

Effects of stifled cooking on the quality and lipid-lowering potential of oils extracted from two species of pumpkin seeds (*Citrullus lanatus* and *Cucumeropsis mannii*)

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Abstract

Background: Hyperlipidemia is a major risk factor for cardiovascular diseases. *Cucumeropsis mannii* and *Citrullus lanatus* commonly called pumpkin seeds or egussi, the oil-rich seeds, have already demonstrated hypolipidemic activity. In Africa, these seeds are popularly used in the preparation of local foods. During that thermal process, the fatty acid content of pumpkin seeds' oils may be altered in their functionality. Thus, this work aims at studying the effect of stifled cooking on the quality and the lipid-lowering potential of oils' extracts from *Citrullus lanatus* (CL) and *Cucumeropsis mannii* (CM).

Methods: The oils were extracted from the pumpkin seeds (raw and cooked) by a mixture of chloroform and methanol (2/1). The acid, iodine, peroxide and thiobarbituric acid value of the oils were assessed. For 28 days, the oils were subsequently administered by oral intubation to high-fat diet induced hyperlipidemic rats. At the end of the experimentation, the lipid profile, the markers of the hepatic and kidney function were determined.

Results: The oils extracted from raw CL and CM significantly reduced ($p < 0.05$) serum triglyceride, total cholesterol, Low Density Lipoprotein (LDL) cholesterol, uric acid, serum transaminases, creatinine, urea and increases the serum High Density Lipoprotein (HDL) cholesterol level relative to the oils extracted from cooked CM and CL. Moreover, the oil from cooked CL significantly ($p < 0.05$) reduced some lipid profile and toxicity parameters (triglycerides and Alanine aminotransferase) while increasing the serum HDL compared to the oil extracted from cooked CM. On the other hand, all the quality parameters of the raw materials followed the limits for vegetable oils, as opposed to cooked samples.

Conclusion: Stifled cooking affects the lipid-lowering potential of CM oil compared to that of CL oil.

Keywords: *Citrullus lanatus*; *Cucumeropsis mannii*; Cucurbitaceae, Hyperlipidemia, Stifled cooking.

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Background

Hyperlipidemia is a serious chronic disease characterized by elevated serum levels of total cholesterol, low density lipoprotein (LDL), very low-density lipoprotein (VLDL) and low serum of high-density lipoprotein (HDL) [1]. It is a major risk factor for cardiovascular diseases (CVD), which is a serious public health problem in the world [2]. These diseases are among the leading causes of death in the world, particularly in developing countries, with nearly 37 million deaths in 2015 [3]. In Cameroon, an estimated 14.2% of CVD causes 23.3% of deaths [4]. In recent decades, there has been a steady increase in the number of cases of hyperlipemia and the prevalence of this disease, which is estimated at 31% in 2016 in the United States compared to 27% in 2008 [2]. In Cameroon, the prevalence of hyperlipidemia is 39% and this is mainly represented by hypercholesterolemia (31%) [5].

Hyperlipidemia, if left untreated, causes serious complications, being responsible for various problems such as atherosclerosis, cardiovascular diseases, and many others. Thus, maintaining lipid levels close to normal can help delay or prevent complications of hyperlipidemia. It is in this perspective that there are now several methods and strategies to fight or limit the evolution of this disease through the use of drugs but however, it is expensive and also has serious side effects [6].

In view of these adverse health effects, the scientific community has shown a growing interest in functional foods in recent decades, among which, are pumpkin seeds belonging to the *Cucurbitaceae* family [7]. Indeed, advances in the research of edible oils have shown that a regular consumption of omega-6 and omega-3 long-chain polyunsaturated fatty acids, could have therapeutic virtues in the fight against cardiovascular diseases [8].

Thus, special attention was paid to pumpkin seeds oil. Studies of some pumpkin seeds species in Cameroon have shown that they are rich in lipids (44-53%), proteins (28-40%), carbohydrates (7-10%), fiber (3-4%) and minerals (3-4%) [9]. These lipids contain omegas 3 and 6 polyunsaturated fatty acids, giving pumpkin seeds oils high biological properties [10]. Numerous studies have already been carried out on pumpkin seeds oils, their hypolipidemic activities [11], hypoglycemic agents [12], antimicrobial agents [7] and antiatherogenic drugs [13]. Pumpkin seeds generally undergo one or more culinary treatments before their consumption, which could reduce the efficiency of their oil. Thus, this work aimed to determine the effect of stifled cooking on the quality of oils extracted from two species of pumpkin seeds (*Citrullus lanatus* (CL) and *Cucumeropsis mannii* (CM)), in relation to their lipid-lowering potential.

Methods

Plant material

The different dried seeds of *Citrullus lanatus* (Voucher No: 42471/HNC) and *Cucumeropsis mannii* (Voucher No: 42485/HNC) were obtained from market in the city town of Dschang (west Cameroon) in January 2019. They were cleaned and distributed into several batches, each of which underwent a specific treatment (Stifled cooking or not) at the Research Unit of Biochemistry of Medicinal Plants, Food Science and Nutrition (URBPMAN) of the Department of Biochemistry of the University of Dschang.

Animal material

Male, albinos *wistar* rats were raised at the animal house of the Department of Biochemistry of the University of Dschang, under standard laboratory conditions (12 hours in the light and 12 hours in the darkness) and fed a diet as elaborated by [14].

Stifled cooking

Pumpkin seeds were cleaned and milled using an electric blender (Royalty line, Model No: SME-600.6; Order No: 16-RL-942) and divided into batches. For the preparation of pumpkin seeds dishes, 120 g of powder from each pumpkin seed's species were mixed with 125 ml of water to form the pumpkin seed paste. To make the mixture of the two species, 30g (25% of 120g) of powder of CL was added to 90g (75% of 120g) of powder of CM (from a survey conducted in the city of Dschang; unpublished data) and were also mixed with 125 ml of water to form a combined paste. After flattening, they were wrapped in clean banana leaves and put in a pot (5L capacity, diameter 27 cm) on a hot plate (HP-211 PEM) for 150 minutes.

Extraction of oils

The oils were extracted from the raw (control) and cooked (pumpkin seeds) samples following the method described by [15]. Briefly, 100 g of sample were introduced into the electric blender. Thereafter, 200 ml of chloroform / methanol (2/1, v / v) were added. After grinding for 10 to 15 min (one minute = one engine running cycle at full speed), an additional 100 mL of chloroform was added. After homogenization of the mixture for 1 min, the homogenate was filtered with Whatman No. 1 paper, and the filtrate was introduced into a funnel to separate the phases. The lower phase consisting of the chloroform / oil mixture was collected and then the chloroform was evaporated under reduced pressure at 60°C using a BÜCHI R-124 rotary evaporator. The oils obtained were concentrated in an air oven at 45°C for 6 hours, then stored in opaque glass flasks in the freezer for subsequent studies.

Chemical characterization of oils

The acid, iodine and peroxide values were determined using standard methods as described by [16]. The determination of the thiobarbituric acid number was performed according to the method prescribed by [17].

Induction of hyperlipidaemia

Hyperlipidaemia was induced in rats with a high-fat diet, consisting of basic diet enriched with beef tallow (20%) and cholesterol (1%) for 28 days as described by [18].

Experimental design

Forty-eight *Wistar* rats aged three months and weighing between 150 and 200 g were divided into 8 groups of 6 rats each. The controls groups received distilled water (4 ml/kg) and staple food (negative control) or high-fat diet food (positive control), and the test groups received the high-fat diet and oil (200 mg/Kg) or Atorvastatin (from Sigma) (10 mg/Kg) by oral intubation. After 28 days of animals' feeding, the rats were anaesthetised with chloroform vapours and blood collected by cardiac puncture into a tube (without anticoagulant). The serum was separated from the erythrocyte by centrifuging the whole blood at 3000 rpm/min for 10 min and was frozen at -20°C until required for analysis. All

experiments were carried out according to the local regulations and ethical approval.

Determination of biochemical parameters

The serum lipid profile was determined using colorimetric methods (CHRONOLAB kit). The method described by [19] was used to determine the triglycerides. The standard protocols as described by [20] and [21] were used for the determination of total and HDL cholesterol respectively, while LDL cholesterol was estimated using the formula established by [22]. The atherogenic index was also estimated using the formula of [23]. SPINREACT kits based on colorimetric methods were used for the assays of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to the standard protocol described by [24] as well as for the determination of creatinine and urea according to the method of [25]. Uric acid was determined according to the colorimetric method of [19] using the CHRONOLAB kit.

Statistical analysis

All data were entered using the Microsoft Excel 2013 software. The results were expressed as mean \pm standard deviation. The influence of the factor was tested through analysis of variance. The post-Duncan test allowed to highlight the significant differences between the means at the threshold 0.05 using the SPSS statistics 22 software.

Results and discussion

The lipid quality of *Cucumeropsis mannii* and *Citrullus lanatus* oils

Table 1 below illustrates the lipid quality of the different oil samples. It appears that stifled cooking had variable effects on the acid, iodine, peroxide and thiobarbituric acid values of the oils of *CM* and *CL*. In fact, this cooking decreased the acid value of cooked samples (cooked *CL* (1.01 ± 0.30), cooked *CM* (0.82 ± 0.50), cooked mixture of *CM* and *CL* (1.00 ± 0.40)) compared to raw samples (raw *CL* (1.10 ± 0.10), raw *CM* (1.10 ± 0.20)) and with significant difference between cooked *CM* and other samples. The decrease in the free fatty acid content during stifled cooking could be due to the denaturation of the lipolytic enzymes by heat thus stopping the hydrolysis of triglycerides in the cooked product and the transformation under the effect of the heat of free fatty acids initially present into primary or secondary oxidation products (hydroperoxides or malonaldehydes) [26]. These results are like those of [27] who obtained an acid value of 1.6 ± 0.8 with raw *Curcumeropsis pepo* oils. Moreover, the acid value of all the oils obtained remains in the normal range (less than 4 mg KOH / g of oil) [28].

Similarly, the iodine value of cooking oils were low (cooked *CL* (80.85 ± 0.80), cooked *CM* (76.74 ± 0.60), cooked mixture of *CM* and *CL* (71.36 ± 0.30)) compared to the value of raw sample (raw *CL* (87.09 ± 0.50), raw *CM* (84.60 ± 0.40)) and with a significant difference between all samples ($P < 0.05$). It could be explained by the thermal deterioration of the double bonds of the polyunsaturated fatty acids which are transformed into primary and secondary oxidation products [29]. In addition, the low iodine value of the oil from the raw sample of *CM* (84.60 ± 0.40 g I_2 / 100 g) obtained in this study compared to those obtained by [10] (101.5 - 108.85 g I_2 / 100 g) could be due to auto-oxidation (catalyzed by temperature or free radicals) of unsaturated fatty acids via

oxidative photolysis reactions which lead to the formation of peroxides [29].

The peroxide value of the oils from the cooked samples were high (cooked *CL* (18.18 ± 0.05), cooked *CM* (28.57 ± 0.76), cooked mixture of *CM* and *CL* (24.28 ± 0.65)) compared to the raw samples (raw *CL* (9.09 ± 0.01); raw *CM* (10.10 ± 0.02)) and with a significant difference between all the samples ($P < 0.05$). It could be due to the transformation of fatty acids (more precisely those unsaturated) under the effect of the high temperatures (catalysts of thermo-oxidation alterations) into the very unstable hydroperoxide molecules [30, 31]. It could also be explained by the activity of water because water molecules interact with metal cations, making them available in the catalysis of auto-oxidation reactions [31]. These results are similar to those of [10]. In addition, it should be noted that all oil samples except for the cooked *CM* remained within the standard value (less than 15 meq of O_2 / kg) [28].

The same observation applied to thiobarbituric acid value, which was high (cooked *CL* (1.68 ± 0.06), cooked *CM* (1.55 ± 0.05), cooked mixture of *CM* and *CL* (1.67 ± 0.07)) in the cooked samples compared to the raw samples (raw *CL* (0.17 ± 0.01), raw *CM* (0.27 ± 0.03)) and significantly different between all samples (raw and cooked) ($P < 0.05$). This could be explained by the transformation of unstable primary oxidation compounds (hydroperoxides) under the effect of the high temperatures into volatiles type (aldehyde, ketone, alcohol) during propagation and termination reactions [30, 32].

Effect of *Cucumeropsis mannii* and *Citrullus lanatus* oils on serum lipid profile

Figures 1, 2, 3, 4, 5 respectively illustrate the serum levels of the biochemical parameters (Triglycerides, Total Cholesterol, HDL, LDL, Atherogenic index) for the rats following the treatment with the oil of the two pumpkin seeds' species (*CM* and *CL*).

Figure 1 shows that the untreated hyperlipidaemic rats' group of positive control has the highest serum triglyceride levels. These values are significantly ($P < 0.05$) different from those observed in all other groups. In addition, the groups treated with the oil of raw *CM* and raw *CL* had lower triglyceride levels compared to those treated with the oil of cooked *CL*, cooked *CM* and cooked mixture of the two species, with a significant difference between these groups ($P < 0.05$). It could be attributed to the abundance of unsaturated fatty acids in raw *CL* and raw *CM* oils which significantly reduces triglyceride levels by reducing their synthesis in the liver or by activating β -oxidation (mitochondrial or peroxisomal) [10]. These results are in accordance with those of [33] who showed that pumpkin seeds oil lowers triglyceride levels.

Figure 2 shows that untreated hyperlipidaemic rats have the highest total cholesterol levels compared to other groups. Even though the oil-treated groups still had high cholesterol levels compared to those of the Atorvastatin and non-hyperlipidemic rats, those values were significantly reduced, with raw *CL* being the most potent. This could be explained by the richness of these oils in essential fatty acids (linoleic acid (n-6) and α -linolenic acid (n-3)) which have a lipid-lowering effect [10]. Diets rich in polyunsaturated fatty acids (PUFA) are well known for their cholesterol-lowering action [34]. Thus, replacing saturated fatty acids (SFA) with n-6 PUFA (or having a diet enriched with n-6 PUFA) leads to a substantial decrease in total cholesterol [34]. However, the high cholesterol level in untreated hyperlipidemic rats is thought to be due to the richness of hyperlipidaemic cholesterol diet (1%) which could have significantly increased the serum total cholesterol levels [33]. These results are similar to those of [18] who found that rats fed a high cholesterol diet (1%) and pumpkin seeds oil had low

cholesterol levels compared to those who did not receive oil and also corroborate results of [35].

Figure 3 shows the HDL cholesterol level of the animals. We noted that HDL cholesterol levels are lower and differ significantly ($P < 0.05$) in untreated hyperlipidaemic rats compared to all other groups. We also noted that animals treated with Atorvastatin and non-hyperlipidaemic rats had significantly different HDL cholesterol levels compared to animals treated with oils. Moreover, the groups treated with the oil of *CL* (raw and cooked) had higher HDL cholesterol levels compared to those treated with the oil of *CM* (raw and cooked) and cooked mixture of the two species, with a significant difference between these groups ($P < 0.05$). The high concentration of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) as well as the essential fatty acids of *CL* and *CM* oils could be responsible for the increase in serum HDL-cholesterol in treated hyperlipidaemic rats and a significant decrease in this concentration in untreated hyperlipidaemic rats [10]. In fact, unsaturated fatty acids stimulate the production of HDL to allow degradation of serum total cholesterol [11]. These results are consistent with those of [18] who showed that pumpkin seeds oil increases HDL cholesterol levels.

Figure 4 shows that LDL cholesterol levels are higher and differ significantly ($P < 0.05$) in untreated hyperlipidaemic rats compared to all other groups. In addition, the groups receiving the oil of raw *CM* and raw *CL* had lower levels of LDL cholesterol with significant difference ($P < 0.05$) from groups receiving the oil of cooked *CM*, cooked *CL* and cooked mixture of the two species. It may be explained by the presence (in greater quantity) of cholesterol and triglyceride lowering compounds in these oils, particularly essential fatty acids (n-6 and n-3) [10]. Moreover, this decrease in LDL cholesterol may be correlated with the increase in HDL level in these animals. Indeed, HDL can cause either activation of the bile acid pathway or inhibition of the enterohepatic cycle, which lead to activation of LDL receptors and consequently a decrease in cholesterolemia and LDL-cholesterol [33]. This work is consistent with that of [33] who showed that LDL levels decreased in hyperlipidemic rats treated with pumpkin seeds oil.

Figure 5 shows the atherogenic index in rats, it was found that the group of untreated hyperlipidemic rats had the highest value. This figure also shows that the groups treated with the oil of raw *CM* and raw *CL* had lower levels of atherogenic index compared to those treated with the oil of cooked *CL*, cooked *CM* and cooked mixture of the two species, with a significant difference between these groups ($P < 0.05$). This is consistent with the results obtained by [13] who revealed that pumpkin seeds oil has a hypolipidemic effect (hypocholesterolemic and hypotriglyceridemic) and antiatherogenic. So, *CL* oil and *CM* could protect against the risk of cardiovascular disease by decreasing the atherogenic index.

The differences in efficacy observed in all parameters for the different oils used (raw pumpkin seeds oils and cooked pumpkin seeds' oils) are a consequence of their differences in composition. Indeed, this study shows that the oils of *CL* and *CM* raw have a significant difference in certain parameters (total cholesterol, HDL, LDL and atherogenic index) in terms of lipid-lowering efficacy that would be related to their different composition of fatty acids [10]. Similarly, the significant reduction in the lipid-lowering potential of the oil extracted from the *CM* dish compared to the lipid-lowering activity of *CL* oil in parameters (such as HDL cholesterol and triglycerides) could be related to the high antioxidant content of *CL* oil in tocopherol and its lower concentration of unsaturated fatty acids compared to the oil of *CM* [36]. In addition, the absence of a significant difference between the oil of the cooked mixture of the two species and the oil of the dish of *CM* as well as the marked reduction in the lipid-lowering

potential of the oil of this mixture, compared to the oils of the other dishes in all the parameters, are due to the fact that this mixture contains *CM* than *CL* and is therefore more affected by heat. These data are consistent with those obtained by [37] who found that *CL* oil was very heat-stable compared to that of *CM*.

Effect of CM and CL oils on markers of hepatic and renal impairment

Figures 6 and 7 show the serum transaminase levels obtained for the animals following treatment with oil of *CM* and *CL*. Figure 6 illustrates that untreated hyperlipidemic (PC) rats had higher serum Aspartate aminotransferase (AST) levels than the other groups with significant difference ($P < 0.05$) from non-hyperlipidemic rats and groups treated with the oils of cooked *CL* and *CM*. However, the cooked *CL* group (CLC) had lower AST levels than the other groups receiving oil and differ significantly ($P < 0.05$) from group treated by raw *CL* (CLR). Figure 7 shows that untreated hyperlipidemic rats had higher serum Alanine aminotransferase (ALT) levels with significant difference ($P < 0.05$) from other groups. However, the cooked *CL* group (CLC) had lower ALT levels than the other groups treated with pumpkin seeds oil with significant difference ($P < 0.05$) from them. All of this could reflect the hepatoprotective effect of the different oils and the thermostability of *CL* oil [38].

Figure 8 shows serum uric acid levels in animals. It appears that the serum uric acid level is higher in untreated hyperlipidemic rats than the other groups of rats and differ significantly ($P < 0.05$) from them. This could be explained by the lipid-lowering potential of oils and Atorvastatin and would therefore suggest a decrease in the serum VLDL level [33]. In addition, raw *CL* and *CM* oils show a significant difference in uric acid levels in the different treated groups that could be related to their different fatty acid composition [10].

Figures 9 and 10 illustrate serum creatinine and urea levels of animals. These figures show that the untreated hyperlipidemic rats had the highest serum creatinine and urea levels and differ significantly ($P < 0.05$) from the other groups. We also found that animals receiving the oil from the mixture of the two pumpkin seeds species (ME) revealed higher levels of creatinine and urea than those receiving raw *Citrullus lanatus* (CLR), cooked *Citrullus lanatus* (CLC) oils, raw *Cucumeropsis mannii* (CMR), cooked *Cucumeropsis mannii* (CMC), Atorvastatin (AS) and negative controls (NC) and is significantly different ($P < 0.05$) from them. The reduction of serum creatinine in treated rats could imply that the oils interfered with creatinine metabolism and therefore would have promoted its excretion into the blood [39]. Moreover, increased serum urea levels in untreated hyperlipidemic rats may reveal renal dysfunction such as the plasma urea excretion mechanism [39]. In addition, the increase in creatinine and urea levels in the rats treated with the oil from ME compared to the levels of the *CL* and *CM* oil-fed rats could be related to thermal instability of *CM* which mainly constituted this mixture [37].

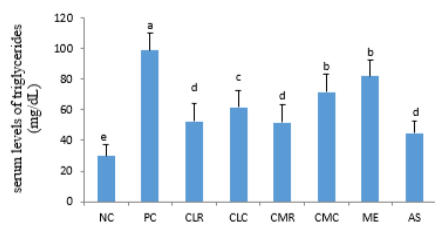


Figure 1. Serum levels of triglycerides.

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

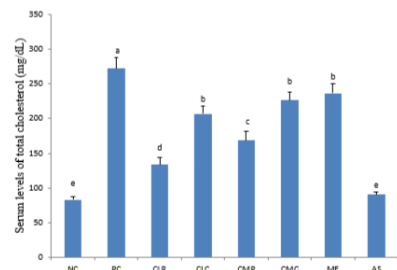


Figure 2. Serum levels of total cholesterol.

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

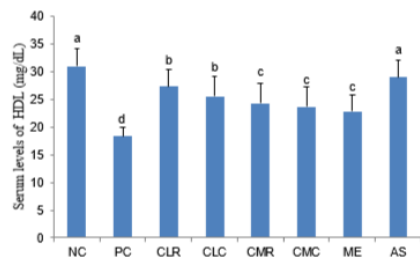


Figure 3. Serum levels of HDL.

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

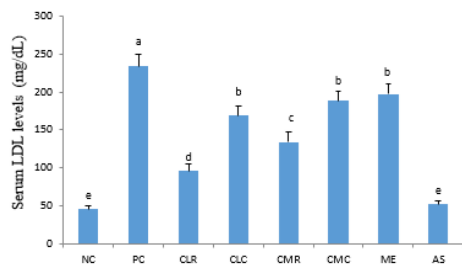


Figure 4. Serum LDL levels.

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

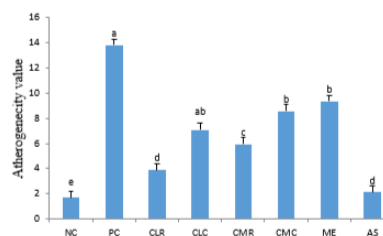


Figure 5. Atherogenicity Value

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

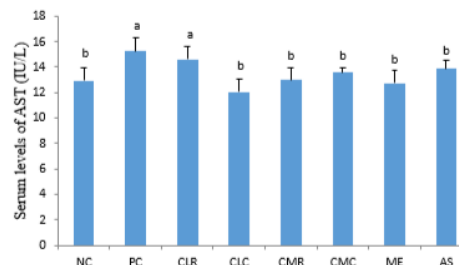


Figure 6. Serum levels of AST.

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

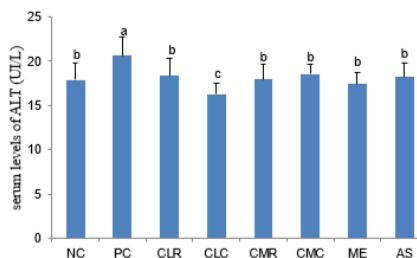


Figure 7. Serum levels of ALT.

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

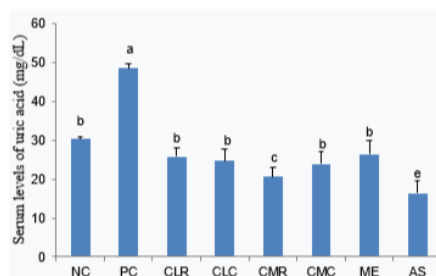


Figure 8. Serum uric acid levels

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

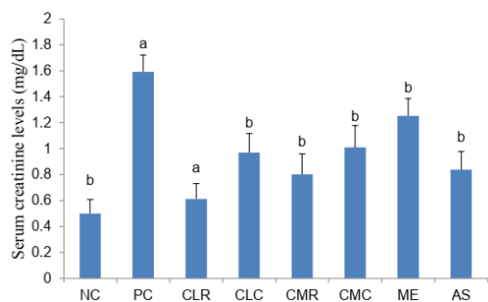


Figure 9. Serum creatinine levels.

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

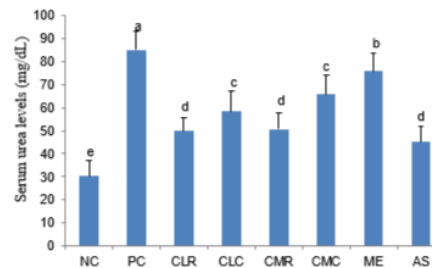


Figure 10: Serum Urea levels

NC: Negative control; PC: positive control; CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%) ; AS: Atorvastatin. a, b, c, d and e: on figure bearing the different letters are significantly different at $p < 0.05$.

Table 1. Lipid quality of the oils of *Cucumeropsis mannii* and *Citrullus lanatus*

Samples	Acid value (mg KOH/g)	Iodine value (gl ₂ /100g)	Peroxide value (meq d'O ₂ /Kg)	Thiobarbituric acid value (µmol MDA/Kg)
CLR	1.10±0.10 ^a	87.09±0.50 ^a	9.09±0.01 ^e	0.17±0.01 ^e
CLC	1.01±0.30 ^a	80.85±0.80 ^c	18.18±0.05 ^c	1.68±0.06 ^a
CMR	1.10±0.20 ^a	84.60±0.40 ^b	10.10±0.02 ^d	0.27±0.03 ^d
CMC	0.82±0.50 ^b	76.74±0.60 ^d	28.57±0.76 ^a	1.55±0.05 ^c
ME	1.00±0.40 ^a	71.36±0.30 ^e	24.28±0.65 ^b	1.67±0.07 ^b

CLR: raw *Citrullus lanatus* ; CLC: cooked *Citrullus lanatus* ; CMR: raw *Cucumeropsis mannii* ; CMC: cooked *Cucumeropsis mannii* ; ME: cooked mixture of *Citrullus lanatus* and *Cucumeropsis mannii* (25% and 75%). a, b, c, d and e: in the table bearing the different letters are significantly different at $p < 0.05$.

Conclusion

Stified cooking affects the quality and lipid-lowering potential of *CL* and *CM* oils. Indeed, the physicochemical analysis (iodine, acid, peroxide, and thiobarbituric acid values) of the oils of the different samples (raw and cooked) showed that all the quality parameters of the raw materials follow the CODEX STAN (2009) limits for vegetable oils, as opposed to cooked samples that do not always respect these standards. In addition, the administration of raw *CL* and *CM* to animals leads to a reduction in serum triglyceride, total cholesterol, LDL cholesterol, uric acid, serum transaminases (ALT and AST), creatinine, urea and increases the serum HDL cholesterol level relative to the oil extracted from cooked *CM* and *CL*, thus reflecting a hypolipidemic potential of these oils. Moreover, the oil from cooked *CL* significantly reduces some parameters of the lipid profile (serum triglyceride, total cholesterol and LDL cholesterol) and the toxicity parameters (ALT, AST, uric acid, urea and creatinine) while increasing the serum HDL cholesterol compared to the oil extracted from cooked *CM* and the cooked mixture of both species, thus indicate that stifled cooking affects more the lipid-lowering potential of *CM* oil than that of *CL* oil.

Abbreviations

LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; HDL: High Density Lipoprotein; CVD: Cardiovascular diseases; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; PUFA: Polyunsaturated fatty acids; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids.

Authors' Contribution

DK is involved in designing the experiment. RON provided the pumpkin seeds. RON, RZ, PFD, CYW, FAT and PTN performed the experimental work. APNK and PFD were involved in statistical analysis. RON and DK wrote the first and final draft. All the authors read and approved the final draft.

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Conflict of interest

The authors declare that they have no conflict of interest.

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