

Functional properties of gluten-free gathotan noodle: lipid profile and satiety power

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Abstract

Gathotan flour had been made into gluten-free gathotan noodle with acceptable texture and sensory characteristics. This work deals with examining profile, and satiety power of gathotan noodle. Lipid profile was determined using fifteen wistar mice for each control or treatment group. Lipid profile was expressed as concentration of total triglyceride, total cholesterol, LDL, and HDL. Satiety power was studied using 30 volunteers and expressed as time to return to baseline pre-prandial level. Consumption of gathotan noodle reduced all lipid profile parameters, with total triglycerides, total cholesterol, HDL, and LDL were 32.46, 20.4, 19.8, 8.46 mg/dL in treatment mice, and 61.67, 71.6, 34.93, 12.46 mg/dL, in control mice, respectively. Consumption of gathotan noodle showed that fullness was reached by consuming about half (100 g cooked noodle) of that of control noodle (200 g of cooked wheat noodle). In either gathotan or control noodle, hunger started at 60 min after consumption.

Keywords

Noodle

Cassava

Gluten-free

Lipid profile

Satiety

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Introduction

Overweight and obesity has been a significant problem globally. According to WHO (2015) 39% of adults, equals to 1.9 billion, suffered from overweight, while 13% of them, equals to 600 million, were obese. Even 42 million children under 5 years old also showed obesity. The problem has led to some chronic diseases such as cardio vascular related disorders, diabetes, musculoskeletal, and some types of cancer. Obesity also lead to death. Obesity has also been linked to hyperlipidemia (Frank, 1988, Akiyama *et al.*, 1996) due to conversion of dietary fat from skeletal muscle to adipose tissue leading to raise in serum lipid (Roberts *et al.*, 2002). Changing sugar diet into complex carbohydrate reduced both ailments (Roberts *et al.*, 2002).

There are several ways to ameliorate cholesterol problem through diet, including incorporation of polysaccharides from various sources, such as *Liriope spicata* tuber (Chen *et al.*, 2009); pumpkin (Zhao *et al.*, 2014), *Ganoderma atrum* mushroom (Zhu *et al.*, 2013), *Laminaria japonica* seaweed (Zha *et al.*, 2012), guar gum, xanthan gum, or pre-gelatinised corn starch (Castro *et al.*, 2003), and *Enteromorpha prolifera* (Tang *et al.*, 2013). Some substances were also reported to improve lipid profile in testing animals, including β -cyclodextrin or resistant starch (Trautwein *et al.*, 1999), chitosan

(Zhang *et al.*, 2012), levan (Yamamoto *et al.*, 1999), rice bran extract (Revilla *et al.*, 2009), and yam and boxthorn noodle mixture (Liu *et al.*, 2006).

Obesity is prevented by limiting calorie intake, which includes low calorie diet, or high satiating food. Satiating has drawn considerable attention, where publications in this topic was doubled during the last ten years (Fizman and Varela, 2013). High satiating diet is an approach to control hunger and food intake. There are several food components that showed satiety power, including dietary fibres of many sources (Jørgensen *et al.*, 2010, da Silva *et al.*, 2012, Sun *et al.*, 2015). Satiety has been correlated to energy content of food, digestion, and food structure (Fizman and Varela, 2013), but its correlation to blood glucose level was considered debatable (Hlebowicz, 2009, Fizman and Varela, 2013). Using several types of pancake, it was shown that higher glycemic index gave lower satiety, and that glycemic response did not correlate to gastric emptying (Clegg *et al.*, 2012). However, it was also reported that satiety did not show any correlation to glycemic index in bread (Holt *et al.*, 2001). Slow gastric emptying rate was attributed to diet rich in fibre which can increase swelling and water binding in the stomach (Jørgensen *et al.*, 2010). Therefore, some food or drink had been added by hydrocolloid to give satiating effect, such as β -glucan added to biscuit or fruit juice (Pentikäinen *et al.*, 2014), and

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yoghurt (Perrigue *et al.*, 2009). Texture of food also seems to affect satiety (Fiszman and Varela, 2013). Solid food was more satiating than liquid drink of the same energy density (O'Neill *et al.*, 2012). Viscosity, however, did not show consistent effect on satiety (Logan, 2013).

This work deals with evaluating the effect of gathotan noodle consumption on postprandial lipid profile and satiety. Gathotan noodle is a gluten-free noodle made from fungal fermented cassava (Purwandari *et al.*, 2014a), which showed antioxidant activity and hypoglycemic effect upon consumption (Purwandari *et al.*, 2014b).

Materials and Methods

Materials

Moldy dried cassava (gathotan) chunks were prepared following a method described by Purwandari *et al.* (2014a). In brief, cassava tubers bought from local market in Kamal, Madura, Indonesia, were peeled and washed, then sun-dried until half-dry. Half-dry cassava tubers were then mixed with 50% suspension of gathotan flour (in potable water) taken from previous batch. Gathotan suspension was lightly spread on surface of each piece of tuber. The tubers were then put in a plastic container, covered with the lid, and let it stand in room temperature for 3 days for fermentation. Every day, tubers were taken out of container, and sun-dried for four hours, and then put back in the container. The process was repeated until 10 days when inside tuber turned black. This black colour tuber is called gathotan. Gathotan tubers were then dried in a cabinet drier at 35°C until fully dry (moisture content around 14%), then ground using an electric grinder, and accordingly passed through a 60 mesh sieve. Gathotan flour was then kept in an air tight container, at room temperature, until used.

Noodle making

Gathotan noodle was made following a previously described method (Purwandari *et al.*, 2014a). Gathotan flour was mixed with distilled water to form 1:1 (w/w) suspension. The suspension was heated in a water bath at 80°C with continuous stirring until all part of flour was gelled. Gathotan gel was then cooled down to room temperature. The gel was then mixed with dry gathotan flour (5:5.5, w/w, for gel and dry gathotan flour, respectively) to form smooth dough. The dough was then rolled into 1 mm thick sheet which was then steamed for about 15 min until all part of the sheet was gelled. At the end of steaming, the sheet was cooled down to room temperature and cut into strips using a noodle roller.

Noodle strips were dried in a cabinet drier at 35°C, until fully dry (moisture content around 14%), then stored in an air tight container until used. Before consumption, noodle was put in boiling water until fully cooked, and drained.

Lipid profile examination

Lipid profile was determined according to a previous method by Lin *et al.* (2006) with some modifications. Wistar mice of 6 weeks old were fed with laboratory standard meal for two weeks before treatment. The standard meal was made from mixed of corn grit, rice bran, fish flour, coconut cake, wheat, peanut cake, canola, and dried mixed leaves. The meal contained 13% moisture, 13-15% protein, minimum of 3% fat, minimum of 8% fibre, maximum of 6% ash, minimum of 0.8% calcium, and minimum of 0.6% phosphorous. Mice were divided into two groups with each group consisted of 9 mice. Mice in the control group were fed commercial pellet for mice, while mice in treatment group were fed gathotan noodle, for three consecutive weeks ad libitum. Every three days, weight of food intake and mice body were measured, throughout testing period. At the end of testing period, mice were fast for 14 hours, and then anaesthetized with ether. Blood was collected and kept in a 1.5 mL vial, and let to stand at room temperature for two hours, before centrifuged at 12,000 g for 15 min at 4°C for serum separation. The animals were then sacrificed using CO₂ inhalation. Serum triglyceride, total cholesterol, and low-density lipoproteins were determined using a commercial kits (Liquicheck™ Lipids Control, Level 1, Bio-Rad).

Satiety power examination

Determination of satiety power was carried out according to a method used previously by Schuring *et al.*, (2012) and Pentikainen *et al.*, (2014) with some modifications. Thirty volunteers consisted of University students within Faculty of Agriculture, age 18-21, and body mass index of 18-23, with no diabetes history within family, non-smokers nor alcohol drinking, were recruited. They were given the testing procedure in brief, especially to understand questionnaire. Before start testing, they were asked to do fasting for 10 hours, allowed only limited water drinking during fasting period until one hour before testing. They arrived to the testing venue 30 min before testing. Prior to the testing, they were asked to fill in a form on self-rating of hunger and desire to eat, to establish a baseline. The questionnaire consisted of four questions for rating in scale of 1-9 about: (1) hunger feeling, (2) feeling of fullness, (3) desire to eat, (4) how much food

Table 1. Lipid profile of mice as affected by gathotan noodle consumption

Parameter	Control mice	Treatment mice	Significance
Pre-treatment body weight (g)	205.00 ± 4.84	206.00 ± 4.84	n.s.
Post-treatment body weight (g)	241.25 ± 3.09	186.83 ± 3.09	*
Total triglycerides (mmol/dL)	89.50 ± 7.54	25.50 ± 7.54	*
Total cholesterol (mmol/dL)	77.08 ± 3.33	40.58 ± 3.33	*
HDL (mmol/dL)	43.67 ± 2.22	24.75 ± 2.22	*
LDL (mmol/dL)	15.58 ± 1.94	10.58 ± 1.94	n.s.
TG/HDL	2.29 ± 0.41	1.08 ± 0.17	*
TC/HDL	1.80 ± 0.08	1.67 ± 0.05	n.s.
TC/LDL	6.16 ± 0.86	3.93 ± 0.22	*
HDL/LDL	3.44 ± 0.40	2.41 ± 0.19	*
Non-HDL-C (mmol/dL)	33.41 ± 2.99	15.83 ± 0.97	*

Data were expressed as mean ± standard error of means, from 12 replications
n.s.: no significant difference ($P \geq 0.05$), *: significant difference ($P < 0.05$)

wanted to be taken. We used two types of meal to be tested: dried commercial wheat noodle as control meal, and gathotan noodle as testing meal. Testing of each meal was separated by five days. After finish filling base line questionnaire, volunteers were asked to consume the meal in around 15 min until they felt full. At the end of eating, they were again asked to rate their hunger, fullness, desire to eat, and quantity of meal wanted to be taken, using similar questions and scale as in the first questionnaire. Then every 15 min, similar questions as in the questionnaire were addressed to establish baseline, until 120 min. Left over noodle in each plate was weighed.

Data processing

All data were processed using a statistical package SPSS® version 16 (SPSS, Inc.), for analysis of variance. Duncan method at 5 % confidence level was then used to analyse any statistical difference among means.

Result and Discussion

Lipid profile examination

Body weight of untreated or treated group of mice was not different ($P > 0.05$) at the beginning of treatment (Table 1), 205.00 and 206.00 g, respectively. However, after treatment, mice fed with gathotan noodle showed significantly ($P < 0.05$) lower body weight (183.83 g) than that of treated mice (241.25 g). This means about 20% difference in body weight of the two groups of mice. There was no considerable difference in food intake between control and treatment mice (data not shown). At the end of testing period, total triglyceride of treated mice (25.50 mmol.dL⁻¹) was about one-third of that

of untreated mice (89.50 mmol.dL⁻¹). Similarly, total cholesterol and HDL of treated mice (40.58 and 24.75 mmol.dL⁻¹, respectively) were lower than that of control mice (77.08 and 43.67 mmol.dL⁻¹, respectively). LDL of both group of mice (10.58 and 15.58 mmol.dL⁻¹, for treated and control mice, respectively) was not significantly different ($P \geq 0.05$). Ratio of total triglycerides to HDL (TG/HDL) was significantly ($P < 0.05$) higher in control mice (2.29) than in treatment mice (1.08). Similarly, ratio of total cholesterol to LDL (TC/LDL) in treatment mice (3.93) was only half of that of control mice (6.16). Ratio of HDL to LDL (HDL/LDL) in control mice was surprisingly higher (3.44) than that of treatment mice (2.41). Both groups of mice did not differ significantly ($P \geq 0.05$) in their ratio of total cholesterol to HDL (TC/HDL). Concentration of non-HDL cholesterol (non-HDL-C) was significantly ($P < 0.05$) higher in control mice (33.41 mmol.dL⁻¹) than in treatment mice (15.83 mmol.dL⁻¹).

Compared to a functional noodle made from purple yam and boxthorn (Lin *et al.*, 2006), gathotan noodle seems to show better lipid profile where gathotan noodle did not only reduce TC, and TG, but also showed significant reduction on HDL. Similar to yam and boxthorn noodle, gathotan noodle did not significantly ($P \geq 0.05$) affect LDL concentration. Several previous studies showed that hypolipidemic effect of some functional materials was prominent only in diabetic animal (Chen *et al.*, 2009, Shah *et al.*, 2011, Zhu *et al.*, 2013), or high-fat diet animal (Castro *et al.*, 2003, Revilla *et al.*, 2009, Zha *et al.*, 2012, Zhang *et al.*, 2012).

While ratio among TG, TC, HDL and LDL in this work was not easy to comprehend, data on non-HDL-C (non-HDL-cholesterol) showed higher

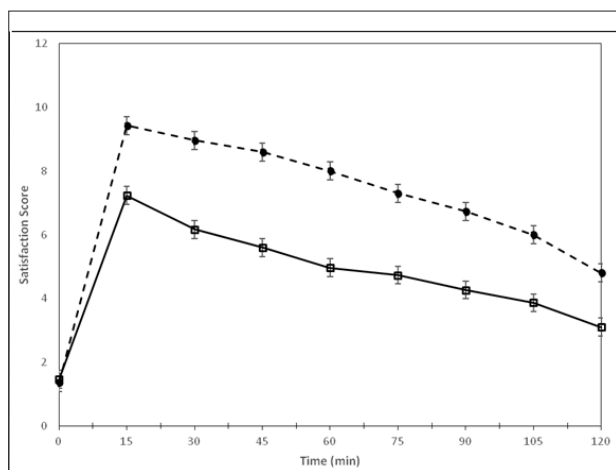


Figure 1. Self-rating score of satisfaction upon consumption of gathotan noodle. (—) is for control commercial dried wheat noodle, and (-----) is for gathotan noodle.

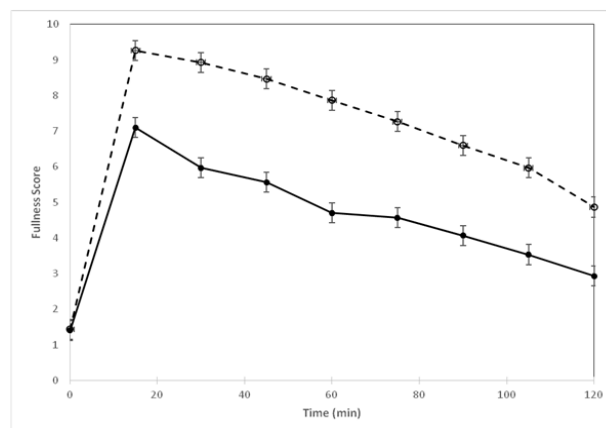


Figure 2. Self-rating score of fullness upon consumption of gathotan noodle. (—) is for control commercial dried wheat noodle, and (-----) is for gathotan noodle

result for control mice compared to that of treatment mice, indicating the possibility of gathotan noodle to reduce risk of coronary heart disease. Non-HDL-C is derived by subtracting LDL from TC. Non-HDL-C, and was reported to be more valuable in indicating the risk of coronary heart disease (CHD) (Kilgore *et al.*, 2014), where higher non-HDL-C correlates to higher risk of CHD (Kilgore *et al.*, 2014). Relying only on LDL may underestimate the risk (Kilgore *et al.*, 2014).

As gathotan noodle can substantially alter lipid profile, it may mean that gathotan noodle is a potential hypolipidemic food. We could not explain lipid lowering mechanism of gathotan noodle, but several reasons are possible. Firstly, gathotan noodle may contain polysaccharide, possibly in the form of dextrin which was able to give hypolipidemic effect. Several types of polysaccharides reduced TG and TC, such as β -cyclodextrin (Trautwein *et al.*, 1999), fungal polysaccharide from *Ganoderma atrum* (Zhu *et al.*, 2013), polysaccharide from a seaweed *Laminaria japonica* (Zha *et al.*, 2012), levan (Yamamoto *et al.*, 1999), chitosan (Zhang *et al.*, 2012), polysaccharide from pumpkin (Zhao *et al.*, 2014), and polysaccharide of *Liriope spicata* (Chen *et al.*, 2009). When using normal healthy test rodents, polysaccharide also increased HDL and reduced LDL, such in the case of pumpkin polysaccharides (Zhao *et al.*, 2014). There are several mechanism of lipid lowering of polysaccharides, including inhibition of adsorption of sterol into serum, as in the case of levan (Yamamoto *et al.*, 1999) or β -cyclodextrin (Trautwein *et al.*, 1999). Secondly, antioxidant activities in gathotan noodle (Purwandari *et al.*, 2014b) may play role in the prevention of oxidation of LDL-cholesterol. Certain type of polysaccharide showed that antioxidant properties improved lipid profile of high fat diet test animals (Tang *et al.*, 2013). Some plant material with

antioxidant activities also inhibited LDL oxidation, and lowering LDL cholesterol in the serum of high fat diet rodents (Luo *et al.*, 2009, Weng *et al.*, 2014). Prevention of LDL oxidation was proposed to hinder development of arterosclerosis (Barter, 2006).

Satiety power determination

Satisfaction of volunteers consuming gathotan noodle was significantly ($P < 0.05$) higher than those consuming commercial wheat noodle (Figure 1). Right after finished consumption of noodle, at 15 minutes, those consuming gathotan noodle scored satisfaction at 9.4, while those consuming wheat noodle rated satisfaction at 7.2. We also noted that the quantity of noodle consumed was different between the two. All volunteers consuming wheat noodle finished 200 g of cooked noodle served during the test. One of volunteers took more (about 100 g) noodle. However, volunteers consuming gathotan noodle only take 140 g of cooked noodle in average (data not shown), and no one took more noodle. It took 1 hour for gathotan noodle volunteers to reached satisfaction (7.3), a score similar to 15 min after consumption of wheat noodle (7.2). Similarly, at the end of testing period at 120 min, satisfaction for gathotan noodle consumption was higher (4.8) that that of wheat noodle (3.1).

Feeling of fullness in the stomach after consumption of gathotan noodle was also significantly ($P < 0.05$) higher than that of wheat noodle (Figure 2). Upon consumption of gathotan noodle, score of fullness was 9.3 which was higher than that of wheat noodle (7.1). After 1 hour of consumption of gathotan noodle, fullness (7.2) was scored similar ($P > 0.05$) to that of 15 min after consumption of wheat noodle. At the end of 120 min, feeling of fullness upon gathotan noodle consumption (4.9) was significantly ($P < 0.05$) higher than that of wheat noodle consumption (2.9).

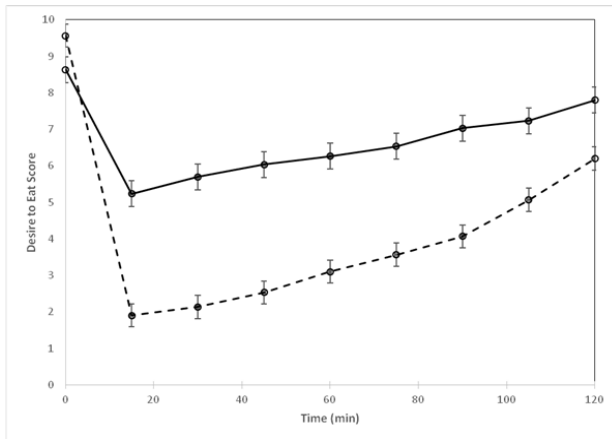


Figure 3. Self-rating score of desire to eat upon consumption of gathotan noodle. (—) is for control commercial dried wheat noodle, and (-----) is for gathotan noodle.

In other word, consumption of gathotan noodle gave more intense and longer feeling of fullness in the stomach.

Correspondingly, the score for desire to eat was lower in volunteers consuming gathotan noodle than those consuming wheat noodle (Figure 3). Desire to eat dropped after noodle consumption. However, desire to eat right after consumption of gathotan noodle was scored significantly ($P < 0.05$) lower (1.9) than that of wheat noodle consumption (5.2). After 105 min of gathotan consumption, the rate of eating desire was the same (5.1, $P < 0.05$) as that at 15 min after wheat noodle consumption. Consequently, after two hours of consumption, the score for desire to eat in volunteers consuming gathotan noodle was significantly ($P < 0.05$) lower (6.2) than those consuming wheat noodle (7.8). This may indicate that consuming gathotan noodle can lower eating desire, as compared to consuming wheat noodle.

The amount of food wanted to be taken went down right after consumption of noodle (Figure 4), with that of gathotan noodle consuming volunteers (2.0) was significantly ($P < 0.05$) lower than that of volunteers consuming wheat noodle (5.0). The score for gathotan noodle volunteers (4.4) at 90 minutes, was the same ($P \geq 0.05$) as that of wheat noodle volunteers at 15 minutes (5.0). Accordingly, at the end of testing period, volunteers consuming gathotan noodle wanted less food (6.3) than those consuming wheat noodle (7.7).

Gathotan had harder texture (Purwandari et al., 2014a) than dried wheat noodle, and consequently needs more chewing. Longer chewing is correlated to high satiety (Hogenkamp and Schiöth, 2013). Texture can affect palatability and food intake (Pritchard et al., 2014). In this work, the amount of gathotan noodle taken by volunteers was lower almost half-

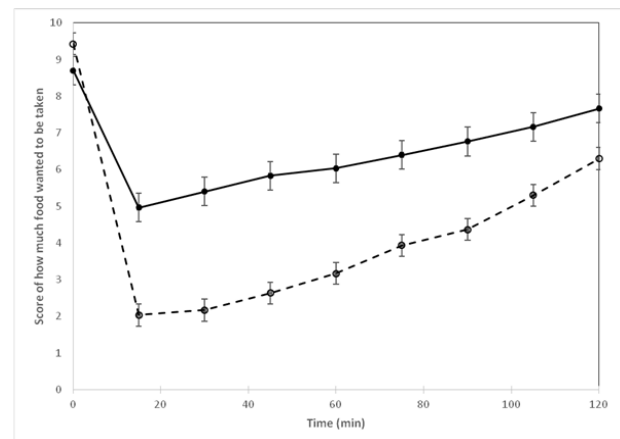


Figure 4. Self-rating score of how much food wanted to be taken upon consumption of gathotan noodle. (—) is for control commercial dried wheat noodle, and (-----) is for gathotan noodle.

the weight of dried wheat noodle. Ad libitum intake of food to achieve the feeling of fullness is called satiation (Hogenkamp and Schiöth, 2013). Although we did not examine satiation, this can be considered as an indication of high satiation of gathotan as compared to dried wheat noodle. High satiety food may have low palatability (Willis et al., 2009). Satiating power of food is closely related to consumers' sensory and cognitive appraisal (Chambers et al., 2014). Overall preference of gathotan noodle (5.5) was substantially lower than that of dried commercial wheat noodle (7.6) (Purwandari et al., 2014a). This may affect less gathotan noodle being taken by volunteers.

Gathotan noodle is a new product. A previous study indicated that familiarity of eating food affected satiety response (Irvine et al., 2013). It was suggested to familiarise volunteers to a new product to increase expected satiety (Irvine et al., 2013). We did not study gathotan components. However, enzymic starch degradation products such as non-starch polysaccharide may present in gathotan as a result of fungal fermentation during gathotan making. Non-starch polysaccharides in gel state can slow down digestion in the stomach, form gel lump to hinder gastric juice digestion (Lundin et al., 2008), which in turn can prolong fullness.

Conclusion

Gathotan noodle showed hypolipidemic effect in rats, in term of total triglycerides, total cholesterol, HDL, and LDL concentration. All parameters of postprandial satiety of gathotan noodle were also lower than those of reference meal, although the amount of gathotan noodle consumption was only about half of the amount of reference meal.

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