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original scientific paper

Fruit Extract Derived from a Mixture of Noni, Pineapple and Mango Capable of Coagulating Milk and Producing Curd with Antidiabetic Activities

Running title: Milk-Clotting Fruits Capable of Producing Antidiabetic Curd

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SUMMARY

Research background. *Morinda citrifolia* L. (noni), *Ananas comosus* L. cv. Sarawak (pineapple) and *Mangifera indica* L. cv. Apple (mango) represent fruits capable of coagulating milk, forming a curd. Plant-derived milk coagulants have antidiabetic phytochemicals that enrich the curd. Hence this work evaluated the dual benefits of the fruits in coagulating milk and the antidiabetic activities found in the curd produced by them.

Experimental approach. The three fruits were mixed to form a supercoagulant (a milk coagulant mixture of the extracts at a ratio of 1:1:1), and the milk coagulation time was measured. The milk was coagulated by the supercoagulant, and the fortified curd was tested for its ability to inhibit α -glucosidase

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and α -amylase. Then, the fortified curd was fed daily to streptozotocin-induced diabetic rats and compared with the untreated diabetic rats and normal rats related to their biochemical markers such as blood glucose level, aspartate aminotransferase, alanine transaminase etc and histopathology of their liver and kidney tissues.

Results and conclusion. The supercoagulant had a milk coagulation time of (28 ± 3) s at a 50 mg/mL concentration. Its fortified curd inhibited α -glucosidase and α -amylase, with IC_{50} values of (4.04 ± 0.03) mg/mL and (3.42 ± 0.02) mg/mL, respectively. The average mass of the streptozotocin-induced diabetic rats fed daily with curd formed by the supercoagulant (SC) was (201 ± 10) g on day 20 in comparison to diabetic control rats (DC), (149 ± 16) g. The blood glucose level after fasting for SC was (15 ± 1) mmol/L compared to DC rats, (26 ± 2) mmol/L. Blood tests on SC for aspartate aminotransferase, alanine transaminase, gamma-glutamyl transferase and alkaline phosphatase (liver function tests) levels were shown to be (214 ± 78) U/L, (91 ± 13) U/L, 3 U/L and (510 ± 138) U/L, respectively, while the total protein test and renal function tests showed the levels of albumin, globulin, urea and creatinine to be (37 ± 2) g/L, (30 ± 2) g/L, (11 ± 1) mmol/L and (42 ± 3) μ mol/L, respectively. These levels were found to be approximately similar to those of the normal rats on day 20. Furthermore, a histopathological study performed on the liver and kidney of the rats found no apparent damage.

Novelty and scientific contribution. This supercoagulant derived from a mixture of fruits is able to coagulate milk rapidly, and its curd is fortified with safe antidiabetic agents. The supercoagulant potentially useful in producing functional dairy food to prevent diabetes or as supplement for diabetics to control their blood sugar. Such products capable to replace dairy products derived from animal enzymes or add to existing functional dairy products for selection by consumers.

Keywords: milk coagulation; antidiabetic curd; streptozotocin-induced diabetes; *Morinda citrifolia*; *Mangifera indica*; *Ananas comosus*

INTRODUCTION

Milk and dairy products are known for their health benefits due to the biologically active components they contain, including bioactive peptides, organic acids, vitamins and others. Curd is a type of dairy product formed from the curdling of milk through the coagulation process. The most common protein found in mammalian milk is casein. The clotting of milk can be described as the clumping of casein due to the distorting effects of the coagulant that eventually cause the formation of gel-like structures

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capable of absorbing some water as well as trapping fat globules (1). In general, milk coagulation can be achieved by the addition of enzymes, acid treatment, or heat-acid treatment. In cheese manufacturing, enzymatic coagulation of milk by chymosin (rennet) is widely used, an enzyme typically isolated from the stomachs of young ruminant animals that may be considered to be unethical. Other sources of milk coagulant are extracted from animal sources, including adult cows and pigs, which have come into conflict with religious beliefs. Moulds, mostly genetically engineered, that have the capability to produce proteolytic enzymes, are used to produce microbial rennet as an alternative to animal-sourced coagulants. However, these products have raised serious concerns, as genetically modified (GM) foods in food production have been claimed to cause life-threatening allergic reactions and they have been banned in France, Germany, and the Netherlands (2).

The increasing demand for dairy products such as cheese, yogurt and others globally has led to research seeking an alternative source of coagulant to form curd, and one such source is plants. Many plant coagulants have been identified to have milk-coagulating capabilities and therefore they can be used as an alternative to commercial coagulants (3). However, plant-derived coagulants come with problems such as the longer time needed for a complete coagulation process compared to rennet, a potential bitter flavour and an inconsistent texture of the curd (2). These challenges can potentially be overcome by combining a number of plants known for their ability to coagulate milk. Therefore, a 'supercoagulant' of plant extracts was established to enhance and improve the coagulation of milk. Supercoagulant is a simple term given within this report to describe a combination of three plant extracts made up of mango (*Mangifera indica*), pineapple (*Ananas comosus*) and noni (*Morinda citrifolia*). The supercoagulant, apart from coagulating the milk, is expected to enrich the curd formed with secondary metabolites or phytochemicals with medicinal properties.

Secondary metabolites play important roles in plants, including defence or preventive mechanisms against infections and diseases. Once plants are consumed by humans and animals, phytochemicals are useful and they have many biological capabilities. One such potential is in the prevention of diabetes, a disease that is becoming a major public health concern. Diabetes, especially type 2 diabetes, is of concern to people consuming food rich in sweeteners. The International Diabetes Federation (IDF) stated that the prevalence of diabetes among Malaysian adults over 18 years of age was 16.9 %, with 3.49 million reported cases of diabetes in 2017 (4). α -Glucosidase and α -amylase are the key enzymes involved in the hydrolysis of carbohydrates to glucose and other monosaccharides. Due to the absence or ineffectiveness of insulin found in diabetic patients, the hydrolysis of carbohydrates can

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cause an accumulation of glucose, leading to hyperglycaemia (5). To counter this, these enzymes need to be inhibited, and it has been found that the phytochemicals made by plants are capable of doing this (6-8). Many studies have been performed on the antidiabetic properties of plants, but none have investigated the curd that is formed by them by coagulation of milk. Hence, this study focuses on milk coagulation by the supercoagulant to form curd and the antidiabetic properties that it incorporates within the curd after coagulation. Supercoagulant is the term used for a coagulant mixture that contains the three plant extract samples of *M. citrifolia* L., *A. comosus* L. cv. Sarawak and *M. indica* L. cv. Apple.

MATERIALS AND METHODS

Sample

Mangifera indica L. cv. Apple, *Morinda citrifolia* L. and *Ananas comosus* L. cv. Sarawak were obtained from various cultivators at region of Kuantan, Malaysia and their voucher specimens were deposited at the Institute of Biological Sciences, University of Malaya, Malaysia with voucher numbers of HI1445, HI1446 and HI1447, respectively. The seeds (seed coat removed) of *M. indica* and the fruits of *A. comosus* and *M. citrifolia* were washed thoroughly to remove unwanted contaminants and then cut into pieces. The *M. indica* seeds were dried in an oven (Universal Oven UN Model Size 55, Memmert GmbH, Schwabach, Germany) with convection at 50 °C until dry and brittle enough to be pulverized into powder. *A. comosus* and *M. citrifolia* were used fresh to avoid spoilage by fungi under drying.

Preparation of extracts

A total of 10 % (m/V) powdered *M. indica* seeds were mixed in water for aqueous extraction. The mixture was spun at a constant speed in a magnetic stirrer (Nuova II Stir Plate, Thermolyne Corporation, USA) for 5 hours before removing large particles with a coarse muslin cloth filter followed by fine filtration with Whatman Grade 1 filter paper (Sigma-Aldrich, St. Louis, USA). The supernatant was freeze-dried (Labconco™ FreeZone™ Console Freeze Dryer, Fisher Scientific, Kansas City, USA) and kept until further use. Each sample of *A. comosus* and *M. citrifolia* at 10 % (m/V) in water were squeezed in a perforated stainless-steel filter and consequently fine filtered in Whatman Grade 1 filter paper (Sigma-Aldrich, St. Louis, USA) for their saps before being freeze-dried.

Super-coagulant formation

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M. citrifolia, *M. indica* and *A. comosus* extracts were combined at a ratio of 1:1:1, i.e. 10 g each was homogenized and dissolved completely in 500 mL of deionized distilled water. This mixture was then freeze-dried to obtain the supercoagulant powder, which was used throughout this study. Type II rennet with chymosin as the main constituent sourced from *Rhizomucor meiheii* (Sigma-Aldrich, St. Louis, USA) was used as the coagulant control for comparison.

Determining the milk coagulation time

The time was recorded after confirming three parameters: the change in viscosity and colour as well as white spots appearing in a drop of milk supplemented with coagulant were verified under a light microscope (Nikon Eclipse E100, NY, USA, fitted with a digital imager, DinoEye Eyepiece Camera Software) at intervals of every 5 s. The milk mixture tested included 1 mL of 10 % (*m/V*) skim milk (BD Difco, US) with 0.5 mL of either 5 % (*m/V*) supercoagulant or 1 % (*m/V*) rennet exposed to conditions of pH=6.5, 35 °C and 10 mM CaCl₂ (Sigma-Aldrich, St. Louis, USA). Experiments were performed in quadruplicate. The pH=6.5 was used as it is the natural pH of milk while CaCl₂ functions as a cofactor for milk coagulation by promoting the aggregation of casein micelles (9). The coagulant concentrations used was 5 % (*m/V*) supercoagulant and 1 % (*m/V*) rennet and this is to achieve a standard coagulation time of about 30 s for both the coagulants as it is ideal to be managed in laboratory conditions for the purpose of analysis. A choice of too rapid coagulation time was found to complicate the analysis and comparison between the coagulants.

Determining the visible changes and measuring the calcium content of the coagulated milk

The observations were performed on supercoagulant coagulated milk, rennet coagulated milk and milk alone (control). In each test, 1 mL of 10 % (*m/V*) skim milk (BD Difco, Sparks Maryland, USA) was treated with 0.5 mL of either 5 % (*m/V*) supercoagulant or 1 % (*m/V*) rennet or milk alone under conditions of pH=6.5, 35 °C and 10 mM CaCl₂ (Sigma-Aldrich, St. Louis, USA). The samples were centrifuged at 10 062×*g* for 60 s using a microcentrifuge (Z 216 MK, Hermle, Germany) to separate the curd and whey for comparison with each other. Next, in separate tests, structural changes were observed using a standard light microscope (Nikon Eclipse E100, NY, USA, fitted with a digital imager, DinoEye Eyepiece Camera Software) at low magnification. Finally, the structural changes were also viewed at a higher resolution using a scanning electron microscope (Fei Quanta 50, Thermo Fisher Scientific, Cleveland, USA).

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For determining the amount of calcium, all parameters were similar to those for the evaluation of the visible changes except without the addition of CaCl_2 , as this study investigated the contributions of the *in situ* calcium present in milk. After being left overnight for coagulation, the curds together with whey and the milk alone were freeze-dried before measuring the calcium content by using an ICP-OES (Optima 7300 DV, Perkin Elmer Inc., Shelton, USA).

Curd fortified with super-coagulant for determining antidiabetic activities

The supercoagulant curd (Fig. S1a) was prepared by allowing 5% (m/V) of the supercoagulant to react completely overnight with 10% (m/V) skim milk (BD Difco, USA) in conditions of pH=6.5, 35 °C and 10 mM CaCl_2 . Centrifugation (Z 216 MK, Hermle, Germany) was performed at $10\,062\times g$ for 2 min to remove the whey, and only the curd was freeze-dried and then used for further study. Similarly, the prepared curd formed by 1% (m/V) rennet (Fig. S1b) was used as a control.

α -glucosidase inhibitory assay

This assay was a modification of a previous method (10). Briefly, an equal volume of various sample aqueous solutions was mixed with 1 U/mL of the enzyme (α -glucosidase isolated from *Saccharomyces cerevisiae*, Sigma-Aldrich, St. Louis, USA) in potassium phosphate buffer, pH=6.9, and incubated for 30 min at 37 °C. This was followed by an additional 15 min of incubation after adding 10 mM *p*-nitrophenol- α -glucopyranoside (Sigma-Aldrich, St. Louis, US). The reactions were stopped by adding 100 mM Na_2CO_3 (Merck, Germany) to the mixture. The samples tested included supercoagulant curd, rennet curd and acarbose (100 mg of Glucobay tablet, Bayer Inc., USA), plus a control without any sample. All of the samples were conducted in multiples of five for the calculation of standard errors. The absorbance was measured at 405 nm using microplate reader (Infinite 200 PRO equipped with Magellan software, Tecan, Switzerland) and the % inhibitory activity was calculated with the following equation:

$$\text{Inhibitory activity} = \left(\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \cdot 100 \quad /1/$$

where *A* is the absorbance reading at the specified wavelength.

A graph of % enzyme inhibitory activity versus the concentration of the sample was used in estimating the IC_{50} value.

α -amylase inhibitory activity assay

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The assay was a modification of a previous method (11). Briefly, 250 μ L samples in increasing concentrations were mixed with 50 μ L of 2 U/mL of the enzyme (α -amylase isolated from pig, Sigma-Aldrich, St. Louis, USA) in potassium phosphate buffer (Sigma-Aldrich, St. Louis, USA), pH=6.9, and incubated for 30 min at 25 °C. Next, the cells were incubated for an additional 10 min once starch (1 % *m/V*) (Sigma-Aldrich, St. Louis, USA), was added as a substrate for the reaction. The reaction was terminated by adding 50 μ L of 3,5-dinitrosalicylic acid or DNS (Sigma-Aldrich, St. Louis, USA) at 85 °C and immediately cooling for 5 min to room temperature. The samples tested included supercoagulant curd, rennet curd and acarbose (100 mg of Glucobay tablet, Bayer Inc., USA), and a control without any sample. All samples were conducted in multiples of five for the calculations of standard errors. The absorbance was measured at 540 nm using microplate reader (Infinite 200 PRO equipped with Magellan software, Tecan, Switzerland), and the % inhibitory activity was calculated with Eq. 1. A graph of % enzyme inhibitory activity versus the concentration of the sample was used for estimating the IC₅₀ value.

Determining the effects of the super-coagulant curd in rats induced with diabetes

Approval to conduct an *in vivo* study was obtained from the Animal Ethics Committee of Universiti Malaysia Pahang (Approval No. UMPIACUC/2020/02) for the use of 12 Sprague-Dawley male rats. The rats, weighing between 200 and 300 grams, were allowed to acclimatize for 2 weeks to laboratory conditions. The experiment involved three groups of rats with 4 in each group: normal control (NC), diabetic control (DC) and supercoagulant curd (SC). Diabetes conditions were induced by streptozotocin (Sigma-Aldrich, St. Louis, USA) using a previously described method (12). Briefly, the rats in the DC and SC groups were injected IP (intraperitoneally) with 60 mg/kg streptozotocin dissolved in 50 mM sodium citrate buffer (Sigma-Aldrich, St. Louis, USA) at pH=4.5. Throughout the day, the rats were given 10 % (*m/V*) sucrose (Sigma-Aldrich, St. Louis, USA) in water and standard food pellets (Gold Coin Feed Mills Sdn. Bhd. Kuala Lumpur, Malaysia) for the initiation of the hypoglycaemia effect. The next day, they were given water and food *ad libitum*. Their initial mass and blood glucose levels were measured before (day 0) and after (day 5) inducing diabetes, with the blood glucose level of the latter being greater than 22 mmol/L.

The experiment commenced by giving the NC and DC groups 2 mL of water alone while SC was dosed with 300 mg/kg supercoagulant curd daily using oral gavage for a total of 20 days. Consequently, the mass and blood glucose after fasting were measured (Accu-Check Performa Glucometer, Roche, Germany) for each rat at intervals of every 5 days. On day 20, the rats were sacrificed humanely by

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overdose with a ketamine and xylazine mixture (LASAM, UKM, Malaysia). A total of 1.0 mL blood was drawn directly from the heart once the midline of the abdomen was cut open to reveal the abdomen cavity. Next, the liver and right kidney were dissected and preserved in 10 % formalin (Merck, Germany). The blood was sent in vacutainers for biochemical parameter diagnostics at Gribbles Pathology Laboratory, Malaysia. The organs were xylene processed, embedded in paraffin wax, microtome sliced into 5-micron sections, and then stained with haematoxylin and eosin, and the prepared slides were carefully graded under a light microscope (Nikon, Eclipse TS100) by an expert pathologist as described previously (13).

Statistical analysis

Data analysis was performed using IBM Statistical Product and Service Solutions (SPSS) Statistics version 25 tools (IBM Corp, NY, USA) (14). All of the samples were conducted in multiples of five for the calculation of standard errors and data were expressed as mean \pm standard error. The results were considered significant at p-values less than 0.05. An independent t-test was used to compare the data within the group while one-way ANOVA was used to compare the data between groups, followed by Tukey's post hoc test for multiple groups comparison.

RESULTS AND DISCUSSION

Milk coagulation and fortification of the curd

The supercoagulant contains extracts from *M. citrifolia* L., *A. comosus* L. cv. Sarawak and *M. indica* L. cv. Apple mixed in a ratio of 1:1:1. The ratio was selected as the protein and phytochemical content within the fruits vary from batch to batch and therefore, an equal mixing of the three fruits is required so that they can contribute equally to the milk coagulating ability and biological activity of the supercoagulant. These fruits have the ability to coagulate milk due to the presence of milk-coagulating proteases. It was found that 5 % (*m/V*) supercoagulant had a coagulation time of (28.33 \pm 2.89) s, while 1 % (*m/V*) rennet had a coagulation time of (31.67 \pm 2.89) s (data not shown). Rennet (a commercial milk coagulant) was chosen to be used randomly at a concentration five times lower for testing than supercoagulant, as the former is a purified protease, while the supercoagulant crude plant extracts are known to contain mostly non-protein constituents. Earlier studies have proven that milk-coagulating proteases are present in each of the fruits that make up the supercoagulant. Studies have shown that *M. citrifolia* contains proteases that are able to coagulate milk, while bromelain, a protein commonly found

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in *A. comosus*, has also been found to have milk-coagulating capability (15-16). Previous studies have also shown that *M. indica* L. cv. *Apple* has milk-coagulating capability due to the proteases found in it (17).

Fig. 1 shows the visible changes in pure milk, supercoagulant coagulated milk and rennet coagulated milk after centrifugation, light microscope analysis and SEM analysis. There was no separation observed for pure milk (Fig. 1a), while clear separation was observed for milk supplemented with supercoagulant (Fig. 1b) and rennet (Fig. 1c) after centrifugation. The centrifuging performed on the supercoagulant and rennet coagulated milks showed that coagulation has occurred as the milk components consist of the solid curd and the liquid whey, which only separate after the coagulation of milk (18). Additionally, for microscopic analysis, the pure milk (Fig. 1a) shows a confluent milk structure with no dark spots or aggregation of casein, while the supercoagulant (Fig. 1b) and rennet (Fig. 1c) coagulated milks show obvious dark spots and colloidal structures of aggregation of casein micelles. For the SEM analysis, the pure milk in Fig. 1a has a smooth surface with hair-like structures that can be seen, which could be casein micelles with kappa-casein (19). Spaced-out structures can be seen that show no aggregation of molecules and thus no coagulation. On the other hand, for the supercoagulant Fig. 1b and rennet Fig. 1c coagulated milk, instead of a smooth surface, both display a rough surface with no clear see-through spaces or translucent areas between the structures because the particles have aggregated together due to coagulation. The dark aggregated spots during light microscopy and SEM analysis represent the aggregated casein micelle that would form the curd, while the clear spaces between them represent the whey (20). It can also be seen that rennet forms a denser curd than supercoagulant, as there are fewer spaces between the structures. This is because rennet is a purified protease, and therefore, it cleaves κ -casein at the Phe₁₀₅–Met₁₀₆ bond in the casein micelles more effectively than supercoagulant, forming a more complex three-dimensional network of chains and clusters (21). A study also reported that low proteolytic activity forms a denser protein network, which is why the rennet coagulated milk structure is denser (22).

The casein micelles are made up of casein submicelles that contain colloidal calcium phosphate clusters and a “hairy” layer of protruding κ -casein, which gives the micelle steric and electrostatic stability (23). The function of calcium phosphate nanoclusters in milk is to prevent precipitation and calcification of the milk and to interlock the protein strands within the casein micelle (24). During cheese-making, a specific protease is added to the milk to cleave κ -casein on the surfaces of the casein micelles, which will lower the repulsive forces between them and cause aggregation (23). Theoretically, when κ -casein

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is cleaved, calcium phosphate nanoclusters might also become disrupted and solubilize, which would lead to an increase in the total calcium content within the curd during coagulation of milk. Calcium is an essential element in the secondary stage of coagulation, where it helps with the aggregation of casein micelles (25). Fig. 2 shows the pure milk, supercoagulant coagulated milk and rennet coagulated milk after determination of the calcium content using ICP-OES. The concentration of calcium in pure milk was 1654 mg/kg, and this value is similar to a previous study, which found that commercial skim milk in South Korea contains 1184 mg/kg calcium (26). Accordingly, a surge in calcium release was detected, and its content in rennet coagulated milk was almost twice as high as the calcium content of supercoagulant coagulated milk. This may be because rennet is a purified form of protease; therefore, the cleavage and coagulation it carries out is much more efficient and specific than supercoagulant, which is in a crude form.

In vitro antidiabetic activities of fortified curd

The inhibition of two important polysaccharide-degrading enzymes, α -glucosidase and α -amylase, by the supercoagulant curd is shown in Fig. 3. The inhibition by supercoagulant was compared with Acarbose, a common drug used to treat type 2 diabetes mellitus. The IC_{50} values for α -glucosidase for acarbose and the supercoagulant curd were (0.05 ± 0.02) and (4.04 ± 0.03) mg/mL, respectively. The IC_{50} values for α -amylase were (0.03 ± 0.02) and (3.42 ± 0.02) mg/mL, respectively. The supercoagulant curd was found to have significant antidiabetic properties even though its IC_{50} value was comparably higher than that of acarbose. The supercoagulant curd had an IC_{50} value compared to the no IC_{50} value achieved for the rennet curd. This is due to the presence of phytochemicals within the plant extracts that give the supercoagulant curd its antidiabetic properties. *M. indica* seed extract and the fruit extracts of *M. citrifolia* have been reported to possess excellent antidiabetic activity based on studies conducted in tissue culture and animal models where they found to inhibit the α -glucosidase and α -amylase enzymes and bring down the blood sugar level (27).

For α -glucosidase inhibitory activity, *M. indica* seed extract and fruit extracts of *M. citrifolia* have been found to have IC_{50} values of 0.34 and 0.2 mg/mL, respectively (27-28). Similarly, for α -amylase inhibitory activity, *M. indica* seed extract and fruit extracts of *M. citrifolia* have been found to have IC_{50} values of 0.71 and 2.62 mg/mL, respectively (27-29). When compared with the literature, the supercoagulant curd's α -glucosidase and α -amylase inhibitory activities were not as strong as those of its individual fruits. The possible differences between the IC_{50} values may be due to several factors, and

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one factor is the leaching out of the antidiabetic components into the discarded whey during curd production (30). Other factors include differences in ripeness, varieties of fruits, and growth conditions (31). Nevertheless, the supercoagulant curd was still able to provide significant antidiabetic activity as opposed to rennet curd, which has no IC_{50} value.

In vivo antidiabetic activities and safety evaluations of the fortified curd

The supercoagulant curd was then subjected to *in vivo* testing of its antidiabetic properties in rats. Fig. 4 shows the mean body mass and glucometer measurements for the fasting blood glucose level in normal control (NC), diabetic control (DC) and supercoagulant curd (SC) rats. The parameters showed no changes on day 0 for NC or for both DC and SC prior to the diabetes induction of the rats ($p > 0.05$). The mass progressively increased for NC rats, being the heaviest on day 20 with a mean body mass of (355 ± 24) g. Growth can be defined as a progressive increase in body mass of an animal during a specific time period due to the accumulation of protein, fat and bone in the animals, which is why there is a constant increase in body mass in the NC group (32). On day 20, the DC group of rats had a mean body mass of (149 ± 16) g, an unfavourable reduction of 34 % compared to the initial mean body mass, (227 ± 13) g. The SC group of rats also showed a reduction of mean body mass but only by approximately 11 %. The mean body mass of diabetic rats was found to decrease over time as the pancreatic beta cells were damaged, causing less insulin production. This prevents the breakdown of glucose in body cells; therefore, the body cannot use glucose as a source of energy but instead uses proteins and fats in the body, which leads to a loss of body mass (33). However, oral treatment of the rats with supercoagulant curd (SC) reduced the drastic mass loss observed in the untreated animals (DCs).

For the fasting blood glucose levels, the measurements showed elevated DC and SC once the rats were induced to be diabetic at day 1. The DC blood glucose level remained high and somewhat flat throughout the measurements until a final reading of (26 ± 2) mmol/L in comparison to SC, where it was reduced to (15 ± 1) mmol/L; however, this value was still found to be almost twice the value of NC, (8 ± 1) mmol/L on day 20. All of the differences in measurements for DC and SC were found to be statistically significant compared to NC ($p < 0.05$). The administration of streptozotocin destroys pancreatic beta cells and causes less insulin to be released into the body, which leads to the accumulation of glucose and the development of constant hyperglycaemia (34). The hydrolysis of carbohydrates to glucose and other monosaccharides in the body is caused by enzymes such as α -glucosidase and α -amylase. Due to the absence or ineffectiveness of insulin, the hydrolysis of carbohydrates causes an accumulation of glucose.

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Antidiabetic drugs such as acarbose are used to inhibit these enzymes so that the glucose level remains low in the body (5). Supercoagulant curd was found to have a similar effect to these drugs, as it was also found to lower the blood glucose level in the SC group rats. Studies have shown that certain plants have the capability to inhibit the α -glucosidase and α -amylase enzymes to lower blood glucose levels, and similarly, the supercoagulant curd was found to have this capability during *in vitro* antidiabetic studies (35). The supercoagulant was able to reduce the increasing fasting blood glucose level, as evident in the diabetic rats after day 20.

Several biochemical diagnostic parameters were evaluated in the rat blood and are shown in **Table 1**. The levels measured for aspartate aminotransferase, alanine transaminase, gamma-glutamyl transferase and alkaline phosphatase were significantly higher between the DC and NC groups of rats ($p < 0.05$). The levels of aspartate aminotransferase, alanine transaminase and gamma-glutamyl transferase were similar to those in SC rats, while alkaline phosphatase levels were found to be low compared to those in untreated DC rats.

When liver tissues are damaged, additional enzymes are released into the bloodstream and raise the enzyme level in the blood (36). The enzymes in the DC group were significantly higher than those in the other groups due to liver cell injury by streptozotocin in diabetic rats, which increased the release of liver enzymes into the blood (37). On the other hand, the SC group had enzyme levels that were significantly lower than those of the DC group ($p < 0.05$), which shows that even though the SC group was initially diabetic, treatment with the supercoagulant curd helped lower the diagnostic enzyme levels. The treatment was effective in lowering all of the enzyme levels of the diabetic rats to almost normal levels except for alkaline phosphatase. The alkaline phosphatase level of the SC group did not decrease as much as that of the other enzymes, but it still showed a significant reduction in alkaline phosphatase levels compared to the DC group.

The same trend was also observed for the protein levels tested, whereby the albumin, globulin and total protein values were similar between NC and SC but were significantly reduced for the DC group of rats ($p < 0.05$). A total protein test was used to determine the amount of protein in the blood and to diagnose liver and kidney functions. If the total protein level is low, there may be a liver or kidney problem because the protein is not digested and absorbed properly by the body (38). The serum albumin level in the DC group was significantly lower than that in the NC group, possibly due to increased urinary excretion or low hepatic synthesis of albumin (39). However, this was not found for the SC group, as the albumin level was found to be significantly higher ($p < 0.05$) than that in the DC group and similar to that

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in the NC group. The DC group also showed lower levels of globulin than the NC and SC groups, but the difference was not significant ($p>0.05$). The total protein level is the measure of the total amount of albumin and globulin in the body, and the total protein level of the DC group was significantly lower than that of the NC and SC groups ($p<0.05$). This is because in diabetic rats, intense changes in protein metabolism and loss of nitrogen from organs take place, which causes a negative nitrogen balance (40). This was not seen in the SC group, as the protein level was found to be significantly higher due to the therapeutic effects of the supercoagulant curd.

For renal function diagnostics, urea and creatine were the highest in DC rats compared to NC and SC rats. A renal function test was used to determine the function and damage that occurred in the kidney due to diabetes in the rats. Urea and creatinine are waste products in the blood that are eliminated by the kidney. A high level of these waste products in the blood indicates damage to the kidney due to diabetes (41). The DC group had a significantly higher urea level than the NC group ($p<0.05$), which shows that the urea level in the blood is high due to significant renal damage. The rats in the SC group showed significantly lower urea levels ($p<0.05$) than those in the DC group. The DC group also showed the highest creatinine level compared to the other groups due to renal damage, which causes an accumulation of creatinine in blood. These levels of SC were comparable to those of the NC group, which shows that the treatment was effective in improving kidney function.

The livers of all of the rats from the NC, DC and SC groups, as shown in Figs. 5a, 5b and 5c, respectively, had normal morphology and no signs of damage. The central vein was prominent, and the hepatic sinusoids were well arranged amidst healthy parenchyma, which consisted of hepatocytes with prominent round nuclei and abundant cytoplasm. Untreated diabetes is usually associated with damage to the liver, as it increases oxidative stress and causes an aberrant inflammatory response that activates the transcription of proapoptotic genes and damages hepatocytes in the liver (42). It was found that the DC group had no overall structural changes visible as evaluated by the pathologist. These observations are common in an acute study. The duration of the current study was only 20 days and hence generalized as an acute study rather than a chronic study, which is usually 8 to 10 weeks (43-44). Even though the liver function test revealed a preliminary elevation of biochemical markers in the blood, an acute study period of 20 days is insufficient to cause observable liver damage. According to a previous study, enlargement of the sinusoids and focal microvesicular fatty degeneration with vacuolization of liver cells could be seen only in the 6th week of diabetes (45).

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The kidneys of the rats from the NC, DC and SC groups (Figs. 5d, 5e and 5f, respectively) showed adequate glomeruli that were well spaced with normal orientation of the tubules. The interstitium had no inflammation or oedema, and the tubules were empty with no casts or haemorrhage. The main function of the kidney is to remove waste from the blood and return the cleaned blood back to the body. Diabetes causes an accumulation of glucose in the body, and over time, a high level of glucose will damage the blood vessel clusters called glomeruli in the kidney, which filters waste from the blood (46). This was not seen for the diabetic rats, as this study was an acute study instead of a chronic study. The glucose in the blood of the DC group rats did not cause observable damage to the kidney 20 days after induction of diabetes, and a chronic study is required for significant damage to take place even though the renal function test revealed a preliminary elevation of biochemical markers of the urea and serum creatinine levels in the blood. Likewise, the SC group of rats showed similar normal morphology without any observable damage. Such an outcome is attributed to the ability of medicinal plants that are considered to have antidiabetic capabilities to ensure that blood glucose is low. Generally, most medicinal plants have been indicated to be protective towards vital organs such as the liver rather than toxic (47). However, such protection or toxicity, if any, is best evaluated over a long duration, *i.e.* in chronic assessment of the liver. Additionally, in both liver and kidney histopathology evaluations the curd containing the plant phytochemicals were also concluded having no immediate toxicity to these organs.

CONCLUSIONS

This study focused on finding a plant-based coagulant with dual effects of coagulating milk to form curd and giving the curd medicinal properties at the same time. *M. citrifolia*, *A. comosus* and *M. indica* were mixed to form a supercoagulant. The supercoagulant demonstrated good milk coagulation capability, which was evident upon comparison to rennet. Supercoagulant-fortified curd was also found to have significant *in vitro* and *in vivo* biological activities in rats. Histopathological study was also performed on the liver and kidney of the rats, and no damage was observable, which indicates that the curd is safe for consumption. Having evaluated all of these results, it can be concluded that the supercoagulant can serve as a raw material for preparing functional dairy food with dual effects of coagulating milk and antidiabetic activities. Hence the functional dairy food capable to substitute or add to the commercial animal dairy products and as supplements for the prevention of diabetic as well as for diabetics to control their blood sugar level.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary materials are available at www.ftb.com.hr.

AUTHORS' CONTRIBUTION

Jaya Vejayan contributed in the co-conception and the overall supervision of the project. He also contributed to rewriting, editing, formatting, revisions and finalizing the initial draft of the article to the final article. Rupbansraaj Bathmanathan in performing all of the experiments for results, data analysis and authoring the first draft. Sharifah Aminah Tuan for repeating selected experiments for confirmation of the results and improving the standard error calculations. Srikumar Chakravathy: contributed as the expert pathologist on all histology work. Last but not least, Halijah Ibrahim contributed in the co-conception of the project and critical revision of the article.

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REFERENCES

1. Dalgleish DG. On the structural models of bovine casein micelles—review and possible improvements. *Soft matter*. 2011;7(6):2265-72.
<https://doi.org/10.1039/C0SM00806K>
2. Shah MA, Mir SA, Paray MA. Plant proteases as milk-clotting enzymes in cheesemaking: a review. *Dairy Sci Technol*. 2014;94(1):5-16.
<https://doi.org/10.1007/s13594-013-0144-33>. Bathmanathan R, Yahya Y, Yusoff M, Vejayan J. Utilizing coagulant plants in the development of functional dairy foods and beverages: A Mini Review. *J. Biol. Sci.* . 2019;19(3):259-71.
<https://doi.org/10.3923/jbs.2019.259.271>
4. International Diabetes Federation. Diabetes facts & figures. International Diabetes Federation; 2019. Available from <https://idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html>.
5. Poovitha S, Parani M. *In vitro* and *in vivo* α -amylase and α -glucosidase inhibiting activities of the protein extracts from two varieties of bitter melon (*Momordica charantia* L.). *BMC Complem Altern M*. 2016;16(1):1-8.
<https://doi.org/10.1186/s12906-016-1085-1>
6. Bahadoran Z, Tohidi M, Nazeri P, Mehran M, Azizi F, Mirmiran P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. *Int J Food Sci Nutr*. 2012;63(7):767-71.
<https://doi.org/10.3109/09637486.2012.665043>
7. Crawford P. Effectiveness of cinnamon for lowering hemoglobin A1C in patients with type 2 diabetes: a randomized, controlled trial. *J Am Board Fam Med*. 2009;22:507-12.
<https://doi.org/10.3122/jabfm.2009.05.080093>
8. Fazel Nabavi S, Thiagarajan R, Rastrelli L, Daglia M, Sobarzo-Sanchez E, Alinezhad H, Mohammad Nabavi S. Curcumin: a natural product for diabetes and its complications. *Curr Top Med Chem*. 2015;15(23):2445-55.
<https://doi.org/10.2174/1568026615666150619142519>
9. Lucey, J., & Fox, P. (1993). Importance of calcium and phosphate in cheese manufacture: a review. *J Dairy Sci*. 1993;76(6):1714-1724.
[https://doi.org/10.3168/jds.s0022-0302\(93\)77504-9](https://doi.org/10.3168/jds.s0022-0302(93)77504-9)
10. Yilmazer-Musa M, Griffith AM, Michels AJ, Schneider E, Frei B. Grape seed and tea extracts and

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catechin 3-gallates are potent inhibitors of α -amylase and α -glucosidase activity. J Agr Food Chem. 2012;60(36):8924-29.

<https://doi.org/10.1021/jf301147n>

11. Ademiluyi AO, Oboh G. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (alpha-amylase and alpha-glucosidase) and hypertension (angiotensin I converting enzyme) *in vitro*. Exp Toxicol Pathol. 2013;65(3):305-9.

<https://doi.org/10.1016/j.etp.2011.09.005>

12. Di Filippo C, Marfella R, Cuzzocrea S, Piegari E, Petronella P, Giugliano D, D'Amico M. Hyperglycemia in streptozotocin-induced diabetic rat increases infarct size associated with low levels of myocardial HO-1 during ischemia/reperfusion. Diabetes. 2005;54(3):803-10.

<https://doi.org/10.2337/diabetes.54.3.803>

13. Vejayan J, Yahya YAC, Chakravarthi S, Bathmanathan R, Ibrahim H, Yun A. Tongkat Ali plants of *Eurycoma longifolia* and *Stema tuberosa* stimulate sexual arousal in domestic cocks. MJS. 2020;39(1):1-14.

<https://doi.org/10.22452/mjs.vol39no1.1>

14. IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

15. de Farias VA, da Rocha Lima AD, Costa AS, de Freitas CDT, da Silva Araújo IM, dos Santos Garruti D, de Oliveira HD. Noni (*Morinda citrifolia* L.) fruit as a new source of milk-clotting cysteine proteases. Food Res Int. 2020;127:108689.

<https://doi.org/10.1016/j.foodres.2019.108689>

16. Komansilan S, Rosyidi D, Radiati LE, Purwadi P, Evanuarini H. The physicochemical characteristics and protein profile of cottage cheese produced by using crude bromelain enzyme extracted from *Ananas Comosus*. Curr Res Nutr Food Sci. 2021;9(2).

<https://doi.org/10.12944/crnfsj.9.2.21>

17. Vejayan J, Munir N, Lian YL, Bathmanathan R, Ibrahim H, Chakravarthi S. Tropical fruits of mango and noni having dual effects of coagulating milk and enriching the curds with micro-constituents of medicinal potential. MJS. 2019;38(3):34-7.

<https://doi.org/10.22452/mjs.vol38no3.4>

18. Hallén E, Lundén A, Allmere T, Andrén A. Casein retention in curd and loss of casein into whey at chymosin-induced coagulation of milk. J Dairy Res. 2009;77(1):71-6.

<https://doi.org/10.1017/S0022029909990434>

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19. McMahon DJ, Brown R, Ernstrom CA. Enzymic coagulation of milk casein micelles¹. J Dairy Sci. 1984;67(4):745-8.
[https://doi.org/10.3168/jds.S0022-0302\(84\)81364-8](https://doi.org/10.3168/jds.S0022-0302(84)81364-8)
20. Vejayan J, Zulkifli AA, Syed M, Saufi NM, Ibrahim H, Ambu S. Uncovering a protease in snake venom capable to coagulate milk to curd. IJABR. 2017;8(4):409-23.
<https://drive.google.com/file/d/0B8lgDBwI33UqelJDRkVSYzITQ3c/view?resourcekey=0-Ny9z7RG-cA5WyHmeYyQPDw>
21. Amira AB, Besbes S, Attia H, Blecker C. (2017). Milk-clotting properties of plant rennets and their enzymatic, rheological, and sensory role in cheese making: A review. Int J Food Prop. 2017;20(1):S76-S93.
<https://doi.org/10.1080/10942912.2017.1289959>
22. Soltani M, Boran O, Hayaloglu A. Effect of various blends of camel chymosin and microbial rennet (*Rhizomucor miehei*) on microstructure and rheological properties of Iranian UF White cheese. LWT-Food Sci Technol. 2016;68:724-8.
<https://doi.org/10.1016/j.lwt.2016.01.028>
23. Sandra S, Ho M, Alexander M, Corredig M. Effect of soluble calcium on the renneting properties of casein micelles as measured by rheology and diffusing wave spectroscopy. J Dairy Sci. 2012;95(1):75-82.
<https://doi.org/10.3168/jds.2011-4713>
24. McMahon DJ, Oommen BS. Casein micelle structure, functions, and interactions. In: McSweeney P, Fox P, editors. Advanced dairy chemistry. Boston, MA: Springer, 2013. pp 185-209.
https://doi.org/10.1007/978-1-4614-4714-6_6
25. Nollet LM, Toldrá F, Benjakul S, Paliyath G, Hui Y, editors. Food biochemistry and food processing. NJ, USA: John Wiley & Sons; 2012.
<https://doi.org/10.1002/9781118308035>
26. Khan N, Choi JY, Nho EY, Hwang IM, Habte G, Khan MA, Kim KS. Determination of mineral elements in milk products by inductively coupled plasma-optical emission spectrometry. Anal Lett. 2014;47(9):1606-13.
<https://doi.org/10.1080/00032719.2013.878842>
27. Irondi EA, Oboh G, Akindahunsi AA, Boligon AA, Athayde ML. Phenolic composition and inhibitory activity of *Mangifera indica* and *Mucuna urens* seeds extracts against key enzymes linked to the

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pathology and complications of type 2 diabetes. Asian Pac J Trop Med. 2014;4:903-10.

<https://doi.org/10.12980/APJTB.4.201414B364>

28. Westendorf J, Mettlich C. The benefits of noni juice: an epidemiological evaluation in Europe. J. Med. Food Plants. 2009;1(2):64-79.

<https://sites.google.com/a/jmedfoodplants.com/www/vol-1-1-jul-dec-2009>

29. Horsfall AU, Olabiyi O, Aiyegbusi A, Noronha C, Okanlawon A. *Morinda citrifolia* fruit juice augments insulin action in sprague–dawley rats with experimentally induced diabetes. Nig Q J Hosp Med . 2008;18:162-5.

<https://doi.org/10.4314/nqjhm.v18i3.45020>

30. Chuah AM, Lee Y-C, Yamaguchi T, Takamura H, Yin L-J, Matoba T. Effect of cooking on the antioxidant properties of coloured peppers. Food Chem. 2008;111(1):20-8.

<https://doi.org/10.1016/j.foodchem.2008.03.022>

31. Isabelle M, Lee B, Lim M, Koh W-P, Huang D, Ong C. Antioxidant activity and profiles of common fruits in Singapore. Food Chem. 2010;123:77-84. <https://doi.org/10.1016/j.foodchem.2010.04.002>

32. Lonergan SM, Topel DG, Marple DN, editors. The Science of Animal Growth and Meat Technology. Academic Press; 2018.

<https://doi.org/10.1016/C2017-0-02648-4>

33. Pournaghi P, Sadrkhanlou R-A., Hasanzadeh S, Foughi A. An investigation on body weights, blood glucose levels and pituitary-gonadal axis hormones in diabetic and metformin-treated diabetic female rats. Vet Res Forum. 2012; 3(2):79–84.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4312800/>

34. Seedeve P, Ganesan AR, Moovendhan M, Mohan K, Sivasankar P, Loganathan S, Shanmugam A. Anti-diabetic activity of crude polysaccharide and rhamnase-enriched polysaccharide from *G. lithophila* on Streptozotocin (STZ)-induced in Wistar rats. Sci Rep-UK. 2020;10(1);1-12.

<https://doi.org/10.1038/s41598-020-57486-w>

35. Nair SS, Kavrekar V, Mishra A. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Euro J Exp Bio . 2013;3(1):128-32.

<https://www.imedpub.com/articles/in-vitro-studies-on-alpha-amylase-and-alpha-glucosidase-inhibitory-activities-of-selected-plant-extracts.php?aid=11467>

36. Hasan KMM, Tamanna N, Haque MA. Biochemical and histopathological profiling of Wistar rat treated with *Brassica napus* as a supplementary feed. Food Sci. Hum. Wellness.. 2018;7(1):77-82.

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<https://doi.org/10.1016/j.fshw.2017.12.002>

37. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *Cmaj*. 2005;172(3):367-79.

<https://doi.org/10.1503/cmaj.1040752>

38. Silverstein D, Hopper K, editors. *Small animal critical care medicine*. Philadelphia, USA: Saunders, Elsevier; 2008.

<https://www.elsevier.com/books/small-animal-critical-care-medicine/9781416025917>

39. Park KT, Yun C-H, Bae C-S, Ahn T. Decreased level of albumin in peripheral blood mononuclear cells of streptozotocin-induced diabetic rats. *J Vet Med Sci*. 2014;76(8):1087–92.

<https://doi.org/10.1292/jvms.13-0631>

40. Ayeleso A, Brooks N, Oguntibeju O. Modulation of antioxidant status in streptozotocin-induced diabetic male Wistar rats following intake of red palm oil and/or rooibos. *Asian Pac J Trop Med* 2014;7(7):536-44.

[https://doi.org/10.1016/S1995-7645\(14\)60090-0](https://doi.org/10.1016/S1995-7645(14)60090-0)

41. Kazeem MI, Akanji MA, Yakubu MT. Amelioration of pancreatic and renal derangements in streptozotocin-induced diabetic rats by polyphenol extracts of Ginger (*Zingiber officinale*) rhizome. *Pathophysiology*. 2015;22(4):203-9.

<https://doi.org/10.1016/j.pathophys.2015.08.004>

42. Mohamed J, Nafizah AN, Zariyantey A, Budin S. Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. *Sultan Qaboos Univ Med J*. 2016;16(2):e132–e41.

<https://doi.org/10.18295/squmj.2016.16.02.002>

43. Pourghasem M, Nasiri E, Shafi H. Early renal histological changes in alloxan-induced diabetic rats. *IJMCM* . 2014;3(1):11.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3927393/>

44. Volper BD, Huynh RT, Arthur KA, Noone J, Gordon BD, Zacherle EW, Broderick TL. Influence of acute and chronic streptozotocin-induced diabetes on the rat tendon extracellular matrix and mechanical properties. *Am J Physiol-Reg I*, 2015;309(9):R1135-R43.

<https://doi.org/10.1152/ajpregu.00189.2015>

45. Bilal HM, Riaz F, Munir K, Saqib A, Sarwar MR. Histological changes in the liver of diabetic rats: A review of pathogenesis of nonalcoholic fatty liver disease in type 1 diabetes mellitus. *Cogent Medicine*. 2017;3(1):1275415.

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<https://doi.org/10.1080/2331205X.2016.1275415>

46. Gross JL, De Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes care*. 2005;28(1):164-76.

<https://doi.org/10.2337/diacare.28.1.164>

47. Abou Seif HS. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. *Beni-Suef Univ J Basic Appl Sci*. 2016;5(2):134-46.

<https://doi.org/10.1016/j.bjbas.2016.03.004>

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Table 1. Changes in blood biochemical parameters on day 20

Blood parameters	Units	Normal control (NC)	Diabetic control (DC)	Supercoagulant curd (SC)
Aspartate aminotransferase	U/L	221.5±86.9	926.0±143.49	214.0±78.8
Alanine transaminase	U/L	52.0±10.20	460.75±94.17	91.0±12.57
Gamma-glutamyl transferase	U/L	3.5±1.00	13.0±2.94	3.0±0.00
Alkaline phosphatase	U/L	180.5±40.74	944.5±37.83	509.75±38.32
Albumin	g/L	38.5±2.89	26.0±1.15	37.25±1.89
Globulin	g/L	29.5±4.65	25.25±2.06	30.25±2.22
Total protein	g/L	68.0±1.83	51.25±2.99	67.5±1.73
Urea	mmol/L	7.05±1.18	19.65±1.86	11.05±1.08
Creatinine	μmol/L	40.25±4.11	44.0±3.37	42.25±2.75

The values are expressed as the mean±standard error, where $p < 0.05$

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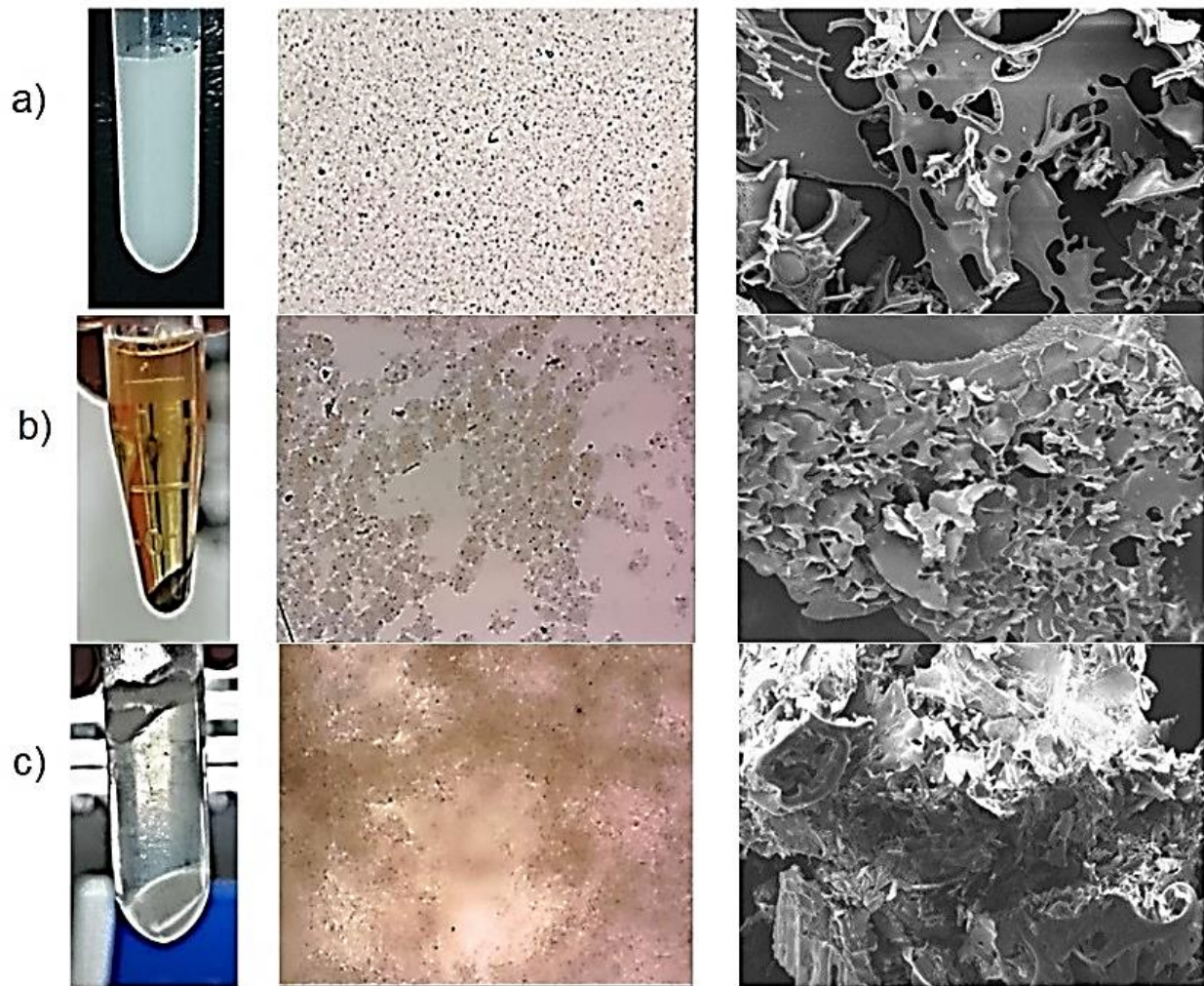


Fig. 1. Pure milk (a), super-coagulant coagulated milk (b), and rennet coagulated milk (c); from left to right: after centrifugation at 10 000 rpm for 60 s, under a light microscope at 100x magnification and SEM micrograph at 500x magnification, respectively.

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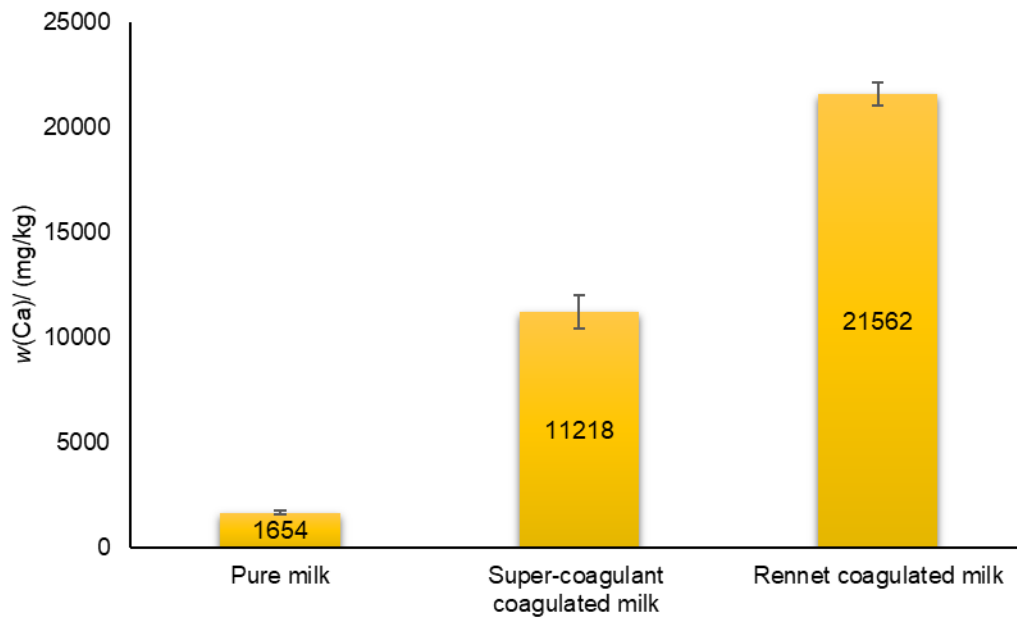
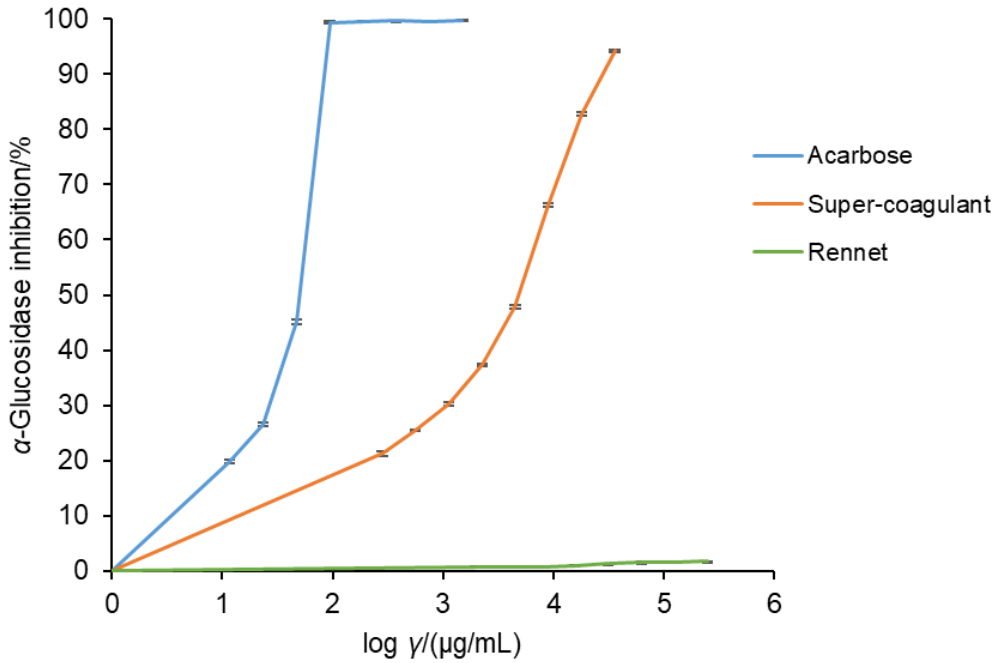


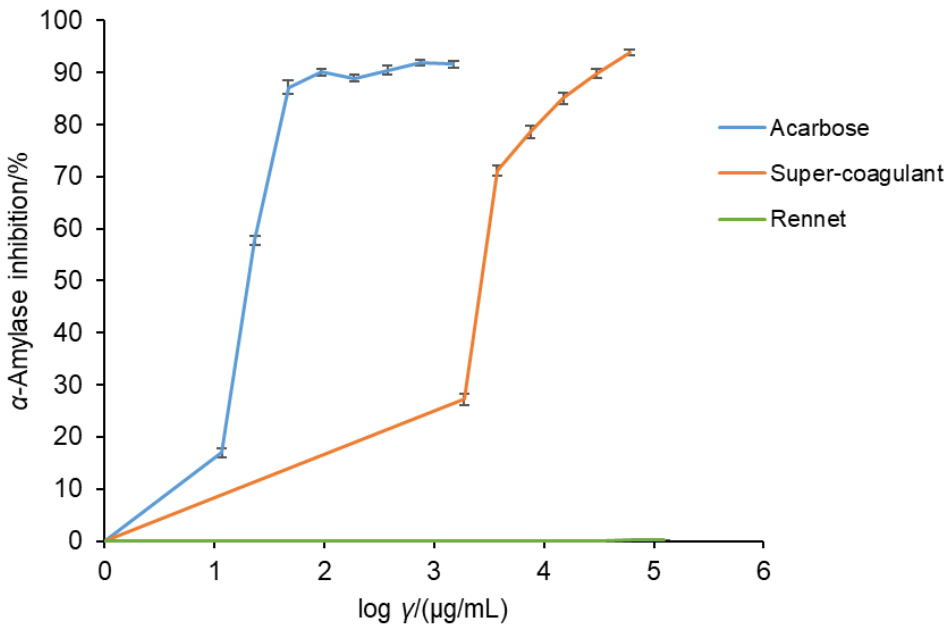
Fig. 2. Calcium content (mg/kg) determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). The experiment was performed in quadruplicate, and the values are expressed as the mean \pm standard error, where $p < 0.05$

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a)



b)



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Fig. 3. The % α -glucosidase inhibition versus the \log_{10} concentration of the tested samples (a) and the % α -amylase inhibition versus the \log_{10} concentration of the tested samples (b). The experiments were performed in quadruplicate, and the values are expressed as the mean \pm standard error, where $p < 0.05$

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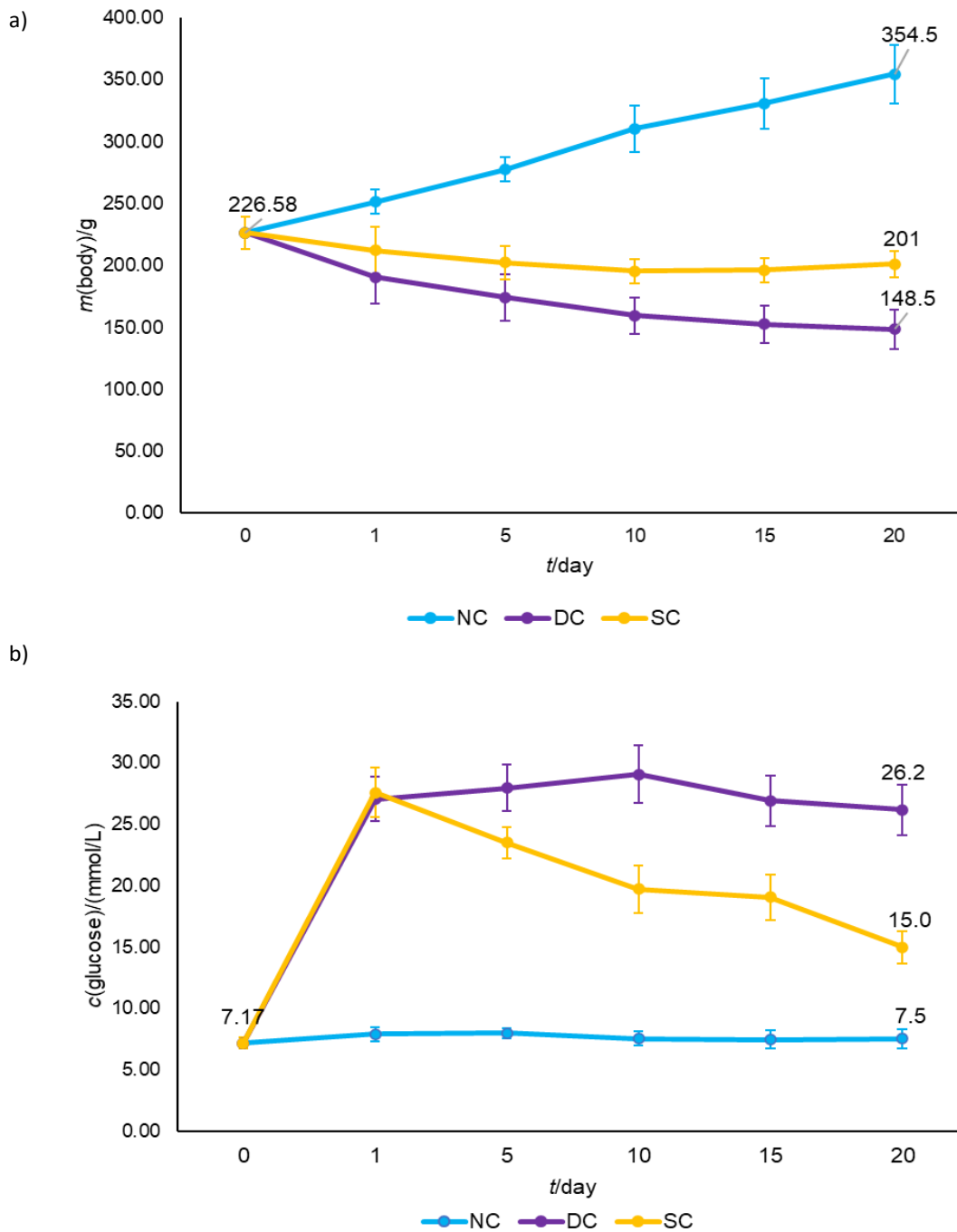


Fig. 4. Mean body mass of the rats (in g) (a) and mean fasting blood glucose (in mmol/L) (b) over the course of 20 days. The experiments were performed in quadruplicate, and the values are expressed as the mean±standard error, where $p < 0.05$

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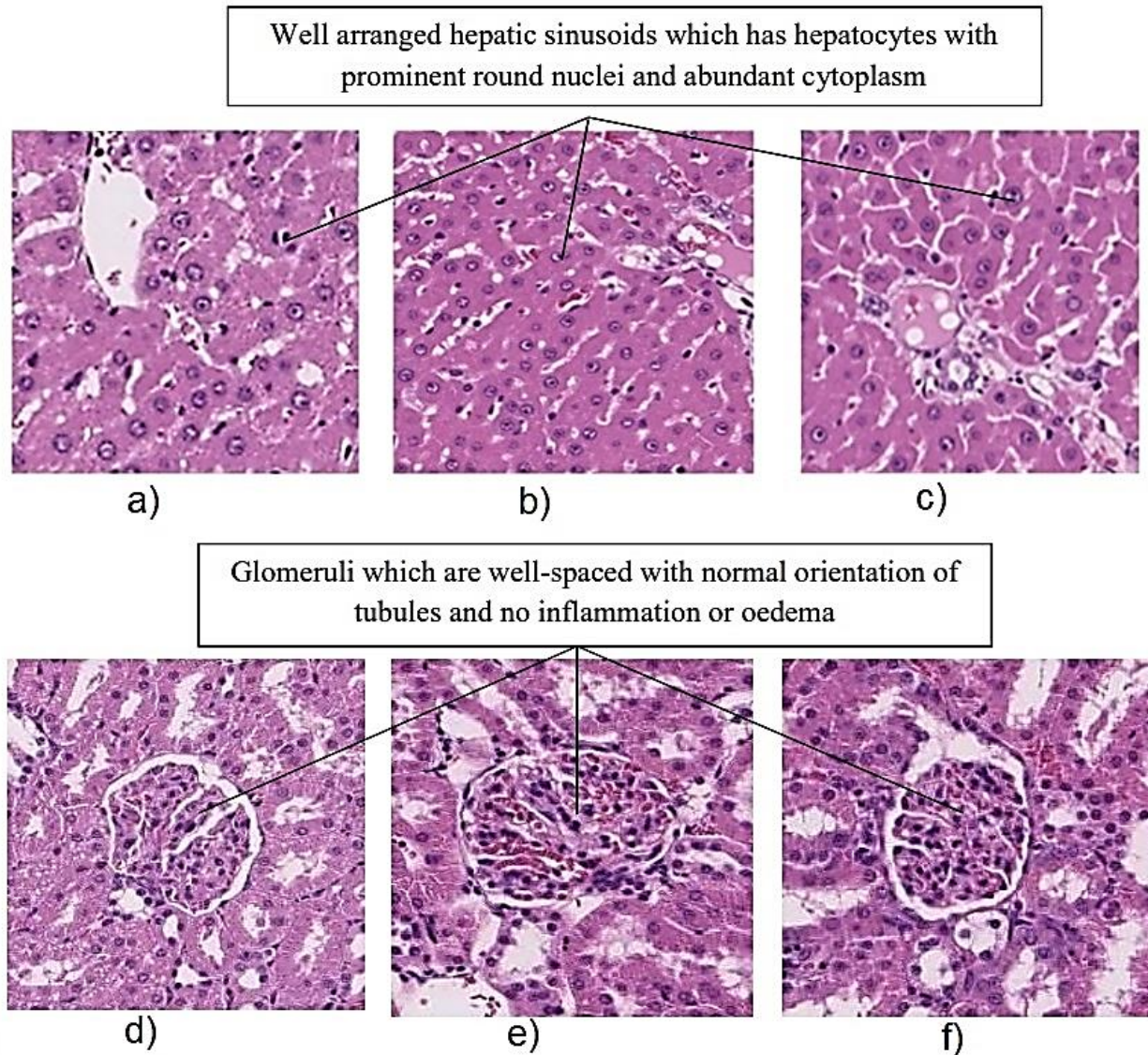


Fig. 5. Photomicrographs of liver cross-sections of NC (a), DC (b), SC (c) and kidney cross-sections of NC (d), DC (e) and SC (f) rats at 10× magnification

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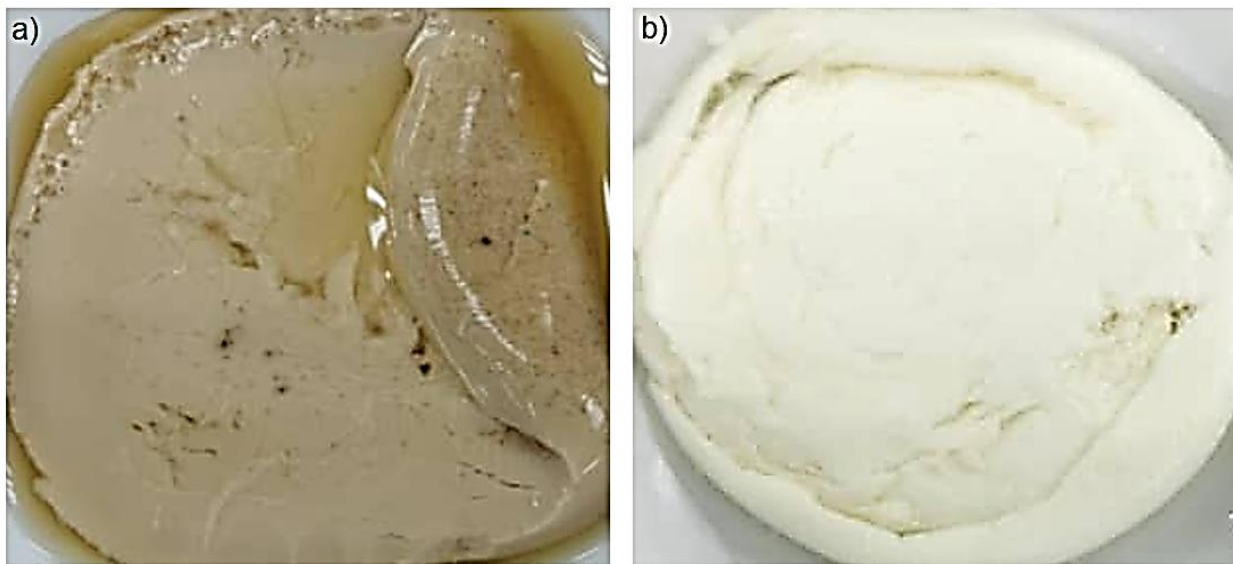


Fig. S1. Supercoagulant curd (a) and rennet curd (b) obtained after coagulation of milk and removal of whey