THE EFFECTS OF GIVING CURCUMA COOKIES ON BLOOD SUGAR LEVELS, MALONDIALDEHYDE, HEMOGLOBIN, AND BODY WEIGHT OF TYPE 2 DIABETES MELLITUS RATS

Isti Handayani, Puspita Amaliyah Dewi*, Nur Aini, Aisyah Tri Septiana
Food Tecnology/Faculty of Agriculture, Universitas Jenderal Soedirman, Banyumas, Indonesia.

*Email: Puspitaamaliyahdewi@gmail.com

Abstract. Type 2 diabetes mellitus is a metabolic disease characterized by high blood sugar levels, leading to oxidative stress. This condition may result in abnormal levels of malondialdehyde, hemoglobin, and body weight. The antioxidants in curcuma cookies can be beneficial for individuals with type 2 diabetes mellitus by aiding in the prevention of oxidative damage. The study aimed to assess the chemical properties and effects of giving cookies with added curcuma microcapsules, using 20% curcuma extract comprising 6% of the total cookie ingredients and 10% curcuma extract comprising 12% of the total cookie ingredients, on blood sugar levels, malondialdehyde, hemoglobin, and body weight in type 2 diabetes mellitus rats. In vitro testing of the cookies included the observation of curcumin, total phenolic content, and free radical scavenging capacity. In vivo testing involved observing blood sugar levels, malondialdehyde, hemoglobin, and body weight of the rats. The results showed that cookies with the addition of curcuma microcapsules from 20% curcuma extract as much as 6% led to a decrease in blood sugar and malondialdehyde levels by 96.59 mg/dL and 3.23 nmol/g, respectively, as well as an increase in hemoglobin and rat body weight by 4.66 g/dL and 26.83 grams.

Keywords: curcuma, microcapsules, cookies, diabetes mellitus

A. Introduction

Type 2 diabetes mellitus is a condition of hyperglycemia due to abnormalities in insulin secretion by pancreatic beta cells or impaired metabolic response to insulin action (insulin resistance). This leads to glucose not being properly stored in muscle and fat cells. According to the International Diabetes Federation (IDF) in 2021[1], type 2 diabetes mellitus is the most prevalent type, accounting for over 90% of diabetes cases globally. The worldwide prevalence of diabetes among 20-79 years old is estimated to be 10.5% (536.6 million people) in 2021, projected to rise to 12.2% (783.2 million people) by 2045. In 2021 and 2045, Indonesia ranks as the 5th country with the highest number of adults aged 20-79 years with diabetes.

Under hyperglycemic conditions, patients with type 2 diabetes mellitus experience increased oxidative stress, characterized by elevated production of free radicals and reduced antioxidant activity. Antioxidants have a role in inhibiting oxidative stress by binding free radicals and reactive molecules, thereby protecting cells from damage. Consuming food sources rich in antioxidants can effectively contribute to this preventive effort. Curcuma, a rhizome, is one such natural source of antioxidants. Research has shown that curcuma extract exhibits antioxidant activity of 87.01 ppm, making it a promising natural antioxidant [2].

Curcuma (Curcuma xanthorrhiza Roxb) has significant potential as a medicinal ingredient for use in functional food additives. In 2022, the harvest area of curcuma in Indonesia is

12,295.2 hectares. This figure demonstrates the productivity and potential of curcuma for utilisation in Indonesia across a range of sectors. The starch fraction constitutes the largest component of curcuma, representing approximately 48.18-59.64% of the total composition, the curcuminoids account for 27.19% of the total, while the essential oils represent a minor fraction of 3-12% [2]. Furthermore, curcuma contains secondary metabolite compounds, including alkaloids, flavonoids, and phenols.

The curcumin content in curcuma can help lower blood sugar levels. Curcumin can restore the sensitivity of insulin secreted by pancreatic β-cells by inhibiting the production of Reactive Oxygen Species (ROS). Curcumin also contains a phenolic OH element in its chemical structure which can prevent oxidation of hemoglobin and the lysis of red blood cells [3]. Antioxidants from curcuma can reduce malondialdehyde by increasing the activity of endogenous antioxidant enzymes and protecting pancreatic cells from ROS damage in diabetic conditions. Curcuma is used in processed foods as an appetite enhancer, and it also offers anti-cholesterol, anti-inflammatory, anti-anemia, and antioxidant[4].

To incorporate curcuma rhizomes into cookie products, the curcuma extract is microencapsulated at a specific concentration and then added to the cookie dough at a certain level. The purpose of microencapsulating is to preserve the bioactive compounds in the rhizome and to reduce the distinctive aroma and bitter taste of curcuma [5].

In this study, microcapsules were utilized, incorporating 10% and 20% curcuma extract, with varying weight ratios of curcuma (in grams) to the distilled water solvent. The escalating curcuma extract concentration correlates with heightened bioactive content, consequently necessitating a diminished addition of curcuma microcapsules to the biscuit dough. Subsequently, the study proceeded with in vivo testing to examine the influence of curcuma cookies on parameters such as blood glucose, hemoglobin, malondialdehyde, and body weight in type 2 diabetes mellitus.

This study aimed to investigate the chemical characteristics of cookies and the impact of feeding cookies containing curcuma microcapsules. These capsules contained either 20% curcuma extract, constituting 6% of the total cookie ingredients, or 10% curcuma extract, constituting 12% of the total cookie ingredients on blood glucose, hemoglobin, malondialdehyde, and body weight in rats with type 2 diabetes mellitus.

B. Methods

1. Time and Place

The research was conducted from May to November 2023 at the Agricultural Technology Laboratory, Faculty of Agriculture, Jenderal Soedirman University and animal testing at the Centre for Food and Nutrition Studies, Gadjah Mada University. The research comprised 5 stages: preparation of curcuma extract, microencapsulation, production of curcuma cookies, in-vitro testing of cookies (including analysis of curcumin content, total phenols, free radical scavengin capacity, proximate), and in vivo testing (including analysis of blood sugar levels, malondialdehyde, hemoglobin, and rat body weight).

2. Preparation of curcuma extract and microencapsulation

Two different concentrations, 10% and 20%, were utilized for the extraction process. The curcuma extraction involved initial steps of peeling, washing, and slicing the curcuma peel, followed by the maceration of the curcuma pulp and extraction through microwave heating (MAE). Microencapsulation was executed by adding gum Arabic and Tween 80 to the curcuma extract, followed by mixing on magnetic stirrer. The foam mat drying procedure was then applied to the microcapsule liquid, utilizing a mixer resulting foam sample was leveled on a glass plate and dried in a cabinet dryer. The dried samples were crushed and sieved through a 100 mesh sieve.

3. Curcuma cookies

According to Aini et al. (2022)[6] modified, the ingredients required for making cookies are flour, margarine, sugar, egg yolk, egg white, baking powder, and salt. Three types of cookies were made: cookies with the addition of microcapsules of 20% curcuma extract up to 6% of the total cookies ingredients (C1), cookies with the addition of microcapsules of 10% curcuma extract up to 12% of the total cookies ingredients (C2), and cookies made from wheat flour (K) as a control.

4. Determination of curcumin content

Standard curcumin was used to compare the curcumin content. A standard curve was created by making various dilutions of curcumin in ethanol and then measuring the absorbance at 425 nm. To test the curcumin content of the cookies, the cookie samples were diluted with ethanol then the absorbance of the sample filtrate was measured using a spectrophotometer at 425 nm. The curcumin concentration was determined by plotting the absorbance of the curcumin standard against the sample filtrate concentration [7].

5. Determination of total phenol

Gallic acid served as the standard comparison solution. The phenol standard curve was prepared by varying the dilution concentrations. The absorbance of the standard solution was then measured at a wavelength of 765 nm. The extract solution was obtained by dissolving the curcuma cookies sample. The filtrate was collected and combined with folin ciocalteu and Na_2CO_3. This mixture was incubated for 60 minutes and the absorbance was measured at a wavelength of 765 nm [8].

6. Determination of free radical scavenging capacity (DPPH)

The test solution was created by dissolving the curcuma cookie sample in methanol. After that, the filtrate was combined with 0.16 mM DPPH solution. The mixture was then shaken and left in the dark for 30 minutes. The absorbance was measured at 517 nm. The ability to scavenge DPPH radicals was calculated using the following equation:

$$\text{Radical scavenging capacity (\%)} = \frac{\text{control absorbance - sampel absorbance}}{\text{control absorbance}} \text{ x } 100\%$$

7. Proximate measurement

Proximate analyses of the cookies included moisture and ash content by the thermogravimetric method, protein by the kjeldahl method, fat by the soxhlet method, and carbohydrate using by difference method. All five analyses are based on AOAC 2005.

8. Determination of blood sugar level

Blood glucose levels were measured using the GOD-PAP (*Glucose Oxidase-Peroxidase Aminoantypirin*) method with a spectrophotometer. Blood was collected and placed in a tube containing EDTA (*Ethylene Diamine Tetra Acetat*). The blood was then centrifuged to obtain blood serum. The GOD-PAP reagent was added to the samples and then incubated. After that, the samples and standards were read against a blanko using a spectrophotometer at a wavelength of 500 nm [9]. The absorbance value obtained was then calculated using the formula:

$$\text{Blood sugar level (mg/dL)} = \frac{\text{A sampel}}{\text{A standart}} \times \text{standardised concentration } (100 \frac{\text{mg}}{\text{dl}})$$

9. Determination of malondialdehyde

Malondialdehyde (MDA) was determined using the thiobarbituric acid reactive substances (TBARS) assay. Blood samples were collected and concentrated by centrifugation. Then, a solution containing folic acid and TEP (1,1,3,3 tetraethoxypropane) was added to the concentrated samples. Subsequently, 250 µL of 40 mM TBA solution and 450 µL of distilled water were added to the mixture. The resulting solution was heated at 100°C for 1 hour, followed by transfer to an ice bath to cool. The sample was then injected into a Sep-Park C18 column, and the absorbance was measured using a visible spectrophotometer at a wavelength of 532 nm. MDA levels were calculated using the formula:

$$\text{MDA content (mmol/l)} = \frac{\text{Absorbance of sample at } \lambda\text{max-intercept standar at } \lambda\text{max}}{\text{standardised slope at } \lambda\text{maks}}$$

10. Determination of hemoglobin

Hemoglobin measurement was conducted using the oxyhemoglobin method. Blood samples from rats were collected and mixed with a liquid containing EDTA. Subsequently, a Na-Carbonate solution was added to the test tube, followed by the addition EDTA. The test tube was then closed and shaken. The absorbance was measured using a spectrophotometer at 540 nm [10]. The hemoglobin concentration reading was obtained using a previously prepared calibration cuvette.

Hemoglobin level= sampel absorbance×36,8 g/dL

11. Determination of body weight

Body weight measurements were conducted using digital scales during the induction period, before and once a week during the intervention until the end of the intervention [11].

C. Results And Discussion

1. Invitro Cookies Analysis

The analysis of the cookies was conducted in vitro by measuring curcumin content, total phenolics, free radical scavenging capacity, and proximate analysis. The findings of these analyses are shown in Table 1.

Table 1. Physicochemical content of curcuma cookies
Notes: K= cookies made from wheat flour, C1= cookies with the addition of microcapsules of 20% curcuma extract up to 6% of the total cookies ingredients, C2= cookies with the addition of microcapsules of 10% curcuma extract

Data	Curcumin content	Total phenolic	Free radical scavenging capacity (DPPH)	Moisture content	Ash content	Fat content	Protein content	Carbohydrat content
	(mg/ml)	(mg GAE/g)			%			
K	0,613	5,184	67,750	2,129	0,999	18,197	15,933	62,742
C1	1,809	6,171	74,576	2,215	1,233	17,067	11,993	67,491
C2	2,268	8,701	76,688	4,484	1,466	15,606	9,747	68,697

up to 12% of the total cookies ingredients

According to Table 1, decreasing the curcuma concentration in extracts by adding more microcapsules to the dough has a significant effect on increasing the curcumin content of curcuma cookies. It occurs as the ratio of curcuma powder to ethanol solvent increases, the solvent can extract more constituents [12]. Additionally, curcuma has a starch fraction of about 48.18-59.64% [13], so high concentrations of curcuma extract have high starch content, which can increase the viscosity of curcuma extract. The high viscosity of the solution may affect the

masking of curcumin by gum arabic. Research has found the higher the viscosity, the lower the encapsulation efficiency [14].

The amount of curcumin in the cookies aligns with the total phenol content and the DPPH free radical scavenging rate. However, the total phenol measurement of the three cookie variations did not show a significant effect in the analysis of variance. This could be attributed to the minor differences in extract concentrations, which may not have been substantial enough to demonstrate a significant difference in each treatment. This is in line with research that shown about the amount of dissolved material does not impact phenolic compounds because they have limited solubility in water [13]. Nonetheless, treatment C2 had the highest average total phenol value of 8.701 mg GAE/g.

Based on Table 1, when the concentration of curcuma is reduced to produce microcapsules, the DPPH free radical scavenging rate of cookies has a significant effect similar to that of curcumin. This is because curcumin can influence the total phenolic [15]. As a phenolic compound, curcumin acts as an antioxidant due to its ability to convert reactive oxygen into a more stable and harmless compound through its OH group. The increase in the average value of DPPH radical scavenging in the cookies reflects the level of antioxidant activity.

The results of the proximate analysis from Table 1 indicate that the addition of microcapsules in treatment C2 results in a higher starch content. The hygroscopic character of starch causes the moisture content in treatment C2 (4.484%) to be higher than in C1 (2.215%). Furthermore, the addition of microcapsules leads to a higher product density, which in turn hinders optimal cookie baking and increases the moisture content of the cookies.

The moisture content is related to the ash content, as higher moisture levels can lead to increased ash content due to the presence of mineral salts in water. This is supported by the measurement results showing that treatment C2 has the highest average ash content (1.466%). Furthermore, curcuma starch contains minerals such as potassium (K), sodium (Na), magnesium (Mg), iron (Fe), manganese (Mn), and cadmium (Cd) which contribute to the increase in ash content due to the greater proportion of microcapsule addition in [16].

The addition of curcuma microcapsules to the cookies led to a reduction in the protein and fat content. Specifically, Treatment C2 exhibited the lowest average protein and fat content at 9.747% and 15.606% respectively. The decrease in fat content is attributed to the antioxidant activity. Measurements of antioxidants such as curcumin content, total phenolics, and DPPH free radical scavenging showed higher results in treatment C2, which aligns with the decrease in fat content in this treatment.

The determination of carbohydrate content is conducted using the difference method, where the carbohydrate content is influenced by other nutritional components. Consequently, a lower carbohydrate content corresponds to higher levels of other nutritional components. The average carbohydrate content for the C2 treatment is the highest at 68.697%.

2. In Vivo Cookies Analysis

Data	Blood sugar (mg/dl)	Malondialdehyde (nmol/g)	Hemoglobin (g/dl)	Body weight grams
Control negative	72,16	1,671	15,16	228,33
Control positive	267,42	10,090	9,08	155,50
P3	152,15	8,057	10,76	197,33
P4	114,84	5,126	12,06	214,17
P5	96,59	3,233	13,67	213,83

Notes: P3= diabetes mellitus rats treated with K, P4= diabetes mellitus rats treated with C2, P5= diabetes mellitus rats treated with C1

The in vivo tests demonstrated that giving curcuma cookies to the P4 and P5 groups led to a significant reduction in average blood glucose levels. This can be attributed to the presence

of curcumin, phenolic compounds, and other secondary metabolites in curcuma, which have the potential to lower blood glucose levels. Phenolic compounds act as antioxidants by engaging in electron transfer and binding free radicals, thereby converting phenolic groups into stable phenoxyl radicals [17]. Moreover, higher concentrations of curcuma extract are associated with greater inhibition of glucose absorption, leading to a higher percentage of glucose reduction. Phenolic and flavonoid compounds demonstrate a linear role with antidiabetic activity, indicating that higher levels of these compounds correspond to better antidiabetic effects.

According to Table 2, the MDA test result for P5 is 3.233 nmol/gram. This result indicates a decrease from the pre-test results due to the presence of antioxidant compounds in curcuma. These compounds act as scavengers of hydrogen peroxide (H2O2), preventing it from further reacting into hydroxyl radicals (OH). By inhibiting free radicals, the antioxidant compounds prevent lipid peroxidation, leading to a reduction in MDA levels in rats [18]. In addition to the activity of antioxidant compounds, the reduction in MDA levels may also be influenced by the presence of minerals in curcuma cookies. Curcuma starch contains nutrients including crude fiber, potassium (K), sodium (Na), magnesium (Mg), iron (Fe), manganese (Mn), and cadmium (Cd) [16]. Iron (Fe) and manganese (Mn) can act as cofactors that influence the antioxidant activity of superoxide dismutase (SOD) enzymes through Fe-SOD and Mn-SOD isoforms. Endogenous antioxidants inhibit fat oxidation reactions in the body by decomposing peroxides and preventing damage to macromolecular cell components, or by absorbing or giving an electron to free radicals to inhibit oxidative stress [18]. Furthermore, the enzyme superoxide dismutase (SOD) converts superoxide free radicals (O2-•) into hydrogen peroxide (H2O2) and O2. It is noted that the higher the concentration of curcuma used to make microcapsules, the more starch is extracted by water. Therefore, treatment P5 may lead to a greater reduction in MDA levels compared to treatment P4.

The data presented in Table 2 indicates that the hemoglobin level of P5 is 13.67 g/dL, closely resembling the negative control rat hemoglobin level of 15.16 g/dL. This similarity is attributed to the presence of flavonoids in curcuma, which contribute hydrogen atoms to bind free radicals and prevent haemolysis of red blood cells [19]. Moreover, the addition of 20% curcuma extract microcapsules (C1) resulted in increased protein content in the cookies. A high level of hemoglobin is strongly influenced by the adequacy of protein used for hemoglobin synthesis. Protein serves as an iron carrier to form transferrin, which is then transported to the bone marrow to form hemoglobin [20]. Additionally, the mineral iron (Fe) in the cookies containing curcuma starch also contributes to the increase in hemoglobin levels.

The results from Table 2 showed that both P4 and P5 led to an increase in the average body weight of the DM rats compared to the positive control. However, there was no significant difference between the two. Weight loss in diabetes mellitus is caused by insulin deficiency, which leads to disturbances in fat and protein metabolism. Therefore, the curcumin and phenolic content of curcuma may restore insulin sensitivity. The weight gain in Wistar rats suggests that consuming curcuma cookies may have a beneficial effect on diabetes mellitus.

D. Conclusion

The addition of microcapsules containing 20% and 10% curcuma extracts, up to 6% and 12% of the total cookie ingredients, respectively, had a significant effect on increasing curcumin, total phenolic and radical scavenging (DPPH) levels. In vivo tests showed that the addition of microcapsules had a significant effect on reducing blood glucose and malondialdehyde levels and increasing hemoglobin and body weight in rats with type 2 diabetes mellitus.

E. References

[1]. IDF (2021) IDF Diabetes Atlas 10th edition.

[2]. Rosidi A, Khomsan A, Setiawan B, Riyadi H, Briawan D (2014) Potensi Temulawak (Curcuma xanthorrhiza Roxb) Sebagai Antioksidan. Prosiding Seminar Nasional dan Internasional

[3]. Mahendra D, Santosa PE, Erwanto, Muhtarudin (2024) Pengaruh Pemberian Tepung Kunyit (Curcuma domestica) terhadap Sel Darah Merah dan Hemoglobin Kambing Jawarandu Jantan. Jurnal Riset dan Inovasi Peternakan 8:248–256

[4]. Khamidah A, Antarlina SS, Sudaryono T (2017) Ragam Produk Olahan Temulawak Untuk Mendukung Keanekaragaan Pangan. Jurnal Penelitian dan Pengembangan Pertanian 36:1–12

[5]. Nugraheni A, Yunarto N, Sulistyaningrum N (2015) Optimasi Formula Mikroenkapsulasi Ekstrak Rimpang Temulawak (Curcuma xanthorrhiza Roxb.) dengan Penyalut Berbasis Air. Jurnal Kefarmasian Indonesia 5:98–105

[6]. Aini N, Sustriawan B, Mela E, Lestari LF (2022) Physical and Chemical Properties of Corn-Almond Cookies Affected by Mung Bean Supplementation and Source of Fat. In: 6th International Conference of Food, Agriculture, and Natural Resource (IC-FANRES 2021). Atlantis Press International B.V., pp 66–74

[7]. Delfiya DSA, Thangavel K, Natarajan N, Kasthuri R, Kailappan R (2015) Microencapsulation of Turmeric oleoresin by Spray Drying and In vitro Release Studies of Microcapsules. J Food Process Eng 38:37–48

[8]. Dewantara LAR, Ananto AD, Andayani Y (2021) Penetapan Kadar Fenolik Total Ekstrak Kacang Panjang (Vigna unguiculata) dengan Metode Spektrofotometri UV-Visible. Jurnal Ilmu Kefarmasian 2:13–19

[9]. Herlina, Amriani A, Solihah I, Damayanti P, Rais SW (2019) Effectiveness of Ethanolic Extract Ketepeng cina Leaves (Senna alata L.) As Antidiabetic Activity Test in Male Wistar Rats Induced by Alloxan. In: J Phys Conf Ser. Institute of Physics Publishing, pp 1–8

[10]. Sari EK, Wihastuti TA, Ardiansyah W (2018) Probiotik Meningkatkan Konsentrasi Hemoglobin pada Tikus Putih yang Diinduksi Lipopolisakarida Escherichia Coli. Majalah Kesehatan 5:18–25

[11]. Fitria L, Lukitowati F, Kristiawati D (2019) Nilai Rujukan Untuk Evaluasi Fungsi Hati dan Ginjal pada Tikus (Rattus norvegicus Berkenhout, 1769) Galur Wistar. Jurnal Pendidikan Matematika dan IPA 10:243–258

[12]. Luviana A, Putri A, Alatif IA, Nurulgina R, Permatasari RP, Sihombing RP, Paramitha T (2023) Pengaruh Pelarut dan Daya Microwave terhadap Hasil Ekstrak Daun Pepaya dengan Metode Microwave Assisted Extraction. Prosiding The 14th Industrial Research Workshop and National Seminar 213–217

[13]. Oktaviana PR, Atmaka KW (2015) Kadar kurkuminoid, total fenol dan aktivitas antioksidan ekstrak temulawak (Curcuma xanthorrhiza) pada berbagai teknik pengeringan dan proporsi pelarutan. Biofarmasi 13:41–49

[14]. Nurlaili FA, Darmaji P, Pranoto Y (2014) Mikroenkapsulasi Oleoresin Ampas Jahe (Zingiber officinale var.Rubrum) dengan Penyalut Maltodekstrin. AGRITECH 34:22–28

[15]. Septiana A tri, Handayani I, Winarsi H (2019) Aktivitas Antioksidan dan Sifat Fisikokimia Madu Temulawak (Curcuma zanthorrhiza Roxb) yang Ditambah Ekstrak Jahe (Zingiber officinale Rosc). Jurnal Aplikasi Teknologi Pangan 8:155–160

[16]. Wruin KM, Sodik MA (2018) Temulawak Pendeteksi Boraks dalam Makanan dan Bahaya Boraks bagi Kesehatan Manusia. OSFPreprints. https://doi.org/https://doi.org/10.31219/osf.io/rt3hv

[17]. Adriansyah I, Handito D, Widyasari R (2020) Efektivitas Bubuk Kopi Robusta Fungsional Difortifikasi Bubuk Daun Kersen Terhadap Penurunan Kadar Gula Darah Mencit DM. Pro Food (Jurnal Ilmu dan Teknologi Pangan) 6:581–590

[18]. Rosidi A (2014) Pengaruh pemberian ekstrak temulawak (Curcuma xanthorrhiza Roxb) terhadap stres oksidatif dan kesegaran jasmani atlet. Disertasi, Institut Pertanian Bogor

[19]. Tandi J, Rahmawati, Isminarti R, Lapangoyu J (2018) Efek Ekstrak Biji Labu Kuning terhadap Glukosa, Kolesterol, dan Gambaran Histopatologi Pankreas Tikus Hiperkolesterolemia-Diabetes. Talenta Conference Series: Tropical Medicine (TM) 1:144–151

[20]. Hasan AEZ, Husnawati H, Zulaikha SW (2022) Hasil Uji Toksisitas Subkronis Temulawak Terhadap Nilai Hemoglobin, Hematokrit, dan Leukosit Tikus. Jurnal Pharmascience 9:234–247

[21]. Thamrin H (2021) Hubungan antara Pengetahuan, Tingkat Konsumsi Protein, Zat Besi, dan Vitamin C dengan Kadar Hemoglobin pada Mahasiswi Kebidanan. Jurnal Penelitian Kesehatan Suara Forikes 12:30–33