P-006	**Isolation and characterization of oil bodies from *Gevuina avellana* and *Madia sativa* seeds**	

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INTRODUCTION AND OBJECTIVES

Oleosomes from plant seeds are oil bodies that act as energy stores for post-germinative growth. These oil bodies consist of an oil core with a matrix of triacylglycerol bounded by a phospholipid monolayer embedded with proteins known as oleosins (Huang, 1992). Oleosins are considered highly stable with natural self-emulsifying properties derived from alternating amphipathic and hydrophobic domains. Oil bodies are remarkably stable and do not aggregate or coalesce (Beisson, 2001).

Oil bodies obtained from oilseeds have been exploited for several biotechnological applications such as emulsifying agent for a wide variety of products (ranging from vaccines, food, cosmetics and personal care) (Bathla, 2010). Oil bodies-based pharmaceutical formulations include therapeutic, diagnostic and delivery agents (Delgado-Vargas, 2003). Oil bodies have been used as carriers of flavouring agent, chelating agents, cosmetic products (Deckers, 2004), hydrophobic molecules such as nutraceutical compounds (Murphy, 2001), pesticides (Boucher, 2008) and pharmaceutical drugs, and they have been successfully tested as a biocapsule for encapsulating lactic acid products (Huang, 1992). Oil bodies and oleosins have been purified and characterized in many plant species such as *Brassica napus* (Murphy, 1991), however, up to date, no purification and characterization of oil bodies from native seeds such as *Gevuina avellana* and *Madia Sativa* has been performed.

Chilean Amerindians have used *G. avellana* and *M. sativa* seeds as oil sources since pre-Columbian times and only few scientific reports about their bioactive molecules have been already found. In addition, both are native species and are economically exploitable in the Araucanía Region of Chile.

G. avellana Mol., a Chilean hazelnut, belongs to a monospecific genera of the Proteacea family from the native forest of the Andes and the Coastal mountains in South of Chile. The seeds contain 12% proteins, 24% carbohydrates and high oil content (40-49%) (Yáñez, 2004). Furthermore, the high content of oil from *G. avellana* seeds suggests that it would be a potential source of oil bodies.

M. sativa Mol., called also "madi" (Mapuche name), has been classified as a weed from Central Chile and their seeds have been processed to obtain edible oil by the Araucanians. *M. sativa* belongs to the Astereacea family and presents approximately 29% of protein, 26% lipid, 24% fibre and 14% of total carbohydrates. This specie is rich in the unsaturated fatty acids such as palmitic, stearic and oleic acids (Shmeda-Hirschmann, 1995). The oil content of *M. sativa* seeds may render these seeds as a suitable source of oil bodies.

The objective of this study was to isolate and characterize oil bodies from Chilean native mature seeds of *G. avellana* and *M. sativa* as possible encapsulation carriers. Extraction and characterization of oleosomes of rapeseeds were run in parallel.

MATERIALS AND METHODS

Plant Materials

Mature seed from *G. avellana*, *M. sativa* were obtained in fields near Villarrica in the Araucanía Region (Chile). Seeds from *G.a avellana* were dehulled.

Isolation of Oil bodies

In this study, the oleosomes from mature seeds were purified using a flotation-centrifugation method according to the method of Tzen (1993) with some modifications.

Characterization of Oil bodies

Oil bodies were characterized by means a proximate composition and particle size by light and transmission electronic microscopy (TEM). Neutral lipids, phospholipids and proteins of oil bodies from mature seeds were separated according to Tzen (1993). Lipid content was determined by diethylether extraction. The protein content of the defatted dried OL was determined by the Bradford method. Proteins were resolved by SDS-PAGE by Laemmli method using 12% and 5% polyacrylamide gels in the separating and stacking gel, respectively.

Fatty acid composition of neutral lipids from oil bodies was obtained by GC analyses. The chloroform fraction containing PL was spotted on a TLC plate (silica gel 60 F254, Merck). The plate was developed in chloroform: acetic acid: methanol: water (70:25:5:2, v/v/v/v) and bands were visualized after development with iodine. Standard phospholipids used for identification were phosphatidylethanolamine, phosphatidylcholine, and phosphatidic acid.

Isolated oil bodies were imaged using transmission electron microscopy (TEM).

RESULTS AND DISCUSSION

The oil bodies can be extracted from *G. avellana* seed

and *M. sativa* seed with an extraction yield of 5.6%, 5.9% respectively. However the higher extraction yield (16.8%) was obtained from *B. napus* seeds. The final washed oleosomes preparation contained 85.9% lipid and 1.9% protein for *G. avellana* seeds, 77.1% lipid and 3.1% protein for *M. sativa* and 86.9% lipid and 5.6% protein for rapeseed. The identified oleosins showed molecular mass values between 14 and 25 kDa, depending on the seed species. The identified phospholipids in oil bodies were phosphatidylcholine (PC) for *G. avellana* and phosphatidic acid (PA) for *M. sativa*. *B. napus* contained PC, PA and PE (phosphatidylethanolamine). High amounts of poly- and monounsaturated fats (omega 3 and 6) were found in oil bodies from native seeds (Table 1). The structures were globular and compact in shape, and ranged in size from 2 to 10 µm. The monolayer of oil bodies from *M. sativa* seeds was thicker than *G. avellana* and *B. napus*.

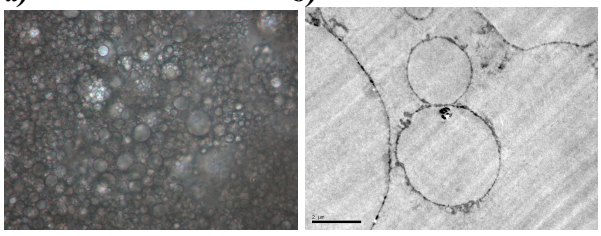
CONCLUSIONS

Our findings support a potential functionality for native Chilean seeds as sources of oleosomes for possible biotechnological applications as microencapsulation carriers.

FIGURES AND TABLES

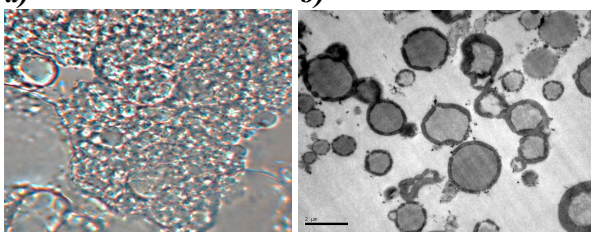
G. avellana

a) ***b)***



M. sativa

a) ***b)***



B. napus

a) ***b)***

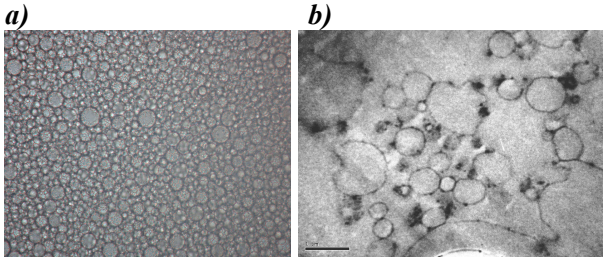


Figure 1. a) Light microscopy (x60) and b)TEM of oil bodies fraction, showing spherical oil bodies of heterogeneous sizes

Table 1. GC analyses of fatty acids from oil body from seeds

Fatty Acid Composition	(mg fatty acid/g oil)	
	G.avellana	*M. sativa*
12:0	-	1.32
14:0	-	1.46
15:0	-	0.48
16:0	1.57	33.2
16:1	46.47	1.36
17:0	-	0.05
18:0	0.65	14.6
18:1n9c	30.07	36.4
18:2n6c	33.07	581
18:3n3	6.73	2.24
20:0	5.01	3.68
20:1n9	8.56	0.22
20:2	-	0.44
20:3n3	10.53	-
20:3n6	4.33	-
21:0	4.07	1.09
22:1n9	8.56	-
22:6n3	-	16.2
23:0	31.04	-
24:0	5.40	1.26
24:1n9	7.10	0.06

REFERENCES

- Huang A.H. (1992) *Oil bodies and oelosins in seeds*. Annual Review of Plant Physiology and Plant Molecular Biology 43: 177-200
- Beisson F. et al. (2001) *Oil-bodies as substrates for lipolytic enzymes*. Biochimica et Biophysica Acta 1531: 47–58
- Bathla et al. (2010) *Use of oil bodies and oleosins in recombinant protein production and other biotechnological applications*. Biotechnology Advances 28 : 293–300
- Delgado-Vargas F. et al. (2003) *Natural colorants for food and nutraceutical uses.* CRC Press Bca Raton, Florida, USA. pp: 113-166, 257-309
- Deckers et al. (2004) *Sembiosys Genetics Inc. Immunogenic formulations comprising oil bodies*. United States patent US 6761914
- Boucher et al. (2008) Sorption of hydrophobic organic compounds (HOC) in rapeseed oil bodies. Chemosphere 70: 1452–1458
- Murphy et al. (1992) *Oil bodies and oelosins in seeds*. Annual Review of Plant Physiology and Plant Molecular Biology 43: 177-200
- Yáñez L.E. (2004) *Prospección del comercio del fruto de la avellana y sus productos derivados, en la región metropolitana*. Memoria para optar al Título Profesional de Ingeniero Forestal. Universidad de Chile. pp. 62
- Shmeda-Hirschmann G. (1995) *Madia Sativa, a potential oil crop of central Chile*. Economic Botany 49: 257-259

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