Testing edible mushrooms to inhibit the pancreatic lipase activity by an *in vitro* digestion model

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Running title: Pancreatic lipase activity of mushroom

ABSTRACT:

One of the strategies in prevention or treatment of obesity is altering metabolism of lipids by inhibition of dietary fat absorption. The extracts obtained with methanol, water and methanol:water (1:1) from 21 mushroom species were screened as potential sources of pancreatic lipase (PL) inhibitors using a standardized *in vitro* test. *Lepiota procera* methanol:water (1:1) extracts showed the highest inhibition activity closely followed by *Grifola frondosa, Pleurotus eryngii* and *Lyophyllum shimeji*. Other mushroom strains such as *Morcella conica, Marasmius oreades, Lentinula edodes, Amanita ponderosa* and *Boletus edulis* also showed a certain inhibitory activity. However, when the PL inhibitory activity was evaluated using an *in vitro* digestion model mimicking gut conditions, none of the selected mushroom extracts were able to inhibit PL activity. On the contrary, stimulation of the lipase activity levels was observed and it was not due to endogenous mushroom lipases activities.

Keywords: Mushroom, fruiting bodies, pancreatic lipase, inhibitors, *in vitro* digestion.

Introduction

Obesity is the sixth most important risk factor contributing to the overall burden of disease worldwide reaching the category of epidemic. Nowadays, pharmacotherapy arises as useful tools to approach obesity. So far, there are three major drug options for the long-term treatment of obesity: orlistat (a gastric and lipase inhibitor), sibutramine (a monoamine reuptake inhibitor) and rimonabant (an endocannabinoid receptor blocker) (Padwal & Majumdar, 2007). However, obese and specially overweighed population is reluctant to assume obesity as a medical problem and, before turning to a health professional, starts his/her own therapy by using special foods, such as reduced fat content (light) products and nutritional supplements (including herbal extracts) and more often, diets without scientific evidence.

Therefore, foods containing active principles with clear metabolic targets and scientific evidence of their activity may help in the self-fight against obesity, reaching to a higher number of individuals and in an earlier stage of their own obesity. A wide range of natural products (including crude extracts) mainly obtained from plants have been reported as effective pancreatic lipase (PL) inhibitors. For instance, berry polyphenols (McDougall *et al*., 2009), triterpenes from *Sapindus* sp (Morikawa *et al*., 2009), monoterpenes from *Monarda punctata* (Yamada *et al*., 2010), abietanes from *Salvia* sp (Ninomiya *et al*., 2004) and more than 70 plant extracts (Sharma *et al.*, 2005) showed PL inhibitory activity. The list of compounds and sources could be further extended with the findings of Birari & Bhutani (2007) and Slanc *et al*., (2004). The latter publication pointed fungi as a potential new source of PL inhibitors since within 60 edible and non-edible fungi species, PL inhibitory activities were found ranging from 1% till 97% depending on the specie considered.

Only a few interesting PL inhibitors were isolated from edible fungi, two of them were β-lactones with unusual configurations named percyquinin (obtained from *Stereum complicatum*) and vibralactone (*Boreostereum vibrans*) with similar IC_{50} (0.4 μg/mL) (Liu *et al.,* 2006; Birari & Bhutani 2007). For a few mushroom species, the observed activities were also effective *in vivo* according to the results obtained with animal models. Ahn *et al.,* (2007) reported the antiobesity effects of *Isaria sinclairii* fruiting bodies and Mizutani *et al.,* (2010) demonstrated the PL inhibitory activity of water extracts (polysaccharide-rich fraction) obtained from *Pleurotus eryngii* fruiting bodies.

However, most of the former results were obtained from biochemical tests and no further studies to evaluate them under gut conditions were carried out. Positive scientific results in dead end may be mislead and produce misuse. For instance, a published scientific evidence of lipase inhibitory activity in some raw foodstuff or an herb does not mean that will have effect on fat absorption but can be interpreted like that, and wrongly used for that purpose.

Thus, in this work the PL inhibitory activities of several extracts obtained from edible mushrooms were determined using a fast and standardized method and compared to the PL inhibitory activities obtained using an *in vitro* digestion model simulating the conditions in which the human PL would be involved in order to confirm or reject, in a step ahead, their lipase inhibitory capacity before animal testing.

Materials and Methods

Biological material

Mushroom species used in this investigation were *Lentinula edodes* S. (Berkeley), *Cantharellus cibarius* (Fr.), *Lactarius deliciosus* (Fr.), *Boletus edulis* (Bull. Ex Fr.), *Pleurotus ostreatus* (Jacq.Ex Fr.) Kummer, *Agaricus bisporus* L. (Imbach), *Amanita caesarea (*Scop. Ex Fri.) Pers.

Ex Schw., *Morchella esculenta* (Pers Ex Amans), *Agaricus blazei* Murill ss. (Heinem), *Grifola frondosa* (Dicks.) Gray, *Ganoderma lucidum* (Curtis) P.Karst., *Flammulina velutipes* (Curt. Ex Fr.) Singer, *Pleurotus eryngii* (D.C. Ex Fr.) Quel, *Lyophyllum Shimeji* (Kawam.), *Morchella conica* (Pers.), *Agrocybe aegerita* (Briganti) Singer, *Auricularia judae* (Bull. Ex St.Amans) Berck, *Amanita ponderosa* Malençon & R. Heim, *Craterellus cornucopioides* (L. Ex Fr.) Pers, *Marasmius oreades* (Bolt. Ex Fr.) Fr. and *Lepiota procera* (Scop. Ex Fr.) Singer. Fruiting bodies were purchased from the local market in Madrid, Spain. Refined Sunflower oil, milk powder, walnut, dehydrated Cereals, dried banana were also obtained from local markets. All the experiments were performed using the same bottle, box or lotus.

Reagents

Pancreatine (P-1750), lecithin, bile salts, maleic acid, 2,3-Dimercapto-1-propanol tributyrate (BALB), sodium dodecyl sulfate, 5,5´-Dithiobis (2-nitrobenzoic acid) (DTNB), D-mannitol, phenylmethylsulfonyl fluoride (PMSF) were procured from Sigma-Aldrich Co. Steinheim, Germany. Orlistat was purchased from Glaxo Group Limited, Middlesex, UK. All other reagents and solvents were used of analytical grade.

Sample preparation

Dried fruiting bodies dehydrated as described in Ramírez-Anguiano *et al*., (2007). The fruiting bodies were ground into fine powder using liquid nitrogen in a mortar. The mushroom powders (10 mg) were mixed with 1 mL of absolute methanol, a mixture methanol: water (1:1) or water, shacked on a Vortex (Heidolph Reax top, Germany) during 3 min and centrifuged (10000 rpm 2 min) in a mini Centrifuge (Eppendorf, Minispin, Germany). The supernatants were screened as source of pancreatic lipase inhibitors.

Pancreatic lipase activity using an standardized lipase assay kit

Pancreatic lipase activity in the presence of mushroom extracts was measured using a QuantiChrom^{TM} Lipase assay kit (DLPS 100) from BioAssay Systems (Hayward, USA). A pancreatic lipase solution was prepared by mixing 19µg porcine pancreatin with 1 ml 50mM Trizma-maleate pH 7.5 and it was used as a lipase source. Mushroom supernatants (10 µl) were mixed with 40 µl of the pancreatic solution and 100 µl of a mixture including 5 mg DTNB, 8 µl BALB in ethanol and 100 µl tris(hydroxymethyl)aminoethane buffer pH 8.5 in a clear flat bottom 96-well plate according to the user´s manual. Absorbance increase during 20 min at 412 nm and 37ºC was recorded using a microplate reader (Tecan Group Lt, Switzerland). Lipase activity was expressed as indicated by the user´s manual. An Orlistat solution (0.75mg/mL) was also applied (10 µl) and used as a positive inhibitor control.

Pancreatic lipase activity using an *in vitro* digestion model

The samples (different concentrations of mushroom extracts with/without 1 g sunflower oil) were submitted to an *in vitro* intestinal digestion model carried out following the method described by Martín *et al.,* (2010). The samples were mixed with 54 mL of 50mM Trizma-maleate buffer pH 7.5 in a titrator device (Titrino plus Metrohm 877, Switzerland) maintaining temperature (37ºC), stirring and pH (7.5) constant for 120 min. Simulation of intestinal digestion was started by addition of 5mM $CaCl_2$, 150 mM NaCl, and 6 mL of a pancreatin solution (1000 IUB/mL, 11.8 mM bile salts and 1.3mM lecithin in the Trizma-maleate buffer). Every 30 minutes (including time 0 immediately after the mixture of the mushroom sample with the pancreatic mixture), 100 µl of the digestion suspension were withdrawn, diluted with 1.8 ml 50 mM trizma-maleate buffer pH 7.5 and assayed for lipase activity (50 µl) according to the procedure of Furukawa *et al.*, (1982). Briefly, diluted solutions were mixed with 1 ml of 0.3 mM DTNB and 20 µl PMSF. The mixture was incubated at 37ºC for 5 min. Afterwards, 100 µl of a BALB solution (20 mM BALB

and 20 mM SDS in ethanol) were added and incubated at 37°C for 30 min. The reaction was stopped by adding 2 ml acetone. Concomitantly, a zero sample of each assay was prepared as above described but with no substrate addition. Absorbance increase at 412 nm was recorded using a spectrophotometer (Evolution 600 Thermoscintific, England). *In vitro* digestions of mushroom extracts were also carried out as before described but without lecithin and bile salts and the effect of these compounds was also evaluated on the test performance. Orlistat (1.48 mg/g oil or 10 mg/ml depending on the type of experiment) was used as a positive inhibitor control.

Mushrooms lipase activities

Mushroom powders (10 mg/mL) were mixed with methanol:water (1:1) and diluted in buffer as described from samples obtained from the *in vitro* digestion model. Obtained mixtures (50 µl) were directly mixed with all the reagents to measure their lipase activity using the protocol described above by Furukawa *et al.*, (1982) except for the pancreatin addition in order to evaluate the potential residual lipase activity that might occur due to endogenous mushroom lipases.

Results and Discussion

Screening of pancreatic lipase inhibitory activity

Several extracts were obtained from mushroom fruiting bodies using methanol, methanol:water (1:1) and water as solvents (eight different concentrations ranging from 666 to 5.2 µg/mL) screened for pancreatic lipase (PL) inhibitory activity using an standardized lipase assay kit and a pancreatin solution.

Extract concentrations lower than 166 µg/mL (corresponding to an enzyme:inhibitor ratio of 1:9 in the final reaction volume) showed no PL inhibitory activity and the percentage of inhibition observed for these or higher concentrations was strain and solvent dependent. None of the water

extracts showed significant PL inhibitory activities compared to controls. The highest inhibitory values were found on the methanol:water extracts except for *Marasmius oreades* (Table 1) and *Morchella conica* showing higher inhibition on their methanol than methanol:water (1:1) extracts (respect. 19.4% and 15.5%). Thus, results were in concordance with those of Slanc *et al.,* (2004) pointing methanol:water as the best solvent mixture to extract PL inhibitors.

The methanol:water (1:1) extracts obtained from the 21 selected mushroom strains were screened using the *in vitro* lipase assay kit for PL inhibitory capacities (Table 2) and many of them lacked inhibitory capacity or it was very low. However, *Boletus edulis*, *Amanita ponderosa*, *Lentinula edodes*, *M. oredades*, *M. conica*, *Lyophyllum shimeji*, *P. eryngii* and *Grifola frondosa* showed between 10% to 20% and *Lepiota procera* a PL inhibitory activity equivalent to the 25% of the selected orlistat concentration.

Although, the PL inhibitory activities found in the latter species were four or five folds lower than the selected orlistat concentration (0.75 mg/mL) results might be of high interest. In fact, a foodstuff with a quarter of the orlistat activity could be recommended by nutritionists as part of a diet to lose weight. Therefore, the same mushroom extracts were tested under simulated gut conditions.

Effect of in vitro digestion on the PL inhibitory activity of mushroom extracts

In order to evaluate whether the mushroom extracts that showed positive PL inhibitory activity would still be able to carry out their potential effect on pancreatic lipase during human digestion, mushroom extracts were mixed with a fatty food matrix such as oil and submitted to an *in vitro* digestion model mimicking the intestinal digestion.

The pancreatic lipase activity was monitorized in an *in vitro* digestion model during 2h using a sunflower oil digestion as control (Figure 1). As expected, the lipase activity levels showed a

constant value of approx. 400 IUB/mL during the selected digestion period and conditions. The increasing volume of NaOH added into the digestion vessel by the tritiation device during the digestion indicated that the pancreatic lipases were degrading the oil triacylglycerides and releasing free fatty acids (provoking a reduction of pH that was compensated by the NaOH) (Martín *et al.*, 2010). Under the selected *in vitro* conditions, the lipase reactions followed a hyperbolic curve needing approximately one hour to liberate most of the fatty acids although, a slight degradation was still observed until 2h digestion. This effect could correspond to the Ca^{2+} extinction in the media needed for the proper lipase activity (Porter *et al.*, 2007). When the same digestion was performed in the presence of orlistat (1.48mg/g oil), activity was reduced by approx. 6 folds during the complete digestion time. However, when the oil was mixed with mushroom extracts of *G. frondosa* (51 mg/g oil dw), *M. oreades* (91 mg/g), and *L. Procera* (189 mg/g) and submitted to *in vitro* digestion during 2 hr no inhibitory effect similar to orlistat was observed. A slightly but significantly lower PL activity was noticed at the beginning of the digestion when the samples containing the *M. oreades* and *L. procera* extracts were analyzed. However, as the digestion proceeded, in the samples containing *M. oreades* extracts, after 1h PL activity reached values similar to control and in those samples containing *L. procera* (applied in higher concentration) the PL activity values increased up to levels even higher than control. Thus, the PL inhibitory capacity detected for some mushroom species using the lipase activity kit might not have significant relevance when the lipase assay is performed simulating the digestion conditions in which pancreatic lipase might be involved *in vivo*. Therefore, the PL inhibitory capacity of all the selected mushroom strains was measured again but adding the mushroom extracts into a pancreatic lipase solution simulating the *in vitro* digestion conditions.

Screening for pancreatic lipase inhibitors under simulated digestion conditions

The methanol:water extracts of the selected edible mushrooms were screened again against pancreatic lipase activity but maintaining the *in vitro* digestion conditions and adjusting the inhibitor concentrations to similar conditions than those of the kit (enzyme:inhibitor ratio 1:8 in the final reaction volume).

Results differed from those obtained when the enzymatic assay was performed using the lipase standardized kit (Table 2). Only *Pleurotus eryngii* extract showed an almost insignificant PL inhibitory activity under the simulated gut conditions, being lower than 7% of the orlistat inhibitory capacity. Those methanol:water extracts that showed PL inhibitory activity using the *in vitro* biochemical kit did not show activity when assayed simulating the gut conditions. Wise to mention was the fact that many of the mushroom species showed higher lipase activity than the negative control (methanol: water (1:1) solution instead of the extract).

Since the obtained results appeared so different than those obtained using the standardized lipase assay and because practically none of the prepared mushroom extracts seemed to contain PL inhibitors, extractions with other solvents were assessed again. Therefore, some mushroom extracts were obtained using 100% methanol or water as a second attempt to screen for PL inhibitors under simulated digestion conditions (Table 3). Also in this case, none of the samples showed any interesting PL inhibitory activity except for orlistat and *P. eryngii* methanol extracts (with an almost insignificant inhibition) and on the contrary, some of the mushroom extracts showed lipase activity values higher than their control including only water, methanol or a methanol:water (1:1) mixture. Moreover, if the extracts were applied doubling the concentration, as it was tested for *M. conica* methanol extracts, *P. eryngii* water extracts and *G. lucidum* methanol:water extracts, the lipase activity was increasing and not decreasing as it would have been expected if the extracts would have contained any inhibitory compound. After the above

described results it could be concluded that none of the tested mushroom extracts contained compounds in sufficient quantities able to reduce the pancreatic lipase activity under the simulated gut conditions.

However, still remains unexplained the reason for increasing PL values in the presence of some of the mushroom extracts. Thus, further studies were carried out in order to clarify this unexpected behavior. The pancreatic lipase source used to carry out this experiments was not an isolated enzyme but a porcine pancreas extract (including co-lipases and other enzymes) so it could be hypothesized that the presence of lipids, lipid-related or lipidic fractions from the mushroom extracts might activate, induce or enhance the lipase activity as it was described for compounds such as triacylglicerides, etc. (Porter *et al.,* 2007) since the presence or absence of lecithin nor bile salt in the reaction medium influenced the PL activity values. Another possible explanation could be that the mushroom extracts might also contain endogenous lipases that might interfere with the reaction since fungal lipases have been previously described in some of the mushroom strains (Krügener *et al.*, 2009).

Thus, in order to further clarify the obtained results, the lipase activity of the mushroom extracts was measured and on the other hand, the pancreatic lipase assay mimicking the gut conditions was carried out in the presence of various food extracts and pure compounds with different lipidic composition.

Methanol:water mushroom extracts were submitted to the lipase activity test under similar conditions than the *in vitro* digestion assay but without pancreatin addition. Thus, the observed activity could be only due to fungal lipases. As expected, results indicated that none of the selected strains showed significant lipase activity compared to the control with pancreatin (Table 4) since the extraction solvent contained methanol and mushroom enzymes (mainly

exoenzymes), although they can tolerate certain level of organic solvents, they exert their activity using water or specific buffer. Moreover, their lipase activity values did not correlate with the mushroom species which showed higher PL activity (in Table 2). Thus, the endogenous mushroom lipases were not the responsible for the increasing of lipase activity observed during *in vitro* digestion of the extracts.

Sucrose, starch or dehydrated wheat (as breakfast cereals) was selected as example of carbohydrates-rich foodstuff. Serum bovine albumin (BSA) and defatted milk powder were selected as protein rich sources. When the methanol:water (1:1) extracts were obtained from these poor lipid-containing samples and assayed for lipase activity no significant differences in PL activity levels were observed compared to control (Table 5). However, when dehydrated banana and walnut extracts (selected as example of lipid-containing foodstuff) were tested, pancreatic lipase activities increased up to similar levels than those observed for some of the mushroom extracts (for instance *M. conica*). Moreover, when the powdered walnut or *M. conica* were mixed with a drop of sunflower oil (285mg/g) an even higher PL activity increase was observed suggesting that the enhancing of lipase activity is related to lipidic compounds.

Edible mushrooms are highly recommended in diets for overweighed people particularly because they are considered as low-fat food (Kalač, 2009). Thus, a minor compound or group of compounds (of lipidic nature) could be the responsible for the stimulation of PL activity, perhaps sterols since cholesterol was described as compound able to mediate pancreatic lipase activity (Ros, 2000). Mushroom fruiting bodies contain ergosterol as the major sterol constituent of the fungal membrane and it was present in the selected mushroom strains in a concentration range between 5.69 to 0.69 mg/g (Gil-Ramirez *et al.*, 2011). However, when the ergosterol levels were plotted toward PL activity measured by the *in vitro* digestion model no correlation was found

leaving the responsible compound / group of compounds still unidentified. Other compounds such as saponins have been described as stimulating compounds of pancreatic lipases, however, edible mushrooms do not contain this type of compounds (Sroka *et al.*, 1997).

Conclusions

Thus, it could be concluded that, although some mushroom extracts showed PL inhibitory activity in an *in vitro* test, under the *in vitro* digestion model utilized mimicking the intestinal human conditions, the selected mushroom species were not able to effectively inhibit the pancreatic lipase when mixed with a food matrix as sunflower oil or directly applied as mushroom extract. These results strengthen the need of more detailed studies before concluding the ability of natural extracts to perform certain biological activities by using only *in vitro* biochemical tests. Therefore, the use of mushroom extracts *i.e.* as ingredient to formulate functional foods able to act as orlistat is, in any case, of limited significance. On the other hand, further studies are, at the present, being conducted to clarify the stimulation of PL activity observed.

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Table 1, Pancreatic lipase activity (U/L) using the standardized lipase activity kit of *Marasmius oreades* (166 mg/mL) methanol, methanol:water (1:1) and water extracts.

	Control	**Extract**
Water	252.14 ± 3.34	244.25 ± 6.08
Methanol: water (1:1)	255.68 ± 0.15	229.22 ± 2.84
Methanol	294.38 ± 56.76	212.83 ± 7.54
ORL		44.13 ± 0.73

Table 2, Pancreatic lipase (PL) activity in the presence of methanol:water (1:1) extracts obtained from several fruiting bodies. The PL activity was measured by two different methods. Enzymatic activity is expressed as percentage compared to a control sample including only a methanol:water (1:1) solution.

Mushroom species	**PL activity assessed using an enzymatic kit (%)**	**PL activity assessed using an *in vitro* digestion model (%)**
Cantharellus cibarius	106.89 ± 12.03	124.62 ± 1.62
Lactarius deliciosus	106.55 ± 0.96	123.21 ± 17.32
Amanita Caesarea	105.08 ± 0.80	122.98 ± 7.03
Flammulina velutipes	103.01 ± 6.55	165.47 ± 11.24
Morchella Esculenta	100.22 ± 0.53	131.12 ± 8.49
Ganoderma lucidum	100.61 ± 0.80	221.61 ± 9.49
Agrocybe aegerita	96.16 ± 4.00	121.56 ± 6.67
Agaricus blazei	96.13 ± 4.58	99.36 ± 12.88
Craterellus cornucopioides	95.75 ± 0.96	181.04 ± 9.83
Auricularia judae	93.42 ± 1.28	104.97 ± 6.31
Agaricus bisporus	93.27 ± 1.01	127.42 ± 5.95
Pleurotus ostreatus	92.53 ± 2.92	123.98 ± 0.47
Boletus edulis	87.35 ± 2.74	106.01 ± 16.45
Amanita ponderosa	86,18 ± 2.74	167.30 ± 24.35
Lentinula edodes	86.10 ± 3.77	165.71 ± 10.19
Marasmius oreades	85.81 ± 1.06	159.19 ± 9.81
Morcella conica	84.46 ± 0.00	96.21 ± 20.70
Lyophyllum shimeji	83.14 ± 1.01	220.87 ± 14.41
Pleurotus eryngii	82.69 ± 1.12	95.44 ± 0.94
Grifola frondosa	82.46 ± 4.00	118.22 ± 19.25
Lepiota procera	79.07 ± 0.29	152.42 ± 0.23
Orlistat	16.45 ± 1.33	33.09 ± 15.25

Table 3, Pancreatic lipase activity (IUB/mL) in the presence of several mushroom extracts (using water, methanol and methanol:water (1:1)) simulating the *in vitro* digestion conditions.

Mush.extracts	**Water**	**Methanol:water (1:1)**	**Methanol**
Control	961.38 ± 99.79	1076.04 ± 23.11	1253.91 ± 45.74
G. lucidum	979.02 ± 112.26	1362.69 ± 130.97	1772.82 ± 128.90
M. conica	1290.66 ± 66.52	1858.08 ± 274.41	1571.43 ± 68.60
P. eryngii	1121.83 ± 174.13	997.89 ± 64.11	892.61 ± 158.11
L. edodes	1646.40 ± 332.62	1166.89 ± 26.19	1073.69 ± 205.81
ORL	247.62 ± 48.39	161.41 ± 71.59	155.11 ± 5.27

Table 4, Endogenous lipase activity determined in several mushroom extracts using the *in vitro* digestion conditions but in absence of pancreatin

Mushroom species	**PL (IUB/mL)**
Control (MeOH:water 1:1)	126.4 ± 8.3
Lyophillum shimeji	132.3 ± 8.3
Craterellus cornucopioides	117.6 ± 12.5
Pleurotus eryngii	111.7 ± 8.3
Agaricus blazeii	123.5 ± 8.3
Amanita caesarea	147.0 ± 8.3
Morchella conica	113.2 ± 10.4
Control (with pancreatine)	1123.1 ± 198.4

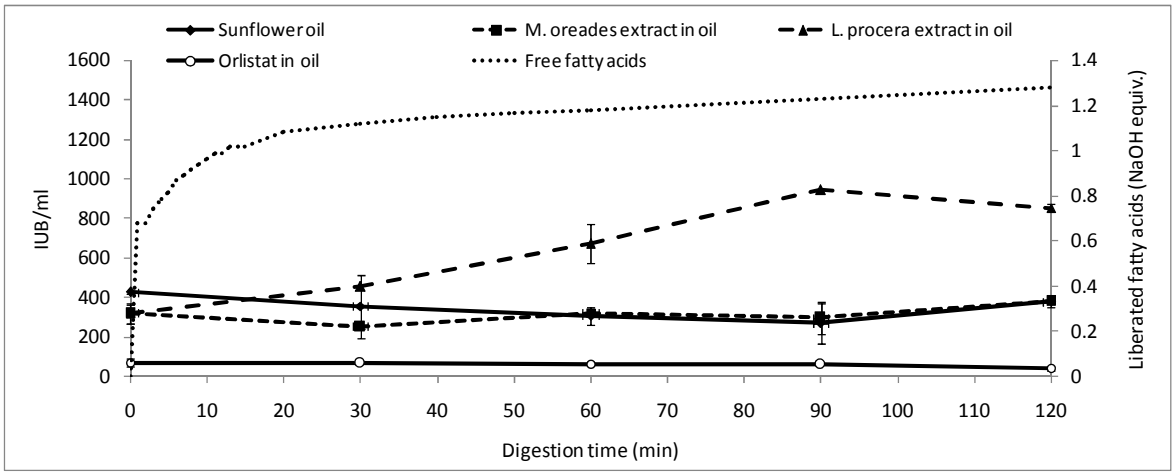
Table 5, Pancreatic lipase activity of some lipidic and non-lipidic food matrices and pure compounds using the simulated *in vitro* digestion conditions.

Food extracts	**PL activity (IUB/mL)**
Control	1123.08 ± 198.42
Sugar	1261.26 ± 41.58
Starch	1149.54 ± 158.00
BSA	1145.13 ± 172.55
Dehydrated wheat (Cereal)	1056.93 ± 81.08
Defatted milk powder	1331.08 ± 76.90
Dehydrated banana	1718.59 ± 2.08
Walnut	1822.80 ± 49.90
Walnut with oil	2335.83 ± 76.90
Morchella conica	1858.08 ± 274.41
Morchella conica with oil	2475.48 ± 241.15
ORL	155.11 ± 5.27

Figure legends

Figure 1, Pancreatic lipase activity during an *in vitro* digestion model of mushroom extracts mixed with sunflower oil. Digestion of sunflower oil was used as not inhibited control and orlistat digestion as inhibited control.

Figure 1


Sunflower oil
M. oreades extract in oil
L. procera extract in oil
Orlistat in oil
Free fatty acids
IUB/ml
Liberated fatty acids (NaOH equiv.)
Digestion time (min)
0
200
400
600
800
1000
1200
1400
1600
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
10
20
30
40
50
60
70
80
90
100
110
120