3-ols compounds have been noticed in enriched samples; as reported in previous studies, barley contains these kinds of flavonoids (Verardo et al., 2011b; Gómez-Caravaca et al., 2014; Verardo et al., 2011c; Gómez-Caravaca et al., 2015). Two prodelphinidin trimers with m/z 593 and molecular formula $C_{30}H_{26}O_{13}$ were identified according to several authors (Gómez-Caravaca et al., 2014; Gómez-Caravaca et al., 2015). Catechin and epicatechin were identified by co-elution with their respective analytical standards and according to their mass data. Two compounds (2 and 5) with molecular formula $C_{27}H_{34}O_{16}$ and m/z 613 were assigned to catechin-dihexoside and its presence in barley was

noticed by Gangopadhyay et al., (2016). Moreover, compounds 4, 6 and 7 were identified as catechin-3-glucoside, procyanidin B3 and procyanidin trimer, respectively and they were previously described in barley (Gangopadhyay et al., 2016; Verardo et al., 2011c; Gómez-Caravaca et al., 2015).

Finally, nineteen flavone-glycoside compounds were described. Among them several apigenin-derivatives were identified; compounds 10, 11, 12, 19, 21, 22 and 23 showed a molecular ion at 593 m/z and a molecular formula $C_{27}H_{30}O_{15}$; according to Dinelli et al. (2009) they were assigned to apigenin-pentoside-hexoside isomers. According to the same authors (Dinelli et al., 2009) compounds 15, 18 and 20 were identified as apigenin-6-C-arabinoside-8-C-hexoside isomers (shaftoside/isoshaftoside). Compound 26 with m/z 431 was assigned to vitexin/isovitexin and compound 27 with m/z 577 and molecular formula $C_{27}H_{30}O_{14}$ was attributed to isovitexin-2''-O-rhamnoside (Dinelli et al., 2009). Two compounds (13 and 14) with m/z 579 and molecular formula $C_{26}H_{28}O_{15}$ were identified as luteolin derivatives named lucenin-1/3 (luteolin-6/8-C-xyloside-8/6-C-glucoside) according to Dinelli et al. (2009). According to the same authors, another luteolin derivative was found (compound 28) and it was assigned to methylisoorientin-2''-O-rhamnoside. Compounds 24 and 25 were identified as flavones-O-glycoside; they reported a molecular ion at 533 and a molecular formula $C_{25}H_{26}O_{13}$, according to Dinelli et al. (2009) they were assigned to isomers of glycosylated and acetylated 3',4',5'-trihydroxy-3,7-dimethylflavone. Finally, two additional flavones (compound 29 and 30) were detected with mass 491 and deduced molecular formula $C_{23}H_{24}O_{12}$; they were tentatively identified as of 3',4',5,-trihydroxy-3,7-dimethylflavone glycosylated forms.

In table 6.3 is reported the free phenolic content of couscous samples. Thirty-one free phenolic compounds were quantified in couscous sample, among them only ten were determined in control couscous which is made with 100% semolina, this mean that the remaining twenty-one compounds derive from barley or they are lost or "blocked" in the matrix during the

technological process. Phenolic acids such as vanillic acid and ferulic acid, represent, about the 47% of total free phenolic compound. The residual 53% is represented by flavonoids, belonging to the group of flavones, such as apigenin, luteolin derivatives.

Overall the final amount of free phenolic compound is 182 μg/100g d.m. in control couscous; this amount increases significantly when enriched barley flour is added reaching the value of 2273 and 2978 μg/100g d.m. for 80/20 couscous and 70/30 couscous, respectively. These data underline that the barley coarse fraction used during the couscous formulation increased from 11 to 15 times the final content of free phenolic compounds compared to the control. These values apparently disagree with data reported by Carcea et al., (2017) that report for semolina couscous and barley couscous value of free phenols of 80.4 mg/100g d.m. and 476.2 mg/100g d.m. respectively, that are much higher than those reported in this work, in terms of magnitude. However, these differences could be due to different wheat and barley varieties but above all to the different method used for phenolic determination (HPLC-MS versus Folin-Ciocalteu procedure). Nevertheless, the trend is the same in fact, also Carcea et al. (2017) show a higher content of free polyphenols in barley couscous with increments of over 11 times compared to the semolina couscous. In this work this increase is due to the barley contribution of flavonoids such as flavan-3-ols that represent about 88% of total free phenolic compound for 80/20 couscous and about 89% of total free phenolic compound for 70/30 couscous increasing according to the increase of barley flour percentage. This trend is in accordance with what is reported by Verardo et al., (2011c) and Verardo et al., (2011d) that describe the highest amount of flavan-3-ols in the barley coarse fraction respect fine fraction and whole barley flour.

Among flavan-3-ols the most abundant are catechin and epicatechin ranging from 209 μg/100g d.m. for 80/20 couscous to 267 μg/100g d.m. for 70/30 couscous, and from 1034 μg/100g d.m. for 80/20 couscous to 1348 μg/100g d.m. for 70/30 couscous, respectively. Similar trend was reported by catechin-glucosides and prodelphinidins. Rao et al. (2018) found catechin-5-O-

glucoside and prodelphinidin B3 as the most abundant polyphenols in the seven Australian barley varieties followed by caffeic acid while, other studies by Hao and Beta (2012), Quinde-Axtell and Baik (2006), Yoshida et al. (2010) have reported ferulic acid to be the most abundant phenol present in barley. These differences can be attributed to factors such as variety, environment and extraction procedure.

With regards to individual free phenolic compound the most abundant, present in all sample, is vanillic acid which content is grater in barley-based couscous. Shaftoside/isoshaftoside is another main free phenolic compound and it is generally higher in control than enriched samples because it is present in wheat. Briefly, phenolic acids were higher in enriched samples than control thanks to the use of barley coarse fraction; among the enriched samples, the total phenolic acids increased according to the amount of barley coarse fraction added in couscous.

Table 6.2 MS data of free phenolic compounds identified by HPLC-ESI-TOF-MS in couscous samples

No	Compound	Retention time (min)	Molecular formula	m/z experimental	m/z calculated	Error (ppm)
1	Prodelphinidin dimer	3.83	$C_{30}H_{26}O_{13}$	593.1298	593.1295	0.5
2	Catechin dihexoside	4.88	$C_{27}H_{34}O_{16}$	613.1761	613.1769	-1.3
3	Catechin	4.93	$C_{15}H_{14}O_6$	289.0696	289.0712	-5.5
4	Catechin-3-glucose	5.04	$C_{21}H_{24}O_{11}$	451.1230	451.1240	-2.2
5	Catechin dihexoside	5.26	$C_{27}H_{34}O_{16}$	613.1774	613.1769	0.8
6	Procyanidin B3	5.96	$C_{30}H_{26}O_{12}$	577.1344	577.1346	-0.3
7	Procyanidin trimer	6.23	$C_{45}H_{38}O_{18}$	865.1988	865.1980	0.9
8	Epicatechin	6.30	$C_{15}H_{14}O_6$	289.0697	289,0712	-5.2
9	Vanillic acid	6.53	$C_8H_8O_4$	167.0335	167.0344	-5.4
10	Apigenin-pentoside-hexoside	7.84	$C_{27}H_{30}O_{15}$	593.1517	593.1506	1.9
11	Apigenin-pentoside-hexoside	9.63	$C_{27}H_{30}O_{15}$	593.1517	593.1506	1.9
12	Apigenin-pentoside-hexoside	9.75	$C_{27}H_{30}O_{15}$	593.1481	593.1506	-4.2
13	Lucenin 1/3	9.91	$C_{26}H_{28}O_{15}$	579.1359	579.1350	1.6
14	Lucenin 1/3	10.09	$C_{26}H_{28}O_{15}$	579.1379	579.1350	5.0
15	Shaftoside/isoshaftoside	10.37	$C_{26}H_{28}O_{14}$	563.1387	563.1401	-2.5
16	*trans* ferulic acid	10.50	$C_{10}H_{10}O_4$	193.0504	193.0501	1.6
17	*cis* ferulic acid	10.77	$C_{10}H_{10}O_4$	193.0489	193.0501	-6.2
18	Shaftoside/isoshaftoside	11.03	$C_{26}H_{28}O_{14}$	563.1406	563.1401	0.9
19	Apigenin-pentoside-hexoside	11.40	$C_{27}H_{30}O_{15}$	593.1492	593.1506	-2.4
20	Shaftoside/isoshaftoside	11.45	$C_{26}H_{28}O_{14}$	563.1413	563.1401	2.1
21	Apigenin-pentoside-hexoside	11.73	$C_{27}H_{30}O_{15}$	593.1509	593.1506	0.5
22	Apigenin-pentoside-hexoside	11.76	$C_{27}H_{30}O_{15}$	593.1540	593.1506	5.7
23	Apigenin-pentoside-hexoside	11.80	$C_{27}H_{30}O_{15}$	593.1509	593.1506	0.5
24	Glycosylated Acetylated flavone isomer	12.23	$C_{25}H_{26}O_{13}$	533.1293	533.1295	-0.4
25	Glycosylated Acetylated flavone isomer	12.27	$C_{25}H_{26}O_{13}$	533.1293	533.1295	-0.4
26	Vitexin/isovitexin isomer	12.39	$C_{21}H_{20}O_{10}$	431.0960	431.0978	-4.2
27	Isovitexin-2-O-rhamnoside	14.08	$C_{27}H_{30}O_{14}$	577.1547	577.1557	-1.7
28	Methylisoorientin-2-O-rhamnoside isomer	14.55	$C_{28}H_{32}O_{15}$	607.1669	607.1663	1.0
29	Glycosylated 3,4,5-trihydroxy-3,7,dimethylflavone isomer	15.17	$C_{23}H_{24}O_{12}$	491.1190	491.1190	0.0
30	Glycosylated 3,4,5-trihydroxy-3,7,dimethylflavone isomer	15.80	$C_{23}H_{24}O_{12}$	491.1190	491.1190	0.0
31	Prodelphinidin B3	23.73	$C_{30}H_{26}O_{13}$	593.1270	593.1295	-4.2

Table 6.3 Content of free phenolic compounds and relative classes in couscous samples (μg/100g d.m.)

PHENOLIC COMPOUND	CTR CC	80/20 CC	70/30 CC
Prodelphinidin dimer	N.D.	60.9 ± 0.4^{a}	119 ± 5^{b}
Catechin dihexoside	N.D.	102 ± 9^{a}	144 ± 14^{b}
Catechin	N.D.	209 ± 3^{a}	267 ± 3^{b}
Catechin-3-glucose	N.D.	251.5 ± 0.6^{a}	323 ± 2^{b}
Catechin dihexoside	N.D.	15 ± 2^{a}	19.1 ± 0.6^{b}
Procyanidin B3	N.D.	299 ± 21^{a}	413 ± 7^{b}
Procyanidin trimer	N.D.	8.8 ± 0.10^{a}	9.413 ± 0.004^{b}
Epicatechin	N.D.	1034 ± 38^{a}	1348 ± 5^{b}
Vanillic acid	85.4 ± 0.8^{a}	129 ± 8^{b}	133.9 ± 0.6^{b}
Apigenin-pentoside-hexoside	N.D.	$3.15 \pm 0,02^{a}$	5.1 ± 0.2^{b}
Apigenin-pentoside-hexoside	0.4143 ± 0.0004^{a}	0.46 ± 0.03^{a}	1.27 ± 0.05^{b}
Apigenin-pentoside-hexoside	1.22 ± 0.06^{a}	0.93 ± 0.10^{a}	1.2 ± 0.2^{a}
Lucenin 1/3	N.D.	< L.O.Q.	< L.O.Q.
Lucenin 1/3	1.56 ± 0.07^{a}	2.2 ± 0.2^{b}	$1.76 \pm 0.09^{a,b}$
Shaftoside/isoshaftoside	24.85 ± 0.72^{a}	18.5 ± 0.6^{b}	20.23 ± 0.05^{b}
trans ferulic acid	N.D.	13 ± 2^{a}	37 ± 5^{b}
cis ferulic acid	N.D.	15 ± 2^{a}	18.5 ± 0.8^{b}
Shaftoside/isoshaftoside	12.90 ± 0.04^{a}	22.9 ± 0.6^{b}	25.6 ± 0.7^{c}
Apigenin-pentoside-hexoside	N.D.	11.79 ± 0.05^{a}	16.9 ± 0.6^{b}
Shaftoside/isoshaftoside	54 ± 4^{a}	$49.09 \pm 0.07^{a,c}$	$44 \pm 2^{b,c}$
Apigenin-pentoside-hexoside	0.40 ± 0.03^{a}	1.87 ± 0.05^{b}	1.7 ± 0.1^{b}
Apigenin-pentoside-hexoside	0.90 ± 0.06^{a}	1.9 ± 0.2^{b}	2.42 ± 0.08^{c}
Apigenin-pentoside-hexoside	0.15 ± 0.03^{a}	$0.3 \pm 0,1^{a}$	0.4 ± 0.1^{a}
Glycosylated Acetylated flavone isomer	N.D.	0.892 ± 0.002^{a}	0.606 ± 0.003^{b}
Glycosylated Acetylated flavone isomer	N.D.	$4.09 \pm 0,30^{a}$	3.7 ± 0.1^{a}
Vitexin/isovitexin isomer	N.D.	0.8 ± 0.2^{a}	0.82 ± 0.07^{a}
Isovitexin-2-O-rhamnoside	N.D.	2.41 ± 0.04^{a}	3 ± 0.1^{b}
Methylisoorientin-2-O-rhamnoside isomer	N.D.	2.4 ± 0.4^{a}	3.412 ± 0.005^{b}
Glycosylated 3,4,5-trihydroxy-3,7,dimethylflavone isomer	N.D.	2.6 ± 0.1^{a}	3.6 ± 0.2^{b}
Glycosylated 3,4,5-trihydroxy-3,7,dimethylflavone isomer	N.D.	0.187 ± 0.005^{a}	0.44 ± 0.03^{b}
Prodelphinidin B3	N.D.	9.2 ± 0.2^{a}	9.2 ± 0.2^{a}
Total (μg/100g)	**182 ± 6**	**2273 ± 41**	**2978 ± 8**
Total Flavan-3-ols	N.D.	1989.7 ± 45.4	2651.9 ± 6.1
Total Phenolic acids	85.4 ± 0.8	157.2 ± 1.3	189.2 ± 1.8
Total Flavons	96.6 ± 5	126.5 ± 3.0	136.8 ± 0.3

CTR CC: Control couscous made with 100% semolina; 80/20 CC: couscous made with 80% of semolina and 20% of enriched barley flour; 70/30 CC: couscous made with 70% of semolina and 30% of enriched barley flour.
N.D.: not detected; L.O.Q.: limit of quantification.
Analyses were carried out in triplicate. Different letters in the same line indicate significantly different values ($p<0.05$) by ANOVA LSD.

6.3.2 Determination of bound phenolic compounds in couscous samples

Table 6.4 shows the MS data of bound phenolic compounds identified by HPLC-MS in couscous samples. This table includes compound, retention time (min), molecular formula, m/z experimental, m/z calculated and error (ppm).

Two aldehyde derivatives named vanillic and benzoic aldehyde were identified according to Dinelli et al. (2009). As expected, few flavonoids have been detected in bound form; among them catechin, shaftoside/isoshaftoside, and vitexin/isovitexin were identified according to their mass data and previous works (Gómez-Caravaca et al., 2014; Dinelli et al., 2009; Verardo et al., 2011c; Gómez-Caravaca et al., 2015). Compound 15 reported a molecular formula $C_{26}H_{32}O_{12}$ and a molecular ion at 535 m/z; according to Dinelli et al., (2009) it was identified as a stilbene named pinosylvin. Two hydroxybenzoic acid derivatives were also noticed in bound form; thus vanillic and syringic acid were found according to other authors (Dinelli et al., 2009; Montevecchi et al., 2019). Compounds 7 reported a molecular ion at 223 m/z and according to Dinelli et al. (2009) it was identified as sinapic acid; compounds 12 and 13 showed the same molecular ion because of that, they were assigned as sinapic acid derivatives. Compound 14 showed a molecular ion at 401 m/z, moreover it reported a molecular formula $C_{22}H_{22}O_{10}$ corresponding to $[M\text{-}H\text{-}CO_2]^-$, thus it was identified as disinapic acid (Grúz et al., 2015).

Several ferulic derivatives were also described; compounds 9 and 10 showed a molecular formula $C_{10}H_{10}O_4$ and a molecular ion at 193 m/z, because of that, these compounds were identified as *trans* and *cis* ferulic acid, respectively (Gómez-Caravaca et al., 2014; Verardo et al., 2011c; Gómez-Caravaca et al., 2015). Three compounds (2, 18 and 21) with m/z 577 and molecular formula $C_{30}H_{26}O_{12}$ were identified as dihydrotriferulic acid (Jilek and Bunzel, 2013). Compounds 16, 17, 19 and 20 reported a molecular ion at m/z 385 and molecular formula

$C_{20}H_{18}O_8$; as previously reported by other authors (Jilek and Bunzel, 2013) they were assigned to diferulic acid.

Twenty-two bound phenolic compounds were found in couscous sample with particular relevance of phenolic acids that represent, in all samples, more than 99% of bound phenolic compounds (table 6.5). The total amount of bound phenolic compound is 5242 μg/100g d.m. for control couscous while, this amount, increases proportionally with the partial substitution of semolina with barley coarse fraction reaching value of 19057 and 27092 μg/100g d.m. for 80/20 and 70/30 couscous, respectively. These data confirm what reported by Lempereur, Surget and Rouau (1998) which they affirm that bound phenols are localized in the external layer (aleurone, bran) of the kernel so, because of their hardness, during milling are crushed in bigger particles, becoming part of coarse fraction. Moreover, these results are comparable with the results obtained by Martini et al. (2017) that reported a higher concentration of bound phenolic acids in micronized product respect semolina obtained from traditional milling process although overall the micronization preserves the natural endowment of total phenolic compound.

As previously mentioned, the main class of bound phenolic compounds in all couscous samples is represented by phenolic acids and among these the most abundant are diferulic acid with a range between 1265 and 21325 μg/100g d.m. and ferulic acid that ranging from 2629 μg/100g d.m. to 4159 μg/100g d.m. with higher value in barley-based couscous followed by sinapic acid (range 411-535 μg/100g d.m.), disinapic acid (range 379-388.8 μg/100g d.m.) and benzoic aldehyde (range 283-391 μg/100g d.m.). Results not entirely similar have been obtained by Nicoletti et al. (2013) which found, on dried pasta sample, ferulic acid and sinapic acid the predominant bound phenolic compounds with average value of 47.55 and 13.20 mg/kg d.m. respectively that, only for ferulic acid are comparable with the values found in this work. Other bound phenolic compounds present in all sample were flavones such as vitexin/isovitexin and

shaftoside/isoshaftoside while, only in barley-based couscous, was found catechin in concentration of 13 μg/100g d.m. for 80/20 couscous and 59.65 μg/100g d.m. for 80/20 couscous.

Table 6.4 MS data of bound phenolic compounds identified by HPLC-ESI-TOF-MS in couscous samples

No	Compound	Retention time (min)	Molecular formula	m/z experimental	m/z calculated	Error (ppm)
1	Vanillic aldehyde	4.56	$C_8H_8O_3$	151.0392	151.0395	-2.0
2	Dihydrotriferulic acid	5.91	$C_{30}H_{26}O_{12}$	577.1373	577.1346	4.7
3	Benzoic aldehyde	6.32	$C_7H_6O_2$	121.0272	121.0290	-4.9
4	Catechin	6.33	$C_{15}H_{14}O_6$	289.0705	289.0712	-2.4
5	Vanilic acid	6.69	$C_8H_8O_4$	167.0327	167.0344	-6.2
6	Syringic acid isomer	7.82	$C_9H_{10}O_5$	197.0441	197.0450	-4.6
7	Sinapic acid	8.02	$C_{11}H_{12}O_5$	223.0569	223.0606	-3.7
8	Shaftoside/isoshaftoside	10.38	$C_{26}H_{28}O_{14}$	563.1407	563.1401	1.1
9	*trans* ferulic acid	10.78	$C_{10}H_{10}O_4$	193.0492	193.0501	-4.7
10	*cis* ferulic acid	10.90	$C_{10}H_{10}O_4$	193.0477	193.0501	-7.4
11	Shaftoside/isoshaftoside	11.05	$C_{26}H_{28}O_{14}$	563.1411	563.1401	1.8
12	Sinapic acid	11.34	$C_{11}H_{12}O_5$	223.0581	223.0606	-6.2
13	Sinapic acid	11.39	$C_{11}H_{12}O_5$	223.0593	223.0606	-5.8
14	Disinapic acid	11.79	$C_{22}H_{22}O_{10}$	401.1233	401.1236	-0.7
15	Pinosylvin	12.17	$C_{26}H_{32}O_{12}$	535.1822	535.1816	1.1
16	Diferulic acid	12.18	$C_{20}H_{18}O_8$	385.0924	385.0923	0.3
17	Diferulic acid	13.40	$C_{20}H_{18}O_8$	385.0915	385.0923	-2.1
18	Dihydrotriferulic acid	14.29	$C_{30}H_{26}O_{12}$	577.1339	577.1346	-1.2
19	Diferulic acid	16.61	$C_{20}H_{18}O_8$	385.0911	385.0923	-3.1
20	Diferulic acid	17.58	$C_{20}H_{18}O_8$	385.0903	385.0923	-5.2
21	Dihydrotriferulic acid	18.92	$C_{30}H_{26}O_{12}$	577.1324	577.1346	-3.8
22	Vitexin/isovitexin	19.79	$C_{21}H_{20}O_{10}$	431.0975	431.0978	-0.7

Table 6.5 Content of bound phenolic compounds and relative classes in couscous samples (µg/100g d.m.)

PHENOLIC COMPOUND	**CTR CC**	**80/20 CC**	**70/30 CC**
Vanillic aldehyde	< L.O.Q.	< L.O.Q.	< L.O.Q.
Dihydrotriferulic acid	N.D.	13 ± 0.4^{a}	42 ± 2^{b}
Benzoic aldehyde	391 ± 20^{a}	300 ± 7^{b}	283 ± 6^{b}
Catechin	N.D.	13 ± 2^{a}	59.65 ± 0.01^{b}
Vanillic acid	80 ± 6^{a}	141 ± 3^{b}	137 ± 2^{b}
Syringic acid isomer	83 ± 7^{a}	$87 \pm 0.5^{a,b}$	97.7 ± 1.0^{b}
Sinapic acid	N.D.	40 ± 0.7^{a}	50 ± 5^{b}
Shaftoside&Isoshaftoside	0.14 ± 0.02	< L.O.Q.	N.D.
trans ferulic acid	1676 ± 31^{a}	2267 ± 54^{b}	2683 ± 28^{c}
cis ferulic acid	953 ± 36^{a}	1197 ± 28^{b}	1476 ± 24^{c}
Shaftoside/isoshaftoside	0.9 ± 0.1^{a}	< L.O.Q.	0.11 ± 0.02^{b}
Sinapic acid	268 ± 8^{a}	352 ± 13^{b}	352 ± 17^{b}
Sinapic acid	143 ± 4^{a}	116.0 ± 0.5^{b}	133 ± 6^{a}
Disinapic acid	379 ± 4^{a}	395.8 ± 0.6^{b}	388.8 ± 0.2^{b}
Pinosylvin	N.D.	< L.O.Q.	< L.O.Q.
Diferulic acid	N.D.	147 ± 7^{a}	197 ± 2^{b}
Diferulic acid	75 ± 5^{a}	417 ± 6^{b}	544 ± 7^{c}
Dihydrotriferulic acid	N.D.	11.8 ± 0.2^{a}	16 ± 2^{b}
Diferulic acid	939 ± 30^{a}	12242 ± 587^{b}	18974 ± 1699^{c}
Diferulic acid	251 ± 5^{a}	1168 ± 45^{b}	1610 ± 11^{c}
Dihydrotriferulic acid	N.D.	149 ± 13^{a}	49 ± 2^{b}
Vitexin/isovitexin	0.2 ± 0.1^{a}	0.22 ± 0.10^{b}	0.30 ± 0.06^{a}
Total (µg/100g)	**5242 ± 155**	**19057 ± 715**	**27092 ± 1763**
Total Flavan-3-ols	N.D.	12.8 ± 1.6	59.7 ± 0.01
Total Phenolic acids	5240.4 ± 155	19043.4 ± 716.9	27032.4 ± 1762.7
Total Flavons	1.22 ± 0.25	0.25 ± 0.10	0.41 ± 0.09

CTR CC: Control couscous made with 100% semolina; 80/20 CC: couscous made with 80% of semolina and 20% of enriched barley flour; 70/30 CC: couscous made with 70% of semolina and 30% of enriched barley flour.
N.D.: not detected; L.O.Q.: limit of quantification.
Analyses were carried out in triplicate. Different letters in the same line indicate significantly different values ($p<0.05$) by ANOVA LSD.

6.3.3 Determination of alkylresorcinols in couscous samples

In table 6.6 is reported the concentration of alkylresorcinols in the samples of couscous analysed. The obtained result shows that, among alkylresorcinol, C23:0 and C25:0 increase with the use of higher percentage of barley flour while, C19:0 and C21:0 do not show great difference between the two barley-based couscous although their content is higher than the control. According to Ross (2012) these lipidic compounds are higher in barley than in wheat, thus their increase is justified.

Regarding individual alkylresorcinol compounds, the most abundant, in experimental couscous are C25:0 (range 0.603-0.96 mg/g d.m.) and C19:0 (range 0.51-0.66 mg/g d.m.) followed by C21:0 (range 0.33-0.54 mg/g d.m.), while C21:1 and C23:0 show, for almost all samples, values less than 0.3 mg/g d.m.. About semolina couscous the results are different with what reported by Ciccoritti et al. (2017) which indicate C21:0 as the most abundant alkylresorcinol compound following by C23:0, C19:0 and C25:0 while in this work, control semolina couscous shows lower concentration of C21:0 (0.33 mg/g d.m.) and C23:0 (0.122 mg/g d.m.) and higher concentration of C19:0 (0.51 mg/g d.m.). These differences are probably due to different working process but especially different raw materials, in fact, Ciccoritti et al. (2017) highlight a high variability of alkylresorcinol content amongst different wheat cultivars confirming the importance of genotype and environment on the alkylresorcinol accumulation in grain. Overall the content of alkylresorcinols increased from 1.01 mg/g d.m. for control couscous to 2.46 mg/g d.m. for 70/30 couscous thanks to the addition of barley coarse fraction to refined semolina confirming what reported by Giambanelli et al. (2018) which affirm that all the plants showed similar performance, with higher alkylresorcinol decreases due to the loss of specific parts of kernel, such as germ and bran. Moreover, as previously reported by Gómez-Caravaca et al. (2015), air classified barley coarse fraction showed higher alkylresorcinol amounts than the respectively whole barley flours. In this study, couscous formulated with different percentage

of barley coarse fraction was compared with a control couscous made with 100% semolina. The results confirmed that the partial replacement of semolina with barley coarse fraction allows obtaining a couscous that not only result to be enriched in fiber and ß-glucans, like previously reported by Messia et al. (2019), but also report high amounts of alkylresorcinols.

Table 6.6 Content of alkylresorcinol compounds in couscous samples (mg/g d.m.)

ALKYLRESORCINOL COMPOUND	**CTR CC**	**80/20 CC**	**70/30 CC**
C19:0	0.51 ± 0.04^{a}	0.66 ± 0.03^{b}	$0.597 \pm 0.007^{a,b}$
C21:1	0.053 ± 0.005^{a}	0.1005 ± 0.0006^{b}	0.0857 ± 0.0002^{c}
C21:0	0.33 ± 0.01^{a}	0.528 ± 0.009^{b}	0.54 ± 0.02^{b}
C23:0	0.122 ± 0.003^{a}	0.250 ± 0.002^{b}	0.277 ± 0.007^{c}
C25:0	< L.O.Q.	0.603 ± 0.008^{a}	0.96 ± 0.03^{b}
Total	**1.01 ± 0.06**	**2.14 ± 0.01**	**2.46 ± 0.05**

CTR CC: Control couscous made with 100% semolina; 80/20 CC: couscous made with 80% of semolina and 20% of enriched barley flour; 70/30 CC: couscous made with 70% of semolina and 30% of enriched barley flour.
L.O.Q.: limit of quantification.
Analyses were carried out in triplicate. Different letters in the same line indicate significantly different values ($p<0.05$) by ANOVA LSD.

6.4 Conclusions

The formulation of couscous with barley coarse fraction permitted to introduce the flavan-3-ol compounds, which are not contained in wheat, in couscous; it was demonstrated that this class of compounds is useful for human health, especially on the prevention of metabolic syndrome diseases. Generally, free and bound phenolic content is enormously higher in barley couscous compared to the control. Moreover, also the alkylresorcinol content doubled in the functional couscous.

Barley coarse fraction obtained by air classification is a valuable ingredient to formulate functional couscous. Further work could be developed in order to evaluate the bioactivity of these new products in *in vivo* models.

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GENERAL CONCLUSIONS

The obtained results show that the produced functional couscous contains quantities of fibre (at least 6 g/100g) and ß-glucans (at least 1 g per served portion) that are compatible with the provisions of the EC Reg. 1924/2006 and the EU Reg. 432/2012, thus allowing the assertion of the nutritional claim "Food with high fibre content" and the health claim "Beta-glucans contribute to the maintenance of normal blood cholesterol levels". Moreover, the final product result to be enriched also in free and bound phenolic compounds with a final increase from 11 to 15 times and from 3 to 4 times respectively, compared to semolina couscous; alkylresorcinols are also increased in the functional couscous, with a twice content than the control sample. Thanks to its composition, the developed couscous permits to introduce, in the diet, different compounds useful for human health to help prevention of chronic diseases related to oxidative stress. It is also a product that shows cooking properties comparable with values found for semolina couscous highlighting that the incorporation of coarse fraction in semolina only slightly affects the quality parameters related to couscous rehydration behaviour, preserving the quality of the cooked product resulting, for these reasons, appreciable by consumer. It follows that innovative couscous, can be considered functional products with improved nutritional value and good cooking qualities that preserve simplicity and versatility typical of traditional couscous, therefore, it is a possible successful product on the market with economic advantages for the companies that would produce it.

On the basis of what has been said so far, it is advisable to extend the concept of functional food to that of functional diet, in order to promote the intake of different types of food (couscous, pasta, biscuits, bread) characterized by significant amounts of bioactive compounds. Only in this way is possible to reach, during the different occasions of food intake (breakfast, lunch, snack, dinner), the quantity of dietary fiber and beta-glucans necessary to have the desired physiological effects.

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