

THE EFFECT OF DIETARY OILS AND HYDROLYZED COCONUT OIL ON MINERALS ABSORPTION IN RATS

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ABSTRACT

Objective: The objective of this study was to investigate the effect of coconut oil (virgin coconut oil; VCO), corn oil, palm oil, and hydrolyzed VCO (HVCO) on minerals (calcium and magnesium) absorption in rats.

Methods: The oils used were corn oil, palm oil, VCO, and HVCO. VCO hydrolysis was done using lipase from *Rhizomucor miehei* (active on sn-1,3 position). The effect of oils and HVCO on minerals absorption was carried out on 25 male rats, weighing approximately 150–200 g. Rats were housed in air-conditioned room at 22–25°C, under 12-light/dark cycle, fed on basal diet and tap water *ad libitum*. After 1-week acclimatization, rats were given oils and HVCO for 21 days. The effect of oils on the minerals absorption were determined over the 4-day metabolic balance study. Mineral concentrations in diet, feces, urine, and serum were measured using atomic absorption spectrometry. Minerals absorption are expressed as apparent minerals absorption, apparent minerals absorption rate, and apparent minerals balance.

Results: Calcium absorption in rats given with VCO, corn oil, palm oil, and HVCO is 47.09%, 45.46%, 44.48%, and 49.33%, respectively, whereas, magnesium absorption in rats given with VCO, corn oil, palm oil, and HVCO is 34.87%, 32.08%, 29.39%, and 37.11%, respectively. The results of this study show that minerals absorption in rats given with dietary oils is significantly lower than the control group (51.79% for calcium and 42.34% for magnesium). Mineral absorption in rats given with HVCO results in the highest rate of all rats given with the other oils tested.

Conclusion: Corn oil and palm oil, which belong to the long chain triglycerides oil, lower calcium, and magnesium absorption more significantly than VCO as a medium chain triglyceride oil does. HVCO results in significantly higher calcium and magnesium absorption compared to non-HVCO.

Keywords: Virgin coconut oil, Enzymatic hydrolysis, *Rhizomucor miehei*, Calcium, Magnesium, Absorption.

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INTRODUCTION

Minerals are classified as macrominerals and microminerals. Our bodies need more than 100 mg macrominerals per day and <100 mg microminerals per day. Macrominerals include sodium, potassium, calcium, phosphorous and magnesium, while microminerals include ferrous, iodine, zinc and copper. Minerals play important roles in cells, tissues, organs, or overall body function. Low intake of these nutrients can be harmful. Calcium and magnesium are found in dairy products, vegetables and nuts [1-4].

More than 99% of total calcium in the body are found in bones and teeth, where calcium acts as the structural part, and the other 1% functions in the metabolism as a signal for a vital physiological process such as vascular contraction, blood coagulation, muscle contraction, and nerve transmission. Low intake of calcium is related to the increased risk of osteoporosis, nephrolithiasis (kidney stones), colorectal cancer, hypertension, stroke, coronary vascular disease, insulin resistance, and obesity [2,4,5].

Magnesium is the fourth and the second most abundant cation in the body and intracellular fluid, respectively. Magnesium is a cofactor of 350 cellular enzymes that involve in energy metabolism. Magnesium is also involved in protein and nucleic acid synthesis, normal vascular function, insulin sensitivity, nerve impulses transmission, body temperature regulation, detoxification, and formation of healthy bones and teeth. Magnesium deficiency is associated with endothelial dysfunction, increased vascular reaction, increased circulating C-reactive protein, and decreased insulin sensitivity. Insufficient intake of magnesium is

known to be involved in hypertension, coronary heart disease, Type 2 diabetes mellitus, and metabolic syndrome [2,4-7].

In the intestine, calcium and magnesium can interact with some food components causing disruption in their absorption. Those food components include oxalic acid, phytic acid, dietary fiber, and fat. Fatty acids, mainly from the hydrolyzed long-chain triglycerides (LCTs), can react with calcium and magnesium in the intestine to form insoluble salts. These salts are unable to be absorbed, and hence, excreted through the feces [8-10].

Coconut oil and virgin coconut oil (VCO), which composed mainly of medium chain triglyceride (MCT), are hydrolyzed rapidly in the mouth and stomach, while oils or fats composed of LCT are hydrolyzed slowly in the small intestine into long chain fatty acids (LCFAs). The products of hydrolyzed MCT, which are monoacylglycerol and medium chain fatty acids, are quickly absorbed through gastrointestinal mucous and then enter the circulation through the portal vein and directly to the liver [11,12].

Rezq *et al.* reported the difference in calcium absorption influenced by some dietary oils and fats, soybean oil, corn oil, palm oil, olive oil, sunflower seed oil, butter, animal fat, and margarine [13]. Tadayyon and Lutwak also reported that there were differences in calcium and magnesium absorption affected by some triglycerides such as triolein, tripalmitin, and tristearin [14]. However, a study on the effect of hydrolyzed oil on calcium and magnesium absorption has not been reported. Therefore, the objective of this work was to study the effect of some dietary oils and hydrolyzed oil on calcium and magnesium

absorption in rats. Oils used in this study were VCO, LCT oils which include palm oil (saturated oil), and corn oil (unsaturated oil). Hydrolysis of oil was done for VCO using lipase from *Rhizomucor miehei* which is active on sn-1,3 position in triglyceride molecule.

MATERIALS AND METHODS

Materials

VCO (Palem Mustika®, Indonesia), palm oil (Bimoli®, Indonesia), corn oil (Tropicana Slim®, Indonesia), lipase from *R. miehei* 200 U/g (Strem Chemicals, Inc., US), buffer tris, sodium hydroxide, concentrated hydrochloric acid, distilled water, n-hexane, sodium sulfate anhydrous, potassium hydrogen phthalate, phenolphthalein, ethanol, and demineralized water were used. All chemicals and reagents used in this work were of analytical grade.

Enzymatic hydrolysis of VCO

Preliminary study of enzymatic hydrolysis was done to determine the optimum hydrolysis condition which was tested at several temperatures (40°C and 50°C) and pH (7, 8, 10) for 8 h. To obtain the best incubation period, VCO hydrolysis was done for 14 h with every 2 h of acid value determination. The best incubation period was indicated by the constant acid value obtained during the time of hydrolysis (0–14 h) [15].

30 g of oil was weighed into 250 ml Erlenmeyer flask, and then, 30 ml distilled water, 12.5 ml 0.063 M CaCl₂, 25 ml buffer Tris-HCl 1 M pH 8, and 3 ml lipase *R. miehei* were added. The mixture was made 8 times in 8 separated Erlenmeyer flasks. All mixtures were stirred at 200 rpm for 10 min of every 1 h incubation time. Each mixture was incubated at 50°C with various incubation time of 0, 2, 4, 6, 8, 10, 12, and 14 h [14,16]. At the end of each mixture's incubation time, the mixture was transferred into the separating funnel, and then, 50 ml n-hexane was added, and extraction was done. The mixture was allowed to stand for some time until two layers were formed. The upper layer (n-hexane fraction) was separated as the first extract, while the bottom layer (water fraction) was extracted again with 50 ml n-hexanes above and separated as the second extract. The first and the second extracts were mixed, and then, 250 g sodium sulfate anhydrous was added to absorb the water residue. The combined extract was allowed to stand for 15 min, filtered, and the n-hexane was evaporated using the water bath [15-18].

Hydrolyzed VCO (HVCO) incubated for 0, 2, 4, 6, 8, 10, 12, and 14 h were labeled and their acid values were determined. HVCO with constant acid value [18] was then used to test its effect on calcium and magnesium absorption in rats.

Acid value determination

5 g of HVCO was weighed and transferred into 250 ml Erlenmeyer flask. 25 mL of neutral ethanol 90% was added and then heated for 10 min in a water bath while being stirred, then 3–5 drops of phenolphthalein were added into this solution. Titration was done with 0.1 N NaOH until the solution turned pink (color did not change for 15 min). The acid value and free fatty acid (FFA) percentage of HVCO were calculated using the following equation [15-20]:

a. Acid value

$$\text{Acid value} = \frac{A \times N \times BM_{\text{NaOH}}}{G}$$

Where,

A=NaOH solution volume (ml)

N=Normality of NaOH solution

G=Sample mass (g)

Molecular weight of NaOH = 40 g/mol

Acid value expressed as mg NaOH/g oil was then converted to mg KOH/g oil.

b. Percentage of FFA

$$\text{CFFA (wt\%)} = \frac{V_{\text{NaOH}} \times M_{\text{NaOH}} \times MM_{\text{FFA}}}{10 \times M_s}$$

Where,

CFFA=Content of free fatty acid (wt%)

V_{NaOH}=NaOH solution volume (ml)

M_{NaOH}=Molarity of NaOH solution (mol/L)

MM_{FFA}=Average molar mass of fatty acids (200.32 g/mol)

M_s=Sample mass (g).

Experimental design

The experiment was carried out on 25 male rats, weighing approximately 150–200 g. Rats were housed in an air-conditioned room at 22–25°C, under 12-light/dark cycle, fed on basal diet and tap water *ad libitum*. Animals were acclimatized for 1 week before starting the experiment. The protocol was approved by the Animal Research Ethics Committees of the Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Indonesia (bearing number: 881/KEPH-FMIPA/2016).

After acclimatization period, rats were divided into five groups with five animals each and were given as follows: (1) Without oil (control group), (2) 2 ml/kg body weight of VCO, (3) 2 ml/ kg body weight of palm oil, (4) 2 ml/ kg body weight of corn oil, and (5) 2 ml/ kg body weight of HVCO, for 21 days. In the last week of experimental period (day 15–21), rats were housed in 25 individual metabolic cages containing a grid-floor and a facility for separate collection of feces and urine. Rats were acclimatized in the new environment for 2 days before the beginning of 4 days metabolic study. During the 4-day metabolic balance study period, each rat was given 7 g/day basal diet, so all of them consumed all the diet. Urine and fecal samples (24 h) from each animal were collected, and the volume of urine was recorded. Portions of the urine samples were acidified with 12 N HCl about 3% of its volume and stored at 221–20°C until required for analysis [13,21].

Quantitative Analysis of Calcium and Magnesium in Samples

Calcium calibration curve was prepared from standard solutions with the concentration of 0, 2, 4, 6, 8, and 10 ppm, while magnesium calibration curve was prepared from standard solutions with the concentration of 0, 0.2, 0.4, 0.6, 0.8, and 1 ppm. Each standard solution contains lanthanum chloride 1% and an aqueous solution containing lanthanum chloride 1% was used as blank. Calcium and magnesium in standard solutions were measured using atomic absorption spectrometry (AAS) at the wavelength of 422.7 nm and 285.2 nm, respectively [22,23]. Calibration curves prepared were linear with the correlation coefficient of 0.9998 and 0.9995 for calcium and magnesium, respectively. The regression equation for calcium was $Y = 0.0722x + 0.0061$, while for magnesium was $Y = 0.9791x + 0.01565$.

The diet and fecal samples were ash-dried at 700°C for 12 h and then solubilized with 6 N HCl solution. Diet, fecal, and urine samples were then diluted with an aqueous solution of lanthanum chloride 1% w/v. Calcium and magnesium content in diet consumed, feces, and urine excreted per day were measured using AAS as the procedure mentioned above and calculated using the following equations [13,21-23]

$$\text{Ca or Mg intake (mg/day)} = \frac{X \times V \times DF}{W_s}$$

$$\text{Fecal Ca or Mg (mg/day)} = X \times V \times DF$$

$$\text{Urinary Ca or Mg (mg/day)} = \frac{X \times V \times DF}{V_s} \times V_u$$

Where,

X = Concentration of the diluted sample (µg/ml)

V = Volume of the dilution (ml)

DF = Dilution factor (250 for diet and fecal sample; 1 for urine sample)
 Ws=Weight of the sample (7 g)
 Vs=Volume of the measured sample (ml)
 Vu=Volume of the urine collected (ml).

Apparent absorption, apparent absorption rate, and apparent balance of each mineral (calcium or magnesium) were calculated using the following equations [13].

Apparent Ca or Mg absorption (mg/day) = Ca or Mg intake in diet-fecal Ca or Mg

$$\text{Apparent Ca or Mg absorption rate (\%)} = \frac{\text{Ca or Mg absorption}}{\text{Ca or Mg intake in diet}} \times 100$$

Apparent Ca or Mg balance (mg/day) = Ca or Mg intake in diet-(fecal Ca or Mg+urinary Ca or Mg).

At the last day of experimental period, rats were fasted for 12 h and sacrificed. Blood was drawn directly from the heart and centrifuged at 3000 rpm for 15 min to obtain serum (supernatant). Serum (0.5 ml) was diluted with an aqueous solution of lanthanum chloride 1% w/v. Calcium and magnesium concentration (mg/dl) in serum were measured using AAS and calculated using the following equation [13,14,23]:

$$\text{Serum Ca or Mg (mg/dl)} = \frac{X \times V \times DF}{V_s \times 10}$$

Where

X=Concentration of the diluted sample (µg/ml)

V=Volume of the dilution (ml)

DF=Dilution factor (1)

Vs=Volume of the sample (ml).

RESULTS AND DISCUSSION

Optimum condition of VCO enzymatic hydrolysis

VCO enzymatic hydrolysis was done using lipase from *R. miehei* which is specific for acyl groups at sn-1 and sn-2 position in triglyceride molecule. In this study, the optimum condition for VCO enzymatic hydrolysis was done at several temperatures and pH [24]. To determine the optimum temperature (40°C or 50°C), hydrolysis was done at pH 8 and incubation time of 8 h. Acid value and %FFA are shown in Table 1.

Acid value and %FFA of VCO hydrolyzed at 50°C are higher than VCO hydrolyzed at 40°C. This indicates that lipase from *R. miehei* is more active at 50°C. At temperature of 50°C, this enzyme hydrolyzes fatty acids on sn-1 and sn-3 position in triglyceride molecule which generates two FFA and one 2-monoglyceride. From Table 1, it is shown

Table 1: Effect of temperature on VCO enzymatic hydrolysis

Temperature	Acid value (mg KOH/g oil) n=3	FFA (%) n=3
40°C	83.29±0.12 ^a	29.79±0.05 ^a
50°C	126.11±0.39 ^b	45.33±1.82 ^b

Means±SE in each column with different superscript letters differ significantly at p<0.05. VCO: Virgin coconut oil

Table 2: Effect of pH on VCO enzymatic hydrolysis

pH	Acid value (mg KOH/g oil) n=3	FFA (%) n=3
7	91.3438±0.4114 ^a	32.68±0.14 ^a
8	126.1098±0.3931 ^b	45.33±1.82 ^b
10	126.1798±0.1329 ^b	45.14±0.05 ^b

Means±SE in each column with different superscript letters differ significantly at p<0.05. VCO: Virgin coconut oil

that the optimum temperature for enzymatic hydrolysis is 50°C. Hence, to determine the optimum pH (7, 8 or 10), hydrolysis was done at 50°C and incubation time of 8 h. Effect of pH on acid value and %FFA of HVCO is shown in Table 2.

From Table 2, it is shown that the lowest acid value and %FFA are obtained at pH 7 which are 91.34 Mg KOH/g oil and 32.68%, respectively, while the acid value and %FFA obtained at pH 8 and 10 are not significantly different, and hence, the hydrolysis VCO was done at pH 8.

Optimum incubation time of VCO enzymatic hydrolysis

Enzymatic hydrolysis of VCO was carried out for 14 h, and acid value was determined in every 2 h of incubation time. The optimum incubation time indicated by the constant acid value obtained, after which there is no more FFA elevation because every triglyceride molecules from VCO has been hydrolyzed by lipase from *R. miehei* specifically at sn-1 and sn-3 position. Acid value determines the amount of FFA in fats or oils. It is defined as mg KOH used to neutralize FFA contained in 1 g of fats or oils. VCO enzymatic hydrolysis results in two FFA and one 2-monoglyceride from one triglyceride molecule contained in VCO [15,19]. The results show that there is an increasing acid value from 0 to 14 h incubation time, while constant acid value was obtained after 10 h of incubation time. This indicates that FFAs are not increasing significantly anymore after 10 h of incubation time. Acid value and FFA (%) obtained from various incubation time are shown at Table 3 and Fig. 1.

Lipase (triacylglycerol acylhydrolases) catalyzes the hydrolysis and synthesis of esters formed from glycerol and fatty acids. Lipase is activated only when adsorbed to an oil-water interface. Commercially useful lipases are usually obtained from microorganisms. Lipase from *R. miehei* is an enzyme which hydrolyzes triglycerides specifically on sn-1 and sn-3 position, and hence, this enzyme works similarly to the lipases in human's gastrointestinal tract. Oils and fats are hydrolyzed enzymatically in the body by lipases found in the mouth (lingual lipase), stomach (gastric lipase), and intestine (pancreatic lipase). These lipases hydrolyze triglycerides at sn-1 and sn-3 position [9,25,26].

Effect of oils and HVCO on calcium and magnesium absorption

Effect of some dietary oils and HVCO on fecal, urinary, serum calcium, and magnesium are presented in Tables 4 and 5.

Results show that the oils tested and HVCO cause significant increases in fecal calcium and magnesium and urinary calcium and magnesium, while significant decreases in serum calcium and magnesium compared to the control group. The highest to lowest fecal and urinary calcium are in rats given with palm oil, corn oil, VCO, and HVCO, respectively. The highest to lowest serum calcium

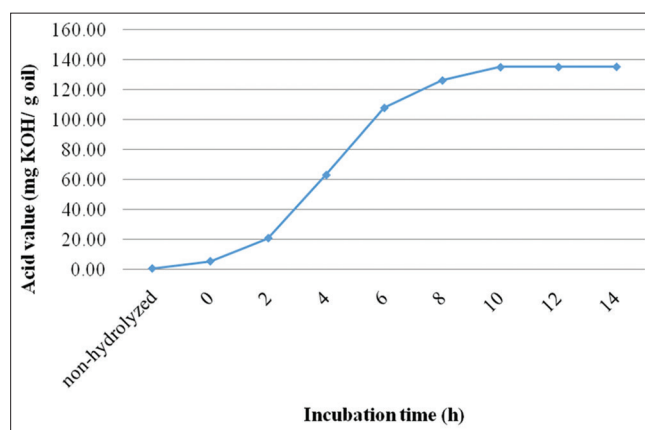


Fig. 1: The effect of incubation time on acid value of hydrolyzed virgin coconut oil

and magnesium are found in rats given with HVCO, VCO, corn oil, and palm oil, respectively. Minerals absorption can be shown from the fecal, urinary, and serum minerals.

Elevation of fecal calcium and magnesium in groups fed on oils indicates the elevation of calcium and magnesium excretion which can be caused by disruption of calcium and magnesium absorption from the diet. Fatty acids, the product of hydrolyzed oil in gastrointestinal tract, react with calcium and magnesium from the diet and form salts of minerals and fatty acid. This salt is insoluble and hardly absorbed by the intestine mucous, therefore, it is excreted through the feces. Reduction of serum calcium and magnesium in rats given with oils can be explained by the elevation of calcium and magnesium excretion through the urine and feces [5,8,27,28].

Effect of oils and HVCO on apparent minerals absorption, apparent minerals absorption ratio, and apparent minerals balance is shown in Tables 6 and 7.

Results show that dietary oils given to rats cause significant decreases in minerals absorption compared to the control group. The highest to lowest minerals absorption in rats given with 2 ml/kg body weight of oils per day are HVCO, VCO, corn oil, and palm oil, respectively.

Difference between the effect of oils on calcium absorption and magnesium absorption is shown in Fig. 2.

As seen in Fig. 2, calcium absorption is higher than magnesium absorption in all groups including groups fed with and without oil.

Table 3: The effect of incubation time on acid value of HVCO

Sample	Incubation time (h)	Acid value (mg KOH/g oil) n=3	FFA (%) n=3
Non-HVCO	-	0.69±0.13 ^a	0.25±0.05 ^a
Enzymatically HVCO	0	5.52±0.29 ^b	1.97±0.10 ^b
	2	20.91±0.74 ^c	7.48±0.27 ^c
	4	63.02±0.67 ^d	22.56±0.14 ^d
	6	107.79±0.14 ^e	38.56±0.05 ^e
	8	126.11±0.39 ^f	45.33±1.82 ^f
	10	134.96±0.23 ^g	48.28±0.10 ^g
	12	135.07±0.11 ^g	48.32±0.03 ^g
	14	135.20±0.44 ^g	48.36±0.17 ^g

Means±SE in each column with different superscript letters differ significantly at p<0.05. HVCO: Hydrolyzed virgin coconut oil

Table 4: Effect of dietary oils and HVCO on fecal, urinary, and serum calcium

Treatment	Fecal Ca (mg/day) n=5	Urinary Ca (mg/day) n=5	Serum Ca (mg/dl)n=5
Control	57.95±0.27 ^a	0.04±0.001 ^a	11.07±0.14 ^a
VCO	63.60±0.37 ^b	0.07±0.001 ^b	10.46±0.07 ^b
Corn oil	65.56±0.49 ^c	0.09±0.001 ^c	10.24±0.07 ^c
Palm oil	66.73±0.22 ^d	0.10±0.001 ^d	10.05±0.10 ^d
HVCO	60.91±0.43 ^e	0.05±0.001 ^e	10.59±0.07 ^e

Means±SE in each column with different superscript letters differ significantly at p<0.05. HVCO: Hydrolyzed virgin coconut oil

Table 5: Effect of dietary oils and HVCO on fecal, urinary, and serum magnesium

Treatment	Fecal Mg (mg/day) n=5	Urinary Mg (mg/day) n=5	Serum Mg (mg/dl) n=5
Control	8.61±0.001 ^a	0.02±0.0001 ^a	4.19±0.03 ^a
VCO	9.72±0.03 ^b	0.03±0.0001 ^b	4.12±0.02 ^b
Corn oil	10.14±0.12 ^c	0.03±0.0004 ^b	3.97±0.04 ^c
Palm oil	10.54±0.04 ^d	0.03±0.0003 ^b	3.38±0.03 ^d
HVCO	9.39±0.04 ^c	0.03±0.0002 ^b	4.19±0.04 ^c

Means±SE in each column with different superscript letters differ significantly at p<0.05. HVCO: Hydrolyzed virgin coconut oil

Table 6: Effect of oils and HVCO on calcium absorption

Treatment	Apparent Ca absorption (mg/day) n=5	Apparent Ca absorption ratio (%) n=5	Apparent Ca balance (mg/day) n=5
Control	62.25±0.27 ^a	51.79±0.22 ^a	62.21±0.27 ^a
VCO	56.60±0.37 ^b	47.09±0.31 ^b	56.53±0.37 ^b
Corn oil	54.64±0.46 ^c	45.46±0.38 ^c	54.55±0.46 ^c
Palm oil	53.47±0.22 ^d	44.48±0.18 ^d	53.37±0.22 ^d
HVCO	59.29±0.43 ^e	49.33±0.36 ^e	59.24±0.43 ^e

Means±SE in each column with different superscript letters differ significantly at p<0.05. HVCO: Hydrolyzed virgin coconut oil

Table 7: Effect of oils and HVCO on magnesium absorption

Treatment	Apparent Mg absorption (mg/day)n=5	Apparent Mg absorption ratio (%) n=5	Apparent Mg balance (mg/day)n=5
Control	6.32±0.05 ^a	42.34±0.32 ^a	6.30±0.05 ^a
VCO	5.21±0.03 ^b	34.87±0.25 ^b	5.18±0.03 ^b
Corn oil	4.79±0.12 ^c	32.08±0.79 ^c	4.76±0.12 ^c
Palm oil	4.39±0.04 ^d	29.39±0.30 ^d	4.36±0.04 ^d
HVCO	5.54±0.04 ^e	37.11±0.26 ^e	5.51±0.04 ^e

Means±SE in each column with different superscript letters differ significantly at p<0.05. HVCO: Hydrolyzed virgin coconut oil

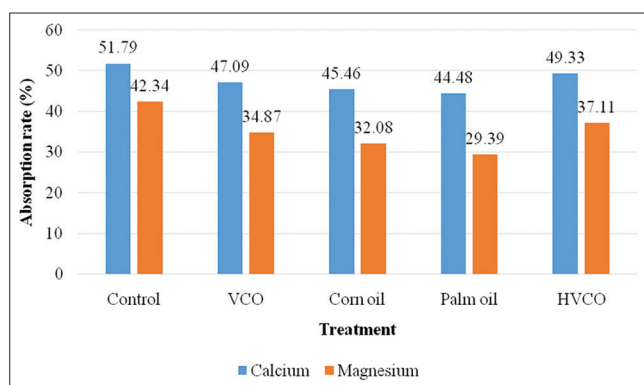


Fig. 2: Effect of oils on calcium and magnesium absorption

However, the effect of oils on magnesium absorption is more significant than on calcium absorption. The highest reduction of calcium and magnesium absorption is in the group fed on palm oil. The effect of palm oil on the reduction of calcium absorption is 7.41%, while the reduction of magnesium absorption is 12.95%.

From the results obtained in this study, treatment with dietary oils whether MCT, LCT, or hydrolyzed oil significantly decreases calcium and magnesium absorption compared to the control group (without oil). LCT (corn oil and palm oil) causes lower mineral absorption than MCT (VCO) and HVCO. Our results agreed with the previous study reported that MCT causes higher calcium absorption rather than LCT. These results may be possibly explained by the basis that LCT is hydrolyzed in the small intestine by pancreatic lipase and absorbed slowly through lymphatic system into the blood circulation. On the other hand, MCT is quickly hydrolyzed, begins in the mouth by lingual lipase, and continues in the stomach by gastric lipase then absorbed into the liver through portal vein, and hence, most hydrolyzed product do not reach the small intestine. Calcium and magnesium can react with LCFA generated from LCT oils hydrolysis to form insoluble mineral salts, which are not absorbed by intestine mucous, hence excreted through the feces [9,10,29,30].

Concerning the effect of corn oil and palm oil on minerals absorption, palm oil causes lower minerals absorption than corn oil does. These results are in accordance to the previous studies reported. Calcium and magnesium absorption decreases significantly in animals fed on saturated fats than animals fed on unsaturated fats. Our results show that apparent calcium absorption in rats fed on corn oil and palm oil is 54.64 and 53.47 mg/day, respectively. While apparent magnesium absorption in rats fed on corn oil and palm oil is 4.79 and 4.39 mg/day, respectively. This may be caused by the difference between fatty acid composition in corn oil and palm oil. Corn oil contains unsaturated fatty acids (mainly linoleic acid) more than palm oil does, while palm oil contains more saturated fatty acids (mainly palmitic acid) [13,14,31].

Non-HVCO causes lower calcium and magnesium absorption compared to HVCO because enzymatically HVCO is readily and quickly absorbed through portal vein, hence, decreasing its chance to react with calcium and magnesium in gastrointestinal tract to form the insoluble salt. It means that insoluble salt is very low or not produced, and hence, mineral absorption is not disrupted.

CONCLUSION

Optimum temperature and pH of VCO enzymatic hydrolysis using lipase from *R. miehei* are 50°C and pH 8, respectively. The best incubation time for VCO enzymatic hydrolysis is after 10 h. LCT (corn oil and palm oil) causes a significant decrease in calcium and magnesium absorption compared to MCT (VCO). HVCO causes a significant increase in calcium and magnesium absorption compared to non-HVCO.

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