Phenolic Compounds: Their Role During Olive Oil Extraction and in Flaxseed – Transfer and Antioxidant Function

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A thesis submitted to the Faculty of Agromical Engineer of the University of Lleida in partial fulfillment of the requirements of the degree of Doctorate of Philosophy

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To

My parents

Jesús María
Luz Celina

My sister

María del Pilar

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Abstract

Biophenols encompass a major group of secondary plant metabolites that display a wealth of structural variety and a large diversity of significant biological activities. An area of special interest concerning olive and flaxseed is their phenolic profile and its direct influence on the quality of derived-products and the production of potential functional foods.

In response to this opportunity, the role of phenolic compounds during olive oil extraction process and their presence in flaxseed was the basis of the doctoral investigation.

Two important areas: transfer and antioxidant function of phenolics during olive oil extraction were investigated. The influence of irrigation treatments, ripening index of olive fruit and different technological aspects in the production of olive oil allowed having a better understanding of the transference and transformations of the phenolic compounds. Quantitative data, in terms of transfer, were established for concentrations of phenolics in olive paste, pomace, wastewater and olive oil phases. Some of these products and by-products have been studied and proven to be effective source of phenolic antioxidants. Therefore, isolation and purification of particular phenolics were performed to evaluate their individual antioxidant activity through the oxidative stability parameter. The bitter index, a sensorial attribute, was also evaluated as function of the addition of phenolic compounds. Simple alcohols, phenolic acids, secoiridoids, secoiridoid derivatives, flavonoids and lignans were studied. The addition of the mentioned phenolic compounds on refined and extra virgin olive oil had a remarkable effect on the oxidative stability of the oils. Gallic acid, hydroxytyrosol (3,4-DHPEA), caffeic acid, luteolin and the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) were found to be effective antioxidants.

Flaxseed phenolic fraction was also studied. Diverse studies on the extraction of phenolics provided a basis for establishing an analytical method to evaluate the phenolic composition of flax. A solvent extraction followed by hydrolysis treatments resulted in the identification of the main phenolic compounds occurred in flaxseed including secoisolariciresinol diglycoside (SDG). A subsequent study to understand the flaxseed antioxidant system was developed using reconstituted flour meals with commercial oil and analyzing hulls and dehulled flour meals. Peroxide and aldehyde values were determined during two weeks of storage. It appeared that SDG and phenolic acids (ferulic and *p*-coumaric glycosides) might not be responsible of the main flaxseed antioxidant function.

Resumen

Los biofenoles abarcan un amplio grupo de metabolitos secundarios de diversa estructura y reconocida actividad biológica. Específicamente el interés en el fruto de la oliva y en la semilla de lino está relacionado con su perfil fenólico y con su influencia directa en la calidad de productos derivados, así como con su posible uso en la producción de alimentos funcionales.

Como respuesta a este reciente planteamiento, la tesis doctoral se centró en la investigación del papel de los compuestos fenólicos en el proceso de extracción de aceite de oliva y en la semilla de lino.

Se tuvieron en cuenta dos importantes aspectos: la transferencia y la función antioxidante de los compuestos fenólicos implicados en el proceso de obtención de aceite de oliva. La influencia de tratamientos de irrigación, el índice de madurez y otros asuntos tecnológicos del procesamiento del fruto de la oliva trajeron como consecuencia un mejor entendimiento de la transferencia y transformación de los compuestos fenólicos. Se determinaron, entonces, datos cualitativos y cuantitativos en términos de transferencia, correspondientes a las concentraciones de fenoles en la pasta de oliva, orujo, alpechín y aceite de oliva. Algunos de estos productos y subproductos se han reconocido como valiosa fuente de compuestos antioxidantes. Por esta razón se procedió al aislamiento y purificación de determinados fenoles con el fin de evaluar su capacidad antioxidante a través del parámetro de estabilidad oxidativa. Igualmente se determinó el efecto de los compuestos fenólicos sobre el atributo sensorial de índice de amargor. Se estudiaron alcoholes simples, ácidos fenólicos, secoiridoides, derivados secoiridoides, flavonoides y lignanos, encontrándose un significativo efecto al adicionar dichos compuestos a distintas matrices de aceite de oliva (refinado y extra virgen). El ácido gálico, el hidroxitirosol (3,4-DHPEA), el ácido cafeico, la luteolina y la forma dialdehídica del ácido elenólico ligada al hidroxitirosol (3,4-DHPEA-EDA), mostraron tener efectividad como antioxidantes.

De igual forma se investigó la semilla de lino en función de su composición fenólica. La existencia de disímiles métodos de extracción de los fenoles se convirtió en el punto de partida para el estudio analítico de la fracción fenólica de la linaza. La extracción con solventes orgánicos, seguida de un proceso de hidrólisis, permitió la identificación de los principales compuestos característicos de la semilla de lino, como el Secoisolariciresinol diglucosídico (SDG). Posteriormente se pasó al estudio del sistema antioxidante de la linaza, para lo cual se evaluó el contenido de peróxidos y aldehídos en muestras de harina de semilla reconstituidas con aceite comercial de lino después de una y dos semanas de almacenamiento. También se analizaron harinas procedentes de semilla sin cascarilla y con cascarilla. La investigación preliminar permitió concluir que el SDG y otros compuestos, tales como las formas glucosídicas de los ácidos ferúlico y *p*-cumárico, no están implicados directamente en el sistema responsable de los procesos antioxidantes de la semilla de lino.

Resum

Els biofenols comprenen un gran grup de metabòlits secundaris que presenten una àmplia varietat d'estructures i una gran diversitat d'activitats biològiques. Específicament, l'interès en l'oliva i la llavor del lli (llinassa) està relacionat amb el seu perfil fenòlic i amb la influència directa d'aquest en la qualitat de productes derivats, Així com en el possible ús en aliments funcionals.

Com a resposta a aquest plantejament, la tesi s'ha centrat a la investigació del paper dels compostos fenòlics durant el procés d'extracció de l'oli d'oliva i de llinassa.

Es van tenir en compte dos importants aspectes: la transferència i les funcions antioxidants dels compostos fenòlics relacionats en el procés d'obtenció d'oli d'oliva. La influència dels tractaments d'irrigació, de l'índex de maduració i d'altres aspectes tecnològics del processament de l'oliva es traduí en un millor enteniment de la transferència i transformació dels compostos fenòlics. S'establiren les concentracions de fenols, en termes de transferència, corresponents a les fraccions de pasta d'oliva, pinyola, oliassa i oli d'oliva. Es reconegueren alguns d'aquests productes i subproductes com a valuosa font de compostos antioxidants. És per això que es va procedir a l'aïllament i purificació de determinats fenols amb la finalitat d'avaluar la seva capacitat antioxidant a través del paràmetre d'estabilitat oxidativa. També es va determinar l'efecte dels compostos fenòlics sobre l'atribut sensorial "índex d'amargor". Es van estudiar els alcohols simples, àcids fenòlics, secoiridoids, derivats secoiridoids, flavonoids i lignans. Es trobà un efecte significatiu a l'addicionar aquests compostos sobre diferents matrius d'oli d'oliva (refinat i verge extra). L'àcid gàlic, l'hidroxitirosol (3,4-DHPEA), l'àcid cafeic, la luteolina i la forma aldèhidica de l'àcid elenòlic lligat a l'hidroxitirosol (3,4-DHPEA-EDA), mostraren tenir una important activitat antioxidant.

Es va investigar també la llinassa en funció de la seva composició fenòlica. L'existència de diversos i no comparables mètodes d'extracció de fenols es va prendre coma punt de partida per a l'estudi analític de la fracció fenòlica de la llinassa. L'extracció mitjançant solvents orgànics prèvia a una hidròlisi va permetre la identificació del principals compostos fenòlics de la llinassa, com ara el secoisolariciresinol diglucòsid o SDG. Posteriorment es va passar a l'estudi del sistema antioxidant de la llinassa, pel què es va avaluar el contingut de peròxids i aldèhids en mostres de farina de llavor reconstituïdes amb oli comercial de llinassa després d'una i dues setmanes de magatzematge. També s'analitzaren farines procedents de llavor sense clofolla i amb clofolla. La investigació preliminar va permetre concloure que l'SDG i d'altres compostos

com ara les formes glucosídics del àcids ferúlic i *p*-cumàric no estan implicats directament al sistema responsable dels processos antioxidants de la llinassa.

Preface and Justification

Polyphenolic compounds are among the most talked about dietary ingredients nowadays. Hence, they have been focus of much research activity. Studies on their transfer and function during olive oil extraction process and in flaxseed were performed to contribute to a better knowledge of their role in food matrices and help in recognizing their value in a healthy diet.

Scientific studies on olive oil and its process are the great importance for several countries. Spain maintains its international prominence as the major olive oil producer and exporter in the world (FAOSTAT, 2005). Moreover, recent studies have been shown the potential effects of olive oil on human health (Visioli & Galli, 2002; Tuck & Hayball, 2002, Owen *et al*, 2000b). Understanding the composition of flaxseed is also a significant contribution to the industrial processing. Flaxseed is an economically important oilseed crop, especially for Canada, which produces about 40% of the world's flaxseed and is the world's largest exporter of flaxseed (Thomson & Cunnane, 2004; Oomah, 2001).

Theory:

Polyphenols belong to a class of phytochemicals found in high concentrations in wine, tea, grapes and a wide variety of other plants. They have been associated with cardiovascular disease and cancer prevention (Manach, *et* al, 2004; Hollman, 2001, Hertog *et al*, 1995; Hertog *et al*, 1993) In general terms, phenolic compounds or polyphenols, have a similar basic structural chemistry including an aromatic ring structure. It is also important to note that at least 8.000 phenolic compounds have already been identified in a dozen chemical sub-categories. Phenolic compounds are responsible for the brightly colored

pigments of many fruits and vegetables. They protect plants from diseases and ultraviolet light helping preventing damage to seeds until they germinate.

One of the more nutritionally important classes of polyphenols widely distributed in plant foods includes:

- Lignins (nuts, whole grain cereals)
- Proanthocyanins (grapes, pine bark)
- Anthocyanins/Anthocyanidins (brightly colored fruits and vegetables, berries)
- Isoflavones – genistein/daidzein (soybeans)
- Catechins (tea, grapes, wine)
- Tannins (tea, nuts)
- Quercetin (grapes, wine, onions)
- Naringenin/Hesperidin (citrus fruits)

Natural polyphenols can range from simple molecules such as phenolic acid to large highly polymerized compounds such as tannins. Conjugated forms of polyphenols are the most common forms, where various sugar molecules, organic acids and lipids (fats) are linked with the phenolic ring structure (Bravo, 1998). Differences in this conjugated chemical structure account for different chemical classification and variation in the modes of action and health properties of the various compounds.

Flavonoids are among the most potent phenolic plant antioxidants. They can form complexes with reactive metals such as iron, zinc and copper – reducing their absorption. This might seem to be a negative side effect (reducing nutrient absorption), but excess levels of such elements (metal cations) in the body can promote the generation of free radicals and contribute to the oxidative damage of cell membranes and cellular DNA. In addition to their chelating effect on metal cations, polyphenols also function as potent free radical scavengers within the body, where they can neutralize free radicals before they can cause cellular damage. Epidemiologic studies have shown a relationship between high dietary intakes of phenolics and reduced risk of cardiovascular disease and cancer. In general, polyphenols are thought to deliver health benefits by several mechanisms, including: (1) direct free radical quenching, (2) protection and regeneration of other dietary antioxidants, (3) chelation of metal ions (Bravo, 1998; Cieslik *et al*. 2006)

Besides competing for moisture, light and soil nutrients, plants have developed chemical defense mechanisms (allelopathy), through the use of simple-structured, low molecular weight secondary metabolites, whose effectiveness depend on the speed with which soil micro-organisms are able to detoxify and metabolize them (Harborne, 1993). Chemical interactions involving plants and micro-organisms may be compatible or incompatible and begin with elicitor-mediated genetic recognition, which triggers signal amplification and gene activation, and culminate by the activation of defense mechanisms, such as hypersensitive response, systemic acquired resistence and phytoalexin induction. This latter can also be triggered by stress conditions, intense cold, ultraviolet radiation, tissue injury, osmotic stress, abiotic agents (fungicides, metallic ions, fosfite, ethylene and glutation) and biotic agents (Cordeiro & Sá, 2000).

Claims:

Antioxidant activity (Manach *et al*, 2004)
Antibiotic / Antiviral activity (Bravo, 1998)
Anti-inflammatory activity (Bravo, 1998)
Protection from diseases (Scalbert *et al*, 2005)

Contribution to original knowledge:

This manuscript provides useful information on the study of phenolic compounds during olive oil extraction process and the role of phenolic compounds in the flaxseed system. The document has been organized into chapters and sections to offer a better flow of material. The Literature Review reports a state-of-the-art of the definition, classification and occurrence in foods in a general view as well as their role in the olive fruit and flaxseed. The result of the whole investigation during the doctoral process is presented in papers published and submitted in recognized scientific journals. A global discussion is also provided as a supplementary text for the general interest to senior undergraduate and graduate students in food engineer or food system. Conclusions are given in a last chapter. Additionally, extensive references have been provided to facilitate further reading of the original reports.

Objectives

The general aim of this doctoral thesis was to investigate the presence, behaviour and biological importance of phenolic compounds in two different food matrices: virgin olive oil and flaxseed. The work presented here derives from two different projects, each one with defined objectives:

Project: Phenolic Fraction of Virgin Olive Oil: Identification, function and transfer from olive fruit to oil

The overall objectives of this project included two main objectives:

1 To study the transfer of phenolic compounds fraction during the extraction process of virgin olive oil (*Arbequina* cultivar) considering some technological variables.

This was accomplished by:

1.1 Determination of the partition of phenolic compounds between the olive paste, pomace, olive oil and wastewater in relation to ripening stage of the olive fruit during olive extraction

1.2 Evaluation of the effect of irrigation practices on the partition of phenolic compounds between different fractions during extraction process of olive oil considering Natural Micro-Talc (NMT) addition.

1.3 Study of the partition of phenolic compounds between olive paste and by-products resulting from olive oil extraction process at industrial level.

2. To investigate the antioxidant activity of diverse compounds of the olive oil phenolic fraction and their effect of on the bitter sensorial attribute.

This objective encompasses:

2.1 Isolation and purification of phenolic compounds from virgin olive oil by semipreparative liquid chromatography.

2.2 Evaluation of individual antioxidant activity of the compounds from virgin olive oil phenolic fraction by Rancimat method.

2.3 Study of the effect of different phenolic compounds on the bitter sensorial attribute by the bitter index (K_{225}).

Project: Phenolic Compounds in Flaxseed (*Linum usitatissimum)*

This project was developed attending to the next objectives:

3. To develop and validation of analytical methods to determine phenolic compounds in flaxseed *(Linum usitatissimun)*.

This first objective includes:

Optimization of methods to extract, hydrolyze, and quantify phenolic compounds in flaxseed.

4. To investigate the role of the phenolic compounds on flaxseed antioxidant system

The objective was reached by:

4.1 Measure of evolution of oxidation parameters (peroxide and aldehyde) of flaxseed from breeder lines to asses the antioxidant properties of seeds.

4.2 Application of the new developed method to analyze phenolic compounds in flaxseed breeder lines having different antioxidant properties.

4.3 Study of the relation between phenolic compounds and antioxidant activity.

Working Plan

Projects on Scientific Research and Technological Development in Food Science Field
Doctoral Thesis: January 2003 - December 2006

Project Reference: AGL2002-00289ALI Ministry of Education and Science (Spain)

PROJECT TITLE: Phenolic Fraction of Virgin Olive Oil: Identification, Function and Transfer from Olive Fruit to Oil	Starting date	Finishing date
Product: Scientific papers published in recognized journals	January, 2003	January, 2006

GLOBAL CONTEXT

Justification and Goals	
Justification	Goals
Fats & Oils Laboratory is a recognized research group of the Food Technology Department (University of Lleida). It has been working during the last years on different projects related to the composition of virgin olive oil obtained from *Arbequina* cultivar in Catalonia, especially in Les Garrigues, a region located in the province of Lleida (Catalonia, Spain). *Arbequina* virgin olive oils are characterized by their excellent sensorial characteristics. However, they show some drawbacks in relation to their resistance to the autoxidation of the oil. Several studies have put in evidence the direct involvement of the phenolic fraction in the high resistance against oxidation and in the sensory attributes of virgin olive oils.	1. To evaluate the phenolic fraction during the processing of olive oil considering different technical variables 2. To complete the identification of the different compounds of the phenolic fraction in olive oil, and once they are isolated and purified, evaluating their involvement, as individual components, in oil properties such as oxidative stability and sensory attributes.

METODOLOGY

1. Preliminary Scientific Study Actions: Apprehension of deep knowledge of the research topic			
Activities	Responsible	Dates	Centers involved
1. Study of the investigations related to the project	L.S. Artajo	January, 2003 April, 2003	UdL
2. Learning of the laboratory established methodologies for experimental work		May, 2003 October, 2003	

2. Analytical Procedures Actions: Evaluation of the phenolic compounds transfer during extraction process in Pilot Plant			
Activities	Responsible	Dates	Centers involved
1. Sampling Design	L.S. Artajo T. Ramo J.R. Morelló	November, 2003 January, 2004	CRDOP/ UdL
2. Extraction of virgin olive oil: ABENCOR System Experimental variables: Ripening index of olive fruit Irrigation Treatment	L.S. Artajo M.J. Motilva M.P. Romero		UdL
In processing: a. Addition of coadyuvants			
3. Extraction of phenolic compounds in olive paste and phases obtained by processing	L.S. Artajo M.J. Motilva M.P. Romero		
4. Analysis of phenolic fraction by HPLC		November, 2003 February, 2004	

Actions: Determination of the phenolic compounds function in virgin olive oil			
Activities	Responsible	Dates	Centers involved
1. Isolation and purification of phenolic compounds from virgin olive oil	L.S. Artajo M.J. Motilva M.P. Romero J.R. Morelló	May, 2004 August, 2004	UdL
2. Identification of phenolic compounds		September, 2004 October, 2004	
3. Enrichment of different virgin olive oil matrices with phenolic compounds	L.S. Artajo M.J. Motilva M.P. Romero Student of agronomical engineering	October, 2004 December, 2004	CRDOP/ UdL
4. Measurement of the oxidative stability: Rancimat System			UdL
5. Quantification of the bitter index in the oil matrices			

Actions: Determination of the phenolic compounds transfer during olive oil industrial process			
Activities	Responsible	Dates	Centers involved
1. Sampling Design Experimental variables: Production period In Processing: a. Malaxation time b. Horizontal Centrifugation c. Vertical Centrifugation 3. Extraction of phenolic compounds in olive paste and phases obtained by processing: wet pomace, wastewater and olive oil 4. Analysis of phenolic fraction by HPLC	L.S. Artajo M.J. Motilva M.P. Romero Student of agronomical engineering	November, 2004 January, 2005 November, 2004 March, 2005	CRDOP/ UdL UdL
3. Analysis of Results Actions: Interpretation of Experimental Data			
Activities	Responsible	Dates	Centers involved
1. Organization of Data 2. Statistical Analysis 3. Interpretation of Results 4. Internal Discussion	L.S. Artajo	October, 2005 February, 2006	UdL

4. Elaboration of Papers

Actions: Writing the research papers:

1. "Transfer of phenolic compounds during olive oil extraction in relation of ripening stage of the fruit"[a]
2. "Effect of irrigation applied to olive trees (Olea europaea L.) on phenolic compound transfer during olive extraction process"[b]
3. "Partition of phenolic compounds during the virgin olive oil industrial extraction process."[c]
4. "Enrichment of refined olive oil with phenolic compounds: Evaluation of their antioxidant activity and their effect on the bitter index"[d]
5. "Enhancement in the oxidative stability of extra virgin olive oil matrices by adding phenolic compounds"[e]

Activities	Responsible	Dates	Centers involved
1. Bibliographic Revision	L.S. Artajo M.J. Motilva	March, 2004 December, 2005	UdL
2. Organization of the information	M.P. Romero		
3. Preparation of the document			
4. Presentation to internal revision			
5. Submission of the paper to Scientific Journal		[a] September, 2004 [b] July, 2005 [c] February, 2006 [d] February, 2006 [e] 2007	

FINANCING SUPPORT

Cost-Budget and Financing	
Cost- Budget	Financing
Experimental Work	Ministry of Education and Science - Interministerial Commission on Science and Technology, Spain
Pre-doctoral scholarship for the training of research personnel (first and second years) FI 00801 Contract for Junior Researcher (third and fourth years) FIC 00397	Agència de Gestió d' Ajuts Universitaris i de Recerca – AGAUR. Interdepartmental Commission on Research and Technologic Innovation – CIRIT, Catalonia European Social Fund

Project Reference: CGC 4579 Canadian Grain Commission (Canada)

PROJECT TITLE: Investigation of the role of phenolic compounds in flaxseed antioxidant system	Starting date	Finishing date
Product: Scientific papers published in recognized journals	August, 2005	February, 2006

GLOBAL CONTEXT

Justification and Goals	
Justification	Goals
The Grain Research Laboratory (GRL) is an internationally known research centre and the Canadian centre for research on the quality of grain. Its scientists ensure that the processing quality of grain is maintained from cargo to cargo and from year to year. They engage in research to expand our scientific knowledge of what constitutes quality in grain. With the recent concern in "healthy food", consumers are seeking foods that have potencial antioxidant activity. Currently, attention is focused on antioxidants and phytoestrogens as evidenced by the interest in soy isoflavones. An important phytochemical that has both phytoestrogenic and antioxidant properties is the lignan "Secoisolariciresinol Diglucoside" (SDG) occurring in flaxseed. Flaxseed (*Linum usitatissimum*) is an ancient crop used for fiber (linen), oil (linseed oil) and food. It can be grown in North and South America, Europe, Asia, and Australia. Canada is a major producer and exporter country. Flaxseed also contain phenolic acids such as *p*-coumaric and ferulic in the free form and glycosilated forms. However, research on the identification of some compounds is so far from being concluded. The presence of other phenolic compounds have also been reported in flaxseed, phenylpropanoids such as *p*-hydroxibenzoic acid, gentisic acid, vanillic acid and sinapic acid.	1. To quantitatively extract, identify and measure phenolic compounds (free, esterified and etherified) in flaxseed. 2. To evaluate the effect of the phenolic compounds on the antioxidant properties of flaxseed.

METODOLOGY

1. Preliminary Scientific Study Actions: Apprehension of deep knowledge of the research topic			
Activities	Responsible	Dates	Centers involved
1. Study of the investigations related to the project	L.S. Artajo	August, 2005 September, 2005	CGC UdL

2. Analytical Procedures Actions: Method development and validation for extracting phenolic compounds in flaxseed			
Activities	Responsible	Dates	Centers involved
1. Extraction of phenolic compounds in flaxseed and solin seed by different methods: stirring, using homogenizer and using vortex. 2. Application of hydrolysis treatments: Alkaline Hydrolysis Acid Hydrolysis Experimental variables: a. Time and Temperature b. Concentration of the acid or alkali 3. Measure of total phenolic content by Folin-Ciocalteau assay 4. Analysis and identification of phenolic compounds by HPLC	L.S. Artajo V.J. Barthet R. Bacala	September, 2005 November, 2005	CGC–GRL / UdL

Actions Determination of antioxidant mechanism of the flaxseed system			
Activities	Responsible	Dates	Centers involved
1. Extraction of phenolic compounds of different fatty acid compositions using breeder lines. 2. Measure of total phenolic content in breeder lines by Folin-Ciocalteau assay 3. Identification of phenolic compounds in the breeder lines samples by HPLC Analysis 4. Induction of oxidation in reconstituted breeder samples by artificial light and O_2 exposition 5. Measure of peroxide and aldehyde values in the breeder samples oil.	L.S. Artajo V. J. Barthet R. Bacala V. Barthet L.S. Artajo	November, 2005 February, 2006	CGC / UdL

3. Analysis of Results Actions: Interpretation of Experimental Data			
Activities	Responsible	Dates	Centers involved
1. Organization of Data 2. Statistical Analysis 3. Interpretation of Results 4. Internal Discussion	L.S. Artajo	October, 2005 February, 2006	UdL

4. Elaboration of Papers Actions: Writing the research paper: 1. "Analysis of flaxseed (*Linum usitatissimum*) phenolic compounds"[a] 2. "Flaxseed antioxidant system"[b]			
Activities	Responsible	Dates	Centers involved
1. Bibliographic Revision 2. Organization of the Information 3. Preparation of the document 4. Presentation to internal revision 5. Submission to Scientific Journal	L.S. Artajo V.J. Barthet	March, 2006 June, 2006 July, 2006[a] 2007[b]	UdL

FINANCING SUPPORT

Costs- Budget and Financing	
Costs- Budget	Financing
Experimental Work Scholarship and grant for research stay outside of Catalonia (6 months). 2005BE 00116	Canadian Grain Commission (Canada) Agència de Gestió d' Ajuts Universitaris i de Recerca – AGAUR, Catalonia European Social Fund

Actions: Elaboration of the Thesis Document and Defense			
Activities	Responsible	Dates	Centers involved
1. Bibliographic Revision	L.S. Artajo	April, 2006 - August, 2006	UdL
2. Preparation and writing of the document			
3. Presentation to internal revision		August, 2006	
4. Corrections			
5. Submission of the Final Document to the Food Technology Department		October, 2006	
6. Thesis Defense		December, 2006	