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Application of organic soybean tempeh in purple sweet potato raisin cookie

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Abstract Organic soybean tempeh or tempeh kedelai is made by fermentation with *Rhizopus oligosporus* was used to produce cookies, as a plant-based protein snack. Four samples with various recipes - purple sweet potato cookie, purple sweet potato raisin cookie, tempeh purple sweet potato cookie and tempeh purple sweet potato raisin cookie – were prepared to determine the physicochemical and sensory features. Chemical analysis found that the tempeh purple sweet potato raisin cookie has the highest moisture (18%) and total proteins (29%). However, the purple sweet potato cookies showed the highest ashes content (2%) and tempeh purple sweet potato cookie showed the highest total lipids (29%). The tempeh purple sweet potato raisin cookie was hardest (61 N). The tempeh purple sweet potato raisin cookie had the highest scores for food acceptability of all qualities in appearance, texture, aroma, colour, taste and general acceptability using a 9-point hedonic scale ranging from slightly liked to very much liked. Therefore, the tempeh purple sweet potato raisin cookie can be accepted as a new snack, that includes a good level of protein and thus a healthy food alternative. Furthermore, if all steps of the production are carefully controlled by USDA or other certifying bodies, thus this product may be labelled as organic product.

Keywords: Tempeh, Organic soybean, Purple sweet potato cookie

Introduction

According to the Codex Alimentarius or food code (Food & Agriculture Organization-World Health Organization, 2017), tempeh (CXS 313R-2013) is a compact, white, cake-form product, prepared from dehulled boiled soybeans through solid-state fermentation with *Rhizopus* spp. The standard of *Rhizopus* spp. are *R. oligosporus*, *R. oryzae* or *R. stolonifer*. Tempeh originated in Indonesia – spelled as "tempe" in Indonesian and the local name is tempeh kedelai (Soybean). Tempeh is an indigenous food that has been consumed as a staple source of protein for more than 300 years. A variety of materials, e.g. beans, pressed peanut cake, pressed coconut cake, etc., are used to make tempeh (Ahnan-Winarno *et al.*, 2020; Gandjar, 1999; Nout and Kiers, 2005;

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Shurtleff and Aoyagi, 2020). Tempeh usyally has a maximum moisture content of ~65% (w/w), Ash content of ~1.5% (w/w) and fiber content of ~2.5% (w/w), a minimum lipid content of ~7% (w/w), protein content of ~15% (w/w), fiber content of ~2.5% (w/w) (Ahnan-Winarno *et al.*, 2020; Food & Agriculture Organization-World Health Organization, 2017). Nowadays, tempeh is a widely accepted as a fermented product, nutritious, affordable and sustainable functional source of high levels of protein (Ahnan-Winarno *et al.*, 2020). Tempeh is one choice allowing people to eat delicious plant-based protein sources – particularly advantageous for vegetarians and vegans. Therefore, we used organic soybean tempeh fermented by *R. oligosporus* to produce cookies as a plant-based protein snack with organic ingredients, analysed the physical and chemical properties and added a sensory evaluation for food acceptance.

Materials and methods

Fungal culture and starter for tempeh

R. oligosporus was recieved from the Thailand Institute of Scientific and Technological Research (TISTR) culture collection services, Thailand (https:// www.tistr.or.th/tistr_culture/service_en.php). *R. oligosporus* was subcultured to potato dextrose agar (PDA) plate and incubated at room temperature (~30 ℃). *R. oligosporus* isolate was identified initially on the base of morphological characteristics on PDA by and growth rate of five replicates were observed after one day at room temperature. To prepare starter, *R. oligosporus* was a culture on PDA for 24 hr and incubated at room temperature. Then, the culture was transferred to rice medium and and incubated at room temperature for three days. It was harvested and dried in a hot air oven at 45 ℃. Finally, driedy fungal biomass was ground and kept at 4 ℃ to be used as a tempeh starter for fermentation.

Preparation of organic soybean tempeh

Organic soybean was cultivated and harvested in Si Sa Ket, Thailand. The tempeh was prepared following Guo (2009): We soaked 500 g of dehulled organic soybean in water overnight. After that, we discarded the water and washed again. Then we boiled the soybean for 60 min in potable water. Then we drained the water and let allowed the soybean to cool. Inoculated 1% of *R. oligosporus* starter and packed organic soybean in plastic bags or banana leaves. The packages were perforated at distance of 10 mm and then incubated for about 24-48 hr at 30 ℃ to tempeh fermented.

Cookie formulation and baking

Formulation standardization based on the purple sweet potato cookie and most ingredient composition based on organic products were achieved as Table 1. Four cookie formulations (purple sweet potato cookie = A, purple sweet potato raisin cookie = B, tempeh purple sweet potato cookie = C and tempeh purple sweet potato raisin cookie =D) were combined with purple sweet potato, raisin and tempeh in different proportions. Mix ingredients by stand mixer speed, not over number two. Drop cookie dough 20 g onto the baking sheet. Bake in a preheated oven of 170 ℃ for 10 min and then drop the temperature to 150 ℃ for 40 min. Remove from the oven and leave the cookies to cool. Seconde bake the cotkies at 150 ℃ for 10 min. Finally, remove the oven and leave the cookies to cool and keep in the individual package for analysis.

Table 1. Formulation of cookies

Ingredient	Formulation (g)				Organic product	% Organic ingredient
	A	B	C	D		
Plain flour	350				Yes	36
Egg	100±5				Yes	10*
Butter	200				No	-
Fresh milk	40				Yes	4
Brown sugar	100				Yes	10
Himalayan salt	8				Yes	1
Vanilla extract	4				No	-
Purple sweet potato	180	90	90	60	Yes	18/9/6
Raisin	-	90	-	60	No	-
Tempeh	-	-	90	60	Yes	9/6
Total % organic ingredient (including salt)	79	70	79	73	-	-
Total % organic ingredient (excluding salt)**	78	69	78	72	-	-

*****Calculated from 100 g., ******Defined following USDA rules for organic labels: percentage of all organic ingredients (excluding salt and water).

Chemical analysis

Three replicates was experimented, Chemical analyses used standard methods of the Association of Official Analytical Chemistry (AOAC, 2000) for pH and moisture content (105 ℃). Crude protein content was determined with the Kjeldahl method. Fat content was measured using the Soxhlet method. Ash content was measured by gravimetry.

Physical analysis

A 20 × 20 × 20 mm^3 sample was taken from three piece of the cookies and measured using a texture analyzer. Texture profile analysis (TPA) pressed two in succession under the printing plate. The compression test used a 5 mm/s test speed with a cylinder probe (Ø 50 mm) and a wait for 5 s before the second compaction.

Sensory acceptance of the cookies

The sensory evaluation of the four-types cookie used 30 untrained participants, randomly recruited among employees and students at the School of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL). They received individual cookie packages with coded samples at room temperature. The acceptance test used a hedonic scale with 9 points – ranging from “liked very much” (score 9) and “disliked very much” (score 1). The evaluated attributes were different parameters such as appearance, texture, aroma, colour, taste, and general acceptability was described by Everitt (2009).

Statistical analysis

Data were analyzed using one-way ANOVA with DUNCAN ALPHA post-hoc test comparison ($p < 0.01$), was performed using SPSS version 16.0.

Results

Morphology of *R. oligosporus* is shown in Figure 1. The average of growth rate on PDA at one day was 35.51 mm, and the four cookie sample appearances are shown in Figure 2. Total fraction of organic ingredients ranged from 70%-79% (net weight including salt) and slightly less if the USDA rules are followed, e.g. purple sweet potato raisin cookie was 69%, tempeh purple sweet potato raisin cookie was 72%, and tempeh purple sweet potato cookies was 78% (Table 1).

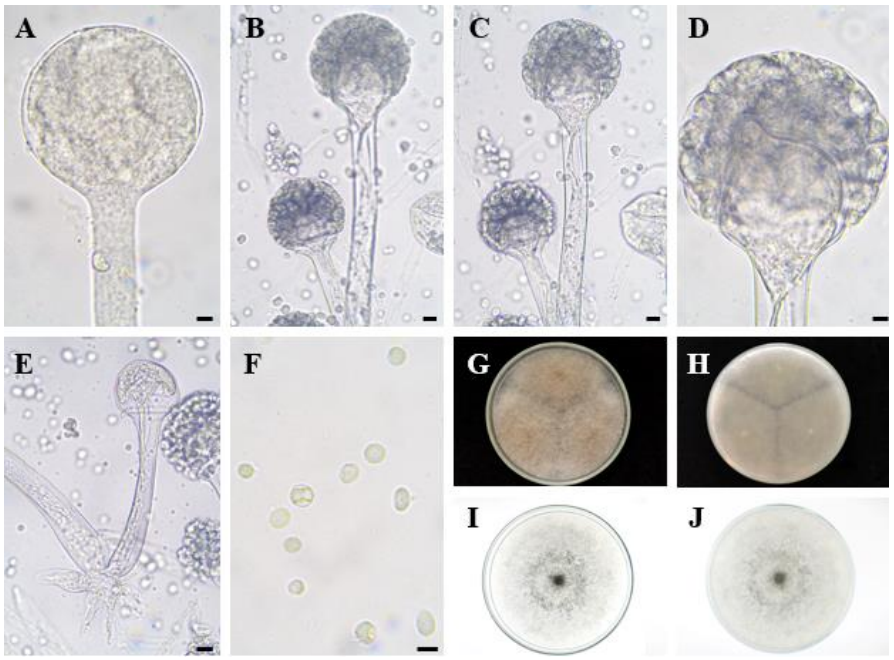

Figure 1. *Rhizopus oligosporus*. A. young sporangium, B-D. mature sporangia, D. rhizoid, F. sporangiospores, G & I front side on PDA plate and H & J back side on PDA plate. Bar: A,D & F = 5 µm and B,C & E= 10 µm

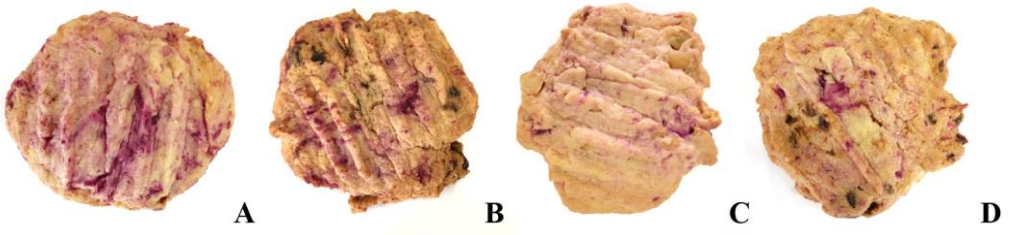

Figure 2. Cookie samples: A = purple sweet potato cookie, B = purple sweet potato raisin cookie, C = tempeh purple sweet potato cookie and D = tempeh purple sweet potato raisin cookie

The chemical and physical properties analysis of the ingredients and cookie samples are shown in Table 2. The tempeh recipes had the highest total proteins (32.7%). For cookie samples, the pH ranged from 5.27-6.85, proximate analysis revealed that moisture ranged from 11-18%, total proteins from 16.8-28.9%, ash was 1.3-2.2%, total lipids were 9.8-28.9 and hardness from 41-61 N. The sensory evaluation (appearance, texture, aroma, colour, taste and general acceptability) of the cookie showed that all the cookies had sensory

qualities ranging from liked slightly (score 6) to liked very much (score 8) (Table 3). The overall assessment from low to high quality was purple sweet potato cookie (●), purple sweet potato raisin cookie (●), tempeh purple sweet potato cookie (●) and tempeh purple sweet potato raisin cookie (●), as shown in Figure 3.

Table 2. Mean physical properties of ingredients and cookies

Parameters	Ingredients		
	Purple sweet potato	Raisin	Tempeh
pH	5.94 ±0.003[b]	3.62 ±0.009[c]	7.33 ±0.173[a]
Moisture (%)	5.32 ±0.148[c]	12.63 ±0.130[a]	6.95 ±0.132[b]
Total proteins (%)	4.67 ±0.467[a]	8.87 ±0.467[b]	32.67 ±0.933[a]
Ashes (%)	2.97 ±0.007[a]	1.76 ±0.118[b]	1.33 ±0.000[c]
Total lipids (%)	1.10 ±0.022[b]	14.27 ±0.940[a]	0.33 ±0.000[c]
Hardness (N)	14.37 ±0.487[b]	66.95 ±0.584[a]	13.29 ±1.425[b]

Parameters	Cookie samples			
	A	B	C	D
pH	6.10 ±0.200[c]	5.27 ±0.003[d]	6.85 ±0.048[a]	6.32 ±0.153[b]
Moisture (%)	16.91 ±0.876[a]	11.03 ±0.496[b]	18.20 ±0.339[a]	18.27 ±0.505[a]
Total proteins (%)	17.73 ±0.467[c]	16.80 ±0.808[c]	26.13 ±0.467[b]	28.93 ±0.467[a]
Ashes (%)	2.22 ±0.111[a]	2.00 ±0.000[a]	1.33 ±0.000[b]	1.56 ±0.111[b]
Total lipids (%)	9.78 ±0.223[c]	17.22 ±0.800[b]	28.89 ±1.110[a]	21.56 ±1.777[b]
Hardness (N)	47.65 ±5.111[a]	51.40 ±3.955[a]	40.70 ±8.627[a]	60.67 ±20.574[a]

[abcd]Columns that do not have a common superscript are statisficant difference ($p<0.01$); Mean ± Standard error (SE)

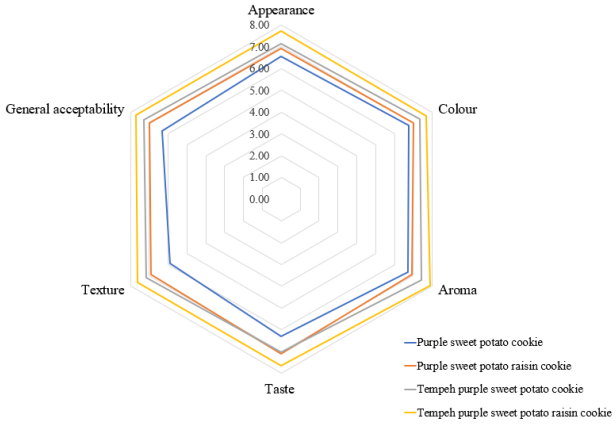

Figure 3. Sensory acceptability average scores for four cookie samples

Table 3. Sensory acceptability scores by 9-point hedonic scale ranging from 30 participants

Quality	Cookie sample	Preception (30)									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**x̄**
Appearance	A	0	0	0	3	4	7	7	7	2	6.6 ± 0.261^{b}
	B	0	0	0	1	2	8	9	7	3	6.9 ± 0.225^{ab}
	C	0	0	0	0	2	4	14	8	2	7.1 ± 0.1778^{ab}
	D	0	0	0	1	3	0	6	9	11	7.7 ± 0.258^{a}
Colour	A	0	0	0	2	4	6	6	11	1	6.8 ± 0.248^{b}
	B	0	0	0	1	1	8	9	9	2	7.00 ± 0.209^{ab}
	C	0	0	0	0	1	4	12	10	3	7.3 ± 0.175^{ab}
	D	0	0	1	0	3	0	4	14	8	7.7 ± 0.264^{a}
Aroma	A	0	0	2	0	1	9	9	8	1	6.7 ± 0.250^{b}
	B	0	0	0	1	2	9	7	9	2	6.9 ± 0.222^{b}
	C	0	0	0	0	2	1	11	14	2	7.4 ± 0.171^{ab}
	D	0	0	0	1	3	0	3	10	13	7.9 ± 0.260^{a}
Taste	A	0	0	2	2	3	9	6	8	0	6.3 ± 0.272^{b}
	B	0	0	0	1	1	9	7	7	5	7.1 ± 0.237^{ab}
	C	0	0	0	2	2	3	11	10	2	7.0 ± 0.232^{ab}
	D	0	0	1	1	2	1	4	11	10	7.6 ± 0.290^{a}
Texture	A	0	1	2	3	4	8	7	5	0	5.9 ± 0.297^{b}
	B	0	0	0	1	1	11	7	7	3	6.9 ± 0.222^{a}
	C	0	0	0	1	2	2	14	8	3	7.2 ± 0.209^{a}
	D	0	1	1	0	2	1	3	11	11	7.6 ± 0.327^{a}
General acceptability	A	0	0	2	2	4	7	8	6	1	6.3 ± 0.280^{b}
	B	0	0	0	1	0	11	7	8	3	7.0 ± 0.214^{ab}
	C	0	0	0	0	2	3	12	11	2	7.3 ± 0.179^{a}
	D	0	0	1	0	3	1	4	10	11	7.7 ± 0.280^{a}

Key: 1 = Dislike extremely, 2 =Dislike very much, 3 = Dislike moderately, 4 = Dislike slightly, 5= Neither like nor dislike, 6 = Like slightly, 7 = Like moderately, 8 = Like very much and 9 = Like extremely. ^{abcd}Columns that do not have a common superscript are statisficant difference ($p<0.01$); Mean ± Standard error (SE)

Discussion

Tempeh fermented by *R. oligosporus*, a wild fungus and and important major or commonly used to cook soybean tempeh (Ahnan-Winarno *et al*., 2020; Food & Agriculture Organization-World Health Organization, 2017; Handoyo and Morita, 2006). This organic soybean tempeh had "high in protein" as well as nutritional claims. The protein content was higher than the expected minimums at 7% or 10%. Therefore, commercially available tempeh in the United States ranged from 10.9% to 17.7% (Ahnan-Winarno *et al*., 2020).

It concluded that two types of cookies (tempeh purple sweet potato cookie and tempeh purple sweet potato raisin cookie) were obtained from an organic soybean tempeh based cookies, which had nutritious and sustainable sources of high protein levels, as a plant-based protein snack. Both tempeh

cookie types was being good food acceptability on all qualities. Nevertheless, the application of tempeh was able to add more experiments such as a variety of substrates (Erkan *et al*., 2020), or modify from tempeh flour (Angulo-Bejarano *et al.*, 2008). In this study, the tempeh cookie products can claim with "made with organic" which followed regulation of organic USDA or other certifying bodies. However, products can be claimed as "organic" label by using all ingredients with organic certification.

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