Effect of solid-state fermentation on properties of jackfruit seed powder

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Summary

Chemical and physico-chemical properties of raw and solid-state fermented jackfruit seed powders (JSP) were determined and compared. With 2.70×10^8 CFU·ml^{-1} *Lactobacillus* sp. at 37 °C, 80% initial moisture content, pH 7 and 120 h of fermentation, crude fat and saccharides decreased by 35.8% and 2.6%, respectively, while total dietary fibre remained unchanged. Swelling, water holding, water retention, oil holding and organic molecule absorption capacities decreased by 46.4%, 50.6%, 34.6%, 16.5% and 31.5%, respectively. Solid-state fermentation changed the chemical and physico-chemical properties of jackfruit seed powder. This information is important in exploring and determining the feasibility of incorporating raw and solid-state fermented JSP in food applications.

Keywords

chemical properties; jackfruit seed powder; *Lactobacillus*; physicochemical properties; solid-state fermentation

Jackfruit seed comes from jackfruit trees (*Artocarpus heterophyllus* Lam.) that grow wild in rain forests of tropical countries [1]. The seed accounts for 10% to 15% of the total fruit weight [2, 3], and have found usage in local culinary preparations [4] and as a substrate in solid-state fermentation for pigment production [3]. However, unused seeds are often discarded [5]. Researchers in recent years have focused their studies on chemical and physico-chemical properties of jackfruit seeds in different forms for food applications. The different forms could be generalized into jackfruit seed [1, 2], seed flour [5, 6] and seed starch [5, 6]. While the chemical properties are well documented [1, 2, 5, 6], the physico-chemical properties are only sparsely reported in the literature. Information on swelling, water holding, water retention, oil holding and organic molecule absorption capacities for jackfruit seed powder are unavailable yet.

This paper reports on results of a study that involved the determination of chemical and physico-chemical properties of raw jackfruit seed powder (JSP), and a newly developed powder via solid-state fermentation (SSF) with *Lactobacillus* sp. at a single fermentation condition, namely the solid-state fermented JSP. SSF is a fermentation that allows growth of microorganisms on natural/inert substrate as support in the absence of free-flowing water, and capable of adding values and improving nutritional qualities of agricultural by-products [3, 7]. SSF was employed in this study to add values to JSP, in view of expanding its food applications, since the unutilized and discarded seeds become solid wastes.

Hence, this paper includes:
- the comparison of the chemical and physico-chemical properties of raw and solid-state fermented JSP,
- physicochemical properties of JSP that are lacking in the literature
- suggestions on how the raw and solid-state fermented JSP could be incorporated in food applications.

MATERIALS AND METHODS

Preparation of jackfruit seed powder

Jackfruit seeds were collected from fruits bought from a local night market in Melaka, Malaysia. Aril of the seeds was manually peeled off. The seeds were sliced into thin chips (2–3 mm

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thick) and dried at 60 °C for 24 h [3]. The dried seed slices were grounded into powder (particle size < 0.813 mm) using a miller (Quadro Comil, Ontario, Canada) in Forest Research Institute, Selangor, Malaysia. The jackfruit seed powder (JSP) was dried again at 60 °C for 24 h prior to further use.

Isolation of pure culture and seed culture development

A volume of 10 ml of a commercial cultured milk drink containing pure *Lactobacillus* sp. was mixed with 90 ml sterilized distilled water in a 250 ml conical flask. Serial dilutions (10^{-2} to 10^{-9}) were performed in 1.5 ml microtubes by transferring 0.1 ml from previous dilution into 0.9 ml sterilized distilled water. Volumes of 0.1 ml of dilutions 10^{-4} to 10^{-9} were plated onto Trypticasein Soy Agar (TSA, Pronadisa, Madrid, Spain). Using spread plate method and incubation at 37 °C for 48 h numbers of colony forming units (CFU) per ml were recorded. Seed culture was prepared by inoculating one loop of the isolated microbial culture into 25 ml sterilized Tryptic Soy Broth (BD Bacto, Franklin Lakes, New Jersey, USA) in a 100 ml conical flask with the flask opening stuffed with sterilized cotton wool, and incubated at 37 °C and 2 Hz for 48 h, a modified condition [8]. Plate count was performed instantly on TSA after incubation. A volume of 10 ml of the seed culture was used as an inoculum for SSF.

Solid-state fermentation of jackfruit seed powder

SSF was performed aseptically with 50 g JSP, 10 ml fresh seed culture, 21.5 ml salt solution (2 g·l^{-1} KH_2PO_4, 5 g·l^{-1} NH_4NO_3, 1 g·l^{-1} NaCl and 1 g·l^{-1} $MgSO_4 \cdot 7\,H_2O$), and 8.5 ml 1 mol·l^{-1} NaOH in a 1 000 ml conical flask stuffed with cotton wool. Operating condition was 37 °C, 120 h, initial pH 7 and initial moisture content 80% [3, 9]. Plate count (1 g fresh sample; 9 ml sterilized deionized water) was used for bacterial growth analysis. Oven drying method (3 g fresh sample; 105 °C) was used for moisture content analysis [10]. For pH analysis, 1 g of fresh sample was mixed with 9 ml deionized water (pH 7) using Vortex mixer (VTX-3000L, Harmony, Tokyo, Japan) and measured with pH meter (pH 510, EUTECH Instruments, Singapore, Singapore). Remaining JSP was autoclaved (121 °C; 15 min), dried at 60 °C for 24 h, and stored at 4 °C.

Chemical properties analysis

Raw and fermented JSP (autoclaved, dried and mortar-crushed) were analysed for chemical properties. Crude protein was analysed with Kjeldahl method (conversion factor 6.25) [10]. Crude fat was determined with a Soxhlet extraction method (using hexane as the extraction solvent) [10]. Ash content was determined by incineration at 550 °C [10]. Total dietary fibre content was determined according to the method AOAC 985.29 [11]. Saccharides content was estimated by difference [10]: saccharides [%] = 100% – (crude protein [%] + crude fat [%] + ash [%]).

Physico-chemical properties analysis

Raw and fermented JSP (autoclaved, dried and crushed) were analysed for swelling capacity (SC), water holding capacity (WHC), water retention capacity (WRC), oil holding capacity (OHC) and organic molecule absorption capacity (OMAC) [12–14].

SC was measured with 1 g sample in 20 ml of deionized water for 18 h, expressed in ml·g^{-1}.

WHC was measured with 1 g sample in 20 ml deionized water for 24 h, expressed in grams of water per gram of dried sample.

WRC was measured with 1 g sample in 10 ml of deionized water for 18 h, centrifugation at 3 000 ×*g* for 20 min, and expressed in grams of water per gram of dried sample.

OHC method was modified slightly, with 1 g sample blended in 10 ml corn oil (density, 0.91 g·ml^{-1}), centrifugation at 3 000 ×*g* for 20 min, and expressed in grams of oil per gram of sample.

OMAC was measured with 3 g sample blended in 10 ml corn oil for 24 h, centrifugation at 25 °C and 2 000 × *g* for 15 min, and expressed in grams of organic molecules held per gram of sample.

Statistical analysis

All experiments were done in triplicate. Data were analysed statistically with independent sample *t*-test (Statistical Package for Social Sciences version 11.5, SPSS, Chicago, Illinois, USA). Significance level was set at 0.05.

RESULTS AND DISCUSSION

Isolation of pure culture and solid-state fermentation of JSP

Pure culture counts of *Lactobacillus* sp. isolated were (6.33 ± 0.20) × 10^8 CFU·ml^{-1}. After incubation at 37 °C and 2 Hz for 48 h, the plate counts were (2.70 ± 0.83) × 10^8 CFU·ml^{-1}. These counts indicated the initial counts for SSF inoculum. After SSF, the bacterial counts were (8.97 ± 0.13) × 10^8 CFU·g^{-1}, and were significantly higher than the initial counts, indicating good microbial growth. The final moisture content of the fermented JSP was (80.4 ± 2.5)%, which was insignificantly different from the initial moisture content. Moisture content was maintained in SSF. On the other hand, pH of fermented JSP dropped to pH 4.54. This could be attributed mainly to lactic acid production [15]. Lactic acid analysis is not reported here because the focus of the study was on the chemical and physico-chemical properties.

The increase in microbial counts after SSF indicates that JSP was a good substrate that was able to support the growth of *Lactobacillus* sp. at the described SSF conditions. This could imply that since JSP could support the growth of *Lactobacillus* sp. well, fermented JSP could possibly be used as means for the introduction of probiotics into various food products [16].

Effect of SSF on the physical appearance of JSP

There was a large variation in particle size before and after SSF (Fig. 1) due to agglomeration and clumping of JSP particles when hydrated with 80% moisture content. It was observed that the JSP particles agglomerated and clumped together once hydrated, and this was very likely to have resulted from formation of liquid bridges among the particles, like in tea powder [17]. The agglomeration happened rapidly and could not be avoided. It increased the particle size unevenly and we believe that it changed the particle structure through the formation of liquid bridges. Due to the changes in particle size, fermented JSP had to be crushed with mortar and pestle for size reduction in an attempt to even up the particle size and reduce the occurrence of analysis error where surface area played a significant role, such as in the physico-chemical properties. Colour change in JSP was caused by autoclaving before and after SSF. There could be chemical reactions like non-enzymatic browning reaction as a result of heating [18].

Chemical properties of raw and fermented JSP

Chemical properties of raw JSP determined in this study (Tab. 1) were comparable to those reported by TULYATHAN and co-workers [5]. While crude protein and ash of raw JSP were lower than those of jackfruit seed [1, 2], the saccharide content was higher. These differences were attributed to the difference in species, environment conditions and maturity of the used materials [1]. Crude fat content determined by AJAYI was extracted with petroleum ether [1], and was higher than the hexane-extracted crude fat in this study. Different solvents could result in different amounts of extracted fat [19]. When compared with jackfruit seed flour, chemical properties of raw JSP varied slightly. This could be due to different sample preparation methods. Jackfruit seed flour had been lye-peeled to remove the brown spermoderm of the seed [5, 6]. The presence or absence of the spermoderm might have contributed to the differences. When compared with jackfruit seed starch, the values of chemical properties of raw JSP were higher, which might be due to different preparation methods.

Comparing the raw and fermented JSP, crude protein and ash increased significantly after SSF. However, it is uncertain at this stage what were the reasons for the increase in both crude protein and ash. The increase in protein and ash could be due to protein enrichment and single-cell protein production in SSF, as demonstrated in SSF using fungal strains [20] and in microbial fermentation [8], respectively. It could also be caused by the salt so-

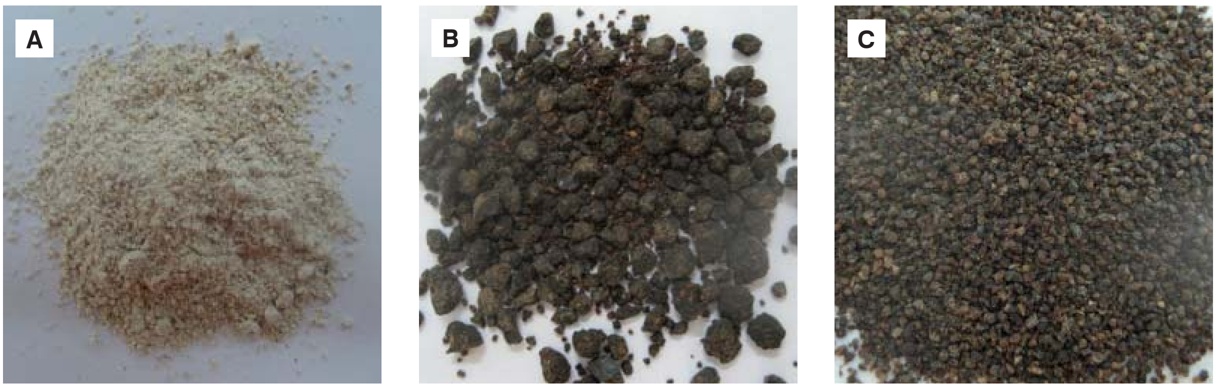

Fig. 1. Jackfruit seed powder (JSP).
A – Dried raw JSP, B – fermented JSP after autoclaving and drying, C – crushed fermented JSP after autoclaving and drying.

Tab. 1. Chemical properties of various forms of jackfruit seed.

Chemical properties (% of dry weight basis)	Jackfruit seed		Jackfruit seed powder				Jackfruit seed flour			Jackfruit seed starch
	Cotyledons		Raw	Fermented			Dry milled	Wet milled		
Crude protein [%]	31.95	20.19	11.68 ± 0.10 ^{a}	13.22 ± 0.08 ^{b}	11.02	11.17	12.63	10.40	0.37	0.90
Crude fat [%]	1.30	11.39	1.09 ± 0.21 ^{a}	0.70 ± 0.12 ^{b}	1.01	0.99	2.34	2.12	0.73	1.00
Ash [%]	–	6.72	3.95 ± 0.01 ^{a}	4.83 ± 0.05 ^{b}	3.97	3.92	3.43	3.51	0.25	0.19
Total dietary fibre [%]	–	–	3.56 ± 0.06 ^{a}	3.83 ± 0.19 ^{a}	–	–	–	–	–	–
Saccharides [c] [%]	65.19	51.82	83.39 ± 0.27 ^{a}	81.24 ± 0.19 ^{b}	81.64	82.25	–	–	–	–
References	2	1	Present study *		5 [d]	5	6		2	6

* Values for JSP are reported as (mean ± standard deviation) and values in each row that are followed by different superscripted letters (a and b) are significantly different ($p < 0.05$).

[c] By difference [10]: saccharides [%] = 100% – (crude protein [%] + crude fat [%] + ash [%]).

[d] Jackfruit seed flour (with brown spermoderm) [5] is reported as JSP due to a similar processing method to avoid confusion.

lution supplemented to JSP prior to SSF. NH_4NO_3 as nitrogen source could have caused overestimation of the crude protein if it was not fully utilized by the microorganism after 120 h of SSF. Similarly for ash, the various mineral salts could have affected the accuracy of analysis. This uncertainty will be further addressed in future works involving SSF with two types of JSP, viz. supplemented and non-supplemented JSP. Strategies that will be employed will include optimization of SSF conditions, determination of microbial growth kinetics and monitoring changes in chemical properties over time.

Crude fat and saccharides of fermented JSP decreased significantly while total dietary fibre (TDF) showed no significant difference. The drop in crude fat might be due to the breakdown of lipids, as *Lactobacillus* sp. is weakly lipolytic [21]. The drop in saccharides was most likely caused by fermentation of saccharides for microbial culture growth and production of metabolites during SSF. The unchanged TDF could possibly result from no utilization of fibre components of JSP, due to the lack of a suitable enzyme to break down the fibre.

Few implications could be drawn here for the potential use of JSP in food applications. Low fat content of both raw and solid-state fermented JSP suggests their use as ingredients in the development of low fat food products, though there is a limit for their use in food that requires the functionality of lipid component [18]. High saccharides content of raw JSP suggests its higher potential as substitute for flour in baked products, and this explains why the jackfruit seed had been processed into jackfruit seed flour or jackfruit seed starch [2, 6].

Physico-chemical properties of raw and fermented JSP

Information on SC, WHC, WRC, OHC and OMAC for JSP is sparse in the literature, but water and oil absorption capacities for jackfruit seed flour had been reported [5]. They were actually equivalent to WRC and OHC, respectively, because samples were subjected to centrifugation in both analyses. WRC of raw JSP ($3.76\ g \cdot g^{-1}$) was higher than jackfruit seed flour ($2.05\ g \cdot g^{-1}$), and OHC of raw JSP ($1.09\ g \cdot g^{-1}$) was lower than the seed flour ($1.41\ g \cdot g^{-1}$). This could be attributed to the absence of brown spermoderm in the seed flour.

An attempt was made to compare JSP with other fibre sources reported in the literature, as presented in Tab. 2. Fibre sources are known to be mainly associated with soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) components, and physico-chemical properties are known to be mainly associated with the IDF [14, 22]. As presented in Tab. 2, values of all physico-chemical properties of JSP decreased significantly after SSF. SC, WHC, WRC, OHC and OMAC decreased by 46.4%, 50.6%, 34.6%, 16.5% and 31.5%, respectively. The main reason for the decrease in values of all physico-chemical properties could be predominantly due to the increase in particle size of JSP as a result of particle agglomeration (Fig. 1). Larger sizes are known to correspond to the reduction in theoretical surface area and total pore volume, where less liquid is bound by surface tension in the pores or by hydrogen bonding, ionic bonding and/or hydrophobic interactions with particle surface area [14, 22, 23]. It is uncertain if SSF had altered the fibre structure such as pore size and decreased the liquid binding ability of the fibre. In fact, it is difficult to ascertain this because in order to do so, fermented JSP would have to be ground to the same particle size (< 0.813 mm) prior to measuring the physico-chemical properties. Grinding the fermented JSP will change the fibre structure of the sample because grinding itself is an independent factor of the physico-chemical properties [23]. Nevertheless, to even up the sizes of agglomerated JSP while minimizing the effect of size reduction on fibre structure, the fermented JSP was only subjected to crushing with mortar and pestle. Another possible reason for the decrease in values of physico-chemical properties was heating of JSP before and after SSF through autoclaving, where possible alteration of physical properties could have taken place [24]. OMAC was higher in raw JSP probably because IDF of raw JSP was higher than that of the fermented JSP. High IDF is known to be able to absorb organic molecules more effectively [14].

Since raw JSP had better physico-chemical properties than fermented JSP, it was compared with other fibre sources (Tab. 2). SC was in the range of SC values reported for bamboo [13], maize bran, oat bran [24] and pea [22], lower than fibre of apple, carrot and wheat [22], and higher than palm kernel cake [25]. WHC was higher than apple fibre, carrot, pea [22] and bamboo [13]. WRC was in the range of values of WRC for apple fibre [22], bamboo [13] and oat bran [24], lower than chia seed, shoyu mash fibre and soya fibre [14], and higher than maize bran [24], palm kernel cake [25], pea and wheat [22]. OHC was lower than barley fibre, chia seed, jackbean fibre, pea fibre and shoyu mash fibre [14]. OMAC was lower than chia seed, but higher than passion fruit fibre and soya fibre [14].

Based on the physico-chemical properties of raw and fermented JSP, some implications may be drawn. SC, WHC and WRC of raw JSP were higher, suggesting the feasibility of incorporating

Tab. 2. Physico-chemical properties of raw JSP, fermented JSP and other fibre sources.

Sample	SC [$ml \cdot g^{-1}$]	WHC [$g \cdot g^{-1}$]	WRC [$g \cdot g^{-1}$]	OHC [$g \cdot g^{-1}$]	OMAC [$g \cdot g^{-1}$]	References
Apple fibre	9.00	4.50	3.50^{c}	–	–	22
Bamboo	5.69	4.83	3.45^{c}	–	–	13
Barley fibre	–	–	–	2.00	–	14
Carrot	7.50	3.80	3.10^{c}	–	–	22
Chia seeds (fibre rich fraction)			15.41^{d}	2.02	1.09	14
Jack bean fibre	–	–	–	2.30	–	14
Maize bran	5.70	–	2.40	–	–	24
Oat bran	5.53	–	3.50	–	–	24
Palm kernel cake	3.44	–	3.08	–	–	25
Passion fruit fibre	–	–	–	–	0.28	14
Pea	5.50	3.50	2.70^{c}	–	–	22
Pea fibre	–	–	–	6.93	–	14
Resistant starch	5.60 – 7.40	–	3.10 – 3.50	–	–	24
Shoyu mash residue	–	–	10.85^{d}	5.36	–	14
Soybean fibre	–	–	4.90^{d}	–	0.65	14
Wheat	7.50	3.10	2.50^{c}	–	–	22
Raw JSP*	5.78 ± 0.26^{a}	6.01 ± 0.11^{a}	3.76 ± 0.14^{a}	1.09 ± 0.00^{a}	0.73 ± 0.02^{a}	Present study
Fermented JSP*	3.10 ± 0.10^{b}	2.97 ± 0.10^{b}	2.46 ± 0.13^{b}	0.91 ± 0.00^{b}	0.50 ± 0.00^{b}	Present study

SC – swelling capacity, WHC – water holding capacity (grams of water per gram of dried sample), WRC – water retention capacity (grams of water per gram of dried sample), OHC – oil holding capacity (grams of oil per gram of sample), OMAC – organic molecule absorption capacity (grams of organic molecules held per gram of sample).
* Values are reported as (mean ± standard deviation) and values in each column that are followed by different superscripted letters (a and b) are significantly different ($p < 0.05$).
c – Due to similar analysis method (sample subjected to centrifugation), the water binding capacity (WBC) [13, 22] is reported as water retention capacity (WRC) to avoid confusion.
d – Due to similar analysis method (sample subjected to centrifugation), the water holding capacity [14] is reported as water retention capacity (WRC) to avoid confusion.

raw JSP in food applications that require ingredients with better water binding characteristics, such as starch gelatinization in baked food products [13]. OHC of fermented JSP was lower, suggesting better potential for fermented JSP to be incorporated into fried food products in order to avoid absorption of excessive fat and to provide non-greasy sensation [14]. Higher OMAC of raw JSP suggests that when raw JSP is incorporated into food and consumed by humans, it could have better capacity in eliminating fats, bile acids, cholesterol, drugs and carcinogenic compounds from the faeces by binding to these substances in the intestines [14, 24].

CONCLUSIONS

1. Solid-state fermentation of jackfruit seed powder (JSP) with *Lactobacillus* sp. improved the chemical properties but decreased the values of physico-chemical properties of the powder.
2. Data on swelling, water holding, water retention, oil holding and organic molecule absorption capacities of JSP, that were previously lacking in the literature, are reported in this paper, and may be used as reference for food applications involving JSP.
3. Selection between raw and solid-state fermented JSP for food applications should be based on the desired chemical or physico-chemical properties. Solid-state fermented JSP should be chosen for applications that require improved chemical properties, likewise raw JSP for higher values of physico-chemical properties.

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