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Abstract. This study was conducted to determine the bioactive components in food ingredients that have as potential natural antioxidants source, it can be used as an alternative to reduce degenerative diabetes mellitus. The potential natural antioxidant of black cumin seed and sesame seed extract by encapsulation methods was carried out by fermentation. This research used a complete randomized design, consisted of two factors: the ratio of black cumin seed extract with sesame seed extract (E) consist of 4 levels: 100:0; 75:25; 50:50; 25:75 and 0:100 (%) and type of coating material (N): maltodextrin-soy protein, maltodextrin-gelatine and maltodextrin-casein. The best antioxidant activity (IC50) was 81.45 µg/mL, which found in the ratio of black-sesame cumin extract (75%:25%) with maltodextrin-soy protein coating material.

1. Introduction

One non-infectious disease (degenerative disease) in the last decade known as diabetes mellitus, generally ends with death. Although the deaths from diabetes mellitus are relatively smaller than the other diseases, but according of World Health Organization report, deaths from diabetes mellitus have increased in the many developed and developing countries for 20 years in the last decade. The pattern of food consumption were higher fat, protein, sugar and salt, but lower fibre could triggered various degenerative diseases. The science and modern technology has been found the phytochemical compounds that it had been a function as antioxidants and a positive effect on healing various degenerative diseases, therefore the research of natural antioxidants were growing [21]. Natural antioxidants have protected the body against damage caused of reactive oxygen species, they are able to prevent the degenerative diseases and the fat oxidation in foodstuffs.

Black cumin seeds (*Nigella sativa*) and sesame (*Sesamum indicum* L) as functional food have not been explored optimally to diabetes mellitus. The mount of bioactive compounds of black cumin seeds such as nigellone and thymoquinone had the ability as anti-inflammatory, anticancer, antidiabetic and antibacterial [14]. The ability of black cumin in reducing blood sugar levels or as an anti-hyperglycemic had been tested through alpha glucosidase enzyme inhibition test (in vitro) and antioxidant activities by IC50 was 172.81 µg/mL [22]

Sesame seeds (*Sesamum indicum* L) contain the bioactive compounds as antioxidants, consisted of fat-soluble and water-soluble antioxidants. Fat-soluble antioxidants such as tocopherols and fat-soluble such as lignans (sesamin, sesamol and sesamolin), lignans have a higher antioxidant activity than

tocopherols [8]. Water-soluble lignans are lignan glucoside (sesaminol triglucoside, pinoresinol glucoside and sesamolinol glucoside). Sesaminol triglucoside was the dominant glucoside lignan [18; 11].

Efforts to extend the shelf life of black cumin and sesame seed extract were the encapsulation technology. Currently, the researchers had developed to improve the quality of functional food. Some of bioactive compounds had been the lipophilic and hydrophilic properties and their absorption in the digestive tract were limited. They can be overcome by the encapsulation method, so that the absorption system can be increased into the body, the bioactive compounds that are less soluble in water can be protected and the regulation of the release of bioactive compounds that are adsorbed in food was more effective. Encapsulation of oil in water emulsions is more effective to protect the stabilities of bioactive compounds [19].

2. Materials and methods

2.1. Materials

Black cumin seeds, sesame seeds, tempeh culture was purchased from Traditional Market in Medan, Indonesia. The coating materials of maltodextrin, soy protein, gelatine, casein and surfactant tween 80 were obtained from CV. Rudang Jaya, Medan, Indonesia. Material analysis are: 70% methanol, 96% alcohol, absolute alcohol, DPPH (2.2-diphenyl-1-pycrilhydrazyl) solution, aquadest, $CuSO_4$, K_2SO_4, H_2SO_4 concentrated, hydrochloride, magnesium powder, $FeCl_3$, Natrum carbonate pentahydrate, sodium sulfate anhydrous, potassium iodate, sodium thiosulfate, mengsel indicator, starch indicator.

2.2. Sample preparation

2.2.1. Black cumin and sesame seeds extract. To prepare extracts of cumin and sesame seeds, each material was cleaned and selected whole seeds in good condition, washed with running water, drained. Black cumin or sesame seeds are soaked in 1% tempeh culture solution (1:2) for 24 hours, dried by oven blower for 12 hours at 50^{o}C, grounded using a seed grinder, obtained the black cumin and or sesame powder. Black cumin and sesame powder were extracted at twice by maceration method with shaker for 20 hours at room temperature: first extraction using 96% ethanol (black/sesame cumin: ethanol = 1:6 w/ v) and second extraction with the same solvent (black/sesame cumin: ethanol = 1:4 w/v). Extraction was carried out for 20 hours by maceration method. The filtrate was separated from the solvent with rotary evaporator at 40^{o}C until a thick extract was obtained.

2.3. The formulation of black cumin and sesame seed encapsulate

One unit experiment consisted of 75 mL of ion-free water, tween 80 as much as 3%, stirred at 50^{o}C for 30 minutes, 5 g of black cumin and sesame extract ratio as the factor 1 (E1 = 100:0, E2 = 75:23, E3 = 50:50; E4 = 25:75 and E5 = 0:100%). The mixture was homogenized at 40^{o}C for 30 minutes to form emulsion. The emulsion was made encapsulate with 15g coating materials ratio as factor 2 (N1 = maltodextrin: soy protein; N2 = maltodextrin: gelatine; and N3 = maltodextrin: casein), both of N1 and N2 ratio of coating material were (4:1), while N3 was (7:1). Then homogenized for 5 minutes with a magnetic stirrer, hydrated at 4^{o}C for 18 hours, dried by oven at 50^{o}C for 12 hours, blended by seed mill, packed with polypropylene plastic, the encapsulates were ready for analysis.

2.4. Observation

The observations were analysed by determination of water content [20], activity antioxidant by DPPH method [12], reduction sugar of Luff Schoorl Method [20], a qualitative test of flavonoid [2] and a qualitative test of phenolic [13].

2.5. Test of antioxidant activity by DPPH method

The antioxidant activity was determined by the free radical method of DPPH [3,12]. DPPH solution was prepared by dissolving 4.7 mg in 100 mL absolute ethanol (pro analysed), it got the concentration of 0,12mM and kept in the dark room for 20 minutes. Making a sample solution with concentration of 3.12 µg/ml, 6.25 µg/ml 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml from stock solution. Each was transferred into the cuvette (5 mL) and then added 1 mL DPPH solution and absolute alcohol, stir well and immediately made visible light spectra (400-600 nm), the absorbance of solution determined on 517 nm.

$$\% \text{ Inhibition} = \frac{\text{Absorbance control-Absorbance sample}}{\text{Absorbance control}} \text{ x } 100\% \quad (1)$$

3. Results and Discussions

3.1. The characteristics of chemical, physical and functional of black cumin and sesame powder

Determination of chemical composition of black cumin and sesame seed powder were fermented using a tempeh culture for 24 hours include water content, ash content, protein content, fat content, fibre content, reducing sugars and antioxidant activity. Physical, chemical and functional characteristics of black cumin seeds and sesame seeds can be seen in Table 1.

Table 1. The characteristics of Chemical, physical and functional of black cumin and sesame seed powder.

Quality	Black cumin	Sesame
Water contain (% dry base)	7.3481±0.6673	5.2855±0.3020
Mineral contain (% dry base)	4.6805±0.0293	4.9238±0.0895
Protein contain (% dry base)	23.3763±0.3944	22.9721±0.2440
Fat contain (% dry base)	25.6466±0.6673	43.1980±0.4022
Crude fibre content (% dry base)	6.1012±0.1261	6.3798±0.1788
Reduction sugar (% dry base)	1,7363±0,1236	1.5180±0.0604
Yield (%)	7.8989±1.0486	5.2848±0.8873
Antioxidant activity IC50 (µg/mL)	67.3125±2.8371	78.3130±1.4274

Note: The test was carried out 3 times, (±) showed the standard deviation value.

IC 50 value of black cumin and sesame powder had a strong antioxidant activity: 67.3125 ± 2.8371 and 78.3130 ± 1.4274 (µg/mL) obtained from the linear regression equation of antioxidant standard curve, but black cumin is relative stronger than sesame because it has a lower value. Antioxidant activity is expressed with IC50 values. This value showed that the concentration of samples to inhibit 50 percent of free radical activity. The higher the IC50 value, the lower the antioxidant activity [12] (Table 2).

Table 2 showed that the testing of phenol and flavonoid content of black cumin and sesame powder were a positive results, qualitatively both of them contain phenol and flavonoid compounds. Positive results for testing of phenol content was indicated by change in colour to green [2] and testing of flavonoid content was indicated by change in colour to yellow [13].

Table 2. Linier Regression equation of standard curve and antioxidant activity value (IC_{50}) (μg/mL) of black cumin and sesame powder.

Replication	Linear Regression equation	IC 50 (μg/mL)
Black cumin		
Repeated I	y = 0.2068x + 35.856	68.39458
Repeated II	y = 0.247x + 32.846	69.44939
Repeated III	y = 0.2223x + 35.752	64.09357
Average		67.31251±2.8371
Antioxidant content	phenolic (+)	Flavonoid (+)
Black cumin powder extract	Change to green colour	Change to Yellow colour
Sesame		
Repeated I	y = 0.2222x + 32.15	79.56121
Repeated II	y = 0.2241x + 32.381	78.62115
Repeated III	y = 0.2294x + 32.392	76.75676
Average		78.31304±1.4274
Antioxidant content	phenolic (+)	Flavonoid (+)
sesame powder extract	Change to green colour	Change to green colour

Note: y = absorbance value of sample; x = IC50 value sample.

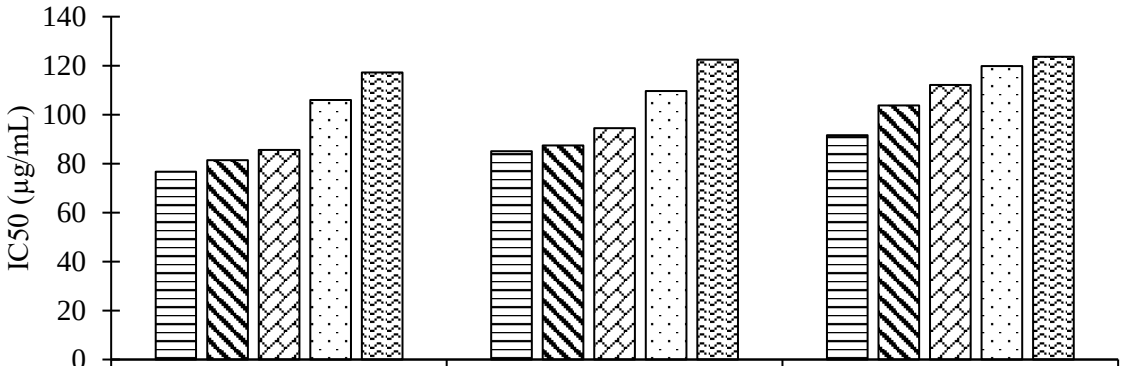
3.2. *Antioxidant activity*

The results of encapsulate analysis of black cumin with sesame extract and type of coating material can be seen Figure 1. The strongest IC 50 value was found in the comparison of black and sesame seed extract (75:25) obtained from the linear regression line equation. IC50 value is the concentration of a test solution (sample) which gives a reduction of DPPH by 50% [12]. Based on the calculation, the IC50 value of was 81.4452 (μg/mL). According to [12] that a compound is very strong antioxidant if IC50 values are less than 50 ppm, strong activity if IC50 values are between 50-100 ppm, moderate activity if IC50 values are between 100-150 ppm and weak if IC50 values between 150- 200 ppm. These showed that the encapsulation of black and sesame seed extract (75: 25%) had a strong activity in reducing free radicals.

Figure 1 shows that the highest antioxidant activity (IC50) was expressed with the lowest IC 50 values found in E1 (100: 0%) and N1 (maltodextrin: soy protein) treatments, while the lowest was found in E5 (0: 100%) and N1 (maltodextrin –soy protein). The lower the black cumin extract and the higher the sesame extract, the lower of antioxidant activity.

Determination of antioxidant potential was based on free radical capture (DPPH = 1,1-diphenyl-2-ficrylhidrazil) by antioxidant compounds through a mechanism of donation of hydrogen atoms so as to produce reduced DPPH or non-radical DPPH (DPPH-H = diphenyl picrylhydrazyl). The parameters for determining antioxidant potential can be seen from the IC50 value. IC50 values are used to express the potential or antioxidant activity of a compound by DPPH free radical reduction methods [12]. DPPH (2.2-diphenyl-1-pycrilhydrazyl) was a free radical compound that acts as an oxidizer when reacted with an antioxidant. The sample solution contains a compound that can function as an antioxidant when reacted with DPPH, the DPPH solution which was originally purple turned yellow, this identified that DPPH was reduced so that DPPH was converted to DPPH-H (diphenyl picrylhydrazyl).

The value of the antioxidant activity of E1N1 was relatively higher than E2N1, but both are not significantly and after being tested with De Garmo effectiveness test, E2N1 treatment was the best treatment. The treatment with encapsulate able to increase antioxidant activity was higher, this was seen from the use of extract ratio with coating material of 1:4. The maltodextrin-soy protein coating agent is more effective at binding to core material than other coatings, it can protect and maintain the stability of the core material from damage during storage and suspected that the soy protein isolates contributed to reduce free radicals so that antioxidant activity increases [5].



	N1	N2	N3
E1	76,6768	85,1244	91,5784
E2	81,4452	87,4199	103,8008
E3	85,6063	94,4897	112,1273
E4	105,9902	109,5955	119,7844
E5	117,2045	122,3961	123,6377

Coating material

Note: E1 (100:0%); E2 (75:25%); E3 (50:50%); E4 (25:75%); E5 (0:100%)

Figure 1. The Effect of ratio black cumin extract with sesame to encapsulate antioxidant activity

3.3. Qualitative determination of total flavonoids and phenolic encapsulate

Phytochemical screening is an examination phase to detect the presence of bioactive compound of plant material that contains natural antioxidants [15]. The bioactive compounds of the encapsulate product had tested are flavonoids and phenolic which had been role as antioxidants. The content of flavonoids and phenolic encapsulate can be seen in Table 3.

Based on Table 3, it can be seen that the all encapsulated products with various coating materials in the form of maltodextrin: soy protein, maltodextrin: gelatine and maltodextrin: casein gave positive results, it meant all encapsulated products contain flavonoid and phenolic compounds. The statement of [15] that flavonoids are commonly found in plants and are bound to sugar as glycosides and flavonoid aglycones. Flavonoids are compounds that dissolve in polar solvents such as ethanol. Based on the results of research by [6] that the longer of soaking by ethanol, the higher the phenol content produced from the extraction process. Phenolic compounds found in black cumin are carvacrol, gallic acid and vanillic acid [16]. Flavonoids contained in black cumin are flavonol triglycosides which are quercetin and routine flavonoid compounds [9]. Phenolic compounds of sesame are sesamol and sesamin [17]. Flavonoid compounds contained in sesame seeds have pharmacological activities such as antioxidants [1].

Based on the antioxidant activity test of black cumin and sesame seed powder, black cumin was relatively higher than sesame seed extract respectively, i.e: 67.3125 $\pm$ 2.8371 and 78.3130 $\pm$ 1.4274 (µg/mL). This can be caused not all flavonoid compounds react $AlCl_3$ [14], there may be other polyphenol compounds that could affect the IC50 value. The difference value is due to the dosage form of the product being analysed, there are the form of juice, flour or yogurt. Based on this analytical method the measured of total flavonoids are contributions from flavones and flavonols, because only these two flavonoid classes can form sTable complexes with AlCl3 on the C4 keto groups and C3 or C5 in the hydroxyl groups [8].

Table 3. The results of the encapsulate products of flavonoid and phenolic content by qualitative method

Product	Flavonoid	Phenolic
E_1N_1	+++	+++
E_1N_2	+++	+++
E_1N_3	+++	+++
E_2N_1	+++	+++
E_2N_2	+++	+++
E_2N_3	++	+++
E_3N_1	+++	++
E_3N_2	++	++
E_3N_3	++	++
E_4N_1	++	++
E_4N_2	++	++
E_4N_3	++	++
E_5N_1	+	+
E_5N_2	+	+
E_5N_3	+	+

Note:
Flavonoid: +++: yellow/dark orange, ++: yellow; +: yellowish
Phenolic: +++: dark green; ++: green; +: greenis

4. Conclusion

Encapsulate of black cumin and sesame extract with kind of coating materials were containing natural antioxidants, there are have been proven to protect the bioactive flavonoid and phenolic compounds with a ratio of 1: 3 (extract: coating material = 1: 3) compared to the extract of the powder. The best result of antioxidant activity was found on the treatment of black cumin and sesame extract (75:25%) with maltodextrin and soy-protein as coating material.

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