Effect of temperature on α-glucosidase, lipase inhibition activity and other nutritional properties of *Moringa oleifera* leaves: Intended to be used as daily antidiabetic therapeutic food

LAXMI PARWANI – YASHASVI BOHRA – SAKSHAM GUPTA – RAJESH KUMAR

Summary

Leaves of *Moringa oleifera* have proven antidiabetic efficacy and are commonly consumed in the form of powder, capsules and tablets. The present study was designed to evaluate the temperature sensitivity of α-glucosidae, lipase inhibition activities and other nutritional properties like total chlorophyll, carbohydrate, protein, phenolics, flavonoid contents and antioxidant activities of *M. oleifera* leaves with an objective to be used as palatable therapeutic food for easy and effective treatment of type 2 diabetes. The dried leaves powder of the plant was exposed to two different temperatures (100 °C and 150 °C) at different time intervals of 5, 10, 15 and 30 min. The findings revealed that exposure at 150 °C temperature for 15 min can be safely used for the preparation of processed foods from powdered leaves of *M. oleifera.* At this point of treatment, less detrimental effects on α-glucosidase and lipase inhibition activity, flavonoid content and antioxidant activity were observed. On the other hand, total phenolic content, content of free amino acids and radical-scavenging activities were increased. However, sharp reduction in carbohydrate content was observed at this point, which makes this temperature and time suitable for the preparation of processed food with sustainable antidiabetic properties.

Keywords

diabetes; *Moringa oleifera*; therapeutic food; α-glucosidase inhibition; temperature sensitivity

Diabetes is the third most prevalent severe chronic disease across the world. According to International Diabetes Federation, in 2014 there were 387 million people affected with diabetes worldwide. This number is predicted to reach 592 million by 2035, of which 46% will still be un-diagnosed [1]. It is a metabolic disorder critically affecting the population of both developed and developing countries. India has the highest number of diabetic patients, and is being called the diabetic capital of the world [2]. The complications related to diabetes pose a significant health care burden (2.3-times higher) and are deterrent to overall quality of life, which makes this disease 8th leading cause of death across the world and resulting in huge economic losses [3, 4]. Recently, its prevalence has reached epidemic levels in many parts of the world. However, effective control of the onset of diabetes and its complications has not been established yet, which is a cause of worry for experts and researchers.

Although there are several drugs available in the market, their long-time use may cause various side effects. Hence, a large number of studies is in progress to find new natural sources, which are effective in reducing the intensity of diabetes along with showing less or no side effects. In the last few years, there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity worldwide because of their natural origin and less or no side effects. The World Health Organization (WHO) has listed 21 000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are

Laxmi Parwani, Yashasvi Bohra, Saksham Gupta, Rajesh Kumar, Department of Biotechnology, Dr. B. Lal Institute of Biotechnology, Jaipur 302017, Rajasthan, India.

Correspondence author:
Laxmi Parwani, tel.: +919982216197, e- mail: laxmi.bibt@gmail.com

available in India, out of which 150 species are used commercially on a fairly large scale. There are more than 400 different plants that have been described as reputedly beneficial for the treatment of diabetes [5]. Most of these plants have been claimed to possess hypoglycemic properties but most claims are anecdotal and few have received adequate medical or scientific evaluation.

Moringa oleifera Lam. is a multipurpose and exceptionally nutritious vegetable tree with a variety of potential uses. It is also known as nature's medicine cabinet as different parts of this plant are used in the indigenous systems of human medicine for the treatment of a variety of human ailments. Leaves of this plant are popularly used for their remarkable nutritional and medicinal qualities along with their hypocholesterolemic and hypoglycemic effects in traditional medicine practices due to the presence of 46 antioxidants [6]. It also boosts the immune system, which usually becomes compromised in those who suffer from type 1 and 2 diabetes, as it is a rich source of ascorbic acid and it also helps in insulin secretion [7]. The protective effect of leaf powder of *M. oleifera* in controlling diabetes has been studied extensively but no study till date was reported on the use of *M. oleifera* leaves powder (MLP) in the form of processed food for the management of diabetes. Processing of the material is required for longer shelf life and for enhanced palatability. Daily consumption of MLP is limited to the form of tablets or capsules. Thus, the present study was designed to evaluate the optimum temperature and time for MLP processing, in a safe way for the preparation of therapeutic food for type 2 diabetic patients, which would have sustainable antidiabetic properties.

MATERIALS AND METHODS

Harvesting of *M. oleifera* leaves, washing and powder preparation

Locally available *M. oleifera* plants were identified and their young and old healthy leaves were harvested in the morning. Initial phase of processing was performed on the same day to avoid any loss of moisture from the leaves. Collected leaves were washed thoroughly in running tap water to remove the dirt, and then soaked in 1% saline solution (NaCl) for 5 min. Leaves were further washed with 70% ethanol and distilled water to remove dust and microorganisms present on the leaves surfaces. After removal of excess water, leaves were dried in shed at room temperature. Grinding was done by domestic grinder for powder preparation.

Temperature treatment

The effect of temperature on MLP was studied by treating the leaves at 100 °C and 150 °C for 5, 10, 15 and 30 min in a hot air oven. Stocks of treated samples (50 mg·ml^{-1} concentration) were prepared in 0.1 mol·l^{-1} NaOH. All experiments were conducted in triplicate and values are presented as arithmetic mean. Standard deviation (*SD*) was calculated using Microsoft Excel (Microsoft, Redmond, Washington, USA) statistical tool.

Total chlorophyll estimation

Total chlorophyll was estimated by the method of Porra et al. [8] using 96% methanol as an extraction solvent. Absorbance of the extracted chlorophyll was measured at 652 nm and 665 nm by UV-Visible spectrophotometer (Systronics, Mumbai, India) using methanol as a blank. Chlorophyll *a* (*Chla*), chlorophyll *b* (*Chlb*) and total chlorophyll (*TC*) contents were calculated by the following equations:

$$Chla = (16.29 \times A_{665}) - (8.54 \times A_{652}) \quad (1)$$

$$Chlb = (34.09 \times A_{652}) - (15.28 \times A_{665}) \quad (2)$$

where concentrations of *Chla* and *Chlb* are expressed in micrograms per millilitre, numbers 16.29, 8.54, 34.09 and 15.28 represent exponent coefficients, A_{665} and A_{652} are absorbance values measured at 665 nm and 652 nm, respectively.

$$TC = \frac{(Chla + Chlb)}{m_{\mathrm{MLP}}} \quad (3)$$

where m_{MLP} is weight of MLP. *TC* is expressed in milligrams per kilogram.

Estimation of carbohydrates

Total carbohydrates content in MLP was estimated by the method of Roe [9] using a standard curve of glucose (0–100 μg·ml^{-1}).

Total proteins estimation

Proteins content of the sample was estimated by the method of Lowry et al. [10] using bovine serum albumin solution as a standard (0–200 μg·ml^{-1}).

Total phenolics content

For determination of total phenolics content, 1 ml Follin-Ciocalteu Reagent (1:10 dilution) was added to 50 mg dried MLP sample. Further, 4 ml of Na_2CO_3 (75 g·l^{-1}) was added in the reaction mixture followed by incubation for 2 h at room temperature. Mixture was centrifuged for 10 min at 2000 ×*g* and absorbance was measured at 760 nm. Gallic acid (0–400 μg·ml^{-1}) was used

as a standard for preparation of the calibration curve, and water was used as a blank. Total phenolics content was calculated as gallic acid equivalents (GAE) [11].

Total flavonoids content

Total flavonoids content was estimated by the method of HANSAWASDI et al. [12]. Dried MLP was mixed (50 mg) with 2.0 ml of $AlCl_3$ (2% ethanolic solution) and 3.0 ml of sodium acetate (50 $g \cdot l^{-1}$). After 2.5 h of incubation at room temperature, the absorbance was measured at 440 nm. Quercetin was used as a standard (0–400 $\mu g \cdot ml^{-1}$) and total flavonoids content was expressed as quercetin equivalents (QE) in milligrams per kilogram of dried leaves powder.

Measurement of total antioxidant activity using phosphomolybdenum assay

The total antioxidant activity of heat-treated and untreated MLP was evaluated by phosphomolybdenum method as described by PRITO et al. [13]. For this purpose, 0.3 ml sample from stock was mixed with 3 ml of the reaction solution (consisting of 0.6 $mol \cdot l^{-1}$ sulfuric acid, 28 $mmol \cdot l^{-1}$ sodium phosphate and 4 $mmol \cdot l^{-1}$ ammonium molybdate in 100 ml distilled water). The tubes were incubated at 95 °C for 90 min and absorbance was measured at 695 nm. A blank with 0.3 ml methanol was used. Total antioxidant activity was expressed in terms of Trolox equivalents (TE) in milligrams per kilogram using a calibration curve of Trolox standard (0–30 $\mu g \cdot ml^{-1}$).

•DPPH radical-scavenging activity

The reduction of 1,1-diphenyl-2-picrylhydrazyl radical (•DPPH) solution in the presence of hydrogen-donating antiradicals of the sample was tested by the method of MENSOR et al. [14]. In reaction mixture, 0.1 ml sample was mixed with 3.9 ml of 0.06 $mmol \cdot l^{-1}$ methanolic •DPPH solution in test tubes. The test tubes were incubated for 60 min at room temperature and absorbance was measured at 515 nm. The •DPPH free radical scavenging activity (*RSA*), expressed as percentage of radical-scavenging activity, was calculated by the following equation:

$$RSA = \frac{(A_0 - A_S)}{A_0} \times 100 \quad (4)$$

where A_0 is the absorbance of control (0.06 $mmol \cdot l^{-1}$ methanolic •DPPH only) and A_S is the absorbance of the reaction mixture.

$ABTS^{\bullet+}$ radical-scavenging activity

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay was performed by the modified method of RE et al. [15]. The assay is based on the ability of antioxidant molecules to quench the long-lived $ABTS^{\bullet+}$ cations. $ABTS^{\bullet+}$ stock solution (7 $mmol \cdot l^{-1}$) was prepared with 2.4 ml of 5 $mmol \cdot l^{-1}$ potassium persulphate followed by incubation for 12–16 h in the dark at room temperature. The $ABTS^{\bullet+}$ solution was diluted with deionized water: 95% ethanol (1:1) to an absorbance of 0.70 (+0.02) at 734 nm. Further, 20 μl of the test sample was mixed with 6 ml of the diluted $ABTS^{\bullet+}$ solution and absorbance was recorded after 1 min. The percentage of *RSA* was calculated using Eq. 4 and compared with Trolox standard (0–400 $\mu g \cdot ml^{-1}$). Data were expressed as TE in milligrams per kilogram.

Estimation of α-glucosidase inhibition

For determination of α-glucosidase inhibition activity, 500 μl phosphate buffer (0.1 $mol \cdot l^{-1}$, pH 6.9) was mixed with 100 μl of the test sample. To this mixture, 250 μl α-glucosidase (1.25 $U \cdot ml^{-1}$) was added, followed by incubation at 37 °C for 15 min. For the initiation of reaction, 250 μl *p*-nitrophenyl-α-D-glucopyranoside (PNPG, 0.5 $mmol \cdot l^{-1}$) was added as a substrate in the reaction mixture. The mixture was incubated again at 37 °C for 15 min, followed by addition of 1 ml Na_2CO_3 (0.2 $mmol \cdot l^{-1}$) for termination of the reaction. Absorbance was measured at 410 nm. In control, the same reaction mixture was used but the test sample was replaced by the buffer. In blank, equal amount of buffer was added in place of α-glucosidase and PNPG [16]. The percent α-glucosidase inhibition (*AGI*) activity was calculated by following equation

$$AGI = \frac{(A_C - A_S)}{A_C} \times 100 \quad (5)$$

where A_C is absorbance of the control and A_S is absorbance of the sample.

Pancreatic lipase inhibition

Pancreatic lipase inhibition activity was determined by the modified method of BUSTANJI et al. [17]. Lipase enzyme (500 μl, prepared in Tris.HCl buffer, pH 7.4) was added to a 250 μl sample and incubated at 37 °C for 1 min. Volume of the mixture was adjusted to 2 ml using Tris.HCl buffer. To this, 4-nitrophenyl acetate (NPA; 5 $mmol \cdot l^{-1}$, 0.2 ml) was added and absorbance was recorded at 410 nm. In control, the same reaction mixture was used but with buffer. Blank was prepared using the same reaction mixture with enzyme and NPA replaced by the buffer. Percent pancreatic lipase inhibition was calculated by Eq. 5.

Tab. 1. Temperature sensitivity of total chlorophyll, carbohydrates, proteins, phenolics and flavonoids content of *M. oleifera* leaves powder.

Temperature	Time [min]	Total chlorophyll [mg·kg^{-1}]	Total carbohydrates [mg·kg^{-1}]	Total proteins [mg·kg^{-1}]	Total phenolics content [mg·kg^{-1}]	Flavonoids content [mg·kg^{-1}]
Untreated		6.40 ± 0.02	557.87 ± 19.93	352.41 ± 0.86	28.85 ± 0.01	33.87 ± 1.44
100 °C	5	4.60 ± 0.14	460.27 ± 11.47	351.45 ± 0.15	33.79 ± 0.80	33.22 ± 2.56
	10	4.47 ± 0.04	288.67 ± 6.90	351.85 ± 0.17	31.85 ± 3.78	31.03 ± 2.03
	15	4.32 ± 0.12	264.13 ± 9.45	351.18 ± 0.14	32.87 ± 0.05	33.87 ± 1.44
	30	3.19 ± 0.02	224.13 ± 11.73	350.33 ± 0.02	28.82 ± 3.56	32.74 ± 2.51
150 °C	5	3.77 ± 0.07	216.13 ± 6.61	350.58 ± 0.12	32.12 ± 1.31	33.87 ± 1.44
	10	3.27 ± 0.23	206.73 ± 3.91	350.93 ± 0.31	30.19 ± 2.32	32.20 ± 0.00
	15	2.94 ± 0.36	198.47 ± 1.09	358.86 ± 1.99	30.61 ± 0.02	32.39 ± 2.23
	30	1.29 ± 0.10	188.20 ± 3.05	358.12 ± 2.97	26.92 ± 4.94	30.38 ± 6.35

Total phenolics and flavonoids content is expressed as milligrams of gallic acid and quercetin equivalent, respectively.

RESULTS AND DISCUSSION

Effect of temperature on chlorophyll, carbohydrates and proteins contents

Chlorophyll is widely present in plants and blue green algae and plays an important role in photosynthesis. Chlorophyll possesses alkalizing, detoxifying and anti-inflammatory, antioxidant and wound-healing properties, thus its assessment is important when diabetes is concerned [18]. In *M. oleifera* leaves, total chlorophyll content in untreated sample was found 6.4 mg·kg^{-1} of dry weight. Treatment of leaves at temperatures of 100 °C or 150 °C for 5, 10, 15 and 30 min decreased the content of chlorophyll gradually. Minimum chlorophyll content (1.29 mg·kg^{-1}) was observed in sample treated at 150 °C for 30 min (Tab. 1). The results showed the temperature sensitivity of chlorophyll molecules, confirming suitability of shed drying of *Moringa* leaves for maintenance of the activity of biomolecules.

On the other hand, carbohydrates are energy biomolecules playing a significant role in diabetes because hyperglycemic effects are directly related to the faulty digestion of carbohydrates in the body. So the high protein-low carbohydrate diet is recommended for diabetic patients to decrease the carbohydrates absorption in alimentary canal. MLP is a rich source of carbohydrates havings 557 mg·kg^{-1} total carbohydrates content. When effect of temperature was evaluated on carbohydrates content in MLP, it was found that the content was gradually lowered with respect to temperature and time. Sample treated at 100 °C for 5 min exhibited 460 mg·kg^{-1} total carbohydrates content, which further decreased and remained 188 mg·kg^{-1} in the sample, treated at 150 °C for 30 min (Tab. 1). The decrease in carbohydrates content is beneficial for the preparation of therapeutic food which can be recommended for diabetic patients.

Large content of proteins in diet slows down the absorption of carbohydrates in intestine and it can also prevent malnutrition in diabetic patients. *Moringa* dried leaves powder contains 35.2% of total proteins in the untreated material (Tab. 1). The results are comparable to Moyo et al. [19] who reported the presence of 30.2% crude proteins in dried *Moringa* leaves. The proteins content remained unaffected by heat treatment at a temperature of 150 °C for 10 min, as no major changes in the proteins content were observed. A slight increase in the content was observed at a higher exposure, at times of 15 min and 30 min at 150 °C (358 mg·kg^{-1}). Similar findings were reported by Kirana et al. [20], where authors reported the effect of blanching methods, namely boiling, steaming, and boiling + sodium bicarbonate, on amino acids and proteins contents in leaves of *M. oleifera*. The results showed that blanching had a great effect on amino acids and scores of *Moringa* leaves, which further increased their digestibility. Blanching treatment can increase the content of essential amino acids in *Moringa* leaves. It can be stated that high temperature can cause breakdown of peptide bonds in proteins, which leads to an increase in the content of free amino acids, reflected by the increased absorption at 660 nm by Lowry method. Presence of free amino acids can further increase the digestibility of proteins present in the sample.

Effect of temperature on phenolics and flavonoids contents

Plant-derived antioxidants, in particular phenolics, have gained considerable interest due to their potential health benefits. Epidemiological studies have shown that consumption of plant parts containing antioxidants is beneficial for health because it down-regulates many degenerative processes and can effectively lower the incidence of diabetes, cancer and cardio-vascular diseases [21]. Phenolics are responsible for reducing power or electron-donating ability of the materials. Tab. 1 shows the effect of temperature on total phenolics content of MLP, expressed as GAE. In untreated material, the content was 28.85 mg·kg^{-1}, which increased at 100 °C and 150 °C till the 15 min exposure (33 mg·kg^{-1} and 30 mg·kg^{-1}, respectively). However, treatment at both temperatures for 30 min reduced the content (29 mg·kg^{-1} and 27 mg·kg^{-1}, respectively). The increase in content can be explained by the browning of pigments and decrease was related to thermal oxidation as previously described by WANGCHAROEN and GOMOLMANEE [22]. Their study explained that the increase in phenolics content was related to the formation of antioxidant products by thermal reactions, such as non-enzymatic browning reactions, and also by thermal degradation of insoluble and bound phenolic compounds. Kim et al. [23] also reported that total phenolics content in whole grapeseed extract and powdered grapeseed extract was significantly increased by heat treatment, however, heating at 200 °C decreased the total phenolics content in samples.

Flavonoids are naturally occurring plant secondary phenolic metabolite compounds. They have a wide range of biological activities and a considerable amount of research was carried out on their potential role in treating diabetes and other diseases. Most importantly, flavonoids and related natural compounds are known to encompass antidiabetic potential due to their antioxidant and anti-inflammatory properties. They play a protective role in diabetes, as demonstrated in various animal models. It is supposed that consumption of flavonoids and flavonoid-rich specific foods and beverages can reduce the risk of incident diabetes [24]. Effect of temperature on flavonoids content in MLP was evaluated and flavonoids content was measured as QE. The maximum flavonoids content of 33.87 mg·kg^{-1} was observed, which was further reduced to 30.38 mg·kg^{-1} in a sample treated at 150 °C temperature for 30 min (10.3% reduction in content). The reduction was only 4.3% in the sample treated at 150 °C for 15 min (Tab. 1).

Effect of temperature on total antioxidant capacity and radical scavenging activities

Estimation of total antioxidant capacity was based on the reduction of Mo(VI) to Mo(V) by the sample, and subsequent formation of green phosphate/Mo(V) complex at acidic pH. The method evaluates both water- and fat-soluble antioxidants [25]. Total antioxidant activity was evaluated as Trolox equivalent. The untreated material showed a total antioxidant activity of 0.017 mg·kg^{-1}. After heat treatment, a change in activity was observed in a range of 0.012–0.20 mg·kg^{-1} (Tab. 2). Initial decrease in activity (at 100 °C, 5 min and 10 min) can be attributed to a decrease in phenolics content, which further increased at 100 °C, 15 min, due to increase in browning. Material treated at 100 °C for 30 min and at 150 °C for 15 min or 30 min showed gradual decrease in activity, which could

Tab. 2. Temperature effects on total antioxidant and radical scavenging activities of *M. oleifera* leaves powder.

Temperature [°C]	Time [min]	Total antioxidant activity [mg·kg^{-1}]	$^{\bullet}$DPPH radical-scavenging activity [%]	ABTS$^{\bullet+}$ radical-scavenging activity [mg·kg^{-1}]
Untreated		0.017 ± 0.21	88.3 ± 0.19	5.6 ± 0.42
100	5	0.013 ± 0.23	88.8 ± 0.16	4.41 ± 0.10
	10	0.012 ± 1.45	88.9 ± 0.92	4.38 ± 0.15
	15	0.015 ± 0.73	90.5 ± 1.51	3.42 ± 0.09
	30	0.013 ± 1.89	91.3 ± 0.75	3.01 ± 0.16
150	5	0.018 ± 2.02	89.6 ± 0.16	4.14 ± 0.18
	10	0.020 ± 1.17	90.9 ± 0.99	3.89 ± 0.03
	15	0.016 ± 4.68	90.8 ± 0.66	2.21 ± 0.16
	30	0.014 ± 1.19	93.5 ± 0.43	1.19 ± 0.02

Total antioxidant and ABTS$^{\bullet+}$ radical-scavenging activities are expressed as milligrams per kilograms of Trolox equivalent and $^{\bullet}$DPPH radical-scavenging activity is expressed as percent radical-scavenging activity.

be attributed to thermal oxidation and decomposition of non-phenolic antioxidant substances in *M. oleifera* leaves, as previously reported by WANGCHAROEN and GOMOLMANEE [22]. Phenolics are powerful antioxidants and act in a structure-dependent manner. They can scavenge reactive oxygen species and chelate transition metals, which play vital roles in the initiation of deleterious free radical reactions [26]. Total phenolic content could be regarded as an important indication of antioxidant properties of plant extracts [27]. SURH [28] reported that consumption of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity of these compounds. The present findings indicate a correlation between total antioxidant activity and phenolics content of MLP, which will be beneficial for preparation of therapeutic food.

Estimation of •DPPH and ABTS•+ scavenging ability is related to the radical-scavenging or antiradical activity of the test sample. According to TIRZITIS and BARTOSZ [29], antiradical activity characterizes the ability of compounds to react with free radicals (in a single free radical reaction), but antioxidant activity represents the ability to inhibit the process of oxidation. The two activities do not necessarily co-incide. •DPPH and ABTS•+ free radicals are commonly used to assess antiradical activity in vitro, however, both of these radicals are foreign to biological systems. The principle of •DPPH method is based on the reduction of •DPPH in the presence of a hydrogen-donating antioxidant. Samples reduce the colour of •DPPH due to their hydrogen-donating ability [30]. •DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers [31]. ABTS•+ is generated by the reaction of a strong oxidizing agent (e.g., potassium permanganate or potassium persulfate) with the ABTS•+ salt. Reduction of the blue-green-coloured ABTS•+ solution by hydrogen-donating antioxidant is measured by the suppression of its characteristic long-wave (734 nm) absorption spectrum [32]. The ABTS•+ method has the extra flexibility in that it can be used at different pH levels (unlike •DPPH, which is sensitive to acidic pH) and thus it is useful to study the effect of pH on antioxidant activity of various compounds [33].

Evaluation of the effect of temperature on •DPPH scavenging activity of MLP showed that the untreated sample had 88.3% radical scavenging ability. At initial stage of drying, 100 °C for 5 min or 10 min, and 150 °C for 5 min, the activity was 88.7%, 88.8% and 89.6%, respectively, which slightly increased (to 91.2–93.5%) at later stages, when the sample was treated for 15 min or 30 min (Tab. 2). Similar results were reported by WANGCHAROEN and GOMOLMANEE [22]. Their study investigated antioxidant activities of three varieties of *M. oleifera* leaves during drying in hot-air oven at 50 °C and 100 °C by 3 different methods, namely, ferric reducing antioxidant power (FRAP), •DPPH scavenging activity and ABTS•+ decolourization. Authors found that the values of •DPPH and ABTS•+ assays rapidly decreased in the first 2.5 h and then were quite constant, although some values slightly increased at the end of the drying process. They correlated the increased activity with the occurrence of antioxidant substances or phenolic compounds by thermal reactions such as non-enzymatic browning reactions. Similar possibilities are expected in the present study.

ABTS•+ activity of untreated sample was 5.6 mg·kg^{-1} of Trolox equivalent, which slightly decreased as temperature and time of treatment were increased. It decreased by 31.7% when duration of treatment was increased from 5 min to 30 min at 100 °C, whereas at 150 °C, the decrease in content was 71.2% for the same time interval (Tab. 2). These results do not follow the trend of phenolic and flavonoid content, thus it is shown that ABTS•+ activity of the material is not dependent on the phenolic and flavonoid contents, but it may be related to the content of other non-phenolic compounds in the material, which are affected by heat treatment.

Effect of temperature on α-glucosidase and pancreatic lipase inhibition activity

Alpha-glucosidase inhibitors are used as oral anti diabetic drugs for treatment of type 2 diabetes mellitus. The intestinal α-glucosidases hydrolyse complex carbohydrates to glucose and other monosaccharides in the small intestine. Inhibition of these enzyme systems helps to reduce the rate of carbohydrate digestion in alimentary canal [34]. Presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhoea, flatulence or abdominal bloating [35]. Therefore, natural glucosidase inhibitors from the dietary plants can be used as an alternative for effective treatment of hyperglycemia with minimal or no side effects.

Assessment of lipase inhibition activity in MLP was aimed to identify the potential of the product to cure obesity and cardiovascular diseases, which are known to cause high risk of type 2 diabetes [36]. Pancreatic lipase plays a key role in triglyceride absorption in the small intestine. This enzyme is secreted from the pancreas into the in-

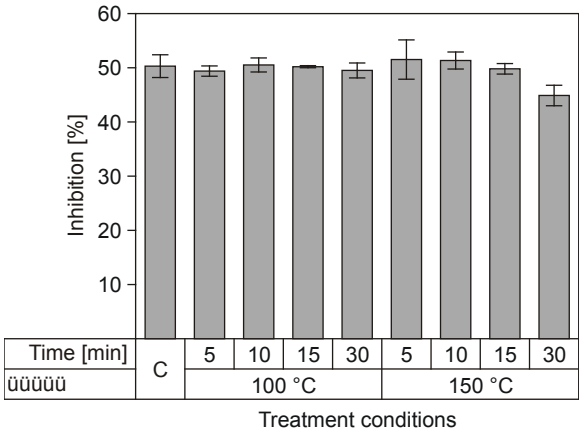

Fig. 1. Temperature effects on α-glucosidase inhibition activity of *M. oleifera* leaves powder.

C – control, untreated sample.

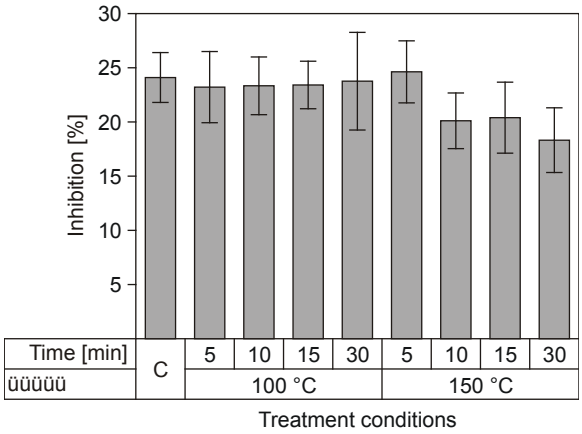

Fig. 2. Temperature effects on pancreatic lipase inhibition activity of *M. oleifera* leaves powder.

C – control, untreated sample.

testine and there it hydrolyses triglycerides into fatty acids. Thus, pancreatic lipase inhibitors are considered to be valuable therapeutic agents for treating diet-induced obesity and associated risks in humans [37].

Alpha-glucosidase inhibition activity of untreated material was 50.3%, which remained unaltered when the material was treated at 100 °C for 5, 10, 15 or 30 min, and at 150 °C for 5, 10 or 15 min (Fig. 1). At 30 min, the activity was decreased by 45%. This shows that the enzymatic inhibition activity is affected by the duration of treatment. This may be caused by inactivity and instability of the respective compounds at longer heat treatments. Similarly, when the effect of temperature on pancreatic lipase inhibition activity was assessed, it was found that untreated material exhibited 24% inhibition activity, which remained unaltered during the treatment of material at 150 °C for 5 min (Fig. 2). Longer heat treatment decreased the values and activity, which remained 20% and 18% when treated at 150 °C for 15 min and 30 min, respectively. These results demonstrate instability of the related compounds at higher temperatures at longer treatment times.

CONCLUSIONS

The present study explored the effect of temperature on α-glucosidase, lipase inhibition activity along with other nutritional properties of MLP, which are associated to antidiabetic efficacy of the material. Findings revealed that heat treatment at 150 °C for 15 min can be used safely for processed food preparation as, at this point of treatment, least detrimental effects were observed in flavonoid content, $ABTS^{\bullet+}$ scavenging ability as well as α-glucosidase and pancreatic lipase inhibition activities. Also the increased values of free amino acids content, phenolics content and $^{\bullet}$DPPH activity at this temperature and time makes it most suitable for therapeutic antidiabetic food preparation. Thus, the knowledge about the optimal temperature and time used for preparation of therapeutic food from *Moringa* leaves will help to increase the palatability of the material, which can be consumed daily for easy and effective management of type 2 diabetes.

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LAXMI PARWANI – YASHASVI BOHRA – SAKSHAM GUPTA – RAJESH KUMAR

The values expressed in article in milligrams per kilogram should read as grams per kilogram.

The authors would like to apologize for any inconvenience caused.

Laxmi Parwani, Yashasvi Bohra, Saksham Gupta, Rajesh Kumar, Department of Biotechnology, Dr. B. Lal Institute of Biotechnology, Jaipur 302017, Rajasthan, India.

Correspondence author:
Laxmi Parwani, tel.: +919982216197, e- mail: laxmi.bibt@gmail.com

