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CARACTERIZACIÓN DE AVENANTRAMIDAS SINTÉTICAS Y DE EXTRACTOS DE AVENAS PRODUCIDAS EN DURANGO, MÉXICO

PRESENTED BY: FAVIOLA ORTIZ ROBLEDO

TO OBTAIN THE DEGREE OF DOCTOR IN SCIENCE IN BIOTECHNOLOGY

DIRECTORS OF THESIS

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Caracterización de Avenantramidas sintéticas y de extractos de avenas producidas en Durango, México

Presentada por el alumno:

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Nombre y firma

This work was performed in three different places, at the CIIDIR-IPN unit Durango, in the laboratory of Pharmacokinetics, in the Cereal Crops Research Unit, United States Department of Agriculture, Agricultural Research Service, Madison, Wisconsin, USA and in the Pacific Agri-Food Research Centre, Agriculture Agri-Food Canada, Summerland, British Columbia, Canada.

DEDICATION

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RESUMEN

La avena (*Avena sativa* L.) es una cereal que se cosecha por su semilla rica en nutrientes que se consume como grano entero, saludable a los humanos por el contenido de sus antioxidantes como las avenantramidas (Avns), encontradas en el grano y la cascarilla. Hay más de 30 Avns pero las más abundantes son Avns 2c, Avns 2f y Avns 2p. Este estudio tuvo como objetivo determinar las propiedades físicas y químicas de las Avns sintéticas y las propiedades nutricionales y químicas de extractos de avenas de cuatro variedades (Avemex, Karma, Cuauhtémoc y Chihuahua) cultivadas en Durango, México. Por esta razón se sintetizaron las tres principales Avns y determinaron algunas de sus propiedades físicas y químicas, después éstas fueron usadas como estándares para determinar el contenido de Avns en extractos de avenas. En los extractos de avenas se determinaron contenido fenólico y capacidad antioxidante y en las harinas de avena contenido de ácido fítico y componentes nutricionales. Los resultados mostraron que los granos de avena Avemex tuvo el más alto contenido de las tres Avns, así como también el más alto contenido de proteína y ácido fítico. Las cascarillas de avena Chihuahua, Cuauhtémoc y Karma mostraron los más altos valores de contenido fenólico y capacidad antioxidante. La Avn 2p mostró una mayor estabilidad a la oxidación y al calor y también mayor capacidad antioxidante que las otras Avns. Los grano de de avena Avemex pueden ser usados para producir alimentos nutritivos y saludables.

ABSTRACT

Oat (*Avena sativa* L.) is a cereal grain grown for its seed rich in nutrients that is consumed as whole grain, healthy to humans for its antioxidant content such as avenanthramides (Avns), found in both oat hull and groats. There are more than 30 Avns but the most abundant are: Avns 2c, Avns 2f and Avns 2p. This study had as aim to determine physical and chemical properties of synthetic Avenanthramides and nutritional and chemical of oat extracts of four oat varieties (Avemex, Karma, Cuauhtémoc and Chihuahua) grown in Durango, México to develop in a future new healthy food products based on oat grains and to determine biological properties of synthetic Avns. For this reason was synthesized the three main Avns and determined some physical and chemical properties and after these was used as standards to determine the content of Avns in oat extracts. In oat extracts were determined phenolics content and antioxidant capacity and phytic acid and nutritional components and oat flours. The results showed that Avemex groats had the highest content of three Avns, as well as the highest content of protein and phytic acid. Chihuahua, Cuauhtémoc and Karma hulls showed the highest phenolic content and antioxidant capacity. Avn 2p showed a major stability to heat and oxidation and also higher antioxidant capacity than other Avns. Avemex groats could be used to produce nutritional and healthy foods.

CHAPTER I. INTRODUCTION

The close relationship between health and food has generated considerable interest nowdays, because there are foods that nurture and can provide health benefits acting on physiological processes that reduce the risk of diseases. Nutraceuticals are defined as any food or part of a food (chemical compounds) that provides human health benefits. The nutraceuticals can be classified according to their biological properties, which are directly related to their chemical structure. A wide variety of nutraceuticals are found in fruits , vegetables, legumes and cereals as flavonoids (flavonols, flavanones, flavones, flavan-3-ols, anthocyanins and isoflavones) phenolics acids, hydroxycinnamic acids, lignans, stilbenoids, terpenes (carotenoids, monoterpenes, saponins and lipids), betalains, organosulfides, phytic acid and other.

Whole grains contain many phytochemicals (chemical compounds present in plants) that present human health benefits. Oat is a rich source of nutrients such as proteins, carbohydrates, oils, soluble fiber, vitamins, minerals and antioxidants (Peterson *et al.,* 2002) and has the advantage of being consumed as wholegrain cereal, which has great nutritional benefits as well as human health because the bran, rich in antioxidants is not removed (Chen *et al*., 2004). Antioxidants play an important role in human health to prevent membrane damage, cardiovascular disease, age-related deterioration and cancer (Nie *et al*., 2006; Bratt *et al*., 2003), due to its function as free radical scavengers, reducing agents and quenchers of singlet-oxygen formation (Emmons *et al*., 1999). A variety of compounds with antioxidant activity, were identified in oat such as tocols, phytic acid, phenolic acids, flavonoids, sterols and avenanthramides (Peterson, 2001). Avns have shown to have antioxidant activity (Dimberg *et al*., 1993; Peterson *et al*., 2002; Bratt *et al*., 2003; Ji *et al*., 2003; Lee-Manion *et al*., 2009; Fagerlund *et al*., 2009), anti-inflammatory activity (Liu *et al*., 2004; Guo *et al*., 2008; Sur *et al*., 2008), vasodilatory effect (Nie *et al*., 2006); anti-atherogenic activity (Liu *et al*., 2004), anti-irritant activity (Sur *et al*., 2008), anti-proliferative activity (Guo *et al*., 2010) and improves cardiovascular disease (Ryan, 2007).

A brief literature review showed that studies on Avenanthramides have not been conducted in México neither in the State of Durango. Therefore, the aim of this work was: (i) to determine physical and chemical properties of synthetic Avenanthramides and (ii) to determine nutritional and chemical properties of oat extracts grown in Durango, México. To this end, was needed to synthesize the Avns 2p, 2f and 2c to use as standards to determine the concentration of Avns in oat extracts of four varieties cultivated in Durango, México. Moreover to synthetic Avns were analyzed by Infrared and UV-Visible spectroscopy, Calorimetry and their antioxidant capacity were determined. Oat extracts were also used to determine phenolics content and antioxidant capacity and protein, fat, ash, crude fiber and phytic acid.

CHAPTER II. LITERATURE REVIEW

II.1 Oat

Oat is a cereal of the family Gramineae (Poaceae), the species more cultivated is *Avena sativa*. There are other oat species that are cultivated in a lesser extent as *Avena bizantina* and *Avena strigosa* (Zwer, 2010). Although its center of origin is unknown, the greatest genetic diversity was found in the Mediterranean, the Middle East and the Himalayan regions. In United State of America and Canada, the oat was introduced for immigrants from Great Britain between 1500 and 1600 as animal feed (Zwer, 2010). In Mexico, the oat was introduced in the early twentieth century for Mennonites as forage for work animals and grain for the production of milk and meat of the cattle (Herrera *et al*., 2006). Oat is better adapted to different soil types than many other cereals but can grow better on acid soils. This cereal grows mostly in cool, moist climates and it can be sensitive to hot and dry weather (Strychar, 2011).

Oat production is found ranked sixth in the world, behind rice, corn, wheat, barley and sorghum. Russia, Canada, the United States, the 27 states of the European Union (EU), and Australia are the largest producer of oat in the world with a percentage of 77 %. Russia is the largest worldwide oat producer of oat worldwide with 20% of the total global production. Roughly two-thirds of oat production is used as feedstock and one-third for human consumption. Almost all Russia´s production is consumed in the country so that import and export very few oat tonnage. Meanwhile Canada is the largest commercial producer and exporter of oat in the world with 15% of total global production and 60% of global exports (Strychar, 2011). México during 2010 produced 111,126.64 metric ton of oat grain and 10,014,936.55 metric ton of oat forage. The State of Durango ranks second in national production of oat forage and third of oat grain (SIAP, 2010).

Oat crop (*Avena sativa L.*) is used as animal feedstock for horses, cattle and sheep, and oat groats are specially used as feedstock for poultry and pigs because they do not have the enzymes to digest the hull (Zwer, 2010). In industry the oat is used to produce food, cosmetics, pharmaceuticals and nutraceuticals (Strychar, 2011). Oat

as human food has shown benefits to human health because it is consumed as whole grain. These benefits are attributed to its content of proteins, lipids, fiber, vitamins, minerals and phytochemicals (Peterson *et al*., 2002). Oat contains one-third more protein than wheat and almost four times fatter and less starch (Strychar, 2011b). The consumption of whole grain decreases the incidence of diseases as cancer, heart diseases and diabetes (Welch, 2011; Lee-Manion *et al*., 2009).

II.1.1 Oat in Mexico

Oat in México is used mainly as livestock feed (forage and hay) and to produce a variety of healthy foods (Jimenez, 2009). In 1960 were introduced to México commercial oats of USA and Canada to perform genetic improvement to obtain genotypes with high yield in forage and grain, nutritional quality and disease resistance under the environmental conditions of México. In 1964 were released forage varieties AB-177, Saia and Opalo, in 1965 Putnam 61 and in 1967 Perla variety. In 1962 was performed the first varieties crossing and among crosses were recombined the varieties AB-177 and Putnam 61 and 1967 were obtained the first Mexican varieties, Chihuahua and Cuauhtémoc, suitable to drought environment and presence of stem rust (Jimenez, 2009).

In 1968 appeared race 31 of *Puccinia graminis avenae* what produced damages to the oat crops and the Chihuahua and Cuauhtémoc varieties showed susceptibility to stem rust (*Puccinia graminis*). The program of genetic improvement of oat of INIFAP developed the Avemex and Karma varieties, which are considered moderately resistant to stem rust (*Puccinia graminis avenae*) and crown rust (*Puccinia coronate*) and 1998 these varieties were provided to the producers (Jimenez, 2009). The characteristic of oats used in this research are given below.

Chihuahua. It is a variety that presents forage qualities and measures between 90 to 110 cm , but irrigation may to measure 135 cm. Grain is large and with white hull. This variety can fit under irrigation and temporary, and its flowering occurs between 55 to 66 days and reaches maturity between 95 to 110 days (Herrera, *et al*., 2006). It si considered susceptible to stem rust (*Puccinia graminis avenae*), to crown rust

(*Puccinia coronate*) and to powdery mildew (*Erysiphe graminis*) (Mariscal-Amaro *et al., 2009*).

*Cuauhtémoc***.** It is a forage variety with broad adaptation to irrigated areas and temporary. It has a growth cycle late, flowering time is between 57 and 66 days and reaches its maturity between 99 and 111 days. Plant grows to 105 to 150 cm height. Grain is large and has a hull of light cream color. It is susceptible to crown rust (*Puccinia coronate*) and stem rust (*Puccinia graminis avenae*) (Herrera *et al*., 2006).

Karma. This is a variety of spring habit, erect growth, its flowering reaches 50 -57 days and its maturity varies between 90-93 days and considered intermediate cycle. The height of the plant can be between 70 and 134cm. In this variety the grain hull is brown (Herrera *et al*., 2006). It variety is considered moderately resistant to stem rust (*Puccinia graminis avenae*) and to crown rust (*Puccinia coronata*) (Mariscal-Amaro *et al*., 2009).

*Avemex***.** This variety is considered as a variety semi-late cycle, flowering at 55-60 days and matures between 99 and 100 days, the average height of the plant is 136 cm, described as tall, stem is hollow, which gives little resistance to lodging. Color of grain hull is yellow (Herrera *et al*., 2006). It is considered moderately resistant to stem rust (*Puccinia graminis avenae*) and crown rust (*Puccinia coronata*) (Mariscal-Amaro *et al.,* 2009).

II. 2 Morphology and biochemical components of oat

Oat grain consists of a protective layer called hull and a caryopsis (Figure 1). The hull is composed of two parts, the lemma and the palea, that encloses and protects the groats. The inner bract, the palea, covers the ventral groat surface, which contains the crease. The outer bract, or lemma, surrounds the rest of the groats and overlaps the palea. The groats (or caryopsis) can be divided into three main components: the bran, germ and starchy endosperm (Miller and Fulcher, 2011).

The major chemical constituents of the hull are cellulose and hemicellulose, with lesser amounts of lignin or related phenolic compounds. The endosperm contains the

food supply for the germ and provides energy for the rest of the plant (Okarter and Liu, 2010). The endosperm, which is the largest component contains starch, which is major source of stored energy (Welch, 2011), proteins, vitamins and minerals. The percentage of protein in the groat is 6 to 18% and there are four storage proteins in the oat, globulins that are the main storage protein in oat (52-57% of the total protein), albumins (11-19%), prolamins (7-13%) and glutelins that are the second source of protein storage (21-27%). The globulins have the highest content of lysine, which is limited found in the cereals. High contents of this amino acid give the oat proteins a superior balance of amino acids compared to other cereals (Zwer, 2010). The bran is the multi-layered outer skin of the grain that protects germ and endosperm from damage due to sunlight, water, and diseases. The bran contains antioxidants, fiber, vitamins, and minerals. Soluble fiber is found in form of β-glucans (55% of oat dietary fiber) and present benefits to human health as reduce cholesterol levels in the blood and reduce the glucose and insulin levels and promotes satiety decreasing intake of foods high in fat or sugar (Zwer, 2010). Minerals and vitamins serve as enzymatic cofactors and are also found in the bran layers (Welch, 2011). Germ contains the highest fat concentration (2-11%) (Zwer, 2010) and about 94% of oat fat is constituted by fatty acids (Welch, 2011). The three main fatty acids in oat are linoleic, oleic and palmitic acids. Linoleic and oleic acids (unsaturated acid) are important for human and animal nutrition and palmic acid (saturated acid) increases oil stability against peroxidation (Zwer, 2010). The germ also contains vitamins, some proteins and minerals (Okarter and Liu, 2010).

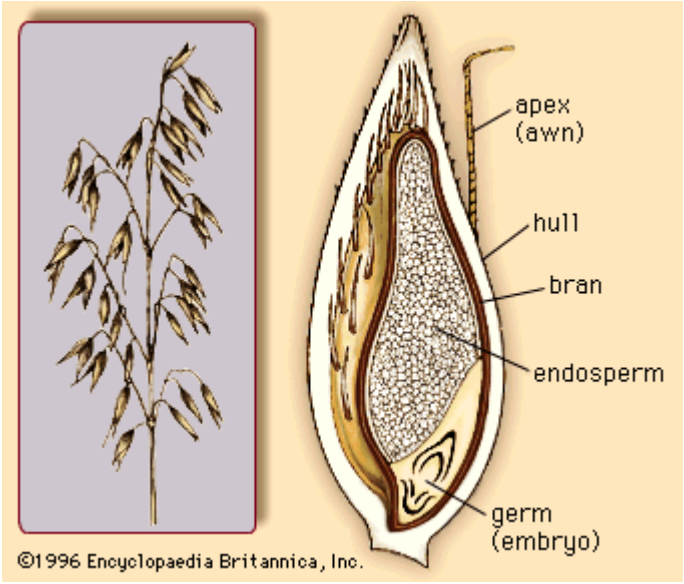


Figure 1 Oat grain structure

II.3 Classification of phenolics compounds

Phenolic compounds or phenolics are considered as secondary metabolites that are synthesized by plants during normal development and under stress conditions such as infection, wounding, UV radiation, among other (Stalikas, 2010). Phenolics are compounds that have one or more hydroxyl groups attached to an aromatic ring and are found in vegetables, fruits, seeds, juices, tea, coffee, chocolate, vinegar and wines (Paixão *et al*., 2008). In plants, phenolics may act as phytoalexins, antifeedants, pollinator's attractants, contributors to plant pigmentation, antioxidants and protective agents against UV light, among others. Phenolics are a diversified group of phytochemicals derived from phenylalanine and tyrosine. In food, phenolics may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability of food (Naczk and Shahidi, 2006). Insoluble phenolics are the components of cell walls, while soluble phenolics are compartmentalized within the plant cell vacuoles (Stalikas, 2010). The content of phenolics may increase under stress conditions such as UV radiation, infection by pathogens and parasites, wounding, air pollution and exposure to extreme temperatures. The level of phenolics in plant sources also depends on such factors as cultivation techniques, cultivar, growing conditions,

ripening process as well as processing and storage conditions, among others (Naczk and Shahidi, 2006)

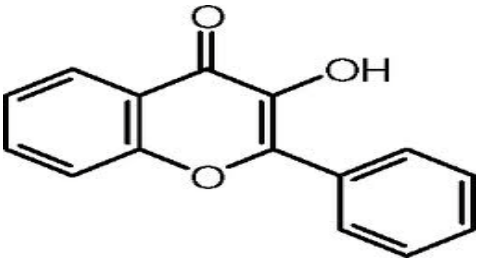
Phenolics have different biological properties as antioxidants, anti-inflammatory, anti-allergic and anti-carcinogenic and many of these functions have been attributed to its antioxidant activity (Fagerlund et al., 2009).

There are two main groups of phenolics termed flavonoids and nonflavonoids. The flavonoids group comprises the compounds with a C_6-C_3-C_6 structure: flavanones, flavones, flavonols, flavan-3-ols, anthocyanidins, isoflavones and proanthocyanidins. The nonflavonoids group is classified according to the number of carbons that they have and comprises the following subgroups: simple phenols, phenolic acids, hydrolysable tannins, acetophenones, cinnamic acids, coumarins, benzophenones, xanthones, stilbenes, chalcones, lignans and secoiridoids (Andrés *et al*., 2010).

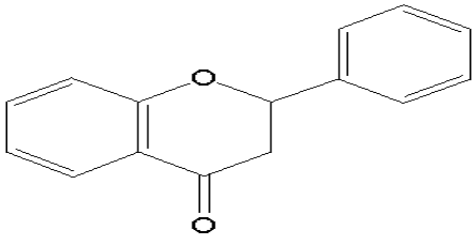
II.3.1 Flavonoids

Flavonoids have a skeleton of diphenylpropanes, two benzene rings (A and B) connected by a three-carbon chain forming a closed pyran ring with the benzene A ring. Flavonoids in plants usually are glycosylated mainly with glucose or rhamnose, but they can also be linked with galactose, arabinose, xylose, glucuronic acid, or other sugars. The number of glycosyl moieties usually varies from one to three (Andrés *et al*., 2010).

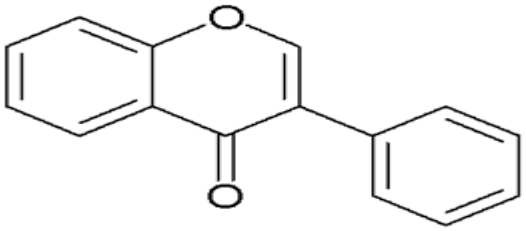
Flavonols and flavones have a double bond between C_2 and C_3 in the flavonoid structure and an oxygen atom at the C_4 position. Furthermore, flavonols also have a hydroxyl group at the C_3 position (Andrés *et al*., 2010).



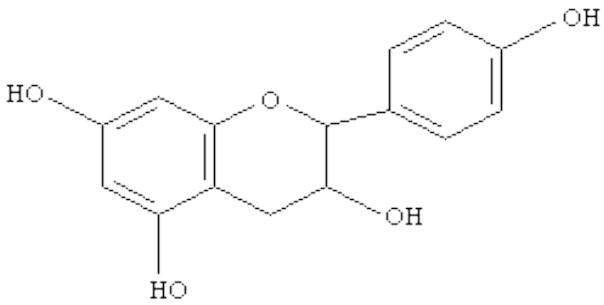
Flavanones are represented by the saturated three-carbon chain and an oxygen atom in the C_4 position (Andrés *et al*., 2010).



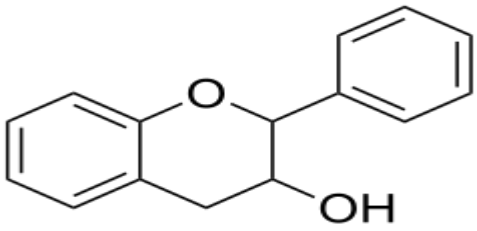
Isoflavones also have a diphenylpropane structure in which the B ring is located in the C_3 position (Andrés *et al*., 2010).



Anthocyanins are based on the flavylium salt structure and are water-soluble pigments in plants. They are found in the form of glycosides in plants and foods of their respective aglycones, called anthocyanidins. The most common sugars are glucose, galactose, rhamnose, xylose, arabinose and fructose, which are linked mainly in the C_3 position as glycosides and in C_3, C_5 as diglycosides (Andrés *et al*., 2010).

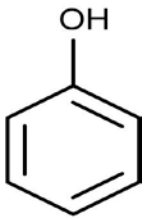


Flavan-3-ols or flavanols have a saturated three-carbon chain with a hydroxyl group in the C_3 position. In food they are present as monomers or as proanthocyanidins, which are polymeric flavanols (4 to 11 units) known also as condensed tannins. In foods they are never glycosylated (Andrés *et al*., 2010).

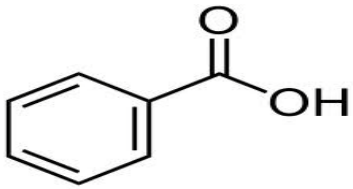


II.3.2 Nonflavonoids

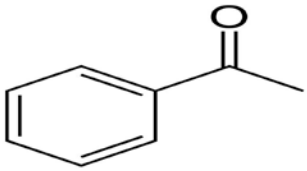
Simple phenols (C_6) are substituted phenols. The ortho, meta and para nomenclature refers to a 1,2-, 1,3- and 1,4-substitution pattern of the benzene ring, respectively, where the functional groups is the hydroxyl group. Also benzene ring can have three substituents 1,3,5, which, when all three substituents are identical, is designated as meta-trisubstitution pattern, whereas the 1,2,6, substitution pattern is indicated by the prefix “vic” (Vermerris and Nicholson, 2006) .



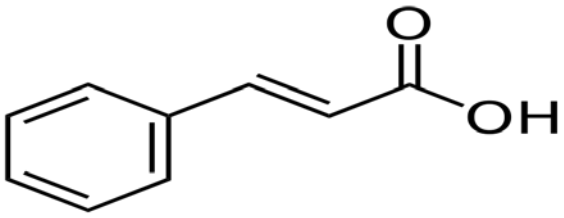
Phenolic acids (C_6-C_1) are hydroxy-benzoic acids and are characterized by the presence of a carboxyl group substituted on a phenol. Examples of this group are gallic acid, salicylic acid and vanillic acid (Vermerris and Nicholson, 2006). Hydrolyzable tannins are mainly glucose esters of gallic acid (Andrés *et al*., 2010).



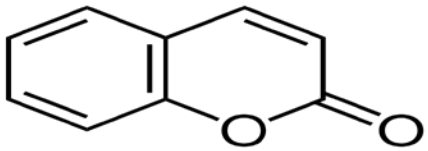
Acetophenones are aromatic ketones and phenylacetic acids; these have a chain of acetic acid linked to benzene. Both have a C_6-C_2 structure (Andrés *et al*., 2010) .



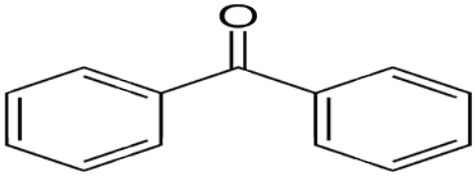
Hydroxycinnamic acids (C_6-C_3) are included in the phenylpropanoid group. They are formed with an aromatic ring and a three-carbon chain. There are four basic structures: the coumaric acids, caffeic acids, ferulic acids and sinapic acids (Lacueva *et al*., 2010). Cinnamic acids are commonly found in plants as esters of quinic acid, shikimic acid and tartaric acid as for example an ester of caffeic acid (Vermerris and Nicholson, 2006).



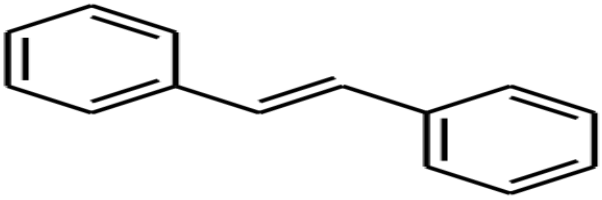
Coumarins are a group of compounds known as the benzopyrones, all of which consist of a benzene ring joined to a pyrone (Andrés *et al*., 2010).



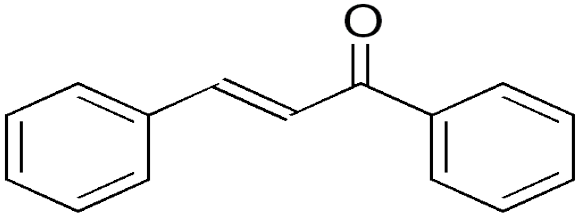
Benzophenones and xanthones have the C_6-C_1-C_6 structure. The basic structure of benzophenone is a diphenyl ketone, and that of xanthone is a 10-oxy-10H-9-oxaanthracene (Andrés *et al*., 2010).



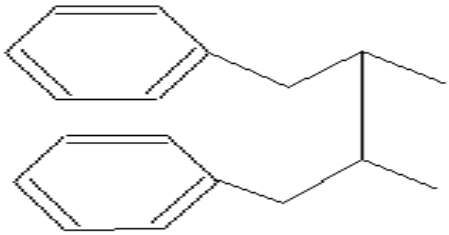
Stilbenes have a 1,2-diphenylethylene as their basic structure (C_6-C_2-C_6). Resveratrol, the most widely known compound, contains three hydroxyl groups in the basic structure and is called 3,4',5-trihydroxystilbene (Andrés *et al*., 2010).



Chalcones with a C_6-C_3-C_6 structure are flavonoids lacking a heterocyclic C-ring. Generally, plants do not accumulate chalcones. After its formation, naringetin chalcone is rapidly isomerized by the enzyme chalcone isomerase to form the flavanone, naringenin (Andrés *et al*., 2010).



Lignans are compounds derived from two β-β'-linked phenylpropanoid (C_6-C_3) units and are widely distributed in the plant kingdom. They are classified into eight subgroups: furofuran, furan, dibenzylbutane, dibenzylbutyrolactone, aryltetralin, arylnaphthalene, dibenzocyclooctadiene and dibenzylbutyrolactol. These subgroups are based upon the way in which oxygen is incorporated into the skeleton and the cyclization pattern (Andrés *et al*., 2010).



Secoiridoids are complex phenols produced from secondary metabolism of terpenes as precursors of several indole alkaloids. They are characterized by the presence of elenolic acid in its glucosidic or aglyconic form in their molecular structure (Andrés *et al*., 2010).

II.4 Oat antioxidants

An antioxidant is defined as any substance that, when is present at low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate (Fagerlund *et al*., 2009). Antioxidants protect the body from membrane damage, cancers, heart disease and age-related deterioration (Zwer, 2010). In humans, radicals are formed during normal metabolism as well as from environmental radiation. These free radicals can damage the DNA molecule or cell membranes (lipid peroxidation) leading to cancerous cell and atherosclerosis (Peterson, 2001). Many compounds that occur in the plants have antioxidant activity in the body as vitamin E, vitamin C, lignans, flavonoids, β-carotenes. Vegetables, fruits and grains are the most rich sources of antioxidants (Peterson, 2001).

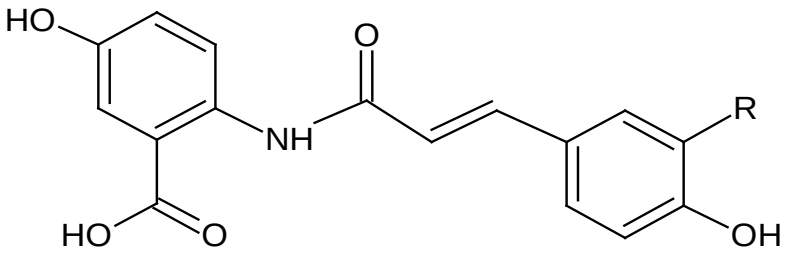
Oat is a grain cereal rich in antioxidants as tocopherols and tocotrienols, phenolic acids, flavonoids, sterols, phytic acid and avenanthramides (Peterson, 2001). This section is focused on avenanthramides and phytic acid.

II.4.1 Avenanthramides

Avenanthramides (Avns) are phenolic compounds formed for a cinnamic acid linked with an amide bond to an anthranilic acid and the enzyme hydroxycinnamoyl CoA: hydroxyanthranilate *N*-hydroxycinnamoyl transferase (HHT) catalyses the final condensation to form Avns in the oat (Wise *et al*., 2009). Avns are present among cereals, exclusively in oat (Meydani, 2009). Although they are also found in butterfly eggs and in carnation (*Dianthus caryophyllus*) (Wise *et al*., 2009). Collins (1989) isolated more 25 Avns, but only three are the most abundant in grain 2p, 2c and 2f (Figure 2) (2 indicating 5-hydroxyanthranilic acid and p, c and f indicating the p-coumaric, caffeic or ferulic acid) (Bratt *et al*., 2003). Avns have been found in the grain (oat groats and hulls) (Collins, 1989; Bratt *et al*., 2003) and leaves (Ishihara *et al*., 1999; Peterson and Dimberg, 2008; Dimberg and Peterson, 2009) and are considered phytoalexins because they are synthesized by plant in response to attack of pathogens such as fungi (Mayama *et al*., 1981; Mayama *et al*., 1982). Avns content can vary greatly among oat cultivars and different growing environment (Emmons and Peterson, 2001; Dokuyucu *et al*., 2003; Peterson *et al*., 2005). Oats grown in different locations in Wisconsin, USA, showed for Avn 2p a concentration range of 9.3-51.9 mg kg^{-1}, for Avn 2f of 13.2-77.8 mg kg^{-1} and for Avn 2c of 25.4-145.3 mg kg^{-1} (Emmons and Peterson, 2001).

Previous studies have demonstrated that Avns present antioxidant activity with different methods and in vitro and in vivo systems. Dimberg *et al*. (1993) studied the antioxidant activity of two Avns using a linoleic acid- oxygen consumption system; Peterson *et al*. (2002), tested the antioxidant activity of Avns using inhibition of β-carotene bleaching and free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH); Bratt *et al*. (2003) performed the study using DPPH and linoleic acid oxidation methods. Ji *et al*. (2003) demonstrated the antioxidant effect of Avn 2c attenuated lipids peroxidation in specific tissues of rats. Chen *et al*. (2007) determined antioxidant activity of Avns on resistance of human LDL to Cu^{2+} induced oxidation. Fagerlund *et al*. (2009) evaluated antioxidant activity of fifteen Avns for DPPH and linoleic acid oxidation, Lee-Manion *et al*. (2009) tasted antioxidant activity of synthetic Avns, Tranilast (oral drug with

chemical structure similar to Avns), hydroxycinnamic acids and ascorbic acid using two assays, DPPH and ferric reducing antioxidant potential (FRAP). Antioxidant activity of Avns increases with the number of radical-stabilizing groups ortho to the phenolic hydroxyl group (Fagerlund *et al*., 2009). Avns also exhibit anti-inflammatory (Guo *et al*., 2008; Sur *et al*., 2008), anti-atherogenic (Liu *et al*., 2004; Nie *et al*., 2006), anti-irritant (Sur *et al*., 2008) and anti-proliferative (Guo *et al*., 2010) activities, prevents coronary heart disease and improves cardiovascular disease (Ryan *et al*., 2007). Avns have also been associated with crown rust (*Puccinia coronata*) incidence and genetic resistance to the disease (Wise *et al*., 2008).



Avenanthramide	Chemical name	R
Anv 2p	N-[4'-hydroxy- (E)-cinnamoyl]-5- hydroxyanthranilic acid	H
Avn 2f	N-[4'-hydroxy-3'-methoxy- (E)-cinnamoyl]-5- hydroxyanthranilic acid	OCH_3
Avn 2c	N-[3', 4'-dihydroxy- (E)-cinnamoyl]-5- hydroxyanthranilic acid	OH

Figure 2 Structure of the main three Avenanthramides in oats

II.4.2 Phytic acid

Phytic acid or also called *myo*-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate or *myo*-inositol hexakisphosphate is a plant component that constitutes 1-5% by weight of most cereals, nuts, legumes, oil seeds, roots and tubers (Graf and Eaton, 1990; Hídvegi and Lásztity, 2002). Phytic acid is generally regarded as the primary storage form of both phosphate and inositol in seeds. Phytic acid phosphorus constitutes the major portion of total phosphorus in several seeds and grains. It accounts for 50-80% of the total phosphorus in different cereals. The phytic acid content is influenced by cultivar, climatic conditions and year. The accumulation of phytic acid in monocotyledonous seed is in the aleurone layer, particularly the aleurone grain. Phytic acid content of cereals varies from 0.5 to 2.0% (Hídvegi and Lásztity, 2002). Phytic acid usually occurs as a mixed calcium-magnesium-potassium salt in discrete regions of the seeds (phytic acid salts are generally referred as phytate) (Graf and Eaton, 1990).

Because of high density of negatively charged phosphate groups, the phytic acid can form salts with minerals cations. The binding can result in very insoluble salts with poor bioavailability of minerals. Besides its well-known negative properties IP6, by complex iron, may bring about a favorable reduction in the formation of hydroxyl radicals in the colon (Garcia *et al*., 1999). Antioxidant property of phytic acid derives from its relatively high binding affinity for iron being that free iron and certain iron chelates greatly facilitate the production of radical species with reactivity similar to that of OH radical (Graf and Eaton, 1990).

Metal complexes of *myo*-inositol-hexaphosphate (IP6) and lower inositol phosphates (IP5-IP1) are poorly soluble at the pH of the gastrointestinal tract and may reduce the bioavailability of minerals such as Fe, Zn, Ca and Cu. The affinity of IP4 and IP3 for mineral elements is lower than that of IP6, and the solubility of metal complexes formed with IP4 and IP3 is higher than with IP6 (Ekholm *et al*., 2003).

II.5 Methods to determine antioxidant capacity

It is well-known that oxidation damages various biological substances and subsequently causes many diseases. These diseases may be cancer, liver disease, Alzheimer´s diseases, aging, arthritis, inflammation, diabetes, Parkinson´s disease and atherosclerosis. Antioxidants have been used to treat or prevent oxidative damage (Moon and Shibamoto, 2009).

Mechanisms of antioxidant action include serving as (1) physical barriers to prevent reactive oxygen species (ROS) generation or ROS access to important biological sites, e.g., UV filters, cell membranes; (2) chemical traps that absorb energy and electrons, quenching ROS, e.g., carotenoids, anthocyanidins; (3) catalytic systems that neutralize or divert ROS, e.g., the antioxidant enzymes SOD (superoxide dismutase), catalase, and glutathione peroxidase; (4) binding/inactivation of metal ions to prevent generation of ROS e.g., ferritin, ceruloplasmin and catechins and (5) chain-breaking antioxidants which scavenge and destroy ROS, e.g., ascorbic acid (vitamin C), tocopherols (vitamin E), uric acid, glutathione, flavonoids (Karadag *et al*., 2009).

On the basis of the inactivation mechanism involved, major antioxidant capacity methods have been generally divided into two categories: (1) hydrogen atom transfer (HAT) reaction and (2) electron transfer (ET) reaction (Karadag *et al*., 2009).

II.5.1 Oxygen radical absorbance capacity assay (ORAC)

ORAC measures antioxidant inhibition of peroxyl-radical-induced oxidations and reflects classical chain-breaking antioxidant activity by H-atom transfer. In the basis assay, the peroxyl radicals generated from thermal decomposition of AAPH in aqueous buffer or hydroxyl radicals generated from Cu^{2+} -H_2O_2 react with a fluorescent probe, an oxidizable protein substrate, to form a nonfluorescent product, which can be quantified easily by fluorescence. Probe reaction with peroxyl radicals is followed by loss of the intensity of fluorescence by time. In general samples, control and standard (Trolox) are mixed with fluorescein solution and incubated at constant temperature (37 °C) before AAPH solution is added to initiate the reaction. Under this

reaction conditions, 1 mol of AAPH loses a dinitrogen to generate 2 mol of AAPH radical. The loss of fluorescence of the probe is an indication of the extent of damage from its reaction with the peroxyl radical. The fluorescence intensity [485 nm (excitation)/525 nm (emission)] is measured every minute for 35 min at ambient conditions (pH 7.4, 37 °C). In the presence of antioxidant, the fluorescence decays is prevented (Karadag *et al*., 2009).

II.5.2 β-Carotene or crocin-bleaching assay

Carotenoids bleach via autoxidation induced by light or heat or peroxyl radicals (e.g., AAPH or oxidizing lipids). The addition of an antioxidant-containing sample, individual antioxidant, or plant extracts causes the inhibition of β-carotene bleaching. The assay measures the decrease in the rate of β-carotene or crocin decay provided by antioxidants. Color loss followed optically at 443 nm in phosphate buffer (pH 7.0), so no special instrumentation is required. The bleaching rate becomes linear at ~1 min after the addition of AAPH and is monitored for 10 min (Karadag *et al*., 2009).

II.5.3 Total phenol assay by using the Folin-Ciocalteu reagent (FCR)

The exact chemical nature of the Folin-Ciocalteu reagent is not known, but it is accepted that it contains phosphomolybdic/phosphotungstic acid complexes. The chemistry behind the FCR assay counts on the transfer of electrons in alkaline medium from phenolics compounds and other reducing species to molybdenum, forming blue complexes that can be monitored spectrophotometrically at 750-765 nm. The phenolics compounds react with FCR only under basic conditions (Karadag *et al*., 2009).

II.5.4 Trolox equivalent antioxidant capacity assay (TEAC)

TEAC assay was first reported by Miller *et al*., (1993) and has been improved and widely used in testing antioxidant capacity in food samples. ABTS is a peroxidase substrate, which when oxidized by peroxyl radicals or other oxidants in the presence of H_2O_2 generates a metastable radical cation $ABTS^{\cdot+}$, which is intensely colored and can be monitored spectrophotometrically in the range of 600-750 nm. The antioxidant

capacity is measured as the ability of test compounds to decrease the color reacting directly with $ABTS^{\cdot+}$ radical and expressed relative to Trolox (Karadag *et al*., 2009).

II.5.5 DPPH radical scavenging capacity assay

The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) is a long-lived organic nitrogen radical and has a deep purple color. It is commercially available and does not have to be generated before assay. In this assay, the purple chromogen radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine. The reducing ability of antioxidants towards DPPH can be evaluated by electron spin resonance or by monitoring the absorbance decrease at 515-528 nm until the absorbance remains stable in organic media (Karadag *et al*., 2009).

II.5.6 Ferric reducing/antioxidant power assay (FRAP)

FRAP assay is based on the ability of phenolics to reduce yellow ferric tripyridyltriazine complex [Fe(III)-TPTZ] to blue ferrous complex [Fe(II)-TPTZ] by the action of electron-donating antioxidants. The resulting blue color measured spectrophotometrically at 593 nm is taken as linearly related to the total reducing capacity of electron-donating antioxidants (Karadag *et al*., 2009).

II.5.7 Total antioxidant potential assay using Cu(II) as an oxidant

The method is based on reduction of Cu(II) to Cu(I) by reductants (antioxidants) present in a sample. It has been introduced as Bioxytech AOP-490 and CUPRAC developed by Apak *et al*., (2004). In the Bioxytech AOP-490 assay, bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) forms a 2:1 complex with Cu(I) producing a chromophore with maximum absorbance at 490 nm. Reaction rate and concentration of products are followed by bathocuproine complexation of the Cu(I) produced (Karadag *et al*., 2009).

II.5.8 Methods that measure the inhibition of induced lipid autoxidation

This method artificially induces autoxidation of linoleic acid or LDL (low-density lipoprotein) particles or serum proteins without prior isolation of LDL particles, by either a transition element such as Cu (II) or thermal decomposition of AAPH, a water-soluble diazo $ROO^{\cdot}$ initiator and peroxidation of the lipid components

determined through the formation of conjugated dienes followed at 234 nm spectrophotometrically (Karadag *et al*., 2009).

II.5.9 Chemiluminescence and photochemiluminescence assays

In general principle of chemiluminescence assay is based on the ability of luminol and related compounds to luminescence under the attack of free radicals. Oxidants sources of peroxyl radicals include the enzyme horseradish peroxidase and H_2O_2-hemin. Luminol is the most widely used marker compound to trap oxidants and convert weak emissions into intense, prolonged and stable emissions (Karadag *et al*., 2009).

Photochemiluminescence assay was described by Popov and Lewin, was commercialized by Analytik Jena AG (Jena, Germany), and is commercialized as a complete system under the name PHOTOCHEM. This assay involves the photochemical generation of superoxide $O_{2\times}$ free radicals combined with chemiluminescence (CL) detection. The optical excitation of a photosensitizer results in the generation of the superoxide radical. The free radicals are monitored with a CL reagent. Luminol acts as a photosensitizer as well as an oxygen radical detection reagent (Karadag *et al*., 2009).

II.5.10 Total oxidant scavenging capacity assay

Total oxidant scavenging capacity (TOSC) assay is based on oxidation of α-keto-γ-methiolbutyric acid to ethylene by peroxyl radicals produced from AAPH. The antioxidant capacity of a molecule is quantified from its ability to inhibit ethylene formation relative to a control. The time duration of ethylene formation is followed by gas chromatography (Karadag *et al*., 2009).

III. JUSTIFICATION

In recent years, the consumption of whole grain foods has come incremented due to its association with a lower risk for coronary heart, diabetes, cancer, atherosclerosis and other diseases. Oat is one cereal that have reported great benefices to human health due to its high protein, lipid, dietary fiber content and for being a source rich in antioxidants.

The mainly use of oat in México is as animal feed and the human consumption is low in our country. Durango ranks second in national production of oat forage and third of oat grain (SIAP, 2006). This show that oat is used in our state basically as animal feed and is not use to manufacture food that in addition to nurture to help to prevent diseases. The main focus of this investigation is to study the nutritional components of oats grown in state of Durango and their chemical and physical properties to develop future healthy foods and nutraceutical ingredients.

IV. OBJECTIVES

General objective: To determine physical and chemical properties of synthetic avenanthramides and nutritional and chemical properties of oat extracts grown in Durango, México.

Specific objectives

- ✓ To synthesize the avenanthramides 2p, 2f and 2c to use them as standards to identify avenanthramides in oat extracts
- ✓ To determine UV-VIS and FT-IR spectra of synthetic avenanthramides to confirm their structures
- ✓ To determine thermal properties of avenanthramides to predict their stability (degradation) during food processing
- ✓ To evaluate antioxidant capacity of synthetic avenanthramides
- ✓ To determine nutritional components of oats
- ✓ To determine avenanthramides, phytic acid and inositol hexakisphosphate (IP6) content in oat extracts
- ✓ To determine phenolic content and antioxidant capacity of oat extracts

V. MATERIALS AND METHODS

V.1 Sample preparation

Four oat varieties were used in this study, Chihuahua, Cuauhtemoc, Avemex and Karma. Two susceptible varieties to stem rust (*Puccinia graminis*) and crown rust (*Puccinia coronata*) (Chihuahua and Cuauhtémoc), and two varieties moderately resistant to these diseases (Avemex and Karma) were used because Avns content have been associated with crown rust (*Puccinia coronata*) incidence and genetic resistance to the disease (Wise *et al*., 2008).

Chihuahua and Cuauhtémoc were provided by MC Jesus Herrera, they grown during 2010 in Nuevo Ideal in the state of Durango, Mexico (24° 53´ 51.3" N; 105° 02´ 32" W; altitude 1990 m). Soil type was sandy clay loam and pH 8.28. Soil was not fertilized and no pathogens were observed. Avemex and Karma were provided by INIFAP Unit placed in Durango, Mexico, these varieties were genetically modified by the Instituto Nacional de Investigación Forestal, Agrícola y Pecuaria (INIFAP) in Mexico, to confer moderate resistance to stem rust (*Puccinia graminis*) and crown rust (*Puccinia coronate*) (Mariscal-Amaro *et al*., 2009), and were grown during 2010 in the Valle del Guadiana, Durango, Mexico (23° 59' 12.4" N; 104° 37' 38.7" W; altitude 1878 m). The soil had pH 8.0, loam texture, fertilized with nitrate 120 kg ha^{-1} and phosphate 60 kg ha^{-1}. No pesticides were used. Neither pathogens were observed.

A total of 500 g of each oat were separated in groats (grain without hull) and hulls (Figure 3) by hand and stored to room temperature and away from light.


Chihuahua Hulls (CHH)


Chihuahua Groats (CHG)


Cuauhtémoc Hulls (CUH)


Cuauhtémoc Groats (CUG)


Karma Hulls (KH)


Karma Groats (KG)


Avemex Hulls (AH)


Avemex Groats (AG)

Figure 3 Hulls and groats of different oat varieties

V.2 Nutritional composition of oats

Samples (25 g of each sample) were ground in a blender until pass through a 1mm sieve and after stored at -20 ºC until use.

V.2.1 Determination of Moisture (Dry matter)

Moisture content was determined according to AOAC (2000), briefly, in a melting pot (previously weighed) was added 1.0 (±0.01g) of sample and introduced to oven at 105 ºC ± 5 ºC during 24 h, after melting pot was placed in the desiccator until to achieve room temperature and finally weighed again. The values obtained were used to calculate in dry basis the nutritional composition of oats.

$$\%\ \mathbf{Moisture}\ (\%\mathbf{M}) = \frac{\mathbf{weight\ loss\ on\ drying\ (g)}}{\mathbf{g\ of\ sample}}\ \mathbf{x\ 100}$$

$$\%\ \mathbf{Dry\ matter} = \mathbf{100} - \%\mathbf{M}$$

V.2.2 Ash determination

A total of 1 g of sample was weighed into a porcelain crucible and placed in a furnace to 550 ± 5 °C for approximately 5 h. Crucible was removed from the furnace and placed in an oven at 110 °C during 2 h. It was there after transferred to a desiccator and cooled and weighed immediately (AOC, 2000).

$$\mathbf{Ash}\ (\%) = \frac{\mathbf{weight\ of\ sample\ (g)} - \mathbf{weight\ loss\ on\ ashing\ (g)}}{\mathbf{weight\ of\ sample\ (g)}}\ \mathbf{x\ 100}$$

V.2.3 Determination of protein content

Protein content was analyzed according to AOAC (2000). A total of 0.1 g of sample and 0.6 g of selenium were placed into a 100 ml kjendahl flask, adding 2 mL of concentrated H_2SO_4. Flask was heated in the digester maintaining constant boiling until the solution clarifies. Flask was cooled and added 25 mL distillated water and 10 mL of NaOH (40%). In a 100 mL beaker , 10 mL of H_3BO_4 and 3 drops of indicator No 5 were added. The solution was distillated until complete a volume of 30 mL and change purple to green color. The beaker that contains the distillated solution was added dropwise 0.02 N HCl until change green to purple color.

$$\%\ \mathbf{Nitrogen} = \frac{\mathbf{mL\ HCl\ x\ Normality\ \ HCl\ x\ (0.014)}}{\mathbf{g\ of\ sample}}\ \mathbf{x\ 100}$$

$$\%\mathbf{Crude\ protein} = \%\ \mathbf{Nitrogen\ x\ 6.25}$$

V.2.4 Determination of fat content

Crude fat content was determined according to AOAC (2000). A total of 1.5 g of dry sample was deposited in a filter paper. It was folded and placed in a cellulose thimble. In an aluminum cup (constant weight) was added 70 mL of petroleum ether, thimble and cup were placed in the Soxhlet extractor. When the extraction was completed, The cup was placed in the oven at 110°C during 2 h. The cup was transferred to desiccator and weighed.

$$\%\ \mathbf{Crude\ fat} = \frac{\mathbf{Weight\ of\ ether\ extract\ (g)}}{\mathbf{g\ of\ sample}}\ \mathbf{x\ 100}$$

V.2.5 Determination of crude fiber

The crude fiber content was determined using the method of AOAC (2000). A total of 1g of defatted sample was placed in a 600 mL Berzelius beaker, where 200 mL boiling 0.255 N H_2SO_4 was added. The beaker was heated until constant boiling, during 30 min (starting from boiling). The beaker was retired from the hot plate and mixture was filtered through a fabric and washed with hot water until all acid was eliminated. Residue was transferred to the breaker again and was added 200 mL boiling 0.313N NaOH. Mixture boiled for 30 min and was filtered and washed with hot water to eliminate NaOH. Fiber was placed in a crucible and dried in the oven at 110 °C during 24 h. Crucible was transferred to desiccator and weighed. Ignite at 550 °C during 4 h and after cooled in the desiccator and weighed.

$$\%\ \mathbf{Crude\ fiber} = \frac{\mathbf{loss\ in\ weight\ on\ ignition\ (g)}}{\mathbf{g\ of\ sample}}\ \mathbf{x\ 100}$$

V.3 Synthesis of avenanthramides

The synthesis of Avns was performed in Cereal Crops Research, ARS-USDA in Madison, Wisconsin, USA, with the supervision of Dr Mitchell Wise. The process is described as following. Avns 2c and 2p were synthesized following the method of Wise (2011) and Avn 2f was provided by Dr Wise. Reaction from Avn 2c started with cinnamic acid without acetylating for what was necessary previously performed the protection of hydroxyl group. A total of 5 mmol of caffeic acid in 2 mL of pyridine was acetylated in 10 mL of acetic anhydride during 5 h, after 50 mL of cold water was added to the reaction and solution was left in the fridge for one hour. The precipitant was collected and washed several times with cold water and dried at 50 °C all night. Acetylated acid was dissolved in 10 mL of dimethylformamide with 1.25 mL of triethylamine, and then an equimolar amount of benzotriazol-1-yloxy-tris-(dimethylamino) phosphonium hexafluorophosphate (BOP) dissolved in 5 mL of CH_2Cl_2, was added to the solution while stirring on an ice bath. After a solution of 5-

hydroxyanthranilic acid in 20 mL of dimethylformamide, (equimolar to the acetylated acid) was added dropwise to the solution and was left 30 min in addition in ice bath. Solution was removed of ice bath and was continuosly stirred to room temperature for 2 h. The reaction was stopped with 80 mL of 0.5 M HCl and stored overnight in the frigde. Acetoxy avenanthramides was extracted with ethyl acetate and solvent was removed by rotary evaporation, and acetyl group was removed with 40 mL of 5% pyrollidine in CH_2Cl_2 for 50 min at room temperature. The reaction was stopped with 80 mL of 1M HCl and avenanthramide 2c extracted with ethyl acetate. Purification was performed by LH-20 column chromatography using solvent IV (chloroform-ciclohexane-methanol-acetic acid, 50:25:20:5 respectively), described by Collins (1989). To synthesize Avn 2p, the reaction started with acetylated acid and then the procedure described to Avn 2c was followed. For the purification to Avn 2p was used solvent III (chloroform-ciclohexane-methanol-acetic acid, 50:30:15:5 respectively) described by Collins (1989). Avenanthramides structures were confirmed by LC-MS. LC-MS analysis was performed on an Agilent 1100 liquid chromatography system with a 1946 series ion-trap mass spectrometer. A 2.1 x 30 mm C-18 column (Zorbax SB-C18, Agilent, Santa Clara, CA) as described by Wise, (2011). The mobile phase used was H_2O with 5 % acetonitrile and 0.1 % formic acid as Buffer A and acetonitrile with 0.1 % formic acid as Buffer B. A gradient of 13 to 30 % B over 20 min at a flow of 0.2 mL min^{-1}. The column was operated at 30 °C with 2.0 µL injections. Detection was made by diode array spectrometry monitoring absorbance at 280 and 330 nm and by ion-trap mass spectrometry. Electrospray ionization was operated in positive ion mode using nebulizer gas (N_2) 30 psi, dry gas flow at 6.0 L min^{-1} at 350 °C with a capillary voltage set at 3500 V, scanning from *m/z* 100 at 1000 at 13000 *m/z* s^{-1}.

V.4 Extraction and analysis of avenanthramides

Extraction and analysis of Avns were performed as described Bryngelsson et al. (2002). The process is described as following. Groats and hulls were separated by hand and ground in a blender until the powder passed through a 0.5 mm sieve. 5g of each sample (in triplicate) were used to extract Avns with methanol (36mL) by stirring for 30 min twice at room temperature the same day that the grinding was performed.

The mixture was centrifuged for 10 min, the supernatants pooled, filtered through Whatman filter paper No. 41, and the solvent evaporated under vacuum at 40 °C. The residue was suspended to 2 mL in methanol and stored at -20 °C until further analysis was performed. Avns were analyzed by HPLC in an Agilent HPLC 1100 equipped with a degasser, quaternary pump and diode-array detector using a reversed phase column (Waters C-18 Symmetry 3.9x150mm). The mobile phase consisted of two solvents: Solvent A (0.01M phosphoric acid in acetonitrile:water 5:95, v/v) and acetonitrile as Solvent B. Samples were run with a linear gradient for 60 min from 0-40% B at a flow rate of 1 mL min^{-1} flow rate. The analytes were detected at 340 nm, with a bandwidth of 8 nm, and 500 nm as reference with a bandwidth of 50 nm. Synthetic standards of each Avns were used to identify and quantify oats Avns. The determination of Avns content in oat extracts was performed wet basis.

V.5 Analysis of oat extracts and synthetic avenanthramides

This part of the investigation was performed in the Bioproducts and Bioprocess Program of Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, in Summerland, British Columbia, Canada.

Samples (40 g of each sample, groats and hulls) were ground in a coffee mill (they were not sieved) and stored at -20 ºC until use.

V.5.1 Determination of the dry matter content

The dry matter content was determined again using the official Method 934.06 of AOAC (2000) to perform the calculations on dry basis of all determinations performed during the internship. In aluminum pans (3 pans per sample) weighed previously, were weighed around 1 to 1.1 (±0.1 g) of ground sample. Then pans were placed into the vacuum drying oven (the temperature used in this analysis was ~65 °C and vacuum of 23.5 in Hg) around 20h. After pans were placed in the desiccators to cool them down and then weighed. Pans returned to the oven around 6h and weighed again (if values are not stable, the pans must be placed in the oven until constant weigh). The final values were recorded.

V.5.2 Extraction of phenolic compounds with 80% ethanol

Extraction of phenolic compounds was performed according to the procedure described by Oomah et al. (2010). Into a 25 mL Erlenmeyer Flask, previously labeled and weighed, was weighed 200 mg of ground sample (the samples were not sieved) and added a stir bar to each flask. The flask was sealed with aluminum covered cork and placed in the fume hood. A total of 8 mL of 80% ethanol were added to the flask (a 5000 µL Eppendorf pipette was used to add the solvent in 2 times 4mL). The sample was stirred magnetically at speed 6 at room temperature for 2 hours, using a magnetic stirrer with 15 stirring positions (RT 15 power IKAMAG). After the content was transferred to a 10 mL Evergreen screw cap plastic vial and centrifuged at full speed for 10 min (IEC HN-SII Centrifuge). The supernatant was transferred to 10 mL Evergreen screw cap plastic vial and stored at -20 ºC until use. The extractions were performed for triplicate.

V.5.3 Analysis of phenolic compounds

The phenolic content of oat extracts was performed following the method described by Oomah et al. (2005). To determine phenolics in the oat extracts was used a UV plate, 96 well flat bottom. First, the empty plate was read to ensure that it is clean, and then 150 µL of 80% EtOH in 2% HCl was placed in each well, after 100µL of 80% EtOH was placed in each well of the blank columns, 100 µL extract in each of sample columns and 100 µL standard solution in each well of standard column(s) (See Figure 4). When the plate was prepared, it was mixed and shaken for 210 seconds and later absorbance was read to 280 (Catechin), 320 (Caffeic acid), 360 (Quercetin) and 520 (Cyanidin-3-glucoside) nm using a spectrophotometer (spectramax Plus 384, Molecular Devices Corp., Sunnyvale, CA). In addition, was read the absorbance at 710 nm to check turbidity. The standards were prepared using Catechin (0-602.5 mg L^{-1}) (Total phenolic), Caffeic acid (0-90 mg L^{-1}) (Tartaric esters), Quercetin (0-79 mg L^{-1}) (Flavonols) and Cyanidin-3-glucoside (0-20 mg L^{-1}) (Anthocyanins).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	CHH1	CHH3	CUH2	KH1	KH3	AH2	CHG1	CHG3	CUG2	Caf 10
B	Blank	Blank	CHH1	CHH3	CUH2	KH1	KH3	AH2	CHG1	CHG3	CUG2	Caf 10
C	Blank	Blank	CHH1	CHH3	CUH2	KH1	KH3	AH2	CHG1	CHG3	CUG2	Caf 10
D	Blank	Blank	CHH1	CHH3	CUH2	KH1	KH3	AH2	CHG1	CHG3	CUG2	Caf 10
E	Blank	Blank	CHH2	CUH1	CUH3	KH2	AH1	AH3	CHG2	CUG1	CUG3	Caf 51
F	Blank	Blank	CHH2	CUH1	CUH3	KH2	AH1	AH3	CHG2	CUG1	CUG3	Caf 51
G	Blank	Blank	CHH2	CUH1	CUH3	KH2	AH1	AH3	CHG2	CUG1	CUG3	Caf 51
H	Blank	Blank	CHH2	CUH1	CUH3	KH2	AH1	AH3	CHG2	CUG1	CUG3	Caf 51
	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
80% EtOH in 2% HCl	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL
80 % EtOH	100 µL	100 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL
Sample	0 µL	0 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	0 µL
Standard	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	0 µL	100 µL

Figure 4 Explanation of a plate that was prepared with blanks, samples and standards to analyze phenolic compounds

The software (SoftMax pro 5.3) calculated the results in mg of standard equivalent per L (mg L^{-1}) and then these were converted to mg of standard per g of dry matter except to anthocyanidin that were converted to µg of standard per g of dry matter.

$$\textbf{Standard}\ (\textbf{mg/g}) = \frac{\textbf{standard}\ (\textbf{mg/L})\ \textbf{x dilution factor x volume of extract}\ (\textbf{mL})}{\textbf{sample weight}\ (\textbf{mg})\ \textbf{x dry matter}\ (\textbf{g})}$$

V.5.4 Antioxidant capacity (ORAC)

Antioxidant capacity of oat extracts and Avns was measured as described Oomah *et al*. (2008), based on the method described previously by Prior *et al*. (2003).

The antioxidant capacity was measured using Oxygen Radical Absorbance Capacity (ORAC), which is described below. A day before of analysis was prepared a 75 mM Phosphate buffer with 10.11 g of Potassium Phosphate dibasic (K_2HPO_4), and 2.3383 g of Sodium Phosphate monobasic monohydrate (NaH_2PO_4 .H_2O). These

were diluted in a 1000 mL volumetric flask with Milli-Q water and measured pH (this should be 7.4 or 7.39). On day of analysis, AAPH radical was weighed (94.92 mg) into a 10 mL volumetric flask and stored in a drawer until use to protect of sunlight, then a 91.85 µM Fluorescein solution was prepared taking 50 µL of 87 µM Fluorescein solution (This solution had already been prepared with 75 mM phosphate buffer) and diluted to 25 mL in a volumetric flask with 75 mM phosphate buffer. The solution was placed in a water bath to 37°C until use. The samples and Trolox standard were diluted with 75 mM phosphate buffer (concentration range of trolox, 0-6 mg/L). 100 µL of buffer, standard or sample were dispensed into a 96-well black microplate (Fluotrac 200, Greiner Bio-One Inc.) (as shown Figure 5) and read in a SpectraMax GeminiEM microplate fluorescence reader (Molecular Devices Co.) to temperature 37°C, excitation 485nm and emission 530nm (the empty plate was read previously to ensure that it is clean). Then 35 mM AAPH solution was prepared (while plate was being read), adding warm buffer to flask that contains the AAPH and placed water bath again until its use. A total of 100 µL of 91.85 µM Fluorescein solution were added in each well (the solution was added from right to left), and plate was mixed in the GeminiEM for 120 seconds at 37°C and read. After 100 µL of 35 mM AAPH solution were add in each well (as shown Figure 5) and immediately the plate was read (plate was shaken for 180 seconds before reading) to 485 nm (excitation wavelength) and 530 (emission wavelength). The readings were taken every 2 minutes for 150 minutes.

	1	2	3	4	5	6	7	8	9	10	11	12
A	*Gr02	Gr02	Gr02	Gr02	Gr02	Gr02	Gr02	Gr02	Gr02	Gr02	Gr02	Gr02
B	Gr01	BL	Tr0	CHH1	CHH1	CHH1	Tr0	KH1	KH1	KH1	Tr0	BL
C	Gr01	BL	Tr0.50	CHH2	CHH2	CHH2	Tr0.50	KH2	KH2	KH2	Tr0.50	BL
D	Gr01	BL	Tr01	CHH3	CHH3	CHH3	Tr01	KH3	KH3	KH3	Tr01	BL
E	Gr01	BL	Tr02	CUH1	CUH1	CUH1	Tr02	AH1	AH1	AH1	Tr02	BL
F	Gr01	BL	Tr04	CUH2	CUH2	CUH2	Tr04	AH2	AH2	AH2	Tr04	BL
G	Gr01	BL	Tr06	CUH3	CUH3	CUH3	Tr06	AH3	AH3	AH3	Tr06	BL
H	Gr01	BL	BL	BL	BL	BL	BL	BL	BL	BL	BL	BL
	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
Buffer, sample or standard	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
Fluorescein	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
AAPH	0µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
*Note: instead of adding sample and AAPH to Gr01 and Gr02 was added buffer												

Figure 5 Explanation of how a plate was prepared with blanks, samples and standards to analyze antioxidant capacity

Results from SoftMax Pro 5.3 Software are calculated on the basis of Trolox Standard.

$$\frac{\textbf{Trolox mg equiv}}{\textbf{g of dry matter}} = \frac{\textbf{Trolox equiv (mg/L) X extract volume (mL)} \Big/ \textbf{sample weight (mg)}}{\textbf{Dry weight of sample g/g}}$$

V.5.5 Phytic acid content

Phytic acid content was determined in flours of oat groats and hulls using a modification of procedure of Latta and Eskin, (1980). 0.1 g of ground sample was extracted with 2mL of 2.2% HCl. Samples were stirred magnetically at room temperature (speed 9.5, RT15 POWER IKAMAG), and after were centrifuged at 15000 rpm for 20 min (Mini Spin plus, Eppendorf). 1 mL of supernatant was diluted to 25 mL with Milli-Q water and frozen at -20ºC until further use. A total of 10 mL of the

diluted supernatant was loaded to an anion exchange resin column (Poly-Prep Prefilled Chromatography Columns, AG 1-X8 Resin 100-200 mesh chloride form 0.8 x 4 cm, BIO-RAD). After 10 mL of Milli-Q water were added to a column to remove interfering compounds. Then 15 mL of 0.1 M NaCl were added to remove inorganic phosphorous (elutes obtained in different fractions were collected in the same tube). And then 30 mL of 0.7 M NaCl were added to elute phytic acid (this fraction was collected in other tube). An aliquot of eluted phytic acid (1.5 mL) was mixed with 0.5 mL of Wade reagent (0.03% Iron chloride III and 0.3% sulfo-salicylic acid) and then 250 µL of this solution were transferred to a 96-wells Microplate (Greiner Bio-One). Absorbance was read at 500 nm in a microplate reader (spectra Max Plus384, Molecular Devices). The concentration of phytic acid and IP6 were calculated from standard curves obtained of Phytic Acid sodium Salt (0-50 µg/mL) and D-myo-inositol 1,2,3,4,5,6-hexakisphosphate, Dodecasodium salt (0-50.4 ug/mL).

$$\mathbf{PA\ (\mu g\ in\ extract)} = \frac{\mathbf{[PA\ (\mu g/mL)\ x\ Mass\ in\ 2\ mL/1.5\ mL\ of\ eluent\ x\ 30\ mL\ eluent]\ x\ 25\ mL\ solution}}{\mathbf{10\ mL\ of\ \ solution\ \ loaded\ in\ the\ column}}$$

$$\mathbf{PA\ (mg/g\ dry\ matter)} = \frac{\mathbf{[PA\ (\mu g\ in\ extract)/1\ mL\ x\ 2\ mL\ of\ extract/Mass\ of\ sample/1000\ \mu g}}{\mathbf{g\ dry\ matter}}$$

$$\mathbf{IP6\ (\mu g\ in\ extract)} = \frac{\mathbf{[IP6(\mu g/mL)\ x\ Mass\ in\ 2\ mL/1.5\ mL\ of\ eluent\ x\ 30\ mL\ eluent]\ x\ 25\ mL\ solution}}{\mathbf{10\ mL\ of\ solution\ loaded\ in\ the\ column}}$$

$$\mathbf{IP6\ (mg/g\ dry\ matter)} = \frac{\mathbf{[IP6(\mu g\ in\ extract)/1\ mL\ x\ 2m\ L\ of\ extract/Mass\ of\ sample/1000\ \mu g}}{\mathbf{g\ dry\ matter}}$$

$$\% \mathbf{IP6} = \frac{\mathbf{IP6\ (mg/g\ dry\ matter)}}{\mathbf{PA\ (mg/g\ dry\ matter)}} \mathbf{x\ 100}$$

V.6. Determination of the physical properties of synthetic avenanthramides

V.6.1 IR spectrum of synthetic avenanthramides

The avenanthramides were analyzed for FT-IR spectroscopy to provide information about their chemical bonds and molecular structure, in a Nicolet 380 spectrometer (Thermo Electron Corp., Madison, WI) with SMART diamond attenuated total reflectance (ATR accessory) with a 45º aperture angle generating a single bounce and a deuterated triglycine sulphate (DTGS) detector, scanning over the frequency range of 4000-400 cm^{-1} at a resolution of 4/cm. Spectra were collected by using a rapid san software running under EZOMNIC 8.0.342 (Nicolet, Madison, WI) and the spectrum for each sample was calculated from the average of 32 repetitive scans. A little amount was placed directly onto the ATR crystal and compressed using a standard pressure tower equipped with a pointed flat tip before the spectra collection.

V.6.2 UV-Vis spectrum of synthetic avenanthramides

To determine the UV-Vis spectrum of Avns was prepared 10 mL of each solution (preparation of solutions was performed using the extinction coefficients that Dr Wise provided for the development of this experiment, the solutions were prepared with methanol). Then 300 µL of each solution were placed in a UV plate with 96 well flat bottom and performed a scanning of 190 to 1000 nm with increments 5 nm in a spectrophotometer (spectramax Plus 384, Molecular Devices Corp., Sunnyvale, CA), to select the range where appear the absorption.

V.6.3 Differential scanning calorimetry of synthetic Avenanthramides

The thermal characteristics of Avns were measures using a modulated differential scanning calorimeter (Modulated DSC-2910, TA Instruments, New Castle, DE). A flow of nitrogen gas of 145mL/min was used in the cell cooled by helium (145 ml/min) in a refrigerated cooling system. The instrument was calibrated for temperature and heat flow with indium (mp = 156.6 °C, ΔH = 28.71 J/g, Aldrich Chemical Crop.). A total of 2 mg of sample (Avns) were weighed into a open aluminum pan (T022707 TA Instruments) and as reference was used empty pan. Both pans were placed inside calorimeter and heated 25 to 325 °C at a rate of 1°C/min with modulation at a period of 60 s and temperature amplitude of 0.16 °C. After temperature decreased until 25°C again at the same rate. For thermal oxidation, the pans were cooled to 10 °C and scanned by heating at 10 °C/min to 400 °C in the presence of oxygen (100 ml/min).

III.7 Statistical analysis

Three determinations were made for all nutritional and chemical analysis. An analysis of variance by the general linear models (GLM) procedure means comparison by Duncan´s test and Pearson correlation were performed according to Statistical Analysis System (SAS Institute Inc, 1990).

Models

Nutritional components = variety + fraction

Avn content = variety + fraction

Phenolic and antioxidant capacity = variety + fraction

Phytic Acid and IP6 = variety + fraction

Antioxidant capacity = Avenanthramides

VI. RESULTS AND DISCUSSION

VI.1 Nutritional composition of oats

The Chemical composition of the four oat varieties showed significant differences ($p<0.05$) among varieties and between fractions (Table 1). Hulls had higher ash content than groats . Chihuahua hulls had the highest ash content (10.21%) whereas Cuauhtémoc groats had lowest ash content. Protein content was higher in groats than hulls, being Avemex groats the richest in protein content, whereas Cuauhtémoc hulls showed the lowest protein content. Respect to the fat content, the Groats had higher fat content than hulls. Chihuahua groats had the highest fat content, whereas Karma, Cuauhtémoc and Avemex hulls had the lowest values of fat content. Crude fiber was higher in hulls than groats, having the Cuauhtémoc variety the highest value in Crude fiber. Shewry *et al*. (2008) determined protein, ash and lipids content in wholemeal samples of five oat varieties grown in Hungary. Protein and ash content reported by them were lower than those reported in this work, whereas lipid content was similar to values reported by us. Shewry *et al*. (2008) also determined dietary fiber and their values were lower than reported in this work for crude fiber (fiber crude is part of dietary fiber) Dimberg *et al*. (2005) determined protein content in oat samples of three cultivars grown in two different cropping systems for 3 years and under two levels of N and their results also showed values lower than those we reported.

Table 1 Chemical composition (% dry basis) of four oat varieties

Fraction	Ash	Protein	Fat	Crude Fiber
Groats				
Chihuahua	2.44a	12.8c	9.72a	1.1ab
Cuauhtemoc	2.05b	15.65b	7.93b	2.06a
Karma	2.43a	19.8a	6.59c	1.01b
Avemex	2.32a	20.73a	7.52bc	1.72ab
Hulls				
Chihuahua	10.21a	5.14b	3.03a	21.41c
Cuauhtemoc	8.53b	4.91b	1.57b	33.08a
Karma	7.16c	7.63a	1.5b	26.53b
Avemex	6.9d	6.62a	1.53b	29.19ab

Means in a column within each fraction with different letters are significantly different ($p < 0.05$).

VI.2 Avenanthramides content in oat extracts

Structure of synthetic Avns was confirmed for LC-MS and retention time (Figure 6). Protonated molecular ions $[M + H]^+$ were produced with *m/z* 299.9, 315.9 and 329.9 for Avns 2p, 2c and 2f respectively. The peaks of three Avns were identified by retention time with authentic Avns standards. The determination of Avns content 2c, 2f and 2p (mg kg^{-1}) by HPLC in extracts from oat groats and hulls of the four oat varieties was performed using as standards the synthezised Avns. Avns concentration was significantly different ($p < 0.05$) among varieties and between fractions (groats and hulls) (Table 2). Avemex variety showed the highest value of Avns content, in oats groats (Figure 7) and hulls. The lowest concentration for Avns 2c occurred in Chihuahua hulls. Whereas Chihuahua, Cuauhtemoc and Karma hulls showed similar values for Avn 2f and respect to Avn 2p, Karma and Chihuahua hulls showed the lowest values. Several studies (Dimberg et al., 1993; Emmons and Peterson, 1999; Shewry et al., 2008; Emmons and Peterson, 2001; Emmons et al., 1999; Dimberg *et al*., 2005) report that Avns concentration depends on variety and fraction which was also observed in this work. Avns concentrations (in groats and

hulls) in this study were lower than those reported previously (Bratt *et al*., 2003; Dimberg *et al*., 1993; Shewry *et al*., 2008; Emmons and Peterson, 2001; Emmons *et al*., 1999; Dimberg *et al*., 2005; Dokuyucu *et al*., 2003), but similar to those observed by Peterson *et al*. (2005) These differences can be explained by the genotype and the strong influence of growing environment that influence the Avns synthesis.

Table 2 also shows the hulls-groats ratio (%) which clearly reveals the difference Avns concentrations among varieties and fractions. Cuauhtémoc had greater amount of Avn 2c in hull than groats (188.2%). Content of Avn 2f in Chihuahua variety was similar in groats and hulls, whereas Chihuahua and Cuauhtémoc had higher amount of Avn 2p in hulls than groats (173 and 127% respectively). This indicates that Chihuahua and Cuauhtémoc varieties, susceptible to stem and crown rust, present Avns content lower than the resistant varieties (Avemex and Karma), and their concentration in groats is similar or lower than hulls. Wise *et al*., (2008) performed a study where determined Avns content in 18 oat genotypes with a wide range of crown rust (*Puccinia coronate*) resistance in environments with different incidence of crown rust, and observed that the most genotypes with strong crown rust resistance also had higher Avns concentration in crown rust environments.

VI.3 Correlation among nutritional components and Avenanthramides in oats

Correlation among nutritional components of oat grain and Avns is shown in Table 3. Protein in oats showed positive correlation with fat ($r = 0.78$, $p<0.0001$) and Avns 2f and 2c ($r = 0.54$ and 0.56, $p<0.05$ and $p<0.005$, respectively) while ash and fiber was inversely correlated ($r = -0.90$ and -0.89 respectively, $p<0.0001$). Dimberg et al. (2005) found correlation among protein and Avns in a study of oat grain grown in conventional and organic system. Correlation among protein and Avns 2f and 2c suggests that oat grain rich in protein will also be rich in Avns. Fat also showed negative correlation with ash and fiber ($r = -0.87$ and -0.95 respectively, $p<0.0001$), whereas Avns showed high positive correlation among them ($r =0.93$ to 0.90, $p<0.0001$). High correlation among the three Avns indicates that their production in oat grain is related, this is that if any one of the Avns is found in grain it is very likely that the other two Avns are also found.

In oat hulls (Table 3), Avns showed positive correlation ($r = 0.81$ for Avn 2f and 2p, $p<0.005$ and $r = 0.71$ for Avn 2c and 2p, $p<0.05$) but showed no correlation with protein, this may be due to low amount of protein in the hulls. Protein was inversely associated with ash content ($r = -0.73$, $p<0.05$), while fat showed positive correlation with ash content ($r = 0.88$, $p<0.0001$) and inverse correlation with fiber ($r = -0.76$, $p<0.005$) In groats, protein content showed a negative correlation with fat ($r = -0.80$, $p<0.005$), while protein content showed positive correlation with Avns 2p and 2c ($r = 0.65$ and 0.694 respectively $p<0.05$). Correlation among protein content and Avns suggests again that oat grains with high protein content produce higher concentrations of Avns than oat grains with low protein content. The three Avns showed a high positive correlation among them ($r = 0.93$ to 0.98, $p<0.0001$) which indicates that if any of these Avns is synthesized in the groats, it is also likely that the other Avns will also be synthesized.

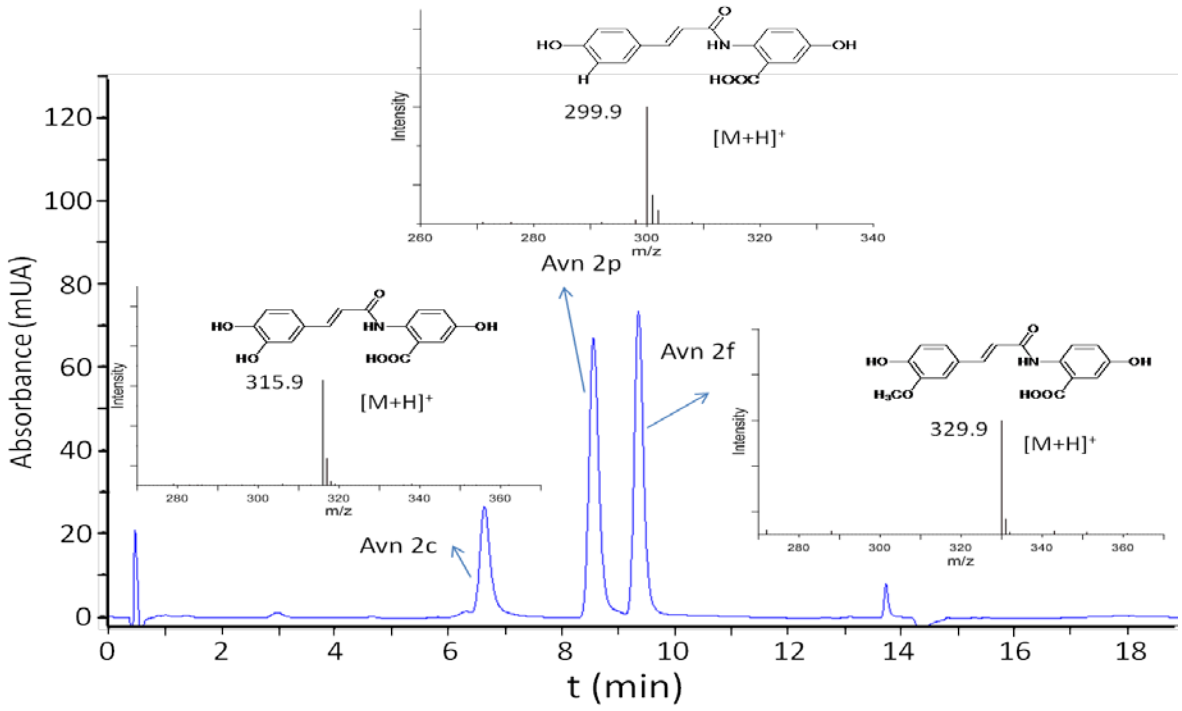


Figure 6 LC-MS chromatogram of Avns 2c, 2p and 2f to UV33O and (inserted) the positive ionization MS spectra

Table 2 Avenanthramides content of oats (mg kg-1).

Fraction	Concentration*			Hulls-groats ratio (%)		
Groats	Avn 2c	Avn 2f	Avn 2p	Avn 2c	Avn 2f	Avn 2p
Avemex	3.6a	3.0a	3.3a	29.4c	54.0c	59.9c
Chihuahua	0.4c	0.7c	0.7c	76.8b	99.1a	172.7a
Cuauhtemoc	0.5c	1.4b	1.2b	188.2a	57.1bc	126.9b
Karma	1.2b	1.0c	1.2b	38.9c	71.6b	87.8c
Mean	1.4	1.5	1.6	83.3	70.4	111.8
Hulls						
Avemex	1.0x	1.6x	1.9x			
Chihuahua	0.3y	0.7y	1.2yz			
Cuauhtemoc	1.0x	0.7y	1.5y			
Karma	0.5y	0.7y	1.1z			
Mean	0.7	0.9	1.4			
Overall Means	1.06	1.21	1.51			

Means followed by different letters within rows or columns are significantly different (p <0.05).

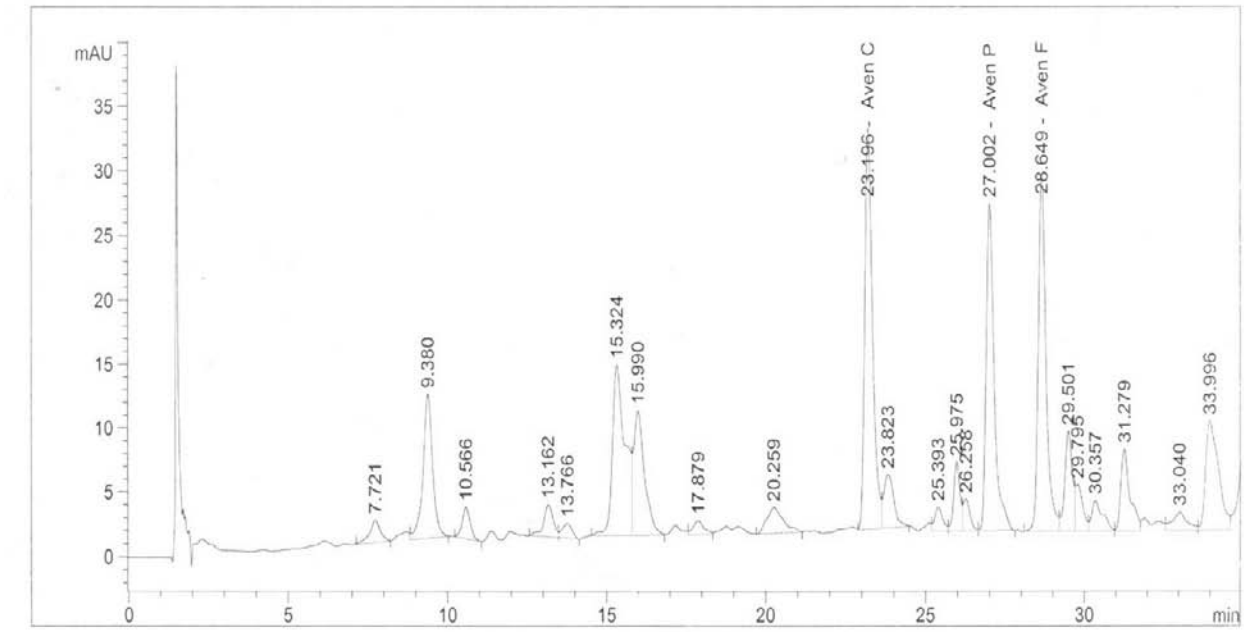


Figure 7 Chromatogram of Avenanthramides 2c, 2p and 2f in methanolic extracts of Avemex oat groats

Table 3 Correlation coefficients for chemical components of oats and avenanthramides

	Ash	Protein	Fat	Fiber	Avn 2p	Avn 2f	Avn 2c
Overall (n=24)							
Protein	-0.899*		0.776*	-0.885*	ns	0.543***	0.56**
Fat	-0.867*	0.776*		-0.949*	ns	ns	ns
Fiber	0.888*	-0.885*	-0.949*		ns	ns	ns
Avn 2f	-0.43***	0.543***	ns	ns	0.923*		0.901*
Avn 2p	ns	ns	ns	ns		0.923*	0.926*
Hulls (n=12)							
Protein	-0.731***		ns	ns	ns	ns	ns
Fat	0.882*	ns		-0.759**	ns	ns	ns
Fiber	ns	ns	-0.759**		ns	ns	ns
Avn 2p	ns	ns	ns	ns		0.81**	0.709***
Groats (n=12)							
Protein	ns		-0.802**	ns	0.653***	ns	0.694***
Avn 2p	ns	0.653***	ns	ns		0.977*	0.981*
Avn 2f	ns	ns	ns	ns	0.977*		0.933*

*, p<0.0001; **, p<0.005; ***, p<0.05; ns, not significant.

VI.4 Phenolic content and antioxidant capacity of oat extracts

Phenolic content in oat hulls was significantly ($p<0.05$) higher that oat groats (Table 4). Cuauhtémoc hulls obtained the highest total phenolic content (7.97 mg g^{-1}) of all hulls, whereas Chihuahua hulls had the highest values of tartaric esters, flavonols and anthocyanins (0.92, 0.45 mg g^{-1} and 21.48 µg g^{-1}, respectively). In groats, total phenolic, tartaric esters and flavonols content was higher in Chihuahua variety (4.2, 0.36 and 0.18 mg g^{-1}) that in the other varieties. Karma groats had highest Anthocyanins content (7.91µg g^{-1}) that other groats. This study reported higher values of caffeic acid (tartaric esters) in oat grain that reported by Emmons and Peterson (1999), although unlike they, this work showed concentrations of caffeic acid higher in hulls than in groats. Dimberg *et al*. (1993) and Emmons and Peterson (2001) also showed lower caffeic acid values in oat groats than those reported here. Guo-Xu et al. (2009) determined phenolics content of cultivars of hulless oats from china and caffeic acid content was lower than that showed in our study. Explanation of why Phenolic content was higher in hulls that groats is perhaps because oat plant is exposed to intense heat what elicits the production of phenolic compounds in hulls to protect grain of environment effect.

Table 4 Phenolic content and antioxidant activity of oat extracts [a]

Sample	Concentration[b]				TEAC[d] (ORAC)
	Total phenolic	Tartaric esters	Flavonols	Anthocyanins[c]	Mg g^{-1}
Hulls					
Chihuahua	7.68b	0.92d	0.45a	21.48a	14.71ab
Cuauhtemoc	7.97a	0.83b	0.41b	16.48b	15.40ab
Karma	7.59b	0.67c	0.40c	13.71b	16.10a
Avemex	3.87d	0.43d	0.20d	12.0c	9.85f
Groats					
Chihuahua	4.2c	0.36e	0.18e	5.28d	13.99bc
Cuauhtemoc	3.91d	0.31f	0.16f	4.59d	12.91cd
Karma	2.72e	0.23g	0.13g	7.91c	11.51de
Avemex	2.65e	0.24g	0.17f	4.92d	10.54ef

[a]Means in a column with different letters are significantly different ($p<0.05$)

[b]Concentrations are expressed as mg standard g^{-1} dry matter

[c]Concentrations are expressed as µg standard g^{-1} dry matter

[d]Trolox equivalent antioxidant capacity

Respect to the antioxidant capacity of oat extracts (Table 4), there were significant differences ($p<0.05$), between groats and hulls. Antioxidant capacity in hulls was higher that groats, except for Avemex hulls. Karma, Chihuahua and Cuauhtémoc hulls showed similar values of antioxidant capacity (16.10, 14.71 and 15.40 mg g^{-1} respectively). Chihuahua Groats showed similar value (13.99 mg g^{-1}) to Chihuahua and Cuauhtemoc hulls (14.71 and 15.40 mg g^{-1} respectively) although these showed a phenolic content higher that Chihuahua groats. Avemex cultivar showed lowest values of antioxidant capacity in groats and hulls.

VI.5 Correlation among phenolics content and antioxidant capacity of oat extracts

The comparison of phenolic content with antioxidant capacity of oat grain (Table 5) revealed a positive correlation. Total phenolic content showed strong correlation with tartaric esters and flavonol contents ($r = 0.962$ and 0.974, $p<0.0001$, respectively) indicating that oat ethanolic extracts have high content tartaric esters and flavonols. Total phenolic and flavonol content showed highest positive correlation with antioxidant activity ($r = 0.706$ and 0.631, $p<0.0001$, respectively) that indicates that antioxidant activity is mainly attributed to flavonoids. In groats, total phenolic content revealed strong positive correlation with tartaric esters ($r = 0.965$, $p<0.0001$) (Table 5) that indicates that ethanolic extracts of oat groats have very high tartaric esters. Antioxidant activity showed positive correlation with total phenolic and tartaric esters ($r = 0.667$ and 0.637, $p<0.0001$, respectively), which indicates that antioxidant activity of ethanolic extracts is due to tartaric esters content. In hulls, total phenolic content revealed positive correlation with tartaric esters content (Table 5) ($r = 0.884$, $p<0.0001$) and strong positive correlation with flavonol content (and 0.972, $p<0.0001$). Total phenolic and flavonol contents showed highest correlations with antioxidant activity ($r = 0.798$ and 0.737, $p<0.0001$, respectively), which indicates that antioxidant activity of ethanolic extracts of oat hulls is due to flavonols content.

Table 5 Correlation coefficients for phenolic content and antioxidant activity of oat extracts

	Tartaric esters	Flavonols	Anthocyanins	ORAC
Oat grain (n = 94)				
Total phenolic	0.962*	0.974*	0.790*	0.706*
Tartaric esters		0.978*	0.868*	0.593*
Flavonols			0.836*	0.631*
Anthocyanins				0.425**
Groats (n=47)				
Total phenolic	0.965*	0.607*	ns	0.667*
Tartaric esters		0.731*	ns	0.637*
Flavonols			ns	ns
Anthocyanins				ns
Hulls (n = 47)				
Total phenolic	0.884*	0.972*	0.579*	0.798*
Tartaric esters		0.944*	0.796*	0.610*
Flavonols			0.713*	0.737*
Anthocyanins				ns

*p<0.0001; **p<0.0005; ns, not significant

VI.6 Antioxidant capacity of synthetic avenanthramides

Antioxidant capacity of Avns showed significant differences among them ($p < 0.05$) (Table 6). Synthetic Avns revealed a very high antioxidant capacity, which was higher in Avns 2p and 2c than in the Avn 2f. Several studies (Bratt *et al*., 2003; Fagerlund *et al*., 2009; Lee-Manion *et al*., 2009) evaluated for different methods the antioxidant capacity of Avns and these revealed that Avns present different activity order, in other words that antioxidant efficiency of Avns vary among antioxidants assays because of different reaction mechanisms and conditions. Fagerlund *et al*. (2009) also observed in the DPPH assay that when Avns present a methoxy group in ortho position to the donor hydroxy group increased the antioxidant capacity, and adding a second methoxy group, also in ortho position or changing the methoxy group to a hydroxy group resulted in an even greater activity. The study also showed that A ring (5-hydroxyanthranilic acid moiety) had greater importance for activity than B ring (cinnamic acid moiety). They also observed that with the linoleic acid assay the reactivity order was completed different that with DPPH assay, this is that methoxy

group in position ortho to the hydroxy group decreased the activity and that B ring had an important effect in activity of Avns. Our study is in agreement with this part of results, because Avn 2f, which have a methoxy group in position ortho to the donor hydroxy group, showed the lowest antioxidant capacity. Further only the B ring (cinnamic acid moiety) had influence on the activity. An explication to this could be to the conjugation of double bonds between cinnamic acid moiety and amide bond and inductive effect of substituent group in position ortho to the hydroxy group.

Table 6 Antioxidant activity of Avns[a]

Avenanthramides	TEAC[b] (ORAC)	
	g g^{-1}	µmol g^{-1}
2p	8.85a	35358.98
2f	7.29b	29126.21
2c	8.17a	32642.14

[a]Means in a column with different letters are significantly different ($p<0.05$)

[b]Trolox equivalent antioxidant capacity

VI.7 Phytic acid and IP6 content

Oat groats and hulls showed significance differences ($p<0.05$) in the phytic acid and IP6 content (Table 7). Oat groats revealed higher values of phytic acid and IP6 that oat hulls. Avemex and Chihuahua groats revealed the highest values of Phytic acid and IP6 whereas Karma hulls recorded the lowest values of phytic acid and IP6. The percentage of IP6 revealed that over 70% phytic acid in oat groats is found in form IP6 and over 40% phytic acid in oat hulls is found in form IP6. Garcia *et al*. (1999) determined phytic acid content in whole oat flour, oat brans and oat bread and found that oat bran samples had phytic acid content higher than whole oat flour and bread. Tian *et al*., (2010), determined phytic acid content in oat grains grown in China to observe the effect of germination in their content. They observed that phytic acid content decreased from 0.35 to 0.11% during the germination. Our work reported values higher than them, what suggest that our oats, especially Avemex groats are a good material to produce healthy foods.

Table 7 Phytic acid and IP6 content in oat extracts

Sample	PA(mg g^{-1})	IP6 (mg g^{-1})	% IP6
Hulls			
Chihuahua	20.73de	10.61de	50.7b
Cuauhtemoc	20.47de	10.34de	50.24b
Karma	17.91e	7.83e	42.59c
Avemex	21.83d	11.61d	52.57b
Groats			
Chihuahua	38.54ab	28.62ab	73.87a
Cuauhtemoc	37.16bc	27.13bc	72.61a
Karma	34.44c	24.36c	70.65a
Avemex	41.3a	31.19a	75.33a

[a]Means in a column with different letters are significantly different ($p<0.05$)

Phytic acid revealed a strong positive correlation with IP6 and %IP6 ($p<0.0001$) in oat grain and its fractions (Table 8). Results of phytic acid, IP6 content, %IP6 and the correlation among them indicate that if phytic acid is synthesized in oat grain is highly probable to be found the highest content in oat groats and major part in form IP6.

Table 8 Correlation coefficients for pythic acid content, IP6 and %IP6 of oat extracts

	IP6	%IP6
All samples (n =72)		
Phytic Acid	0.999*	0.962*
IP6		0.963*
Groats (n = 36)		
Phytic Acid	0.999*	0.986*
IP6		0.988*
Hulls (n = 36)		
Phytic Acid	0.997*	0.971*
IP6		0.978*
*p<0.0001		

VI.8 Analysis of FT-IR and UV-VIS spectra of synthetic Avenanthramides

The FT-IR spectra (Figure 8) of synthetic Avns showed several absorption bands that confirm their structure. The three Avns reveals a broad band in the typical region of O-H of carboxylic acids (3300 and 2500 cm^{-1}) (3018.58 cm^{-1} Avn 2p, 2939.17 cm^{-1} Avn 2f and 3134.87 cm^{-1} Avn 2c). Avns 2p and 2f also revealed a rounded band to 3286.46 and 3265.31 cm^{-1}, respectively and Avn 2c a small absorption between 3500 and 3250 cm^{-1}. These absorptions are associated N-H group of amides (3500-3100 cm^{-1}). In the spectra of Avns appeared the C=O stretching vibration of amides (1680-1630 cm^{-1}) (1646.38 cm^{-1} Avn 2p, 1649.87 cm^{-1} Avn 2f and 1650.36 cm^{-1} Avn 2c) and the absorption of C=C of aromatic group (1600-1580 cm^{-1}) (1594.67 cm^{-1} Avn 2p, 1584.82 cm^{-1} Avn 2f and 1593.96 cm^{-1} Avn 2c). Amide group of Avns (secondary amide) also revealed the absorption band of N-H bending, trans to carbonyl group in the 1570-1515 cm^{-1} region (1538.24 cm^{-1} Avn 2p, 1536.27 cm^{-1} 2f and 1547.35 cm^{-1} Avn 2c). The aromaticity of Avns is supported for the peak that appears in the 1500-1400 cm^{-1} region (1450.68 cm^{-1} 2p, 1440.79 cm^{-1} 2f and 1455.51 cm^{-1} 2c. In the IR spectrum of Avns also appears a strong band in the 1320-1000 cm^{-1} region due to stretching of C-O bond (1220.92 cm^{-1} Avn 2p, 1184.37 cm^{-1} Avn 2f and 1179.05 cm^{-1} Avn 2c). The peaks that appear in the 900- 675 cm^{-1} region prove the existence of benzene rings again.

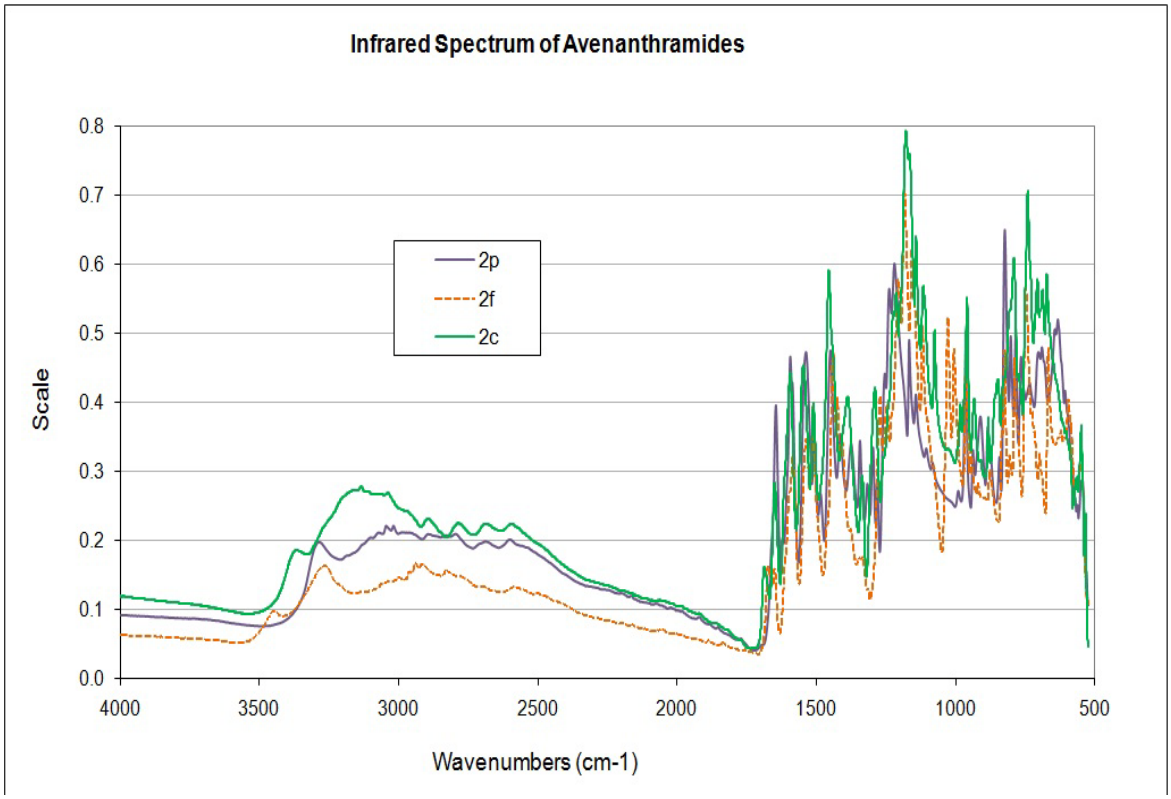


Figure 8 FT-IR spectra of Synthetic Avns

UV-Vis spectra of Avns were recorded 200 - 500 nm (Figure 9). Avns 2p showed two absorption bands in 320 nm and 345 nm, Avn 2f showed absorption maxima to 349 nm whereas Avn 2c showed absorption maxima to 351 nm. Avns reveal absorption maxima in the range 325-355 nm and show a yellow to yellow-green in solution. These absorption rates represent electronic transitions typical of hydroxycinnamoyl functions (Collins, 1989). The absorptions in this region reveal a system with conjugated double bonds ($\pi \rightarrow \pi^*$) (benzene and aliphatic chain of cinnamic acid moiety) and the presence of a carbonyl group, which has a heteroatom (Oxygen) with electrons pairs without bonding that help to maintain the conjugation.

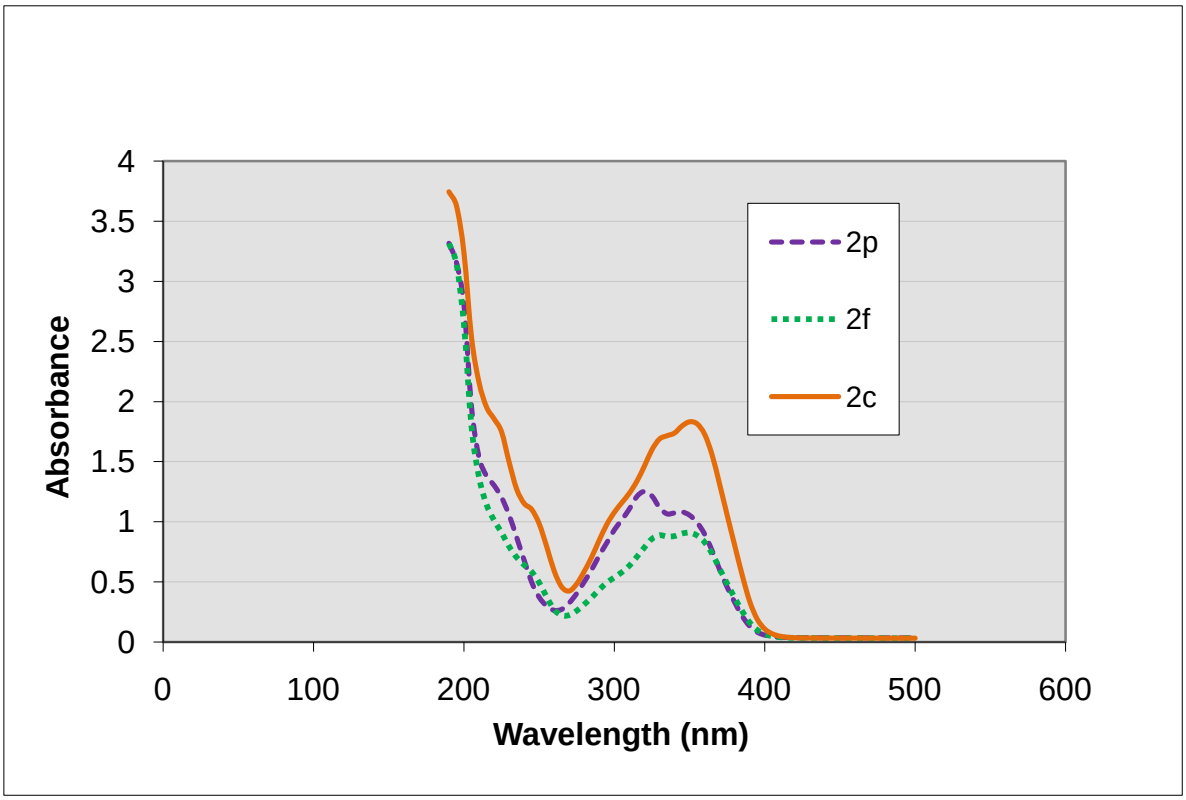


Figure 9 UV-Vis spectra of synthetic Avns

VI.9 Thermal analysis of synthetic Avns

The Avns exhibited reversing transitions (Figure 10). The reversible thermal flow, where typically appear the glass transitions and melting, revealed an endothermic peak at 228 °C in Avn 2f, which indicates the melting of its crystal structure (Table 9). At 265 °C appears another peak, which reveals the decomposition of Avn 2f. The enthalpy values of these peaks (20.2 and 21.8 J g^{-1}) (Table 9) indicate that Avn 2f needed similar energy (heat) to melt and to decompose its structure, which reveals that Avn 2f is very stable to heat. Avn 2p and Avn 2c showed an endothermic peak to 268 and 252 °C respectively. These peaks are due to the melting of crystal structure, being the enthalpy (ΔH) higher in Avn 2p than Avn 2c, what indicates that crystal structure of Avn 2p is stronger than Avn 2c.

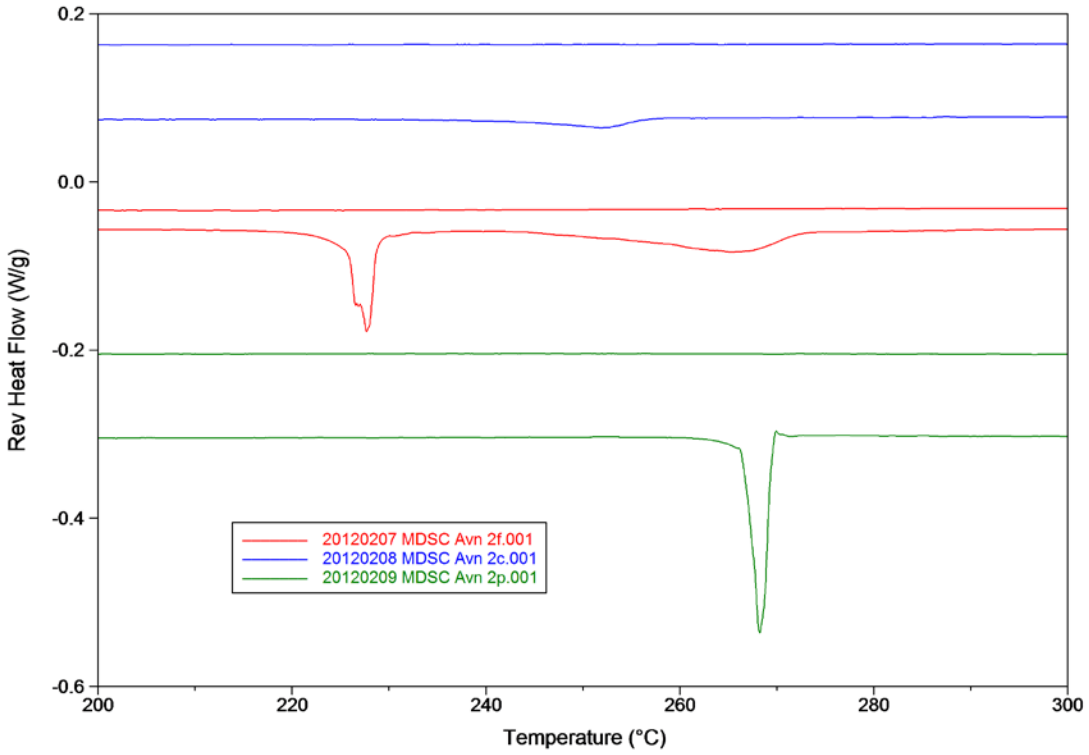


Figure 10 Modulated Differential Scanning Calorimetry reversing components of Avenanthramides

Table 9 Thermal characteristics of avenanthramides (Reversible heat flow, W g^{-1})

	Peak 1		Peak 2		Peak 3	
	Tmax	ΔH	Tmax	ΔH	Tmax	ΔH
Sample	(°C)	(J g^{-1})	(°C)	(J g^{-1})	(°C)	(J g^{-1})
2p	-	-	268	26.8	-	-
2c	-	-	252	5.4	-	-
2f	228	20.2	265	21.8	-	-

In the non-reversing signal tipically appears the crystallization (exothermic transitions), but in this case the Avns showed again the melting of crystal structure (Figure 11). In the non-reversing signal, the Avn 2f showed 3 peaks (122, 227 and 267 °C) (Table 10), whereas Avn 2p and 2c showed a peak to 267 and 252 °C respectively. Peak at 122 °C of Avn 2f revealed impurity. This perhaps because the crystal structure of Avn 2f contained solvent as part integral of its structure. The peak at 227 °C is due to the melting of the crystal structure of Avn 2f whereas the peak at 267 °C reveals again the decomposition of its structure. The Avns 2p and 2c showed a peak of melting at 267 and 252 °C respectively. The enthalpies of all peaks (Table 10) in the non-reversing signal were very high, which indicates that Avns are very stable to heat.

The signal heat flow of avenanthramides (Table 11) revealed the transitions that showed the Avns in the reversible and non-reversible signals. These transitions showed enthalpies similar to the sum of enthalpies obtained during reversing and non-reversing transitions.

The thermal analysis of Avns in an oxidant environment (Figure 12), exhibited an endothermic peak in the Avn 2p and 2c at 291 and 273 °C, respectively, which was produced for the melting of crystal structure. Avn 2f maybe showed an endothermic peak at 148 °C, product of an impurity. Avn 2f showed another endothermic peak at 244 °C, due to the melting crystal structure (Figure 12). Enthalpies of Avns 2p and 2f were higher than Avn 2c (Table 12), which indicates that crystal structure of Avn 2p and 2c is very strong and needs a great amount of energy to break it. Thermogram of Avns also reveals a high stability to oxidation, which remains until the melting but immediately after the melting, they begin to decompose.

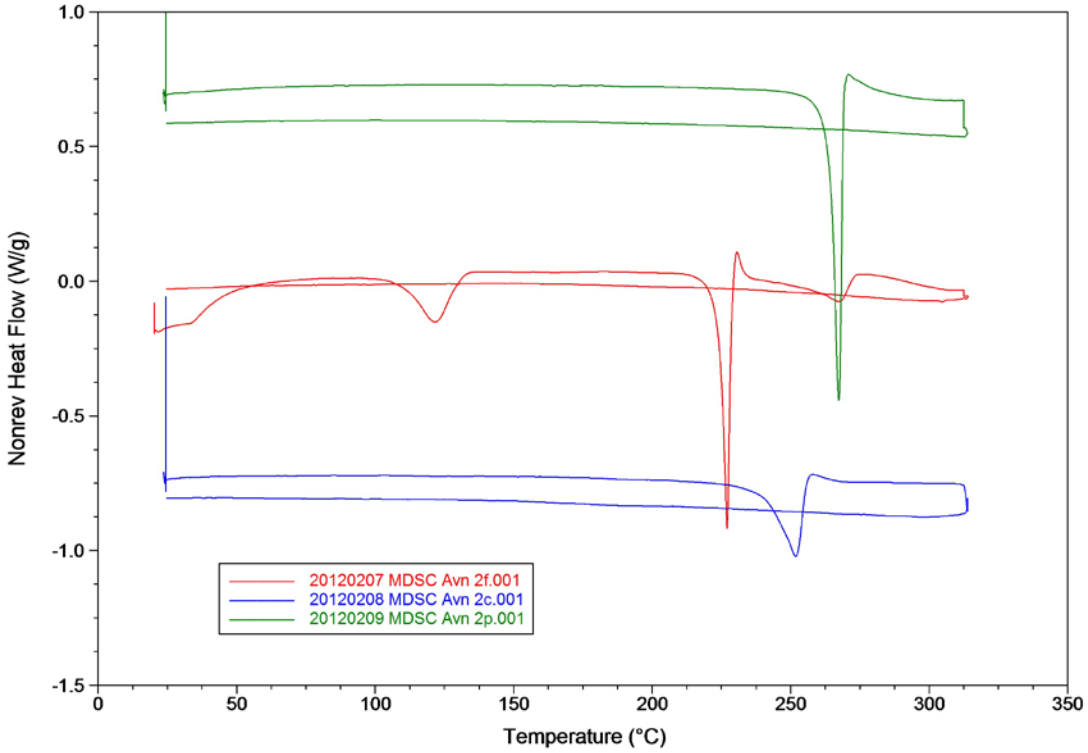


Figure 11 Modulated Differential Scanning Calorimetry nonreversing components of Avenanthramides

Table 10 Thermal characteristics of Avns (Non-reversible heat flow, W g^{-1})

	Peak 1		Peak 2		Peak 3	
	Tmax	ΔH	Tmax	ΔH	Tmax	ΔH
Sample	(°C)	(J g^{-1})	(°C)	(J g^{-1})	(°C)	(J g^{-1})
2p	-	-	-	-	267	335
2c	-	-	-	-	252	234
2f	122	151	227	261	267	217

Table 11 Thermal characteristics of Avenanthramides (Heat flow, W g^{-1})

	Peak 1		**Peak 2**		**Peak 3**	
	Tmax	ΔH	Tmax	ΔH	Tmax	ΔH
Sample	(°C)	(J g^{-1})	(°C)	(J g^{-1})	(°C)	(J g^{-1})
2p	-	-	-	-	268	345
2c	-	-	-	-	252	288
2f	122	158	227	319	267	237

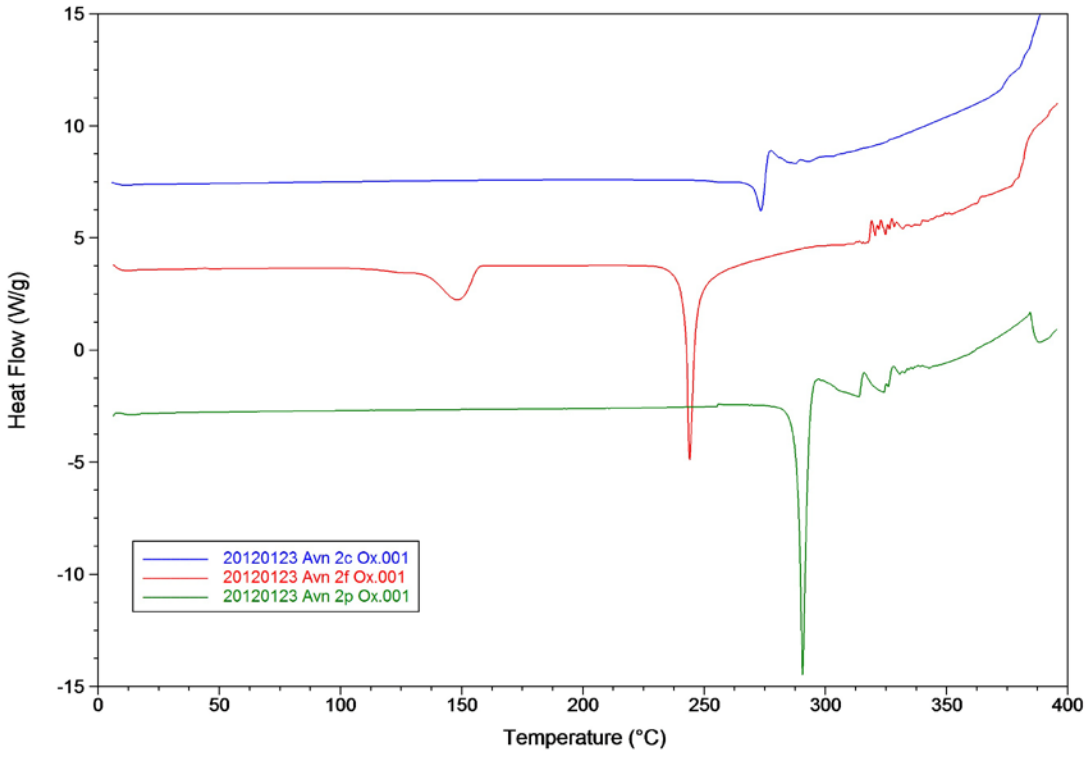


Figure 12 Differential Scanning Calorimetry of Avenanthramides thermo-oxidation

Table 12 Thermal characteristics of avenanthramides under oxidant environment (Heat flow, W g^{-1}).

	Peak 1		Peak 2	
	Tmax	ΔH	Tmax	ΔH
Sample	(°C)	(J g^{-1})	(°C)	(J g^{-1})
2p	-	-	291	348
2c	-	-	273	68
2f	148	150	244	252

VII. CONCLUSIONS

The results of this investigation showed that oat groats had higher content of proteins and lipids than hulls, whereas oat hulls had higher content of ash (minerals) and crude fiber than groats. In general, Avns content was higher in methanolic extracts of groats than in methanolic extracts of hulls, whereas phenolic content and antioxidant activity of ethanolic oat extracts was higher in hulls than groats. On the other hand, phytic acid and IP6 were higher in groats than hulls. The correlations revealed that Avns content was high in varieties with high protein content, that the antioxidant capacity of extracts of oat hulls was mainly due to the flavonoids, and that antioxidant capacity of extracts of oat groats was due to the tartaric esters. Also the correlation showed that phytic acid in groats is mainly in form IP6. Synthetic Avns showed very high antioxidant capacity and great stability to the heat and oxidation.

Avemex oat, a variety resistant to crown rust and stem rust, demonstrated to be the variety most nutritious and healthy. Avemex, particularly the groats can be an ingredient to develop new healthful and nutritious foods. Karma, Chihuahua and Cuauhtemoc hulls could be used to extract nutraceutical ingredients.

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Avenanthramides and Nutritional Components in four Mexican Oat Varieties (*Avena sativa* L.) cultivated in Durango

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Abstract

Oat (*Avena sativa L*.) is a cereal grain grown for its seed rich in nutrients that is consumed as whole grain, healthy to humans for its antioxidant content such as Avenanthramides (Avns), found in both oat hulls and groats. There are more than 30 Avns but the most abundant are: *N*-(3', 4'-dihydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (Avns 2c), *N*-(4'-hydroxy-3'-methoxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (Avns 2f) and *N*-(4'-hydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (Avns 2p). In this study was evaluated the content of Avns 2c, 2f and 2p by HPLC in groats and hulls of Chihuahua, Cuauhtémoc, Karma and Avemex varieties cultivated in Durango, México and their correlation with nutritional components of oat. Avns contents differed significantly ($p<0.05$) among oat varieties, groats and hulls. Avemex groats had the highest concentrations of Avns 2c (3.6±0.73), 2f (3.0±0.48) and 2p (3.3±0.65) mg kg^{-1} and protein content. Avns in oat grain showed positive correlation with protein content, what indicates that Avns likely are found in oat varieties with high protein content.

Keywords: Oats; antioxidants, polyphenols, avenanthramides

1. Introduction

The benefit of consuming Oat (*Avena sativa L.*) for human and livestock are its content of proteins, lipids, carbohydrates, fiber, vitamins, minerals and antioxidants [1], as the Avenanthramides (Avn), which are compounds formed from an anthranilic acid linked to hydroxycinnamic acid with an amide bond [2]. These compounds are present among cereals, exclusively in oat and are biosynthesized through the enzyme hydroxycinnamoyl CoA: hydroxyanthranilate *N*-hydroxycinnamoyl transferase [3]. The most abundant Avns found in grain are *N*-(3', 4'-dihydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (2c), *N*-(4'-hydroxy-3'-methoxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (2f) and *N*-(4'-hydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (2p), 2 indicating 5-hydroxyanthranilic acid and p, f and c indicating *p*-coumaric, ferulic and caffeic acids, respectively [4]. Avns were first referred as Avns A, B and C for Collins [5] that searching for phenolic content of oats, found over 40 different avenanthramide-like compounds in methanolic extracts of oat groats and hulls. Later, other studies reported the presence of these phenolic compounds [6-10]. Besides oat, Avns are found in carnation leaves and eggs of white cabbage butterfly [3] and oat leaves [2, 11-13].

Studies have demonstrated that Avns possess biological properties as antioxidant activity [1, 4, 6, 14-17] which is increased with the number of radical-stabilizing groups ortho to the phenolic hydroxyl group [17]. Avns also have anti-inflammatory activity [18-20], vasodilatory effect [21], anti-atherogenic activity [18, 22], anti-irritant activity [20], anti-proliferative activity [23] and improves cardiovascular disease [24]. Avns have also been associated to crown rust (*Puccinia coronata*) incidence and genetic resistance [25].

Although oat has been recognized as a nutrient-rich cereal with human health benefits, mainly by the presence of antioxidants, there is limited information about Avns content in oats and its anatomical fractions from different parts of the world, so that the objective of this study was to determine the content of Avns 2p, 2f and 2c in four oat varieties grown in the State of Durango, México as well as their correlation

with chemical composition of grain, to provide information for a future to develop functional foods and nutraceutical ingredients.

2. Materials and Methods

Four varieties of oat were used in this study, Chihuahua, Cuauhtemoc, Avemex and Karma. Chihuahua and Cuauhtémoc were grown during 2010 in the location called Nuevo Ideal in the state of Durango, Mexico (latitude, 24° 53´ N; longitude 105° 04´ W; altitude 1990 m). Soil type was sandy clay loam and pH 8.28. Soil was not fertilized and pathogens were not observed. Avemex and Karma varieties were genetically modified by Instituto Nacional de Investigación Forestal, Agrícola y Pecuaria (INIFAP) in Mexico, to confer moderate resistance to stem rust (*Puccinia graminis*) and crown rust (*Puccinia coronata*) [26], and were grown during 2010 in the Valle del Guadiana in the state of Durango, Mexico (latitude 23° 59' N; longitude 104° 37' W; altitude 1878 m). The soil had pH 8.0, loam texture, fertilized with nitrate 120 kg ha^{-1} and phosphate 60 kg ha^{-1}. No pesticides were used. Neither pathogens were observed.

Chemicals. Synthetic Avns *N*-(3', 4'-dihydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid (2c), *N*-(4'-hydroxy-3'-methoxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (2f) and *N*-(4'-hydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (2p) were provided by Dr. Mitchell L. Wise (Cereal Crops Research, ARS, USDA, Madison, WI, United States). Methanol and orthophosphoric acid were obtained from Fermont (Monterrey, México) and acetonitrile from Aldrich (D.F., México).

Proximate analysis of oat groats and hulls. Ash (942.05), crude protein (960.52), crude fat (920.39) and crude fiber content (962.09) were determined according to AOAC [27]. All chemical analysis was performed in triplicate.

Avns extraction and analysis. Extraction and analysis of Avns were performed as described Bryngelsson et al [28]. Groats and hulls were separated by hand and ground in a blender until the powder passed through a 0.5 mm sieve. 5g of flour of each sample (for triplicate) were used to extract Avns, with methanol (36 mL) by

stirring for 30 min twice at room temperature the same day the grinding was performed. The mixture was centrifuged for 10 min, the supernatants pooled, filtered through a filter paper Whatman No. 41, and the solvent evaporated under vacuum at 40 °C. The residue was suspended to 2 mL in methanol and stored at -20 °C until further analysis. Avns were analyzed by HPLC in an Agilent HPLC 1100 equipped with a degasser, quaternary pump and diode-array detector using a reversed phase column (Waters C-18 Symmetry 3.9x150mm). The mobile phase consisted of two solvents: Solvent A [0.01M phosphoric acid and acetonitrile (95:5, v/v)] and acetonitrile as Solvent B. Samples were run with a linear gradient for 60 min from 0-40% B at a flow rate of 1 mL/min. The analytes were detected at 340 nm, with a bandwidth of 8 nm, and 500 nm as reference with a bandwidth of 50 nm. Synthetic standards of each Avn were used to identify and quantify the Avns.

Statistical analysis. Analysis of variance was performed with the general linear model (GLM) procedure and means comparison with Duncan's test to determine variety, fraction, and variety-fraction effects, Pearson correlation were performed according to statistical Analysis System, SAS 9.1 for Windows.

3. Results and Discussion

Chemical composition of four oat cultivars are shown in Table 1. Chihuahua hulls had the highest ash content (10.21%), while Chihuahua groats showed the highest fat content (9.27 %). Protein content was highest in Avemex groats (20.73%), and the hull fraction richest in crude fiber was Cuauhtémoc hulls (33.08%). Our results are consistent with those reported on ash and fiber rich oat hulls [29] and protein and lipid rich groats [30, 31].

Table 2 shows the concentration of Avns 2c, 2f and 2p (mg kg^{-1}) of extracts from oat groats and hulls of the four oat varieties. Avns concentration was significantly different ($p < 0.05$) among varieties, and between fractions (groats and hulls), being Avemex the variety with the highest concentration of three avns, While the fraction where was found the highest concentration of avns was groats. The lowest concentration for Avn 2c occurred in Chihuahua hulls. Chihuahua, Cuauhtemoc and Karma hulls showed

the lowest values for Avn 2f and Karma hulls for Avn 2p. Table 2 also shows the hulls-groats ratio (%) which reveals more clearly the difference among varieties and fractions for the concentrations of Avns. Hulls-groats ratio (%) showed that Avemex, Karma and Chihuahua varieties synthesized greater amount of Avn 2c in groats than hulls (29.4, 38.9 and 76.8%), except Cuauhtemoc with higher amounts in hulls (188 %) than groats. Avn 2f content was higher in groats than hulls. Avemex and karma varieties synthesized greater amount of Avn 2p in groats (59.9 and 87.8% respectively) than hulls, while Chihuahua and Cuauhtémoc, synthesized a greater amount of Avn 2p in hulls (172.7 and 126.9 % respectively) than groats. Various authors [6, 7, 29, 32-34] have reported that the Avns concentration depend on the variety and fraction which was also observed in this work. Although this study presented concentrations (in groats and hulls) lower than those reported by Dimberg et al [6], Emmons et al [33], Emmons and Peterson [32], Bratt et al [4], Dokuyucu et al [35], Dimberg et al [34] and Shewry et al [29], and only Peterson et al [36], reported similar results. These differences can be explained by the genotype and the strong influence of growing environment that unchain the synthesis of avns.

Correlation among chemical components of oat grain and Avns is shown in Table 3. Protein in oat grain showed positive correlation with fat ($r = 0.78$, $p<0.0001$) and Avns 2f and 2c ($r = 0.54$ and 0.56, $p<0.05$ and $p<0.005$, respectively) while with ash and fiber was inversely correlated ($r = -0.90$ and -0.89 respectively, $p<0.0001$). Dimberg et al [34] in a study of oats grown in conventional and organic system found correlation among protein and Avns. Correlation among protein and Avns 2f and 2c suggest that when oat grain is rich in protein there are Avns in the grain. Fat also showed negative correlation with ash and fiber ($r = -0.87$ and -0.95 respectively, $p<0.0001$), whereas Avns showed high positive correlation among them ($p =0.93$ to 0.90, $p<0.0001$). High positive correlation among three Avns indicates that their production in oat grain is related, this is that if any one of the Avns is found in grain it is very likely that the other two Avns are also found.

In oat hulls (Table 3), Avns presented positive correlation among them ($r = 0.81$ for Avn 2f and 2p to $p<0.005$ and $r = 0.71$ for Avn 2c and 2p to $p<0.05$) but showed no

correlation with protein, this may be due to the low amount of protein in the hulls. Protein was inversely associated with ash content ($r = -0.73$, $p<0.05$), while fat showed positive correlation with ash content ($r = 0.88$, $p<0.0001$) and negative correlation with fiber ($r = -0.76$, $p<0.005$) In groats, protein content showed a negative correlation with fat ($r = -0.80$, $p<0.005$), while protein content showed positive correlation with Avns 2p and 2c ($r = 0.65$ and 0.694 respectively, $p<0.05$). Correlation among protein content and Avns suggests again that oat grains with high protein content produce higher concentrations of Avns than oat grains with low protein content. Three Avns showed again a high positive correlation among them ($r = 0.93$ to 0.98, $p<0.0001$) which indicates that if any of these is synthesized in the groats is also likely that other Avns may also be synthesized.

4. Conclusions

Oat groats had higher Avns content than oat hulls. Avemex variety had the highest concentration of Avns 2c, 2f and 2p and present the highest protein content. Avemex oat, a variety resistant to crown rust and stem rust proved to be the variety most nutritious and healthy. Oat Avemex, particularly Avemex groats can be an ingredient to develop of new healthful and nutritious foods.

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Table1. Chemical Composition (%) of Oat Varieties

Fraction	Ash	Protein	Fat	Crude fiber
Groats				
Chihuahua	2.44 ± 0.08*	12.8 ± 0.90	9.72 ± 0.08	1.1 ± 0.69
Cuauhtemoc	2.05 ± 0.09	15.65 ± 0.44	7.93 ± 0.97	2.06 ± 0.55
Karma	2.43 ± 0.17	19.8 ± 1.08	6.59 ± 0.02	1.01 ± 0.32
Avemex	2.32 ± 0.07	20.73 ± 1.67	7.52 ± 0.30	1.72 ± 0.32
Hulls				
Chihuahua	10.21 ± 0.06	5.14 ± 0.63	3.03 ± 0.14	21.41 ± 3.42
Cuauhtemoc	8.53 ± 0.07	4.91 ± 0.32	1.57 ± 0.03	33.08 ± 2.71
Karma	7.16 ± 0.06	7.63 ± 0.19	1.5 ± 0.10	26.53 ± 1.26
Avemex	6.9 ± 0.12	6.62 ± 0.86	1.53 ± 0.12	29.19 ± 1.01

*Mean ± stardard desviation

Table 2. Avenanthramides content (mg/kg) of oats

Fraction	Concentration*			Hulls-groats ratio (%)		
Groats	Avn 2c	Avn 2f	Avn 2p	Avn 2c	Avn 2f	Avn 2p
Avemex	3.6a	3.0a	3.3a	29.4c	54.0c	59.9c
Chihuahua	0.4c	0.7c	0.7c	76.8b	99.1a	172.7a
Cuauhtemoc	0.5c	1.4b	1.2b	188.2a	57.1bc	126.9b
Karma	1.2b	1.0c	1.2b	38.9c	71.6b	87.8c
Mean	1.4	1.5	1.6	83.3	70.4	111.8
Hulls						
Avemex	1.0x	1.6x	1.9x			
Chihuahua	0.3y	0.7y	1.2yz			
Cuautemoc	1.0x	0.7y	1.5y			
Karma	0.5y	0.7y	1.1z			
Mean	0.7	0.9	1.4			
Overall Means	1.06	1.21	1.51			

Means followed by different letters within rows or columns are significantly different (p <0.05).

*Concentration of avenanthramides are expressed as milligram per kilogram of sample (as is basis).

Table 3 Correlation coefficients for chemical components of oats and Avenanthramides

	Ash	Protein	Fat	Fiber	Avn 2p	Avn 2f	Avn 2c
Overall (n=24)							
Protein	-0.899*	ns	0.776*	-0.885*	ns	0.543***	0.56**
Fat	-0.867*	0.776*	ns	-0.949*	ns	ns	ns
Fiber	0.888*	-0.885*	-0.949*	ns	ns	ns	ns
Avn 2f	-0.43***	0.543***	ns	ns	0.923*	ns	0.901*
Avn 2p	ns	ns	ns	ns	ns	0.923*	0.926*
Hulls (n=12)							
Protein	-0.731***	ns	ns	ns	ns	ns	ns
Fat	0.882*	ns	ns	-0.759**	ns	ns	ns
Fiber	ns	ns	-0.759**	ns	ns	ns	ns
Avn 2p	ns	ns	ns	ns	ns	0.81**	0.709***
Groats (n=12)							
Protein	ns	ns	-0.802**	ns	0.653***	ns	0.694***
Avn 2p	ns	0.653***	ns	ns	ns	0.977*	0.981*
Avn 2f	ns	ns	ns	ns	0.977*	ns	0.933*

*, p<0.0001; **, p<0.005; ***, p<0.05; ns, not significant.

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