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Mini Review

Phenolic acids identification by capillary electrophoresis in *Lupinus* ssp. flours for application in the foods

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Abstract

The free phenols and phenolic acids are best considered, since they are usually identified together during plant analysis. Acid hydrolysis of plant tissues releases a number of ether-soluble phenolic acids, some of which are universal in their distribution. These acids are either associated with lignin combined as ester groups or present in the alcohol-insoluble fraction bound as simple glycosides. Universal among the angiosperms are p-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid. Gentict acid is also wide spread; less common are salicylic and o-protocatechuic, two acids found characteristically in the Ericaceae. Gallic acid is found in many woody plants, bound as gallotannin, but it is a very reactive substance. By contrast with the above acids, free phenols are relatively rare in plants. Hydroquinone is probably the most widely distributed; others, such as catechol, orcinol, phloroglucinol and pyrogallol, have been reported from only a few sources. The simple phenols are included here, because their identification is important in relation to determining the structure of flavonoids. The *Lupinus* is one of the most complex among the legumes, and its species grow in habitats ranging from sea level to the alpine tundra. Of the almost 500 known species worldwide, twelve are found in Europe and North Africa, and over three hundred in America. Some body authors identified 111 *Lupinus* species in Mexico, accounting for 22% of the described *Lupinus* taxa, although none of these have been cultivated or domesticated for food use. Use of *Lupinus* seeds is limited, however, by non-nutritional factors such as tannins, alkaloids and oligosaccharides. The alkaloids in *Lupinus* species are quinolizidine alkaloids (QA), which have very broad biological activity; for example, they can inhibit virus multiplication, bacteria proliferation and growth in certain fungi. This Legumen is promoted by health authorities in Western countries as a means of reducing the risk of diseases such as cancer, diabetes, and coronary heart disease. The capillary gel electrophoresis (CGE) was used to identify the protein fractions in the *L. montanus, L. barkeri* and *L. albus* isolates, using polymeric-coated commercial capillaries.

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Introduction

The phenolic compounds are very important in vegetation biochemistry, where they have diverse functions: from the coloration of flowers and fruits to the impregnating of lignin of 20 pecto-cellulose walls. The chemical point of view is that phenolic compounds consist of a benzene ring that contains one or more diverse hydroxyl groups. According to their chemical structure, these compounds can be subdivided in flavonoids or nonflavonoids that derive from the structure of basic fluoroglucinol, characterized by a skeleton of 2 benzene rings united by a chain of 3 carbon atom cycle in heterocycle oxygenated (Dykes and Rooney, 2007; Ignat *et al*., 2011). The phenolic compounds have their origin in the vegetation world. These are the main secondary metabolite's of the plants and its presence in the animal kingdom. The phenols are synthesized by the plants and are regulated genetically, so qualitative as quantitative, although at this level also the environmental factors exist. In addition, they act as phytoalexins (the wounded plants exert phenols to defend themselves of possible fungy or bacterial attacks) and contribute to the pigmentation of many parts of plant (the anthocyanins are responsible of charge of the red, orange, blue, purple or violet colors that we found in the skins of the fruits and vegetables). On the other hand, when the phenols are oxidized, they give rise quinones giving brown color that often is undesirable. The phenols are almost in

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all foods of vegetable origin. Onion, tea, red wine, cacao, virgin olive oil, and etc. are rich foods in phenols. These substances influence in the quality, acceptability and stability of foods, since they act like coloring, antioxidants and provide flavor. Thus, for this reason, the olives contain phenolic compounds that content in small proportion to the oil during the period of extraction. The virgin olive oil is the most unique oil that contains remarkable amounts of natural phenolic substances, since the rest of oils when being consumed and refined loose these compounds. By this motive, the virgin olive oil contains a typical imperceptible flavor in the refined oils (Dai and Mumper, 2010).

In Mexico the consume of cereals is increasing, in particular wheat of which infinity of products are elaborated as the breads, pastes, cereals, cookies, desserts, and snacks, etc; this food provides in general, an important quantity of the calories to the diet of the population. The content of protein contained in wheat flour is approximately 10% and patron of aminoacids indicates that it is deficient in lysine, threonine and tryptophan. In order to correct these deficiencies of quantity and biological quality of the protein in wheat, one has tested the addition of such diverse protein concentrates as fish meal single-cell proteins, and flours of different leguminous. The above mentioned is one of the important nutrients for humanity (Jímenez-Martínez and Dávila, 2006; Mora-Rochin *et al*., 2010).

The interest in the use of legumens are high protein content, two or three times more than the majority of the cereals, contributing 20% of protein consumed in the world. Nevertheless, these are deficient in sulfur aminoacids as the methionine and the cysteine. They contain a good quantity of essential minerals, as calcium and iron. Soy bean is one that high in protein and nutritional value, the first place worldwide regarding nutritional value, nevertheless, for the countries in which cultivation is not feasible for ecological reasons, an alternative is to find, varieties that adapt to extreme climates and poor soils, since it is the case of the *Lupinus*, which content protein (40-50%), it is similar to the soy bean (Jiménez *et al*., 2003). From the decade of the thirties, proteins have joined of leguminous in the human supply, across the obtaining and use of concentrates and isolated protein, as forms with major content and purity of protein. In these conditions they are possible to diminish the content of fiber, carbohydrates, fat and to diminish or to eliminate the toxic factors and/or antinutritional presents in all the legumens. The previous processing increase the original content (42-45%) of protein from flours of legumens to 60-70 % obtained for concentrates or to form purity with the isolated and that present 90-95%.

At present, a great diversity of investigations exists where they characterize the incorporation of flours different varieties of regional *Lupinus* in Mexico with excellent results, as component or substitute of wheat flours. The results with isolated of *L. mutabilis* and flour they have presented up to 52% of protein, very similarly to other *Lupinus* species. There are reports it brought 41. 5% for *L. Luteus* and for species since *L. albus* there have been brought 43.2%. Güemes 2012, in his works has brought similar percentages in the elaboration of regional breads with the incorporation of *L. mutabilis* and *L. albu*s (Jiménez-Mártinez and Dávila, 2006; Güemes, 2009; Porras 2013; Sirtori *et al*., 2010).

The phenolic compounds find in the different foods constitute a very complex fraction formed by a very big number of compounds, some still not identified. The concentration in polyphenols of any food also is very variable, because it depends on many such factors as the variety or the degree of mature of the vegetable. Also bioavailability is very variable: many of them are metabolized by microorganisms of the colon before being absorbed. In addition, the technological processes and the culinary habits of the consumer can reduce a larger part of the phenols in the food. Polyphenols traditionally have been considered to be antinutritional by the nutrition of animals and humans, due to the adverse effect of the tannins on the digestibility of the proteins that provokes a minor growth in cattle and a minor laying of eggs. Nevertheless, nowadays there is an increasing interest due to antioxidant capacity, so much as capture of radical free as q wheat chelating of metals. These antioxidant properties are the motives of possible implications in the human health, since they are the prevention of the cancer or enclosed, of the cardiovascular diseases of neurodegenerative diseases as Alzheimer. Actually many investigations exist on the substances with activity estrogenic (phytoestrogens) as the isoflavones, the lignans and the resveratrol, and others with antimicrobial properties. In case of resveratrol of the wine or the isoflavones of the soy bean they are a clear example of the recent trend for phenolic compounds in the food due to protective effects opposite to the cardiovascular diseases and antioxidant properties, even proven in animals (Benito, 2001; Frias *et al*., 2005; Dykes and Rooney, 2007).

Of all the phenolic compounds, the group of the flavonoids is most extended in the nature and inside themselves; the flavonols are those who possess a major antioxidant activity. Epidemiological studies

have demonstrated that a rich ingestion in flavonoids correlates with a minor risk of cardiovascular disease and has been observed that they act at different levels. On the one hand, they diminish the rates of cholesterol and of lipoproteins of low density (LDL) oxidized due to antioxidant properties as strong chelating of metals and as donors of hydrogen (across the groups hydroxyl).In the cereals from grains as well as the flours of oats, they find significant levels of antioxidants, and these are using as preservers a long before the antioxidant synthetic BHT and BHA before the market (at the beginning of the year 1900). The consumption of this food reduces the incident of chronic degenerative diseases including cardiac problems and some cancers. The antioxidant in addition have the function to eliminate radical free, agents reducers, agents quelants, quenchers of singlet oxygen molecules, activators of antioxidative defense enzyme systems, to suppress radical damage in biological Systems (Zielinski and Kozlowska 2000).

Phenolic components in the prevention of the cancer

The mechanisms of phenolic compounds can anticipate the cancers. Laboratory studies in animals of experimentation have revealed effects and biological varied activities, which summarize in table 3. On the other hand, Steinmetz and Potter (1996) gathered information proceeding from 206 epidemiological studies, which revealed that high consumptions of fruits and vegetables are related to a low incidence of different types of cancer, as those of stomach, lung, oral cavity, pharynx, endometrium, pancreas and colon. Nevertheless, in these studies it is very difficult to discern whether the effect is due specifically to a compound or more likely, is due to synergistic effect of different phytochemestry in these food as they are, in addition to polyphenols, vitamins C and E, carotenoids, folic acid, fiber, and soon (Scalbert *et al.*, 2005; Speisky *et al*., 2006).

In summary, natural phenolics have been found to intervene at all stages of cancer development. In addition to their antioxidant action, the inhibition of cancer development by phenolic compounds relies on a number of basic cellular mechanisms, involving a spectrum of cellular basic machinery. Therefore, the extensive studies of this class of compounds will provide clues about their possible pharmaceutical exploration in the field of oncology (Yu *et al*., 2002; Dai and Mumper, 2010).

Aromatic plants have also been used since ancient times, mainly in food flavours, pharmaceuticals, cosmetics and perfumes. Herbs, aromatic plants and natural herbs contain antioxidants of proven efficacy against oxidative degradation and some human diseases such as cancer, inflammatory disorders, neurological degeneration or coronary heart disease (Herrero *et al.*, 2005).

Virgin olive oil (VOO) has been widely produced and consumed since ancient times in the Mediterranean region, where it is highly appreciated for its fine taste and aroma, as well as for its nutritional properties. VOO is obtained from the fruit of the olive tree (*Olea europea* L.), using purely mechanical or other physical means under conditions that in no way detract from its properties and require no additional treatment other than washing, decantation, centrifugation or filtration. Chemically, VOO consists of an unsaponifiable fraction of monounsaturated and polyunsaturated fatty acids, and a polar fraction of natural antioxidants including carotenoids, phytosterol, flavonoids and phenolic compounds (phenolic acids, phenylethyl alcohols, secoiridoids and lignans, mainly). The content of phenolic compounds is an important factor to be considered in evaluating VOO quality (Berzas-Nevados *et al*., 2012); in fact, phenols are powerful antioxidants and a significant contributor to its stability against oxidants, and hence to extending its shelf-life relative to other vegetable oils. Moreover, phenolic compounds possess analgesic, anti-inflammatory and anticarcinogenic (skin, breast and colon) properties that are of essential for human health (López-Miranda *et al*., 2010; Berzas-Nevados *et al.*, 2012). Phenolic acids (basically benzoic and cinnamic derivatives such as vanillic, coumaric and hydroxyphenylacetic acid) constitute one of most important groups of phenols present in VOO (Ballus *et al*., 2014b).

Bioavailability

In spite of these effects having been demonstrated in many studies, others have not achieved significant results. These discrepancies may be due, among other factors, deficiencies in the methods of assessing intake of phenolic compounds. Also the matrix of the food and constituent other factors of the diet that they accompany the polyphenols can have a relevant effect on bioavailability and metabolism. It is known the absorption, bioavailability and metabolism of phenolic compounds and it is likely that each group has a different kinetic. Chemical structure determines the rate of absorption, the nature of the circulating metabolites and their elimination. In this sense despite conjugates flavonoids no found in plasma of these, found an increase in the plasmatic antioxidant capacity in rats fed alcohol-free. These results would demonstrate that the derivatives of the flavonoids

keep circulating antioxidant in plasma activity. Also it is assume that due to hydrophilic nature, phenolic compounds are eliminated very rapidly from the circulation. In the case of the olive oil for example, the phenolic compounds are absorbed by the intestine and are transported by a separate system for chylomicrons formation, therefore this causes rapid presence in urine (Kinsella *et al*., 1993; Benito, 2001).

Another aspect to be consider in the analysis of phenolic compounds, is the antioxidant effect of the vitamin E is major or greater or not than the phenolic compounds. Gimeno (2004) mentioned that the flavonoids are excellent antioxidant vitamin E and that they can change color the effect of α-tocopherol and breaking the chain of radicals free in the microsomals membranes hepatic. Evidently the only different is in lipoavailability, each antioxidant has it's special place of action. It is believed that the major antioxidant in the LDL is vitamin E. However, some phenolic compounds had more antioxidant power then α -tocoferol in vitro. For example, the mechanisms by which phenolic compounds may protect LDL is still unknown. Gimeno (2004), mentioned that the LDL contains phenolic compounds of which were identified from the quercetin 2. It has been suggested that phenolic compounds locate in the surface of the bilayer lipidic and neutralize free radicals presents in the mid water, delaying the consumption of endogenous antioxidants such as vitamin E.

Polyphenols are recognized as anti-nutrients of different minerals, since they have the ability to chelate cations divalents, like Fe and Zn, through binding to the group's hydroxyl and carboxyl, reducing the bioavailability of the same intestine. Phenolic compounds are released during digestion and it can join the iron in the intestinal lumen making them not bioavailable. The capacity of chelating this cation changes from a few phenolic compounds to others having been directly related to the chemical structure. Thus, the reduction in the bioavailability of Fe is related to the presence in the diet of monomeric flavonoids (catechins) and esters of gallic acid present in the tea, cocoa catechins, chlorogenic acid in coffee, acids phenolic, flavonoids monomeric and polimeric tannins and sorghum and beans. Studies realized by Hurrell *et al.* (1997) have demonstrated that the presence of polyphenols in tea and varies herbal tea, cause a reduction in the bioavailability of iron from enriched bread with ferrous sulfate, when administered at breakfast, this reduction being more pronounced in the tea other infusions as a result of higher content of polyphenols.

Currently, despite of the realized investigations, it little is known on the bioavailability, absorption and metabolism of phenolic compounds in the humans, but it is known that the different groups of flavonoids possess different pharmacokinetics properties. It is evident that some phenolic compounds, so much the poliphenolic extracted or the soluble are metabolized in the gastrointestinal tract. Aglycons and free simple phenolic compounds, flavonoids (quercitin and genistein) and phenolic acids can be directly absorbed through the lining of the small intestine. Free phenolics compounds (acids cinamics and his derivatives, acid p-cumaric, ferelic, caffeic, etc.) have been absorbed in to the intestinal tract both much in vivo test with rats as *in vitro* experiments perfomed with jejuno isolated. The mediation of the bacterial enzymes in the bioavailability of the phenolic glycosides has been proved by Griffiths and Barrow, who showed that flavonoids glycosides were excreted in the feces of germ-free rats.

Bacterial fermentation of carbohydrates may also release phenolic compounds bounded to the fiber, which could be metabolized as polyphenols extra. In the colon, the aglycones are absorbed through the intestinal epithelium and methylated and / or conjugated with glucuronic acid or sulfate in the liver. The main organ involved in metabolism is the liver polyphenols, but also the kidneys and the mucous intestinal are which, contain enzymes involved in the metabolism of polyphenols.

Phenolic compounds that have been studied are the flavonoids. However, the absorption of flavonoids in the diet is major problem and hasn't been resolving, despite their potential beneficial health effects. In fact, it has been widely suggested that flavonoids in food cannot be absorbed in the intestine because they are attached to glycosides proteins. Only sugar free flavonoids, that is to say the aglycone, are capable of crossing the intestinal wall, but they neither are synthesized nor are present in internal enzymes that can break hydrolyser ß-glycosides unions. The nature hydrophilic glycosides and their relative high molecular weight excludes the absorption in the small intestine. In addition, the flavonoids ß-glycosides resist the action of the intestinal hydrolases, so you pass unchanged glycosides to the large intestine. Hydrolysis occurs in the colon by microorganisms, which degrade flavonoids with time. The flora in the intestine produces glycosidase able to release liberating the aglycone of sugar. Moreover, this flora can break the pyrone ring (ring C) provined phenyl acetic and phenyl propionic and other derivatives. Therefore there is an accumulation of aglycones in the intestine which it may be absorbed through the intestinal wall. However, recent studies suggest that

the absorption of flavonoids does not occur via the aglycone. Hollman and Katan (1997), conducted a study on the absorption of flavonoids in healthy volunteers to who had undergone an ileostomy. The results of this investigation was that the quercitin glycosides are absorbed directly through the wall of the small intestine and a faster rate the corresponding aglycone, and also speculated about the possibility that the absorption of glycosides could happen through awards and tributes glucose/Na^+.

Previous studies were conducted by Mizuma *et al*. (1994), showed them that absorption of naphtol glycoside in the small intestine of rats depended on the nature of the sugar and presence of Na^+ consistent with relationship with the system glucose/Na^+. The absorption of these glycosides is inhibited by phloridzin, an inhibitor of transport of glucose and of itself, a glycoside structurally related to the flavonoids. If in fact this provides that the absorption of flavonoids glycosides in the small intestine occurs in a specific mechanism of absorption, showing selectivity between the different glycosides of flavonoids, it will require detailed studies of the chemical nature of the glycosides and their levels in plant foods in the chemical form in which the normally eat such foods.

Little is known on the destination of the majority of the phenolic compounds after intake. The metabolism of flavonoids and flavonones in the diet it remains still without making concrete information exists on pharmacokinetic, probably due to the lack of selective methods for the determination of these compounds in fluids in the body. There has been demonstrated that the catechins are absorbed in the human intestine after the administration of 3-0-metilcatequin marked radioactivity, later found metabolites in urine (glucurnid of 3-3-0-dimetylcatequin and glucuronid and sulphate of 3-0-metylcatechin).

Rutin and quercitin are poorly absorbed whereas caffeine acid appears to be well absorbed, although human studies have been identified only in the urine between 1 and 5 metabolites. However, these studies were developed by pure crystalline substances, whereas in the food substances are generally in the shape of glycosides or of esters, or in solution in the component lipidic terphen of the food. Some studies have been done on quercitin. This way, there is knows that the ingestion of a quantity known about this compound revealed less then 11% is absorbed in the intestine. Over 150% of the given dose was degraded by the microorganisms in the colon, whereas the rest got lost in the feces. Urine samples taken at 24 hours of the ingestion in volunteers who administered 4 grams of quercitin, proved to be neither the above mentioned compound (Martinez-Valverde *et al.*, 2000).

The presence of quercitin, proceeding from the diet not absorbed in the colon or alternative, the liberation of quercitin glycoside rutin when attacked by the microflora of the colon could act as an agent to protect, anticipate or disable the carcinogenesis in place. Therefore experimental studies in which a mammary cancer is induced in rats by feeding them quercitin reveal a decrease in the incident of tumors, through the quantity distributed to the mammary gland by this route still is questionable. Finally, it is known that the quercitin in the diet is absorbed in humans and is eliminated slowly throughout the day, is able to contribute hereby to the antioxidant defenses present in the plasma. The absorption of these glycosides is inhibited through phloridzin, an inhibitor of the transport of the glucose, a glycoside structurally related to flavonoids. In fact it is found that the absorption of flavonoids glycosides happens in the small intestine through a specific mechanism of absorption, showing selectivity between the different glycosides of flavonoids, they will be necessary detailed studies of the chemical nature of the glycosides and levels in foods for the chemical form in which they are normally consumed (Martinez-Valverde *et al*., 2000) (Figure 1).

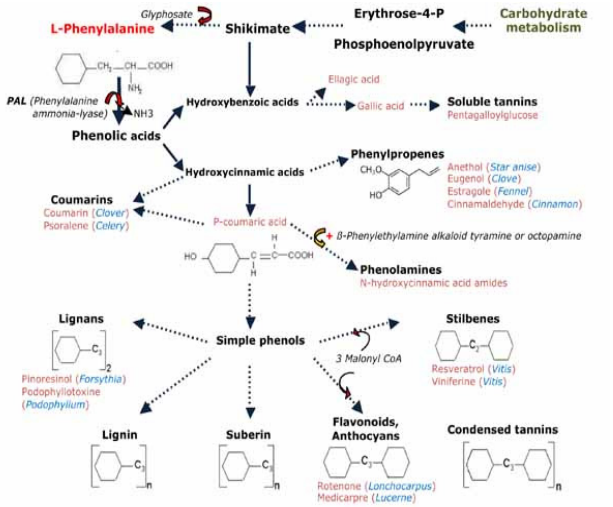

Figure 1. Simplified pathway of phenolic compounds synthesis (Adadpted from Douce, R. 2005)

Toxicity of phenolic compounds

Regardless the phenols may have anti-nutritional effects because they can interact with some elements of the diet. For example, a very high acute and chronic ingestion of these compounds can interfere with the absorption of iron, causing anemia. However general, the toxicity of phenols in moderate ingestion is very small due to it's low absorption, rapid metabolism and the presence of a very effective system of

detoxification. The problem is that the majority of studies are made *in vitro* or in experimental animals, which limits the extrapolation of results in human. It has been show that polyphenols can be toxic if your intake is between 1 and 5% of the total diet, which is possible under normal conditions, since it is customary to eat from, approximately, between 25 mg^{-1} g/day. Even this way, it suits to be prudent and to recommend consumption by raising of phenolic compounds until bioactivity is not better understood. In short, since the antioxidant play a role in preventing various diseases, the recommendation for the general population (primary prevention) is to enrich the diet of naturals antioxidants (fruits and fresh vegetables, dried fruits, virgin olive oil, etc.). Prophylactic use of antioxidants in high doses is still in discussion and needs more studies, though, in some cases high risk of cardiovascular disease (secondary prevention), it is possible to use supplements, fundamentally of vitamin E, since a difficultly diet balanced will reach more than 30 mg/dairy (Gimeno, 2004).

Analytical determination and quantification of the phenolic compounds

Quantification and identification of components diphenelics in the diet has aroused great interest because nutritional importance, it has done that every day more data you can find it, the scientific literature on the phenolic profile of foods. In addition, the great diversity of phenolic compounds in the plants tissues scattered, as well as their different chemical structures, has brought and obtain the need to develop a great number of analytical techniques for identification and quantification (Peng *et al.*, 2005; Peng *et al*., 2008; Mahugo *et al*., 2009; Ignat *et al*., 2011).

The first techniques developed were spectrophotometrics techniques, which though are interesting from the stand point of quality control, do not contribute the sufficient information from a nutritional point of view, it has been necessary to use more precise techniques, as the chromatographic, that allow the individual identification of each polyphenols of nutritional interest. The ultraviolet techniques have been in use as rapid quantification of phenolic compounds due to the fact that every group is characterized by one or more maximum absorbance at different wave lengths within the ultraviolet spectrum.

Chromatography techniques

The chromatographic technologies allowed the separation, isolation, purification and identification of phenolic compounds, as well as the study of the interaction between the polyphenols and other components of the food. The technologies of chromatography in paper and in thin layer, are used for the purification and isolation of phenolic compounds in the food, especially for the determination of and the determination of phenolic acids respectively.

Nowadays, the technologies of liquid chromatography of high resolution (HPLC) are more employees for the separation and quantification of phenolic compounds. There exist different supports and mobile phases that allow the analysis of anthocyanins, procyanidins, flavonones and phenolic acids. The utilization of the detector of photo-diode array facilitates the detection of these compounds for HPLC, on having used of joint form the time of retention and the spectrum ultra violet for the identification of the beaks. By means of HPLC's employment, we can determine a great number of polyphenols of nutritional interest, as simple phenols, phenolic acids and his derivatives, and the different flavonoids, though this technology asks the utilization of methods of extraction optimized from each of the compounds that are going to be analyzed. HPLC's technologies have been used for the characterization of the polyphenols in a great variety of vegetable extracts, fruits juices, olive oil, wines and other drinks.

Nowadays, HPLC's utilization with detection photo diode array and connected to a detector of mass has been used for the quantification of flavonols, flavons and flavonoids in food. This technique, the areas of the beak of each of compounds to investigating use for the quantification, whereas the detector of masses is used for increasing the specificity of the method. Moreover Analytical techniques commonly used in the determination of phenols are high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) in combination with ultraviolet detection (UV), electrochemical detection or mass spectrometry detection (MS). Liquid chromatography of phenols is generally carried out with the addition of acids or buffers to the mobile phase. Their function is to suppress the ionisation of both, the analytes and the residual silanols of the stationary phase base material, which otherwise would either decrease retention on the analytical column or lead to interactions of the analytes and the stationary phase, resulting in lower separation efficiencies.

Also, gas chromatography (GC), using several detection methods like flame ionisation detection (FID), electron-capture detection (ECD) or mass spectroscopy detection (MS), have been used, although in the case of CG, a derivatization step is

needed. The high polarity of free phenols hinders their correct chromatographic resolution because they produce broad, tailed peaks; this limitation can be circumvented by derivatising free phenols to less polar compounds, such as acetylated derivatives. They are commonly derivatised either before or after extraction, or with an on column reagent in the GC injector port. Nevertheless, the detection limits imposed by environmental quality legislation can only be achieved by using appropriate sample preparation techniques, which provide high enrichment factors of these analytes. Current official analytical methods for phenolic compounds extraction are liquid extraction (LLE) for liquid samples, and Soxhlet extraction, for solid samples. These methods require expensive and hazardous organic solvents, which are undesirable for health and disposal reasons, and they involve a long time per analysis. For these reasons, these traditional extraction sample methods have been replaced for other methodologies, more sensitive, selective, fast and environmentally friendly (Peng *et al*., 2005; Ignat *et al*., 2011).

Electromigration techniques including capillary electrophoresis (CE), capillary zone electrophoresis (CZE), and micellar electrokinetic chromatography coupled with UV, and to a less extent EC and MS detection are also employed for phenolics analysis (Dai and Mumper, 2010). In the figure 2 shower the different technologies used for the determination of phenolics components where the efficiency is observed.

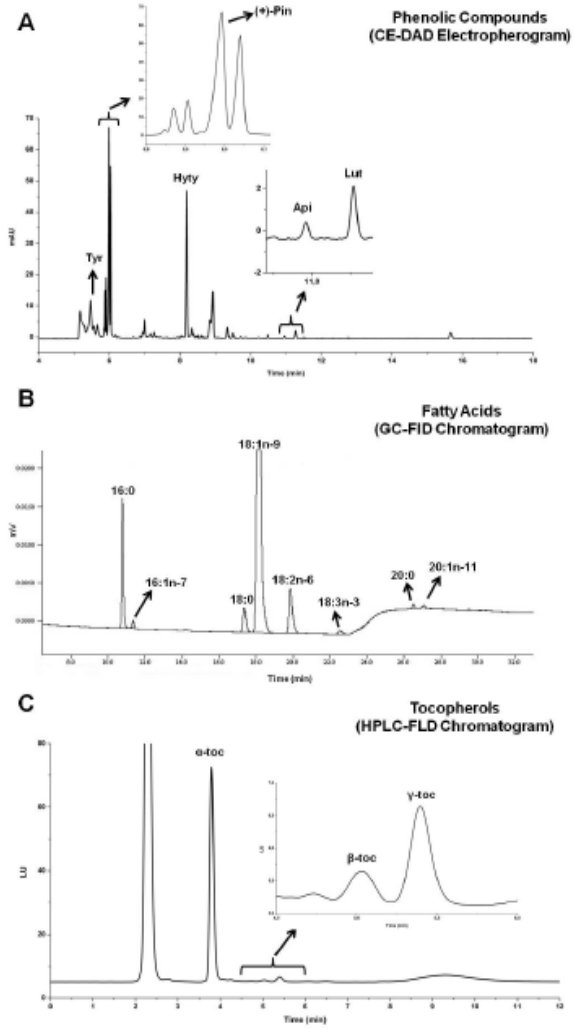

Figure 2. Representative electropherogram for the analysis of phenolic compounds (A), and representative chromatographic profiles for the analysis of fatty acids (B) and tocopherols (C) in Brazilian virgin olive oil (Taken from Ballus *et al*., 2014b).

Capillary electrophoresis

Capillary electrophoresis (CE), which is an alternative separation technique to HPLC, is especially suitable for the separation and quantification of low to medium molecular weight polar and charged compounds, the resultant separations being often faster and more efficient than the corresponding HPLC separations (Cifuentes, 2006; Ignat *et al*., 2011). Capillary electrophoresis (CE) is increasingly becoming a versatile analytical tool for the routine determination of a wide variety of phenolic compounds in different types of samples due to its high separation efficiency, high resolution power, short analysis time and low consumption of sample and reagents. On the other hand, one of the major limitations of CE, compared to other techniques like GC or HPLC, is its low sensitivity in terms of solute concentration, and worse reproducibility compared to chromatographic techniques which is caused by the short optical path-length of the capillary used as detection cell and also by the small volumes that can be introduced into the capillary (normally, a few nanoliters). There are a few examples of CE used to separate and determine the levels of naturally occurring flavonols in plant material (Ronda *et al.*, 2008). The use of CE in the separation of anthocyanins is a quite recently developed technique, scarce, but promising due to the high hydrosolubility of these compounds. CE is suitable technique for the separation, identification and quantification of anthocyanins. CE has also been used to create correlations between the content of anthocyanins content and the ageing of red wine (Saenz- Lopez *et al.*, 2004). CE with ESI–MS coupling has been used for monitoring anthocyanins and flavonoids in wine (Castañeda-Ovando *et al*., 2009). Micellar electrokinetic capillary chromatography (MECC) has extended the utility of capillary electrophoresis to the separation of neutral analytes under the influence of an electric field. The fractionation of monomeric

and polymeric pigments of higher molecular mass by gel permeation chromatography (GPC) improved the analysis of these compounds by CE (Ignat *et al*., 2011).

Natural phenolics are of interest from many viewpoints (antioxidants, astringency, bitterness, browning reactions, color, etc. Selection of the proper analytical strategy for studying phenolics in plant materials depends on the purpose of the study as well as the nature of the sample and the analyte. The assays used for the analysis of phenolics are usually classified as either those measuring total phenolics content, or those quantifying a specific group or class of phenolic compounds. Quantification of phenolic compounds in plant extract is influenced by the chemical nature of the analyte, as well as assay method, selection of standards and presence of interfering substances (Dai y Mumper, 2010).

Two modes of capillary electrophoresis (CE) — free-solution capillary zone electrophoresis (CZE) and sodium dodecyl sulfate capillary electrophoresis (SDS-CE) using a non-gel sieving matrix — have been developed for comparative analysis of low-molecular-mass 2S albumin isoforms from lupins. The albumin fraction and 2S albumins were separated in uncoated fused-silica capillary by CZE with 0.02 M phosphate buffer, pH 7.3, containing the sodium salt of phytic acid. The use of phytic acid (0.025 M) as buffer modifier and ion-pairing agent improved migration reproducibility, peak shape and separation efficiency. The reduced 2S albumins were separated by SDS-CE using a high concentration (0.3–0.5 M) mixture of tris (hydroxymethyl) aminomethane and borate buffers in uncoated fused-silica capillary. Of the various polymers used as non-gel sieving matrix, SDS-CE with a 10% dextran solution was found to be suitable for separation of 2S albumin polypeptides with molecular masses of 4000–7000 and 8000–11 000. The addition of glycerol or ethylene glycol to the SDS separating buffer improved the resolution of polypeptides. The examined *Lupinus* species showed species-specific CZE and SDS-CE migration profiles of the 2S (Salmanowicz, 2000).

Kronholm *et al.* (2003), they compared two techniques of gas chromatography–mass spectrometry and capillary electrophoresis in analysis of phenolic compounds extracted from solid matrices with pressurized hot water, obtained values by GC–MS and CZE were generally of similar magnitude. In the other hand, Cala-Molina *et al*. (2007), they characterized the antioxidant activity of native plant from Central America *(Phyllantus acuminatus)* using electroforesis capillary. Peng *et al*. (2008), bring results where they determined the phenolic components and ascorbic acid of different fractions of tomato using capillary electrophoresis with electrochemistry detection. In the same year, Zamora-Natera *et al.* (2008) determined the composition of alkaloids in seeds of Ganzera *Lupinus* mexicanus as well as the evaluation antifungal and allelophatic of alkaloid extract.

Recently CE is becoming increasingly recognized as an important analytical separation technique for its speed, efficiency, reproducibility, ultra small sample volume, and minimal consumption of solvent. In addition, with electrochemical detection (ED), CE-ED offers high sensitivity and good selectivity for electroactive analytes, and this method has been applied to analyze some phenols in foods. To our knowledge, so far the CE-ED method has not been applied to determine phenolic compounds, such as (2)-epicatechin, rutin, hyperoside, and quercetin. In this work, we first developed a simple, rapid, and dependable method for the determination of phenolic compounds in buckwheat *(Fagopyrum esculentum* Moench*)* hull and flour (Peng *et al.*, 2005; Mahugo *et al*., 2009; Dai and Mumper, 2010; Ganzera *et al*., 2010).

Lee *et al*. (2011), described a rapid CZE method suitable for the separation and quantification of phenolic acids present in broccoli and *Brassica oleracea*. A simplified extraction and rapid CZE method was developed for the isolation and separation of the four main hydroxycinnamic acids present in both. Solid phase extraction is used to isolate phenolic acids from broccoli extracts and baseline resolution is achieved in less than 7 min using an optimized borate buffer system which enabled accurate and reproducible quantification of phenolic acids in broccoli extracts. A linear relationship was observed for the method (r = 0.9997–0.9999) with detection limits ranging from 1.1 to 2.3 mg/kg of vegetables for the analytes. This method demonstrated good reproducibility with coefficients of variation of less than 5% for peak area and less than 1% for migration time (n = 7). The method was successfully applied to quantitatively determine the phenolic acid contents in a range of brassica vegetables and the results were in good agreement when compared to those from high performance liquid chromatography analysis (HPLC).

Bizzotto *et al.* (2012), separated, identified, and quantified the phenolic compounds present in the extracts of four commercial types of mate herb *(L. paraguariensis)* before and after hydrolysis by capillary zone electrophoresis with a diode array detector. The analysis method was adapted and validated and the hydrolysis process was optimized

by central composite design for obtaining the greatest concentration of phenolic compounds in the hydrolyzed mate herb extract. The multivariate optimization was fundamental, with only 18 experiments, it was possible to obtain the optimal condition of hydrolysis for the quantification of the caffeic acid and 3,4-dihydroxybenzoic acid, which were initially present in glycosylated forms. The quantification of rutin and of caffeic and 3,4-dihydroxybeonzoic acids before and after acid hydrolysis showed that mate herb has high amounts of these compounds. High levels of rutin were detected before hydrolysis, and after hydrolysis, 3,4-dihydroxybenzoic acid was detected, as well as high levels of caffeic acid. The contents found in the present experiments indicate that the matrix mate herb should be studied as a source of rutin, caffeic acid, and 3,4-dihydroxybenzoic acid. In this study, it demonstrate that mate herb should be further investigated as a major source of these compounds.

Berzas-Nevado *et al.* (2012), optimized and validated the used capillary zone electrophoresis method for determinate phenolic compounds from virgin olive oil (VOO). They used three different procedures to enrich VOO with natural antioxidants from herbs. One involved simply placing the studied herbs in the VOO for 190 days; another keeping the herb–VOO mixture under stirring at room temperature (25ºC) for 11 days; and the third stirring at temperatures above room level (35–40ºC). Subsequently, the phenolic compounds were separated and determined by using a fast and reliable capillary zone electrophoretic (CZE) method. Also, an SPE procedure was developed to isolate the target compounds from olive samples.

The efficiency of each procedure was assessed by using a reproducible, efficient, reliable analytical capillary zone electrophoresis (CZE) method to separate and determine selected phenolic compounds (rosmarinic and caffeinic acid) in the oil. Prior to electrophoretic separation, the studied antioxidants were isolated from the VOO matrix by using an optimised preconcentration procedure based on solid phase extraction (SPE).

Ballus *et al.* (2012), optimized a capillary zone electrophoresis method to simultaneously separate 16 phenolic compounds present in wines (In less than 19 minutes), as well as to evaluate sample on-line preconcentration for detectability improvement. The separated compounds were narirutin, (−)-epicatechin, (+)-catechin, rutin, kaempferol, myricetin, quercetin, morin, trans-resveratrol, cinnamic acid, ferulic acid, p-coumaric acid, vanillic acid, caffeic acid, gallic acid, and 3,4-dihydroxybenzoic acid. The method

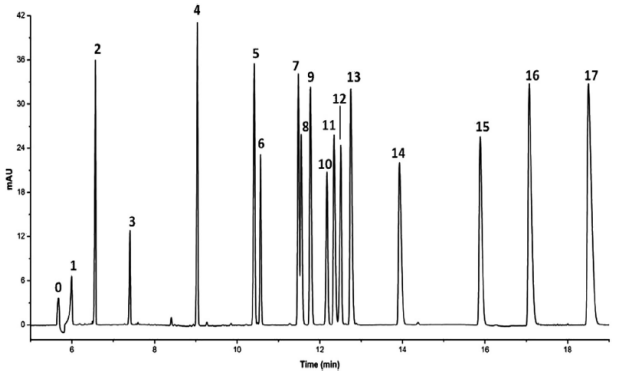

Figure 3. Electropherogram for the optimal separation of 17 phenolic compounds. Fused-silica capillary of 50 lm internal diameter and 72 cm of effective length with extended light path, 101.3 mmol L^{-1} of boric acid electrolyte at 9.15 pH, 30 kV, 25 _C, injection of 0.5 Pa for 5 s, and detection at 210 nm. Peak identification: 0, solvent; 1, tyrosol; 2, (+)- pinoresinol; 3, oleuropein glycoside; 4, hydroxytyrosol; 5, cinnamic acid; 6, sinapinic acid; 7, syringic acid; 8, ferulic acid; 9, o-coumaric acid; 10, apigenin; 11, p-coumaric acid; 12, luteolin; 13, vanillic acid; 14, p-hydroxybenzoic acid; 15, caffeic acid; 16, gallic acid; 17, 3,4-dihydroxybenzoic acid.

was validated and applied with success in a total of 23 samples of red, white, and rose wines. The developed method showed excellent applicability due to the simple extraction procedure and the low volume of reagents used, reducing expenses for reagents and technicians. This method presented a fast analysis, low consumption of reagents, minimum generation of residues, and, consequently, reduced costs, without causing negative environmental impacts. Moreover, the method employing large volume injection with on-line preconcentration exhibited elevated analytical reliability and avoided exhaustive and onerous extractions.

Recently Ballus *et al*. (2014b), analyzed 17 compounds comprised palmitic acid (6-12%), stearic acid (1.6-2.2%), oleic acid (70-84%), linoleic acid (3.2-11.7%), α-linolenic acid (0.6-1.4%), tyrosol (NQ-155 mg kg^{-1}), (+)-pinoresinol (2.9-23 mg kg^{-1}), hydroxytyrosol (ND-38 mg kg^{-1}), luteolin (ND- 2.2 mg kg^{-1}), α-tocopherol (29-233 mg kg^{-1}), β-tocopherol (ND-9.6 mg kg^{-1}), and ɣ-tocopherol (ND-19 mg kg^{-1}). There was a significant difference in the contents of almost all of the analyzed compounds between the two crop years. Principal component analysis demonstrated that some varieties can be differentiated from one another by chemical composition. The results indicated that some Brazilian monovarietal VOOs are promising and that further studies will help to improve the quality of Brazilian VOO. In the figure 3, are observed 17 components analyzed in VOO demonstrating the efficiency and viability

using technique CE in the determination of phenolic compounds.

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Conclusions

Various studies that have been conducted on phenolic compounds on seed Lupins, these substances influence in the quality, acceptability and stability of foods, since they act like coloring, antioxidants and provide flavor. These antioxidant properties are the motives of possible implications in the human health, since they are the prevention of the cancer or enclosed, of the cardiovascular diseases of neurodegenerative diseases as Alzheimer. Actually is new techniques for determination these compounds like chromatography, HPLC and capillary electrophoresis, the use of this technique is mainly to the bioactives compounds capable of preventing degenerative chronic diseases.

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