Amaranth supplementation prevents the increase in activity of dipeptidyl peptidase IV in plasma of streptozotocin-induced diabetic rats

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Abbreviations: ACE, angiotensin-converting enzyme; **apo**, apolipoprotein; **ANOVA**, analysis of variance; **DA**, diabetic rats fed with control diet supplemented with amaranth; **DC**, diabetic rats fed with control diet; **DPPIV,** dipeptidyl peptidase IV; **DPPIVi**, DPPIV inhibitors; **HDL-c**, high density lipoprotein cholesterol; **IPG**, immobilized pH gradient; **NA**, normoglycemic rats fed with control diet supplemented with amaranth; **NC**, normoglycemic rats fed with control diet; **PON1**, paraoxonase/arylesterase 1; **T2D**, type 2 diabetes

ABSTRACT

Scope: Amaranth grain is a source of several bioactive compounds such as cancer-preventive and ACE inhibitory peptides. *In vitro* analysis of amaranth proteins has also demonstrated the presence of bioactive peptides, which exert inhibitory activity upon dipeptidyl peptidase IV (DPPIV), a new target for type 2 diabetes, but this potential activity has not been tested *in vivo*. The aim of this work was to evaluate whether chronic amaranth consumption has a beneficial effect in streptozotocin-induced diabetic rats, particularly in DPPIV activity and changes in plasma proteome profile.

Methods and results: Diabetic rats were fed with 20% popped amaranth grain diet for 12 weeks. Changes in lipid profile, DPPIV activity in plasma and kidney, as well changes in plasma proteome were evaluated. Total cholesterol and DPPIV activity in plasma were increased in diabetic rats but this was ameliorated by amaranth consumption. Diabetic rats fed with amaranth showed the highest levels of HDL and this correlated with an accumulation of apolipoprotein (apo) A-II. Furthermore, amaranth diet stimulated the up-accumulation of the antioxidant protein paraoxonase 1(PON1).

Conclusion This study provides molecular evidence about the role of amaranth as a potential functional food with DPPIV inhibitory activity and with a positive effect in plasma lipoproteins.

1 Introduction

Type 2 diabetes (T2D) is a complex metabolic condition characterized by chronic hyperglycemia that leads to inflammation and increases the risk of vascular diseases, nephropathy, neuropathy, and infectious processes [1, 2]. T2D is a multifactorial disease in which genetic predisposition, diet and energy expenditure play crucial roles [3, 4].

Due to its high prevalence in industrialized countries, prevention, treatment and control of T2D have become major priorities in health systems worldwide. In recent years, several antidiabetic drugs that reduce blood glucose levels with a lower risk of presenting hypoglycemic episodes have been developed. Thus, the use of incretin-based therapy has increased, particularly dipeptidyl peptidase IV (DPPIV) inhibitors because they act at different levels providing several benefits with lower adverse effects [5, 6]. Incretins are peptidic hormones responsible for insulin release (>70%) after food intake and are rapidly inactivated by DPPIV; hence, the use of DPPIV inhibitors increases the action time of these endogenous hormones [7]. Besides insulin release stimulation, incretins also increase proliferation and surveillance of pancreatic beta cells, increase blood irrigation, stimulate satiety, among other effects [8, 9]; this make DPPIV inhibitors a useful drug for T2D treatment.

In silico and *in vitro* studies have reported the presence of DPPIV inhibitory peptides in several food proteins like milk and dairy products, fish, meat, and some seeds like soybean, wheat, and barley [10-18]. Recently, we reported the presence of DPPIV inhibitory peptides encrypted in amaranth seed proteins, which were released by enzymatic cleavage with trypsin and in a simulated gastrointestinal digestion *in vitro* [19]. Amaranth is a prehispanic food cultivated and consumed in several countries due to its high nutritional value and presence of bioactive compounds with various

physiological functions [20]. These include antioxidant activity [21], and presence of angiotensin-converting enzyme (ACE) inhibitory peptides [22] and lunasin-like peptide [23]. The aim of this investigation was to determine whether the incorporation of amaranth in the diet had any beneficial effect on streptozotocin-induced diabetic rats. Changes in rat plasma proteome, DPPIV activity in plasma and kidney and levels of DDPIV gene expression in kidney were also evaluated.

2 Materials and methods

2.1 Handling of biological specimens

Animals were provided by the animal warehouse of the School of Medicine of the Universidad Autónoma de San Luis Potosí. The protocol was approved by the University Ethics Committee. All the animals were treated according to the ethic recommendations of the Mexican Official Norm for the production, use and care of laboratory animals (NOM-062-ZOOO-1999) and the regulations contained in the Mexican General Health Law.

2.2 Amaranth-containing diet

Natural popped amaranth seeds were obtained at the local market (Arantto, Mexico) (16% protein, 4% fat, 27.6% carbohydrates, 3.4 kcal/g). 2018S Teklad Goblal 18% Protein Rodent Diet (Harlan, Mexico) was used as a control diet (18.6% protein, 6.2% fat, 44.2% carbohydrates, 3.5% crude fiber, 3.1 kcal/g). Both, amaranth and rodent foods were milled in an industrial blender (Industrial, Mexico). Amaranth diet consisted in standard rodent food supplemented with 20% popped amaranth; water was added to each formulation in order to obtain a paste that was later dried in the oven at 40-45 ºC (Felisa, Mexico) for 24 h.

2.3 Animal experiments and diabetes induction

Three weeks old-Wistar male rats (n=40) were used as experimental units with a weight ranging between 200 and 280 g. All the animals were kept in individual propylene cages at a temperature between 18 and 26 ºC, 30-70% relative humidity with proper ventilation and a 12 h dark/light cycle.

After 5 days of adaptation, 20 rats were randomly selected for diabetes induction, by administering a single intraperitoneal dose of 40 mg/kg of streptozotocin freshly prepared in 0.01 M sodium citrate buffer pH 4.5 (Sigma-Aldrich, St. Louis, MO, USA). Hyperglycemic state was confirmed 3 days after induction by blood sampling of the caudal vein in the fasting state (glucose >350 mg/dL). During the experiment, glucose levels were maintained between 250-350 mg/dL by subcutaneous administration of long-acting insulin glargine (Sanofi-Aventis, Germany).

The experiment consisted in 4 groups of 10 rats each: normoglycemic rats fed with control diet (NC), normoglycemic rats fed with control diet supplemented with amaranth (NA), diabetic rats fed with control diet (DC), and diabetic rats fed with control diet supplemented with amaranth (DA). Rats were fed daily with 30 g of their corresponding formulation and water disposition *ad libitum* for 12 weeks. Food intake and body weight were registered daily and every 2 weeks, respectively. Fasting blood serum and plasma samples were collected by caudal vein punction every 2 weeks. A postprandial sample was taken at week 11 and after 1 h of feeding. At the end of the experiment, all the specimens were euthanized with a pentobarbital overdose; blood was collected from cardiac punction and kidneys were dissected, frozen and finely ground in liquid nitrogen. All biological samples were stored at -80 ºC until analysis.

2.4 Biochemical parameters in blood

Fasting serum glucose, total cholesterol, triacylglycerols and HDL-cholesterol (HDL-c) levels were determined by commercial kits (BioSystems®, Spain) according to

manufacturer's instructions using a BS-300 Chemistry Analizer (Shenzen Mindray Bio-Medical Electronics Co., Ltd., China).

2.5 DPPIV activity and expression

2.5.1 DPPIV activity in plasma and kidney

DPPIV activity was measured in plasma samples using the chromogenic substrate Gly-Pro-pNitroanilide (Sigma-Aldrich) as previously reported [19] (**Supplementary Information**). Results were expressed as µmol of nitroaniline/min based on a p-nitroaniline (Sigma-Aldrich) standard curve.

Kidney membrane-bound proteins were extracted from pooled frozen tissue (**Supplementary Information**). The protein extract was used in a 1:10 dilution (v:v) following the same method as for activity in plasma. Results were expressed as µmol of nitroaniline/min/g of tissue.

2.5.2. DPPIV expression in kidney by RT-PCR

Total RNA from kidney was extracted using Trizol® reagent (Thermo Fisher Scientific Inc.) and 50 mg of pooled frozen tissue following the manufacturer's instructions. cDNA and dsDNA were synthetized from total RNA (1.5 ug) and RT-PCR reactions were conducted with 150 ng of DNA. Expression levels of DPPIV were normalized with β-actin (**Supplementary Information**). The expression levels of DPPIV were analyzed based on the intensity of the bands by Quantity One™ v4.5.0 (BioRad).

2.6 Plasma proteome profile

In order to determine changes of protein profile in blood due to amaranth supplementation, plasma samples from each group or rats were pooled and high-abundance proteins were depleted and collected using the ProteoMiner™ Enrichment Kit (BioRad) ending with two protein fractions for each group. Both fractions, high-

abundance and low-abundance proteins were precipitated as reported by Santos-González [24] with slight modifications (**Supplementary Information**). IEF of high-abundance proteins was carried out onto 11 cm immobilized pH gradient (IPG) linear gradient strips of pH 4-7 (BioRad). For IEF of low-abundance proteins, 7 cm IPG linear gradient strips of pH 4-7 (BioRad) were used. Gels were scanned at 100 μm resolution with a PharosFX™ PLUS Molecular Imager (BioRad). Image analysis was performed with MELANIE 2D Gel Analysis Software v7.0 (GENEBIO SA, Geneva, Switzerland). Proteins were considered as differentially accumulated when their normalized volume displayed a fold change ≥ 2 when control and treatments were compared. Significant changes were determined using ANOVA ($P<0.01$).

Differentially accumulated protein spots were excised from the gels and digested with trypsin. Separation of tryptic peptides and mass spectrometric analysis were performed with a nanoACQUITY UPLC System coupled to a SYNAPT-HDMS (Waters, Milford, MA, USA). MS/MS spectra data sets were used to generate PKL files using Protein Lynx Global Server v2.4 (PLGS, Waters). Proteins were then identified using PKL files and the MASCOT search engine v2.3 (Matrix Science, London, UK). Searches were conducted against the *Rattus* subset of the NCBInr protein database (68 575 sequences). Protein identification criteria included at least two MS/MS spectra matched at 99% level of confidence, and identifications were considered successful when significant MASCOT scores (>25) were obtained, indicating the identity or extensive homology at $P<0.01$ and the presence of a consecutive *y* ion series of more than three amino acids. Identified proteins were classified into different categories of biological processes in which they are involved according to Gene Ontology (http://www.geneontology.org/). For detailed information see **Supplementary Information**.

2.7 Statistical analysis

All extractions and determinations were performed in triplicate. Statistical analyses were performed by using the GraphPad Prim Software v5.01 (GraphPad Software Inc., La Joya, CA, USA). One- way ANOVA and Tukey's multiple comparison test were performed to detect statistical differences at the end of the experiment ($P<0.01$). Two- way ANOVA and Bonferroni's test were performed to detect statistical differences throughout the experiment. All data were expressed as mean of triplicates ± SD.

3 Results

3.1 Biochemical parameters

Changes in body weight and blood glucose during the experimental period are shown in **Supplementary Figure S1**. Normoglycemic and diabetic rats experienced a gradual weight increase. Although normoglycemic rats had a slightly higher body weight than diabetic rats, there were not statistical differences among treatments. Fasting glucose levels were stable (around 150 mg/dL) in normoglycemic rats. On the other hand, diabetic rats revealed an increased and variable glucose levels up to 400 to 500 mg/dL. Glucose levels reflect the differences in the metabolic state due diabetes induction confirming the hyperglycemic state produced by streptozotocin. Similar results with vildagliptin have been previously reported [25].

Because diabetes not only affects glucose levels but also produces an unbalance in lipid metabolism due to the deregulation in carbohydrate processing, a general lipid profile was carried out. **Supplementary Figure S2** shows changes in triacylglycerols, HDL-c, and total cholesterol of the experimental groups during the experiment. **Table 1** summarizes the results obtained at the end of the experiment. In normoglycemic rats, triacylglycerol levels were constant throughout the experimental period with no statistical differences among diets. DA group showed the highest triacylglycerol levels;

three times higher than NC group ($P<0.01$). Interestingly, in the diabetic state there was a gradual increase in HDL-c and by week 4 these differences became statistically different (50% higher) ($P<0.001$) comparing with normoglycemic groups (**Supplementary Figure S2B, Table 1**); moreover, the DA group showed the highest levels ($P<0.001$). Regarding total cholesterol (**Supplementary Figure S2C, Table 1**), there were no differences between diets when comparing the same metabolic state, but it was observed that diabetes induction increased cholesterol levels about 40% ($P<0.001$) and that amaranth supplementation slightly counteracted this effect ($P<0.05$).

3.2 DPPIV activity and transcript expression analysis

In previous *in vitro* studies, we reported the presence of DPPIV inhibitory peptides in amaranth seed protein hydrolysates [19]. In this study the plasma DPPIV activity was determined in order to establish the *in vivo* effect of amaranth inhibitory peptides. **Figure 1A** shows fasting plasma DPPIV activity among experimental groups. In normoglycemic rats no differences were found among diets, however, in the DC group there was an increase in DPPIV activity since week 10 ($P<0.001$); these differences were not observed in the DA group. This trend was also observed in the postprandial sampling 1 h after feeding ($P<0.001$) (**Figure 1B**). These results are consistent in different conditions and may confirm the action of DPPIV inhibitory peptides encrypted in amaranth proteins that are released by gastrointestinal digestion.

DPPIV is an ubiquitous enzyme with a wide substrate range and is expressed in several tissues, mainly in kidney where it helps on the clearance of different compounds [26]. Membrane proteins from kidney were extracted and DPPIV enzymatic activity and DPPIV transcript levels were determined (**Figure 2**). Unlike plasma activity, the activity of DPPIV in kidney was different. Although there was a slight tendency of higher DPPIV activity in diabetic rats, no statistical differences were found among experimental groups

(**Figure 2A**). Interestingly, as shown in **Figure 2B**, amaranth supplementation in normoglycemic rats induced a down-regulation of DPPIV transcript ($P<0.01$). No DPPIV transcript was detected in the DC group, whereas the DPPIV transcript levels in the DA group were similar to the NA group. The differences between the groups with control diet are drastic (**Figure 2B**), since the normoglycemic control group showed the highest transcript levels ($P<0.001$).

3.3 Analysis of plasma proteome profile

Blood is one of the most dynamic tissues that keep a connection and communication with organs and helps maintaining body homeostasis. In order to determine the possible changes that a diet supplemented with amaranth may produce in two different metabolic states, a plasma proteomic analysis was carried out. One of the main challenges when working with blood proteins is the presence of highly abundant proteins that mask other proteins present in lower magnitude ranges but are still important and may be useful as biomarkers in a particular condition [27]; hence, the plasma was fractionated in order to obtain the high- and low-abundance proteins. Proteomic analysis from both fractions was performed and the differential proteins among groups were determined.

In order to establish the differential accumulation of protein spots, three statistical comparisons were made to observe the effect of diabetes induction (NC vs. DC), the effect of amaranth supplementation in health specimens (NV vs. NA), and the effect of amaranth supplementation in diabetic specimens (DC vs. DA). Representative 2-DE protein maps of low- and high-abundance proteins of rat plasma are shown in **Supplementary Figure 3**. In total, 141 spots were detected in low-abundance protein gels and 150 spots in high-abundance protein gels. From the total protein spots, 37 were differentially accumulated and 29 proteins were successfully identified and classified according to its biological process from Gene Onthology (**Supplementary**

Figure 4). Several proteins were identified in more than one spot and in some spots more than one protein were detected.

Regarding the proteins classified in lipid metabolism (**Table 2**), the changes in protein accumulation were heterogeneous, but these changes were only observed in rats fed with amaranth in both metabolic conditions. Amaranth supplementation in both control and diabetic groups induced the down-accumulation of apolipoprotein E (spots 19 and 43 low-abundance proteins). Beta-2-glycoprotein (spot 78 in high-abundance proteins) was down-accumulated in the NA group. Apolipoprotein A-II (spot 86 in low-abundance proteins) and paraoxonase/arylesterase 1 (spot 49 in low-abundance proteins) were up-accumulated in the DA group.

4 Discussion

An increase in serum DPPIV activity in type 1 diabetes with moderate or severe hyperglycemia has been reported and it is believed that this is related to insulin resistance [28, 29]. In this study, hyperglycemia was achieved by a direct β-cell damage obtaining an insulin-dependent diabetes; hence, it was expected to observe an increase in DPPIV activity in the diabetic group. The fact that amaranth supplementation in diabetic rats slightly reduced DPPIV activity at an intermediate level between the diabetic, control and normoglycemic groups shows that amaranth has a protective effect in diabetes due to the presence of DPPIV inhibitory peptides.

On the other hand, no changes were observed in DPPIV enzymatic activity in kidney and the activity observed did not correlate with the transcript levels of the enzyme. It has been previously reported that streptozotocin (30 mg/kg) produces an increase in DPPIV activity and expression in rat kidney [30]. Moreover, another study showed that the same dosis of streptozotocin in rats decreased DPPIV activity in kidney

but increased its expression, suggesting that DPPIV is involved in renal function [31]. The absence of transcript in the DC group could reflect a greater renal damage due to the streptozotocin dose used in this research and it seems that amaranth prevents this effect. The lack of correlation between expression and enzymatic activity in our investigation may indicate that regulation of DPPIV expression is independent of catalytic activity of the enzyme in this organ. Besides, this may indicate that DPPIV function in blood is very different than on the kidney, suggesting a greater relevance of DPPIV on the body. DPPIV is a very ubiquitous protein and it is responsible for the inactivation of regulatory peptides like incretins, chemokines, neuropeptides and hormones [26]. DPPIV can be found in a soluble or membrane-bound form where it can interact with other proteins like adenosin deaminase, collagen and fibronectin [32]. Particularly, it is known that DPPIV modulates the activity of a Na^+/H^+ exchanger in proximal tubule cells [33] but little is known about DPPIV in kidney aside its catalytic activity and its relation with renal function.

Amaranth seeds contain around 9% fat [34], composed mainly by unsaturated fatty acids, tocopherols and sterols like squalene [35]. Squalene is a direct precursor of cholesterol and its administration has positive effects in the regulation of plasma lipids by promoting cholesterol secretion through bile acids [36, 37]. We have shown that the streptozotocin-induced diabetic state increased serum total cholesterol and that amaranth slightly counteracted this effect. Furthermore, there was a considerable increase in HDL-c levels in diabetic rats, particularly the DA group. Recently, an increase in HDL-c in type 1 diabetic patients has been reported [38] and this is related with age and probably disease progression.

Several studies have focused on determining the effect of amaranth squalene upon lipid profile and the results are diverse. It has been reported that extruded amaranth

consumption reduces LDL cholesterol and total cholesterol in hypercholesterolemic rabbits [39]. In hamsters, amaranth grain and its oily fraction did not significantly affect plasma lipids [35]. Besides, it has been shown that amaranth oil and squalene consumption increases fecal excretion of bile acid on hamsters but had no hypocholesterolemic effect [40]. The addition of amaranth oil in the diet of diabetic patients with cardiovascular disease reduced total cholesterol, triacylglycerols, LDL and VLDL [34]. These results apparently depend on the species analyzed and in addition, the role of the proteins present in amaranth seed has to be considered. Mendonca et al. [41] reported that amaranth protein consumption resulted in a decrease in plasma lipids in hypercholesterolemic hamsters, but the mechanisms of action of amaranth are still unknown and proteomics is a very useful research tool.

Proteomic analysis showed changes in lipoproteins only in the rats fed with amaranth. There was a down-accumulation of beta-2-glycoprotein (Apo H) by amaranth in normoglycemic rats. Apo H can be considered as a cardiovascular disease marker as it is involved in atherosclerotic plaque progression, triacylglycerols clearance and in the regulation of LDL-ox degradation. A rise in Apo H in diabetic patients has been reported and it is believed that its expression may be related to insulin resistance and metabolic syndrome, however its physiological function is not clear [42, 43]. In our particular case, a decrease in Apo H expression could indicate a possible preventive effect of amaranth upon atherosclerotic plaque formation and progression in a non-diabetic state; although further experimentation is needed to corroborate this statement. On the other hand, apo A-II and apo E give stability to lipoproteins [44-46]. Apo A-II is the second major lipoprotein in HDL and, in rat, most Apo E is associated with these lipoproteins [47]. Despite that, in our experiment, Apo E presented a down-accumulation and the up-

accumulation in Apo A-II in diabetic rats fed with amaranth correlated with changes observed in HDL-c levels.

Paraoxonase 1 (PON1) was absent in the rats fed with control diet, but the protein was detected in the DA group. PON1 is a hydrolytic enzyme synthesized in the liver with a wide substrate range [48]. In the blood, this protein is associated with HDL and LDL where it prevents their oxidation and presents an athero-preventive function [49]. It is known that nutritional antioxidants like glabridin, quercetin and even pomegranate juice increase PON1 activity and therefore reduce peroxidation of lipids within these lipoproteins [50-52]. Furthermore, a positive correlation between apo A-II-containing HDL and PON1 activity has been reported [53], which explains the accumulation of these two proteins in the current experiment. Together, these results show that amaranth had a positive effect upon cholesterol regulation in streptozotocin-induced diabetic rats. The molecular mechanism may rely in a promotion of cholesterol reverse transport by HDL and presumable in a decrease in oxidation of lipids present in these particles.

This is the first study that shows some of the possible molecular mechanisms by which amaranth exerts a positive health effect *in vivo*. Our results support the potential use of amaranth as a functional food in diabetes with the advantage of no adverse effects. Furthermore, we showed the possible role of some antioxidant proteins like paraoxonase 1, area where further investigation is needed.

Authors contributions: AJVS, experimental design, DPPIV and 2-DE analysis, manuscript writing; ERR handling of animals, lipid analysis; ABP mass spectrometry analysis, BIJF handling of animals, EGM revision of manuscript, APB experimental design and revision of manuscript.

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Figure legends

Figure 1. Changes in DPP4 activity from rat plasma (A) Fasting DPPIV activity during the experiment (B) Postprandial DPPIV activity 1 h after feeding at week 11. NC: Normoglycemic rats fed with control diet, NA: Normoglycemic rats fed with control diet supplemented with amaranth, DC: Diabetic rats fed with control diet, DA: Diabetic rats fed with control diet supplemented with amaranth. Reaction was performed with 500 μM of Gly-Pro-p-nitroanilide as substrate for 1 h at 37 °C. λ=415 nm. Mean ± SD. ***$P<0.001$.

Figure 2. Changes in DPPIV in kidney (A) DPPIV activity from kidney homogenate. Reaction was performed with 500 μM of Gly-Pro-p-nitroanilide as substrate for 1 h at 37 °C. λ=415 nm (B) DPPIV expression in kidney by RT-PCR. 1.5 μg of RNA were used for reverse transcription. β-actin was used as loading control. NC: Normoglycemic rats fed with control diet, NA: Normoglycemic rats fed with control diet supplemented with amaranth, DC: Diabetic rats fed with control diet, DA: Diabetic rats fed with control diet supplemented with amaranth. Mean±SD. **$P<0.01$ ***$P<0.001$.

Table 1. Lipid profile levels of normoglycemic and diabetic rats with a control diet or an amaranth supplemented diet at the end of the experiment

Lipid	NC	NA	DC	DA
Triacylglycerols (mg/dL)[1]	73.4 ± 20.4^{a}	122.7 ± 27.8^{ab}	159.83 ± 84.1^{ab}	235.66 ± 116.0^{b}
HDLc (mg/dL)[1]	35.7 ± 4.8^{a}	39.6 ± 4.2^{a}	56.4 ± 8.0^{b}	67.7 ± 5.2^{c}
Total colesterol (mg/dL)[1]	86.9 ± 13.1^{a}	97.1 ± 17.6^{a}	123.6 ± 22.4^{b}	107.0 ± 15.0^{ab}

NC: Normoglycemic rats fed with control diet, NA: Normoglycemic rats fed with control diet supplemented with amaranth, DC: Diabetic rats fed with control diet, DA: Diabetic rats fed with control diet supplemented with amaranth. [1] Values with different letters indicate statistical differences and represents the mean of triplicates ± SD.

Table 2. Differential plasma proteins detected by LC-MS/MS

Protein	Accession number[a]	Gene ID[a]	Spot[b]	Exp kDa/pI[c]	Theor kDa/pI[d]	Mascot score[e]	PM/%C[f]	Fold Change[g] NC vs NA	NC vs DC	DC vs DA
Apolipoprotein E precursor	gi|162287337	25728	19 (L)	40,00/5,1	35,78/5,23	63	9/28	-	-	-5,2192
			43 (L)	180,00/5,47		36	2/5	↓	-	-
Apolipoprotein A-II preprotein	gi|6978517	25649	86 (L)	3,97/4,14	11,49/6,23	40	2/19	-	-	↑
Beta-2-glycoprotein 1 precursor (Apo H)	gi|57528174	287774	78 (H)	17,50/6,13	39,74/8,58	25	2/5	↓	-	-
Serum paraoxonase/arylesterase 1 precursor	gi|54292130	84024	49 (L)	45,40/4,17	39,56/5,06	26	2/5	-	-	↑

For detailed information see **Supplementary tables 1** and **2**.[a] According to NCBInr database, [b]Spots numbers as indicated in figure 3 (L) low-abundance proteins (H) high-abundance proteins, [c]Experimental mass (kDa) and *pI* of identified protein spots, [d]Theoretical mass (kDa) and *pI* of identified proteins retrieved from NCBInr database, [e]Mascot score reported after database search. Individual ion scores >25 are statistically significant (*P*<0.01), [f]Number of peptides matched and protein coverage percentage, [g]Fold change is expressed as a ratio of the vol% and each value represents the mean value of three independent measurements. For some spots, fold change cannot be accurately calculated because of a complete absence of the spot; this is noted as ↑ and ↓, indicating the presence or absence of the spot, respectively. NC: Normoglycemic rats fed with control diet, NA: Normoglycemic rats fed with control diet supplemented with amaranth, DC: Diabetic rats fed with control diet, DA: Diabetic rats fed with control diet supplemented with amaranth.