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Fruit quality in almond: Chemical aspects for breeding strategies

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Abstract. Almond (*Prunus amygdalus* Batsch) kernel composition is a basic aspect for almond quality evaluation, but, with the exception of kernel taste, only nut and kernel physical traits have been considered so far in almond evaluation. Kernel bitterness, due to the presence of amygdalin, has been thoroughly studied from the physiological and genetic points of view, but not the other chemical traits, which, however, have a great importance in the organoleptic appreciation of the kernel, in its industrial utilization, in its keeping performance, and in its nutritional and healthy value. The major component of almond kernels is the lipid fraction, ranging from 35 to 70% of the total kernel dry weight. More important is the different proportion of the main fatty acids, with a predominant role of oleic acid, the most interesting from the nutritional and health point of view, ranging from 62 to 78% of the total fatty acids. Some confectioneries, such as nougat, require a high proportion of fatty acids whereas others, such as marzipan and almond flour, require a low proportion. Protein content ranges between 13 and 29%, being inversely proportional to the percentage of fatty acids. Soluble sugars range from 3 to 8%, with a predominant presence of sucrose. Ash residue ranges from 3 to 4.5%, with K, Ca and Mg as predominant elements. Fibre content ranges from 2 to 6%. Mostly interesting is the presence of tocopherols, due to their antioxidant activity. They are the source of vitamin E and also avoid, or retard, the rancidity process in almond kernels. Large differences in tocopherol content have been found between cultivars and selections, allowing an effective selection process for this trait in breeding programmes because of their implication in the industrial, nutritional and healthy value of the almond kernels. All these parameters are more or less heritable and must be considered in the design of crosses and in the evaluation during a breeding programme.

Keywords. Almond – *Prunus amygdalus* – Fruit quality – Fruit composition – Breeding – Nutritive value – Healthy food.

Qualité du fruit chez les amandes : aspects chimiques pour les stratégies d'amélioration génétique

Résumé. *La composition de l'amandon est un aspect de base pour l'évaluation de la qualité chez l'amandier (*Prunus amygdalus *Batsch). Pourtant, à l'exception du goût de l'amandon, seulement les caractères physiques de la coque et de l'amandon ont été considérés pour le moment dans l'évaluation des cultivars d'amandier. Le goût amer, dû à la présence d'amygdaline, a été profondément étudié du point de vue physiologique et génétique, mais pas les autres caractères chimiques, qui ont malgré tout une grande importance pour l'appréciation organoleptique de l'amandon, pour son utilisation industrielle, pour son stockage, et pour sa valeur nutritive et favorable à la santé. La composante majeure de l'amandon est la fraction lipidique, qui va de 35 à 70% du total du poids sec de l'amandon. Plus importante est la différente proportion des acides gras les plus présents, avec un rôle prédominant de l'acide oléique, le plus intéressant du point de vue nutritif et de la santé, qui va de 62 à 78% du total des acides gras. Quelques produits, comme le nougat, demandent une proportion élevée d'acides gras, mais d'autres, comme le massepain et la farine d'amande, en nécessitent une proportion faible. La teneur en protéine va de 13 à 29%, étant inversement proportionnelle à la teneur en acides gras. Les sucres solubles vont de 3 à 8%, avec une présence prédominante du saccharose. Le résidu de cendres va de 3 à 4,5%, avec K, Ca et Mg comme éléments prédominants. La teneur en fibre va de 2 à 6%. Très intéressante est la présence de tocophérols, étant donné leur activité anti-oxydante. Ils sont la source de la vitamine E et aussi évitent, ou retardent, le processus de rancissement des amandons. De grandes différences dans la teneur en tocophérols ont été trouvées parmi les cultivars et les sélections, ce qui permet un processus de sélection effectif pour ce caractère dans les programmes d'amélioration génétique à cause de son implication pour la valeur industrielle, nutritive et de santé des amandons. Il faut considérer tous ces paramètres, qui sont plus* *ou moins héritables, dans la conception des croisements et dans l'évaluation pendant un programme d'amélioration.*

***Mots-clés.** Amandier –* Prunus amygdalus *– Qualité du fruit – Composition du fruit – Amélioration – Valeur nutritive – Nourriture favorable à la santé.*

I – Introduction

Almond is a major tree nut cultivated in areas of Mediterranean climate. The kernel is the edible part of the nut and is considered an important food crop, with a high nutritional value. It may be consumed raw or cooked, blanched or unblanched, combined and/or mixed with other nuts. It can also be transformed to be incorporated into other products or to produce marzipan and nougat (Socias i Company *et al*., 2008).

Almond kernel quality has so far been defined exclusively by physical parameters: size, shape, double kernels, etc. However, the different uses of almond may require kernels with a specific composition, depending on each commodity. The high nutritive value of almond kernels arises mainly from their high lipid content, which constitutes an important caloric source but does not contribute to cholesterol formation in humans, due to their high level of unsaturated fatty acids, mainly monounsaturated fatty acids, because these acids are negatively correlated with serum cholesterol levels (Sabate and Hook, 1996). Kernel tendency to rancidification during storage and transport is a quality loss and is related to oxidation of the kernel fatty acids (Senesi *et al*., 1996). Thus, oil stability and fatty acid composition are considered an important criterion to evaluate kernel quality.

Until recently, almond breeding has been focused on selecting self-compatible and late-blooming cultivars with fruits of a high physical quality (Socias i Company *et al*., 2008). Consequently, very little information on chemical evaluation on the almond kernel has been found and the studies carried out to determine the chemical components of the almond kernel and their variability are scarce. Incorporation of such analyses in the evaluation of new plant material would be of great interest in determining the possible commercial and industrial use of the product, since the specific use of the kernel depends primarily on its chemical composition (Socias i Company *et al*., 2008).

In recent years, food and health aspects are receiving special attention from the general public. The determination of food authenticity and origin is a crucial issue in food quality control and safety (Barile *et al.*, 2006). The objective of this report is to review the different chemical components of the almond kernels in order to stress their importance in considering almond quality and the possibilities of their consideration during the evaluation of seedlings in a breeding program.

II – Chemical components

1. Kernel taste

The particular bitter taste in almond kernels is due to the presence of the cyanogenic glycoside amygdalin and the enzyme amygdalase (Conn, 1980), making them impalatable. Kernel bitterness was the first qualitative trait described in almond. Heppner (1923, 1926) reported a 3:1 distribution of sweet and bitter seedlings, concluding that sweet kernel was dominant over bitter kernel and that most cultivars were heterozygous for this trait. These results were further confirmed (Socias i Company, 1998), although Spiegel-Roy and Kochba (1974) suggested that three genes could be involved in the determination of kernel taste, but they later discarded this three-gene hypothesis and accepted a monofactorial determination (Spiegel-Roy and Kochba, 1977, 1981).

Dicenta and García (1993) have reported that certain sweet-kernel cultivars produce nuts with distinct "amaretto" taste owing to the presence of trace amounts of amygdalin. Barbera *et al*. (1988) showed that American and Russian cultivars had amygdalin contents ranging from 0.33 to 0.84% whereas Italian cultivars varied from 0.73 to 1.95%. However, it has not been possible to genetically differentiate the trace amygdalin content in the slightly bitter "amaretto" cultivars from the nondetectable amounts in completely sweet cultivars. The "amaretto" to slightly bitter taste is associated with the heterozygous genotype for most but not all genotypes evaluated (Arrázola Paternina, 2002). Thus, for seedling evaluation, the phenotypic characterization of each plant is required. In all cases, it is the seed parent alone that determines seed bitterness. The pollen parent does not affect the taste of the kernel, and all kernels of a tree will have either sweet or bitter kernels (Frehner *et al*., 1990). Only Crane and Lawrence (1952) mentioned a case of xenia in almond taste, but their results have not been confirmed by any other further research (Kumar and Das, 1996).

2. Lipids

The lipid portion is the main component of the almond kernel, and is a major determinant of kernel flavor particularly following roasting. Lipids may constitute 50% or more of the almond kernel dry weight, but the specific fatty acids in almond are very similar in composition to olive oil and generally considered to be desirable for a healthy diet. Lipid content and composition is also very important in the confectionery industry, because higher oil contents result in less water absorption by the almond paste (Alessandroni, 1980). Kernels with a high percentage of oil could be used to produce nougat or to extract oil, which is used in the cosmetic and pharmaceutical industries. However, kernels with a low percentage of oil are required to produce almond milk, a dietetic product, because its caloric level must be similar to that of cow's milk (Cotta Ramusino *et al*., 1961). A low lipid content is also suitable for production of marzipan, almond flour and several kinds of food because of their correlated high protein content (Longhi, 1952).

Lipid content and composition is also the major determinant of oil stability since component fatty acids differ in their vulnerability to oxidation (Senesi *et al*., 1996). Thus, both total kernel lipids and the proportion of the component fatty acids (particularly the ratio between oleic and linoleic acids) are considered very important criteria for almond kernel quality evaluation (Kester *et al*., 1993). The degradation of the fatty acids to peroxides results in the production of several products affecting the quality of the almond kernel (Sung and Jeng, 1994), including a rancid taste (Harris *et al*., 1972). Oil oxidation is affected by several factors, such as the percentage of unsaturated fatty acids, light, oxygen, metallic ions, temperature, and enzymes (Gou *et al*., 2000; Zacheo *et al*., 2000). However, 'Guara' shows a profile of fatty acid composition opposite to that considered as resistant to oxidation, yet is resistant to oxidation and rancidity (Berenguer-Navarro *et al*., 2002). This indicates that resistance to rancidity not only depends on the fatty acid composition but also on the presence of natural antioxidants, such as tocopherols.

Fatty acid content and composition vary significantly with genotype and origin. Thus, oil content on dry weight basis was found to range between 36 and 53% in the Californian cultivars (Abdallah *et al*., 1998) between 35 and 61% in Australian cultivars (Vezvaei and Jackson, 1996) and between 40 and 68% in European cultivars (Souty *et al*., 1971; Romojaro *et al*., 1977; Saura Calixto *et al*., 1981, 1988; Barbera *et al*., 1994; Schirra *et al*., 1994; Kafkas *et al*., 1995; Aslantas *et al*., 2001; Cordeiro *et al*., 2001; Kodad and Socias i Company, 2008). The differences among cultivars are highly significant. Some studies have found the year effect to be not significant (Saura Calixto *et al*., 1981; Romojaro *et al*., 1988a; Kodad and Socias i Company, 2008) whereas others (Barbera *et al*., 1994; Kafkas *et al*., 1995; Abdallah *et al*., 1998) found significant differences, possibly due to the specific climatic conditions of years tested.

Oleic acid is the main component of the lipid fraction, ranging from 62 to 78% of the total amount. This content is inversely correlated to the amount of linoleic acid (Saura Calixto *et al*., 1981; Schirra *et al*., 1993; Abdallah *et al*., 1998; Kodad and Socias i Company, 2008) with both constituting more than 90% of the total lipid fraction. The high content of unsaturated fatty acids, mainly of oleic acid, increases the phytonutrient value of the almond because this type of fatty acids does not contribute to the formation of cholesterol.

Oil content variability, approximately 3%, has been shown to be higher among genotypes than among years (Kodad, 2006). Individual fatty acids show different levels of year-to-year variability, but oleic acid, which is the most desirable fatty acid from a quality perspective, shows the highest content and the lowest variability (Kodad, 2006).

High percentages of oleic acid and high ratios of oleic/linoleic acids were observed in some breeding progeny having 'Felisia' as a parent (Kodad *et al*., 2005). Since total lipids as well as the proportion of oleic acid are occasionally higher than in the parents, opportunities for improving kernel oil quality are supported.

3. Antioxidants

Almond nuts possess high levels of phenolics, an important class of antioxidants in human nutrition, with most of this diverse range of compounds being found in the seed coat (Frison and Sporns, 2002). Most are thought to have powerful effects on health. Little is currently known concerning the genetic variability and inheritance of these compounds in almond, although the variability in total polyphenols is very high in almond and is genotype dependent (Berenguer-Navarro and Prats-Moya, 2003; Kodad, 2006). Particularly high antioxidant activity has been reported in the cultivar 'Ferragnès' while a much lower activity was observed in 'Marcona' (Kodad, 2006). Since 'Marcona' also demonstrates resistance to rancidity during long storage periods (García-Pascual *et al*., 2003), this resistance must be due to other physical and chemical traits, such as the tocopherol content (Zacheo *et al*., 2000; García-Pascual *et al*., 2003; Kodad *et al*., 2006) or the proportion of unsaturated fatty acids (Kester *et al*., 1993; Zacheo *et al*., 1998).

Almond kernels are a good source of α-tocopherol. The main biochemical function of tocopherols is believed to be the protection of polyunsaturated fatty acids against peroxidation (Kamal-Eldin and Appelqvist, 1996). Tocopherol concentrations have been determined in many oils and have been correlated with their antioxidant activity, considering the importance of the ratio of % total tocopherols / % polyunsaturated fatty acids (Senesi *et al*., 1996). In almond, tocopherol concentration plays an important role in protecting lipids against oxidation and thus lengthening their storage time (Senesi *et al*., 1996; Zacheo *et al*., 2000; García-Pascual *et al.*, 2003).

Alpha-tocopherol is the form of vitamin E that is most efficiently used by the human body yet is often deficient in modern diets (Pongracz *et al*., 1995; Krings and Berger, 2001). The vitamin E from almonds has been shown to be efficiently absorbed and produces a significant increase in plasma α-tocopherol levels (Sabate and Haddad, 2001). Higher α-tocopherol levels are also associated with resistance to kernel rancidity with storage, probably owing to its inhibition of auto-oxidation (Fourie and Basson, 1989). In almond, the concentration of α-tocopherol ranges between 187 and 490 mg/kg oil, followed by those of γ and δ isomers (Kodad *et al*., 2006). Relative concentrations of the three isomers may be genotype specific. There is a significant positive correlation between α-, γ-, and total tocopherol concentrations, but no correlation was found between oil content and the concentration of any of the tocopherol isomers. The high concentrations of α-tocopherol and the medium-high concentrations for the other isomers found in 'Marcona' may have contributed to its very high value when roasted, used in almond confectioneries, and especially when used for making nougat.

The heritability of almond kernel tocopherol has not been determined, although Kodad *et al.* (2006) have shown that the contents of α-, δ-, and γ-tocopherol in most selections of several breeding progenies have been clearly higher than those of their parents for each isomer. The continuous distribution of tocopherol concentrations suggests polygenic control, thus allowing a positive selection process because a high tocopherol concentration is an attainable objective in almond breeding (Kodad *et al*., 2006). However, when evaluating the oxidative stability of new almond genotypes, both the fatty acid profile and the activity of other natural antioxidants need to be considered.

4. Proteins

Almonds are a very good source of dietary protein, nearly as much as red meat, both of high quality and highly digestible. The major protein components are albumin, globulin, glutelin, and prolamin (Schirra, 1997). Protein content of almond kernels ranged from 13 to 29% on a dry weight basis depending on authors (Saura Calixto *et al*., 1988; Schirra, 1997; Kodad *et al*., 2004). The average total nitrogen value of the different almond samples ranges between 3% (Saura Calixto *et al*., 1988), 3.35% (Kodad, 2006), 3.6% (Riquelme Ballesteros *et al*., 1982), 3.69% (Casares and López, 1952), 4.23% (Aslantas *et al*., 2001), 4.28% (Romojaro *et al*., 1977), 4.4% (Souty *et al*., 1971), and 4.62% (Barbera *et al*., 1994).

The most common amino acids in almond proteins are glutamic acid, aspartic acid, and arginine. Small amounts of nonprotein nitrogen have also been found (Schirra, 1997). There is no information on the genetic control of protein content in almond, but the variability coefficient among years and among geographical origins is lower than that among cultivars (Saura Calixto *et al*., 1988), supporting the possibility of a positive response to breeding selection.

5. Carbohydrates

Carbohydrates are present in almond kernels as soluble sugars and as polysaccharides often associated with fibre. Soluble sugars, while present in relatively low amounts, are sufficient to make kernels sweet-tasting (Schirra, 1997). Published values range from of 3.3 (Vidal Valverde *et al*., 1979), 4.3 (Riquelme Ballesteros *et al*., 1982), 4.4 (Woodroof, 1979), 5.6 (Saura Calixto *et al*., 1988), and 7.1 (Casares and López, 1952). Part of the reported variation results from different cultivars being evaluated. Most soluble sugars are nonreducing, with sucrose representing more than 90% of the total. Other sugars include raffinose, glucose, fructose, sorbitol, and inositol (Schirra, 1997).

Data on carbohydrate composition are mostly descriptive, but differences among cultivars can be significant, particularly when considering the relatively low amount of carbohydrates present. Variability among cultivars is much greater than among years and among geographical locations (Saura Calixto *et al*., 1988), thus suggesting good potential for genetic selection. The predominance of sucrose masks the possible influence of other sugars on taste. However, the relatively low amount of carbohydrates in the almond kernel and the frequent addition of sugar during processing reduce the overall importance of sugar content in almond quality evaluations.

Along with the polysaccharides in almonds, there are 10 g of dietary fibre per 100 g of almond kernel. About 80% of the fibre is insoluble and 20% is soluble fibre. This mixture positively affects colonic health and cholesterol levels (Saura Calixto *et al*., 1988). Fibre concentrations in the kernel show a large variability. Initial determinations showed low amounts of crude fiber, in the order of 2 to 3% dry weight (Casares and López, 1952; Bauzá, 1980; Riquelme Ballesteros *et al*., 1982; Saura Calixto *et al*., 1988), while more recent results showed higher amounts, of 4.92 (Mateos Otero and Castañer Gamero, 1993) and 5.81% (Kodad, 2006), probably due to improvements in analytical methods. The large variability of this trait among genotypes and years shows a strong environmental influence, which would act to confound genetic selection.

6. Minerals

Published measurements of the mineral (% ash dry weight) content of almond kernels have been very similar with estimates of 3.1% (Souty *et al*., 1971), 3.17% (Saura Calixto *et al*., 1988), 3.27% (Kodad, 2006), and 3.45% (Romojaro *et al*., 1977) reported. Kodad (2006) determined variability coefficients of approximately 4% among the genotypes with slightly higher values among the years. Because the genotype and environment effects were similar, it does not appear feasible to select for higher mineral content based solely on individual phenotype (Saura Calixto *et al*., 1988).

The high contents of macroelements make almond an important dietary source for these essential elements. Processed almond has become commercially important as a natural food additive in milks, margarines, cereals, and baked goods. Almond milk is considered a vegetable milk substitute recommended in cases of intolerance to cow's milk (Cotta Ramusino *et al*., 1961). The most abundant minerals are potassium and phosphorus, representing over 70% of the total (Schirra, 1997). Potassium predominates with values ranging from 0.6 to 1.2% dry weight among tested genotypes and showing a variability coefficient higher than for the ash content, indicating a high variability among samples (Kodad, 2006). Almond kernels are an important source for calcium, magnesium, and manganese (Souty *et al*., 1971; Saura Calixto and Cañellas, 1982; Schirra, 1997), though phosphorus and calcium show a negative correlation (Schirra, 1997). Calcium levels are often higher than magnesium, but individual genotypes may show an inverse relationship (Souty *et al*., 1971; Saura Calixto and Cañellas, 1982; Saura Calixto *et al*., 1988; Cordeiro *et al*., 2001; Kodad, 2006). The genetic variability coefficients for calcium and magnesium tend to be higher than potassium but tend to be less significant among years, suggesting greater opportunities for genetic manipulation.

7. Vitamins

In addition to the presence of vitamin E (α-tocopherol), almond kernels are also a source of vitamins B1 (thiamine), B2 (riboflavin), B6, and niacin. Their loss is dependent on kernel processing, much less when blanching than when roasting due to the temperature effect on vitamin degradation.

III – Conclusion

The chemical composition of almond kernels has been reviewed (Saura Calixto *et al*., 1988; Schirra, 1997), but these overviews are primarily descriptive without attempts to relate the presence of each component to a particular aspect of quality. Extensive variability in chemical composition has been demonstrated among cultivars (Souty *et al*., 1971; Saura-Calixto and Cañellas, 1982; Romojaro *et al*., 1988a,b; Schirra *et al*., 1988; García-López *et al*., 1996; Abdallah *et al*., 1998). The importance of differences in geographical origin (Abdallah *et al*., 1998) as well as climatic and growing conditions (Gall and Grasselly, 1977; Grasselly and Crossa-Raynaud, 1980; Barbera *et al*., 1994) have been demonstrated. Despite this extensive information, little is known concerning the genetic control and inheritance of biochemical components of almond quality. However, the information presented in this report has shown that all parameters considered for almond quality are moss or less heritable and must thus be considered in the design of crosses in a breeding program, although individual selection is essential during seedling evaluation.

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