Development of nugget analogue from filamentous fungi cultivated using left over boiling water of tempeh factory

AN EVALUATION

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Abstract

The circular economy is about rethinking the definition of waste into resource. Tempeh boiling water is cheap and would otherwise be washed into the river and pollute the water which would affect the environment badly. Tempeh boiling water is going to be used as substrate to produce mycoprotein with the fungi *Rhizopus oligosporus*. This study is about making a mycoprotein nugget and evaluate it with a sensory evaluation and to evaluate the protein content in the nugget and chemical oxygen demand of the boiling water. The sensory evaluation will have 65 panellists to assess the liking of the nugget with two control samples.

The purpose of this project was to evaluate the potential of tempeh boiling water for the circular economy as substrate. To produce high mycoprotein nugget that will be accepted by the community. Assess the protein content in the mycoprotein nugget and assess the carbon used by the fungi with chemical oxygen demand analyse. The target group for evaluating the fungal nugget was students studying at Gadjah Mada University, Yogyakarta, Indonesia.

The project was done in multiple following stages: Finding best formula of mycoprotein, mycoprotein production, sensory evaluation, and protein analyse with Kjeldahl method and COD analyse of the boiling water.

The result of this study is that the mycoprotein nugget were not liked nor disliked with the average score of 3,9 out of 7. The overall characteristics (appearance, colour, texture, and taste) were 4,0 out of 7. The COD before and after fermentation were 6,6 g/L. The most COD were removed by pre-treatment of the boiling water from 172 to 121 g/L.
The protein content of the mycoprotein nugget were 23,8%.
The social aspect to produce healthy foods to a low cost at the same time improve water quality by removing foods for toxic microorganisms.

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INTRODUCTION

To meet the United Nations Sustainable Development goals and the Paris Climate accord by working towards the goals for Zero Hunger, Good Health and Well-being, Responsible Consumption and Production and Climate Action. These goals can be met by increasing the production of sustainable foods like filamentous fungi as a substitute for traditional meat protein sources. Red meat is a common protein source and has been for a long time, some down effects has been recorded with increasing low-density lipoprotein (LDL) which leads to coronary artery disease. But the loss of protein has a bad impact on our health like protein energy undernutrition (PEU).(Rousta et al., 2021)

Fungi is a good alternative to meat because it contains all essential amino acids and high amounts of protein that human body needs. The protein in fungal biomass can be retrieved by our bodies in the same amount as protein in meat. High amounts of crude protein (60-90%) is obtained when its dried. Proteins in fungi biomass doesn't need to be extracted but can be used directly as food after cultivation. The chitinous walls is a good source of dietary fibre and can be easily digested. Fungi biomass is low on cholesterol and fat which gives fungi another promising advantage.(Awasthi et al., 2023)

The fungi Rhizopus Oligosporus has been used for production of fermented protein rich foods and the genus Rhizopus is particularly important for proteins with high digestibility, hinder the formation of unwanted toxic substances and has the Generally Recognized As Safe (GRAS) status by Food and Drug administration.(Canedo et al., 2016)

The increasing interest of leaving linear economy by implying the circular economy, where overexploitation of natural resources and the environmental effect of accumulation of wastes, doesn't need to continue. The circular economy where the resources are closed in a loop, where waste is a resources. (Wikandari et al., 2022)

Bio-waste is organic waste made from microorganisms like filamentous fungi and bacteria or comes from biological waste such as sewage, food leftovers, agrarian, sawdust, forestry matter manure which is majority made of organic materials et; cellulose, hemicellulose, protein, lipids and starch. (Awasthi et al., 2021)

These liquid wastes go through physical, chemical, and biological changes which produce harmful substances. The medium can be a suitable home for different kinds of bacteria which can multiply and be a threat for us humans and/or the tempeh by diseases or harmful by itself.

The bacteria will change the liquid wastes colour to black and produce a harmful odour which can cause reparatory disease. The toxic waste can spread in the rivers and even to water wells in the area. The water can cause diarrhea and other diseases if it's used. (Puspawati et al., 2019)

By taking care of bio-waste in a profitable way the environment can be guarded from pollution and the living standard can be increased. Biomaterials, chemicals, and renewable energy can be produced by these different bio-wastes. Because most of the bio-waste is created from biomass, aerobic and anaerobic fermentation can be used to decompose the biomass to useful substances. By sorting the bio-waste and using different pre-treatments high-value products can be produced. (Awasthi et al., 2021)

The human population is growing fast and estimated to reach 9,7 billion in 2050. In the developing countries the demand for animal protein is increasing which rises concerns for food security and sustainability. The increasing demand for animal protein will impact the greenhouse gas emissions and biodiversity negatively due to increasing demand for agricultural land. The land will be taken from natural Greenlands, forests and wetlands. (Wikandari et al., 2023)

The problem

A continuing growing industry with over 81000 tempeh factories in Indonesia, using 12,8 million tons of soybean each year and consuming 200-300 L water for each 300 kg soybean. Because the nutritional value of the water from these factories this is ideal for using as substrate for mycoprotein. (Wikandari et al., 2023)
Residual water like soybean boiling water from tempeh factories in Indonesia release a big stream of nutritional substrate that can be used to cultivate edible filamentous fungi. This possibility can benefit the economy for the factory owner by making a second income and provide a healthy food for the community which is resource efficient and at the same time lower the water pollution in the river.
By reducing the liquid waste or reusing it in a circular way (like producing mycoprotein for nugget production) the environment can improve by reducing the pollutant from the factories into the rivers. (Wikandari et al., 2023)
The impact on the ecological is connected to bio-waste which needs to be reduced to continue with sustainable growth. This can be done with treatment strategies and LCA (life cycle assessment). (Awasthi et al., 2021)

Novelty

The new work being proposed.

The novelty of this thesis is to assess mycoprotein as a protein source cultivated on boiling water from a tempeh factory. To assess and improve the texture by adding jackfruit for its fibre content. To assess the consumer acceptance of a mycoprotein nugget cultivated on factory residue.

The goal of the research

By investigate mycoprotein and jackfruit mix to improve texture with a Texture analyzer.
Evaluate mycoprotein nugget with a sensory evaluation and it's liking with a panellist of 65 people.
To assess the protein content of different mixes of mycoprotein and jackfruit nuggets.
To assess the COD and indirectly evaluate the amount of organic compounds in the tempeh wastewater used by the filamentous fungi.
Assess the yield by weighting and drying a portion of the nugget.

Materials and methods

The project was done in multiple following stages: Finding best formula of mycoprotein, mycoprotein production, sensory evaluation, and protein analyse with Kjeldhal method and COD analyse of the boiling water. The inoculation were conducted in a UV laminar flow cabinet with a flame.
The goal of the research was achieved by trying out different combinations of biomass and jackfruit to find the best tasting nugget and the hardest bite (evaluated by a texture analyser). Plan and execute a sensory evaluation with mycoprotein, chicken and soy-protein nuggets. Measure the protein content with Kjeldahl method. Analysing the COD by titration from tempeh factory before pre-treatment, before fermentation and after fermentation.

Substrate characterisation

The substrate characterization was done with a high performance liquid chromatography (HPLC, Water corporation, Milford, CT, USA) at University of Boras with 4 samples under a period of 24 hours. The samples were taken at 0, 3, 20 and 24 (hours). The HPLC showed readings that the substrate contained glucose, mix sugar, acetic acid, lactic acid, glycerol, and ethanol.

Fermentation - Variables and conditions in the experiment.

Working culture

An agar solution (100 mL) was prepared and sterilized in an autoclave for 60 minutes. The fungi were transferred from a reaction tube which was kept in the freezer (-18 °C) to a 250 mL working culture that contained 39 g/L liquid agar. The fungi grew in the solid agar for three days until it was transferred in an airflow cabinet with a glass spreader, 20 mL measuring glass and 100 mL tween to the growth culture of 500 mL. The 1,0 L cotton-plugged Erlenmeyer flask containing 500 mL sterilized substrate were put on shaking at 120 rpm and normal room temperature (25-32 °C). The biomass were harvested with a cotton cloth in a funnel, washed with AC condense water and pressed with a potato press to remove water. The biomass were put in a plastic bag and kept in the freezer (-18 °C) until use.

The majority of the fungal production were done in 1 L Erlenmeyer flasks with cotton plugs with 500 mL substrate, 10 g/L yeast extract and 4,84 g/L lactic acid. In the first step a control of fungal production was done with D-glucose at the concentration 50 g/L, 100 g/L and 150 g/L, and 5 g/L yeast extract.

A test was also done with sucrose, yeast extract and lactic acid with the same concentration.

Nugget preparation protocol

A preliminary chicken nugget recipe was done before hand to make it easier to switch chicken with fungal biomass without starting from scratch.
From the texture analysis the best performing nugget with the “hardest bite” was the nugget with 75% biomass and 25% jackfruit.

After harvest the biomass was pressed in a cloth in a potato ricer 5 times to remove excess water. The biomass was stored in a freezer until used.

The ingredient for the nugget is the following:
300g fungal biomass, 100g jackfruit, 48,6 g white bread (without crust), 78,7g steered egg, 60,7g onion, 15,8g garlic, 9,7g corn starch, 0,7g ground pepper, 0,87 nutmeg, 5,8g chicken seasoning, 19,4g sugar, 48,6g Tapioca flour and 1,52g Sodium tripolyphosphate (STPP)*.

*The binder STPP was calculated by 2,2 multiplied by total weight (biomass, jackfruit and ingredients) of equals the amount STPP in milligram.

The fungal nugget recipe:

The biomass was taken out of the freezer and put in a water bath. Onion was cut into smaller pieces and fried in a pan with 1 tablespoon (tbsp) vegetable oil until yellow/brown. Garlic was pressed with the broad side of the knife and cut into smaller pieces. The bread crust was cut and removed, the white bread was put in a mixer with the onion, garlic, and egg for 3 minutes. The dry ingredients were added to the mixer and mixed another 2 minutes. The biomass and jackfruit were added last and a third mixing was done for max 1 min. The steamer was turned on and prepared with baking paper. 1 tablespoon of dough was shaped on the baking paper with the thickness of 0,8-1,0 cm. The nuggets were steamed for 18 minutes and later cooled down. A pan with 0,4 L vegetable oil were heated and the nuggets were put in (in the following order shown in figure 1): Protein flour, steered egg, breadcrumbs, and the frying pan for 4 minutes and 2 on the other side. The fried nuggets were put on tissues to dry from the oil on the counter for 10 minutes.

Figure 1: Picture of the preparations before frying the mycoprotein nugget.

Texture analysis

The texture analysis were done with the texture analyser (Lloyd Instruments, Texture Analyser TA1, figure 3) with six different samples and three replicas each. The texture analyser measured: adhesion force, adhesiveness, chewiness, cohesiveness, firmness, fracturability, gumminess, hardness, modulus, resilience and springiness. The texture analyse were used to make a choice between the samples and evaluate which one would have the hardest bite, which would simulate a bite and give the most satisfying result from sensory evaluation.
The nuggets were prepared the day before with the recipe above with the ratio 50/50 biomass/jackfruit, 75/25 biomass/jackfruit and 100/0 biomass/jackfruit. The nuggets were kept in the refrigerator until use.

Figure 2: Pictures of the mycoprotein nugget in the texture analyser TA1.

Sensory analysis

The nuggets were prepared and put in the freezer one day each before the sensory test.
A poster was made and sent out on social media (whatsapp story) two days before the sensory test. Panellists were able to register and book a time on the morning or afternoon. While at the sensory test panellists were checked off from the list of registered panellists.
A tray (figure 4) with three samples, water, straw, napkin, and a cracker were brought to the panellist at their cubical. They were informed about the sensory and how to evaluate the nuggets. The hedonic test is scaled from 1-7, 7 = like very much, 6 = like moderately, 5 = like slightly, 4 = neither like nor dislike, 3 = dislike slightly, 2 = moderately dislike and 1 = dislike very much.

37 panellists used their phone and accessed the evaluation form through QR-code and 26 panellists used a paper form. After the evaluation the panellists were given a present as a compensation.
The majority of the participants for the evaluation was students at Gadjah Mada universitas. The control samples for the fungal nugget were a soy protein nugget and a chicken nugget. Soy-protein is a food product from Indonesian made from extracted soybeans made to a dough and dried. Both soy protein and chicken nugget were done with in the same way as the fungal nugget.

Figure 3: Picture of the tray that was given to each panellist.

Chemical oxygen demand (COD)

Three samples with three replicas except for after fermentation (6 samples) were prepared from boiling water (BW), pre-treated water (PW) and after fermentation (F). The samples were taken and kept in the refrigerator (+4 °C) in 100 mL Erlenmeyer flasks with plastic film to cover the top. The samples were diluted BW 200 times, PW and F 100 times. 2,5 mL of the sample were pipetted to each reaction tube with a plastic cap. 1,5 mL potassium dichromate ($K_2Cr_2O_7$) and 2,5 mL silver sulphate were pipetted (inside airflow cabinet). The samples were heated at 150 °C for 120 minutes and removed to cool down in room temperature for 30 minutes. The samples were poured into 100 mL Erlenmeyer flasks and titrated with ferrous ammonium sulphate (FAS) and three drops of indicator Ferroin until "brick red". The COD were calculated with equation 1.

$$COD\ \left(\frac{mg}{L}\right) = \frac{(b-a)x8000xNxDF}{V_s}$$

Equation 1: Where b is the blank sample (mL), a is the sample volume used (mL), N is normality of FAS (0,18N), DF is dilution factor (100/200 times) and V_s is sample volume (2,5 mL)

Protein content with Kjeldahl method

Three samples with three replicas and two samples as blank were prepared. 0,10 g of sample were weighed with 0,70 g of catalysator ($H_2O \cdot K_2SO_4$). They were wrapped in filter paper and put in a Kjeldahl flask. The blank contained 0,70 g of catalysator. 3 mL concentrated (95%) sulfuric acid (H_2SO_4) were pipetted in each Kjeldahl flask and put on a heater at 200 C° in an airflow cabinet until the liquid became clear (60-90 minutes).
Each sample were distilled by the Kjeldahl method with 20 mL of sodium thiosulphate ($Na_2S_2O_3 \cdot NaOH$) and in a 100 mL Erlenmeyer flask containing 5 mL 4% boric acid (H_3BO_3) and 3 drops of indicator (BCG-MR) to collect the nitrogen. The nitrogen flasks were titrated with 0,2N HCL until pink/cream.

The protein content were calculated by the following equations:

$$Nitrogen\ \% = \frac{(a-b)xNHCLxM_N x100}{W_s}$$

Equation 2: a is the sample volume in mL, b is the blank volume mL, NHCL is the normality of titrant (mmol/mL), M_N is the molar mass of nitrogen and the W_s is the sample weight (mg).

$$Protein\ \%\ (wet\ based) = Nitrogen\ \%\ x\ CF$$

Equation 3: The protein content was calculated with a conversion factor (CF) of 6,25 (Wainaina et al., 2019)

$$Protein\ \%\ (dried\ based) = \frac{Protein\ \%\ (wet\ based)}{1-c}$$

Equation 4: c is the sample water content.

Result and discussion

Substrate characterization results

Glucose and mix sugars in airlift fermentor and Erlenmeyer flask increase the first few hours but later decrease and mix sugars are almost totally consumed in airlift fermentor (figure 6). In the end of the fermentation the Erlenmeyer flask (figure 5) has an increasing glucose concentration. This could be the enzymes breaking down other nutrients to glucose because the cells produce a lot of enzymes under fermentation et, amylase, glucosidase, protease and endoglucanase. (Yin et al., 2023)
Protein was not analysed with the HPLC which could explain why the acetic acid was increasing in the end. Different proteins could be broken down with protease to nutrients for enzymes.
Ethanol, acetic acid and lactic acid are increasing in the airlift fermentor (figure 6) except for in the end where ethanol in the Erlenmeyer flask and lactic acid in the airlift decrease.

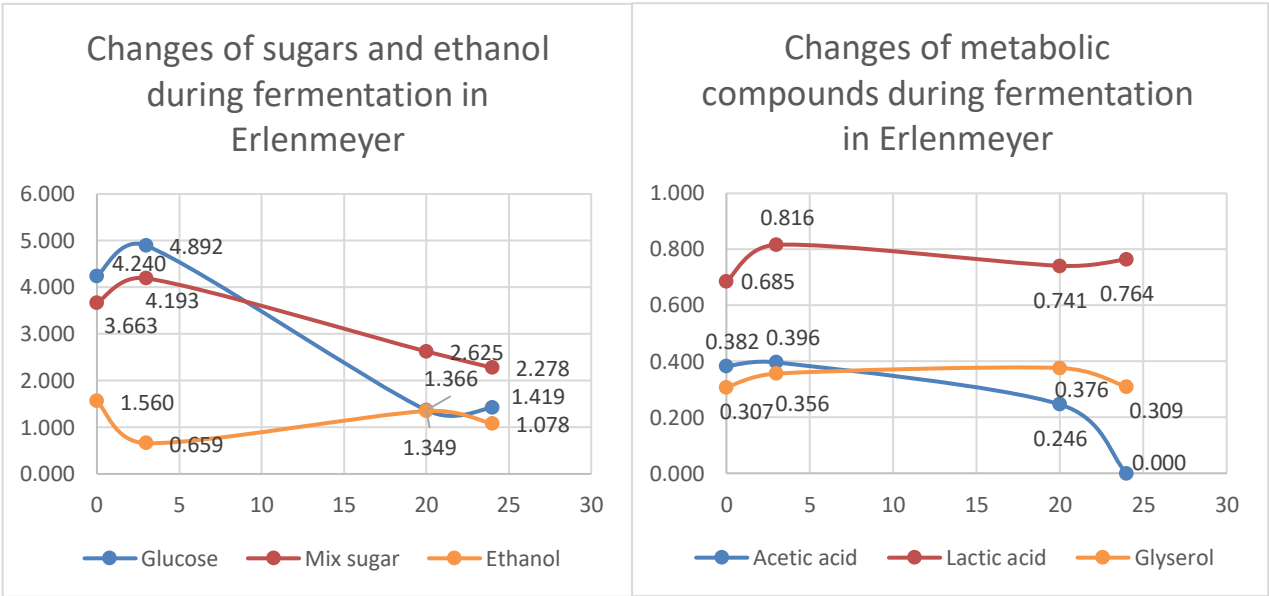

Figure 4: Data from the HPLC in University of Boras from Erlenmeyer flask.

Lactic acid, glycerol and acetic acid in Erlenmeyer flask is increasing in the beginning but slightly decrease where acetic acid is being consumed by enzymes. The fungi are consuming the sugars and producing ethanol and acids in the airlift fermentor (figure 6) while in the Erlenmeyer flask (figure 5) the fungi also consume acetic acid and in the end some of the glycerol. The decrease for lactic acid and acetic acid is because the fungi produce enzymes to break down the acids. This is also shown in research with sweet potato residue (Yin et al., 2023)

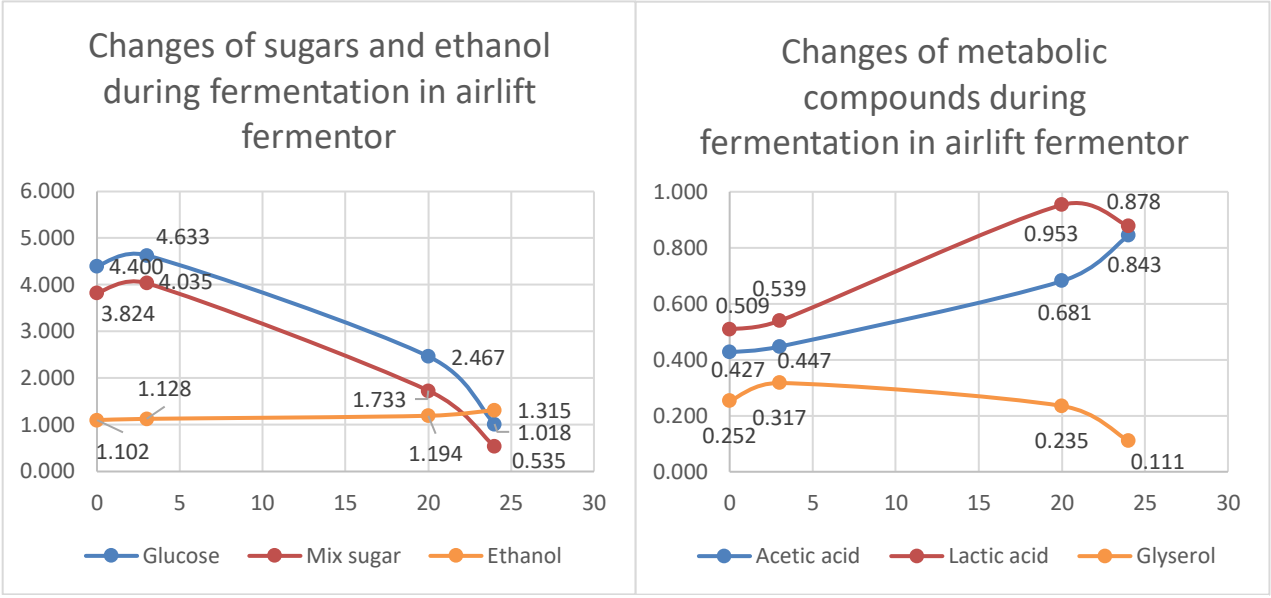

Figure 5: Data from the HPLC in University of Boras from Airlift fermentor.

Fermentation results.

As seen in (figure 5) the substrate consumption of glucose concentration from 4,4 g/L to 1,4 g/L equals a reduction of 67% and the consumption of mix sugars concentration from 3,7 g/L to 2,3 g/L equals a reduction of 38%. R. oligosporus favours glucose over other sugars.
The colour of the inoculum with boiling tempeh water is darker than the inoculum with glucose. Both contains yeast extract and the same volume of substrate. The colour of the fungi in the right picture (glucose) is white while the fungus in the boiling water is darker like the substrate. This shows that the fungi absorb the colour of the substrate in its environment.
The mycelium grew different, the mycelium on the right picture in (figure 7) grew less in number but larger in size, and the mycelium in boiling water grew smaller in size but with more colonies. This could be because the optimum temperature and pH for the fastest hyphal expansion is 42 °C and pH 5,85 (Awasthi et al., 2023). Because the temperature used for this

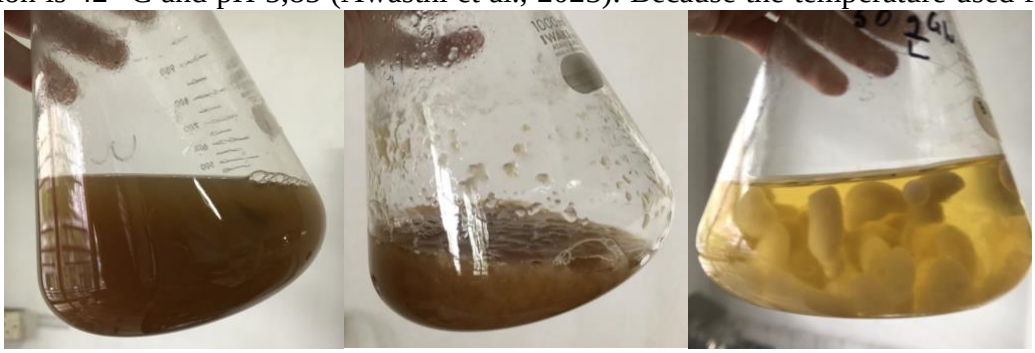

Figure 6: Fermentation with Boiling tempeh water at 0h (left), 48h (middle) and glucose 48h (right)

experiment was 25-32 °C this has some effect on the growth and that explains the short hyphal.

Chemical oxygen demand of boiling water

In (figure 8) the difference between the chemical oxygen demand (COD) of pre-treated water (PW) and after fermentation (F1-F3) is 6,6 g/L which is a 5,4% difference. With the dried biomass of 0,58 g/L and the difference (per litre) in COD the yield is 0,088g dried biomass per gram COD removed. The largest difference is the pre-treated water from the boiling water (BW) with a reduction of 70% COD. This is because the pre-treatment removes a cheese-like (figure 8) by-product containing a lot of carbon.

COD (g/L)			
BW1	BW2	BW3	Average BW
176,3	167,0	171,6	171,6
PW1	PW2	PW2	Average PW
116,9	123,8	122,7	121,2
F1 (1)	F1 (2)	Average F1	
104,3	114,6	109,4	
F2 (1)	F2 (2)	Average F2	Average F1-F3
112,3	108,9	110,6	114,6
F3 (1)	F3 (2)	Average F3	
123,8	123,8	123,8	

Figure 7: Data from COD (Left) and picture of the by-product for pre-treating the boiling water (right).

Production of carbon in fungi vs carbon dioxide released.

$$C + O_2 = CO_2$$

The molar mass ratio between oxygen (32 g/mol) and carbon (12 g/mol) is 2,7. From the data in figure 8 amount oxygen needed to produce the fungi is 6,6 g/L. If that value is divided by the molar ratio than the answer is 2,4 g carbon/L.
Fungi contains ~50% carbon which is half of the carbon used (1,2 g fungi/L substrate). 1,2 g/L is the theoretical amount of mycoprotein produced.

Figure 8: Picture taken at Gadjah Mada Universitas of the before steamed mycoprotein

Characteristics of fungal nugget.

The nugget was moist but firm dough when placed on the baking paper (figure 9). It didn't have any structure and could be poked through easily.
After the nugget was steamed it was firm but a bit squishy. This can be presented by the gumminess at 41,7 N, springiness at 3,5 mm and resilience at 0,38. The gumminess is the energy needed to be chewed before it can be swallowed. The springiness shows that the nugget can be squeezed and return to its original shape. And resilience shows that the nugget fights to get back into original shape.

Biomass production

The produced biomass obtained at 0,58 g/L dried biomass. This is low to compare with (Wikandari et al., 2023) research which gave the best result at pH 4,5 and 125 rpm. To compare with *Aspergillus oryzae* the yield was obtained at 6 g/L cultivated on oat flour. (Rousta et al., 2021) This could be improved by checking the pH of the growing medium and optimize it to pH 4,5. The optimum temperature for R. oligosporus is 37 °C (Awasthi et al., 2023).
To increase the biomass productivity smaller Erlenmeyer flasks with less medium could give better result. The ratio between the medium and gas above could not be the best for mass transfer between the two mediums.

Addition of jackfruit to mycoprotein nugget.

The jackfruit added texture by the long fibres seen in figure 10. Before the mycoprotein nugget was too moist and without any resistance when pressed. The mycoprotein didn't add any long fibres because it was not cultivated the perfect way to grow for long hyphal explained in the article (Awasthi et al., 2023).
The jackfruit contains a lot of liquid and by pressing the jackfruit with a cloth in a potato press 36,7% liquid was removed.

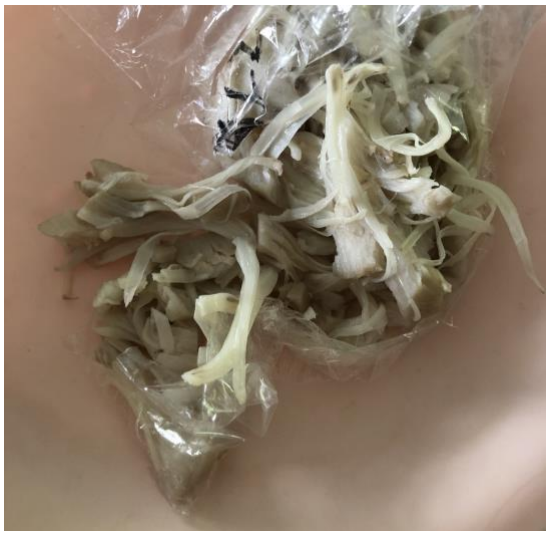

Figure 9: Picture of the fibres of the cut jackfruit.

Texture analysis

The texture analysis was done to evaluate the hardness bite for six samples and the data is presented in table 11. The best result was the 75% biomass nugget. Both the fried nugget with the average of 105 Newton (N) and the no fried with the average of 90 N. 105 N with a standard deviation (STD) of 39 is questionable. Otherwise, it would be the 50% fried nugget with the

hardness bite of 96 N and STD of 18. The most important result is the no fried nugget (75% biomass) with 90 N because it focuses on the mycoprotein and not involves the bread coating.

	100% No fried				75% No fried				50% No fried			
	sample 1	sample 2	sample 3	Mean ± SD	sample 1	sample 2	sample 3	Mean ± SD	sample 1	sample 2	sample 3	Mean ± SD
Hardness bite 1 (N)	51,778	37,152	41,814	43,58 ± 7,47	78,108	87,873	103,71	89,90 ± 12,92	63,112	61,474	61,90	62,16 ± 0,85
Cohesiveness	0,59542	0,573	0,59128	0,59 ± 0,01	0,45503	0,40657	0,51922	0,46 ± 0,06	0,40038	0,47324	0,43184	0,44 ± 0,04
Adhesiveness (Nmm)	-0,1939	0,38858	0,00294	0,07 ± 0,30	1,6388	-0,0236	4,9558	2,19 ± 2,54	0,41809	0,00445	0,01455	0,15 ± 0,24
Gumminess (N)	30,83	21,288	24,724	25,61 ± 4,83	35,542	35,726	53,848	41,71 ± 10,52	25,268	29,092	26,732	27,03 ± 1,93
Resilience	0,47983	0,48978	0,50501	0,49 ± 0,01	0,33938	0,3533	0,4449	0,38 ± 0,06	0,36823	0,39826	0,3661	0,38 ± 0,02
Fracture (N)	2,0902	1,9635	2,0128	2,02 ± 0,06	2,4621	2,2708	2,106	2,28 ± 0,18	2,4958	2,2866	2,0947	2,29 ± 0,20
Springiness (mm)	2,1568	2,1337	0,54842	1,61 ± 0,92	4,3347	2,0031	4,1116	3,48 ± 1,29	1,8272	0,54635	1,5411	1,30 ± 0,67
Chewiness (N) Graph	23,9	16,72	18,852	19,82 ± 3,69	26,628	26.656	41,86	34,24 ± 10,77	18,667	21,245	18,95	19,62 ± 1,41
Chrispiness peaks	8	11	9	9,33 ± 1,53	10	8	9	9,00 ± 1,00	10	9	6	8,33 ± 2,08
Crispiness	189,9	135,5	149,08	158,16 ± 28,31	283,93	316,7	377,7	326,11 ± 47,59	243,86	217,6	217,05	226,17 ± 15,32
Crunchiness (Nmm)	105,45	99,371	90,356	98,39 ± 7,59	197,87	241,01	220,2	219,69 ± 21,57	158,98	115,85	150,8	141,88 ± 22,91

	100% Fried				75% Fried				50% Fried			
	sample 1	sample 2	sample 3	Mean ± SD	sample 1	sample 2	sample 3	Mean ± SD	sample 1	sample 2	sample 3	Mean ± SD
Hardness bite 1 (N)	65,305	68,516	99,988	77,94 ± 16,15	80,111	150,21	84,203	104,84 ± 39,34	100,18	110,36	75,91	95,48 ± 17,70
Cohesiveness	0,62546	0,53865	0,72112	0,63 ± 0,09	0,55589	0,64481	0,61343	0,60 ± 0,05	0,3535	0,52907	0,59863	0,49 ± 0,13
Adhesiveness (Nmm)	-0,0945	0,02896	0,02699	-0,01 ± 0,02	-0,0024	-0,9671	-1,0231	-0,66 ± 0,57	-1,0466	0,04389	0,01845	-0,33 ± 0,62
Gumminess (N)	40,846	36,906	72,103	49,95 ± 17,79	44,533	96,856	51,653	64,35 ± 28,38	35,315	58,389	45,442	46,38 ± 11,57
Resilience	0,50979	0,472	0,66645	0,55 ± 0,10	0,48803	0,53577	0,49645	0,51 ± 0,03	0,4719	0,45519	0,49359	0,47 ± 0,02
Fracture (N)	2,2266	2,1556	2,0918	2,16 ± 0,04	2,1876	2,2049	2,0972	2,16 ± 0,06	2,2645	2,128	2,1241	2,17 ± 0,08
Springiness (mm)	-4,2944	1,2048	1,5807	-0,50 ± 1,11	-0,7129	7,0827	8,6976	5,02 ± 5,03	5,3376	-0,7369	1,7328	2,11 ± 3,05
Chewiness (N) Graph	34,619	30,253	63,312	42,73 ± 16,69	36,075	82,343	42,106	53,51 ± 25,15	29,863	48,12	37,04	38,34 ± 9,20
Chrispiness peaks	10	9	11	10,00 ± 1,00	10	9	13	10,67 ± 2,08	10	10	9	9,67 ± 0,58
Crispiness	237,01	250,52	379,77	289,10 ± 66,35	301,6	546,23	316,59	388,14 ± 137,11	374,32	413,2	271,93	353,15 ± 72,98
Crunchiness (Nmm)	164,96	228,7	185,41	193,02 ± 23,11	266,7	334,6	190,11	263,80 ± 72,29	277,85	307,84	207,74	264,48 ± 51,37

Table 11: The data from the texture analyser with six samples and three replicas each.

Sensory analysis

65 participants between the age of 19 and 50, mostly students but some staff as well participated in the sens8ory evaluation.

The result from the sensory evaluation can be found in figure 13. The most liked nugget was the chicken nugget with the average score 4,9, second soy-protein nugget with 4,1 and the mycoprotein nugget with 3,9 out of 7.

The overall characteristics score was the chicken with 5,2, and the second was the soy-protein with 4,3 and mycoprotein with 4,0 points out of 7. The score with the averages between 3,9 and 4,7 out of 7 shows that the panellists did not love or hate any of the nuggets. The first noticeable thing about the score is that the fungal nugget has the lowest score for the appearance and colour. The nuggets differ here, the fungal nugget is more dark (black/grey) in colour than the soy-protein nugget, which is more brown/golden, and the chicken nugget is bone white (figure 12). The colour of the mycoprotein nugget could be explained with the ratio or biomass and jackfruit. Some parts in the jackfruit are darker than others. The biomass was also pressed to remove excess water. Another explanation is the substrate which is darker than glucose (which is yellow).

Figure 12: The nuggets from the sensory evaluation, soy-protein (left), fungal (middle) and chicken (right)

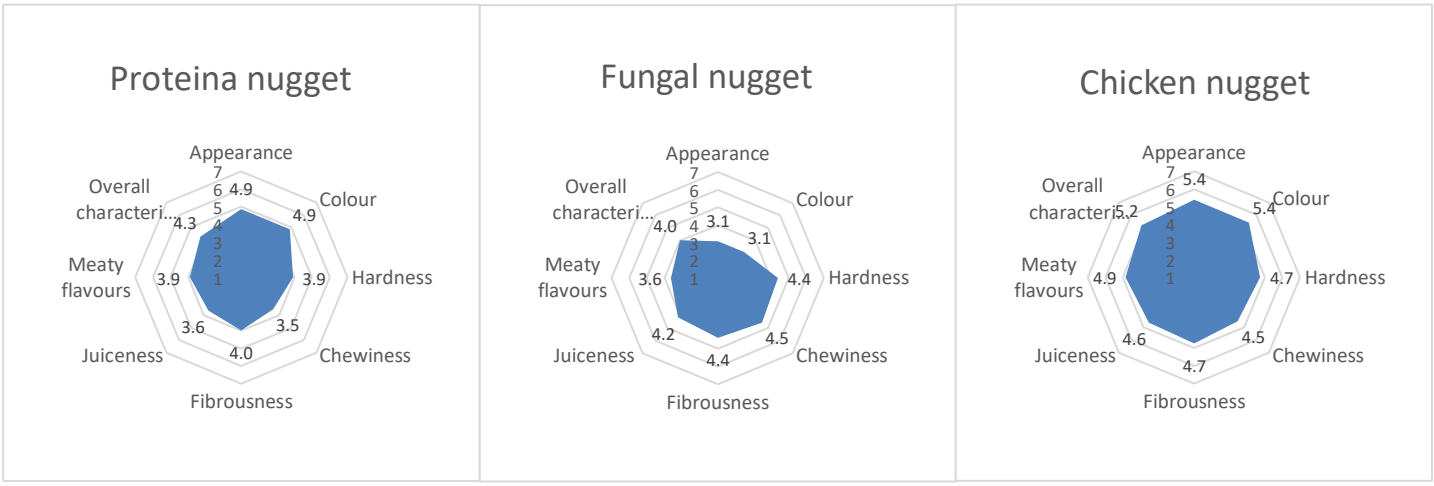

Figure 13: The radar-graph for each nugget with the score 1-7 (1 dislike very much, 7 like very much) from the sensory test.

Results of the sensory evaluation

The chicken nugget has the highest score in every category except one shared score. This shows that the product is accepted and liked by many in comparison with mycoprotein and soy-protein. 5,4 in score for appearance and colour for chicken. 4,9 in appearance and colour for the soy-protein nugget and 3,1 in appearance and colour for fungi. The mycoprotein nugget needs a colour change, to either bone white or brown. For the hardness the mycoprotein nugget (4,4) is in the middle and close to the chicken at 4,7. The hardness could be improved by removing more water with heating in the oven instead of steaming.

For the chewiness the score 4,5 is shared between the chicken and the mycoprotein nugget. The soy-protein has a lower score at 3,5. For the mycoprotein nugget this is the highest value and could increase if the hardness is being improved with heating.

When it comes to fibrousness the mycoprotein nugget is once more in the middle with the soy-protein with the lowest score (4,0). This could be because the soy-protein nugget didn't have much fibres but a more consistency of etc, pressed bulgur. The fibres in the mycoprotein nugget could be improved by mixing the mycoprotein and jackfruit a shorter time so that the fibres is still intact and uncut by the knives in the mixer.

The juiceness is related to hardness for the liquid content. The score for the nuggets; soy-protein (3,6), mycoprotein (4,2) and chicken (4,6). If the juiceness and hardness for the mycoprotein nugget has a score a few points above 4 (nor like or dislike) the hardness needs to increase.

For the meaty flavour the mycoprotein nugget had the lowest score (3,6), soy-protein (3,9) and chicken (4,9). This could be because the chicken seasoning enhanced the meaty chicken taste in the chicken nugget and neutralised the taste in the mycoprotein and soy protein nuggets. Some other seasoning could be tried to increase the taste.
For the overall characteristics (appearance, colour, texture, and taste) the most liked was the chicken nugget at 5,2 just a few scores above "like slightly". The mycoprotein with the lowest score at 4,0 and soy-protein at 4,3. The score is low for every nugget, and no one is really liked by the panellists.

Suggested improvements

For the mycoprotein nugget the appearance is something that needs to be improved to be liked. This could be an additive to make the colour lighter like the chicken nugget or browner in colour like the soy-protein. The additive Brown HT (E 155) could be used to colour the mycoprotein nugget browner.
The meaty flavour could be improved with another seasoning for example beef.

Protein of the mycoprotein nuggets.

The protein content was measured with Kjeldahl method at Gadjah Mada universitas with the same samples as for the texture analyse; 50/50 biomass/jackfruit, 75/25 biomass/jackfruit and 100/0 biomass/jackfruit.
The result gave the 50/50 nugget a content of; 16,2% protein, 75/25 nugget; 23,8% protein and 100/0 nugget: 31,8% protein.

Social aspect of using boiling water

The boiling water from the tempeh factory is a liquid waste stream that goes to the river or into the sewers right outside the factory. This nutritional stream contains a lot of carbon and other nutrients which is washing away and pollute the rivers by increasing the COD in the rivers which was explained in the introduction by research of (Wikandari et al., 2023). The waste stream help bacteria and other microorganism to grow and multiply. These bacteria and other microorganism can be harmful for the community living close to the rivers. The liquid waste will interfere with its surroundings and produce foul odours and make the water unwanted to live in (Puspawati et al., 2019). The community living close to the rivers can be affected by this by experience a reduction of fish and other animals in or close to the river.
The increasing value of the boiling water from residual water that were going to be washed out in the river to a protein rich source food is a great thing and circular.
Indonesia is a developing country and have a huge population and the majority (65,4%) of the people eat plant-based protein which are mostly derived from rice and bean. Animal proteins from poultry and red meat is connected to higher socio-economic status and people living in urban areas. The most animal protein came from meat and poultry 12% and fish 9,8% (Khusun et al., 2022). To secure food, especially protein rich foods this is a good way to use boiling water from tempeh factory to cultivate mycoprotein and increase the amounts of protein rich foods. By using the boiling water which would otherwise be washed out and use it to produce cheap foods. Because the substrate is cheap this could be a competition with mycoprotein grown on glucose.
It could be a positive spiral by lowering the pollution by lowering the carbon to the river. The water quality could improve and make the environment for fish a better and healthier place to live which would improve the number of fish and could increase the fish eaten.

Conclusion

To use the boiling water for a circular economy and to reduce the pollution in rivers this can have an impact on the environment. The sensory evaluation showed that the mycoprotein nugget was not liked nor disliked with the average score of 3,9. And the overall characteristics was 4,0 out of 7. The COD gave a result of 6,6 g/L before and after fermentation. The most COD was removed by pre-treatment of the boiling water from 172 to 121 g/L. Based on the sensory test, the best mycoprotein nugget was obtained with the ratio 75:25 (fungal biomass/jackfruit). This nugget contains 23,8% protein.

The social aspect to produce healthy foods to a low cost at the same time improve water quality by removing foods for toxic microorganisms.

Three mixtures of mycoprotein and jackfruit was analysed by the texture analyser. But it was not compared with the mycoprotein without any jackfruit. A sensory evaluation with 65 participants were done the 20th of April 2023. The protein content were assessed for three different mixtures of biomass/jackfruit. The COD and carbon used by the filamentous fungi were done. The dry biomass was weighed but the yield could not be calculated because the reducing substrate wasn't analysed.

It is possible to produce a mycoprotein from tempeh boiling water and this is a good way to make a nugget as good as a chicken nugget. Some improvements could do their tricks for colour and taste. It was a successful first try!

Ethical statement

Written information was given to all participants in the sensory evaluation and their concent for participant in this research.

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