

BENEFICIAL EFFECTS OF CAROB PULP (*CERATONIA SILIQUA*) ON LIPIDS PROFILE AND OXIDANT/ANTIOXIDANT STATUS IN OBESE RATS

SOUR Souad^{1*}, FRIDI CHAHINEZ¹ and TAIF Amel¹

1. University of Blida-1, Faculty of Nature and Life Sciences, Biology Department., Algeria

Reçu le 30/05/2019, Révisé le 28/06/2019, Accepté le 30/06/2019

Abstract

Description of the subject: Obesity is generally accompanied by a chronic state of oxidative stress, implicated in the development of different complications. Carob pulp naturally rich in antioxidants, represent a real preventive potential in this context.

Objective: The aim of the present study was to investigate the anti-obesity effects of carob (*Ceratonia siliqua*) pulp administration in cafeteria diet-induced obesity in rat model.

Methods: Male wistar rats weighing 120 to 150g were fed a standard chow or a cafeteria diet enriched or not with carob pulp (20%) for two months. Different biochemical parameters and oxidant / antioxidant status were determined at the end of the experiment.

Results: Our results revealed that the cafeteria diet caused an alteration of the metabolic and the balance oxidant / antioxidant. However, the consumption of diet enriched with carob pulp, lead to a reduction in body weight, adipose tissue weight, and glycemia. Additionally, carob pulp enriched diet was also found to improve the lipids profile and oxidant / antioxidant status. In fact, there was a reduction in plasma levels of malondialdehydes and hydroperoxides, and an increase in the levels of erythrocyte catalase activity and total antioxidant ability of plasma.

Conclusion: The different alterations of the metabolism and the oxidant/antioxidant balance associated with nutritional obesity can be corrected by the regular consumption of carob pulp.

Keywords: obesity; cafeteria diet; Wistar rat; carob pulp; lipids profile; oxidant/antioxidant status

EFFETS BÉNÉFIQUES DE LA PULPE DE CAROUBE (*CERATONIA SILIQUA*) SUR LE PROFIL LIPIDIQUE ET LE STATUT OXYDANT/ANTIOXYDANT CHEZ LE RATS OBÈSE

Résumé

Description du sujet : L'obésité s'accompagne généralement d'un stress oxydant chronique, participant à l'installation de nombreuses complications. La pulpe de caroube naturellement riche en anti-oxydants, représente ainsi un réel potentiel de prévention dans ce contexte.

Objectifs : Le but de la présente étude était d'étudier les effets anti-obésité de l'administration de la pulpe de caroube (*Ceratonia siliqua*) dans un régime cafétéria qui induit l'obésité chez le rat.

Méthodes : Des rats mâles Wistar pesant entre 120 et 150g ont reçu pendant deux mois un régime standard ou cafétéria enrichi ou non en pulpe de caroube (20%). Différents paramètres biochimique et du statut oxydant/antioxydant ont été déterminées à la fin de l'expérience.

Résultats : Nos résultats ont révélé que le régime cafétéria provoquait de nombreuses altérations métaboliques et de la balance oxydant/antioxydant. Cependant, la consommation du régime enrichi en pulpe de caroube, entraînait une réduction du poids corporel, du poids du tissu adipeux, et de la glycémie. En outre, l'enrichissement du régime en pulpe de caroube améliore également le profil lipidique et le statut oxydant/antioxydant. En effet, on a noté une réduction des teneurs plasmatiques en malondialdéhydes et en hydroperoxydes, et une augmentation de l'activité érythrocytaire de la catalase et de pouvoir antioxydant total de plasma.

Conclusion : Les différentes altérations du métabolisme et de la balance oxydant/antioxydant associées à l'obésité nutritionnelle peuvent être corrigées par la consommation régulière de la pulpe de caroube.

Mots clés: Obésité ; régime cafétéria ; rat Wistar ; pulpe de caroube ; profil lipidique ; statut oxydant/antioxydant

* Auteur correspondant: SOUR souad, E-mail:souadphysiologie@hotmail.fr

INTRODUCTION

Obesity is characterized by abnormal fat accumulation that impairs health [1]. It is a chronic metabolic disease caused by numerous factors, such as a high fat diet, and is associated with many disorders of metabolic dysregulations including dyslipidemia, type 2 diabetes mellitus, cardiovascular diseases, liver diseases and certain types of cancers [2, 3]. Obesity is characterized by a state of chronic oxidative stress, an imbalance between the generation of reactive oxygen species (ROS) and antioxidant reactions [4, 5]. This oxidative state is associated with metabolic abnormalities, including hyperinsulinemia, lipid metabolism alterations, increased adipose tissue mass and triglyceride storage [6]. These lipid alterations are risk factors contributing to the prevalence and severity of atherosclerosis and subsequent coronary heart disease [7].

Faced with the side effects of surgery and the adverse effects of synthetic drugs for weight loss, current research in the treatment of obesity is concerned with the use of natural products [8]. These last contain vitamins, minerals, fibers, polyphenols, sterols and alkaloids that can increase energy expenditure, decrease caloric intake and act as a regulator of fat metabolism in the body [9]. Among these products *Ceratonia siliqua* commonly known as carob tree which is frequently utilized in our local culinary and medicinal traditions, it is a thermophilic species cultivated in the Mediterranean climate, but originating from the Arab countries. *Ceratonia siliqua* is a rich source of minerals, such as potassium, calcium, magnesium, sodium, copper, iron, manganese and zinc [10]. It also contains amino acids (alanine, glycine, leucine, proline, valine, tyrosine and phenylalanine), sugars, and dietary fibers such as cellulose, hemicelluloses and lignin, as well as water insoluble polyphenols [11, 12, 13].

Interestingly, carob pods were reported to exert several pharmacological properties such as antioxidant [14] anti-bacterial [15], anti-ulcer and anti-inflammatory effects [16, 17]. Beside those effects, it has also been found to play an effective role in the suppression of some parasites as well as in the treatment of diarrhea, diabetes, and hyperlipidemia [18, 19]. To the best of our knowledge, the antioxidant effects of carob pulp have never been previously investigated in a high-fat diet-induced obesity model.

In this study, animal model of obesity has been used, induced by “a cafeteria diet” that consists of various foods consumed by humans, in order to study the effect of carob pulp on obesity-related biomarkers (body weight, plasma glucose, lipid profile and oxidative stress parameters). Supplementation with carob pulp could be considered as an efficient treatment that may correct obesity-related alterations, especially hyperlipidemia and oxidative stress.

MATERIAL AND METHODS

1. Animals and experimental protocol

The study was conducted in accordance with the national guidelines for the care and use of laboratory animals. All the experimental protocols were approved by the Regional Ethical Committee. Adult male wistar rats weighing 120 to 150g were obtained from the Pasteur Institute of Algiers (Algeria). Animals were housed at 25±1°C individually in plastic cages, and maintained on a 12:12 h light/dark cycle. Rats were randomly assigned to one of four experimental diets. The control group (control, C, n=5) was fed standard laboratory chow (ONAB, Algeria). The second group (control carob, CC, n=5), rats were on standard chow supplemented with carob pulp powder (20%). In group 3 (cafeteria group, CAF, n=5) was fed a fat-rich hypercaloric diet, and in group 4 (cafeteria carob (CAFC, n=5), rats were on cafeteria diet supplemented with carob pulp powder (20%). The control diet (386 kcal/100g) was composed of 20% of energy as protein, 20% of energy as lipids and 60 % of energy as carbohydrates. The components of the cafeteria diet were pate, cheese, bacon, chips, cookies, and chocolate (in a proportion of 2:2:2:1:1:1, by weight) and control diet (mix/control diet, W/W) [20]. The composition of the cafeteria diet (523 kcal/100 g) was 16% of energy as protein, 24% of energy as carbohydrates and 60% of energy as lipids.

The mature carob pods were collected from the region of Tipaza (Algeria) during October 2017. The seeds were separated from the pulp; wick was air-dried and powdered in an electric blender. The powder was stored in dry sterilized containers, and stored at 4°C until further use.

2. Blood samples

At the end of experimental period (two months), the animals were fasted overnight and sacrificed.

They were anaesthetized with intra-abdominal injection of sodium pentobarbital (60 mg/kg of body weight). Blood samples were collected from abdominal aorta in two tubes, with and without anticoagulant, for plasma and serum separation. Serum was used for separation of different lipoprotein fractions and plasma was used for biochemical determinations and oxidant/antioxidant status parameters. After removal of plasma, erythrocytes were washed with isotonic saline. Erythrocytes were lysed with ice-cold distilled water and stored at 4°C for 15 min, and the cell debris was removed by centrifugation (2000g for 15 min). Erythrocyte lysates were assayed for catalase enzyme activity

3. Determination of biochemical parameters

Plasma glucose was measured using the Trinder glucose kit (Sigma). Serum lipoproteins were separated by sequential ultracentrifugation. Serum total cholesterol (TC) and triglycerides were measured using enzymatic kits (Quimica Clinica Aplicada S.A., Amposta, Spain). HDL-cholesterol, VLDL-cholesterol and LDL-cholesterol concentrations were also measured by enzymatic kits.

4. Determination of oxidant / antioxidant markers

Plasma levels of hydroperoxides (lipoperoxides, LOOH) were measured by the ferrous ion oxidation-xylenol orange assay – using the specific LOOH reducer triphenylphosphine – according to the method of Nourooz-Zadeh *et al.* [21]. The plasma malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were determined by the method of Nourooz-Zadeh *et al.* [21].

Based on the reaction of MDA with thiobarbituric acid (TBA). Carbonyl proteins were measured in plasma by the 2,4 dinitrophenylhydrazine reaction described by Levine *et al.* [22]. Vitamin C was measured in plasma by the method of Jagota & Dani [23]. Catalase (CAT; EC 1.11.1.6) activity was determined in erythrocyte by measuring the decrease in hydrogen peroxide (H₂O₂) absorption. It is based on the consumption of H₂O₂ by CAT and loss of absorbance at 240 nm according to Aebi [24]. The total antioxidant ability of plasma (oxygen radical absorbance capacity, ORAC) was estimated by the capacity of erythrocytes to resist free-radical-induced haemolysis, according to the method of Blache and Prost [25].

5. Statistical analysis of data

All values were expressed as the mean ± SD. Significant differences among the groups were analyzed statistically by a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The significance level was set at $P < 0.05$. These calculations were performed using STATISTICA version 8.1 (STATSOFT).

RESULTS

1. Effects of carob pulp on body weight, food intake and adipose tissue weight

Cafeteria diets (CAF and CAFC) consumption led to significantly higher body weight and daily food intake as compared with control diets (C and CC) (Table 1). However, supplementation with carob pulp reduced body weight in both normal and obese rats (CC and CAFC). The obese rats had a higher relative adipose tissue weight compared with normal rats. In contrast, carob pulp had reduce relative adipose tissue weight in normal and obese rats.

Table 1: Body weight, food intake and adipose tissue weight

Parameters	Control rats		Cafeteria obese rats	
	C	CC	CAF	CAFC
Initial weight (g)	135.34±3.60	137.12±7.48	132.8±2.28	130.15±3.24
Final weight (g)	256.4±7.09 ^c	239±6.15 ^d	298.2±9.12 ^a	267.33±8.55 ^b
Food intake (g/day/rat)	21.16±2.5 ^b	19.03±2.9 ^b	27.69±2 ^a	24.18±2.2 ^{ab}
Relative Adipose tissue weight	2.90±0.41 ^c	2.41±0.34 ^d	4.80±0.61 ^a	3.21±0.32 ^b

Values are presented as means ± standard deviations (SD). C: control diet; CC: control diet supplemented with carob pulp; CAF: cafeteria diet; CAFC: cafeteria diet supplemented with carob pulp.

2. Effects of carob pulp on plasma glucose, lipids and lipoprotein cholesterol levels in rats

Plasma glucose, serum cholesterol and triglycerides levels were significantly high in the rats administered CAF and CAFC diets

than in those given C and CC. Similarly, in obese group, LDL-C and VLDL-C were high, and HDL-C low compared to control group (Table 2).

However, Carob pulp supplementation reduced plasma glucose, triglycerides, total cholesterol, VLDL cholesterol, and LDL cholesterol in obesities and control rats. For HDL cholesterol,

carob pulp had no effects in control groups while it induced a significant increase in obese group (Table 2).

Table 2: Plasma glucose, lipids and lipoprotein cholesterol levels in rats

Plasma/serum	Control rats		Cafeteria obese rats	
	C	CC	CAF	CAFC
Glucose (g/l)	1,015 ± 0,10 ^c	0,734±0,05 ^d	1,476 ± 0,27 ^a	1,091±0,12 ^c
TC (g/l)	1,824±0,04 ^c	1,598±0,10 ^d	2,468±0,14 ^a	1,892±0,10 ^c
LDL-C (g/l)	1,0932±0,05 ^c	0,8656±0,08 ^d	1,7168±0,16 ^a	1,1344±0,10 ^c
VLDL-C (g/l)	0,3108±0,008 ^c	0,2764±0,012 ^d	0,4292±0,02 ^a	0,3356±0,02 ^c
HDL-C (g/l)	0,42±0,02 ^{ab}	0,456±0,05 ^a	0,322±0,02 ^c	0,392±0,008 ^b
TG (g/l)	1,554±0,04 ^c	1,282±0,06 ^d	2,146±0,11 ^a	1,678±0,13 ^c

Values are presented as means ± standard deviations (SD). C: control diet; CC: control diet supplemented with carob pulp; CAF: cafeteria diet; CAFC: cafeteria diet supplemented with carob pulp. TG, triglycerides; TC, total cholesterol; HDL-C, HDL cholesterol; VLDL-C, VLDL cholesterol; LDL-C, LDL cholesterol. Values with different superscript letters (a, b, c, d) are significantly different (ANOVA)

3. Effects of carob pulp on oxidant/antioxidant markers in rats

Plasma total antioxidant status (ORAC), catalase activity and plasma vitamin C were lower, whereas plasma MDA, LOOH and carbonyl proteins levels were higher in obesities (CAF and CAFC) rats compared with control (C and CC) rats (Table 3).

Carob pulp significantly decreased plasma markers of lipid peroxidation (TBARS and LOOH) in the control and obese rats. Plasma ORAC and CAT activity were significantly increased in carob treated rats, whereas, carbonyl proteins and vitamin C concentrations remained unmodified (Table 3).

Table 3: Plasma markers of lipid oxidation and antioxidant status

Plasma/erythrocyte	Control rats		Cafeteria obese rats	
	C	CC	CAF	CAFC
LOOH (µmol/l)	0.95±0.04 ^b	0.69±0.03 ^d	1.85±0.06 ^a	0.81±0.04 ^c
MDA (µmol/l)	2.95±0.04 ^c	1.79±0.06 ^c	4.50±0.21 ^a	3.10±0.15 ^c
CAPR (mmol/l)	2.07±0.25 ^b	1.96±0.41 ^b	3.47±0.31 ^a	3.22±0.43 ^a
ORAC (AU)	1,3±0,22 ^b	1,5±0,15 ^a	0,9±0,08 ^d	1,26±0,15 ^{bc}
Vitamin C (mg/ml)	11.5±0.35 ^a	11.9±0.63 ^a	9.06±0.70 ^c	9.52±0.84 ^c
CAT (U/mg Hb)	146.35±8.29 ^b	165.19±6.93 ^a	117.23±5.12 ^d	129.45±5.67 ^c

Values are presented as means ± standard deviations (SD). C: control diet; CC: control diet supplemented with carob pulp; CAF: cafeteria diet; CAFC: cafeteria diet supplemented with carob pulp. LOOH, lipoperoxides; MDA, malondialdehyde; CAPR, carbonyl proteins; ORAC, oxygen radical absorbance capacity; CAT, catalase; AU, arbitrary units; Hb, hemoglobin. Values with different superscript letters (a, b, c, d) are significantly different (ANOVA)

DISCUSSION

In the present work, we aimed to check whether diet supplementation with carob pulp has the potential to counteract the effects of cafeteria diet on body weight, lipids profile, and oxidant/antioxidant markers. In this study, cafeteria diet was given to rats for 8 weeks to induce dietary obesity. As a result, this diet induced an increase in body weight, food intake, and accumulation of adipose tissue. Moreover, elevated plasma glucose, cholesterol and triglycerides concentrations have also been reported. These findings are in agreement with those found by previous investigations [26, 27].

However, consumption of diet enriched with carob pulp for two months, lead to a reduction in body weight accompanied by a decrease in adipose tissue weight, in favor of its anti-obesity effect. This result agrees with Brennan [28], who found that the soluble fiber in carob powder has shown a potential benefit for enhancing weight loss. Similarly, El Rabey and coll. [19], have shown that a diet supplemented with methanolic carob extract for eight weeks decreased body weight in hypercholesterolemic male rats.

Different anti-obesity mechanisms for carob polyphenol were proposed, such as the suppression of fat absorption from the gut,

inhibition of differentiation of pre-adipocytes to adipocytes, and stimulation of apoptosis of mature adipocytes [29].

Supplementation with carob pulp decreased plasma glucose and improved the serum lipids profile parameters by decreasing the total cholesterol, triglycerides, LDLc, and VLDLc, and increasing HDLc. The hypolipidemic effect of carob pulp may be due to the presence of phenolic antioxidants and dietary fibers [30]. Our results are in agreement with those of Hassanein *and coll.* [31], who reported that consumption of carob powder lowered serum total cholesterol and LDL cholesterol in hypercholesterolemic male Sprague -Dawley rats. In parallel, Ruiz-Roso *et al.* [32], reported that feeding of carob fiber led to a significant decrease in total and LDL cholesterol levels. They stated that polyphenols found in carob may be partly responsible for carob fiber's cholesterol-lowering effects.

The resulted hypoglycemic effect of carob pulp might be due to its high content of fibers which provoked feeling of satiety [33] or the polyphenols content of carob could chelate sugars, lipids and fibers leading to reducing their intestinal absorption [34, 35]. It has recently been established that the immature carob bean prevents intestinal glucose absorption by the inhibition by electrogenic sodium-dependent glucose transport in mice by using a technique of Ussing chamber, which participates in the hypoglycaemic effect [18]. Moreover, carob bean gum increases the dietary fiber in food products without increasing the calories and increases the swelling of the food once in the stomach, which encourages a feeling of fullness, so it is considered a natural appetite suppressant and effective in prevention and treatment of hypercholesterolemia [36, 37].

These obese rats presented also an oxidative stress. In fact, the elevated levels of plasma Hydroperoxides, malondialdehyde and carbonyl proteins suggested an increased in lipid peroxidation and protein oxidation, which is in agreement with previous studies [26, 27]. In addition, total antioxidant ability of plasma and levels of erythrocyte catalase, one of the most important cellular antioxidant defense mechanisms, were reduced in obese rats compared to controls. The levels of plasma vitamin C were lower in obese rats than controls.

In our study, low levels of vitamin C could reflect their high utilization rate, suggesting that this vitamin may be used to reduce oxidative stress in obese rats. Oxidative stress in cafeteria fed rats may be generated by exacerbated nutrient oxidation, as previously reported [38].

In our work, carob pulp administration caused significant reduction in lipid peroxidation (LOOH and MDA) and an increase in antioxidant defense (ORAC and catalase activity). Previous studies have shown the richness of polyphenols in carob [39, 40]. These molecules are the major source of antioxidant ability of carob, by scavenging free radicals [41]. The antioxidant properties of the methanolic extract of carob may be act through its ability to neutralize reactive oxygen species and myeloperoxidase [17, 18].

Furthermore, it has been shown that leaf extract of carob protects against CCl₄-induced hepatic oxidative damage in rats [42]. Also, in a study conducted by Souli [43], the protective effect of carob extract on oxidative stress in liver induced by alcohol was investigated. The levels of malondialdehyde decreased while the level of enzyme superoxide dismutase and catalase increased in the carob group. Rtibi *and coll.* [17], investigated the capacity of carob to inhibit the phosphorylation of p47phox-Ser-328. The phosphorylation was changed by the carob extracts at various doses, which induced a modulation of NADPH-oxidase activity and reduced the superoxide anion overproduction. In addition, it was reported that the carob aqueous extract protects the cells from disturbances provoked by lipid peroxidation caused by dextran sulphate sodium and ethanol.

These antioxidant activities could be assigned to a synergistic action of the different compounds contained in carob pulp including, polyphenols and other antioxidant compounds [39, 40]. Carob pulp protected against lipid peroxidation and depletion of catalase activity and ORAC levels may act together as a compensatory mechanism to counteract excessive oxidative stress induced by obesity.

CONCLUSION

Data showed that carob pulp administration induced weight loss and reduced adipose fat accumulation via its protective role against obesity-related metabolic alterations and its hypolipidemic and antioxidant effects in cafeteria diet induced obese rats.

This could be mediated by the antioxidative properties of carob pulp because it prevented an increase in oxidative stress, as reflected via preventing lipid peroxidation and increasing catalase activity and ORAC levels. Thus, this in vivo study in an animal model supports the use of carob pulp as a potential nutritherapeutic agent to correct obesity-related alterations.

REFERENCES

- [1]. Apovian C.M. and Mechanik J.I. (2013). Obesity is a disease!, *Curr. Opin. Endocrinol. DiabetesObes.* 20 (5) :367–368.
- [2]. Kyle T.K., Dhurandhar E.J. and Allison D.B. (2016). Regarding obesity as a disease: evolving policies and their implications, *Endocrinol. Metab. Clin. North Am*, 45(3) : 511–520.
- [3]. Pozza C. and Isidori A. M. (2018). What's Behind the Obesity Epidemic. In A. Laghi, & M. Rengo (Eds.). *Imaging in Bariatric Surgery Cham*: 978-3-319-49299-5_1.
- [4]. Ndisang J.F., Vannici A. and Rastogi S. (2014). Oxidative stress and inflammation in obesity, diabetes, hypertension, and related cardiometabolic complications. *Oxid Med Cell Longev*, ID506948, 3p.
- [5]. Verdile G., Keane K.N., Cruzat V.F., Medic S., Sabale M., Rowles J. and Newsholme P. (2015). Inflammation and Oxidative Stress: The Molecular Connectivity between Insulin Resistance, Obesity, and Alzheimer's Disease. *Mediators Inflamm*, ID 105828, 17 p.
- [6]. Santilli F., Guagnano M.T., Vazzana N., La Barba S. and Davi G. (2015). Oxidative Stress Drivers and Modulators in Obesity and Cardiovascular Disease: From Biomarkers to Therapeutic Approach. *Current Medicinal Chemistry*, 22(5), 582-595.
- [7]. Ståhlman M., Fagerberg B., Adiels M., Ekroos K., Chapman J.M. and Kontush A. (2013). Dyslipidemia, but not hyperglycemia and insulin resistance is associated with marked alterations in the HDL lipidome in type 2 diabetic subjects in the DIWA cohort: Impact on small HDL particles. *BBA – Molecular and Cell Biology of Lipids*, 1831:1609–1617.
- [8]. Niyah Njike G., Watcho P., Nguenefack T.B. and Kamanyi A. (2005). Hypoglycaemic activity of the leaves of *Bersama englerianain* rats. *Afr J Trad*, 2(3): 215-221.
- [9]. James B. (2017). Use of Nutraceutical and Natural Compounds Containing Anti-Obese Properties for the Prevention and Treatment of Obesity. *EC Nutrition*, 6 (5) : 184-186.
- [10]. Ayaz F.A., Torun H., Ayaz S., Correia P.J., Alaiz M., Sanz C., Grúz J. and Strnad M. (2007). Determination of Chemical Composition of Anatolian Carob Pod (*Ceratonia Siliqual.*): Sugars, Amino and Organic Acids, Minerals and Phenolic Compounds. *Journal of Food Quality*, 30(6), 1040-1055.
- [11]. Haber B. (2012). Carob fiber benefits and applications. *Cereal Foods World*, 47: 365-369.
- [12]. Goulas V., Sylos E., Chatziathanasiadou M.V., Mavromoustakos T. and Tzakos A.G. (2016). Functional components of carob fruit: linking the chemical and biological space. *Int. J. Mol. Sci.*, 17: 1875.
- [13]. Stavrou I.J., Christou A. and Kapnissi-Christodoulou C.P. (2018). Polyphenols in carobs: a review on their composition, antioxidant capacity and cytotoxic effects, and health impact. *Food Chem*, 15(269):355-374
- [14]. Custódio L., Escapa A.L., Fernandes E., Fajardo A., Aligué R. and Alberício F. (2011) Phytochemical profile, antioxidant and cytotoxic activities of the carob tree (*Ceratonia siliqua* L.) germ flour extracts. *Plant Foods Hum Nut.*, 66(1):78-84.
- [15]. Meziani S., Oomah B.D., Zaidi F., Simon-Levert A., Bertrand C. and Zaidi-Yahiaoui R. (2015). Antibacterial activity of carob (*Ceratonia siliqua* L.) extracts against phytopathogenic bacteria *Pectobacterium atrosepticum*. *Microb Pathog*, 78:95102.
- [16]. Rtibi K., Jabri M.A., Selmi S., Souli A., Sebai H., El-Benna J., Amri M. and Marzouki L. (2015A). Carob pods (*Ceratonia siliqua* L.) inhibit human neutrophils myeloperoxidase and in vitro ROS-scavenging activity. *RSC Advances*, 5:84207–84215.
- [17]. Rtibi K., Jabri M. A., Selmi S., Souli A., Sebai H., El-Benna J., Amri M. and Marzouki L. (2015B). Gastroprotective effect of carob (*Ceratonia siliqua* L.) against ethanol-induced oxidative stress in rat. *BMC Complementary and Alternative Medicine*, 15:292.
- [18]. Rtibi K., Selmi S., Grami D., Saidani K., Sebai H., Amri M., Eto B. and Marzouki L. (2017). *Ceratonia siliqua* L. (immature carob bean) inhibits intestinal glucose absorption, improves glucose tolerance and protects against alloxan-induced diabetes in rat. *Journal of the Science of Food and Agriculture*, 97(8): 2664-2670.
- [19]. El Rabey H.A., Al-Seeni M.N. and Al-Ghamdi H.B. (2017). Comparison between the Hypolipidemic Activity of Parsley and Carob in Hypercholesterolemic male rats. *Bio Med Research international*, ID 3098745, 9 p.

- [20]. Darimont C., Turini M., Epitoux M., Zbinden I., Richelle M. and Macé K. (2004). β 3 Adrenoceptor agonist prevents alterations of muscle diacylglycerol and adipose tissue phospholipids induced by a cafeteria diet. *Nutrition & Metabolism*, 1: 4–13.
- [21]. Nourooz-Zadeh J., Tajaddini-Sarmad J., Ling K.L.E. and Wolf P.S. (1996). Low density lipoprotein is major carrier of lipid hydroperoxyde concentrations. *Biochem J*, 313: 781- 786.
- [22]. Levine R.L., Garland D., Olivier C.N., Amica A., Climent I., Lenz AG., Ahn B.W., Shantiel S. and Stadtman E.R. (1990). Detrmination of carbonyl content in oxidatively modified protein. *Ehod Enwy.*, 186: 464-478.
- [23]. Jacota SK. and Dana H.M. (1982). A new colorimetric technique for the estimation of vitamin C using folin phenol reagent. *Analytical Biochemistry*, 172: 178-182.
- [24]. Aebi H. (1974). Catalase. In *Methods of Enzymatic Analysis*, 2nd ed., pp. 673–684 [HU Bergmeyer, editor]. Weinheim: Verlag Chemie.
- [25]. Blache D. and Post M. (1992). *Free radical attack: biological test for human resistance capability*. In *Proceedings of the College Park on Chemical Evolution*, pp. 82–98. Washington, DC: Chemical Analysis Laboratory.
- [26]. Imessaoudene A., Merzouk H., Berroukeche F., Mokhtari N., Bensenane B. and Elhabiri M. (2016). Beneficial effects of quercetin–iron complexes on serum and tissue lipids and redox status in obese rats. *Journal of Nutritional Biochemistry*, 29:107–115
- [27]. Rahoui W., Merzouk H., El Haci I.A., Bettoui R., Azzi R. and Benali M. (2018). Beneficial effects of Aloe vera gel on lipid profile, lipase activities and oxidant/antioxidant status in obese rats. *Journal of Functional Foods*, 48 : 525–532
- [28]. Brennan C.S. (2005). Dietary fiber, glycaemic response and diabetes. *Mol Nutr Food Res*, 49:560–570.
- [29]. Meydani M. and Hasan S.T. (2010). Dietary polyphenols and obesity. *Nutrients*, 2:737 -51.
- [30]. Raninen K., Lappi J., Mykkänen H. and Poutanen K. (2011). Dietary fiber type reflects physiological functionality: comparison of grain fiber, inulin and polydextrose. *Nutr Reviews*, 69: 9–21.
- [31]. Hassanein K.M.A., Youssef M.K.E., Ali H.M. and El-Manfaloty M.M. (2015). The influence of carob powder on lipid profile and histopathologyof some organs in rats. *Comp. Clin. Pathol*, 24 : 1509–1513.
- [32]. Ruiz-Roso B., Quintela J. C., De La Fuente E., Haya J. and Perez-Olleros L. (2010). Insoluble carob fiber rich in polyphenols lowers total and LDL cholesterol in hypercholesterolemic subjects . *Plant Foods for Human Nutrition*, 65 : 50–56.
- [33]. Papakonstantinou E., Orfanakos N., Farajian P., Kapetanakou A.E., Makariti I.P., Grivokostopoulos N., Ha M.A. and Skandamis P.N. (2017). Short-term effects of a low glycemic index carob-containing snack on energy intake, satiety, and glycemic response in normal-weight, healthy adults: results from two randomized trials. *Nutrition*, 42:12–19.
- [34]. Lattimer J.M. and Haub M.D. (2010). Effects of dietary fiber and its components on metabolic health. *Nutrients*, 2:1266–1289.
- [35]. Barak S. and Mudgil D. (2014). Locust bean gum: Processing, properties and food applications—A review. *International Journal of Biological Macromolecules*, 66: 74-80.
- [36]. Martin O.W. and Andreas F.H.P. (2008). Metabolic Effects of Dietary Fiber Consumption and Prevention of Diabetes. *Am Society Nutr*, 138: 439–442.
- [37]. De Bock M., Derraik J.G.B and Cutfield W.S. (2012). Polyphenols and Glucose Homeostasis in Humans. *J Acad Nutr Diet*, 112: 808–815.
- [38]. Bouanane S., Merzouk H., Benkalfat N.B., Soulimane N., Merzouk S.A. and Grest J. (2009). Time course of changes in serum oxidant/antioxidant status in overfed obese rats and their offspring. *Clinical Science*, 116 : 669–680.
- [39]. Durazzo A., Turfani V., Narducci V., Azzini E., Maiani G. and Carcea M. (2014). Nutritional characterisation and bioactive components of commercial carobs flours. *Food Chemistry*, 153: 109-113.
- [40]. Rached I., Barros L., Fernandes I.P., Santos-Buelga C., Rodrigues A.E., Ferchichi A., Barreiro M.F. and Ferreira I.C.F.R. (2016). *Ceratonia siliqua* L. hydroethanolic extract obtained by ultrasonication: antioxidant activity, phenolic compounds profile and effects in yogurts functionalized with their free and microencapsulated forms. *Food & Function*, 7(3): 1319- 1328.
- [41]. Kumazawa S., Taniguchi M., Suzuki Y., Shimura M., Kwon M.S. and Nakayama T. (2002). Antioxidant Activity of Polyphenols in Carob Pods. *Journal of Agricultural and Food Chemistry*, 50(2): 373-377.
- [42]. Hsouna A.B., Saoudi M., Trigui M., Jamoussi K., Boudawara T., Jaoua S. and Feki A.E. (2011). Characterization of bioactive compounds and ameliorative effects of *Ceratonia siliqua* leaf extract against CCl4 induced hepatic oxidative damage and renal failure in rats. *Food and Chemical Toxicology*, 49(12): 3183-3191.
- [43]. Souli A., Sebai H., Chehimi L., Rtibi K., Tounsi H. and Boubaker S. (2013). Hepatoprotective effect of carob against acute ethanol-induced oxidative stress in rat. *Toxicol Ind Health*, 31(9):802-10.